The Secretariat of the Pacific Community

AusAID/SPC
TARO GENETIC RESOURCES:
CONSERVATION AND UTILIZATION

TARO CONSERVATION STRATEGY WORKSHOP REPORT

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1.0 Contents

2.0 Introduction

The Taro Genetic Resources: Conservation and Utilisation (TaroGen) is a three-year project, funded by the Australian Government (AusAID), and implemented by the Secretariat of the Pacific Community (SPC). In collaboration with the International Plant Genetic Resources Institute (IPGRI), and the University of the South Pacific (USP), TaroGen is working with national programmes in developing a regional strategy for taro genetic resource conservation and improvement. The impact of taro leaf blight (TLB) on taro cultivation in the Samoas, the loss of taro genetic resources occurring in the Pacific Island countries, (PICs), and continuing vulnerability of other PICs to TLB was the major impetus behind the development of TaroGen. A unit has been established within SPC to provide the expertise required in conservation, plant breeding and project management. This unit includes the Regional Germplasm Centre, (RGC), which has a mandate to conserve and promote utilization of various crop species in the region important for food security. The RGC currently holds collections of taro, sweet potato, banana, bele and yam, and is researching into improved methods of multiplication and conservation for these crops.

The goal of TaroGen is to improve food security and rural incomes in PICs through the conservation of taro genetic resources and support to regional taro improvement programmes. The project has three objectives:

?? To describe and conserve the majority of taro genetic diversity in the Pacific region.
?? To provide farmers in PICs with taro varieties, which have improved resistance to TLB.
?? To provide support to implementing agencies so they can effectively and efficiently manage the project.

TaroGen has been working with PICs and IPGRI in the collection and characterization of regional taro germplasm. By September 2001, over 2,400 taro accessions have been collected and described. Using morphological characterisation data, IPGRI has assisted the project in the rationalization of this collection, and the selection of a taro core subset. This subset has been further analysed by the University of Queensland (UQ), using DNA fingerprinting technology to select a final taro core collection. This core collection will be conserved *in vitro* at the SPC RGC. As well as investigating *in vitro* technologies, TaroGen has also been evaluating seed storage and on farm conservation.
The TaroGen workshop brought together participants from the region to review and discuss results from the rationalization process. The analyses used by IPGRI and UQ to select the core were presented and the final composition of the core examined by the countries. Countries were given the opportunity to modify the accessions within their country core, if they felt that other accessions should be included. In addition to the rationalization work, the results of the various investigations into conservation methodologies were also discussed. There were two outcomes from these discussions, namely the taro complementary conservation strategy paper, and recommendations for further action. These recommendations focussed on validation and utilization of the core, so that this collection benefits growers from the PICs. Recommendations were also made on future investigations into conservation methodologies.

3.0 Collection of taro from the Pacific

For most countries in the Pacific, the opportunity to collect, document and exchange their taro varieties came with assistance from three UNDP/FAO root crop projects in the 1980s. Prior to this, only Fiji, PNG, New Caledonia and the Solomon Islands had assembled significant collections of root crops. By 1986, most countries had established collections of taro, although there were differences of opinions as to how representative each collection was. A follow up survey of taro collections in the region in 1994 (Jackson, 1994) recorded total losses in the Cook Islands, Niue, Solomon Islands, Tonga and Vanuatu. Collections still remained in Fiji, PNG, New Caledonia and several smaller countries. Documentation and evaluation of the collections was also a problem.

As many of these collections were either completely lost, or had lost a significant number of accessions, the TaroGen project had to generate collections from all the countries participating in the project.

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3.1 Collection, characterization and documentation of taro collections from the Pacific

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Introduction

A taro collecting strategy workshop was held at the commencement of the TaroGen project in December 1998. The detailed outcomes of this workshop have been reported in various project publications. A regional approach was adopted to enable a large part of the crop gene pool to be sampled at one time and common methods used for collecting, describing and documenting germplasm. This approach has assisted in the rationalization of national collections and the identification of a core collection representing the taro diversity in the region which will be conserved in the RGC based at SPC, Suva. During this workshop collecting strategies and plans for PICs were devised and agreement reached on a set of IPGRI descriptors to be used for germplasm characterization. Training was also provided on genetic resource databases. By September 2001 the number of accessions collected and described within the TaroGen project was over 2,400 (Table 1).

Papua New Guinea

Collecting was carried out by four teams each comprising two research staff and an extension officer familiar with the area. Collecting was completed by October 1999 and accessions were forwarded to Bubia Agricultural Research Centre (BARC) in Lae, where the national collection was established. Ethnobotanical information collected during the missions was entered into a database.

In order to compare the accessions at the same stage of growth the collection was replanted in February 2000. Morphological description of the accessions was completed and a database prepared using a set of IPGRI descriptors. The database was forwarded to IPGRI for analysis and selection of a core sub-set. Accessions from this sub-set were sent to UQ for molecular analysis and final selection for the core. A total of 151 accessions were used for molecular analysis and selection of the final core.

Solomon Islands

Four teams comprising of three staff, which included an extension agent from the collecting area, carried out the collecting. Collecting started in July 1999 and, in spite of the ethnic tensions in the country, was completed by the end of the year in all provinces except Guadalcanal. It was not possible to collect in Guadalcanal Province due to unrest in the area. The accessions were planted at Fote Experiment Station in Malaita where the national collection was established. The ethnobotanical information recorded during collecting was entered into a database. As in PNG, the Solomon Islands collection was also replanted in February 2000 to compare accessions at the same stage of growth.

Unfortunately, the Solomon Islands 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) decided to re-collect the taro in Solomon Islands and this commenced with an initial planning meeting at Fote Research Station, Malaita from 22-27th June, 2001 attended by TaroGen, an NGO (Planting Materials Network/PMN) and the Agriculture Research Division. Two-three day awareness raising and planning workshops were subsequently held in Malaita, Guadalcanal, Temotu and Choiseul provinces through the month of July and into early August. The workshops included farmers (PMN members and others), local agriculture extension staff and research division resource people (Malaita and Choiseul), and PMN facilitators. Workshop participants were trained in and planned the collection process, field genebanks and diversity fairs. Following the planning workshops collection of taro was undertaken by teams of PMN members assisted where possible by Research Division and Agriculture Officers in the four provinces. In Guadalcanal and Temotu no research officers were available so the PMN allocated extra personnel and support visits. Collection teams recorded passport and morphological descriptors in the field during collection. As expected, morphological descriptors caused some difficulties due to many taros not being mature and the difficulty of visiting gardens to observe all taros in the field. Field genebanks have been established in four provinces and currently hold a total of 824 accessions: Malaita (313); Guadalcanal (220); Choiseul (245) and Temotu (46). The collections held in the field genebanks, will be redescribed in early 2002, prior to data analysis by IPGRI and UQ.

Vanuatu and New Caledonia

Vanuatu undertook to collect under its national programme and maintained a collection of 502 accessions. The New Caledonia collection, also collected and maintained by its national programme, had 82 accessions. Both these collections were described using the Taro Network for South East Asia and Oceania (TANSAO) descriptors, which differed from the set used by TaroGen. The six-monthly project review meeting in Lae, PNG recommended to use the TANSAO descriptors to select the country cores in these countries. The databases for these collections were obtained from the TANSAO project and forwarded to IPGRI to select a core sub-set. This core sub-set was forwarded to UQ for molecular analysis before deciding on the final core sample. A total of 89 accessions from Vanuatu and 18 from New Caledonia were used for molecular analysis.

Fiji

The Fiji collection of 72 accessions was maintained by the national programme. Samples of 70 accessions were forwarded to UQ for molecular analysis and selection of accessions for the core collection.

Polynesia

In all, 67 accessions were collected from Cook Islands, Niue, Samoa and Tonga. Ethnobotanical information collected during the visits was entered in a database. In addition, 12 Palau accessions with resistance to TLB were obtained from Samoa and included in the RGC. The Polynesian accessions will need to be virus indexed at QUT before they can be grown in Fiji for morphological description. A total of 68 accessions from Niue, Tonga, Samoa, Cook Islands and Palau were forwarded to UQ for molecular analysis and final selection of accessions for the core.

Table 1. The location of taro collections and number of accessions in the Pacific Islands.

Country	Location of Collection	Number of Accessions
Papua New Guinea	BARC, Lae	859
Solomon Islands	Malaita (313), Guadalcanal (220), Choiseul (245) and Temotu (46)	824
Vanuatu	VARTC, Santo	502
New Caledonia	-	82
Fiji	Koronivia Research Station	72
Niue	SPC, Nabua, Fiji	25
Samoa	SPC, Nabua, Fiji	15
Tonga	SPC, Nabua, Fiji	9
Cook Islands	SPC, Nabua, Fiji	18
Palau	SPC, Nabua, Fiji	12
Total		**2418**

3.1 *In Vitro* collecting: the transfer of taro from the field to the tube.

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Introduction

In recent years there has been an increasing awareness of the presence of endogenous bacteria in tissue cultures. The following genera have been isolated from plant cultures: *Acinetobacter, Agrobacterium, Bacillus, Corynebacterium, Enterobacter, Erwinia, Flavobacterium, Lactobacillus, Micrococcus, Pseudomonas, Staphylococcus, Torulopsis, Xanthomonas*, and yeasts (Leggatt *et al.*, 1988; Buckley *et al*., 1995). There is a general consensus that some of these bacteria are introduced into tissue culture with the explant. Populations of viable bacteria can exist in substomatal cavities or intercellular spaces. In these cells, bacteria will be protected from contact with chemicals used to disinfest the plant surface. Certain bacteria have also been reported to reside endophytically in vascular tissues of apparently healthy plants. These bacteria are more difficult to eradicate due to their internal nature. Bacterial contamination is often difficult to detect. With some bacteria, their presence is detected within the first stage of culture. It is likely that these are gram negative species, commonly associated with field-grown plants and the soil. However, because plant tissue culture medium does not necessarily support the growth of bacteria, and often exudates from the plant itself are required for bacterial growth, bacteria can remain undetected for several subcultures. Contaminated plants may lack obvious symptoms, but have reduced multiplication rates or reduced rooting rates. With others, death may occur for no seemingly obvious reason.

Methodology

Some taro tissue culture samples were sent to the Central Science Laboratory in 1994 for bacteria identification. The bacteria identified were *Pseudomonas* spp. and *Methylobacterium* spp. In 1999, taro tissue cultures were tested by CABI, UK. The bacteria found were *Bacillus* spp. *Pseudomonas* spp. *Curtobacterium* spp. *Micrococcus* spp. and an unknown coryneform (gram +ve bacteria). All of these bacteria with the exception of *Micrococcus*, are widely found in nature. *Bacillus* and *Pseudomonas* are found primarily in the soil. *Pseudomonas* spp are commonly found to be early contaminants of tissue cultures, probably because they are often the dominant bacteria associated with aerial surfaces of plants grown under field conditions.

Although it is argued that antibiotics can be used for the treatment of bacteria, it is not recommended. Antibiotics can be used but often their effect is only bacteriostatic, so only masking of the contaminants is achieved through their use. In addition, selecting drug-resistant strains of bacteria from long-term exposure to antibiotics is also of concern. Permanent use of antibiotics was found to select for antibiotic resistant bacterial species such as *Pseudomonas fluorescens*, (Leifert *et al*., 1994), one of the bacteria identified in taro samples in 1994. Treatment of unidentified mixed populations of microorganisms and the prophylactic use of antibiotics should, therefore be avoided.

For all of the taro collections now established in tissue culture in the SPC RGC taro plants were sampled directly from the field. In many of the countries there was no tissue culture facility so suckers had to be harvested and shoot-tips initiated into tissue culture at a later stage. The shoot-tip is sampled from plant tissue, which is very near to the soil. Soil-borne bacteria are very likely to be found in tissue taken from plant parts at or below soil level. With all of the collections, bacteria contamination was a problem, but with some collections, the problem was worse than with others. One of the recommendations when sampling plants for tissue culture is that the stock plants should be kept as dry as possible. On both occasions, when suckers were taken from the field collection in Santo, Vanuatu, it had been raining heavily (on one occasion, there had been a cyclone the day before the suckers were harvested). Accessions initiated into tissue culture from the Vanuatu field collection were heavily infested with bacteria.

It is also recommended that explants for tissue culture should be taken from very young tissues, as these are often found to be free of detectable internal populations of micro-organisms. There were two collections made in PNG. For the first collection, the explants were taken from suckers that were more than six months old, whereas with the second collection, the suckers were about four months old. In addition, with the second collection, suckers were harvested in the morning and explants from these were initiated into tissue culture on the same day. With the second collection in PNG, bacterial contamination was at a minimum, unlike the first collection, where accessions were lost because of bacterial problems. It is the same story with Vanuatu. Not only were suckers harvested in wet conditions but, at the time of harvesting the suckers for tissue culture, the crop itself was ready for harvesting. In addition, there are no tissue culture facilities in Santo, and so there was a significant delay between harvesting of the suckers and initiation of tissue cultures.

Bacterial contamination can be a major problem in establishing tissue cultures of taro directly from field material. This can be very wasteful of resources, especially when collections are made from other countries and then transferred elsewhere, as in the case of the TaroGen project. Lessons have been learnt from this exercise, and the following recommendations should be taken into account when field-grown suckers are going to be used for the initiation of tissue cultures.

Recommendations

?? If possible suckers should be screened using a microbial detection medium, (523 medium) before they are used for tissue culture.

?? Suckers for use as the source of explants should be as young as possible. Using mature plants as the source of explants should be avoided.

?? Every attempt should be made to harvest in dry weather. If this is unlikely then a solution would be to harvest suckers and plant in a screenhouse until it is time for tissue culture initiation.

?? There should be as short a delay as possible between harvesting the suckers and excising the explant for tissue culture.

?? As bleach loses its activity rapidly at room temperature, (all activity is lost after 12 months), and this loss accelerates with increasing temperature, solutions of calcium hypochlorite should be used for sterilization.

?? Tween, as a wetting agent, should always be used in the sterilization solution as it does aid the penetration of hypochlorite into small cavities on the plant surface.

?? It could be useful to swab the sucker with alcohol as it is being trimmed down for explant removal, as this avoids the smearing of surface micro-organisms onto the cut surfaces during excision.

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4.0 Rationalization of Pacific Island Collections

The concept of a core collection was first proposed by Frankel, (1984), in response to the realization that the size of many plant germplasm collections hindered the use of that collection. The original definition of a core collection was " a limited set of accessions representing, with a minimum of repetitiveness, the genetic diversity of a crop species and its wild relatives". From this definition, two more operational definitions have evolved:

- ?? For an individual genebank, a core collection consists of a limited number of the accessions in an existing collection, chosen to represent the genetic spectrum in the whole collection. It should include as much as possible of its genetic diversity. (Brown, 1995).
- ?? For a whole crop species, a core collection consists of a limited number of entries chosen to represent the genetic diversity of the whole crop species and its wild relatives. This collection is assembled cooperatively by national and international genebanks. It is also supplemented with fresh samples of wild or crop populations, where needed to fill the gaps

Since Frankel's proposal, the concept of a core collection has become accepted. The Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (FAO, 1996) recommends core collection development as one of the activities required to improve the use of plant genetic resources. A core collection will always be substantially smaller than the collection from which it is formed. Brown (1989b) suggested that it should be no more than 10 per cent of the whole collection, and always less than 2,000 entries.

As discussed, the total number of taro accessions in the collections made under TaroGen exceeded 2,000. It was not realistic to expect all of the countries to have sufficient resources to maintain their individual collections. In addition, the RGC did not have sufficient capacity overall to allocate the resources necessary to conserve this number of taro accessions. Finally, the relatively large size of the entire collection would have had a negative impact on its effective evaluation and utilization, especially in a region where each country has very limited resources with which to carry out this work. Therefore, it was agreed that a core collection would be developed from the entire collection, using morphological and molecular data.

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4.1 Selection of a taro germplasm core subset using morphological descriptors

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Introduction

The passport and characterization data recorded for taro collections from New Caledonia, Papua New Guinea and Vanuatu was used for data analysis and identification of core sub-sets for each country collections. It was also agreed that a sample of 20 per cent core sub-set would be identified based on information available for passport and characterization data. These identified core sub-sets would then be further analyzed for molecular characterization and a final selection of 10 per cent core sub-set would be identified based on molecular data. The methodology used for developing these core sub-sets are discussed below:

Methodology used for identifying Vanuatu taro core collection

The Vanuatu taro collection contained 452 accessions, and data had been recorded for 22 descriptors. All these descriptors were recorded on a qualitative scale of 1 to 8, except for dry matter yield. Based on literature survey, the collections were first stratified using meaningful characters viz. formation of stolen (present and absent), growing conditions (flooded and rain fed) and maturity period (early – 4 to 8 months and late – 8 to 11 months). Further sub-grouping was done based on maturity period recorded as early types, (4 to 8 months) late types, (8 to 11 months) The final sub-classification based on these three descriptors resulted in 8 groups. However, two of these groups contained over 100 accessions, and so it was decided to further classify these groups into sub-groups using Ward's method of the hierarchical clustering and the distance used was the Euclidean. The various descriptors used for this classification 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. As a result of this a total of 22 clusters were identified and final selection of the core collection 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. The SYSTAT programme was used for clustering analysis using Ward's method of clustering and S-Plus was used for PCA. As a result of these analyses a total of 93 collections (20 per cent) were identified as a sub-set of Vanuatu taro collection.

Methodology used for identifying New Caledonia taro core collection

22 descriptors were used to describe the 81 accessions in the New Caledonia collection. Since there was no variation with regards to characters such as growing type, growing conditions, botanical variety, growth habit, presence of stolen, initial stratification, based on these characters, was not possible as in the case of Vanuatu collections. Hence, different clustering methods were evaluated and it was found that Ward's method gave a clearer clustering pattern compared to other methods. Therefore, Ward's method using Euclidean distance was used for clustering these accessions. As a result, 10 clusters were identified, which were analysed for PCA scores to identify the most diverse accessions

within each cluster. Based on PCA scores, a total of 19 collections were identified as a core sub-set for the New Caledonia taro collection.

Methodology used for identifying Papua New Guinea taro core collection

For the PNG taro collection, passport and characterization data for 814 taro accessions was obtained. Initial groupings were based on three morphological characters, as they were shown to have significant differences in the taro collection. These characteristics were corm flesh colour, presence of stolon and leaf blade colour variegation. These initial groupings resulted in 12 distinct groups, however, seven groups had relatively large numbers of accessions, and so these were further divided into sub-groups. To achieve this, characterization data was used, and subjected to cluster analysis using Ward's method of hierarchical clustering. As with the other collections, the distance used was Euclidean. This analysis resulted in 39 clusters. These clusters were further analyzed for PCA scores. As a result of these analyses, a total of 163 accessions were identified as a core sub-set of the PNG taro collection. As with the Vanuatu collection, the SYSTAT programme was used for the clustering analysis and S-Plus 2000 software was used for PCA.

Other Pacific Island country collections

Collections from the Polynesian countries, and from Fiji could not be analyzed using this methodology as there was no characterization data available. These collections will be analyzed and a 10 per cent core sub-set identified using molecular data.

4.2 Core collections and molecular markers.

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Introduction

Advances in molecular and cell biology over the past ten years have led to the development of a wide range of techniques for manipulating and describing genomes, collectively referred to as biotechnology. These molecular techniques are being increasingly used to address issues relating to plant genetic resources conservation and utilisation.

The new molecular tools available offer many advantages over the traditional methods for managing plant genetic resources. Classical methods used in germplasm management have relied upon morphological characters. However, these characters can be influenced by environmental factors. Molecular markers avoid many of the complications of environmental effects acting upon characters by looking directly at variation controlled at the genetic level. Molecular markers therefore represent a powerful and rapid method for managing diversity of plant genetic resources.

The advantages of using molecular techniques as compared to the more traditional, morphological approaches can be summarized as follows:

?? high levels of polymorphism; the exact level of polymorphism detected varies depending on the method.
?? co-dominant inheritance; the different forms of the marker should be detectable in diploid organisms to allow the discrimination of homozygotes and heterozygotes.
?? occur throughout the genome; except when a marker is a specific locus, the marker should be evenly and frequently distributed throughout the genome.
?? easy, fast and cheap to detect
?? reproducible/ transferable within and between laboratories.

Unfortunately, there is no single marker that meets all these needs. Some will be useful because they are co-dominant and can be used in plant breeding, others can detect variation in a wide range of different species and are therefore widely applicable, and some will be much more reproducible and transferable than others.

What molecular tools are currently available?

Currently, there are five main molecular tools used in the management of plant genetic resources. These are: RFLPs; RAPDs; ISSRs; AFLPs; microsatellites. Microsatellites is one of the most popular techniques, (Morgante and Olivieri, 1993). Microsatellites exploit hypervariable regions in the genome, which are comprised of tandemly repeated simple sequences, e.g. 'AAAAAA', 'CACACA'. Hence, microsatellites are also known as Simple Sequence Repeats (SSRs), and also, more generally, VNTRs (variable number of tandem repeats). These repeats vary in number and length, and are known as microsatellites when the basic repeat unit is around two to eight base pairs in length.

Microsatellite markers have many advantages, namely that they are often highly polymorphic, because of their high mutation rate, they are co-dominant, highly reproducible, they don't require the use of radioactivity for their detection and multiplex reactions can be run to speed up the process where the products have non-overlapping size-ranges. The main drawback of this technique lies in the fact that prior sequence knowledge of the genome is required to design the probes, which can be costly and time-consuming.

When deciding which technique to use, many other factors need to be considered as well as simply comparing the various merits of the markers available. In particular these are:

?? What is the specific question being addressed - taxonomic or more ecological based, or is the focus inter-specific or intra-specific.
?? The capital investment and skills available
?? The 'starting position' of the plant in question, in terms of whether previous molecular investigations have been carried out.

Different techniques sample the genome differently and provide different information with respect to the diversity questions being raised.

Application of microsatellite methodology to taro genetic resources

The DNA fingerprinting part of the project was undertaken as part of the ACIAR-funded project, 'Virus indexing and DNA fingerprinting for the international movement and conservation of taro germplasm.' The overall aims of the molecular marker work are:

?? to facilitate identification and management of taro genetic resources
?? to develop Pacific Island countries capabilities in DNA fingerprinting

The specific objectives are:

?? to develop and apply various markers to taro germplasm to develop cultivar genotype fingerprints. The markers to be used will be initially microsatellites, but it is envisaged that other techniques such as ISSRs and AFLPs will be used too.
?? to use DNA marker information for genetic analysis of Pacific Island taros
?? to establish a computer database incorporating DNA fingerprint data

<u>Marker Development.</u>

Microsatellites

Following optimization of the protocol described by Edwards *et al*., (1996) for the creation of microsatellite enriched genomic libraries, the following synthetic oligonucleotide fragments have been used to enrich for microsatellite containing DNA fragments: [CT]15, [CA]20, [GA]15, [ACC]10, [GT]15, [GAC]10, [CAT]10, [AGC]10, [GATA]8, [GAG]10, [GAA]10, [AGA]12, [ACT]10, [TAA]10. In total, 269 putatively positive clones have been sequenced. Overall, 77 of the 269 clones contained SSRs (28.6%). The majority of the repeat motifs were either dinucleotide or trinucleotide repeats, of which 40.3 per cent were compound perfect repeats and the remaining 59.7 per cent were compound imperfect repeats. One heptamer was also recorded with two repeats. The dinucleotide repeats had, on average, higher numbers of repeats (nine) than the trinucleotide repeats (seven). The most common motif found in taro was GT/CA. In total, primers have been designed for 41 SSRs and 16/41 (39 per cent) show polymorphism, when screened against a small representative sample of germplasm from the region.

ISSRs

The ISSR fingerprinting technique has also been studied for its use in distinguishing taro genotypes. ISSR differs from the microsatellite marker approach in that no prior sequence knowledge is required. The technique can therefore be applied immediately to the germplasm, with only a small amount of optimization necessary for different taxa. However, the ISSR approach does have limitations, principally that they are dominant markers, and hence homozygotes can not be distinguished from heterozygotes (Godwin *et al*., 1997).

The technique has been applied to many different plant taxa for genetic diversity analysis e.g. lupin (Gilbert *et al.,* 1999), chickpea (Ratnaparkhe *et al*., 1998), wheat (Nagaoka and Ogihara, 1997) and rice (Blair *et al*., 1999). Primers based on a repeat sequence, such as (CA)n, are designed with a 3'-anchor, such as (CA)8RG or (AGC)6TY (where R=purines: G or A; Y=pyrimidines: C or T). The resultant PCR reaction amplifies the sequence between two SSRs.

33 primers were initially screened, and four were selected, based on the clarity of the bands, the presence of polymorphism, and repeatability of the banding patterns. These four primers were based on (GA)n repeats and (ACC)n repeats. The PCR products were separated by both agarose gel and polyacrylamide gel electrophoresis. With the agarose gels, the bands were visualised by staining with ethidium bromide, and with the polyacrylamide gels, the bands were visualised through silver staining. A Masters student, (Ms Nur Zuhairawaty) carried out most of the ISSR work. The ISSR methodology could be applied to the 450 TaroGen samples if the SSRs fail to distinguish between all the accessions.

<u>Use of DNA marker information for genetic analysis of Pacific Island taros</u>

The data sets obtained from the two molecular marker techniques can be applied to germplasm management in the identification of duplicates, mislabelling and rationalization of collections, both within and between countries in the Pacific Island region. They can also be used for phylogenetic analysis, genetic diversity analysis, analysis of parentage and analysis of genetic integrity after tissue culture.

Database Development

One of the other major objectives of the DNA-fingerprinting component of the ACIAR project is to develop a data module that will handle DNA fingerprint data. The database management system ICIS (the International Crop Information System; http://www.cgiar.org/icis/homepage.htm) has been investigated for its potential to handle this type of information. This is a database system for the management and integration of global information on genetic resources and crop improvement for any crop. Currently, it is being used for crops such as maize, wheat, barley, rice, and potato. ICIS was initially developed for the unique identification of germplasm, the management of nomenclature and the management of pedigree information. However, the scope of ICIS has now been expanded to also handle molecular marker data.

A new module, GEMS (the Gene Management System), has recently been developed to handle various types of molecular marker data. The key data that need to be integrated are:

?? Raw data (the presence / absence of each band on the gel)
?? Accession information
?? Molecular methodologies
?? Gel images

GEMS provides a controlled vocabulary that describes these key data. These data can then be integrated with the morphology databases that have been developed for each country's taro collection.

Summary

A microsatellite enriched genomic library for taro has been created. Overall, 28.6 per cent of clones sequenced contained a microsatellite sequence. Primers were designed for 41 (53.2 per cent) of the SSRs and sixteen (39 per cent) revealed polymorphism between a core set of taro genotypes from the Pacific Islands. ISSR fingerprinting has been assessed for use with taro germplasm. Database of molecular fingerprint data has been created that can be integrated with the morphology database for each country's taro collections.

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4.3 Development of recommended taro core collection based on molecular characterisation

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Accessions used

416 accessions from the TaroGen collection were fingerprinted. The entire collections of the Polynesian countries and Fiji were included in the fingerprinting study. With these collections there were small discrepancies between the collection size and the fingerprinting subset due to either samples not surviving the transfer to the University of Queensland (UQ), or samples being missing or too small at the time of collection. The larger national collections of Papua New Guinea (PNG), Vanuatu and New Caledonia were first analyzed using passport and morphological data. This data was analyzed and the 20 per cent most diverse accessions from each country were selected for further analysis using molecular markers.

Microsatellite markers used

Seven SSR primers were selected, based on preliminary assays of amplification quality and product length polymorphism in taro genotypes. When applied to the 416 accessions received, they revealed a total of 36 alleles, with an average of 5.2 alleles per locus.

Results

Samples with a high proportion of missing values were left out of analysis. These were:

- PNG: 12 (7.4 per cent overall)
- Fiji: 1 (1.4 per cent overall)
- Cook Islands: 1 (7.1 per cent overall)
- Niue: 2 (8.3 per cent overall)

Consequently, the total number of accessions in the final data set was 399

<u>Data Analysis</u>

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's (1979) definition of similarity: $Sij = 2a/(2a + b + c)$, where Sij is the similarity between two individuals, i and j, a is the number of bands present in both i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i; this is also known as the Dice coefficient (1945); Jaccard's coefficient (Jaccard, 1908): $Sij = a / a + b + c$; the simple matching (SM) coefficient (Sokal and Michener, 1958): $Sij = a + d / a + b + c + d$, where d is the number of bands absent in both i and j. 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, 1993).

Cluster analyses were carried out for:

?? the entire data set (399 accessions from nine PICs)

?? within each country

?? with all the Polynesian countries.

For each country's data set, three different similarity coefficients were calculated and a dendrogram created from each different measure. In each case, the dendrogram with the highest correlation coefficient was used.

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

Use of cluster analysis to select core accessions

For each country's taro collection, the dendrogram with the highest correlation coefficient was used to make the selections of the suggested core collection, i.e. 10 per cent of the accessions in the dendrogram for Fiji, Palau and Polynesia, and 50 per cent of the accessions in the dendrogram for PNG, Vanuatu and New Caledonia.

Cut-off points were assigned to group the accessions into clusters on all dendrograms produced by selecting an appropriate similarity measure. The cut-off points, and consequently the number of clusters, varied between the countries and depended on the number of accessions and the level of diversity within each country. Therefore, the cut-off point had to be flexible in order to take account of the variations between accessions within each country.

While studying the level of diversity within each cluster for each country, a second factor, morphological groupings, was taken into account where possible. This could only be done for three country's data sets; PNG, Vanuatu and New Caledonia. In these three cases, the suggested core accessions identified within each cluster were cross-checked with the morphological groupings generated from multivariate analysis. Where possible, at least one representative accession from each morphological group was selected for final core recommendations.

Ideally approximately 50 per cent of each morphological group should be included in the final core, thereby reducing the 20 per cent to 10 per cent overall. However, genetic diversity as revealed by the SSR analysis did not always correlate with the morphological diversity. For example, in some cases, accessions from the same morphological grouping were very diverse and were distributed across the entire dendrogram, and in other cases accessions from different morphological groupings were found to be identical. In the latter case, it did not make sense to select both accessions, even though they were from different morphological groupings. Therefore, ultimately, the selection of the final core relied more heavily on the genetic analysis.

In all cases, for the selection of each country's suggested final core, a third factor was taken into account: duplication between countries. In order to avoid including between country duplicates in the suggested final core, it was necessary to cross check to the cluster analysis carried out for the entire data set (399 individuals from nine countries).

86 per cent of the total variation accounted for in the data set was found within each country and only 14 per cent between each country. These AMOVA results verified selection of core from cluster analyses carried out on each country individually. However, cross checking to cluster analysis of entire tree was also carried out to ensure that identical accessions between countries were not selected for the final core.

Country-by-country analysis

PNG

In total, 151 accessions were used in the data analysis, and from these, 81 had to be selected for inclusion in the suggested final core. The dendrogram created using the SM coefficient and UPGMA clustering was initially sub divided into 23 separate clusters. From these 23, 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.

Vanuatu

In total, 89 accessions were used in the data analysis, and from these 44 had to be selected for inclusion in the suggested final core. The dendrogram created 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,

In total, 18 accessions were used in the data analysis, and from these eight had to be selected for inclusion in the suggested final core. The dendrogram created using Jaccard's coefficient and UPGMA clustering was initially subdivided into eight separate clusters. From these eight clusters, eight accessions were selected, one from each cluster, following the same procedure as detailed for PNG.

Fiji

In total, 70 accessions were used in the data analysis, and from these seven had to be selected for inclusion in the suggested final core. The dendrogram created using Jaccard's coefficient and UPGMA clustering was initially subdivided into seven separate clusters. From these seven clusters, eight accessions were selected, one from each cluster, based on the level of diversity within each country and also the cluster analysis of the entire data set. No morphological analysis was available to use in selection of recommended final core.

Palau
In total, 11 accessions were used in the data analysis, and from these two had to be selected for inclusion in the suggested final core. The dendrogram created using Jaccard's coefficient and UPGMA clustering was initially subdivided into four separate clusters. From these four clusters, five accessions were selected, following the same procedure as detailed for Fiji. Five accessions were selected overall, rather than only two, because not only was the level of diversity within Palau found to be very high, but also the level of diversity between Palau and other PICs was very high.

Niue
In total, 22 accessions were used in the data analysis, and from these three had to be selected for inclusion in the suggested final core. The dendrogram created using Jaccard's coefficient and UPGMA clustering was initially subdivided into six separate clusters. From these six clusters, six accessions were selected, one from each cluster, following the same procedure as detailed for Fiji.

Tonga
In total, nine accessions from the national collection and three additional accessions from Pacific Biotech, based in Nuku'alofa, were used in the data analysis, and from these one had to be selected for inclusion in the suggested final core. The dendrogram created using the simple matching coefficient and UPGMA clustering was initially subdivided into three separate clusters. From these three clusters, three accessions were selected, one from each cluster, following the same procedure as detailed for Fiji

Cook Islands
In total, 13 accessions were used in the data analysis, and from these one had to be selected for inclusion in the suggested final core. The dendrogram created using Jaccard's coefficient and UPGMA clustering was initially subdivided into three separate clusters. From these three clusters, three accessions were selected, one from each cluster, following the same procedure as detailed for Fiji.

Samoa
In total, 13 accessions were used in the data analysis, and from these two had to be selected for inclusion in the suggested final core. The dendrogram created using Jaccard's coefficient and UPGMA clustering was initially subdivided into four separate clusters. From these four clusters, four accessions were selected, one from each cluster, following the same procedure as detailed for Fiji.

Conclusions
The results from SSR analyses allowed the selection of the suggested final core. These selections were based primarily on cluster analysis within country. However, the selections also took two other factors into account, where possible:

?? to ensure that between country duplicates were not included in final core, there was a comparison between the country cluster analysis and the cluster analysis for entire data set

?? within country clusters were compared with morphological analyses, (this was only possible for PNG, Vanuatu and New Caledonia) In these cases, where possible, at least one representative accession from each morphological data grouping was selected.

Overall there were very few indistinguishable accessions based on the SSR data set. Fiji's national collection had the lowest rate of unique DNA fingerprints, with only approximately 60 per cent of accessions being differentiated using the SSR data set. This contrasted with the results from other Pacific Island countries, but particularly Samoa and the Cook Islands, where over 92 per cent of the national collections had unique DNA fingerprints.

In order to try and differentiate between accessions currently identical, there are a number of options. Firstly, the remaining polymorphic primers that were developed could be applied. In total, 16 polymorphic markers were developed, but only the top seven, in terms of quality and level of polymorphism, were used in this study. Consequently, the remaining nine could be applied to limited data sets. Secondly, the ISSR technique, which has been optimized for taro germplasm, 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 applied to the remaining identical samples.

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 were introduced to the region from PNG. It also suggests that regional breeding programs using taro leaf blight resistant lines from PNG could be highly successful, as there should be no barriers to breeding, as they originally came from a common source.

The fact that many lines from the Pacific Island countries can be traced back to PNG raises the issue of whether PNG is really the centre(s) of origin. However, without SE Asian accessions included in analysis, it is very difficult to draw any firm conclusions. Consequently, it would be very interesting to analyze the TANSAO taro core collections for SE Asian countries with the developed SSR markers, to compare the level of diversity in SE Asia with that in Oceania. Further work required is the DNA analysis of the Solomon Islands taro collection. Currently, the Solomon Islands collection is believed to consist of approximately 800 accessions. Therefore, following morphological analysis and selection of 20 per cent, approximately 160 samples will be sent to UQ for analysis.

Currently, the recommended final core collection for the Pacific Island region, minus the Solomon Islands collection, consists of 164 accessions. This core needs to be validated and various options are available:

?? Is the core selection similar to a 10 per cent selection that would have been made from morphological/passport data only?

?? DNA fingerprint a random 10 per cent sample from PNG (81 accession) and comparing the level of diversity with that of the selected core. The assumption is that the selected core will encompass more genetic diversity than a random 10 per cent sample.

A final cluster analysis was carried out based on the164 core accessions alone, in order to verify that each accession in the core was unique. The suggested final core has been circulated to each country for review and consideration of other factors unavailable at the time of analysis, such as farmer's preferences, breeder's observations, taste quality. The suggested final core can be modified to take these factors into account, upon advice from each country.

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4.2 Managing, validating and utilizing the core collection.

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Introduction

Once the entries for the core collection have been selected, decisions have to be made on the management of the core collection. These decisions include how to store the core, regeneration procedures, and documentation. In addition, it will be necessary to determine how much diversity the core has captured, a process known as validation. Finally, procedures for altering the core collection will have to be established to take account of new knowledge or new accessions.

Management of the core collection.

Maintaining the core collection

One question that has to be addressed is whether it is necessary to maintain the core collection separate from the whole collection. Physical separation is desirable for a number of reasons, namely:

?? Minimizes the possibility of errors by guaranteeing that a single standard source is used.

?? Simplifies distribution and use.

?? Provides added security

This decision has already been taken with the taro core, in that it will be maintained as a separate collection under slow growth conditions. The entire (base) collection will be maintained under liquid nitrogen, once the cryopreservation protocols have been developed.

Distributing the core collection

There is likely to be a relatively high demand for the core collection, and so any storage methodology should allow for this. If large number of samples cannot be maintained *in vitro* then the regeneration protocols should be optimized so that material can be multiplied "on demand". Maintaining the taro core under slow growth conditions satisfies this requirement. Any accessions requested, can be taken out of slow growth storage and easily multiplied to provide the number required. The multiplication rates of each accession in the core collection will be noted and the number of replicates per accession stored can take this into account. Eventually all of the accessions in the core will be pathogen-tested, and so there will be no quarantine problems in distributing this material. Prior to that, a proposed virus survey could indicate that some countries have common viruses, and therefore distribution/exchange of germplasm would not be a problem.

Information Management

As the core collection is developed and used, more information will become available on the entries. This has to be documented, though no special procedures are necessary. It should be the first material used when new characters are assessed. The process of core collection establishment and entry itself should be documented. Information on the core collection can provide valuable information on the whole collection. The stratification procedures, which were used to establish the core collection can be used in reverse to provide information about other accessions in the group from which a specific entry was obtained.

Validating the core collection.
The selected core has to be validated, that is, there has to be a check to see how much of the diversity in the whole collection has been captured by the core. The process of validating the core collection usually involves comparing it with the original collection from which it was developed. A comparison can be carried out using either the characteristics that were used to develop the core, or using the characteristics that were not utilized in the development of the core. Biochemical and molecular markers can also be used to assess the success of the core in capturing the diversity of the whole collection. Core collections of about 10 per cent should possess about 70 per cent of the alleles found in the whole collection. Therefore a random sample of the core can be taken, and compared with the core itself to determine whether they have broadly similar biochemical or molecular marker alleles.

Altering the core collection.
Once established, the core should remain fairly stable. If it is constantly changing, then management will be difficult, and utilization will be inhibited. However there may need to be changes in the number and identity of the entries in a core collection from time to time. These changes could be for the following reasons:

- ?? Need to acquire accessions with specific desirable traits, such as a new form of resistance
- ?? Meeting requests from users to include particular accessions on the grounds that they have important characteristics for the user community.
- ?? Obtaining additional accessions with new diversity that should be represented in the core.

Whatever the reason for change, the process of altering the core should be carried out in such a way as to minimize any problems. Users should be informed of any changes and the reasons for the changes. As the concept of a core collection was developed because of the size of germplasm collections, any alterations should ideally be a replacement rather than an addition of new material.

5.0 Conservation strategies for taro.

In the past the favoured method of conservation for taro has been field genebanks, but these have either failed or have had major problems in sustainability. *In vitro* storage has been tested to some degree by the Pacific Regional Agriculture Project (PRAP) in Samoa, and has been further evaluated, and costed by the TaroGen project. Cryopreservation as a conservation option has also been evaluated. Similarly there have been preliminary studies on seed and *in situ* storage by TaroGen. When formulating an overall conservation strategy for a particular species it is vital to incorporate a combination of *in situ* and *ex situ* methods in order that they complement one another. A number of techniques are available for the conservation of the gene pool of any particular plant species. Each method has its own strengths and weaknesses but if used in an appropriate complementary manner a combination of techniques can offer the most effective and safest strategy for long term conservation. The combination of methods used will depend on a number of factors but is usually characteristic for a particular crop type. Therefore, a cereal species may have seed storage and on-farm conservation as the dominant methodologies, whereas for a vegetatively propagated root and tuber crop, such as taro, *in vitro* and field genebanks may predominate. In establishing a complementary conservation strategy for an species, the choice has to be based on an approach that offers security of germplasm, relative ease of access and optimum use of resources.

5.1 The Role of the RGC in Taro Conservation

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Introduction

The use of tissue culture for conservation of some of the Pacific Island crops began in the mid-1980s when assistance was given from international agencies for the collecting of regional root crops. By 1987 collections existed in many of the countries, and efforts had been made to characterize and evaluate the collections. Because of the resources involved and the information that had been amassed, there was a need to facilitate the sharing of this germplasm between countries. Sharing of this germplasm necessitated plant propagation and distribution. Both of these requirements could be satisfied by tissue culture. The majority of Pacific Island crops are vegetatively propagated, and therefore an efficient tissue culture system enables these species to be multiplied. In addition, through meristem culture, these crops can be 'cleaned' and as pathogen-tested cultures, can be disseminated throughout the region without any implications for quarantine. For this reason, tissue culture laboratories were established within regional institutes, and pathogen-testing schemes developed. A tissue culture laboratory was established at the then, South Pacific Commission (SPC), in Suva, Fiji. This laboratory held collections of pathogen-tested accessions of taro, cassava, yam, banana, sweet potato and vanilla.

In 1986 the Commonwealth Fund for Technical Cooperation established a small tissue culture laboratory at the University of the South Pacific (USP), Samoa. This laboratory collaborated with the SPC laboratory in various aspects of tissue culture research pertinent to the crops of the Pacific Island region. In 1990, the European Union, through the Pacific Regional Agricultural Programme (PRAP), started funding the laboratory at USP. The laboratory was the focus of a PRAP project, 'Provision of Tissue Culture Services for the Region'. Within the first year of funding the laboratory was modified so that it could conserve some 20,000 cultures under normal and slow growth conditions. The EU funded this project until December 1999. During this time regional staff were trained in tissue culture techniques, tissue culture laboratories were established in some of the ACP countries, and the collections within the USP laboratory were increased.

An important output of both the SPC laboratory, and the PRAP project (in its first phase, 1990-94) was raising the awareness of how tissue culture can be used to benefit agriculture. These benefits come from being able to propagate plants at a rate that is usually quicker than that achieved in the field, distribute pathogen-tested germplasm, and conserve germplasm. An indication of the advantage to be gained from using tissue culture for multiplication is demonstrated by taro. A method was developed at the PRAP laboratory in Samoa for significantly improving the multiplication rate of taro. This method is now being used by the Ministry of Agriculture in Samoa in their national tissue culture laboratory for bulking up improved, taro leaf blight resistant varieties, so that they can be made available to the farmers. In addition, the importance of tissue culture as a conservation method became apparent. As stated earlier, collections had been made in the mid-1980s, and much of this collected material was maintained as field genebanks in the various countries. However, over the ten years that followed, many of these collections were either completely lost, or either significantly depleted in terms of accession numbers. Vanuatu, for example, lost all of its taro collection as a result of extreme climatic conditions and a workers' strike. This same taro, however, was still available as pathogen-tested tissue cultures at both the USP and SPC laboratories. Finally, access to pathogen-tested germplasm was shown to be very important in a region that is composed of many islands, each with their own strict quarantine regulations. The existence of a regional tissue culture laboratory, maintaining pathogen-tested tissue cultured germplasm therefore facilitated distribution and utilization, as well as being the best option for conservation.

The second phase of the EU-funded PRAP project commenced in 1995. The concept behind the second phase of the PRAP tissue culture project was that a regional tissue culture centre existed as a unit providing conservation, expertise, and germplasm. This regional centre would support the national, low cost tissue culture laboratories that were basically propagation and distribution units for individual countries. In addition to this, it was also felt that there should be a germplasm centre for Pacific Island crops that were not conserved in any of the International Agricultural Research Centres (IARCs). For example, none of the IARCs has an international mandate to conserve taro. Similarly, with yams, IITA, Nigeria, maintains collections of the African yams, but has no Pacific yams in its collection.

With the realization that there should be a regional centre for germplasm conservation and research, decisions had to be made as to where that centre should be located. For many reasons it was considered that SPC was the most sustainable location for a regional germplasm centre. The rationale for this was strengthened by the decision that two germplasm-based projects were to be located at SPC. These were the AusAID-funded taro project (TaroGen), and the EU-funded yam project (SPYN).

How the RGC operates.

The SPC Regional Germplasm Centre has been functioning since September 1998 but was officially opened in March 1999. Funding for the establishment of the Centre came from the Australian government through AusAID and ACIAR projects, and from the EU through the Pacific Regional Agricultural Programme. The Centre is presently being supported by funds from different projects, operating in the Centre. The EU supports the Plant Protection Service (PPS) within the SPC. In addition, there is support from AusAID through the Taro Genetic Resources project (TaroGen), and from the EU through the South Pacific Yam Network (SPYN project), and there is also funding from INIBAP and COGENT.

The RGC originally operated within the Plant Protection Service (PPS), but now is operating within the Crop Improvement programme. With the growth of PGR-related activities and the advent of the PGR network, the RGC will become more self-sustaining, providing a service to most of the sections in the SPC.

Objectives
The RGC aims to assist PICs to conserve the region's genetic resources and to provide access to improved crop germplasm. Through this effective conservation and utilization, dynamic, sustainable agricultural systems can be achieved, which will reduce poverty, increase food security, and assist in the protection of the environment. The RGC will continually seek better ways to safeguard and evaluate these resources, and build a strong base of knowledge and expertise for effectively preserving and using plant genetic resources. Access to these genetic resources will recognize countries' sovereign rights under the Convention on Biological Diversity.

The current situation regarding taro.
The RGC currently holds approximately 416 accessions, which were collected from PNG, Vanuatu, New Caledonia, Fiji, Tonga, Samoa, Niue, Cook Islands and Palau. The accessions from PNG, Vanuatu and New Caledonia represent 20 per cent of the national collections, whereas those from the other countries (with the exception of Palau) represent almost 100 per cent of what was collected from those countries. Palau is the exception and the accessions from Palau are varieties that were sent to Samoa in response to the TLB problem. These accessions are currently being bulked up so that there are sufficient numbers for virus indexing and also for any cryopreservation studies. Representative samples of each accession were sent to UQ for DNA fingerprinting. After the core collection has been identified the RGC will have some 240 accessions to maintain. This will be the core collection and will be representative of the genetic diversity that has been collected during the course of the TaroGen project.

Future plans?
Some work is still in progress in the RGC to determine which slow growth method for taro best suits the widest number of varieties. In the meantime, once the taro core has been identified, it will be put into slow growth storage using reduced temperature and a medium conducive to slowing down growth. The core collection will also be duplicated at a location chose by the region for safety purposes. Resources permitting work will continue in cryopreservation to determine if the current method can be modified to achieve recovery rates in the order of 40 per cent with a larger number of accessions. If this does not prove to be successful, then some preliminary studies will be carried out to evaluate another method, which is similar. This method is known as encapsulation/dehydration, and although it is more labour intensive, it is less demanding of the size and quality of the meristem. It is hoped that a cryopreservation protocol will be developed that will be applicable to a wide range of varieties, and so could be used to conserve the base collection. The initial costs of cryopreserving material are relatively high, but after that maintenance costs are lower than slow growth storage. Added to this, there are the advantages of security, both with respect to loss of accessions, and genetic integrity.

Any improved clones from the breeding programme will be established in tissue culture in the RGC and so will be available for distribution to the region, once exchange policies have been agreed on. Some material from the Samoa breeding programme at USP is already in the RGC and approval has been given to receive some of the PNG clones. The quarantine implications of distributing this material will be clearer, once agreement has been reached on the viruses in the region, and the virus status of this material. In anticipation of the need for virus indexing all of the improved clones are meristem cultured when they come into the RGC.

As we are all aware there is little point in conserving any crop unless there is active distribution and utilization associated with its conservation. Tissue culture plants are easier to distribute, and are usually acceptable to quarantine, unlike vegetative propagules. With the use of tissue culture it is possible to improve on the multiplication rates that are achieved in the field, and so this can be an aid to distribution. Within the INIBAP programme at SPC, there is active distribution of FHIA bananas resistant to Sigatoka disease. Different FHIA accessions are multiplied in the RGC and sent out to SPC member countries. Where possible, 10 tubes of each accession are sent out. If the country has a tissue culture laboratory, such as Samoa, then they are sent proliferating cultures. Alternatively, if the country wants to plant out these accessions immediately, then well rooted cultures are disseminated. The same procedure could be adopted for taro, which would facilitate utilization of the improved material. Tissue cultures of taro could be sent in a greater number because of their smaller size.

Material held in the RGC will only be used if countries know that it exists. The RGC plans to have documentation available for 'advertising' this germplasm. This documentation will be aimed at different clients, with extensive, informative databases for breeders, and a more condensed form, outlining traits such as taste, yield etc to other users such as growers. Again using bananas as an example, a publication has recently been produced by DPI, Australia, entitled 'Banana Varieties, The ACIAR Years 1987-1996. This has one page devoted to text with all essential agronomic information, and another to pictures of the described varieties. It would be an excellent way to promote some of the taro varieties.

As well as continuously trying to improve existing tissue culture methods in the crops that the RGC works with, such as taro, other avenues can also be explored. An issue that is often discussed but has yet to be clarified is what are the advantages of tissue cultured, or pathogen-tested material. The PRAP sweet potato project found that higher yields could be maintained with sweet potato varieties, not only if the plants had been derived from pathogen-tested tissue cultures, but also if they had just gone through the tissue culture process. In a recent edition of the New Scientist, (March, 2001), an article described how meristem culture and virus indexing (for feathery mottle) techniques were being used in two provinces of China, (Shandong and Anhui). Last year, 30 million tonnes of sweet potatoes were produced on 800,000 ha. The difference in yield was 30-40 per cent. Enough plants are produced by the methods to plant 25,000 ha within 2 years, but after 3 years the selections once more succumb to viruses, so the process has to be done all over again. The Shandong Academy of Agricultural Sciences in Jinan, in collaboration with CIP, are responsible for this work. There was a report of a similar finding but with taro from Costa Rica. It was shown that taro pathogen-tested for dasheen mosiac virus would be re-infected with the virus within the first few months of planting out. However, even though the symptoms of the virus were expressed, yields remained significantly higher with these plants than with plants that had not been pathogen-tested. This is an area that requires investigation, and is one where there could be collaboration between SPC and Koronivia Research Station. If the benefits on yield of tissue culture can be confirmed, this would facilitate distribution of clones that have originated from tissue culture, and could also assist in defining a valuable role for tissue culture laboratories attached to national agriculture stations.

The RGC through its contacts overseas, and its involvement in networks will ensure that all operational practices within the RGC are of the most appropriate and highest standard. Methodologies will take on board new developments in tissue culture that are relevant to the activities within the RGC. Some of these new methodologies could be relevant to taro, and so the RGC being part of a regional organization, and the soon to be formalised regional PGR network, is in a position to capitalise on these new methodologies. These could be of benefit to all aspects of the RGC from conservation through to utilization.

Conclusion

A few of the IARCs have a mandate to conserve some crops that are important to the Pacific Island region, such as sweet potato, cassava and yam. However, none of the IARCs is mandated with conserving the region's most important crop, taro. This provides the RGC with an opportunity to take up this much-needed role and establish itself as a Centre for the conservation of taro. As the RGC develops and it asserts itself as a Centre of excellence, this can only have benefits for future developments in taro research. The RGC will obviously also conserve other crops. Currently a collection of sweet potato is maintained, and with the SPYN project, there will be a collection of *Dioscorea alata*. In the future, it is planned that a cassava collection could be established, possibly through importing some varieties that would be useful from CIAT. Although, the intention is to focus on major root and tuber crops, the RGC will also attempt to establish collections of a number of minor crops that are endemic to the region, and important nutritionally, (such as *Abelmoschus manihot* or bele).

The Pacific Island region, which is very vulnerable to global climatic and economic changes, and suffers from internal pressures such as pests, disease, and civil unrest, needs to preserve its genetic diversity in order to meet the challenges of food security and income generation. All of the major root and tuber crops of the region have been identified as crops of food security importance by countries through the International Understanding. Therefore, in providing a Centre for conserving these crops, the RGC is assisting the region in meeting these challenges

5.2 *In vitro* genebank study.

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Background and Justification

One of the significant outputs from the TaroGen project is the collection of over 2,400 taro accessions from several PICs. The strategy adopted by the project was to reduce this collection to a core collection of approximately 240 accessions, using morphological descriptors and DNA markers. Although this core collection is significantly less in number than the original collection, it is still relatively large and has to be conserved by as safe and secure a method as possible.

As taro is a vegetatively propagated crop, it is traditionally conserved in field collections. This is not an effective conservation strategy for medium to long term preservation of taro genetic resources. Field collections are notorious for their demands on resources, with the safe maintenance of the accessions being reliant on constant vigilance and upkeep. Pest and disease outbreaks can be disastrous reducing the number of accessions in a collection to a level that is not representative of genetic diversity. Similarly, the climatic extremes experienced in the Pacific can also have a very significant negative impact on a field collection. More recently, as in the Solomon Islands, civil strife can be added to the list of events, which can seriously impact on field collections.

As well as the need to identify an appropriate conservation system for this taro collection, the project also wanted to provide information to the countries on the conservation methodologies for taro, so that should they wish to maintain national collections they would have the information required for decision making. One key area of importance here is costs. There is very little information available on the cost of implementing any conservation strategy. Consequently, as part of the conservation component within the TaroGen project, a pilot *in vitro* genebank (IVAG) study was established at the SPC RGC in parallel with a field genebank at Koronivia Research Station (KRS). The aim of the study was to determine all the inputs required for an *in vitro* genebank, and to compare this with the inputs necessary for a field genebank with the same accessions. In addition, there was also a need to evaluate the slow growth methodologies that had been developed for taro in another laboratory. Other issues that required consideration were genetic stability, safety and management. Taro is a crop that is amenable to tissue culture. Propagation systems have been developed that are optimal for a wide range of cultivars. In addition, it also seems to be a relatively stable crop, both in the field, and in tissue culture, so is suited to *in vitro* storage.

A recent status report on the use of *in vitro* techniques for the conservation and use of plant genetic resources (Ashmore, 1997) concluded that slow growth techniques are now successfully and routinely applied to a range of species and across a range of genotypes within species. The use of these techniques allows storage of healthy germplasm with extended subculture intervals, thus reducing time and costs for maintenance. However, the report also identified gaps in the development of techniques, and limitations in basic scientific knowledge. Some of the gaps highlighted are relevant to this study, in that the proposed outputs would generate the information required to fill these gaps for taro. These were:

?? More studies are needed on genetic stability after relatively long periods of storage, to establish the safety of slow growth storage, when compared with other methods.
?? There is a need for the development and application of characterization systems, including molecular genetic markers for initial identification as well as monitoring of the genetic stability of stored accessions.
?? Reproducible, simple and more widely applicable techniques are required.
?? Cost analyses of *in vitro* conservation is needed to allow comparison with other conservation methods, particularly the comparison between *in vitro* storage and field genebanks.

In vitro conservation methodologies offer an opportunity to preserve taro within a controlled environment, thereby eliminating risks from pest and disease outbreaks, and climatic extremes. Although various methods exist for reducing the growth rate of plants *in vitro*, the most widely applied slow growth storage technique is temperature reduction, often combined with a decrease in light intensity or culture in the dark. There are reports in the literature of taro being stored for more than eight years at 9?C in total darkness, with transfer intervals of approximately three years. (Bessembinder *et al*., 1993). Similarly, Staritsky *et al*., (1986), reported that taro (*Colocasia esculenta*) could be conserved for three years at 9?C, and still be viable. Research carried out in the regional tissue culture laboratory at the University of the South Pacific, Samoa, when it was funded by the European Union, under the Pacific Regional Agricultural Programme, demonstrated that temperature reduction was the most practical method for slowing down the growth rate of taro. Taro could be maintained at 20?C, for 9 to 12 months, without subculturing, depending on the variety. Other parameters, besides reduced temperature, were also investigated. These were reduced light and supplementing the culture medium with osmoticums. The inclusion of mannitol in the culture medium did suppress growth, but some morphological changes in the resulting plantlets were observed. In addition, when mannitol was used with cultures initiated directly from the field, a phytotoxic effect was observed. As a result of the work carried out in Samoa, together with evidence from the literature, it was decided that temperature reduction would be used in this IVAG study.

This pilot study was modelled to some extent on a study carried out by CIAT in collaboration with the then IBPGR over a three year period. (IBPGR/CIAT, 1994). The study covered the principal steps involved in an *in vitro* active genebank, from the selection and sampling of material from the field to the evaluation of genetic stability of retrieved material growing under slow growth conditions. The overall objective was to assess and demonstrate the technical and logistical aspects of establishing and operating a pilot *in vitro* active genebank using cassava as a model crop. The pilot genebank used 100 clones selected from the 4,250 cassava accessions maintained in the field. At the end of the study several recommendations were made regarding *in vitro* conservation by slow growth. These were:

?? The value of this approach should be balanced against other conservation strategies, based on knowledge of the genepool, reproductive behaviour, range of variability (geographic and genetic), and costs. It should complement other conservation strategies for the same crop species, such as seed, field genebank, and *in situ* conservation.
?? There should be a thorough knowledge of the *in vitro* culture behaviour, such as culture initiation, explant, micropropagation, and requirements of the species, prior to any *in vitro* conservation strategy.
?? Depending on the crop and its agronomic and economic importance, two different levels of *in vitro* conservation should be envisaged: a fully implemented system compared with a minimum input system.
?? All the steps, procedures, and data required throughout the experiment should be taken into account.
?? The rate at which accessions are introduced into the *in vitro* genebank should be balanced against the risks of losing accessions from genetic erosion, pest and disease attack or climatic extremes in the field.
?? It is important to introduce 'clean' material into the *in vitro* genebank despite the delays this might impose on the study.
?? Genetic stability is an important consideration of any *in vitro* conservation strategy. Monitoring techniques for stability will depend on the history of the crop species regarding this trait. Sophisticated, high-technology monitoring has value, when there is sufficient base to assume instability, either intrinsic, or due to the culture system and subculture frequency. In other instances, visual observation of morphological changes may be sufficient in comparison with appropriate controls.
?? If variants are observed in the *in vitro* system, the reason for the variation has to be determined, and in this case the best option is to refer back to the original field collection.
?? A field collection should exist for as long as the *in vitro* genebank has not been duplicated elsewhere for security reasons. Once a collection has been duplicated, only material for evaluation should be held in the field.
?? Decisions regarding the number of replicates will depend on: size of the collection, size of vessels, risk of losses during *in vitro* multiplication, subculturing and storage. In the case of cassava, a minimum of one and a maximum of three replicates were lost, and therefore a replication of three to five per accession is recommended.
?? The information system required for the management of the collection will depend very much on the size of the collection.

These recommendations were taken into consideration when the pilot genebank study for taro was established under the TaroGen project.

Objectives of the TaroGen pilot *in vitro* gene bank (IVAG) study

The overall objective of this project is to investigate the technical and logistical aspects of establishing and running an IVAG, using taro as a model.

Specific objectives

- To select a sample of Fijian taro accessions, and to process these samples into *in vitro* storage under conditions of slow growth.
- To provide a cost analysis for both field and *in vitro* maintenance of taro
- To evaluate the applicability and reliability of available slow growth methodologies for taro.
- To monitor genetic stability (through visual observation), and viability during slow growth in *in vitro* storage.
- To determine the needs for laboratory facilities, equipment, consumable items, and technical staffing involved throughout the operation of the *in vitro* genebank.
- To provide recommendations for establishing and running an *in vitro* active genebank (IVAG) on the basis of the experience gained with taro.

In vitro genebank

Selection and multiplication of accessions

50 of the non-export taro accessions were initially selected from the field collection at KRS. However, because of delays in obtaining sufficient suckers for initiation into tissue culture, only 44 accessions were eventually established in tissue culture. Shoot-tips were excised from these suckers and subjected to a three stage sterilization procedure. This consisted of 70 per cent alcohol for two minutes, followed by 20 per cent domestic bleach (1 per cent active chlorine) for 15 minutes, and after trimming, another wash with 10 per cent domestic bleach (0.5 per cent active chlorine) for 10 minutes. The final stage was three to four washes with sterile distilled water.

From previous experience with *in vitro* culture of taro, and the information from the CIAT study on cassava, it was decided that for every accession in the pilot study there should be five replicates. Bulking up was achieved by using the three-stage multiplication system, which utilizes the growth regulators thidiazuron (TDZ) and benzylaminopurine (BAP). This involves culture on a medium containing TDZ for three to four weeks, followed by culture on a medium supplemented with BAP for a further three to four weeks. For the next stage the explants are transferred to a medium containing a low concentration of TDZ. Depending on the degree of multiplication achieved, the cycle can begin again after the third stage, or if the multiplication rates are sufficient, the explants can be cultured on a medium containing the basic macro- and micro-nutrients, and no growth regulators (Murashige and Skoog, 1962)

Multiplication rates were affected by the presence of endogenous bacteria in the explants. These bacteria are in the plant tissue and are not eliminated as part of the surface sterilization procedure. Tissue cultures of taro were analyzed by CABI, UK in mid-2000, and the bacteria identified as *Pseudomonas*, *Bacillus*, *Micrococcus*, *Curtobacterium* and an unknown coryneform. Some taro cultures had been sent to the UK in the early 90's for bacteria identification, and at this time the bacteria present were identified as

Pseudomonas and *Methylobacterium* spp (D. Stead, pers. comm). In the IVAG, these bacterial contaminants did not express themselves immediately, and so the problem was not apparent until after several subcultures, and some varieties were affected more than others. Because of the need to generate numbers for the IVAG, the possibility of eliminating the bacteria through meristem culture could not be determined. For this reason, antibiotic treatment only was investigated. Rifampicin was the first antibiotic investigated, as it had proved effective in control of bacteria in sweet potato cultures at CIP and also in the USP laboratory in Samoa. Treating contaminated taro cultures with 80mg/l rifampicin eliminated the contamination from 89 per cent of the 65 explants tested. However, after culturing with 0.5mg/l thidiazuron for multiplication, contamination reappeared in the majority of the explants. The effectiveness of gentamycin was also evaluated. This was chosen as it had been shown to be effective with cultures of other plant species, where *Pseudomonas* spp was present. Using gentamycin with rifampicin, it was possible to eliminate the bacteria from the cultures and proceed with the multiplication process.

Slow growth storage system

The RGC has one growth room, which runs at a temperature of 20?C, and so establishing an IVAG at a reduced temperature presented no problems. Shoot-tips of approximately 1cm in size were excised from the cultures that had been on the multiplication cycle. Prior to their excision all cultures after the multiplication period, were grown on basal medium without any growth regulators for one month, to reduce any possible carry-over of the growth regulators into the IVAG. In the IVAG taro cultures were grown in glass jars containing 20mls of Murashige and Skoog basal medium (1962), supplemented with 3 per cent sucrose. This medium was changed after three months of culture as it was apparent that growth was too vigorous, especially root production. The IVAG was re-established using the same basal medium but supplemented with benzylaminopurine (BAP) and napthaleneacetic acid (NAA).

Evaluation of viability

Some guidelines were necessary for when the security/survival of an accession was under threat. It was therefore decided that cultures would be replaced when three or more cultures no longer looked healthy, that is, were not viable. Viability here refers to cultures that had either (a) not grown from initiation, or (b) had outgrown the culture container and the culture medium, and were starting to senesce, or (c) were affected by any of the viability factors below. This was a parameter set in an IPGRI-coordinated sweet potato slow growth experiment. Once three or more of the replicates are no longer viable the security of that accession is affected. Viability 'descriptors' were recorded every month. These were:

?? shoot tip necrosis: this would be observed as a form of dieback from the main shoot, and would be recorded as absent (0) or present (1).

?? stunting: stunting is a concern for the reasons outlined above. Cultures that are too stunted would not grow into plants.

- ?? contamination: if contamination was present it was recorded as 1, if absent, then 0 would be recorded. A separate sheet was kept for noting what the contaminant was (bacterial or fungal), and what the treatment was.
- ?? senescence: this was recorded as number of leaves that had senesced out of the total number of leaves.

These non-viable cultures would be removed, and that accession would be 'replaced' with five new replicates, either generated from the cultures in the IVAG, or from the cultures maintained outside of the IVAG. Contaminated cultures similarly were removed from the growth room. If the problem was fungal contamination, then the culture would be 'cleaned' (provided fungal growth was not excessive), and returned to the IVAG. All inputs required for that cleaning process were recorded. Any cultures with bacterial contamination were destroyed and replaced with new cultures from the multiplication phase.

Morphological characterization *in vitro.*

With the IBPGR/CIAT study on cassava, morphological characters were observed after six months of storage. The parameters examined were selected as being relevant for cassava, and included etiolation, shoot number, callus formation, rooting, aerial roots, and special observations such as different leaf shape, pigmentation etc. Different parameters were chosen for taro. These were:

- ?? sucker number: the number of suckers per replicate was recorded.
- ?? callus formation: although callus is uncommon in shoot-tip cultures of taro, it was included because of its importance in terms of genetic stability. The values used were 0, indicating absence, and 1 for presence.
- ?? rooting: rooting was evaluated in terms of relative amount, 1, 2, 3, which corresponded to poor, medium and high respectively.
- ?? hyperhydricity: this was included as it can be a problem with cultures that have had exposure to TDZ, and can affect the establishment of that culture in the soil.
- ?? stunting: this was a problem experienced with an exotic variety in Samoa, in that the cultures could not be induced to develop, and elongate into viable plantlets.
- ?? leaf shape: leaf shape can be an indication of some form of 'change'. When experiments were carried out using mannitol with taro, leaf shape was affected.

These parameters were monitored after three months of culture

Genetic stability

It was hoped that the cultures subjected to slow growth storage could be analysed using the DNA fingerprinting system developed for the taro collections. However, this was not possible, and so stability was monitored through observation of morphological characters. It is also intended that a random sample is planted out in the field, and morphological descriptors recorded for these, and compared with those plants that have experienced no *in vitro* processing and have been grown continuously in the field. It is worth noting here that one of the recommendations from the IBPGR/CIAT report, stated that the use of DNA technology is only really relevant if there is sufficient basis to suspect instability, otherwise visual observation of morphological changes in comparison with appropriate controls is adequate. Taro is a crop that appears to be relatively stable in the field, and has not demonstrated loss of genetic integrity in tissue culture. With some work in Samoa a change in petiole colour was observed with one of the varieties in tissue culture. This variety was monitored and characteristics such as yield weight, shape and taste recorded. These all gave the same results as those with 'normal' petiole colour.

Associated field genebanks

In the IBPGR/CIAT study, Associated Field Genebanks (AFG) were established. These consisted of (a) 100 clones without any *in vitro* processing, (b) the same 100 clones but subjected to a micropropagation stage and (c) accessions after the *in vitro* storage period. Although the rationale behind the need for AFGs is sound, one of the comments from the report was that the establishment and running of the AFG is expensive and time consuming and that its function can be replaced by the original field collection. Considering the limited resources of the project, and the even more limited resources available at KRS, the only AFG established was the accessions without any *in vitro* processing.

Costs of *in vitro* culture

Although it is generally agreed that *in vitro* storage is a more secure method of conservation, it is often argued that it is too expensive to consider, especially in regions and/or countries where resources are limited. However, there is very little evidence to support this argument, therefore there is a need to provide more cost analysis information for the various conservation strategies that are in use. In addition, as the number of accessions increase, and organizations/institutes make a commitment to the conservation of countries' germplasm, cost analysis becomes more crucial. A cost analysis identifies areas where cost controls and reductions can be implemented. Such analyses suggest ways to achieve cost effectiveness. Perhaps certain consummables can be changed so that costs are reduced; until an accurate cost analysis is carried out, unnecessary expenditures might slip by unnoticed. Cost analysis also assists with charging for tissue cultures, if some aspect of the tissue culture laboratory is to be commercialised.

A standard cost analysis encompasses total, variable and fixed costs. Variable costs involve inputs that are easily varied in the short term, usually a period of less than one year. Fixed costs involve items that cannot be varied in the short term, for example, buildings and machinery. Straight-line depreciation can be used to determine annual depreciation expenses for capital goods such as buildings, machinery and equipment. This type of cost analysis was carried out by CIAT for both *in vitro* and field collections (Epperson, *et al*.,1997). The total number of accessions for both collections was 5,992. The total cost per accession in the field was US$17.09 with variable costs running at US$10.50. With the *in vitro* collection the total costs/accession were US$26.22 with variable costs running at US$1.85. Variable costs usually refer to the costs that will change depending on the number of accessions you have.

The costs of the pilot genebank were assessed in two different ways:

?? Using spreadsheets devised by the ACIAR funded project, 'Economics of preserving genetic diversity in PNG' specifically for the cost analysis of the SPC RGC. These sheets cover all costs for *in vitro* conservation and distribution of taro at SPC, and consist of (a) a price list for variable costs, (b) a summary table, (c) key variables table, (d) initiation and maintenance budget, and (e) multiplication and distribution budget. The cost budgets contain data and formulae developed to estimate the total cost of inputs employed in the conservation of *in vitro* collections, and the multiplication and distribution of the germplasm material. The summary table provides an annual estimate of variable costs, medium term variable costs, fixed costs and total costs for maintaining the *in vitro* taro collection at SPC. This summary table, which is included in this report, allows users to examine the cost budget without having to view the entire spreadsheet. Costs are estimated for the whole taro collection, per accession, and per plant replicate.

?? A simpler system was devised so that all inputs in the IVAG were recorded at the time of carrying out the activity. This shows what resources (labour, equipment, consummables) are required for the different operations, and also confirmed the cost analysis from the spreadsheets. It also gives an indication of immediate costs. This costing does not take into account fixed costs.

Results and Discussion:

In vitro genebank

Availability of material for genebank

The availability of suckers for initiation into the multiplication cycle was a problem. With varieties that easily produce suckers there was sufficient material for introduction into the IVAG. However, some of the varieties chosen for the genebank were poor at suckering, and so it was difficult to obtain an adequate number of suckers for the experiment. It was also found that some of the selected accessions would not respond well to the multiplication system and so could not be used in the genebank. This reduced the number of actual accessions used to 44, instead of the original 50 selected. This same problem was experienced by CIAT in their pilot genebank experiment, of the 100 accessions selected for the study, three would not respond to tissue culture, and so could not be used.

Contamination also affected the multiplication rates of the selected accessions. As noted earlier, this was contamination due to endogenous bacteria. Although for the purpose of the multiplication cycle, these bacteria were eliminated using the combination of the antibiotics, rifampicin and gentamycin, bacterial contamination caused a problem later in the study. Contamination occurred with both these 'cleaned-up' cultures and also with other cultures where bacterial contamination had not been a problem in the initial stages of the study. It would seem therefore that in the cases where antibiotics had been used, they had merely suppressed the expression of the bacteria. In the plants where contamination was not visible initially, the growth regulators used in the multiplication process, and the rate of subculturing, encouraged the expression of the bacteria. Using antibiotics is not a viable option for cultures that are going to be used in a slow growth storage system. Evidence shows that generally the antibiotics merely suppress bacterial activity, and can lead to resistance problems at a later stage. In addition, there is the possibility that the use of antibiotics in combination with a system that induces stress through slow growth will result in genetic instability. Antibiotics are also costly, and so add an extra cost to the total *in vitro* conservation cost.

The recommended strategy for addressing endogenous contamination in cultures is to screen the material before it is initiated into tissue culture. A microbial detection medium is easy to make and this can be used to determine whether or not a sucker from a particular accession is contaminated. Any plants testing positive can be rejected and only those that test negative can be introduced into tissue culture. Initiating clean material into tissue culture will guarantee that multiplication rates are not affected, and that there will be no bacterial contamination problems with those cultures at a later date. This need to screen obviously has implications for plant numbers and again means that there has to be availability of planting material. This planting material should be easy to access so that there is a minimum delay between harvesting and the tissue culture process. Any extended delay will only exacerbate any bacterial contamination problems.

Response to slow growth storage system

When an *in vitro* genebank is established, one technique has to be selected and applied to all the different genotypes under investigation. Varietal influence can be responsible for very different responses to the selected slow growth methodology. CIAT found that of 48 varieties, 50 per cent had to be subcultured after one year of storage, about 18 after 15 months, and six required subculturing after eight to nine months. In this study all of the varieties would grow without requiring subculture for six months, but after that 70 per cent required subculturing whereas the remainder could be maintained in culture for a further three months. The need to subculture reflects to a large extent the vigour of the plant, in that the culture medium has been fully utilized, and nutrient deficiency is therefore a potential problem. In addition, with older cultures, defoliation and senescence become a more common occurrence, increasing the chance of fungal contamination from rotting leaves in the culture vessel. This lack of uniformity across the range of genotypes being investigated merely adds to the labour input required in successfully maintaining the genebank. Culture viability has to be checked regularly, and decisions made as to when subculturing is required. If this is necessary with individual accessions, or small groups of accessions, too often, then this will place a strain on resources.

The system that was initially used with the taro from Fiji in this study was the same system used in Samoa with the regional taro collection held in the laboratory at the University of the South Pacific. Using the system there, the taro could be maintained without subculture for 9-12 months. With the Fijian taro, the need to subculture had been reduced to an average of six months instead of nine months. This is possibly due to the length of time the accessions in the USP laboratory have been in tissue culture, and so a reflection of their reduced vigour. The Fijian accessions, on the other hand, had been initiated into the IVAG more or less directly from the field. This shows that one cannot assume that a system that works in one laboratory, will work in the same way in another laboratory. Some of the laboratories in the region carried out an experiment on slow growth storage of sweet potato in collaboration with IPGRI, and the results from each laboratory where the same varieties were used with the same methodology, were quite different.

The inherent vigour in the explants resulted in excessive growth from the majority of the accessions, especially when the medium used was basal Murashige and Skoog. The IVAG was re-initiated with the same medium but supplemented with growth regulators, BAP and NAA. This did reduce the growth compared to the initial medium used, but as stated, the interval between subcultures was shorter than that experienced in the laboratory in Samoa. As a result of this different response to the culture medium used in the USP laboratory in Samoa, some smaller experiments were established in the RGC looking at other parameters for slowing down growth. These include culture size, reduced nitrogen in the medium and sucrose concentration in the medium.

Contamination

After each subculture, or re-initiation of the IVAG, fungal contamination was recorded within one to two months. The rate of fungal contamination was 4 per cent, which is relatively high. However, once these cultures were removed, and replaced with new cultures, fungal contamination did not re-occur. It is likely that this fungal contamination was due to using old cultures, where fungal spores were present, but not visible, as the source of the explant. Bacterial contamination was less obvious, but was detected in approximately 8 per cent of the cultures. However, when all cultures were screened for the presence of bacteria to determine whether reduced growth was due to genotype or the presence of bacteria, 20 per cent were tested positive. CIAT found that contamination largely occurred in seventh month of storage, and was much higher in some varieties than others. A problem that can occur with maintenance of cultures for a long period is defoliation and senescence, then cultures can become contaminated. This might not be obvious until the cultures are subcultured for reintroducing back into the IVAG. Therefore, there is a need to identify when a balance is achieved between having to subculture because of contamination risks, and efficient use of resources. Senescence occurred fairly early on in the growth of the cultures (after two to three months), but remained at 30-50 per cent throughout the culture period.

Cultures that suffered from fungal contamination were either cleaned up, or they were replaced with new cultures. All inputs required for the cleaning process were recorded. Any cultures with bacterial contamination were removed, and replaced with other clean cultures.

Genetic stability

It was intended that a sample from the IVAG could be sent for DNA fingerprinting, and the resulting fingerprints compared with fingerprints from the same accessions that had not been through the genebank. However, this was not possible. The cultures were monitored for selected characteristics for the duration of the experiment. At no time, was there any indication from these recordings that genetic stability was a problem. A random sample of accessions from the genebank has been retrieved from slow growth storage, transplanted to screenhouse, and will be planted in the field for morphological evaluation.

Costs

These summary tables have been extracted from the ACIAR spreadsheets, and provide an annual estimate of variable costs, medium term variable costs, fixed costs and total costs for maintaining the *in vitro* taro core collection at SPC, and also the IVAG. Summary tables allow users to examine the cost budget without having to view the entire spreadsheet. Costs are estimated for the whole taro collection, per accession, and per plant replicate.

Using this system the cost to maintain one accession for one year has been calculated at F$98.3. (US$49), if the RGC is maintaining the entire taro core collection of 206 accessions. (Table 1). This is a relatively high cost but as can be seen by a closer study of the costs involved, it is the fixed costs that are the major contributor to this figure. These fixed costs are very extensive, and include the original cost of converting the barracks to the buildings now used by SPC, and also the rent that SPC has to pay for the present location of the library, which used to be housed in this building. This is what is known as an opportunity cost. In addition, costs are also included for support given to the RGC by administration, finance and information technology, and these are quite high. These costs were taken from a 1999 budget for these departments, and then divided according to the number of staff at SPC. The actual tissue culture costs are relatively low. The cost of *in vitro* storage quoted by CIAT is slightly lower than this at US$26.22 per cassava accession, and this is probably due to CIAT being a much larger scale operation, and so the lower cost reflects economies of scale.

Reducing the actual tissue culture costs for maintaining one accession would be quite difficult as they are already relatively low. Since this costing was first done, (approximately 12 months ago) the RGC has taken action to reduce costs by changing some of the consummables that are being used. Further attempts to reduce the cost would impact on the high standards being practiced in the RGC.

The impact of fixed costs is still seen when comparing Tables (1) and (2), where the number of accessions differ between the core collection of 206 and the IVAG of 44 accessions. Fixed costs with a collection of 44 accessions are still high. Cost per accession, when 206 accessions are in storage is F$98.3, (US$49), whereas when 44 accessions are in storage the cost is F$146.9 (US$73.08) per accession. This does show the effect of economies of scale. Once a laboratory is established, it is important to put it to full use to make best use of resources.

Table 1: Summary Table showing all costs for maintaining the taro core collection of 206 accessions at the SPC RGC

Costs	Cost of Dalo Collection/Year ($FJ)	Cost per Accession/Year ($FJ)	Cost per Plant Replicate/Year ($FJ)
Variable Costs			
(A) Pathogen Testing	0	0.0	0.00
(B) Initial Medium Preparation	129	0.6	0.12
(C) Cleaning of Plant Material			
(i) Isolation from the field	15	0.1	0.01
(ii) Ongoing cleaning of material	12	0.1	0.01
(D) Preparation of Materials for Sub-Culture	3,101	14.9	2.98
(E) Laboratory Hygiene	113	0.5	0.11
(F) Multiplication	0	0.0	0.00
(G) Distribution	0	0.0	0.00
Total Variable Cost	**3,369**	**16.2**	**3.24**
Medium Term Variable Costs			
(A) Cleaning of Material	1	0.0	0.00
(B) Laboratory Hygiene	5	0.0	0.00
(C) Stationery	35	0.2	0.03
Total Medium Variable Cost	**41**	**0.2**	**0.04**
Fixed Costs			
(A) Capital Costs			
(i) Laboratory equipment	1,285	6.2	1.24
(ii) Office equipment	1,579	7.6	1.52
(iii) Miscellaneous	8,388	40.3	8.07
(B) Labour Costs	5,777	27.8	5.55
Total Fixed Cost	**17,029**	**81.9**	16.37
Total Cost	**20,439**	**98.3**	**19.65**

Table 2: Summary Table showing all costs for maintaining the taro core collection of 44 accessions at the SPC RGC

Costs	**Cost of Dalo Collection/Year ($FJ)**	**Cost per Accession/Year ($FJ)**	**Cost per Plant Replicate/Year ($FJ)**
Variable Costs			
(A) Pathogen Testing	0	0.0	0.00
(B) Initial Medium Preparation	29	0.7	0.13
(C) Cleaning of Plant Material			
(i) Isolation from the field	15	0.3	0.07
(ii) Ongoing cleaning of material	10	0.2	0.05
(D) Preparation of Materials for Sub-Culture	920	20.9	4.18
(E) Laboratory Hygiene	34	0.8	0.15
(F) Multiplication	0	0.0	0.00
(G) Distribution	0	0.0	0.00
Total Variable Cost	**1,008**	**22.9**	**4.58**
Medium Term Variable Costs			
(A) Cleaning of Material	0	0.0	0.00
(B) Laboratory Hygiene	2	0.0	0.01
(C) Stationery	10	0.2	0.05
Total Medium Variable Cost	**12**	**0.3**	**0.06**
Fixed Costs			
(A) Capital Costs			
(i) Laboratory equipment	385	8.8	1.75
(ii) Office equipment	474	10.8	2.15
(iii) Miscellaneous	2,516	57.2	11.44
(B) Labour Costs	2,067	47.0	9.40
Total Fixed Cost	**5,443**	**123.7**	24.74
Total Cost	**6,463**	**146.9**	**29.38**

Table 3: Simple cost analysis (F$) for maintaining 20 taro accessions (5 replicates each) in slow growth storage (6 month subculture interval).

Acc Nos	Activity	Time	Labour pa (F$)	Cons cost pa (F$)	Overall cost pa (F$)
20	Plant preparation	3h 20min	26.34	0	26.34
20	Shoot-tip excision	3h 20min	26.34	41.16	67.50
20	Media preparation	3h	35.70	26.88	62.58
20	Subculturing	5h	59.50	84.64	144.14
20	Cleaning up contaminated plants	1h	5.95	8.12	14.07
20	Monitoring the genebank	5h	119	3.00	122.00
			Initiation and maintenance		**436.63**
			Maintenance		**342.79**

These costs were obtained from the pilot genebank experiment and so reflect the amount of time that was involved in monitoring the experiment. With a reliable slow growth system cultures would only have to be checked regularly for contamination and viability. Subculturing costs are high because of the cost of the culture containers. Cost could be reduced here if old jars were obtained free of charge.

This table only allows for variable costs, it excludes all medium term variable costs and fixed costs. There is also no allowance for electricity. This would vary significantly with each laboratory.

Resources Used

Table 4: Activity table to show resources used for maintaining one taro accession (5 replicates each) in slow growth storage (6 month subculture interval)

Activity	Time required for 1 accession (5 replicates)
Plant preparation	10mins
Excision of shoot-tip and initiation of culture (includes sterilization)	10mins (based on 4 accessions taking 40mins)
Preparation of 1l of medium	9mins
Subculturing	15mins
Cleaning up contaminated plants for 1 plant only (unlikely that entire accession requires cleaning)	15min
Recording	1 5min

The first two activities would be required every time a plant was initiated into tissue culture. Once the collection had been established, only the last three activities are required. Any replacement of plants in the *in vitro* genebank by plants from the field would require the first two activities again.

Chemicals
With the exception of the antibiotics required at the beginning of this study, the only chemicals required were those used in preparation of the culture medium. These include all the basic macro- and micro-nutrients as defined by Murashige and Skoog (1962), supplemented with sucrose and a gelling agent. For the multiplication phase of the study, Stage 1 and Stage 3 media were supplemented with thidiazuron (TDZ), whereas Stage 2 medium contained the growth regulator, benzylaminopurine. All of these chemicals, with the possible exception of TDZ would be found in any basic tissue culture laboratory. TDZ would be present if the laboratory was involved in active multiplication of taro, such as the lab at Nu'u in Samoa. For the maintenance medium used in the IVAG, the basal medium was supplemented with benzylaminopurine and napthaleneacetic acid.

Glassware
Glassware used in the IVAG mainly comprised beakers, graduated cylinders, pipettes, conical flasks, glass jars (culture containers) etc., These would all be available in any basic tissue culture laboratory.

Equipment
Basic tissue culture equipment was required for this study, such as, electronic balance, pH meter, hotplate/magnetic stirrer, autoclave (pressure cooker can suffice) distilled water unit, LAF cabinet etc. Any reasonably equipped tissue culture laboratory would contain this equipment.

Facilities
The following SPC RGC facilities were used in the IVAG:

?? Tissue culture preparation room.

?? Transfer room

?? Growth room at 20?C.

?? Screenhouse

The field collections maintained at Koronivia Research Station were also used for the supply of the taro accessions used in the IVAG.

Staffing.
One technician had the responsibility of the IVAG. Because of other duties, the time allocation was not 100 per cent. On average, the time spent on this study was two per cent, but the technician had to be able to make larger amounts of time available when it was required. Obviously, the establishment of the genebank required a large time input and whenever the accessions had to be subcultured.

The TaroGen Tissue Culture specialist supervised the study. Less supervision would be required if there had not been problems with contamination and plant vigour. If a system is established with all the necessary recommendations and all the parameters calculated minimum supervision would be necessary.

Field genebank
The political situation in Fiji last year did emphasize the problems that can occur with field genebanks, if they cannot be given the attention they need. The genebank went through two cycles, but with both cycles there were problems. Some of the problems encountered were largely due to the political situation. These were:

?? Reduction of budget allocated; hence,
?? No casual labourers
?? Untimely arrival of materials such as fertilizers and chemicals.
?? High incidence of theft
?? Trampling by cattle
?? Increase in labourers absenteeism
?? Inefficient control and management of labourers
?? Extended wet weather.

The *in vitro* genebank also suffered at this time due to lack of observation and monitoring. There were several days when staff had to stay at home because of security reasons, and when at work it was difficult to focus on work with the day's events. However, because the *in vitro* genebank is within a far more controlled and self-sustaining environment, these problems had minimum impact, as indicated by no accessions lost from the *in vitro* genebank, compared to four accessions lost from the field genebank.

Table 5: Summary showing all costs for maintaining 50 accessions in the field at KRS

Activity	Cost of Collection/ Year (F$)	Cost of Each Accession/Year (F$)	Cost of Each Plant/Year (F$)
Variable costs			
[A] Land Preparation			
- Slashing (Machinery)	0.00	0.00	0.000
- Ploughing (Machinery)	23.18	0.46	0.077
- Harrowing (Machinery)	21.06	0.42	0.070
- Field Marking	12.85	0.26	0.04
Total Cost of Land Preparation	57.09	1.14	0.19
[B] Planting and Replanting			
- Planting	55.67	1.11	0.19
- Plant Rescue[a]	0.00	0.00	0.00
- In-filling	11.00	0.22	0.04
Total Cost of Planting and Replanting	66.67	1.33	0.22
[C] Crop Maintenance			
- Weeding	180.45	3.61	0.60
- Fertiliser	208.76	4.18	0.70
- Insecticides	0.00	0.00	0.00
- Fungicides	98.61	1.97	0.329
Total Cost of Crop Maintenance	487.82	9.76	1.63
[D] Harvesting	98.00	1.96	0.33
Total Variable Costs	710.00	14.19	2.37
Medium Term Variable Costs			
[A] Equipment	30.93	0.62	0.10
[B] Stationery	19.89	0.40	0.07
Total Cost of Medium Term Variables	50.00	1.02	0.17
Fixed Costs			
[A] Capital	80	1.60	0.27
[B] Labour	9993	199.86	33.31
Total Fixed Costs	10073	201.46	33.58
Total Costs	**10830**	**216.67**	**36.11**

This table shows all the costs for maintaining the 50 accessions at KRS for one year. As can be seen when comparing the costs here with the costs as shown in Table 2, there is very little difference between field and *in vitro* costs for a collection of 40 to 50 accessions. Again the impact of fixed costs is seen. The labour input in the fixed costs for the field genebank is fairly high and it is possible that reductions could be made here.

Assuming these costs are correct and labour input has been calculated accurately then, it does indicate quite clearly that the costs of the two forms of conservation are very similar. Depending on the circumstances, *in vitro* conservation could be a more sustainable use of resources because there are the added advantages of *in vitro* storage.

Conclusion

During the period of the IVAG, important components of the establishment and operation of such a genebank were assessed. These included: sampling of material from the field for the genebank, micropropagation of the accessions for storage, the applicability of the storage system, and the cost of maintaining an IVAG. Because of the problems generating sufficient material for storage, the IVAG only ran for 16 months. As a result, components of the IVAG, such as genotypic stability were not fully evaluated. Logistical aspects of *in vitro* storage like equipment needs, supplies and technical staff requirements were also determined in the period of the IVAG.

Using the methodology evaluated in this study of low growth temperature and a basal medium supplemented with the growth regulators, BAP and NAA, the subculture frequency ranged from six to nine months; this variability was attributed to genotypic effects. No accessions were lost from the IVAG, but because of the genotypic effect and also the problem with bacterial contamination, replicates had to be replaced from outside the IVAG, that is, from the multiplication phase attached to the IVAG.

Very little suckering was observed in any of the cultures. As the sample of accessions in the IVAG covered a range of different varieties with differing suckering ability, this was obviously due to the conditions of the IVAG, and was not the result of genotype. A preferable system could be one that encourages some suckering, yet at the same time, reduces apical dominance and rooting. In this way, the IVAG could always be supplied by material from the genebank, should replicates be lost. There would be less concern too, about old cultures becoming sources of contamination for the next culture.

Morphological characterization relied on shoot number, callus formation, rooting, hyperhydricity, stunting and leaf shape. No changes were observed in these characters to indicate a problem with genetic stability.

Actual maintenance costs for both systems are very similar, and the spreadsheets prepared for both types of genebanks enable a full analysis of all costs. As stated with the *in vitro* genebank it would be very difficult to reduce the actual tissue culture costs further. The major cost is with the fixed costs. This is the same with the field genebank, and so if reductions in costs are to be made with either method, it must be in the fixed costs (labour?). It could be argued that where conservation is concerned, *in vitro* is better value, as the accessions are protected from any pest and disease outbreak, or climatic disaster, unlike with the field genebank.

Recommendations

?? The reason why *in vitro* storage is being used has to be clearly defined. The value of this approach should be balanced against other conservation strategies. *In vitro* storage enables germplasm to be maintained in a pathogen-tested state, which could provide farmers with a yield advantage over several years. This might also be important in larger island countries where certain diseases are found in only some areas. It also offers advantages on space and vulnerability to pest and disease attack, and climatic extremes.

?? Any slow growth storage system will not work for all varieties to the same extent. There has to be allowances for this and this will impact on resources with the need to subculture different accessions at different times.

?? Only those accessions that respond well to the existing multiplication system, and also the slow growth storage system should be put into *in vitro* storage. Using accessions with low responses to both will jeopardize the security of that accession.

?? Accessions should be screened for bacterial contamination prior to being introduced into *in vitro* storage. This will eliminate any problems with bacterial contamination later.

?? There should be relatively easy access to the material required for the genebank, as this will facilitate the sampling and screening process.

?? A decision has to be made as to when is the optimum time to subculture. If cultures are left for too long, then this increases the risk of fungal spores being present to contaminate the next culture. A balance has to be achieved between this risk and use of resources, as the more subculturing required, the more resources will be used.

?? Five replicates would be the minimum number of replicates recommended for an *in vitro* genebank of taro. However, if sufficient material is available for initiation into the genebank, then using more replicates would increase the security of that accession and lengthen the time period before subculturing is required.

?? Whichever genebank system is selected, the manager/curator must be aware of all the costs and carry out a full cost analysis to ensure that the resources available will sustain all activities.

?? The documentation system for managing the process depends to a large extent on the size of the collection and the resources available. It can be simple cards or a computerized system.

?? Proper labelling of plants throughout the system is necessary. Accessions should be given accession numbers that are specific to that genebank at the time of introduction to the genebank. Ideally these accession numbers should include letters and numbers, as this helps to prevent mis-labelling problems. The use of variety names should be avoided, as this can lead to errors.

?? Close monitoring and accurate recording is required.

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5.3 Cryopreservation of taro using vitrification.

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Introduction.

Cryopreservation has been recognized as a practical and efficient technique for long-term storage of vegetatively propagated plants, requiring minimum space and relatively low costs. Added to this, it provides a conservation method, which preserves genetic integrity. Many of the International Agricultural Research Centres (IARCs) are looking at cryopreservation as the means to store large numbers of accessions on a long-term basis, with the minimum input of resources, while at the same time guaranteeing the genetic integrity of these accessions.

Cryopreservation methodology has advanced significantly in recent years and new procedures, such as vitrification, encapsulation-dehydration and encapsulation-vitrification, have led to an increase in the number of species that can be cryopreserved.

The method used with taro in the TaroGen project was developed in Japan (Takagi *et al*., 1997), initially for *Colocasia esculenta* var. *antiquorum*, but was later used with var. *esculenta*. Good recovery rates were obtained with both, though the recovery rate was higher for var. *antiquorum* than for var. *esculenta*. This method is a vitrification procedure, which enables direct immersion in liquid nitrogen, after osmotically dehydrating the cells and meristem in a highly concentrated vitrification solution. Vitrification is achieved through using a glycerol-based, low-toxicity solution (PVS2), which sufficiently dehydrates cytosols without causing injury. As a result they are converted into a stable glass when plunged into liquid nitrogen. The vitrification procedure involves several stages. These are: a preculture or preconditioning stage, treatment ('loading') of samples with cryoprotective substances, dehydration with highly concentrated vitrification solutions, rapid freezing and thawing, removal of cryoprotectants ('unloading'), and recovery.

It has been found that a preculture or preconditioning stage on a culture medium, which incorporates cryprotectants, can enhance the survival of meristems after freezing. Several theories have been put forward to explain this. One suggestion is that the high osmotic pressure resulting from the high level of sucrose in a culture medium can trigger certain responses in plant cells, such as the accumulation of ABA and/or proline. The presence of these substances can protect against freezing damage. Loading consists of treating explants in a liquid medium supplemented with cryoprotective substances. These substances reduce the sensitivity of the material to the highly concentrated vitrification solutions, which follow later.

After this dehydration step with the vitrification solution (PVS2), specimens are then cooled rapidly by direct immersion in liquid nitrogen, in order to achieve vitrification of the internal solutes. Similarly rewarming is carried out as quickly as possible to avoid devitrification, as this would lead to the formation of ice crystals detrimental to cellular integrity. The unloading part of the process aims at progressively removing the vitrification solution, thus reducing the osmotic shock.

Methodology and Results

The procedure used by Takagi *et al.,* (1997) is as follows:

?? The first stage which requires some conditioning of the tissue prior to the loading stage was either:
(a) Preculture; excised shoot-tips were cultured on solid Murashige and Skoog (1964) medium supplemented with 0.3M sucrose overnight in the dark, or
(b) Preconditioning; excised shoot-tips were cultured on MS + 60, 90 or 120g/l sucrose for 1-3 months
?? Loading; explants were loaded with a mixture of 2M glycerol + 0.4M sucrose for 20mins at 25?C
?? Dehydration; explants were exposed to PVS2 (30%w/v glycerol + 15% w/v ethylene glycol (EG) + 15% w/v dimethyl sulphoxide (DMSO) + 0.4M sucrose) for 12mins at 25?C
?? Immersion in liquid nitrogen
?? Thawing; 1 min @ 40-45?C followed by 1.5min @ 25?C.
?? Unloading; 3 washes with 1.2M sucrose
?? Recovery; MS + 0.1M sucrose. Cultures are maintained in a dim light for 10 days before exposure to full light conditions

Eight varieties from four different countries were used in this study. Of the eight, successful recovery was only achieved with three varieties. The average recovery rates for these three varieties were 29, 20, and 29%. Higher recovery rates (> 70 per cent) were obtained in individual trials with these same varieties. The optimum exposure time for the vitrification solution, PVS2, was found to be 12 mins.

This study showed that the quality of the meristem, and the quality of the stock plants play a very significant role in cryopreservation success. It was found that using older donor plants as sources of the shoot tips gave better results, than using recently subcultured plants. With the variety Tausala ni Samoa, plants that had been cultured for seven weeks on a preconditioning medium supplemented with 90g/l sucrose gave the optimum recovery rate (100 per cent). In contrast, the optimum results were achieved with two other varieties (CPUK and E399), when they were cultured for three months on medium supplemented with 30g/l sucrose, followed by an overnight preculture on a medium supplemented with 0.3M sucrose. The quality of the plant, and of the meristem was also affected by the culture container in that plants cultured in the larger glass jars produced meristems, which were larger, less succulent, more rigid and more homogenous, than those grown in the small glass jars.

This investigation has demonstrated that cryopreservation of Pacific Island taro is possible, but for realistic recovery rates to be achieved, further research is required to improve the quality of the source plants, and to optimize conditioning treatments for different varieties.

Implementing a cryopreservation strategy

Cryopreservation costs for the RGC have been estimated at F$270 for labour and F$75 for consummables, for 200 taro shoot-tips per week. Assuming that 40 shoot-tips would be cryopreserved for every accession this would be a cost of F$69 (US$30) to cryopreserve one accession. This cost just takes into account variable costs and does not include medium term variable or fixed costs, except for the actual labour involved in the procedure. As we have seen from the spreadsheet produced for slow growth storage *in vitro*, fixed costs can be a major part of the cost of any conservation strategy. Although initial costs are high, in the long-term cryopreservation is less costly than slow growth storage because maintenance costs are lower. Fixed costs would be less with a cryopreserved accession, because in maintaining that accession, far less equipment and staff are involved. In addition, in order to see the real difference in costs between different conservation strategies, one needs to construct budgets over time (say 20 years). The budgets currently being analyzed are only annual budgets. There are also indirect costs, which are difficult to calculate, and these can be high. These costs result from losses of accessions, and changes in the genome due to genetic instability. Such risks are greater with slow growth storage than with cryopreservation.

?? Initial decisions

What accessions do we want to place in *in vitro* storage and which ones should we cryopreserve? Generally *in vitro* storage starts with ‘at-risk’ plants, that is, those plants that are likely to be lost in the field genebank. With the taro collection, the strategy that has been discussed is to put the core collection into slow growth storage, and cryopreserve the base collection, assuming that the protocol can be developed to the level at which it is applicable to this wide range of varieties. Therefore, as the decision about what to store has been made, the next decisions involve those of logistics. How many samples of each accession should be stored? This will vary depending on the recovery rate, how many recoveries over time will be required and if periodic testing is anticipated.

?? Developing a testing and storage protocol

Before reaching this stage the protocol being used should result in at least a 40 per cent recovery rate, preferably higher, with the varieties that have been tested, and ideally the varieties tested should have originated from different countries. This would mean that for every 40 samples cryopreserved, 16 would survive. Then it is a case of applying this protocol to other, as yet untested, varieties. There are two schools of thought on this. One recommends storing five vials of 10 propagules, and using one as a control. If the control recovers with a high % (>40 per cent), then four vials remain in storage. Alternatively, a small number of propagules can be stored in two vials, and both thawed after a short time. If the recovery rate is >40 per cent then another group is processed and stored. If the percentage is lower, then improvements are made to the culture or cryopreservation protocols to improve the recovery rate. CIP in Peru have adopted a strategy in that they are utilizing a protocol that has shown some promise with potato. They tested 80 genotypes, and for each genotype 120 shoot-tips were stored. For each evaluation 20 shoot-tips were checked, with the first evaluation at one month, and the second at three months. This same assay was repeated with accessions where success was very limited, and if after repeating it failed to work then the assay was performed again but with different dehydration times. CIP has chosen a lower recovery rate, and have stored larger numbers to allow for this. They can cryopreserve eight genotypes on a weekly basis. For this purpose, 290 shoot-tips are isolated, 20 are used as controls, and the remaining 270 are frozen. 20 were thawed one day after freezing. This left 250 to be stored under LN with a survival rate of 20 per cent plus. CIP's system permits storage of 960 accessions (250 shoot-tips organized in five cryovials containing 50 shoot-tips each) in a LN storage tank of 130l. The cost to date for one accession is US$35. This cost is very similar to the cost calculated for cryopreserving one taro accession in the RGC. It is not clear whether the CIP costs were calculated using a full cost analysis. One would expect that with CIP there should be economies of scale. Added to this, it is easier to excise a potato shoot-tip than a shoot-tip from taro, which would enable greater numbers to be processed with potato, thus reducing labour costs. CIP has cryopreserved 197 potato accessions, and eventually expect to be able to conserve the Potato Base Collection (4,000 accessions). Accessions from this base collection are not often requested.

Because of the size of CIP, devoting resources, both labour and facilities to cryopreserving large numbers of shoot-tips is not a problem. However, this is not feasible with the resources available at SPC. The storage capacity presently within the RGC is limited to two dewars. These two dewars will store approximately 320 accessions, provided we are working at a rate of 40 shoot-tips per accession. The current method is giving recovery rates that are averaging around 30 per cent with the varieties that have been tested. It is planned that further testing will be done with this method, and at the same time, the technique of encapsulation-vitrification or encapsulation-dehydration will be investigated. Ideally recovery rates of 50 per cent are required before any germplasm is cryopreserved for storage. This rate would enable the resources of SPC to be used sensibly and is the rate at which the costs have been calculated.

?? Maintenance of a back-up collection in slow growth

Slow growth storage has been proposed as a back up for cryopreservation only until all of the accessions have been cryopreserved, and there has been sufficient evaluation of the system in terms of regeneration and genetic integrity. This is the normal procedure adopted by any germplasm centre utilizing cryopreservation as a conservation technique. When a cryopreservation technique is implemented, it takes time because of the methodology involved and also because varietal differences occur, and so techniques have to be adjusted. Consequently the slow growth collection can be used to maintain the accessions until the cryopreservation methodology can be applied to all accessions. Even when all accessions have been cryopreserved, there will, of course, be a duplicate of this collection elsewhere for security purposes.

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5.4 Taro Seed Storage and Germination Protocols used by PNG Breeding Program

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Introduction

Taro (*Colocasia esculenta*) is vegetatively grown root crop. However, seed production is not uncommon, especially in PNG. The breeding programme of PNG regularly produces seed for crop improvement. A range of genotypes flowers naturally in crossing nurseries and fields in PNG climate. Flowering can easily be induced in genotypes which do not flower naturally using gibberellic acid (0.3 - 0.5 g/l). Seed production is, however, highly specific to genotypes (G), environment (E) and GXE interactions.

Taro is a monoecious plant. The plants are self-compatible, but artificial cross-pollination is readily accomplished because the male and female flowers develop in different parts of the spadix, separated by a sterile region and because of protogynous condition. Taro genotypes are usually classified as self-compatible, partly self-compatible or self-incompatible. Self-compatible genotypes tolerate self-pollination, resulting in normal seed production in isolation. However, isolated partly self-compatible plants rarely produce seed because a fruit does not develop if only a few female flowers in the inflorescence are fertilised. Self-incompatible genotypes never produce seeds in isolation (Ivancic *et al*., 1994).

The viability of seeds depends upon various harvesting, storage and germination methods. This paper highlights various protocols and factors affecting seed germination under PNG environment.

Harvesting of seeds

Fruit is harvested when berries turn lighter in color and softer in appearance (generally four to five weeks after pollination). Seed germination was found to decline if harvesting was done before three weeks. Seeds are extracted from fruit by washing in sterilized water. Clean seeds are dried under room temperature. Drying of the seeds under direct sunlight is avoided because it was found to reduce viability.

Storage of seeds

After harvesting, the seeds are stored in a desiccator inside the refrigerator. The seeds can stay in refrigerator for about one year with 60-70 per cent viability, but germination does reduce with time. The best germination rate is achieved within the first three months. Seeds can also be stored at cooler temperature. However, the germination frequency is less than that under refrigerator storage. Seeds stored at room temperature under BARC conditions were found to have further reduced germination.

Planting of seeds

Seeds are planted in pots filled with sterilized soil taken from deep under-ground and pots placed in special waterbeds. Waterbed germination was found to be simple, cheap and very reliable. It was found to be 90 per cent efficient. Waterbed germination can be further improved by covering pots with transparent plastic sheets, immediately after sowing. Germination of seeds directly in petri dishes was relatively less efficient than direct planting in pots, but still comparable.

Conclusion

Germination of taro seeds is affected by genotypes, environmental factors, harvesting conditions, storage conditions and germination protocols. The results presented here are based only on routine observations and not on any scientific and statistically designed tests. There is a need for more detailed testing in order to understand the factors leading to improved germination, especially if taro seeds are to be cryopreserved as one of the conservation strategies.

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5.5 The Collection, Description, Documentation and On-farm Conservation of Taro in the Solomon Islands

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Introduction

The previous taro collection, collected in 1999, and maintained at Fote, Malaita, Solomon Islands, was lost for two main reasons:

?? Disease (alomae) affecting the field genebank (the area where the field genebank was established is particularly prone to alomae and bobone virus')

?? The ethnic tension, which led up to and continued after the coup of June 2000 disrupted management of the field genebank

Civil unrest in Solomon Islands has had far-reaching effects, severely disrupting government services and creating a high level of uncertainty and instability throughout the country. Some of the major effects of unrest have been:

?? As estimated 30-50 thousand people have been internally displaced. More than 20,000 thousand of these have returned to North Malaita.

?? Near collapse of the national economy with economic recovery estimated to take ten to fifteen years

?? National unity is uncertain with calls for independence from some provinces and negotiation of a new state government system. Ethnic divisions have drawn lines between Solomon Islands people as never before.

This situation is classified as a 'post conflict' period with the signing of a peace agreement between the two militant groups. It is this environment in which the assignment is being carried out by the Solomon Islands Planting Material Network (PMN). PMN is a non government organisation (NGO) with over 400 members across Solomon Islands and the Agriculture Research Division of the Ministry of Agriculture (MAPI).

Objectives of the assignment:

1. To recollect, describe and document taro in Solomon Islands
2. To promote awareness of taro diversity in Solomon Islands and involve rural communities in the use and on farm conservation of taro genetic resources

The PMN's organisational mission and objectives are:

'To encourage and assist village farmers and organisations working directly with village people to conserve and sustainably manage a diverse variety of crops needed to maintain household food security and a diverse and sustainable village agriculture'

The objectives of the network are:

1. Share planting materials among village farmers
2. Encourage self reliance and conservation of planting materials by farmers and for farmers
3. Encourage diversity which leads to food security

The network was formed in 1995, and now has over 400 members in a national network made up of farmers, teachers, extension workers, NGO's, community and women's groups. The network conducts awareness raising activities, practical village level agriculture training, and seed production training for village service organisations such as rural training centres as well as producing and distributing seed to rural farmers.

Recollection of Taro

Due to limited funds and time constraints only four provinces were chosen for recollection. The selection of the provinces was based on PMN membership patterns and also as a rough cross section of taro diversity in the country based on the previous collection data. The network had the capacity to carry out the collection through its members working in partnership with agriculture officers as technical advisers despite the impacts of civil unrest.

The main interest expressed by the board and members of PMN when consulted about participation in the TaroGen project were:

?? Opportunity for some *in situ* conservation activities (diversity fairs) to promote on farm conservation – the primary focus of the PMN

?? Participation in a regional programme with the chance to develop regional links to strengthen the network

?? Capacity building of the network through training of members and new links with the Agriculture research and extension in the participating provinces.

The following provinces are participating in the collection: Malaita, Guadacanal, Choiseul and Temotu.

<u>Process of collection</u>

The following steps were/are being carried out for the assignment:

1. An initial planning workshop with the TaroGen Technical Director and PMN/Ag. Research resource people
2. Planning workshops were held in each province over two to three days. The workshops included PMN members, local agriculture and research officers where available, and PMN resource people from Honiara.
3. Collection by PMN members: passport data, morphological descriptors; conduct participatory rural appraisal (PRA) exercises with selected groups
4. Establish field genebanks for one season
5. Diversity fairs connected with field genebanks and organised by network members

Each of these steps is outlined in more detail below.

<u>Planning workshops</u>

?? Two to three days workshop in each province that involved PMN members (farmers) and local taro expert farmers

?? Agriculture division from that province (except Guadalcanal where they were not contactable)

?? Introduction to the TaroGen project and its objectives

?? Discussion on conservation of taro diversity through trial of participatory rural appraisal (PRA) tools to be used in the community during collection
?? Training on how to record morphological descriptors and passport data
?? Planning of collection target areas and the location and management of the field genebank
?? Establish diversity fair planning committee

Collection

Teams of two to three PMN members in each group carried out collection supported by Research Division and Extension officers where possible. In all provinces except Temotu at least two different collection teams were working in different areas. Farmers provided taro in exchange for network membership (which entitles them to seeds) in Choiseul and Guadalcanal provinces. In Malaita and Temotu provinces farmers received payment following the precedent set by the previous collection in 1999. Taro donors have been suggested for the core group of farmers involved in awareness activities and in the establishment of an on-farm conservation network through the diversity fairs.

Provincial field genebanks

The field genebanks have been established in each province at the following locations:
?? Malaita – Busurata Village – PMN members, farmer group
?? Guadalcanal – St Martins Training Centre – vocational training centre for youth
?? Choiseul – Choiseul Bay Demonstration Farm – provincial extension services demonstration farm
?? Temotu – Field genebank with farmer family on Santa Cruz island

Collection results

Province	1999 collection (lost)	2001 Collection (no. of accessions)	2001 Collection areas	Field Gene Bank planting completed
Malaita	173	313*	Kwa'arae-bush, Lau – bush and coastal, Toambaita – bush and coastal villages.	29/08/01
Guadalcanal	10	220	Guadalcanal Plains – bush and coast villages Longgu (North East) – bush and coastal villages.	31/08/01
Choiseul	72	245	Six areas in north-west and south-west. Coastal villages.	17/08/01
Temotu	291	46*	Santa Cruz Island – details not available at this time	24/08/01
TOTAL	(all of SI – 594)	824		

*Exact figures for Malaita and Temotu are still being finalized as passport data has not yet reached Honiara.

Numbers of taro collected have been higher than expected. Duplicates are expected within and between the provincial collections, especially where the same variety has been given different names in different language areas in the same province. Many farmers commented that if relationships and trust were built further over time then more taro varieties would have been collected as rare taros were not being handed over.

Weakness

?? Carrying out morphological descriptors at the same time as collecting. Many taros are not mature and it is difficult to visit gardens to observe all the taros collected. Many are collected in the villages where farmers have bought them down from gardens when they heard about the collection programme. However, given the circumstances the collectors have done a very good job.
?? Recording of PRA data by PMN members / facilitators has been poor. This has been expected as it takes more than two to three days training to be able to carry out this work accurately. Experienced facilitators are interviewing the collectors on their return about farmers' comments on taro conservation and diversity to overcome this problem.
?? Tight timeframe due to TaroGen external constraints and logistical difficulties have affected the collecting
?? Insufficient time to build relationships with farmers to collect ' rare and hidden' taro varieties
?? Use of local terminology and farmers perceptions/ criteria in collection forms would most likely have led to more accurate results with descriptors and passport data.

Strengths

?? Farmers involved in the collecting and in the field genebanks with high level of interest and ownership
?? Opportunity for farmers to share local knowledge
?? The collecting is building the capacity of the local network and local organizations involved
?? Building links between the network and extension in the provinces concerned
?? Exchange of taro for seeds has led to a very good response
?? Awareness raising about on farm conservation through PRA data collection exercise
?? Taro donors and collectors will form the beginning of an *in situ* conservation network
?? Local field genebanks are accessible to farmers and the farmers involved have some degree of ownership

In situ / on-farm conservation: some preliminary results

The data from the PRA exercises is still being collected and analysed. However, some trends have been observed, and are noted below.

Rapid Rural Appraisal Approach

These are the steps being used (each step is described in more detail below)

?? Collectors were trained to carry out PRA exercises during the three day planning workshops in each province.
?? PRA exercises were carried out in a minimum of two villages in each province.
?? Qualitative data from PRAs is recorded and then analysed
?? To fill in any gaps in recording or data collection, the PRA facilitators are then interviewed in focus group discussions by experienced facilitators from the PMN. This allows recording of information that the facilitators heard or observed but did not actually write down in their notes.
?? Extra PRAs will be carried out based on the analysis and comments from the focus group discussions. This might occur in one to three villages to fill in any gaps in the data.
?? Code the data into thematic groups
?? Prepare a draft report
?? Facilitate feedback on the draft report through comment from collection groups in each province and from the key Agriculture Research and PMN people involved
?? Produce the final report

PRA Tools

Three PRA tools were used. They were selected and adapted at the first assignment planning meeting:
?? Change over time (a historical matrix)
?? Garden diagram
?? Taro varieties brainstorm and focus group discussion

A checklist of the main areas to be covered during that tool/ group exercise accompanies each tool. The tools are exercises to promote discussion, reflection and analysis of the topic by participants. Recording of the participants' discussions is very important. Likewise, good facilitation skills are important to ensure that the facilitator does not overly influence discussions with his/her opinions, instead carefully guiding discussions through the key areas.

Changes or trends

Some areas appear to have increased in taro diversity in recent decades but most have declined. The decline in diversity in coastal areas is more severe than in bush communities. Areas where there is very high land pressure (short fallow periods) are less likely to be growing taro in any significant amount.

Influencing factors

?? Market opportunities and demand. Often other crops with a high market value are displacing taro production.
?? Soil fertility and fallow periods were an often mentioned limitation to growing taro as opposed to sweet potato, which produces well in less fertile soils and is now the major staple food crop.
?? Increase in diseases, especially taro beetle but dieback and other diseases were also mentioned

?? Changes in diet preferences. New preferences are for rice and sweet potato especially with the younger generation. They have grown up eating these new foods/crops and do not have the strong desire that older people have for taro.
?? Labour/yield of taro relative to sweet potato is such that sweet potato is now the most important food crop. In some areas sweet potato is followed by cassava in importance.

Why are taro varieties being lost?

?? New varieties have displaced old varieties of taro
?? Lack of customary or cultural practices to 'respect' taro, for example, traditional magic to protect taro, use of traditional shell for cutting of taro, traditional garden layouts. Some people, especially the elderly, believe that the breakdown in these practices has resulted in increased disease and problems affecting taro production and hence its decline.
?? Hiding and lack of sharing of special varieties leads to eventual loss of those taro varieties
?? There are less people growing taro and so the risks of loss are higher
?? Many people are only growing a small quantity of taro and so do not maintain very many varieties
?? It is hard work to grow taro compared to other crops.

Who is responsible for taro?

?? Gender differences were apparent in different provinces in regard to different roles in taro cultivation. In Malaita men are the main growers of taro while in Choiseul women are responsible for taro.
?? Old people maintain the taro tradition
?? Middle aged people are the main collectors and holders of taro diversity at this time.

New Varieties

?? Many people expressed interest to try new varieties if given the opportunity. Many people also expressed an interest to get back old varieties that have been lost, with some identifying particular varieties they would like.
?? Sweet potato has increasing pest problems so some people are turning back to growing more taro, especially in areas where there is suitable soil.
?? Some older people do not want any new varieties of taro, they prefer to continue growing their traditional varieties.

How are new cultivars found

Many new varieties are bought from other villages, islands or provinces. They are usually named after the place they come from or the person who introduced it. In Choiseul province farmers reported that new varieties are found growing in old garden sites after a long fallow of 10-20 years. Taro had been planted there previously. When the garden site is cleared taro seedlings are reported to grow and some experienced farmers look for new varieties among the seedlings and keep those that are of interest. These new varieties are typically named after the person who found them as 'gaden bilong' (name of person). One example was given of a taro found growing high in a forest tree in the canopy when the tree was cut down for timber. That taro is now widely grown and is named after that tree. People observed different types of parrots eating taro flowers and seeds and assumed that these birds must drop the seeds in different places such as in the tree example above.

Ideas to encourage and support on farm conservation

The following ideas came from farmers and PMN members as ways to encourage the revival and maintenance of taro diversity:

?? Diversity fairs (as proposed by the project)
?? Market development and linkages – the current market price is SBD$5-10kg in Honiara. A problem is that the areas of high diversity are often remote and isolated areas where transportation to market is difficult.
?? Community diversity registers
?? Focus on the current custodians of taro diversity and assist and encourage them to continue to maintain that diversity.
?? Soil improvement methods, for example, improved fallows, to allow taro cultivation in areas where its decline is associated with soil degradation.
?? Improved pest and disease management
?? Awareness raising on the role of diversity for food security and hence increased appreciation of diversity
?? Revival of traditional knowledge and traditional values related to taro production and use.

Diversity fairs

The following points are a summary of the main recommendations of the workshop groups in planning the diversity fairs for early 2002.

?? Will involve taro donors from the collecting as the core group of farmers to begin with
?? A range of community activities to coincide with taro harvest at field genebanks (cooking, competitions, crafts etc)
?? Exchange of taro among the donors. This will be recorded in community diversity register that will be started at the diversity fair
?? Training and awareness raising in practical areas such as taro disease and breeding
?? Sharing of kastom knowledge related to taro

Recommendations for the safe and secure duplication of the taro core collection.

Mary Taylor
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Introduction.

The core collection representative of the genetic diversity in the taro collected by several countries under the TaroGen project is currently maintained *in vitro* at the SPC Regional Germplasm Centre (RGC). For each accession, at least five replicates are being maintained. This collection will continue to be maintained by SPC, either under slow growth storage or cryopreservation. None of the accessions have been tested using the cryopreservation protocol. This will be carried out gradually, and only those accessions where a 40 per cent recovery rate is achieved will be cryopreserved. Slow growth storage will be the main conservation strategy used for preserving the taro regional core collection. Although it has been shown in the Pacific, and with other international organizations concerned with germplasm conservation, that *in vitro* storage is safer than maintenance of germplasm in a field genebank, there is still a need to conserve this germplasm elsewhere, that is, maintain a duplicate core collection. As well as the taro core collection, the rest of the RGC collection has also got to be duplicated.

Duplication of any collection is essential and is an important component of a complementary conservation strategy. In a consultation meeting on management of field and *in vitro* collections (Engelmann, 1999), safety duplication was considered and the following recommendations were made:

?? One complete duplication in one location or dispersed over several locations
?? *In vitro*, if possible; cryopreservation where feasible; or field germplasm collection.
?? Distant from the original, preferably in another country because of civil risks
?? Under own management, that is, black box, or collaborator's management
?? Secure and appropriate.

It is possible that some accessions, currently in the regional core collection will be maintained in national collections. However, there is still a need to replicate the core collection in a formal duplicate collection. Several locations have been considered for this purpose, and they fall into three categories, namely a national institute, a regional institute and an international institute.

National Institute.

At the last TGRC meeting in Nadi, (May 2001), when the issue of duplication was discussed, the possibility of conserving the taro core collection at the Coffee Research Institute, Aiyura, PNG, was raised. CRI has a very well equipped tissue culture laboratory, which was established in the early 90s with Australian aid for the purpose of multiplying a coffee variety resistant to coffee rust. The laboratory is a self-contained building containing all the essential tissue culture equipment, as well as having HEPA filtered air circulating in the transfer rooms. The laboratory consists of two large growth rooms, and one small growth chamber. The growth rooms can hold 23,270 vials of 6.5mm diameter, and 35,320 vials of 3.5mm diameter. Therefore, space and equipment are not a problem. Discussions have been held between NARI and CRI regarding the possibility of the laboratory at CRI holding the duplicate core, and in principle, CRI is willing to share its laboratory facilities with NARI/SPC, but detailed arrangements on sharing would have to be worked out.

CRI lacks the capacity to fully utilize the laboratory. Future activities include the multiplication of selected Robusta coffee lines. Staffing could be a problem in that the most experienced member of staff, with tissue culture skills could be leaving. This would mean only one graduate officer in the laboratory with tissue culture skills. However, there is a planned tissue culture training workshop for next year, which would result in more staff with tissue culture skills. The European Union has indicated a willingness to assist CRI with funding to restore the laboratory to a fully operational condition, but the details of this project are not known. As indicated by the size of the growth rooms, the facility is quite large, and as such, may be difficult to sub-divide, therefore basic running costs may be high. Some costs have been provided and these are electricity costs at K10,000 (US$3044), and maintenance costs at K4,500 (US$1370) per annum. Details of these costs were not specified.

Aiyura is in the Highlands of PNG and is three to four hours by road from Lae, which may be a problem if trying to access good maintenance services for equipment breakdowns. In addition, there have recently been some vehicle hold-ups on the road between Aiyura and Kainantu, which would make it difficult for staff, especially female staff, and also non-Highlanders. The laboratory itself has not been the target of rascal activities in the area.

In conclusion, the laboratory is basically an excellent tissue culture facility, and should have no problems with contamination rates because of the nature of the design of the building. However, there are certain issues that would need careful consideration if the laboratory was selected as a site for duplication and these would be:

?? Staffing
?? Security
?? Access to good maintenance services
?? General lack of use of the facility by CRI. This is likely to mean that any staff working there would be working very much in isolation which could have a deleterious effect on motivation

Regional Institute

A tissue culture laboratory was established in the School of Agriculture campus of the University of the South Pacific, Samoa, in the late 80s. This laboratory was the centre for regional tissue culture services under the Pacific Regional Agricultural Programme (PRAP), from 1990 to 1999, and collaborated with the SPC tissue culture laboratory in the provision of these services. When EU funding under PRAP finished, the laboratory became the responsibility of the university. The laboratory no longer maintains the regional collection it had under PRAP, but is currently working with a number of local crops. The university has not yet developed a work programme for the laboratory, but is willing for the laboratory to be used for duplicating the RGC collection.

There are currently four well-trained staff working in the USP laboratory. All are funded by USP but on short-term contracts. These short-term contracts have been supported with funds from other university positions that have not been filled. It is likely that these positions will be filled by the end of this year. The university has yet to commit itself fully to the laboratory, and to formally establish it as a working facility within the university, supporting teaching and research.

The laboratory is fully equipped, with two growth rooms, measuring approximately 2.5m by 2.5m by 3m. One growth room can be run at a cooler temperature to facilitate slow growth storage. When the laboratory was constructed, there was little room for creating an optimum environment for sterility. The laboratory had to be accommodated within a very old building, and rooms could not be positioned so that there was movement directly from one to the other. This lack of a unilateral flow has meant that contamination rates are high, because staff and equipment have to move across a very dirty corridor. During the course of PRAP, a lot of time and resources were spent ‘cleaning’ contaminated cultures. The contamination rates are currently running at 10 to 20 per cent, when the acceptable rates in a tissue culture laboratory are 2 to 5 per cent. If the USP laboratory is to be effective as a duplication site for the RGC collection, then there would have to be some relatively major modifications to the laboratory, in order to reduce the current contamination rates. Costs have been obtained from the USP laboratory, which have taken into account the need for upgrading the facilities. Maintenance costs for the RGC collection for one year have been calculated at ST$52,535.39, (US$15,616), which include consummables, labour and electricity. Two quotes have been obtained, both of which would improve the layout and therefore the flow of the facility. For ST$16,000, (US$4,755), the function of an existing room can be changed so that it can be incorporated into the tissue culture facility, and thereby enabling a unidirectional flow in the facility. Alternatively, for ST$34,384.35, (US$10,403), an extension can be built adjoining the unit, which again would bring about a unidirectional flow.

In conclusion, the USP laboratory is a well-equipped laboratory, with all the essential requirements for duplicating the RGC collection. However, there would be some concerns, which would have to be addressed, if it was selected as the duplication site. These are:

?? High contamination rates

?? Lack of any programme for the laboratory so that it can be incorporated into the university. This would have implications for the future of the staff currently working in the laboratory.

International institute.
The region does have an option to store its germplasm in one of the international agricultural research centres (IARCs), within the CGIAR system. With this in mind, discussions have been held with IPGRI, IITA, Nigeria and CIP, Peru. As IITA has the mandate for conserving yam germplasm, IITA would be willing to hold a duplicate collection of the region's yam germplasm. CIP is the IARC with the mandate for sweet potato conservation, and therefore the sweet potato accessions currently held at the RGC could be duplicated there. CIP have also indicated a willingness to duplicate the taro and yam collections.

Discussions regarding duplication of the RGC collection within one of the international centres began with a request to IITA regarding the possibility of a black box arrangment, that is, where the material is just held as a security back-up with no availability to the Centre or a third party. Although IITA did not completely reject this proposal, they did point out that their policy was to hold germplasm thus ensuring its long-term conservation, yet at the same time make it freely available to researchers from national programmes from any part of the world. This response reflects the policy of the CG Centres. It is the policy of the CG centres to attempt to acquire and hold germplasm without conditions that would restrict its future availability. This means that the Centre would have Prior Informed Consent and Mutually Agreed terms with the provider of the germplasm. This will allow the Centre to designate the germplasm in-trust under the Centre-FAO agreement, thereby placing it in the International Network of *Ex Situ* collections under the auspices of FAO, and held in-trust for the world community. The consequence of this arrangement is that the Centre undertakes to conserve the material for the long-term, and not to claim ownership or IPR over it. At the same time it is able to use the germplasm and make it available, and any related information, to any third party for conservation, research and breeding. However, this availability is subject to a Material Transfer Agreement, which restricts the recipient from claiming ownership, or seeking IPR on the material and any related information, and to obtain similar commitments from any further recipients. Under this arrangement, the germplasm becomes a part of the genebank collection at the Centre, and the accession records appear in the genebank database and SINGER (System-wide Information Network on Genetic Resources). This does provide for safety duplication, as the Centre is required to conserve the material on a long-term basis, and restore it to the provider.

Although this is the policy of the CG system, Centres may consider holding germplasm, which they cannot designate under the FAO agreement. This would either be under (a) a black box arrangement or (b) as a deposit in the Centre genebank. With the latter, the germplasm is managed by the Centre (stored, documented, regenerated, etc etc), and is available to the Centre. However, its third party distribution and use is under restrictions imposed by the provider, that is, it is part of the genebank collection, but cannot be designated as in-trust under the FAO agreement. Under a black box arrangement, the holder is just providing a store in a different location for a security back-up of the material. The material is not registered as part of the genebank collection, and the accession records do not appear in the genebank information system. The holder has no rights to access/open the consignment, nor should they need to, since it is the responsibility of the provider to replace the consignment when its viability is threatened, and to judge when this is needed. This obviously works quite well with seeds, but would create technical problems for *in vitro* material, which needs at the least regular monitoring, if not relatively regular replacement. Cryopreservation could solve this problem of black box storage of *in vitro* material, depending on the cryogenic facilities available at the Centre, and the reliability of the cryopreservation methodology for that particular crop/species. Holding *in vitro* material under a black box arrangement would require a condition in the safety duplication agreement, that the Centre has to monitor and re-culture the material. This would generate costs, and the coverage of these costs may need to be taken into account in the agreement.

The option where the Centre almost acts like a deposit bank, in that the germplasm is registered in the genebank, and managed by the Centre, but has restrictions imposed by the provider on its use and further distribution, is not an option preferred by the Centres. The Centres prefer to take material that can be designated in-trust under FAO, consistent with the CGIAR's position for the unrestricted availability of genetic resources. The CGIAR guidelines do propose however, that the Centres should evaluate on a case-by-case basis, whether the provider's terms imposing restrictions are acceptable.

If germplasm is deposited in one or more of the CGIAR genebanks for designation in-trust, then it does become the responsibility of that CG Centre in conformity with the terms and conditions of the FAO Agreement, with distribution under the terms of the standard CGIAR Centre MTA. However, the Agreement and the MTA are documents approved by the FAO Commission on Genetic Resources for Food and Agriculture, subject to policy decision by the Commission member countries, (more than 100 countries), so there is a form of intergovernmental control in terms of the content of the documents. If the region wants more 'control', and so wants to impose restrictions on the use and distribution of the germplasm, then this would be considered in relation to the political, scientific and management difficulties that these restrictions might cause.

Basically there are three options. Germplasm can be given as designated in-trust germplasm, and if this is the case, it is likely that there will be no cost, especially for the crops that the Centres manage anyway, i.e. yams, cassava and sweet potato. The alternative is to choose maintenance with restrictive use but this will incur costs. Costs could be reduced if the Centre has limited rights to use the material for research, such as parents in breeding programme, or for international trials. In this situation it would be just use by the Centre and no distribution would be allowed. Any benefit sharing is likely to be limited to cases of direct release in significant commercial production systems. Finally, there could be a black box arrangement, such as the arrangement used at CIP (it is possible that only CIP has this arrangement). With their system the duplicate collection would be shipped from here. CIP would maintain, but would only observe, and record. They would not subculture any of the accessions. The RGC would have to judge by the monitoring information provided by CIP, when the cultures had reached their 'sell-by' date. At this time, RGC would instruct CIP to destroy the cultures, and then a new batch would be sent and the process repeated. This would incur some costs, though they would be minimal. If a black box arrangement involved the Centre having to subculture the region's duplicate collection, then obviously the costs that would be incurred would be far greater.

In conclusion, duplication of the RGC collection at any one of the CG Centres would be in excellent facilities, where the technicalities of duplicating the collection, and maintaining it at the highest standards would not be in question.

International Understanding.

The International Understanding is an agreement, which could have implications on exchange of certain crop species. The IU was established in 1983 as a non-binding agreement to promote conservation, exchange and the use of PGR as a common heritage of humankind. In recent years there have been discussions to revise the IU to bring it into harmony with the CBD (sovereign rights, access and benefit sharing). The basis of the IU is the special nature of PGR for food and agriculture, in their importance for food security and sustainable agriculture for all, and the interdependence of countries in their use. The revised IU creates a multilateral system for facilitated access to a negotiated selection of PGR for Food and Agriculture, and for a fair and equitable sharing of benefits arising from their use. If the revised IU is adopted at the FAO Conference in November, it then has to be ratified by 40 countries. It will come into force 90 days after being ratified by the 40th country. The ratification process can take some time, and until ratified the IU has no legally binding effect. But once this treaty has come into force all collections of all crops on the multilateral list will be available under conditions of multilateral access. Access to these will be for the purpose of conservation and use for food and agriculture only. Access will be provided under a MTA, which specifies benefit sharing should commercialization occur. The MTA "shall include a requirement that a recipient who commercializes a product that is PGRFA, and that incorporates material accessed from the Multilateral System, shall pay to the (financial) mechanism …an equitable share of the benefits arising from the commercialization of that product, except whenever such a product is available without restriction to others for further research and breeding. Countries do not have to adhere to the IU if they have not ratified it.

The question of utilization and distribution.

This question of who uses the region's germplasm, once it is stored outside of the RGC arises with all of the proposed duplication sites. With both the national and regional institutes, it is likely that the region would require storage under a black box arrangement. Contracts would have to be drawn up, which stated precisely under what conditions the germplasm would be stored. Although both institutes would adhere to these conditions in management of the germplasm, there is no guarantee that an unauthorised user will not access this material. If this happens with either of these two institutes, it will be very difficult to monitor anything beyond the confines of the two institutes. As well as difficulties that would be incurred in practically trying to do this, it is likely that the legal agreements would only be operative within the institutes. If the RGC collection is duplicated in a CG Centre as designated material, material will be distributed. However, there will be close tracking and monitoring of that material, so that any unauthorised use would be detected either through the FAO system, or through a NGO, such as RAFI, which monitors germplasm flow. Under existing FAO agreements CG system is obliged to follow up on any violations of a MTA, undertake to check and also to contact violator. In late 1997 and early 1998, several cases came to light and were publicized wherein recipients of designated germplasm from a CGIAR Centre sought plant breeders' rights for that germplasm in alleged contravention of the 1994 FAO/CGIAR agreements. In response to these abuses, the Chair of CGIAR, called for a moratorium on the granting of IP rights on designated plant germplasm held in collections of CGIAR Centres throughout the world. The parties seeking PBRs withdrew their applications. Admittedly this should never have occurred, but what matters is that the attempt to acquire PBR on this material failed because of good monitoring. It has also shown that there is a need to strengthen and enforce the international agreement that places germplasm "in trust" for the world community.

The benefits of sharing germplasm have to be acknowledged. Once germplasm is shared, it can be evaluated, and with that evaluation, comes information, which can be of great benefit to agriculture in the Pacific. Placing germplasm in a CG Centre provides an opportunity for evaluation of the regions' germplasm that is not possible with the region's resources. This could open up avenues that could remain closed without this global opportunity. Sharing germplasm is not a one-way activity. Access to all of the germplasm maintained within the CG Centres will be possible. IITA, for example, have more than 3,200 accessions of *D. alata,* some of which could be useful for Pacific countries.

<u>Possible disadvantages and advantages of the three options.</u>

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Site	Management	Technical	Activities	Funding	Level of sterility	Maintenance costs (US$)	Other Costs (US$)
CRI, PNG	Not guaranteed	One TC technician	Multiplication of Robusta	Limited, possible support from EU	Facility should support low contamination rates	4,414 for 500 mixed accessions (includes power)	Not known
USP, Samoa	Tissue Culture Manager present	One TC technician plus 2 supportive staff	Low level of activity with local crops	No source of funding yet identified	Contamination rates high; Facility requires upgrading	15,616 For 500 mixed accessions	For upgrading: 4,755 Or 10,403
CG Centre	No problems	No problems	Full research programme supported by CG system	Full funding under CG system	Low contamination rates.	Would depend on the system chosen	Not known.

Recommendation

The initial reaction regarding duplication of the taro core collection, and the entire RGC collection is to store it within the region. This would seem to be the safest option. However, we need to look at why we are duplicating the RGC collection. We are duplicating the RGC collection, so that if some disaster strikes that collection at SPC, we would be able to replace that collection by sufficient replicates being regenerated from the duplicate collection. We are duplicating for security, so we need to identify the location that will provide long-term secure conservation. Any of the CG Centres can provide state-of-the-art facilities, where resources are not under any pressure and where funding is guaranteed. There are other advantages in entrusting the region’s germplasm to the CG Centres. As one of the conditions of the FAO/CGIAR agreement, FAO will assist Centres in evacuating and/or transferring collections in case of emergencies. In addition, FAO will also assist Centres through the provision of expertise, if so requested. It is unlikely that either the national, or the regional institutes, which could duplicate the collection, can offer this level of security.

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6.0 Summary of recommendations from Taro Conservation Strategy Workshop.

The following recommendations and actions were agreed on at the Taro Conservation Strategy Workshop.

- ?? The core collection should be structured on a needs basis to promote better utilization.
- ?? For rationalization of the Solomon Islands collection, the initial clustering will be for each province, and then will be further divided by location and morphological characters. Dendrograms will be prepared for each province.
- ?? In the Solomon Islands, the taro was described as it was collected in the field. IPGRI has recommended that accessions be re-described in the provincial genebanks around February/March 2002.
- ?? Based on the analysis of passport and morphological data, leaf samples from a sub-set of accessions will be forwarded to UQ for DNA analysis in April/May 2002.
- ?? If countries wish to maintain their national collections, they could reduce the size of the collection using the PCA scores. This would reduce the numbers per cluster. Countries can also remove duplicates to reduce collection size. Preferably TaroGen and PICs should keep all collections, and when possible this base collection should be cryopreserved.
- ?? Validation is necessary to determine whether 70 per cent of the genetic diversity has been captured in the core collection. This can be achieved by taking a 10 per cent sample based on morphological descriptors, and comparing this with the core collection. This can be carried out for PNG, Vanuatu, New Caledonia and Fiji.
- ?? Similarly a random 10 per cent sample can be taken for DNA fingerprinting and compared with the core collection. The core collection should contain at least as much diversity as the random sample. These investigations will depend on the availability of resources at UQ.
- ?? The core collection can be grown in countries and evaluated. This will provide more information on the core and promote its use.
- ?? If funds are available, and after virus indexing, the core should be tested across the region, to make comparisons and estimate G&E interactions. This will help determine wide adaptability of the germplasm.
- ?? The core collection should be actively promoted through publications, (scientific and popular), internet/CD ROM, diversity fairs, agriculture shows and farmers' days.
- ?? There is a need for a full documentation of the core, and the whole collection.
- ?? Intellectual property issues relating to the sharing of the core collection require urgent resolution, and should be addressed through the regional PGR network
- ?? There is a need to have access to the TANSAO collection and also information on comparison of DNA fingerprinting between the two collections.
- ?? The participants at the workshop gave support to the seed storage study by NARI, USP and Koronivia Research Station (KRS). Based on the outcome of this study TaroGen can decide on the role of seed storage in a complementary conservation strategy.
- ?? The workshop participants supported the preparation of a project proposal for further research into cryopreservation.

7.0 Complementary Conservation Strategy for taro.

Introduction

Taro (*Colocasia esculenta* (L). Schott) is a vegetatively propagated crop with edible tubers and leaves belonging to the family Araceae. Two botanical varieties – var. *esculenta* and var. *antiquorum*, are recognised. *C. esculenta* var. *esculenta*, commonly known as dasheen, is grown widely throughout the tropics with greatest importance in the Pacific Islands. *C. esculenta* var. *antiquorum*, known as eddoe, is more important in the West Indies and is also commonly grown in China and Japan (Purseglove, 1972). Taro is one of the most ancient cultivated crops and domestication may have occurred independently in the Indo-Malayan and the Pacific regions. Taro spread eastwards into the Pacific, probably reaching the Polynesian islands 2,000 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 but were domesticated from wild sources existing in the Melanesian region (Lebot 1992). Worldwide, taro ranks fourteenth among staple vegetable crops 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 an everyday 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 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, Cook Islands and Samoa (pre-1993).

Assistance from three UNDP/FAO root crop projects in the 1980s provided most countries in the region with the opportunity to collect, document and use their taro genetic resources (Ramanatha Rao *et al*., 1999). These studies were summarized by Jackson (1994). Prior to this, only Fiji, Papua New Guinea (PNG), New Caledonia and the Solomon Islands had assembled significant collections. Collections of taro had been assembled in most countries by 1986, although there were differences of opinions as to how representative each collection was. However, a follow up survey of taro collections in the region in 1994 (Jackson, 1994) recorded total losses in the Cook Islands, Niue, Solomon Islands, Tonga and Vanuatu. Collections still remained in Fiji, PNG, New Caledonia and several smaller countries (Table 1). Documentation and evaluation of the collections was also a problem.

Table 1. Collections of taro in nine Pacific Island countries in 1986 and 1994.

Country	1986	1994
Papua New Guinea	307	521
Solomon Islands	31	2
Vanuatu	138	0
New Caledonia	?	86
Fiji	72	78
Niue	52	0
Samoa	20	17
Tonga	14	21
Cook Islands	57	0
Total	**691**	**725[1]**

Source: Adapted from Ramanatha Rao *et al*., 1999
[1]collections were also recorded in American Samoa, CNMI, French Polynesia, Guam, Hawaii, Kiribati, Marshall Islands, Palau and Tuvalu.

Organizations working on taro in the Pacific

Many of the PI countries have a long tradition of taro research and some continue to maintain national collections and to carry out research. The Universities of the South Pacific (USP), Hawaii (UH), Guam (UG), Queensland (UQ), Sydney (US) and Queensland University of Technology (QUT), Wageningen Agricultural University (WAU) and the University of Technology (UNITECH) in PNG are all involved in taro related activities covering breeding, agronomy, pathology, molecular characterization and conservation. Donors and other organisations active in supporting activities relating to taro conservation, and related issues, include: the Australian Agency for International Development (AusAID), Australian Centre for International Agricultural Research (ACIAR), CIRAD, European Union (EU), the Secretariat of the Pacific Community (SPC), NZODA, HortResearch, Food and Agriculture Organisation (FAO), International Plant Genetic Resources Institute (IPGRI) and Agricultural Development in the American Pacific (ADAP). Because of declining funds and resources for Pacific Island crops, such as taro, regional crop networks are being seen as the approach for PI countries to take. This approach offers a more effective use of funding, standardisation of cultivar descriptions, development of core collections, secure storage, central databases and mechanisms for dealing with germplasm exchange, sharing and protection from external exploitation (Lebot *et al*., 2001).

The Taro Genetic Resources: Conservation and Utilisation (TaroGen) project, is such a regional network, which aims to improve the welfare of Pacific Islanders by facilitating taro improvement and conservation. The impact of taro leaf blight on taro cultivation in the Samoas, the loss of taro genetic resources, as shown in Table 1, and the continuing vulnerability of other PI countries to the disease were the major factors in the development of TaroGen. In recognition of the urgency of the problem, three regional meetings to discuss disease control, loss of genetic resources and ways to prevent further spread of the disease were held in the region between 1993 and 1995. Outcomes from these meetings contributed to the formulation of the TaroGen project, which commenced activities in 1998. The project is implemented by the SPC and funded by AusAID. The project represents collaboration with IPGRI, National Agricultural Research Institute (NARI) and USP, and is working with national programmes to develop a regional strategy for taro genetic resource conservation and crop improvement. A unit has been established within SPC to provide the expertise required in conservation, plant breeding and project management. This unit includes the Regional Germplasm Centre, (RGC), which has a mandate to conserve and promote utilization of crops in the region important for food security. The RGC currently holds collections of taro, sweet potato, banana, bele and yam, and is researching improved methods of multiplication and conservation for these crops. TaroGen works closely with the ACIAR-funded project on virus indexing and DNA fingerprinting of taro. Through support from this project QUT is developing reliable and sensitive methods for detection of viruses in order to facilitate the international transfer of taro germplasm. DNA fingerprinting of regional taro collections at UQ has helped TaroGen develop a core collection, representative of the diversity present in the region.

The Taro Network for Southeast Asia and Oceania (TANSAO) is another network with similar aims, to improve the competitive position of taro in cropping systems and markets and facilitate the sharing of information and resources. TANSAO focuses on the characterisation of genetic resources and their utilisation for crop improvement in Thailand, Vietnam, Malaysia, Philippines, Indonesia and PNG. TANSAO commenced activities in 1998 and is funded by the European Union.

Collecting efforts in the Pacfiic

As previously outlined root crop collections were established in the 1980s with project funds through UNDP/FAO. As Table 1 shows many of these collections were either completely lost, or a significant number of accessions were lost. With the establishment of the TaroGen project, collections had to be generated from all the countries participating in the project. TaroGen, in collaboration with IPGRI, held a taro collecting strategy workshop in December 1998 (Anonymous, 1999). A regional approach was adopted to enable a large part of the taro gene pool to be sampled at one time. In addition, the strategy that was developed promoted the use of common methods for collecting, describing and documenting germplasm. It was agreed that this approach would assist in the rationalization of national collections and the identification of a core sample representative of taro diversity in the region. This core sample would be conserved in the SPC RGC. During the workshop collecting strategies and plans for PI countries were devised and agreement reached on a set of standard descriptors to be used for germplasm characterisation. Training was also provided by IPGRI on recording of information on the agreed descriptors, documentation of information and database development. Within three years after this initiative, almost 2406 taro accessions with well-documented data were held in Pacific Island collections (Table 2).

Table 2. The location of taro collections and number of accessions in nine Pacific Islands.

Country	Location of Collection	Number of Accessions
Papua New Guinea	BARC, Lae	859
Solomon Islands	Fote Research Station, Malaita	824
Vanuatu	VARTC, Santo	502
New Caledonia	-	82
Fiji	Koronivia Research Station	72
Niue	SPC, Nabua, Fiji	25
Samoa	SPC, Nabua, Fiji	15
Tonga	SPC, Nabua, Fiji	9
Cook Islands	SPC, Nabua, Fiji	18
Total		**2406**

Source: TaroGen Annual Report 1999/2000

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 (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 collections amounting to 2,406 accessions. It is unlikely that country partners will be able to sustain these collections beyond the duration of the TaroGen project. In addition, unless cryopreservation protocols are developed to 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. This core collection would be maintained *in vitro* in the SPC RGC. In general it was recognized that the core collection is not meant for conservation, nevertheless, given the conditions noted above, such use of a core collection for conservation was felt justified.

The taro core was developed with the assistance of IPGRI and UQ. Where country collections existed, and had been described, (PNG, Vanuatu and New Caledonia), 20 per cent of the total collection was selected on the basis of morphological data. Initially groupings were made on the basis of distinct morphological characters, but this gave groups containing too many accessions. Therefore, a cluster analysis was carried out using Ward's method of hierarchical clustering, which gave more groups, but with fewer accessions. The process was applied to both PNG and Vanuatu. New Caledonia, however, could not be subjected to the same procedure because an initial stratification was not possible. No pattern was observed with regards to distinct morphological descriptors, therefore stratification was done with multivariate analysis.

It was decided that 20 per cent would be selected from each group, and that this 20 per cent would be chosen on the basis of Principal Component Analysis (PCA). PCA ensures that the maximum sample of diversity is selected, and was possible because extensive characterization data was available. Therefore accessions with the highest PCA score were chosen for the 20 per cent. In the case of Fiji, and the Polynesian collection (Cook Islands, Niue, Samoa and Tonga), 20 per cent could not be selected as no morphological data were available. In contrast, the entire collections from these countries were subjected to molecular analysis.

These selected accessions and the entire collections from Fiji and the Polynesian islands were sent as leaf samples or tissue cultures to UQ for DNA analysis. Molecular markers are becoming a powerful tool for managing diversity of plant genetic resources, as they avoid the complications of environmental effects by looking directly at variation controlled at the genetic level.

Currently, there are five main molecular tools used in the management of plant genetic resources. These are: Restriction Fragment Length Polymorphism (RFLPs), Random Amplified Polymorphic DNA (RAPDs), Inter-simple sequence repeat amplification (ISSRs), Amplified Fragment Length Polymorphism (AFLPs). and Microsatellites. Microsatellites is one of the most popular techniques (Morgante and Olivieri, 1993). Consequently microsatellites were developed to enable further rationalization of the taro collections. Using these markers it was anticipated that the final core would be approximately 10 per cent of the collections.

From the DNA analysis, the recommended final core collection for the Pacific Island region, minus the Solomon Islands collection, consists of 164 accessions. Although this final core relied heavily on genetic analysis, morphological groupings were taken into account. Where possible, at least one representative from each morphological group was selected for the final core. In addition, between countries duplication was avoided by cross checking to the cluster analysis for the entire data set. The selected core still requires input from the Solomon Islands collection, where recollecting has only recently been completed. As this collection consists of 824 accessions, the final core will be in the order of 246 accessions.

Approaches to conservation

As noted in the foregone discussions, there have been several attempts in collecting and conserving taro genetic resources. Conservation efforts using field genebanks has been the main option so far. However, there are many constraints to this method. A review of different conservation methods available for taro genetic resources has been carried out in order to develop an integrated approach applicable to the Pacific region.

There are two approaches to conservation of PGR, *ex situ* and *in situ*. The *ex situ* approach to conservation includes storing seed in cold rooms, maintaining plants in field genebanks or botanical gardens, storing cells, tissues or pollen *in vitro* and storing DNA. In contrast, the *in situ* approach is about conserving genetic resources in the plants' natural habitat and includes the maintenance of reserves/protected areas, conservation on-farm and in home gardens. These different approaches are reviewed below with regard to their relevance for taro conservation and also in the light of recent developments under TaroGen.

Ex Situ Conservation

Seed storage

Conservation of seeds is the technique most commonly employed for genetic resources conservation, since approximately 90 per cent of the 6.1 million accessions stored *ex situ* worldwide are conserved as seed (FAO, 1996). Seed storage is an efficient and reproducible technique and IPGRI has supported developments in this technology and produced several technical guidelines. Seeds are dried to appropriate low moisture contents before low temperature storage. This method is almost universally applied for conservation of orthodox seed species. Recalcitrant seeds, which do not tolerate drying to low moisture contents, cannot be stored using this technique.

Seed storage of taro, and other root crops, has received little attention because they are usually vegetatively propagated and certain species are difficult to propagate by seed. However, seed can be produced by taro and preliminary studies have shown that taro has orthodox seed storage characteristics (Jackson *et al*., 1977). Therefore the potential exists to develop seed storage as a useful method of conservation of taro genes, but not particular gene combinations. Wilson (1989) has demonstrated that taro seed can be stored for up to two 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 one year, and 100 per cent germination being obtained after storage. Prior to storage the seeds were dried at room temperature (Lebot, pers. comm).

Seed storage is one of the conservation strategies under investigation by TaroGen, and this has necessitated importing seeds from the breeding programmes in Samoa and PNG. However, attempts to work with seeds in Fiji imported from either country have failed. All of the seeds had poor viability, and as a result no storage experiments were established. A preliminary study carried out in PNG has 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 per cent were obtained with seeds less than three months old, however only 40-50 per cent 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 compared to germination of seeds directly in petri dishes. This work and the problem with viability of imported seeds points to the need to identify procedures for handling seeds prior to storage, so that they are stored with maximum viability, and to optimise germination methods. In addition, the orthodox behaviour of seeds should be confirmed using the protocol as described by Hong and Ellis (1996). If conditions can be optimised seed storage will offer a convenient method for long term conservation of taro genes. In addition, if suitable storage methods can be determined for taro seed it will offer an alternative avenue for the international movement of plant germplasm by minimising the risk of transmitting pathogens. This will be useful for crop improvement programmes.

Field genebanks

Many field crops, horticultural and forestry species are either difficult or impossible to conserve as seeds as they do not set seed, or the seeds they produce are recalcitrant, or these species reproduce vegetatively. Hence, they are conserved in field genebanks. There are several disadvantages with field genebanks. They run a risk of being damaged by natural calamities, attacks by pests and pathogens, neglect or abuse. It is well known that *ex situ* conservation of taro using field genebanks may not require a substantial number of plants and large area but it requires a considerable amount of labour. In addition, the plant material remains exposed to biotic and abiotic stresses and field genebank maintenance is relatively expensive (Florkowski and Jarret, 1990; Jarret and Florkowski, 1990; Ramanatha Rao, 1998). Although it is argued that field genebanks are less expensive to maintain than *in vitro* genebanks, there is little information on the costs of *ex situ* conservation. CIAT has shown with cassava that the costs are similar for both field and *in vitro* genebanks (Epperson *et al*., 1997). In a study carried out by TaroGen, it was shown that costs for both field and *in vitro* collections of taro were similar. However, with accessions maintained *in vitro*, rather than in the field, there is the advantage of security, ease of distribution and the ability to exchange pathogen-tested material.

For a number of plant species, alternative methods to field genebank conservation have not been fully developed (Chin *et al*., 1999), and even if alternatives are developed, 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, accessiblity for characterization, evaluation, multiplication and breeding. In addition, field genebanks, of manageable size, can be applied in resource-poor conditions, and the linkages between field genebank conservation, use and improvement can be very functional (Eero Nissilä *et al*., 1999).

Due to the vegetatively propagated nature of the crops, the common conservation method utilised in PI countries for root and tuber crops has been field genebanks. Historically, field genebanks have not proved sustainable for the reasons outlined above, however, they have been important for characterization, evaluation and utilization of germplasm. Maintenance of national taro field genebanks in PI countries has been instrumental in the work of TaroGen in developing a regional taro core collection.

In vitro methods

In vitro methods are suited to those species, which are vegetatively propagated, and *in vitro* methods include both slow growth storage and cryopreservation. Slow growth storage is generally used for short to medium term storage, whereas cryopreservation is a long-term option.

Storing material *in vitro* can provide additional advantages to those specifically relevant to conservation. These are the ability to produce disease-free stocks through meristem culture. This facilitates quarantine procedures for the international exchange of germplasm. Tissue culture can enable higher multiplication rates to be achieved than in the field, which assists in the distribution of new improved clones. Despite the advantages provided by *in vitro* storage, there can be technical constraints in its application. Firstly, these methods are often considered too technical for routine application by many national plant genetic resources (PGR) programmes. Concern exists too regarding the demand for relatively sophisticated equipment, a reliable electricity supply and trained staff. Secondly, for some species, risks of somaclonal variation increase with prolonged time in slow growth storage. There is a general consensus that species prone to somaclonal variation in the field will show a similar susceptibility in tissue culture, and that the type of slow growth methodology used, can exacerbate the problem. However, for an increasing number of species, tissue culture maintenance is becoming more relevant due to improvements in methodology. When *in vitro* conservation techniques are further refined, their large-scale adoption will be possible (Engelmann and Ramanatha Rao, 1996; Ramanatha Rao, 1998; Chin *et al.*, 1999; Engelmann, 1999).

The use of *in vitro* methods for conservation of root and tuber crops such as yams and taro overcomes many of the problems of field genebanks. Tissue culture methods exist for the majority of root and tuber crops, grown in PI countries, including taro, though they vary in their efficiency and reproducibility. Slow growth methods, employing the use of additives or growth retardants in media or reduced storage temperature or oxygen tension (Taylor, 1996), provide a medium-term storage option for conservation and can allow subculture intervals to be extended significantly.

Slow Growth: Subculture intervals of three years for taro stored in the dark at a temperature of $9^{o}C$ 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 was carried out in this area, under the EU-funded Pacific Regional Agriculture Programme (PRAP). Using a storage temperature of 20?C, *in vitro* cultures of taro could be maintained 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 six to nine 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. Results to date indicate that there is an interaction between the culture container size and reduced nitrogen levels, which could extend the subculture interval beyond nine months. Further work is needed in this area to determine a method which provides the optimum storage time for the maximum number of cultivars. The methodology chosen must take into account genetic integrity and resources. As previously stated, somaclonal variation can be a problem with slow growth storage systems. More research is required 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 six to seven years (Ng, 1991).

Cryopreservation: For long-term *in vitro* storage, cryopreservation, storage at ultralow temperatures, using liquid nitrogen (-196^{o}C), is employed. 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. It is essential to recognize that, due to the various problems and limitations encountered with both protected areas and field genebanks (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.

Cryopreservation protocols are now available for cell suspensions, callus, apices, zygotic and somatic embryos of several hundred species of temperate and tropical origin (Engelmann, 1997). There are an increasing number of cases where techniques can be considered operational on a routine and large-scale basis. However, there are only a limited number of cases where cryopreservation is routinely used in a genetic resources conservation context. These include *in vitro* shoot tips of potato, cassava, *Musa* and pear, seeds of some short-lived or endangered orthodox species, dormant buds of various tree species and pollen of some horticultural with species (Engelmann and Engels, 2001).

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 per cent have been reported for taro using a vitrification procedure (Takagi *et al*., 1997). TaroGen has been using this cryopreservation protocol, and with modifications, recovery rates of 70 and 100 per cent were obtained with three varieties. However, these high success rates have not been reproducible and on average 20 to 30 per cent recovery rates are obtained with the varieties tested. This investigation has demonstrated that cryopreservation of Pacific Island taro is possible, but for realistic recovery rates to be achieved, further research is required to improve the quality of the source plants, and to optimize conditioning treatments for different varieties.

Conservation of pollen

Pollen storage is considered an emerging technology for genetic conservation (Harrington, 1970; Roberts, 1975; Zhang *et al*., 1993; Towill and Walters, 2000). Cryopreservation storage of pollen has been used to assist with hybridization of species with asynchronous flowering. Consequently this method can help in enhancing utilization of available genetic resources. Pollen can be easily collected and cryopreserved in large quantities in a relatively small space. Exchange of germplasm through pollen would pose fewer quarantine problems compared with seed or other propagules. In the last 10 years, cryopreservation techniques for pollen have been developed for a number of species (Towill, 1985; Bhat and Seetharam, 1993; Hanna and Towill, 1995). This method has not been evaluated for taro, but it is unlikely that it would be useful, because of the general lack of flowering.

DNA Storage

In principle, storage of DNA is simple, widely applicable and appears to be relatively easy and cheap. Progress in genetic engineering has resulted in breaking down species and genus barriers, which have previously prevented gene transfer (Council, 1993). However, strategies and procedures have to be developed for the utilization of material stored in the form of DNA. A better understanding of the role and value of this method for PGR conservation is required. This relatively new technology has not yet been investigated for taro.

Botanical gardens

There are about 1600 botanic gardens and arboreta worldwide (Bramwell *et al.*, 1987; WWF-IUCN-BGCS, 1989) containing almost four million accessions of between 80–100,000 species. Though the role of botanical gardens in the context of crop germplasm conservation and propagation appears to be limited, they may have a greater role in the conservation of wild, rare and endangered plant species, public awareness and education, by bringing people and plants together. As there is a serious limitation on the number of plants (population size) that can be maintained in botanical gardens, it will be difficult to conserve genetic diversity, for example, the National Tropical Botanical Gardens in Hawaii has only nine taro accessions. Their role, therefore, in a taro conservation strategy programme is likely to be one of public awareness and education.

***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; Ledig, 1992; Jarvis, 1999; Sthapit and Jarvis, 1999; Jarvis *et al.*, 2000). However, given the fact that human activities can cause habitat destruction and loss of biodiversity in some cases, it is necessary to complement *in situ* conservation with *ex situ* conservation. In any *in situ* conservation effort (wild or on-farm), assessing, locating and monitoring aspects are most important. In the final analysis, it is the information on genetic diversity - how much and where it is located and how it is changing over space and time – that is most important for conservation and more so for utilization of that diversity. Monitoring also helps maintain constructive contact with farmers and communities and assists in the transfer of new information to them on relevant aspects of conservation and use. IPGRI’s current focus is on *in situ* conservation of forest species and wild relatives of crops and on-farm conservation of crop genetic diversity (Jarvis, 1999; Sthapit and Jarvis, 1999; Jarvis *et al.*, 2000).

Biosphere reserves/protected areas

It is well recognized that biodiversity at the species and ecosystem level can only be conserved through *in situ* conservation (McNeely, 1996). Various types of protected or semi-protected areas (IUCN, 1994) are in place in several regions of the world identified as rich in diversity of ecosystems and/or species. This is the most important method for conservation of fruit and forestry species and their relatives. However, it must be noted that genetic resources conserved in such reserves are often not easily accessible for use. Additionally, monitoring and management may not be optimal due to the difficult conditions under which these need to be performed. For the same reason, characterization and evaluation will be limited. The reserves are also vulnerable to natural and human-made disasters. Although reserves could be a means for conserving wild relatives of crop species, they do not have this as a specific aim. It is often Departments of Environment that are responsible for implementation of reserves and future consideration of wild relatives conservation will require more input and collaboration from Ministries of Agriculture (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 committees, on which there is representation from the Environment and Agriculture. With the recent establishment of the PGR network in the Pacific, there will be more interactions between all the sectors involved in agrobiodiversity.

On-farm conservation

In situ conservation of agrobiodiversity, or on-farm conservation, involves the maintenance of traditional crop cultivars or landraces and farming systems by farmers within traditional agricultural systems (Hodgkin *et al*., 1993; Jarvis, 1999). This method of conservation has been gaining importance in recent years, though farmers have been using it for centuries (Sthapit and Joshi, 1996). In the evolution of diversity on-farm, the effects of growers-practices are of paramount importance, the baseline diversity being the one that is determined by the local adaptation of the genotype. In most cases, there is little information available on the status of the genetic diversity on-farm. It is now possible to monitor and estimate genetic diversity using molecular markers, in conjunction with agro-morphological traits (Hodgkin and Debouck, 1992; Neale, 1998). Use of molecular markers is expected to assist in better understanding of the structure of genetic diversity both at a specific site and across regions.

In situ conservation programmes must benefit local communities. Management by local communities can be developed to effectively link conservation and use (McNeely, 1996; Sthapit and Jarvis, 1999). In addition, it is important to consider indigenous knowledge (IK), people's participation and cooperation between local people, researcher and conservationists and non-governmental organizations (NGOs). Several efforts by IPGRI are in progress (Jarvis, 1999).

Conservation of taro on-farm is an important consideration in an overall complementary conservation strategy. TaroGen has been investigating the feasibility of on-farm conservation for taro genetic resources. A pilot study carried out in Vanuatu indicated that if on-farm taro conservation is to be successful areas selected should be remote and isolated, with no or limited access to markets. Awareness of the benefits and value of maintaining taro landraces also need to be explained to farmers. A further study is underway in the Solomon Islands, linked to collecting activities. The original Solomon Island collection was lost because of disease, and consequently recollecting had to take place. The Ministry of Agriculture and a NGO (Planting Materials Network/PMN), have collected successfully in four provinces and four decentralised, farmer linked field genebanks have been established. Diversity fairs are planned in each of the four provinces. Taro from 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 the project has been experienced in the four provinces especially in regard to locally based field genebanks. This work in Solomon Islands and the study in Vanuatu demonstrate that potential for this approach exists in particular areas of the Pacific where diversity of landraces is high. This approach might also be specifically suited to those countries, where a decentralised system would be appropriate. TaroGen is in the process of submitting a proposal to the Global Environment Facility (GEF) to implement an on-farm taro conservation project involving Vanuatu, Solomon Islands and PNG

Home gardens

Home garden conservation is similar to on-farm conservation but on a smaller scale. Home gardens, especially in rural areas, tend to contain a wide spectrum of species, such as vegetables, fruits, medicinal plants and spices (Evenson, 1986; Michon *et al.*, 1986). Home gardens, as single units, may be of little value in terms of conservation, but a community of them in a given area could contribute significantly to the conservation and direct use of genetic diversity.

Root crops are important components of species diverse home gardens and such locations can be an important dynamic conservatory of genetic diversity maintained by rural households. Women are often responsible for maintenance of home gardens and the diversity present is often correlated to multiple uses of plants. Surveys in the Pacific have shown that taros are among the most dominant plants found in home gardens in PNG, Fiji and Tonga (Thaman, 1982). In Asia and the Pacific, different cultivars of taro are often maintained in close proximity to rural households to meet staple, vegetable and animal food, medicinal and cultural exchange needs. Although little is known about the extent of taro diversity in home gardens in the Pacific there is evidence from elsewhere to suggest that they are important sites for diversity. Twenty-four taro landraces were reported in a baseline survey of home gardens in Nepal with eight 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). This suggests that home gardens have an important role to play in maintaining diversity of taro landraces. Encouraging home garden and urban taro production could provide immediate nutritional, economical and social benefits in addition to enhancing genetic conservation and could be achieved through community, school or youth groups or other networks to exchange planting materials.

Integrated Approach to Taro Conservation

The range of methodologies available for conservation can be divided into two strategies: *ex situ* and *in situ*. It is important to emphasize that no one method of conservation can meet all conservation needs and that the two approaches or strategies are complementary in nature.

Article 9 of the CBD promotes complementary approaches for the conservation of plant germplasm. Therefore, for any crop there is a need to employ a combination of methods, from nature reserves to genebanks to ensure as much genetic diversity as possible is conserved. The balance between different methods employed depends on factors such as the biological characteristics of the gene pool, infrastructure and human resources, number of accessions in a given collection and its geographic site and the intended use of the conserved germplasm. For any given gene pool the extent of a particular method used may differ from that used in another gene pool (Maxted *et al*. 1997, Ramanatha Rao 1996). Once the target genepools, considering both taxonomic and geographical aspects, are determined and their genetic structures identified, the next steps in developing a complementary conservation strategy (CCS) can be taken.

From a functional point of view CCS appears to be an amalgamation of different conservation methods, however, in reality it is a decision-making process leading to conservation of genetic diversity existing in a target gene pool. A large amount of information on various parameters is required for establishing the knowledge base to develop a CCS that is most appropriate for a given gene pool and these details are discussed elsewhere (Maxted *et al*., 1997; Ramanatha Rao, 1996; Ramanatha Rao and Arora, 1999). Such information is not only scientific or technical but addresses political and practical issues including:

?? Information on the importance of the gene pool;
?? Extent and distribution of genetic diversity within the gene pool;
?? Life history traits and reproductive biology of target species;
?? Storage characteristics of these species;
?? Knowledge about those who are presently using and conserving diversity of the species;
?? Location and size of the germplasm collections;
?? Cost-effectiveness of the methods used;
?? Conservation objectives;
?? Human resources and infra-structural facilities available; and
?? Legal issues and access to material conserved, etc.

TaroGen has compiled information about many of these issues. It is known that taro is one of the most economically and culturally important plants in the region. With the assistance of IPGRI and UQ there is now a good understanding of the extent of genetic diversity within the regional taro gene pool. TANSAO has provided additional information on taro genetic diversity in Asia. Further, valuable information has been gained on the applicability and cost-effectiveness of different conservation methods.

At the TaroGen Conservation Strategy (TCS) Workshop, held at SPC, Suva, in September 2001, a conservation strategy for taro was considered. All aspects of conservation from rationalization through to utilization were discussed, and recommendations made (Taro Conservation Workshop Report, in press). As some conservation methods were still being evaluated, for example, on-farm conservation, it was not possible to make recommendations as to how such methods should be used. It was more a case of recommending that studies should be continued and suggesting how these different approaches could play a role in the overall strategy.

The taro core has, in essence captured the diversity present in the collections currently maintained either in the field and/or in the RGC. This core collection of accessions must be readily available for distribution. It has to be evaluated by the countries so that more information can be obtained about the accessions, thereby promoting its use. Its distribution must also not impose any quarantine risks. *In vitro,* namely meristem culture, can eliminate viruses, so that *in vitro* storage associated with the relevant virus indexing techniques can facilitate distribution. Slow growth storage *in vitro* satisfies these requirements, consequently this was the approach recommended for the core. Countries could also maintain their own core collections, but this would depend on resources available. They might choose to maintain other varieties in field genebanks knowing that the SPC RGC was conserving the core collection for the region. The establishment of the RGC at the 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 regional organization eliminates some of the arguments often put forward against *in vitro* conservation, such the level of technical skills and resources required.

Having an *in vitro* taro collection at SPC also enables further research into cryopreservation. As stated earlier, this is the only effective long-term method of conservation for vegetatively propagated species. With an effective and reproducible cryopreservation technique, large collections can be maintained in a small space with low maintenance costs. In addition, the risk of losing accessions due to contamination, or somaclonal variation is minimised. 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, ensuring they are safeguarded for future generations.

Field genebanks have played a major role in the work of TaroGen in developing a regional taro collection. It was agreed that the role of national field genebanks in a taro conservation strategy would be very much a decision made by each country. Field genebanks require resources therefore it is primarily a country's decision as to whether they have the resources and what accessions they wish to maintain in field genebanks. Countries could conserve their cores, or perhaps they might choose to conserve varieties most difficult to recollect. Where there are geographical restrictions when collecting germplasm, such as PNG, this could be an option. Alternatively field genebanks could be used for maintaining those varieties, frequently utilized. Spreadsheets for field genebanks developed by ACIAR, have been used by PNG and Fiji to determine the costs of field genebanks. These are available for all countries to use. Spreadsheets for *in vitro* storage are also available. These could be used to compare costs between the two methods, if countries feel they have the capacity to embark on *in vitro* storage. Field genebanks provide the means to maintain working collections for breeding and evaluation work in country. They may also be the method best suited for conservation of each country's elite varieties or farmer-preferred varieties, many of which have not been selected for inclusion in the core collection. Using field genebanks PI countries can maintain relatively small collections, which are used either for breeding, evaluation and/or distribution to farmers and which would require minimal resources. Such an approach would help broaden the genetic diversity conserved in the region.

Storage of taro seeds provides a method that is more dynamic, in that the genes in the seeds can be used by countries to provide varieties suited to their specific needs, assuming resources allow for evaluation. Seed viability requires certain conditions, which need yet to be defined, therefore there is a need for further research. Once pre-storage treatments can be specified, another method of storage for taro is available. This will allow countries to maintain taro genes at low cost, especially from those varieties, rarely used. It will allow countries, with no breeding programmes to evaluate seedlings and to select plants best suited to their needs.

In situ approaches, will promote conservation of taro landraces that may not be part of *ex situ* collections and therefore enable 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, unlike plants stored *ex situ*. The feasibility study in Vanuatu, and the on-going work in Solomon Islands highlight the potential of on-farm conservation. On-farm conservation as demonstrated by PMN and the Ministry of Agriculture in the Solomon Islands may be a model for other countries, where a centralised system for PGR might not be appropriate. Preliminary studies show that there is a need to determine the natural and human managed environmental factors influencing on-farm conservation. Similarly it has been demonstrated that for farmers to maintain taro diversity they must see benefits through increased income or other advantages. This may involve adding value to traditional landraces by improved methods of processing or marketing. TaroGen has considerable experience in participatory plant breeding and this option could directly improve landraces by increasing disease resistance or quality. On-farm conservation requires close monitoring of varieties cultivated over time, and this will require collaboration with national programmes.

The TCS workshop agreed on the need to acquire more information on on-farm conservation and fully supported the submission of a proposal to the GEF. TaroGen will continue to collaborate with IPGRI on this important activity. There is potential to link into an IPGRI home gardens study, and to assess their role in a CCS for taro. Field, *in vitro* and seed methodologies provide *ex situ* conservation. If models for *in situ* conservation can be determined this will provide a dynamic system in which a wider range of genotypes can be conserved and which remain directly available to farmers for their own use.

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

Any CCS for taro in the region must seriously consider security and duplication of the core collection. This avoids accidental loss of material as a result of fire or civil unrest. TaroGen has explored the options for duplication, and a paper was presented at the TCS workshop, which considered duplication within a national institute, a regional institute, and an International Agricultural Research Centre (IARC). Both the national and regional institutes could not provide the level of security required for safe duplication. The workshop recommended that further information be obtained on duplication within a IARC but with restricted use or black box arrangements.

Any CCS must have a strong utilization component. The taro core collection will be evaluated in PI countries to provide information on the accessions. If funds are available, (and after virus indexing), the core collection will be tested across the region, so that comparisons can be made and G?E interactions examined to determine adaptability of the germplasm. In the first instance, countries will be provided with the existing information on all the accessions in the core collection, and from this can select a subset to evaluate.

A conservation strategy should include provision for dissemination of information on the germplasm to promote its utilization. The TCS workshop recommended the core collection be promoted through publications (scientific and popular), internet, CD-Rom, diversity fairs, agriculture shows and farmers' days. Documentation should be extended to the whole collection, and not confined to the core. Documentation should be suitable for all users, and not just targeted at breeders. Language barriers should also be addressed.

Two initiatives on PGR will have a significant impact on taro conservation activities in the region. NZODA is funding a project to promote the conservation and use of PGR for crops of local importance. The aim of this project is to ensure long-term conservation and access to genetic resources by Pacific Islanders, thereby contributing to sustainable development, food security and income generation. Some expected outputs of the project include:

?? A functional, efficient and sustainable network
?? IPR/*sui generis* models for the protection of traditional crops.
?? Policies for access and benefit sharing in the region.
?? Documentation of existing germplasm within and outside the Pacific region, and compilation and publication of a PGR inventory
?? Needs-based collecting and characterization
?? Public awareness raising
?? Human resource development

A complementary project to commence in early 2002, has been submitted to ACIAR for consideration. This project aims to develop complementary conservation technologies and to establish the position of a PGR Adviser based at SPC to ensure efficient and effective coordination of PGR network activities in the region.

A planning meeting to establish the PGR network was held at SPC, Suva in September 2001. It was recommended that existing networks such as TaroGen link into the PGR network, to strengthen activities. The work of TaroGen in developing a core collection was seen as an approach to be taken with other crops of importance in the region. Further research into cryopreservation, as the most effective method for long-term conservation of vegetatively propagated species, and the only realistic conservation approach for base collections, was also recommended. The meeting discussed on-farm and home gardens conservation and, and agreed that they had a very important role to play in conservation of agricultural PGR in the region.

Implementation of these projects will significantly increase PGR activities in the region. More information will become available on different technologies, and national and regional programmes will be strengthened. This will help countries fine tune conservation strategies and sustain activities. These broad initiatives will benefit taro conservation activities.

Summary
This paper highlights the organisations involved in taro research and conservation in the region and reviews efforts in taro germplasm collecting and conservation in the last few decades. Conservation strategies for plant germplasm are discussed and the current status of taro conservation is reviewed, highlighting the advantages and disadvantages of various *ex situ* and *in situ* methods. The paper outlines the need for a complementary conservation strategy for taro genetic resources, and suggests how the various approaches can be integrated so that taro diversity is conserved and utilized. Establishing national taro field genebanks and regional collections within the SPC RGC are important steps in the conservation and use of taro genetic resources. However, this alone will not be sufficient if we are to safely conserve the genetic diversity of taro and promote its utilization. Since it is impossible to collect and conserve all taro genetic diversity *ex situ*, on-farm conservation 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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8.0 Acronyms and Abbreviations

ACIAR	Australian Centre for International Agricultural Research
ADAP	Agricultural Development in the American Pacific
AFG	Associated Field Genebank
AFLPs	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
AusAID	Australian Agency for International Development
BAP	Benzylaminopurine
BARC	Bubia Agriculture Research Centre
CABI	CAB International
CBD	Convention on Biological Diversity
CCS	Complementary Conservation Strategy
CGIAR	Consultative Group on International Agriculture Research
CIAT	Centro Internacional de Agricultura Tropical
CIP	International Potato Centre
CIRAD	Centre de cooperation internationale en recherche agronomique pour le developpement
COGENT	Coconut Genetic Resources Network
CRI	Coffee Research Institute
DNA	Deoxyribonucleic acid
DPI	Department of Primary Industries
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GEF	Global Environment Facility
GEMS	Gene Management System
Hort-Research	Horticulture and Food Research Institute of New Zealand Ltd.
IARC	International Agriculture Research Centre
ICIS	International Crop Information System
IITA	International Institute of Tropical Agriculture
IK	Indigenous Knowledge
INIBAP	International Network for the Improvement of Banana and Plantain
IPGRI	International Plant Genetic Resources Institute

IPR	Intellectual property rights
ISSRs	Inter-simple sequence repeat amplification
IU	International Undertaking
IVAG	*In Vitro* Active Genebank
KRS	Koronivia Research Station
LAF	Laminar Airflow Cabinet
LN	Liquid Nitrogen
MTA	Material Transfer Agreement
NAA	Napthaleneacetic acid
NARI	National Agricultural Research Institute
NGO	Non-government organisation
NZODA	New Zealand Overseas Development Administration
PBR	Plant Breeders Rights
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PGR	Plant Genetic Resources
PGRFA	Plant Genetic Resources for Food and Agriculture
PI	Pacific Island
PICs	Pacific Island Countries
PMN	Planting Materials Network
PPS	Plant Protection Service
PRA	Participatory Rural Appraisal
PRAP	Pacific Regional Agricultural Programme
QUT	Queensland University of Technology
RAFI	Rural Advancement Foundation International
RAPDs	Random Amplified Polymorphic DNA
RFLPs	Restriction Fragment Length Polymorphism
RGC	Regional Germplasm Centre
SINGER	System-wide Information Network on Genetic Resources
SM	Simple Matching Coefficient
SPC	Secretariat for the Pacific Community
SPYN	South Pacific Yam Network
SSRs	Simple Sequence Repeats

TANSAO	Taro Network for South East Asia and Oceania
TCS	Taro Conservation Strategy
TDZ	Thidiazuron
TGRC	Taro Genetic Resources Committee
TLB	Taro Leaf Blight
UG	University of Guam
UH	University of Hawaii
UNDP	United Nations Development Programme
UNITECH	University of Technology, Papua New Guinea
UPGMA	Unweighted Pair Group Method
US	University of Sydney
USP	University of the South Pacific
UQ	University of Queensland
VNTRs	Variable Number of Tandem Repeats
WAU	Wageningen Agricultural University

9.0 TARO CONSERVATION STRATEGY WORKSHOP

9.1 Taro Conservation Strategy Workshop Programme

Suva, Fiji

5 - 7 September 2001

Participating countries: Cook Islands, Fiji, Niue, PNG, Samoa, Solomon Islands, Tonga, and Vanuatu

Objectives of the workshop

?? To explain how the Pacific taro collections were rationalized and the accessions for the core collection were selected.

?? To present information on the various conservation strategies investigated by TaroGen.

?? To develop a complementary conservation strategy for taro.

Day 1: Wednesday 5th September

0830-0845 **Welcome:** *Jimmie Rodgers*

Chair: *Grahame Jackson*

0845-0915 **Introduction to the workshop:** *Danny Hunter, TaroGen*

0915- 0945 **Collection, characterization and documentation of taro collections from the Pacific**: *Danny Hunter, TaroGen*

0945-1015 **Teabreak**

1015-1115 **Rationalization of Pacific taro collections: its need, scope and prospects:** *Prem Mathur, IPGRI.*

1115-1200 **Barking up the right tree: a consumers guide to cluster analysis:** *Ian Godwin, UQ*

1200-1230 **Discussion**

1230-1330 **Lunch**

Chair: Davinder Singh

1330-1415 **Concept of core collection and methodologies used (a) morphological descriptors and (b) molecular descriptors:** *Emma Mace and Prem Mathur, UQ/IPGRI*

1415-1500 **Development of final taro core collection based on morphological and molecular characterization:** Emma Mace and Prem Mathur, UQ/IPGRI

1500-1530.1 **Teabreak**

1530-1615 **Proposed methodology for further validation and improvement of the designated core collection, and ways to enhance its utilization**: *Prem Mathur, IPGRI*

1615-1700 **General discussion about the strategy adopted for core identification**

Day 2: Thursday September 6th

Chair: *Danny Hunter*

0830-0845 ***In vitro* collecting: transfer of taro from the field to the tube;** *Mary Taylor, TaroGen*

0845-0915 **Overview of the conservation strategies available to the region**: *Mary Taylor, TaroGen*

0915-0945 **The potential role of the RGC in taro conservation:** *Mary Taylor, TaroGen*

0945-1015 **Tea Break**

1015-1115 **Comparison of the inputs required for field genebanks and *in vitro* genebanks**: *Mary Taylor, TaroGen*

1115-1200 **Status of taro cryopreservation, and what are the possibilities:** *Rajnesh Sant and Mary Taylor, TaroGen*

1200-1230 **Discussion on morning's session**

1230-1330 **Lunch**

Chair: *Tom Osborn*

1330-1415 **Review of seed storage:** *Danny Hunter and Davinder Singh, TaroGen*

1415-1500 **Review of *in situ* conservation studies:** *Tony Jansen, APACE, Solomons; Peter Koah, FSA, Vanuatu; Grahame Jackson, TaroGen*

1500-1530 **Tea break**

1530-1700 **Discussion on seed storage and *in situ* conservation studies**

Day 3: Friday September 7th

Chair: *Grahame Jackson and Prem Mathur*

0830-0915 **Conservation strategy for taro core collections and recommendations for the safe and secure duplication of the taro core:** *Mary Taylor. TaroGen*

0915-0945 **How to develop a complementary conservation strategy (CCS) for the region:**. *TaroGen*

0945-1015 **Teabreak**

1015-1230 **Discussion on CCS**

1230-1330 **Lunch**

1330-1400 **Recommendations for CCS for taro to be adopted by the region**

1400-1430 **Close of workshop**

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