INCO-DC: International Cooperation with Developing Countries

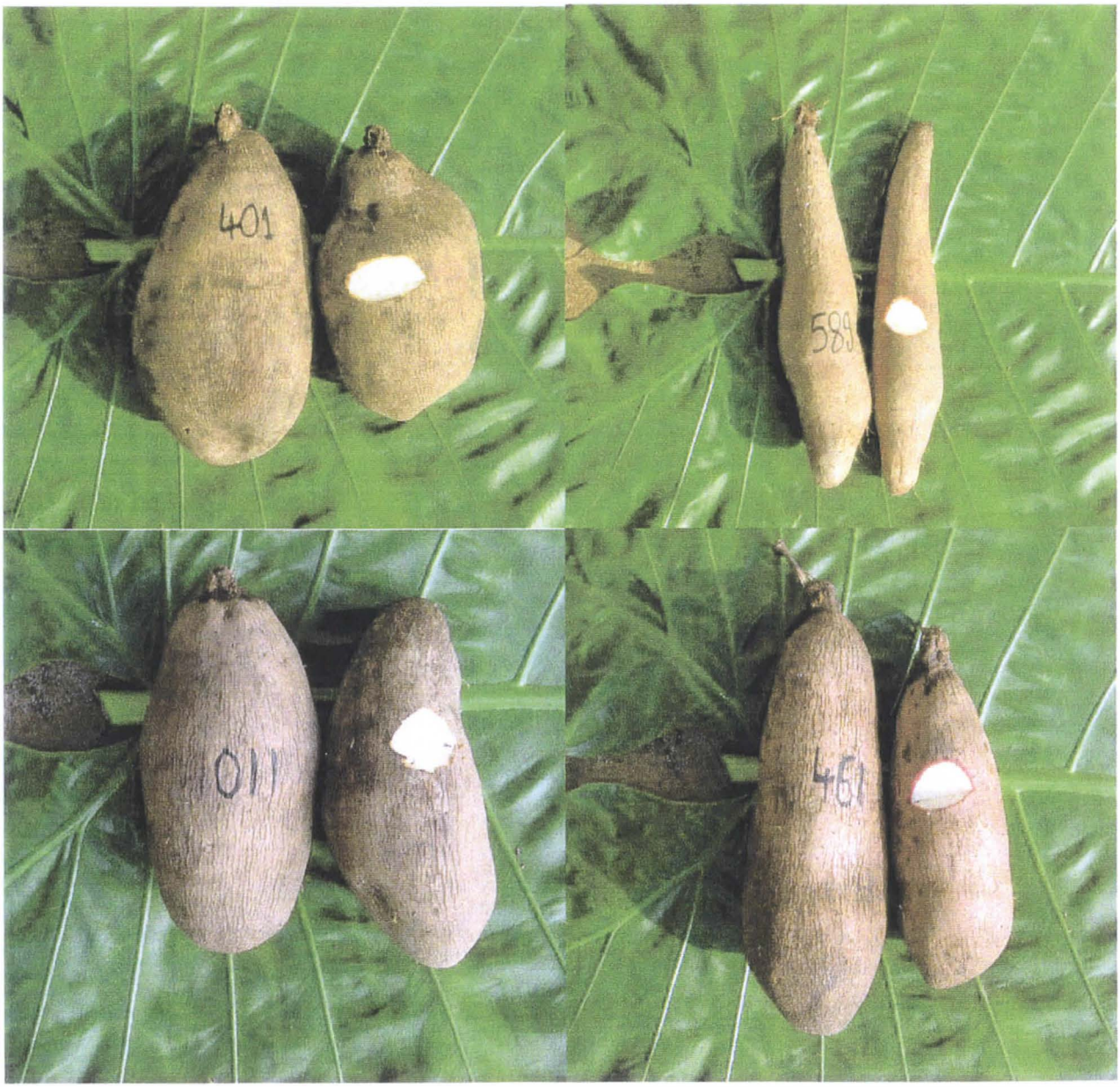
Contract number: ERBIC18CT980309

Final Report

Covering period from 01 Feb. 1999 to 31 Jan. 2003

Yam: Cultivar Selection for Disease Resistance & Commercial Potential in Pacific Islands

SPYN



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Abbreviations:

AFLP	Amplified Fragment Length Polymorphism
BAP	Benzyl adenine purine
CABI	Centre of Agriculture and Bioscience International
CGIAR	Consultative Group for International Agriculture Research
ChYNMV	Chinese yam necrotic mosaic virus
CMV	Cucumber mosaic virus
DABV	*Dioscorea alata* bacilliform virus
DaV	*Dioscorea alata* virus
DBBV	*Dioscorea bulbifera* bacilliform virus
DDV	*Dioscorea dumetorum* virus
DFID	Department for International Development
DLV	*Dioscorea* latent virus
DNA	Deoxyribonucleic acid
EC	European Commission
ELISA	Enzyme linked immunosorbent assay
EU	European Union
IARC	International Agriculture Research Centre
INCO-DC	International Cooperation with Developing Countries
IRD	Institut de recherches pour le développement
ISEM	Immunosorbent electron microscopy
JYMV	Japanese yam mosaic virus
MTA	Material transfer agreements
NAA	Naphthalene acetic acid
NARI	National Agricultural Research Institute, Papua New Guinea
NRI	Natural Resources Institute
PNG	Papua New Guinea
PPPO	Pacific Plant Protection Organisation
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism.
RGC	Regional Germplasm Centre
RT-PCR	Reverse transcriptase polymerase chain reaction
SPC	Secretariat of the Pacific Community
SPYN	South Pacific Yam Network
SSR	Single Sequence Repeat
UCL	University College of London
UK	United Kingdom
UPMGA	Unweighted Pairwise Method
VARTC	Vanuatu Agricultural Research & Technical Centre
YMV	Yam mosaic virus

Contents

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Abstract: Yam (*Dioscorea alata*) is a crop with potential for increased commercial exploitation. However, several problems are limiting its development: tuber shape is often irregular making harvest time-consuming and anthracnose disease, caused by the fungus *Colletotrichum gloeosporioides*, is always a threat. In addition, the lack of information on starches hinders the prospective utilisation of yam as a high quality vegetable. The SPYN project had 5 objectives: 1- Characterisation and evaluation of germplasm to rationalise collections and to select cultivars of interest in Fiji, Vanuatu, the Solomons and Papua New Guinea. 2- Development of *in vitro* conservation strategies. 3- International exchange of virus-tested genotypes. 4. Identification of sources of anthracnose inoculum; and 5. Agronomic evaluation of selected cultivars. Overall, 1,040 accessions were collected (Fiji = 108, PNG = 209, Solomons = 392, Vanuatu = 331). Based on tuber shape, tolerance to anthracnose and palatability properties, the best varieties were selected and propagated.

Samples were taken on 719 plants representing nine *Dioscorea* species from seven South Pacific countries. ELISA was used to examine the presence of YMV, DAV, DDV, DABV, DBBV, DLV, and CMV. Some samples were later tested for two more viruses of Asian origin: JYMV and CYNMV. DAV (*Potyvirus*) was the virus most commonly detected (69% of samples) by serology (ELISA) in *D. alata, D. esculenta, and D. bulbifera,* from across the South Pacific region. YMV (*Potyvirus*) was commonly found in *D. rotundata* plants from Africa, but has not been demonstrated in the South Pacific.

Eighty-one isolates of *C. gloeosporioides* were characterised according to radial growth, the morphology of reproductive structures in culture, and 49 isolates according to their pathogenicity on *D. alata* leaves. Molecular fingerprints of 39 isolates from yam leaves and tubers, and from other plants were obtained using ISSR primers by PCR amplification following extraction of DNA. There were significant differences in pathogenicity among *C. gloeosporioides* isolates. However, there was no clear association between morphological data and pathogenicity among isolates studied. The detection of *C. gloeosporioides* in yam tubers (4.8%) confirms that the fungus is able to infect and survive in tuber tissue from season to season. It is likely that the infected tubers could act as a primary source of inoculum playing an important role in the epidemiology of the pathogen in the field. *C. gloeosporioides* is a polyphagous pathogen with a wide host range and, under optimum conditions, is able to cross-infect different host species.

AFLP study on the Vanuatu collection indicated that *D. alata* was closer to *D. nummularia* than *D. persimilis*, one of the putative parents of *D. alata*. *D. nummularia* accessions fall into two groups, with three cultivars of intermediate type closely associated. The data suggest that they are a distinct species from *D. alata* and *D. nummularia*, but share a common ancestor. Comparisons of these varieties and *D. transversa* from New Caledonia suggest they belong to that species. *D. alata* accessions from different parts of the world clustered together indicating that there has been wide distribution of clones. Tetraploids, hexaploid and octoploid varieties of *D. alata* are present in the collection, although hexaploids are rare. Hexaploids and tetraploids are probably auto-polyploids.

Analyses were made for percentage starch, amylose, lipids, minerals, proteins, sugars and gelatinisation temperature range on 110 accessions. Significant variation exists within each country for each of these major characteristics. Good varieties are characterised by high dry matter, starch and amylose contents. Chemotypes are genetically controlled

SPYN has produced scientific data that will be used in the long term: one Ph.D. thesis and one MSc thesis have been completed and another Ph.D. thesis will be defended in 2004. The project has emphasised collaboration among participants. Countries are encouraged by the results to date and resolved to continue the work initiated by SPYN. No doubt that the constraints resulting from the numerous viruses identified will cause some practical difficulties.

Summary of final report

Yam is a traditional crop in Melanesian societies and methods of cultivation are consistent with maintaining the fragile ecosystems of the lowland areas where they are cultivated. Yam is also a crop with potential for increased commercial exploitation. However, several problems are limiting its development: tuber shape is often irregular making harvest time-consuming and labour-intensive, anthracnose disease, caused by the fungus *Colletotrichum gloeosporioides*, is always a threat, and staking is expensive and demands materials that are often in short supply. In addition, the lack of information on starches hinders the prospective utilisation of yam as a high quality vegetable. As a first step, a thorough evaluation of genetic resources of the crop is required.

Objectives

To achieve the aim of project, SPYN had five objectives; these and the respective results, were:

1. Review of yam genetic resources in five Pacific Island countries, and characterisation and evaluation of germplasm to rationalise collections and to select cultivars of interest.
   - a computerised database developed containing morphological and molecular descriptors on at least 1,250 cultivars, allowing: a) comparison and rationalisation of collections; and b) selection of 150 cultivars for tuber shape, unstaked cultivation, tolerance to disease and acceptable cooking and processing characteristics.
2. Development of *in vitro* conservation strategies.
   - a regional conservation strategy implemented, involving active *in vitro* national genebanks and cryopreserved base collections (limited to 150 accessions initially).
3. International exchange of virus-tested, selected, genotypes.
   - at least 150 yam cultivars cultured *in vitro*, tested for viruses found in Pacific Island countries and subjected to therapy treatments if infected.
4. Identification of sources of anthracnose (*Colletotrichum gloeosporioides*) inoculum;
   - molecular characterisation of *Colletotrichum* isolates from yam tubers and from at least 10 common weeds and crop plants to estimate the potential of tuber-borne and alternative hosts as sources of inoculum for epidemics of anthracnose disease.
5. Agronomic evaluation of selected cultivars;
   - assist countries multiply yam germplasm rapidly using *in vitro* and field-based (mini-sett) technologies, sufficient for at least one trial per country.

Materials and Methods

Members of SPYN were MAFF, Fiji (Ministry of Agriculture, Forestry and Fisheries); MAL, Solomon Islands (Ministry of Agriculture and Livestock); NARI, Papua New Guinea (National Agricultural Research Institute); DARD, Vanuatu (Department of Agriculture and Rural Development; CIRAD, France and Vanuatu (Centre International de Recherches Agronomiques pour le Développement), NRI, (National Research Institute, University of Greenwich) and University of Reading (Department of Agriculture), UK.

A thorough evaluation of genetic resources of the crop was conducted in each country, followed by intensive evaluation to select a small number of commercially acceptable cultivars for exchange and commercial exploitation in Pacific partners. At the same time, an

investigation was made into sources of inoculum of the anthracnose fungus, *Colletotrichum gloeosporioides*, to assist farmers in the control of this serious pathogen. Molecular analyses were carried out on the selected country accessions to avoid redundancy within a core sample for Melanesia. DNA fingerprinting was also done on isolates of *Colletotrichum* taken from yam and associated plants to determine similarities which might indicate sources of inoculum. Conservation strategies employed *in vitro* methods as well as cryopreservation for long term maintenance. New methods of virus diagnosis were used, based on molecular and serological methods.

Results achieved

Morpho-agronomic variation

Overall, 1,040 accessions were collected (Fiji = 108 acc., PNG = 209 acc., Solomons = 392 acc., Vanuatu = 331 acc.). They were planted at national research stations, according to traditional cultivation techniques and when tubers sprouted, data on 32 morphological characteristics were collected based on the IPGRI published list: *Descriptors for Yam (Dioscorea spp.) 1997*. Some countries took the opportunity to collect other species of yam. Data bases were computed on Excel format, duplicates were identified. Based on tuber shape, tolerance/resistance to anthracnose and palatability properties, the best varieties were selected and included in national core samples.

Virus survey

Samples were taken on 719 plants representing nine *Dioscorea* species from seven South Pacific countries. ELISA was used to examine the presence of *Yam mosaic virus* (YMV), *Dioscorea alata potyvirus* (DAV), *D. dumetorum potyvirus* (DDV), *D. alata badnavirus* (DABV), *D. bulbifera badnavirus* (DBBV), *Dioscorea latent potexvirus* (DLV), and *Cucumber mosaic virus* (CMV). Some samples were later tested for two more viruses of Asian origin: JYMV (*Japanese yam mosaic virus*) and CYNMV (*Chinese yam necrotic mosaic virus*). DAV (*Potyvirus*) was the virus most commonly detected (69% of samples) by serology (ELISA) in *D. alata, D. esculenta, and D. bulbifera,* from across the South Pacific region.

The large number of samples that tested positive with both DABV and DBBV antisera in ELISA suggests that they recognise some strains of both these Badnaviruses. Also, the incidence of samples positive to both DBBV and DLV was higher than would be expected by chance, suggesting that DBBV antiserum has affinity for some DLV strains. Further, some of the sequences obtained show nucleotide deletions that would result in the translated gene product being inactive, and that there are sequences integrated into the genome of some yam varieties. Further study is required to determine if these integrated sequences can recombine to form active virus.

YMV (*Potyvirus*) was commonly found in *D. rotundata* plants from Africa, but has not been demonstrated in the South Pacific. Both antisera against JYMV and CYNMV from Japan gave some positive reactions when used in ELISA on some samples from the South Pacific. Thirty-three plants associated with yam cultivation were found infected with yam viruses. Among these, *Piper methysticum* (Vanuatu) and *Colocasia esculenta* (Fiji) tested positively for badnavirus, and *Pueraria* species for DAV (Solomon Islands) and *Colocasia esculenta* for JYMV (Vanuatu). This suggests that some viruses may have spread from one crop to another.

There is still much to do in yam virology in the South Pacific, but a good start has been made. The DAV and *Dioscorea badnavirus* sequences obtained in the study revealed the presence of strains and/or species of viruses, which seem to be present in all South Pacific countries. The next step is to undertake nucleotide sequencing for classification of the viruses and taxonomic studies, and for the development of more reliable virus indexing tests.

One of the aims of SPYN is to get to the stage where selected elite yam germplasm can be exchanged between South Pacific countries for evaluation and promotion. The virology component of the project has shown that exchange will not be easy because of the prevalence of a wide range of viruses infecting yam in the region. Despite the great diversity and genetic variability of the viruses, diagnostic tests are now available for several of the viruses. For many, these involve both ELISA and PCR.

Cryopreservation

Dr. Bernard Malaurie, IRD, Montpellier visited the RGC, SPC, Fiji, 7-24 May 2001 to train staff in encapsulation-dehydration cryopreservation technique for yams and other crops. He provided illustrated manuals on the cryopreservation protocols and media preparation, and gave a seminar on yam germplasm conservation for SPC and Fiji agricultural research staff. Overall, the cryopreservation technique has been disappointing. Meristems from one cultivar showed some swelling but failed to produce shoots and roots. As the control meristems (*ie* without cryopreservation) also failed to grow, it is not clear whether the difficulty is with the meristems or the medium. However, it is difficult to grow meristems from Pacific yams, in contrast to those from the Caribbean and Africa.

Anthracnose study

Eighty-one isolates of *C. gloeosporioides* were characterised according to radial growth, the morphology of reproductive structures (conidia, ascospores and appressoria) in culture, and a selection (49 isolates) according to their pathogenicity on *D. alata* leaves. A selection of *Colletotrichum* isolates was further characterised on non-yam hosts (okra, chilli pepper, tomato, cowpea, kidney beans and citrus). Molecular fingerprints of 39 isolates from yam leaves and tubers, and from other plants were obtained using ISSR primers by PCR amplification following extraction of DNA using standard procedures.

The overall mean growth rates recorded in this study were in general agreement with those of published studies, and spore and appressoria measurements were consistent with descriptions of *C. gloeosporioides*, although individual variations were noted. The studies showed that there were significant differences in pathogenicity among *C. gloeosporioides* isolates, and this agrees with findings of others. However, there was no clear association between morphological data and pathogenicity among isolates studied.

The detection of *C. gloeosporioides* in yam tubers from Vanuatu and Papua New Guinea (4.8%) confirms that the fungus is able to infect and survive in tuber tissue from season to season. It is likely that the infected tubers could act as a primary source of inoculum playing an important role in the epidemiology of the pathogen in the field. The results corroborate earlier work on the occurrence of *C. gloeosporioides* tuber infection under natural conditions in the Caribbean and West Africa.

The molecular tree generated by ISSR-PCR analysis correlated well with the dendrogram of morphological data. There was also a good correlation the ISSR-PCR data and ability of

isolates to produce sexual spores. By contrast, there were few similarities between the AFLP results and morphological data.

The high heterogeneity and complex patterns, at the molecular level, exhibited by isolates of *C. gloeosporioides* indicates the existence of a complex population structure in which sexual recombination probably plays a major role in generating variation. The genetic diversity revealed looks similar to isolates from natural habitats rather than those of agro-ecosystems. *C. gloeosporioides* is a polyphagous pathogen with a wide host range and, under optimum conditions, is able to cross-infect different host species.

AFLP and SSR diversity

The detailed study on the Vanuatu collection showed that several accessions belonging to the section Enantiophyllum were difficult to classify within *D. alata* or *D. nummularia* as they possessed characters identified with both species. The molecular analysis, while confirming the integrity of the botanical classification of the several species compared, indicated that *D. alata* was closer to *D. nummularia* than *D. persimilis*, one of the putative parents of *D. alata*. *D. nummularia* accessions fall into two groups, with three cultivars of intermediate type closely associated. The data suggest that they are a distinct species from *D. alata* and *D. nummularia*, but share a common ancestor. Comparisons of these varieties and *D. transversa* from New Caledonia suggest they belong to that species, and that this is the first time *D. transversa* has been recorded from Vanuatu. *D. alata* accessions from different parts of the world clustered together indicating that there has been wide distribution of varieties as clonal material.

The results of the ploidy studies showed tetraploids, hexaploid and octoploid varieties of *D. alata* present in the collection, although hexaploids are rare. Hexaploids and tetraploids are probably auto-polyploids. It was also found that morphologically similar accessions have similar ploidy levels and *vice-versa*, indicative of the highly variable nature of the species.

A preliminary study was done by CIRAD, Montpellier using chloroplastic simple sequence repeat markers (microsatellites). Overall, about 300 DNA fragments were sequenced successfully, 51 microsatellite markers were identified and 16 were selected for their clear polymorphism. Amplification and migration on acrylamide gel can detect alleles that are different by one or two bases. The amplification with the marker NTcp19 obtained one to four alleles per accession of the LSC part of the chloroplastic genome, whereas usually only one copy of the sequence would be expected. The presence of 4 alleles per accession could be explained by the paternal transmission of chlorplast with the conservation of the maternal chloroplast.

Physico-chemical characteristics

Analyses were made for the following: percentage starch, amylose, lipids, minerals, proteins, sugars and gelatinisation temperature range. Overall, 110 accessions were analysed (Vanuatu = 48, Fiji = 19, Papua New Guinea = 43), and samples were analysed twice to confirm the results. Significant variation exists within each country for each of these major characteristics. Good varieties are characterised by high dry matter, starch and amylose contents. In Vanutu, varieties suitable for *laplap* the national dish (a pudding) appear to have high amylose versus starch ratio (>0.18). Chemotypes are genetically controlled and these traits will have to be taken into consideration when recommending a variety and/or for future breeding programmes because they determine the cultivars likely to be sought by farmers.

Agronomic evaluation of cultivars

In Papua New Guinea, Fiji and Vanuatu, selected varieties are being propagated for distribution to farmers. In Vanuatu, agronomic trials are being conducted with recommended varieties to test different levels of fertilisation in different geographic locations with different soil types.

Problems encountered

There were several changes to the agreed work plan devised at the outset of the project. These were necessary due to unforeseen political events as well reduced institutional capacities within Pacific Island countries. There was also a delay in fulfilment of the work by one of the European partners. The University of Reading would not only assist with the DNA fingerprinting of *Colletotrichum* isolates, but also make molecular comparisons of the DNA from the core sample of tubers. In 2001, CIRAD assumed responsibility, and recruited a ni-Vanuatu student to do the work as part of a Ph.D. programme on the diversity of yams in Melanesia.

The transfer of this work from the University of Reading allowed it to devote more attention to the study of anthracnose. However, this, too, ran into problems. An initial survey for *Colletotrichum* associated with yams, other crops and weeds, was undertaken by the University of Reading in January 1999, but was not followed up. Later, it became apparent that the DNA study, required to substantiate the morphological study in the quest to determine the origin of *Colletotrichum* epidemics on yams (tuber borne or from associated crops and weeds), could not be done by the University of Reading. In 2001, the University College, London was asked to assist, but that proved unsatisfactory, so the work was sub-contracted to CAB International. It was then successfully completed.

Management

Country visits by NRI personnel to SPYN partners were well supported. Yam tubers were willingly sent to SPC and NRI for tissue culture and indexing (except Solomon Islands. Others were sent to CIRAD, for DNA analysis (except Solomon Islands). Fresh leaf samples with symptoms of virus infection were also sent to NRI. There was a general understanding within the project for the need to share germplasm.

In Fiji, a coup occurred in May 2000. This was followed a year later by an investigation into accusations of fraud within the Ministry, and suspension of several senior staff. Funds to the Ministry were reduced, making it difficult to carry out research activities under SPYN. In Solomon Islands, so-called ethnic tension began before the start of the project, but it was by no means understood that it would have such dramatic and long-term consequences. In 1998, it became apparent that certain parts of the country were 'no-go areas'. Events escalated in 1999, and in May 2000, a coup occurred, mirroring events in Fiji at the time. Most research stations were abandoned (the main one was destroyed by fire), and staff dismissed

Meetings

Four annual meetings were organised during the four-years project:

Port Vila, Vanuatu, 23-26 February 1999.
Suva, Fiji, 24 January 2000.
Port Vila, Vanuatu, 24–26 January 2001.
Port Vila, Vanuatu, 9-10 April 2002.

Consolidated scientific report

Introduction

Yam: Cultivar Selection for Disease Resistance and Commercial potential in Pacific Islands was designed as a four-year project, beginning in 1998, to enhance the competitive position of yam in traditional cropping systems. Five Pacific Island countries, plus an inter-governmental organisation combined with three European partners to carry out the work. The association between the partners became known as SPYN, the *South Pacific Yam Network.* Members of SPYN were MAFF, Fiji (Ministry of Agriculture, Forestry and Fisheries); MAL, Solomon Islands (Ministry of Agriculture and Livestock); NARI, Papua New Guinea (National Agricultural Research Institute); DARD, Vanuatu (Department of Agriculture and Rural Development; CIRAD, France and Vanuatu (Centre International de Recherche Agronomique pour le Développement), NRI, (National Research Institute, University of Greenwich) and University of Reading (Department of Agriculture), UK.

Yam, *Dioscorea alata*, has the potential for increased commercial exploitation. However, several problems limit its development: tuber shape is often irregular making harvest time-consuming and labour-intensive, anthracnose disease, caused by the fungus *Colletotrichum gloeosporioides*, is always a threat, and staking is expensive and demands materials often in short supply. In addition, the lack of information on starches hinders potential utilisation of yam as a high quality vegetable. *D. alata* rarely flowers, and if flowers are produced, they are mostly male, so the possibility of breeding does not yet exist to make improvements in this crop. However, the wealth of germplasm has the potential of selecting varieties that comply to modern-day requirements, and those of commercial exploitation in particular.

Past attempts to collect, evaluate and conserve the genetic resources that exist, have been largely unsuccessful. Collections have been made and lost before evaluations were complete. Countries did not have the resources to maintain *ex situ* field collections for long periods due to costs and the presence of diseases. Consequently, farmers were not provided with the opportunity to test the best selections in their own countries, nor share those selected elsewhere. The potential to share was also hindered by the presence of pathogens of quarantine concern, and a lack of technologies to eliminate them.

To overcome these problems, the project considered that a thorough evaluation of genetic resources of the crop was required in each country, followed by intensive evaluation to select a small number of commercially acceptable cultivars for exchange and commercial exploitation in Pacific partners. At the same time, an investigation was made into sources of inoculum of the anthracnose fungus, *Colletotrichum gloeosporioides*, to assist farmers in the control of this serious pathogen. In order to do this work efficiently in the limited time available, modern technologies were applied – technologies that were not available to countries in the past, because of lack of funds or because they had not been developed at the time.

Molecular analyses were carried out on the selected country accessions to avoid redundancy within a core sample for Melanesia. DNA fingerprinting was also done on isolates of *Colletotrichum* taken from yam and associated plants to determine similarities which might indicate sources of inoculum. Conservation strategies employed *in vitro* methods, active growth in tissue culture as well as cryopreservation for long term maintenance, avoiding past

losses of germplasm. New methods of virus diagnosis were used, based on molecular and serological methods, which gave insight into the diversity of several viruses and their relationships to those in Asia and Africa. And finally, selections made on morphological assessments were subjected to statistical analysis to look at relationships between *D. alata* and other yam species, the nature of which had not previously been possible.

To achieve the aim of project, SPYN had five objectives; these and the respective results, were:

4. Review of yam genetic resources in five Pacific Island countries, and characterisation and evaluation of germplasm to rationalise collections and to select cultivars of interest.
- a computerised database developed containing morphological and molecular descriptors on at least 1,250 cultivars, allowing: a) comparison and rationalisation of collections; and b) selection of 150 cultivars for tuber shape, unstaked cultivation, tolerance to disease and acceptable cooking and processing characteristics.

5. Development of *in vitro* conservation strategies.
- a regional conservation strategy implemented, involving active *in vitro* national genebanks and cryopreserved base collections (limited to 150 accessions initially).

6. International exchange of virus-tested, selected, genotypes.
- at least 150 yam cultivars cultured *in vitro*, tested for viruses found in Pacific Island countries and subjected to therapy treatments if infected.

4. Identification of sources of anthracnose (*Colletotrichum gloeosporioides*) inoculum;
- molecular characterisation of *Colletotrichum* isolates from yam tubers and from at least 10 common weeds and crop plants to estimate the potential of tuber-borne and alternative hosts as sources of inoculum for epidemics of anthracnose disease.

5. Agronomic evaluation of selected cultivars;
- assist countries multiply yam germplasm rapidly using *in vitro* and field-based (mini-sett) technologies, sufficient for at least one trial per country.

The project was supported by the European Union INCO-DC programme with a grant of €600,600 (contract No. IC18CT980309).

1. Changes to the work plan

There were several changes to the agreed work plan devised at the outset of the project. These were necessary due to unforeseen political events as well reduced institutional capacities within Pacific Island countries. There was also a delay in fulfilment of the work by one of the European partners. Initially, it had been intended that Papua New Guinea would take the lead role amongst the Pacific partners in DNA fingerprinting 150 selected yams. Papua New Guinea and Solomon Islands would collaborate in the collection, purification and identification of *Colletotrichum* isolates. Solomon Island scientists would be responsible for indexing the core sample for viruses, receiving cultures *in vitro* from the SPC Regional Germplasm Centre in Fiji or directly from other partners. It was also the intention that the

selected genotypes would be grown in New Caledonia in a controlled environment to avoid genotype/environment interactions affecting starch quality.

The University of Reading would not only assist with the DNA fingerprinting of *Colletotrichum* isolates, but also make molecular comparisons of the DNA from the core sample of tubers. The original intention had been to develop methods at the University and then carry out the analyses in NARI, PNG. If the analyses could not be undertaken in the Pacific because, for instance, more sophisticated methods were needed for DNA tests, then Pacific scientists would do this at European institutes. However, staff changes at the University of Reading prevented the start of the work to develop methods.

In 2001, CIRAD assumed responsibility, and recruited a ni-Vanuatu student to do the work as part of a Ph.D. programme on the diversity of yams in Melanesia. CIRAD also took on the starch study as quarantines prevented transfer of the tubers to New Caledonia for analysis.

The transfer of this work from the University of Reading allowed it to devote more attention to the study of anthracnose. However, this, too, ran into problems. An initial survey for *Colletotrichum* associated with yams, other crops and weeds, was undertaken by the University of Reading in January 1999, but was not followed up. Staff in Fiji, Papua New Guinea, Solomon Islands and Vanuatu undertook limited national surveys and sent samples. Subsequently, the University decided to do a comprehensive and detailed morphological description of the isolates, preferring to delay the start of the DNA analysis. Later, it became apparent that the DNA study, required to substantiate the morphological study in the quest to determine the origin of *Colletotrichum* epidemics on yams (tuber borne or from associated crops and weeds), could not be done by the University of Reading. In 2001, the University College, London was asked to assist, but that proved unsatisfactory, so the work was sub-contracted to CAB International. It was then successfully completed.

Changes were also made in the work programme of NRI. Indexing was originally a function for Papua New Guinea and/or Solomon Islands after NRI had developed methods. In the event, indexing was done entirely at NRI. There were good reasons for this: the main research station in Solomon Islands was destroyed by arson in October 2000, the virus situation in yams of the Pacific turned out to be far more complex than imagined at the time of developing the proposal (more species, more strains), and this not only delayed the development of indexing protocols, but also made the task of indexing more complicated due to the number of tests needed, many of which required sophisticated equipment and expertise greater than exists in the region.

To progress work on one of the important virus groups found in yams, an NRI scientist spent time at CIRAD developing methods of detection. In addition, and to assist the growing workload of NRI, so that the Institute might complete the virus indexing protocols in the allotted time, SPC was asked to establish *in vitro* cultures of the yam selections made by the five Pacific partners.

Finally, it was the intention of the programme to assist national tissue culture laboratories to conserve national yam collections, and to multiply the selections shared among partners. This proved to be over ambitious. Tissue culture laboratories no longer exist in Solomon Islands and Vanuatu, and that those of Fiji and Papua New Guinea are on a care and maintenance basis, suffering from an absence of trained staff, equipment and supplies. The annual SPYN meeting at Port Vila, Vanuatu, 2001, agreed that assistance from SPYN would not make any

significant difference to these laboratories, and that *in vitro* conservation could only be done at the SPC Regional Germplasm Centre. SPYN's policy has been to assist the SPC RGC, which was established in 1998. This is well equipped and able to conserve plant genetic resources of crops specified by Pacific Island governments. SPYN has provided funds for a RGC research assistant to investigate the cryopreservation of yam, as well as funds for an *in vitro* collection.

2. Methods

2.1 Collection and description of germplasm

A review was made of the *D. alata* collections already established at the start of SPYN as well as the descriptions and evaluations carried out. Based upon the results, strategies were formulated in Fiji, Papua New Guinea, Solomon Islands and Vanuatu for collecting from farmers' fields and characterising the accessions at national research stations. In some countries germplasm was collected to meet SPYN criteria of potential commercial importance, in others, collections were made of all varieties available. In both cases, they were planted at national research stations, according to traditional methods and when tubers sprouted, data on 32 morphological characteristics were collected based on the IPGRI published list: *Descriptors for Yam (Dioscorea spp.) 1997*. Some countries took the opportunity to collect other species of yam.

The accessions of *D. alata* were screened first for resistance to anthracnose, caused by the fungus, *Colletotrichum gloeosporioides,* for compact, spherical tubers with firm skin, few roots, ease of harvest and desirable palatability. Later, they were assessed for the ability to perform satisfactorily unstaked. The results led to the selection of core samples in each country, and databases containing the information.

2.2 Rationalisation of selections

At CIRAD Montpellier, France, a DNA extraction protocol was developed and applied to yam selections from each country, which were then analysed by AFLPs (Amplified Fragment Length Polymorphisms) to identify duplicates and to determine the SPYN core sample for Melanesia. The data were analysed by UPGMA clustering with the DICE coefficient. A database, was developed, which included morphological and molecular data of the chosen accessions.

As a sub-component of this study, an AFLP analysis was carried out on the genetic relationships between several yam species: *D. abyssinica, D. alata, D. cayenensis-rotundata, D. bulbifera, D. esculenta, D. nummularia, D. persimilis,* and *D. transversa.* This study also undertook comparisons of ploidy levels within *D. alata* accessions of Vanuatu. Chromosome counts were made on root tips, and flow cytometry on the nuclei of leaf cells.

Preliminary work using chloroplastic Single Sequence Repeats (cpSSR) was carried out at CIRAD in order to identify polymorphic microsatellites.

2.3 Anthracnose epidemiology

The relative importance of *Colletotrichum* inoculum from yam tubers and alternative hosts was investigated by the University of Reading and CABI Bioscience. Leaves were collected from *D. alata* (and other yam species) as well as a variety of crops and weeds growing in the vicinity of yam plots in all Pacific countries. Yam tubers were also sampled. The species of fungi commonly associated with leaf and tuber lesions were isolated and identified, and for *Colletotrichum*, single-spore isolates were obtained. Eighty-one isolates were characterised according to radial growth, the morphology of reproductive structures (conidia, ascospores and appressoria) in culture, and a selection (49 isolates) according to their pathogenicity on *D. alata* leaves. A selection of *Colletotrichum* isolates was further characterised on non-yam hosts (okra, chilli pepper, tomato, cowpea, kidney beans and citrus). Tubers from Papua New Guinea and Vanuatu were grown in greenhouse trials to determine if tuber-borne inoculum resulted in systemic vine infection.

Molecular fingerprints of 39 isolates from yam leaves and tubers, and from other plants were obtained using ISSR primers by PCR amplification following extraction of DNA using standard procedures. AFLP fingerprints were also prepared.

To elucidate the relationship among *C. gloeosporioides* isolates and to establish links between their morphology, DNA fingerprinting and pathogenicity, the data on morphology and cultural characteristics were subjected to cluster analysis using multivariate statistical programmes. Dendrograms was generated from the similarity matrix obtained by Gower's linear similarity coefficient using UPMGA. Standard statistical procedures were used to compare lesion size data, and correlations between radial growth and mean lesion size.

2.4 Physico-chemical characteristics of starches

The original intention was to grow all the accessions in the core samples in one country – New Caledonia – to determine starch content and quality, but this was not possible because of quarantines and the lack of acceptable virus-indexing protocols. Instead, tubers were sent to CIRAD, Montpellier, where the analyses were made. It was not expected that the results would be affected by the fact that tubers were produced in different countries.

Analyses were made for the following: percentage starch, amylose, lipids, minerals, proteins, sugars and gelatinisation temperature range. Overall, 110 accessions were analysed (Vanuatu = 48, Fiji = 19, Papua New Guinea = 43), and samples were analysed twice to confirm the results.

2.5 Germplasm conservation

Conservation and distribution of germplasm were the tasks of SPC, the Secretariat of the Pacific Community, using its expertise in *in vitro* technologies. SPC took over the role of establishing country core selections in tissue culture from NRI. Tubers were sent to the Regional Germplasm Centre, Fiji, from each country, stored, and nodal cuttings were excised as shoots sprouted, and grown *in vitro*. Tests were made to reduce the build up of phenolic compounds.

Protocols were developed for the cryopreservation of yam shoot tips at IRD, Montpellier and applied at the RGC, Fiji. Ways of enhancing the success of cryopreservation were investigated.

2.6 International exchange

NRI worked with SPC to produce plants that were safe for exchange between partners, overcoming quarantine concerns about the viruses present in yam germplasm. SPC investigated factors influencing the growth of meristems on artificial media, taking explants from *in vitro* cultures as well as from shoots from tubers in storage and in the field. Several treatments of nodal cuttings were also investigated to produce plantlets from infection, including chemotherapy (viricides placed in the culture media), electrotherapy (heating tissues *in vitro*), and thermotherapy (growing shoots at high temperature before culture of the apical portion). Hot water treatments were also tested.

The project supported a study at NRI comparing serological and molecular techniques for the detection of yam viruses, their prevalence and diversity, and the genetic variability of the main types. This work was submitted as part fulfilment of the requirements for a Ph.D at the University of Greenwich in 2001(*Bénédicte Lebas*). At CIRAD, Montpellier, research was done on the molecular variability of yam badnaviruses, and to determine if badnavirus-like sequences are integrated into the yam genome (Susan Seal).

The isolation and characterisation of the viruses found in the South Pacific was done by NRI in collaboration with CIRAD. In order to have a better understanding of the distribution of yam viruses in the South Pacific, extensive surveys were carried out to observe symptoms and to collect leaves. Samples were taken mostly from collections at national research stations. ELISA was used to examine the presence of *Yam mosaic virus* (YMV), *Dioscorea alata potyvirus* (DAV), *D. dumetorum potyvirus* (DDV), *D. alata badnavirus* (DABV), *D. bulbifera badnavirus* (DBBV), *Dioscorea latent potexvirus* (DLV), and *Cucumber mosaic virus* (CMV) in leaf samples from 719 plants representing nine *Dioscorea* species from seven South Pacific countries.

Some samples were later tested for two more viruses of Asian origin: JYMV (*Japanese yam mosaic virus*) and CYNMV (*Chinese yam necrotic mosaic virus*).

The studies provided information on the diversity, variability and prevalence the viruses in *D. alata* and other species. Two viruses, DAV (*Dioscorea alata* virus) and a group referred to as yam badnaviruses were investigated in more detail as they were the most common viruses detected. These tests were done to determine the presence of different strains, their distribution and the reliability of the antiserum used in serological tests. Comparisons were made with viruses isolated from Asian and African yams.

2.7 In-country evaluations

The virus-tested selections were multiplied in field plots sufficient to carry out comparisons between the accessions chosen for the country core samples. At the outset, it was expected that these trials would continue beyond the life of the project.

3. Results

3.1 Collection and selection of core samples

For most countries the review of yam collections found that most had lost a majority of the cultivars collected a decade or so earlier, and in some instances, information on the accessions could not be found. Recollecting was required, but the late transfer of funds to Fiji, Papua New Guinea (plus uncertainties within NARI) and Solomon Islands in 1999 delayed fieldwork until 2000. The crop is seasonal and there is only one period in the year when yams can be collected.

However, collections were complete in all countries by year 2, described by year 3, and most of the potentially useful characteristics (good tuber shape, resistance to anthracnose, good taste, etc) identified in all countries by the end of the project. These formed the core collections of the countries.

The numbers of accessions collected and the core samples selected are presented in Table 1.

Table 1. Collections of *D. alata* and core samples based on SPYN criteria of commercial acceptability in five Pacific Island countries

Country	No of accessions collected	Described	No. in core sample[1]	Evaluations still in progress?
Fiji	108	108	19	Yes
Papua New Guinea	209	209	43	Yes
Solomon Islands	392	238 (by 2001)	Unknown	Unknown
Vanuatu	331	331	14	Yes
Total	1040			

[1]It is expected that the composition of the core samples will change based on evaluations still on-going

Where there were omissions in the data collected, they involved disease resistance and its compounding affects on tuber shape. Some countries found it difficult to manage anthracnose. In Papua New Guinea for instance, harvests were not until June and this meant that replanting was relatively late, towards the beginning of the rainy season in October and November. Consequently, anthracnose epidemics were more severe than if collections had been planted in the drier part of the year, and plants had reached the tops of their supports before the onset of rains. If epidemics had been allowed to progress naturally, the collection of morphological data would have been sacrificed due to blackening and defoliation of leaves and vine dieback. It would also have caused early death of the plants and tuber shape would have been impossible to evaluate. Consequently, plants were sprayed frequently with fungicides. This has meant that the core sample has not yet been adequately assessed for anthracnose resistance.

In Fiji, political events, especially those of 2000, delayed planting and consequently tuber shape was difficult to assess. In addition, the collection was planted initially at Koronivia, in the wet zone (3500 mm rainfall), where early death of the plants due to anthracnose is commonplace, and where in 2000 and 2001 the wet seasons had unusually high rainfall. In contrast to Papua New Guinea, anthracnose was not controlled by fungicides, and this meant that tubers were not always sufficiently well formed at harvest to indicate their proper shape if plants had been healthy. More evaluations are required, and the collection is now being grown at Dobuilevu Research Station where rainfall is 2500 mm.

By contrast, plantings in Vanuatu were early, in August of each year. Growth was luxurious due to lack of disease, and also because large tuber pieces were available for planting. This allowed a full characterisation of the germplasm and the inclusion into the core sample of accessions with compact tubers ideal for the market.

The situation in Solomon Islands remains unclear. The political difficulties in that country were protracted and the social repercussions severe. Communications were difficult from 2000 onwards, and it is not certain whether the collection at Fote Experimental Station, Malaita still exists. There have been indications that a core sample has been selected, but so far it has not been sent to SPC, Fiji, for safekeeping or to CIRAD for starch analysis. Previously, recordings were made on the collection for anthracnose resistance, and leaves with virus symptoms were despatched to NRI.

3.2 Yam viruses of the South Pacific

3.2.1 Survey results

A comparison of ELISA, ISEM, and PCR was carried out in order to identify the most reliable technique for the detection of yam viruses. The studies revealed that there was poor comparison between ELISA and PCR techniques. PCR tests have greater sensitivity but the genetic variability of the viruses will need to be studied to develop primers that detect the strains that exist. However, there were occasions where ELISA tests out-performed PCR.

DAV (*Potyvirus*) was the virus most commonly detected (69% of samples) by serology (ELISA) in *D. alata, D. esculenta, and D. bulbifera,* from across the South Pacific region. The DAV antiserum gave positive reactions in ELISA with samples from all the countries included in the study. RT-PCR was generally more sensitive than ELISA for detecting DAV. However, several primer pairs had to be used to be sure of detecting all strains of DAV, as sequence analysis suggests that many are present. There was no strong association between strains and where they were isolated.

Antiserum designated as anti-DDV (*Potyvirus*) also commonly gave positive reactions when used in ELISA. The frequency of samples testing positive for both DAV and DDV was higher than would be expected by chance, suggesting that either the DDV antiserum was cross-reacting with some DAV strains, or that plants infected with DAV are more prone to infection from DDV (or *vice-versa*). None of the *Potyvirus* sequences from the South Pacific clustered with putative DDV sequences from Sri Lanka and Bangladesh.

The antiserum against DLV (*Potexvirus?*) gave many positive reactions. However, when RNA from some of the DLV ELISA positive samples was tested by RT-PCR with universal *Potexvirus* antiserum, results were negative. It is uncertain what the DLV antiserum is detecting. Similar results have been obtained in Guadeloupe.

The large number of samples that tested positive with both DABV and DBBV antisera in ELISA suggests that they recognise some strains of both these Badnaviruses. Also, the incidence of samples positive to both DBBV and DLV was higher than would be expected by chance, suggesting that DBBV antiserum has affinity for some DLV strains. The results of sequencing of PCR products found at least 12 different *Badnavirus*-like sequence clades in yam samples. These are sufficiently different that they might be thought to be different *Badnavirus* species or different strains, depending upon interpretation. Further, some of the

sequences obtained show nucleotide deletions that would result in the translated gene product being inactive, and that there are sequences integrated into the genome of some yam varieties. Further study is required to determine if these integrated sequences can recombine to form active virus.

YMV (*Potyvirus*) was commonly found in *D. rotundata* plants from Africa, but has not been demonstrated in the South Pacific. The triple antibody sandwich ELISA using antiserum from IITA Nigeria is very sensitive, but still it is probably safest to test accessions both with this ELISA and by RT-PCR before allowing germplasm export or exchange.

CMV (*Cucumovirus*) has a very wide host range, and some (probably many) strains will infect yam in the South Pacific. The variability of CMV strains means that as yet no single antiserum can be used with certainty to detect all strains. Several different PCR primer pairs have been designed for detection of CMV by RT-PCR, but more work is required to determine if any of these is capable of detecting all strains of the virus.

Both antisera against JYMV and CYNMV from Japan gave some positive reactions when used in ELISA on some samples from the South Pacific. PCR primers for the specific detection of JYMV have recently been published, and the development of CYNMV-specific primers is a possibility.

Thirty-three plants associated with yam cultivation were found infected with yam viruses. Among these, *Piper methysticum* (Vanuatu) and *Colocasia esculenta* (Fiji) tested positively for badnavirus, and *Pueraria* species for DAV (Solomon Islands) and *Colocasia esculenta* for JYMV (Vanuatu). This suggests that some viruses may have spread from one crop to another.

3.2.2 Identification of yam viruses

Thirty-three plants associated with yam cultivation were found infected with yam viruses. Among these, *Piper methysticum* (Vanuatu) and *Colocasia esculenta* (Fiji) tested positively for badnavirus, and *Pueraria* species for DAV (Solomon Islands) and *Colocasia esculenta* for JYMV (Vanuatu). This suggests that some viruses may have spread from one crop to another.

The detailed study of DAV isolates from the Pacific and those in other regions showed that although there are several different "strains" of DAV, these were not separated by geography. Most of the DAV sequences were obtained from *D. alata* samples, although some came from *D. rotundata* and *D. esculenta*. Contrary to previous suggestions, there did not appear to be strains unique to *D. esculenta*.

From the study of DAV variability, there is a good chance that primers can be developed that will detect all strains. This is not so for the badnaviruses where there is evidence that DNA fragments of badnavirus are integrated into the host genome. Whether or not these sequences can form virus particles is not known.

Phylogenetic analyses were carried out on badnavirus sequences obtained from the CIRAD collection that contains yams from Africa, the Caribbean and the Pacific, and comparisons were made with sequences from closely related viruses: *Banana streak virus* (BSVOnne; BSVMys), *Cocoa swollen shoot virus* (CSSV), *Commelina yellow mottle virus* (CoYMV), *Sugarcane bacilliform virus* (SCBV), *Rice tungro bacilliform virus* (RTBV), and *Citrus yellow mosaic virus* (CitYMV). The results showed that there were high sequence similarities

between samples from different countries – for instance, *D. esculenta* from Fiji, Papua New Guinea and Solomon Islands – or even from different continents, indicating past exchange of badnavirus-infected yam plants or tubers. This appeared to be confirmed by a greater association of sequences from different *Dioscorea* host species than with geographical origin. The dissimilarity between the clusters may indicate different species, although a definition of this term for Badnavirus is wanting. Interestingly, several sequences from yams cluster closely with BSV, suggesting convergent evolution of yam and banana, or transfer of virus between these plants.

Two of the sequence groups appearing on the dendrogram may not represent badnavirus sequences as they share a similar level of identity with RTBV. RTBV is not considered as a *Badnavirus* member but forms a separate group within the *Caulimoviridae* family. Further sequencing as well as biological characteristics needs to be obtained to determine if these groups represent novel viruses or are divergent integrated sequences.

There is still much to do in yam virology in the South Pacific, but a good start has been made. The DAV and *Dioscorea badnavirus* sequences obtained in the study revealed the presence of strains and/or species of viruses, which seem to be present in all South Pacific countries. The next step is to undertake nucleotide sequencing for classification of the viruses and taxonomic studies, and for the development of more reliable virus indexing tests.

3.3 Therapies, indexation and safe germplasm transfer

Chemotherapy, thermotherapy, electrotherapy, and hot water therapy were assessed to gauge their potential to produce plants free from virus. The studies used both meristems and nodal cuttings. The efficiency of the methods was tested against plants that were known to be infected by DAV. Chemotherapy is the inclusion of viricides (quercitin dihydrate, ribavirin, Virkon) in the medium; thermotherapy is the growth of plants at temperatures greater than 34°C prior to extracting young nodes or meristems, and electrotherapy, the heating of vines by passing an electric current through them, with nodes reaching temperatures of up to 39°C.

Regenerated of plants from meristems was very low (7%), and further work is required on the development of suitable method for their establishment *in vitro*. In contrast, plantlets grown from node cuttings had a regeneration rate of 58%.

A newly described method – electrotherapy – was used on node cuttings, and proved to be more efficient on DAV eradication (64% DAV-free plant) compared to chemotherapy (6% DAV-free plant) and thermotherapy (43% DAV-free plants). This method has considerable potential: it avoids the use of toxic chemicals (chemotherapy), long delays in growing plants at high temperature (thermotherapy), and poor performance (hot water). Also, node cuttings can be used as explants, where the rate of regeneration is much greater than meristems. It does have the disadvantage in that only one stem can be treated at a time. Much more work is required to substantiate the findings made so far since indexing of the plantlets was done while they were still very young, and it is well recognised that virus titres are often very low in newly established tissue cultured plantlets. Also, only the elimination of DAV was studied here; elimination of other viruses may require different approaches since other viruses may be more resilient or may be confined to different tissues of the plant. The method also needs to be used on other yam species.

3.3.1 Indexing: development and application

One of the aims of SPYN is to get to the stage where selected elite yam germplasm can be exchanged between South Pacific countries for evaluation and promotion. The virology component of the project has shown that exchange will not be easy because of the prevalence of a wide range of viruses infecting yam in the region. In many plants, combinations of viruses occur, the most common being: DAV+DDV; DAV+badnaviruses; and DDV+badnaviruses, and there is great diversity among the viruses, in particular DAV and the badnaviruses. Furthermore, the results from the badnavirus study mean that reliable indexing for active *Badnavirus* in yam accessions to permit germplasm exchange is not currently possible since it is likely that even a mix of all available antisera will not detect all strains, while PCR with universal *Badnavirus* primers is likely to result in many "false" positive reactions due to amplification of integrated sequences.

Despite the great diversity and genetic variability of the viruses, diagnostic tests are now available for several of the viruses. For many, these involve both ELISA and PCR. The original plan was that selected yam accessions would be established in tissue culture either at SPC, Fiji, and sent to NRI, or tubers would be sent directly to NRI for culture of emerging shoots. Each plant would then be indexed after 3 and 6 months growth in soil. Those that tested negative for all viruses would be further sub-cultured in tissue culture, and then returned to the SPYN partners as *in vitro* plantlets. However, the work is still incomplete, and discussions are underway as to how the tissue culture and virus therapy can continue. There is a possibility that the tissue culture aspects can continue at SPC and virus indexing at USP, Fiji.

3.4 Conservation: *in vitro* and cryopreservation

3.4.1 Growth of yams *in vitro*

It is not expected that countries will be able to retain field collections of yams without loss. If past experiences are anything to go by, losses will be substantial and swift. Thus, it was important to develop reliable alternative methods. Tissue culture was investigated as means of long-term conservation of active collections. Although there is much work on African yams, *D. rotundata-cayenensis*, in particular, there has been less research on *D. alata*, especially those from the Pacific.

Nodal cuttings of most varieties were successfully grown in tissue culture. Experiments using citric acid (an anti-oxidant) to reduce phenol build up at the cut ends did not lead to useful results; where phenolic compounds were produced explants were sub-cultured frequently to fresh medium, but even this was relatively ineffective. It was found that shoots need to have at least five nodes before the nodes or shoot tips are cultured, otherwise they are too soft and contaminated with bacteria. Overall, more than 70% of the nodes were successfully cultured, representing a majority of the accessions tested. A few failed due to inadequate surface sterilization or the composition of the medium was unsatisfactory.

The culture of shoot tips from plants taken from the field was less successful, with only about 5% developing into plantlets. A number of protocols reported in the literature were used, but none were successful. The results confirmed previous workers' experiences that it is difficult to culture meristems from Pacific Island yams *in vitro*. However, much greater success was

achieved when meristems were cultured from *in vitro* plantlets, especially those on a medium with 3% sucrose. The success rate using two Fiji cultivars was more than 50%.

The advantage of *in vitro* maintenance is that it will facilitate the exchange of plant materials free from pests – fungal and bacterial pathogens, in particular. To date, most of the core collections of countries are in tissue culture (Fiji, 19; New Caledonia, 4; PNG, 47; and Vanuatu, 30), safely maintained at the SPC RGC, Fiji. This leaves the following still to do: PNG (3), Vanuatu (3), Fiji (1), and all those from Solomon Islands. This is a considerable achievement. The challenge now is to produce plants that are in harmony with national plant quarantines. That means establishing plantlets from meristems or using other therapies to remove virus infections.

3.4.2 Cryopreservation

Dr. *Bernard Malaurie*, IRD, Montpellier visited the RGC, SPC, Fiji, 7-24 May 2001 to train staff in encapsulation-dehydration cryopreservation technique for yams and other crops. He provided illustrated manuals on the cryopreservation protocols and media preparation, and gave a seminar on yam germplasm conservation for SPC and Fiji agricultural research staff.

Overall, the cryopreservation technique has been disappointing. Meristems from one cultivar showed some swelling but failed to produce shoots and roots. As the control meristems (*ie* without cryopreservation) also failed to grow, it is not clear whether the difficulty is with the meristems or the medium. However, as explained above, it is difficult to grow meristems from Pacific yams, in contrast to those from the Caribbean and Africa.

3.5 DNA analysis of yam and *Colletotrichum*

3.5.1 Molecular analyses and relationships between yam species

The DNA analysis of the core samples is incomplete. It was been carried out for the Vanuatu collections but not for the others. This work will be done in June 2003. It could not be completed during the project period because of the later start to the project, the need for countries to recollect in the first two years, and the time taken to make selections before they were sent to CIRAD, Montpellier for analysis. However, the work done at CIRAD on the Vanuatu collection has shown that AFLPs are useful for germplasm management purposes as they allow distinction of *D. alata* duplicates. Results from that collection indicate that rationalization needs to be done using both morphological and genotypic characterization due to the phenotypic plasticity of the species.

The detailed study on the Vanuatu collection showed several interesting results. First, the selected IPGRI descriptors revealed great variation in foliar characteristics, tuber shape, size, weight, flesh colour, etc, and when subjected to multivariate analyses, revealed a continuum of morphological variation. The accessions belonging to the section Enantiophyllum were difficult to classify within *D. alata* or *D. nummularia* as they possessed characters identified with both species.

Second, the molecular analysis, while confirming the integrity of the botanical classification of the several species compared, indicated that *D. alata* was closer to *D. nummularia* than *D. persimilis*, one of the putative parents of *D. alata*. In fact, *D. persimilis* was genetically closer to *D. nummularia*, an endemic species of Oceania, than to *D. alata*.

Third, the *D. nummularia* accessions fall into two groups, with three cultivars of intermediate type closely associated. The data suggest that they are a distinct species from *D. alata* and *D. nummularia*, but share a common ancestor. Comparisons of these varieties and *D. transversa* from New Caledonia suggest they belong to that species, and that this is the first time *D. transversa* has been recorded from Vanuatu.

Four, *D. alata* accessions from different parts of the world clustered together indicating that there has been wide distribution of varieties as clonal material.

Five, species from Africa (*D. abyssinica*, *D. cayenensis-rotundata*) are more distant from the Asian-Oceania species (*D. alata*, *D. nummularia, D. persimilis* and *D. transversa*).

The results of the ploidy studies showed tetraploids, hexaploid and octoploid varieties of *D. alata* present in the collection, although hexaploids are rare. Tetraploids had narrow leaves, whereas leaves of hexaploids and octoploids were more likely to be thick, dark green, waxy, and cordate. Hexaploids and tetraploids are probably auto-polyploids. It was also found that morphologically similar accessions have similar ploidy levels and *vice-versa*, indicative of the highly variable nature of the species.

A preliminary study was done by CIRAD, Montpellier using chloroplastic simple sequence repeat markers (microsatellites). Amplification and migration on acrylamide gel can detect alleles that are different by one or two bases. The amplification with the marker NTcp19 obtained one to four alleles per accession of the LSC part of the chloroplastic genome, whereas usually only one copy of the sequence would be expected. The presence of 4 alleles per accession could be explained by the paternal transmission of chlorplast with the conservation of the maternal chloroplast.

Although it is too soon to formulate definite conclusions, it appears that the genetic base of the species *D. alata* is narrow, *D. nummularia* is closely related to *D. transversa* and to *D. alata*, and that some varieties may be auto-polyploids.

3.5.2 Sources of *Colletotrichum* inoculum

C. gloeosporioides was isolated from leaf lesions in all yam-growing provinces sampled throughout Fiji, Vanuatu and Papua New Guinea, but not from anthracnose lesions from Solomon Islands, although the fungus has been reported there from extensive past studies. The absence may be attributed to the high incidence of other leaf fungal pathogens present.

The overall mean growth rates recorded in this study were in general agreement with those of published studies, and spore and appressoria measurements were consistent with descriptions of *C. gloeosporioides*, although individual variations were noted. However, the tuber isolate, YTPNG-044-1 was unusual in that it clustered with other slow-growing, non-ascigerous forming isolates; it has abnormally large conidia and forms abundant sclerotia on PDA as well as on leaves. Further studies are required on its identity, pathogenicity and geographical distribution. So far, sclerotia forming isolates have not been reported in *C. gloeosporioides*.

The studies showed that there were significant differences in pathogenicity among *C. gloeosporioides* isolates, and this agrees with findings of others. These differences require further investigation in order to have a comprehensive knowledge of the nature of pathogenic

variability. This is a prerequisite for adequate disease management particularly in implementing a successful breeding programme for disease resistance, and suggests that all cultivars selected under SPYN for commercial exploitation should be tested for their resistance to a wide range of *C. gloeosporioides* isolates. However, there was no clear association between morphological data and pathogenicity among isolates studied. There was no indication that those isolates with the slowest growth rate (in culture) were also the most virulent as suggested by work in Nigeria.

Although the occurrence of the teleomorphic state, *Glomerella cingulata*, has been widely reported on yam, there is little information on its importance in the epidemiology of yam anthracnose. Half of the isolates tested in this study produced sexual structures on host tissue suggesting that *G. cingulata* occurs more frequently in nature than previously thought. There was no clear link between pathogenicity and the production of the teleomorph form. Hence, it is most likely that the teleomorph stage plays a vital role as a mechanism for genetic variability as well as in the perination of the fungus between seasons.

Both yam and non-yam isolates of *C. gloeosporioides* were able to infect yam as well as a other non-yam hosts, inferring that of a number of crops and associated natural flora could act as an inoculum reservoir posing a serious threat to yam production. Hence, in yam growing areas, farming practices such as intercropping, or mixed cropping, with known *C. gloeosporioides* hosts should be minimised, whereas weeding and other sanitation measures should be promoted.

The detection of *C. gloeosporioides* in yam tubers from Vanuatu and Papua New Guinea (4.8%) confirms that the fungus is able to infect and survive in tuber tissue from season to season. The isolates were all highly pathogenic to both yam and non-yam host species, despite some variations in their cultural morphological and cultural characteristics. It is likely that the infected tubers could act as a primary source of inoculum playing an important role in the epidemiology of the pathogen in the field. The results corroborate earlier work on the occurrence of *C. gloeosporioides* tuber infection under natural conditions in the Caribbean and West Africa. The mechanism of spread of *C. gloeosporioides* from tuber to canopy is still yet to be fully understood, but systemic spread is not suspected.

Analyses at the molecular level revealed some interesting results. The dendrograms produced from using ISSR-PCR and AFLP markers did not give similar fingerprints, even where multiple *Colletotrichum* isolates were compared from the same leaf. However, the molecular tree generated by ISSR-PCR analysis correlated well with the dendrogram of morphological data. There was also a good correlation the ISSR-PCR data and ability of isolates to produce sexual spores. By contrast, there were few similarities between the AFLP results and morphological data.

The high heterogeneity and complex patterns, at the molecular level, exhibited by isolates of *C. gloeosporioides* indicates the existence of a complex population structure in which sexual recombination probably plays a major role in generating variation. The genetic diversity revealed looks similar to isolates from natural habitats rather than those of agro-ecosystems. This fits the nature of the yam cultivation in the South Pacific islands with tiny plots within forest rather than large crop monocultures. In addition, the lack of clear linkages between molecular fingerprints and host and the fact that isolates from different host species have a higher degree of homology than isolates from the same host species adds weight to the argument that *C. gloeosporioides* is a polyphagous pathogen with a wide host range. It also

points to the likelihood that plants surrounding yam plots are acting as disease reservoirs. Moreover, the lack of clear linkages between molecular patterns and the geographical origin coupled with the evidence that closely similar strains are present in widely separate localities probably reflects the historic movement of germplasm between the islands.

No correlation was apparent between isolate pathogenicity and their molecular fingerprint patterns: isolates showing marked differences in pathogenicity appeared to be closely related in molecular terms. This might be expected as the DNA regions investigated in the study were unlikely to be linked to pathogenicity.

In conclusion, the results of the current work confirm that under optimum conditions, *C. gloeosporioides* is able to cross-infect different host species. This underpins the real threat that yam cultivations could face from neighbouring crops as well as from the nearby natural flora. The results further highlight the importance of *C. gloeosporioides* as a tuber-borne pathogen under field conditions and that tuber-borne infection poses a threat to yam production both as a source of infection and as a means of dispersal. The results also show that *C. gloeosporioides* isolates are highly heterogeneous indicating a complex population structure. However, a high degree of similarities between *C. gloeosporioides* isolates from widely different islands also suggests that populations of this pathogen from different South Pacific islands are not entirely distinct.

3.6 Starch analyses

It was agreed at the 2001 Annual Meeting, that countries would send tuber samples to Vanuatu at harvest, and that a combined consignment of yams would be forwarded to CIRAD for starch analysis. So far samples have been sent from Vanuatu (48), Fiji (19) and PNG (43). None were sent from Solomon Islands.

Significant variation exists within each country for each of these major characteristics. Good varieties are characterised by high dry matter, starch and amylose contents. In Vanutu, varieties suitable for *laplap* the national dish (a pudding) appear to have high amylose versus starch ratio (>0.18). Chemotypes are genetically controlled and these traits will have to be taken into consideration when recommending a variety and/or for future breeding programmes because they determine the cultivars likely to be sought by farmers.

4. Project management

4.1 Collaboration and sustainability

Country visits by NRI personnel to SPYN partners and other South Pacific countries were well supported. Yam tubers were willingly sent to SPC and NRI for tissue culture and indexing (except Solomon Islands), even though some countries were concerned at the start of the project about intellectual property issues. Others were sent to CIRAD, Montpellier for DNA analysis (except Solomon Islands). SPC took charge of the development of MTAs and these were agreed by Pacific heads of agriculture and are now being revised). Fresh leaf samples with symptoms of virus infection were also sent to NRI. There was a general understanding within the project for the need to share germplasm: none of the countries has a unique advantage over the others, and there is much to gain if yam genetic resources are shared.

However, collaboration between the Pacific Island partners was difficult to establish and maintain. There was little if any direct contact between them. Partly this was to do with the paucity of scientists in the national agriculture agencies of the region and with the political events from 1998-2002 that created considerable uncertainty and lack of focus amongst SPYN participants. If economic situations in all the countries were problematic before the start of the project, they worsened as it progressed and as political instability become commonplace. Vanuatu and New Caledonia were least affected, but here SPYN was supported by CIRAD, with sufficient independent resources to overcome deficiencies in the local budget.

In PNG, a major difficulty occurred in the National Agricultural Research Institute in 1998, at the start of the project. At that time, it seemed unlikely that NARI would survive, and it would be reabsorbed into the Department of Agriculture and Livestock. Funds were reduced, staff laid-off, and work placed on a care and maintenance basis. The uncertainties that this caused, delayed yam collecting, which was also affected by the late arrival of funds in the first year. The situation improved with a change of government in 1999.

In Fiji, a coup occurred in May 2000, and destabilised the country for several weeks. This was followed a year later by an investigation into accusations of fraud within the Ministry, and suspension of several senior staff. During the period of investigation, funds to the Ministry were reduced, making it difficult to carry out research activities under SPYN.

In Solomon Islands, so-called ethnic tension began before the start of the project, but it was by no means understood that it would have such dramatic and long-term consequences. In 1998, it became apparent that certain parts of the country were 'no-go areas' where yam collecting was impossible. Events escalated in 1999, and in May 2000, a coup occurred, mirroring events in Fiji at the time. Most research stations were abandoned (the main one was destroyed by fire), and staff dismissed. However, MAL was able to collect yams from some areas and a national collection was established, partly described but not evaluated properly. The Solomon Islands did not attend the annual meetings of 2001 and 2002.

Further attempts to assist Solomon Islands were made in 2002, and agreement was reached between CIRAD as project manager and the EU that Solomon Islands need not submit a detailed acquittal of the first tranche of funds. A sum of €10,000 was offered so that evaluations of the core collection could proceed. However, a work plan was not produced and the funds were not transferred.

It is not surprising, therefore, that SPYN did not develop into a viable network sharing resources, staff and information, a network that would have been to mutual benefit through cooperation and complementary actions. It was not possible for research staff from PNG and Solomon Islands to visit other partners to carry out surveys for anthracnose and viruses as originally intended. Nor was it possible to establish indexing capabilities and those for DNA analysis in the region. It was very much left to the European partners to take over the research in order to achieve the project objectives.

Although the network concept of SPYN may not have resulted in the outcomes anticipated, nevertheless, parts of its work will continue under the auspices of SPC. The project gave support to the development of the Regional Germplasm Centre, and as a result an *in vitro* collection of yams was made. As yet, accessions from this collection cannot be distributed

due to the need to remove virus infection. However, SPC intends to continue the work. Therapies will be investigated and an indexing facility will be developed in collaboration with the University of the South Pacific. In addition, the training on cryopreservation at SPC has provided a foundation for further research.

4.2 Meetings

Port Vila, Vanuatu, 23-26 February 1999. The Scientific Coordinator outlined the programme for the four-day meeting, with discussions on the administration and work plan of the Network interspersed with field visits on Efate and Santo to see yams in farmers' fields and the Department of Rural Development's collections. He mentioned the unique role of *D. alata* in Pacific cultures: it the only root crop that is seasonal and as such it is a biological clock. There is great variation in yam genetic resources, but also great variation in cropping techniques. It deserves to be better researched and conserved. Countries gave statements and a work plan for the year was agreed.

Suva, Fiji, 24 January 2000. During the first year of the project, collections had been made in some countries, morphological descriptors chosen and applied to collections, *Colletotrichum* and virus surveys carried out, characterisation of fungal and virus isolates initiated, and cryopreservation protocols investigated. The specific objectives of the workshop were to review the progress made during 1999, and formulate a work plan for 2000 for the selection of 150 yam cultivars for virus indexation and sharing; assist national *in vitro* genebanks; develop/test yam cryopreservation methods at CIRAD, Montpellier for transfer to SPC Fiji; DNA fingerprint 150 yam cultivars; DNA fingerprint *Colletotrichum* isolates and carry out pathogenicity tests; continue development of virus indexing procedures and therapy treatments on virus-infected yams; and agree on MTAs to facilitate movement of yams between countries. Difficulties were noted in the extraction and analysis of DNA from cultivars as well as *Colletotrichum*, and the complexity of viruses from yams of the region was far greater than envisaged.

Port Vila, Vanuatu, 24–26 January 2001. All partners attended the meeting, except and Solomon Islands and the University of Reading (represented by Dr Richard Strange, University College, London, who had been asked to take on the DNA fingerprinting of *Colletotrichum* when the principal investigator left the University). During the two years of the project, collections have been made in all countries, morphological descriptors chosen and applied, *Colletotrichum* and virus surveys carried out, characterisation of fungal and virus isolates initiated, and cryopreservation protocols investigated. Although some aspects of the work have been successful, overall, progress has been slower than anticipated.

A core sample of 150 accessions from the five South Pacific countries had not been identified, protocols for comparing DNA fingerprints of *Colletotrichum* had not been produced, development of indexing techniques were constrained by the large number of viruses encountered, and therapy treatments applied to remove infections were not effective. There was a possibility that SPYN would not meet its objectives. Collections would not be shared between participants and evaluated in different agro-ecological areas, and starches would not be assessed. The meeting provided the opportunity for a mid-term review, and a chance to re-orientate the project in order to achieve its goal. It was decided that CIRAD, Montpellier would do the starch analyses, and also take over the work on DNA fingerprinting the selected varieties (a student from Vanuatu was hired to do the work at the University of Rennes and CIRAD, Montpellier), and that SPC would help NRI establish collections *in vitro*.

Port Vila, Vanuatu, 9-10 April 2002. All partners, except Solomon Islands were present. The University of Reading was represented by Dr P Cannon, CABI Bioscience. The meeting discussed the several problems: a core sample of 150 accessions from the five South Pacific countries had not yet been identified, only recently have DNA fingerprint of *Colletotrichum* been produced, development of indexing techniques have been constrained by the large number of viruses found, and therapy treatments to remove infections have proved elusive. Political events in Solomon Islands have affected its work programme. The meeting thought it unlikely that SPYN would meet its main objective to share collections between participating South Pacific countries and evaluate them in different agro-ecological areas. The meeting provided the opportunity to summarise progress, discuss the shortcomings and to determine a work plan for the final year that will maximise the chances of successfully completing a majority of the project's objectives.

4.3 Staff exchanges/training

Mr *Roger Malapa* successfully completed his Diploma d'études approfondies, at the University of Rennes, in France, and is registered for a Ph.D. in the same University. He has made several visits to CIRAD, Montpellier, where the laboratory studies are carried out. He will complete his research in June 2004, defend his thesis and return to Vanuatu.

Training in cryopreservation of yams, using encapsulation-dehydration, was provided by Dr *Bernard Malaurie*, IRD, Montpellier, from May 7-24, 2001. All RGC staff received this training, so that they would be better equipped for future conservation and research on yams, or on any other species using the same technique. A technician, *Eliki Lesione*, was recruited to work on yam tissue culture and cryopreservation.

4.4 Problems

The problems that occurred during the course of the project were both of a technical and administrative nature. The difficulties of establishing a viable sustainable network have already been mentioned. There was also a difficulty at the University of Reading resulting from staff changes and the problem of finding a suitable contractor to take on the *Colletotrichum* fingerprinting. Eventually, CABI Bioscience took on the work, and provided some extremely interesting results. The morphological studies done by the University of Reading went ahead without hindrance, and were done well.

The main technical problems that beset the project were related to the unexpected number of viruses encountered by NRI in the course of the work, the difficulty of removing the infections, and also the problem of finding a suitable cryopreservation technique. However, it is good to report that this work will continue in the region under the auspices of SPC.

It is likely, too, that the recently established transit centre supported by CIRAD will also pay attention to the consequences of viruses in its attempts to transfer the better yam cultivars internationally.

5. Technology implementation plan

In the short term, the genotypes included in the core sample will be used in all countries for distribution directly to farmers. In the long term, it is hoped that therapies will be successful and genotypes will be exchanged between countries. At this stage, it is not intended to patent these varieties.

6. Publications and papers

CIRAD (& IRD):

Lebot V., Trilles B., Noyer J.L. and Modesto J., 1998. Genetic relationships between *Discorea alata* L. cultivars. *Genetic Resources and Crop Evolution*, 45 (6): 499-509.

Malaurie, B. 1999. *Technical guidelines for the cryopreservation of Yam apices by the encapsulation/ dehydration process*. IRD-GeneTrop. Montpellier, France.

Roger Malapa. 2000. Evaluation de la diversité génétique de la grande igname (*Dioscorea alata*) par AFLP. DEA de l'Université de Rennes.

Malapa R., Arnau G., Noyer J.L. & V. Lebot. 2002. Genetic relationship between *D. alata* and *D. nummularia* as revealed by AFLP. *Origins, Evolution and Conservation of Crop Plants: A molecular Approach.* Society for Economic Botany. New York Botanical Garden, Bronx N.Y.

SPYN, 1999. Annual report, CIRAD, Montpellier, France

SPYN, 2000. Annual report, CIRAD, Montpellier, France

SPYN, 2001. Annual report, CIRAD, Montpellier, France

NRI, University of Greenwich:

Lebas, B.S.M. (2002) Diversity of viruses infecting *Dioscorea* species in the South Pacific. *PhD Thesis, Natural Resources Institute, University of Greenwich, UK.*

Kenyon, L., Seal, S.E. and Lebas B. (2003) Detection and elimination of viruses infecting *Dioscorea* yams. *Poster No 1440 (Abstract 7.85) presented at the 8th International Congress of Plant Pathology (ICPP2003), Christchurch, New Zealand* 2-7 February 2003.

Kenyon, L., Lebas, B., Seal, S. and Lebot, V. (2002) Viruses infecting Dioscorea yams in the South Pacific islands. P.395-397. In *Proceedings of the Twelfth Symposium of The International Society for Tropical Root Crops: Potential of Root Crops for Food and Industrial Resources,* Ed. M. Nakatani and K. Komaki. ISTRC.

Lebas, B.S.M., Kenyon, L. and Seal, S.E., (2001) Prévalence et Variabilité Génétique des Virus Infectant les Ignames du Pacifique Sud. *Les Rencontres de Virologie Végétale, Aussois, France March 2001.* (Oral presentation)

Lebas, B.S.M., Kenyon, L., Seal, S.E., Canning, E.S.G. (1999). Viruses of yam in South Pacific Islands. *Poster presented at Association of Applied Biologists (AAB)-virology meeting, York,* 8-9 April 1999.

Lebas, B.S.M., Canning, E.S.G. Kenyon, L., Seal, S.E., (1999). Yam Viruses of the South Pacific Islands. Poster presented at BSPP/AAB-Presidential meeting "Biotic interactions in plant-pathogen associations" Oxford, 19-22 December 1999.

Lebas, B.S.M., Kenyon, L., Seal, S.E., (?) Identification of viruses infecting *Dioscorea* yams from countries of the South Pacific. (submitted to *Australasian Plant Pathology*)

Lebas, B.S.M., Seal, S.E., Kenyon, L., Bousalem, M., Marchand, J-L. (?) Diversity of yam badnaviruses. (submitted to *Archives of Virology*)
Lebas, B.S.M., Seal, S.E., Kenyon, L. (?) Diversity of yam potyviruses. (*Archives of Virology*)
Seal, S.E., Kenyon, L., Iscra-Caruana, M-L., Muller, E. (?) Integration of yam badnavirus sequences in *Dioscorea* spp. (*Molecular Plant Pathology*)

Papua New Guinea:

Gunua T. G. and P. A. Gendua (2001). Anthracnose (*Colletotrichum gloeosporides):* A possible cause for the loss of varieties and the decline of True Yam (*Dioscorea alata)* production in Papua New Guinea. In Bourke, R.M, Allen, M.G and Salisbury, J.G, (eds). Food Security for PNG. Proceedings of Food and Nutrition 2000 Conference, University of Technology, Lae, Papua New Guinea, June 26-30, 2000. ACIAR Proceedings No.99, Pp 775 - 782.
Risimeri, J.B. P.A.Gendua and J.B. Maima (2001) The status of Introduced White Yam in PNG. In Bourke, R.M, Allen, M.G and Salisbury, J.G, (ed). Food Security for PNG. Proceedings of Food and Nutrition 2000 Conference, University of Technology, Lae, Papua New Guinea, June 26-30,2000. ACIAR Proceedings No.99, Pp 783 - 787.
Risimeri, J.B. (2001) Yam and food security in the lowlands. In Bourke, R.M, Allen, M.G and Salisbury, J.G, (ed). Food Security for PNG. Proceedings of Food and Nutrition 2000 Conference, University of Technology, Lae, Papua New Guinea, June 26-30,2000. ACIAR Proceedings No.99, Pp 768 - 774.
Peter A. Gendua and Tony G. Gunua (*In press*) Yam Germplasm Collection in Papua New Guinea. NARI Technical Bulletin Series.

Vanuatu:

Malapa R., Arnau G., Noyer J.L. & V. Lebot. 2002. Genetic relationship between *D. alata* and *D. nummularia* as revealed by AFLP. *Origins, Evolution and Conservation of Crop Plants: A molecular Approach.* Society for Economic Botany. New York Botanical Garden, Bronx N.Y.

7. Conclusions

SPYN has achieved more than what was initially planned by the technical annex of the INCO contract. The project has successfully established yam genebanks in all countries (except the Solomons), complete with passport and characterisation data. From the 1,040 accessions collected, a core sample representative of the best varieties existing within the region has been selected based on tuber shape and tolerance to anthracnose. These are outstanding varieties as demonstrated by their yield performance and palatability qualities.

The project, aimed to assist yam growers, present ones, who require access to selected germplasm to overcome severe disease problems or the vulnerability to disease, and also those of future generations, who will benefit from the conservation of genetic resources for as yet undefined needs. A number of male and female tetraploid accessions have been identified and will represent the base for future breeding work. The benefits will accrue to rural peoples wishing to improve yam production for subsistence purposes in order to maintain a traditional food of high nutritional quality and cultural significance. But this project will also benefit in

the short term to growers producing tubers for domestic and export markets, especially those of Sydney and Auckland where important Pacific Islanders communities consume yams.

In Melanesia, a fast growing population is continuously increasing the pressure on arable land (*i.e.* in Vanuatu, the population is going to double over the next 20 years). There is an increasing need for crop improvement in order to maintain productivity and to protect the environment. There is also a need for crops which can produce food under less-than-favourable conditions as well as crops which can help sustain the environment under increased pressure. The greater yam has much potential and is consistent with maintaining fragile ecosystems. With limited resources available for yam breeding and rising costs, international cooperation is to be encouraged and SPYN has shown that networking is a relevant approach for neglected tropical rootcrops. Genetic resources and their exploitation are the common base for this. Such a network encourage the sharing of information and the development of new ideas on which future progress depends.

SPYN has produced scientific data that will be used in the long term. In fact, one Ph.D. thesis (*B. Lebas*) and one MSc thesis (*R. Malapa*) have been completed thanks to SPYN and another Ph.D. thesis (*R. Malapa*) will be defended in 2004.

Yam is a traditional crop with a long history of cultivation in Melanesia. It was not the aim of the SPYN project to increase substantially the areas planted to the crop: it was primarily to develop methods of studying, rationalizing and using the existing germplasm and ultimately to provide farmers with greater diversity of germplasm to ensure sustainability and stability of the crop against adverse biotic and abiotic conditions, and increase production per unit area.

SPYN has emphasised collaboration among participants. Melanesian countries are encouraged by the results to date and resolved to continue the work initiated by SPYN. No doubt that the constraints resulting from the numerous viruses identified will cause some practical difficulties. The challenge then is now to develop a co-ordinated international network for yam genetic improvement that will encourage and support national breeding programmes and facilitate the sharing of germplasm in a form that can be distributed rapidly for evaluation by growers.

INDIVIDUAL PARTNER FINAL REPORTS

1- CIRAD, France

1.1- Genetic diversity

R. Malapa, G. Arnau, J. L. Noyer and V. Lebot

Dioscorea alata L. or the greater yam, is the most widely cultivated species of yam throughout the tropics. This annual crop is cultivated for its starchy tubers which are harvested from 6 to 9 months after planting. The origin of the species is still controversial but it is generally accepted to be derived from the genetic pool of the Enantiophyllum section in Southeast Asia. De Candolle (1886) firstly placed the origin of *D. alata* within the Indo-Malayan peninsula. Prain and Burkill (1939) suggested a domestication process involving closest wild relatives *D. hamiltonii* Hook. and *D. persimilis* Prain. & Burk. Although there is no further evidence to support this hypothesis, it has been widely accepted by various authors since (Alexander and Coursey 1969, Martin and Rhodes 1974, Degras 1986, Hahn 1991, Mignouna 2002).

There is however, some confusion on the exact geographical area where domestication took place since *D. alata* is exclusively a cultivated species and wild forms are unknown. Surprisingly, its greatest morphological variation is not found within the geographical range of distribution of the two putative progenitors (Alexander and Coursey 1969, Martin and Rhodes 1977). *Dioscorea hamiltonii* is naturally distributed from East India to West Burma, while *D. persimilis* thrives in Indochina (Alexander and Coursey 1969) but it has been demonstrated by various authors that the area of diversification for *D. alata* is Melanesia (Martin and Rhodes 1977, Degras 1986, Lebot *et al.* 1998). A comprehensive comparison of morphological variation in a germplasm collection of 235 accessions from different geographical origins including the Caribbean, West Africa, India, Southeast Asia and Melanesia led Martin and Rhodes (1977) to identify Papua New Guinea as the center of greatest variation before Indonesia. In the former region, the gene pool includes a particularly confusing array of local cultivars with primitive forms and more than a thousand clones maintained in traditional gardens and in the feral state through the reproduction of aerial bulbils.

Several authors have attempted to study the intra specific classification of *D. alata* using morphological description of aerial and underground organs (Bourret 1973, Martin and Rhodes 1977, Velayudhan *et al.* 1989, Lebot *et al.* 1998, Cruz and Ramirez 1999). No clear structure was revealed within the species and instead an "anastomosing patterns like branching of a tropical banian tree" was suggested (Martin and Rhodes 1977). The use of morphological traits for classifying cultivars seems to be rather unreliable within *D. alata* because it is extremely widespread and variable. It is also because no investigator had the opportunities to see more than a small fraction of the existing variation.

Prain and Burkill (1939) and Burkill (1951) suggested an allopolyploid origin for the species but no further investigations were carried out to confirm this hyptothesis. Ploidy levels analyses have demonstrated that tetraploid, hexaploid and octoploid cultivars exit with x=10 as basic chromosome number but no diploids were identified (Abraham and Nair 1991, Hamon *et al.* 1992, Gamiette 1999a). Abraham and Nair (1991) described an unbalanced sex ratio within tetraploids where male plants are dominant (3:1).

Lebot *et al.* (1998) used isozymes for genetic relationships studies among 269 accessions of *D. alata* originating from the South Pacific, Asia, Africa and the Caribbean. They found 66 zymotypes using four polymorphic enzyme systems. However, the rather low polymorphism of these markers revealed no correlation between genetic groups, geographical origins, or phenotypic traits. The tremendous morphological variation existing within *D. alata* is suggested to derive from somatic mutations, sexual recombinations, and polyploidy (Lebot *et al.* 1998, Gamiette 1999b).

Neutral molecular markers have proved to be reliable for fingerprinting accessions across collections in spite of environmental influences. Random Amplified Polymorphism DNAs (RAPD) have been used to assess the genetic relationships in *D. alata* (Asemota *et al.* 1996) and *D. bulbifera* L. (Ramser *et al.* 1996). Amplified Fragment Length Polymorphisms (Vos *et al.* 1995) have also been used successfully to study genetic relationships between Guinea yams and wild relatives (Mignouna *et al.* 1998).

As collections of yams were assembled in Southeast Asia, Melanesia and Polynesia, curators frequently reported misidentifications between *D. alata* and *D. nummularia* cultivars (Sastrapradja 1982, Cruz and Ramirez 1999). In Vanuatu, the *South Pacific Yam Network* (SPYN) has recently established a collection of 379 accessions, including local and introduced accessions of different edible yam species. As the possible confusion between *D. alata* and *D. nummularia* was also observed in Vanuatu (SPYN annual report 2001), it was decided :

1- to assess the potential of AFLPs for accurate taxonomical clarification between *Dioscorea* species and especially, to differentiate similar morphotypes belonging to *D. alata* and closely related Melanesian species *D. nummularia.* Lam.,
2- to evaluate the ploidy levels among *D. alata* cultivars,
3- to assess the performance of AFLP markers to elucidate genetic relationships between *Dioscorea alata* cultivars.

Materials and Methods

Indigenous knowledge:

In Vanuatu, the classification of yams is based on a phenetic approach of similarity or difference using phenotypic and organoleptic criteria. Traditionnal farmers use the combination of chemical composition, growth cycle, specific phenotypic traits (leaf shape and texture, underground organ shape and texture, tuber outer skin appearance, presence or absence of spines), and organ pigmentation (leaf color, tuber inner skin colour) to classify their yams.

The chemical composition of the tuber is the most important criterion as it defines the character of edible/unedible or palatability/unpalatability. All yams are classified into "soft yams" or "strong yams" according to the tuber flesh consistency when cooked. Soft yams include yams with soft, moist and mealy flesh (low dry matter content) whereas strong yams include yams with firm and dry flesh (high dry matter content). Within each group, yams are grouped into "families" of similar morphotypes using specific morphological traits and growth cycle. Such classification distinguished 6 families of yams in the island of Malekula (Table 1). The following order *Nimb*, *Nowerenbow*, *Nimbow*, *Romets*, *Batubapa* and *Nambour* corresponds to families of yams from the lowest to the highest tuber flesh consistency. The families *Nimb*, *Nowerenbow*, *Nimbow*, *Romets* and *Batubapa* present an

annual growth whereas *Nambour* develops a semi-perennial system of its aerial vegetative organs. The five families *Nimb*, *Nowerenbow*, *Nimbow*, *Romets* and *Nambour* correspond to the Linnean species *D. pentaphylla*, *D. bulbifera*, *D. esculenta*, *D. alata* and *D. nummularia* (Table 1). The family *Batubapa* is elusive because it includes an array of morphotypes with intermediate phenotypic traits between *D. alata* and *D. nummularia*. Moreover some of these morphotypes (i.e. *Lapenae*, *Net* and *Timbek*) are clearly identifed by their floral characteristic as cultivars of *D. nummularia*.

Table 1: Traditional classification of local *Dioscorea spp.* of Vanuatu

Traditionnal classification					Linnean classification	
Tuber flesh consistency	**"Family"**	**"Kaen"**	**Status***	**Islands**	**Species**	**Sections**
Strong yam	*Nambour* "wild yam"		P/ W	Malekula	*D. nummularia*	Enantiophyllum
	Batubapa "strong yam"	*Timbek*	C	Malekula		
		Net	C	Malekula		
		Lapenae	C	Malekula		
		Maro	C	Malo	*D. transversa*	
		Langlang	C	Malekula		
		Nassar	C	Malekula	Undefined taxa	
		Rull	C	Malekula		
	Romets "true yam"	*Basa* *Mombri* (> 40 cvs)	C/F	Malekula	*D. alata*	
Soft yam	*Nimbow* "sweet yam"		C/P/F	Malekula	*D. esculenta*	Combilium
	Nowerenbow		C/ W	Malekula	*D. bulbifera*	Opsophyton
	Nimb		P/ W	Malekula	*D. pentaphylla.*	Botryoscysios

*C : cultivated ; (e) : edible ; F : Feral ; (n.e.) : non edible ; n.d. : non determined ; W : Wild ; P : Protocultivated

Within each family, yams are devided into "kaen". A "kaen" designs a botanical label with a specific chemotype combining the consistency (dry matter content) and the organoleptic quality of the tuber flesh (flavor). All "kaen" are assigned to a specific culinary and socio-cultural usage. They correspond to the variety and are identified by a vernacular system of nomenclature. Within each "kaen", the nomenclature uses a binomial system to identify the "kaen kaen" of yams where the first lexicon identifies the variety and the second lexicon specifies its variant based on tuber characteristics (shape, flesh colour or, hairiness). A "kaen kaen" is mainly used to classify the phenovariants of a cultivated "kaen".

In Vanuatu, the traditional usages and alimentary importance of yams assigns a socio-economical value to each variety and species which can be appreciated on their prevalence under cultivated or uncultivated states. A yam variety or a yam species will be used as a staple if it combines an appreciated chemotype with an ease of harvest. Staple yams belong exclusively to the families of *romets* and *strong yams* which also present the greatest phenotypic variability. Non staple yams present either non appreciated organoleptic qualities (*Nimb*, *Nowerenbow*), an underground network of spines (*Nimbow*) or very deep hard digging tubers (*Nambour*). Hence, only staple yams have a socio-economic value and are exclusively cultivated while non staple food yams are spontaneous, feral or protocultivated.

Plant Material:

The germplasm collection is maintained at VARTC (Vanuatu Agronomic Research and Training Center) on Santo. Accessions were collected with passport data throughout Vanuatu with emphasis on *D. alata* cultivars. Accessions are maintained in the field on stakes with four replicates per accession. The replicates are planted together on 1 x 1 m spacing. The stakes are assembled to form pyramids of 1.5 m height for each accession. All accessions were planted on ridges of 50 cm at the beginning of August and were harvested the following year from April to June. Accessions of *D. alata* were described using 28 IPGRI-type international morphological descriptors (IPGRI/IITA 1997). Morphological descriptions were conducted during three years from 1999 to 2002. A core sample of *D. alata* accessions was selected for DNA analysis based on morphological variation and geographical origin. The selection was conducted according to statistical analysis of morphological data using the Dice index and the UPGMA clustering method (Malapa, 2000).

AFLP studies:

Overall, 12 distinct couple of primers were assayed. The aims were: 1- to check the robustness of the AFLP technique for classifying *Dioscorea* species, 2- to choose few couple of primers revealing clear polymorphic banding patterns for fast an easy scoring of numerous accessions of *D. alata*. For each analysis, two *D. alata* accessions were used as internal controls. The purpose was not only to check the repeatability of the data, but also to calibrate each gel on already known band levels, in order to avoid any shift or confusion from one gel to another for a given primer pair. In order to confirm the performance of AFLPs for genetic relationship analysis, four successive studies were carried out using a different set of samples (Table 2).

Table 2: Number of polymorphic bands revealed per primer pair and per study

	Study 1	Study 2	Study 3	Study 4
E-AAC/M-CTA	39	-	-	-
E-AAC/M-CAG	53	40	-	-
E-ACA/M-CAT	52	-	38	-
E-ACC/M-CTA	55	-	-	-
E-ACC/M-CAT	46	54	-	38
E-ACA/M-CAA	40	-	-	-
E-ACG/M-CTA	43	-	-	-
E-AAC/M-CAT	32	31	-	-
E-ACT/M-CTA	40	-	-	-
E-ACT/M-CTC	53	-	-	-
E-ACA/M-CAC	40	-	-	-
E-ACA/M-CAG	-	31	37	30
Total	493	156	75	68
Mean	44.8	39	37.5	34

DNA extraction and AFLP fingerprinting

Total genomic DNA was extracted from 5 g of young green leaves from field-grown plants. A MATAB procedure (Risterucci *et al*. 2000) was used with minor modifications as follow: a leaf sample for each accession was put in plastic tube (15 ml) and kept in transportable container of liquid nitrogen. Each sample was ground in liquid nitrogen with a mortar and

pessel. The fine powder was quickly transferred to 50 ml Falcon tubes and 15 ml of the extraction buffer: 100 mM Tris HCl (pH8); 1.4 M NaCl; 20 mM EDTA (pH8); 2% MATAB; 2% PEG (6000); 0.5% sulfite Na. Extraction was done at 74°C for 1 hour. Proteins were precipitated with 15 ml of a 24:1 solution of chloroform: isoamylalcool and the mixture was centrifuged at 5000 rpm for 30 mn using a "Universal 32" centrifuge (Hettich Zentrifugen).

DNAs were precipitated with cold ethanol (2.5v) and hooked with a sterile Pasteur pipette. They were dissolved in 1 ml buffer (50 mM Tris HCl-pH 8; 0.70 mM NaCl; 10 mM EDTA). Purification of DNAs were done using the QIAGEN-tip Protocol, with mini prep-volumes. Purified DNA was checked on agarose gel (0.8%) and the concentration determined by Hoeschst staining using a DNA fluorometer. Extracts were then diluted in Merck water to obtain DNA concentrations of 50 ng/µl. Commercial Gibco BRL kits (Life Technologies™) with the given pair of restriction enzymes (*Eco*RI and *Mse*I), were used for AFLP procedure. Radioactive labeling was done using $\gamma^{33}P$ and the amplified fragments were separated on a 5% urea-PAGE.

Analysis of data:

Binary data matrix (1 = present; 0 = absent) were coded for each band level and for each individual accession. Very weak bands were excluded from analysis and band intensity was not taken into account. Only unambiguous signal intensities and banding patterns after two independent readings of AFLP profiles were retained. Studies one and two and three were conducted by two different persons in order to assess the robustness of AFLP. Pairwise distances matrices were computed using the Dice coefficient of similarity (also Nei and Li index) for the first and second analysis and the Simple Matching coefficient (also Sokal and Michener) for the third analysis. The resulting matrices were subjected to UPGMA (*Unweigthed Pair Group Method Analysis using Average*) to construct dendrograms with the software package of NTSYS-PC (Rohlf, 1995). As the Winboot program is not available on this sofware package, the distance matrices were also subjected to the software DARwin 4.0 in order to calculate the bootstrap values which were then reported for the main branches of the dendrograms.

Ploidy level analysis:

Fifty-three accessions of *D. alata* were planted in pots in a greenhouse at Montpellier, France. Chromosomes were counted on slide preparations of root protoplasts from adapted protocol described by D'Hont *et al.* (1996). Young root tips (5 mm in length, 0.5 mm thick) were removed from pot plants with fine forceps and immersed directly into a 0.04% solution of 8-hydroxyquinoline. They were kept 2 hours at room temperature and 2 hours at 4°C. They were fixed in a freshly prepared ethanol: acetic acid solution (3:1) for 48 hours at room temperature.

Protoplasts were prepared as follow: root tips were rinced for 10 mn in distilled water and immersed into a hydrochloric acid solution (0.25N) for 10 mn. They were then rinced for 10 mn in distilled water and immersed into a citrate digestion buffer (1.47g trisodium citrate-dihydrate; 1.05 g citric acid-monohydrate; 500 ml distilled water) for 10 mn at room temperature. Root tips were cut off and reduced to 1 mm length then immersed into a enzyme solution of 5% cellulase (Onozuka R-10, Yakult Honsha Co. Ltd., Japan) and 1% pectolyase (Y-23, Seishin Pharmaceutical Ltd., Japan) for 1 hour hydrolysis at 37°C into Eppendorf microtubes. Tips were removed with a Pasteur pipette and rinced in distilled water for 1 mn.

Chromosomes were spread on a slide with a drop of 3:1 solution and stained with 12 µl of DAPI (4,6-diamidino-2-phenylindole). Slides were observed under UV fluorescent light (340-380 nm) using a Leica DMRXA microscope equiped with a wavelength filter passing under 430 nm.

For flow cytometry, the protocol used is adapted from Arugumunathan and Earle (1991). About 0.5 cm^2 of an adult green leaf was used for nuclei isolation. Each sample was chopped with a new razor blade in a plastic Petri dish in 2 ml of ice-cold extraction PBS buffer. The suspension was filtered through a 30 µm mesh nylon filter into a microfuge tube to which ice-cold propidium iodide was added to a final concentration of 50 ppm. The sample was incubated for 5 mn at room temperature. The fluorescence of the nuclei was measured using a Faxscan flow cytometer equiped with an argon ion laser tuned to 488 nm. Two different cultivars of *D. alata* with ploidy level (4x, 8x) determined by chromosome counting were used successively as internal standards and gave consistent results of ploidy level using the formula given in Gamiette (1999a). Overall, ploidy levels were determined for 53 accessions.

Results and discussion

Morphological variation

Frequencies of 28 phenotypic traits were scored on 331 accessions of *D. alata* (see table in appendix). Tremendous variation is observed for pigmentation which varied from white to brown depending on variety, organ, tissus and/or growth stages. Hence, according to traditional knowledge, different cooking preparations are necessary for different cultivars. The shape and size of organs, yields and maturity period were also highly variable depending on the variety, the quality of the planting material and the planting date. Yields/plant and number of tubers/plant varied from 0.5 kg to 11 kg and 1 to 10 respectively. Very few accessions produced bulbils (7.4%) or flowered (6%). In general, most of the accessions produced cylindrical (46.6%) or irregular (38.2%) tuber shape, all with white flesh (73%). Moreover, flowering plants are mainly of sex male indicating a sex-biaised ratio. The 28 phenotypic traits were coded for each modality and the resulting matrix was subjected to various multivariate analyses. They revealed that a vast continuum of morphological variation exists within *D. alata* cultivars in Vanuatu (Malapa, 2000).

Such variation was also observed within the section Enantiophyllum of Vanuatu at both morphological and chemical levels. In this country, the section Enantiophyllum is traditionnally divided into three "families" including *Romets*, *Batubapa* and *Nambour* according to the consistency and organoleptic quality of tuber flesh when cooked. The family *Batubapa* includes yams which are highly appreciated for their firm, dry and white tuber flesh. The most representative are the traditionnal cultivars *Langlang*, *Maro*, *Nassar*, *Net* and *Timbek* which are commonly known as "*strong yam*" (cf. Table 1). Among them, the former three cultivars are difficult to classify in *D. alata* or *D. nummularia*. Leaves are alternate basally on stems and opposite distally on stems. They are similar in shape to those of *D. alata* (ellongate to cordate) but with thick and shiny laminas similar to those of *D. nummularia*. The stems tend to be lignified and developped no wings like those of *D. nummularia* or very discret wings (*Nassar*) like those of *D. alata*. Spines are few at stem base and present at both side of junction between stem and petiole like *D. nummularia*. These cultivars are late maturing compare to *D. alata* (9-12 months) and are highly appreciated for their high dry matter content and organoleptic quality which are similar to those of the *D. nummularia*

cultivars *Net* and *Timbek*. However, *D. nummularia* has been reported as being a very polymorphic species in the Pacific (Cable and Wilson 1984).

AFLP studies

All AFLP primer pairs resulted in distinct, polymorphic banding pattern for all accessions and raised species-specific profiles. Banding patterns were highly complex including intensity and size of AFLP fragment. The sizes of the AFLP fragments were determined by comparing control DNA patterns to AFLP sample patterns and gave fragment sizes ranging from approximately 50 to 350 bp. Overall, the higher polymorphism and the complexity of AFLP banding patterns revealed whithin *Dioscorea* species offered less ambiguous bands and a higher scorability in the intraspecific level. The number of polymorphic bands scored for the four successive studies gave averages of 44.8, 39, 37.5 and 34 respectively (Table 2).

Study 1: Eleven combinations of primer pairs were used to assess the genetic relationships among 17 accessions belonging to 8 species of *Dioscorea* (cf. Table 3).

Table 3: Number of accessions analysed per species and per study

Species	Sections*	Origin*	Study 1	Study 2	Study 3	Study 4
D. abyssinica	Enantiophyllum	West Africa	-	1	-	-
D. alata	Enantiophyllum	SE Asia (?)	4	26	83	20
D. bulbifera	Opsophyton	Gondwana	1	-	-	1
*D. cayenensis***	Enantiophyllum	West Africa	2	2	-	-
D. esculenta	Combilium	Asia (?)	3	-	-	1
D. nummularia	Enantiophyllum	Oceania	-	4	-	7
D. pentaphylla	Botryosicyos	SE Asia (?)	2	-	-	1
D. persimilis	Enantiophyllum	Indochina	1	1	-	-
D. transversa	Enantiophyllum	Australia	2	7	-	8
D. trifida	Macrogynodium	S. America	2	-	-	-
Total			17	41	83	38

Overall, AFLP raised 493 polymorphic and scorable bands with an average of 44.8 bands. Comparison of pairwise distances averages between *D. alata* and the other species showed the decreasing genetic similarities of 53.7%, 25.7%, 14.7%, 8.8%, 8.4%, 5.7% and 5.5% with *D. nummularia* (N), *D. persimilis* (P), *D. cayenensis-rotundata* (C), *D. pentaphylla* (Pn), *D. bulbifera* (B), *D. esculenta* (E) and *D. trifida* (T) respectively (Table 4).

This result is consistent with the biosystematic of the genus *Dioscorea* in respect to the botanical sections and the geographic origins of these species. However comparison of genetic similarity between *D. alata*, *D. nummularia* and *D. persimilis* did not support previous hypothesis involving *D. persimilis* in the origin of *D. alata* (Prain and Burkill 1939, Mignouna *et al.* 2002). Moreover, *D. persimilis* is genetically more closer to the Oceanian endemic species *D. nummularia* (31.4%) than to *D.alata* (25.7%). This discrepancy is also confirmed by the cluster analysis of the distance matrix and conforted by strong boostrap values ranging from 87% to 100% (Fig. 1).

Table 4: Average of genetic distances ans similarities (%) among 8 *Dioscorea* spp. (Study 1)

	A	N	P	C	Pn	B	E	T
A	87.7							
N	53.7	94.1						
P	25.7	31.4	-					
C	14.7	14.7	13.0	78.3				
Pn	08.8	10.9	07.0	02.6	84.7			
B	08.4	06.8	10.1	05.8	02.2	-		
E	05.7	05.2	05.0	03.9	06.2	03.0	94.2	
T	05.5	05.1	04.9	00.3	01.1	03.3	01.3	74.5

A: D. alata ; B: D. bulbifera ; C: D. cayenensis-rotundata ; E: D. esculenta ; N: D. nummularia Pn: D. pentaphylla ; P: D. persimilis ; T: D. trifida

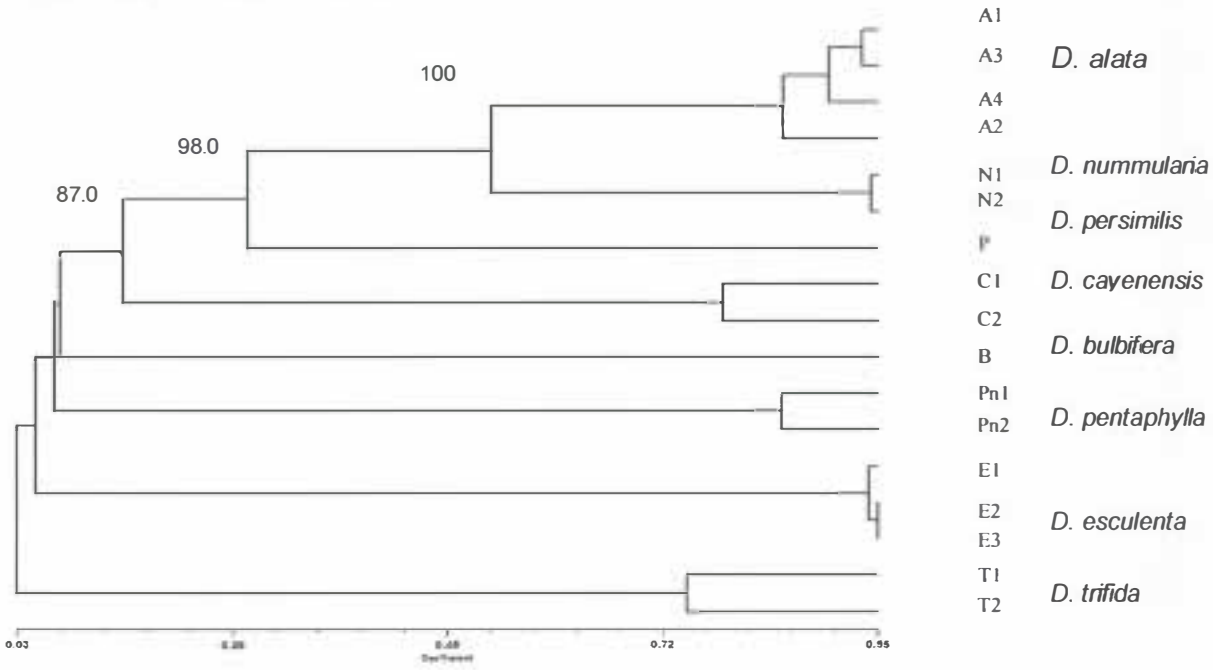

Fig. 1 Strict consensus tree from an AFLP data matrix of 8 Dioscorea species using 11 primer pairs combinations (UPGMA and Dice coefficient). Boostrap values are indicated upon lines. Numbered letters correspond to species identification as used in Table 5.

Study 2: Using 4 primer pairs combinations to screen 41 accessions belonging to the species *D. abyssinica, D. alata, D. cayenensis-rotundata, D. nummularia* and *D. persimilis*, AFLP generated 156 polymorphic bands with an average of 39. Cluster analysis of the distance matrix results in a tree topology which is divided into 5 major clusters and supported by high bootsrap values (Fig. 2).

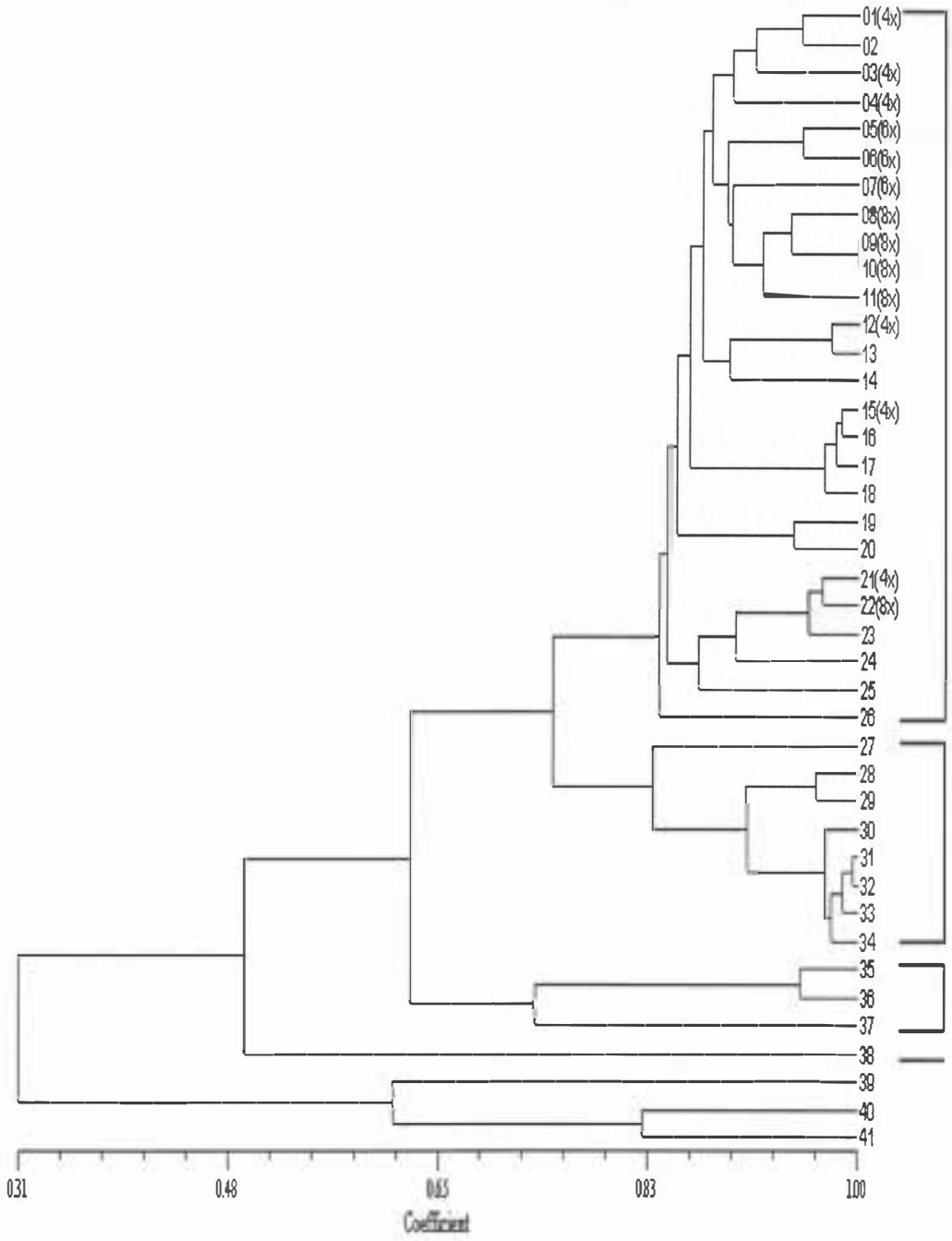

Fig. 2: UPGMA analysis using four combinations of primer pairs and 41 accessions belonging to five Enantiophyllum species

Cluster 1 2, 3 and 4 include *D. alata* (nos 1-26), *D. nummularia* (nos 27-34), *D. nummularia* (nos 35-37) and *D. persimilis* (no 38) respectively. In cluster 5 were included the African species *D. abyssinica* (no 39) and *D. cayenensis-rotundata* (nos 40, 41). The separation of clusters 1-4 from cluster 5 is supported by a high bootstrap value of 99.7% and indicates a divergent gene pool for African Enantiophyllum section species.

The general topology of the tree revealed an interspecific relationship which is consistent with the previous results of study one. *Dioscorea alata* is very distant to *D. persimilis* as compared

to *D. nummularia* and this result is supported by a bootstrap value of 98.3%. However, another high boostrap value of 99.7% separated the species *D. nummularia* into cluster 2 and cluster 3. This result suggests that the group of *D. nummularia* accessions studied here belong to two taxa. In fact cluster 2 groups the traditionnal cultivars *Nassar* (no 27), *Lanlang* (nos 28, 29) and *Maro* (nos 30-34) whereas cluster 3 includes three accessions of *Nambour* (nos 35-37). The first three cultivars shared more than 83% of genetic similarity and are genetically intermediate between *D. alata* and *D. nummularia* although more closer to the former species as supported by a lower bootstrap value (72.7%). Moreover, AFLP markers allow to fingerprint the cultivars *Lanlang*, *Maro* and *Nassar* into three distinct genotypes with *Langlang* and *Maro* being more closely related to each other. These two cultivars are similar morphologically and could be distinguished from the cultivar *Nassar* by the absence of discret wings along stems.

AFLP profiles revealed common banding patterns between local species of the Enantiophyllum section of Vanuatu. Moreover, the intermediate phenotypic position between *D. alata* and *D. nummularia* of the cultivars *Langlang*, *Maro* and *Nassar* (cluster 2) is also confirmed at the genomic level with AFLP. However, the dominant nature of these markers could not really define the nature and phylogeny of the relationships existing between these taxa. Numbers of specific band levels per primer pairs and per clusters gave averages of 5.25, 8.2 and 10 bands for cluster 1, cluster 2 and cluster 3, respectively (Table 6). Taken together, these findings suggest that the high number of specific band levels revealed within the cultivars *Langlang*, *Maro* and *Nassar* could identified them as being a distinct species from *D. alata* and *D. nummularia* but sharing a common ancestor with them as revealed by their common genetic background.

Table 6: specific aflp bands for *D. alata*, "Maro" types and *D. nummularia* (study two)

Primer pairs	Total bands	Polymorphic bands	Specific bands		
			Cluster 1*	Cluster 2**	Cluster 3***
E+AAC/M+CAG	47	40	4	10	15
E+AAC/M+CAT	43	31	8	9	6
E+ACA/M+CAG	43	31	6	7	4
E+ACC/M+CAT	58	54	3	7	15
Mean	47.75	39	5.25	8.25	10
SD	7.09	10.86	2.22	1.50	5.83

**D. alata*, **cultivars *Langlang*, *Maro* and *Nassar*, ***cultivar *Nambour*

Study 3: Using 2 primer pairs combination to screen 83 accessions of *D. alata* originating from Melanesia, Southeast Asia (Vietnam) and West Africa (The Republic of Guinea), AFLP generated 75 polymorphic and scorable bands with an average of 37.5 bands per primer pairs.

Pairwise genetic distances between individuals using the Simple Matching coefficient raised genetic similarities varying from 49.3% to 100%. The analysis of the distance matrix with the UPGMA clustering method showed that only few accessions are duplicates (100% similarity) whereas most of them could be uniquely fingerprinted (Fig. 3). However, the overall structure of the tree suggests a continuum of genomic variation which could hardly reflect a strict and clear genetic structure of *D. alata* into homogenous group of similar genotypes. A close look at the Vanuatu data base did not show any obvious agreements between clustering patterns, geographical origin, specific phenotypic traits or ploidy levels.

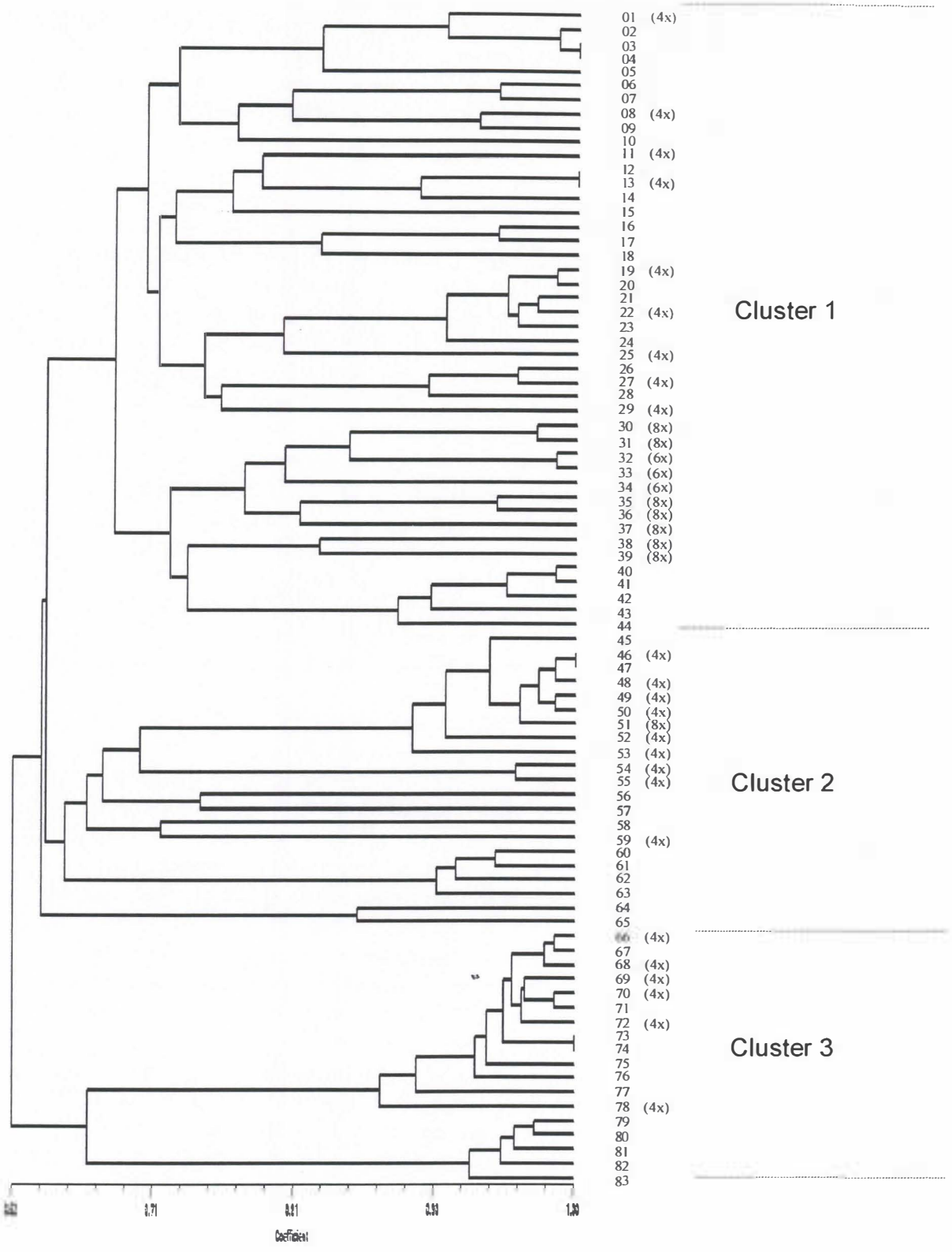

Fig. 3: Strict consensus tree derived from an AFLP data matrix of 83 accessions of *D. alata* using two primer pairs combination (UPGMA and Simple Matching coefficient). Ploidy levels are indicated in brackets.

However field observations revealed that in general, all cultivars with broad leaves or with very developed and curled wings appeared to be in clusters 1 and 2. Moreover and notwistanding pigmentation features, groups of genotypes with a genetic similarity of over 85% expressed generally a similar morphotype though some exceptions may be observed. We thereby divided the dendrogram into three main clusters in order to identify subclusters with homogenous morphotypes/genotypes.

Cluster 1 (nos 01-44) includes 53% of the accessions analysed. This cluster is highly heterogenous with 44 accessions sharing from 68.5% to 100% genetic similarity. Field observations on general plant appearance revealed a continuum of leaf shape and size from ovoid and narrow to round and large in a downward direction. Accessions 19-24 formed a homogenous group of morphotype with 90.8% of similarity. They are highly appreciated for the firm consistence, white colour and good organoleptic quality of their tuber flesh. Tubers are round shaped, shallow and thus easy to harvest. Accessions 30-44 are morphologically heterogenous but having hearth shaped leaves. Male (nos 31, 32, 33) and female (35, 36, 39) are found within this subgroup. All produced regular cylindrical shaped tubers in tilled soil, all with white or purple flesh. Accessions also formed a homogenous group of morphotype with hearth shaped leaves. All other subclusters included one to four accessions with homogenous morphotype except for accession 03. Surprisingly, this individual is 100% genomically similar to accession 04 though phenotypically very different but similar to accessions 79-83 of cluster 3. This discordance could be explained by the misidentification of accession number during the sampling of leaves for DNA extraction. This hypothesis is supported by the fact that all other 3 duplicates which are identified here (i.e. 12/13 ; 46/47 ; 73/74) were phenotypically conformed to other accessions whitin their respective subclusters.

Cluster 2 includes 21 accessions (nos 45-65) with a genetic similarity of 63.6%. Though accessions 64 and 65 may be regarded as an outstanding group, they are closer to cluster 2 than to cluster 1 and cluster 3. Hence, we include them in the former cluster. These two accessions subclustered in respect to their homogenous morphotype sharing almost 85% of genetic similarity. Accessions 45, 56, 57, 58 and accession 59 originated from Vietnam and Africa, respectively. They clustered with accessions from Vanuatu which produced irregular tuber shape with white or anthocyaned tuber flesh. Wings are obviously developed and curly. Accessions 45, 46, 47, 48, 49, 50, 52 and 53 belong to the same morphotype and shared more than 89.6% genetic similarity. Their tuber flesh is characterized by an elastic consistency when cooked. Accession 51 is phenotypically different with big heart shaped leaves. Stems are quadrangular in shape and with discret wings. Spines are numerous and very developed at stem base.

Cluster 3 includes 18 accessions sharing 67.2% genetic similarities grouped into two distinct subclusters 3a and 3b. All accessions are late maturing plants. Subcluster 3a groups 13 accessions (nos 66-78) with 87.5% genetic similarity. They produced irregular, ovoid or cylindrical shapes of tubers, all having white to purple flesh. All cultivars of this subcluster are male flowering plants. Subcluster 3b includes 5 accessions (nos 79-83) with of narrower genetic basis (93,2%). They are non flowering, produce the most diverse tuber shape (round, ovoid, cylindrical, flattened, triangular and irregular) and are resistant to anthracnose. Flesh colour is also variable from white to purple. Leaves are ovoid and narrow.

Study 4: Using two primers pairs combinations to screen 38 accessions, AFLP generated 68 markers. 20 *D. alata* cultivars were studied: 1 from Africa, 3 from Vietnam, 5 from New

Caledonia (including acc. No A100= Florido) and 11 from Vanuatu, were compared to *D. nummularia, D. transversa, D. bulbifera, D. esculenta* and *D. pentaphylla.*

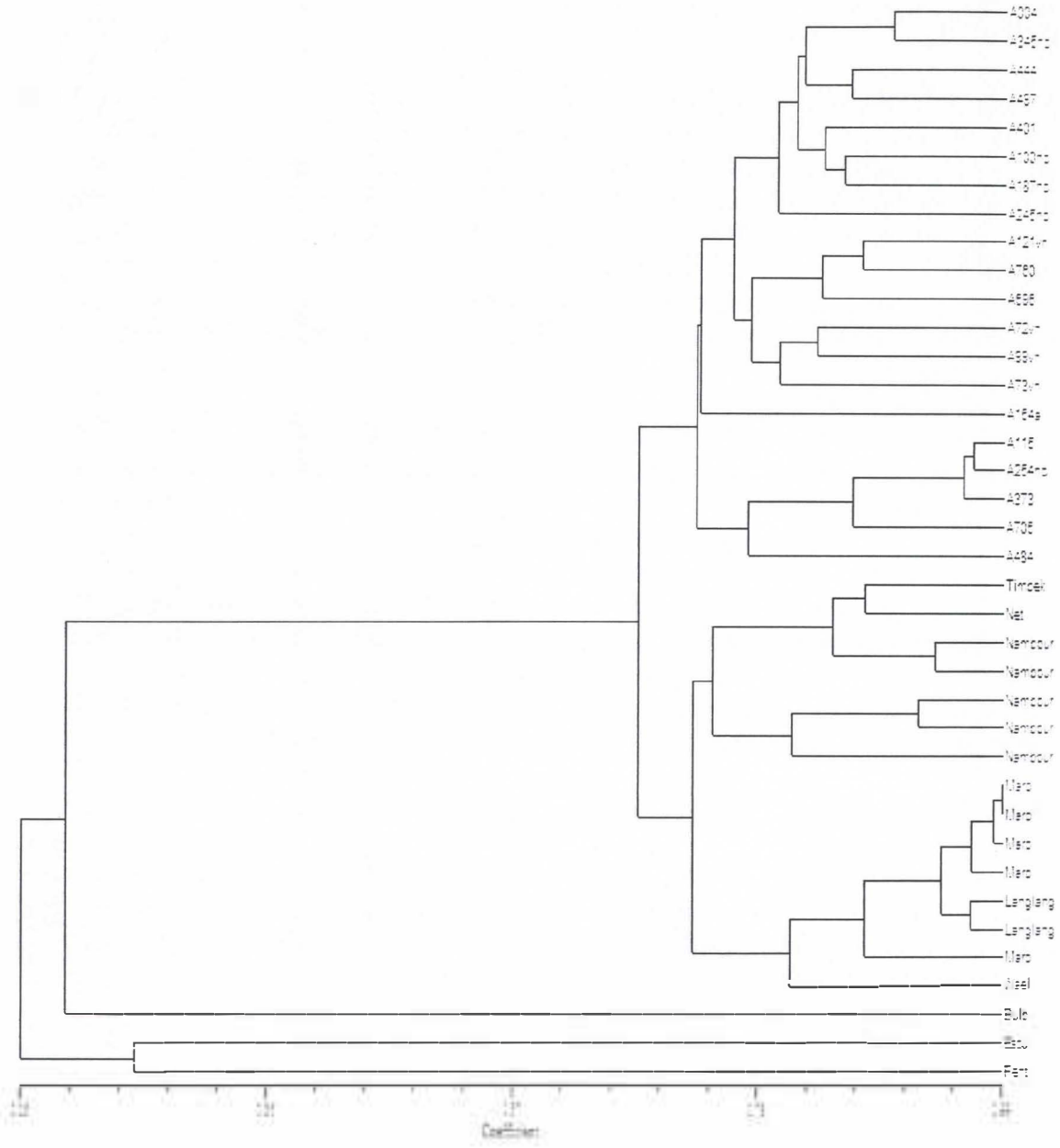

Fig. 4: UPGMA and Dice coefficient of 38 accessions including *D. alata, D. nummularia, D. bulbifera, D. esculenta* and *D. pentaphylla*

Overall, only two primer pairs combinations allowed to separate the species *D. alata* (the A numbers), *D. nummularia* (cultivars *Nambour*, *Net* and *Timbek*) and *D. transversa* (cultivar *Wael* from New Caledonia) from each others (Fig. 4). AFLPs revealed that cultivars *Maro* and *Lanlang* clustered together with cultivar *Wael* which was been described in New Caledonia as being *D. transversa* (Bourret, 1973). According to Lebot (1997), it has all the morphological characteristics of the "*Maro*" taxon of Vanuatu. However, since no one has ever reported the existence of *D. transversa* in Vanuatu, it is somewhat difficult to rely only

on morphological description for accurate taxonomical identification. AFLPs confirmed that the cultivars *Langlang*, *Maro* and *Wael* belong to the same botanical species *D. transversa*. These results also confirmed the observed morphological similarity between the wild accession *Nambour*, and the cultivars *Net* and *Timbek* as forming the *D. nummularia* taxon. Hence, AFLPs revealed that the traditionnal family of *strong yam* is highly heterogenous and include the 2 distinct species *D. nummularia* and *D. transversa*.

Comparision of *D. alata* genetic diversity confirmed the absence of clustering pattern with the geographic origins as accessions from Africa (A164a), New Caledonia (nc numbers), Vanuatu, and Vietnam (vn numbers) clustered together (cf. Fig. 4). Interestingly, accession A100nc (variety *Florido*) has been selected by the USDA department at Mayaguez (Puerto Rico) and introduced by CIRAD in New Caledonia. This accession clustered with the cultivar *Basa* (A401) from Vanuatu which was also selected for the rounded shape and smooth outer-skin of its tuber. The two accessions have similar morphotypes with dark green triangular leaves and are tetraploids. A similar tree is also obtained with the Simple Matching coefficient (dendrogram not shown).

In conclusion AFLPs proved to be highly powerfull in identifying *D. alata* duplicates for germplasm management purposes. However the rationalization needs both morphological and genotypic characterization due to the phenotypic plasticity of the species. Analysis of the genetic diversity revealed also a continuum of genomic variation which could be hardly divided into three main groups of genotypes. Comparison of pairwise genetic distances revealed that the two most distant accessions shared 49.3% genetic similarity (Study 3). They belong to cluster 2 (no 45) and cluster 3 (no 83) in Figure 3 and originated from Vietnam and Vanuatu, respectively. As accessions from Vietnam and West Africa clustered together with accessions originating from Vanuatu, one can suggest that the species *D. alata* have been distributed widely as clonal material.

Ploidy levels

Overall, 53 accessions of *D. alata* cultivars were analysed and their ploidy levels confirmed with both root tips counts and flow cytometry. Twenty nine tetraploids, five hexaploids and nineteen octoploids were identified. No diploids were found. Tetraploids and octoploids were widely distributed geographically throughout the Vanuatu archipelago. The five hexaploids originated all from the South.

Comparison between morphotypes and cytotopes revealed that tetraploids, hexaploids and octoploids tend to assemble very distant morphotypes but with narrow leaves for tetraploids and thick, dark green waxy and cordate lamina for hexaploids and octoploids. Flowering plants include tetraploids (nos 18, 68, 69, 70, 73, 75, 77), hexaploids (nos 32, 33) and octoploid (no 31, 35, 36), among which two plants are female (nos 35, 36), (Fig. 3).

Comparison between genotypes and cytotypes did not revealed any obvious grouping pattern according to ploidy levels. However, 3 hexaploids (nos 32, 33, 34) and 7 octoploids (nos 30, 31, 35, 36, 37, 38, 39) subclustered together with 72% genetic similarity and 1 octoploid (no 51) which clustered with tetraploids (cf. Fig. 3). Screening of AFLP profiles did not reveal any grouping pattern according to specific bands or the total number of bands within each ploidy levels. Taken together, these results indicate that hexaploids and octoploids cultivars resulted from autopolyploidization through endomitosis or production of unreduced gametes.

Conclusion

Melanesia is the area of morphological diversification of the species *D. alata*. Numerous morphotypes exist, are recognized in local classification and have different uses. They exhibit tremendous morphological variation expressed by different colours and shapes for both aerial and underground organs. This was already observed by Martin and Rhodes (1977) and confirmed by Lebot *et al.* (1998). Our study revealed that a vast continuum of morphological variation exists whitin *D. alata*. This is also the case between *D. alata, D. nummularia* and *D. transversa*. Within and between these species, noble cultivars are traditionnaly distinguished according to the chemical composition of their tubers and their plant growth cycle.

Genetic diversity study using AFLPs clearly indicate that the *Dioscorea* genus can be uniquely fingerprinted from species to variety levels owing to specific banding patterns and to the high polymorphism generated. This high level of polymorphism reflects the outcrossing mating system of *Dioscorea* species. Furthermore, using only two primer pairs, AFLPs are powerful tools for fingerprinting individual accessions of *D. alata*. In general, accessions with 85% genetic similarity (using SM) can be considerate as potential duplicates. However field observation is a pre-requisite to the rationalization of *D. alata* germplasm due to the phenotypic plasticity of the species.

Ploidy levels analysis indicated that hexaploids are rare and tetraploids and octoploids are dominant. Where 4 and 2 AFLP primer pairs combinations were used on respectively 26 and 83 accessions of *D. alata*, tetraploid, hexaploid and octoploid accessions clustered together with very narrow genetic distances between them. These results indicate that hexaploids and octoploids are autopolyploids. Furthermore, these accessions have similar morphotypes although they have different ploidy levels and *vice-versa* have different morphotypes within the same ploidy level. Hence, *D. alata* is highly plastic at genomic and phenotypic levels.

Interspecies genetic relationships analysis within the Enantiophyllum section revealed that this section is consistant at the molecular level. These results agreed with the botanical classification which included species with an anticlockwise twining vine direction in the Enantiophyllum section. AFLPs also revealed that species from Africa (*D. abyssinica*, *D. cayenensis-rotundata*) are more distant from the Asian-Oceanian species (*D. alata*, *D. nummularia, D. persimilis* and *D. transversa*). Hence they are on line with the hypothesis put forward by Burkill (1960) that African and Asian Enantiophyllum section formed divergent genepools since the dessication of southwestern Asia about 10 million years ago.

A major finding of this study is the genetic relatedness between *D. alata, D. nummularia* and *D. transversa*. AFLPs molecular markers revealed a common genetic background between these three species but also showed that *D. persimilis* is genetically distant from them (Figure 2). Consequently, these results contradict the previous hypothesis involving the Asian species *D. persimilis* in the origin of *D. alata* (Prain and Burkill, 1939). Furthermore, they confirm the existence of intermediate taxa between *D. alata* and *D. nummularia* and among which the most representative cultivars is locally called *Maro* in Vanuatu. These intermediate forms appear to belong to the species *D. transversa*. In the present study, we attempted to demonstrate several points:

1 - The area of origin of *D. alata* occured somewhere within the geographic range of distribution of *D. nummularia* and *D. transversa* which are restricted to Indo-Melanesia and

Australia. According to Barrau (1956) and Degras (1986), the high polymorphism of *D. nummularia* is observed between Indonesia and Melanesia.
2- The existence of an intermediate taxon, *D. transversa*, between *D. alata* and *D. nummularia* indicates that these three species have most likely a common ancestor existing in this region.
3 - As *D. alata* and *D. nummularia* are genetically very distant to *D. persimilis*, one can suggest that species related to *D. alata* have evolved independantly from Asian species and formed a divergent genepool. The geographic isolation separating these two gene pools probably occurred 10 000 years ago since the last glaciation period which isolated the southeast Asian peninsula from the Sunda plate and New Guinea from Australia.
4- The combinations of various cytotypes and genotypes indicate that hexaploid and octoploid cultivars of *D. alata* are autopolyploids.
5- The non distinction between Asian, African and Melanesia cultivars of *D. alata* confirms that clones have been widely distributed but diversification process is still going on involving fixed somatic mutations, polyploidization and sexual recombination. Hence, the tremendous variation observed at the morphological level could also reflect a genomic plasticity which is magnified by the outcrossing mating system imposed by dioecism.

Other powerfull tools such as microsatellites and genomic *in situ* hybridization may bring more insight on the genetic relationships at the inter- and intra-specific levels.

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Appendix

Frequencies (in%) of accessions exhibiting morphological traits

Descriptors	Categories	Frequencies
Young stem color	Green	09.1
	Purplish green	44.6
	Brownish green	01.4
	Purple	16.2
	Yellowish green	27.7
	Others	01.0
Young wing color	Green	05.6
	Green with purplish edge	61.4
	Purple	21.4
	Other	11.6
Young leaf color	Yellowish	13.0
	Pale green	05.6
	Purplish green	42.1
	Purple	35.1
	Others	04.2
Young leaf vein color	Yellowish	11.9
	Pale green	09.8
	Dark green	54.0
	Purplish green	22.8
	Others	01.5
Young leaf petiole color	Green with purple base	07.4
	Green with purple leaf junction	00.4
	Green with purple at both ends	06.7
	Purplish green with purple base	08.4
	Purplish green with purple at both ends	34.0
	Green	31.5
	Purple	09.5
	Brownish green	00.3
	Others	01.8
Mature stem color	Green	11.6
	Purplish green	09.8
	Brownish green	77.9
	Purple	00.7
Mature stem wing color	Green	17.9
	Green with purple edge	75.4
	Purple	06.0
	Others	00.7
Mature leaf color	Pale green	51.0
	Dark green	48.4
	Purple	00.3
	Others	00.3
Mature leaf vein color	Yellowish	01.4
	Green	84.6
	Dark green	09.1

	Purplish green	04.9
Mature leaf petiole color	Green with purple base	12.3
	Green with purple leaf junction	00.4
	Green with purple at both end	36.1
	Purplish green with purple base	00.3
	Purplish green with purple at both ends	03.5
	Green	47.4
Skin color at tuber head	White	09.8
	Yellow	33.3
	Orange	01.0
	Pink	22.8
	Purple	33.1
Flesh color at central section	White	73.0
	Yellowish white	01.0
	Yellow	00.4
	Light purple	01.0
	Purple	03.9
	Purple with white	07.7
	White with purple	12.6
	Outer purple, inner yellowish	00.4
Flesh color of lower part	White	71.2
	Yellowish white	03.9
	Yellow	00.7
	Light purple	02.8
	Purple	06.3
	Purple with white	03.5
	White with purple	11.2
	Outer purple, inner yellowish	00.4
Mature leaf shape	Elongate	39.2
	Ovoïd	05.3
	Cordiform	53.0
	Curled under	00.7
	Cupped	01.8
Distance between lobes	Intermediate	63.9
	Very distant	36.1
Petiole length	6-9 cm	55.4
	3 >10 cm	44.6
Number of stems	Single	04.3
	Few	40.3
	Many	55.4
Mature spines on stem base	None	82.1
	Few	15.1
	Many	02.8
Mature stem wing size	≤1 mm	78.2
	>1 mm	21.8
Internode length mature stem	<9 cm	07.0
	>9-18 cm	87.4
	>18 cm	05.6

Leaf size	Narrow (<10cm)	10.1
	Medium (10-15cm)	08.0
	Large (>15cm)	81.9
Tuber shape	Round	03.2
	Oval	08.1
	Cylindrical	46.6
	Flattened	01.4
	Triangular	02.5
	Irregular	38.2
Number of tuber per plant	0-2.5	74.3
	2.5-5	22.1
	5-7.5	02.5
	≥ 7.5	01.1
Flowering	Absent	94.0
	Present	06.0
Sex	Female	11.8
	Male	88.2
Aerial tubers	Absent	92.6
	Present	07.4
Maturity of tuber	< 6 months	06.3
	> 6-9 month	93.7
Yield per plant (Kg)	≤ 0.5	18.9
	0.5-2.5	57.2
	2.5-4.5	17.2
	4.5-6.5	03.2
	≥ 6.5 kg	03.5

1.2. Development of microsatellites markers (cpSSR) to study *Dioscorea spp.* phylogenetics

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Introduction:

AFLP have revealed that the species *D. nummularia* and *D. alata* are closely related and that they both exhibit significant molecular diversity. It appears interesting to confirm this relationship with co-dominant markers. Chloroplastic Simple Sequence Repeats (cpSSR) were developed to study genetic diversity within and between *Dioscorea spp.*

Materials and methods:

96 accessions from the Pacific and West Africa were studied (see table 1). The characteristics of the primers used are presented in table 2. The most polymorphic primers (NTcp8, NTcp9, NTcp19, RCt10) were applied to all accessions. A dendrogram was constructed using SM coefficient with the DARWIN 4.0 and NJTree softwares. Sequences alignment was realised using BIODET and ClustalW softwares.

Results:

Study of the allelic diversity using acrylamid gel

Primers were tested in radioactive conditions to allow migration on acrylamid gel so that accuracy is improved to one base. This level of accuracy is not possible with agarose gel. The technique allows to distinguish different alleles with each 4 microsatellites markers. Sixteen alleles have been identified, 8 with NTcp19, 3 alleles were identified with NTCP8 and 5 with NTCP9 (see figure 1).

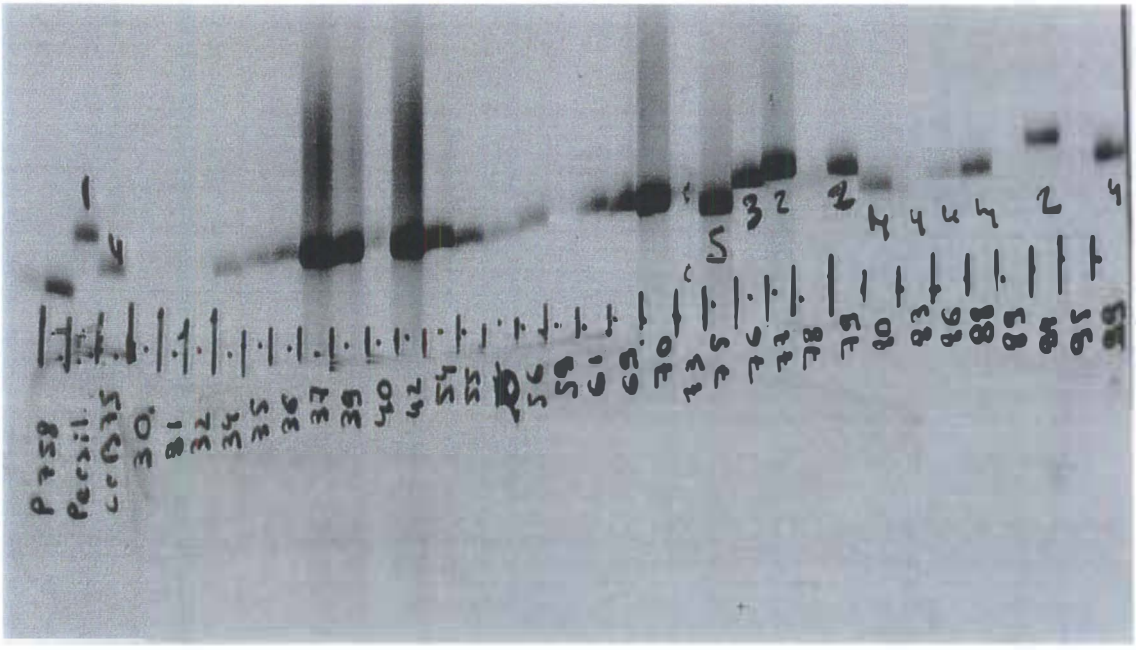

Figure 1 : Amplification profile of locus NT cp9 on acrymamide gel.

Table 1: List of accessions:

No	Species	Varietal group	Section	Country	Collection
A605	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A643	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A676	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A678	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A693	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A695	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A700	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A727	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A731	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A734	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A740	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A753	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A760	*D. alata*		Enantiophyllum	Vanuatu	CARFV
A121vn	*D. alata*		Enantiophyllum	Vietnam	Vietnam
Abys	*D. abyssinica*		Enantiophyllum	Guinée	
B619	*D. bulbifera*		Opsophyton	Vanuatu	CARFV
B620	*D. bulbifera*		Opsophyton	Vanuatu	CARFV
B675	*D. bulbifera*		Opsophyton	Vanuatu	CARFV
E627	*D. esculenta*		Combilium	Vanuatu	CARFV
E743	*D. esculenta*		Combilium	Vanuatu	CARFV
Nc100	*D. alata*		Enantiophyllum	Brésil	Port Laguerre
Nc310 (1)	*D. alata*		Enantiophyllum	Nouvelle Caledonie	Port Laguerre
Nc345	*D. alata*		Enantiophyllum	Nouvelle Caledonie	Port Laguerre
Nd027	*D. nummularia*	*Dimbek*	Enantiophyllum	Vanuatu	Beverly Hill
Nd028	*D. nummularia*	*Dimbek*	Enantiophyllum	Vanuatu	Beverly Hill
Nm10	*D. nummularia*	*Marou (N662)*	Enantiophyllum	Vanuatu	CARFV
Nr26	*D. nummularia*	*Rul/nambour*	Enantiophyllum	Vanuatu	Stade
Ns20	*D. nummularia*	*Nassar (N702)*	Enantiophyllum	Vanuatu	CARFV
Nt29	*D. nummularia*	*Net*	Enantiophyllum	Vanuatu	Beverly Hill
Nr21	*D. nummularia*	*Rul*	Enantiophyllum	Vanuatu	Beverly Hill
Nw34	*D. nummularia*	*Nambour*	Enantiophyllum	Vanuatu	Charles Rodgers
Nw35	*D. nummularia*	*Nambour*	Enantiophyllum	Vanuatu	Charles Rodgers
Nw39	*D. nummularia*	*Nambour*	Enantiophyllum	Vanuatu	Charles Rodgers
Pers	*D. persimilis*		Enantiophyllum	Vietnam	Vietnam
P758	*D. pentaphylla*		Lasiophyton	Vanuatu	CARFV
Prahen	*D. prahensilis*		n.d.	n.d.	n.d.
Nm02	*D. nummularia*	*Marou*	Enantiophyllum	Vanuatu	Beverly Hill
Nm03	*D. nummularia*	*Marou*	Enantiophyllum	Vanuatu	Beverly Hill
9	*D. abyssinica*	*6 éme année de domestication*	Enantiophyllum	Bénin	UAC
10	*D.abyssinica*	*6 éme année de domestication*	Enantiophyllum	Bénin	UAC
11	*D.abyssinica*	*6 éme année de domestication*	Enantiophyllum	Bénin	UAC
12	*D.abyssinica*	*6 éme année de domestication*	Enantiophyllum	Bénin	UAC
13	*D.abyssinica*	*6 éme année de domestication*	Enantiophyllum	Bénin	UAC
14	*D. abyssinica*	*6 éme année de domestication*	Enantiophyllum	Bénin	UAC
18	*D.rotundata*	*Ahimon*	Enantiophyllum	Bénin	UAC
20	*D.rotundata*	*kpakara*	Enantiophyllum	Bénin	UAC
22	*D.rotundata*	*Kponan*	Enantiophyllum	Bénin	UAC
26	*D.rotundata*	*Soussou*	Enantiophyllum	Bénin	UAC
28	*D. abyssinica*	*1ére année de domestication*	Enantiophyllum	Bénin	UAC
29	*D.abyssinica*	*Sauvage*	Enantiophyllum	Bénin	UAC
30	*D.abyssinica*	*Sauvage*	Enantiophyllum	Bénin	UAC
31	*D.abyssinica*	*Sauvage qui va être domestiqué*	Enantiophyllum	Bénin	UAC
32	*D. abyssinica*	*non domesticable*	Enantiophyllum	Bénin	UAC
34	*D. abyssinica (à l'origine de 9)*	*Sauvage utilisé pour la domest.*	Enantiophyllum	Bénin	UAC
35	*D. abyssinica (à l'origine de 9)*	*Sauvage utilisé pour la domest.*	Enantiophyllum	Bénin	UAC
36	*D.rotundata*	*Banioure*	Enantiophyllum	Bénin	UAC
37	*D.rotundata*	*Banioure*	Enantiophyllum	Bénin	UAC
39	*D. abyssinica*	*3 éme année de domestication*	Enantiophyllum	Bénin	UAC
40	*D. abyssinica*	*3 éme année de domestication*	Enantiophyllum	Bénin	UAC
42	*D.abyssinica*	*3 éme année de domestication*	Enantiophyllum	Bénin	UAC
54	D.rotundata	*kokoro*	Enantiophyllum	Bénin	UAC
55	*D.rotundata*	*Singou*	Enantiophyllum	Bénin	UAC
56	D.rotundata	*kponan*	Enantiophyllum	Bénin	UAC
59	*D.rotundata*	*Ahimon*	Enantiophyllum	Bénin	UAC
61	D.rotundata	*morokourou*	Enantiophyllum	Bénin	UAC
69	*D. abyssinica (à l'origine de 34,35)*	*Sauvage utilisé pour la domest.*	Enantiophyllum	Bénin	UAC
70	*D.abyssinica (àl'origine de 45)*	*Sauvage utilisé pour la domest.*	Enantiophyllum	Bénin	UAC
73	*D. praehensilis*	*Sauvage*	Enantiophyllum	Bénin	UAC
75	*D. bulbifera*	*Sauvage*	Opsophyton	Bénin	UAC
76	*D. dumetorum*	*Sauvage*	Lasiophyton	Bénin	UAC
77	*D. togoensis*	*Sauvage*	Enantiophyllum	Bénin	UAC
78	*D. praehensilis*	*domesticable*	Enantiophyllum	Bénin	UAC
79	*D. preussi*	*Sauvage*	Macrocarpaea	Bénin	UAC
80	*D. praehensilis*	*domesticable*	Enantiophyllum	Bénin	UAC
83	*D.abyssinica/D. praehensilis*	*Sauvage*	Enantiophyllum	Bénin	UAC
86	*D.cayenensis*	*IKENI*	Enantiophyllum	Bénin	UAC
87	*D.praehensilis*	*1ere année de domestication*	Enantiophyllum	Bénin	UAC
88	*D.cayenensis*	*IKENI*	Enantiophyllum	Bénin	UAC
89	*D.praehensilis*	*5 éme année de domestication*	Enantiophyllum	Bénin	UAC
94	*D.alata*	*Florido*	Enantiophyllum	Bénin	UAC
95	*D.rotundata*	*Gnidou*	Enantiophyllum	Bénin	UAC
99	*D.praehensilis*	*1 ére année de domestication*	Enantiophyllum	Bénin	UAC
100	*D.praehensilis*	*1 ère année de domestication*	Enantiophyllum	Bénin	UAC
101	*D. praehensilis*	*1ére année de domestication*	Enantiophyllum	Bénin	UAC
102	*D.praehensilis*	*1ére année de domestication*	Enantiophyllum	Bénin	UAC
103	*D. togoensis*	*Sauvage*	Enantiophyllum	Bénin	UAC
104	*D.rotundata*	*Gnidou*	Enantiophyllum	Bénin	UAC
108	*D. praehensilis*	*3 éme année de domestication*	Enantiophyllum	Bénin	UAC
109	*D.praehensilis*	*3 éme année de domestication*	Enantiophyllum	Bénin	UAC
121	*D.rotundata*	*Mondji mâle*	Enantiophyllum	Bénin	UAC
122	*D.rotundata*	*Linduin mâle*	Enantiophyllum	Bénin	UAC
123	*D.rotundata*	*linduin femelle*	Enantiophyllum	Bénin	UAC

Among 21 loci tested, 12 were amplified and loci Rct10, NTCP19, NTCP8 and NTCP9 were found to be polymorphic (table 2).

Table 2 : Results of amplification tests

Codes	Nucleotide sequences 5'-3'	Amplification	Polymorphism
NTCP2	CTCGCCTACTACATTCC AAGGAGAGGTTATTTTCTTG	-	
NTCP3	AAGTCAAAAGAGCGATTAG TGATACATAGTGCGATACAG	-	
NTCP4	TTGGATTAGATTTGTAGTTCCA ATCCACTTCATTTATCACAATG	-	
NTCP6	GGTTCGAATCCTTCCGTC GATTCTTTCGCATCTCGATTC	-	
NTCP7	TGATCCCGGACGTAATCC CGAATCCCTCTCTTTCCG	-	
NTCP8	ATATTGTTTTAGCTCGGTGG TCATTCGGCTCCTTTATG	Yes	Yes
NTCP9	CTTCCAAGCTAACGATGC CTGTCCTATCCATTAGACAATG	Yes	Yes
NTCP10	TGCTGAATCGACGACCTA AATATTCGGAGGACTCTTCTG	Yes	
NTCP11	AGTGAATATTCATTGAGACGAACG ATCTAGAGTGATAGCAAAAA	-	
NTCP12	CCTCCATCATCTCTTCCAA ATTTATTTCAGTTCAGGGTTCC	-	
NTCP13	TTTCCTGTTCCTGGTGGTA TTGGGGTAGATACACAAATCAC	Yes	
NTCP14	AATCCGTAGCCAGAAAAATAAA CCGATGCATGTAATGGAATC	-	
NTCP18	CTGTCTTTCCATGACCCCTC CCACCTAGCCAAGCCAGA	Yes	
NTCP19	AATGTTGTTTTAGACGATGC GAAACCCATTCTTACCACAAG	Yes	Yes
NTCP20	TCCTCGTAAGACTGAGAGAAAT TTACGAGTAATTCCGACAACTT	-	
NTCP21	AAAAAGATCCCACAAAGAAAA CTTATCGATTCCTGTCAAAAAG	-	
NTCP22	TATCAGAAAAAGAAAAAGAAGG GTCAAAGCAAAGAACGATT	-	
NTCP23	CAAAGGAACATTATCAATCATC TAGCTCAGAGGTTAGAGCATC	Yes	
NTCP24	GACCGATGATTTGGACGAC GCTAGCGGACATTTATTTTGAA	Yes	
NTCP28	TCCAATGGCTTTGGCTA AGAAACGAAGGAACCCAC	Yes	
RCt3	TAGGCATAATTCCCAACCCA CTTATCCATTTGGAGCATAGGG	Yes	
RCt4	ACGGAAAAGGAACTTCTTTGG AAAAGGAGCCTTGGAATGGT	Yes	
RCt5	ATTTGGAATTTGGACATTTTCG ACTGATTCGTAGGCGTGGAC	-	
RCt9	ATAAGGTTATTCAATAAGAGTA AAATTGGGGGAATTCGTACC	-	
RCt10	TCTTCATTTGGAATCTGGGC CTATTGATGCAAACGCTGTACC	Yes	Yes

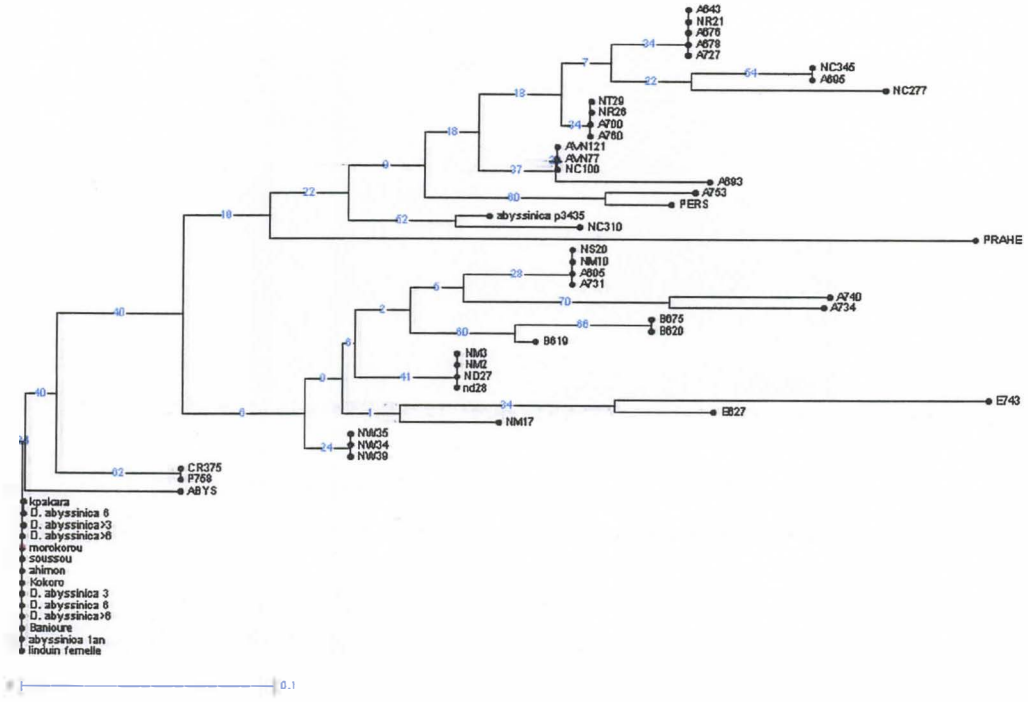

Figure 2 : Dendrogram (NJTree) obtained using loci NTcp8, 9 and 19. The dissimilarity matrice was obtained using Simple Matching. La matrice de dissimilarité a été obtenue avec l'indice Simple Matching.

Accessions from Benin (West Africa) appear to be grouped in one cluster and only one accession was kept for the analysis at the NTcp19 locus. This locus corresponds to the intron II of the *ycf3*gene (LSC component of the chloroplastic genome) and presents some interesting characteristics. As the chloroplastic genome is haploid, only one allele is usually observed per accession. However, in this particular case, each accession present 1 to 4 bands and 8 distinct levels of bands have been observed in the sample studied. The fact that some accessions exhibit several alleles raise interesting questions. It was therefore decided to clone at leadt each of the eight levels and to clone all alleles exhibiting profiles with numerous bands (table 3).

Table 3 : Accessions and level of bands selected for cloning

	PRAHE	B620	E743	ABYS	A693	A734	A740	Linduin F
1								
2								
3	prahe2		E74332	aby12				
4								
5			E74342					
6							A7402	
7					A69312	A7342		
8		B620 32		aby22+23	A69322			1232

Sequencing is an important step. Amplification with NTcp19 revealed several alleles. Polymorphic accessions and/or those presenting peculiar levels of bands were selected for sequencing. Overall, 6 accessions were selected representing a total of 18 DNA fragments.

PCR and a control gel were realised to verify the presence of DA fragment in slices of the gel. Results presented in figure 3 indicate that all fragments presented DNA with the expected size of approx. 165pb and a polymorphism in the length of the fragments is clearly observed.

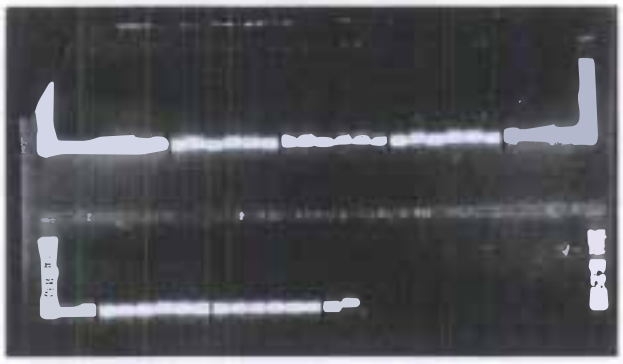

Figure 3 : agarose gel showing the presence of DNA and the polymorphism of PCR products obtained using primers pairs NTCP19

Once bacteria $DH_5\alpha$ were transformed by plasmids, 1 to 10 clones per fragment were identified and were either bleue or white. Only the white bacteria were preserved as their colour is related to βgalactosidase gene expression.

A second gel was realised after the bacteria were transformed. All colonies that were selected allowed amplification (figure 4) and are therefore all transformed. However, one colony present a band lower than the others indicating that the plasmid does not present the insert and was therefore excluded.

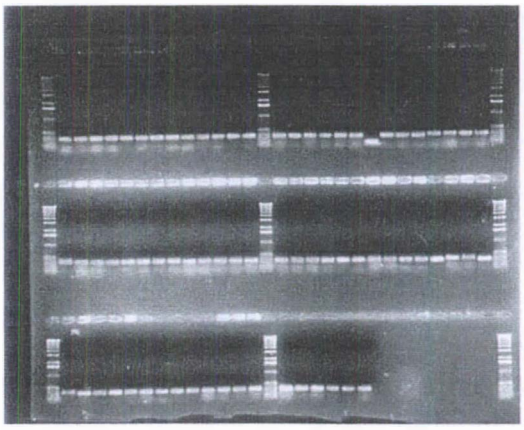

Figure 4 : agarose gel showing the resulting amplification using SP6 and T7

One to six colonies per DNA fragment were tested. Figure 5 present the different levels of cloned bands, all fragments were expected except band no 12. Two bands appear to be present although the bacteria colonies were transformed by only one plasmid containing only one insert. Therefore this fragment will not be sequenced. Band no 13 is also excluded from the study as it was not matching the expected size equivalent to nos 6 & 7.

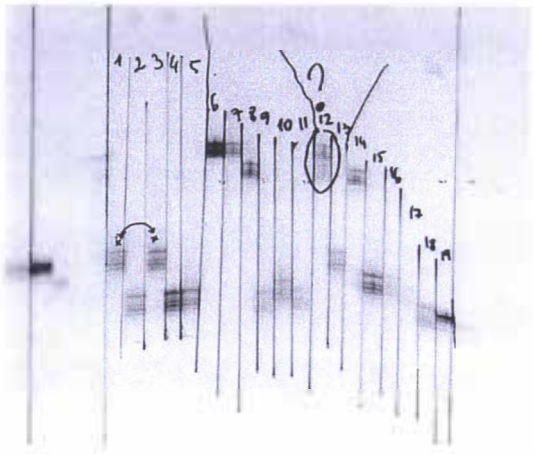

Figure 5 : acrylamid gel showing different sizes of the cloned fragments

Sequences analysis

The characteristics of the sequences presented in figure 6 reveal the origin of their length variation observed on acrylamid gel. We observe variation for the number of repeated units within a micorsatellite locus and insertion/deletions in the flanking sequences. Furthermore, the alignment of sequences shows that identical coded bands present differences in their sequences (table 4).

	PRAHE	B620	E743	ABYS	A693	A734	A740	Linduin F
1		B62012						
2		B62022	E7432					
3	prahe2		E74332	aby12				
4								
5			E74342					
6							A7402	
7					A69312	A7342		
8		[illegible]		aby23	A69322			[illegible]

Table 4 : the analysis of the sequences obtained indicates that alleles having identical length present differences in sequences.

Discussion:

This study has revealed intra and inter specific polymorphism. Amplification and migration on acrylamide gel can detect alleles that are different by one or two bases. It is therefore possible that loci which appear monomorphs on agarose gel could present some polymorphism on acyrlamid gel. The limited polymorphism observed within the group of *D. alata* accessions originating from Benin (West Africa), indicates that the African sample should be broaden in order to be representative of the diversity existing in Africa. Regarding the amplification obtained with NTcp19, one to four alleles per accession were obtained. This locus is located in the LSC part of the chloroplastic genome and there is usually only one copy of the sequence.

Several hypotheses can be discussed to explain these results. Chimeras can be maintained and propagated vegetatively. Some yams could be chimeras and several cultivation techniques could induce such chimeras. The plantation of different species in the same hole could be an explanation as wild species present only one allelic level. The transfert of some or part of the chloroplastic genome could also have occurred. The *ycf3* gene could have been transferred

with a modification. The presence of 4 alleles per accession could be explained by the paternal transmission of chlorplast with the conservation of maternal chloroplast. In this latter case, it would be possible to study past hybridizations.

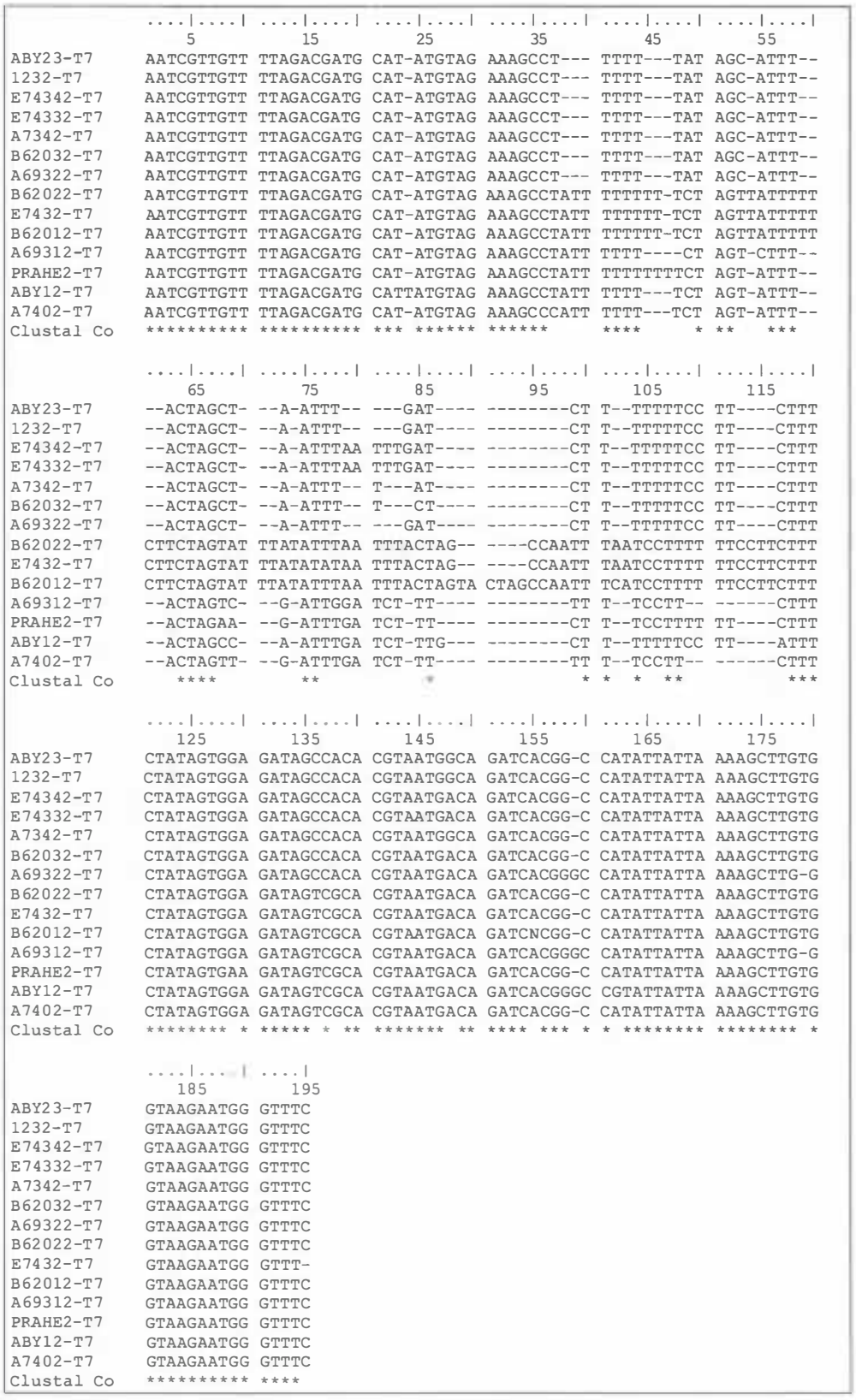

Figure 6: Allelic sequences for the locus amplified with NTcp19

Perspectives:

To confirm the hypothesis of chimeras, it would be possible to use somatic embryogenesis. A somatic embryo originating from a single cell, the application of well selected microsatellites could be interesting to compare amplified DNA fragment with those of the mother plant.

In order to study the transmission of chloroplastic genome, it could be interesting to realise crosses and to study using the polymorphic markers developed in this study, the profiles of the progenies.

1.3 - Physico-chemical variation of yam (*Dioscorea alata* L.) tubers

B. Pons, G. Piombo, JL. Marchand and V. Lebot

Introduction

Yam is also a crop with potential for increased commercial exploitation. Tubers are increasingly sold on urban markets, returning much needed cash to rural communities, and they are also being exported to meet the needs of Pacific-rim island communities. Yam, alone among the tropical root crops, can be stored for long periods, and because of this and its robust nature it can be transported with relative ease. However, several problems are limiting its development: tuber shape is often irregular making harvest time-consuming.

Although some preliminary work has been done at the species level (Bradbury and Holloway, 1988), the lack of information on the chemical variation within *D. alata* hinders the prospective utilisation of yam as a high quality vegetable. Previous work conducted on a germplasm collection exhibiting limited genetic variation (Lebot *et al.*, 1998) has shown that different cultivars present highly variable characteristics of their tubers but no correlations could be connected to taste or specific palatability.Indigenous knowledge claims that there is tremendous variation between the culinary and palatability properties of *D. alata* cultivars, some being suitable for certain types of preparation while others are not and some being cooked much faster than others. In Vanuatu for example, only few varieties are suitable for the preparation of the national dish, laplap, a pudding made from freshly and finely grounded tubers steam cooked in *Heliconia indica* leaves.

The present study aims to provide information on the tuber characteristics of *D. alata* cultivars in an area of major genetic diversity, in Vanuatu, Fiji and Papua New Guinea, so that the extent of variation existing within this species can be assessed. It appears interesting to study the physico-chemical characteristics of the tubers from selected cultivars in order to compare these with indigenous knowledge related to palatability and cooking properties.

Materials and Methods

In Vanuatu, 48 different varieties were selected because of their distinct morphological and palatability characteristics (table 1). These varieties were planted the same day (500g per seed set). For each accession, four plants were established with 1 x 1 m spacing and a pyramidal staking system. Plants were harvested when mature and at harvest. A thick slice was cut from the centre of each tuber, peeled and bulked with those from the other 3 plants to calculate the mean dry matter weight of the variety. Fresh tuber flesh was then cut into small 2 X 2 cm cubes and oven dried at 60-80°C until complete dehydration. Dried pellets were then converted into flour using and electrical hammer mill and flour samples were sent to CIRAD laboratories in Montpellier, France. Analyses included: percentage moisture content, percentage starch yield on a dry basis, gelatinisation temperature range, amylose content, minerals, lipids, proteins and sugars. Dry samples from selected varieties were received from Fiji (19 acc.) and Papua New Guinea (43 acc.) and were analysed following the same protocols. All samples were analysed twice and the means were recorded.

Results

The list of varieties originating from Vanuatu is presented in Table 1.

Table 1: List of varieties from Vanuatu analysed

Acc No.	Island	Village	*Local name*	Tuber shape	Flesh color	Status	% DM
655	Vanua Lava	Bangcap	*Turea*	cylindrical	white		20.00
049	Maewo	Narovorovo	*Malabong hivo*	cylindrical	white		29.26
657	Maewo	Marino	*Sovwa*	deformed	white		23.68
661	Maewo	Marino	*Ririho*	deformed	white		23.40
415	Pentecost	Marteli	*Obal*	oval	white	recommended	22.91
418	Pentecost	Marteli	*Tahirao*	cylindrical	white		28.16
419	Pentecost	Marteli	*Kilman*	deformed	white		27.77
633	Pentecost	Lolbuavatu	*Bwev Mudani*	deformed	white		22.22
639	Pentecost	Lolbuavatu	*Bwalanvara*	deformed	white		21.21
645	Pentecost	Lolbuavatu	*Shulniu*	round	white		22.00
373	Ambae	Waisala	*Bughi toa*	deformed	purple		22.91
247	Santo	Chapuis	*n.a.*	cylindrical	white		27.27
400	Santo	Fanafo	*Rave*	cylindrical	white		27.58
402	Santo	Fanafo	*Raranaeolo*	oval	white		24.07
404	Santo	Fanafo	*n.a.*	cylindrical	pink		25.74
452	Santo	Ipayato	*Uratavue*	flattened	white		23.21
453	Santo	Ipayato	*Livusivari*	cylindrical	white		28.00
491	Santo	Fanafo	*Riprip*	deformed	white		29.41
510	Santo	Fanafo	*Aga*	deformed	white		13.68
514	Santo	Fanafo	*Tageu*	deformed	purple		16.21
592	Santo	Chapuis	*n.a.*	cylindrical	white		20.00
596	Santo	Chapuis	*n.a.*	cylindrical	white		24.00
597	Santo	Chapuis	*n.a.*	oval	white		19.64
463	Malo	Avunamalae	*Balabalavuvuha*	deformed	white		14.81
459	Malo	Avunamalae	*Basa*	oval	white	recommended	20.37
008	Malakula	Unmet	*Navilu*	deformed	purple		31.42
009	Malekula	Fau	*n.a.*	triangular	purple		25.71
016	Malakula	Brenwe	*Pirai*	deformed	white		19.04
265	Malakula	Lorlow	*Nabulalas*	deformed	white		23.52
286	Malakula	Wintua	*Nivikilmlak*	cylindrical	white		25.00
531	Malakula	Unmet	*Visn*	deformed	white		20.23
533	Malakula	Potinweo	*Nikelpo woman*	cylindrical	white		21.95
536	Malakula	Pinalum	*Romb soso*	cylindrical	white		20.68
551	Malakula	Lavalsal	*Mombri*	cylindrical	white	recommended	26.31
562	Malakula	Losinwe	*Tumas*	deformed	white		22.60
567	Malakula	Orap	*Homb*	deformed	white		18.60
577	Malakula	Rori	*Baksan*	deformed	white		28.57
578	Malakula	Orap	*Letslets maser*	oval	white	recommended	24.21
579	Malakula	Orap	*Letslets nambas*	deformed	yellow		21.42
589	Malakula	Lavalsal	*Tapa*	cylindrical	white	recommended	18.75
602	Malekula	Faou	*n.a.*	deformed	purple		29.98
623	Ambrym	Reverger	*Masinruburo*	deformed	white		25.00
475	Efate	Mélé	*Salemanutetea*	oval	white	recommended	22.44
700	Tanna	Imanaka	*Tumas*	deformed	white		26.51
701	Tanna	Imanaka	*Wasu*	deformed	white		27.94
704	Tanna	Imanaka	*Nawanurunkimanga*	deformed	purple		28.23
706	Tanna	Imanaka	*Rosapin*	deformed	white		19.69

The results of the analyses are presented in Table 2.

Table 2: Physico-chemical characteristics of 48 different varieties grown in Vanuatu

Acc no.	DM %	Ratio A/S	Amy %	Starch %	Min %	Lip %	Pro %	Sugars %	Gluc %	Fruct %	Sacc %	n.d. %	Raffi %	Malt %	T. gel °C	T. gel °C
596	24.00	0.21	20.7	73.4	3.0	0.2	13.3	1.17	0.00	0.01	0.49	0.02	0.59	0.06	74.8	87.1
643	21.42	0.21	20.5	67.0	3.0	0.3	10.7	0.58	0.02	0.00	0.40	0.00	0.12	0.04	78.8	87.2
452	23.21	0.20	20.4	74.8	2.8	0.3	9.2	1.9	0.06	0.12	1.63	0.02	0.04	0.03	79.2	86.9
475	22.44	0.20	20.0	75.2	3.6	0.3	10.8	1.07	0.04	0.05	0.81	0.04	0.08	0.05	78.9	87.9
578	24.21	0.20	19.9	78.2	3.0	0.3	9.5	0.64	0.02	0.05	0.43	0.00	0.09	0.05	78.9	86.7
704	28.23	0.20	19.8	75.6	2.6	0.3	12.1	0.28	0.01	0.03	0.12	0.00	0.07	0.05	78.6	86.0
701	27.94	0.19	19.4	73.8	2.8	0.2	8.8	2.39	0.00	0.03	2.28	0.00	0.03	0.05	77.8	89.2
597	19.64	0.19	19.2	78.2	3.1	0.3	11.5	2.11	0.03	0.03	1.89	0.06	0.04	0.06	79.1	86.7
247	27.27	0.19	19.2	71.3	2.5	0.3	9.8	0.83	0.04	0.02	0.64	0.03	0.04	0.06	80.0	91.6
562	22.60	0.19	19.0	71.5	3.4	0.3	10.6	1.32	0.04	0.04	1.09	0.04	0.06	0.05	78.8	88.1
645	22.00	0.19	19.0	74.9	2.9	0.3	10.3	2.96	0.10	0.16	2.57	0.05	0.03	0.05	79.8	88.0
404	27.74	0.19	18.9	75.8	2.5	0.2	11.5	1.44	0.08	0.03	1.19	0.03	0.07	0.04	77.2	89.4
415	22.91	0.18	18.4	70.1	3.8	0.3	11.6	3.22	0.12	0.15	2.75	0.10	0.06	0.04	77.8	86.7
551	26.31	0.18	18.2	78.6	3.0	0.3	11.2	0.83	0.00	0.03	0.70	0.01	0.04	0.05	79.7	87.8
491	29.41	0.18	18.0	76.4	3.1	0.3	9.9	1.62	0.06	0.09	1.31	0.04	0.07	0.05	78.8	86.2
402	24.07	0.18	17.8	75.7	3.0	0.3	12.4	0.81	0.02	0.03	0.59	0.00	0.12	0.05	78.3	87.1
453	28.00	0.18	17.8	78.2	2.8	0.2	9.3	0.87	0.00	0.02	0.76	0.01	0.04	0.04	80.4	89.1
623	25.00	0.18	17.8	77.4	3.9	0.3	15.7	1.23	0.05	0.04	0.94	0.10	0.04	0.06	77.1	85.5
533	21.95	0.18	17.8	69.0	3.8	0.3	12.1	0.25	0.00	0.00	0.10	0.00	0.09	0.06	79.2	87.3
459	20.37	0.18	17.7	74.8	2.5	0.2	9.1	2.6	0.11	0.17	2.24	0.01	0.03	0.04	80.2	88.5
602	29.98	0.18	17.7	75.7	3.3	0.2	12.1	0.76	0.02	0.03	0.49	0.01	0.16	0.05	79.3	88.7
373	22.91	0.18	17.7	76.1	2.8	0.3	11.7	0.72	0.03	0.02	0.47	0.00	0.16	0.04	79.4	88.6
655	20.00	0.18	17.7	76.2	3.4	0.3	12.8	2.05	0.02	0.04	1.91	0.00	0.03	0.05	76.8	87.4
008	31.42	0.17	17.4	75.5	2.9	0.2	9.9	0.86	0.09	0.04	0.62	0.03	0.05	0.03	77.6	85.8
592	20.00	0.17	17.4	72.6	3.1	0.3	9.2	3.78	0.16	0.30	3.22	0.01	0.07	0.02	80.7	91.2
633	22.22	0.17	17.2	75.9	3.2	0.3	10.4	5.71	0.12	0.12	5.31	0.07	0.04	0.05	78.7	87.4
657	23.68	0.17	17.2	67.1	3.3	0.3	10.9	2.62	0.03	0.04	2.44	0.02	0.04	0.05	77.4	85.2
016	19.04	0.17	17.1	71.4	3.5	0.3	11.5	4.51	0.14	0.10	4.12	0.09	0.00	0.06	78.1	85.6
706	19.69	0.17	17.0	69.4	3.2	0.4	12.1	5.2	0.11	0.17	4.78	0.01	0.06	0.07	77.5	88.4
531	20.23	0.17	16.8	72.1	3.9	0.3	14.2	0.8	0.07	0.00	0.51	0.10	0.05	0.07	78.6	86.1
536	20.68	0.17	16.7	75.5	3.3	0.3	10.6	1.92	0.03	0.03	1.72	0.05	0.03	0.06	78.8	88.6
049	29.26	0.17	16.5	76.3	3.0	0.2	11.0	1.12	0.07	0.02	0.96	0.02	0.00	0.05	79.3	87.4
009	25.71	0.16	16.4	74.8	3.3	0.2	10.4	0.29	0.02	0.00	0.14	0.00	0.09	0.04	81.3	90.7
661	23.40	0.16	15.9	71.8	3.2	0.3	10.7	2.12	0.05	0.08	1.90	0.00	0.04	0.05	80.4	89.7
400	27.58	0.16	15.8	73.6	3.2	0.2	14.6	0.76	0.01	0.03	0.47	0.16	0.05	0.04	78.2	86.3
418	28.16	0.16	15.6	73.2	3.2	0.2	15.1	1.45	0.01	0.04	1.20	0.14	0.00	0.06	78.2	86.2
577	28.57	0.16	15.5	75.6	3.1	0.3	11.6	2.45	0.09	0.10	2.13	0.05	0.03	0.05	79.1	88.5
639	21.21	0.15	15.4	68.8	3.7	0.3	15.0	1.04	0.02	0.03	0.91	0.03	0.00	0.05	75.7	84.2
286	25.00	0.15	15.2	68.0	3.2	0.3	13.2	3.0	0.07	0.07	2.68	0.12	0.00	0.06	78.5	88.3
589	18.75	0.15	15.1	72.1	4.3	0.4	15.6	2.4	0.05	0.06	2.09	0.09	0.04	0.07	78.9	88.0
579	21.42	0.15	15.1	68.3	3.1	0.3	10.2	4.91	0.29	0.32	3.87	0.31	0.04	0.08	80.3	87.9
700	26.51	0.15	15.0	71.1	4.0	0.3	14.8	0.6	0.01	0.01	0.36	0.00	0.18	0.04	78.6	86.9
463	14.81	0.14	14.4	64.9	3.8	0.4	15.8	3.28	0.06	0.06	2.77	0.32	0.03	0.04	77.6	85.2
510	13.68	0.14	14.2	66.9	4.2	0.4	17.0	1.26	0.01	0.02	0.87	0.26	0.04	0.06	78.9	86.5
514	16.21	0.14	14.1	63.6	4.9	0.5	16.4	2.12	0.14	0.23	1.41	0.19	0.07	0.08	79.6	88.2
419	27.77	0.14	13.8	75.0	3.2	0.3	11.7	0.67	0.03	0.03	0.55	0.02	0.00	0.04	79.5	88.0
567	18.60	0.14	13.7	69.8	3.2	0.4	11.2	3.97	0.40	0.38	2.85	0.23	0.05	0.06	80.7	88.4
265	23.53	0.13	13.4	73.1	3.8	0.3	12.4	0.36	0.02	0.00	0.18	0.00	0.12	0.04	81.5	90.3
Max	**31.42**	**0.21**	**20.7**	**78.6**	**4.9**	**0.5**	**17.0**	**5.71**							**81.5**	**91.6**
Min	**13.68**	**0.13**	**13.4**	**63.6**	**2.5**	**0.2**	**8.8**	**0.6**							**78.8**	**87.6**
Mean	**23.44**	**0.172**	**17.2**	**73.1**	**3.3**	**0.3**	**11.95**	**1.85**							**74.9**	**84.2**
Std	**4.02**	**0.020**	**2**	**3.67**	**0.5**	**0.1**	**2.13**	**1.37**							**1.3**	**1.6**
CV%	**17.15**	**11.62**	**11.6**	**9.12**	**15.2**	**33.3**	**17.82**	**91.3**							**1.7**	**1.8**

It appears that all characteristics are quite variable, including the amylose vs starch ratio which is an important palatability trait. Appreciated varieties present a high A/S ratio (> 0.18) and are suitable to produce *Laplap*, the national dish.

Results of the analyses conducted on varieties received from Fiji, are presented in Table 3. The starch and amylose contents are not significantly variable.

Table 3: Cultivars from Fiji

No.	Cultivar	DM %	Sta %	Amy %	Sugars %	Pro %	Min %	Total %	Temp °C	Temp °C
	Taniela Vula Leka	n.a.	69.5	19.2	2.34	9.0	3.6	84.4	77.0	85.5
	Niumadu I	n.a.	64.7	18.3	3.14	6.6	4.0	78.4	76.4	84.8
	Damuni Balavu	n.a.	70.4	18.2	2.54	7.4	3.9	84.3	79.0	88.3
34	Vurai	31.3	72.8	20.2	1.93	6.6	3.2	84.6	77.6	86.5
19	Muropoi	30.3	61.9	17.2	2.58	8.2	4.1	94.0	75.7	85.7
1	Taniela Damu II	33.6	70.4	18.5	1.51	11.3	4.6	87.9	78.9	87.7
23	Kivi	35.4	70.7	18.2	1.65	9.2	3.9	85.5	77.1	88.8
9	Veiwa	25.1	63.3	18.8	1.72	11.6	6.8	83.4	78.1	87.1
20	Kuro Round	33.8	67.7	19.9	2.45	8.4	5.2	83.8	77.1	85.5
	Taniela Damu Leka	n.a.	64.7	19.0	3.50	7.6	3.6	79.4	76.7	86.1
21	Reado	30.1	66.1	19.8	2.05	7.4	4.0	79.6	77.6	85.5
26	Sisiwa	33.4	66.9	18.6	2.73	6.5	4.4	80.5	77.1	86.2
	Voli Balavu	n.a.	66.1	18.2	2.30	6.2	4.0	78.6	78.9	87.5
	Vurai Vula Leka	n.a.	73.2	16.6	4.02	7.2	3.9	88.2	76.8	86.1
13	Voli	31.2	73.7	17.7	1.91	6.1	3.5	85.2	77.4	85.4
6	Taniela Vula Leka A	29.2	73.3	20.5	2.27	7.1	4.3	87.0	76.7	84.0
15	Uvi ni Futuna	31.4	64.5	16.9	2.89	11.4	5.1	100	76.2	85.0
7	Taniela Vula Leka B	33.5	76.7	19.0	3.06	7.0	4.0	90.8	75.8	83.7
14	Futuna Vula Leka	29.3	64.3	19.1	2.11	7.8	4.6	97.9	76.6	84.8
	Minimum		**61.9**	**16.6**	**1.5**	**6.1**	**3.2**	**78.4**	**75.7**	**83.7**
	Maximum		**76.7**	**20.5**	**4.0**	**11.6**	**6.8**	**100.0**	**79.0**	**88.8**
	Mean		**68.5**	**18.6**	**2.46**	**8.03**	**4.25**	**85.97**	**77.2**	**86.0**
	Standard deviation		**4.21**	**1.07**	**0.65**	**1.74**	**0.80**	**6.15**	**0.98**	**1.37**
	Coeff. of variation		**6.14**	**5.75**	**26.42**	**21.67**	**18.8**	**7.15**	**1.26**	**1.59**

Results of the analyses conducted on the varieties received from Papua New Guinea are presented in Table 4.

Overall, 110 samples were analysed. In the three countries surveyed, starch content appears to be the less variable characteristic. Amylose, proteins, minerals and sugars are very variable and are apparently controlled genetically.

Country	n	Starch	Amylose	Proteins	Minerals	Sugars
Vanuatu Mean	48	73.1	17.2	11.9	3.3	1.85
Vanuatu CV%	*48*	*9.1*	*11.6*	*17.8*	*15.2*	*91.3*
Fiji Mean	19	68.5	18.6	8.03	4.25	2.46
Fiji CV%	*19*	*6.1*	*5.7*	*21.7*	*18.8*	*26.4*
PNG Mean	43	67.5	17.5	12	5.1	3.3
PNG CV%	*43*	*7.8*	*11.4*	*32*	*14.7*	*49.1*

Recommended varieties are characterised with high dry matter, starch and amylose contents and low proteins and minerals contents. This is often correlated to a white flesh which is not susceptible to oxidation when exposed to air. Further screening of germplasm and/or breeding will have to take into consideration these characteristics, important for farmers adoption.

Table 4: Cultivars from Papua New Guinea

Acc No.		DM %	Starch %	Amy %	Pro %	Min %	Sugars %	Total	Temp. °C	Temp. °C
001	Takua Yavu	28.5	82.3	15.4	4.6	4.2	2.82	94.0	76.6	84.5
002	Takua Kupmi	19.15	65.2	13.7	12.3	6.0	6.08	89.6	82.2	91.2
003	Kipmora	n.a.	71.1	18.4	9.6	5.6	3.84	90.2	78.0	86.5
009	Yavovi	n.a.	70.3	16.8	6.5	5.3	6.80	89.0	80.0	88.8
012	Modavateu	n.a.	73.8	16.0	11.4	4.8	3.20	93.3	78.2	86.8
014	Shek I	n.a.	69.9	16.6	8.6	6.4	6.26	91.2	79.5	88.1
018	Naso	n.a.	65.2	16.3	17.3	6.0	2.74	91.2	79.5	88.4
022	W. Fergusson I	18.02	65.3	15.8	14.0	5.4	1.50	86.3	81.7	89.0
024	W.Fergusson III	22.21	59.0	17.1	15.0	5.2	4.28	83.5	81.1	90.5
033	Mui (female)	21.76	56.3	16.3	17.8	5.6	2.31	82.0	83.3	91.5
035	Napo Goning	n.a.	68.8	17.3	10.7	4.0	2.34	85.9	79.3	87.9
042	Gai	n.a.	67.2	17.9	13.6	5.2	3.50	89.5	76.2	86.3
043	Barai	23.85	69.2	18.1	18.2	5.8	1.71	95.0	80.5	88.6
047	Simbang	19.71	59.7	14.8	19.7	6.3	2.80	88.5	82.7	91.5
048	Kusin De	n.a.	72.4	20.7	10.0	3.3	3.23	88.9	77.5	85.8
052	Tobo	28.79	75.2	20.7	8.4	4.6	2.37	90.6	78.4	86.3
053	Kaiore Rau	28.86	75.4	20.0	10.6	4.7	1.84	92.6	78.9	86.5
054	Kisi	23.49	70.2	16.6	13.5	4.2	3.13	91.1	80.7	89.1
073	Suasua	26.31	64.5	16.4	13.7	4.9	4.37	87.4	79.7	87.6
078	Kilekile	n.a.	62.1	14.7	16.5	5.7	3.25	87.6	77.7	88.6
080	Meloba	23.39	64.5	15.3	17.4	5.5	3.70	91.1	79.9	88.6
083	Lobaloba	22.37	64.8	16.1	16.7	4.7	2.92	89.1	81.0	90.3
094	Noh	26.09	63.3	14.7	14.2	4.3	3.98	85.7	80.2	88.0
106	Makuia (white)	19.93	63.2	15.4	18.6	5.2	3.83	90.9	80.7	90.0
108	Kwadesera	24.33	64.5	16.4	14.3	5.4	4.03	88.2	80.7	88.7
110	Mainina	22.67	65.5	15.3	13.2	5.7	2.95	87.3	79.9	87.9
112	Gumanum	n.a.	71.4	20.8	6.3	3.8	3.84	85.4	79.6	88.1
140	Yabedua(female)	n.a.	67.6	20.2	9.2	4.9	3.34	85.1	78.1	87.2
143	Dininibu	n.a.	68.8	20.5	8.4	5.0	2.92	85.1	78.5	87.8
156	Kokoroku	n.a.	65.7	19.5	8.4	5.1	2.61	81.8	78.5	86.6
167	Meaku I	23.11	64.1	16.9	12.7	6.2	3.11	86.0	79.5	88.8
170	Davi	24.70	69.5	19.9	7.8	4.0	4.23	85.6	79.3	87.0
180	Maireba (red)	23.16	67.0	17.9	6.6	4.6	5.47	83.7	79.5	87.2
55		n.a.	79.6	18.2	9.0	5.1	1.89	95.6	77.7	86.5
56		n.a.	74.6	18.3	13.3	5.1	2.10	95.2	77.0	86.7
79		n.a.	67.2	17.1	14.3	5.3	1.31	88.1	77.8	86.9
88		n.a.	69.1	20.4	11.4	5.1	2.30	87.9	76.4	85.0
93		n.a.	69.2	19.8	10.6	3.9	2.00	85.7	81.9	92.2
95		n.a.	67.7	19.0	14.5	5.1	1.14	88.5	79.2	90.6
124		n.a.	61.3	18.1	7.3	4.2	9.84	82.7	77.5	88.6
142		n.a.	60.9	17.0	11.4	6.2	2.63	81.2	78.7	88.1
146		n.a.	62.6	17.2	11.8	5.9	2.30	82.7	78.0	89.1
190		n.a.	68.1	20.9	6.5	4.1	2.67	81.3	80.1	89.9
	Minimum		**56.3**	**13.7**	**4.6**	**3.3**	**1.1**	**81.2**	**76.2**	**84.5**
	Maximum		**82.3**	**20.9**	**19.7**	**6.4**	**9.8**	**95.6**	**83.3**	**92.2**
	Mean		**67.5**	**17.5**	**12.0**	**5.1**	**3.3**	**87.9**	**79.3**	**88.2**
	Std deviation		**5.27**	**1.99**	**3.85**	**0.75**	**1.62**	**3.86**	**1.68**	**1.76**
	Coeff variation		**7.80**	**11.4**	**32.0**	**14.7**	**49.1**	**4.39**	**2.11**	**1.99**

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2. NRI, University of Greenwich, UK

Lawrence Kenyon and Susan Seal (Natural Resources Institute, UK.)
Associated Student: Bénédicte Lebas (University of Greenwich, UK.)

2.1 - Virus component of the project

Virus diseases cause significant economic losses in yam production systems worldwide. Because such diseases are generally tuber-borne, they are a major impediment to international germplasm exchange. The role of NRI in the South Pacific Yam Network (SPYN) was to provide virology support to the project to:

- assess the diversity, variability and prevalence of the viruses infecting yams in the different SPYN countries
- determine what are the appropriate tools to diagnose the virus diseases, and if necessary to try to improve these tools so they can be used within a germplasm health certification scheme
- use the appropriate virus indexing tools to assess if selected South Pacific yam germplasm can be safely exchanged both within and outside the region
- explore the use of chemotherapy and thermotherapy with meristem-tip-culture for the elimination of virus from selected South Pacific yam germplasm.

Activities and outputs during the reporting period

- Bénédicte Lebas completed the practical work for her PhD study on "The Diversity of Viruses Infecting Dioscorea species in the South Pacific" and successfully defended her thesis (Lebas, 2002) in a viva on 18 February 2002. She has now moved to take up a position with the New Zealand Ministry of Agriculture and Food in Auckland. Chapters of Bénédicte's thesis are available online at: http://www.gre.ac.uk/~fd03/students/bene/bene.htm
- Lawrence Kenyon participated in the 4th annual project (SPYN) meeting in Port Vila, Vanuatu in April 2002.
- Susan Seal continued her secondment at CIRAD Montpellier through until July 2002. Her aims were to assess the molecular variability of badnaviruses infecting yam and to determine if badnavirus-like sequences are integrated into the yam genome. (A summary of Dr Seals work and results is attached as Annex 1)
- Lawrence Kenyon visited project partners in Fiji and delivered 30 accessions of yam in tissue culture (Table 1) to the SPC Regional Germplasm Centre in January 2003. He also presented a poster on the virology component of the project at the 8th International Congress of Plant Pathology (ICPP-2003) in Christchurch, New Zealand in February 2003 (see attached reprint).

Diversity of viruses in Yams in the SPYN countries

About 15 different viruses have been described infecting members of the Dioscoreacea in different parts of the world. In order to know what viruses yam germplasm from the South Pacific countries should be indexed for prior to movement within or outside the region, it is necessary to ascertain the range of viruses present in each country. Since the start of this project, leaf samples have been collected from different yam plants in each of the SPYN partner countries, as well as other countries in the region. Although it was originally intended

to have a stratified sampling procedure, because of logistical problems, many of the samples came from the yam germplasm collections, especially those from Vanuatu and Fiji. Each sample was tested by Enzyme-linked immunosorbent assay (ELISA) for the presence of each of seven different viruses using specific antisera (Table 1). Some of the samples obtained later in the project were also tested by ELISA for *Chinese yam necrotic mosaic virus* and *Japanese yam mosaic virus*.

Table 1. The main viruses known to infect *Dioscorea* yams and the specific antisera used in this study

Virus	**Antiserum (source)**
Chinese yam necrotic mosaic virus (ChYNMV), genus *Macluravirus*	ChYNMV Japan (Kondo)
Cucumber mosaic virus (CMV), genus *Cucumovirus*	CMV (Sanofi and IITA)
Dioscorea alata bacilliform virus (DaBV), genus *Badnavirus* (?)	DaBV Nigeria (HRI 500)
Dioscorea alata virus (DAV), genus *Potyvirus* (=*Yam virus I* (YVI) or *Yam mild mosaic virus* (YMMV))	YVI Papua New Guinea (HRI 316, 428)
Dioscorea bulbifera bacilliform virus (DbBV), genus *Badnavirus*	DbBV Puerto Rico (HRI 465)
Dioscorea dumetorum virus (DDV), genus (?) *Potyvirus*	DDV Papua New Guinea (HRI 508)
Dioscorea esculenta virus (DEV), genus (?) *Potyvirus*	N/a
Dioscorea latent virus (DLV), genus *Potexvirus*	DLV Puerto Rico (HRI 438)
Dioscorea mottle virus (DMoV), genus (?) *Comovirus*	N/a
Dioscorea trifida virus (DTV), genus (?) *Potyvirus*	N/a
Japanese yam mosaic virus (JYMV), genus *Potyvirus*	JYMV Japan (Fuji & Nakamae, 1999a)
Yam internal brown spot virus (IBSV), genus (?) *Badnavirus*	N/a
Yam mosaic virus (YMV), genus *Potyvirus*	YMV Nigeria (HRI 433)(IITA PaB, MaB)

(N/a = no antiserum available to this study)

***Dioscorea alata virus* (DAV)**

Because DAV (genus *Potyvirus*) appears to be very common in all the South Pacific countries when samples are tested using the available polyclonal antiserum, work was initiated to determine how variable this virus is in the region and how specific and reliable the antiserum is. Reverse transcriptase polymerase chain reaction (RT-PCR) was tested with different combinations of "universal" *Potyvirus* oligonucleotide primers (Table 2) using RNA extracted from DAV-ELISA positive samples as template. PCR amplicons of the expected molecular weight were only produced with certain sample and primer combinations. Based on alignment of the nucleotide sequences of some of these amplicons, more specific DAV PCR primers were designed (Figure 1).

PCR primer DAVCP01-F paired with oligo d(T)-R allowed amplification of the region from the WCIEN box to the end of the coat protein region of many of the South Pacific DAV ELISA positive samples. These amplicons were digested with the restriction enzyme *Alu*1.

which resulted in 36 unique restriction fragment patterns for the different samples, which suggests the presence of considerable genetic variability among the samples. When the molecular weights of each of the restriction fragments were summed, the total for each sample was generally in the expected region of 750 bp. However, for some samples the total was well over 800 bp suggesting the presence of some mixed *Potyvirus* infections. Eighteen of the amplicons from South Pacific samples were cloned and then sequenced using the DAVCP01-F primer.

The sequences were aligned using Clustal V (DNAstar) with the sequences from the same coding region of a range of other yam-infecting potyviruses and some DAV sequences from samples from Africa and Asia. The estimated phylogenetic tree (Bootstrap analysis by maximum parsimony using a heuristic search) is presented in Figure 1. In this tree the four yam potyviruses (DAV, YMV, JYMV and DDV) cluster separately with branch points at greater than 15% dissimilarity, reaffirming that these are probably different *potyvirus* species.

Table x. PCR primers used for detecting viruses infecting *Dioscorea* yams.

Virus	PCR primers	Reference
ChYNMV (*Macluravirus*)	ODTNOTIM / CYV790P	Kondo *et al* (Submitted)
CMV (*Cucumovirus*)	CMV1-F / CMV1-R	Quemada *et al*., (1989)
Badnavirus (DaBV DbBV IBSV)	Badna-F / Badna-R	Yang *et al* (In Press)
Potexvirus (DLV)	PotexCP1-R / PotexCP2-F Potex 1-6	Mumford (unpublished) van der Vlugt & Berendsen (2002)
Potyvirus (DAV DDV, DEV, DTV, JYMV)	CN48-F / oligo d(T)-R Pot2Nib-F/ Pot1CP-R	Pappu *et al.* (1993) Colinet & Kummert (1993)
DAV	YMMVCP2F / YMMVUTR1R YMMVS1-F DAVCP01-F	Mumford & Seal (1997) Fuji *et al.* (1999) Lebas (this study)
JYMV	JYMVF/R	Fuji & Nakamae (1999b)
YMV	YMVCP1F / YMVUTR1R	Mumford & Seal (1997)

(Note: Primers in rows shaded grey were not tested in this study)

Within the DAV cluster there are isolates from across the Pacific region and from South East Asia (Sri Lanka), Africa and the Caribbean (Martinique), and although there are several different "strains" of DAV represented in this tree, there is little evidence for there being different strain clusters confined to different regions of the world. Most of the DAV sequences were obtained from *D. alata* samples. However, there are also DAV sequences obtained from *D. rotundata* and *D. esculenta* samples. With the inclusion of more sequences in the analysis (compared to the year 2000 SPYN report), there is now less suggestion of a *D. esculenta* specific sub-cluster of DAV strains.

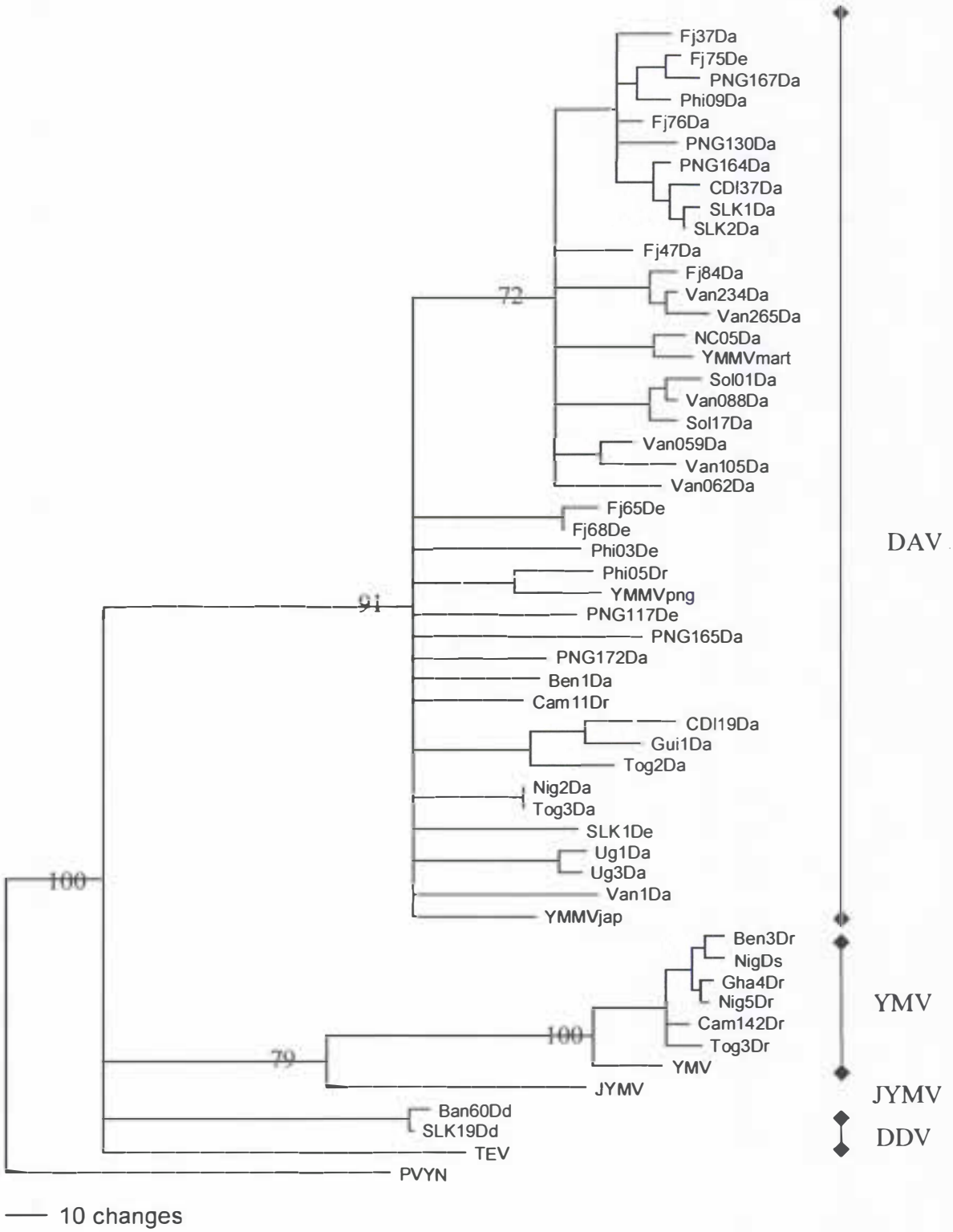

Figure 1. Phylogram of aligned nucleotide sequences of part of the coat protein coding region of *Potyvirus* isolates from the South Pacific islands and Africa. Branch lengths are proportional to percentage dissimilarity and numbers at branch points are bootstrap values (from 100 replicates).

Yam mosaic potyvirus (YMV) was not detected in any of the samples from the South Pacific region. All the YMV sequences included in the phylogenetic analysis (Figure 1) were obtained from *D. rotundata* samples from Africa. Although YMV has occasionally been detected in *D. alata* plants in Africa, it is more commonly associated with *D. rotundata*, and the apparent absence of this virus in the South Pacific region may be a reflection of the small number of *D. rotundata* samples from the South Pacific region collected and tested in this study. In order to say with greater confidence that YMV is not in the South Pacific region, a far greater number of *D. rotundata* samples from the region would have to be tested and all should test negative for this virus.

Many samples from the South Pacific region tested positive by ELISA for *Dioscorea dumetorum potyvirus* (DDV) (see draft manuscript on virus diversity). However, although *Potyvirus* sequences were amplified by RT-PCR from many of these samples, all such sequences clustered with the DAV sequences in the phylogenetic tree (Figure 1), and none clustered with the DDV sequences obtained from samples from Sri Lanka and Bangladesh. This suggests that the DDV antiserum used in ELISA is detecting a subset of DAV isolates and that the sequences designated as DDV used in the phylogenetic analysis may represent a different *Potyvirus* to that recognised by the antiserum. Unfortunately, there is no leaf material of the Sri Lanka or Bangladesh samples available for testing with the DDV antiserum.

A small volume of *Japanese yam mosaic virus* (JYMV; genus *Potyvirus*) antiserum was obtained at the end of 2000. Of the 164 leaf samples from the South Pacific region tested using this antiserum, 56 presented weak positive reactions. However, none of the *Potyvirus* sequences obtained from the South Pacific samples clustered with the JYMV sequence (from Japan), though the PCR primers used in obtaining the sequences were not the optimum for amplifying JYMV sequences. Thus, more time and resources are required to take work forward on this virus. Similarly, a few samples tested weakly positive for *Chinese yam necrotic mosaic virus* (ChYNMV; genus *Macluravirus*) by ELISA, but time and resources have not been available to evaluate a recently published PCR-based diagnostic test for ChYNMV (Kondo, 2001) to use this to verify the ELISA results.

Badnavirus

After DAV, the next most common group of viruses detected by ELISA in the samples from the South Pacific was the badnaviruses (either DABV or DBBV or both). In order to start to assess the specificity and reliability of the two badnavirus antisera, a number of badnavirus PCR primers were tried using DNA from the ELISA-positive samples as template. Only primers BadnaF and BadnaR (Yang *et al.*, in press) have consistently primed PCR amplification of *Badnavirus* DNA (part of the reverse transcriptase gene) from some of the ELISA-positive samples. As in the DAV study, first the genetic diversity within these PCR-positive samples was assessed by digesting the amplicons with restriction enzymes (*Alu*1 and *Taq*1) and comparing the restriction fragment patterns produced. These patterns indicated that many of the samples contained more than one badnavirus sequence type.

The BadnaF/BadnaR PCR products from many of the samples were cloned and then sequenced, and the aligned sequences were analysed to produce a phylogenetic tree (Figure 2). The sequences from yam samples group into about 12 distinct clusters, each as different from the other sequences from yam as they are from the other *Badnavirus* sequences included in the analysis (*Sugarcane bacilliform virus* [SCBV], *Rice Tungro bacilliform virus* [RTBV],

Cocoa swollen shoot virus [CSSV], *Commelina yellow mottle virus* [CYMV]). The high level of dissimilarity between the clusters suggests that each cluster represents a different yam *Badnavirus* species, though in the light of these results, it may be necessary to reassess the definition of what delimits a different *Badnavirus* species. Several of the yam-derived sequences cluster more closely with Banana streak virus (BSV) sequences, and we can start to speculate whether there has been convergent evolution of yam and banana species, or whether there has been horizontal transfer (transmission) of virus from banana to yam, and/or *vice-versa.*

The complexity of the yam *Badnavirus* sequence phylogenetic tree (Figure 2) means that it is difficult to assess the significance of any apparent association between particular sequence clusters (species) and geographic or host species origin. All the sequences from African samples fall in one loose cluster that contains mainly sequences from *D. rotundata* and *D. alata* samples. Other sequences, such as those from *D. trifida* or *D. nummularia* samples group separately. More sequences from different host species and different countries should be included in the analysis to obtain a better understanding of the significance of the molecular genetic variability within the *Badnavirus* genus.

Amino acid sequence analysis (translated from the nucleic acid sequences) reveals that some of the amplified sequences have nine or ten amino acid deletions that would render the gene product (the reverse transcriptase) inactive. This was the first indication that some of the *Badnavirus*-like sequences amplified from the yam samples might not have been derived from active virus. I.e. the plants may contain virus-like sequences in a form other than active virus. Work by Dr Susan Seal during her secondment at CIRAD Montpellier has now confirmed that there are *Badnavirus*-like sequences integrated into the genomes of some (possibly all?) yam varieties. This finding has significant implications for the development of appropriate virus indexing procedures for use in ensuring the safe movement of yam germplasm. A summary of Dr Seals work and findings from her period in Montpellier is attached as Annex 1.

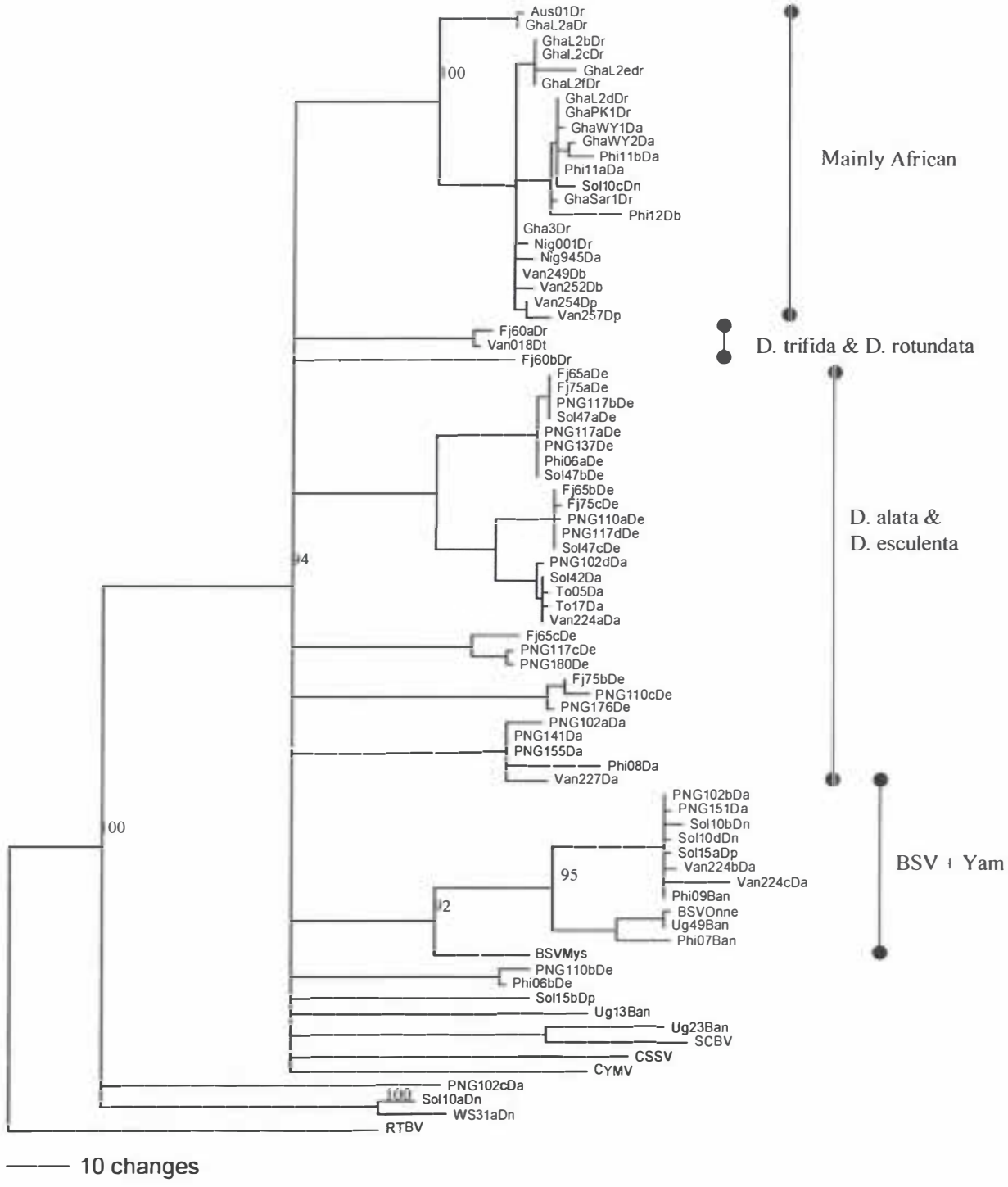

Figure 2. Phylogram for aligned badnavirus deduced amino acid sequences estimated by maximum parsimony with heuristic search. For each yam and banana sample, a region of the reverse transcriptase gene was amplified and sequenced using BadnaF/BadnaR primers. Genebank sequences for CYMV, CSSV, RTBV and SCBV were included to root the tree. Branch lengths are proportional to percentage dissimilarity, and numbers at branch points represent bootstrap % values (from 100 replications).

Tissue culture and virus elimination by chemotherapy and thermotherapy

Some of the NRI effort in 2001 was aimed at improving the rate of establishment of node cuttings of South Pacific yam accessions in tissue culture. Plants were grown in the quarantine glasshouse at NRI from small tuber pieces received from Vanuatu and Papua New Guinea. Nodes taken from these plants were surface sterilised and transferred to a range of tissue culture media of different composition and incubated in a controlled light and temperature room (14 h light at 29°C and 10 h darkness at 25°C). The medium that gave the most consistent establishment of plantlets was composed of Murashige and Skoog (MS) basal medium supplemented with 20g/l sucrose, 0.5mg/l 6-benzyl-aminopurine and 20 mg/l cycteine, and solidified with 2g/l phytagel. However, despite using this medium and taking great care over the surface sterilisation of nodes, several accessions failed to establish in tissue culture. Many nodes simply failed to grow and were subsequently overgrown with what were believed to be endophytic fungi, bacteria or yeasts.

The medium that gave the most consistent regeneration rates for meristem tips was composed as follows: Murashige and Skoog basal medium, 30 g/l sucrose, 20mg/l cysteine, 100mg/l inositol, 80 mg/l adenine, 0.2 mg/l naphthaleneacetic acid (NAA), 0.15 mg/ml 6-benzyl-aminopurine, and 0.08mg/l gibberelic acid (GA3). Dissected meristem tips were either placed on filter paper bridges in liquid medium or on the surface of medium of the same composition but solidified with 2g/l phytogel.

Attempts to establish *in vitro* cultures of yam from meristem tips ran into the same problems of lack of growth and contamination, as occurred with the node cuttings. Even plantlets that did appear to have established well *in vitro* often were killed by the suddenly emergence of contaminating organisms. A selection of Fiji yam accessions recently transferred to tissue culture at SPC-Fiji was carried back to NRI for sub-culturing and virus indexing. Disappointingly, within three weeks of transfer all had died due to contamination with fungi and bacteria.

Once the conditions for tissue culture had been established at NRI, tests were started on the incorporation of viricides into the tissue culture medium to promote the elimination of virus infections. Stock solutions of the viricides quercetin dihydrate (Sigma Q0125) and ribavirin (Sigma 9644) were made up in dimethylsulphoxide (DMSO) and incorporated into either node cutting or meristem tip medium at a final concentration of 10mg/l. Virkon (Scientific Lab Supplies, UK) was made up in water and filter sterilised before adding to tissue culture medium at a final concentration of 1g/l. Unfortunately, very few meristem tips regenerated when placed on/in medium with viricides and all the nodes that grew on quercitin or ribavirin medium still were positive for DAV when tested by RT-PCR. 20% of nodes of accession PNG189 surviving on Virkon medium tested negative for DAV by RT-PCR on first testing, but these plantlets have subsequently died. Table 3 lists the South Pacific yam accessions being held in tissue culture at NRI as of 20/01/03.

Preliminary results suggested that thermotherapy, whereby plants are grown at an elevated temperature (>34°C) in controlled conditions prior to young nodes or meristems being transferred to tissue culture, could be an efficient means of eliminating DAV from infected yam accessions. Up to 40% of nodes that regenerated in tissue culture following this treatment tested negative for DAV on first screening.

A procedure whereby lengths of yam vine were heated by passing an electric current through them, known as "Electrotherapy" was also investigated. Some nodes on these vines reached temperatures up to 39°C during this treatment. When treated nodes were transferred to tissue culture, a small proportion of the surviving plantlets tested negative for DAV at first testing.

All the methods tested for virus elimination were based on regenerating plantlets in tissue culture from treated nodes or meristems taken from plants infected with DAV. Because of lack of time and human resources, the plantlets that regenerated in the experiments described above were tested for DAV by RT-PCR while still *in vitro* (after only about 2 months growth). However, it is well known that virus titres are often very low in newly established tissue culture plantlets, and it is often only after the plantlets have been grown on in soil for several months that the virus titre increases again to detectable levels. Thus, the results reported here should be interpreted with some caution.

Table 1. South Pacific *Dioscore*a accessions held in tissue culture at NRI as of 20/01/03 and transferred to the SPC regional Germplasm Centre on 25/01/03

TC ID*	Original ID	Variety name
NC 002	NC 002	Noumea Rouge
NC 003	NC 003	Koupet
NC 004	NC 004	Noumea Rouge
NC 007	NC 007	Louis"241"
PNG 183	Y008	Napo Goning
PNG 184	GL051	Kisi
PNG 185	RL009	Mui (female)
PNG 186	Y006	Barai
PNG 187	GG013	Kusin de
PNG 188	CMG001	Naso
PNG 189	MFA004	West Fergerson 1
PNG190	Y004	Gai
PNG 191	GL050	Kaiore rau
PNG 192	MFA005	West Fergerson 2
PNG193	GL049	Tobo
PNG194	PNG194	??
PNG195	GG005	Simbang
Van 004	Da 0003.1	Tuber 3
Van 005	Da 0004.1	Tuber 4
Van 070	VU401	Basa
Van 085	VU424	Buntun ankapkap
Van 087	VU426	Warereo
Van 127	VU480	Patapata
Van 145	VU500	Not
Van 208	VU578	Letslets nambas
Van 226	VU597	??
Van 232	VU603	Lakon
Van 252	VU632	Bwev Mudani
Van 284	VU675	??(bulbifera)
Van 311	VU630	Bwevu

* Prefix NC = New Caledonia, PNG = Papua New Ginea, Van = Vanuatu

Summary of findings and implications for germplasm movement in the South Pacific

DAV (*Potyvirus*) was the virus most commonly detected by serology (ELISA) in the important yam species (*D. alata, D. esculenta, and D. bulbifera*) from across the South Pacific region. The DAV antiserum gave positive reactions in ELISA with samples from all the countries included in the study. RT-PCR was generally more sensitive than ELISA for detecting DAV. However, although primer pair DAVCP01F/Oligo d(T)-R is capable of detecting many of the DAV strains present, other primer pairs have also to be used to be sure of detecting all strains of DAV. Sequence analysis suggests that there are many different strains of DAV present in the South Pacific region, but there is no strong association between particular sequence types (strains) and geographic or host origin.

Antiserum designated as anti DDV (*Potyvirus*) also commonly gave positive reactions when used in ELISA with South Pacific samples. A chi squared test (χ^2) indicated that the frequency of samples testing positive for both DAV and DDV was higher than would be expected by random chance ($p < 1.5\%$) suggesting that either the DDV antiserum was cross-reacting with some DAV epitopes/strains, or that plants infected with DAV are more prone to being infected with DDV (or *vice-versa*). *Potyvirus* sequences obtained from DDV-ELISA positive samples clustered with DAV sequences in phylogenetic analysis, further supporting the suggestion that the DDV antiserum is detecting a subgroup of DAV strains. None of the *Potyvirus* sequences obtained from the south pacific samples clustered with what have been designated as DDV sequences from Sri Lanka and Bangladesh, and it may be that these sequences represent a different (contaminating) virus in the Sri Lanka and Bangladesh samples.

The antiserum against DLV (*Potexvirus?*) gave many positive reactions when used to test the South Pacific samples. However, when RNA from some of the DLV ELISA positive samples was tested by RT-PCR with universal *Potexvirus* primers (van der Vlugt and Berendsen, 2002), no amplicons were produced. Thus it is still uncertain what the DLV antiserum is detecting; perhaps DLV is not a typical *Potexvirus*. Recently, a *Potexvirus* that does not appear to react with the DLV antiserum was detected by RT-PCR in a yam sample from Guadeloupe (Denis Filloux, CIRAD, personal communication, January 2003).

The large number of samples that tested positive with both the DABV and DBBV antisera in ELISA suggests that these two antisera recognise some epitopes common to some strains of both these Badnaviruses. Also the incidence of samples positive for both DBBV and DLV by ELISA was higher than would be expected by random chance, and it is likely that the DBBV antiserum has affinity for some DLV strain epitopes. This suggestion is further supported by the observation that the DBBV antiserum can appear to trap significant numbers of filamentous particles (that might be DLV) when used in immunosorbent electron microscopy with some samples. Recently, a cocktail of different antisera against different strains of *Banana streak virus* (another *Badnavirus*) has been compared with the DBBV and DABV antisera in ISEM and ELISA with some yam samples from the South Pacific and some from Guinea. The results indicated that there are other strains/serotypes of *Badnavirus* in yam that are not recognised by the DABV or DBBV antisera. Sequencing the products of PCR using universal Badnavirus primers (Badna-F and Badna-R) and DNA from yam samples as template confirms that there are many (at least 12) different *Badnavirus*-like sequence clades detectable in yam samples. These clades are sufficiently different for some people to regard them as representing different *Badnavirus* species; others regard them as representing different strains. Further complication is added by the finding that some of the *Badnavirus*-

like sequences obtained from yam samples display 27-30 nucleotide deletions which would result in the translated gene product (reverse transcriptase) being inactive. This was the first indication that yam may contain *Badnavirus*-like sequences not associated with active *Badnavirus*, and recent findings have confirmed that there are *Badnavirus*-like sequences integrated into the genome of some yam varieties. Further study is required to determine if these integrated sequences can recombine to form active virus, as is the case for some integrated BSV sequences. Either way, it means that reliably indexing for active *Badnavirus* in yam accessions to permit germplasm exchange is not currently possible since probably even a cocktail of all available antisera will not detect all strains of *Badnavirus*, while PCR with universal *Badnavirus* primers is likely to result in many "false" positive reactions due to amplification of integrated sequences.

YMV (*Potyvirus*) was commonly found in *D. rotundata* plants from Africa, but has not been conclusively demonstrated to be present in the South Pacific region. The triple antibody sandwich ELISA using antiserum from IITA Nigeria is very sensitive, but still it is probably safest to test accessions both with this ELISA and by RT-PCR before allowing germplasm export or exchange.

CMV (*Cucumovirus*) has a very wide host range, and some (probably many) strains will infect yam in the South Pacific region. However, the genetic/serological variability of CMV strains means that as yet no single antiserum can be used with certainty to detect all the strains of CMV that could be present in the South Pacific yams. Again several different PCR primer pairs have been designed for detection of CMV by RT-PCR, but more work is required to determine if any of these is capable of detecting all strains of the virus.

Both antisera against JYMV and CYNMV from Japan gave some apparent positive reactions when used in ELISA on some samples from the South Pacific. PCR primers for the specific detection of JYMV have recently been published, and it has recently been shown that CYNMV is a *Macluravirus*, so it should be possible to design universal *Macluravirus* PCR primers that could be used in the first stages of developing CYNMV-specific primers. As yet there has been insufficient time or human resources to develop these PCR diagnostics for these viruses, so the ELISA results have not been corroborated by any other technique.

Meristem tip culture and thermotherapy have previously been reported as being useful methods for eliminating virus infections from yam accessions. Unfortunately, it took much longer and more effort than expected to find the conditions required for the establishment of South Pacific yams in tissue culture. Preliminary results from this study using DAV-infected plants indicate that electrotherapy (heating meristems by passing an electric current through them) may also increase the rate of virus elimination. However, much more work is required to substantiate these findings since indexing of the plantlets was done while they were still very young, and it is well recognised that virus titres are often very low in newly established tissue culture plantlets. It is often only after the plantlets have been grown on in soil for several months that the virus titre increases again to detectable levels. Also, only the elimination of DAV was studied here; elimination of other viruses may require different approaches since other viruses may be more resilient or may be confined to different tissues of the plant.

One of the aims of the SPYN project was to get to the stage where selected elite yam germplasm could be exchanged between the South Pacific countries for evaluation and promotion. This virology component of the project has shown that this exchange will not be

so straightforward because of the prevalence of a wide range of viruses infecting yam in the region. Despite the great diversity and genetic variability of the viruses (and virus strains), diagnostic tests are now available for several of the viruses, though it would generally be safest to use two different tests (e.g. ELISA and PCR) for each virus. The original plan was that selected yam accessions would be established in tissue culture either at SPC Fiji, or at NRI and then transferred to NRI. Here they would be subcultured and a proportion of the cultures of each accession would be transferred back into soil in the quarantine glasshouse. Each plant would then be tested after 3 and 6 months growth in soil with the best tests available for each virus. Only the accessions for which all the glasshouse-grown plants tested negative for all viruses would be further subcultured in tissue culture, and then returned to the SPYN partners as *in vitro* plantlets. However, because of delays in sending tubers to NRI from the South Pacific, and then the difficulties mentioned above in establishing many of the accessions in tissue culture, there is no time left in the project to continue this work at NRI. Discussion is underway as to how the tissue-culturing and virus therapy can continue at the SPC Regional Germplasm Centre (SPC-RGC) with virus indexing being carried out at a proposed SPC – USP plant biotechnology and virus indexing lab at USP, Suva.

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2.2- Studies on badnaviruses infecting yams

CIRAD-NRI Collaborative project : Poste d'accueil CIRAD: 5/3/2001- 8/7/2002

Dr Susan Seal, NRI, University of Greenwich at Medway, UK

Background

Yam plants are generally propagated through their tubers and this has resulted in the acumulation of viruses. Two viruses of the family *Potyviridae* (YMV and DAV) are reported to be the most widespread and economically important viruses worldwide. However, the relative importance of potyviruses may have been distorted by potyviruses being more easily detected than the other viruses infecting yams such as badnaviruses, cucumoviruses, potexviruses and comoviruses. Yam badnaviruses, due to their very low virus titres and ability to not produce any clear symptoms often go undetected. The latter together with the extreme heterogeneity of badnaviruses and yam being an under-researched crop, have resulted in a very scarce knowledge of this group of viruses.

Badnavirus particles were first reported in yam in association with a flexuous virus, causing internal brown spot (IBS) disease in *D. alata* and *D. cayenensis* in the Caribbean. Badnavirus particles isolated in *D. alata* from Nigeria were partially characterised and named as *Dioscorea alata badnavirus* (DABV). DABV was found to be transmitted mechanically and also by the mealybug *Planococcus citri* from *D. alata* to other *Dioscorea* species. DABV is the only yam badnavirus for which the complete nucleotide sequence of 7.4 kb has been published. However the presence of another yam bacilliform virus was indicated by DABV being serologically related to a badnavirus from *D. bulbifera* named as *Dioscorea bulbifera badnavirus* (DBBV).

Badnaviruses have received increased research attention since the discovery that they can be present as integrated sequences in some plant host genomes. *Banana streak virus* (BSV) integrated sequences have been demonstrated to be able to lead to BSV infections when activated by stresses such as tissue culture. The possible phenomenon of integration of badnavirus sequences in the yam host genomes together with their high genetic variability complicates the development of reliable indexing tests.

Virus infections currently paralyse yam germplasm movement worldwide and thus hinder international exchange of selected varieties. This study based at CIRAD (Montpellier) was initiated to tackle this problem by improving our knowledge of yam badnaviruses and the detection thereof. The specific objectives of Susan Seal's "poste d'accueil" at CIRAD (led by Dr Jean-Leu Marchand) were as follows:

- To screen the CIRAD yam collection for badnaviruses, concentrating on samples from West Africa and the Caribbean
- To obtain sequence information from a wide range of badnavirus isolates
- To use the sequences obtained to develop PCR primers for badnavirus detection, and to then adapt these primers to NRI room-temperature stable «kit» format
- To carry out preliminary studies to determine if badnavirus sequences have integrated in to yam plant host genomes

The outputs of these objectives were expected to not only result in improved diagnostic tests for yam badnaviruses, but also generate a mutual transfer of expertise between NRI and CIRAD.

Results

Seven different primer pair combinations for yam badnaviruses were tested on known badnavirus-infected yam DNAs from Africa and the South Pacific to determine which set was most suitable for detecting badnavirus infections. Only primer pair BadnaF/R was found to detect all badnavirus strains, and was selected as the best primer pair with which to screen CIRAD yam plants. Total DNAs were prepared from selected CIRAD samples using Qiagen DNeasy kits and screened for badnavirus sequences by PCR. Due to the number of Badna F/R positives being exceptionally high (48 out of 60 samples) it was decided that confirmation of these infections should be sought through use of diagnostic techniques that detect virion particles such as ELISA and immunosorbent electron microscopy (ISEM). Seventeen samples were examined by all three techniques (there was insufficient leaf material and time to perform more ISEM tests) and results for these samples are shown below.

Sample	Origin	*Dioscorea* species	Badna PCR	ISEM	BenL ELISA	DaBV ELISA	DbBV ELISA
A 103	Burkina Faso	Pilimpikou	++	-/-	-	-	-
A 105	Burkina Faso	Pilimpikou	++	-	-	-	-
BF 54	Burkina Faso	Pilimpikou	++	-	-	-	-
CFP R1	Martinique	*D. alata*	-	-	-	-	-
CFP C3	Martinique	*D. alata*	-	-	-	-	-
B 39	Benin	*D. sansibarensis*	+	++	++	++	+++
Cuba 1	Cuba	*D. alata*	+	+low	-	-	-
Guinée 35	Guinea	*D. cay-rotundata*	+	+	+++	+++	+++
Guinée 43	Guinea	*D. cay-rotundata*	+	+	++	+	+
Guinée 44	Guinea	*D. cay-rotundata*	+	+	++	+	+
Guinée 50	Guinea	*D. cay-rotundata*	+	++	++	++	++
Guinée 84	Guinea	*D. cay-rotundata*	+	++	+	++	++
Guinée 144	Guinea	*D. cay-rotundata*	+	-	+++	+	-
Guinée 155	Guinea	*D. cay-rotundata*	+	++	+	-	-
Guinée 158	Guinea	*D. cay- rotundata*	+	++	-/+*	-	-
Guinée 163	Guinea	*D. cay- rotundata*	+	++	-	-	-
Guinée 164	Guinea	*D. alata*	+	++	+	+	+

** different results obtained in repeat ELISA runs*

PCR, ISEM and ELISA are in agreement for samples CFPR1 and CFPC3 not containing virus particles or genomes, and samples B39, Guinea 35, 43, 44, 50, 84, 144 (ISEM negative, but ELISA positive) and 164 being badnavirus-infected. Pilimpikou samples from Burkina Faso were positive by PCR but no badnavirus particles were detected by ISEM or ELISA. Sequences generated from these PCR products confirmed that the PCR amplicons did represent badnavirus sequences and so were not false positives. This suggested that these samples represented either very weak infections only detected by the very sensitive PCR technique, or that these plants contained badnavirus sequences integrated in their genomes.

The best antibody for badnavirus detection was the general badnavirus polyclonal mixture produced in Ben Lockhart's laboratory (BenL). However this antisera nevertheless failed, using the PAS-ELISA format, to detect badnavirus infections in *D. alata* sample Cuba1 and *D. cayenensis-rotundata* samples Guinea 163 and 158 (in some tests). Badnavirus infections

were not detected using antisera DaBV and/or DbBV in Guinea 144, 155, 158 and 163. Hence none of these antisera can be relied on being used in ELISA format as a definitive diagnostic test for yam badnavirus particles.

ELISA

Many of the samples tested for badnaviruses were also screened for the presence of another six yam viruses (potyviruses YMV, DAV, DDV, and JYMV, potexvirus DLV, and CNYMV thought to be a macluravirus) using available antisera and either TAS-ELISA (for YMV) or PAS-ELISA (for all other viruses). Results (Table 1) show that overall badnaviruses are the most prevalent (94/159 samples), followed by DAV (27/144 samples) and then YMV (10/144 samples). The prevalence of the viruses is, however, biased by the origin of samples with 112 out of the 159 samples screened being from West African *D. cayenensis/rotundata*. The low level of DAV in these samples is therefore not surprising as DAV is only found occasionally in *D. cayenensis/rotundata* samples (unpublished data, NRI). As the name suggests, DAV's common host is *D. alata*, supported by 70% of tested Vanuatu samples (predominantly *D. alata*) scoring as DAV-ELISA positive. This is very similar to previous results of 65% obtained at NRI for 287 Vanuatu samples tested from the EU SPYN project's collection. The percentage of badnavirus infections is also very similar with NRI finding 16% infection in 287 samples and 16.7% of 30 samples scoring positive in this study.

YMV is reported to be very prevalent in West Africa. However it is only present in 11% of Guinea samples and around 4.5% of Benin samples tested in this study. This is thought to be due to this virus causing pronounced mosaic symptoms and attempts presumably having been made by collectors to select healthy-looking YMV-free material.

The BenL antisera is a mix of around 30 different polyclonals to "non-yam badnaviruses", but nevertheless a better antisera for detecting yam badnavirus infections than the two yam badnavirus polyclonals DaBV and DbBV. The latter both only react with some yam badnaviruses, whilst the DbBV antiserum was shown by ISEM to also react with filamentous particles, and so is of little diagnostic use. The DaBV antisera reacts with some samples (Vu567a, Vu579a, Benin19) that are missed by the BenL polyclonal mix, and hence it is essential to screen with both antisera.

The diagnostic value of ELISA is highlighted by one (Guinea 1563) of the two *D. abyssinica* samples tested scoring BenL- and DaBV-ELISA positive, but PCR negative. Although there was insufficient leaf material available of this wild yam isolate to carry out ISEM, results with two different ELISA tests suggest that the PCR result is wrong. The DNA extracted from this sample was high in PCR-inhibitory substances and had to be diluted a further ten-fold to get a clear PCR result. It is therefore possible that the PCR, even on the diluted DNA, was not working effectively and hence insufficiently sensitive.

Analysis of the ELISA results obtained with antisera to DDV, DLV, JYMV and CNYMV is futile as their sensitivity and specificity was not determined, and the reactions observed were generally weak or non-reproducible (Guinea 14 and 22 scored positive in their first screening with JYMV, but negative on retesting). It was not determined whether the non-reproducible results were due to non-specific cross-reactions or an uneven distribution of the reacting virus in the leaf samples.

Table 1: Summary of BadnaF/R PCR and ELISA results for CIRAD-IRD yam plants.

Yam collection	Badna PCR	BenL	DaBV	DbBV	YMV	DaV	DDV	DLV	JYMV
IRD Africa	13/13	3/11	1/11	1/11	1/6	0/6	0/6	0/6	0/6
C/S. America	21/32	3/17	1/17	1/17	0/7	6/7	0/7	0/7	0/7
Guinea	14/15	74/78	52/78	51/78	8/78	2?/78	2?/78	8?/78	5/78
Vanuatu	4/9	5/30	2/30	2/30	0/30	21/30	0/30	0/30	0/30
Benin	10/10	9/23	10/23	2/23	1/23	0/23	2/23	0/23	1/23
Totals	**62/79**	**94/159**	**66/159**	**57/159**	**10/144**	**27/144**	**2/144**	**8?/144**	**6/144**

CNYMV was not detected in any of the 115 samples tested.

To obtain sequence information from a wide range of badnavirus isolates

Prior to this study, Benedicte Lebas had obtained 50 partial ORFIII sequences (579 bp amplified by BadnaF/R primer pair) from 28 samples from the South Pacific, seven African samples from Nigeria and Ghana, and one Australian sample. No sequence information had been determined or published for any Caribbean or South American yam badnaviruses. Forty seven readable BadnaF/R sequences were obtained from the CIRAD-IRD yam collection representing Caribbean and African sequences and 12 new host-country origins. Three sequences were also obtained from a wild *D. sansibarensis* plant collected in Benin.

FASTA3 software was used to compare all sequences obtained to those present in the EMBL genebanks and retrieve those sequences with the highest level of nucleotide identity. All sequences, but one, had the highest level of identity with either DaBV sequences or other badnaviruses (generally BSV or CitYMV). One *D. alata* clone from Guadeloupe, had the best EMBL bank match with *Oryza sativa* genomic DNA (62.5% identity) but this rice sequence represents a stretch of rice DNA known to be very similar to the ORF3 protein of rice tungro bacilliform virus. Further interest in this *D. alata* sequence is generated by it being 6 bp shorter than expected, and from an ELISA-negative plant. Hence this sequence either represents a distinct *Caulimoviridae* family virus, or is an integrated sequence of viral origin.

To carry out phylogenetic analyses of the yam badnavirus samples

Phylogenetic analyses were carried out on the sequences together with the following sequences from closely related viruses as comparison: *Banana streak virus* (BSVOnne; BSVMys), *Cocoa swollen shoot virus* (CSSV), *Commelina yellow mottle virus* (CoYMV), SCBV, *Rice tungro bacilliform virus* (RTBV), and *Citrus yellow mosaic virus* (CitYMV). Nucleotide sequences were aligned using the clustering software CLUSTAL (W1.82) and the grouping obtained from this is shown in Figure 1. The coding system used for samples is that the first two letters denote the country of origin, the middle letters are an abbreviation of the actual sample name, and the last two letters denote the *Dioscorea* host species.

Sequences were found to fall into 16 groups each sharing less than 80% nucleotide identity with the other groups. Sequences belonging to up to three different groups have in one case originated from a single leaf sample (Gn163Dr). A greater association of groupings was observed to *Dioscorea* host species (e.g. groups A, I, J and N) than with geographical origin. The yam badnavirus groups showed greater identity to other yam virus groups than they did

to non-yam badnaviruses, with the exception of Groups I, J and L showing identities slightly closer to BSV, CitYMV and CSSV than some of the other yam badnavirus groups.

Two of the 16 sequence groups, Groups O and P, may not represent badnavirus sequences, as they share less than 60% nucleotide identity with the other yam sequence groups. They share a similar level of identity with RTBV. RTBV is not considered as a *Badnavirus* member but forms a separate group within the *Caulimoviridae* family. Consequently, isolates belonging to Groups O and P might represent previously uncharacterised viruses within the family *Caulimoviridae*. Further sequencing as well as biological characteristics need to be obtained to determine if these groups do represent novel viruses or are divergent integrated sequences.

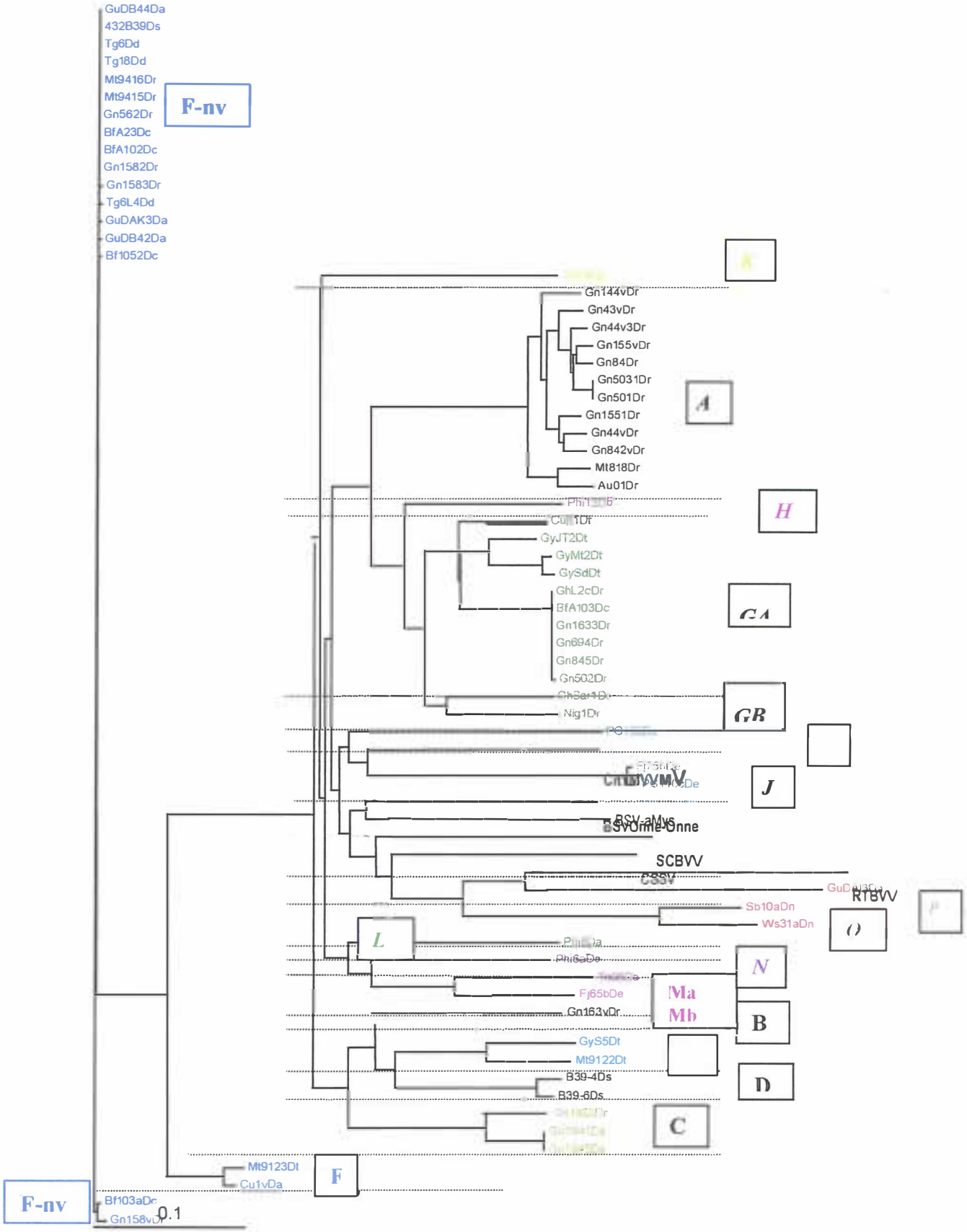
The high sequence similarities between samples from different countries (e.g. Group J, *D. esculenta* from Fiji and Papua New Guinea) or even continents (Mt818Dr and Aus01Dr to Guinea material), indicates past exchange of badnavirus-infected yam plants or tubers.

The RT-RNaseH region of the badnavirus genome analysed above represents only around 530 bp of the total DNA genome of around 7400 bp. It would be preferable to study sequences from more of the genome and also from different genes that did not suffer from the possible disadvantage of having potentially high homologies with putative yam plant retrotransposon sequences.

As described above it was desirable to get non RT-RNaseH badnavirus sequences for the diverse range of sequence groups obtained. Using the sequence information available, primers were designed in reverse orientation to try to amplify up the remainder of the circular badnavirus genome. Primers were designed to amplify up badnaviruses in Guinea 155, Guinea 163 and B39, as these sequences all shared <75% nucleotide identity with the DaBV sequence present in the genebank. A great deal of optimisation of reaction conditions, eventually led to the amplification of ~7kb bands for all three viruses. Cloning of the long PCR products was achieved using an Invitrogen Topo-XL kit exactly according to the manufacturer's recommendations.

Sequences obtained to date indicate that the B39 clones are the desired product but that for Guinea 155 a sequence with genetic rearrangements and non-badnavirus DNA has been amplified. Screening of further clones for this sample and Guinea 163 is still in progress with the assistance of Dr Emmanuelle Muller (leader of CIRAD ATP badnavirus project).

Figure 1. ClustalW (version 1.82) phylogenetic tree of partial RT-RNaseH sequences generated from yam samples, and sequences of BSV, CitYMV, CSSV, RTBV and SCBV.



To use sequences obtained to develop PCR primers, and a diagnostic kit

One valuable component to PCR tests is immunocapture (IC)-PCR as it avoids the need to extract DNA (or RNA for RT-PCR) and can assist in concentrating virus particles on the sides of PCR tubes using the bound antisera. Effective immunocapture protocols have been developed at CIRAD for banana badnaviruses and at NRI for yam potyviruses. The lack of antisera that detects all yam badnaviruses currently complicated the development of IC-PCR protocols to detect these badnaviruses. Moreover, the CLUSTALW alignment of all BadnaF/R sequences revealed that there was too much diversity in yam badnaviruses to design improved or more specific PCR primers for their detection. Primers to detect the different groups of strains could be designed but this is not considered to be of value, especially if knowledge on which sequence groups represented integrated sequence is lacking. Hence the latter area was investigated further.

Studies to determine if yam badnavirus genome can integrate

The existence of PCR-positive, but ISEM/ELISA negative results suggests that these plants may contain integrated badnavirus sequences. Further support for this was given through some of the sequences generated clearly containing either deletions resulting in non-functional viral replicases (Bénédicte Lebas' results) or genetic rearrangements. To determine if such non-functional sequences were present in viral or plant genomes, nucleic acid hybridisations were undertaken. Several membranes and probes were prepared. Results indicated that the sequences represented by groups B and D did represent viral particles and no plant sequences hybridised to these probes.

However for other probes selected from groups A, F-nv, and GA, the probes hybridised to both viral and plant bands. The inclusion of plants without viral particles allowed evidence of plant sequences with >85% homology to badnavirus RT-RNaseH sequences. Together with sequence information obtained, these sequences have been denoted as integrated badnavirus sequences. However it was not possible to determine whether they represented dead integrants or activatable sequences that could pose a threat to yam germplasm health and movement.

Transfer of techniques to CIRAD staff

Training was given to Denis Filloux and Gemma Arnau in TAS- and PAS-ELISA, immunocapture reverse transcription PCR (IC-RTPCR) methods for the potyviruses, and in the BadnaF/R PCR.

Publications

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Lebas, B.S.M., Seal, S.E., Kenyon, L. (??) Diversity of yam potyviruses. (Archives of Virology??)

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Executive Summary

A primary objective of the present study was to investigate sources of infection of anthracnose of yams. The results demonstrate that under high humidity and optimum temperature, yam isolates of *Colletotrichum gloeosporioides* were pathogenic to a number of other crop species. Equally, isolates derived from chilli, cassava, kava, *Mikania micrantha*, pigeon pea and yard long bean were also capable of inciting typical anthracnose lesions on yam. These cross-infections reveal that *C. gloeosporioides* is a polyphagous (non-host specific) pathogen with a wide host range. It thus appears that in nature the disease not only spreads from non-yam hosts to yam but also yam crops could act as sources of infection to other non-yam host species in close proximity to yam cultivations. The results of the present study also clearly demonstrate that in both Vanuatu and Papua New Guinea, *C. gloeosporioides* is able to infect yam tubers under field conditions. This demonstrates that these tuber infections could act as primary sources of inoculum from season to season. It further highlights the risk that through tuber-borne infections, the pathogen could inadvertently disperse from region to region.

A second major objective of the current study was to investigate the variability of *C. gloeosporioides* isolates from yam, and other hosts, by linking morphology and DNA fingerprinting to their pathogenicity. The results of this study revealed that *C. gloeosporioides* isolates are highly variable. At the morphological level, isolates of *C. gloeosporioides* were able to cluster into three main groups that were consistently distinguishable by their colony and conidial characteristics and on their ability to produce sexual reproductive structures. Strong similarities were observed between these morphological groups and with the clusters generated by DNA fingerprinting obtained by using ISSR-PCR. However, only a limited correlation was detected between morphological types and those groupings based on AFLP analysis. There are some similarities between patterns produced by ISSR-PCR and AFLP techniques.

Significant ($P<0.001$) differences in pathogenic ability were found amongst *C. gloeosporioides* isolates. However, there was no clear relationship linking isolate pathogenicity to morphological and molecular data.

Finally, the genetic variability has no clear linkages either to host or to geographic origin. Despite the existence of notable genetic diversity amongst the isolates within a given location, the close genetic relatedness observed among some isolates from different South Pacific countries indicates that *C. gloeosporioides* populations in these islands are not entirely distinct populations.

Background

The greater yam is the most widely distributed *Dioscorea* species in the humid and semi-humid tropics. It is an important food in the Pacific Islands and the Caribbean, where it has considerable social and cultural importance, and it is also a crop of significance in parts of upland Asia (Coursey, 1967). Traditionally, farmers maintained a wide range of genetic

diversity, growing yams in small areas of land that were relatively isolated one from the other. As human populations have expanded in recent years, pressures on land availability have increased and yam diseases, particular anthracnose, caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., have intensified. In addition, yams need to be staked, but suitable materials are often in short supply and labour costs are high. It has been estimated that harvesting alone can account for 20 per cent of total production costs (Leach, 1988). As a consequence of all these factors, production is in decline (Lebot, 1992).

The potential of the crop is realised by many countries in the Pacific: it has good nutritional qualities and, unlike other root crop staples, the tubers can be stored for several months which is important for food security. Countries are keen for research and development on traditional root crop staples, yams in particular, as it is a much neglected crop. At a meeting of Ministers of Agriculture of the Pacific ACP countries in Suva, Fiji, 8-9 October, 1996, Recommendation 4 stated, that they resolve to put in place both in their countries and through regional co-operation, policies to conserve, protect and best utilise their plant genetic resources. One of the ways of doing this is to build on the work that has been done on genetic resources in the past.

Collections of yam have been made in Fiji, New Caledonia, Papua New Guinea, Solomon Islands and Vanuatu and as part of regional root crop programmes of the 1980s (Jackson, 1994). Most have been described in detail using descriptor lists formulated by the International Board of Plant Genetic Resources (Guarino & Jackson, 1986). In some cases, evaluations have been made for yield and anthracnose resistance (Jackson, 1981; Jackson & Linton, 1983; 1984; Lebot, 1997), and data from Vanuatu also includes an ease of harvest index (Van Wijmeersch & Bule, 1988).

The South Pacific Yam Network (SPYN) project was formed to enhance the competitive position of yams in traditional cropping systems of five Pacific Island countries: Fiji, New Caledonia, Papua New Guinea, Solomon Islands and Vanuatu. The aim is to improve yam production by selecting improved germplasm, using existing germplasm collections (see above), for distribution throughout the region. Improved varietal characteristics include desirable tuber shape, flavour, and resistance to anthracnose.

Yam anthracnose is one of the main causes of economic loss in yam crops worldwide. However, in the South Pacific along with other yam growing countries, the epidemiology of the disease has not been fully elucidated. The causal organism, *Colletotrichum gloeosporioides* is known to have a wide host range (Murdue, 1977; Jefferies, 1990). The disease has been shown to be tuber–borne in both West Africa (Nwankiti & Okpala, 1981) and West Indies (Green & Simons, 1994). However, the role of the alternate hosts of the pathogen needs to be ascertained in order to calculate the risk of infecting clean germplasm distributed to growers. In order to minimise the risk of cross-infection, it is important that the germplasm released to farmers is tolerant to the most virulent forms of the pathogen.

The role of the University of Reading in the South Pacific Yam Network (SPYN) project is to:

- Collect yam and non-yam tissue showing symptoms of anthracnose.
- Isolate *C. gloeosporioides* from sample tissue.
- Test the relative pathogenicity of isolates on yam.
- Develop *Colletotrichum* DNA extraction protocols.

- Study the variability of the pathogen using morphological characters and DNA fingerprinting
- Link patterns in isolate morphology and DNA fingerprinting to pathogenicity in order to identify potential sources of infection and assess variability in pathogen virulence.

Project reports submitted

Before his departure from the University of Reading in January 2001, J Peters submitted 1st and 2nd Annual Report on isolation work, cross inoculation tests by *C. gloeosporioides* on yam and other host species and a complete literature review on *C. gloeosporioides* host range. During the same month, Dr R. Strange of University College London (UCL) whose laboratory was informally subcontracted to carry out the molecular work was delegated to present a detailed midterm review of the project at the 2nd SPYN meeting. July 2001, a six-month report based on preliminary studies highlighting the first clear differences in morphological and cultural characteristics among *C. gloeosporioides* isolates from yam and other host species was submitted.

The molecular work was formally transferred from UCL to CABI-Bioscience, Egham. In April 2002 a 3rd Annual Report consisting of comprehensive studies on morphology, pathogenicity and molecular variability of *C. gloeosporioides* was presented by Dr P. Cannon from CABI-Bioscience at the 3rd SPYN meeting. In July 2002, a six-month report summarising the results on tuber and stem isolations, the final trials of pathogenicity test and host range of *C. gloeosporioides* as well as the use of new additional molecular techniques was submitted.

Materials and Methods

Research activities in the South Pacific

Between January 1999 and January 2000, Dr J. Peters, in collaboration with project collaborators, carried out extensive collections of yam and non-yam leaf samples throughout four of the five Melanesian countries covered by this project (namely in Fiji, Vanuatu, Papua New Guinea [PNG] and Solomon Islands). At each locality, sampling was conducted hierarchically at leaf, plant, field and locality level. Between May and August 2001 limited consignments of tuber samples were sent to Reading from Vanuatu and PNG. During his visit to Vanuatu, Drs P. Cannon and L. Kenyon, collected tuber and leaf/stem trash samples for more *C. gloeosporioides* isolation work.

Research activities in UK

Isolations of *Colletotrichum gloeosporioides*

Isolation from yam and non-yam host leaves and stems.

Small pieces were excised from leaves of yams and a number of other hosts bearing characteristic anthracnose symptoms and then washed in sterile distilled water (SDW) in 200 ml universal bottles for 20 minutes. Following washing, the pieces were surface sterilised for 5 min in a 0.35 % aqueous solution of sodium hypochlorite, rinsed three times in sterile distilled water and dried on sterile Whatman filter papers. Surface sterilised leaf pieces were placed onto 9-cm Petri dishes containing quarter-strength potato dextrose agar (PDA) medium

described in **Table 1.** For each Petri dish four to six leaf pieces were placed with two to three replicate plates per leaf sample. All the plates were incubated at 25 ± 1^{0}C under 12/12 h alternating light/darkness regimes and were examined periodically under a stereomicroscope for growth and sporulation of *C. gloeosporioides*.

To obtain pure cultures, small mycelial blocks were cut from the growing margins of all parent colonies of *C. gloeosporioides* emerging from the leaf pieces. The blocks were re-plated centrally on to fresh plates of the amended PDA medium and incubated again under the above temperature and light regimes. After 5 days, all subcultures from the same leaf were divided into groups based on their morphological structures and cultural characteristics and from each group, the most vigorous and/or heavily sporulating subculture was selected.

To make single spore culture isolates, a sterile bacteriological loop was first moistened by stabbing into the sterile medium. Then with the aid of a binocular microscope, the loop was gently touched on heavily sporulating zones of C. *gloeosporioides* sub-cultures and streaked out onto plates containing fresh medium. The plates were incubated overnight at 25 ± 1 ^{0}C under 12/12 h light/darkness and then germinating single spores were transferred on to the centre of pure fresh medium under a low power dissecting microscope with a sterile fine inoculating needle. From each *C. gloeosporioides* subculture, one single spore was selected as parent culture. These parent isolates were multiplied then preserved as stock cultures in autoclaved mineral oil (paraffin specific gravity 0.84 ml/g, Sigma Chemical Co., Sigma-Aldrich Co., Germany) or SDW in universal bottles without cap liners. A copy of each isolate has been also preserved by means of freeze-drying or liquid nitrogen.

A detailed description of all single-spore isolates throughout obtained including their place of origins, codes, and hosts are presented in **Appendix Table 1.**

Table 1 A quarter-strength potato dextrose agar (PDA) medium employed for isolations of this research work.

Potato dextrose agar (Oxoid Ltd, Baskingstoke, Hampshire, England).	9.5 g
Bacto-Agar (Difco laboratories Detroit, Michigan, USA).	15 g
Streptomycin sulphate* (Sigma Chemical Co. Sigma- Sigma-Aldrich Co.).	0.1 g
Distilled water	1 litre

* To suppress bacteria, streptomycin sulphate was added to cooled molten agar medium.

Isolation from yam tubers

A total of 49 tubers were received from Vanuatu (Van) and Papua New Guinea (PNG) between 2000-2001. All the tubers were washed under running tap water until clean and then dried with clean paper towels. Then each tuber was cut into halves with half subdivided into small cubes of 5-10 mm^3 and surface-sterilised in 3% sodium hypochlorite for 10 minutes. The pieces were then rinsed three times in sterile distilled water, dried on sterile Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, UK) before plating onto Petri dishes containing the medium described in **Table 1**. With six cubes/dish, 6-20 dishes/tuber, depending on the size of the tuber. The Petri dishes were incubated at 25 ±1 ^{0}C near UV-light to induce sporulation and examined periodically for fungal growth and sporulation.

The other set of halves of the tuber samples were grown in 5-litre plastic pots containing sterile Multi-purpose (peat-based) compost and then kept under semi-quarantine conditions in glasshouse with additional heating and light. The plants were watered every other day and monitored weekly for disease development. Leaf materials showing disease symptoms were collected and then cultured as described above to detect the presence of pathogenic fungi.

Identification of isolates

All the isolates were identified following the descriptive keys of Sutton (1980) and of Baxter (1984). In addition 71 of isolates were further confirmed using DNA sequencing whereas the identity of 33 isolates were further confirmed using primers specific for *C. gloeosporioides.*

Morphological and cultural characteristics

Sixty of the 81 isolates listed in **Appendix Table 1** were grown on PDA for detailed study of morphological and cultural characteristics using a descriptive key based on the morphological structures and cultural characters presented in **Appendix Table 2.**

Cultural characteristics

Radial growth

For each of the sixty isolates, four discs of 5 mm-diameter were cut from the colony edge and placed separately to the centre of Petri dishes containing 25ml PDA amended with 0.01% streptomycin sulphate. The Petri dishes were sealed with insulation tape to ensure high relative humidity (RH) and avoid contamination. After 5 days' incubation at 25 ^{0}C under 12 h alternating light/darkness, the linear growth (mm) was measured by measuring each colony across two diameters at right angles to one another. These measurements were then averaged for each colony and mean growth rate was calculated for each isolate.

After ten days, the cultures of all isolates were also visually compared using the characters detailed under categories of mycelium and overall colony appearance in the descriptive key presented in **Appendix Table 2.** The colour of each colony, its mycelial hyphae and colony colour from the reverse were described by using a colour chart for the flora of British fungi (Royal Botanical Garden, Edinburgh, 1967).

Reproductive structures

After 15 days, the cultures of all isolates were again visually examined for the reproductive structures listed in the descriptive key in **Appendix Table 2.**

Conidia, ascospore and appressoria measurements

Finally, the cultures of each of the isolates were washed with sterile distilled water and filtered through three layered muslin cloths before droplets of the conidial suspension were placed on microscopic slides. For each isolate, 30 conidia were randomly selected, their length and width were measured and their conidal shapes were recorded. The length and width of 30 randomly selected ascospores were also measured for each of all the isolates producing perithecia. Each isolate measurements of 30 appressoria were also recorded.

Pathogenicity testing of *C. gloeosporioides* isolates

Pathogenicity of *C. gloeosporioides* isolates on *D. alata*

Tubers of *D. alata* variety, **White Lisbon,** were grown in plastic pots containing a mixture of 1:1 Multiple-purpose and John Innes (loam-based) composts. The plants were then maintained in a polyethylene tunnel for three months to produce sufficient canopy.

Preparation of inoculum and inoculation

Cultures of the 49 isolates listed in **Appendix Table 3** were prepared as described above. After 10 days, the cultures were flooded with sterile distilled water and conidia were further dislodged by sterile bent glass rod before filtering through three layers of muslin cloth to separate the mycelium and conidial fractions. The concentration of conidial suspensions were determined by a haemocytometer, adjusted with SDW to $1x10^6$ conidia ml^{-1} and then supplemented with 0.05% Tween 20 (Polyoxyethylene 20 sorbitan monolaurate, Sigma Chemicals).

Healthy, medium aged leaves were collected, surface sterilised in 0.5% sodium hypochloride for 5 min. and then rinsed in three changes of sterile distilled water. On each leaf, six inoculation sites per leaf were made by wounding the surface of leaf tissues with a sterile needle. The leaves were inoculated by applying a 10µl of conidial suspension on each inoculation site. The control treatments received 10µl of SDW with 0.05% of Tween 20 added. The leaves were then sealed in large plastic sandwich boxes lined with sterile towel papers moistened with sterile distilled water to maintain in a high humidity at 25 ^{0}C. The treatments were arranged in a complete randomised design with six replicates per isolate. This method of inoculation was modified from that described by others (Winch *et al.*, 1984; Vagelas, 1999; Peters, 2000).

Disease assessment

Seven days after inoculation, lesion sizes were determined by measuring two perpendicular diameters for each lesion that were then averaged. Then mean lesion diameter for individual leaves was calculated from the sum of the individual lesion diameters by number of lesions per leaf.

Nine days after inoculation, with the aid of a binocular microscope, in all isolates the numbers of sporulating lesions out of total lesions per leaf were counted. The presence/absence of acervuli and setae as well as perithecial structures of *Glomerella cingulata* were also recorded.

Pathogenicity of *C. gloeosporioides* isolates on other host species.

Plant growth and maintenance

Seeds of okra, chilli pepper and tomato were bought from the local market whereas seeds of cowpea, kidney beans were obtained from stocks maintained in the Plant Environment Laboratory (PEL) at Shinfield, Reading. Seeds of cotton were also received from Syngenta (Jealots Hill, Bracknell, UK) while citrus seeds were extracted from locally purchased citrus

fruits. All the seeds were then planted in 2.5 litre plastic pots containing Roffey Multi-purpose compost (Roffey Brothers Ltd, England). The pots were then transferred in an environmental cabinet (Fi-totron 600, Fisons, England) illuminated with both fluorescent and tungsten lights under 12h light/darkness regimes at 27 ^{O}C.

Preparation of inoculum and Inoculation

Conidial suspensions of the four single-spore isolates: **YTPNGBA-044, DaF9-2, DaV21-4** and **DaPNG-013-4** whose detailed descriptions are given in **Appendix Table 1,** were prepared as the manner described in section 4.1. Then healthy, 3-wk old leaves of each the nine host species presented in **Table 4** were collected, surface sterilised and then inoculated with conidial suspensions of the four isolates in 145mm diameter Petri dishes as described in section 4.1. The treatments were arranged in a complete randomised block design with four replicates per isolate-host combination and then incubated at 26± 2^{0}C.

Disease assessment

Fourty-eight hours after inoculation both inoculated and non-inoculated leaves were assessed using a stereomicroscope on daily basis for symptom development, lesion type and sporulation for up to 3 weeks in a manner similar to that described by Pring *et al.* (1995). Then on each host species, isolates were classified as pathogenic or non-pathogenic.

Molecular fingerprinting

DNA extraction

The 34 isolates presented in **Fig. 6** were grown up in flasks 200ml of potato dextrose broth in a shaking incubator at 25 ^{0}C for 3 days. The resulting fungal growths were washed, dried and then transferred to sterile Petri dishes for storage at –80 ^{0}C. Prior to extraction the isolates were crushed with liquid nitrogen using a pestle and mortar. DNA was extracted using a modified Reader and Broda technique. This involved:

1. Add ground, frozen mycelium to 500-800 µl (depending on amount of material available for the extraction) of extraction buffer.
2. Add 10µl of 20mg/ml solution of proteinase k and 0.5µl of Ribonuclease a solution.
3. Incubate at 37^{0}C for one hour.
4. In a fume hood add 500µl to 800µl of phenol/choroform/isoamyl alcohol and mix gently on the wheel for 10 minutes.
5. Centrifuge at maximum speed for 10 minutes at room temperature.
6. In a fume hood slowly remove the upper aqueous layer and transfer to a clean 2.0ml tube.
7. Add an equal volume (500µl to 800µl) of cold chloroform and mix gently on the wheel for 10 minutes.
8. Centrifuge at 13,000g for 5 minutes
9. Slowly collect the upper aqueous layer and transfer to a 2.0ml tube.
10. Add an equal volume of chloroform and mix gently on the wheel for 5 minutes.
11. Centrifuge at 13,000g for 5 minutes
12. Slowly collect the upper aqueous layer and transfer to a 1.5ml tube.
13. Precipitate DNA with 0.54volume (250µl to 400µl) of cold isopropanol
14. Pipette off as much liquid as possible or centrifuge at 13,000g for 5 minutes.
15. Collect pellet with a short (5-10 second) centrifugation and drain all liquid.
16. Wash pellet in 70% ethanol. dry, and re-dissolve in 20-100µl of TE buffer (depending on yield).

Inter-simple sequence repeat (ISSR) PCR amplication

Having tried variations on a number of reaction parameters, diluting the DNA by a factor of 1/1000 was found to produce good quality banding patterns with the ISSR primers. The primers used for ISSR were 5'-BDB$(ACA)_5$, 5'-BDB$(CGA)_5$ and 5'-BDB$(CCA)_5$ (where B= not A, D= not H= not G). They are degenerate with a random assortment of 3 bases at the 5'end so the primer will target the outside of the microsatellite.

Reactions were carried out on volumes of 20µl, containing 2.0µl of 10x Buffer, 2.0µl of $MgCl_2$ (25mM), 2.0µl of dNTP mix (10mM), 0.5µl of primer (100pmol/µl), 0.2µl of Taq polymerase (5U//µl) and 1.0µl of (diluted) DNA sample. For each primer a negative control was set up in the same volume.

The reaction conditions were the same for all primers apart from annealing temperature which was 46^0C when using the 5'-BDB$(ACA)_5$ primer and 53^0C when using the other two primers. The conditions were as follows; 94^0C for 2 min followed by 35 cycles of 94^0C for 30 s, 46^0C or 53^0C for 60s,72^0C for 2 min and a final step of 72^0C for 10 min. to ensure full strength of final products. To each reaction 2µl of loading buffer was added and 14µl of loading buffer was added and 14µl of the reaction mix was loaded on a 1.5% (w/v) agarose gel and run for 3 hours at 100V and stained with ethidium bromide. The molecular weight marker was the low DNA Mass Ladder from GibCo, band sizes 2000, 1200, 800, 400, 200, and 100 base pairs. The gel were photographed and scanned prior to analysis with Gelcompar.

Modified Raeder and Broda Reagents:

Extraction buffer (EB). A total of 10ml, 6.375ml of sdH20, 200mM tris- HCL (pH8.5) 2ml of 1M 250mM NaCl 625ml of 4M, 25mM EDTA (pH8.0) 0.5ml of 0.5M 0.5% sodium dodecyl sulphate 0.5ml of 10%. [If making up large quantities of EB, mix everything, apart from the sodium dodecyl sulphate, and aliquot into 10mls per tube prior to freezing. Add the sodium dodecyl sulphate when the rest of the EB has reached room temperature, do not freeze with the sodium dodecyl sulphate in the mixture as freezing will cause it to precipitate.]

TE buffer. 10mM Tris-HCL (pH8.0), 1mM EDTA, Cold isopropanol, Cold 70% ethanol, Saturated phenol/chloroform/isoamyl alcohol [Sigma 100ml of P2069].

Ribonuclease solution: Ribonuclease A (70U/mg) made up at 20mg/ml in TE and boiled for 5 minutes when first made up Proteinase K solution: [Sigma P2308 - 100mg]
10ml of a 20mg/ml solution.

Amplification fragment length polymorphism (AFLP)-PCR fingerprinting

AFLP fingerprinting were produced for the 31isolates listed in **Fig. 7.** by a simplified AFLP protocol using single enzyme pst system (Muller *et al.*,1996). The protocol is divided into the following stages:

Stage 1. DNA restriction digestion and adapter ligation.
1) Make up reactions on ice.
Per reaction: 10x multicore enzyme buffer 2.0µl, 0.2µg adapter 1.0µl, 0.5mM ATP 0.1µl,20 Units PST I 1.33µl,1 Unit T4 DNA ligase 0.17µl.Add 4.6µl to each tube and make up to 20µl

with DNA and water (about 0.5 to1.0µg of DNA per reaction).
2) Incubate at 37^{0}C overnight. Add 230µl water and store at -20^{0}C.

Stage 2. Pre-selective amplification with Adapter primer AD A
1) Set up reactions on ice.
Per reaction: 10x PCR (Taq) buffer 2.5µl, 25mM MgCl2 2.5µl, dNTP mix (20mM each) 2.0µl, Primer AD A 2.5µl, Taq polymerase (1 unit) 0.2µl,dH20 12.8µl
Mix gently, spin down briefly and aliquot 22.5µl per tube. Add 2.5µl diluted digestion/ligation mix to each tube. Amplify on these cycling conditions, 94^{0}C 5 minutes, 40 cycles of [94^{0}C 1 minute,600c 1 minute, 72^{0}C 2 minutes 30 seconds], 72^{0}C 5 minutes and 4^{0}C hold temperature.
2) Dilute 1:10 with dH20. Stored at -20^{0}C.

Stage 3: PCR amplification with AFLP primers
1) Set up reactions on ice. Per reaction: 10x PCR (Taq) buffer 2.5µl, 25mM MgCl2 2.5µl, dNTP mix (20mM each) 2.0µl
Primer (30pmols) 2.5µl, Taq polymerase (1 unit) 0.2µl, dH20 12.8µl.
Mix gently, spin down briefly and aliquot 22.5µl per tube. Add 2.5µl diluted pre-amplification mix to each tube. Amplify on these cycling conditions 94^{0}C 5 minutes, 40 cycles of [94^{0}C 1 minute, 60^{0}C 1 minute, 72^{O}C 2 minutes 30 seconds]
72^{0}C 5 minutes, 4^{0}C hold temperature,
2) Add 2µl of loading buffer (40% glycerol, 0.25% bromophenol blue) to each sample.
3) Run 12µl on a 1.5% w/v agarose gel (to visualise fragments) at 100volts for 6 hours with marker.

Primer sequences

Adapter A 5'CTCGTAGACTGCGTACATGCA3' and Adapter B 5'TGTACGCAGTCTACGGAG3'
To reconstitute the adapter mix together in equal amounts, heat at 940c for 10 minutes and cool slowly to room temperature.

Selective primers used

AFLP-A 5'GACTGCGTACATGCAGGT3', AFLP-D 5'GACTGCGTACATGCAGAC3'
APLP-E 5'GACTGCGTACATGCAGAG3', AFLP-H 5'GACTGCGTACATGCAGAA3'
AFLP-I 5'GACTGCGTACATGCAGAT3' and AFLP-J 5'GACTGCGTACATGCAGTA3'

Statistical analysis

To elucidate the relationship among *C.gloeosporioides* isolates and to establish links between their morphology, DNA fingerprinting and pathogenicity, the data on morphology and cultural characteristics were subjected to cluster analysis using MultiVariate Statistical Package (MVSP) (*hht//www.kovcom.co.uk/MVSP/indexhtml*). Dendrograms was generated from the similarity matrix obtained by Gower's linear similarity coefficient using Unweighted Pair Group Method of Arithmetic Average (UPMGA). Lesion size data was subjected to analysis of variance performed with the Genstat programm (Payne *et al.*, 2001). The data on proportion of sporulating lesions per leaf were arcsine transformed prior to analysis of variance (Mead & Currnow, 1989). To identify homogenous groups or to detect significant differences between the means, the Student –Newman-Keuls (SNK) test was used. Linear

correlation coefficient analysis was also performed between mean radial growth and mean lesion size whereas simple regression analysis was performed between mean lesion and sporulation using Microsoft Excel 2000.

Results

Isolations of *C. gloeosporioides* from leaf and stem samples

Approximately 31% of the 168 leaf and stem samples collected from Vanuatu, Fiji, and Papua New Guinea and the Solomon islands between 1999-2002, were found to be infected by *C. gloeosporioides* thus generating a total of 81 isolates listed in **Appendix Table 1.** There were marked differences in disease incidence among the four islands with 61% samples from Fiji showing *C. gloeosporioides* infections whereas those from Vanuatu and Papua New Guinea, *C.gloeosporioides* infections of 45.8% and 30% were respectively recorded. Nevertheless, despite the presence of some typical anthracnose symptoms, samples from the Solomon Islands, have apparently, failed to produce any *C. gloeosporioides* infections. Instead, a number of other pathogens especially *Curvularia* spp. *Colletotrichum capsici* and *Cercospora pyrilifoliae* and *Botryodiplodia theobromeae* that cause symptoms which initially resemble to those by *C. gloeosporioides,* were often isolated from most of these samples from the Solomon Islands. Furthermore among yam species collected, the pathogen was isolated from *D. alata, D. esculenta,* and *D. pentyphylla* but not from *D. nummularia* while among other 34 non-yam species, the fungus was detected from the samples of citrus, cassava, melon and chilli crops as well as from the leaf samples of kava, *Mikania micrantha*, and yard long bean as shown in **Table 2.**

Table 2. Non-yam host species from four South Pacific countries which were assessed for *C. gloeosporioides* infection from 1999-2002

Country	Host species	No of samples	No of samples with *C. gloeosporioides*
Fiji	Kava	4	2
	Chilli pepper	1	0
Vanuatu	Kava	1	1
	Cassava	1	1
	Mikania micrantha	1	1
	Citrus spp.	1	1
Pupa New Guinea	Tobacco	4	0
	Tomato	4	0
	Weeds	5	0
	Yard long bean	4	1
	Alocasia sp.	1	0
	Cassava	3	2
	Kava	3	1
	Sweet potato	1	0
	Coconut	1	0
	Melon	2	1
	Chilli pepper	2	1
Solomon Islands	*Mikania micrantha*	3	0
	Pueraria sp.	1	0
	Tomato	1	1

Isolations of *C. gloeosporioides* from tuber samples

Seventeen genera of fungi were isolated from the tubers and their shoots as shown **Table 3** with 4.8% of all tuber samples from Papua New Guinea and Vanuatu islands showing infection by *C. gloeosporioides* isolates which, despite some variations in their cultural morphological and cultural characteristics, were all highly pathogenic to both yam and non-yam host species as presented in this study.

The most commonly recovered fungi were *Penicillium* spp., *Fusarium* spp., *Rhizopus* spp., and *Rhizoctonia* spp. particularly *Penicillium sclerotigenum, F. solani, F. oxysporium, Rhizopus stolonifer, Rhizoctonia solani and Aspergillus* spp. which are regarded either as important soil-borne pathogens or causal agents of tuber rotting in storage.

Other frequently encountered fungi included *Botryodiplodia theobromae, Phompsis* sp, *Phoma* spp, *Alternaria alterata, Cladosporium* sp., *Bipolaris* spp, *Nigrospora* sp. and *Curvularia eragrostidis*, which are all regarded as minor leaf pathogens in many yam-growing regions.

Moreover, approximately 24% of the tubers also showed extensive infestation by various nematodes with almost all nematode infested tubers showing either *Colletotrichum* or *Fusarium* infection or both.

Table 3 Organisms isolated from tuber samples and their respective shoots obtained from Vanuatu & Papua New Guinea between 2000-2

Organisms	Tuber tissue	% incidence	Shoots from tubers	% incidence
C. gloeosporioides	v	4.8	-	-
Rhizopus sp	v	25.2	-	-
Fusarium spp	v	64.6	v	25
Penicillium spp	v	9	-	-
Aspergillus spp	v	89.6	-	-
B. theobromae	v	36.6	-	-
Cladosporium sp	v	10.8	-	-
Phoma sp	v	15.1	-	-
Curvularia sp	v	2	-	-
Phompsis sp	v	43.8	-	-
Rhizoctonia solani	v	20	-	-
Alternaria sp	v	2	-	-
Chaetomium sp	-	-	v	15
Nematodes	v	23.8	-	-
Bipolaris spp	v	9	-	-
Nematospora	v	2	-	-
Trichoderma spp	v	9	-	-
Nigrospora sp	v	2	-	-

No. of samples = 49 tubers
- = Absent and v = present

Morphology and cultural characteristics

The dendogram tree generated from similarity matrix based on 27 morphological and cultural characters grouped the 61 isolates of *C. gloeosporioides* into the following three clusters each further divided into a number of sub-clusters:

Cluster 1. Contains mostly fast growing isolates with mean radial growth rate ranging from 9.10 - 14.75 mm with overall mean of 13.10 mm day $^{-1}$ with colony colour grey to dark with low or slightly elevated grey to dark aerial mycelium and olivaceous black or dark from the reverse (**Plate 1a**). All isolates produced grey to creamy white spore masses in dark setose melanized acervuli.

The conidia of this group were mostly round at apex and their mean conidial dimensions varied from 12.3-16.6 x 3.8 – 6.7µm and an overall mean length and width of 14 x 5.6 µm (**Plate 2a**). The acervuli were often associated with numerous fertile perithecia with ascospores measuring 14.4-18.8 x 4.1-6.2 µm and overall means of 16.8 x 5.1µm. The mean length and width of their appressoria varied from 9.6 –17.3 x 6-9.8µm with an average of 11.3 x 7.1µm.

Cluster 2. Contains fast growing isolates with mean daily growth rates ranging from 11.16 - 16.27 and an overall mean of 14.07mm day^{-1}. Colony colour white to grey producing highly elevated white to grey aerial mycelium and white to orange from the reverse (**Plate 1b**). All the isolates produced abundant pink (salmon) to orange conidial masses with mean conidial length and width ranging from 12.6 – 17.4 x 3.8 – 5.2 µm and overall mean of 13.9 x 4.7 µm with high percentage of acute apex produced either in simple saucer-like acervuli or only masses of conidia with little or no setae (**Plate 2b**). Almost all isolates in this group failed to form perithecia but produced abundant appressoria with mean length and width ranging 8.1-15.4 x 5.3 - 8.3 µm and average means of 10.5 x 6.8 µm

Cluster 3. Contains of a small group of slow growing isolates with mean radial growth rates varying from 8.94 - 12.4 mm and overall mean of 10.41mm day $^{-1}$ (**Plate 1c**). Most of the isolates produced dark colony colour with low to moderately elevated grey or dark aerial mycelium. The isolates produced orange or grey to white conidial masses with both acute and round apex conidia with mean conidial size of 14-22.2 x 4-8µm. and overall mean of 15.9 x 5.2µm (**Plate 2c**). .In this group, conidial masses were produced in either typical melanized acervuli or in dark complex acervuli (conidiomata) with typical setae or abundant hair-like setae. No teleomorphs were formed although appressorial bodies measuring 10.6-16.4 x 5.7-8.6µm and overall mean of 13.6 x 7.3µm were recorded.

As shown in **Fig. 6** the cluster analysis failed to categorise the isolates into clusters according to their geographical location or host origin with representative isolates from all localities occurring in each of three clusters. For instance, cluster 3 which shares similarities with both cluster 1 and cluster 2 contains two yam isolates from Fiji, one *Mikania micrantha* isolate from Vanuatu and a chilli and two yam isolates from three provinces from Papua New Guinea.

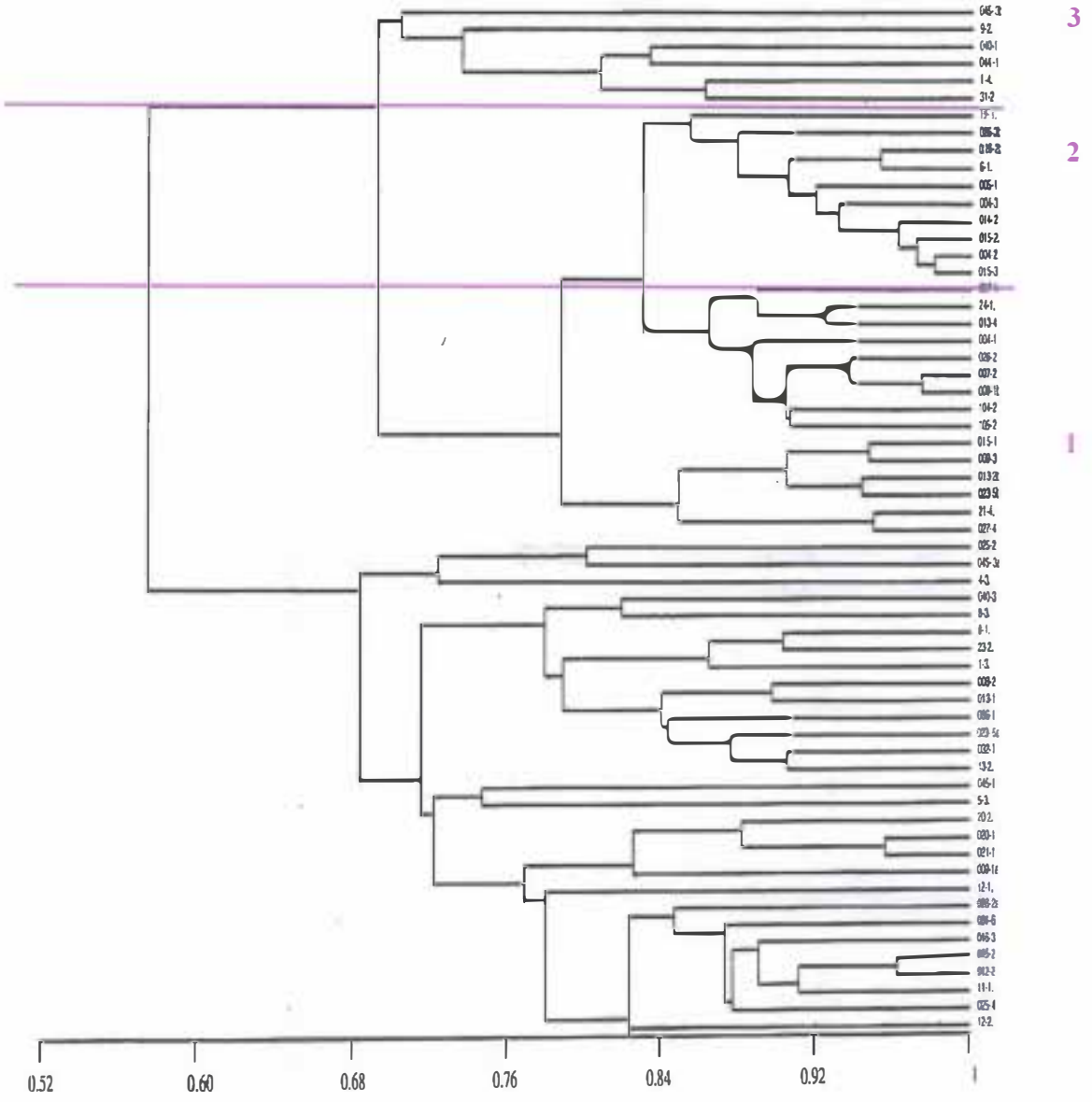


Fig. 1. Hierarchical classification of 60 *C. gloeosporioides* isolates based on the 27 described morphological and cultural characteristics.

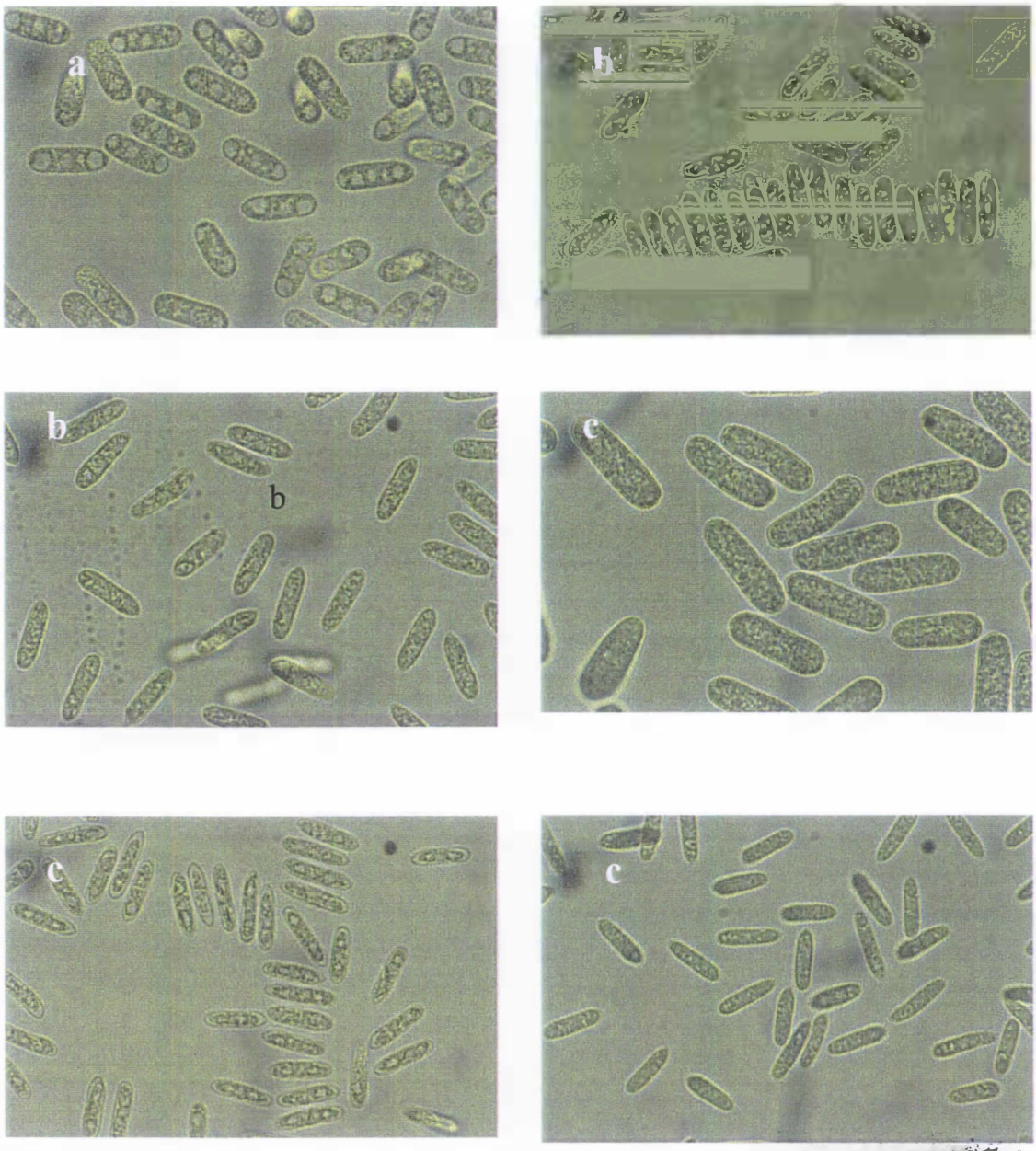


Plate 2. Conidia of C. *gloeosporioides* isolates (x 1000): (a) yam leaf isolate in cluster 1, (b) yam isolate and cassava isolate in cluster 2 (c) yam tuber isolate, *Mikania micrantha* isolate and yam leaf isolate in cluster 3.

Studies of pathogenicity tests

Pathogenicity of yam and non yam isolates on yam leaves

All isolates produced sporulating lesions within 7 days after inoculation (**Plate 3 a-d**). However there were significant differences ($P<0.001$) among *C. gloeosporioides* isolates in their mean lesion diameter on the yam variety White Lisbon with mean lesion values ranging from 11.57 - 33.33 mm as shown in the **Appendix Table 3**. However, there was no obvious link between these variations and the nature of host species from which different isolates were derived from or their different geographical origins. As presented in **Fig 2 a-c,** differences in mean lesion size among isolates from Vanuatu or Fiji were as highly variable as those produced by *C. gloeosporioides* isolates obtained from different provinces in Papua New Guinea. Similarly, as shown in non-yam isolates derived from various host species were as pathogenic as yam isolates with differences in disease severity among them as widely variable

as those incited by yam isolates. For non-yam isolates, the mean lesion varied from 13.24 - 30.06 mm while those produced by yam isolates, mean lesions were between 11.57-33.33 mm.

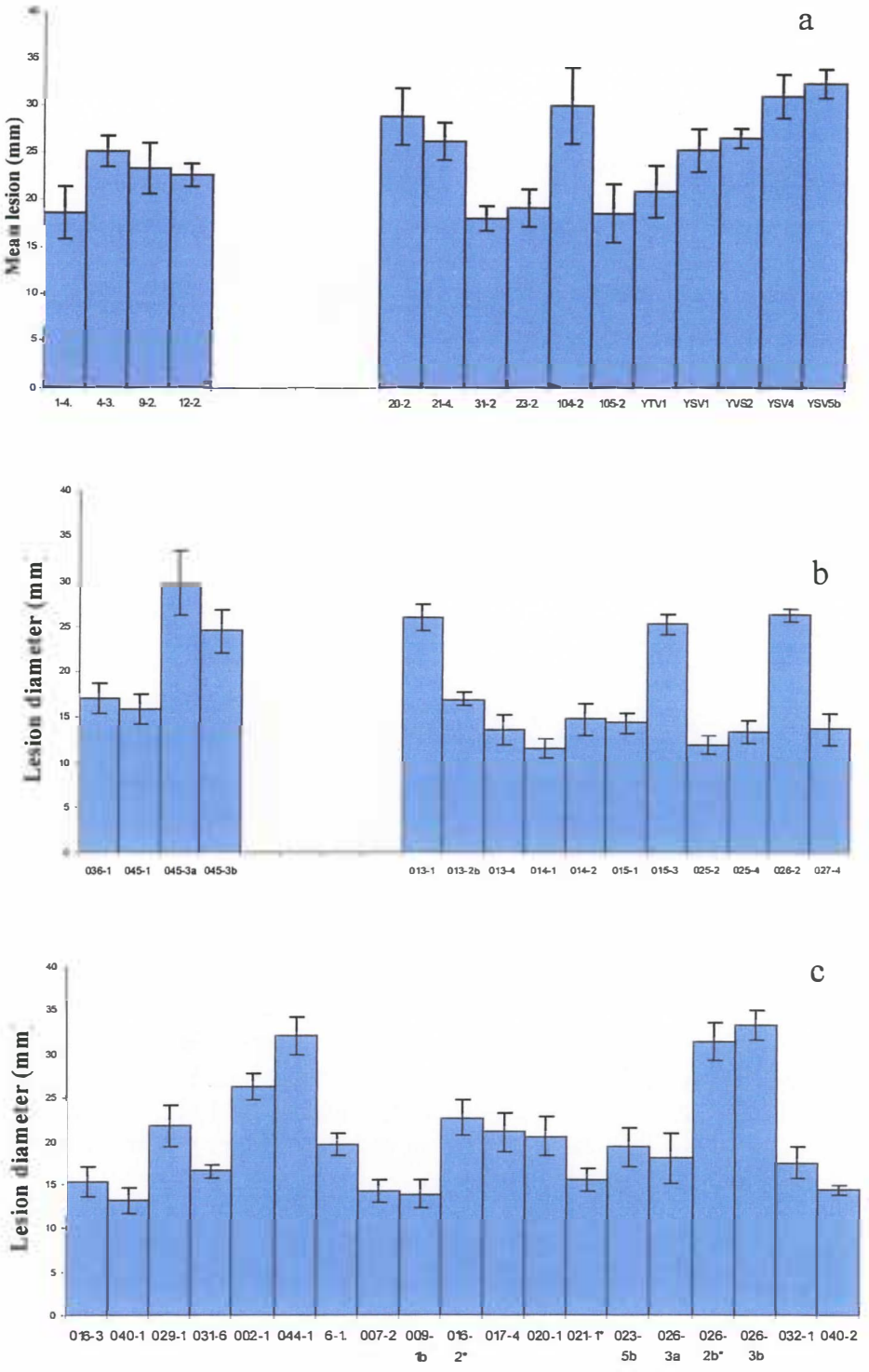


Fig 2. Mean lesion size of *C. gloeosporioides* isolates on *Dioscorea alata cv* White Lisbon 7 days after inoculation. (**a**) Fiji & Vanuatu isolates, (**b**) West New Britain & New Ireland isolates and (**c**) Mainland Papua New Guinea. Each mean is average of six replicates. Bars are standard error of means

It is worth noting that on the basis of their pathogenicity, C. *gloeosporioides* isolates failed to cluster into patterns similar to the morphological groups illustrated in the dendrogram tree **Fig. 1** with each morphological group represented at each level of pathogenicity.

Moreover, there was no significant linear correlation (r_{df50}=0.04; *P>0.05*; **Fig.3. & Appendix Table 4**) between mean radial growth and mean lesion size of the 49 isolates tested for pathogenicity.

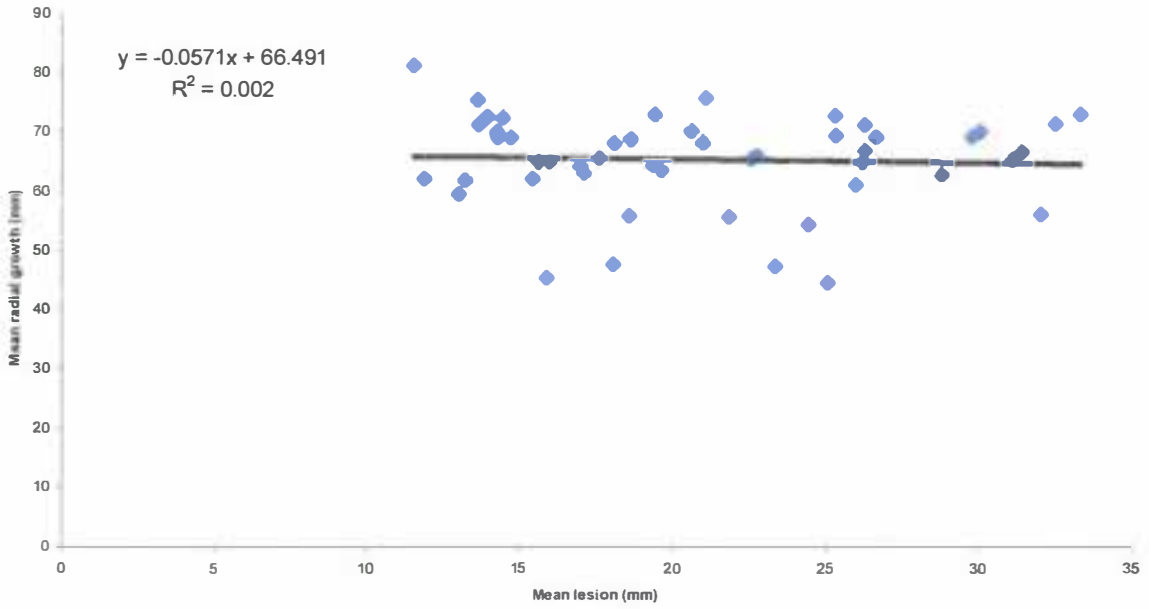


Fig. 3. Relationship between the lesion and radial growth of 49 *C. gloeosporioides* isolates from yam and six other host species.

Furthermore, in all isolates spore production was recorded within 6-9 days with spore size and shape similar to those produced in culture media. Most of the isolates had high mean numbers of sporulating lesions/leaf but the difference among them was significant (P<0.001) as shown in **Appendix Table 5.** However these differences were neither based on the host species nor geographic location from which isolates were obtained with both the highest and lowest mean number of sporulating lesions/leaf recorded among yam isolates from Vanuatu and from Papua New Guinea as shown in **Fig 4.**

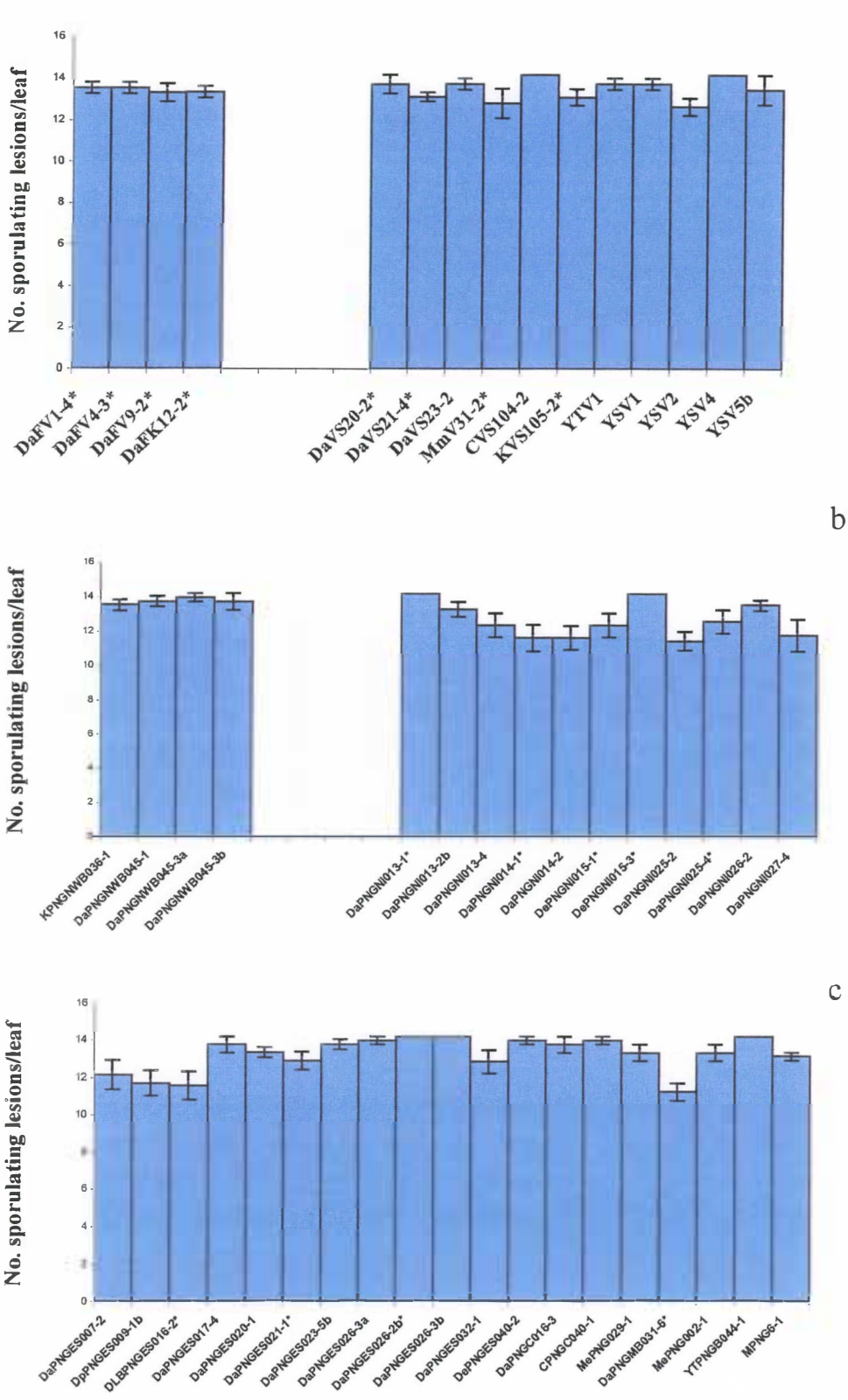


Fig. 4.

Mean no. of sporulating lesions/leaf of *C. gloeosporioides* isolates on *Dioscorea alata cv* White Lisbon 7 days after inoculation **(a)** Fiji & Vanuatu isolates, **(b)** West New Britain & New Ireland isolates and **(c)** Mainland Papua New Guinea. Each mean is average of six replicates. Bars are standard error of means

Moreover, there was a significant positive linear relationship (r_{df49}=0.60; P< 0.05) between mean number of sporulating lesions per leaf and mean lesion size of the 49 isolates tested for pathogenicity with 36% of all variations accounted for the simple linear regression between the two variables (**Fig. 5**). This indicates that lesion size has a positive effect on the number of sporulating lesions.

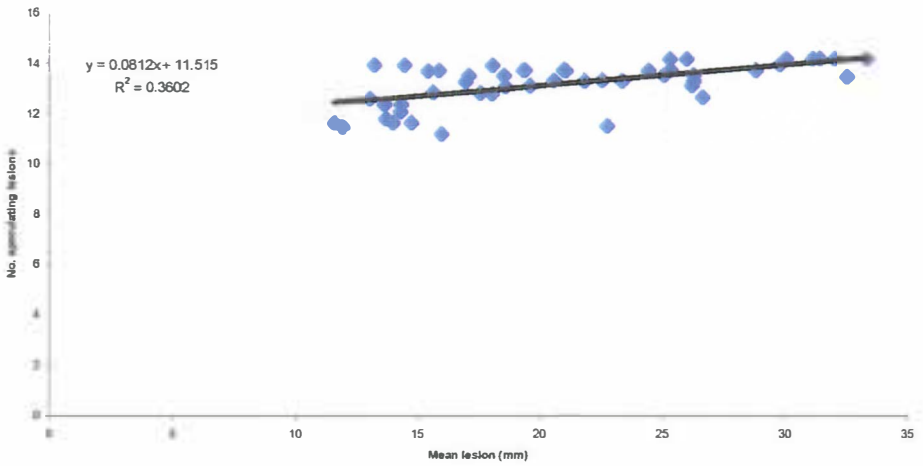
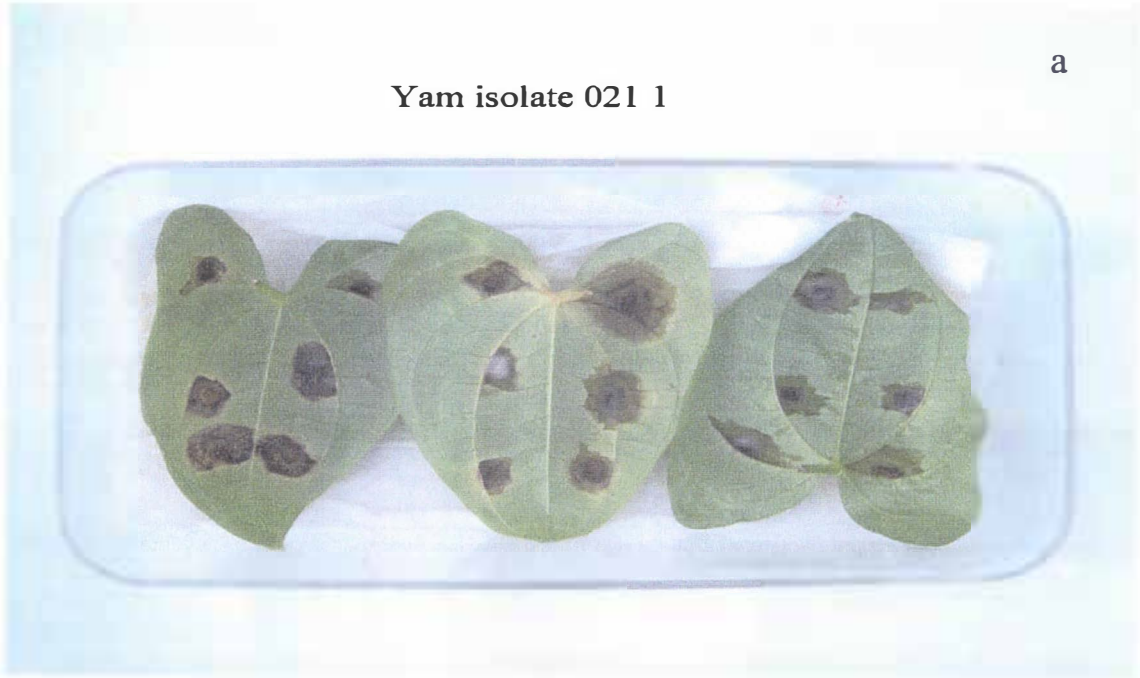


Fig. 5. Relationship between the lesion size and no of sporulating lesions per leaf of 49 *C. gloeosporioides* isolates from yam and six other host species

a

Yam isolate 021 1



b

Yam isolate 026 2

c

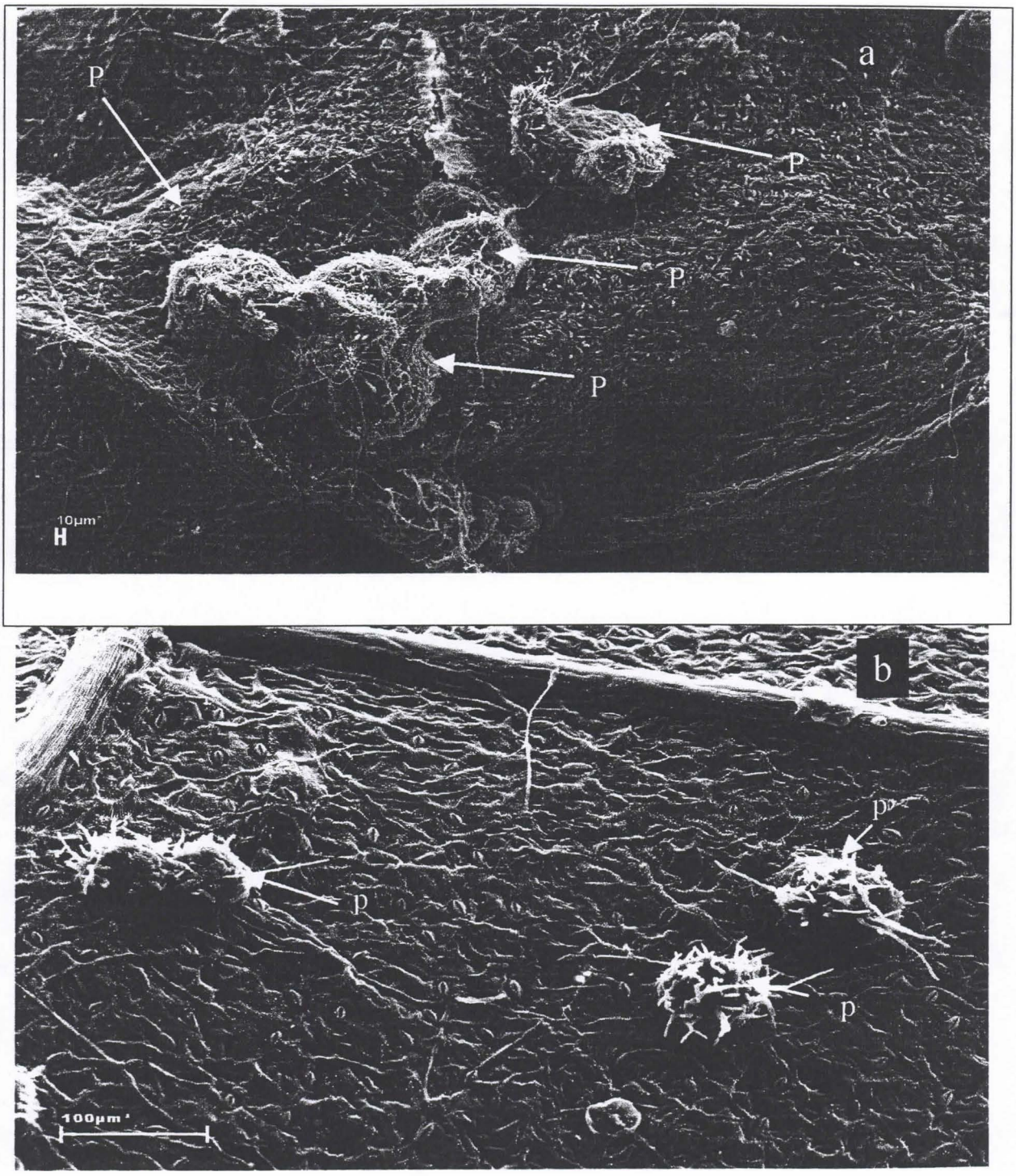
Cassava isolate 24 1

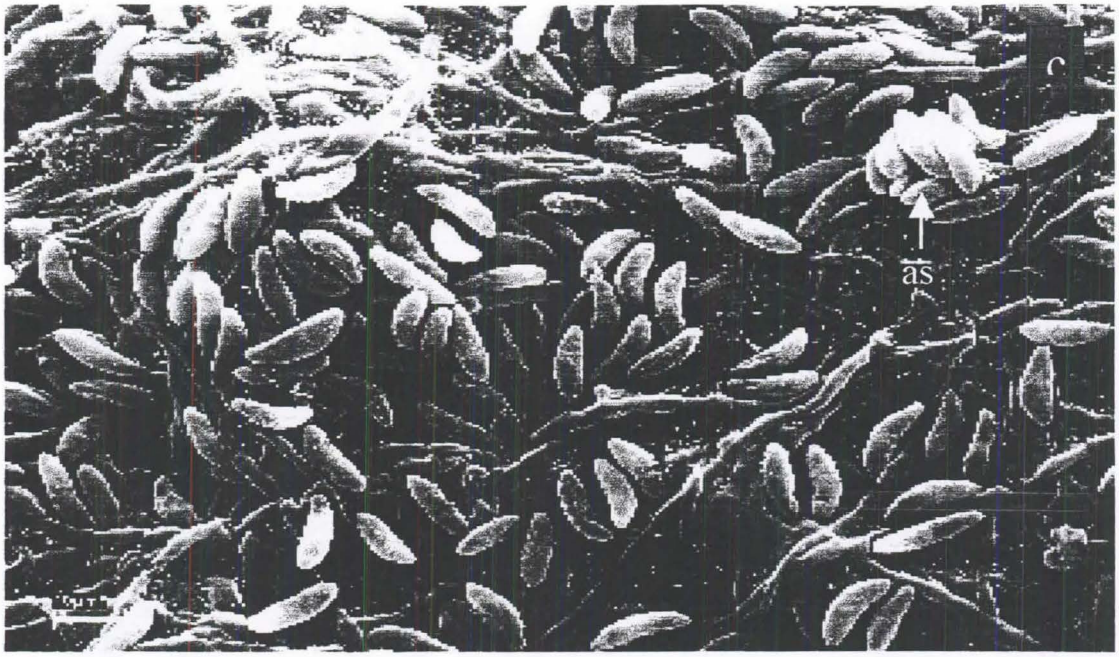
d

Control

Plate 3. Leaves of on *Dioscorea alata cv* White Lisbon inoculated *with C. gloeosporioides* isolates 7 days after inoculation. (a) **DaPNGMB021-1**, (b)**DaPNGNI026-2**, (c) **MePNGMB02-1** and (d) **Control**

On leaf tissue, acervulii, setae and perithecia with mature asci and ascospores formed abundantly also within 7-9 days. However, as shown in and **Appendix Table 6**, only 22 of 49 isolates tested for pathogenicity, produced setae, perithecia and asci/ascspores on leaf tissues **Plate 4**. Moreover, it is interesting to note that almost all of these 22 isolates also produced these sexual and asexual fruiting structures on artificial culture medium (i.e. PDA).

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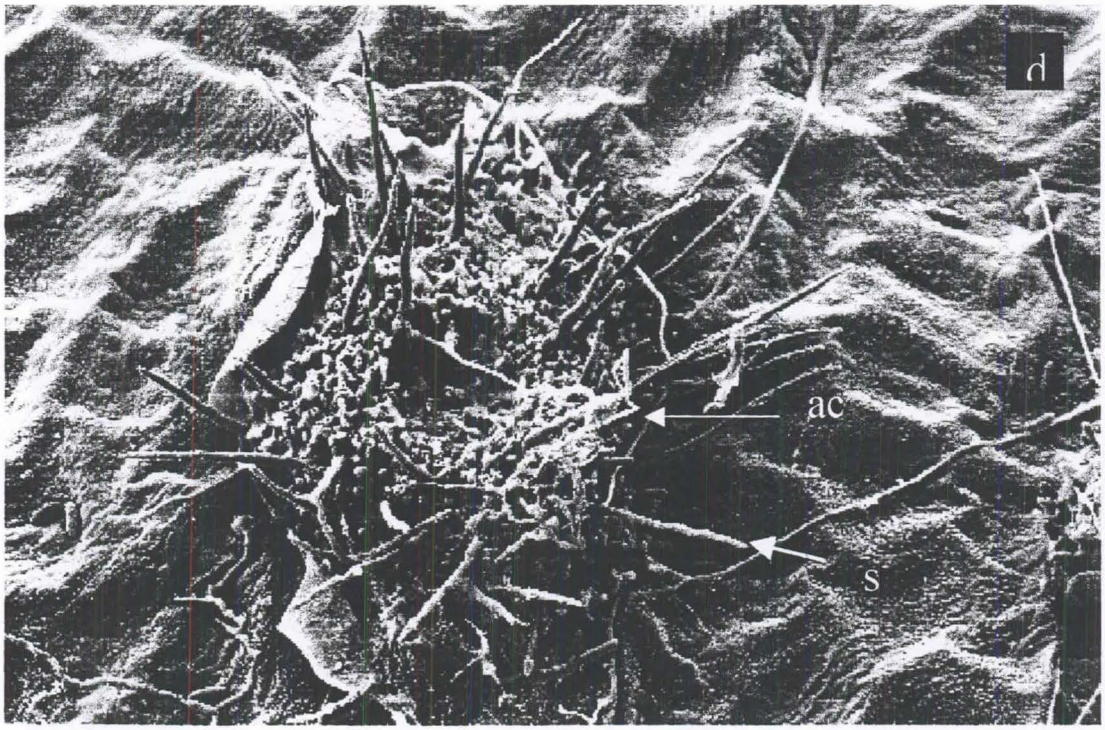
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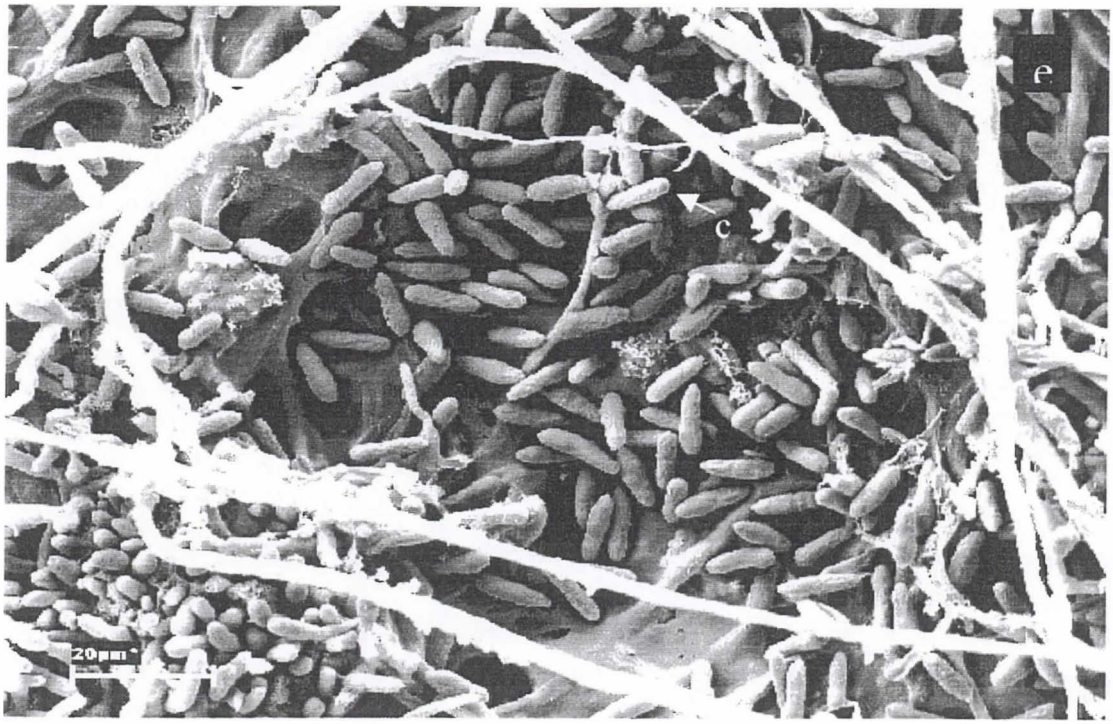


Plate 4. Sexual and asexual structures of *Glomerella cingulata* on *D. alata* White Lisbon (**a & b**) Mature perithecia at different stages (p), (**c**) Ascospores (as), (**d**) Acervuli (ac) with setae (s) and (**e**) Conidia (**c**).

Pathogenicity of yam isolates on other host species

All the four isolates were pathogenic to cowpea, chilli pepper, okra and yam with no obvious differences in symptom development and spore production on each individual host among the four isolates examined. Moreover, differences in disease development were observed among the hosts with symptoms on cowpea, okra and pepper appearing as small non-pigmented spots in and around the inoculation sites. On cowpea and okra, these spots began to enlarge producing large water-soaked lesions with profuse sporulation, which often extended over the entire leaf surface within 4-5 days while on pepper, symptom expansion was mainly confined within and along the leaf veins with profuse sporulation and disintegration of tissue occurring within 9-10 days as shown in **Table 4.**

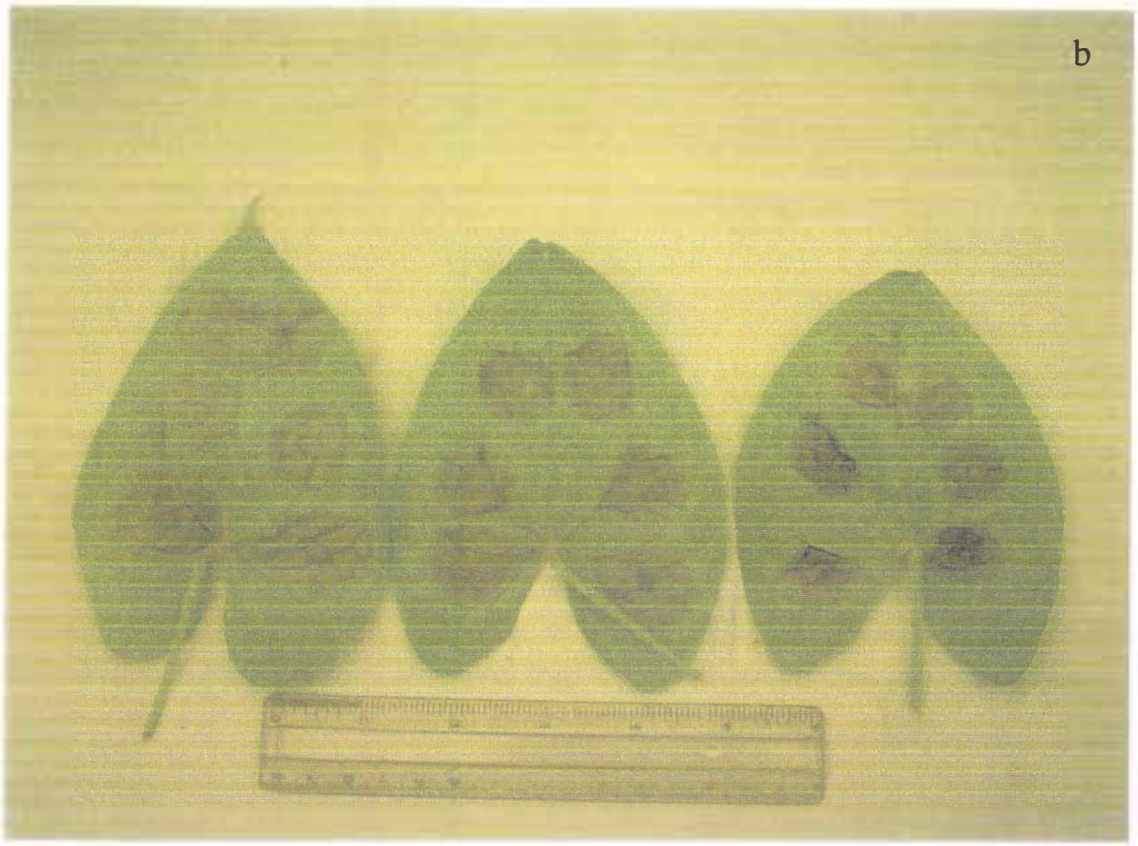
On yam and on tomato, symptoms appeared as small strongly pigmented spots, which in yam after 36-48 h begin expanding as translucent chlorotic lesions around inoculation site. On yam, coffee and kidney bean leaves, these lesions start coalescing within 5-6 days and heavy sporulation and total rotting of large parts or whole leaves takes were observed within 7-9 days. However, on tomato, the tuber isolate was more pathogenic than the other three isolates causing abundant sporulation and complete destruction within 9-10 days whereas other isolates incited only few translucent lesions with limited sporulation. Hence of the nine hosts tested, okra and cowpea appear to be the most susceptible whereas *Citrus* spp., and cotton were the only hosts resistant to *C. gloeosporioides* with all isolates causing either no symptoms or only small superficial browning of the tissue even after three-weeks.

Table 4. Number of days after inoculation when each of the isolates produced water-soaked lesions with profuse sporulation invaded large parts or the entire leaf tissue.

Host	Disease development			
	Isolate 044.	Isolate 013-4.	Isolate 21-4.	Isolate 9-2.
Cowpea (*Vigna unguiculata*)	4	4	4	4
Okra (*Abelmoschus moschatus*)	4	4	4	4
Chilli pepper (*Capsicum annum*)	9	10	10	10
Yam (*Dioscorea alata*)	7	8	8	8
Tomato (*Lycopersiconm esculentum*)	9	>[a]14	>14	>14
Citrus spp. (*C. paradisi, C..limon, C. sinensis*)	>[b]21	>21	>21	>21
Kidney beans (*Phaseolus vulgaris*)	8	7	8	8
Coffee (*Coffea arabica*)	6-9	6-9	7	8
Cotton (*Gossypium* sp.)	>21	>21	>21	>21

>[a] =limited water-soaked lesions and sporulation were observed within 14 days.

>[b]= Water-soaked lesion and sprulation were not observed within 21 days.


b


c

Plate 5. Leaves of various host species showing various disease symptoms (a, b & c) coffee, yam, and kidney beans showing susceptible lesions 8 days after inoculation and (d) citrus leaves with resistant pinpoint lesions 14 days after inoculation.

The growing conditions and extraction technique used in this study produced DNA of suitable quality for PCR allowing to achieve good amplification and therefore ensuring reproduceable and readable results.

The two dendrograms **Fig 6** and **Fig 7.** produced following the ISSR-PCR and AFLP molecular techniques respectively both form a complex pattern, with no identical fingerprinting patterns even where multiple isolations of *Colletotrichum* isolates from the same leaf took place, the colonies concerned were not genetically identical. However despite some differences, there does appear to be some similarities between these two ISSR-PCR and AFLP data. Furthermore, the molecular tree generated by ISSR-PCR analysis correlated fairly well with the dendrogram representing all morphological data with the major clusters corresponding to with cultural major types. There was also a good correlation between molecular results generated by ISSR-PCR and with ability of the isolates to produce sexual spores. However, there were few similarities between the AFLP results and morphological data.

No correlation was apparent with data on pathogenicity with isolates showing significant differences in pathogenicity appearing to be closely related in molecular terms. Moreover, there are no clear patterns of variation and no clear linkages either to host and to geographical origin. Interestingly, in some cases, isolates from different host species have a higher degree of homology than isolates from the same host species.

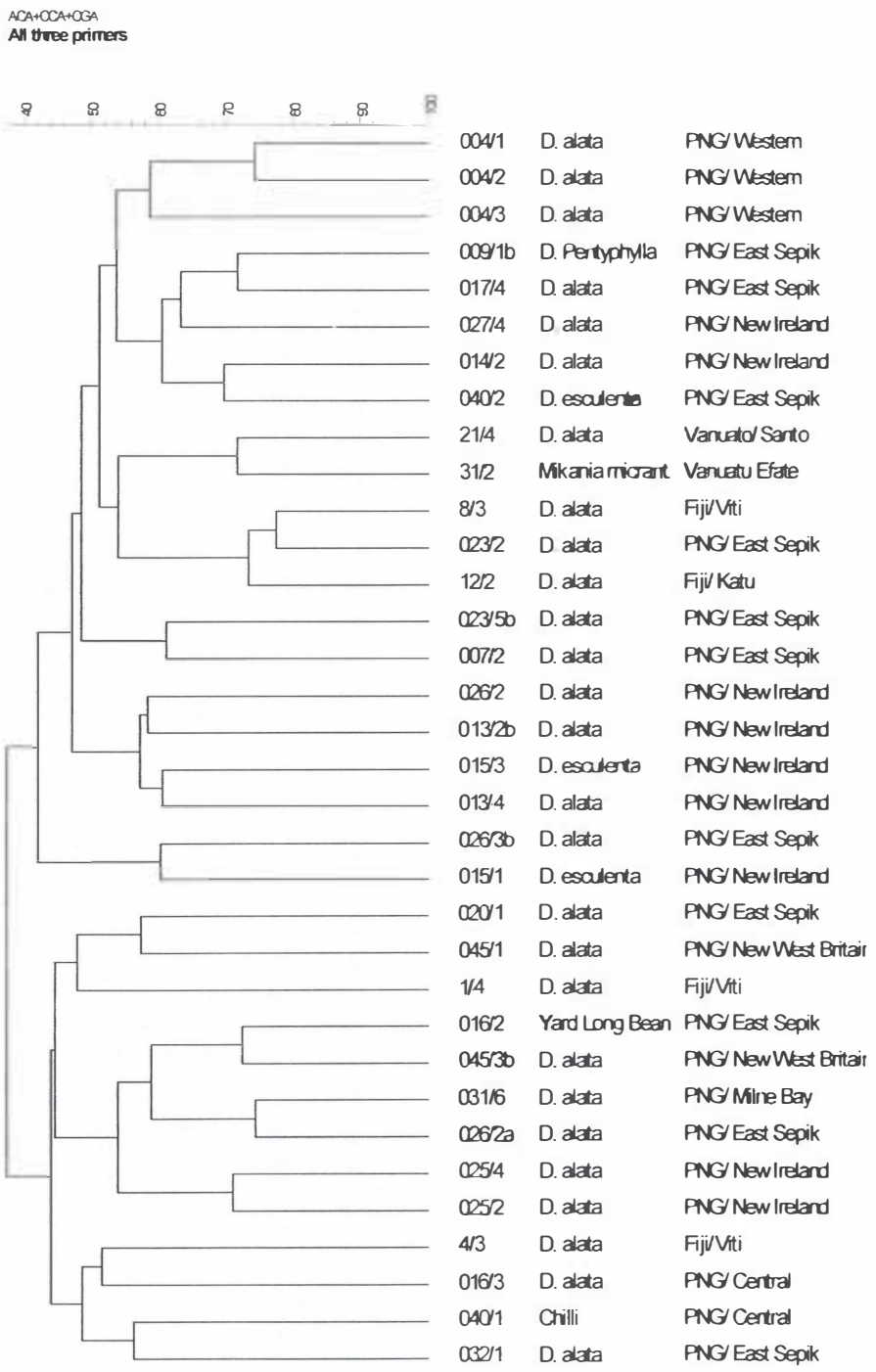


Fig. 6. Unweighted Pair Group Method Arithmetic Average (UPGMA) dendrogram of ISSR similarity among 34 C. gloeosporioides isolates derived from yam and other species

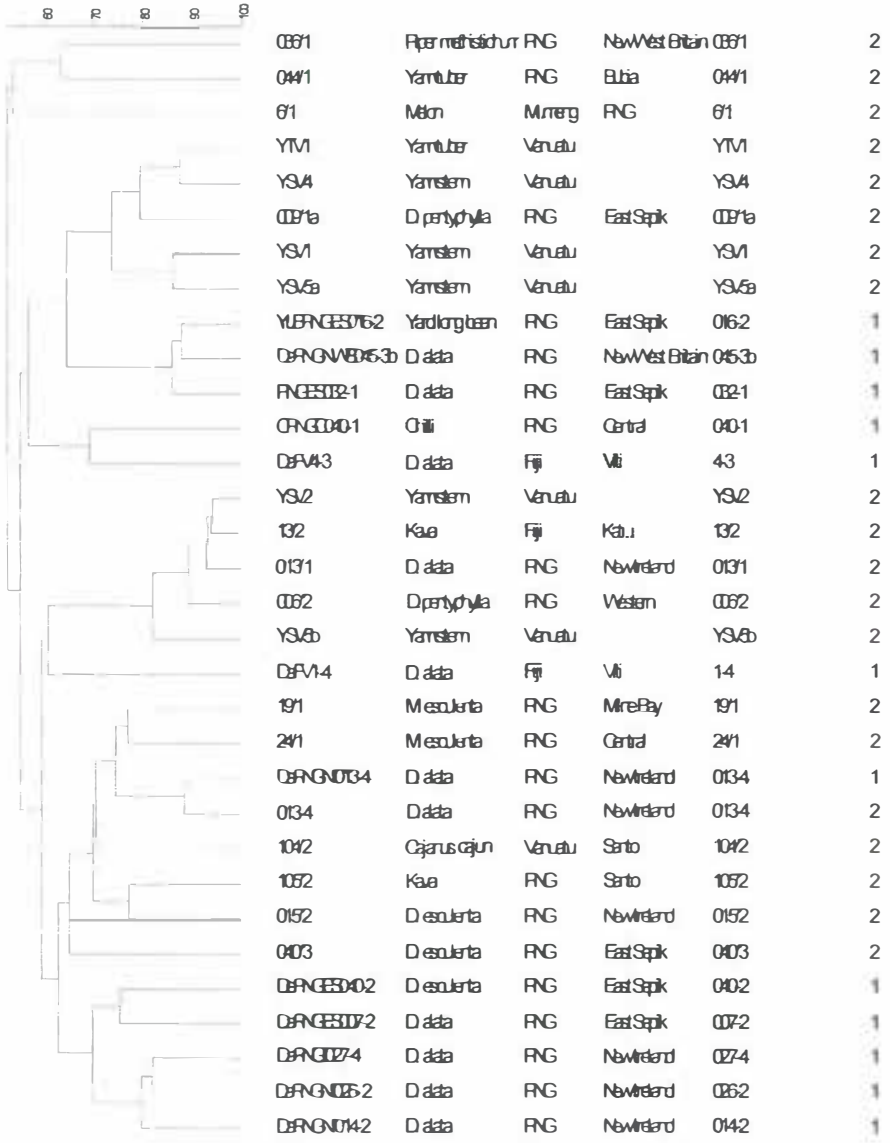


Fig. 7. Unweighted Pair Group Method Arithmetic Average (UPGMA) dendrogram of AFLP similarity among 31 C. gloeosporioides isolates derived from yam and other species

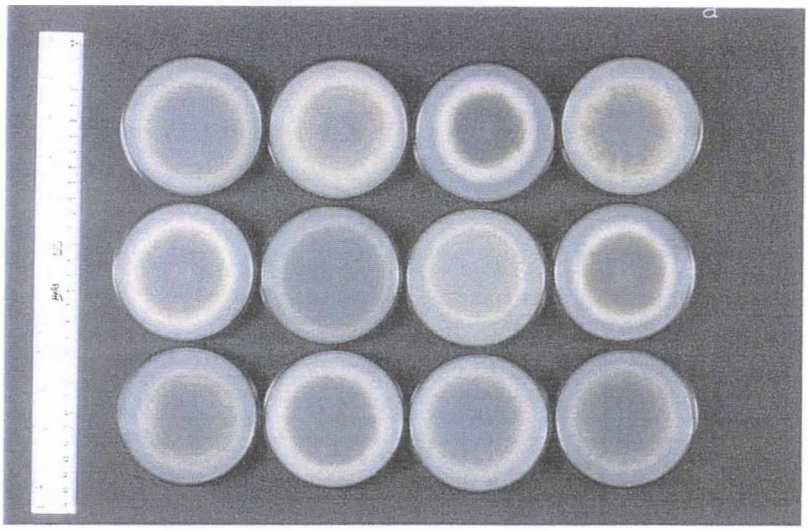


Plate 1. Cultures of 30 *C. gloeosporioides* from various host species in Fiji, Vanuatu and Papua New Guinea. (a) Fast growing dark-grey isolates (Cluster one), (b) Fast growing white-salmon isolates and (c) Slow growing mostly dark-grey isolates (Cluster three). For details refer Fig. 1

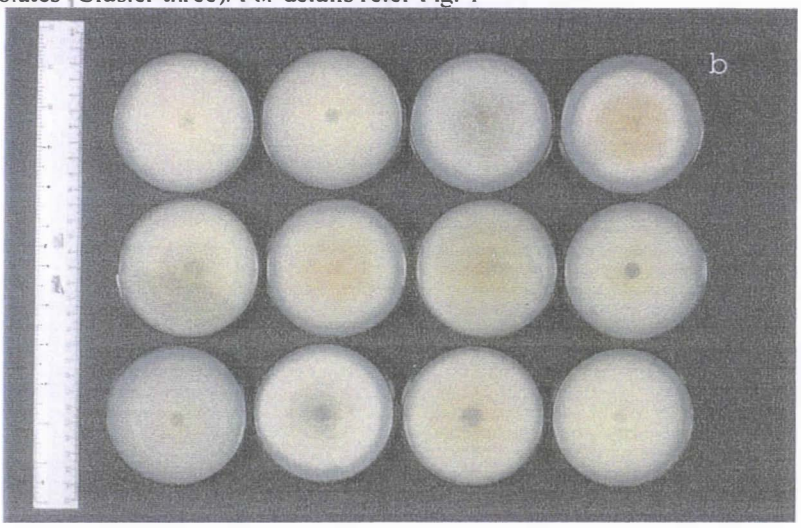


Plate 1. Cultures of 30 *C. gloeosporioides* from various host species in Fiji, Vanuatu and Papua New Guinea. (a) Fast growing dark-grey isolates (Cluster one), (b) Fast growing white-salmon isolates and (c) Slow growing mostly dark-grey isolates (Cluster three). For details refer Fig. 1

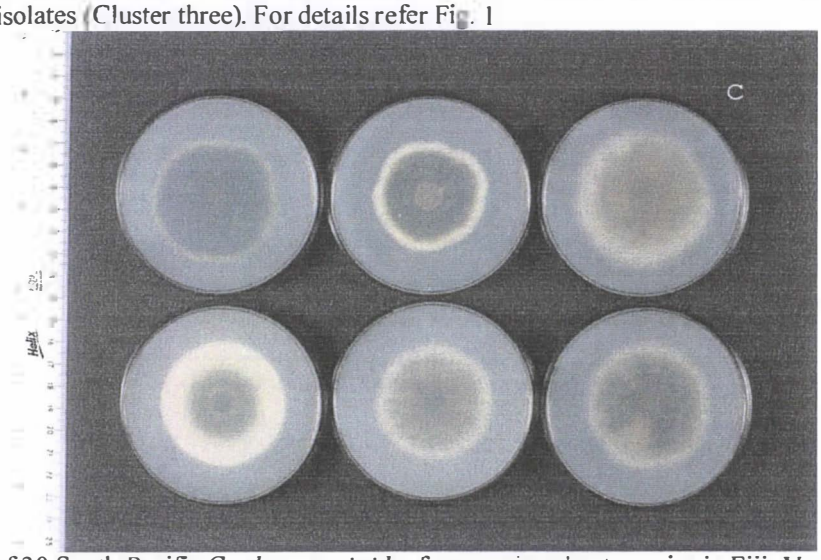


Plate 1. Cultures of 30 South Pacific *C. gloeosporioides* from various host species in Fiji, Vanuatu and Papua New Guinea. (a) Fast growing dark-grey isolates (Cluster one), (b) Fast growing white-salmon isolates and (c) Slow growing mostly dark-grey isolates (Cluster three). For details refer Fig. 1

Discussion and Conclusions

The high percentage of C. gloeosporioides infection on yam foliar lesions examined reveals the importance and the wide distribution of this pathogen in almost all yam-growing provinces sampled throughout Fiji, Vanuatu and Papua New Guinea. Colletotrichum gloeosporioides was not isolated from yam anthracnose lesions in samples collected from the Solomon Islands. However, the importance of yam anthracnose in the Solomon islands has already been well documented (Jackson and Newhook, 1979; Winch et al.,1984). This rules out any suggestion that the disease is less serious in that country than in the other Pacific islands. Hence, the unusual absence of C. gloeosporioides infections on the samples from the Solomon Islands could be partially attributed to the high incidence of other leaf fungal pathogens (particularly C. capsici, Curvularia spp. and B. theobromeae that have been frequently encountered in this study. Antagonism by these other leaf pathogens on C. gloeosporioides colonisation on yam leaves has been recently elucidated by Wharton (1995).

The overall mean growth rates recorded in this study were in agreement with those by Manaut et al. (2001) but were slightly higher than those given by Singh and Prasada (1966) and Abang et al. (2002). Despite notable variation in mean conidial length and width observed among individual isolates, in all isolates these measurements fitted with C. gloeosporioides descriptions (Arx et al. 1957a; Mordue, 1971; Baxter, 1983; Sutton, 1992). Mean length and width of ascospores were also within the range given by Arx et al. (1957a), Baxter et al. (1983) and Sutton (1980, 1992). Also, appressorial measurements obtained in the current study were also within the range given by Mordue (1971) and Sutton (1980, 1991). However, it should be pointed out that although the tuber isolate, YTPNG-044-1, has closely clustered with the other slow-growing, non-ascigerous forming isolates, because of its unusually large conidial size and abundant sclerotial formation on PDA as well as on leaves, further studies are required on its identity, pathogenicity and geographical distribution. So far, sclerotial forming isolates have not been reported in C. gloeosporioides (Murdue1971; Sutton, 1992).

The significant differences in pathogenicity among C. gloeosporioides isolates encountered in this study are in agreement with the findings by Winch et al. (1984) who observed similar differences in pathogenicity among C. gloeosporioides on both detached and attached yam leaves. The results also support the more recent studies by Green et al. (2000) and those by Abang et al. (2002). Abang et al. (2002) identified three distinct virulence phenotypes of Colletotrichum isolates from yam in Nigeria. From a practical point of view, these differences in pathogenicity among C. gloeosporioides isolates requires further investigation in order to have a comprehensive knowledge of the nature of the pathogenic variability. This is a prerequisite for adequate disease management (particularly in implementing a successful breeding programme for disease resistance. The large range in pathogen virulence has implications for the commercialisation of elite yam cultivars as proposed by SPYN. All cultivars should be tested for their resistance to a wide range of C. gloeosporioides isolates.

The lack of a clear association between morphological data and pathogenicity among isolates studied in this work confirms other previous studies by Winch et al. (1984) who failed to reveal any cultural characters that enabled them to detect pathogenic isolates on yam. The results presented in this report also support those by Denham and Waller (1981) who pointed out that pathogenic isolates of C. gloeosporioides from citrus did not fall into distinct morphological strains. Abang et al. (2002) identified four morphologically different forms of Colletotrichum representing three distinct virulence phenotypes from yam in Nigeria. Furthermore, the authors pointed out that isolates with the slowest growth rate were also the

most virulent. However the results of the study presented here failed to verify these claims as there was no significant simple linear correlation between mean lesion size and mean radial growth of C. gloeosporioides isolates tested for pathogenicity in this study.

Although the occurrence of the teleomorphic state, Glomerella cingulata (Stonem.) Spauld & Schenk has been widely reported on yam (Baudin, 1956; Mwankiti and Ente, 1978; Abang et al., 2001), there is still a paucity of information on its importance in the epidemiology of yam anthracnose. The fact that almost half of the isolates tested in this study produced sexual structures on host tissue reveals that G. cingulata occurs more frequently in nature than previously thought. The results also reveal that despite the fact that these G. cingulata isolates have, by and large, clustered into a single morphological group, there was no clear link between pathogencity and the production of the teleomorph form. Hence it is most likely that the teleomorph stage plays a vital role as a mechanism for genetic variability as well as acting as an over-wintering structure for survival from season to season. Furthermore, it is not clear whether the isolates producing only conidia are heterothallic or have lost the ability to reproduce sexually in a rapidly changing environment. Hence to appreciate the significance of this distinction, more research on the genetics of the species is required.

Both yam and non-yam isolates of C. gloeosporioides tested in this study were able to infect yam as well as a number of other non-yam hosts. This demonstuates the existence of isolates of C. gloeosporioides with a wide host range. These results contrast with previous findings by Singh et al. (1966) who had reported that isolates of C. gloeosporioides from D. alata were highly specific. The results presented here, however, are consistent with those by Winch et al. (1984), Jefferies et al., (1990) and of more recent findings by Vagelas (1999) and by Peters (2001). Moreover, it is interesting to note that Aiyere and Strange (2003) found that fresh cells of yam and cassava were both susceptible to toxins produced by C. gloeosporioides culture filtrates (using isolates generated during this South Pacific study). From a practical point of view, these results suggest that C. gloeosporioides from yam is a potential pathogen of a number crops and associated natural flora which could act as inoculum reservoir posing a serious threat to yam production. Hence, in yam growing areas, farming practices such as intercropping, or mixed cropping, with known C. gloeosporioides hosts should be minimised whereas weeding and other sanitation measures should be promoted.

Since only a limited number of isolates were tested, the total lack of infection by yam isolates of C. gloeosporioides on cotton and Citrus spp. needs further investigation involving more isolates from various yam species. Baudin (1956) obtained symptom reactions with yam inoculum on a number of hosts including citrus trees.

The detection of C. gloeosporioides in yam tubers from Vanuatu and Papua New Guinea confirms that the fungus is able to infect and survive on the tuber tissue from season to season. These infected tubers could act as a primary source of inoculum playing an important role in the epidemiology of the pathogen in the field. The results also corroborate earlier works by Green and Simons (1992) and Peters et al.(1997) who reported the occurrence of C. gloeosporioides tuber infection under natural conditions in the Caribbean and West Africa. The mechanism of spread of C. gloeosporioides from tuber to canopy is still yet to be fully understood. However, the failure to re-isolate or detect the pathogen from shoot parts of plants raised from heavily infected tubers in this study, rules out any systemic spread of this pathogen.

The high incidence of soil-borne pathogens such as Fusarium spp., R. solani, and nematodes as well as tuber rotting agents such as Rhizopus spp., Penicillium spp. and Aspergillus spp. further confirms the importance of these organisms both in field and in storage (Ogundana et. al., 1981; Augustus, 2001). Further studies to determine whether these pathogens play any role in predisposing the process of tuber infection by C. gloeosporioides are required. Moreover, the high degree of contamination by these fast-growing fungi particularly Rhizopus, Rhizoctonia and Mucor might have inhibited the growth of C. gloeosporioides on agar hence underestimating its frequency. Therefore, to minimise these contaminants in future, both semi-selective medium (Ekefan et al., 1999) and monoclonal antibodies (James, 1998; Peters et al., 1997) could prove valuable tools for C. gloeosporioides isolations from soil as well as from yam tubers. In addition, detection of B. theobromeae, Phoma spp., Phomopsis spp., C. capsici and Curvularia spp. which cause a number leaf spot diseases on yam (Toribio et al., 1980; Torres-Lopez et al., 1986; Peters et al., 1997) gives an indication of the potential risk these pathogens, including C. gloeosporioides, could pose on tuber quality.

The high heterogeneity and complex patterns, at the molecular level, exhibited by isolates of C. gloeosporioides obtained from the South Pacific indicates the existence of a complex population structure in which sexual recombination probably plays a major role in generating variation. Interestingly, it is not unusual for such complex patterns of colonisation to occur in this genus, bearing in mind the genetically complex nature of the inoculum. The results further reveal that there is little or no evidence of clonality and the genetic diversity looks more similar to the isolates from natural habitats than to the standard agri-ecosystem pattern of relatively little genetic variation and lots of clonality. This fits the nature of the yam cultivation in the South Pacific islands with tiny plots within forest rather than large fields of monoculture crops. In addition, the lack of clear linkages between molecular fingerprints and host and the fact that isolates from different host species have a higher degree of homology than isolates from the same host species adds weight to the argument that C. gloeosporioides is a polyphagous pathogen with a wide host range. It also points to the extreme likelihood that the surrounding plants are acting as disease reservoirs. Moreover, the lack of clear linkages between molecular patterns and the geographical origin coupled with the evidence that closely similar strains are present on widely separate localities probably reflects the historic movement of germplasm between the islands.

The close correlation between the molecular trees generated by ISSR-PCR and the dendrogram representing all morphological data suggests that variation in morphology is at least partly genetically controlled. It thus appears that despite their limitations, morphological and cultural characteristics could still prove useful tools which will complement modern techniques in understanding the genetic variability of this pathogen.

No correlation was apparent between isolate pathogenicity and their molecular fingerprint patterns: isolates showing marked differences in pathogenicity appeared to be closely related in molecular terms. Perhaps this lack of correlation is not unsurprising since the DNA regions investigated are unlikely to be linked to pathogenicity in the fungus. It might be useful in the future to analyse DNA regions known to be associated with pathogenicity (for example by using AFLP analysis of the cDNAs of pathogens showing differences in virulence).

In conclusion, the results of the current work confirm that under optimum conditions, C. gloeosporioides is able to cross-infect different host species. This underpins the real threat that yam cultivations could face from the other neighbouring crops as well as from the natural flora in the proximity. The results further highlight the importance of C. gloeosporioides as a

tuber-borne pathogen under field conditions and the severe implications that tuber-borne infections could pose on yam production both as sources of infection and as a means of dispersal. The results also show that C. gloeosporioides isolates are highly heterogeneous indicating a complex population structure. However, a high degree of similarities between C. gloeosporioides isolates from widely different islands also suggests that populations of this pathogen from different South Pacific islands are not entirely distinct.

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APPENDIX

Table 1. C. gloeosporioides isolates isolated from yam and non-yam samples collected from various of the South Pacific countries between January 1999-April 2002.

Country	Region	Sample Code	Host	Collection date	Isolate code
Fiji	Viti	1	*D. alata*	03.03.99	DaFV1-3
Fiji	Viti	1	*D. alata*	03.03.99	DaFV1-4
Fiji	Viti	4	*D. alata*	03.03.99	DaFV4-3
Fiji	Viti	5	*D. alata*	03.03.99	DaFV5-3
Fiji	Viti	6	*D. alata*	03.03.99	DaFV6-1
Fiji	Viti	7	*D. alata*	03.03.99	DaFV7-3
Fiji	Viti	8	*D. alata*	03.03.99	DaFV8-1
Fiji	Viti	8	*D. alata*	03.03.99	DaFV8-3
Fiji	Viti	9	*D. alata*	03.03.99	DaFV9-1
Fiji	Viti	9	*D. alata*	03.03.99	DaFV9-2
Fiji	Viti	10	*D. alata*	03.03.99	DaFV10-1
Fiji	Katu	11	*D. alata*	03.03.99	DaFK11-1
Fiji	Katu	12	*D. alata*	03.03.99	DaFK12-1
Fiji	Katu	12	*D. alata*	03.03.99	DaFK12-2
Fiji	Katu	13	Kava	03.03.99	KFK13-1
Fiji	Katu	13	Kava	03.03.99	KFK13-2
Vanuatu	Efate	102	*D. alata*	24.02.99	DaVE102-3
Vanuatu	Efate	31	*Mikania micrantha*	26.02.99	MmVE31-2
Vanuatu	Santo	20	*D. alata*	24.02.99	DaVS20-2
Vanuatu	Santo	21	*D. alata*	24.02.99	DaVS21-4
Vanuatu	Santo	23	*D. alata*	24.02.99	DaVS23-2
Vanuatu	Santo	104	*Cajanus cajan*	24.02.99	CcVS104-2
Vanuatu	Santo	105	Kava	24.02.99	KVS105-2
Vanuatu	?	Van1	Yam tuber	.04.2002	YTV1
Vanuatu	?	Van1	Yam stem	.04.2002	YSV1
Vanuatu	?	Van2	Yam stem	.04.2002	YSV2
Vanuatu	?	Van4	Yam stem	.04.2002	YSV4
Vanuatu	?	Van5	Yam stem	.04.2002	YSV5a
Vanuatu	?	Van5	Yam stem	.04.2002	YSV5b
Pupa New Guinea	Western	OO4	*D. alata*	21.05.00	DaPNGW004-1
Pupa New Guinea	Western	OO4	*D. alata*	21.05.00	DaPNGW004-2
Pupa New Guinea	Western	OO4	*D. alata*	21.05.00	DaPNGW004-3
Pupa New Guinea	Western	OO6	*D.pentyphylla*	21.05.00	DpPNGW006-2
Pupa New Guinea	Central	OO5	*D. alata*	29.02.00	DaPNGC005-1
Pupa New Guinea	Central	OO5	*D. alata*	29.02.00	DaPNGC005-3
Pupa New Guinea	Central	O16	*D. alata*	29.02.00	DaPNGC016-3
Pupa New Guinea	Central	O4O	*Chilli*	29.02.00	CPNGC040-1
Pupa New Guinea	Central	O29	*M.esculenta*	03.03.00	MePNGC029-1
Pupa New Guinea	Milne bay	O31	*D. alata*	26.02.00	DaPNGMB031-6
Pupa New Guinea	Milne bay	O2	*M.esculenta*	26.02.00	MePNGBM02-1
Pupa New Guinea	New Ireland	O12	*D. alata*	30.03.00	DaPNGNI012-2
Pupa New Guinea	New Ireland	O13	*D. alata*	30.03.00	DaPNGNI013-1
Pupa New Guinea	New Ireland	O13	*D. alata*	30.03.00	DaPNGNI013-2a

Pupa New Guinea	New Ireland	O13	*D. alata*	30.03.00	DaPNGNI013-2b
Pupa New Guinea	New Ireland	O13	*D. alata*	30.03.00	DaPNGNI013-4
Pupa New Guinea	New Ireland	O14	*D. alata*	30.03.00	DaPNGNI014-1
Pupa New Guinea	New Ireland	O14	*D. alata*	30.03.00	DaPNGNI014-2
Pupa New Guinea	New Ireland	O15	*D. esculenta*	30.03.00	DePNGNI015-1
Pupa New Guinea	New Ireland	O15	*D. esculenta*	30.03.00	DePNGNI015-2
Pupa New Guinea	New Ireland	O15	*D. esculenta*	30.03.00	DePNGNI015-3
Pupa New Guinea	New Ireland	O25	*D. alata*	30.03.00	DaPNGNI025-2
Pupa New Guinea	New Ireland	O25	*D. alata*	30.03.00	DaPNGNI025-4
Pupa New Guinea	New Ireland	O26	*D. alata*	30.03.00	DaPNGNI026-2
Pupa New Guinea	New Ireland	O27	*D. alata*	30.03.00	DaPNGNI027-4
Pupa New Guinea	New West Britain	OO9	*D. alata*	07.04.00	DaPNGNWB009-3
Pupa New Guinea	New West Britain	OO9	*D. alata*	07.04.00	DaPNGNWB009-4
Pupa New Guinea	New West Britain	O36	*Kava*	04.04.00	KPNGNWB036-1
Pupa New Guinea	New West Britain	O38	*D. alata*	04.04.00	DaPNGNWB038-2
Pupa New Guinea	New West Britain	O45	*D. alata*	04.04.00	DaPNGNWB045-1
Pupa New Guinea	New West Britain	O45	*D. alata*	04.04.00	DaPNGNWB045-3a
Pupa New Guinea	New West Britain	O45	*D. alata*	04.04.00	DaPNGNWB045-3b
Pupa New Guinea	East Sepik	OO7	*D. alata*	15.06.00	DaPNGES007-2
Pupa New Guinea	East Sepik	OO9	*D .pentyphylla*	15.06.00	DpPNGES009-1a
Pupa New Guinea	East Sepik	OO9	*D .pentyphylla*	15.06.00	DpPNGES009-1b
Pupa New Guinea	East Sepik	O16	Yard long bean	15.06.00	YLBPNGES016-2
Pupa New Guinea	East Sepik	O17	*D. alata*	15.06.00	DaPNGES017-1
Pupa New Guinea	East Sepik	O17	*D. alata*	15.06.00	DaPNGES017-4
Pupa New Guinea	East Sepik	O2O	*D. alata*	15.06.00	DaPNGES020-1
Pupa New Guinea	East Sepik	O21	*D. alata*	13.06.00	DaPNGES021-1
Pupa New Guinea	East Sepik	O23	*D. alata*	13.06.00	DaPNGES023-2
Pupa New Guinea	East Sepik	O23	*D. alata*	13.06.00	DaPNGES023-5a
Pupa New Guinea	East Sepik	O23	*D. alata*	13.06.00	DaPNGES023-5b
Pupa New Guinea	East Sepik	O26	*D. alata*	13.06.00	DaPNGES026-2a
Pupa New Guinea	East Sepik	O26	*D. alata*	13.06.00	DaPNGES026-2b
Pupa New Guinea	East Sepik	O26	*D. alata*	13.06.00	DaPNGES026-3a
Pupa New Guinea	East Sepik	O26	*D. alata*	13.06.00	DaPNGES026-3b
Pupa New Guinea	East Sepik	O32	*D. alata*	17.06.00	DaPNGES032-1
Pupa New Guinea	East Sepik	O4O	*D. esculenta*	14.06.00	DePNGES040-2
Pupa New Guinea	East Sepik	O4O	*D. esculenta*	14.06.00	DePNGES040-3
Pupa New Guinea	Mumeng	6	Melon	31.01.00	MPNGM6-1
Pupa New Guinea	Bubia	Bda-044	Tuber yam	01.08.01	YTPNGB044-1

Table 2. Morphological and cultural characters used to compare isolates of *C.gloeosporioides* on PDA.

Characters	State	Ranking
1.Whole colony appearance		
1. 1. Colony radial growth	< 50	1
	51-60	2
	61-70	3
	71-80	4
	>80	5
1.2. Form of colony margin	Uniform	1
	Irregular	2
1.3. Colony annulation	Distinct	1
	Indistinct	2
	Absent	3
1.4.. Colony colour	Grey	1
	Grey dark	2
	White	3
	Brown	4
1.5.. Colony colour from reverse	Olivaceous black	1
	Grey dark	2
	White	3
	Brown	4
	Orange	5
1.6. Sector development	Present	1
	Absent	0
1.7. Sector colour	Olivaceous black	1
	Grey dark	2
	Grey	3
	Dark	4
	White	5
	Orange	6
	Not applicable	7
2. Nature of mycelium		
2.1. Colour of hyphae	Hyaline	1
	Dark	2
	White	3
	Grey and hyaline	4
	White and grey	5
	grey	6
2.2. Abandance of hyphae	Sparse	1
	Moderate	2
	Abundant	3
2.4. Mycelium elevation	surface	1
	low	2
	Moderate	3
	High	4
2.5 Sclerotial bodies	present	1
	absent	0
3. Reproductive structures		
3.1. 1.Acervuli	Absent	0
	Rare	1
	Moderate	2

	Abundant	3
3.1.2. Position of Acervuli	throughout colony	1
	Centrally located in the colony	2
	Not apllicable	3
3.1.3. Setae in Acervuli	present	1
	Absent	0
3.1.4.. Conidia	present	1
	Absent	0
3.1.5. Conidial mass colour	Orange to pink	1
	Whitish creamy	2
	Brown	3
	Not applicable	4
3.1.6. Number of conidial mass	Rare	1
	Moderate	2
	Abundant	3
	Not applicable	4
3.1.7. Position of conidial masses	throughout colony	1
	Centrally located in the colony	2
	Not apllicable	3
3.1.8. Conidial length	< 12	1
	12-15.	2
	16-19	3
	19-22	4
	>22	5
3.1.9. Conidial width	< 4	1
	4-6.	2
	>6	3
3.1.11. Appressoria	present	1
	Absent	0
3.1.12. Appressorial size	< 5	1
	5-8	2
	9-12	3
	13-15	4
3.2 Sexual stage		
3.2.1. Perithecia	present	1
	Absent	0
3.2.2. Position of perithecia	throughout colony	1
	Centrally located in the colony	2
	Not applicable	3
3.2.3. Number of perithecia /dish	Rare	1
	Moderate	2
	Abundant	3
	Not applicable	4
3.2.4. Ascospore length	< 15	1
	15-17	2
	18-20	3
3.2.5. Ascospore width	5-6.	1
	5-6.	2
	> 6	3

Table 3. Mean lesion diameter of 49 yam and non-yam isolates of *C. gloeosporioides* tested for relative pathogenicity on *D. alata*, **White Lisbon.**

Host species	Isolate code	Mean lesion (mm)
Control	Control	4.49
Yam	DaPNGNI014-1	11.57 |
Yam	DaPNGNI025-2	11.91 |
Chilli	CPNGC040-1	13.24 ||
Yam	DaPNGNI025-4	13.40 ||
Yam	DaPNGNI013-4	13.67 ||
Yam	DaPNGNI027-4	13.72 ||
Yam	DpPNGES009-1b	14.00 ||
Yam	DaPNGES007-2	14.31 |||
Yam	DaPNGNI015-1	14.35 |||
Yam	DaPNGES040-2	14.48 |||
Yam	DaPNGNI014-2	14.75 |||
Yam	DaPNGC016-3	15.45 ||||
Yam	DaPNGMB021-1	15.65 ||||
Yam	DaPNGNWB045-1	15.90 |||||
Yam	DaPNGMB031-6	16.60 ||||||
Yam	DaPNGNI013-2b	17.01 ||||||
Kava	KPNGNWB036-1	17.12 ||||||
Yam	DaPNGNI032-1	17.61 ||||||
Minute-a-mile	MmVE31-2	18.07 ||||||
Yam	DaPNGES026-2a	18.10 ||||||
Yam	DaFV1-4	18.57 ||||||
Kava	KVS105-2	18.64 ||||||
Yam	DaVS23-2	19.36 |||||||
Yam	DaPNGES023-5b	19.42 |||||||
Melon	MPNGM6-1	19.63 |||||||
Yam	DaPNGES020-1	20.61 ||||||||
Yam	YTV1	20.99 ||||||||
Yam	DaPNGES017-4	21.09 ||||||||
Cassava	MePNGC029-1	21.85 |||||||||
Yam	DaFV12-2	22.56 |||||||||
Yard Long Bean	YLBPNGES016-2	22.75 |||||||||
Yam	DaFV9-2	23.26 ||||||||||
Yam	DaPNGNWB045-3b	24.44 ||||||||||
Yam	DaFV4-3	25.08 |||||||||
Yam	DaPNGNI015-3	25.32 |||||||||
Yam	YSV1	25.35 |||||||||
Yam	DaPNGNI013-1	26.00 ||||||||
Yam	DaVS21-4	26.21 |||||||
Yam	DaPNGNI026-2	26.29 |||||||
Cassava	MePNGMB002-1	26.30 |||||||
Yam	YSV2	26.67 |||||||
Yam	DaVE20-2	28.81 ||||||
Yam	DaPNGNWB045-3a	29.78 |||||
Pigeon pea	CcFE104-2	30.06 |||||
Yam	YSV4	31.12 ||||
Yam	DaPNGES026-2b	31.42 ||||
Yam	YTPNGB044-1	32.03 |||
Yam	YSV5b	32.52 ||
Yam	DaPNGES026-3b	33.33

Means in the column followed by the same number of vertical bars (lines) are not significantly different according to the Student-Newman-Keuls test ($P < 0.05$). Each mean is the average of six replicates.

Table 4. Mean lesion diameter and radial growth of 49 yam and non yam isolates of *C. gloeosporioides* tested for relative pathogenicity on *D. alata*, **White Lisbon.**

Host species	Isolate code	Lesion size (mm)	Radial growth (mm)
Pigeon pea	CcVE104-2	30.056 (4.036)	70.19(1.66)
Kava	KVS105-2	18.639 (3.098)	68.88(1.44)
Yam	DaFV12-2	22.562 (1.198)	65.50(0.54)
Yam	DaFV1-4	18.572 (2.767)	55.94(0.34)
Yam	DaVE20-2	28.812 (2.987)	62.88(0.95)
Yam	DaVS21-4	26.206 (1.960)	65.00(1.06)
Yam	DaVS23-2	19.361 (1.975)	64.50(0.37)
Minute-a-mile	DaVE31-2	18.069 (1.323)	47.75(0.55)
Yam	DaFV4-3	25.079 (1.617)	44.69(2.07)
Yam	DaFV9-2	23.264(2.660)	47.44(1.69)
Yam	YTV1	20.990(2.74)	68.62(0.38)
Yam	YSV1	25.350 (2.27)	69.50(1.59)
Yam	YSV2	26.670 (1.03)	69.25(1.11)
Yam	YSV4	31.120(2.33)	65.38(2.13)
Yam	YS5b	32.520(1.51)	71.63(0.90)
Yam	DaPNGNI014-2	14.749 (1.780)	69.19(1.35)
Yam	DaPNGC016-3	15.451 (1.679)	62.25(1.70)
Yam	DaPNGNI026-2	26.297 (0.809)	66.94(1.58)
Chilli	CPNGC040-1	13.236 (1.544)	62.00(1.14)
Yam	DaPNGNI015-1	14.320 (1.158)	69.19(0.93)
Yam	DaPNGNI015-3	25.32 (1.16)	72.88(0.46)
Yam	DaPNGNI025-2	11.910 (1.020)	62.25(0.16)
Yam	DaPNGNI025-4	13.403 (1.235)	59.63(0.81)
Yam	DaPNGNI013-1	25.998 (1.452)	69.29(3.32)
Yam	DaPNGNI013-4	13.667 (1.690)	75.56(1.33)
Yam	DaPNGNI014-1	11.569 (0.990)	81.38(0.52)
Yam	DaPNGMB031-6	16.604 (0.828)	65.06(0.56)
Yam	DaPNGNI013-2b	17.014 (0.654)	64.48(0.62)
Yam	DaPNGES007-2	14.306 (1.274)	70.13(1.01)
Yam	DpPNGES009-1b	14.000 (1.518)	72.69(1.07)
Yard long bean	YLBPNGES016-2	22.745 (1.984)	65.99(1.18)
Yam	DaPNGES017-4	21.090 (2.228)	75.94(1.08)
Yam	DaPNGES0020-1	20.611 (2.314)	70.31(1.10)
Cassava	MePNGMB02-1	15.646 (1.347)	65.06(0.78)
Yam	DaPNGNI027-4	13.722 (1.801)	71.38(1.84)
Kava	KPNGNWB036-1	17.118 (1.646)	63.19(1.27)
Yam	DaPNGNWB045-1	15.896 (1.724)	45.50(2.58)
Yam	DaPNGNWB045-3a	29.783 (3.523)	69.25(0.59)
Yam	DaPNGNWB045-3b	24.440 (2.438)	54.50(1.89)
Yam	DaPNGES023-5b	19.417 (2.252)	73.07(1.64)
Yam	DaPNGES026-2b	31.420 (2.12)	66.69(1.37)
Yam	DaPNGES026-3a	18.100 (2.94)	65.69(0.75)
Yam	DaPNGES026-3b	33.390 (1.71)	73.19(0.39)
Yam	DaPNGES032-1	17.610 (1.18)	65.69(1.68)
Melon	MPNGM6-1	19.630 (1.24)	63.69(0.61)
Yam	YTPNGB044-1	32.030 (2.10)	56.31(0.56)
Yam	DePNGES040-2	14.480 (0.54)	72.50 (1.94)
Cassava	MePNGC029-1	21.850 (2.31)	55.81(0.78)
Cassava	MePNGMB02-1	26.290 (1.47)	71.38(1.60)

Lesion size is the mean of six replicates and Radial growth is the mean of four replicates. Numbers in parentheses are the standard errors (s.e)

Table 5. Mean no. sporulating lesions/leaf of 49 yam and non-yam isolates of *C. gloeosporioides* tested for relative pathogenicity on *D. alata*, **White Lisbon.**

Host species	Isolate code	Mean of no. sporulating lesion	
Yam	DaPNGNI014-2	3.500[a]	10.68 | b
Yard Long Bean	YLBPNGES016-2	3.833	11.17 ||
Yam	DaPNGMB031-6	3.833	11.25 |||
Yam	DaPNGNI025-2	4.000	11.48 ||||
Yam	DaPNGNI014-1	4.167	11.66 |||||
Yam	DpPNGES009-1b	4.167	11.69 |||||
Yam	DaPNGNI027-4	4.333	11.84 |||||
Yam	DaPNGES007-2	4.500	12.13 |||||
Yam	DaPNGNI015-1	4.667	12.39 |||||
Yam	DaPNGNI013-4	4.667	12.39 |||||
Yam	DaPNGNI025-4	4.833	12.62 |||||
Yam	YSV2	4.833	12.67 |||||
Minute-a-mile	MmVE31-2	5.000	12.83 ||||
Yam	DaPNGNI032-1	5.000	12.85 ||||
Yam	DaPNGMB021-1	5.000	12.88 ||||
Kava	KVS105-2	5.167	13.11 ||||
Melon	MPNGM6-1	5.167	13.13 ||||
Yam	DaVS21-4	5.167	13.13 ||||
Yam	DaPNGNI013-2b	5.333	13.32 ||||
Cassava	MePNGMB002-1	5.333	13.32 ||||
Yam	DaFV9-2	5.333	13.32 ||||
Cassava	MePNGC029-1	5.333	13.32 ||||
Yam	DaPNGES020-1	5.333	13.34 ||||
Yam	DaFV12-2	5.333	13.34 ||||
Yam	YSV5b	5.500	13.48 ||||
Yam	DaPNGNI026-2	5.500	13.55 |||
Kava	KPNGNWB 036-1	5.500	13.55 ||||
Yam	DaFV1-4	5.500	13.55 ||||
Yam	DaFV4-3	5.500	13.55 ||||
Yam	DaPNGC016-3	5.667	13.74 ||
Yam	DaPNGES017-4	5.667	13.74 ||
Yam	DaPNGNWB045-3b	5.667	13.74 ||
Yam	DaVE20-2	5.667	13.74 ||
Yam	DaPNGES023-5b	5.667	13.76 ||
Yam	DaPNGNWB045-1	5.667	13.76 ||
Yam	DaVS23-2	5.667	13.76 ||
Yam	YSV1	5.667	13.76 ||
Yam	YTV1	5.667	13.76 ||
Yam	DaPNGES02 6-2a	5.833	13.97 |
Yam	DaPNGNWB045-3a	5.833	13.97 ||
Yam	DaPNGES040-2	5.833	13.97 ||
Chilli	PNGC040-1	5.833	13.97 ||
Yam	DaPNGNI013-1	6.000	14.18 |
Yam	DaPNGNI015-3	6.000	14.18 |
Yam	DaPNGES02 6-2b	6.000	14.18 |
Yam	DaPNGES02 6-3b	6.000	14.18 |
Yam	YTPNGB044-1	6.000	14.18 |
Pigeon pea	CcFE104-2	6.000	14.18 |
Yam	YSV4	6.000	14.18 |

a = Original data
b = Arcsine-square-root transformed data

Means in the column followed by the same number of vertical bars (lines) are not significantly different according to the Student-Newman-Keuls test ($P < 0.05$). Each mean is the average of six replicates.

Table 6. Formation of acervuli, setae and perithecia and mature with ascospoes on *D. alata* **White Lisbon** after 7- 9 days after inoculation with *C. gloeosporioides* isolates from yam and other host species.

Host species	Isolate code	Acevuli	Setae	Perithecia	Asci/Acospores
Yam	DaFV1-4	P	P	A	A
Yam	DaFV 4-3	P	P	P	P
Yam	DaFV 9-2	P	P	A	A
Yam	DaFV12-2	P	P	P	P
Makania micrantha	VE31-2	P	A	A	A
Yam	DaVE20-2	P	A	A	A
Yam	DaVS21-4	P	P	A	A
Yam	DaVS23-2	P	p	P	P
Pigeon pea	CcVE104-2	p	A	A	A
Kava	KVS105-2	P	A	A	A
Yam	YSV1	P	P	P	P
Yam	YSV2	P	P	P	P
Yam	YSV4	P	P	P	P
Yam	YS5b	P	P	P	p
Yam	YTV1	P	P	P	P
Yam	DaPNGC016-3	P	p	P	P
Chilli	CPNGC040-1	P	A	A	A
Cassava	MePNGC029-1	P	A	A	A
Yam	DaPNGMB031-6	P	P	P	P
Cassava	MePNGMB02-1	P	A	A	A
Yam	DaPNGNI013-1	P	A	A	A
Yam	DaPNGNI013-2b	P	A	A	A
Yam	DaPNGNI013-4	P	P	P	P
Yam	DaPNGNI014-1	P	A	A	A
Yam	DaPNGNI014-2	P	A	A	A
Yam.	DaPNGNI015-1	P	A	A	A
Yam	DaPNGNI015-3	P	A	A	A
Yam	DaPNGNI025-2	P	P	P	P
Yam	DaPNGNI025-4	P	P	P	P
Yam	DaPNGNI026-2	P	A	A	A
Yam	DaPNGNI027-4	P	P	P	P
Kava	KPNGNWB036-1	P	P	P	P
Yam	DaPNGNWB045-1	P	A	A	A
Yam	DaPNGNWB045-3b	P	A	A	A
Yam	DaPNGNWB045-3a	P	P	P	P
Yam	DaPNGES007-2	P	A	A	A
Yam	DpPNGES009-1b	P	A	A	A
Yard Long Bean	YLBPNGES016-2	P	P	P	P
Yam	DaPNGES017-4	P	A	A	A
Yam	DaPNGES0020-1	P	P	P	P
Yam	DaPNGES0021-1	P	P	P	P
Yam	DaPNGES023-5b	P	A	A	A
Yam	DaPNGES026-2b	P	A	A	A
Yam	DaPNGES026-3a	P	P	P	P
Yam	DaPNGES026-3b	P	A	A	A
Yam	DaPNGES032-1	P	P	P	P
Yam	DePNGES040-2	P	p	P	P
Melon	MPNGM6-1	P	A	A	A
Yam	YTPNGB044-1	P	P	A	A

P = Present, A= Absent and d= for details of each isolates see Appendix Table 1.

4. Secretariat of the Pacific Community, Fiji

E. Lesione, V. Tuia, M. Taylor & T. Osborn

Introduction

The role of the Secretariat of the Pacific Community (SPC) in the SPYN is to serve as focal point for the *in vitro* multiplication, conservation, and distribution of selected disease free yam cultivars to other national partners in SPYN. In addition it is expected that SPC will conduct research relevant to the conserve the SPYN yam collection *in vitro* and hopefully by cryopreservation for the Pacific region where yams are a significant crop. The SPC Regional Germplasm Center (RGC) is undertaking these tasks in Fiji. The role of SPC RGC in SPYN complements the ongoing activities with other vegetatively propagated crops that are important for the Pacific Islands such as taro, bananas, and sweet potatoes. Valerie Tuia, RGC Curator and laboratory technician Eliki Lesione are responsible for SPYN activities on a part time basis under the supervision of Dr. Mary Taylor with Tom Osborn as the project leader.

Objectives

- To develop *in vitro* conservation strategies; a regional conservation strategy implemented, involving active *in vitro* national genebanks and cryopreserved base collections (limited to 150 accessions initially)
- To establish an international exchange of virus-tested, selected genotypes; at least 150 yam cultivars cultured *in vitro*, tested for viruses found in Pacific Island countries and subjected to therapy treatments

Initially SPC's role was to develop a germplasm centre for *in vitro* conservation of selected yam cultivars (including cryopreservation), and propagate virus-free genotypes for distribution to other partners (and for ACP-member countries of SPC). SPC would develop Material Transfer Agreements to ensure germplasm could be moved internationally under intellectual property rights protection. The Regional Germplasm Centre (RGC) had already been established at SPC, and therefore was able to accommodate the yam core collection. In addition, Material Transfer Agreements were in place for other crops at SPC, and could be used for yam germplasm exchange.

At the SPYN annual meeting in Port Vila, Vanuatu, 2001, it was agreed that SPC's role would be expanded to assist NRI in the establishment of core samples from other countries in tissue culture. This request was made to SPC because the NRI's virus work had increased significantly beyond what was expected. SPC RGC would endeavour to establish the core sample *in vitro,* leaving time for NRI to concentrate on virus indexing and therapy treatments. *A* request was made to Fiji quarantine for permission to import yam tubers directly into Fiji to use for the establishment of tissue cultures. After some discussion of what the appropriate protocol should be for disinfecting the yam tubers, an import permit was processed, which enabled the SPC RGC to directly import tubers from the participating countries.

One of the objectives related to involving the national tissue culture laboratories in the conservation of the yams. However, none of the national genebanks have the potential to conserve national yam collections, Most of the laboratories suffer from an absence of trained staff, equipment and supplies. The annual SPYN meeting at Port Vila, Vanuatu, 2001, agreed

that assistance from SPYN, would not make any significant difference to these laboratories, and that it is unlikely that yams can be conserved as *in vitro* cultures, other than at the SPC RGC. As there are plans to duplicate the RGC taro collection at either one of the IARCs, or at another regional tissue culture laboratory, the yam collection would also be safely duplicated at that time. As yams grow relatively slowly *in vitro*, their period between subcultures can be extended to 9-12 months, if plants are cultured at a temperature of 20°C. Consequently slow growth storage *in vitro* is the strategy adopted by the SPC RGC for the core sample of *D. alata* yams.

Maintaining the yams actively growing, albeit at a reduced temperature, enables multiplication protocols to be easily pursued in response to requests for germplasm. Although cryopreservation was a component of the SPYN project, it is unlikely that this method of long-term conservation would be used for this collection, because of the need to actively distribute. Should the collection be further reduced after country evaluation, then those accessions not selected by the countries, could be cryopreserved.

Activities

Receipt and planting out of yam tubers

The following protocol for disinfecting yam tubers was proposed and accepted by Fiji quarantine:

In the exporting country (Solomon Islands, Vanuatu, PNG and New Caledonia);

- Tubers should be washed free of soil and remnants of stems and roots removed.
- Tubers should be inspected to ensure freedom from cuts, blemishes and other signs of damage.
- Tubers should be inspected to ensure freedom from dry rots that may be associated with the nematode, *Pratylenchus coffeae*, or the fungus, *Colletotrichum gloeosporioides*.
- Tubers should be treated in a mixture of carbaryl (0.1%), malathion (0.1%) and white oil (1%) in order to remove the threat of insect contamination.
- Tubers should not be treated with fungicides, but can be surface sterilized by dipping for a short time (3 min) in a 1% sodium hypochlorite (bleach) to destroy surface contaminants

The SPC RGC's obligation is to:

- Establish *in vitro* cultures using appropriate surface sterilization techniques, and meristem culture. Any material resulting from this process must be autoclaved.
- When yam plants have been used to establish *in vitro* cultures, the remaining foliage and below soil parts, together with the soil should be autoclaved or incinerated under Fiji MAFF Quarantine supervision.

The freshly cut parts of the tuber were dusted with 10g/l of fungicide (Ridomil) and the tubers were then planted in a substrate consisting of sterilized soil: sand (70:30). The pot size was 160mm diameter and 170mm height. Attempts are being made to obtain the selected *D alata* accessions from the Solomon Islands. It is likely that these will be sent from the Solomon Islands to the RGC in February.

Establishment of yam tissue cultures from nodal buds and shoot tips

Dormant tubers were received from Papua New Guinea, Fiji and Vanuatu. They were planted in the RGC screenhouse as described in 2.1. Once the tubers had sprouted, young stems were removed to provide the nodal bud explants for initiation into tissue culture. The selected stems were cut and surface sterilized using the protocol described by Ng (1992). Stems were washed under running tap water. Nodal cuttings (2-3cm long) were excised, and then rinsed in 70% ethanol for 5 min. After ethanol treatment the explants were sterilized using 10% bleach for 20 min, trimmed and then sterilized again in 5% bleach for 10 min. They were then rinsed three times with sterile distilled water (SDW), and kept in the final rinse until subsequent use to prevent dehydration of the nodes. The final size of the explant was 0.5-1cm. With shoot tips the same procedure is followed, except that the disinfection time is reduced. 70% ethanol for 2 min and 5% bleach for 5 min has given the best results with shoot tips.

Experiments have been carried out to optimize the establishment of *D alata* in tissue culture, as some *D alata* cultivars are difficult to grow *in vitro*. In establishing yams *in vitro* the production of phenols can be a problem, and if excessive, can hinder establishment in tissue culture. Phenolic production is more apparent, when samples for tissue culture are taken shortly after the yam has sprouted, therefore nodal explants should only be taken from fully extended stems. The problem of phenolic substances can be addressed in different ways. Rapid transfer to fresh medium is usually imperative if the medium around the explant begins to become discoloured or darkened. The interval between transfers can be adjusted according to the severity of the problem. However, frequent transfers take up time, and can be labour intensive. Explants from plants whose tissues are liable to browning, can be washed in a solution of an anti-oxidant, immediately after excision. The RGC has been investigating this method as an alternative to frequent transfers to fresh medium. Citric acid (100mg/l) can be an effective anti-oxidant, presumably acting as a chelating agent, sequestering metal ions, which are needed to activate the oxidative enzymes. Newly excised explants have been rinsed in citric acid solutions for 10, 20, 40 and 60 min. Although rinsing with citric acid (100mg/l for 10 min) can be effective, generally with most explants, this results in a bleaching effect, which then hinders growth and development of that explant. The procedure now used in the RGC is to transfer explants to fresh medium if necessary.

From the experiments with shoot tips it has been observed that they do not produce phenols in the early stages of culture, but after a period of two to three months, phenolics are apparent in the medium. However, shoot tips being more delicate, require a more careful disinfection period, and the success rate with the establishment of shoot tips (5%) *in vitro* is less than that with nodal buds (70%).

In addition to the anti-oxidant treatment, the RGC has also looked at which nodes respond well in tissue culture. Nodes from the shoot-tip to node 14 have been excised, and cultured. Of the cultivars used, nodes in the range 3- 6 are the most responsive, although with some cultivars, nodes 1-7 can respond equally as well as nodes 3-6. Experiments have also shown that the stems from which these nodes are excised should have at least five nodes present. If less than this then the excised nodes are too soft and contaminated from bacteria. This applies to shoot tips as well as nodes.

The medium used for establishment of both nodal buds and shoot tips is M50 (standard MS + 50g/l sucrose), without activated charcoal. The absence of activated charcoal enables both

phenols and bacterial contamination to be detected. Once the explants have grown, they are transferred to M50 medium containing activated charcoal.

A method that has been used successfully for collecting leaf, stem and bud tissue from a number of plant species, directly from the field in the tropics was investigated for its potential for initiating nodal explants directly from yam field collections. 100mg/l benlate was incorporated into the culture medium as a deterrent against fungal growth. The nodal cuttings were excised from the plants, and disinfected as described above. Once disinfected, they were inoculated onto the culture medium. The explants were then treated with antibiotics by dropping one drop each of vancomycin (0.25mg/ml), and chloramphenicol (0.25mg/ml) onto the explants. The antibiotic treatment proved to be phytotoxic, and none of the explants survived. This experiment will be repeated to see if a non-toxic antibiotic treatment can be determined.

Establishment of yam meristems from screenhouse grown plants.

In an effort to speed up the establishment of meristem-derived cultures, meristems were excised directly from plants in the screenhouse. A total of 50 nodal cuttings were taken from five Fijian cultivars (10 per cultivar), rinsed with tap water, and dipped in soapy water prior to further surface disinfection. Before surface sterilization, the nodal cuttings were soaked in SDW for one hour. They were then subjected to the same disinfection treatment as described in 2.2. The explant was trimmed down between the two bleach treatments, and before rinsing in SDW, so that the final size of the meristem was 0.2-0.5mm long. Liquid and solid media for meristem-tip cultures were modified from Ng (1992) protocols and also that described by Malaurie *et al.*, (1998). Filter paper bridges were used with the liquid medium.

Of the 50 meristems excised for this experiment, all failed to grow. Some of the meristems remained green for some time after excision, but there was no development, and eventually they died. There was no obvious bacterial or fungal contamination, but in many cases, there was excessive phenolic exudation. Attempts were made to reduce the effect of the phenolic compounds on the development of the meristems by regular subculturing, but this was not successful. Other yam researchers have found that the establishment of meristem cultures from plants either in the field, or in the screenhouse is highly problematic.

Establishment of meristems excised from yam tissue cultures

In this experiment, the effect of the sucrose concentration in the culture medium, as described by Malaurie *et al.,* 1998, on meristem response was evaluated. Two Fijian cultivars were cultured on medium containing 3%, 5% and 7% sucrose. Plants grew well on all three media, although as the time on culture was extended, those plants on the media with 5% and 7% sucrose, showed a higher degree of senescence. Meristems were excised from plants cultured on all three concentrations of sucrose as described in 2.3. For each cultivar, 15 meristems were excised from each sucrose treatment. 60% of meristems, excised from plants cultured on medium containing 3% sucrose developed, and grew into plants (ten from one cultivar and eight from the other). Only 10% of the meristems excised from plants cultured on a medium containing 5% sucrose survived. There was no survival of the meristems, which had been excised from plants cultured on medium containing 7% sucrose. These results (Table 1) show that meristem culture from *in vitro* plants has a far higher rate of success, than the culture of meristems excised from screenhouse grown plants. The results also show that meristems

excised from mother plants cultured on medium containing 3% sucrose have optimum survival and development.

Table 1: The effect of varying concentrations of sucrose in the culture medium on the growth and development of meristems

Meristem medium	Sucrose concentration	Rooting of mother plant	Senescence of mother plant (%)	Growth of meristem excised from mother plant (%)
BM-4-50 Macro: 200mls Micro: 5mls Vit: 10mls FeEDTA	3%	-ve	2	60
BAP (1mg/ml) NAA (1mg/ml) Sucrose Agar	5%	+ve	50	10
	7%	+ve	75	0

Results achieved

Yam tubers established in the RGC screenhouse

To date, the following yam tubers have been received and have been planted in the screenhouse.

Table 2. Number of yam tubers received.

Countries	Total No received
PNG	38
Vanuatu	24
Fiji	20

Of these tubers, three accessions from Vanuatu were rotten (VU 498, VU 649, VU 729), and so could not be established in tissue culture. All of the other yam tubers planted in the screenhouse produced sprouts.

Yam tissue cultures established in the RGC

On average, approximately 70% of the nodal explants grew successfully into plantlets with the first culture. With some cultivars, the explant died, and the process had to be repeated. The cultivars that were difficult to establish *in vitro* were those with purple stems. A higher rate of phenolic production was shown by those cultivars with purple stems, compared to those cultivars with green stems.

Table 3. Yam accessions maintained as tissue cultures in RGC, February 2003

Country	No of accessions
PNG	47
New Caledonia	4
Vanuatu	30
Fiji	19

Tissue cultures were also received from NRI. In some cases these were replicates of what was already held in the RGC, but there were also "new" accessions received. There are some accessions that still have to be established in tissue culture. These are PNG (3), Vanuatu (3), and Fiji (1). A detailed database is attached as an appendix at the end of this report.

Yam meristem culture

Of 50 meristems excised directly from screenhouse plants, none developed into plants. Of 90 meristems excised from tissue cultured plants, 21 developed into plants. 86% of these had been excised from plants cultured on medium containing 3% sucrose.

Discussion

D. alata is one of the more difficult yam species to establish *in vitro*, with both phenolic production, and bacterial and fungal contamination causing problems. The disinfection protocol used with these Pacific yams appears to work well, with relatively few losses occurring as a result of contamination. However, all of the plants established in tissue culture were derived from nodal cuttings of screenhouse grown plants. Attempts to establish plants *in vitro* from nodal buds excised directly from field grown plants failed. Growing the plants in a screenhouse environment, avoiding watering from the top, and maintaining a relatively pest and disease free environment all promote the development of "clean" axillary buds.

Phenolic production is an ongoing problem, especially with purple-stemmed plants. As stated, frequent transfer to fresh medium can help, but if the phenolic production is especially excessive then this response is not sufficient to ensure unhindered development. The use of anti-oxidants as a dip, or incorporated in the culture medium can have more impact. The use of anti-oxidants, other than citric acid, both as dips and incorporated in the medium will continue to be investigated in the RGC.

Work is continuing in the RGC to try and improve the establishment *in vitro* of both nodal explants and meristems.

Training

Training in cryopreservation of yams, using encapsulation-dehydration, was provided by Dr Bernard Malaurie, IRD, Montpellier, from May 7-24, 2001. All RGC staff received this training, so that they would be better equipped for future conservation and research on yams, or on any other species using the same technique.

This training complimented the cryopreservation training provided under the TaroGen project, and therefore increases the ability of the SPC RGC to provide a valuable service to the Pacific in the area of PGR conservation (see protocols in Appendix).

The training included medium preparation of all the encapsulation solutions, excision of apices, the encapsulation-dehydration procedure, immersion of apices in liquid nitrogen and the culture of the apices on a specific recovery medium (4-50) containing the growth regulators, NAA and BAP, to facilitate regrowth from the thawed apices.

The encapsulation-dehydration procedure consists of the following steps:

- excised apices;
- apices encapsulated in alginate
- alginate beads pretreated in sucrose liquid medium
- dehydration over silica gel
- dried beads in cryovial, rapidly and directly immersed into liquid nitrogen
- thawing and culture on recovery medium.

Dehydration of the meristems is an important step before cryopreservation because the presence of excess water in the plant tissue will cause ice damage and therefore failure of the technique. Three Fijian varieties maintained *in vitro* were used to investigate the optimum sucrose pre-treatment and dehydration duration. For the sucrose pre-treatment, two concentrations of sucrose were evaluated, 0.75M and 1.0M.

Apices embedded in alginate beads were dropped into standard liquid MS medium containing sufficient sucrose to provide the desired molarity, and cultured on a shaker for three days. After the sucrose pre-treatment, the optimum dehydration period (8h, 10h, 14h, 16h and 24h) was investigated, with the encapsulated apices held in airtight boxes over silica gel for the required length of time.

One of the cultivars tested, showed some growth with the 0.75M sucrose pre-treatment, and both 8h and10h dehydration. However, the swelling of the meristem was not sustained, and there was no differentiation of shoots and roots. This result was no indication of the effect of that treatment as all of the control meristems failed to grow, suggesting there was a problem with the "quality" of the meristems, and/or the recovery medium.

The quality of the meristems is very important and plays a very significant role in determining whether a cryopreservation protocol succeeds or fails. In these experiments, the dissection of good meristems was hindered by the presence of excess mucilage in the yam explants. In addition, the meristems were hyperhydrated, and this has been shown, with yams and taro, to reduce the ability of the meristem to survive cryopreservation.

Documentation was provided on the technical protocols and media preparation, together with photographs of all the stages in the procedure.

Problems encountered

With the change in the project strategy requiring SPC RGC to develop *in vitro* elite national collections, yam tubers had to be imported into Fiji. As Fiji has strict quarantine regulations, a yam tuber importation protocol had to be developed and approved. The SPYN Scientific Director and Technical Adviser worked with the RGC Adviser to develop a protocol acceptable to Fiji quarantine. The review process of the protocol took four months. By this time many of the national collections were being planted in the field.

One shipment of yam tubers was received and subsequently destroyed because it failed to meet the requirements of Fiji quarantine. All these issues have caused a major delay in putting the national elite yam collections into tissue culture.

The problems encountered in the establishment of tissue cultures, both from nodal cuttings and meristems have been described in 4.0 (Discussion).

Conclusions and Perspectives

Plant genetic resources are important for the present and future of agriculture in the Pacific. The Pacific contains important and unique germplasm that is under threat and need to be properly conserved and used. PGR conservation and use in the Pacific offers a difficult challenge in a region with small and very isolated countries.

The SPC Regional Germplasm Centre (RGC) opened in September 1999 with funding support from European Union and Australian governments in response to the need for PGRFA conservation and use in the Pacific. The RGC has been central to SPC's role in PGRFA management, and it is the only *in vitro* conservation facility in the Pacific with a regional multi-crop mandate. SPC is now hosting both a regional genebank and a regional PGRFA network for a complimentary conservation approach to PGRFA conservation and use at a regional and national level. The RGC is playing a central role in SPC and the region for the conservation and distribution of germplasm in the region under a strict code of conduct. Important collections are held in the RGC including taro, bananas, sweet potatoes, and cassava.

As explained in this report, the South Pacific Yam Network (SPYN) has enabled the SPC RGC to undertake research to optimize the tissue culture methodology for *D.alata* yams. This has enabled the establishment of an *in vitro* collection from countries participating in SPYN, and this will be a lasting contribution of SYPN. Although accessions from this collection cannot be distributed as yet, due to the need for virus indexing, once this has been achieved, the distribution of this germplasm will greatly benefit yam production in the region. The cryopreservation training provided during SPYN complimented cryopreservation research on taro and this will provide a foundation for further research in this area so that the yam collection, and any other crops responding to the same methodology, can be cryopreserved, when necessary. The lessons learnt during SPYN and the protocols developed can be applied to other yam species so that the RGC regional yam collection can continue to expand.

In conclusion the SPC RGC component of SPYN has made an important contribution to research on the *in vitro* methodology of this species. A regional *in vitro* yam collection has been established which will make an important contribution to the cultivation of this species in the Pacific beyond the life of the SPYN project.

References

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Appendix 1

Yam database for RGC Feb 2003

RGC ACC NO	ACC NO.	VARIETY	ORIGIN	Source	Received
DA/PNG-01	PNG 184	Kisi (GL 051)	PNG	NRI-UK	12/4/02 as tc
DA/PNG-02	PNG 185	Mui (female)(RL 009)	PNG	NRI-UK	12/4/02 as tc
DA/PNG-03	PNG 186	Barai (Y 006)	PNG	NRI-UK	12/4/02 as tc
DA/PNG-04	PNG 188	Naso (CMG001)	PNG	NRI-UK	12/4/02 as tc
DA/PNG-05	PNG 189	West Fergerson 1 (MFA 004)	PNG	NRI-UK	12/4/02 as tc
DA/PNG-06	PNG 191	Kaiorerau (GL 050)	PNG	NRI-UK	12/4/02 as tc
DA/PNG-07	PNG 192	West Fergerson 2 (MFA 005)	PNG	NRI-UK	12/4/02 as tc
DA/PNG-08	PNG 193	Tobo (GL049)	PNG	NRI-UK	12/4/02 as tc
DA/PNG-09	PNG 194	N/A	PNG	NRI-UK	12/4/02 as tc
DA/PNG-10	Bda 001	Takua Yavu	PNG	NARI-PNG	12/12/01as tb
DA/PNG-11	Bda 002	Takna Kupui	PNG	NARI-PNG	16/9/02 as tb rpl
DA/PNG-12	Bda 012	Modavatere	PNG	NARI-PNG	12/12/01as tb
DA/PNG-13	Bda 022	West fergusson I	PNG	NARI-PNG	16/9/02 as tb rpl
DA/PNG-14	Bda 024	West fergusson II	PNG	NARI-PNG	16/9/02 as tb rpl
DA/PNG-15	Bda 033	Mui (female)	PNG	NARI-PNG	12/12/01as tb
DA/PNG-16	Bda-043	Barai	PNG	NARI-PNG	16/9/02 as tb rpl
DA/PNG-17	Bda 052	Tobo	PNG	NARI-PNG	12/12/01as tb
DA/PNG-18	Bda 053	Kaiorerau	PNG	NARI-PNG	12/12/01as tb
DA/PNG-19	Bda 054	Kisi	PNG	NARI-PNG	16/9/02 as tb rpl
DA/PNG-20	Bda 108	Kwadesora	PNG	NARI-PNG	12/12/01as tb
DA/PNG-21	Bda 110	Mainina	PNG	NARI-PNG	12/12/01as tb
DA/PNG-22	Bda 140	Yabeduae	PNG	NARI-PNG	12/12/01as tb
DA/PNG-23	Bda 180	Maireba Red	PNG	NARI-PNG	16/9/02 as tb rpl
DA/PNG-24	Bda 003	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-25	Bda 009	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-26	Bda 014	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-27	Bda 035	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-28	Bda 047	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-29	Bda 048	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-30	Bda 055	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-31	Bda 056	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-32	Bda 073	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-33	Bda 078	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-34	Bda 079	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-35	Bda 080	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-36	Bda 083	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-37	Bda 088	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-38	Bda 093	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-39	Bda 095	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-40	Bda 106	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-41	Bda 112	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-42	Bda 124	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-43	Bda 142	N/A	PNG	NARI-PNG	16/9/02 as tb

DA/PNG-44	Bda 146	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-45	Bda 156	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-46	Bda 167	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-47	Bda 190	N/A	PNG	NARI-PNG	16/9/02 as tb
DA/PNG-48	PNG 183	Napo Goning (Y008)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-49	PNG 187	Kusin de (GG013)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-50	PNG 190	Gai (Y004)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-51	PNG 195	Simbang (GG005)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-52	PNG 184	Kisi (GL 051)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-53	PNG 185	Mui (female)(RL 009)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-54	PNG 186	Barai (Y 006)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-55	PNG 188	Naso (CMG001)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-56	PNG 189	West Fergerson 1 (MFA 004)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-57	PNG 191	Kaiorerau (GL 050)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-58	PNG 192	West Fergerson 2 (MFA 005)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-59	PNG 193	Tobo (GL049)	PNG	NARI-PNG	28/01/03 as tc
DA/PNG-60	PNG 194	N/A	PNG	NARI-PNG	28/01/03 as tc
DA/NC-01	NC 002	Numea Rouge	N.Caledonia	NRI-UK	12/4/02 as tc
DA/NC-02	NC 003	Koupet	N.Caledonia	NRI-UK	12/4/02 as tc
DA/NC-03	NC 004	Numea Rouge	N.Caledonia	NRI-UK	12/4/02 as tc
DA/NC-04	NC 007	Louis "241"	N.Caledonia	NRI-UK	12/4/02 as tc
DA/NC-05	NC 002	Numea Rouge	N.Caledonia	NRI-UK	28/01/03 as tc
DA/NC-06	NC 003	Koupet	N.Caledonia	NRI-UK	28/01/03 as tc
DA/NC-07	NC 004	Numea Rouge	N.Caledonia	NRI-UK	28/01/03 as tc
DA/NC-08	NC 007	Louis "241"	N.Caledonia	NRI-UK	28/01/03 as tc
DA/VAN-01	VAN 004	DA 0003.1	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-02	VAN 005	DA 0004.1	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-03	VAN 070	Basa (VU 401)	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-04	VAN 085	Buntun ankapkap (VU 424)	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-05	VAN 087	Warereo (VU 426)	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-06	VAN 108	Mere (VU 454)	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-07	VAN 127	Patapata (VU 480)	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-08	VAN 145	Not (VU 500)	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-09	VAN 208	Letslets nambas (VU 578)	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-10	VAN 226	N/A	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-11	VAN 232	Lakon (VU 603)	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-12	VAN 311	Bwevu (VU 630)	Vanuatu	NRI-UK	12/4/02 as tc
DA/VAN-13	VU 011	Malekula	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-14	VU 029	Efate	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-15	VU 232	Epi	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-16	VU 401	Santo	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-17	VU 415	Pentecost	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-18	VU 421	Pentecost	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-19	VU 423	Pentecost	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-20	VU 426	Pentecost	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-21	VU 459	Avunamalae	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-22	VU 461	Avunamalae	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-23	VU 475	Efate	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-24	VU 498	Santo	Vanuatu	Vanuatu	22/4/02 as tb

DA/VAN-25	VU 521	Malekula	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-26	VU 536	Malekula	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-27	VU 562	Malekula	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-28	VU 563	N/A	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-29	VU 578	Malekula	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-30	VU 589	Malekula	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-31	VU 633	Pentecost	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-32	VU 649	Gaua	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-33	VU 650	N/A	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-34	VU 676	Tanna	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-35	VU 699	Tanna	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-36	VU 729	Erromango	Vanuatu	Vanuatu	22/4/02 as tb
DA/VAN-37	Van 252	Bwev Mudani (VU632)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-38	Van 284	n.a. (bulbifera) (VU675)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-39	VAN 004	DA 0003.1	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-40	VAN 005	DA 0004.1	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-41	VAN 070	Basa (VU 401)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-42	VAN 085	Buntun ankapkap (VU 424)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-43	VAN 087	Warereo (VU 426)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-44	VAN 108	Mere (VU 454)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-45	VAN 127	Patapata (VU 480)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-46	VAN 145	Not (VU 500)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-47	VAN 208	Letslets nambas (VU 578)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-48	VAN 226	N/A	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-49	VAN 232	Lakon (VU 603)	Vanuatu	NRI-UK	28/01/03 as tc
DA/VAN-50	VAN 311	Bwevu (VU 630)	Vanuatu	NRI-UK	28/01/03 as tc
DA/FJ-01	YAM/FJ-01	Kivi	Fiji	KRS	25/2/02 as tb
DA/FJ-02	YAM/FJ-02	Murapoi	Fiji	KRS	25/2/02 as tb
DA/FJ-03	YAM/FJ-03	Taniela Vula Leka (rough skin)	Fiji	KRS	25/2/02 as tb
DA/FJ-04	YAM/FJ-04	Taniela Vula Leka A	Fiji	KRS	25/2/02 as tb
DA/FJ-05	YAM/FJ-05	Sisiwa	Fiji	KRS	25/2/02 as tb
DA/FJ-06	YAM/FJ-06	Futuna Vula Leka	Fiji	KRS	25/2/02 as tb
DA/FJ-07	YAM/FJ-07	Uvi ni Futuna	Fiji	KRS	25/2/02 as tb
DA/FJ-08	YAM/FJ-08	Taniela Damu Leka 1	Fiji	KRS	25/2/02 as tb
DA/FJ-09	YAM/FJ-09	Damuni	Fiji	KRS	25/2/02 as tb
DA/FJ-10	YAM/FJ-10	Vurai	Fiji	KRS	25/2/02 as tb
DA/FJ-11	YAM/FJ-11	Vurai Vula Leka	Fiji	KRS	25/2/02 as tb
DA/FJ-12	YAM/FJ-12	Reado	Fiji	KRS	25/2/02 as tb
DA/FJ-13	YAM/FJ-13	Voli Balavu	Fiji	KRS	25/2/02 as tb
DA/FJ-14	YAM/FJ-14	Damuni Balavu	Fiji	KRS	25/2/02 as tb
DA/FJ-15	YAM/FJ-15	Voli	Fiji	KRS	25/2/02 as tb
DA/FJ-16	YAM/FJ-16	Taniela Damu 2	Fiji	KRS	25/2/02 as tb
DA/FJ-17	YAM/FJ-17	Niumadu 1	Fiji	KRS	25/2/02 as tb
DA/FJ-18	YAM/FJ-18	Kuro	Fiji	KRS	25/2/02 as tb
DA/FJ-19	YAM/FJ-19	Taniela Vula Leka B	Fiji	KRS	25/2/02 as tb
DA/FJ-20	YAM/FJ-20	Veiwa	Fiji	KRS	25/2/02 as tb
DR/AF-01	Yam 15	TDR 89/01278	Nigeria Afica	USP Samoa	Rec as tc
DR/AF-02	Yam 27	TDR 747	Nigeria Afica	USP Samoa	Rec as tc
DR/AF-03	Yam 29	TDR 87/00551	Nigeria Afica	USP Samoa	Rec as tc

rpl - replacements				
tc - tissue culture				
tb - tubers				
DA - *Dioscorea alata*				
DR - *Dioscorea rotundata*				
dorm - dormant				
SH - screenhouse				
KRS - Koronivia Research Station				
NRI - National Research Insitute, UK				

5. NARI, Papua New Guinea

Peter A. Gendua, Geoff Wiles and Jimmy B. Risimeri

Background

Yam is an important staple food and ceremonial crop in the South Pacific Island countries. In Papua New Guinea (PNG) it ranks fifth after sweet potato, banana, sago and taro as a staple crop in terms of estimated production. However, the crop's survival and production is threatened by many factors including declining soil fertility, pest and disease problems, and competition from alternative crops. The South Pacific Yam Network (SPYN) project was designed to enhance the competitive position of yam in traditional cropping systems of five Pacific Island countries: Papua New Guinea, Solomon Island, Vanuatu, New Caledonia and Fiji. Without any yam-breeding programme taking place in the region, selecting the best available germplasm for distribution throughout the region will achieve improvements in yam production. Improved varietal characteristics include desirable tuber shape, eating quality (taste preference) and anthracnose resistance.

To select improved varieties and share these between countries, each Pacific partner country including PNG undertook to review the *ex situ* yam germplasm collection of the country and initiate collection of additional yam germplasm if need be, characterize and evaluate the germplasm, rationalize collections and select cultivars of interest. The selection process was not only based on agro-botanical traits but also physio-chemical traits and resistance to anthracnose.

The project was a four-year project funded by the INCO-DC programme of the EU. The project was started in February 1999 and ended in January 2003. This report reviews the achievement, or otherwise, of the planned milestones and describes constraints encountered during the four years of the project. The report also assesses status of the PNG component of the project at the time of project termination.

Objectives

The specific objectives of the PNG component of the project were:

1. To review the *ex situ* yam germplasm collection of the country and initiate collection of additional yam germplasm: characterize and evaluate the germplasm, rationalize the collection and select cultivars of interest using agreed selection criteria. As a result the project would:

    - Develop a computer database containing passport data and morphological descriptors of accessions in the collection for sharing between partner countries.
    - Select cultivars of greater yam (*Dioscorea alata*) with compact round to oval tuber shape and anthracnose tolerance.
    - Provide selected yam tuber samples to collaborators to determine physio-chemical characters.

2. In collaboration with other collaborators conduct surveys and collect virus infected samples from yams and other plants for identification of virus strains present in the region and development of virus indexing methods and their application.

3. In collaboration with other collaborators conduct surveys and collect isolates of anthracnose (*Colletotrichum gloeosporoides*) for DNA fingerprinting of these isolates and to conduct pathogenicity tests.

4. Collaborate with other collaborators by providing selected yam cultivars for *in vitro* conservation of yams.

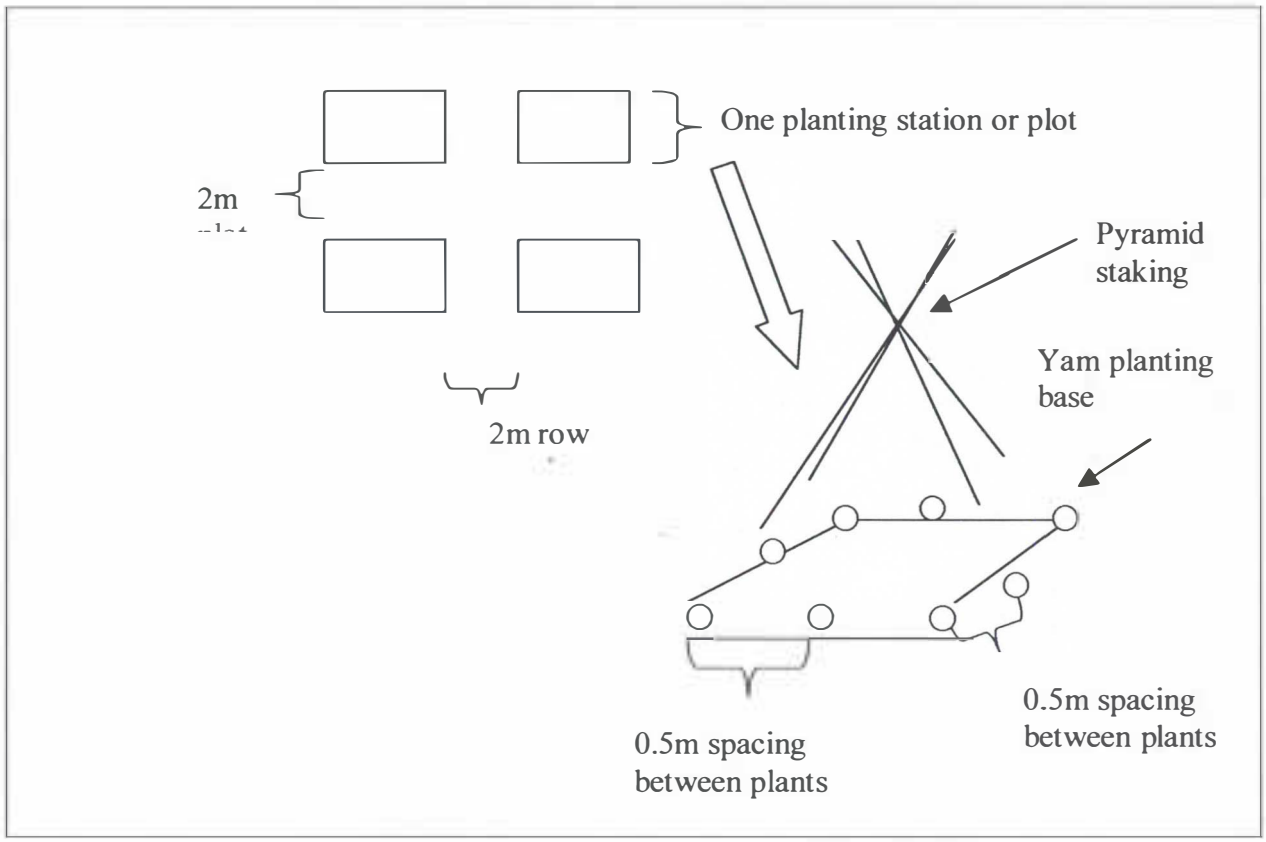
5. To participate in developing *in vitro* conservation strategies and to provide yam samples for an *in vitro* national/regional gene bank and cryopreserved base collection.

6. To conduct agronomic evaluation of selected yam cultivars.

Materials and Methods

To initiate the project, the PNG component of the SPYN project reviewed the *ex situ* yam germplasm collection of the country and initiated collection of additional yam germplasm in November 1999. The project staff undertook yam germplasm collection trips to most of the yam growing areas of PNG and collected additional available germplasm.

Figure1. Field planting arrangements and pyramid staking method



The germplasm was assembled in an *ex situ* gene bank at Bubia. The collection has been replanted in the field every growing season between October and November each year. In the 1999/2000 and 2000/1 seasons the collection was planted in rows of 1m apart and at a plant spacing of 0.5m between plants using the "A" frame staking method. However, due to the high incidence of anthracnose and cross infection of accessions in the field under Bubia conditions, for 2001/2 season and thereafter the individual accessions were planted in separate plots or planting stations (see Figure 1). Each plot area is $1m^2$. The plant spacing is 0.5m between plants; therefore there are 8 plants per plot per accession. The spacing between plots is 2m, which allows a much wider spacing between accessions, and is intended to minimise the spread of anthracnose and avoid cross-infection of accessions. The staking method used is a pyramid method.

The varieties were sorted and grouped according to their different tuber shapes and planted accordingly. The accessions with round and oval-shaped tubers were planted in one block, those with cylindrical tubers in another block, followed by those with flattened, triangular and deformed tubers.

The accessions were scored in the field for anthracnose susceptibility. In the first season (2000/1) yam anthracnose was found to be a major problem for the *D. alata* collection, and resulted in poor growth and premature death of many accessions. In 2001/2 and 2002/3 the collection was sprayed once a fortnight with fungicides, Bravo (chlorothalonil), Copper Champion (copper oxychoride) and Manzeb (Dithane M45) in rotation. For the survival of the collection the accessions will continue to be sprayed every growing season. The collection is also sprayed fortnightly with insecticides Karate® and Orthene® to control the mirid *Harpedona plana* Poppius (Hemiptera: Miridae).

The mirid, *Harpedona plana* Poppius (Hemiptera: Miridae), a pest reported in the 2001 annual report, attacked the yam plants and caused severe defoliation of *D. alata*. It sucks on *D. alata* leaves and vines causing leaf shriveling and leaf fall and consequent death of the plant. The insecticides Karate® and Orthene® were regularly used for the control of this pest. After spraying the pest was successfully controlled. However, we believe the pest must have other alternative host plants, which they feed on during yam off-season, since they attack the yam plants again in the following season. The entomology section at Bubia is looking at the problem with a view to identifying alternative hosts and describing the life cycle of the pest (Masamdu, in press).

The collection was morphologically described using the morphological descriptors adopted by the project. The completed descriptor database is attached as Appendix 1.

A PNG core collection of greater yam (*D. alata*) was intended to be selected according to the desired agronomic characteristics (*i.e.* round to oval tuber shape and resistance to anthracnose). However, the core collection was based on tuber shape and appearance alone and anthracnose susceptibility was not given much consideration.

The core selections were to be further characterized for physico-chemical traits and DNA fingerprinting. Therefore, tuber samples from the core collection were prepared and sent to the different collaborators for starch analysis, DNA fingerprinting and *in vitro* conservation.

Tubers were also sent for starch analysis. Fresh tubers of 300 to 700g of yam varieties from the PNG core collection were peeled and cut into small slices, placed in paper bags and oven

dried at 76°C for 63 hours. At least 150 g of oven dried yam chips of each of the core accessions were packed in separate paper bags, labelled and sent to CIRAD, France for starch analysis.

Also at least one tuber of the available core collection accessions and selected accessions of other yam species including *D. nummuralia*, *D. esculenta*, *D. bulbifera* and some other non-selected *D. alata* accessions were washed under running water and treated with disinfectants (0.2% bleach, 3% Benomyl & 2% karate) for 5 minutes under room temperature (25°C) according to quarantine standards and sent to CIRAD, France for DNA fingerprinting in December 2002.

One to two whole tubers of each of the core collection accessions were washed in 0.2% bleach (household bleach) then submerged in aqueous solutions of 3% Benomyl (fungicide) and 2% karate (insecticide) at room temperature (25°C) for 5 minutes as required by quarantine protocols and sent to SPC – Suva, Fiji for in-vitro preservation at the Regional Gene Bank.

Table 1 shows the different accessions from the core collection accessions that were sent to the different collaborators and Table 2 shows the other non-core accessions of *D. alata* and other species that were sent to CIRAD for DNA fingerprinting.

Table 1. PNG core collection accession samples sent to different collaborators

Accession number	Local name	Tissue culture (SPC)	Starch analysis (CIRAD)	DNA fingerprinting (CIRAD)	*Comments*
2001 selection (35 accessions)					
BDa 001	Takua Yavu	1	1	2	
Bda 002	Takua Kupmi	1, 2	1	2	
Bda 003	Kipmora	2	2	2	
Bda 009	Yavovi	2	2		
Bda 012	Modavateu	1	2	2	
Bda 014	Shek I	2	2	2	
Bda 018	Naso		2		
Bda 022	W. Fergusson I	1, 2	1	2	
Bda 023	W.Fergusson II				*Dead*
Bda 024	W.Fergusson III	1, 2	1		
Bda 033	Mui (female)	1	1	2	
Bda 035	Napo – Goning	2	2	2	
Bda 042	Gai		2		
Bda 043	Barai	1, 2	1		
Bda 047	Simbang	2	1	2	
Bda 048	Kusin De	2	2		
Bda 052	Tobo	1	1	2	
Bda 053	Kaiore Rau	1	1	2	
Bda 054	Kisi	1, 2	1	2	
Bda 073	Suasua	2	1		
Bda 076	Kwatea Duau				*Dead*
Bda 078	Kilekile	2	2		
Bda 080	Meloba	2	1		

Bda 083	Lobaloba	2	1		
Bda 094	Noh		1		*Dead*
Bda 106	Makuia (white)	2	1	2	
Bda 108	Kwadesera	1	1	2	
Bda 110	Mainina	1	1	2	
Bda 112	Gumanum	2	2		
Bda 140	Yabedua	1	2	2	
Bda 143	Dininibu		2	2	
Bda 156	Kokoroku	2	2	2	
Bda 167	Meaku I	2	1	2	
Bda 170	Davi		1		*1 small tuber rescued*
Bda 180	Maireba (red)	1, 2	1	2	
2002 selection (10 accessions)					
BDa 055	Ngarung Tung	2	2		
BDa 056	Wap Ambu	2	2	2	
BDa 079	Tausemo	2	2		
BDa 088	Dandak		2		
BDa 093	Pukan	2	2		
BDa 095	Wasel	2	2	2	
BDa 124	Tomlaweta	2	2	2	
BDa 142	Falai	2	2		
BDa 146	Abo	2	2		
BDa 190	Bugang	2	2		

The number '1' in the table above means that a sample of that accession was sent to the collaborator in 2001 and '2' means the sample was sent in 2002.

Table2. Non-core collection *D. alata* accessions and other species accessions sent to different collaborators

Accession number	Local name	Tissue culture (SPC)	Starch analysis (CIRAD)	DNA fingerprinting (CIRAD)	Comments
BDa 034	Napo Kapi			2	
BDa 105	Laknin Wapi			2	
BDa 146	Abo			2	
BDa 158	Kavonova			2	
BDa 166	Langeai			2	
BDa 178	Laki			2	
BDe 035	Iwokasa			2	
BDb 004	Urukum			2	
BDn 006	Koame			2	

The number '1' in the table above means that a sample of that accession was sent to the collaborator in 2001 and '2' means the sample was sent in 2002.

Virus surveys were conducted and virus infected samples from yam leaves and other plants were collected and sent to collaborating Institutions to identify the different strains present in

the region and develop indexing methods to establish an international exchange of virus-tested material.

Anthracnose surveys were also conducted infected samples from yam leaves and isolates of *Colletotrichum* from yam and other plants were collected and sent to collaborating Institutions for, molecular characterization of the different isolates. The collected accessions were also scored for anthracnose disease resistance in the field.

Thirty-four out of the forty-five Core PNG Collection accessions were planted in replicated plots for the agronomic evaluation of the different varieties or accessions. The trial was planted using a completely randomized design with variable replication of different accessions depending on the amount of planting material available (Table 3). The spacing between plots was 2m apart, which allows a much wider spacing between accessions, and is intended to minimize the spread of anthracnose and avoid cross-infection of accessions. The spacing between plants is 1m apart with 4 plants per plot per accession and each plot area is 1 m^2. The "pyramid" frame staking method was used (similar to Figure 1). The trial was planted on 3rd December 2002 and will be harvested in June 2003. The parameters to be measured are: anthracnose susceptibility score, canopy vigour rating, leaf area index and tuber yield data (marketable and non-marketable grades). The trial is still in progress.

Table 3. Replication of different accessions in first evaluation trial

No. of replicates	No. of Accessions
2	11
3	1
4	5
5	2
6	8
7	1
9	3
10	1
20	2
Total	34

Note: A further eight accessions were planted in unreplicated plots for observation purposes

Results achieved

Yam Germplasm Collection and Maintenance

When reviewing the PNG national yam germplasm collection it was found that the initial national yam germplasm collection, assembled in the 1980s, which used to be kept at the Laloki Research Centre, had 412 cultivars, including 212 *D. alata*. However, these had all been lost by 1995. During the time of review (late October 1999), only about 20 *D. alata* and a few of the other species were in the yam collection at Bubia. These were the remnants of the East Sepik provincial working collection, which was previously kept at the Saramandi Research Station in the East Sepik Province, and was transferred to Bubia in 1995.

Collection of yam germplasm under the SPYN project was initiated in November 1999 and was completed in December 2000. The SPYN project has collected and re-established the PNG national yam germplasm collection with 209 accessions of *D. alata*, 80 accessions of *D. esculenta*, 37 accessions of *D. nummularia* and 9 accessions of *D. bulbifera* (335 accessions

in total). The yam germplasm collection was given permanent accession numbers in February 2001. The collection was replanted in November 2002 and will be harvested in June 2003.

Germplasm evaluation and Selection of the Core sample

Morphological description of the PNG *D. alata* germplasm collection was completed by August 2002. The characterization data is set up in the computer database and is given in *attached Excel file.* This data is available to partner countries of the region.

Table 4: Number of yam cultivars with different tuber shapes.

Tuber shape[1]	Number of accessions	Percentage of accessions
Round (1)	56	26.8
Oval (2)	37	17.7
Cylindrical (3)	74	35.4
Flattened (4)	3	1.4
Triangular (5)	5	2.4
Deformed (99)	26	12.4
No information (plants died)	8	3.8
Total	209	100.0

The collection shows significant morphological variation between the different accessions. These morphological variations will be supplemented by the results of the DNA fingerprinting work carried out by CIRAD. The collection was characterized and grouped according to the tuber shape. Table 4 shows the number of accessions in the collection with the different tuber shapes. Eight (8) accessions (including four from the initial Core collection) out of the total germplasm collection died due to poor field establishment and other pest and disease problems.

Forty-five (45) cultivars with the desired tuber shape (round and oval) and with smooth skin were selected for inclusion in the PNG Core Collection. Four accessions from the core collection died and are indicated as dead in Table1. Although there were 93 accessions with round and oval tubers to select from, only 45 cultivars were selected because most of the cultivars were not good looking (rough skin) and will not look appealing to potential buyers if they are produced for commercial purposes. The core collection was based on tuber shape and appearance alone and anthracnose susceptibility was not given much consideration. Some of the best yielding accessions with the desired tuber shape were highly susceptible to anthracnose. None of the accessions showed immunity to anthracnose. All the cultivars show some degree of susceptibility ranging from low (score = 3) to high (score = 7).

In-vitro conservation (tissue culture), starch and DNA analysis

Between December 2001 and October 2002 samples of 38 PNG core accessions were sent to SPC Regional Germplasm Centre for tissue culture and *in vitro* conservation. The first batch (samples of 14 accessions) was sent in December 2001. However, it was reported that some of them failed to establish in tissue culture. The second batch (30 accessions) was sent to SPC, Suva in September 2002. The second batch includes the new (2^{nd}) selection, additional

[1] Numbers in brackets are the codes used in the Excel database

accessions from the first selection not sent in 2001 and the ones from the first (December 2001) batch that failed to establish in tissue culture.

Oven dried samples of 43 PNG core collection cultivars (100 to 150g each) were packed in separate paper bags, labelled and sent to CIRAD, France for starch analysis. The samples were sent in two batches. The first batch (20 accessions) was sent after the 2001 season and the second batch (23 accessions) was sent after the 2002 season. They were sent in two batches because of the low number of tubers available in 2001. The analysis was to determine the starch content of each variety.

At least one tuber of the available PNG core accessions and sample tubers of other species of yams including *D. nummularia, D. esculenta, D. bulbifera* and other non-selected *D. alata* accessions were washed and treated with disinfectant according to quarantine requirements and sent to CIRAD, France for DNA fingerprinting[2]. Unfortunately, not all the core collection and other morphologically diverse varieties and species that were supposed to be sent were sent, due to a misunderstanding while the project scientist was overseas on a study tour. PNG has not yet received any report of the samples sent to CIRAD (France) for both DNA and starch analysis. The results obtained from the starch and DNA analysis are reported in the CIRAD report.

Agronomic Evaluation of the Core PNG Collection

Thirty-four out of the forty-five Core PNG Collection accessions were planted in replicated plots for the agronomic evaluation of the different varieties or accessions. The trial was planted on 3rd December 2002 and will be harvested in June 2003. The trial is still in progress and NARI cannot recommend any variety at the time of this report.

Problems encountered:

There were some problems or constraints encountered during some stages of the project life that affected the project. Some of the problems were overcome or have been solved and the set goals or milestones have been achieved satisfactorily, while others have not been solved satisfactorily and caused some delay or setback in achieving set milestones on time. Below are some of the constraints encountered by the project and their implications for achievement of the project goals.

There were two problems encountered in the first year of the project that caused initial setbacks which leads to late start of the project in PNG:

- Firstly, delays in the banking system led to the unavailability of the funds for timely implementation and caused the initial setback.
- Another factor affecting project implementation in the first year was an institutional crisis, especially with staffing. NARI's future was uncertain up until the change of the government in July 1999. The scientists to work on the project were laid off or were offered casual employee status for other activities until September 1999, when the actual implementation of the project began.

[2] Yam tubers sent to CIRAD are covered by a Material Transfer Agreement signed by NARI and CIRAD

A number of other constraints were encountered during the project:

- A further constraint realized during the collection exercise was that cultivars generally had to be collected during harvesting season. But during that period there were not many diseased leaf samples available for collection (leaves were already dead). Conversely, if the collection was done during the growing season, diseased leaf samples were available but not much planting material (tubers) to collect. With the late start of the project, combined with this constraint, most germplasm collection was delayed to the second year, since the main harvest season falls in the May – August period. This caused delays in other following activities such as morphological description and core selection.
- Not all the core collection and other morphologically diverse varieties and species that were supposed to be sent to CIRAD were sent, due to a misunderstanding while the project scientist was overseas on a study tour. Some of the tubers that should have been sent to CIRAD were accidentally field planted.
- Because of the delay in the start of the project and delayed germplasm collection and evaluation, the agronomic evaluation of the PNG core collection is still in progress and no variety recommendation can be made before the project termination. However, agronomic evaluation will continue after termination of the project funding.
- Due to the late start of the project and poor access to biotechnology facilities in PNG, planned work on DNA fingerprinting did not take place. In particular training of NARI personnel in DNA fingerprinting techniques was not done and establishment of a NARI capability for DNA fingerprinting was not accomplished.

Publications and papers:

The SPYN project team from Bubia has produced four papers on yam work at Bubia in addition to annual reports of the SPYN project for the last three years. The references for the publications and reports are:

Publications:

1. Gunua T. G. and P. A. Gendua (2001). Anthracnose (*Colletotrichum gloeosporides):* A possible cause for the loss of varieties and the decline of True Yam (*Dioscorea alata)* production in Papua New Guinea. In Bourke, R.M, Allen, M.G and Salisbury, J.G, (eds). Food Security for PNG. Proceedings of Food and Nutrition 2000 Conference, University of Technology, Lae, Papua New Guinea, June 26-30, 2000. ACIAR Proceedings No.99, Pp 775 - 782.

2. Risimeri, J.B. P.A.Gendua and J.B. Maima (2001) The status of Introduced White Yam in PNG. In Bourke, R.M, Allen, M.G and Salisbury, J.G, (ed). Food Security for PNG. Proceedings of Food and Nutrition 2000 Conference, University of Technology, Lae, Papua New Guinea, June 26-30,2000. ACIAR Proceedings No.99, Pp 783 - 787.

3. Risimeri, J.B. (2001) Yam and food security in the lowlands. In Bourke, R.M, Allen, M.G and Salisbury, J.G, (ed). Food Security for PNG. Proceedings of Food and Nutrition 2000 Conference, University of Technology, Lae, Papua New Guinea, June 26-30,2000. ACIAR Proceedings No.99, Pp 768 - 774.

4. Peter A. Gendua and Tony G. Gunua (*In press*) Yam Germplasm Collection in Papua New Guinea. NARI Technical Bulletin Series.

Reports:

1. Gendua, A. P, T.G. Gunua and J.B. Risimeri (1999) South Pacific Yam Network (SPYN) Project. Papua New Guinea component Annual Report.

2. Gendua, A. P, T.G. Gunua and J.B. Risimeri (2000) South Pacific Yam Network (SPYN) Project. Papua New Guinea component Annual Report.

3. Gendua, A. P and Tony G. Gunua (2001) South Pacific Yam Network (SPYN) Project. Papua New Guinea component Annual Report.

Management:

Papua New Guinea has found great difficulty in sustaining research and development work on yams and has tended to work alone. Because of limited human and financial resources, little or no progress has been made. Collections have been assembled and described, but they have not been utilized in plant improvement programmes. These collections have subsequently been lost because of inadequate resources for their maintenance.

The SPYN project has facilitated co-operation between Pacific Island countries and the three European institutions (CIRAD, Natural Resources Institute (UK) and University of Reading). It facilitates the sharing of expertise and provides for the contribution of modern biotechnologies to germplasm characterization and crop improvement in yam.

The project has initiated collaboration between the Pacific countries to share information, genetic resources and expertise. It facilitates regional conservation (*in vitro* and cryo-preservation) of elite planting materials and will provide strategies and protocols for safe movement of materials between countries in the region and other parts of the world.

CIRAD coordinated the project and organized annual meetings. The scientific coordinator (Dr. Vincent Lebot) has ensured that the project activities begin and are completed efficiently and in accordance with the annual work plan. The scientific coordinator keeps regular contacts with country coordinators and project scientists by telephone, e-mail and/or facsimile. The individual scientists and other staff involved with the project in the partner countries and institutions have contacted each other via telephone, e-mail and/or facsimile to discuss technical matters. The partners and collaborators also share materials and information on a regular basis to achieve the set project goals. Some of the collaboration and contacts established will be maintained even after the SPYN project concludes.

The PNG component of the project was managed or overseen by NARI Headquarters in Lae. The NARI Chief Scientist (Dr. Geoff Wiles) ensured that the project scientists Jimmy Risimeri (Principal Agronomist & SPYN coordinator), Peter Gendua (Agronomist & project scientist) and Tony Gunua (Plant Pathologist) carried out the planned activities efficiently and on time. Despite the initial setback and slow start, the staff have put in a lot of their efforts to meet most of the planned targets and milestones on time and efficiently.

The project scientists and the NARI senior management have had 6 monthly in-house meetings or reviews discuss the agreed annual work plans and the progress. The team has

submitted 6 monthly and annual reports for years 1999 to 2002 to the scientific coordinator. Representatives of NARI (SPYN project staff) have attended and taken part in all the annual meetings of the project.

The PNG Yam collection passport database and PNG Yam collection (*D. alata*) morphological Descriptor database are available through the scientific coordinator to the project partners, collaborators and other interested parties wishing to access them.

Meetings:

There were several meetings including SPYN annual meetings attended by the PNG - SPYN project staff in the last four years. The National Agricultural Research Institute (NARI) was represented by Mr. J. Risimeri in the first meeting of SPYN in Port Vila, Vanuatu on 23 –24 February 1999. In early November 1999 Dr Vincent Lebot visited PNG and met with NARI executive management and country coordinator and discussed the status of SPYN activities.

Mr. Tony Gunua represented NARI at the second SPYN annual meeting from the 24 –25 January 2000 at the SPC Headquarters at Nabua, Suva, Fiji.

The third and fourth annual meetings for the project were held in Port Vila, Vanuatu from the 24 – 26 January 2001 and 9 – 10 April 2002. These meetings were both attended by Mr. Peter Gendua on behalf of NARI.

Exchanges and Training:

According to the initial plan, Pacific scientists, including those from PNG, were to be trained in different aspects of the project, either in-country by visiting specialists or through short courses organized in Europe. Trainings were to cover DNA fingerprinting of yam, molecular characterization of *Collectotrichum*, virus indexing and cryopreservation.

After the 2000 meeting scientists from NRI and Reading University (collaborators) visited PNG and conducted virus and anthracnose disease surveys in Morobe and Madang Provinces with the NARI staff. They also collected virus-infected samples of yam leaves and anthracnose disease samples from yams and other host plants during the trip. Some hands-on training for collection of anthracnose disease samples was conducted during this collection trip.

Apart from this none of the other planned training benefited PNG. The plan for University of Reading staff to train and transfer knowledge and methodologies on molecular characterization of yam cultivars did not eventuate. A course on characterization of yam anthracnose inoculums also did not materialize. These activities did not eventuate because NARI has poor access to the biotechnology facilities in PNG.

Conclusion and Perspectives:

Papua New Guinea's effort in yam research and development has been enhanced by the SPYN project. The project has re-established the PNG national yam collection and completed the morphological description of the *D. alata* accessions. The project has set up a computer database containing passport and morphological descriptors for the yam collection, which is available for sharing between the Pacific partner countries. The virus and anthracnose disease

surveys were completed and the samples of yam and other host plants were collected and sent to NRI and University of Reading for virus indexing and DNA fingerprinting of anthracnose strains for pathogencity testing respectively. A PNG core collection was selected and samples sent for starch analysis. Samples of the core collection were also sent to SPC for *in vitro* conservation and CIRAD for DNA fingerprinting. A trial for agronomic evaluation of core accessions has been initiated and is in progress. Upon completion some cultivars will be selected on the basis of yield, anthracnose tolerance and commercial potential.

A major concern is that protocols for safe movement of yam germplasm between Pacific islands were not finalized during the life of the project and as a result there was no exchange of yam germplasm between project partners.

NARI will continue to manage and maintain the yam germplasm collection. The yam research and development work will be continued under NARI and some further variety evaluation and other yam research and development activities will be continued by NARI. The networks, facilities, collaboration and individual contacts established through the project will continue to be used by the yam research and development team in NARI.

6. MAFF, Fiji

Moti Lal, Koronivia Research Station, Nausori, Fiji

1. Introduction

1.1 Background to the Project

Yam (*Dioscorea alata)* is the traditional root crop of the Pacific Islands. Yam is known to the Fijians by fourteen different names and was possibly cultivated by people living in isolated communities for centuries. It is an important source of food produced for home consumption, domestic markets and also for export. The importance of this crop is far greater than its contribution to nutrition and revenue. It is very much part of the peoples custom and assures high cultural status. The production of this crop in the Pacific Islands has been in decline.

Several factors have combined to limit the production. At present this crop is mostly grown in small-scale traditional culture. Tuber shape is often irregular making harvest time-consuming and labour-intensive. Soil fertility has declined and labour availability for subsistence agriculture has decreased and outbreak of disease and pests has occurred. In addition, the lack of information on starches hinders the prospective utilisation of yam as a high quality vegetable. The result has been a change to other root crops and reduction in the number of yam cultivars grown. This concern resulted in setting up a Project for the Pacific Island nation and Dr. Vincent Lebot of CIRAD took the leading role in writing up a project document. "The South Pacific Yam Network (SPYN) project" is to enhance the competitive position of yam in traditional cropping systems of the five countries: Papua New Guinea, Solomon Islands, Vanuatu, New Caledonia and Fiji. The main focus of the project was to review yam genetic resource by collecting, characterizing and evaluating the germplasm, rationalise collection and select cultivars for disease resistance and of commercial potential.

Selecting improved germplasm for distribution throughout the region will do improvements in yam production. Improved varietal characteristics include desirable tuber shape and flavour, and resistance to anthracnose.

The four-year project has resulted in the accomplishment of the major objectives including:

- Collection of cultivars within Fiji Island.
- Completion of morphological descriptors
- Selection of cultivars based on tuber shape
- Collection and shipment of yam leaves specimen to University of Reading for anthracnose disease diagnosis and to University of Greenwich for virus indexing.
- Development of data containing descriptors information
- Multiplication of selected cultivars for further evaluation and distribution

Subsistence agriculture based on root crop staples is a mainstay of Pacific Island economies. Yam, alone among the tropical root, can be stored for long periods, and because of this and its robust nature it can be transported with relative ease. Tubers are increasingly sold on urban markets, returning much needed cash to rural communities, and they are also being exported to meet the needs of Pacific-rim Island communities living aboard. There is need to assist these trends. Modern biotechnologies offer the possibility of overcoming the constraints,

which exist. The project on completion will enhance exchange of yam cultivars as well as assist Pacific Island countries to take advantage of these technologies.

1.2 Objectives and Goals

The objectives of the project were:

- To review yam genetic resources in Papua New Guinea, the Solomons, Vanuatu, New Caledonia and Fiji: to characterise and evaluate the germplasm, rationalise collections and select cultivars of interest. A computerised database will be developed containing morphological and molecular descriptors on at least 1,250 cultivars, allowing:
  a) Comparison and rationalization of collections; and
  b) Selection of 150 cultivars for compact tuber shape, unstaked cultivation, tolerance to anthracnose and acceptable cooking and processing characteristics.
- To develop *in vitro* conservation strategies;- a regional conservation strategy implemented, involving active *in vitro* national genebanks and cryopreserved base collections (limited to 150 accessions initially).
- To establish an international exchange of virus-tested, selected genotypes; at least 150 yam cultivars cultured *in vitro*, tested for viruses found in Pacific Island countries and subjected to therapy treatments if infected.
- To identify sources of anthracnose (*Colletotrichum gloeosporioides*) inoculum; molecular characterisation of *Colletotrichum* isolates from yam tubers and from at least 10 common weeds and crop plants to estimate the potential of tuber borne and alternative hosts as sources of inoculum for seasonal epidemics of anthracnose disease.
- To conduct an agronomic evaluation of selected cultivars; assist all countries multiply yam germplasm rapidly using *in vitro* and field-based (mini-sett) technologies, sufficient for at least one trial per country by the end of the project.
- For Fiji, the task was to assemble the descriptor data, collect germplasm of selected cultivars if not already held in national genebanks, multiply the selections agreed by regional consent, and conduct agronomic trials in different agro-ecological zones to evaluate the cultivars selected.

The specific activities for Fiji included:

- Germplasm collection at different locations and outer islands
- Characterisation and elimination of duplicates collected from farmers
- Collection and Dispatching of infected leaves to U.R. & NRI.
- Send tubers of selected types for therapy & indercing NRI,
- Field planting of collected cultivars.

2.0 Results and Conclusions

2.1 Collection of Cultivars

During the four-year period total of One hundred and eight (108) cultivars were collected mostly from the main Island, Viti Levu, while few cultivars were collected from the Eastern part of the smaller Islands, Lau Group. Further collections were not possible during the year 2000 and 2001 due to political instability in the country. It was also noticed from gathered information from the extension staff of the inter Islands that more than 90% of the varieties grown in the small Islands are available in the main Island (VitiLevu). This is because of rapid movements of people from small Islands to Main Island, mainly urban and sub urban centres.

Initially all cultivars collected were raised at Koronivia Research Station which lies in the wet climatic zone of Fiji where the average annual rainfall exceeds 3,300mm. This was done because of its central location where all agronomy experts were available and secondly, due to the easy access. Later in the year 2001, the replicate of the collection was planted at Dobuilevu Research Station, which falls in the intermediate zone, which receives an average annual rainfall of 2500 mm.

Since most of the cultivars collected were from intermediate climatic zone, its performance in the wet zone was very poor. As a result fourteen cultivars died prematurely at the age of three months before the tuber formation took place. Twelve of these cultivars were regained from farmers but two cultivars are still missing. Efforts are being made to regain these cultivars from Island of Moala this year 2003. It was seen that fungicide, Benlate and Mancozeb had very little or no effect on these cultivars. Weekly spray was carried out but still the cultivars did not survive.

2.2 Completion of Morphological Descriptions

All accessions have been described using SPYN Standardized Morphological descriptors and data base matrix developed. The Database Matrix has been submitted to the Project Co-ordinator Dr. Vincent Lebot, CIRAD for compilation (Refer to appendix I and II)

2.3 Selection of Cultivars based on "Tuber shape"

Cultivars were grouped based on tuber shape using SPYN Standardized descriptor. At times there were difficulties in grouping the tubers. The undersized tubers in poor soil (due to high rainfall and poor drainage) did not give it true shape, for example the short cylindrical tubers appeared to be round/oval, and flat tubers appeared to be oval shape. Efforts were also made to identify and group the cultivars according to its maturity period. The cultivars are grouped into three categories:

Early season: 6-7 month maturity
Mid season: 7-9 month maturity
Late season: over 9 month maturity

The maturity of the cultivar differs according to the climatic condition. The cultivar matures early in intermediate zone and delays for more than a month in wet condition. Selection is also being made to identify suitable cultivars for each climatic zone i.e. wet zone and intermediate climatic zone. Consideration to dry zone is not given because none of the wet zone cultivars will perform well in dry weather condition. Only some of the intermediate zone cultivars will perform with lower yield. So far performance of all cultivars in intermediate climatic zone is the best, wet zone is too wet for yam and outbreak of anthracnose disease is very high, hence, the commercial cultivation is expensive or even may result in losses.

2.4 Collection of Leaf Samples from Yam Plants for Disease Diagnosis

Jeff Peters, University of Reading visited Fiji and collected leaf samples from 108 accessions planted at Koronivia Research Station for Anthracnose Isolation and Dr. Laurence Kenyon, University of Greenwich for virus detection.

2.5 Yam Tubers for DNA Finger Printing

Dried chips of nineteen (19)-core sample cultivars (approx. 1Kg/cultivar) were sent to Dr. Jean L. Marchand, CIRAD Montpellier for DNA Finger Printing. The following core sample tubers from Fiji collection included:

Accession No.	Local Name	Dry Matter %
FJ 1	Taniela Damu II	33.6
FJ 6	Taniela Vula Leka (A)	29.2
FJ 7	Taniela Vula Leka (B)	33.5
FJ 9	Viwa	25.1
FJ 13	Voli	31.2
FJ 14	Futuna Vula Leka	29.3
FJ 15	Uvi ni Futuna	31.4
FJ 16	Vurai Vula Leka	33.6
FJ 19	Muropoi	30.3
FJ 20	Kuro Round	33.8
FJ 21	Reado	30.1
FJ 23	Kivi	35.4
FJ 24	Davui	31.2
FJ 26	Sisiwa	33.4
FJ 33	Voli Balavu (C)	33.4
FJ 34	Vurai	31.3
FJ 55	Beka	35.1
FJ 62	T.V.R	33.9
FJ 63	Damuni	

2.6 Evaluation of Core Samples for Yield Potential

A core sample of nineteen cultivars mostly round/oval shape have been selected and planted at two different sites for evaluation. The sites are Koronivia Research Station (Wet Zone) and Dobuilevu Research Station (Intermediate Zone). These cultivars are planted in a plot size of 40 plants and in 3 replications at both sites and their performance will be evaluated upon harvest in August 2003. The agronomic aspect of the yam crop is that it is planted on flat land under semi-mechanised system where land system preparation and ridging at one meter spacing is done by machine (tractor) and the rest of the operations are manual. Planting is done on the ridge at 0.6m apart, the size of each planting material is 200-250gm. Trellis at Koronivia is on wire at the height of 1.5m from the ridge using pine posts as corner post and smaller bamboo posts are propped at an interval of 10 meters in the furrow. A strong wire (No. 8-12) is slung over the erected pine posts and bamboo posts. Yam vines are stacked on to the top of the wire using reeds.

At Dobuilevu where local materials (bamboo and reeds available), stacking of vines is on bamboo and reeds only. Instead of wire a thinner bamboo is placed on top of the 1.5m high, thicker bamboo posts whose top end is cut in "U" shape for holding the thinner bamboo to form the top of the trellis and reeds are used to train the vines on to the overhead bamboo. The

use of bamboo is economical. The field evaluations of 19 cultivars are doing well at both sites and relevant agronomical practices are being utilized to keep the crop healthy.

2.7 Problems Encountered

The major problem during the project period was the late arrival of funds to initiate field collection of cultivars and the political instability of 2000 in the country. The adverse weather conditions also affected the time of planting and performance of the cultivars. During the year 1999, collection was not done on time due to the late arrival of funds. Collection started well in year 2000 but came to a halt at the middle of the collection period because of political instability in the country when the movement to farmers in the interior became impossible. In this same year severe attack of anthracnose disease in few of the newly collected accessions also encountered difficulties in foliar description work. In the year 2001 the adverse weather (continuous heavy rain) delayed planting and resulted in the loss of fourteen cultivars and a very poor yield from the remaining cultivars.

2.8 Achievements

Fiji has achieved its overall objectives of the Four-year Project. It has successfully managed to describe 108 cultivars (accessions), which will be used for future endeavours. Fiji has also managed to retrieve the lost cultivars to add on to its germplasm collection.

Potential Cultivars have been successfully selected for commercial purpose. Furthermore, Scientists and other technical staff have acquired and broadened their knowledge and skills via this project.

This project has seen the contribution towards the conservation and preservation of the biological diversity, which without SPYN would have resulted in the loss of some of our very important components of biological inheritance.

7. VARTC, Vanuatu

Godwyn Ala, Tari Molisale and Roger Malapa

1. Background

According to the technical annex attached to the contract, VARTC has to assemble the descriptor data, collect germplasm of selected cultivars, if not already held in national genebanks, multiply the selections agreed by regional consent, and conduct agronomic trials in different agro-ecological zones to evaluate the cultivars selected. Varieties have been collected throughout the country and collecting ceased in 2001. A data base of the morpho-agronomical data has been completed. Propagation of selected varieties started in 2002 and is still continuing.

2. Methods

The collection (Table 1) was planted in August every year, at VARTC, Santo, during the four years of the project. Each accession was represented by four plants, planted 1 X 1 m square, with four individual stakes 1.20 m high, tied together at the top. All accessions were described agro-morphologically using 32 SPYN standardised descriptors. Each year, the harvest began in April and lasted until July for the late maturing types. All tubers from the four plants of each of the 331 accessions were weighed individually.

Samples were collected and sent for virus detection (see NRI report) and for anthracnose isolates identification (see University of Reading report). Selected cultivars were analysed for their AFLP polymorphism and for the physico-chemical variation of their tubers (see CIRAD report).

Table 1 : Geographical origin of *Dioscorea* spp. accessions collected in Vanuatu

Island	***Dioscorea* spp.**						
	alata	*nummularia*	*bulbifera*	*esculenta*	*pentahylla*	*cayenensis*	*trifida*
Gaua	3	-	-	-	-	-	-
Vanua Lava	4	-	-	-	-	-	-
Santo	70	7	-	2	-	-	-
Malo	6	-	-	1	-	-	-
Pentecost	24	-	3	9	5	-	-
Ambae	1	-	-	-	-	-	-
Maewo	11	3	1	1	-	-	-
Mallicolo	99	-	-	-	-	1	1
Ambrym	8	4	2	-	-	-	-
Tongoariki	1	-	-	-	-	-	-
Efaté	35	-	-	-	-	-	-
Tanna	32	3	2	2	-	1	-
Futuna	6	-	-	-	-	-	-
Erromango	20	-	-	-	-	-	-
Aneitum	11	-	-	-	-	-	-
Total	**331**	**17**	**8**	**15**	**5**	**2**	**1**

3. Results

Considerable variation exists between cultivars for tuber shape, number of tubers per plant, average yield per plant, anthracnose resistance and maturity period (Table 2). However, numerous accessions were also found to be duplicates (acc. nos. with shade in table 2). The scope for selection within varieties presenting compact tubers is therefore limited.

Table 2 : List of *Dioscorea alata* accessions (ranked by their tuber shapes)

No	Island	Village	Local name	Shape	Tubers	Yield/ plant	Anthracnose	Maturity
617	Ambrym	Wilit	*Bumaso*	compact	2.00	2.15	tolerant	medium
751	Aneitum	Anelghowhat	*Ross*	compact	1.75	1.16	tolerant	late
029	Efate	Tagabe	*n.a.*	compact	1.50	2.48	tolerant	early
033	Efate	Tagabe	*n.a.*	compact	1.75	1.04	tolerant	medium
480	Efate	Mele	*Patapata*	compact	2.50	0.96	tolerant	medium
723	Erromango	Ipota	*Neitpnesi*	compact	2.00	1.20	resistant	medium
648	Gaua	Bangcap	*Pentecost*	compact	5.50	1.04	tolerant	medium
001	Malakula	Unmet	*Hurp*	compact	2.75	2.22	susceptible	early
529	Malakula	Unmet	*Ptris*	compact	2.25	2.08	tolerant	medium
566	Malakula	Losinwe	*Homb*	compact	2.00	0.33	tolerant	medium
461	Malo	n.a.	*Tumas*	compact	1.00	1.77	tolerant	medium
656	Maewo	Marino	*Sovwa (hair)*	compact	n.d.	n.d.	tolerant	n.d.
421	n.a.	n.a.	*n.a.*	compact	1.75	1.98	susceptible	early
070	Pentecost	Baravet	*Bangro*	compact	1.33	0.95	tolerant	medium
422	Pentecost	Marteli	*Maligni*	compact	1.75	1.75	tolerant	medium
423	Pentecost	Marteli	*Manlankon*	compact	2.25	2.64	tolerant	late
633	Pentecost	Lolbuavatu	*Maligni*	compact	2.00	3.12	tolerant	medium
402	Santo	Fanafo	*Raranaeolo*	compact	1.75	3.65	resistant	late
439	Santo	Viorloko	*Thomas molmolia*	compact	1.00	3.11	tolerant	medium
447	Santo	Ipayato	*Vavulararajuju*	compact	2.67	3.28	susceptible	early
454	Santo	Natuy	*Mere*	compact	3.50	1.52	tolerant	medium
497	Santo	Fanafo	*Maliok*	compact	1.50	4.39	tolerant	medium
498	Santo	Fanafo	*Pulmo*	compact	2.50	4.25	tolerant	medium
502	Santo	Fanafo	*Suk*	compact	2.75	3.15	resistant	medium
678	Tanna	Imanaka	*Nowanao*	compact	2.00	0.65	tolerant	late
025	Efate	Tagabe	*n.a.*	compact	2.25	3.55	tolerant	medium
475	Efate	Mele	*Salemanu tetea*	compact	1.00	4.11	tolerant	medium
736	Erromango	Ipota	*Tauva*	compact	1.00	0.43	tolerant	medium
649	Gaua	Namasari	*Lolohe*	compact	1.00	1.85	tolerant	medium
011	Malakula	Brenwe	*Bretis*	compact	3.00	2.68	tolerant	medium
562	Malakula	Losinwe	*Tumas*	compact	1.50	2.72	tolerant	medium
569	Malakula	Dravai	*Maligni*	compact	n.d.	n.d.	tolerant	medium
578	Malakula	Orap	*Letslets nambas*	compact	1.50	2.25	tolerant	medium
459	Malo	Avunamalae	*Basa*	compact	1.75	2.65	tolerant	medium
232	Pentecost	Baravet	*Malini*	compact	1.25	2.70	tolerant	medium
415	Pentecost	Marteli	*Obal*	compact	2.00	2.55	tolerant	medium
425	Pentecost	Marteli	*Maligni*	compact	n.d.	n.d.	tolerant	n.d.
426	Pentecost	Marteli	*Warereo*	compact	1.25	2.01	tolerant	medium
645	Pentecost	Lolbuavatu	*Mutarigete*	compact	2.00	2.55	tolerant	medium
500	Santo	Fanafo	*Not*	compact	1.50	1.83	tolerant	medium
445	Santo	Viorloko	*Nivu*	compact	1.00	7.14	tolerant	medium
401	Santo	Fanafo	*Basa*	compact	1.25	2.82	tolerant	medium
597	Santo	Chapuis	*n.a.*	compact	1.50	2.37	tolerant	medium
492	Santo	Fanafo	*Dam luwotok*	compact	2.75	2.83	tolerant	medium
690	Tanna	Imanaka	*Rosapin*	compact	2.67	1.45	tolerant	medium
676	Tanna	Lowiel	*Nowaneum*	compact	1.00	2.68	tolerant	medium
647	Vanua Lava	n.a.	*Nadas*	compact	1.50	2.00	tolerant	medium

612	Ambrym	Wilit	*Tabaom*	elongate	n.d.	n.d.	tolerant	n.d.
615	Ambrym	Wilit	*Horbee*	elongate	1.00	1.93	tolerant	medium
609	Ambrym	Wilit	*Tabaomung*	elongate	n.d.	n.d.	susceptible	early
610	Ambrym	Wilit	*Tasip*	elongate	n.d.	n.d.	susceptible	early
750	Aneitum	Anelghowhat	*Wanorak*	elongate	9.00	7.23	tolerant	late
754	Aneitum	Anelghowhat	*Noulelcae*	elongate	3.00	5.23	tolerant	medium
003	Efate	Tagabe	*n.a.*	elongate	1.75	2.95	susceptible	early
024	Efate	Mele	*Tepuva*	elongate	2.75	3.38	resistant	late
281	Efate	Tagabe	*n.a.*	elongate	2.50	2.19	tolerant	medium
467	Efate	Mele	*Toufitetea*	elongate	3.00	3.99	susceptible	medium
473	Efate	Mele	*Teuareki tetea*	elongate	4.75	2.94	tolerant	medium
474	Efate	Mele	*Tumas*	elongate	3.25	2.51	tolerant	medium
476	Efate	Mele	*Tepuna*	elongate	2.50	1.75	tolerant	medium
477	Efate	Mele	*Totosake*	elongate	2.00	0.76	resistant	late
478	Efate	Mele	*Teitsi*	elongate	1.25	2.55	resistant	late
479	Efate	Mele	*Pulepule*	elongate	1.75	1.91	tolerant	medium
482	Efate	Mele	*Noai*	elongate	2.25	2.23	tolerant	medium
483	Efate	Mele	*Tenaru*	elongate	2.25	1.69	tolerant	medium
485	Efate	Mele	*Viripoa*	elongate	1.50	2.24	tolerant	medium
486	Efate	Mele	*Nusamu*	elongate	1.50	2.62	tolerant	medium
489	Efate	Mele	*Roru*	elongate	3.75	1.57	resistant	medium
720	Erromango	Ipota	*Netieti*	elongate	2.00	5.22	tolerant	medium
721	Erromango	Ipota	*Nupumori*	elongate	2.50	2.85	tolerant	medium
722	Erromango	Ipota	*Gorevinuwo atma*	elongate	1.33	1.67	susceptible	early
724	Erromango	Ipota	*Potninau*	elongate	2.00	3.29	tolerant	medium
726	Erromango	Ipota	*Orenuwuo*	elongate	2.00	5.30	tolerant	medium
728	Erromango	Ipota	*Nupumori*	elongate	5.00	6.61	tolerant	medium
729	Erromango	Ipota	*Orenuwo kau*	elongate	1.00	5.20	tolerant	medium
731	Erromango	Ipota	*Malie*	elongate	1.00	4.40	susceptible	early
732	Erromango	Ipota	*Nupumori*	elongate	4.00	6.12	tolerant	medium
738	Futuna	Ipau	*Ruruvanua*	elongate	2.00	3.00	tolerant	medium
047	Maewo	Narovorovo	*Malingova*	elongate	2.00	2.32	resistant	late
049	Maewo	Narovorovo	*Malabong hivo*	elongate	1.75	2.54	susceptible	medium
655	Maewo	Bangcap	*Sugar*	elongate	2.00	3.19	tolerant	medium
658	Maewo	Marino	*Reve*	elongate	1.00	3.91	tolerant	late
660	Maewo	Marino	*Ririho*	elongate	1.00	1.10	susceptible	early
663	Maewo	Marino	*Turi*	elongate	2.25	2.65	tolerant	medium
002	Malakula	Unmet	*Vitibergbak*	elongate	1.75	2.20	tolerant	early
004	Malakula	Unmet	*Man Solomon*	elongate	2.25	1.50	susceptible	early
007	Malakula	Unmet	*Masap*	elongate	4.75	2.42	tolerant	medium
012	Malakula	Brenwe	*Blarghlin*	elongate	1.50	0.75	susceptible	medium
015	Malakula	Brenwe	*Drav*	elongate	3.67	0.47	susceptible	early
018	Malakula	Brenwe	*Ravahr*	elongate	6.25	1.81	tolerant	medium
019	Malakula	Brenwe		elongate	4.25	1.68	tolerant	medium
021	Malakula	Unmet	*Vitibarei*	elongate	1.25	2.80	tolerant	medium
023	Malakula	Brenwe	*Neikal*	elongate	1.25	2.40	tolerant	medium
036	Malakula	Vao	*Woiewo*	elongate	2.75	2.63	tolerant	medium
059	Malakula	Vao	*Namsas*	elongate	1.33	2.41	tolerant	medium
280	Malakula	Lorlow	*Nabatebate*	elongate	1.50	2.45	tolerant	medium
286	Malakula	Wintua	*Nivikimlak*	elongate	1.00	3.28	tolerant	medium
287	Malakula	Vao	*Bihuri*	elongate	1.50	2.37	tolerant	early
296	Malakula	Vao	*Tobe*	elongate	1.67	1.97	tolerant	n.d.
298	Malakula	Vao	*Nakal*	elongate	1.50	2.37	tolerant	medium
518	Malakula	Unmet	*Naoul*	elongate	1.50	2.30	tolerant	medium
520	Malakula	Unmet	*Navilou*	elongate	3.00	2.09	resistant	medium
521	Malakula	Unmet	*Napi*	elongate	7.75	3.07	susceptible	early
522	Malakula	Dravai	*Red navos*	elongate	1.75	0.67	tolerant	medium
530	Malakula	Unmet	*Visn*	elongate	1.50	3.27	susceptible	medium
532	Malakula	Potinweo	*Nikelpo woman*	elongate	2.00	2.74	tolerant	medium
533	Malakula	Losinwe	*Naharto*	elongate	1.00	3.01	tolerant	medium

536	Malakula	Unua five	*Rengreng*	elongate	1.25	2.90	tolerant	medium
537	Malakula	n.a.	*Nelpo*	elongate	1.25	2.39	tolerant	medium
539	Malakula	Losinwe	*Behenzen*	elongate	1.25	2.89	susceptible	early
540	Malakula	Lavalsal	*Sot nenuis*	elongate	3.75	2.02	resistant	medium
542	Malakula	Lavalsal	*Nakanma*	elongate	3.00	4.55	tolerant	medium
543	Malakula	Losinwe	*Nahansar*	elongate	1.00	3.52	tolerant	medium
544	Malakula	Tenbul Orap	*Balka*	elongate	1.25	2.38	tolerant	medium
545	Malakula	Lavalsal	*Nambis veveg*	elongate	3.50	2.60	susceptible	early
546	Malakula	Wala	*Big Mombri*	elongate	5.75	2.12	tolerant	medium
550	Malakula	Lavalsal	*Mombri*	elongate	4.50	4.53	tolerant	medium
551	Malakula	Worlep	*Dapa*	elongate	3.75	2.19	susceptible	early
552	Malakula	Dravai	*Svoka*	elongate	2.25	1.88	tolerant	medium
553	Malakula	Tenbul Orap	*Letslets masis*	elongate	n.d.	n.d.	tolerant	medium
555	Malakula	Santa Maria	*Valise*	elongate	1.00	2.43	tolerant	medium
559	Malakula	Lavalsal	*Tursanavul*	elongate	1.00	0.51	tolerant	n.d.
560	Malakula	Lavalsal	*Long makila*	elongate	2.25	1.78	tolerant	medium
561	Malakula	Losinwe	*Daekara*	elongate	1.00	1.42	tolerant	medium
563	Malakula	Losinwe	*Botel*	elongate	1.00	3.41	tolerant	medium
573	Malakula	Orap	*Romsup*	elongate	1.00	2.70	tolerant	medium
575	Malakula	Orap	*Baksan*	elongate	1.50	2.48	tolerant	medium
582	Malakula	Losinwe	*Nevis*	elongate	2.50	1.56	resistant	medium
583	Malakula	Orap	*Letslets meut*	elongate	2.00	3.42	tolerant	medium
584	Malakula	Worlep	*Navo*	elongate	1.75	2.34	tolerant	medium
586	Malakula	Pinalum	*Bip*	elongate	1.25	2.39	tolerant	medium
587	Malakula	Tanmial	*Sombubu*	elongate	n.d.	n.d.	tolerant	n.d.
588	Malakula	Dravai	*Tapa*	elongate	2.00	5.64	resistant	medium
589	Malakula	Lavalsal	*Makila*	elongate	2.00	2.00	resistant	medium
591	Malakula	n.a.	*Maewo*	elongate	2.25	2.59	tolerant	late
600	Malakula	Vao	*Maewo*	elongate	3.00	2.12	resistant	late
457	Malo	Avunamalae	*Viravira*	elongate	1.00	2.81	tolerant	medium
465	Malo	Avunamalae	*Blan bunjo*	elongate	2.00	2.38	tolerant	medium
032	Pentecost	Lorlow	*Nukumosien*	elongate	1.00	1.97	tolerant	medium
115	Pentecost	Panlimsi	*Senap*	elongate	2.50	2.02	resistant	late
332	Pentecost	Alau	*Malintogho*	elongate	n.d.	n.d.	tolerant	n.d.
416	Pentecost	Marteli	*Buramera*	elongate	n.d.	n.d.	tolerant	n.d.
418	Pentecost	Marteli	*Tahirao*	elongate	1.00	2.10	tolerant	late
420	Pentecost	Marteli	*Senap*	elongate	3.00	2.31	tolerant	medium
644	Pentecost	Lolbuavatu	*Shulniu*	elongate	2.00	1.51	tolerant	medium
247	Santo	Chapuis	*n.a.*	elongate	1.50	3.02	tolerant	medium
400	Santo	Fanafo	*Rave*	elongate	2.25	2.20	resistant	late
404	Santo	Fanafo	*n.a.*	elongate	5.25	3.21	resistant	n.d.
406	Santo	Fanafo	*Fotafot*	elongate	2.00	3.75	tolerant	medium
407	Santo	Fanafo	*Awe*	elongate	1.75	2.27	tolerant	medium
408	Santo	Fanafo	*Manioc*	elongate	1.75	1.69	tolerant	medium
410	Santo	Fanafo	*Maltchi*	elongate	3.00	2.99	tolerant	medium
430	Santo	Puama	*Matamata*	elongate	1.00	2.22	tolerant	medium
431	Santo	Puama	*Vusivari*	elongate	1.25	2.15	tolerant	medium
432	Santo	Puama	*Pisuroï*	elongate	5.00	2.82	tolerant	early
433	Santo	Malovira	*Masala*	elongate	2.00	2.19	tolerant	early
435	Santo	Najara	*Vuluvulu*	elongate	5.25	2.17	tolerant	n.d.
436	Santo	Malovira	*Pisu*	elongate	1.50	4.47	tolerant	medium
443	Santo	Ipayato	*Viraiji*	elongate	3.75	2.32	tolerant	medium
444	Santo	Malovira	*Tamate ajuju*	elongate	2.25	2.30	tolerant	medium
450	Santo	Ipayato	*Tupu*	elongate	1.75	1.74	susceptible	medium
451	Santo	Natuy	*Malakula lulu*	elongate	1.75	2.27	tolerant	medium
453	Santo	Ipayato	*Livusivari*	elongate	n.d.	n.d.	susceptible	early
455	Santo	Natuy	*Sevi*	elongate	5.50	1.79	tolerant	medium
456	Santo	Natuy	*Valvalpusa*	elongate	2.00	2.74	tolerant	medium
466	Santo	n.a.	*n.a.*	elongate	6.25	1.05	tolerant	medium
469	Santo	n.a.	*n.a.*	elongate	n.d.	n.d.	tolerant	n.d.
493	Santo	Fanafo	*Malakula*	elongate	1.67	0.49	tolerant	medium

501	Santo	Fanafo	*Wudwudiei*	elongate	1.50	2.89	susceptible	early
507	Santo	Fanafo	*Sulnio*	elongate	2.50	1.76	tolerant	medium
509	Santo	Fanafo	*Bwagura*	elongate	3.25	3.21	tolerant	medium
512	Santo	Fanafo	*Bwagura*	elongate	1.00	2.75	tolerant	medium
590	Santo	Chapuis	*n.a.*	elongate	2.00	2.18	tolerant	late
592	Santo	Chapuis	*n.a.*	elongate	1.75	2.44	tolerant	medium
593	Santo	Chapuis	*n.a.*	elongate	1.00	2.70	tolerant	medium
595	Santo	Chapuis	*n.a.*	elongate	1.50	2.33	tolerant	medium
596	Santo	Chapuis	*n.a.*	elongate	1.50	2.17	tolerant	medium
599	Santo	Chapuis	*n.a.*	elongate	4.67	3.09	tolerant	medium
605	Santo	Chapuis	*n.a.*	elongate	2.25	1.70	resistant	late
606	Santo	Chapuis	*n.a.*	elongate	4.25	1.92	tolerant	medium
668	Tanna	Marino	*Nusua*	elongate	3.75	2.79	tolerant	medium
669	Tanna	Lowiel	*Norafanoa*	elongate	1.00	2.72	susceptible	early
670	Tanna	Lowiel	*Tamoni*	elongate	2.50	2.20	tolerant	medium
671	Tanna	Lowiel	*Nusua Lemanu*	elongate	1.50	1.11	susceptible	early
680	Tanna	Imanaka	*Nolawinu*	elongate	2.00	2.36	tolerant	medium
683	Tanna	Imanaka	*Selemnu*	elongate	3.25	1.14	susceptible	early
691	Tanna	Imanaka	*Pefene*	elongate	2.00	3.16	tolerant	medium
693	Tanna	Imanaka	*Taniru*	elongate	4.75	1.10	tolerant	medium
694	Tanna	Imanaka	*Nielo*	elongate	2.00	2.38	tolerant	medium
695	Tanna	Imanaka	*Talibasa*	elongate	2.75	1.87	tolerant	medium
703	Tanna	Imanaka	*Wambi*	elongate	1.67	1.00	tolerant	medium
604	Tongariki	n.a.	*Natevetev*	elongate	1.75	1.91	susceptible	medium
055	Maewo	Naone	*Dinta*	triangular	2.50	0.37	tolerant	late
598	Malakula	Vao	*Laslas mamba*	triangular	1.50	1.31	tolerant	late
452	Santo	Ipayato	*Uratavue*	triangular	3.00	3.25	tolerant	medium
697	Tanna	Imanaka	*Nowateknempian*	triangular	2.00	1.05	tolerant	late
006	Malakula	Unmet	*Rite*	flat	2.75	2.02	tolerant	medium
009	Malakula	Vao	*Maewo*	flat	1.25	1.78	tolerant	medium
424	Pentecost	Marteli	*Buntun ankapkap*	flat	1.75	1.80	tolerant	n.d.
428	Santo	Ipayato	*Kala*	flat	n.d.	n.d.	tolerant	early
484	Efate	Mele	*Totosake*	flat	2.00	1.10	resistant	late
506	Santo	n.a.	*Buridamu*	flat	2.25	3.40	resistant	late
696	Tanna	Imanaka	*Nonimanaka*	flat	1.25	1.13	tolerant	late
373	Ambae	Waisala	*Bughi toa*	deformed	1.75	2.95	resistant	medium
611	Ambrym	Wilit	*Tamatine*	deformed	3.50	1.56	tolerant	early
613	Ambrym	Wilit	*Peter*	deformed	n.d.	n.d.	resistant	late
614	Ambrym	Wilit	*Tabaom Ten*	deformed	1.50	1.84	tolerant	medium
752	Aneitum	Anelghowhat	*Nowanmerei*	deformed	1.50	4.40	tolerant	medium
753	Aneitum	Anelghowhat	*Wanorak*	deformed	1.00	5.63	tolerant	medium
755	Aneitum	Anelghowhat	*Nepelev*	deformed	1.00	4.72	tolerant	medium
756	Aneitum	Anelghowhat	*Nowanrowod*	deformed	1.00	7.13	tolerant	medium
757	Aneitum	Anelghowhat	*Narouvanua*	deformed	1.00	4.00	tolerant	medium
760	Aneitum	Anelghowhat	*Nureangdan*	deformed	2.00	4.51	tolerant	late
761	Aneitum	Anelghowhat	*Nambi*	deformed	1.00	4.30	tolerant	early
746	Aneitum	Ipau	*Nioutec*	deformed	n.d.	n.d.	tolerant	late
646	Banks	Lolbuavatu	*Lei*	deformed	n.d.	n.d.	tolerant	n.d.
010	Efate	Mele	*Martenik*	deformed	2.00	1.16	tolerant	medium
028	Efate	Tagabe	*n.a.*	deformed	1.25	2.75	tolerant	medium
030	Efate	Tagabe	*n.a.*	deformed	1.50	1.99	tolerant	medium
031	Efate	Tagabe	*n.a.*	deformed	1.00	1.23	resistant	late
035	Efate	Tagabe	*n.a.*	deformed	1.75	2.38	tolerant	medium
231	Efate	Tagabe	*n.a.*	deformed	1.75	2.07	tolerant	medium
265	Efate	Tagabe	*n.a.*	deformed	1.75	3.24	susceptible	early
472	Efate	Mele	*Tepuna*	deformed	1.00	1.97	tolerant	medium
481	Efate	Mele	*Sikara*	deformed	2.25	1.65	tolerant	medium
487	Efate	Mele	*Purufea*	deformed	1.75	4.17	resistant	medium

725	Erromango	Ipota	*Nablokon*	deformed	1.00	8.14	tolerant	medium
727	Erromango	Ipota	*Orenuwuo ve*	deformed	1.00	4.01	tolerant	medium
730	Erromango	Ipota	*Ross*	deformed	9.00	8.20	resistant	late
733	Erromango	Ipota	*Pete*	deformed	1.00	3.80	susceptible	early
734	Erromango	Ipota	*Nablokon*	deformed	1.00	9.30	tolerant	medium
735	Erromango	Ipota	*Noplon*	deformed	2.00	11.20	resistant	late
717	Erromango	Ipota	*Nupumori*	deformed	3.00	4.06	tolerant	medium
718	Erromango	Ipota	*Moite*	deformed	8.00	1.40	resistant	medium
719	Erromango	Ipota	*Potninau*	deformed	3.00	4.30	tolerant	medium
739	Futuna	Ipau	*Kiniroa*	deformed	1.33	1.43	susceptible	early
740	Futuna	Ipau	*Colega*	deformed	1.00	2.50	tolerant	medium
741	Futuna	Ipau	*Kiniroa*	deformed	1.00	4.74	susceptible	early
745	Futuna	Ipau	*Tama tua*	deformed	n.d.	n.d.	resistant	late
650	Gaua	Namasari	*Maravovo*	deformed	1.25	1.61	susceptible	early
657	Maewo	Marino	*Sovwa (smooth)*	deformed	2.75	2.48	resistant	late
659	Maewo	Marino	*Rap*	deformed	6.50	1.90	tolerant	medium
661	Maewo	Marino	*Dam buna*	deformed	2.25	2.55	tolerant	medium
005	Malakula	Unmet	*Valis Bavi*	deformed	4.00	1.32	tolerant	medium
008	Malakula	Unmet	*Navilu*	deformed	1.00	3.16	tolerant	medium
013	Malakula	Brenwe	*n.a.*	deformed	6.75	2.40	tolerant	medium
014	Malakula	Brenwe	*Melesles Tylibel*	deformed	2.25	1.71	tolerant	medium
016	Malakula	Brenwe	*Pirai*	deformed	1.75	2.59	tolerant	medium
020	Malakula	Unmet	*Vitibarei*	deformed	2.50	2.38	tolerant	medium
022	Malakula	Vao	*Maewo*	deformed	2.00	2.93	tolerant	early
026	Malakula	Brenwe	*Dam masis*	deformed	1.50	2.75	resistant	medium
027	Malakula	Brenwe	*n.a.*	deformed	1.00	2.83	tolerant	medium
059	Malakula	Vao	*Namsas*	deformed	1.33	2.41	tolerant	early
264	Malakula	Lorlow	*Nabulalas*	deformed	2.00	3.39	tolerant	medium
297	Malakula	Vao	*Lalas mamba*	deformed	1.00	2.46	susceptible	medium
516	Malakula	Unmet	*Salomon*	deformed	1.50	0.96	resistant	medium
517	Malakula	Brenwe	*Malesles*	deformed	1.00	1.84	tolerant	late
519	Malakula	Unmet	*Salomon*	deformed	1.25	0.83	tolerant	medium
523	Malakula	Unmet	*Nakraï*	deformed	2.00	3.01	tolerant	medium
525	Malakula	Brenwe	*Nakrimet*	deformed	1.25	2.72	tolerant	medium
526	Malakula	Unmet	*Viti*	deformed	2.00	4.12	susceptible	early
527	Malakula	Dravai	*Tacharamivar*	deformed	2.25	3.33	susceptible	early
528	Malakula	Unmet	*Sinoua*	deformed	n.d.	n.d.	tolerant	medium
531	Malakula	Brenwe	*Nikel*	deformed	1.25	3.21	tolerant	medium
534	Malakula	Wala	*Bisro*	deformed	1.25	2.64	tolerant	late
535	Malakula	Pinalum	*Romb soso*	deformed	2.25	1.31	tolerant	medium
538	Malakula	Pinalum	*Romb net tumas*	deformed	1.25	2.85	tolerant	medium
549	Malakula	Pinalum	*Melali taling*	deformed	2.00	3.50	susceptible	early
554	Malakula	Potinweo	*Novoribo*	deformed	1.50	2.81	resistant	late
556	Malakula	Santa Maria	*Red tumas*	deformed	1.00	2.58	resistant	late
557	Malakula	Santa Maria	*Red tumas*	deformed	1.25	2.75	tolerant	medium
564	Malakula	Dravai	*Makila*	deformed	1.50	3.53	tolerant	medium
567	Malakula	Orap	*Letslets bolos*	deformed	1.75	4.25	tolerant	medium
568	Malakula	Pinalum	*Aulo*	deformed	2.25	3.47	tolerant	medium
571	Malakula	Dravai	*Rorosiv*	deformed	2.25	3.47	tolerant	medium
572	Malakula	Unua five	*Betmel*	deformed	1.00	2.03	tolerant	medium
577	Malakula	Rori	*Letslets maser*	deformed	1.50	3.16	susceptible	early
579	Malakula	Orap	*Letslets bokis*	deformed	1.25	4.24	tolerant	medium
580	Malakula	Lavalsal	*Nharn nahahar*	deformed	1.75	2.68	tolerant	early
585	Malakula	Dravai	*Paou*	deformed	1.25	3.48	tolerant	medium
601	Malakula	Vao	*Nuatbabag*	deformed	2.50	1.64	resistant	late
462	Malo	Avunamalae	*Bisu*	deformed	1.25	2.54	resistant	medium
463	Malo	Avunamalae	*Balabalavuvuha*	deformed	1.25	1.76	tolerant	medium
602	n.a.	n.a.	*n.a.*	deformed	1.75	3.47	resistant	medium
034	n.a.	n.a.	*n.a.*	deformed	3.25	3.59	tolerant	medium
066	Pentecost	Vansemakul	*Solomon*	deformed	1.50	2.29	resistant	medium
419	Pentecost	Marteli	*Kilman*	deformed	1.75	3.14	tolerant	medium

623	Pentecost	Reverger	*Veingerker*	deformed	1.75	3.05	tolerant	medium
634	Pentecost	Lolbuavatu	*Tariusi*	deformed	1.50	1.49	tolerant	medium
639	Pentecost	Lolbuavatu	*Malalagi*	deformed	1.25	3.67	resistant	late
643	Pentecost	Lolbuavatu	*Bataihivo*	deformed	3.00	1.43	tolerant	medium
427	Pentecost	Marteli	*Buntun antho*	deformed	n.d.	n.d.	resistant	late
405	Santo	Fanafo	*Rocaptain*	deformed	3.00	0.30	tolerant	n.d.
434	Santo	Viorloko	*Pili*	deformed	1.00	4.10	tolerant	medium
437	Santo	Viorloko	*Mamun*	deformed	2.00	1.30	tolerant	medium
446	Santo	Ipayato	*Vavulararajuju*	deformed	1.75	3.40	tolerant	medium
448	Santo	Natuy	*Vavula*	deformed	1.00	2.28	tolerant	late
490	Santo	Fanafo	*Riprip*	deformed	2.50	1.69	tolerant	medium
491	Santo	Fanafo	*Ragir waet*	deformed	1.00	1.48	tolerant	medium
495	Santo	Fanafo	*Ragir red*	deformed	8.50	2.56	tolerant	n.d.
499	Santo	Fanafo	*Wotonemei*	deformed	2.00	2.15	tolerant	medium
503	Santo	Fanafo	*Lakon*	deformed	1.25	3.34	resistant	late
505	Santo	Fanafo	*Bonboni*	deformed	1.50	1.74	tolerant	medium
508	Santo	Fanafo	*Aga*	deformed	1.75	2.03	susceptible	early
510	Santo	Fanafo	*Buridamu*	deformed	1.50	2.41	resistant	late
511	Santo	Fanafo	*Malogu*	deformed	2.50	3.00	resistant	late
513	Santo	Fanafo	*Tageu*	deformed	1.50	1.64	tolerant	medium
514	Santo	Fanafo	*Kolmo*	deformed	1.50	1.69	susceptible	early
594	Santo	Chapuis	*n.a.*	deformed	2.75	2.06	tolerant	medium
603	Santo	Chapuis	*Lakon*	deformed	1.50	3.25	resistant	late
770	Santo	Fanafo	*n.a.*	deformed	n.d.	n.d.	tolerant	medium
771	Santo	Chapuis	*n.a.*	deformed	n.d.	n.d.	tolerant	n.d.
037	Tanna	Loukupas	*Telipaua*	deformed	1.50	3.77	resistant	medium
199	Tanna	Loukupas	*Telipaua*	deformed	3.50	2.90	tolerant	medium
672	Tanna	Lowiel	*Kuenkopo*	deformed	1.25	3.25	tolerant	medium
677	Tanna	Imanaka	*Rostuan*	deformed	4.75	4.23	tolerant	medium
679	Tanna	Imanaka	*Nonoanoriktunga*	deformed	1.00	3.08	tolerant	late
681	Tanna	Imanaka	*Kaselang*	deformed	1.25	2.61	susceptible	early
682	Tanna	Imanaka	*Yangnamita*	deformed	1.00	1.51	susceptible	early
684	Tanna	Imanaka	*Salamoni*	deformed	1.50	0.45	resistant	late
689	Tanna	Imanaka	*Ifit*	deformed	1.50	4.23	tolerant	medium
698	Tanna	Imanaka	*Nawanaong*	deformed	5.75	1.68	tolerant	medium
699	Tanna	Imanaka	*Nowanalimin*	deformed	1.75	2.14	tolerant	early
700	Tanna	Imanaka	*Tumas*	deformed	2.00	2.98	tolerant	medium
701	Tanna	Imanaka	*Wasu*	deformed	2.00	2.49	susceptible	early
704	Tanna	Imanaka	*Nawanurunkimanga*	deformed	1.25	2.04	tolerant	medium
705	Tanna	Imanaka	*Nouwigo*	deformed	2.00	3.65	resistant	medium
706	Tanna	Imanaka	*Rosapin*	deformed	3.00	3.51	resistant	medium
712	Tanna	Imanaka	*Wiigosu*	deformed	2.00	2.59	tolerant	medium
688	Tanna	Imanaka	*Kahut*	deformed	1.50	3.14	resistant	late
651	Vanua Lava	Namasari	*Nihnacon*	deformed	3.00	3.18	susceptible	early
653	Vanua Lava	Bangcap	*Turea*	deformed	n.d.	n.d.	tolerant	n.d.
	Mean*				**2.16**	**2.64**		
	Minimum				**1**	**0.3**		
	Maximum				**9**	**9.3**		
	Std				**1.3698**	**1.2829**		
	CV%				**63.4**	**48.6**		

* computations do not include accessions no. 617 and 735, yield are in kilograms per plant without fertilisers, maturity is expressed in months of growth cycle (early= 5-6, medium= 7-8, late= 9-10).

It appears that the best varieties (i.e. the 'Basa' group) have been widely distributed throughout Vanuatu. These varieties present an ease of harvest due to the compact shape of their tubers and an excellent multipurpose chemotypes, suitable for boiling, baking and/or laplap. The characteristics guarantee their rapid adoption by farmers, even if the yield is not exceptional or if they are not immune of anthracnose.

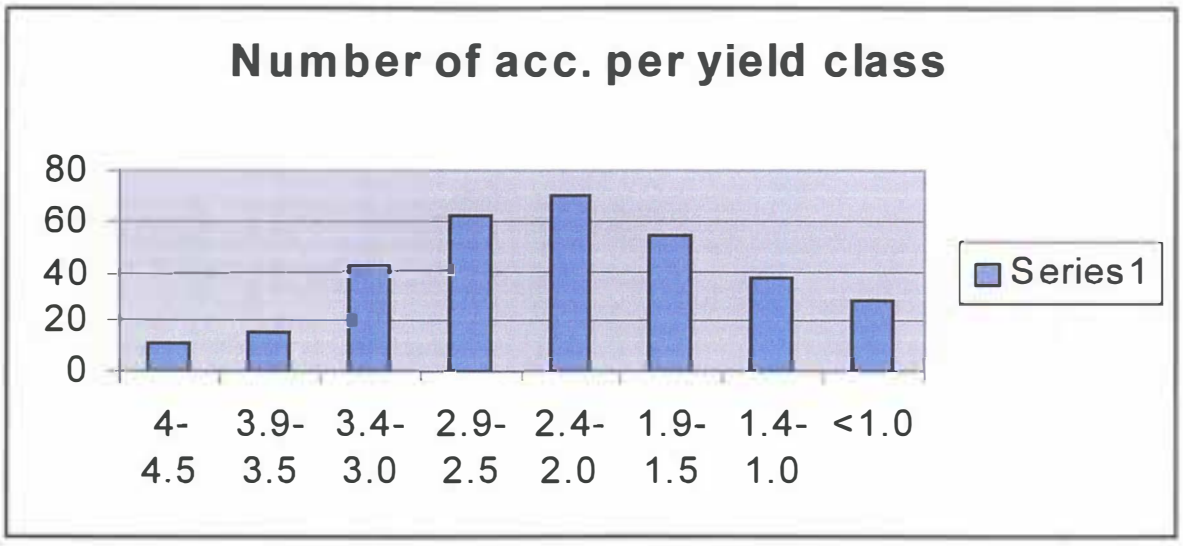


Figure 1. Distribution of *D. alata* accessions from Vanuatu in yield classes of less than 1 to more than 4 kg. These yields were measured with 1 X 1 m spacing (10 000 plants /ha).

Recommended varieties:

In 2002, for the third consecutive year, the germplasm collection of 331 varieties has been fully characterised using SPYN morpho-agronomic descriptors. From the data collected, 14 varieties (Table 2) have been chosen, based on ease of harvest, tuber shape and tolerance to anthracnose and the quality of the tuber flesh (no oxidation and palatability). Recommended *D. alata* varieties for Vanuatu are presented in Table 3.

Table 3: List of recommended varieties for Vanuatu

Acc.	Name	Island	Village	Yield	Shape	Anthracnose
VU 029	*Tagabe*	Efate	Tagabe	2.5	2	tolerant
VU 401	*Basa*	Santo	Fanafo	2.8	2	tolerant
VU 421	*Marteli*	Pentecost	Martelli	2.0	2	tolerant
VU 423	*Manlakon*	Pentecost	Martelli	2.6	2	resistant
VU 461	*Tumas*	Avunamalae	Malo	1.8	2	tolerant
VU 498	*Pulmo*	Santo	Fanafo	4.2	2	tolerant
VU 521	*Napi*	Malekula	Unmet	3.1	3	tolerant
VU 536	*Rengreng*	Malekula	Unua five	2.9	3	tolerant
VU 551	*Mombri*	Malekula	Rose Bay	3.0	3	tolerant
VU 563	*Mendrovar*	Malekula	Dravai	3.4	3	tolerant
VU 589	*Makila*	Malekula	Lavalsal	2.0	3	resistant
VU 633	*Maligni*	Pentecost	Lolbuavatu	3.1	2	tolerant
VU 699	*Nowanalimin*	Tanna	Imanaka	2.1	2	tolerant
VU 729	*Ipota*	Erromango	Ipota	1.3	3	tolerant

Variety 401: *Basa*
Variety 589: *Makila*

Variety 461: *Tumas*
Variety 563: *Mendrovar*


029
421


498
699

These varieties were propagated and distributed to farmers. Propagation is continuing.

Physico-chemical characteristics:

Forty-eight varieties have been selected for the physico-chemical analysis of their tubers. For each accession, dry weights have been determined, and flours produced at the Tagabe laboratory and sent to CIRAD, Montpellier for analysis. There was significant variation in the dry matter (from 13 to 31%) between the varieties, although all were planted and harvested at the same time and in the same location. Variation in physico-chemical characteristics of the tubers is significant. Recommended (and good tasting) varieties appear to have a high starch content and a high amylose vs starch ratio with comparatively low proteins, minerals and sugars.

Publications:

Malapa R., Arnau G., Noyer J.L. & V. Lebot. 2002. Genetic relationship between *D. alata* and *D. nummularia* as revealed by AFLP. *Origins, Evolution and Conservation of Crop Plants: A molecular Approach.* Society for Economic Botany. New York Botanical Garden, Bronx N.Y. (*Economic Botany,* accepted).

Problems encountered:

Tubers of the core sample sent to SPC for tissue culturing were destroyed byt the officers of the Quarantine department in Suva, Fiji.

APPENDIX

Contract number : ERBIC18CT980309 **Year: 2003**

Data sheet
for final report

Dissemination activities	**Totals (cumulative)**
Number of communications in conferences (published)	3
Number of communications in other media (internet, video, ...)	
Number of publications in refereed journals (published)	3
Number of articles/books (published)	
Number of other publications	21

Training	
Number of PhDs	2
Number of MScs	1
Number of visiting scientists	1
Number of exchanges of scientists	1

Achieved results	
Number of patent applications	
Number of patent granted	
Number of companies created	
Number of new prototypes/products developed	
Number of new tests/methods developed	
Number of new norms/standards developed	
Number of new softwares/codes developed	
Number of production processes	

Industrial aspects		
Industrial contacts	yes	no
Financial contribution by industry	yes	no
Industrial partners: - large	yes	no
- SME	yes	no

Comments

Other achievements: : Varieties selected