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Coconut Improvement in the South Pacific

Proceedings of a workshop held in Taveuni, Fiji Islands
10–12 November 1993

Editors: **M.A. Foale and P.W. Lynch**

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Introduction

WHEN the ACIAR Coconut Improvement Project was due for review it was decided to invite coconut research workers with an interest in the South Pacific to a workshop. The aims of the workshop were to:

- hear a presentation of the main achievements of the Coconut Improvement Project;
- enable delegates from South Pacific countries to report on the status of national activity in coconut research, development and extension;
- hear a report on progress in the ACIAR coconut virus and viroid project; and
- explore possibilities for future research in coconut production in the region.

As a result of the workshop, two communiques were drafted to capture the main findings and opinions of delegates on coconut production, and on the problem of viroid-like RNA sequences first observed in coconut in 1987. These communiques are presented here, and in the next paper a statement of my view of the current dilemma regarding the viroid-like RNA sequences; and the associated quarantine issue.

General Communique

1. Reports were made by country delegates on the general situation of the coconut industry in Papua New Guinea, Solomon Islands, Vanuatu, Fiji Islands and Tonga. There was no delegate from Western Samoa, Tuvalu or Kiribati. Declining exports of copra and coconut oil were reported for all countries represented. In some cases, copra production has ceased and nuts surplus to domestic requirements are used solely for livestock.

2. The reasons advanced for this decline included low price, breakdown of marketing arrangements, increased local consumption of coconuts, and, in some cases, the farmers giving attention to more profitable alternatives. Coconut for copra is seen as a worthwhile activity solely by farmers with no other choice for earning cash.

3. In the case of small and remote islands, including many atolls, continued production of copra encourages ships to visit, thereby sustaining communication. Unless copra or other coconut products are presented for sale such regular visits would cease in the future, creating extreme isolation.

4. Marketing of higher-value products, such as fresh mature nuts and drinking nuts, provides a greater financial return than copra. Appropriate new technology for the production of oil or coconut cream, embodying both low complexity and small scale, is urgently needed if coconut is to survive as a cash crop. Value-adding at a household or village level (for example micro-scale extraction of oil recently developed at the Australian National University) is a very promising concept that should be fully assessed by in-country policy makers and by aid donors.

5. From an agronomic point of view, there is a need to optimise the management of crops that are associated with coconut. A place for coconut in most farming systems is assured in the long-term by the continuing demand for food from an expanding human population, and by the use of excess production as feed for domestic animals.

6. The country delegates in particular gave priority to research into the management of the diverse farming systems that include coconut. The introduction of any cash crop requiring intense or expensive inputs (cultivation, fertiliser or pesticide) was seen as a danger, not only to the maintenance of physical and chemical fertility of soil, but also to ground-water and streams through pollution.

7. Three positive developments reported to the meeting were: the advance in embryo culture for the movement of germplasm; the advance in DNA fingerprinting for germplasm; and the development of methods for sequencing of viroid-like RNAs. Research organisations will be seeking to secure further funding to enable applications to flow from these basic results.

Communique on the Implications of Viroid-like RNA for the Movement of Coconut Germplasm in the South Pacific Region

The draft statement below was prepared by the 22 delegates to the meeting to assist national quarantine authorities and other agencies with responsibility for advice or policy relating to the movement of coconut germplasm. There was insufficient time to finalise the communique at Taveuni so the version presented here has been developed after later interaction with delegates. Opinion was divided, among delegates, as to the likely level of risk associated with the presence of viroid-like RNA. Some believed that the viroid-like sequence has been present for some time without obvious harm, whereas others felt that symptomless harm to growth and yield may already be manifested in palms carrying viroid-like RNA.

1. Pending clarification of the problem of viroid-like RNA as recommended below, it is suggested that the South Pacific Commission (SPC) should not recommend the movement of coconut germplasm between countries, unless testing shows the material to be free of viroid-like RNA. In other respects, IBPGR (draft) guidelines should be followed as far as they are applicable to the South Pacific region. (These actually recommend indexing only for germplasm that goes to countries where viroid-like RNA has not been found, but it has been found in every country surveyed so far).

2. Given that there is an urgent need to facilitate germplasm exchange, funding must be found for the following priority activities:

(a) to initiate research on viroid-like RNA to determine whether it is, in fact, pathogenic or not pathogenic to a range of coconut populations;

(b) sequencing of the viroid-like RNA found in coconut to characterise unknown aspects, including the degree of relatedness of RNA found in different coconut populations. (Work on this aspect is under way in ACIAR project 9221 in Adelaide, funded until December 1994);

(c) assessment of the implications for quarantine of the degree of relatedness of variants of viroid-like RNA found in different coconut populations, and assessment of the risk to coconut health of relatedness between variants of viroid-like RNA and CCCVd; and

(d) establishment of an indexing facility for viruses, viroids, and viroid-like RNA.

3. Sufficient research results with respect to viroid-like RNA are not yet available for a definite policy on movement of germplasm to be established. When the important issues listed here have been dealt with a well-informed policy could be drawn up.

4. More support is required to create, through training and collaboration, better understanding and general awareness of the nature of cadang-cadang viroid and of any potential problem due to the presence of viroid-like RNA.

M.A. Foale
CSIRO Division of Tropical Crops and Pastures

A Personal View of the Implications of Viroid-like RNA for the Movement of Germplasm and for Coconut Production

M.A. Foale*

Definitions

COCONUT cadang-cadang viroid (CCCVd) is a pathogenic circular single-stranded RNA molecule or sequence. *Viroid-like RNA* refers to RNA that is chemically similar to CCCVd but which has not been shown to be pathogenic. A *pathogen* is an organism or molecular sequence that has an adverse but not necessarily fatal effect on growth and yield of another organism. A *viroid* is an RNA sequence that is known to be pathogenic. The pathogenicity of the viroid-like RNA found in coconut is unknown.

Introduction

The reaction of many policy-makers to the identification of viroid-like RNA in coconut has been to 'err on the side of caution' and prohibit movement of germplasm. This has been due to: the highly pathogenic nature of the known viroids of coconut, notably CCCVd; the possibility raised by pathologists that a small mutation might be sufficient to change viroid-like RNA to a viroid; and speculation on the basis of experience with viroids in potato and citrus that viroid-like RNA might have a significant negative effect on growth and yield of coconut.

Does Viroid-like RNA Affect Yield?

One cannot dismiss the possibility that viroid-like RNA affects growth and yield. In the absence of evidence one should also accept that there are several possible effects: negative (pathogenic); neutral; or even beneficial (such as the effect of mycorrhiza [VAM] or of *Rhizobium* bacteria in association with certain plants). Because viroid-like RNA might confound the results of basic research in coconut genetics and long-term breeding—since they are generally present in about half the members of a population—there is a persuasive argument in favour of screening it out of breeding material. To achieve these long term goals it is essential that the present research, which will identify and evaluate differences between viroid-like RNA and CCCVd, should continue, and also that an indexing capability be developed to serve the coconut breeders.

Why Move Coconut Germplasm?

There are at least three different reasons for moving coconut germplasm between countries, or indeed between populations within a country. First, there is transfer of seed nuts for field production, such as hybrid seeds or those with a specific trait such as large fruit. Second, there is transfer of pollen or of a small quantity of seed nuts, comprising parent germplasm for the purpose of producing hybrid seed nuts on a commercial scale. Finally, there is selected seed and pollen for use in breeding programs, including individual palm selection, tests of specific combining ability, and study of heritability of traits and the genetics of the coconut palm.

Viroid-like RNA is Already Widely Distributed.

Given that viroid-like RNA has been found in every coconut population tested to date, and given that there is no evidence of its pathogenicity, there seems to be scant reason to restrict the movement of coconut germplasm that is intended for the first two purposes listed above. One would expect an introduced population to contain a proportion of individuals with viroid-like RNA similar to the coconut population at the destination. It is useful to note here that coconut germplasm still moves via the sea 'carrier' (the sea is definitely not a barrier to movement of coconut germplasm), and viable nuts easily pass across the political boundaries between many neighbouring countries in the South Pacific; just as easily as they pass between islands within the island

*CSIRO Division of Tropical Crops and Pastures, 306 Carmody Road, St. Lucia Queensland 4067.

nations. Imposition of strict quarantine is therefore not going to halt the spread of viroid-like RNA, comprising only a partial barrier at best. There has been massive movement of coconut germplasm in the past through natural dissemination and through human intervention. Assessment of the risk involved in future movement must take this past movement into account. The absence of evidence showing how viroid-like RNA spreads should not be allowed to paralyse all decision-making.

A similar argument applies to the introduction of germplasm for the second purpose i.e. the generation of seed nuts for commercial planting, with one or both parents being of exotic origin. It would be conducive to peace-of-mind for those planning to develop a seed-garden, using introduced germplasm, to test for viroid-like RNA on a representative sample of both the proposed material for introduction; and on the coconut palms already present in the target region. There is no evidence to suggest at present there is any difference in potential pathogenicity between the populations of viroid-like RNA found in different countries. However, work on this is being done in the ACIAR project on viruses and viroids of coconut. I suggest that speculation about pathogenicity should not be allowed to influence policy relating to the types of movement of coconut germplasm described in this and the previous paragraph.

There is justification for eliminating viroid-like RNA from a population of palms that is to be used for genetic study or as a source of selections for long-term breeding work. As viroids in some other crop species have indeed been shown to reduce plant performance, without showing severe symptoms, the possibility that viroid-like RNA might affect coconut in this way cannot be ignored.

The Relevance of Coconut Population Dynamics to Concern about Viroids and Viroid-like RNA

In closing, it is useful to think about the relation between the effect of proposed restrictions and possible future gain from that action. I suggest that present evidence is not strong enough to justify the total restriction of movement of germplasm that has been imposed by most quarantine authorities. The question of population dynamics of coconut in the predominant farming system, in relation to possible outbreak of disease such as coconut cadang-cadang, should also be considered. Many smallholders maintain a coconut stand of mixed age and replace any loss as it occurs. There might indeed be very little disruption to the output of the coconut palms maintained by the smallholder, if there were a steady loss of older palms due to a slow-developing disease. Harvesting of the whole dying palm in order to mill the trunk for timber is a potential source of revenue where milling is available.

The amount of anticipated loss due to death of palms following infection with CCCVd may be high in the industrial-crop context, but not on a smallholding. Where there is a stand of mixed age, the low-level attrition of palms due to the disease might not seriously affect the viability of the farm. Because coconut has been phased out as a plantation enterprise in many countries, the level of threat to the survival of coconut smallholders, for the 'worst-case' possibility of the emergence of a pathogenic form, should be reassessed when determining policy relating to viroid-like RNA.

The Current Status of the Coconut Industry in Papua New Guinea

Tore Ovasuru*

PAPUA New Guinea (PNG) has a land mass of about 460 000 km^2. From the 1990 national census it was concluded that Papua New Guinea has a population of about 3 680 000. Topography of the country consists of more than 60% of rough mountainous terrains usually unsuitable for agriculture. The daily mean temperature ranges from a cool 15–20C° on the highland plateaus to hot tropical temperatures of 25–30C° along the coast. There is also marked variability of soil types which range from sandy soils, with low organic matter, on coral atolls to very rich volcanic ash soils. The major portion of the population is rural and coconut provides an extensive proportion of their livelihood. Coconut culture occurs on some of the richest soils as well as on poorer soils of the atolls. Export earnings of copra and some of the major agricultural commodities have dropped due to price fluctuations.

Importance of Coconut

Coconut is a major source of food. In fact, on most atolls, life would be unsustainable without coconut. Livestock, especially pigs, also benefit from its products as feed. Its vegetative parts are extensively used for construction materials for houses. Leaves are also used as thatch for roofs and baskets. The shells are carved into spoons and bowls, and used for carrying water. Copra, a dried endosperm of the mature fruit, has been a vital source of export earnings for 100 years. Until recently, copra was one of the most valuable export products of PNG. For a considerable proportion of the littoral population, copra production provides the only source of cash income. The value of copra lies in its high concentration (65–70%) of lauric oil known in the international trade as coconut oil. Coconut meal, a useful stock feed, is a by-product of oil extraction.

*Papua New Guinea Cocoa and Coconut Research Institute, P.O. Box 1846, Rabaul East, New Britain Province, Papua New Guinea.

Coconut Plantings

The coconut palm is extensively cultivated, throughout coastal mainland areas and on many islands. The total area planted to coconut in PNG has been estimated at 250 000 ha of which an insignificant proportion of palms were derived from selected planting material (Douglas 1965). Smallholders have been actively planting coconuts after World War II up until the 1970s. Also, after independence, the government instituted a plantation acquisition scheme. This scheme allowed foreign-owned plantations to be returned to their original landowners whenever the foreigners agreed to do so. When the properties were acquired they were either subdivided into smallholder blocks (5–10 ha), and each block given back to a family, or run as a plantation. This reduced the number of plantations owned by foreign companies. Most significantly, the management of these properties also returned to the local people who, at that time, were ill prepared to manage large properties. Consequently, generally, these properties were not managed well and this resulted in huge bank debts. It is common to see run-down plantations as you travel around PNG. This can be viewed as one of the major causes of the decrease in the amount of copra reaching depots from the plantation sector. Furthermore, there was an increased activity in the smallholder sector of the industry which has led to smallholders producing more copra than the plantation sector. By 1986, smallholder production had increased to about 60% and by 1988 to about 70%. Another important constraint is that about 50% of plantations remain senile and this has also affected the productivity on plantations, as yields from senile palms are constantly diminishing.

Production

The most important economic products from coconut are tender nuts (Kulaus), mature nuts, copra, coconut oil and copra meal. Tender and mature nuts are very popular and nuts are either sold from roadside stalls or local markets. These two products are consumed in homes as ingredients of some of the local recipes.

Copra is mostly exported to markets in Asia and Europe. For more than about a decade PNG produced an average of about 145 150 t of copra. The copra production comes mostly from the Island regions which contribute about 70% of total production. Depending largely on plant age, copra yield was estimated to vary from 0.5–1.0 t/ha and 0.3–1.1 t/ha and these ranges now appear optimistic.

The industry is facing some difficult times due to market price fluctuations. However, in recognition of the rural population's reliance on the sale of agricultural products, the Government has made a commitment for the next 4 to 5 years to a price support scheme on copra and other major agricultural commodities. The production of copra seems to have declined since the last decade as indicated in Table 1. The decline in the market price has also led to a decline in copra production.

Coconut Intercropping

From 1984 agriculture statistics, about 30 700 smallholders were growing cocoa underneath their coconut stands. Within the plantation sector, where the soil is suitable for both cocoa and coconut, the two crops are always grown together. Smallholders make gardens underneath mature palms which allow sufficient light penetration. Crops such as banana, taro, peanut, pineapple and other green vegetables form part of this intercropping system. No significant scientific research has been done on intercropping. The only work documented is that of Gallasch who demonstrated that banana, pineapple, taro and other vegetables can be successfully intercropped with coconut up until the palms are about 4 years old. Usually, from the first year, many different types of annuals can be cultivated between coconut rows. Intercropping is one of the major components of coconut research that the PNG Cocoa and Coconut Research Institute will be undertaking as part of its coconut research and development program.

Research Activities

The main coconut research activities in Papua New Guinea are:

- germplasm prospection;
- germplasm collection;
- breeding;
- embryo culture; and
- coconut agronomy.

Germplasm prospection

The identification of existing genetic materials has never been scientifically attempted. Earlier workers identified various ecotypes based on fruit characters, especially the size of the fruit and nut. This has led to the identification and selection of tall populations known as Markham, Karkar and Gazelle. For breeding purposes, the genetic materials used have a very narrow genetic base and this needs to be broadened. Past experience has shown that hybrids originating from only two exotic ecotypes failed to survive in the New Guinea islands region due to attack by rhinoceros beetles and palm weevils. The best possible alternative is to broaden the genetic base by identifying

Table 1. Copra production by receiving depot (tonnes) in Papua New Guinea.

Year	Province										
	Milne Bay	New Ireland	West New Britain	North Solomons	Morobe	Manus	Madang	Central	East New Britain	East Sepik	Gulf
1981	5671	18249	8985	21493	2447	1358	20740	6018	58860	3018	0
1982	3935	17962	11399	17782	2614	1257	22641	3822	54071	2645	0
1983	5448	15455	11975	18462	2662	1482	22927	4256	51904	2856	0
1984	7155	16589	13421	26658	3470	1888	22525	4204	58597	3717	0
1985	8424	21700	16400	30782	4450	2184	24568	5224	58923	3179	0
1986	7714	17765	14615	25834	3587	757	25618	4000	55543	2059	0
1987	5546	16566	15454	24790	3230	595	24390	2499	53362	2885	0
1988	4636	15002	12531	27081	2514	667	18735	2119	50362	2205	0
1989	3695	12438	14150	26756	1972	588	20294	1400	50172	2202	71
1990	4257	11426	15349	1801	1806	380	25997	1339	51558	1669	70
1991	2811	8874	10839	1984	773	147	20811	613	42068	1892	12

Source: Copra Marketing Board of Papua New Guinea.

and selecting the existing local materials which may have built up natural tolerance to the local pests and diseases. Because of the susceptible nature of the existing hybrids to rhinoceros beetles and palm weevils, the exploitation of local materials may be desirable.

Progress to date

Prospection by province is shown in Table 2. Germplasm prospection has led to the identification of the following populations:

1. Markham Tall
2. Karkar Tall
3. Gazelle Tall
   —Pellevarua
   —Raulawat
   —Natava
   —New Massava
4. Gazelle Yellow Fruited Tall
5. Gazelle Red Fruited Tall
6. Gazelle Many Fruited Tall
7. Baibara Tall
8. Milne Bay Tall
9. Oro Tall
10. Vailala Tall
11. Namatanai Tall
12. Manus Tall
13. East Sepik Tall
14. Bougainville Large Fruited Tall
15. Nuguria Tall
16. West New Britain Tall
17. Talasea Tall (Red fruited)
18. PNG Yellow Dwarf
19. PNG Red Dwarf
20. Rabaul Red Dwarf
21. Iokea Red Dwarf
22. PNG Brown Dwarf

Future prospections

As opportunities become available, other areas will be prospected.

Germplasm collection

To characterise the populations identified in the prospection, it is necessary to assemble the materials in one area to study the floral biology and other phenotypic characters. This study may lead to the establishment, on the basis of morphological characters, of the genetic distance between populations. The collection will also become a germplasm collection for the inclusion of material from all other Pacific countries, as well as providing coconut breeders with a wide range of genetic material for their breeding programs.

Table 2. Provinces in which prospection for germplasm has been completed.

Province	No of Sites
East New Britain	35
West New Britain	4
Gulf	11
Milne Bay	7
Central	6
Oro	7
Morobe	3
New Ireland	11
Manus	27
Madang	9
East Sepik	26
Sandaun	7
North Solomons	6
Western	5
Total Sites	165

Method of Collection

Collection is done mainly by use of seed nut, as embryo culture methods offered no cost advantage. The following materials have been assembled as a live field collection:

Talls

New Massava
Alexhaffen
Karkar
Pellevarua
Natava
Raulawat
Nagada
Rennell

Dwarfs

PNG Brown
PNG Yellow
PNG Red
Rabaul Red
Malayan Yellow
Malayan Red
Nias Yellow
Nias Green

Collections

Collection of seednuts from the germplasms identified within Papua New Guinea started in March 1993 for the Gazelle area and the distant sites. Seednuts are being collected and sent to Omuru for nursery propagation. Germplasm materials collected so far are shown in Table 3.

Land for Field Planting

The Institute has purchased Murnass and Kaile plantations in Madang for field planting of the germplasm collections. Multiplication and collection blocks, and progeny trials, will be established in these plantations.

Breeding

Proposals for the breeding program of the Institute have been documented by Tan, Ovasuru and Bridgland in 1991. In summary the aim of the coconut breeding program is to create a range of Dwarf x Tall and Tall x Tall hybrids with the following characteristics:

- increased copra yield;
- resistance to *Scapanes australis, Oryctes rhinoceros* and *Rhycophorous bilineatus;*
- precocious;
- ideal for intercropping; and
- acceptable by the smallholders and large estates.

The program aims to utilise the local genetic materials identified in the germplasm prospections.

Planned trials are listed in order of priority:

1. Dwarf x Tall — Progeny trial I
2. Dwarf x Tall — Progeny trial II
3. Dwarf x Tall — Progeny trial III
4. Tall x Tall — Progeny trial IV
5. Dwarf x Dwarf — Progeny trial V
6. Dwarf x Tall (Exotic) and Tall x Tall (Exotic) — Progeny trial VI
7. High Fruit Number x High Fruit Number — Progeny trial VII

Emphasis will be given to testing progeny in as many locations as possible to detect their tolerance to rhinoceros beetles and evaluate possible genotype/environment interactions. This will enable the Institute to identify and recommend to farmers the best hybrids for their respective regions.

Table 3. Coconut seednuts so far collected from germplasms identified within Papua New Guinea.

Province	Type		No. Collected
East New Britain	Gazelle Tall	-(GLT 1)	225
		-(GLT 2)	225
		-(GLT 3)	225
		-(GLT 4)	225
	Gazelle Yellow Fruited Tall	-(GYT 1)	225
		-(GYT 2)	225
		-(GYT 3)	225
	Gazelle Many FruitedTall	-(GMT 1)	225
		-(GMT 2)	225
		-(GMT 3)	225
		-(GMT 4)	225
		-(GMT 5)	225
New Ireland	Tall	-(NLT 1)	225
		-(NLT 2)	225
		-(NLT 3)	225
Manus	Tall	-(MLT 1)	225
		-(MLT 2)	225
		-(MLT 3)	225
West New Britain	Tall	-(WNT 1)	950
		-(WNT 2)	950
		-(TRT)	225
Solomon	Rennell Tall	-(RLT)	700
Oro	Tall	-(OLT 1)	225
		-(OLT 2)	225
		-(OLT 3)	225
West New Britain (Dam)	PNG Yellow Dwarf	-(PYD)	750
	PNG Red Dwarf	-(PRD)	750
	Rabaul Red Dwarf	-(RRD)	750
	PNG Brown Dwarf	-(PBD)	750
	Iokea Red Dwarf	-(IRD)	750

Conclusions

The coconut industry in Papua New Guinea has been given another opportunity by the government to improve its planting materials because of the importance of the industry to the rural population. Papua New Guinea Cocoa and Coconut Research Institute, after its formation in 1986, took on the role of developing technologies suitable for both smallholders and plantations. Its activities include: germplasm identification, collection and establishment; breeding and selection; farming systems research; and downstream processing. It has attracted outside agencies to assist in the germplasm activities and a good germplasm collection is underway. Breeding programs utilising the materials identified in the germplasm prospection are in progress. It is the desire of the Institute to develop good planting material for the smallholder in Papua New Guinea to increase farm productivity.

The government price support scheme has created stable copra prices. This is a very important development as producers will be assured of a good market

price for copra, and will therefore continue to supply the depots with copra at a more constant rate. Consequently, the Copra Marketing Board of Papua New Guinea will in turn meet its international contracts without much constraint.

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Current Status and Development of Coconut in Solomon Islands

Michael Max Oliouou*

SOLOMON Islands is situated in the Southwest Pacific Ocean between latitude of 5 and 12°S and longitude 154 and 162°E. The major islands of the group are Guadalcanal, Malaita, Makira, Choiseul, Isabel and New Georgia. There are numerous small islands, including some atolls. The total land area is 27 566 km^2.

The large islands are all alike in that they have central mountainous ranges falling away through a series of foothills on raised coralline plateux to the coast. Rugged terrain predominates. Only the islands of Guadalcanal have extensive alluvial coastal plains. The small islands include some active volcanoes and one of the largest atolls in the world—Ontong Java.

The climate is hot and humid; pleasantly tropical, with temperatures usually within the range of 22° to 35°C and mid-day heat being moderated by coastal breezes. Cyclones occasionally strike the islands, usually during the November to April monsoon period. The rainfall is 3000 to 4000 mm per annum throughout most of the country.

Due to the mountainous nature of the country, only a small portion of the total land area is suitable for coconut production. This physical factor also brings coconut production into direct competition with other cash crops such as cocoa, oil palm and even food crops which can only ideally be grown on relatively flat land—the coastal plains.

Coconut—the Tree of Life

Few would deny the coconut tree occupies a unique position in the life and culture of Solomon Islanders and indeed many of the South Pacific island nations. Apart from providing logs for timber, it has long been a provider of staple foods and therefore is important to the livelihood of islander people. Yet it is this very ubiquity which often has misled people to accept the existence of 'The Tree of Life' as just a norm.

* Dodo Creek Research Station, Ministry of Agriculture and Lands, P.O. Box G13 Honiara, Solomon Islands.

Importance of Coconut in the Solomons

The total area of land planted to coconuts was estimated in a 1985 survey to be 60 000 ha. Since then however, some new plantings of approximately 850 ha have taken place under the STABEX (Smallholder Development Program), about 100 000 seedlings having been distributed following cyclone Namu in 1989. Apart from this, there also have been informal plantings by smallholders.

Management

The standard of management varies enormously from one farm to another. A survey in 1985 indicated that about 47% of the coconuts owned by smallholders had weeds growing to shoulder height and even more. It was also noted that most coconuts in the smallholder plantations are of the types Rennell and Local Tall with only about 10% being hybrids. Large plantations on the other hand have completed replanting coconuts with the hybrid Malayan Red Dwarf x Rennell Tall (MRD x RT).

Yield

The yield of copra averages from 700 kg/ha on smallholder farms using Local Talls to about 2500 kg/ha on plantations with hybrids.

Where soil is suitable, intercropping coconut with cocoa is advocated but many of the coastal soils are unsuitable for cocoa as they are highly alkaline sands, shallow, and over-lying coral stones.

Coconut Exports

Nearly all export is in the form of copra: the annual average being 30 000 t, 75% of which is produced by smallholders. In 1992 copra fetched about US$5.5 million, 6% of total exports. Nevertheless, the relative contribution of copra to the total exports in Solomon Islands has been steadily declining since the good years of 1984–85. Today copra appears at the bottom of the export list. This is partly due to widely fluctuating prices, with a steadily falling long term trend and partly due to increased exports of these other commodities (Table 1).

Oil Production

At the beginning of 1990 the plantation (Levers Solomons Limited [LSL]) started to convert its copra into oil. In 1992 Levers produced 9412t of copra, 90.8% of which went to the mill to be converted to oil. In the same year the company exported 3267t oil and 3200t of copra cake.

More recently there have been some exports of fresh mature nuts to Australia, about one container per month and mainly catering for the needs of Pacific Island communities in Sydney and Brisbane.

Table 1. Major export commodities in order of importance.

Commodities exported	Qty (kg)	Value (SI $)
Wood(M^3)	551610	330459468
Logs (M^3)	543095	304019525
Sawn (M^3)	8515	26439943
Fish Products	3322549	88193794
Frozen	2784496	48131252
Smoked	428891	3063291
Canned	3448883	6756525
Others	249874	244726
Oil Palm Products (Ct)	34282	33913125
Oil (Ct)	30540	31944613
Kernels (Ct)	3742	1968512
Coconut Products	25742123	31342864
Copra	25739624	27295958
Oil (Ct)	3499	1968512
Cocoa	4454816	13345142

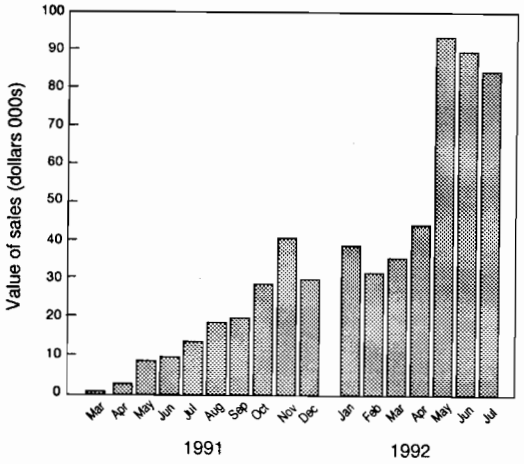
Source: Government Statistics Office, 1992 Report, Honiara, Solomon Islands. (In press).

Domestic Consumption

Domestic consumption of fresh and dry nuts is estimated to be 4.5 nuts per family per day, an equivalent of 14000 M/t of copra per year. This figure is likely to increase as population increases and more nuts are supplied to urban centres.

With respect to green nut consumption, since March, 1991 Levers has sold green nuts to shops and restaurants in Honiara and to the public. The trend of supply and demand is shown in Figure 1.

Smallholder farmers closer to main centres have also increased their green nut supply to town markets. Apparently the farmers find that they make SI$1.00 per nut as green nuts and only 15¢ per nut as copra, so they would rather sell green nuts than cut copra.



Source: Levers Solomons Ltd, Liungga Estate, Honiara, Solomon Islands

Figure 1. Value of sales of green coconuts showing trend in supply and demand.

Copra is purchased and exported by the Commodity Export Marketing Authority (CEMA), a statutory body which buys copra through its nationwide network of copra buying centres (Fig. 2).

Coconut Improvement

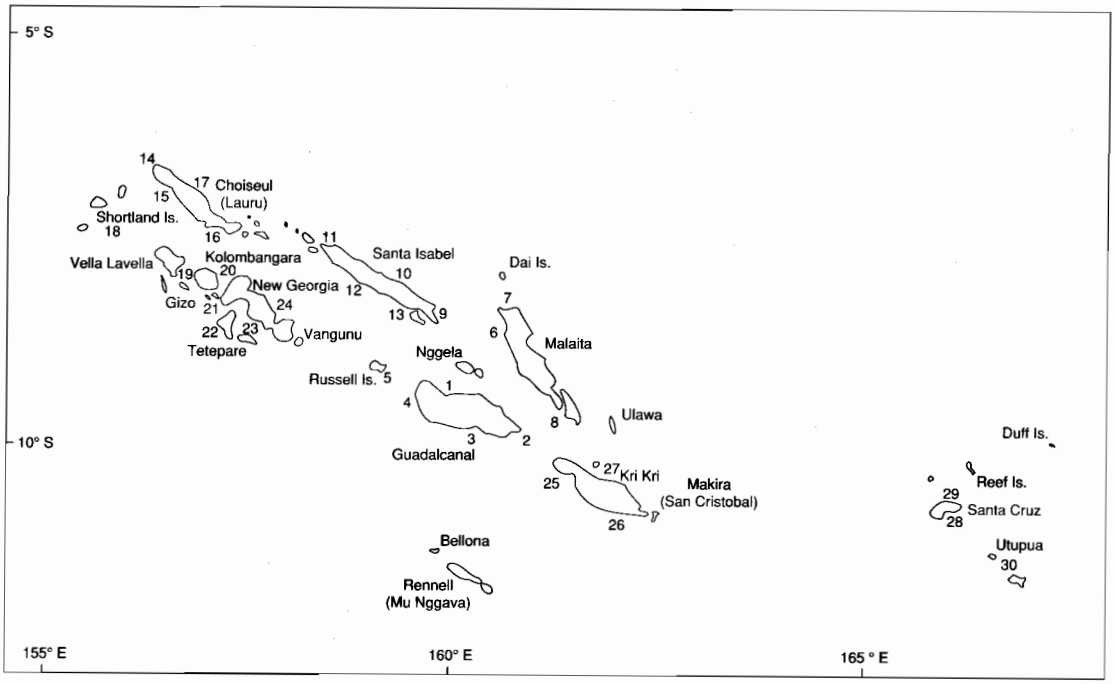
Research

Coconut has been a major focus of research in the Solomon Islands since the 1950s. The research has concentrated on: selection and breeding, entomology, pathology and agronomy (nutrition, spacing, nursery procedures).

A large collection of coconut varieties was established on LSL commercial estate at Yandina in the Russell Islands.

Past breeding work involved crosses between a number of tall varieties (including Rennell, Local Tall, West African Tall and F.M.S [Malaysian Tall]) which were crossed with a number of dwarfs notably the Malayan Red Dwarf (MRD). As a result of the breeding program the Malayan Red Dwarf x Rennell Tall (MRD x RT) hybrid has been recommended, both for smallholders and estate sectors.

There are plans to duplicate the Yandina collection at Dodo Creek Research Station (DCRS) on Guadalcanal as a safeguard against cyclones. To enable this, two officers from the Tree Crops Agronomy Section at DCRS have attended training in



Guadalcanal
1. Honiara
2. Manikaraku
3. Haimarau
4. Marasa

Russell Islands
5. Yandina

Malaita
6. Auki
7. Malu'u
8. Afio

Isabel
9. Buala
10. Estrella Bay
11. Kia
12. Susubona
13. Samasodu

Choiseul
14. Choiseul Bay
15. Katuraselu
16. Posarae
17. Nanago

Shortlands
18. Korovou

New Georgia
19. Vonunu
20. Irigila
21. Gizo
22. Rendova Harbour
23. Noro
24. Karu

Makira/Ulawa
25. Marou Bay
26. Waimarega
27. Kaonasughu

Temotu
28. Calisle Bay
29. Waimarega
30. Nabao

Figure 2. Commodity exporting authority's copra buying centres.

emasculation and pollination. This training has been made possible by the EEC funded Coconut Improvement Program for the South Pacific based in Saraoutou, Vanuatu.

Recently, more serious investigations were undertaken jointly between Levers and the Solomon Islands Government. Although both now run their own research programs, they collaborate closely. The government program is presently investigating performance and fertiliser requirements of several coconut varieties on a range of soil types.

The estates, on the other hand, have plans that include investigating the relationship between the fertiliser requirements of cocoa and Ngali nut (*Canarium* spp), under coconut, at varying plant densities.

Entomological studies include work on the leaf beetle *Brontispa* (*B. froggatti*) on coconut seedlings

and premature nutfall caused by the sucking bug *Amblypelta* (*A. cocophaga*). More recently there has been work on the coconut rhinoceros beetle (*Promecotheca opacicollis*), which occurs only on Santa Cruz island in the Eastern Province of Temotu. Fortunately, the large rhinoceros beetle (*Scapanes oryctes*) has not been found in the Solomon Islands as far as can be known.

Pathological studies include work on *Marasmiellus cocophilus* on coconut seedlings and more recently on a viroid similar to cadang-cadang which was found in an oil palm in 1988 at a commercial plantation near Honiara. Samples have been collected and were sent to Australia for identification, but progress in the Solomon Islands is being frustrated by a lack of funds.

Seed Gardens

The main source of hybrid seeds has been the 40 ha seed garden located at LSL estate in the Russell Islands. The seed garden was established in 1965. More recently, provinces have started to establish their own seed gardens of 2–10 ha to provide hybrid seed nuts to farmers in their region. Six of the eight seed gardens are now bearing and are operational.

Except for the seed garden in Temotu Province (Santa Cruz) all other seed gardens provide Malayan Red Dwarf x Rennell Tall (MRD x RLT), though Yandina seed garden did originally produce a range of hybrids for varietal assessment.

The MRD x RLT is being produced by natural pollination. Two lines of MRD are planted between two lines of Rennell, with three guard rows of Rennell around the outside of the garden. The MRD are emasculated daily to avoid self-pollination. The Yandina seed garden is being operated by the LSL whilst those in the Provinces are operated by the Solomon Islands Government but under the jurisdiction of the provincial administration.

Development Programs

Senility is not a serious problem with Solomon Islands coconuts. A survey in 1985 established that about 50% of the palms are 15 years old or less with only 10% being greater than 40 years old. This is a consequence of the 1962–76 Replanting Scheme in which the Government provided a subsidy in the form of a cash payment of SI$24.70 per hectare for properly planted and well maintained coconut plots. Thus by 1976, about 50% of the total area under coconuts had been replanted.

The major developments that have occurred in the last five years have come about through the activities of the Smallholder Development Program (SDP), financed by the EEC and from STABEX funds. About 50% of SDP funding was designed to increase production from coconuts through a number of activities:

- development of a mini copra drier for smallholder producers. The program adapted and modified a mini multi-purpose drier which was originally designed at the University of Los Banos in the Philippines, for use in the Solomon Islands. Over 100 drier units were supplied to SDP farmers throughout the country;
- a number of demonstration plots were established on plantations to demonstrate the advantages of fertiliser application. The plots also incorporate intercropping other crops, depending on soil type and location;
- provision of transport facilities to extension staff in order to enable them to assist coconut farmers in remote parts of the country, provision of training courses for coconut farmers and in-service training for extension staff; and
- provision of support for the Agricultural Information Unit (AIU) to implement radio programs and produce printed information for distribution to farmers.

The current Smallholder Development Program (SDP 1) finishes in December 1993. It is planned that the second stage of SDP (SDP 2) will begin immediately in January 1994.

SDP 2 is designed to enhance and consolidate the achievements of SDP 1 but to also strive to achieve the following:

- improvement in the present copra processing methods by searching for effective drying methods that are based as far as possible on locally made materials. The quality of copra being exported will have to continue to be improved in order to market the product successfully in an increasingly competitive market;
- increasing production either by increasing the area planted to coconut or by increasing productivity of existing areas. Although some new plantings are inevitable, if only to replace the 10% of palms that are over 40 years of age, further expansion of the existing areas is not considered feasible. This is because of the increasing pressure on land for food gardening and the desire and capability of smallholders to manage an increased acreage. Under such circumstances, the Provincial Development Plans (1993–1997) have indicated an overall increase in areas of about 4% (Table 2) plus some rehabilitation of about 5% which should only maintain the balance at the existing hectarage (60000 ha).

Table 2. Provincial planned expansion and rehabilitation 1993–1997.

Province	Expansion (ha)	Rehabilitation (ha)
Guadalcanal	228	63
Malaita	620	800
Makira	380	790
Temotu	200	250
Central	100	-
Isabel	174	870
Western	415	-
Choiseul	150	350
Solomons	**2277**	**3123**

Source: Smallholder Development Programmes (SDP), first quarterly report, 1993, Honiara, Solomon Islands.

Conclusion

The coconut is an important source of cash income for Solomon Islands (more than SI$31 million in export revenue from coconut products in 1992) and of locally produced food for the rural and urban populations.

The search for better and higher yielding varieties as well as processing methods that would improve the quality of copra, will continue. However, the area planted to coconut (60000 ha) is unlikely to increase or decrease substantially in the foreseeable future.

Growth in this sector must therefore come from increased productivity per unit area rather than expansion of the area planted.

Production and Dissemination of Improved Coconut Cultivars EEC—Pacific Regional Agricultural Program (PRAP)

Gerard Duhamel*

THE paramount importance of the coconut for the Pacific countries from both a social and an economic point of view is well known. The productivity of coconut plantation is very low and unfortunately the sluggish price of copra is not an incentive for replanting.

Due to several causes—senility, typhoons, destruction to make room for other activities—coconut is slowly disappearing in several countries and the time could soon come when, paradoxically, some countries may have to import coconut products to cover the needs of their fast growing populations.

Assessing the situation, and taking into consideration their common needs and conditions, the South Pacific, Asian, Carribean and Pacific (ACP) countries requested the EEC to support a project with the purpose of finding highly productive coconut cultivars to be utilised for replanting programs.

In response to this request the EEC agreed within the framework of its 'Pacific Regional Agricultural Program' (PRAP) to finance the operating costs of a project—'Production and Dissemination of Improved Coconut Cultivars'. The French Government agreed to fund the coordination and technical assistance required by the project and the Vanuatu Government made available the facilities and the coconut collection at Saraoutou, Vanuatu for the implementation of the work. The Vanuatu Government also made available the necessary land for field experiments and tests.

Objective and Methods

Creation and testing of cultivars

The general objective of the project is to advise member countries on the best varieties to use for planting or replanting coconut and of the value of testing varieties already existing in the region; thus maximising the value of adaptation of regional varieties to local conditions. At the same time this should limit or avoid transfers of planting materials.

In the first phase, hybridisation between Dwarf and Tall varieties has been implemented because this has proved to be a faster way of improving production rather than attempting improvement of pure ecotypes. Tall x Tall, although potentially a higher producing cross is less precocious and more difficult to produce. This strategy however could be worked into other phases of the project (Project No. 6100.94.093).

The current hybridisation programs are mainly based on the Tall and Dwarf varieties in the countries. The two Malayan dwarf varieties—Yellow and Red—are included in all programs because they are widespread in the region. Other varieties are selected either for their combining ability, or because they are well established in the country from which the pollen is obtained.

Prior to a decision to create hybrids the countries are visited. Discussions are held with the agricultural officers responsible for coconut improvement and development. In this way the project responds to needs and demands and takes into consideration national specificities. The National officers participate in the implementation.

The hybridisation work is carried out in Saraoutou on existing palms in the germplasm collection. When necessary, Tall variety pollen is imported from the country for which the program is designed.

The hybrids are planted at Saraoutou to a statistical design and compared to permanent controls.

The project program gives emphasis to staff training so that those involved in coconut improvement in the countries can properly implement all operations: sampling, observation and description of varieties, multiplication of varieties, seed garden management, seedling production and selection, and other requirements for proper coconut cultivation.

Training courses are organised either at Saraoutou or in-country during visits that are made by the coordinator. Techniques and methods are then adapted, when necessary, to specific country conditions.

* Centre Agronomique de Recherche et de Formation du Vanuatu, B.P. 231, Banto, Vanuatu.

Description of varieties

The project is also to undertake the description of varieties to ensure that the genetic variation in the coconut populations, in the region, is properly recorded.

The data collected will be analysed in order to obtain a description and classification of the ecotypes. This includes:

- The mean and variance calculated from the measurements carried out over a relatively long period and for a large number of characteristics at young and mature stages to allow a good description of the ecotypes.
- Descriptive multi-variable analyses:—analysis of principal components, discriminating factorial analysis, generalised distances. These checks should enable graphic maps presenting the possibility of discrimination between varieties or groups of varieties to be drawn. In addition, the calculation of genetic distance between varieties might give significant indications for guiding the selection of cross-breeding material.
- Correlations will be sought between important agronomic characteristics (such as precocity and yield) for which the genetic-value-estimation is long and not always accurate, and more heritable vegetative characteristics which are easier and faster to measure. The use of these correlations should help to improve the effectiveness of selection by the use of indirect selection-type methods or based on an index.

Current Achievements of the Project

The project started in June 1989 with a meeting in which the general program was discussed and drawn up with the representatives and national coordinators of the countries involved. Yearly performances and the program are reviewed by a board of the Directors of Agriculture of the South Pacific (ACP) countries and EEC representatives.

Creation of cultivars

The programs that have been implemented are shown in Figure 1.

Training Courses and Visits to Countries

By 1993, 54 agricultural officers from the participating countries had attended training and information sessions at Saraoutou.

All the countries involved in the project have been visited by the coordinator and technical services were provided to meet specific needs of countries. For example:

- pollen production and dispatching for the creation of hybrids has been implemented, after training by officers from Kiribati and PNG;
- a specific technique has been developed and demonstrated to improve the quality of coconut seedlings in atoll conditions;
- technical advice and support on seed garden management has been given in Western Samoa and in Tuvalu;
- a consultancy by an entomologist has been organised in Vanuatu, Solomon Islands and Papua New Guinea to investigate the significance of the pests *Oryctes, Scapanes, Rhyncophorus* and *Sexava* in relation to coconut and recommend means of control. This activity has facilitated the launch of a renewed coconut program in Papua New Guinea;
- technical advice has been given in Tonga on the creation of a coconut collection and the establishment of a seed-garden. Specific training sessions have also been organised; and
- specific work programs have been established with the officers-in-charge of coconut projects in Kiribati, Western Samoa and Tonga.

As indicated already, all the work and steps taken are discussed with the national representatives to answer the questions and needs of the countries. The project receives interest and effective collaboration from these representatives.

Description of varieties

To fulfil this objective of the programs several observations are in progress: germination, seedling growth, and growth in the field (for hybrids).

Phenotypic characteristics and production are recorded for the parental varieties.

All data are collected and recorded in accordance with the established guidelines and will be subsequently dealt with in the appropriate manner. For this work the project receives the support of CIRAD Biometry Division—which is associated with the COGENT (IBPGR) data base. It can also call upon the assistance of another PRAP project involved in biometry. However it is still too early to comment on the results.

Future of the project

A review of the project was carried out in 1993 and continuation of the activities described above was recommended. Emphasis will be given to staff training, adaptation of techniques to country conditions and transfer of information to national research and extension services so as to further support their activities.

	Dwarf ←		Varieties			→ Tall				
	MYD	MRD	CRD	NLD	NBD	AYD	BGD	VRD		
Trial No.									**Pollen from:**	
No 1	X		X	X		X		X	Rennel - RLT	Saraoutou Collection
								X	VTT Control	Saraoutou Collection
No. 2	X		X		X		X	–	Tonga : TGT	Saraoutou Collection
			X						RLT Control	Saraoutou Collection
								X	VTT Control	Saraoutou Collection
No. 3	X		X			X	X	–	Kiribati : KIT	Imported from Kiribati
							X		Polynesia: PY2T	Ivory Coast
								X	VTT Control	Saraoutou Collection
No. 4	X		X	X		X		–	Rotuma : RTT	Saraoutou Collection
			X						RLT Control	Saraoutou
								X	VTT Control	Saraoutou
No. 5	X		X	X		X		–	Markham Valley: MVT	Imported from PNG
			X						RLT Control	
								X	VTT Control	
No. 6	X		X	X		X			Gazelle GZT	Peninsula Saraoutou
			X						RLT Control	
								X	VTT Control	

Note: In trials No. 3 and 5, pollen was produced in Kiribati or PNG and imported. In other trials, pollen was harvested from palms in the Saraoutou (Vanuatu) collection.

MYD	=	Malayan Yellow Dwarf	NBD	=	New Guinea Brown Dwarf (Madang)
MRD	=	Malayan Red Dwarf	AYD	=	Apia Yellow Dwarf
CRD	=	Cameroon Red Dwarf	BGD	=	Brazil Green Dwarf
NLD	=	Niu Leka Dwarf	VRD	=	Vanuatu Red Dwarf
RLT	=	Rennell Tall	RTT	=	Rotuma Tall
TGT	=	Tonga Tall	VTT	=	Vanuatu Tall
KIT	=	Kiribati Tall	PY2T	=	Polynesian Tall

Figure 1. Implemented trials.

Raising Coconut Productivity in the South Pacific: the Australian Connection

M.A. Foale* and G.R. Ashburner†

AUSTRALIA is geographically a part of the South Pacific, forming the western edge of an ocean that stretches across 140 degrees of latitude. The commercial centres of the coconut economies of Melanesia and the Fiji Islands are within a few hours flying time and a few days 'steaming' time of the cities of the east coast of Australia and the Polynesian countries are not much further away. A strong and mutually beneficial association is developing between Australia and its near neighbours in cultural, educational, and scientific fields as well as in business and trade. It is entirely natural for Australia to foster partnerships in the development and movement of technology that contributes to productivity and stability in agriculture in both Australia and the partner country. This is the model that is specifically supported by the Australian Centre for International Agricultural Research (ACIAR).

A coconut industry for the production and export of copra and coconut oil has developed only within the past 110 years. A flurry of development of plantations in the first three decades of the 20th century was followed by depression and war. From the 1950s productivity of the old plantations fell as an inevitable result of loss of palms through chance injury and disease, and the declining ability of the aging coconut palm to intercept light and therefore to remain productive. Whereas most expatriate investors began to lose interest, old plantations were revitalised by the introduction of cocoa and other intercrops, which thrive especially in the moderate shade of an aging stand. At the same time, many indigenous people became smallholders with respect to coconut, meeting their increasing desire for cash from the sale of copra, and of fresh nuts if they lived near urban centres. The coconut has remained an important part of the diet of most Pacific island people, and is absolutely essential to survival of the human population in many places.

* Consultant to ACIAR, CSIRO Division of Tropical Crops and Pastures, 306 Carmody Road, St. Lucia, Queensland 4067, Australia.

† IHD, Private Bag 15, Southeast Mail Centre, Vic 3176, Australia.

The coconut is well adapted to the wide range of South Pacific coastal environments, as, with few exceptions, there is adequate rainfall for its survival and indeed high levels of productivity. It seems clear that the coconut has grown on islands and coastal fringes in the region for millions of years as it is also adapted to the wide range of indigenous pests and diseases. It is generally only when seeds from one place are planted in a distant country that disease and insect pests show themselves in a really destructive mode. The greatest threat to coconut production, however, has been the ever-declining price for coconut oil over the last 50 years with only short periods of respite due to shortage of one of the competing vegetable oils or through manipulation of the market. While the great flavour and nutritive value of coconut flesh, and the incomparable freshness of coconut water, have remained unchanged, the place of coconut oil for use in cooking and in manufactured food such as margarine, has been usurped to a large degree.

The response to a lower return for industrial coconut products (copra and oil) has been to seek higher productivity so that the return on investment of effort is at least sustained. Inevitably the attraction of coconut as a financial investment has disappeared almost completely, but its place in the household, local and regional economies remains secure and indeed grows in proportion to the rise in population of local communities. The future for the coconut therefore appears to lie in its integration into a multiculture cropping system where it constitutes a highly stable perennial component. Coconut palms have the capacity to protect the landscape, maintain a cyclical flow of nutrients through the ecosystem, and provide a microenvironment that is favourable to many other crops grown either for food or cash.

Coconut growers would be expected to look to science for advice on:

- how to protect their palms against disease and insects;
- what seeds to plant to get sturdy, long-living, well adapted and productive palms, which bear fruit that is suitable for the best available market;

- what sort of management of other crops and of residues will sustain adequate nutrition for all cultures in the system with the minimum of requirement for artificial fertiliser; and
- new methods of adding value to coconut products, especially at the household or village level, to maximise the cash return from production that exceeds the local demand for food.

An Australian Contribution to Coconut Science

ACIAR has supported projects in the South Pacific under the first two points listed above; that is, protection against disease and coconut improvement.

The virus and viroid project (which will not be reported on in detail here) seeks ways to manage a virus disease that is found in Vanuatu, and it also supports basic research on viroid-like RNA sequences which resemble a pathogenic viroid found in the Philippines. The Coconut Improvement Project has developed improved methods of culturing coconut embryos to assist germplasm transfer, and has achieved the description of the principle traits and variability of a large number of coconut populations throughout the South Pacific. Many populations have also been characterised both in terms of fruit morphology and molecular variability.

The following projects have come to a conclusion as the Coconut Improvement Project draws to a close.

Germplasm characterisation

AIMS: To use existing and novel techniques to characterise coconut germplasm in the South Pacific region. This will aid in setting priorities and increase the efficiency of germplasm collection and conservation.

- Morphological techniques have been intensively applied within Papua New Guinea and extensively in the Pacific, i.e. Solomon Islands, Vanuatu, Fiji, Nauru, Kiribati, Tuvalu, Samoa (West and East), Niue, Cook Islands, Society Islands, Tuamotu Archipelago, Marquesas Islands, Christmas Island, Phoenix Islands, Tonga and Hawaii. Other regions were sampled as the opportunity arose. Fruit component analysis was performed at each site and vegetative characters were measured at some sites.
- DNA analysis using Random Amplified Polymorphic DNA (RAPD) techniques with Restriction Fragment-Length (Polymorphism (RFLP) analysis in conjunction with Professor Wolfgang Rohde of the Max Planck Institute, Germany. Accessions tested were from Cocos Island (Indian Ocean), Papua New Guinea, Solomon Islands, Rennell Island (Solomom Islands), Vanuatu, Kiribati, Rotuma (Fiji Islands), Fiji Islands, Tonga, Cook Islands, French Polynesia, Christmas Island (Kiribati) and Hawaii (USA).

Germplasm collection

AIMS: To establish a regional germplasm repository in Papua New Guinea.

- A collection of PNG germplasm is underway based on the results of the intensive germplasm survey. Collection is in the form of both seednuts and pollen.
- Collection of germplasm from elsewhere in the Pacific was done using embryo culture to avoid quarantine problems with seednuts. However, due to uncertainty regarding the viroid that has been found in coconut populations throughout the region, no cultured material has been forwarded to PNG. In some remote locations embryo collection has not been feasible due to inadequate facilities. Some basic research was done on the method of culture of embryos in order to resolve inconsistencies between previously published methods.

Dissemination of information

In order to ease the burden for many workers of lack of recent or obscure published information about coconut technology, particularly regarding genetic improvement and management in the field, three bibliographies have been compiled. The bibliographies list about 500 coconut publications or papers or books that are connected with coconut. Each entry consists of the author's name, title of the journal article conference paper or book, the appropriate reference data, and in most cases an abstract. This service has been greatly appreciated by workers in scientifically isolated situations.

Crop physiology

AIMS: Exploring the limits to coconut productivity. While the emphasis in the ACIAR project has been on germplasm characterisation and transfer there has been some opportunity to explore the physiology of yield of coconut. Particular attention has been given to light interception and also to the partitioning of dry matter between the fruit and the vegetative parts on the one hand, and between the endosperm and the remainder of the fruit on the other. A great deal of work remains to be done, especially in the area of light interception, and the evolution of the shape of the coconut crown with age. It appears that there is an inexorable decline in interception beyond the age

of about 20 years as the fronds are forced into a declining position by the expanding base of young emerging fronds. While this is an inbuilt handicap for the coconut, it is also an opportunity for the production of intercrops (Foale, Ashburner and Friend, these proceedings).

Regional breeding project

AIMS: To participate in the PDICC regional breeding objective. There is an opportunity presented by the PDICC project to have 'country' populations tested for their general combining ability with recognised 'mother' populations, such as Cameroun Red Dwarf, Malayan Yellow Dwarf and so forth. The contribution of other funds to create this opportunity is acknowledged.

Publications

An assortment of papers has been published in journals, conference proceedings, and by ACIAR. A complete list of these will appear in these Proceedings of the Regional Coconut Workshop.

What Does the Future Hold?

The future for Australian science in the coconut industries of the South Pacific region is far from clear except for the continuing work on viruses and viroid-like DNAs being done by the group from the Waite Institute, University of Adelaide. ACIAR's charter for developing collaborative projects from which an Australian agricultural industry benefits, as well as an industry in the partner country, cannot be complied with for coconut due to the absence of commercial coconut production on a significant scale in Australia.

On the other hand, there is no shortage of interest and enthusiasm on the part of many Australian scientists to support advances in production science as well as processing and marketing of coconut products. Indeed, there is currently a most promising advance in microscale processing of dried coconut for the extraction of oil at the household and village level that has been achieved by Australian-based scientists. This requires only careful planning of bilateral and perhaps multilateral projects that can be delivered, at the appropriate level, into South Pacific countries to revive the coconut industry as a major contributor to economic activity. There are other scientific groups in Australia that could contribute to multidisciplinary efforts to improve productivity of farming systems that contain coconut, perhaps in a consulting mode or in a short-term collaboration. Such science technology as simulation modelling of complex farming systems might be of great assistance in devising procedures to optimise management of a mixed crop system to achieve the best economic return. Collaboration with in-country scientists will depend on their ability to attract appropriate funding.

Coconut Embryo Culture for Remote Locations

G.R. Ashburner*, M.G. Faure†, P.R. Franz*, D.R. Tomlinson*, P. Pulo†, J.M. Burch*§ and W.K. Thompson*

COCONUT embryo culture has many uses including the collection and transportation of germplasm. Considerable savings can be made in transportation costs since one fruit is the equivalent of 10000 embryos (Harries 1982). Quarantine risks are reduced because all surface pathogens are eliminated. Apart from this, coconut embryo culture has been used for embryo rescue of makapuno type coconuts (Rillo and Paloma 1992a) and for in vitro selection for such traits as drought tolerance (Karunaratne et al. 1991).

However, the technique has many inconsistencies and requires the use of specialised equipment, making it expensive to set up. This paper reports the current protocol used for coconut embryo culture simplified to be more applicable to remote locations. The basic requirements needed for an embryo culture capability is given and its application to germplasm collection discussed. Embryo culture may be divided into three discrete areas: collection and transport of the embryo; in vitro growth; and acclimatisation to free-living conditions. Each of these areas will be discussed and their minimal requirements given.

A number of protocols have been developed for the collection of coconut embryos (Assy Bah et al. 1987; Sossou et al. 1987; Rillo and Paloma 1992b). However, either they require the use of bulky or uncommon equipment or they do not cater for international transfer. The following protocol has been devised in a kit form that is easily dispatched and simply carried out (Table 1). These methods have been extensively tested in various situations in the South Pacific region.

An improvised laboratory may be used for isolation of embryos. It consists of a clean, draught-free room with a work bench that has been disinfected with alcohol, bleach or other disinfectant. A spirit lamp, gas burner or other heat source is required for sterilising forceps. To collect embryos:

- Select mature fruit directly off the palm and dehusk on a sharpened stake.
- Break the coconut around its middle (equator), and working from the inside of the coconut, remove the plug of endosperm that surrounds the embryo. The plug should have a diameter of about 20mm and can be removed with a cork-borer or a sharp-bladed knife.
- Place the plug in a clean container or plastic bag.

The above stages may be done in the field.

- Surface disinfection of the embryo should be done in a laminar flow cabinet and using sterile techniques. If further transportation is required, then this step provides surface sanitation and may be carried out on a bench.
- Wash endosperm plugs in running water and briefly rinse in 70% ethanol, if available.
- Cut the endosperm plug and divide in half to release the embryo.

Table 1. Requirements for embryo collection and transport.

bush knife or machete
cork borer[1,2]
small knife
dehusking stake
plastic bags
20ml disposable syringe[2]
forceps[2]
bleach or sodium hypochlorite
ethanol[1]
culture or transport tubes[2]
sterile water
spirit/gas burner

[1] optional.
[2] supplied with collecting kit, if for collection and transport only.

* Department of Agriculture, Institute for Horticultural Development, Private Bag 15, South Eastern Mail Centre, Victoria 3156, Australia.

†Cocoa and Coconut Research Institute, PO Box 1846, Rabaul, Papua New Guinea.

§Present address: Faculty of Biological and Behavioural Sciences, The University of New South Wales, PO Box 1, Kensington, New South Wales 2037, Australia.

- Place the embryos into a syringe barrel, draw in a 1% active solution of chlorine, eject any air and leave for 15 minutes. Expel the chlorine from the syringe, draw in and expel two changes of sterile water (or water that has been repeatedly boiled on subsequent days).
- Remove the embryos with forceps and place in germination media (if for immediate culture) or transportation tubes.

Resterilisation after transport is carried out as follows: the entire contents of the transportation tubes should be tipped into a 20ml syringe and the water allowed to drain through the outlet. The sterilisation steps above should be repeated and the embryos then inoculated into germination media.

In field experiments throughout the South Pacific, an average 86.1% of embryos were free from contamination and 84.9% germinated. Contamination rates varied from 47.5% with inexperienced operators in improvised laboratories to 9.8% for experienced operators in both a fully equipped and improvised laboratory (Ashburner et al. 1993b). Germination success reaches about 90% of the uncontaminated embryos.

In Vitro Growth

This area requires the use of specialised equipment (Table 2) and specialist knowledge. Therefore it is expensive to set up and is time-consuming to become experienced in using the system. However, once established, good economies of scale occur if many embryos are being cultured. Although the following protocol has been simplified, it is relatively laborious and results come slowly. Some trade-offs have been made between simplicity and percentage outcome.

The germination medium consists of MS macro and micro elements (Murashige and Skoog 1962; Morel and Wetmore 1951) vitamins, 6% sucrose and 0.2% activated charcoal (Assy Bah 1986). A commercially available mixture of plant salts is used with the addition of a vitamin stock solution. If a four-place balance is not available, pre-weighed chemicals prepared at another location could be transferred. Although BMY3 medium is optimal for coconut embryo growth (Maheswaran and Thompson 1988), germination medium can be used for simplicity. It should be gelled with 0.8% agar but with only 4% sucrose as opposed to 6%.

It is possible to manipulate the in vitro plant form by changing sucrose and growth regulator levels. Increasing the sucrose level increases root length but this occurs to the detriment of shoot length (Ashburner et al. 1993a). Assy Bah et al. (1989) report that this improves acclimatisation, whereas we have found that it is severely detrimental. Similarly, continual NAA can be used to stimulate additional root production but this depresses shoot growth (Ashburner et al. 1993a). The value of these manipulations is minimal since Assy Bah et al. (1989) believe that NAA retards growth after acclimatisation, and there is little evidence that an increased root system aids in acclimatisation. Therefore, although manipulation of plant form is possible, it is probably preferable to use the standard hormone-free medium.

Plants should be germinated in 10ml of medium and then transferred to 20ml of growth medium immediately after germination (Ashburner et al. 1991). After six weeks, they should be placed on 40ml of growth medium. After a further six weeks they should be either acclimatised or placed on another 40ml of media for another six weeks. If the plants are still small they should be discarded. Any cultures that are infected, vitrified or dead should also be discarded. Plants that appear white should be placed onto fresh media and should then recover.

Table 2. Requirements for in vitro growth.

refrigerator/freezer	media
balance	media
pH paper/pH Meter and solutions*	media
pressure cooker/autoclave*	media
culture flasks	growth
media dispenser*	media
laminar flow cabinet	transfer
forceps	transfer
scalpels and blades	transfer
spirit lamp/gas burner*/electric steriliser*	transfer
shelves with fluorescent lights	growth
thermostatically controlled air-conditioner	growth
negative-ion generator/filter	growth
glass/plastic ware	media
pipettes	media
magnetic stirrer/hotplate and stirrer bars	media
syringes	transfer
microwave oven*	media
chemicals	media
MS plant mixture	media
activated charcoal	media
sugar	media
agar	media
bleach/sodium hypochlorite*	transfer
still/deioniser*/distilled water	media/ transfer
ethanol/methylated spirits	transfer

*Optional items.

After a transportation stage, losses due to abnormal or retarded growth are approximately 40% and 10% due to contamination. Contamination can destroy most cultures if strict hygiene is not maintained. Therefore, culture rooms and laboratories should be repeatedly cleaned with disinfectant and access should be limited. They should be well isolated and be equipped with a negative ion generator to adsorb dust and spores. Sterile techniques should be rigorously maintained.

Table 3: Requirements for acclimatisation.

clear plastic bags
potting media (sterile sand, sand and organic matter)
pots/polybags
rubber bands
benzimidazol (Benlate) fungicide
shade cloth
soil steriliser
spray gun

Acclimatisation

Plants derived from embryo culture have poor water relations due to growing at 100% relative humidity. They are not photosynthetically active (Malijan and del Rosario 1986) because all their energy needs were met by the basal medium. Furthermore, the plants are extremely sensitive to high light intensity and microorganisms. Therefore, there are problems with desiccation of the plant, slow growth and contamination and a process of acclimatisation is required. This stage may be carried out in locations remote from the location where the embryos were grown, using the equipment outlined in Table 3.

Plants should be acclimatised when they have at least one photosynthetic leaf, a root system and a shoot length of at least 160 mm. Loosen the lids on the embryo cultures three days before acclimatisation. Then carefully remove the plants from containers, wash the media from the roots and trim back any dead leaves and the remnant haustorium. Drench them with 0.025% benzimidazole (Benlate, Du Pont, Wilmington), and put in pots or polybags with damp, sterile sand and firm down. Enclose in a clear plastic bag, with supports to prevent collapse, and seal around the base with a rubber band. Water three times a week or as required to maintain moist conditions. After two weeks, cut a small hole (2 cm diameter) in the top of the bag. After another week, cut a similar hole. One week later, cut the entire top off the bag. The bag should be completely removed after another week. Fungus growth should be treated with more Benlate. Remove any dead or dying plants. After two more months, plants should be put into larger polybags, filled with sterilised sand and organic matter without disturbing the roots. Fertilising with a complete liquid fertiliser should be carried out weekly from the time of removal of the plastic bag. The complete process of acclimatisation should be carried out beneath shade cloth.

During acclimatisation there is the potential for serious loss due to the action of normally harmless microorganisms, therefore strict hygiene must be maintained. Although approximately 80% survival can be achieved, more realistic field conditions give a survival rate of approximately 50%. Complete loss of material due to infection has also occurred. The most important characteristic of plants to be acclimatised is their 'intrinsic' quality (Debergh 1991) and coconut embryos also need to be vigorous in vitro to acclimatise successfully. Unfortunately, this characteristic varies both between as well as within populations and seems to be of genetic origin.

Conclusions

The embryo culture technique can be used in remote locations, but considerable costs in equipment, training and time are incurred. Collection of embryos and acclimatisation are parts of the technique that can be carried out in remote locations, therefore these places can participate in germplasm exchange programs provided that there is cooperation with a fully-equipped laboratory where embryos would be grown. It is estimated that between 400 and 500 embryos need to be collected to generate 100 plants in the field after the embryo culture process. Experience needs to be gained before this process is used because of the potentially devastating losses that may occur at each stage.

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The Use of Zygotic Embryo Culture to Solve the Problem of Low Germination Associated with Malayan Red Dwarf Coconut in Western Samoa

M.B. Taylor, G.V.H. Jackson, C. Smith and S. Efu*

In 1977, a coconut hybrid seed garden was established at Olamanu, Upolu, Western Samoa. The low germination rate of the Malayan Red Dwarf (MRD) seednuts caused concern because hybrid seed production would be affected. Examination of the seednuts in the late 1970s found an abundance of *Marasmiellus inoderma*. Germination rates ranged from 55 to 77% between trials, though generally averaged 60%, whereas the germination rate for the Samoan Local Talls (SLT) seednuts was in the order of 76%, (E.H.C. McKenzie, pers. comm.). At the present time germination rates are still in this order, (Efu, pers. comm.).

M. inoderma causes death of the sprouting seedling, often before the sprout emerges from the husk. A study on the same fungus in Solomon Islands found that infection occurs through the calyx end; the fungus then colonises the fibrous husk tissues and grows beneath the operculum as it is raised by the emerging shoot (Jackson and Firman 1982).

A report in 1986, (McKenzie 1986), stated a number of findings associated with *M. inoderma* in the seed garden. These were namely: the absence of *Marasmiellus* in the husk of MRD x Rennell Tall (RLT) hybrid when first harvested from the mother palms; the presence of *Marasmiellus* in 40% of the seednuts after three months in the nursery; a large variation in seedling loss between cultivars and hybrids, with Malayan Yellow Dwarf (MYD), and RLT hybrids being more susceptible than MRD x RLT, and SLT least susceptible; and seednuts set vertically in the nursery less affected by the fungus, possibly due to a freer penetration of the water to the sprout before it emerges from the husk.

In Malaysia *Marasmiellus* damage was significantly reduced with the use of fungicides, and similarly, in Solomon Islands, a problem with *M. cocophilus* was removed with increased general care and cleanliness in nursery management (Foale 1987). In the mid-1980s fungicidal dipping treatments were carried out in the Olamanu seed garden in Western Samoa, but this appeared to have little effect on the germination rates of the hybrid seednuts, (G.V.H. Jackson, pers. comm.).

The objective of this work was to compare the germination rates of MRD and SLT seednuts both in vivo and in vitro. This would ascertain to some extent the effect that the fungus *M. inoderma* was asserting on the germination rate of the MRD seednuts.

Materials and Methods

Mature seednuts were collected from MRD and SLT trees when at least one seed nut in the bunch had turned from the fresh to the dry colour. Half of the bunch was used for embryo culture, and the remainder was placed in a nursery to monitor natural germination.

Seednuts were dehusked, split in two, and a cylinder of endosperm containing the embryo obtained by using a 20 mm corkborer. Embryo cylinders were sterilised in a filtered calcium hypochlorite solution, (45 g/l), for 20 minutes. Individual cylinders were transferred to sterile sites and the embryo extracted. Each embryo was rinsed once for 15 minutes in sterile distilled water and then inoculated into culture on semi-solid medium (Assy Bah 1986). The culture medium was composed of Murashige and Skoog mineral elements, (1962), Morel and Wetmore's vitamins, (1951), iron EDTA, (41 mg/1), sodium ascorbate, (100 mg/1), activated charcoal, (2 g/1), sucrose, (60 g/1), and agar, (8 g/1). The pH was adjusted to 5.5 and the medium was dispensed into polycarbonate tubes of 80 mm height and 28 mm diameter before sterilisation at 120°C and 1.06 bar for 15 minutes. For both varieties 100 replicates were set up and cultured at 26° C ± 1°C in the dark until the embryos had sprouted; at that point cultures were subjected to a 12 hour photoperiod under 3000 lux light intensity.

* IRETA Tissue Culture Unit, University of the South Pacific, Alafua Campus, Apia, Western Samoa.

At the same time, nursery plots of MRD and SLT seednuts were established in the seed garden. This material was collected at the same time and under the same conditions as those used in tissue culture. For each variety there were 100 replicates. These were set up in 20 plots of 10 seednuts per sub-plot. MRD and SLT were placed randomly adjacent to each other. Within each sub-plot seednuts were placed in a 2 x 5 spacing, with 0.5 m between the replicated sub-plots.

Tissue culture results were assessed after 1, 3, 6 and 12 weeks. The germination of the seednuts in the nursery plots was determined after 16 weeks.

Results and Discussion

Fungal contamination rates in vitro were relatively high; the incidence of Cyclone Val rendered the tissue culture unit with no power for one week, and then limited power—four hours per day—for a further three weeks. High temperature and minimal subculturing led to increased contamination. The seednuts were planted in the nursery bed after the cyclone, and therefore natural germination rates should not have been affected.

Table 1 shows the percentage of uncontaminated embryos. With many of these cultures contamination was present, but as there were indications of germination their culture continued. However, it is possible that the level of contamination in these cultures was eventually inhibitory to complete germination. Embryos were discarded if contamination was considered excessive. The percentages given in Table 1 reflect these discarded cultures.

After one week in culture many of the embryos had expanded in size. Over a period of six weeks in culture embryos continued to increase in mass with eventual emergence of the shoot. This was followed by the development of the haustorium.

In many of the cultures the haustorium surface became brown but this did not appear to affect any further development of the embryo. Root production occurred on the same medium, at approximately the same time as leaf development.

It has been reported that development of the haustorium requires activated charcoal and a high concentration of sucrose, (6%), (Karunaratne et al. 1985). The culture medium used in this experiment contained both requirements; however, complete development of the haustorium occurred when the sucrose concentration in the medium was 2%, (Assy Bah et al. 1989). It is possible that haustorium growth is also influenced by variety and the stage of development of the embryo at time of culture. Varietal differences have been suggested as likely reasons for the varying response to the presence of coconut milk (water) in the culture medium (Fisher and Tsai 1978).

The total percentage of MRD seednuts germinated after 12 weeks in culture was 48%; for SLT seednuts

Table 1. Germination rate and contamination levels of MRD and SLT embryos after 12 weeks in culture.

Replicate number	SLT seednuts			MRD seednuts		
	Germinated nuts (%)	Embryos with developed gemmules (%)	Uncontaminated embryos (%)	Germinated nuts (%)	Embryos with developed gemmules (%)	Uncontaminated embryos (%)
1	25	0	80	25	25	80
2	20	40	100	40	40	100
3	44	11	90	50	0	100
4	33	22	90	37	12	80
5	71	0	70	50	20	100
6	50	12	20	67	22	90
7	67	0	90	44	0	90
8	56	22	40	50	20	100
9	100	0	40	60	20	100
10	43	14	70	56	33	90

Replicate number varied due to contamination levels.
Total representative number for MRD = 93; Total representative number for RLT = 80.

the percentage was 47%. The percentage of MRD seednuts with developed germules was 22% and for SLT seednuts, 18%. Statistical analysis shows that there was no significant difference between the germination rates of the two varieties in vitro. The germination rates achieved in vitro were low but were similar to the rates reported by Assy Bah et al. (1989) using the hybrid of Malayan Yellow Dwarf crossed with West African Tall. In this experiment 49% of embryos were recorded with developed gemmules after six months in culture. As there was no significant difference between the germination rates of the two varieties in vitro, the results infer that germination is not influenced by variety.

Total germination for SLT in the nursery seedbed was 76%. For MRD total germination was 64%. As Table 2 shows some of the seednuts died after germination. With MRD seednuts 70% of the replicated sub-plots were affected compared to 40% with SLT seednuts. The eventual mortality rates of the germinated seednuts were 15% with MRD and 7% with SLT. Death appeared to be the result of a fungal attack. Although there was no identification of the fungus, it is interesting to note MRD appeared to be more susceptible than SLT. None of the non-germinated seednuts was examined to determine whether germination had occurred, followed by death of the sprout before emergence from the husk. These germination rates are in agreement with those of previous trials (E.H.C. McKenzie, pers. comm.).

Statistical analysis indicated that any difference between varieties, both in in vivo and in vitro culture was not significant.

Conclusion

From this experiment it would appear that differences in germination rates are due to some factor(s) other than a varietal influence. The germination rates of both MRD seednuts and SLT seednuts in vitro differed by 1%; in vivo, the difference in germination rates between the two varieties was increased to 12%. Although statistical analysis indicated this, the differences were not significant and in practice would not affect production potential.

The germination rates of both varieties were reduced to a similar degree when the embryos were cultured in vitro. Work with seednuts of Malayan Yellow Dwarf crossed with West African Tall gave a germination level of 91.7% in the field and 49% (embryos with developed germules) in the nursery bed after six months and in culture respectively. It has been observed that embryo development in vitro is slower than that of complete seednuts in the nursery bed (Assy Bah et al. 1989). It is possible, therefore, that extended culture in vitro would have increased germination rate, however, this was not possible because of the problem with contamination.

It is intended that this work will be repeated at a time which does not coincide with the cyclone period. Attempts will also be made to improve the in vitro techniques thus optimising the germination rate, and to examine and identify any fungal infection of the non-germinated nuts in the nursery seedbed.

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Table 2. Germination rate of MRD and SLT seednuts in nursery bed after 16 weeks.

Replicate No.	SLT seednuts		MRD seednuts	
	Germinated nuts (%)	Mortality rate of germinated nuts (%)	Germinated nuts (%)	Mortality rate of germinated nuts (%)
1	40	20	50	20
2		30	30	20
3	50	0	10	20
4	70	0	40	10
5	60	0	30	20
6	70	0	30	40
7	20	10	60	0
8	80	0	50	20
9	50	10	30	0
10	60	0	70	0

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Preliminary Analysis of Coconut (*Cocos nucifera* L.) Germplasm in Papua New Guinea

Tore Ovasuru*

THE coconut palm (*Cocos nucifera* L.) is cultivated extensively throughout coastal, mainland areas and on many islands in Papua New Guinea (PNG) and is a major source of food, and copra, derived from the dried endosperm of the mature fruit, has been a vital source of export earnings for 100 years. For a considerable proportion of the littoral population, copra production provides the only source of cash income. Copra value lies in its coconut oil content as well as in the coconut meal, a by-product of oil extraction.

The total area planted to coconuts in PNG has been estimated at 250 000 hectares of which an insignificant proportion derives from selected planting material (Douglas 1965). Some 50% of commercial plantings were considered to be approaching senility by Sackett and Williamson (1973) and yields have unquestionably declined since that time. There is an urgent need to replace these aging plantations and this should be done with the best varieties available. Also, reports on global warming and the possible rise in sea level have serious implications for the low lying coral atolls, and may lead to their inundation. Furthermore, there is an erosion of the coastline along the Gulf of Papua and other parts of Papua New Guinea which support a lot of old stands of coconut. Figure 1 shows erosion of the coastlines occurring along the Papuan coast. If the coconut genetic materials are not identified and preserved now, there is a danger that they may be lost.

There are two coconut varieties recognised around

Figure 1. Kiwai local tall washed out by the sea tides along the Gulf of Papua.

* Head of Coconut Research, Papua New Guinea Cocoa and Coconut Research Institute, P.O. Box 1846, Rabaul East, New Britain Province, Papua New Guinea.

the world. These are var. *typica* (tall variety), and var. *nana* (dwarf variety). Jay et al. (1989) reported that about 95% of the palm groves around the world are talls The dwarf type, which is normally associated with human settlement and activities, is found throughout the intra-tropical zone. It is known to be highly self-fertile, has slow vertical growth, tightly packed leaf scars and short leaves. The dwarf is precocious; i.e. early flowering and fruiting and produces bunches more rapidly than the tall.

There are two levels of diversity recognisable: diversity found within the allogamous population; and diversity between populations due to selection by both humans and by nature. All the tall varieties are allogamous, although there is some degree of autogamy found among some populations (Bourdeix 1988, Bourdeix et al. 1990; Ashburner, these proceedings). Many populations have increased in genetic diversity because of the introduction by humans of contrasting genetic materials that has resulted in introgression of different types. The dwarf, on the other hand, shows a low level of within-population variability because it is highly self pollinated (autogamous).

Studies on the variability of coconut have been based on morphological characters and this allowed only partial evaluation of the genetic composition of the varieties. In the past, characterisation was based upon characters such as frond production, trunk diameter, frond length, number of pinnae per frond, length of petiole and petiole cross section (width x thickness), crown shape, floral biology, nut production, fruit characters and colour forms. Fruit characters have been the major morphological characters used in identifying different ecotypes. Harries (1982), while using fruit components, classified different ecotypes occurring in India and Ivory Coast into two contrasting tall types. These are the Niu vai type, and Niu kafa type; a name adopted from commonly grown, distinct, tall types occurring in the Pacific. A Niu kafa type has been identified by Harries (1978) as being the primitive type possessing a high proportion of husk, thick shell and low water content which has evolved through natural selection. It also takes slightly longer to germinate. The Niu vai type is characterised by a low proportion of husk and shell and high water content. Harries (1981a) advocates that the Niu vai type is a product of continuous selection by humans for its desirable traits such as high water content, which was used by the Polynesians during long voyages. The intermediate types are considered to be the result of natural hybridisation of the two distinct types (Niu kafa and Niu vai), and are known as the introgressed types.

A wide range of local tall ecotypes is planted through the coastal areas and islands of Papua New Guinea. The present study intends to group the population of coconut palms in Papua New Guinea by cluster analysis in order to understand their phenotypic relationship. This study uses fruit, nut and vegetative characters from a wide range of tall populations surveyed in different regions of Papua New Guinea.

Materials and Methods

Choice of populations. Coconut germ investigations began with the identification of areas to be sampled. Different coconut-cultivating areas were visited non-selectively with the aid of the local inhabitants. Apart from biased samples from sites having palms with superior endosperm weights (otherwise, greater than 500 g were selected), all sites were randomly sampled. Within each population, palms were randomly selected and numbered with paint from 1 to 30. Measurements began immediately after the numbering was completed. Figure 2 represents the sites of the different populations that were measured.

Fruit component analysis. The principal characters recorded were the fruit and nut characters. Fruits were chosen from selected trees, in which most of the fruit colour changes from green (fresh) to brown (dry) with the calyx still remaining green, and the water inside the nut splashing when shaken. Nuts were rejected if they were very heavy and did not splash, or if they were completely dry. Nuts that had germinated, were deformed, or had been damaged by insects or other causes were also rejected. Freshly harvested fruits were selected and labelled with the palm number.

The following procedure was carried out on each tree:

- weigh the whole fruit;
- dehusk;
- weigh the nut (dehusked fruit);
- crack nut, drain water and reweigh;
- measure thickness of endosperm (to nearest mm) in three positions and enter the mean;
- separate endosperm from shell; and
- weigh endosperm.

The following characters were obtained:

- husk weight;
- shell weight;
- water weight;
- % husk to fruit;
- % shell to nut;
- % water to nut; and
- % endosperm to nut.

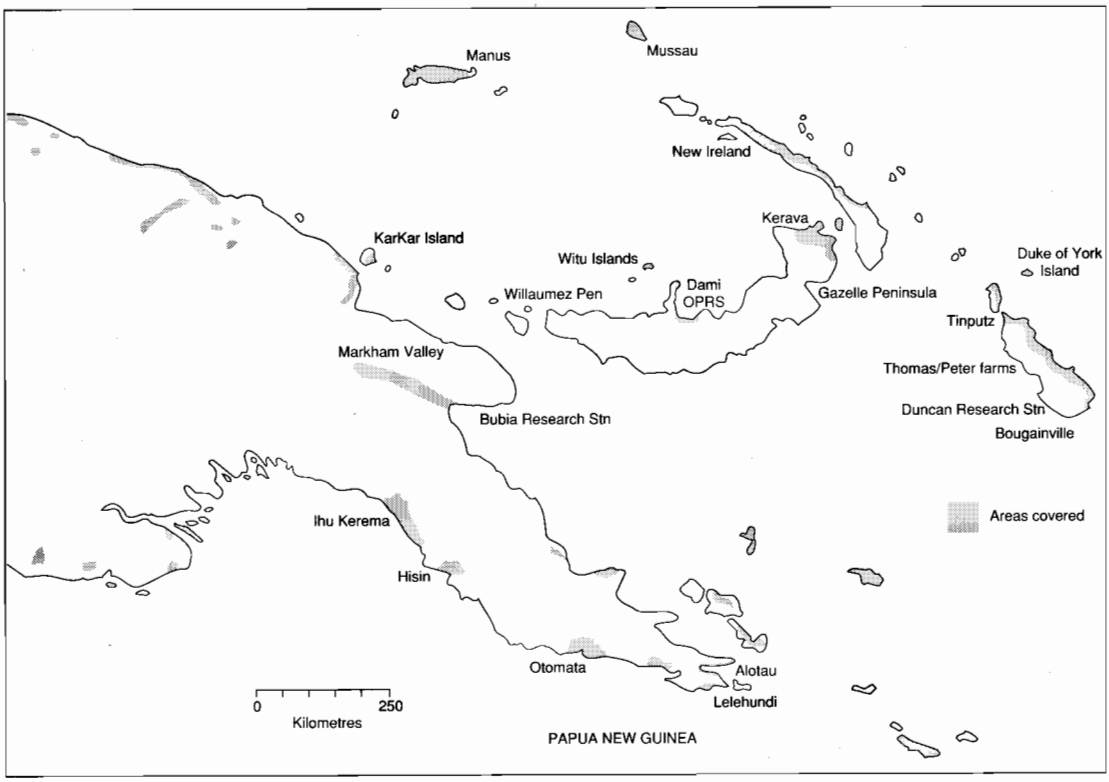

Figure 2. Regions of Papua New Guinea sampled for coconut germplasm analysis.

Vegetative characters. Vegetative measurements were also made at several sites. These include:

- bole or girth diameter;
- trunk diameter (50 cm above the bole);
- trunk internode length (average of 10 internodes);
- trunk height (ground level to base of frond 14); and
- frond
  - — number per palm (A);
  - — petiole length;
  - — petiole cross section (width x thickness);
  - — rachis length;
  - — number of leaflets/frond;
  - — leaflet length (average of six middle portion of leaflets);
  - — leaflet width (average of six middle portion of leaflets);
  - — leaf area/frond (B);
  - — leaf area/palm (A x B);
  - — petiole colour (ranges from 1–5);
  - — colour of frond (ranges from 1–5).

Inflorescence characters. With the vegetative measurements, studies were also made on the inflorescence characters such as:

- Inflorescences
  - — length;
  - — stalk length;
  - — rachis length;
  - — number of female flowers/rachis;
  - — number of female flowers/spade.

Measurements were done once on each palm with each sample represented by 30 palms per population.

Analysis

The unweighted paired-group, based on the arithmetic mean (UPGMA), method of analysis is used. As a preliminary analysis, the aim of this study is to assess patterns of variability in the coconut populations; therefore, there is no need at this stage to test the strength of this classification nor ordination methods to overcome obscuring of individuals which may, in fact, be intermediate between groups.

With the later studies other tests including ordination methods will be employed.

Results and Discussion

The results in Tables 1 and 2 are populations and sub-populations of coconut grouped into different clusters by UPGMA methods. The summary of the cluster analysis is shown in Tables 3 and 4 which are arranged in the order of their fruit weight and the vegetative vigour from the highest to the lowest.

This preliminary cluster analysis indicates that the coconut populations in Papua New Guinea can be classified into five different clusters using fruit characters. The analysis showed that about 72% of the populations measured fall into one cluster and this suggests their relatedness. Within the Gazelle Tall population, about 98% of all sub-populations analysed so far fall into cluster one, except for Rabaul Red Dwarf. This means that their fruit characters are all alike and can be regarded as one tall ecotype. All the Nuguria, Milne Bay, Central, Oro, New Ireland, Manus, Sandaun and Western tall populations also fall into cluster one (Table 1). Harries classified this particular type as the wild type or the original ancestral *Cocos nucifera*, which he later called Niu kafa type palms because of its high husk content, low percentage water and very high proportion of endosperm to nut (Harries 1981b). The Niu kafa type palms were probably not domesticated. However, evidence from the Gulf province tends to indicate that Niu kafa type palms were later domesticated because their shell characters make them ideal for construction of utensils for household uses.

Table 1. Distribution of 78 PNG coconut populations into different clusters using 16 fruit and nut characters.

	Papua New Guinea Tall Coconut Population				
Clusters:	1	2	3	4	5
Province					
East New Britain	Naparpar				Rabaul Red Dwarf
	Tavilo				
	Natava				
	Raulawat				
	New Massava				
	Pellevarua				
	Tobera 1				
	Tavilo (VFT)				
	Tobera 2				
	Bitapaka				
	Baliora				
	Raulawat				
West New Britain		Gaungo			
		Naviro			
East Sepik	Morok	Suanam	Kwakwie		
	Majar	Rabarabo			
	Boikin	Lawain	Yangoru		
	Falala	Mandi	Gwelikum		
	Moem	Moi	Wamain		
	Urip (YFT)				
	Balik				
	Forok				
	Urip (MFT)				
	Meivi				
	Yangoru				
	Krinkrin				
	Urip				
	Krinkrin (YFT)				
New Ireland	Kenapit				
	Karu				

continued on next page

Table 1 continued

Province	Papua New Guinea Tall Coconut Population Clusters: 1	2	3	4	5
Manus	Lawes				
North Solomon	Tikani 2 Peiti Panao Sauma				
Sandaun	Paup Aitape Sowain				
Madang		Ulatava Tovuru Katom Kinim	Guananga Mangar		
Marobe			Riara		Markham Farm
Milne Bay	Tukava Kitava Guleva Lousia Siagara Kunapapau Central	Baibara			
Oro	Ajoa Saiho Kikibator 1				
Marako'of	Kikibator 2 Aratatab				
Gulf	Keakea	Miha Kav			
Western	Severimabu Dirimu Pongarigi Mata Mibu				

When the planters arrived in Papua New Guinea they indiscriminately collected the seed nuts from these ecotypes, and cultivated them for commercial exploitation, since there were large stands of them in existence. This confirms the report by Douglas (1965) who concluded in his study that nearly all the plantations in Papua New Guinea were established with unselected planting materials from the existing local stands.

Two sub-populations in west New Britain, five in east Sepik, four from KarKar and one from Gulf province are classified also as one population (cluster two). This group may represent the introgressed types as a result of natural crossing between the domesticated types such as Markham tall (cluster five) and cluster three populations and the Niu Kafa types such as cluster 1. Four populations from east Sepik, two from KarKar, and one from Markham sub-population are classified into cluster three, a grouping which results from domestication and selection for the desired morphological traits such as high water content and large shell (for use as water containers). The cluster five and cluster three populations were also a result of human selection for large fruit size which indicated high water content; which was convenient for use on long voyages. This particular type is referred to as the Niu vai type palms which are quite common in Polynesia. The archaeological evidence

Table 2. Distribution of 47 PNG coconut populations into different clusters using 17 vegetative and 5 inflorescence characters.

	Papua New Guinea Tall Coconut Population						
Clusters:	1	2	3	4	5	6	7
Province							
East New Britain	1 Tob 2	NMA NYFT Rau Tav	NMA Pel Bal Nap RYFT	RRD	Bit		
West New Britain			Gau	Nav			
New Ireland		Ken	Kau				
Manus			Law				
North Solomon						Tik	
East Sepik	Mor	UYFT UMFT Mal	Yan	Uri Moi			
Madang	Gua Man Kin		Kat	Tov	Ula		
Morobe					Ria		MAF
Milne Bay	Tuk	Gul Sia	Lou	Kun		Kil	
Oro	Sai	Kik					
Gulf	Mia		Kea				
Western		Dir Sev Mat					

indicates that Polynesians were long distance sea travellers (Kirk 1987).

This study agrees with Jay et al. (1989) that genetic divergence does exist between local populations around the world, and Papua New Guinea is no exception. The study shows that, although the majority of the populations fall into the Niu kafa type, there is a geographical difference within this particular ecotype, as indicated by the variability in vegetative and inflorescence traits (Table 2). For instance, among Gazelle Tall sub-populations, which are all Niu kafa type palms, four different clusters were produced using the vegetative and inflorescence traits. All the other Niu kafa type populations also displayed the morphological variability, except the Kiwai Tall ecotype (Western Province).

The results also confirm that the dwarf types are distinct as demonstrated by Rabaul Red Dwarf (cluster 4). The dwarf palms were domesticated since they could not survive without human assistance.

The results indicate that cluster 5 which is the Markham tall sub-population at Markham farm, stands out quite clearly. Its mean fruit weight (3694 g) is much higher than for any other population. In coconuts of the cluster 5 group, about 50% of the nut weight consists of water, but they have the same husk content of 34% as the population in cluster 3 (Figure 3). Nuts of cluster 5 also have a very thin shells (18%). Phenotypically the population in cluster 3 looks very similar in the husk content, but different in other fruit traits.

Further studies will be done on more populations and sub-populations using other methods of analysis including RFLP (molecular) methods to confirm the diversity and also determine the genetic distances between various Papua New Guinea coconut populations.

Table 3. Mean of different clusters from 78 PNG coconut populations based on fruit characters.

	Clusters				
Variables	5	3	2	1	4
Fruit diameter (polar) (cm^3)	29.88	25.85	24.07	22.78	24.27
Fruit diameter (equatorial) (cm^3)	24.42	22.11	19.74	17.15	11.29
Fruit circumference (polar) (cm^3)	83.02	72.87	67.40	65.51	57.15
Fruit circumference (equatorial) (cm^3)	75.52	68.60	62.63	50.48	36.20
Fruit weight (g)	3694.17	2537.40	1981.51	1485.79	875.17
Nut Weight (g)	2444.70	1694.96	1234.71	871.95	422.03
Nut without water (g)	1221.50	1005.67	788.44	601.25	330.33
Endosperm weight (g)	790.80	640.96	501.52	383.02	188.97
Endosperm thickness (mm)	12.35	12.26	11.91	12.02	9.85
Husk Weight (g)	1250.00	842.44	746.80	613.80	453.13
Water Weight (g)	1222.67	689.29	446.27	270.69	91.70
Shell Weight (g)	430.70	364.71	286.92	218.28	141.37
Husk (%)	33.67	32.49	36.94	40.75	51.20
Water (%)	49.94	39.89	35.12	29.77	21.41
Endosperm (%)	32.39	38.28	39.70	44.78	33.72
Shell (%)	17.68	21.84	25.18	25.45	44.87

Table 4. Mean of different clusters from 78 PNG coconut populations based on 17 vegetative and 5 inflorescence characters.

	Clusters						
Variables	7	5	1	3	2	4	6
Girth Diameter (cm^3)	243.47	154.59	153.44	147.95	146.28	138.99	131.76
Trunk Diameter (cm^3)	224.67	137.50	136.26	129.34	130.10	120.15	113.45
Internode Length (cm)	87.80	113.69	108.06	103.13	97.91	89.23	87.80
Trunk Height (cm)	1375.17	1376.71	1445.99	1478.42	1364.35	1240.83	1267.53
Frond (No.)	38.30	33.11	31.52	31.01	30.81	30.56	31.25
Frond length (cm)	584.10	575.92	586.75	550.71	523.36	509.16	457.20
Petiole Length (cm)	141.07	136.98	141.93	130.64	125.68	124.15	114.61
PCS (cm^2)	32.68	26.84	26.93	24.79	22.18	21.70	16.95
Rachis length (cm)	443.03	438.94	444.82	420.42	397.47	385.01	345.27
Leaflet No.	234.70	242.91	232.02	229.28	223.59	217.17	205.51
Leaflet length (cm)	157.20	141.90	142.90	137.10	131.80	128.00	120.20
Leaflet width (cm)	4.83	4.63	4.44	4.35	4.26	4.10	3.81
Leaf area/frond (m^2)	9.27	8.27	7.65	7.09	6.53	5.90	4.91
Leaf area/palm (m^2)	355.04	273.82	241.13	219.86	201.19	180.30	153.44
Petiole colour	5.63	5.12	3.01	3.64	3.83	3.51	3.07
Leaf colour	5.95	5.97	5.65	5.64	5.53	5.55	5.50
Frond colour	4.20	4.53	4.69	4.48	3.91	4.35	4.61
Spadix length (cm)	143.87	134.78	140.85	132.80	123.20	120.89	109.86
Stalk length (cm)	49.70	59.20	50.39	52.79	52.36	49.29	49.67
No. of rachis	46.70	40.23	39.12	37.30	37.54	37.84	30.20
Female flowers/rachis	0.57	0.74	0.72	0.81	0.71	0.77	0.36
Female flowers/palm	26.63	27.59	27.97	29.12	27.70	26.86	10.50

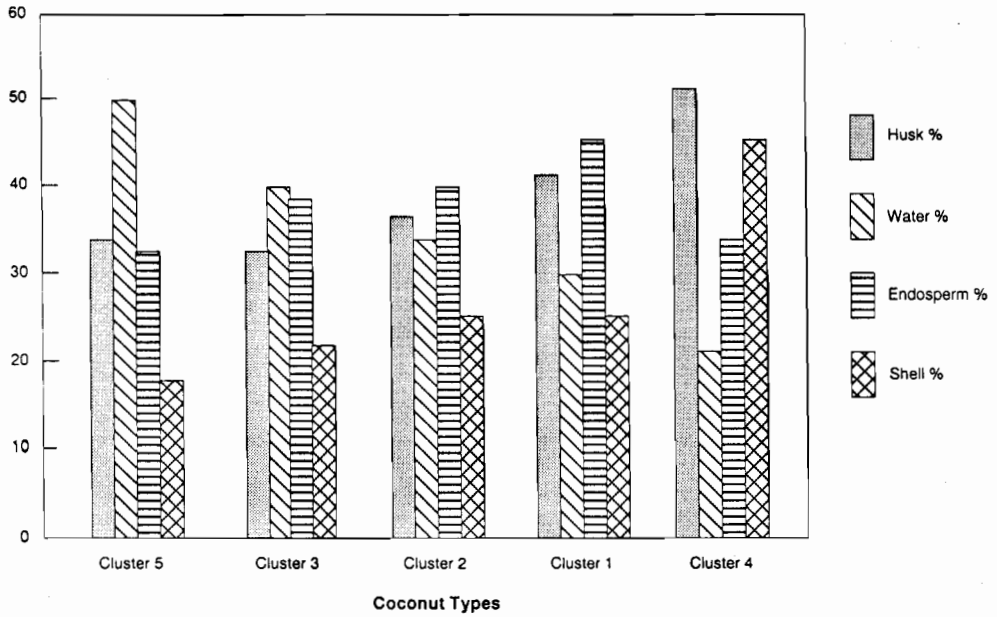

Figure 3. Fruit characters of Papua New Guinea coconut types.

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Methods for Coconut Germplasm Prospection

G.R. Ashburner*, M.G. Faure† and M.A. Foale§

BOTH short and long-term coconut improvement depends upon introducing useful germplasm into breeding programs. Prospecting for potentially useful germplasm is the first stage of this process. Prospection usually involves characterising coconut populations during a single visit to a variety of areas, either locally or internationally. The introduced germplasm should be evaluated further both in trials for combining ability and provenance trials.

Surveys that encompass all common coconut descriptors (IBPGR 1992) are not practical since time is normally limited at each site. Therefore, the characters that can be measured should be prioritised according to their power of discernment. Prospection activities are often coupled with initial germplasm collection activities. The nature of the coconut palm leads to both remote and widely scattered prospection sites, so the protocols and equipment must make allowances for varied environments, facilities and transit times. This paper provides a guide to prospection protocols based upon extensive experience throughout the South Pacific region.

Preparation

Once a survey area has been chosen, the relevant agricultural authorities should be contacted at both the head of department and field level. The assistance of local authorities is imperative for efficient prospection. Initial communication should occur well in advance of the proposed survey so that the necessary permits may be obtained. Quarantine approval must be sought before the event, if samples are being collected.

* Department of Agriculture, Institute for Horticultural Development, Private Bag 15, South Eastern Mail Centre, Victoria 3176, Australia.

† Cocoa and Coconut Research Institute, PO Box 1846, Rabaul, Papua New Guinea.

§ CSIRO, Division of Tropical Crops and Pastures, 306 Carmody Road, St Lucia, Queensland 4067, Australia.

Equipment

Equipment to be used should be commonly available. Specialised equipment should be simple, compact and reliable. Various items of equipment have been evaluated in our prospecting activities and a list of useful equipment is given in Table 1.

Table 1. Prospection equipment and their uses.

Equipment	Use
Bush knife	cutting fruit, fronds, inflorescences, making a dehusking stake
Copra knife	removing endosperm
Tape measure	measuring length, circum ference etc.
Camera	photographing fruit, palms
Steel hook	removal of fruit when no climber is available
Colour charts	recording fruit, flower and petiole colours
Knapsack	carrying equipment
Scales	measuring weight

Measurements

Some coconut germplasm descriptors (IBPGR 1992) are suitable for use in prospection work, but are better suited to provenance trials using common environments and age structure. A full analysis on the utility of these descriptors is being undertaken (T. Ovasuru, pers. comm.). The following measurements, in descending order of importance, may be taken: 1. fruit characteristics; 2. trunk characteristics; 3. leaf characteristics; 4. flower characteristics.

Location, soil type, anecdotal evidence, prevailing biotic and abiotic stresses and crop associations should be recorded at each prospection site. These data may explain deviations from trends and may be useful for identifying adaptive genes from novel environments.

Many of the descriptors are highly influenced by environmental and age effects. They are characters with a low heritability. Fruit number, leaf characters and flower characters fall into this category (Coomans 1975). The effects of both short and long term environmental fluctuations must be minimised by measuring highly stable characters.

Fruit component analysis (FCA) has been widely used in characterising germplasm during prospection (Whitehead 1966; Harries 1978; Foale 1987; Ovasuru et al. 1993). The effects of environmental fluctuations are minimised by the use of ratios instead of absolute values; therefore size should not influence the partitioning in the fruit. Fruit characters are a major component of yield and of great interest to coconut breeders. FCA should be carried out in the following manner: weigh a mature coconut whose epidermis is becoming brown; split it in half from stem to tip with a carpellary ridge pointing upwards, and weigh again; photograph the half fruit; dehusk and weigh again; then remove the endosperm and weigh. The weight of the components can be calculated by subtraction. This method uses one fruit for both FCA and photography. Photographs are useful for comparing shape and may be used in shape analysis techniques (Bookstein 1978).

Further vegetative measurements can be taken if time permits. These values may be used to calculate dry weight partitioning of the palm (D. Friend, pers. comm.) which will become increasingly important in coconut breeding once the physiology of yield is better understood and idiotypes are developed.

The following characters can easily be measured using the equipment outlined in Table 1: trunk girth at 20 cm and 1.5 m; distance between 10 leaf scars taken at a height of 1.5 m from ground; number of green leaves; colour of petiole; petiole length thickness and width; rachis length; number of leaflets; leaflet length and width; leaflet colour; leaf spiral direction; stalk colour; branch colour; female flower colour; male flower colour; length of central axis; length of stalk; stalk girth; number of spikelets with and without female flowers; length of longest branch; length of spikelet; number of female flowers; female flower distribution per rachilla; diameter of female flower; fruit colour; fruit shape; fruit polar section shape; fruit polar and equatorial length; fruit polar and equatorial circumference; nut shape; nut polar length and circumference; nut equatorial length and circumference; endosperm thickness; and shell thickness.

With the advent of molecular methods, accessions can now be characterised in a laboratory after the site has been visited. These characters have little or no environmental influence. Samples may be collected for both isozyme and DNA analysis. Pollen is the optimal tissue for isozyme analysis (G.F. Moran, pers. comm.), whereas leaves are collected for DNA studies (Ashburner and Rohde, these proceedings). The use of molecular techniques to characterise coconut germplasm has been reviewed elsewhere (Ashburner, in press).

Sample Collection

Pollen collection may pose problems in remote regions without adequate drying facilities, although pollen may be collected by drying flowers at 40°C for 40 hours or simply at ambient temperature (Whitehead 1963). However the lack of isozyme diversity between coconut populations (Benoît and Ghesquière 1984; G. F. Moran, pers. comm.) places serious doubts on its use in characterising germplasm. Leaf collection for DNA extraction is a simple task, as leaves from any healthy frond may be removed, cut into reasonable lengths, sealed in plastic bags with tape, placed in a box and dispatched by air freight or air mail. The samples should be refrigerated if there are any foreseeable delays. Quarantine permits should be obtained before collection to avoid any delays at the destination.

Germplasm may also be collected at the same time as prospection, but correspondingly more time is required to collect and dispatch seednuts, pollen or embryos.

Sampling Regime

Choice of prospection areas should be based on the likelihood of finding germplasm that is unique or possesses useful characters. Therefore major influences are: geographic location; environment type; and presence of both biotic and abiotic stresses. Within a particular area, prospection should cover as many varied sites as possible with major emphasis on the predominant environments. Sampling can be either random or biased for specific characters, for example, large fruit size. A complementary random sample should also be taken with any biased sample. It is likely that a germplasm sample will revert to its type population structure in its first generation, especially since coconut has a mixed mating system. If possible, random samples should be taken in populations that have not been established from a common seed source such as plantations or replanting schemes. Sampling should occur from palms further than 200 m away from each other.

Thirty palms per population should be surveyed and fifty palms measured for fruit components (IBPGR 1992). However, the number of samples required to characterise validly a coconut population has never been fully investigated, The number probably varies from population to population depending

on the level of variability. Basically, the power of the analysis increases as the number of replicates increases. No fewer than 25 replicates should be taken.

Analysis and Presentation of Results

The data that are produced by most descriptors, including FCA, are quantitative and are best analysed using multivariate analysis. These techniques will take the population variability into account when comparing populations. Simplification of the data is achieved by using principal component analysis (Namkoong et al. 1988). Fruit component analysis has traditionally been compared graphically by bivariate plots of fruit weight against husk percentage and trivariate plots of endosperm proportion against shell proportion and water proportion (Harries 1987). The value of using liquid endosperm (water) weight in fruit component analysis must be questioned since its weight is greatly reduced before maturation on many atolls and in dry seasons.

Data generated by molecular techniques are amenable to well characterised analytical techniques (Weir 1990). Measures of diversity within population and genetic distance between populations can be calculated. For more details see Ashburner and Rohde (these proceedings).

Conclusions

Germplasm prospection is a simple yet important method for gauging the diversity of germplasm in an area and highlighting the presence of populations that require further evaluation. It is in the interest of all coconut producing nations for extensive prospections to be done. Fruit component analysis should be used along with molecular techniques. The value of using vegetative and floral traits for characterising germplasm in different locations is limited. It is vital for the future to conserve biodiversity (UNCED 1992) and prospection is the first step in that process.

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Coconut Germplasm Characterisation using DNA Marker Technology

G.R. Ashburner* and W. Rohde†

CURRENT coconut characterisation methods are predominantly based on morphological and agronomic characters. These characters are important, but their use in prospection activities is often misplaced. It should be confined to accession trials (Ashburner et al., these proceedings). Molecular characterisation has previously been used in coconut palms through isozyme analysis (Benoît and Ghesquière 1984; G. Moran, pers. comm.) and foliar polyphenol analysis (Jay et al. 1989). Isozyme analysis revealed little population divergence, and polyphenol analysis showed discrete differences between ecotypes although environmental influences on the level of polyphenols have not been quantified (Meunier 1992). DNA analysis is well suited for use in germplasm characterisation because its expression is not affected by environmental conditions, the range of potential characters is large, and DNA is a relatively stable molecule.

DNA may be analysed in many ways, with Restriction Fragment Length Polymorphism (RFLP) analysis and Random Amplified Polymorphic DNA (RAPD) analysis predominating for germplasm characterisation. Both these techniques have been used to characterise coconut germplasm, although RFLP analysis has not been fully utilised. This paper describes the protocols used in characterising coconut germplasm using DNA techniques, and discusses the implications of such techniques for germplasm management and breeding.

RAPD Analysis

RAPD analysis is based on Polymerase Chain Reaction (PCR) technology. PCR enables large quantities of DNA to be generated from a stretch of DNA between chosen sites (Erlich 1988). RAPD amplifies DNA from random regions of the genome using the PCR system. Results are visualised by gel electrophoresis and scored by comparing fragment lengths of products between individual plants. Each locus has two possible alleles: either presence or absence of the fragment; therefore each character is inherited in a dominant manner. A review of RAPD and its application to plant improvement has been published by Waugh and Powell (1992).

DNA is extracted using a method based on Rogers and Bendich (1985). Healthy leaf material is collected in sealed plastic bags and dispatched to its destination by air-freight or air mail. This material is still useable after two weeks in transit, if extremes of temperature are avoided. The material is then stored at minus 20°C until processing. Material kept in this state for two years has still yielded high quality DNA.

DNA is extracted for RAPD analysis in the following way: grind 300 mg of leaf tissue to a fine powder in a mortar and pestle with liquid nitrogen and place in a pre-chilled microcentrifuge tube. Ensure that the material never thaws. Add 500 µl of extraction buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 1% PVP (mw 40 000), 0.1% monothioglycerol (added just prior to using), and shake to form a slurry. Incubate at 65°C for 1–3 min, add 500 l of 24:1 chloroform:iso-amyl alcohol (use fume hood), shake and then spin at 13 000 rpm in a microcentrifuge for 30 sec. Transfer the supernatant to a new microcentrifuge tube, add 1/10 volume of 10% CTAB in 0.7 M NaCl, re-extract with one volume of 24:1 chloroform:iso-amyl alcohol, and centrifuge at 13 000 rpm for 30 sec. Transfer the supernatant to a new microcentrifuge, tube being careful not to take the interphase, and add one volume of precipitation buffer (1% CTAB, 50 mM Tris-HCl pH 8.0, 10 m*M* EDTA) at 65°C. Mix together and centrifuge at 13 000 rpm for 1 min. Discard the supernatant and dissolve the pellet in 300 µl hsTE (10 m*M* Tris-HCl pH 8.0, 1 m*M* EDTA, 1 M NaCl)—this may require heating at 65°C for 5–10 minutes. Add two volumes of 100% ethanol and place at –20°C for 30 min. Centrifuge at 13 000 rpm for 15 min, discard the supernatant and wash the

* Department of Agriculture, Institute for Horticultural Development, Private Bag 15, South Eastern Mail Centre, Victoria 3176, Australia.

† Max Planck Institut für Züchtungsforschung, Carl von Linne Weg 10, 5000 Köln 30, Germany.

pellet twice in 70% ethanol. Dry the pellet, resuspend in 200 µl TE (1 mM Tris-HCl pH 8.0, 0.1 mM EDTA) and then store at –20°C.

DNA amplification is performed in a 25 µl reaction mixture of 67 mM Tris-HCl pH 8.8, 16.6 mM $(NH4)_2SO_4$, 0.45% Triton X-100, 0.02% gelatin, 4 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, dTTP, 0.25 µM of primer (10mer, Operon Technologies, Alameda), approximately 25 µg genomic DNA and 1U of *Taq* DNA polymerase, all overlaid with 1 drop of paraffin oil. Thermal cycling is performed in a thermal cycler programmed for 45 cycles of 60 sec at 94°C, 60 sec at 36°C and 2 min at 72°C. Amplification products are analysed by electrophoresis in a 1.5% TAE agarose gel at 75 V for 4.5 hours. The results are visualised by ethidium bromide staining under UV light (λ = 204 nm).

RAPD visualises an average of 9 loci per assay at an approximate cost of $A0.15 excluding DNA preparation. This compares favourably with starch gel electrophoresis which is approximately $A0.10 per locus. In addition, the technique has the advantage of being very rapid. Since it is a PCR-based assay the amount of leaf material required is small. However, the method suffers from two major problems: characters are inherited in a dominant manner so it is difficult to determine heterozygotes; and the extreme sensitivity to reaction conditions (Meunier and Grimont 1993) makes it difficult to compare results obtained in different laboratories. It is still possible to compare populations with this technique but comparisons must be carried out using exactly the same conditions, equipment and preferably the same operator.

RFLP Analysis

RFLP analysis compares the size of different fragments of the genome after it has been digested with restriction endonucleases which cleave the DNA at specific sites. Results are visualised using a complicated procedure of gel electrophoresis, blotting and DNA hybridisation with a labelled probe.

DNA extraction is similar to that for RAPD except that more material is required. Therefore the method has been scaled up and the DNA is collected with a glass rod instead of centrifugation after the ethanol precipitation. The RFLP procedure is standard but uses a simplified hybridisation buffer (0.5 M phosphate buffer pH 7.2, 7% SDS, 1 mM EDTA) with high stringency conditions as recommended by the membrane manufacturer.

RFLP analysis measures loci in a co-dominant fashion, making heterozygotes simple to determine. Therefore the technique is well suited to studying inheritance topics such as outcrossing rates. The markers are stable and are therefore directly comparable between laboratories. However, only approximately 3 loci are measured per assay, an average cost is difficult to estimate as the methods are not yet routine. The method is also laborious compared with RAPD.

Results

The data generated by these techniques allow direct comparisons between coconut populations growing in different environments. Information on the diversity present within a population and the genetic distance between populations can be calculated. From these statistics, phylogenies can then be constructed (Weir 1990). The statistical tests of Nei and Li (1979) that give a value for genetic similarity are typically used.

A RAPD data set was prepared from 20 coconut populations from the South Pacific region and one from the Indian Ocean (Cocos-Keeling Islands). Initial analysis has shown a moderate and variable level of diversity within populations in the Pacific, but very little population divergence. However, there are relatively large differences between the Pacific and Indian Ocean types. It remains to be seen whether further differences will be found elsewhere. These results indicate the presence of extensive migration of coconut germplasm in the Pacific, which leads to blurring of genetic structure when coupled with a mixed mating system. Natural dispersal by sea has probably played a minor role in this migration, but the influence of human-assisted dissemination and selection has undoubtedly had a major impact. These results tend to support the theory of Harries (1978) on domesticated introgression into a wild-type genepool, but further studies are required to confirm or deny it. Initial RFLP analysis has confirmed these findings.

Conclusions

DNA analysis should not replace currently used characterisation methods, but should be used as an adjunct when formulating conservation and crossing strategies. Analysis of data can distinguish similarities or differences between coconut populations and thus can be used to prevent duplication in conservation blocks and crossing programs. However, if two populations appear similar, major adaptive genes may still exist and these will not be picked up by these analyses. Therefore, collection priorities should still be based on populations from unique environments. However, if differences are detected by these techniques, then there is a greater probability of the

presence of different genes resulting from genetic drift, and priority should also be given to their collection. The data may also assist in setting priorities for crossing programs through maximising genetic distance and taking advantage of heterosis. In the future, the techniques may also be used to tag important genes that will allow the use of marker assisted selection.

Acknowledgments

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Methods for Identifying Viroids in Coconuts and other Commercially Important Palms

R.A.J. Hodgson and J.W. Randles*

THE Australian Centre for International Agricultural Research (ACIAR) has sponsored several research projects into the improvement of coconut production by Pacific island countries. Three of these projects have focussed on a viroid pathogen of coconut—the coconut cadang-cadang viroid (CCCVd). The current project, ACIAR 9221—nucleotide sequence determination of viroids in the Pacific area—aims to determine the nucleotide sequence of viroid-like molecular species found in coconut to resolve their relationship to known viroid pathogens so that Pacific countries can establish a policy for the safe movement of viroid-free coconut germplasm.

Coconut cadang-cadang viroid is the causal agent of the lethal cadang-cadang disease of coconut in the Philippines (Zelazny et. al. 1982; Hanold and Randles 1991a) and the closely related coconut *tinangaja* viroid (CTiVd) is associated with a similar disease of coconut in Guam (Boccardo et. al. 1981). CCVd also infects oil palm in the Philippines (Randles et al. 1980). A recent ACIAR-sponsored survey has shown the occurrence of viroid-like molecules, similar to CCCVd, in many coconut palms in 28 countries (Hanold and Randles 1991b); Hanold and Randles 1993). Thus, the possibility exists that cadang-cadang disease may appear in these countries, either through mutation of the viroid-like molecular species in coconut in that country or through importation of coconut germplasm containing CCCVd-like molecular species. It is therefore essential to compare the nucleotide sequence of the viroid-like sequences found in coconut palms outside the Philippines with that of CCCVd.

Nucleotide sequencing requires the separation of the individual viroid-like sequences from the host nucleic acids. These viroid-like RNAs are in extremely low concentration, so the first step is to prepare molecular probes that specifically detect the viroid-like sequences. These probes can be used to isolate and purify the viroid-like sequences and may also be constructed into primers for the sequencing reactions. This review describes the procedures we are adopting to optimise the detection and characterisation of CCCVd and viroid-like sequences in coconut.

* Department of Crop Protection, Waite Campus, University of Adelaide, Glen Osmond, S.A. 5064, Australia.

Definitions

Viroid. One of about 25 described infectious, small, single-stranded circular RNAs, which maintain a rod-shaped base-paired native structure and occur solely in plants.

CCCVd. The coconut cadang-cadang viroid, which is the smallest of the known viroids with only 246 nucleotides and is the only known lethal viroid. This viroid can occur as 4 different-sized molecules (246, 247, 296, 297 nucleotides) that are seen during advancement of the cadang-cadang disease (Hanold and Randles, 1991a). Dimers of each of these forms can also occur in infected plants.

Viroid-like. RNA which shows both nucleotide sequence homology to a viroid probe(s) and has a size and structure similar to that of described viroids.

CCCVd-like. RNA of viroid size showing homology with probes designed to recognise the unique sequences of CCCVd.

Viroid detection. The separation and visualisation of viroid RNA in gels or on membrane filters using either nucleic acid stains or radioactively labelled viroid probes.

Viroid characterisation. Determination of the nucleotide sequence, the most likely secondary base-paired structure, and the sequence relationship to other characterised viroids.

Target. A nucleic acid (RNA or DNA), generally of unknown sequence that is detected, isolated, or subjected to sequencing.

Probe. A nucleic acid with a defined sequence and which specifically binds to a target sequence (viroid) of interest.

Homology. The degree of nucleotide sequence similarity between a target (viroid-like) molecule and a probe.

Pathogenic viroid. A viroid that interferes with a normal host process(es) to cause expression of disease symptoms such as reduced growth, reduced yield, tissue abnormalities or death.

Non-pathogenic viroid. A viroid that is maintained in the host without causing disease symptoms. Non-pathogenic viroids may become pathogenic if environmental conditions change or if they are inoculated into other host species.

General Detection Methods for Viroids

Comparison of the sequences of a range of viroids has shown that they fall into one of three general groups (Koltunow and Rezaian 1988). In each group viroids maintain homology in regions of their sequence and also have similar structural domains. Between groups however there is much less homology. Sequence comparisons between viroids have revealed five discrete regions which have been termed the pathogenicity region, the conserved central region, the variable region and the two terminal regions (Keese and Symons 1985). A description of these regions in CCCVd is given in an accompanying paper (Hanold and Randles, these proceedings).

Regions with sequence homology within groups and the difference between groups, as well as viroid structure, physico-chemical properties and host range, are all used to help detect and classify viroids. Viroids do not code for any proteins nor are they encapsidated by protein. Therefore, serological procedures cannot be used for viroid screening. However, since viroid structure and pathogenicity are governed by the nucleotide sequence (Visvader and Symons 1983; Keese and Symons 1985; Hecker et al. 1988; Hammond 1992; Rodriguez and Randles 1993) the techniques of molecular biology (Sambrook et. al. 1989) are appropriate for viroid identification. Molecular biology provides the technology for sensitive, rapid, reliable and specific detection of viroids in a safe and relatively simple manner. These diagnostic procedures can be used to determine the geographic distribution of host plants containing a particular viroid, the occurrence and spread of viroids among crop plants and for indexing germplasm or parental breeding stocks for viroid infection. But perhaps the greatest advantage of molecular biology in viroid detection is the ability to determine the precise nucleotide sequence and hence determine the pathogenic potential of viroids.

Detection of CCCVd and Viroid-like Sequences

Molecular hybridisation is used to detect specific nucleic acid sequences amongst the diverse population of sequences found in every cell. Usually a total nucleic acid sample is isolated, purified to some degree and then transferred to a supporting membrane filter before being hybridised with a probe to detect the sequence of interest. The procedure for sample and probe preparation as well as the type of membrane filter used and the buffer conditions used during transfer and hybridisation all influence the detection of the target sequence. A full review of the importance of these conditions has recently been published (Hull 1993).

Dot blot hybridisation of probe to membrane bound samples (Fig. 1. I). The dot blot hybridisation procedure uses either a crude cell extract, total nucleic acids or purified nucleic acids and provides rapid detection and quantification of a target sequence in unfractionated nucleic acid. It is therefore an ideal procedure for screening large numbers of samples. In the schematic example in Fig. 1 (I) the dot blot shows that samples 1 and 2 are positive for a CCCVd-like sequence and that sample 3 is a negative healthy sample. Estimates of the level of viroid-like sequence can also be obtained, either by direct scintillation counting of radioactive spots or by densitometry from the X-ray film. In the example in Figure 1 (I), sample 1 gives a weaker signal than sample 2 due to either a lower concentration of target or less homology between the target and the probe. The disadvantage of the dot blots is that they cannot distinguish between samples containing the viroid-like target sequence(s) of interest and samples containing non-viroid sequence(s) that are partially homologous to the probe. This is because the dot blots give no information on the size or structure of the viroid-like sequence. Until it has been established that the viroid probe(s) to be used for the dot blot assay are specific for only the viroid sequence of interest, the procedure should be used only for preliminary screening of samples. The specificity of probes for detecting only the viroid of interest is evaluated by Northern hybridisation and by sequencing the target molecules homologous to the probe. Once these aspects are accomplished the dot blot procedure may be used in conjunction with one or more specific viroid (CCCVd) probes to rapidly screen large sample numbers with a very high level of confidence.

Northern hybridisation of probe to membrane bound samples (Fig. 1. II). The transfer of fractionated deoxyribonucleic acid (DNA) onto membrane filters

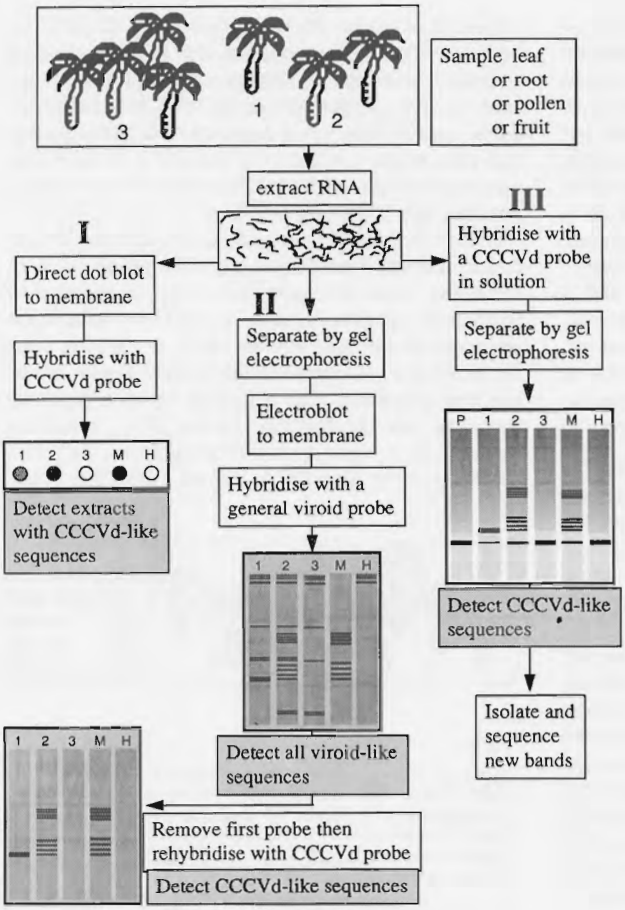


Figure. 1. Flow diagram showing procedures used to detect and isolate CCCVd and viroid-like sequences in extracts from coconut plants. I) Dot blot hybridisation, II) Northern hybridisation and III) In solution hybridisation. 1, 2, and 3 represent coconut leaf extracts, M is a marker sample containing the four common monomer and dimer CCCVd size classes, H is a healthy coconut leaf extract and P is probe sequence.

and the detection of a specific sequences by hybridising an appropriate nucleic acid probe was first described by Southern (Southern 1975). The technique had such wide application that it became known as Southern blotting, or simply Southerns. The equivalent analysis of RNA, therefore, became known as Northerns and analysis of protein as Westerns. There are no Easterns. Northern analysis examines RNA sequences which have been transferred to a supporting membrane filter. The membrane can then be incubated with a radioactively labelled probe and the position on the membrane of the hybridised radioactive probe visualised by exposing X-ray film to the membrane. This Northern hybridisation procedure has been successfully employed following two-dimensional gel electrophoresis to detect palm RNAs that have the size and structural characteristic of viroids (Hanold 1993).

A schematic example of a Northern analysis, for screening RNA from plants suspected of containing CCCVd, is shown in Figure 1 (II). Sample extracts 1, 2 and 3, a CCCVd marker (M) and a healthy plant extract (H) were examined following hybridisation with a full length CCCVd probe and later with a CCCVd diagnostic probe. As with dot blotting, in Northern blotting the target is irreversibly bound to the membrane prior to the hybridisation. Because binding of the probe to the target is reversible, membranes can be repeatedly probed and washed. In this way the homology of precisely the same sequence(s) in a sample to different probes can be examined. In the example of Figure 1 (II), the marker lane shows a region containing all the CCCVd monomer and dimer size classes, which are used as a reference to show the relative mobility of bands binding the general viroid probe in samples. With this general viroid probe all samples show viroid-like bands in the size range for CCCVd. In addition some bands are shown at the top of the gel that are out of the viroid size range and which may be either aggregates of viroid RNA or unrelated sequences of host origin. From the

Northern analysis with a general viroid probe it cannot be determined whether the viroid-like bands are variant size classes of CCCVd, other related viroids or small non-viroid host sequences. To answer this question, characterisation of the RNA bands by hybridisation with diagnostic probes and nucleotide sequencing is necessary. Removal of the general viroid probe followed by rehybridisation with a viroid-specific (CCCVd) probe detects sequences with a particular homology to CCCVd. In the example in Figure 1 (II), this is seen in samples 1 and 2 only. By repeating this rehybridisation with several other CCCVd-specific probes the probability that the detected band(s) is indeed CCCVd is increased to a virtual certainty. This is the basis of diagnostic hybridisation which is discussed in a later section.

Hybridisation of probe to samples in solution (Fig. 1. III). With both dot blotting and Northern analysis, samples are permanently bound to a membrane filter support and therefore are not available for isolation and characterisation. To obtain sequence information about particular viroid-like sequences an alternate detection and isolation procedure can be used.

The in-solution hybridisation procedure allows the probe to bind with target sequence before and during gel separation. When this occurs the target-probe complex has a different size and structure compared to either the probe or the target complex during gel separation compared to the probe alone. Thus, if the probe is radioactively labelled, samples containing the target sequence show new bands in the gel. This in-solution hybridisation is represented in Figure 1 (III). The labelled probe (P) will have a characteristic mobility in the gel. Hybridisation of the probe with healthy coconut extract (H) and then gel electrophoresis shows no change in probe mobility. However, when probe is hybridised with the CCCVd marker (M) new bands appear, which shows how the probe detects the CCCVd target. For the three hypothetical coconut extracts (1, 2 and 3) only two of the samples show new bands. Thus, the samples 1 and 2 are suspected of having viroid-like sequences. Furthermore, since a CCCVd diagnostic-probe was used for the hybridisation, and since the mobility of the bands in the coconut samples matches at least one of the bands in the CCCVd marker line, it is likely that the positive samples contain CCCVd-like sequences. The greatest benefit from in-solution hybridisation is that each new band can be removed from the gel and the RNA extracted for further analysis such as in sequencing. Furthermore, since the CCCVd diagnostic-probe has strong homology with the viroid-like sequence, this probe or a portion of the probe can be used as a specific primer for sequencing or amplification through the polymerase chain reaction.

Polymerase chain reaction (PCR) (Fig. 2. IV). As with hybridisation in solution, the PCR provides a technique to isolate viroid-like material for sequence analysis. The greatest advantage of PCR is its potential to amplify very small amounts of target sequence and thus make possible the detection of even the smallest amount of viroid in tissue at the earliest possible time following infection.

The PCR is used to amplify DNA samples of low concentration. Two oligonucleotide primers—with different sequences that are complementary to regions on opposite strands of the DNA—flank the sequence of interest, and are used as priming sites for extension (polymerisation) of new copies of the original sequence. The reactions involve repeated cycles of heat denaturation of the DNA, annealing the specific primers at lower temperature and then synthesis of the new DNA. Repeat cycles are possi-

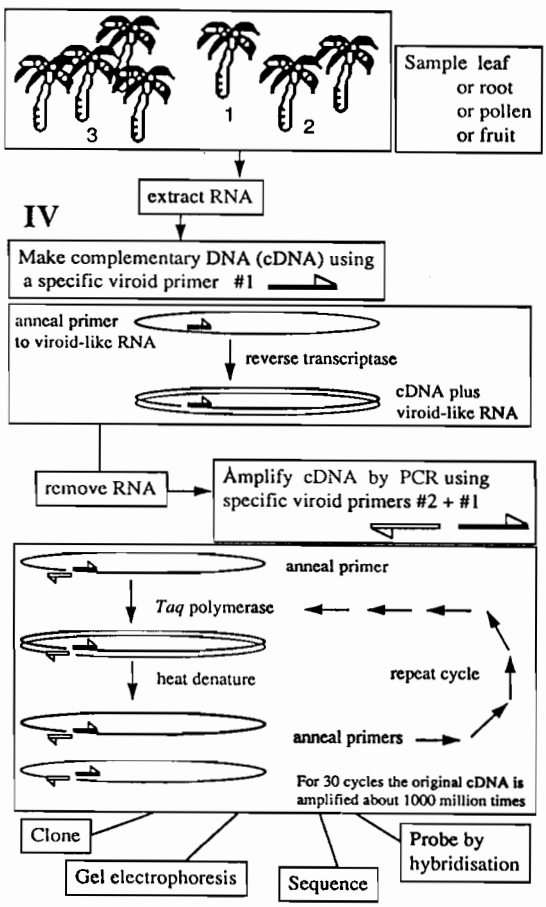


Figure. 2. Flow diagram showing the procedure for PCR amplification (IV) of viroid sequences in a plant extract.

ble by using a heat stable DNA polymerase (*Taq* polymerase) from a thermophilic bacterium (*Thermus aquaticus*). The product from one round of amplification serves as the target for the next, so that each successive cycle essentially doubles the amount of the desired product. Thus, after 30 cycles there are a possible 2^{30} (about 1000 million) copies of the original sequence. For more detailed information on PCR the reader can consult any of the numerous reviews (Innis et al. 1990; Bottema et al. 1993).

PCR of viroids, which are RNA molecules, involves some preliminary reactions to convert the RNA into a complementary DNA (cDNA) copy before the amplification can begin. The procedure for PCR of RNA is schematically shown in Figure 2. The RNA extract containing the viroid or viroid-like sequences is isolated followed by the annealing of a primer whose sequence is complementary to that of the viroid of interest. For CCCVd the primers are based on the unique sequence in the CCCVd diagnostic-probes, which were previously used to detect viroid-like sequences in the Northern analysis. After making the cDNA, with the enzyme reverse transcriptase, amplification of the viroid-like sequence by PCR can proceed as already outlined and as shown in Figure 2. The PCR product can then be cloned for future conversion back to RNA to test infectivity, or the fragment can by analysed by gel electrophoresis, by sequencing and by hybridisation, to identify the product and determine its sequence relationship to CCCVd or other viroids.

Characterisation of CCCVd and Viroid-like Sequences

Diagnostic hybridisation (Fig. 3. V). Diagnostic hybridisation of viroids utilises the information provided by existing viroid sequences to construct a group of probes that together uniquely detect only one type of viroid (Sano et. al. 1988). Diagnostic hybridisation can be used to definitely exclude the occurrence of particular viroids in a sample. Thus, if a sample does not react positively to a viroid-specific diagnostic probe then that viroid can be excluded from being a component of the plant extract. Diagnostic hybridisation cannot unequivocally include a specific viroid in a sample. However, when

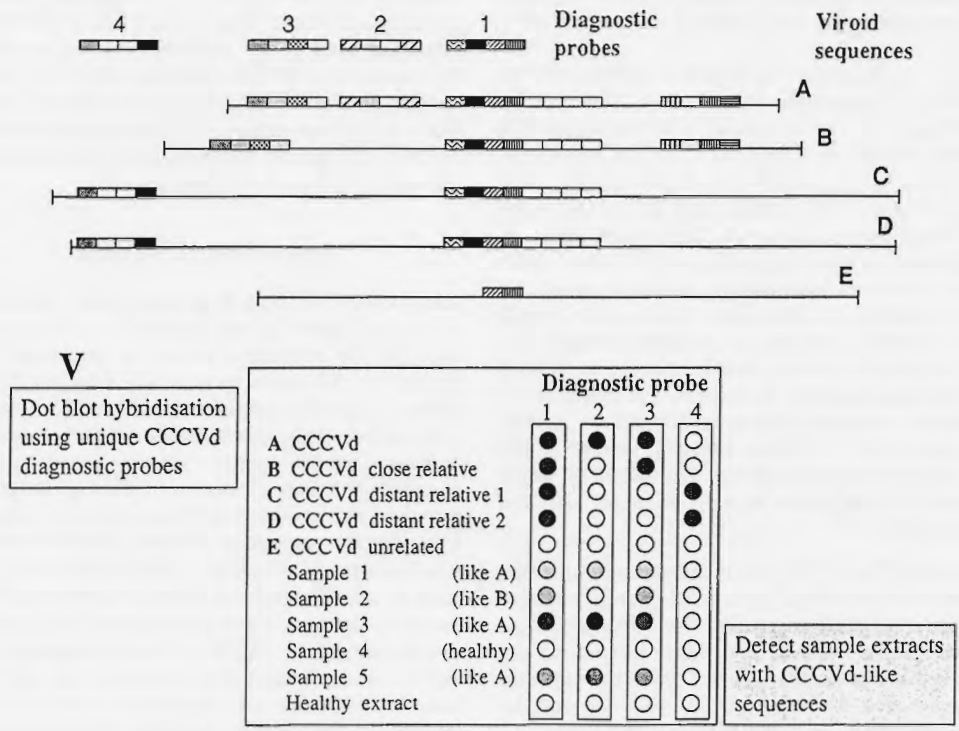


Figure. 3. Schematic representation of the nucleotide sequence relationship between unique diagnostic probes and CCCVd related sequences. V) Visualisation of diagnostic Dot blot hybridisation for 5 coconut samples suspected to contain CCCVd.

the diagnostic hybridisation involves the use of 3, 4 and 5 unique-sequence diagnostic probes to CCCVd and a positive is recorded for each probe, then the probability is extremely high (almost a certainty) that the sample detected does in fact contain CCCVd. The only definitive way to identify a particular viroid in a sample is to isolate and sequence it.

A schematic representation of diagnostic hybridisation using the dot blot procedure is shown in Figure 3 (V). Several viroids are represented as sequences A to E. Sequence A is CCCVd, sequence B, C, and D are different but have regions of sequence homology to CCCVd and sequence E is an unrelated viroid. Regions of sequence homology are shown as boxes with the same shading. Thus, sequences A, B, C and D share a common homologous central region, sequences A and B share three common homologous regions and sequence A contains a unique region not found in any other viroid. To detect these different viroids, probes are designed to match various regions of unique sequence. Diagnostic probe 1 would detect all viroids with sequences A, B, C and D, but not those with sequence E. Probe 2 would only detect sequence A, the CCCVd sequence, and probe 4 would not detect CCCVd or its close relative. Individually the probes give only limited information, but combined they become diagnostic for a viroid.

The dot blots shown in Figure 3 demonstrate an application of diagnostic hybridisation. Using probe 1, samples 1, 2, 3 and 5 appear to have a viroid-like sequence related to sequence A. Probe 2 confirms this result and in addition shows that sample 2 is a negative and so is possibly a close relative of CCCVd as in sequence B. This assumption is strengthened by the result using probe 3 where again all samples except 5 are positive. Probe 4 is used as a negative control to show that if some other viroid type is present it too can be detected. Sample 4 is negative to all probes and is therefore free of CCCVd-like sequences. To increase the specificity of the analysis diagnostic hybridisation can be used in conjunction with Northern analysis, instead of dot blots, to simultaneously obtain information on target homology to the probe as well as target size and structure (Fig.1 II).

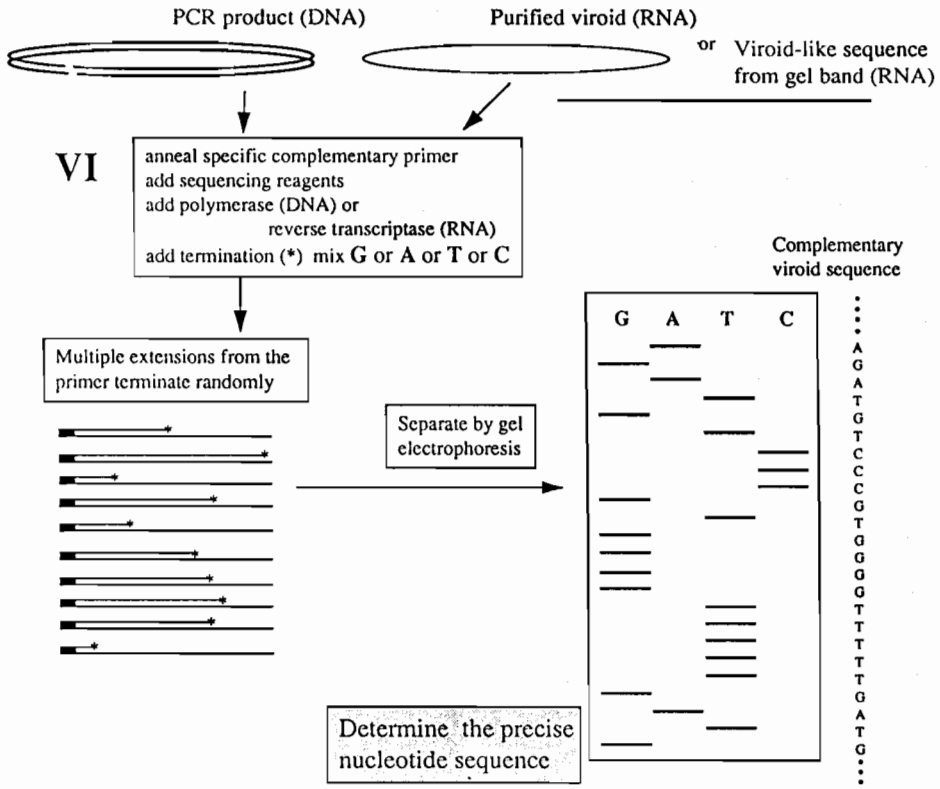
Sequencing (Fig. 4. VI). All techniques shown in the previous sections have their particular advantages and disadvantages for detecting viroid-like sequences in plant samples. But none of these procedures can determine unequivocally whether or not the sequence of interest is a particular viroid. This can only be done by isolating and sequencing the RNA.

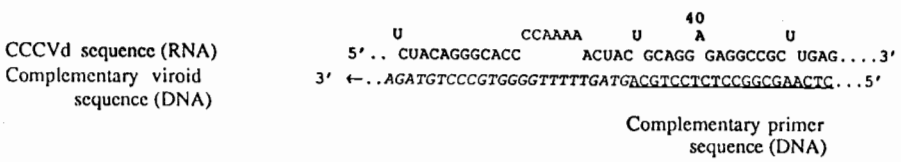
Two procedures for sequencing DNA have been developed. These are the enzymic method of Sanger and the chemical degradation of Maxam and Gilbert, which were both developed in the mid-1970s. Viroids can also be sequenced by a modification of these methods or the RNA can be converted into cDNA which is then sequenced. Enzymic sequencing is most appropriate for the viroid-like samples detected in coconut and will be discussed further.

Sequencing produces a population of radioactively labelled oligonucleotides (short stretches of DNA) that begin from a fixed point (at the primer) and terminate randomly along the target sequence. Every nucleotide in the target sequence has an equal chance of inducing the random termination position so each population of oligonucleotides from a reaction is a mixture of lengths. The populations of oligonucleotides are separated by gel electrophoresis under conditions that can resolve individual DNAs that differ in as little a one nucleotide. Shown in Figure 4 (VI) is a schematic representation of the sequencing procedure. The end result is the determination of the nucleotide sequence of the viroid-like material. In the example in Figure 4 a portion of the sequence is shown. This portion is exactly complementary to the sequence of CCCVd from nucleotide #10 to #33 in the Terminal 1 and Pathogenic regions of the viroid. If the remainder of the sequence also matches the known CCCVd sequence then this example of viroid-like material is CCCVd. The sequence analysis has resolved whether the viroid-like sequence is in fact CCCVd, related to CCCVd, another unrelated viroid or non-viroid host material. The reactions are so specific that variants of CCCVd having only single nucleotide differences can be detected (Rodriguez and Randles 1993).

Experimental Results

The previous sections give background information as well as justifying the choice of particular procedures for the analysis of viroid-like sequences found in coconut. We have successfully developed procedures for dot blot hybridisation, Northern hybridisation and in-solution hybridisation for coconut extracts. This now makes it possible to screen a large number of samples and confidently determine whether viroid-like sequences, closely related to CCCVd, are present in extracts. Furthermore, the development of diagnostic hybridisation has been used to confirm that the viroid-like RNA in many coconut samples from outside the Philippines is closely related to CCCVd. These procedures were the necessary initial developments that had to be achieved before it was possible to correctly detect and isolate the specific viroid-like RNAs in coconut for sequencing analysis. With the isolation of these viroid-like sequences and with the use of sequencing primers based on the sequence of the diagnostic





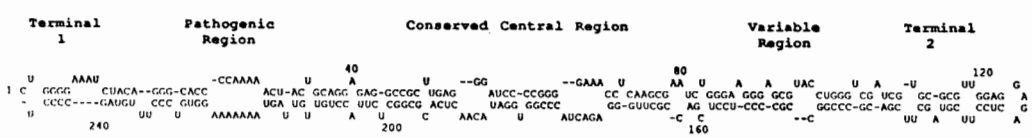


Figure 4. Schematic representation of a procedure for enzymic chain-terminating sequencing (VI) of purified viroid, viroid-like species from gels or PCR amplified products. Also shown are the nucleotide sequence and sequence regions of CCCVd, together with a complementary primer site and the corresponding derived sequence.

probes it is now possible to sequence the viroid-like species in coconut and therefore determine their relationship to CCCVd.

Future Developments

Concurrently with determining the nucleotide composition of the viroid-like sequences in coconut it will be necessary to develop an efficient viroid screening procedure for coconut and other commercially viable palms. This will aim to reduce diagnosis time (quick viroid extract, rapid viroid detection and diagnosis) and develop a diagnostic kit (user friendly, portable, non-radioactive, reasonable cost) for establishing viroid (CCCVd) free germplasm/breeding stock in a standardised indexing procedure.

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A New Viroid Family Infecting Tropical Monocotyledons

D. Hanold and J.W. Randles*

VIROIDS are the smallest known pathogens and have been found only in plants. They consist solely of a small circular single-stranded infectious RNA molecule which can replicate in the host cell and which can be transmitted between plants. Viroids range in size from 246 to approximately 375 nucleotides (Keese and Symons 1987; Herold et. al. 1992) and have strong internal base pairing which gives them a rod-like shape in their native state. They have a characteristic melting pattern with a transitional intermediate due to the arrangement of bases in the central conserved region (Riesner 1987). Most viroids known so far have been found in vegetatively propagated cultivated plants and are transmitted both by propagation and sometimes mechanically by man's cultural practices. Some can spread naturally through the agency of insects, seed and pollen (Diener 1987) or by unknown means (coconut cadang-cadang viroid [CCCVd], coconut tinangaja viroid [CTiVd]; Hanold and Randles 1991).

Little is known about the physiology of pathogenicity and the replicative mechanisms of these pathogens, except that they appear to require RNA polymerase II for replication, have no DNA intermediates (Sänger 1987), and have no messenger RNA function.

A generally accepted model of viroid structure has been developed (Fig. 1). It divides the molecule into a number of regions:

- central conserved region (CCR) which retains a high level of homology among different viroids of the same group and is thus used to classify viroids;
- pathogenicity region (P) to the left of the CCR which has a high level of homology between viroids with a similar host range—mutations in this region can affect infectivity and symptomatology;
- variable region (V) to the right of the CCR which has high variability between isolates; and
- two terminal regions, the left (T1) and right (T2).

* Department of Crop Protection, Waite Campus, University of Adelaide, Glen Osmond 5064 Australia.

Relationships between viroids are determined by their level of sequence homology. For example, both CCCVd and chrysanthemum stunt viroid (CSVd) belong to the potato spindle tuber viroid (PSTVd) group and each have approximately 70% homology with PSTVd in the CCR. However, between each other they have only 64% homology in the CCR and 44% homology overall and are thus only distantly related. CCCVd and CTiVd, in contrast, have 77% homology in the CCR and 64% overall, and are thus closely related (CTiVd causes a disease called tinangaja in Guam which is similar to cadang-cadang; see below).

Pathogenicity of viroids is affected by minor changes in base sequence. For example, PSTVd isolates which are mild differ from severe isolates by changes in only 2 to 4 bases.

Cadang-Cadang

Cadang-cadang is a lethal disease of coconut palms (*Cocos nucifera* L.) in the central Philippines where it has been estimated to have caused losses exceeding 30 million palms (Zelany et al. 1982; Randles and Imperial 1984; Randles 1987; Randles et al. 1992). The causal CCCVd occurs as a small (246–297 nucleotide) form depending on the stage of infection (Imperial et al. 1981; Haseloff et al. 1982). It has also been detected in naturally infected African oil palm (*Elaeis guineensis* Jacq.) and buri palm (*Corypha elata* Roxb.) in the Philippines and has been transmitted to these and other species of palm by mechanical inoculation (Randles et al 1980; Imperial et al. 1985). The related CTiVd was found in coconut palms with tinangaja disease in Guam (Boccardo et al. 1981). CTiVd is a variant of CCCVd differing in symptom expression and size (254 nucleotides) and with about 64% nucleotides sequence homology to CCCVd (Randles and Imperial 1984; Keese et al. 1987). Despite the screening of a wide range of coconut varieties no genetic resistance has been detected (Randles 1987). CCCVd spreads naturally by an unknown means and is regarded as a threat to coconut and oil palm production in Southeast Asia. It is also a concern of quarantine authorities in this region (Randles 1982).

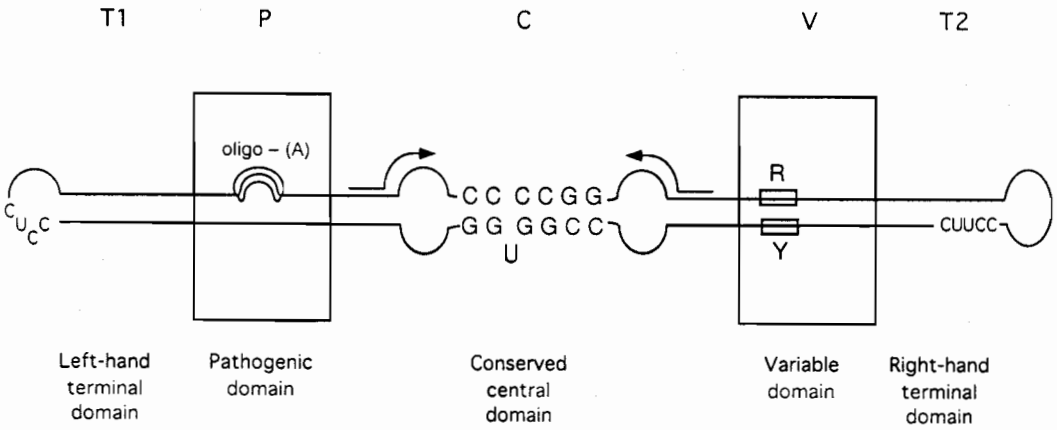

Figure 1. The regions or domains of a typical viroid molecule. (From Keese and Symons 1987).

The severity of disease induced by CCCVd is influenced both by sequence and by variation in the host. For example, a severe form of cadang-cadang viroid probably differs from the common form by only 2–3 bases (see below). On the other hand, seedlings from the same line which were inoculated with the same inoculum of CCCVd showed great variation in response from severe to mild over a seven year period (D. Hanold and J.W. Randles, unpublished results).

The sequence of CCCVd. CCCVd is the smallest of the known viroids. It differs from the other viroids in two respects.

Firstly, it shows duplication of part of the right terminal domain as the disease progresses to produce a large form of the molecule (Haseloff et al. 1982).

Secondly, sequencing of a range of variant RNAs associated with a particularly severe form of the disease where the lamina is almost absent (a symptom described as brooming because fronds comprise mainly midribs and thus look broom-like) has shown that they differ from the normal viroid by mutations at either one or two of 3 sites in the molecule (Rodriguez and Randles 1993). Unlike the other viroids, two of the sites where mutations could occur are in the CCR, and the other is in the pathogenicity domain (Fig. 2).

Identification with molecular probes. Symptomatology is unreliable for disease diagnosis, and serological tests are not appropriate because viroids are not antigenic. Tests for the detection of CCCVd by polyacrylamide gel electrophoresis and molecular hybridisation have therefore been developed (Schumacher et al. 1983; Imperial et al. 1985). These tests are sensitive and when used together as in Northern blots are definitive for the viroid because they test for size, structure and nucleotide sequence. Using this method, viroids closely resembling CCCVd have recently been reported for the first time outside the Philippines/Guam region (Hanold and Randles 1989, 1991a, 1991b).

The Northern blot assay using a complementary RNA probe specific to CCCVd also produces strong signals with CTiVd, but gives no reaction with CSVd (Hanold and Randles 1991b). We therefore concluded that all viroid-like molecules detected in the extensive survey of coconut growing areas are more closely related to CCCVd than CSVd, but at different levels indicated by variability in the strength of their reaction in the assay. The isolate we have shown to be associated with oil palm 'genetic' orange spotting, for example, binds the probe almost as strongly as CTiVd (Hanold and Randles 1991b).

The Survey

The processing method for coconut and other monocots has been described (Hanold and Randles 1991b). Samples were collected from 28 countries, 26 of which are listed in Table 1. Coconut was the principal species sampled, but other monocots were sampled so as to include other palm species, *Pandanus* (Pandanaceae), *Zingiber spp.* (Zingiberaceae), *Maranta spp.* (Marantaceae), *Commelina spp.* (Commeliniflorae), various grasses and others (Table 2).

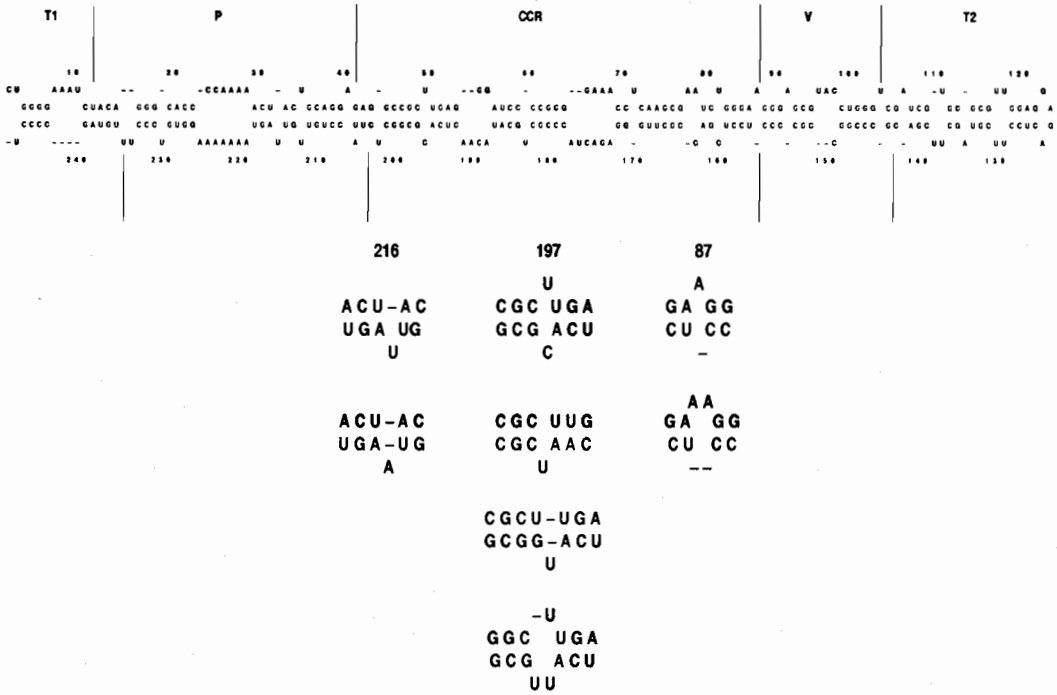

Figure 2. The sequence of CCCVd showing the sites where mutations associated with the severe brooming symptom of the disease are found in the viroid molecule. The regions are shown in Figure 1. (From Rodriguez and Randles 1993).

All samples were subjected to Northern blot analysis, using conditions of low stringency (to detect sequences distantly related to CCCVd). Samples were recorded as definitely positive when they had bands which bound the probe at high stringency and were about the same size as CCCVd. Those which bound the probe in the viroid region only under low stringency were regarded as suspect, and probably contained a less closely related viroid.

Table 1 shows the range of incidence of positives in the 26 countries. None of the sites had been reported to have cadang-cadang disease. Positives ranged in incidence from 0 to 100%. A statistical analysis failed to show a correlation between region, cultivar, presence of yellowing, bearing of nuts, and the presence of bands. However, the relatively small number of coconut samples from each country, and their variation in age, site, and health may explain failure to show any significant correlations.

As shown in Table 2, CCCVd-like sequences were detected in many other monocot species. The difference between results with low and high stringency probing possibly reflects bands which are distantly and closely related to CCCVd, respectively.

Conclusions

Our results have demonstrated that:

- the CCCVd probe does not detect CSVd, and is thus probably specific for CCCVd and CTiVd;
- the large proportion of positive samples detected in coconut, and other monocots where tested, in all of the 28 countries surveyed, indicates that viroids related to CCCVd are widely distributed in the tropics;
- in oil palm, the CCCVd related viroid appears to spread from foci of severely affected palms (Hanold and Randles 1991b); and
- minor mutations in CCCVd, which is the lethal form of the viroid in the Philippines, are associated with an even more severe form of cadang-cadang disease. Mutations are thus possible, and may lead to severe disease in new areas.

The absence of typical cadang-cadang disease in the countries surveyed may be due to the absence of trained observers in these countries, and it may also reflect the presence of non-virulent forms of the viroid. Many questions remain to be answered about the significance of our results.

Table 1. Coconut samples containing nucleic acids related to (CCCVd) according to countries and sites; tested by hybridisation assay at high stringency. The numerical code is used because of the wish of some countries for the results to remain confidential.

Country	Site	Number of samples	Positive	
			number	% of total
1	1	12	2	17
	2	63	36	57
2		38	16	42
3	1	45	32	71
	2	4	3	75
4		10	9	90
5	1	24	8	33
	2	6	5	83
	3	7	5	71
6		10	4	40
7	1	2	2	100
	2	30	13	43
8	1	24	18	75
	2	87	18	21
	3	12	6	50
	4	19	3	16
9	1	43	9	21
	2	4	1	25
	3	4	0	0
10	1	16	9	56
	2	13	9	69
11	1	55	31	56
	2	5	2	40
12	1	32	25	78
	2	19	16	84
	3	23	13	57
	4	31	18	58
13		25	13	52
14		26	11	42
15	1	15	11	73
	2	16	10	63
16	1	26	11	72
	2	21	6	29
	3	17	9	53
17		40	9	23
18		6	4	67
19		43	16	37
20		28	7	25
21		9	8	89
22		2	1	50

(continued on next page)

Table 1 (continued)

Country	Site	Number of samples	Positive	
			number	% of total
23		2	0	0
24		2	2	100
25		10	9	90
26		6	4	67
Total 26	**44**	**932**	**444**	**48**

Explanations and conclusions:

1. The table shows the number of samples that contain nucleic acid molecules closely related to the cadang-cadang pathogen (coconut cadang-cadang viroid, CCCVd) by size and molecular structure.

   Samples from different areas may contain different distinct types of these molecules, all related to CCCVd and to each other, but not identical. Further studies are needed to classify and characterise the various members of the family and to assess what risk they pose to the countries.

2. In most countries, samples of species other than coconut were also collected and analysed, including oil palms and other palms, Pandanus, gingers, grasses, arrowroot etc. Some of these also contained CCCVd-related molecules which need to be compared with the coconut isolates to determine whether the other species could serve as reservoirs. For oil palm, severe orange leaf spotting, reduction of yield, and stunting (previously called 'genetic' orange spotting syndrome) was shown to be associated with a viroid related to CCCVd.

3. Analysis of relationships between viroid detection and origin, cultivar and age of palms is proceeding.

An indexing program needs to be established (Persley 1992) and only viroid free germplasm should be used for planting new areas. Planting of viroid tested material is recommended because of:

- the lack of knowledge of the effect of the viroid-like sequences;
- the risk of mutation leading to an outbreak of a cadang-cadang like disease in new areas;
- the availability of viroid free material in coconut populations;
- the availability of molecular indexing methods for assaying candidate germplasm; and
- the availability of guidelines for the safe movement of coconut germplasm (IPGRI, in press).

It does not appear to be wise to use viroid positive material in breeding programs. Once an indexing program is established, viroid positive and negative material will be available for comparison. Indexing will allow complications arising from interactions between viroids, other pathogens, and host physiology to be avoided.

ACIAR Project 9221 was set up to sequence a representative range of viroids from coconut and allow the range of variation in sequence to be evaluated. A number of benefits will derive from the successful completion of this project, including a data base from which risk assessment can be made, and specific indexing methods optimised.

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Table 2. Incidence of CCCVd-related sequences in other palm species and families. Assays done at both low (LS) and high (HS) stringency; countries identified by code only.

	Other Palm Species					**Pandanaceae**					**Zingiberaceae**					**Marantaceae**					**Commeliniflorae**					**Other Monocots**				
		+ for CCCVd-probe					+ for CCCVd-probe					+ for CCCVd-probe					+ for CCCVd-probe					+ for CCCVd-probe					+ for CCCVd-probe			
country	**total no.**	overall		viroid region		**total no.**	overall		viroid region		**total no.**	overall		viroid region		**total no.**	overall		viroid region		**total no.**	overall		viroid region		**total no.**	overall		viroid region	
		LS	HS	LS	HS		LS	HS	LS	HS		LS	HS	LS	HS		LS	HS	LS	HS		LS	HS	LS	HS		LS	HS	LS	HS
AUS	42	20	15	9	6	16	11	8	11	6	9	7	7	3	2	3	3	3	2	2	8	8	8	6	6	17	8	7	5	4
COI	0	–	–	–	–	4	4	4	4	4	2	2	2	2	1	1	1	1	0	0	1	1	1	1	0	5	5	5	3	3
COS	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
FJ	6	3	3	2	2	24	24	23	24	21	24	11	8	5	5	3	3	3	2	2	2	2	2	2	2	10	9	8	5	3
FPO	5	4	4	4	4	14	14	14	13	11	11	5	2	1	1	1	1	1	1	1	7	7	4	3	2	12	10	10	7	6
GU	6	2	2	2	1	2	2	2	2	2	0	–	–	–	–	0	–	–	–	–	4	2	2	2	0	1	1	1	1	1
IND	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
IDO	0	–	–	–	–	2	2	2	2	2	1	1	1	1	1	3	3	3	3	2	0	–	–	–	–	2	1	1	1	1
KIR	0	–	–	–	–	10	10	10	10	9	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
MAL	0	–	–	–	–	1	1	1	1	1	2	2	1	2	1	1	1	1	1	0	0	–	–	–	–	4	3	3	3	3
MAI	0	–	–	–	–	9	9	9	9	8	0	–	–	–	–	0	–	–	–	–	1	1	0	1	0	0	–	–	–	–
MOZ	0	–	–	–	–	1	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
NU	1	1	1	1	1	1	1	1	1	1	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	1	1	0	0	0
NC	1	0	0	0	0	0	–		–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0–	–	–	–	–
NZ	1	1	1	1	1	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
PNG	0	–	–	–	–	2	2	2	2	2	15	14	8	11	3	2	2	2	2	1	3	3	2	3	2	0	–	–	–	–
PEL	3	0	0	0	0	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
PHIL	2	2	2	2	2	1	1	1	1	1	2	2	0	1	0	2	2	2	2	2	2	2	0	2	0	1	1	0	1	0
PON	1	1	1	1	1	2	1	1	1	1	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	2	2	2	2	2
SI	8	6	4	6	3	2	2	2	2	2	15	12	6	7	3	0	–	–	–	–	8	8	6	6	3	8	7	7	7	7
SRL	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
TAN	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
THA	0	–	–	–	–	1	0	0	0	0	2	2	1	1	0	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
TOG	0	–	–	–		6	6	6	5	4	4	3	1	1	1	0	–	–	–	–	1	1	1	1	1	6	6	5	5	3
TRU	0	–	–	–		0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
TUV	0	–	–	–	–	10	10	10	10	6	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–
VA	11	4	4	3	3	17	15	15	15	15	5	0	0	0	0	1	0	0	0	0	3	0	0	0	0	13	6	6	5	5
WSA	0	–	–	–	–	7	7	7	7	7	10	8	8	4	3	0	–	–	–	–	2	1	0	1	0	1	1	1	1	1
YAP	1	0	0	0	0	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–

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Research on Viroid-like Molecules in Oil Palm

M. Dollet, L. Mazzolini and V. Bernard*

Oil Palm 'Bud Rot' (BR) is rife in Ecuador. The agronomic, mycological, bacteriological, nematological and entomological research work undertaken on the origins of this disease has drawn a blank. The virus hypothesis (virus, or viroid, or mycoplasma-like-organism [MLO]) has therefore logically been considered. As no mechanical transmission has been obtained and no viral particles or MLO could be seen under the electron microscope, it was decided to adopt a research strategy involving nucleic acids. Such a strategy had already proved successful on at least two occasions: cadang-cadang in coconut, and coconut foliar decay (Randles 1975; Randles et al. 1986; Dollet 1992). A comparison of total nucleic acids in diseased and healthy oil palms revealed no difference. We therefore turned to the search for a possible viroid.

Viroid-like molecule (Vd.L.M.)

In our viroid search, we used a selective electrophoresis method—bidirectional or 'return' electrophoresis—isolating viroids, which are small circular RNAs whose electrophoretic migration is highly reduced under denaturing conditions (Schumacher et al. 1983), from the rest of the nucleic acids. After extracting nucleic acids using various techniques, 'return' electrophoresis revealed a retarded migration band on polyacrylamide gel for diseased and ostensibly healthy palms from the same region. This band corresponded to a molecule similar in size to the chrysanthemum stunt viroid (CSVd), a viroid used as a control (Fig. 1). The use of nucleases (DNAse and RNAse) showed that it was an RNA molecule. This molecule was also soluble in 2 M lithium chloride, as are viroids. Once purified, this molecule behaves like the coconut cadang-cadang viroid (CCCVd) under natural conditions: it migrates to the same level as the CCCVd, whereas part of the purified material from this band continues to migrate to the same level as the CSVd. In addition to this major band migrating to the CSVd level, several other smaller bands can sometimes be seen too, on either side. These bands stretch over the distance separating the CSVd (354–356 nucleotides) and the CCCVd (246–247 nucleotides). All these bands behave like viroids. However, they exist both in oil palms affected by BR and in healthy palms from West Africa. We therefore tried to ascertain whether these Vd.L.M. occurred in wild *Elaeis guineensis* populations that had never left Africa. We in fact found them in a population in Cameroon, the 'Widikoum'. Finally, it should be pointed out that Singh et al. (1988) and Beuther et al. (1992) also found these types of molecules in diseased oil palms (affected by spear rot) and in ostensibly healthy palms in Brazil. These Vd.L.M therefore seem to be 'normal constituents' of the oil palm.

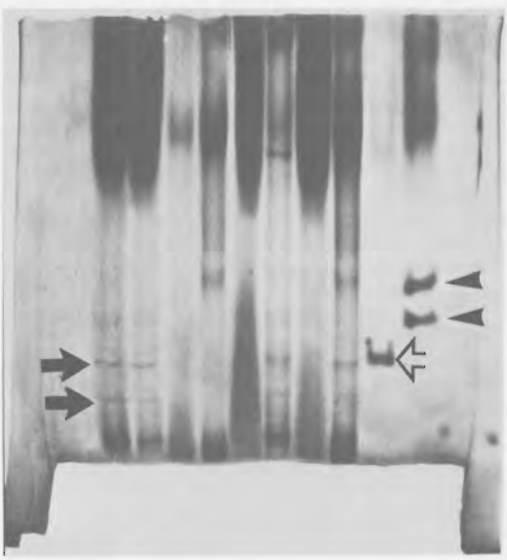

Figure 1. Detection of viroid like molecules in oil palm by bidirectional or 'return' electrophoresis on 5% polyacrylamide gel: black arrow = oil palm viroid like molecule; white arrow = chrysanthemum stunt viroid; arrow head = coconut cadang-cadang viroid.

* Laboratoire De Phytovirologie Des Regions Chaudes Cirad-Orstom, Bp 5035–34032, Montpellier, Cedex 1 France.

Vd.L.M. or double-stranded RNA?

We have seen that return electrophoresis was considered to be a specific method for viroids, linked to their secondary structure. Double-stranded RNA (dsRNA), but with a strong secondary structure, may behave in quite similar ways to viroids. Rosenbaum and Riesner (1987) showed that it was possible to distinguish viroids from double-stranded RNA using thermogradient electrophoresis. Viroids and dsRNA migrate in their native form at the lowest temperatures in the gradient. At a given so-called transition, or denaturing temperature, these molecules migrate more slowly. The migration banding pattern obtained in this way can be used to distinguish between viroids and dsRNA. The pattern obtained with the purified oil palm Vd.L.M., with a transition temperature of 46°C leads us to conclude that this molecule behaves more like dsRNA. Indeed, the different viroids tested under the same conditions by D. Riesner's team begin their denaturation at around 20°C. Schönborn et al. (1991) produced a monoclonal antibody that specifically reacted against dsRNA. We therefore tested this antibody on the Vd.L.M. The Vd.L.M. reacted strongly to this monoclonal antibody, whether after electrophoresis under native conditions or in thermogradient, after transfer to a nylon membrane. This result indicated a dsRNA, rather than viroid, type structure for this oil palm molecule.

Use of molecular probes

Many viroids reveal variable sequence homologies and even have conserved sequences of varying length. Keese and Symons (1987), for example, reported between 35 and 76% overall sequence homologies in 10 studied viroids (including the CCCVd) and homologies of 35 to 99% in the conserved domain of these viroids.

Several laboratories have radioactively or chemically marked molecular probes that are composed either of the actual sequence (RNA) of the viroid, or of a complementary DNA. Diagnosis kits are even available by mail order (Potato Spindle Tuber Viroid [PSTVd] CCCVd, CSVd and Avocado Sun Blotch Viroid [ASBVd]). We therefore set out to ascertain whether sequences identical, or similar to, those of different viroids existed in the total extracts of nucleic acids from diseased and healthy oil palms. We did the same thing after prior separation of the nucleic acids by electrophoresis.

Dot Blot Hybridisation. A set of 30 purified nucleic acid samples from diseased and ostensibly healthy oil palms in Ecuador, from healthy palms in the Ivory Coast and from oil palms affected by ringspot disease were tested. We used probes corresponding to the cloned genome of six viroids: tomato apical stunt viroid (TASVd), hop stunt viroid (HSVd), carnation viroid (CarSAVd), citrus exocortis viroid (CEVd), PSTVd and CSVd. The controls were nucleic acids of chrysanthemum parasitised by the CSVd, potato parasitised by the PSTVd and peach parasitised by an unidentified viroid. The dot blot results of these hybridisations revealed that several blots of nucleic acids from various trees (palms affected by BR or even healthy palms) hybridise with one or other of these probes. (Fig. 2). In particular, three blots corresponding to healthy oil palms from Ecuador (17'18'19) hybridise strongly with the PSTVd and the CarSAVd and, to a lesser degree, with the HSVd. The same type of result was obtained with the non-radioactive probes of the CCVd, the CSVd and the ASBVd.

Northern Blot Hybridisation. On the whole, many fewer hybridisations were obtained after transfer to membrane following return electrophoresis. However, worth noting are hybridisations with the TASVd and an interesting result obtained in conjunction with D. Hanold (mentioned in Hanold and Randles 1991): very strong hybridisations between the nucleic acids of healthy oil palms from Africa and a CCCVd probe used by J. Randles' laboratory (Fig. 3). However, the purified Vd.L.M., which migrates to the CCCVd level under natural conditions, does not hybridise with the non-radioactive marked CCCVd probe supplied by BRESATEC.

Discussion and Conclusion

We have discovered molecules with the same size and electrophoretic properties as viroids in oil palm. However, the behaviour of these molecules in thermogradient electrophoresis suggests that they resemble double-stranded RNAs more than they do a viroid. Moreover, although the largest Vd.L.M. migrates to the level of the CCCVd, it differs from it since it does not hybridise with the probes corresponding to this viroid. Neither the CCCVd nor the Vd.L.M. therefore seems to be responsible for BR. Indeed, although we obtained certain hybridisations between the nucleic acids of diseased palms and the CCCVd probe, we also obtained hybridisations with the nucleic acids of healthy palms from Africa where BR does not exist, and we even obtained hybridisations with various other viroids (TASVd, HSVd, etc.). These results resemble those obtained on coconut by Hanold and Randles (1991), which is not surprising since these two plants are very similar and it is known, for example, that they are susceptible to several common diseases (cadang-cadang, Hartrot, Blast, Dry Bud Rot). It may be that oil palm and

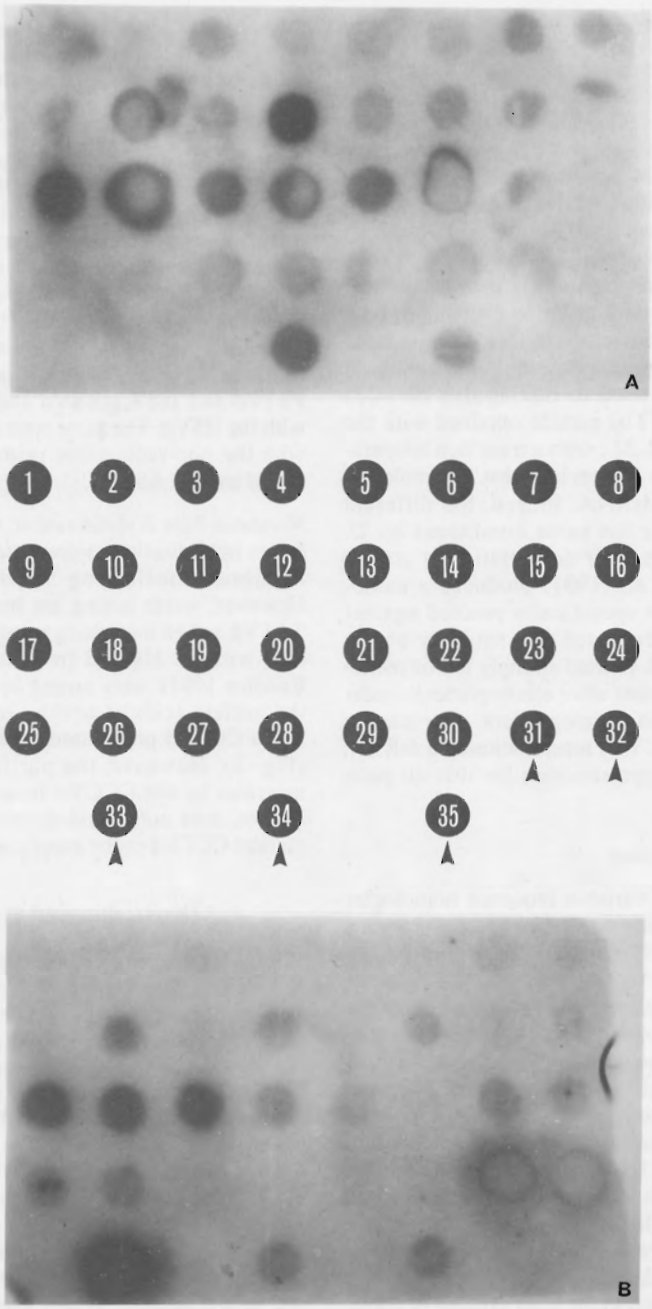

Figure 2. Autoradiographs of cellulose filters carrying oil palm nucleic acid extracts after hybridisation with a probe corresponding to (A) HSVd, (B) PSTVd. Sample types: 1, 6, 7, 13, 15, 17, 18, 19, 23, 27 = healthy palms from Ecuadorean Amazonia; 2, 3, 4, 5, 8, 9, 10, 12, 14, 16, 20, 21, 22, 24, 25, 26, 28, 29, 30 = diseased palms; 11 = healthy palms from West Africa. Controls: 31-32 = Chrysanthemum infected by CSVd; 33 = Potato infected by PSTVd; 34-35 = diseased peach from southern France.

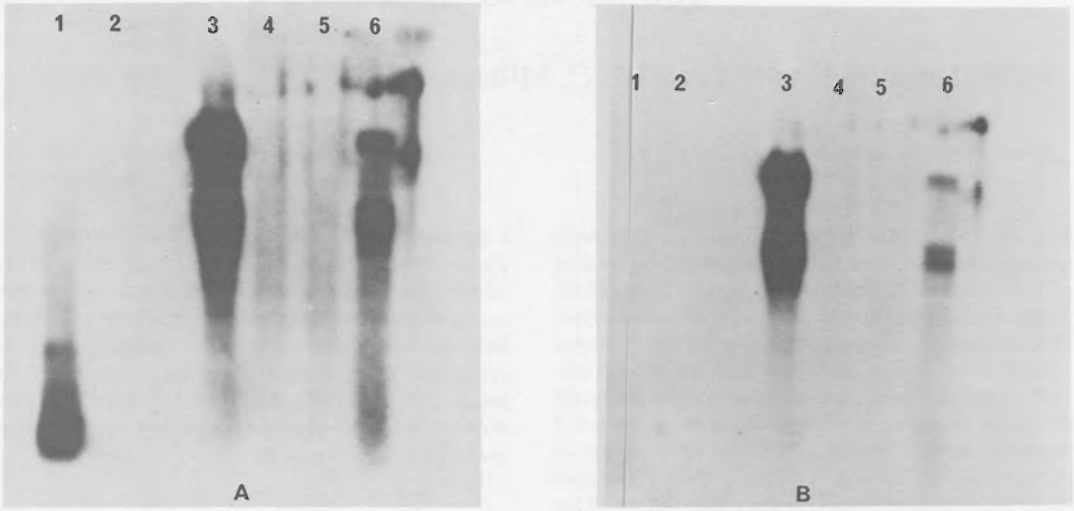

Figure 3. Hybridisation assay of electroblots from agarose gel electrophoresis. Probe: CCCVd. 1 = CCCVd; 2 = CSTVd; 3, 4, 5 = diseased oil palm from Ecuador; 6 = healthy palms from West Africa. A = Wash 1 x SSC, 65, 2 hours; B = Wash 0.1 x SSC, 65, 2 hours.*

coconut have nucleic acids containing sequence homologies with the PStVd group of viroids, a group to which the CCCVd belongs. It would obviously be worthwhile characterising all these molecules to exactly determine their relationships with pathogenic viroids.

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Studies of Coconut Foliar Decay Disease and its Virus Agent

J.W. Randles*, J.P. Morin† D.C. Millar*, D. Hanold* and W Rohde§

FOLIAR decay is a disease of introduced coconut palm cultivars in Vanuatu that is induced by feeding of the cixiid bug (plant hopper), *Myndus taffini* (Julia 1982, Julia et al. 1985). It is also known as New Hebrides coconut disease and foliar decay of coconut caused by *M. taffini*. It has not been recognised outside Vanuatu. The disease is economically important because of its present influence on a regional coconut palm selection and breeding program in Vanuatu. It first appeared around 1964 in introduced lines from Malaysia, causing the death of most of the palms within 3–5 years. Most varieties introduced from other countries show low, medium, or high levels of sensitivity and have been excluded from selection and breeding programs because of their susceptibility.

Symptoms

A range of symptom severity is observed in affected introduced cultivars and their hybrids, whereas the local *Cocos nucifera* L. 'Vanuatu Tall' (VTT) population is symptomless and its hybrids show mild symptoms (Calvez. et al. 1980). The cultivar Malayan Red Dwarf (MRD) is highly susceptible to coconut foliar decay disease (CFD) in the field and is used as an indicator plant for epidemiology and insect transmission studies. In the field, yellowing first appears in several leaflets of the fronds 7 to 11 positions down in the crown from the unopened spear leaf. More general yellowing of the fronds ensues and they develop lateral necrosis of the petiole and die prematurely, hanging from the petiole downward through the canopy. Other fronds become yellow and die as they reach position 7 to 11 in the crown, so that affected palms characteristically show a normal apex, several yellowish fronds, then several upper dead fronds hanging through green older fronds. The trunk generally narrows and may thicken again if remission occurs, as in a tolerant palm. Susceptible cultivars die between 1 and 2 years after symptoms appear. Spathes at the base of yellow fronds rot if the subtending frond dies, or they produce an inflorescence that develops normally but bears fewer nuts than normal.

Experimental Transmission

Seedlings that are experimentally inoculated in insect-proof cages with *M. taffini*, collected in the field, show similar yellowing symptoms on fronds, in positions 3 to 5 below the youngest frond, from 6 to 11 months after inoculation. The frequency of transmission increases as the number of insects, or the duration of the acquisition feed is increased. Transmission with artificially cultured *Myndus* has not yet been demonstrated, and the parameters of transmission are yet to be determined.

A correlation has been observed between the distribution of diseased palms and *M. taffini* in plantations, and this, together with the demonstration of transmission of CFD by adults of *M. taffini*, shows that the disease is caused by a transmissible agent. It seems unlikely that an insect toxin is involved because small groups of *M. taffini* can transmit the disease during a short inoculation feed.

Identification of a Disease-Associated DNA

Determination of the aetiology of CFD is particularly important so that diagnostic procedures may be developed for application to epidemiology and control methods. A single stranded DNA (ssDNA) of low electrophoretic mobility in 5% polyacrylamide gel was found to be specifically associated with diseased Malayan Red Dwarf coconut palms (Randles et al. 1986). This DNA (CFDV-DNA) was extractable in low amounts only. It was further characterised to determine whether it could be used as the basis of a diagnostic assay, and to help in

* Plant Pathology, Waite Institute, Private Mail Bag, Glen Osmond, SA 5064.

† CIRADF-CP, BP 5035, 34032, Montpellier, Cedex 1, France.

§ Max Planck Institut für Züchtungsforschung, Carl von Linne Weg 10, 5000 Köln 30, Germany.

predicting the type of virus particle in which it may be encapsidated.

The DNA was spread for examination by electron microscopy, and showed circular molecules of mean mol. wt. 4.3 x 10^5. Circularity of the DNA was also demonstrated by its unique pattern of migration in two-dimensional polyacrylamide gel electrophoresis (PAGE) and by the absence of free ends in a biochemical test.

Analysis of the DNA by sedimentation gave an S value of 12S to 15S. The detection of two or three bands in non-denaturing gels which have the same electrophoretic mobility in the second denaturing dimension of 2-dimensional gels suggests that under 'native' conditions the DNA occurs as two or three conformers which differ in secondary structure.

All previously described plant viruses with ssDNA are placed in the geminivirus group. Geminiviruses contain either one of two circular ssDNA molecules of mol. wt. approx. 7 x 10^5 to 8 x 10^5, and comprising about 2700 nucleotides (Harrison 1985). The CFDV-DNA is significantly smaller than geminivirus DNA, but is close to the size of the DNA of porcine circovirus (Tischer et al. 1982) which sediments at 12.7S, has a contour length of 633 μm, 1.76 kilobases, and a mol. wt. of 5.8 x 10^5.

A 20 nm Virus Particle Associated with CFDV-DNA

Unusual 20 nm icosahedral particles which copurify with CFDV-DNA are considered to be the particles of coconut foliar decay virus (CFDV).

The purification procedure was as follows (Randles and Hanold 1989): Leaflets from inoculated Malayan Red Dwarf seedlings were chopped and blended, and a polyethylene glycol 6000 precipitable component prepared. This was resuspended in 10 m*M* Tris-HCl (pH 7.2), clarified by centrifugation at 10000 g for 20 minutes, and then centrifuged at 360000 g for 1 hour. The pellet was resuspended in 0.6 ml of the Tris buffer and incubated for 1 hour at 25°C with 0.2 ml of serum from a rabbit immunised against polyethylene gycol precipitated material from healthy coconut leaf. The resulting precipitate was removed by sedimentation at 37000 g for 10 minutes and the supernatant was centrifuged at 270000 g for 1 hour. The pellet was resuspended as above and subjected to isopycnic density gradient centrifugation in a 2.4 ml preformed 30–60% Nycodenz (Nyegaard, Oslo) gradient. Centrifugation was done in a Bechman TLA-100.3 rotor at 103000 g for 15 hours at 5°C. Fractions (0.2 ml) were displaced from the top of the gradient with Fluorinert FC-40 (Sigma), diluted, and subjected to high-speed centrifugation as above. Pellets were resuspended in the tris buffer, and aliquots were assayed for DNA by PAGE (Randles et al. 1987) or dot blot hybridisation (Hanold et al. 1988; Randles et al. 1992). Electron microscope grids (400 mesh) coated with formvar-carbon films were made hydrophilic by high-voltage glow discharge. Samples were diluted in water, placed on the grids for 5 minutes, drained and the grids were washed once with water. They were stained for 10 seconds with 2% uranyl acetate or shadowed at 20° with Pt-Pd. Electron microscope magnification was calibrated with a cross-grating replica 22160 lines/mm (Agar, UK).

Particles 20.0–0.4 nm in diameter were exclusively associated with the CFDV-DNA. The number seen was approximately proportional to the amount of CFDV-DNA detected in the fraction. Some stained particles appeared hexagonal in outline, and shadowed particles also showed an angular outline which would be consistent with an icosahedral structure of 12 vertices and 20 faces (T = 1 symmetry). Smaller spherical particles of 12–15 nm in diameter were also detected in lower density fractions from both healthy and diseased plants and a few were commonly seen in preparations of the 20 nm particles.

The particles could be extracted from fresh or frozen leaves and their morphology was not affected by treatment for 1 hour at 25°C with chloroform, 50% Cs_2SO_4, 100 m*M* sodium acetate buffer (pH 4.0).

Both the DNA and the particles occur in very low amounts in diseased coconut palms. The infectivity of the particles has yet to be tested, but their distribution should allow the development of serological tests for determining the epidemiology of CFD. The particles are similar in size and symmetry to half-geminate (Hatta and Francki 1979) particles and to subterranean clover stunt virus (SCSV) particles (Chu and Helms 1987). They differ from these other plant viruses in that their single-stranded circular DNA is intermediate in size between that of geminiviruses (ca 2700 nucleotides) and SCSV (900–1000 nucleotides). They most closely resemble the porcine circovirus (Tischer et al. 1982).

The Nucleotide Sequence of CFDV-DNA

The number of DNA molecules representing the genome of CFDV is not known. To obtain sequence data the circular single-stranded CFDV-DNA from infected plants was purified, amplified by the polymerase chain reaction, and cloned. The complete sequence was established by analysis of overlapping subgenomic cDNA clones. The complete sequence comprised 1291 nucleotides and contained possible open reading frames for six proteins of molecular

weight larger that 5 kDa. One of these (ORF1, 33.4 kDa) codes for a leucine-rich protein with the nucleoside triphosphate-binding motif GXGKS and may possibly participate in virus replication. The putative viral protein encoded by ORF3 (6.4 kDa) is a positively charged arginine-rich protein with homology to the capsid protein of nuclear polyhedrosis virus, and may represent the CFDV coat protein. The DNA can form a stable stem structure of 10 GC base pairs subtending a loop sequence which in one orientation closely resembles the motif TAATATTAC conserved in a similar structural arrangement within the geminivirus group. Otherwise no sequence homology to DNA-containing plant viruses of the gemini- or caulimovirus groups was found. CFDV, therefore, represents a new taxonomic group of plant viruses (Rohde et al. 1990).

Diagnosis of CFDV Infection using DNA Detection Methods

PAGE

CFDV infection can be recognised in coconut using PAGE to identify CFDV-DNA. It migrates as a single band in denaturing polyacrylamide gels, but generally as two bands in non-denaturing gels (Randles et al 1987).

Routine diagnosis by this method lacks proof that bands have nucleotide sequence similarity to CFDV. Therefore, hybridisation assays for the DNA were set up.

Hybridisation assays with labelled complementary DNA

Hybridisation tests are far more sensitive than PAGE assays for coconut palms, and probably have broader applicability to other species for which the PAGE nucleic acid preparation procedure developed for coconut palms may not be appropriate. We have found that palms assayed just before the appearance of symptoms generally had detectable levels of CFDV-DNA. Diagnosis of CFD by symptoms is not reliable, and only MRD palms show characteristic symptoms of the disease.

Detection of CFDV-DNA independently of symptomatology allows a more reliable evaluation of epidemiology to be undertaken. For example, hybridisation assays indicate that the symptomless VTT coconut palm variety can be infected. This raises the possibility that this variety, which is the main component of plantations in Vanuatu, could act as a latent reservoir of virus infection for sensitive varieties. Neither *Hibiscus tiliaceus*, the host palm of *M. taffini*, nor geminivirus-infected *Digitaria sanguinalis* contained detectable sequences of CFDV-DNA, and neither can therefore be implicated as the natural alternate virus source. Further development of more reliable and faster dot blot assays will allow other species to be assayed on a large scale as potential natural hosts of CFDV.

The hybridisation technique has been used: to locate CFDV-DNA in various tissues of palms and to indicate the level of sampling needed for reliable diagnosis; to show the vascular location of CFDV in rachis and leaf; to show that CFDV-DNA can be detected in the body of the vector; and that in cases where the signal was sufficiently discrete to show that it was located in the abdomen rather than the head.

A non-radioactive probing system has also been developed as an alternative detection method. This uses digoxygenin (DIG) labelled complementary RNA (cRNA).

Generally, all steps in diagnostic tests must be optimised. It was found to be necessary to inhibit tannin production during extraction of leaf and to include an alkali incubation step. Tests with the non-radioactive DIG-cRNA probe utilising antibody against the probe and an alkaline phosphatase-based assay required a more rigorous alkali incubation than tests with the radioactive probe. This was necessary to prevent development of a non-specific endogenous reaction with the non-radioactive probing system. We have previously observed apparent endogenous phosphatase activity in coconut nucleic acid extracts which was highly stable and interfered with colorimetric detection methods.

Applications of the hybridisation assay

CFDV-DNA was distributed unevenly in whole plants and in an apparently non-systematic pattern. Thus, multiple sampling of each plant is needed for reliable diagnosis. The result of the examination of 29 whole palms indicated that root sampling should be combined with leaf sampling, particularly where new areas are being surveyed for CFDV incidence. We conclude that no preferred sampling position on leaflets or on fronds is indicated, but a minimum sampling should include tissue from fronds 1, 3 and 5, and secondary roots.

To localise virus-containing tissue, hybridisation and macro- and micro-autoradiography have been done on unsupported intact sections of coconut rachis. The location of the virus as indicated by the probe, in vascular bundles and phloem in particular, probably accounts for the low recovery of virus from tissue and the need to use alkali maceration to release CFDV-DNA for hybridisation assay.

Pathology

Symptoms of CFD in highly susceptible cultivars are yellowing of leaflets in upper to central fronds, and progressive necrosis of the outer edge of the petiole. This necrosis is the site of collapse, causing the death of fronds. Autoradiography of a diseased palm showed that the virus DNA was present in the rachis of a young asymptomatic frond, and both within and adjacent to the necrotic region at the early stages of lesion development on an affected rachis. It was not detected within the necrotic zone of an older frond of the same tree, but it was detected in the non-necrotic zones either side of this older lesion. These observations would be consistent with CFDV being associated with and causing this necrosis, then becoming degraded several months after the development of necrosis. No reason is known for the localisation of necrosis on petioles in a particular part of the crown. The only other symptom, namely, yellowing of leaflets, may be associated with cytopathic effects in phloem and reduced carbohydrate translocation.

CFDV can be detected in leaves of seedlings within six months of controlled inoculation with *Myndus*, and symptoms appear 1–4 months later. Palms infected by natural exposure in the field show CFDV in fronds sampled within 4–7 months of symptoms first appearing. The disease-free trees, including tolerant cultivars, and palms in disease remission, generally contain CFDV-DNA in leaflets and rachis, and a large reservoir of virus can, therefore, exist in apparently disease-free coconut growing areas.

Although CFDV-DNA has been detected in embryos, no transmission of CFDV through seed has been demonstrated. No CFDV-DNA was detectable in pollen samples. However, husks contain CFDV, and movement of nuts can therefore allow transfer of virus to new sites. It will be necessary to determine whether vectors can acquire virus from green husk to decide whether there are quarantine risks from the movement of nuts.

Future Studies of the Vector

The vector, *M. taffini*, commonly settles on coconut fronds at the junction of leaflet with rachis, where our in situ studies have shown that virus molecules are likely to be available. Observations of feeding sites and behaviour should now be attempted to determine whether virus may be acquired by phloem feeding. The frequent detection of virus in coconut roots raises an alternative possibility that the soil-inhabiting larvae of *Myndus* could acquire virus from or infect, coconut roots during their pre-adult stage. We have not detected CFDV in the dicotyledon host of *Myndus* larvae, *Hibiscus tiliaceus*, but the frequently occurring proximity of coconuts to *Hibiscus* could allow acquisition of virus by larvae from roots of infected coconut palm growing adjacent to roots of *Hibiscus*. The association of CFDV-DNA with the abdominal site of *Myndus* is consistent with accumulation of virus in the gut. Experiments now need to be done to determine whether the virus accumulates or replicates in the insect, and/or its eggs, to determine whether it has a semi-persistent, circulative, or propagative association with its vector.

Control

The eradication of the *Hibiscus* host of *Myndus* has been suggested as a control measure for CFD, but its effectiveness has yet to be demonstrated. The use of hybrids between VTT and the local dwarf, VRD, thus combining the advantages of tolerance to the disease and heterosis, is currently being developed for use in Vanuatu. Although the potential performance of these hybrids is below that of F1 hybrids used in other countries, they are the preferred option.

Future work will be directed towards: mapping the distribution of the virus in Vanuatu and any other countries where it is detected in future; studying the genome of the virus to determine whether cross-protection strategies can be developed; determining the nature of the association of the virus with its vector (i.e. its persistence; ability to replicate in the vector; and whether it can be passed through the egg); and utilising any new techniques in molecular biology and tissue culture of coconut as they are developed.

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Canopy Development and Light Interception of Coconut

M.A. Foale*, G.R. Ashburner† and D. Friend§

THE coconut palm has the potential, under favourable climate and soil conditions, to live for over 100 years. In the absence of seasonal variation in temperature and water supply, the palm produces a new frond every 25 to 30 days throughout much of its life. In spite of this regularity, however, the shape of the coconut crown, and its ability to intercept light, go through a distinct series of changes with age of the palm. A lack of appreciation of the inevitable changes, that are 'built in' to the coconut as it ages, has led to confusion in much of the discussion in the literature about intercropping and yield potential (Corley 1985). It is the objective of this note to describe these changes and propose an hypothesis to explain them.

Shape of the Coconut Crown

Phase 1 — pre-flowering.

Under favourable conditions, the young palm produces in its third year nine or ten new fronds and, by the end of that year, the foundation of the trunk is well-developed. In a palm of the tall type the base of the trunk is up to 0.8 m in diameter, tapering quickly to about 0.4 m. During phase 1, all the fronds maintain throughout their life (of about 2 years) a declination, from the vertical position of the newly-emerged frond, of less than 90 degrees (Fig. 1). Phase 1 ends when flowering begins in the sixth or seventh year.

Phase 2 — early flowering.

At the beginning of this phase, the rate of leaf production has risen to 12 to 15 per year, and the crown assumes a hemispherical shape as each frond descends gradually throughout its life to a maximum declination of 90 degrees (i.e. a horizontal position — Fig. 2). The dense mass of fronds arranged between the vertical and horizontal positions intercepts a high proportion of the incident light. The rate of increase in height of the palm begins to fall during this phase, as assimilate is diverted from trunk extension to fruit growth.

Phase 3 — high productivity.

At about the 15th year, the palm achieves its peak light interception and approaches maximum fruit production. The lower fronds begin to droop and, over a few years, the crown evolves towards a spherical shape (Fig. 3). Under stress conditions induced by a very heavy load of fruit, or sometimes by water deficit, a large number of lower fronds will droop to form a 'skirt' (Fig. 4), resulting in sharp reduction in light interception.

Phase 4 — diminishing productivity.

Beyond about the 30th year there is a gradual reduction in the rate of both frond emergence and frond longevity, resulting in a declining number of fronds in the crown (Fig. 5). The length of the frond also decreases, contributing further to the reduction in light interception caused by the falling number of fronds. Production of fruit falls and the diameter of the trunk diminishes. This phase may last for 30 years or more.

Phase 5 — loss of production, and senescence.

With advancing age beyond 60 years (or later in favourable environments), the decline in rate of frond emergence as well as longevity continues. Fronds also show a progressive reduction in length. A point is reached when little if any fruit can be supported and the newly-formed portion of the trunk is thin and fragile (Fig. 6). The fragile upper trunk of the ageing palm may eventually be broken by a gust of wind.

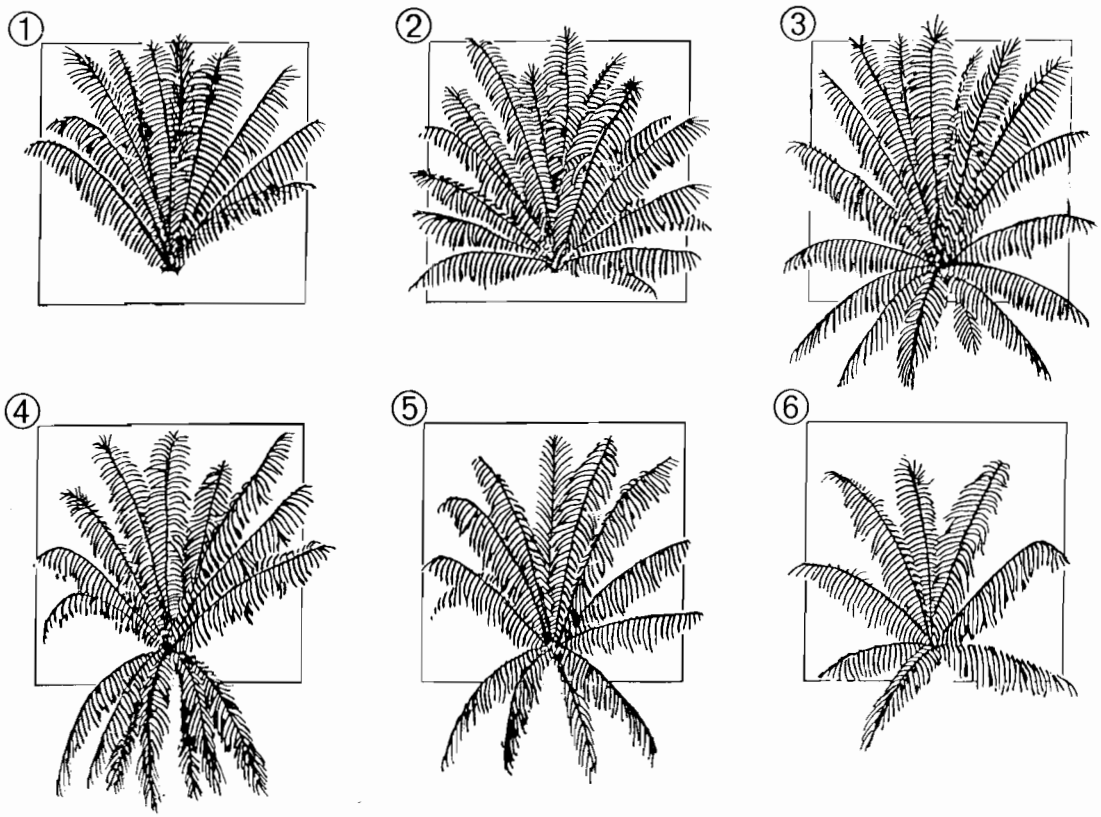
The Anatomy of Frond and Trunk

The evolution of size and shape of the palm crown through the phases described is related to length of frond, number of fronds retained, and the rate of increase in declination from the vertical of each

* Consultant to ACIAR, CSIRO Division of Tropical Crops and Pastures, 306 Carmody Road, St. Lucia, Queensland 4067, Australia.

† IHD, Private Bag 15, Southeast Mail Centre, Vic 3176, Australia.

§ Levers Solomons Ltd, Yandina, Solomon Islands.



Figures. 1–6. This series of drawings of the crown of a coconut palm at different stages of its life illustrates the evolution of shape that takes place: 1. In a young palm less that 8 years old all fronds retain an upward sweep. 2. As the palm begins to produce fruit, physical pressure between the frond bases begins to force them downwards, giving the crown a hemispherical shape. 3. A 'fully mature' palm assumes a spherical shape as few fronds reside in a horizontal position and many decline below that plane. 4. An extreme case of rapid 'drooping' of fronds in response to a heavy load of fruit or to severe water deficit. 5. An older palm showing signs of reduced longevity and size of the fronds, which results in a further decline in light interception. 6. A senescent palm showing a low number of surviving fronds and rapid drooping of new fronds.

frond with age. In a young palm each frond sustains an upward declination indefinitely, but as the palm enters the production phase the declination of the fronds tends to increase gradually during its life, until it finally occupies a horizontal position. The effect of this change is to transform the crown from a loose inverted cone (Phase 1) to a hemispherical shape (Phase 2). Both of these configurations achieve high light interception per unit of leaf area.

The next phase (3) is characterised by the progressive development of a spherical shape in which, with increasing age, more of the lower fronds decline below the horizontal position. The resultant dearth of fronds in a position close to horizontal results in much lower light interception by the whole crown, especially when the sun is at high elevation. As a palm that is located in a stand of moderate to high density tends to be shaded by its neighbours early and late in the day, a spherical crown is a poor type in a plantation. Such a shape in a palm that stands alone, however, achieves enhanced interception when the sun has a high declination early and late in the day.

The Hypothesis of What Brings About the Change with Age

We propose that the reason for the more rapid increase in declination of the older fronds, of both highly productive and older palms, is a weakening of the attachment of the base of the frond to the trunk. As the internode length (i.e. the space between the position of attachment of adjacent fronds) of a young vigorous palm decreases—in response to a reduced supply of assimilate to the trunk—the frond bases, which are broad and thick, become 'crowded' and exert mutual pressure. This pressure can only be relieved by an outwards expansion, during which the fibres holding the thickened frond base to the trunk are stretched and weakened. When the frond is freed from the central cluster, the weak fibres allow it to descend over a few months from a position with a small, to one with a large, declination (Figs. 3, 4).

The small circumference of the coconut trunk, compared to date and oil palms for example, is probably an important adaptation imparting a flexibility to the trunk which protects it against wind damage. This trait, combined with the tightly bound upper cluster of young fronds of the coconut, withstands all but the most severe wind. However, the evolution of this successful vegetative structure, for survival on the hurricane-affected natural coastal habitat of coconut, has certain consequences later on in the life of the palm. In the older palm the weakness that predisposes the older fronds to a rapid increase in declination, and to be detached readily from the palm by cyclonic wind, (which would also contribute to improved survival), has the inevitable consequence of a low potential for light interception.

Confirmation of the Hypothesis

Some study of the arrangement of the basal tissues in the upper cluster of fronds is needed to confirm the hypothesis put forward here. A vertical section cut through the crown in a series palms displaying the range of phases described would reveal the differences with age in the physical relation of the frond bases to each other. We suggest that signs of the outward pressure which weakens the attachment of the frond to the trunk would be apparent and could be confirmed by the insertion of a penetrometer between adjacent frond surfaces, as well as by detection of signs of stretching of the basal fibres.

Full investigation of the changes with age would require the sacrifice of several palms. It would be a worthwhile project, however, as there has been confusion about what constitutes a desirable shape of coconut crown, and little advance on the recommendations of Pieris (1937), cited by Dwyer (1938). If the confounding effect of age of the palm on the shape of its crown can be eliminated, it should be possible to select mother-palms according to more effective guidelines.

Effect of Crown Shape on Productivity of a Farming System

The changes described are of great importance in considering the cultivation of other crops in a farming system in which coconuts are present. It is clear that the potential productivity of intercrops will vary as the coconut stand captures a different share of production resources at different times in its life cycle.

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Prospects for Future Coconut Research in the South Pacific: A Personal View

M.A. Foale*

THERE are many environments in the South Pacific that are highly favourable to the growth and productivity of coconut. They meet the main requirements of the crop for a non-limiting water and nutrient supply, combined with only minor seasonal fluctuation in temperature and a high level of solar radiation. The principal departure from these conditions is due to seasonal and/or episodic water deficit, especially where this is exacerbated by shallow soil or a saline water table.

In this paper, the yield-influencing factors connected with climate, soil and genetic potential of the coconut are reviewed. An attempt is then made to identify research areas in which new knowledge might contribute to improved performance of coconut either alone or in mixed culture. No attempt is made to deal with the vast and complex area of pests and diseases except to allude to examples of adaptation in coconut that have overcome or mitigated the effect of a pest or disease.

What are the Limitations on Coconuts?

Water

The least amount of rainfall that could provide non-limiting water to crops in the equatorial zone is about 2000 mm. If the soil had a high capacity for the retention of water, and if little water runs off due to high rainfall or to soil saturation, coconut grown under such rainfall would rarely experience water deficit. In reality, even where the annual rainfall exceeds 2500 mm, seasonality and runoff result in episodes of mild to severe water deficit in most years. In an environment where annual rainfall exceeds 3000 mm there is likely to be a constraint on productivity due to episodes of excessive cloudiness and low solar radiation.

Nutrients

Many soils in the region are derived from relatively young geological formations, often incorporating either volcanic ash or basalt. These are high in nutrients such as phosphorus and sulphur, and in cations, except that rain-borne sodium may displace these from the clay complex. Potassium is frequently in short supply and therefore often needed in fertilizer to replace that which is removed from the system. Nitrogen is not often deficient on the high islands, due apparently to the high rate of turnover of biomass as well as fixation by leguminous and possibly other plants. Intense land use, especially growing food crops under the coconuts, is likely to reduce the reserve of nitrogen.

On atolls, coconut growth is often constrained by the deficiency of manganese and iron whose availability is lowered by the high level of soil alkalinity. Where rainfall and therefore biomass production is high however, such deficiency is not common, perhaps because the nutrients are taken up from the decomposing organic layer. Nitrogen is sometimes a limiting factor on atolls, especially those with low rainfall and a consequent low level of biomass cycling.

Sulphur deficiency is acute in certain ecosystems where grassland has displaced forest in the distant past, due perhaps to human activity. Frequent firing of the grass has led to loss of sulphur which is especially volatile, so this nutrient must be applied. Some soil types remote from the coast, and therefore from the accession of salt in rainfall, are deficient in chlorine, but I am not aware of any instance of this in the South Pacific.

Plant density

This is a vexed question in coconut, due in large part to a lack of appreciation of the evolution of crown size and shape with age (Foale et al., these proceedings). There is clear evidence in old plantations that they intercept only a small fraction of incoming radiation. Planting anew at a high density, however, is likely to result in intense competition between the young palms as they approach maximum leaf area, resulting in an extended period of rapid stem elongation, to the detriment of yield.

* CSIRO Division of Tropical Crops and Pasture, 306 Carmody Road, St. Lucia Queensland 4067.

A density that allows the early development of fruit and maximises early yield will inevitably appear to be too low 15 years later when the crowns take on a more open shape and frond length begins to diminish. It is advisable to consider coconut as a sole crop only in the first 10 to 15 years and then plan for appropriate intercrops which either respond favourably to shade (such as cocoa) or are not adversely affected (such as pasture in which the production of biomass is the principal concern). Market forces may dictate that intercropping should begin simultaneously with planting of coconut. This is quite feasible for a short while with annual crops, but creates conflict in the case of a perennial crop such as cocoa. Early planting of cocoa under coconut is usually done in association with a reduced density of planting of coconut so that shading will not be excessive when light interception by the coconut palms peaks at around 15 years. Further into the future, however, the density of coconut will become insufficient as its light interception progressively falls. Additional shade would then be required.

Germplasm

Glowing reports of high yield from hybrids (especially in the early years) have led to their adoption on quite a large scale in some parts of the world. Recently, however, there has been reason to question the wide use of a limited range of germplasm in this way because of the widespread fatal attack of disease, in particular *Phytophthora*. There have also been attacks by insects such as *Scapanes*, a type of rhinoceros beetle which is quite devastating when the black palm weevil (*Rhyncophorus*) is present, and also *Brontispa* on introduced hybrids in Papua New Guinea and Indonesia. In Tanzania, hybrids show far less tolerance to water deficit than the local tall population, and in Vanuatu all exotic germplasm shows little or no tolerance to Foliar Decay Virus which induces no symptoms in the local populations.

There has been a misleading interpretation of the high yield that hybrids have demonstrated in some countries. Whereas it seems that their peak yield potential is around 30% higher than for a tall population, their much higher yield than talls in the first few years has caused confusion. In years 4, 5 and 6 from planting talls may yield very little, while a hybrid may accumulate 4 to 5 t/ha of copra production. Clearly this precocity is a valuable trait, but by year 12 the gap in annual production will be reduced to about 30%. Reports that hybrids will produce double or treble what talls do are based on the first two to four years of production, and often refer to young hybrids compared to old talls. Therefore these reports must be placed in the long-term perspective.

An unknown aspect of hybrids is their long-term ability to sustain production. There are indications that light interception in hybrids may suffer as a consequence of their very ability to achieve a high yield. A high yield is usually associated with reduced partitioning of dry matter to support stem elongation, which results in very short internodes and crowding of the frond bases. It is likely that this is responsible for a high proportion of fronds descending to a downward position in the crown not long after they are freed from the central cluster of young fronds at the apex of the palm. On some hybrids the lower fronds form a distinct 'skirt' which has poor light interception. Eventually a decline in overall yield, limited by reduced light interception, is likely to result.

Catastrophic wind damage to a coconut plantation would in many locations be experienced only rarely. Nevertheless, the assurance that the palms are robust enough to cope with such wind would be of great value. There is a danger that some of the high-yielding hybrids may in fact embody reduced strength as a consequence of the high partitioning of biomass to yield. On the other hand, a hybrid that is based on at least one parent with adaptation to high wind would be valuable, even if its peak yield were a little less than the best performers.

What are the Research Needs?

Apart from plant protection what are the most important issues for future research? As one who is placed somewhat apart from the coconut scene in the South Pacific, I can only present here the perception of an onlooker rather than an involved player. In the course of the seven years or so spanned by the ACIAR Coconut Improvement Project, however, it has at least been possible to see some of the issues in a wide context. The following remarks should be taken from that perspective.

Water deficit

It might be possible to develop means to reduce the intensity of water deficit. If there is a problem of runoff of high-intensity rainfall, then improved retention and management of surface residues might assist infiltration. An increase in the amount of water stored in the soil will reduce the duration of periods of deficit.

When there is a predictable seasonal deficit, management should be aimed at reducing water use by associated plants. Annual intercropping should cease or be restrained at that time and perennial intercrops might be pruned, or in the case of pasture it should be grazed or slashed short to minimise water use. It is generally not practicable or economically sound to irrigate coconut.

The density and age of palms influence the rate of water use and therefore the duration and severity of periods of deficit. As coconut palms achieve maximum light interception around year 10 and sustain this for 10 years or more, that is the period when most severe deficit is likely. Research on the soil water balance of plantings of this age would set an upper bench-mark for the rate of water use which could help in devising management of intercrops.

Nutrients

I am not aware of any particular challenges yet to be met in the area of identifying limiting nutrients. Over the past 30 years the effects of deficiency of sulphur, chlorine, manganese and iron have been well documented. The principal challenge in my view is to achieve management that will reduce to a low frequency the occurrence of nutrient limitation to growth and production. A first approach to this is to maximise the return to the soil of biomass from the system, especially components high in major nutrients such as nitrogen and potassium. Work is needed to achieve a good rate of nitrogen fixation by herbs, shrubs and trees intercropped with coconut.

In the case of potassium, a deficiency is harder to remedy without resorting to artificial fertiliser. Appropriate research may identify ways to prolong the retention in the system of added potassium. Different options for the disposition of residues, especially the husk, should be explored in order to maximise the recycling of potassium.

Foliar analysis appears to be an effective tool for identifying nutrient deficiencies. There is a need to compile the total of current knowledge of nutrient levels in the leaf into a simple accessible information resource for the use of farmers and extensionists. Such a reference would assist in the interpretation of raw analysis values for nutrients in the light of the age and genotype and current productivity of the palms. The effect of the local climate, as well as seasonal conditions at the time of sampling, soil type, and the nature of other components in the farming system would also be taken into account.

One of the decisions faced by the coconut farmer is whether to adopt a new crop, or to intensify current intercropping activity. An example of this is the introduction of pumpkins into the coconut farms of Tonga. The short-term financial gain is very attractive and there are no data available to show precisely what long-term detrimental effect may result for soil chemical and physical properties, the vigour of the coconut palms and the productivity of other intercrops such as traditional food crops. Research is needed on key components of the farming system to show the change that is induced in soil organic carbon, level of nutrients in the soil, especially nitrogen, as well as soil water balance, rainfall runoff and soil erosion.

Plant density

Appreciation of the changing significance of the density of the palms is important when considering the choice of intercrop. A coconut stand will achieve peak light interception at around ten years of age, sustaining that level for about ten years until it begins to decline with change in the shape of the crown of the palm. There could be a threefold increase in transmitted light beneath a coconut canopy between year 10 and year 40. Limited work has been done in measuring light transmission under coconut canopies of uniform age whereas the reality is that many farmers appear quite deliberately to contrive a canopy of mixed age. The highly indeterminate growth habit of many intercrops allows them to respond according to the amount of light energy available locally under the coconuts.

More information is needed on the distribution patterns and amount of transmitted light under such informal-looking coconut canopies to predict accurately potential yield from new crops. The farmer has less interest in the matter of 'optimum' density for coconut as he bases his decision-making on an appreciation of the value of all the products generated by the mix of crops that is growing. The fact is that the 'best' density of the coconut component at one age, for maximum profit from the other components as well as the coconuts, will differ from the best density when the coconuts are older.

Germplasm

Before really widespread adoption of high-yielding hybrids is accepted as a desirable long-term objective, closer attention must be given to identifying the important local adaptive traits of the current coconut population. The amount of local adaptation is not always obvious as it is generally expressed by the absence of symptoms. This would apply to insect or disease attack as well as to the effect of strong wind or prolonged water deficit. Tests of high-yielding hybrids should always include as a control treatment some crosses based on local material descended from the 'indigenous' coconut population.

Where there is a need to meet a particular market requirement there may arise a demand for specific traits, especially in the fruit, that could best be met by the introduction of germplasm from another region or even another country. Freedom to carry out appropriate introduction of germplasm will depend on quarantine regulations. Currently the movement of germplasm worldwide is constrained due to uncertainty about the possible pathogenicity of viroid-like RNAs that has been found to be widespread in

coconut populations. The RNAs show a close resemblance morphologically to the pathogenic coconut cadang-cadang viroid of the Philippines. Current ACIAR-supported research aims to determine the degree of relatedness, in a molecular sense, of the RNAs to the viroid, and to develop an inexpensive indexing procedure to distinguish between them in coconut germplasm (Hodgson and Randles, these proceedings).

Conclusion

Apart from research into protection of coconut through better knowledge of diseases and pests, other opportunities for science to contribute to the improved welfare of coconut farmers may well lie in the area of improved management of intercrops. While coconut is not presently an attractive prospect as a cash crop, it lends stability and predictability to a farming system, as well as providing a strong measure of food security for the farmer and his livestock. New developments in micro-scale processing of coconut for high quality oil may lead to a resurgence of its cash value within the local economy rather than for export. In that case there may be a renewal of interest in the development of better farming systems that include coconut, based on affordable and sustainable inputs.

BUROTROP's Global Activities with Particular Emphasis on the Pacific

M. Hazelman*

BUROTROP (the Bureau for the Development of Research on Tropical Perennial Oil Crops) is a network that was created at the initiative of the European Community to strengthen and enhance the coordination of research and development activities in coconut and oil palm. The secretariat is located in Paris, France, where it enjoys excellent support and collaboration from CIRAD (Centre Internationale pour Recherche en Agriculture pour le Developpement).

Being a network, BUROTROP is itself very dependent on, and strongly promotes networking and collaboration with organisations and agencies having similar objectives. For the Pacific, given the small size and isolation of the countries as well as the low number of professionals working on coconut, and considering the importance of coconut to the livelihood of the people, links with BUROTROP and similar bodies is justified and a necessity.

Goals and Objectives

The Articles of Association for BUROTROP adopted at the September 1993 Executive Committee meeting give the goals and objectives of the Bureau as follows:

Goals

To encourage research and development of perennial oil crops and to contribute to the increase in the income of producers, in particular of smallholders.

Objectives

BUROTROP's objectives are to:

- further the exchange of information and experiences between research institutions and organisations concerned with tropical perennial oil crops;
- contribute to the identification of production-constraints and research and development needs;
- offer bilateral and multilateral associations a means to coordinate their operations; and
- identify training needs, analyse and strengthen existing means.

Potential BUROTROP activities

BUROTROP can achieve these goals by:

- encouraging the setting up of national, regional or international producer organisations—to encourage and further their activities; help distribute their products; facilitate dialogue with existing producer associations; increase the interest of donor agencies; and facilitate access to technical and financial resources;
- promoting the constitution of research and development networks on priority issues defined by producers;
- supporting requests for financial support for projects;
- setting up information and data gathering services;
- helping existing information and data gathering services and promoting the distribution of their products;
- sponsoring or financing conferences, workshops, seminars and other meetings; and
- furthering the training of researchers and technicians, and contributing to the financing of these activities.

Organisation and Membership

BUROTROP activities are established and followed up by its Executive Committee where both donor countries and producer countries from Africa, Asia, Latin America and the Pacific are represented. The Pacific region is currently represented by the South Pacific Commission.

The Executive Committee currently includes 15 members (one EEC (European Economic Community), seven from European donor countries, and seven from the producer countries). A Program

* SPC Agriculture Coordinator (acting) and BUROTROP Executive Representative, South Pacific Commission, Private Bag, Suva, Fiji Islands.

Committee of six members is appointed by the Executive and meets biannually to prepare the work program for consideration by the Executive at their annual meetings.

Funding

The operations of BUROTROP are financed by contributions from the EEC, donor countries and other organisations.

Mode of operation

To ensure relevance and as a means to have direct input and guidance from producer countries, BUROTROP has adopted the use of regional meetings and seminars to develop research agendas and projects within regions which it then helps to seek funding. This approach also helps to ensure that outcomes from such meetings are actioned. Thus far, such meetings/seminars have included:

- the First African Coconut Seminar held in Arusha, Tanzania in 1991;
- a seminar on the Constraints to the Development of Oil Palm in Africa in Abidjan, Côte d'Ivoire in 1991;
- a seminar on Coconut held in Kingston, Jamaica in 1992; and
- a seminar on Oil Palm in Latin America and the Caribbean in Santa Marta, Colombia in 1993.

BUROTROP has agreed to support a regional coconut meeting for the Pacific to be held in Fiji in June 1994 which will also be the venue for its Program Committee meeting.

In addition, a number of seminars are also held in collaboration with other organisations. Recent examples include:

- a EUROCOCO (EEC funded coconut) Seminar held in Montpellier, France in September 1993; and
- a Small-scale Technology Seminar to be held in Accra, Ghana in December 1993.

BUROTROP Support for the Pacific

After giving priority attention to the needs of the African, Caribbean and Latin American regions as part of a conscious strategy to bring these regions to a level in coconut development already existing in the Asia and Pacific regions, BUROTROP has now turned its attention to the Pacific. Most significant is BUROTROP's commitment to collaborate with SPC and other appropriate bodies; and to convene a regional coconut meeting in 1994. Other areas of support have included:

- distribution of world maps depicting coconut and oil plam production and consumption.
- distribution to ACP (Asia, Carribean, Pacific) member countries of the semi-annual publication Bulletin Buro Trop;
- sponsorship of four participants (from Solomon Islands, Tonga, Western Samoa and SPC (South Pacific Commission) to the EUROCOCO seminar in 1993;
- sponsorship of an SPC Officer to the BUROTROP-AFOPDA seminar on Small-scale Technology in Africa;
- providing positive evaluation and recommendation for an SPC coconut processing technologies proposal [for which GTZ (German Aid Organisation) has shown interest in funding]; and
- providing general information on coconut with a CARIS-type database on the who, what, where of coconut research which is being finalised and for which a copy will shortly be made available to SPC.

These activities receive financial support provided annually to the Asia and Pacific Coconut Community's (APCC's) COCOTECH (Coconut Technology) meetings.

Although not used at present, the Pacific region can submit project proposals to seek BUROTROP's evaluation as well as to seek their support in securing donor funding. This approach will improve donor acceptance of proposals.

It is strongly recommended, however, that the Pacific representative (SPC) be kept informed of all requests made to BUROTROP as this would enable endorsement and follow-up.

Conclusion

BUROTROP is an international network concerned with the improvement of coconut and oil palm. The South Pacific Commission is the Pacific region's representative on the network's Executive Committee. Although previously not considered a priority, the Pacific region is now actively supported by BUROTROP, which could provide useful links for information, funding and other support.

COGENT and its Global Activities

M. Hazelman*

THE Coconut Genetic Resources Network or COGENT is a recently established network established under the guidance of the International Board of Plant Genetic Resources (IBPGR). The inaugural meeting for COGENT took place in Singapore in December 1992 but its framework came from the guidance of an IBPGR-sponsored meeting in Indonesia in 1991 and a consultancy by Hugh Harris in 1992.

COGENT's secretariat is based in Singapore housed together with the IBPGR's Asia-Pacific Office. However, guidance for policy matters is given by a Standing Committee consisting of two representatives from six regions. The regions and countries represented currently include Africa (Ivory Coast and Tanzania), the Americas (Brazil and Jamaica), South Asia (India and Sri Lanka), East Asia (Indonesia and Philippines), and the Pacific (SPC and Vanuatu).

Goals and Objectives

The goals and objectives for COGENT as agreed to at the first Steering Committee Meeting in Singapore in 1992 are:

Goals. To improve coconut production on a sustainable basis and to increase incomes in developing countries through improved cultivation of coconut and exploration of its products.

Objectives. To further develop an International Coconut Genetic Resources Network to coordinate activities on genetic resources—from exploration to enhancement of germplasm—and so form a basis for collaborative initiatives on broader aspects of coconut research.

Activities

It has been agreed that the Network will function at three levels—national, regional and global—to support the capacity of national programs to conserve and utilise coconut genetic resources. This is to be accomplished through:

1 Establishing and maintaining an International database on existing and future collections (version No. 2 is already with Fiji, PNG and Vanuatu);

2 Encouraging the protection and utilisation of existing germplasm collections (to be actioned);

3 Identifying and securing additional threatened diversity through the development and adoption of suitable technologies and conservation strategies (to be actioned);

4 Promotion of greater collaboration among research groups in producer countries and advanced technology sources in the exchange of germplasm and the development of new techniques (being addressed); and

5 Appropriate training, information dissemination and securing the necessary funding (being addressed).

Current Immediate Concerns

Several matters are being addressed by the Standing Committee (and IBPGR). These include:

1 Recruitment of a Coordinator;

2 Ensuring compatibility of COGENT's goals and objectives with those of IBPGR;

3 Seeking funding;

4 Membership drive and networking to increase the number of member countries and to create awareness about the network;

5 Replacements on Steering Committee for Vanuatu and Sri Lanka;

6 Ensuring the completeness of its germplasm database via the solicitation of further input data (passport and collection data as well as characterisation and evaluation data) from potential countries;

* COGENT Committee Member, South Pacific Commission, Private Bag, Suva, Fiji Islands.

7 Publication via CIRAD of composite photo illustrations;

8 Provision of information to IBPGR/FAO regarding corrections to the draft guidelines for the safe movement of coconut germplasm;

9 Seeking STD3 funding for a project to address molecular biology relating to coconut genetic diversity;

10 Giving priority attention to solving problems related to 'weaning' and acclimatisation of plantlets obtained from embryo culture and from cryopreserved embryos; and to a project on cryopreservation;

11 Assessing regional needs for germplasm collecting and providing logistical support where needed;

12 Facilitating and promoting the establishment of five regional centres for coconut germplasm conservation (with PNG identified as the host country for the Pacific region);

13 Seeking STD3 support for a project to develop techniques relating to somatic embryogenesis;

14 Assisting national programs in screening and identifying indigenous genetic materials that are adapted to conditions prevailing in their environments;

15 Sponsoring a meeting on the standardisation of breeding techniques (to be held in Ivory Coast in early 1994);

16 Facilitate and support research relating to coconut viruses and viroids, *Mycoplasma*-like organisms (or MLO's) and *Phytophthora* and to engage in constructive dialogue with ACIAR and others who are involved with coconut virus studies;

17 Support for research related to coconut physiology;

18 Development of a COGENT brochure for publicity purposes;

19 Provision and support for training in all project activities the network becomes involved in.

Significance and Importance of COGENT

At this stage of COGENT's development, its major interests in the region's research, especially that relating to viruses and viroids, germplasm collection and conservation, are:

i Training and financial support for research on coconut breeding, for general research especially that relating to viruses and viroids, germplasm collection and conservation; and

ii information sharing, including the germplasm databases.

From COGENT's perspective, input from the Pacific region is requested in the following areas:

1 Countries support of the COGENT network by taking up membership of COGENT (the basic requirement being a country's willingness to share freely their coconut germplasm resources and information);

2 Countries to provide input to the germplasm database being coordinated by CIRAD, France;

3 Country participation in identifying their germplasm collecting requirements and relaying these to SPC as a matter of urgency;

4 Fiji, PNG and Vanuatu to identify persons to represent the region at the breeders' meeting to be held in the Ivory Coast in early 1994;

5 Provide support to donors regards COGENT's efforts in securing funding for it's activities and projects; and

6 Provide support to the Pacific representatives on COGENT's Standing Committee (i.e. SPC and Vanuatu) for COGENT's work.

Conclusion

COGENT is a coconut network set up by IBPGR to address the specific needs of coconut germplasm collection, conservation and use. The Pacific region is represented on its Standing Committee by the South Pacific Commission and Vanuatu but wider membership is needed. Pacific countries should consider becoming members and can stand to benefit through membership and involvement with the many projects and activities COGENT is currently promoting.

Participants

Roger Ashburner, IHD, Private Bag 15, Southeastern Mail Centre, Vic, 3176, Australia

Marlene Diekmann, IPGRI, Via Delle Sette Chiese 142, 00145, Roma, Italy

Michel Dollet, CIRAD, BP 5035, 34032 Montpellier, Cedex 1, France

Gerard Duhamel, Director—PDICC, Saraoutou Research Station, PO Box 231, Espiritu Santo, Vanuatu

Matthias Faure, CCRI, Box 1846, Rabaul ENBP, Papua New Guinea

Michael Foale, CSIRO Tropical Crops and Pastures, 306 Carmody Road., St. Lucia, Queensland, 4067, Australia

Hugh Harries, 17 Alexandra Road, Lodmoor Hill, Weymouth, Dorset, DT47QQ, England

Malcolm Hazelman, South Pacific Commission, Private Bag, Suva, Fiji Islands

Richard Hodgson, Plant Pathology, Waite Research Institute, Private Mail Bag, Glen Osmond, SA, 5064, Australia

Joeli Kauvere, Officer In Charge, Taveuni Coconut Centre, Taveuni, Fiji Islands

Peter Lynch, ACIAR Editorial Services, PO Box 1571, Canberra, ACT, 2601, Australia

David Meadows, Agriculture and Rural Development, World Bank, 1818 H Street HW, Washington DC, 20433, USA

Moceiri, Extension Specialist, Taveuni Coconut Centre, Taveuni, Fiji Islands

Michael Max Oliouou, Dodo Creek Research Station, PO Box G13, Honiara, Solomon Islands

Tore Ovasuru, c/- CCRI, Box 1846, Rabaul ENBP, PNG

Col Piggin, Coordinator, Plant Projects, ACIAR, Box 1571, Canberra, ACT, 2601, Australia

Semisi Pone, Plant Protection Officer, South Pacific Commission, Suva, Fiji Islands

John Randles, Plant Pathology, Waite Institute, Private Mail Bag, Glen Osmond, SA, 5064, Australia

Francois Rognon, CIRAD - CP, BP 5035, 34032 Montpellier, Cedex 1, France

Brian C. G. Smith, P.R.A.P, GPO Box 12621, Suva, Fiji Islands

Gabriel de Taffin, Consultant to Coconut Development Project, Koronivia Research Station, PO Box 77, Nausori, Fiji Islands

Mohta'ane Vatuvei, Research Div. Ministry of Agriculture, PO Box 14, Nukua'lofa, Tonga

F.Vilsoni, Plant Pathology, KRS, PO Box 77, Nausori, Fiji Islands

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