PACIFIC REGIONAL AGRICULTURAL PROGRAMME

PROJECT 7- PROVISION OF TISSUE CULTURE SERVICES FOR THE REGION

REGIONAL TISSUE CULTURE WORKSHOP

APIA, SAMOA, 5–13 JUNE 1997

PRAP REPORT No. 7

PRAP

The Pacific Regional Agricultural Programme, Phase II

Pacific Island economies are resource-poor and predominantly agriculturally based. In the Pacific ACP (PACP) states, the agricultural sector accounts on average for 30 per cent of GDP, 50 per cent of export revenues and over 60 per cent of employment (paid and subsistence). Most of the rural population is dependent on subsistence agriculture. The range of crops is very similar: root crops and tubers, coconuts and a range of fruits and vegetables, sometimes very varied, sometimes very restricted (small atolls).

The main technical problem areas facing the region can be summarised as follows: productivity is very low in both subsistence and commercial agricultural production; pest and diseases are increasing as communications improve and islands lose their natural isolation; agricultural research, information and extension services are poor and lack sound technological innovations. There is also a need for on-the-job training and support for post-graduate studies to ensure the long-term sustainability of technological achievements

The Pacific Regional Agricultural Programme (PRAP) addresses these problems through a comprehensive regional approach. The first phase of PRAP (PRAP I) concluded in September 1994 and focused on agricultural research and technology development. The present second phase (PRAP II) consists of eleven components which aim to finalise the research activities under PRAP I and transfer their results to the field in the various PACP countries. PRAP II will contribute to increased productivity by the selection of improved varieties of staple and export crops, by the introduction of appropriate farming systems and biological control of a major pest, by training of regional and national staff and the provision of tissue culture and biometric services. Compared with PRAP I, PRAP II puts much more effort into improving technology transfer from researchers to farmers in rural areas. An information support service is being promoted and strengthened; assistance and training at all levels is being provided and pilot schemes established. Through these means PRAP II is expected to make a significant contribution to rural development throughout the PACP states.

PRAP II is a four year programme with the Forum Secretariat as the overall Regional Authorising Officer. The programme is co-ordinated by a Programme Co-ordinator based in Fiji. The individual components are implemented in the various countries by either the leading regional institutions in agriculture or the respective national ministries. The cost of PRAP II is Ecu 9,265,000, or approximately 12,234,000 US dollars, which is financed as a grant under the Seventh European Development Fund, Pacific ACP Regional Programme.

Regional Tissue Culture Workshop

Apia, Samoa 5–13 June 1997

Front row: Léon Mu, Luseane Taufa, Takako Murikami, Josie Tuia, Samila Devi, Sanitea Tulifau, Prof. Yoneo Sagawa, Valerie Tua, Roseina Toalepai, Helen Tsatsia, Losa Naivalulevu,
Back row: Dr Mary Taylor, Alfred Kembu, Tuaine Turua, Tony Gunua, Vina Holo, Lucia, Tua Mataia, Jean Galo, Fatu Faao'o, Dumont Boe.

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AND

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Introduction

PRAP Project 7: Provision of Tissue Culture Services for the Region has as its overall goal the availability of improved planting material to farmers. To achieve this goal certain activities are essential, one of these being the training of NARS staff in tissue culture techniques. Initially this training is carried out on a one-to-one basis with NARS staff spending a period of up to six weeks at the PRAP/IRETA Tissue Culture Unit in Samoa. However, it is also necessary to hold workshops during which the skills learnt during this training can be reinforced, new techniques can be learnt, and tissue culturists can come together to discuss problems and share experiences.

This workshop was the second to be held since the start of PRAP Project 7. It brought together all the tissue culturists from the ACP countries. Funding from UNESCO and SPC were able to fund tissue culturists from non-ACP countries. The programme of the workshop and the contents of these proceedings reflect the present needs of tissue culture in the region.

The first workshop focussed on tissue culture of the major root and tuber crops, virus testing and quarantine issues. This workshop considered other crops, such as Irish potato and strawberry, in which there is interest from the region. As laboratories are considering ways of income generation, there are also demands for information on the methodology for tissue culture of ornamentals, for example, orchids and anthuriums. We were fortunate in being able to obtain the services of Professor Sagawa of the University of Hawaii for this purpose. In addition to the time spent on tissue culture of specific crops, other issues associated with tissue culture and important to the effective utilisation of tissue culture in the region were also discussed. These included topics such as intellectual property rights and *in vitro* conservation.

The workshop provided the forum for discussing the need for a tissue culture network, and how it would function and channel its information. These discussions led to the official naming of the tissue culture network as the Oceania Tissue Culture Network (OCTN). It was decided that until on-line facilities were more available and reliable, communication would be through the PEACESAT, and through the regular production of a quarterly newsletter.

Tissue Culture of Ornamentals

Professor Yoneo Sagawa[1]

The difficulties experienced with tissue culture of orchids are the same as those experienced in tissue culture of other plants, i.e. initiation and transfer to soil stages. Any method cannot be assumed to be workable until the end product is achieved, i.e. the plant in the field. One cannot assume what works for one species in a genus will work for all species; variation exists between varieties, let alone species, and so methods have to be adapted. Observation is very important so that one can respond to any change in the growth and development of the plant.

ORCHIDS

What is the optimum culture medium for orchids?

The two main media for orchids are Knudson and Vacin & Went (VW); the latter would appear to be more useful. It can be purchased as a premix from Sigma or prepared from stock solutions. One ingredient, calcium triphosphate is both difficult to obtain and requires water to be acidified before it will dissolve. A completely soluble fertiliser can substitute for the constituents in VW medium, but would have to be 100% soluble, and experiments would have to be carried out to assess what strength is required, that is, 0.25, 0.5, etc. VW allows orchids to be stored for approximately six months without subculturing, unlike Murashige and Skoog medium (1962), where orchid cultures deteriorate if left too long without subculturing. Epiphytic orchids are not so demanding in their nutrient requirements. Terrestrial orchids can be problematic and can have specific requirements, and need to be looked at individually. VW medium can also be used for the initiation of anthurium into tissue culture.

Coconut water (CW) is an essential ingredient of most orchid media, usually at a concentration of 150ml/l. Immature coconuts are the optimum source of water providing more volume and being easier to open. The water is usually double filtered, and can then be frozen for about six months. CW turning pink is not a sign of contamination, but a change in sugars. If too much proliferation occurs, then the concentration of CW can be reduced to 25/50ml/l. It is possible to combine CW and banana extract.

Banana powder was first used in a medium for orchid seed germination in Brazil (Graeflinger, 1950). The use of banana in culture media for orchid seedlings became popular after that. Some growers homogenised banana fruit pulp with their media, whereas others stirred puree into their solutions. Banana pulp can also enhance the growth of plantlets obtained from explants *in vitro*. The concentration of banana extract usually required is 50 g/l. Its inclusion in the culture medium appears to specifically encourage rooting.

Initiation of orchids in agitated liquid medium is more successful. Some US companies do not prefer agitation, choosing instead to use a shallow layer of liquid medium, however, the response obtained is slower.

Culture of orchid seeds

Orchid seeds are minute (80–130 m wide and 470–560 m long), and usually undifferentiated. Each seed contains an embryo composed of 8 to 10 cells with the endosperm either underdeveloped or completely lacking. The level of contamination can vary with the source of the seeds but they can be disinfected with Chlorox (domestic bleach). It is best to increase the duration of sterilisation rather than the concentration of Chlorox. A combination that seems to work well is 10% Chlorox for 10 minutes followed by 5% Chlorox for 5 minutes. Rinsing in between is not necessary. Liquid detergent can be used instead of Tween.

[1]University of Hawaii at Manoa, Department of Horticulture, 3190 Maile Way, Honolulu, Hawaii 96822-2279, USA.

The usual pre-treatment methods can work if there are problems in disinfection.

Orchids are unusual in the development of seeds. Post pollination phenomenon exists in that orchids do not have ovaries, these only develop when pollination occurs. Pollen tube production and penetration into the fruit can take 5 to 10 days but the female tissue is not ready and so fertilisation does not occur immediately. The presence of the pollen tubes encourages changes in the female tissue, and the subsequent development of ovaries. After pollination, fertilisation can take from two months, for example, *Dendrobium* spp. or up to six months as in the case of *Cattleya* spp. Orchid fruits can hold from 1000 seeds to several million. Ovule culture is possible, by disinfecting the fruit, and culturing the ovules. This is a good technique to use for wide crosses (embryo rescue). It is thought that hormones are responsible for the regulation of dehiscence of the fruit, and abscission of the fruit from the flower.

Orchid seeds either sink or float depending on species/variety. Seeds are very small, and therefore care has to be used in sterilisation, such as decanting the liquid or using a special dropper. Germination varies depending on the species and can take place within a week. As the seed germinates, the embryo enlarges and breaks through eventually consuming the endosperm. The embryo develops two regions; one forming the shoot, with the root forming later. The small spherical tuber-like bodies formed by the germinating orchid seeds are known as a protocorm and should not be confused with the term protocorm-like-body (PLB) which is used for the structures that resemble protocorms and are formed by tissue explants *in vitro*.

With *Phalaenopsis* spp. the lip is a modified petal which rotates 180° during flowering and ends up at the bottom of the flower. When the fruit is ready the corolla dries up. The fruit should be cut from top to bottom to avoid cross-contamination. Inside there are convoluted ridges which support millions of ovules. Ovules are fertilised at this point. The fruit can be disinfected prior to opening as the inside of the fruit is sterile.

What explants can be cultured?

As already discussed seeds can be used for tissue culture propagation of orchids, however, other explants can also be used.

Axillary buds can be excised, and placed on a simple medium to produce a single plantlet, that can then be modified for proliferation through nodal explants. With most ornamentals ½ strength MS + CW can be used to establish a plantlet from an axillary bud. Often the lower portions of large inflorescence have axillary buds that can remain suppressed unless excised and given the chance to respond. This technique can be used with *Dendrobium* spp. but with *Vanda* spp. usually only one node will respond.

It is best to take nodal buds from this year's growth, and these nodal sections can be disinfected with 10% Chlorox for 10 minutes + Tween followed by several sterile distilled water (SDW) rinses. The bract is then removed and the bud exposed and further treated with 5% Chlorox for 5 minutes + Tween followed by SDW rinses. The explant can then be initiated onto the culture medium after the removal of some basal tissue. It can take two years for flowering to occur. In some laboratories *in vitro* culture of axillary buds of *Dendrobium* spp. is slightly different to that described above. Cubes approximately 5mm wide, 6mm long and 2–3mm thick are taken after surface sterilisation of 10% Chlorox for 15 minutes + Tween followed by SDW rinses. In the procedure used the explants are first cultured in a liquid modification of Vacin-Went medium. After approximately one month on this medium the explants are moved to a high auxin medium. Clusters of PLBs that form at this stage are cultured on a third, solid medium for plantlet formation.

With the local Samoan spider orchid propagation can be done in this way. It is usual to remove all leaf blades and peel off the leaf bases. The stem is then placed on a sterile surface and a cube is cut around the bud. The bud is composed of root initials (coloured) and the axillary bud (green); the root initials are either side of the axillary bud. This explant can be placed in liquid culture. The explant will turn brown but the intercalary meristems respond and develop. With agitation proliferation occurs, and the proliferating bodies on the out-

side of the mass fall off, and can then be transferred to solid medium.

Dendrobium shoots can also be cultured. Ideally small shoots from the base are preferable because the leaf bases enclose the buds, and they are therefore cleaner. The initial size of the explant is quite large, and includes basal tissue where there are axillary buds. This initial explant is disinfected with 10% Chlorox for 10 minute + Tween followed by SDW rinses. It is then reduced in size and further treated with 5% Chlorox for 5 minute + Tween followed by SDW rinses. The explant is initiated in liquid culture that is agitated at 100 rpm. With this system it takes 18 months for the 1st flower to be produced and 24–36 months for good productivity.

Leaf tissue can also be used in some cases; the basal leaf tissue is more responsive. Basal parts of the leaves of flowering plants were used to regenerate large numbers of phenotypically uniform plants of *Renanthera imschootiana* (Red Vanda). Differentiation of up to 10 shoots free of callus and PLBs occurred in 10–12 weeks from the base of a single leaf cultured in a medium supplemented with sucrose, peptone, benzyladenine and naphthaleneacetic acid. Further proliferation took place when the culture medium was enriched with 10% coconut water and 35 g/l banana pulp (Seeni and Latha, 1992). There are some reports that leaf tips can also be used to propagate orchids, however, the precise timing required makes the method not easily reproducible. Success depends on removal of the explants before the leaf tips differentiate fully and lose their ability to form callus (Arditti and Ernst, 1993).

Root tissue can also be used, and with 2,4-D in the medium a tumour-type growth is produced from just below the root tip, which can be subsequently subcultured. However, with root culture there is a high degree of variation. Roots of *Catasetum*, *Rhynchostylis* and *Cyrtopodium* have been cultured successfully and used to produce plantlets (Arditti and Ernst, 1993). When choosing which explant to use the order of prioritisation should be: shoot-tip/axillary bud; inflorescence; leaf; root.

Monopodial and sympodial orchids

Monopodial orchids are vertical and unidirectional. Sympodial orchids grow laterally, with axillary buds producing inflorescence which are determinate, and then vegetative growth continues laterally. Many of the SE Asian orchids are monopodial. Monopodial orchids are sensitive to sucrose, and therefore sucrose should be removed from the medium (there is carbohydrate source in the CW). Sucrose is reintroduced as the PC or PLB start to differentiate.

With monopodial orchids young inflorescence can be used. These should be tight bracts, i.e. do not wait for the flower buds to develop. If the inflorescences are harvested too late then the axes will elongate in culture and flowers will develop. However, these do have nodes, that can be used but the flowers produced from these do not develop well. This method has been reported as successful with *Aranda* 'Deborah'. Developing inflorescence (20–40 cm) were used, and the floral buds excised to provide an explant approximately 3–4mm in length. This work showed that as long as the florescence tips are highly meristematic and actively differentiating floral buds, they are suitable explants (Goh and Wong, 1990).

Types of mutation/variation that occurs in orchid tissue culture

Albinism can be a problem, and these tissues will not survive outside the flask. Stunting can also occur. Chromosome doubling can also be a problem, leading to flowers of different size and texture

The orchid market

Three specific markets can be defined:

- Hobbyist – where new species are desirable (America and Europe);
- Potted plants – mass market (supermarket situation); and
- Cut flower market – local and export.

Different countries have different tastes: Europeans prefer intense colours, Japanese like white, small and delicate flowers, Americans prefer brighter colours.

Transfer to soil

A well-drained substrate is essential. One must remember that most orchids are epiphytic, and therefore it is better not to provide too much water, misting is preferable. Good results with

both terrestrial and epiphytic orchids have been obtained in mixes composed of equal parts by volume of sphagnum peat and perlite. Apparently plants grown in this medium produced more growth, leaves and flowers than in tree fern mixes and bark. In the tropics, coconuts husks also provide a good substrate for orchid growth.

ANTHURIUMS

There are two different techniques available for micropropagation of anthuriums:

1. Use of leaf tissue – leaf sections are taken along lateral veins, callus is produced and plants regenerated from that callus.
2. The axillary bud can be used, but this is a more difficult technique. The axillary bud is used to produce a plantlet that is manipulated for micropropagation.

Anthuriums do not like light, especially strong, direct light – diffuse light is preferable, and so in the growth rooms cultures should be placed on the top or bottom shelves. Initiation of cultures capable of proliferation takes approximately one year. When transferring anthuriums from tissue culture to the soil good drainage and maintenance of humidity is essential. Some anthuriums sucker very readily, and therefore are not desirable for cut flowers, but can be used for pot plants. The Dutch maintain five leaves on their plants and have selected for non-suckering. Flower size can be regulated through crowding of plants/leaves.

FLOWERING GINGER

Floral petals are modified leaves, and inside these are axillary buds. These buds can be initiated to produce a plantlet that can then be manipulated for proliferation. Flowers have to be well rinsed in soapy water. Tight inflorescences must be used because there is less contamination, and the intercalary meristems have not been expressed. Rhizome buds can work well, if they are induced to grow larger before being initiated into tissue culture, and nodal explants can also be used.

GENERAL PROCEDURES FOR SHRUBS

Where possible axillary buds should be used, and young tissue as this is always more responsive.

The system for propagation of *Schefflera* is relatively easy, and the same with *Cordyline*. Shoot-tips can be cultured, developed into a tissue culture plantlet, and then subcultured using nodal explants. *Cordyline terminalis* can be propagated *in vitro* using a modified Murashige and Skoog medium supplemented with 0.5 mg/l 6-benzylaminopurine. No growth regulators were required for the rapid rooting of shoots (Kunisaki, 1975). If the nature of the Cordyline is chimeric then establishing clonal plantlets can be a problem. Crotons are more difficult to tissue culture.

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COUNTRY PAPERS

Report on Tissue Culture in the Cook Islands

Tuaine Turua[1]

INTRODUCTION

The Cook Islands is free of some known serious pests and diseases such as *Phythophthora colocasiae* (taro leaf blight), banana bunchy top virus, and many more. However, the country is vulnerable to many serious pests and diseases due to the easy access via air and sea transportation, and the subsequent free movement of passengers from one place to another. Therefore the use of tissue culture to introduce pathogen-tested material into the country would significantly reduce the possibility of introducing new pests and diseases.

TISSUE CULTURE LABORATORY

A tissue culture laboratory is based at Totokoitu Research Station funded by ACIAR in 1993, under the Banana Development Project. Various problems such as insufficient equipment, have resulted in the laboratory not being fully functional.

RECENT TISSUE CULTURE ACTIVITIES

Some attempt was made recently to establish banana tissue cultures. However due to the shortage of resources this was not very successful. The level of contamination was high and of six plants initiated into tissue culture, only two showed signs of growth.

CONCLUSION

The laboratory could be used to provide clean planting material to the growers. However, for this to be achieved there is a need for further equipment, and also for the Government to understand the potential tissue culture has in improving agricultural productivity in the country.

[1] Ministry of Agriculture, PO Box 96, Rarotonga, Cook Islands.

Micropropagation of Bele (*Abelmoschus manihot*)

Losa Naivalulevu[1]

INTRODUCTION

This project is the result of collaboration between PRAP Projects 3 and 7. The main objective is the selection of superior varieties of bele, and the subsequent establishment of a pathogen-tested collection of these accessions, which would then be available for distribution to other island countries, especially the atolls. Bele compared to other leafy vegetables is high in iron and Vitamin A. This objective is being achieved through the following activities:

- Develop a micropropagation system for bele, using Fijian varieties.
- Import bele varieties from PNG.
- Establish PNG varieties in tissue culture.
- Develop a technique for virus elimination in collaboration with overseas institutes.

TISSUE CULTURE METHODOLOGY

Research is focussing on several areas:

- Optimum sterilisation procedure
- Optimum explant size for growth and contamination.
- Difference in contamination levels between the three types of explant: shoot-tips, meristems and nodal buds, and the different varieties of bele.
- Evaluation of meristem culture media
- Evaluation of multiplication media.
- Ease of rooting on different media.

Optimum sterilisation medium

Disinfecting bele explants proved difficult with explants showing bacterial and fungal contamination. The four different Fijian varieties (pawpaw, round, strapped, cotton), were used in this experiment which looked at three sterilisation procedures:

Procedure	Ster A	Ster B	Ster C
Soapy water rinse	-------	------	5 mins
Tap water rinse	3 mins	5 mins	--------
Sterile water rinse (SDW)	-------	--------	5 mins
70% ethanol	30 secs	60 secs	60 secs
20% bleach + Tween	10 mins	5 mins	5 mins
3 SDW	3 mins	3 mins	5 mins
30% bleach + Tween	20 mins	10 mins	5 mins
3 SDW	3 mins	3 mins	5 mins
40% bleach + Tween	30 mins	10 mins	5 mins
3 SDW	3 mins	3 mins	5 mins

Results	Ster A	Ster B	Ster C
Bacterial contamination	20–28%	33–38%	11–17%
Fungal contamination	10–14%	12–18%	5–10%
Necrotic	60%	35%	2%

As the results show Sterilisation C was the optimum procedure for eliminating contaminants from bele explants.

Vitrification in bele.

Evaluation of explant size

Nodal buds and shoot-tips of the four Fijian varieties were used. Explant size varied from 0.2–0.4 cm with nodal buds, and 0.1–0.3 cm with shoot-tips. The optimum sterilisation pro-

[1] PRAP 3/7, SPC, Private Mail Bag, Suva, Fiji.

Table 1 Evaluation of the level of contamination associated with the four Fijian varieties, and the different explants.

Bele variety	Nodal Bud	Shoot tip	Meristem
Pawpaw (Var 1)	15–18%	14–16%	5–7%
Round (Var 2)	10–13%	13–15%	2–3%
Strapped (Var 2a)	0–3%	0–2%	0%
Cotton (Var 3)	25–28%	18–20%	6–9%

Table 2: Evaluation of meristem culture media.

Okra medium	Medium A	Medium B	Medium C
MS Plant Salts	MS Plant Salts	MS Plant Salts	MS Plant Salts
3% sucrose	3% sucrose	3% sucrose	3% sucrose
0.05mg/l IAA	95 M NaFeEDTA	0.12mg/l BAP	100mg/l inositol
0.10mg/l BAP	220 M AS	0.20mg/l NAA	0.4mg/l thiamine
40.52 mg/l AS	450 M SDH	0.08mg/l GA	40.52mg/l AS
	0.29 M IAA	20mg/l cysteine	
	0.45 M BAP		

AS = Adenine Sulphate; SDH = Sodium Dihydrogen Sulphate; IAA = Indole-3-acetic acid; BAP = 6-benzylaminopurine; NAA = napthaleneacetic acid; GA = gibberellic acid; MS Plant Salts = Murashige & Skoog Plant Salts (1962); NaFeEDTA = Ethylenedinitrilotetraacetic acid Ferric-Sodium salt.

cedure was followed, and with both nodal buds and shoot-tips contamination was higher with the larger explants than the smaller ones.

As shown above meristem culture results in the lowest level of contamination. In addition, there is a varietal effect associated with contamination, with Var 2a (Cotton), having less contaminants associated with its tissue.

The first culture medium to be assessed had proved successful with okra (*Abelmoschus esculentus*) using seedling material and a modified Murashige and Skoog medium (Mangat and Roy, 1986). This medium was compared with three others as described below.

Although all four media supported organogenic growth, the presence of growth regulators encouraged the formation of callus. It was also found that a medium supplemented with growth regulators encouraged hyperhydricity in the plantlets. As a bele tissue culture system will be used for the propagation of desirable varieties and distribution of pathogen-tested material throughout the region, it is important that such a micropropagation system is clonal, and therefore any technique that encourages the formation of callus should be avoided.

CONCLUSION

It is now possible to establish tissue cultures of bele. The medium still requires improvement so that multiplication can be achieved without any problems with callus and/or hyperhydricity. Future research will focus on the use of meristem culture for the eradication of known viruses.

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Micropropagation of Ginger

Samila Devi[1]

INTRODUCTION

Ginger (*Zingiber officinale*) cultivation in Fiji dates back over a century, probably having been introduced from India. Ginger is one of the spice crops widely consumed by Indian and Chinese communities in Fiji. The ginger industry is in decline due to a rhizome rot which affects the plant during the warmer, wetter summer months. It has been estimated that the potential yields in some areas are reduced by up to 30% by nematodes and associated fungal secondary infections. Nematodes are costing the industry dearly in terms of lost production, and through the closure of potential export markets. Many farmers are now turning to alternative crops such as taro and cassava. Assistance with this problem was requested by the growers, and so SPC was asked to produce material through tissue culture to assess if its 'clean' state would be effective against the pest and disease problems. In order to provide clean planting material, the following parameters had to be determined:

- Optimum sterilisation technique
- Suitable culture media.

METHODOLOGY

Ginger rhizomes harvested from Koronivia Research Station were washed thoroughly, dried and stored at room temperature. When the rhizomes had sprouted, apical and lateral buds, 1-3cm in length were excised. Trimmed buds were surface sterilized using the following treatments with 25 buds per treatment.

- 3 secs 70% ethanol followed by 5 mins in 1% bleach + Tween
- 1 min 70% ethanol followed by 10 mins in 1% bleach + Tween
- 2 mins 70% ethanol followed by 15 mins in 1% bleach + Tween

Following these treatments buds were rinsed three times in sterile distilled water. The buds were then asceptically dissected under a microscope in a laminar flow transfer cabinet. The lengths of the dissected meristem tips ranged from 0.3-0.5mm. The shoot-tips were transferred to 80mm by 28mm vials containing 10ml of medium. Three different media were evaluated and for each medium there were ten replicates.

- Medium 1: Murashige and Skoog (MS) Plant Salts (1962); 30g/l sucrose; 1.75g/l gelrite; 0.1, 0.2, 0.3, 0.5, 1.0mg/l BAP
- Medium 2: MS; 30g/l sucrose; 0.1mg/l kinetin; 0.1mg/l BAP; 1.75g/l Gelrite
- Medium 3: MS; 30g/l sucrose; 1.0mg/l BAP; 1.0mg/l kinetin; 1.75g/l Gelrite.

The pH of the medium was adjusted to 5.7 prior to autoclaving. The cultures were grown at a temperature of 25°C-30°C under a 16 hour daylength.

After five weeks plantlets were formed and these were subcultured onto fresh medium. After two transfers on this medium shoots were cultured on one of the following two media:

- Medium 4: MS; 30g/l sucrose; 1.75g/l gelrite; 2.5mg/l BAP.
- Medium 5: MS; 30g/l sucrose; 1.75g/l gelrite; 4.0mg/l BAP.

For rooting plants were transferred to MS medium without any growth regulators.

RESULTS

1. The optimum sterilisation technique was 2 mins 70% ethanol followed by 15 mins 1% bleach + Tween. With this treatment the contamination was less than 3%.

[1] SPC Plant Protection Service, Private Mail Bag, Suva, Fiji.

Micropropagation of ginger.

2. For the first stage the optimum medium was MS + 3% sucrose + 0.1mg/l BAP + 0.1mg/l kinetin. With the other media the explants did not survive.
3. Cultures growing on medium containing 4.0g/l BAP produced more shoots compared to those growing on medium containing 2.5mg/l BAP.

ESTABLISHMENT OF THE PLANTS IN THE SOIL

In vitro grown plants were thoroughly washed in tap water to remove the gelrite from the roots. Any old leaves and roots were also removed. Plantlets were individually planted in plastic pots containing sterilised soil. Polythene bags were placed over the plants which were kept in an airconditioned room. Once new leaves emerged the polythene bags were removed. Plants were then transferred to Koronivia Research Station for eventual transfer to the field.

Tissue-cultured ginger trials in Raki Raki, Fiji.

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Murashige, T and F. Skoog (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*, **15**: 473–497.

Factors Influencing Vanilla Mass Propagation *in vitro*

B. Pett and A.B. Kembu[1]

INTRODUCTION

Because of the increasing interest in natural vanillin in the world market, there has been a corresponding increase in vanilla cultivation. Therefore the demand for vanilla planting material has increased from vanilla growers in Papua New Guinea. During the last two years the potential of this crop has been demonstrated, and it is quickly becoming a significant minor cash crop.

The main objectives in the application of tissue culture techniques to vanilla production are:

- the acceleration of the multiplication rate of vanilla to provide the farmers with adequate planting materials; and
- cloning of selected healthy and vigorous plants.

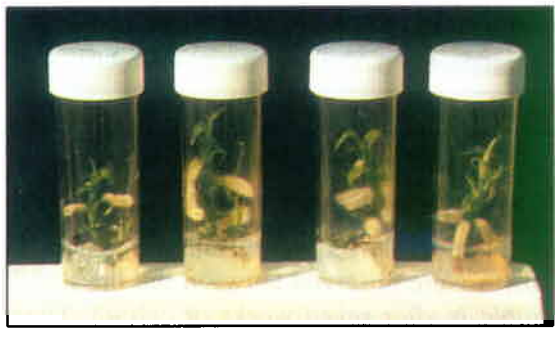
MATERIALS AND METHOD

The two species of vanilla *Vanilla tahitensis* and *V. planifolia* were used in this investigation. Explants were shoot-tips and axillary buds derived from field material, and cultured as single and double node explants. The medium used was Murashige and Skoog (1962) supplemented with vitamins by Nitsch and Nitsch (1969), 30 g/l sucrose and 3 g/l Phytogel. This was the basal medium that was either supplemented with 500 ml/l of deproteinised coconut milk or 0.5 mg/l BAP was used. Culture vessels were either 30 ml or 120 ml in volume.

The following factors capable of influencing growth were investigated:

- donor explant position;
- comparison of selected lines;
- influence of medium;
- artificial and natural illumination;
- influence of twin cultures, i.e. two explants in one vessel; and
- comparison of single and double node explants.

The parameters used for measurement were as follows: length (mm); number of shoots per explant; number of nodes and rooting.



Mass propagation of vanilla.

RESULTS AND DISCUSSION

Explant position

The three positions compared were tip explants (A), middle explants (B), and base explants (C). As shown by Table 1, explants derived from the middle sections of the plant gave superior growth in terms of length and number of nodes. However, explants from the tip and the base of the plant produced more shoots per explant. Both vanilla species responded similarly in relation to explant position. There was also no difference in rooting between the explant positions and the two different species. However, generally *V. planifolia* was more vigorous in culture than *V. tahitensis*. Table 1 shows results after six weeks of culture.

[1]LAES Tissue Culture Laboratory, Keravat, East New Britain, Papua New Guinea.

Table 1. Effect of explant position on the growth of Vanilla *spp. in culture.*

Explant Position	Variety: *V. tahitensis*				Variety: *V. planifolia*			
	length (mm)	shoots/ explant	number of nodes	rooting	length (mm)	shoots/ explant	number of nodes	rooting
A	18.6	2.3	2.8	2.8	26.8	2.8	4.1	3.3
B	23.1	1.3	3.7	3.1	29.5	1.2	4.8	3.2
C	19.8	1.9	3.2	2.9	28.1	1.9	4.4	3.5

A=tip explants; B=middle explants; C=base explants
Rooting scores: 1=no rooting; 2=50%rooting; 3=100% rooting

Table 2 Influence of different lines of Vanilla *spp. on growth in culture.*

Species/lines	length (mm)	shoots per explant	Number of nodes	Rooting
V.tahitensis 14	19.2	1.2	3.2	2.5
V. tahitensis 2	23.0	2.1	3.9	4.0
V. tahitensis 13	26.7	1.6	3.8	3.3
V. tahitensis 20	25.3	1.7	4.0	2.9
V. tahitensis 5	38.8	1.6	6.3	3.7
V. planifolia 18	28.5	1.8	4.5	3.4

Influence of lines

As Table 2 shows significant differences in growth were observed between the lines of *V. tahitensis* after seven weeks of culture. These differences are shown in shoot length and number of nodes with very little difference being shown by the shoots per explant and the rooting.

Other Influencing factors

The basal medium supplemented with BAP resulted in cultures with more shoots than the same basal medium supplemented with coconut milk. With the former the percentage multishoot formation was 54.5% compared with 20.5% when the medium contained coconut milk. Rooting and length of shoots were also less with MS medium containing coconut milk compared to BAP, being 90.9% and 61.5%, and 21.7 mm and 15.9 mm, respectively. These results compare favourably with those obtained by Jarret and Fernandez (1994). However, Pone (1992) found that MS medium supplemented with coconut milk gave optimum results in vanilla culture compared with other media. These differing results could be due to the undefined nature of coconut milk, and therefore the potential inconsistencies of the physiologically active substances within the coconut milk, which are influenced by age of nut, individual palm and seasonal factors. Philip and Nainar (1986) obtained the best growth on Knudson C and other media while MS medium was not suitable for the growth of shoots of vanilla plants.

Illumination

Previous work in the LAES tissue culture laboratory showed that vanilla plantlets tolerate a wide range of light quantity (400–3,500 lux) without any significant growth difference. The comparison between natural and artificial illumination at the level of about 2,000 lux did not have any significant effect on shoot length, though there was a tendency for natural illumination to promote root and multishoot formation. A similar finding was also reported by Havkin-Frenkel *et al.* (1996).

Twin culture and explant size

With two explants in one culture vessel, one explant always seemed to be inhibited by the other resulting in reduced growth, thus making

the use of twin culture unsuitable for a rapid multiplication system. Similarly there was no significant increase in length when double nodes were used as explants instead of single nodes. Consequently the use of a single node is more effective for rapid multiplication.

CONCLUSION

The inclusion in the basal medium of 0.5 mg/l BAP resulted in optimum growth of vanilla plantlets compared to those plantlets cultured on basal medium containing 500 ml/l of deproteinised coconut milk. Significant differences were found in growth between different lines of *V. tahitensis*, however, only one line of the five examined was superior in growth to the one line of *V. planifolia*, which was investigated. It was also shown that explants from the middle section of the donor plant gave better growth with respect to length and number of nodes, whereas those explants derived from the tips of donor plants were superior in multishoot formation. Maximum multiplication rates will be provided by a system which combines optimal length growth and multishoot formation. Future investigations will focus on identifying the factors that can result in this optimum combination. In addition, it will be necessary to ascertain that such a system will yield plantlets that can be established and develop at a favourable rate *ex vitro*.

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Maintenance and Conservation of Taro (*Colocasia esculenta* var *esculenta*) in Tissue Culture

Tony Gunua[1]

INTRODUCTION

In PNG, genetic erosion is occurring in a wide range of crops including taro. Therefore, there is an urgent need to characterise taro accessions now maintained in field collections, and to re-establish these collections *in vitro*. Initially the number of taro accessions maintained in the field were in the order of 800, in 1996 there were 350. It was felt that the original collection could have been restored if there had been a duplicate collection maintained *in vitro*. Storage *in vitro* offers several advantages: low labour intensity; no infection from pests and diseases; no weed competition; provision of optimal growth conditions; ability to store vegetatively propagated crops; less space required.

There is no international centre with the mandate to conserve taro and yam, the two major root crops of the Pacific Island countries. It would therefore seem wise for PNG to maintain its national collection, especially as it would appear that PNG has the greatest diversity of all the Pacific Island countries. As the major taro collection in the country is maintained at Bubia it would seem logical for a tissue culture collection to also be established at the station.

CONSERVATION OF PNG TARO GERMPLASM

Taro is a major staple in the wet lowlands of PNG with the national collection being held at Bubia. The average losses in the field collection is 10% per annum, therefore there is a need to reconsider conservation strategies.

With a tissue culture laboratory on the station the accessions could be maintained *in vitro*, thus adding to the security of the collection.

Taro conservation in the field.

MICROPROPAGATION OF TARO

Smaller farmers in PNG may encounter a shortage of planting materials. Not only can it be a problem of volume but also what is available can be heavily infected with alomae bobone virus. Tissue culture in combination with an effective virus-testing system could solve this problem for the farmers.

SLOW GROWTH STORAGE

Investigations can be conducted to establish minimal growth techniques for taro for medium term conservation as has been reported for other crops (Withers, 1980). Minimal growth techniques available include slow growth temperatures, incorporation of growth retardants/osmoregulatory compounds, and modification of the nutrients in the basal medium. Use of growth retardants were successful with potatoes (Westcott, 1981). High input conservation methods should be avoided.

[1]Bubia Agricultural Research Centre, PO Box 1639, Lae, Papua New Guinea.

CONCLUSION

The tissue culture unit at Bubia has the technical capability to carry out the above tasks mentioned, however, there is a need to modify the unit and to increase and improve on the equipment.

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Tissue Culture Related Activity in the Solomon Islands: A Progress Report

Jean Galo and Helen Tsatsia[1]

INTRODUCTION

The progress of establishing the tissue culture laboratory in the Solomon Islands has been relatively slow. This is mainly due to lack of funding. In the past there have been some attempts at establishing crops such as yam in tissue culture using the Plant Pathology laboratory but the contamination rate was very high. The Plant Pathology staff are very keen to tissue culture various crops but the lack of basic medium components, such as agar, makes this very difficult. The Plant Pathology staff are responsible for any incoming tissue cultures, usually from the PRAP/IRETA unit or SPC.

Consideration has been given to using the tissue culture facilities to generate an income, such as through the sale of ornamentals, however, this is not possible through the present Government system.

STATUS OF THE TISSUE CULTURE LABORATORY IN THE SOLOMON ISLANDS

Most of the basic facilities, such as autoclave, LAF cabinet, shelving, lights, and other smaller pieces of equipment are in place. The major constraint on the operation of the laboratory is finance. At present, only orchids can be found in the laboratory.

GERMPLASM COLLECTION

Different cultivars of all of the major crops are currently being collected by staff, and either bulked at Dodo Creek Research Station, or Field Experimental Stations in the Provinces. These will be described and documented.

Taro

There are 30 local cultivars and 9 selected hybrids being bulked at Dodo Creek Research Station. A bulking plot was also established on Santa Cruz Island, (Eastern Solomons). This collection is very special because the island is known to be free of taro leaf blight.

Banana

A total of 43 cultivars have been collected from around the Guadalcanal Province. This includes both dessert banana and plantains. Two other bulking plots have been established in two other provinces. There are plans to set up a trial to screen for diseases.

Cassava

The collection consists of the top ten high yielding varieties grown in the Solomon Islands.

Yam

There are 26 cultivars in the collection. Some documentation will take place based on studies made two years ago when postharvest qualities and yam dieback tolerance/resistance factors were evaluated.

Ornamentals – orchids

A few hundred plantlets have been maintained in the laboratory for five months. Two indigenous *Dendrobium* spp. have been collected to date.

Ngali nut

The Tree Crops Section is responsible for collecting the different species and varieties found in the country.

[1]Ministry of Agriculture and Forestry, Dodo Creek Research Station, PO Box G13, Honiara, Solomon Islands.

The general Plant Protection Laboratory, Solomon Islands – present site of tissue culture activities.

TRAINING

Experience in the Solomon Islands has shown that training of one officer in a specialised discipline is not sustainable, and so several members of the Plant Pathology staff have been trained in tissue culture techniques. The Head of Plant Pathology remains the Officer-in-Charge of the overall running of the tissue culture laboratory.

The future medium preparation room in the Solomon Islands laboratory.

CONCLUSION

Funding is essential for the tissue culture laboratory to become fully operational. However, the tissue culture team in the Solomon Islands will continue with germplasm collection and documentation, which is essential for future tissue culture work.

Tissue Culture in French Polynesia

Léon Mu[1]

INTRODUCTION

The Department of Agronomic Research has been working extensively on tissue culture since 1990. The main objectives were to provide plantlets of anthuriums, heliconias, tis, monsteras, dendrobiums and alpinias for our horticulture farmers. An average of 25,000 plants a year are now produced and sold for approximately US$ 10,000. A stock of 5,000 virus-indexed *in vitro* plantlets of vanilla is also ready as a multiplication pool for a possible project of 500 acres of screenhouses. Some work has also been carried out on *Etlingera cevuga* which has a high content of methyl-iso-eugenol and on *Apetahia raiateensis*, an endangered species.

Since the December workshop on endogenous bacteria, the main concern in the laboratory has been to obtain clean cultures. It was found that all cultures, with the exception of vanilla, were contaminated. As recommended by Dr Barbara Reed, two different procedures were evaluated. The first was to carry out new explantings and to select clean explants after a week's immersion in a peptone and yeast supplemented medium. The second involved the use of antibiotics. Out of 51 explants of *Etlingera cevuga*, 5 showed contamination on peptone and yeast supplemented medium.

The results on anthuriums are shown in below:

Table 1: Anthuriums contamination levels using peptone and yeast supplemented medium

Explanting	Number of explants	Contaminated explants
N 1	6	5
N 2	10	9
N 3	19	19
N 4	5	5
N 5	7	6

With the anthuriums it seemed harder to obtain clean explants. To obtain Stage 2 cultures was a very slow and time consuming job. As it was difficult to obtain many plants per cultivar, the use of antibiotics was considered a better alternative in obtaining clean cultures.

The first results on bacterial contaminants in some ornamentals were obtained through a medical laboratory. In two different *Dendrobium* cultures the two contaminants identified were *Corynebacterium aquaticum* and *Rhodococcus equi*. Two separate *Anthurium* cultures were found to contain *Bacillus* spp. and *Acinetobacter* spp. In two *Cordyline* cultures examined, *Bacillus* spp. and *Micrococcus kristinae* were identified.

The antibiograms revealed that gentamycin combined with kanamycin or penicillin would be a good choice for an assay. Prior to obtaining the antibiotics through Sigma Chemicals, an experiment on dendrobiums contaminated with *Corynebacterium aquaticum* was carried out. Gentamycin has been used at 25 mg/l, 50 mg/l and 100 mg/l on protocorm-like-bodies that have been either surface sterilised with LD 1:1:10 (Alcide Corp.) or not. The final results are expected in approximately seven months.

The tissue culture facilities are quite satisfactory. Funding was obtained in part from the French government and part from the Polynesian government. The nursery screenhouse may need to be equipped with a new watering system. A computer will soon be purchased with income generated by the sale of plants. However, staffing is a limiting factor in production. At the present time, there is one tissue culturist, myself, and an assistant who is in charge of the medium preparation, the washing-up and the nursery. This is insufficient and can significantly affect the output of the laboratory.

[1]Service Développement Rural, Papeete, Tahiti.

ANTHURIUM MICROPROPAGATION

The plant preparation and selection is of overall importance in order to provide easy to clean planting material, whether axillary buds or leaf explants.

Anthuriums in tissue culture.

Plant preparation

- Provide cultural practices that ensure good and healthy plant growth (fertiliser, water, shade, etc.).
- Provide gravel mulching to reduce splashing of soil onto plant.
- Avoid manure or any organic material that increases micro-organism development.
- Pest and disease control.
- Avoid overhead irrigation. Grow plants under solid cover.

Plant selection

Select healthy, matured plants with a minimum of 6 nodes that have as many leaves as possible left on the plant.

Surface sterilisation

- Remove all leaves and roots from the cuttings.
- Wash entire stem with detergent.
- Allow to air dry for 4 days.
- Excise the buds and remove the first leaf covering.
- Agitate the buds in a detergent solution for 20 minutes. Rinse.
- Agitate in 70% alcohol for 1 minute. Rinse.
- Agitate in 10% Chlorox plus a drop of Tween 20 for 20 minutes. Rinse 3 times in sterile water.
- Under a dissecting microscope, remove 2 or 3 leaf coverings and excise bud to obtain an explant of less than 2 mm in length.
- Agitate in LD 1:1:10 (Alcide Corp.) for 3 minutes. Rinse 3 times in sterile water. (or use 5% Chlorox plus 1 drop of Tween 20 for 15 minutes).
- Transfer to culture medium.
- Place culture under 16-hour light at 25°C.

Multiplication and root formation

The plantlets derived from the explant are transferred onto the multiplication medium which is a modified N69 Nitsch and Nitsch medium supplemented with 0.5 mg/l benzyladenine. They are continuously transferred onto the multiplication medium or onto a N69 medium without hormones if more growth in length is expected.

To get rooted plants, the plants are individually cut from the clump at a length of 2–3 cm and transferred onto a rooting medium. A satisfactory root system should be formed in one month.

Anthuriums planted out from tissue culture.

Weaning off

Roots are washed with tap water and the plants planted out into the weaning off soil mix. After a week under a plastic humidity chamber, they are grown on a bench under an overhead misting watering system. They are liquid fertilised with a Peters 20-20-20 solution twice a week. Plants can be sold two months after weaning off. The farmer can keep them in the same container for two more months before repotting into a commercial orchid mix or more conventional anthurium substrates.

Anthuriums derived from tissue culture.

The plants should flower 18 months after weaning off. The flowers being first quite small and reach their normal size six to eight months later provided the plants are given good cultural practices.

Details of the different media required at the different stages of production are given in Annex 2.

Problems Associated with Kava Tissue Culture

Luseane Taufa[1]

INTRODUCTION

With some plant species contamination is extremely difficult to eliminate. In attempts to initiate kava into tissue culture high levels of contaminants (70–100%) have severely hindered any progress. This is the case despite the use of explants from pre-treated plants and stringent sterilisation procedures including the use of antibiotics. This difficulty in eliminating pathogens is due to the high level of exogenous and endogenous contaminants.

Hawaiian kava in the field.

METHODOLOGY

Different approaches to overcoming contamination have been used with kava. These include the following:

- A number of different methods have been tried as pre-treatments on stock plants: spraying with fungicide combined with bactericide; pruning to induce new growth; use of forcing solutions to encourage the rapid development of new shoots; the inclusion of antibiotics and fungicides in hydroponic solutions.
- The effect of both sodium hypochlorite and/or mercuric chloride, either with or without alcohol, have been evaluated as surface sterilisation techniques.
- The effect of antibiotics either in the medium or as a sterilant dip have been investigated.
- Heat therapy experiments have been carried out to see if high temperatures would have a detrimental affect on external and internal pathogens.
- The plant tissue has been dried to reduce the level of sap that is likely to be a source of contaminants.
- High BAP concentrations in the culture medium have been used in an attempt to encourage the rapid development of 'clean' shoots.
- The effect of filter paper bridges and liquid medium has been examined to see if less handling of the tissue, and limited contact with the medium will reduce contamination rates.
- A decrease in the concentration of macro- and micronutrients in the culture medium was tested as a means of reducing the levels of contamination.

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Kava in the screenhouse being pre-treated prior to initiation into tissue culture.

Despite all these different approaches contamination remained an obstacle to initiating kava into tissue culture. From each experiment, two to three cultures out of 20 replicates remained clean for two to five weeks before contaminants became visible. These 'clean' cultures eventually either became necrotic and died, or became contaminated and further attempts to clean up these explants failed.

BLACKENING OF THE EXPLANT AND/OR THE CULTURE MEDIUM

The release of phenolic compounds was also affecting culture establishment and attempts were made to control this. The explants were either dipped in antioxidants or they were incorporated into the culture medium. The concentrations used were as follows: 2% citric acid; 2% ascorbic acid; 2% citric acid + 2% ascorbic acid overnight; 3% activated charcoal in the medium.

Explants were dipped in 70% alcohol for 2 seconds and rinsed before being placed in the solutions as described. Blackening was not reduced by all treatments, and explants showed phytotoxic effects.

The transfer of apparently clean cultures to fresh media was tried without success to control medium discoloration. Procedures that were successful with *Piper* spp. were followed without success (Bhat *et al.*, 1995).

Determination of the LEC of a range of decontaminants of contaminants isolated from bud and shoot cultures of kava from field-grown material.

Fungicides (ppm) F=fungus B= bacteria	0	15	20	30	50
1. Benlate F	+++	+++	+++	++	+
Benlate B	+++	+++	+++	+++	+++
2. Nystatin F	+++	+++	+++	++	++
Nystatin B	+++	+++	+++	+++	+++
3. Penchloro-nitrobenzene F	+++	+++	+++	+++	+++
Penchloro-nitrobenzene B	+++	+++	+++	+++	+++
Bactericides (mcg)	**0**	**30**	**50**	**70**	**100**
1 Cefotaxime F	+++	+++	+++	+++	+++
Cefotaxime B	+++	++	++	+	+
2. Imipenium F	+++	++	++	++	++
Imipenium B	+++	–	–	–	–
3. Polymycin B F	+++	+++	+++	+++	+++
Polymycin B B	+++	+++	+++	++	++
4. Rifampicin F	+++	+++	+++	+++	+++
Rifampicin B	+++	+++	++	++	++
5. Streptomycin sulphate (SS) F	+++	+++	+++	+++	+++
Streptomycin sulphate (SS) B	+++	+++	+++	++	++
6. Amphotericin F	+++	+++	+++	+++	+++
Amphotericin B	+++	+++	++	++	++

Notes: + = light growth of contaminating agent
++ = medium growth of contaminating agent
+++ = abundant growth of contaminating agent
– = no growth

Effect of nutrient strength on the growth of kava and associated contaminants in tissue culture.

MS strength	% contaminated	% decontaminated	% dead
1. Full strength	85	0	15
2. 1/2 strength	50	0	50
3. 1/4 strength	70	10	20
4. 1/6 strength	30	30	40
5. 1/8 strength	40	30	30

DETERMINATING THE LEAST EFFECTIVE CONCENTRATION OF ANTICONTAMINANTS FOR ELIMINATING CONTAMINANTS IN KAVA

The Least Effective Concentration (LEC) of fungicides (Benlate, nystatin and penchloronitrobenzene), and bactericides (cefotaxime, imipenium, polymixin B, rifampicin, streptomycin sulphate, and amphotericin) was determined by inoculating potato dextrose agar (PDA) and nutrient agar (NA) plates containing a range of concentrations of anticontaminants with samples taken from kava plants. The LEC is considered as the lowest concentration giving no visible growth of contaminants.

The decontamination effects of three fungicides and six bactericides were tested on contaminants isolated from kava bud and shoot cultures. The three fungicides reduced fungal mycelia growth at LECs 30 and 50 ppm. No fungicides, at any of the concentrations tested, were able to completely control fungal growth or reduce bacterial growth. The decontamination effect of the six bactericides tested showed that imipenium could completely control bacterial growth at LEC 15 ppm. The decontaminating effect of benlate and imipenium were further studied by incorporating 75 ppm benlate and 30 mcg imipenium into the culture medium. 30 shoots and 15 buds were cultured, but after 16 days incubation, all explants were dead. Further investigations will have to look at different combinations of these decontaminants, in particular, the fungicides benlate and nystatin, and the bactericides imipenium and cefotaxime.

MEDIUM COMPOSITION

The effect of different strengths of nutrients in the basal Murashige and Skoog medium (1962) on the expression of contaminants was investigated. The sterilisation procedures which had proved successful with *Piper* spp. (Bhat *et al.*, 1995) were used in this experiment. Shoot and bud explants, taken from two-year old field grown kava, were replicated 15 times. Root explants from hydroponic plants were replicated 10 times.

As can be seen from the table above low nutrient strength media (1/6 and 1/8) resulted in the optimum control of contaminants, and the least number of dead explants. Further work is continuing in this area.

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Tissue Culture of Banana

Vina Holo[1]

SOME OF THE REASONS WHY TISSUE CULTURED PLANTING MATERIAL IS USED BY GROWERS

1. To obtain disease-free plants. These plants can be free of any of the following diseases, bunchy-top virus, bract mosaic virus, cucumber mosaic virus, nematodes, beetle borer, and panama diseases.
2. To supply disease resistant plants. It may be possible to breed or select disease resistant plants and make these available via tissue culture, for example, resistance to Black Leaf Streak Disease.
3. To supply plants with better growth and yielding characteristics, such as height, speed of suckering, wind resistance, fruit quality, fruit quantity.
4. To obtain planting material at the correct time of the year.

SOME PROBLEMS ENCOUNTERED IN THE TISSUE CULTURE OF BANANAS

Variability in the cultured plants is a problem which has not been fully analysed. The 'Williams' hybrid clones have shown variability. This variability could have arisen from several sources:

- The selected material sent originally to the laboratory varied markedly, thus causing variability between clones.
- Poor tissue culture techniques could have resulted in the passage of the material through a callus stage, thereby introducing variability.
- The grading and selection process at the final stage of tissue culture may have been inadequate.
- The young material may have been treated poorly in the nursery stage. At least some of the material was weak, spindly and poorly grown.
- Some plants in the field show evidence of viral infection, possibly indicating that the material selected was not clean.
- Banana plants as a rule are genetically unstable. The Williams hybrid clones show a reversion to Cavendish of up to 5%.

Although these problems exist, there are still many good reasons why tissue culture can provide the means by which growers have access to superior and disease free planting material. Many of the problems can be removed or minimised by attention to the following:

- The selection of source material.
- Tissue culture techniques used.
- Problems associated with the intermediary nursery stage.
- The time required from establishing the cultures through to the evaluation of the material in the field. It takes about four months to establish cultures, a further eight months to build thousands of plants, and two to three months for growth in the nursery.

METHODOLOGY FOR BANANA TISSUE CULTURE

The methodology of banana tissue culture is fairly well known. Suckers, approximately 10–12 cm in diameter are used to initiate cultures. However, it is also possible to use small button suckers and even the terminal shoot apex derived from the inflorescence. From this initial material, an explant, 6 cm long by 2 cm in diameter is prepared, and then sterilised in 3% sodium hypochlorite plus detergent for 15 minutes, and then rinsed three times in sterile

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Newly initiated banana explants.

distilled water. The explant is further reduced in size to 4 cm long by 1.5 cm in diameter and sterilised in 3% sodium hypochlorite plus detergent for 5 minutes, followed by three rinses in sterile distilled water. Further trimming removes the bleached material, and putting a cross cut on the top of the explant can assist in the emergence of the shoot.

Tissue culture derived banana plants.

Shoot and sucker growth from the explant can take as long as three months. Usually blackened tissue has to be cut away, and the explant transferred to fresh culture media several times during this development period. For optimal growth bananas need to be subcultured every six to eight weeks.

Yam Tissue Culture

Takako Murikami[1]

EXPERIMENT 1: TO IDENTIFY A SUITABLE STERILISATION PROCEDURE FOR *DIOSCOREA* SPP.

Materials and method

Three different species were used, namely *D. rotundata*, *D. alata* and *D. esculenta*. Nodal buds were excised from screenhouse grown material. All buds were washed in tap water, dipped in soapy water for 10 minutes, rinsed in running tap water for 10 minutes, and finally dipped in 70% ethanol for 5 minutes. The material was then divided and subjected to two separate treatments:

1. 10% Chlorox + Tween for 20 minutes Rinse three times with sterile distilled water (SDW). 5% Chlorox + Tween for 10 minutes. Rinse three times with SDW.
2. 20% Chlorox + Tween for 10 minutes Rinse three times with SDW. 10% Chlorox + Tween for 5 minutes Rinse three times with SDW.

After sterilisation any bleached tissue was removed and then explants were inoculated onto the culture medium (see Annex 2 for details).

Results

	% contamination		% regeneration	
	(1)	(2)	(1)	(2)
D. rotundata	64	70	27	30
D. esculenta	56	0	22	0
D. alata	33	40	83	57

As can be seen from the results, there is much variation in the response of the tissue to the sterilisation procedure depending on which species is used. With *D. rotundata*, there was very little difference in the response of the explants to the two sterilisation procedures. Conversely with *D. esculenta* procedure (1) was optimum, with procedure (2) resulting in death of the explant. The growth of *D. alata* was severely hindered by the presence of phenolics, and although there was regeneration (30–40%), these explants did not develop. As the exudation of phenolics was stronger with this species than the others, it was thought that the poor development of the explant was due to the output of phenolics. In a repeat of the experiment it was found that increasing the rinsing time after sterilisation was very helpful in removing the phenolics. In addition, frequent transfers to fresh medium reduces the impact the phenolics have on the growth of the explant.

Yams in tissue culture

EXPERIMENT 2: TO IMPROVE THE RESPONSE OF *D. ALATA* IN TISSUE CULTURE

Materials and method

The effect of the age of the nodal bud on the establishment of the explant in tissue culture was examined. Nodal buds were numbered from (1) to (16) starting with the shoot-tip. The sterilisation procedure used was as follows:

[1]PRAP/IRETA Tissue Culture Unit, USP, Private Bag, Apia, Samoa.

(a) 20% Chlorox + Tween for 10 minutes.

(b) Rinse three times with SDW for 5 minutes, 5 minutes and 10 minutes, respectively.

(c) 10% Chlorox + Tween for 5 minutes.

(d) Rinse three times with SDW for 5 minutes, 5 minutes and 10 minutes, respectively.

Results

Nodal Position	% regeneration
n1	100
n2–n9	0
n10–n12	6–20
n13	67
n14	67
n15	50
n16	50

Of all the explants other than the shoot-tip (n1), nodal buds (13) and (14) developed well, and at a faster rate than the others. In future work with *D. alata*, this will be taken into account when initiating plants into tissue culture.

Taro Micropropagation Using Thidiazuron (TDZ)

Anthony Palupe[1]

INTRODUCTION

The rapid multiplication of *Colocasia esculenta* var. *esculenta* using *in vitro* shoot-tip culture was studied as the system in use was not optimal. Sucker production varied according to the variety used. The culture medium used for taro multiplication utilised 6-benzylaminopurine (BAP) and napthaleneacetic acid (NAA). Experiments were established to determine if increasing the concentrations of these two growth regulators would significantly increase sucker production. Increasing the concentrations of both growth regulators had no significant affect on sucker production.

TDZ AND TARO MULTIPLICATION

The presence of TDZ in the culture medium successfully induced shoot initiation, but inhibited plant height and root formation, with the optimum concentration of TDZ being 0.025 mg/l for the cultivars investigated. Initiated shoots were fasciated, (fused together and forming a single stem) and cultures had a tendency to be hyperhydrated. Shoot fasciation has been reported in other crops when TDZ is used. For example, multiple shoots generated from leaf explants of *Arachis hypogaea* (peanut) were described as fasciated (Kanyand *et al.*, 1994). It has been suggested that this problem with fasciation is the result of long-term use of TDZ. In observing the taro cultures it would seem that the TDZ-initiated shoots were comprised of adventitious and non-adventitious shoots. TDZ has been reported to stimulate the induction of adventitious shoots in a number of woody plant species, such as roses, carnations (Lu, 1993), and hardy deciduous azaleas (Fellman *et al.*, 1987). TDZ was found to be highly effective in inducing non-adventitious shoot formation in shoot cultures of *Rhododendron* (Fellman *et al.*, 1987), and *Malus domestica* (Fasolo *et al.*, 1989)

No histological examination was carried out during the course of this study. However, based on the morphological differences observed in the regenerated shoots, it is possible that two different forms of TDZ-initiated shoots were present. These two forms consisted of shoots that grew well as distinct, individual plants, and fasciated shoots which comprised of enlarged and flattened petioles with small protruding leaf-like structures giving the appearance of several shoots fused together. When the former were transferred to a secondary medium, vigorous mother plants were formed which produced more shoots. When fasciated shoots were transferred to a secondary medium, colonies of microshoots (small miniature shoots growing close together) grew, eventually losing their fasciated stage and developing into distinct and individual shoots. It is possible that these microshoots were formed *de novo*.

The occurrence of hyperhydricity in cultures which have developed on TDZ-containing medium has been reported with several crops (Huetteman and Preece, 1993). Hyperhydricity has been observed in tissue cultures that have not been exposed to TDZ, although when TDZ is present there is a greater degree of hyperhydricity. Therefore this state could be due to a number of factors and not just TDZ. With the taro tissue cultures in this study these factors could be prolonged use of BAP in the culture medium (the taro accessions are maintained on a culture medium which contains 1.0 mg/l BAP); the frequency of subculturing which increases the exposure of the plant tissue to high levels of growth regulators: decapitation of the plantlet during subculturing.

The addition of NAA to TDZ-containing medium reduced shoot fasciation and hyperhy-

[1]PRAP/IRETA Tissue Culture Unit, USP, Private Bag, Apia, Samoa.

Taro and TDZ multiplication.

dricity, but also reduced shoot production. In addition the presence of NAA lessened the inhibitory effect of TDZ on root formation and plant height. The addition of BAP to TDZ-containing medium, did not improve shoot production, but increased the extent of shoot fasciation. However, it was found that for the development of shoots initiated on TDZ-containing medium it was necessary to transfer cultures to a medium containing BAP, not TDZ. This use of a secondary medium has been successful in inducing shoot elongation with *Rhododendron* (Preece and Imel, 1991), and apple (Fasolo *et al.*, 1989). Further shoot production was encouraged by introducing a third medium containing a relatively low concentration of TDZ (0.005 mg/l). The following three stage system is now recommended for maximising shoot production:

STAGE 1: MSO + 0.025 mg/l TDZ
STAGE 2: MSO + 1.0 mg/l BAP
STAGE 3: MSO + 0.005 mg/l TDZ

With four weeks on each stage approximately 55 shoots can be obtained from one explant. MSO medium is Murashige and Skoog Plant Salts (1962) with the addition of 100 mg/l myo-inositol, 0.4 mg/l thiamine, 30 g/l sucrose and 1.75 g/l Gelrite at a pH of 5.7

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Tissue Culture Activities at Nu'u Research Station

Valerie S. Tua[1]

The present objectives of the tissue culture programme at Nu'u are (a) multiplication of selected varieties of banana and taro for growers; (b) development of strategies for germplasm collection and conservation; (c) production of an operational manual for the tissue culture unit.

The shadehouse at Nu'u.

Crops currently being multiplied are taro, banana, orchids and anthurium, with the emphasis on agricultural crops. With taro, priority is being given to the varieties that have demonstrated tolerance/resistance to taro leaf blight (TLB) such as PSB-G2 from the Philippines, and Toantal from Federated States of Micronesia. In addition, the laboratory has also obtained some varieties from Palau that have shown good tolerance to TLB; these are being bulked up for evaluation.

There is also a large demand for the banana variety 'Goldfinger', and therefore the aim of the laboratory is to deflask about 1,000 bananas every month. Multiplication of other varieties, (FHIA-2 and TG-6) that have shown tolerance to Black Sigatoka disease is also ongoing.

There is interest in the ornamentals from local growers but as stated earlier the agricultural crops have preference. Plants that have been deflasked and are established outside are available for sale to the public.

Fungal contamination in the laboratory is relatively low, but there are always problems with bacterial contamination. Generally this tends to be bacteria originating from the internal tissues of the plant, rather than being introduced from outside.

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POSITION PAPERS

Micropropagation of Agricultural Crops

Mary Taylor[1]

INTRODUCTION

Micropropagation is the true-to-type propagation of a selected genotype using *in vitro* culture techniques. Most often micropropagation is associated with mass production at a competitive price. The advantages of micropropagation are listed in many tissue culture textbooks and are as follows:

- Increased rates of propagation.
- Ability to propagate at any time of year.
- Clonal propagation and therefore the ability to select for desirable characteristics.
- The use of meristem culture can enable pathogen-tested material to be produced: certification schemes can be initiated.

The disadvantages are stated as follows:

- Initial cost of establishing a tissue culture unit.
- Genetic stability.
- Expertise required.

Although a tissue culture unit can be expensive basic units can be established at a minimum cost, using whatever facilities are available. State-of-the-art laboratories can be constructed for US$150,000, for example, the unit at the Coffee Research Institute in Aiyura, PNG. However, it is also possible to equip a tissue culture unit with the basic, essential equipment for US$11,000. Obviously the proposed function of the unit determines to some extent what the cost will be, and also what funds are actually available to use.

Genetic stability has been a criticism of tissue culture for many years, and the scenario with oil palm and Unilever many years ago did little to alleviate the critics. However, over the years studies have shown that certain crops are more susceptible to genetic mutations and the production of off-types, and these will appear with both conventional propagation and micropropagation. Banana is an example of one of these crops, where a 2% production level of off-types is an acceptable limit. Even within the *Musa* spp., some varieties are more susceptible than others to this problem. Studies at the cassava institute (CIAT), in Colombia, have shown that cassava is a very stable crop in tissue culture and in the field. When Unilever had problems with the clonal oil palm material it was due to the technique used where the plantlets had been initiated through a callus phase. Although genetic stability in tissue culture is still a recognised problem, there is a greater understanding as to what can cause this problem, thus tissue culturists are aware of the techniques that can induce genetic stability, and also know the crops where mutations are likely to occur.

ARE THERE MICROPROPAGATION TECHNIQUES AVAILABLE FOR MANY OF THE CROPS IMPORTANT IN THE REGION?

Multiplication systems exist for many crops, and already in some countries tissue culture is being used in the commercial production of these crops.

Musa spp

In Taiwan, the Taiwan Banana Research Institute is producing in the order of two million plantlets resistant to *Fusarium* Race 4 per year for growers. With the micropropagation scheme yearly planting is encouraged so that fruits and plants are harvested at the same time. A higher planting density together with a very significant reduction in the use of chemical inputs offsets the increased costs from annual planting. In addition the growers claim that other advantages result from the use of

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tissue cultured plants such as increased uniformity, reduced losses at the initial stage of planting, increased vigour and increased yields.

Closer to home tissue cultured banana plants are sold to growers in Tonga. Again growers have recognised the advantages of higher quality planting material and the project has been a success. The tissue culture production is linked to field trials that are aimed at determining the 'best' banana for the grower in terms of resistance to Black Leaf Streak, taste and yield. A similar project is underway in Samoa where banana production is hindered by Black Leaf Streak. The cultivar 'Goldfinger' has performed well in field trials on the research station, and so the national tissue culture laboratory is now in the process of producing 15,000 banana plantlets for on-farm evaluation.

Colocasia esculenta var *esculenta* (Taro)

Until recently the taro found in this region was not very responsive to tissue culture, and multiplication rates tended to reflect what was found in the field. The micropropagation system used by the regional laboratories could improve on this slightly but not to any great significance. The taro found in Asia and Africa was more responsive, being var *antiquorum*; consequently this was the variety on which reports were found in the tissue culture literature.

The work of two research assistants at the PRAP laboratory in Samoa, Valerie Saena and Anthony Palupe, has led to the development of a micropropagation system for taro that gives multiplication rates of 35–200 within four months which is a significant increase over the field rate of 15 in six months. This system is now being used in Fiji and Samoa to bulk up taro planting material for the growers.

Pineapples

Although no laboratory in the region has had experience in the micropropagation of pineapples, tissue culture of this fruit is well-established and is used commercially. It is a plant that proliferates more rapidly if established explants are subcultured in liquid medium, and therefore it is a system that responds well to the use of fermentors. As a result high rates of multiplication can be achieved. The multiplication rate can be enhanced by the use of coconut water and casein hydrolysate in the medium; these are relatively simple compounds, and easily obtainable. In addition, pineapples can be rooted in the same medium in which they proliferate as there is no auxin requirement for root initiation and development. Some workers suggest that at least 5,000 plants could be produced in 12 months from a single crown. Another micropropagation technique has been developed in Hungary where the starting material was tissue cultured plants. Using this technique it is predicted that 80,000 plants can be regenerated from one primary plant within one year (Kiss *et al.*, 1995).

Pineapple, like banana is prone to variation, but there is some evidence that this variation is linked to the variety. It has to be remembered, however, that variation can be useful; spineless variants of pineapple have been identified in tissue cultured pineapple.

Strawberry

Although considered a temperate fruit crop, strawberry production is possible in the tropics using varieties specifically bred for the tropical conditions. Improved plant material is available from DPI, Brisbane, at an approximate cost of AUS$50.00 per tube. Strawberry was the first fruit crop to be micropropagated on a large scale. Commercial production began in the mid- to late-70s and was particularly active in Italy where production figures were near 1,000,000 plants per year for several years.

Micropropagated plants are generally used in nurseries as the starting point for one or two years of runner propagation. These runner plants are then used to establish the fruiting fields. The direct use of tissue cultured plants in the field has been avoided as costs were high and fruits from such plants tended to be smaller than from runner-propagated plants. With a crop like strawberries, it is likely that growers would have to obtain 'clean' planting material on a regular basis, as this crop picks up diseases and yields decline after several years.

Some work has been carried out on strawberry tissue culture in the PRAP lab at Samoa, and it is a relatively easy technique. Shoots can be induced from leaf tissue as well as from de-

Newly planted strawberries from tissue culture.

fined axillary buds. Like pineapple roots are formed on the same medium as the shoots thus reducing labour input and costs. Some plants produced from tissue culture have recently been planted out in the screenhouse, and it would appear that transfer from *in vitro* to *in vivo* is not problematic.

Potato

Again potato is considered to be a temperate crop but using varieties developed by CIP potato production in the tropics is a viable option. The possibility of producing potatoes in the tropics has been evaluated in Samoa using a technique developed by CIP and used extensively by Vietnamese farmers. This technique uses tissue cultured plants for the production of apical cuttings. From one tissue culture plant it is possible to produce a crop of apical cuttings every three weeks for a period of six to nine months. These cuttings can either be used for the production of more apical cuttings, for the generation of seed tubers, or can be used directly as planting material in the field. This system negates the reliance on expensive seed tubers from overseas. It would appear to be possible to use the tubers from the first crop for the next season's crop, but persistent use of tubers leads to a poorer quality crop. When the quality of the apical cuttings decreases then the tissue cultured material can be used to generate more apical cuttings. This system has been so successful in Vietnam that many farmers have established very basic tissue culture facilities in their front rooms. Work in Samoa indi cates that using this system potato production can occur all-year-round except when there are very heavy rains. More details are available from the PRAP leaflet on this system.

Strawberries in tissue culture.

Asparagus

Improved material can be obtained from New Zealand. Asparagus was grown at Samoa, and the project indicated that this crop could be cultivated all-year-round providing three crops per year. There is a manual available for the commercial propagation of asparagus which provides details of a technology which is sufficiently advanced to allow commercial field plantings of asparagus clones (Abernethy and Conner, 1993). Using the technique described the authors have been able to produce over 120,000 cloned plants ready for field establishment over the past three years. The system relies on the production of minicrowns. These can take two to six months to produce depending on the variety with the original explant being lateral buds from the upper area of the spear. The technique would appear to be relatively easy, but labour intensive. There is no indication in the manual of costing and the time required for material to be ready for field planting. However it would seem that the total tissue culture period is in the order of three to nine months with a further five to six weeks required for weaning off.

WHAT ARE THE STAGES OF PRODUCTION INVOLVED IN MICROPROPAGATION?

Stage I: Initiation into culture

This is the very first stage in the micropropagation of any plant. Decisions have to be made as to what explant should be used, what will be the sterilisation technique used, what will be the initiation medium, etc., etc? Because it is crucial that clones are produced in micropropagation the selected explant is usually an apical or axillary bud. For some plants other explants are used and shoots are produced from adventitious buds, for example, leaf tissue is used for *Anthurium* spp.

Potato in tissue culture.

Sterilisation to remove all associated pathogens can be problematic especially if the tissue is derived from woody species. This can take up a lot of time in the initial stages of research and development into a crop, and be very frustrating. With some plants it is necessary to use the more toxic substances such as mercuric chloride to obtain effective disinfestation.

With some crops the production of phenolics can hinder establishment of the explant in culture, and can necessitate the pre-treatment of the explant prior to initiation, the incorporation of activated charcoal into the culture medium, and/or frequent changes onto new medium.

Stage II: Multiplication

There are several factors to consider with this stage. Although one can choose whether to have axillary or adventitious bud formation, with micropropagation it is important to maintain genetic fidelity and so the safe system of axillary bud formation is chosen. Associated with this requirement for maintaining genetic stability in the system is another variable, that of the subculture interval. Although short subculture intervals favour multiplication rates, they also favour mutation rates, and so a compromise has to be made.

Obviously of key importance is the culture medium and the growth regulators to use. Again there has to be a compromise between high multiplication rates and genetic stability. It is generally agreed that high levels of cytokinin can induce rapid shoot proliferation, however, high cytokinin levels are also linked to genetic mutations. Some cytokinins are more likely to favour genetic change than others, for example, 2,4-D.

Finally there is the culture environment, which includes light, (quality and quantity), temperature, humidity, culture containers.

Potato apical cuttings from tissue culture.

Although it has been shown with some species that warm red light will benefit shoot development, ordinary fluorescent lights are widespread because of the ease with which they can be obtained and their cheap cost. With quantity there are two considerations, daylength and amount of light that the plant uses. Daylength is usually either 16 h or 12 h depending on whether the species is temperate or tropical. The amount of light that the plant uses varies with laboratory but is always less than the natural light levels found in the field; it can be measured as lux, foot-candles, etc.

Stage III: Elongation and root induction/development

With many plants there is no need for this Stage as all the processes can be achieved in Stage II. However, with many tissue culture systems, when shoots are produced in clusters it is often necessary to introduce a elongation stage so that the shoots are easier to separate without losses occurring. The elongation stage usually necessitates a different medium in which there is either no growth regulators, or lower levels.

Many commercial labs do not root plantlets *in vitro* for several reasons: very labour intensive because single shoots have to be handled; the root system formed *in vitro* is not very functional; the roots can be damaged during the planting process; for difficult to root plants it can be easier and cheaper to induce roots *in vivo* rather than *in vitro*. However, rooting *in vivo* might not be a viable option as depending on the species and facilities, rootless plantlets may die in large numbers when removed from culture, whereas those with roots may have a higher survival rate. If the losses are low, then rooting and acclimatisation can be done simultaneously. This can significantly reduce

costs, and add efficiency to production schemes.

Stage IV: Acclimatisation

As with the other stages described several factors have to be considered with this very important process. Considering all the inputs that have gone into the system prior to this stage it is obviously crucial to minimise/eliminate losses. The factors that require the major considerations are: humidity; light; sanitation; substrate; fertiliser.

Because of the anatomical and physiological condition of the plantlets that develop *in vitro*, maintaining a high level of humidity for the first few days following transplanting has been critical for survival. Some commercial laboratories have installed automatic mist systems but these have their disadvantages in that the mist leaches nutrients; it can cause the medium to become too wet; the plantlets themselves can become dry; an environment is created that favours the growth of algae, fungi and bacteria. Other methods that are used are fogging, humidifiers, or the use of structures that will retain water vapour. Suitable structures include polythene flat covers, humidity boxes or polythene tents. Disadvantages with these structures are heat build-up and the labour required to monitor water loss.

In tissue culture plants have been exposed to relatively low levels of light, therefore their leaves are thin, and resemble shade leaves. If placed under too high a light level, they will become chlorotic and scorched. With some plantlets periods of four weeks in 90% shade is recommended, however, plantlets will benefit from as high a light level as they will tolerate. Shading reduces the transpirational demand and excessive light that can destroy chlorophyll molecules. Following a period of shade, plantlets should be gradually moved to the light level under which they will be grown.

Substrate and containers can be another important variable. Most labs and nurseries will transplant into a uniform medium that adequately supports the plant, has a suitable pH, is well buffered, reproducible, and sufficiently porous to allow adequate drainage and aeration.

To avoid the introduction of diseases, for example, leaf and/or stem decay, good sanitation practices, such as sterilised soil, new or disinfested containers are used. Although many labs discourage the use of pesticides until the plantlets have begun to acclimatise, others apply bactericides and/or fungicides as a prophylactic measure.

Most plant species grow more vigorously when they are fertilised regularly after transplanting, with the optimum fertiliser rate depending on the species.

ECONOMIC CONSIDERATIONS

A production laboratory must have a substantial part of its production in cultivars that are demanded in large quantities at a reasonable price; they help to lower the unit price for themselves and for other cultivars.

In countries such as Australia and the United States labour accounts for 60 to 80% of the costs, with 80% being the appropriate figure for the smaller laboratory. The second most important cost is related to the space needed to grow plants. In the US this is costed at US$4 to 5 per sq.m. per week, which accounts for 10 to 15% of the total cost, 50% being power costs, and 50% depreciation. Ingredients for the preparation of the culture medium accounts for only 5% of the total cost of the operation, therefore the use of cheaper products has little effect on production costs per unit. It is better to improve efficiency than to look for cheaper media ingredients. Increasing the number of shoots per container is far more important. It is likely that in a region such as the South Pacific, where tissue culture laboratories can be run with a relatively small number of technical staff, with little need for complex research, that labour costs can be kept relatively low.

There are possibilities for lowering cost and these should be considered.

Labour

As labour costs can be the largest portion of the production costs it is essential to optimise the outputs from the area where the labour input is the greatest, and that is the transfer area. Factors to consider are spacious work benches, large airflow cabinets; these will make working easier and reduce problems like contamination. Time spent making media can be reduced through making large batches, but then

the larger the batch, the larger the loss if mistakes are made.

Electricity

Lighting and cooling are important factors. The cost of lighting can be reduced if natural light is exploited, and this in turn will reduce cooling costs. As it is the ballasts that generate the heat with the lights some laboratories have these installed outside the culture room. Alternatively, electronic ballasts that produce less heat can be used. Obviously the savings in electricity have to be balanced against the cost of these ballasts.

Planting density

It is worth investigating whether more shoots can be grown in the same container, with the same amount of medium, while maintaining the same quality. Studies have shown that increasing the number of explants per container by 10% results in a total saving of at least 3% on the production costs.

Finally it has to be remembered that although the economics of the system has to be seriously considered, there can be other reasons for establishing a tissue culture unit that are difficult to put a value on. With the national laboratories in this South Pacific region there have always been two other considerations, namely to facilitate the distribution of improved germplasm, and to provide a more secure means of conserving plant genetic resources.

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In Vitro Conservation

Mary Taylor[1]

INTRODUCTION

The importance of genetic diversity to agricultural productivity cannot be disputed. The genetic resources of a crop consists of the total pool of genetic variation which exists in the crop species, and includes the genes of cultivated, weedy and wild related species which are sufficiently close to the cultivated forms for gene exchange to be possible (Holden *et al.*, 1993). Genetic diversity is a necessity if plant breeders are to have the means with which to adapt crops to the ever-changing demands of the environment and the consumer. The generation of new varieties with characteristics different from those in use today will depend on the availability of a genepool from which to select the desirable genes. Although great advances have been made in the field of biotechnology one certainly cannot make any assumptions that at any one time certain genes and gene combinations can be manufactured.

Examples of how genes have come to the rescue in the past in the improvement of crop varieties, often in response to pest or disease epidemics are many. Disease resistance is not the only kind of breeding problem for which genetic resources can be used, but it does provide some excellent examples of the way in which genes from primitive varieties and wild species can be used to treat a problem and improve crop performance. Breeding for potato eelworm resistance led to the availability of varieties which enabled farmers to produce high crop yields on land which had previously produced failures. The banana cultivar 'Goldfinger' is another example of the use of genetic diversity in breeding for resistance to black Sigatoka.

In the same way that the contribution of genetic diversity to agriculture is not disputed, the fact that genetic erosion is occurring at a significant rate is also accepted. The genetic diversity of many crop plants is at risk, and genetic resources are a non-renewable resource. Genetic erosion is the result, largely, of human activity. It occurs either directly through large-scale destruction of plant communities, for example, logging of rainforests, and construction of dams, or indirectly through man-induced climatic changes; the latter having a more significant effect on crop plants than the former. In addition, with crop plants this is exacerbated through the substitution of genetically uniform high-yielding varieties for the more variable landraces.

PROBLEMS WITH PRESENT CONSERVATION STRATEGIES

Much of the conservation of crop genetic resources in the South Pacific is in the form of field genebanks maintained within the individual countries. As with field genebanks throughout the world losses are occurring at an ever-increasing rate. Most of the information that is available from the South Pacific Island countries on field conservation of food crops relates to taro and yams. The following two tables show data for field collections for these two crops for 1985 and 1994.

There are numerous reasons for the losses that have occurred since the collections were made in the 1980s, but generally they are no different from those reported in other regions of the world (Henshaw *et al.*, 1980; Hanson, 1986; Jarrett *et al.*, 1990; Malaurie *et al.*, 1993). Those reasons are stated as cost of maintenance of plants that require frequent harvesting and replanting; inadequate storage facilities for yams; extreme climates, e.g. cyclones and drought, and lethal diseases. As well as loss of germplasm, descriptor records have also been lost.

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Table 1. Collections of taro and yam in nine Pacific Island countries, 1985 (based on data of Guarino and Jackson, 1986)

Countries	Number of Accessions in Collections						
	Taro	Yams					
		D alata	*D. esculenta*	*D. anummularia*	*D. bulbifera*	*D. pentaphylla*	Other species
Cook Islands	57	8	0	0	0	0	0
Fiji	72	89	16	1	0	0	0
Niue	52	23	0	1	0	0	0
Laloki	135	153	94	1	1	5	0
PNG Bubia	120	153	94	1	1	5	0
PNG Aiyura	52	6	0	7	1	1	0
Solomon Islands	31	238	117	31	11	2	15
Tonga	0	0	0	0	0	0	0
Tuvalu	13	0	0	0	0	0	0
Vanuatu	138	194	17	9	12	4	9
Samoa DAFF	20	0	0	0	0	0	0
Samoa USP	28	12	2	6	2	1	0

Table 2. Collections of taro and yams in nine Pacific Island countries, October 1994 (Jackson, G.V.H., 1994)

Countries	Number of Accessions in Collections						
	Taro	Yams					
		D. alata	*D. esculenta*	*D. nummelaria*	*D. bulbifera*	*D. pentaphylla*	Other species
Cook Islands	0	3	0	0	0	0	0
Fiji	78	75	17	1	0	0	0
Niue	0	0	0	0	0	0	0
Laloki	0	167	168	20	20	20	20
Bubia	450	0	0	0	0	0	0
Solomon Islands	2	55	83	0	0	0	0
Tonga	21	0	0	0	0	0	0
Tuvalu	13	0	0	0	0	0	0
Vanuatu	0	*	0	0	0	0	0
DAFF	17	0	0	0	0	0	0
USP	0	7	0	0	0	0	0

*A few accessions may still be present.

As well as losses occurring with collected material, there is relatively strong evidence that general genetic erosion is taking place with both taro and yams. Before taro leaf blight taro and yams were the main crops grown in the Solomon Islands; today 65% of the land is used for sweet potato production. It has been suggested by Kesevan *et al.* (1982), that taro production in Papua New Guinea has declined as a result of population pressure, plant diseases and the introduction of higher yielding crops such as *Xanthosoma*, sweet potato and cassava. Similarly in Micronesia, declining taro production has been attributed to pests and diseases, and ranks behind breadfruit, yam, banana, and imported rice as a staple food (Raynor *et al.*, 1992). In Hawaii, it was estimated that more than 50% of the traditional taro cultivars no longer existed (Whitney *et al.*, 1939).

Yam cultivation is also decreasing with the high costs of staking, anthracnose disease, and storage problems cited as the reasons for the lack of interest in the crop. There is less information available about this crop, and what there is tends to be concerned with the two dominant cultivated species of the region, namely *Dioscorea alata* and *D. esculenta*. It is likely that the other species *D. nummelaria*, *D. bulbifera* and *D. pentaphylla* are the ones at the greatest risk of erosion (Kesevan *et al.*, 1982).

Losses of genetic resources in field collections have not only occurred with taro and yams. Sweet potato and cassava collections have suffered in most countries, though to a lesser extent, possibly because there is less disease pressure on these two crops. What all the crops share in common is high maintenance input and high risk factor associated with field genebanks. The South Pacific region is made up of small island countries, many of which have very limited resources with which to maintain field collections. It is fairly common to find that a staff member responsible for a field collection also has numerous other duties. Low wages are the rule for such staff and so staff turnover is high; with staff turnover continuity and commitment is lost. Land can be a problem in many countries, and land ownership disputes can occur over land on which collections are maintained. Furthermore it has often been said that in most cases funds have been available for collecting but not for maintenance. The maintenance factor is further exacerbated by the fact that everything has been collected, i.e. there has been no collection strategy.

There is an obvious need to look at the conservation strategies for most of the regional food crops, especially within countries such as Papua New Guinea where much genetic diversity exists; there is a vast wealth of genetic resources which agriculture can utilise to its advantage. Loss of these resources would have a negative effect on agricultural output. The question is what is the optimum method of storing plant genetic resources. In defining optimum we are considering security and costs. In the ideal world no one method should exist in isolation, a collection of plant material should be duplicated for security purposes. Conservation strategies for any plant genepool may include a range of both *in situ* and *ex situ* methods. The methods chosen should be carefully considered, taking account of feasibility, practicality, economy and security. Whichever strategies are chosen, a clear and well-organised management system needs to be in place to ensure that linkages between the components of the conservation and use programme are maintained.

IN VITRO CONSERVATION AS AN ALTERNATIVE STRATEGY TO FIELD CONSERVATION

Field conservation

As already stated the conservation strategy most utilised in the South Pacific is field genebanks. The problems associated with this method have already been discussed. Although field genebanks provide easy access to conserved material for use and research, they are very vulnerable systems, easily affected by natural disasters or pest and disease outbreaks. It is also argued that they are highly demanding of space and labour, and are expensive to maintain (Jarret *et al.*, 1990).

In vitro conservation

Tissue culture conservation can offer some distinct advantages over alternative strategies. These include: (i) the potential for virus elimination, that is material can be maintained in a pathogen-tested state, (ii) pathogen-tested material allows for safer distribution across

borders compared to vegetative material, (iii) reduced storage space requirements compared with field storage, (iv) storage facilities may be established at any geographic location, (v) cultures are not subject to environmental disturbances such as cyclones, pest and disease outbreaks etc., (vi) less labour intensive. There are also some limitations in the use of tissue culture for conservation. These are mainly the relatively high cost of establishing tissue culture facilities, and the need for trained staff for management of the tissue culture genebank. The latter is especially important in the more tropical, and less industrialised countries where contamination levels are naturally high, and minor frustrations can become major problems due to poor infrastructure. Finally tissue culture storage techniques have not been developed to the extent that they can be routinely applied across a wide range of crops.

The two major tissue culture storage strategies are (i) slow-growth techniques and (ii) cryopreservation. Slow-growth techniques for medium term storage allow clonal plant material to be stored under tissue culture conditions with extended subculture intervals. Cryopreservation techniques may be applied for the long-term storage of regenerable material, such as zygotic or somatic embryos and shoot-tips.

SLOW GROWTH STORAGE

Slow-growth techniques are used as a medium term storage option, allowing subculture intervals to be extended to between 12 months and four years for many species. There are several different techniques for achieving slow growth in tissue culture. These are: modification of the culture medium through the use of osmotic or hormonal inhibitors, or other growth retardants; reduction of storage temperature; mineral oil overlay; reduced oxygen tension; defoliation of shoots; immature zygotic embryos (Rao *et al.*, 1994). The main disadvantages with these systems are stated as being genetic instability, and limited length of time in storage. However, it is generally agreed that slow growth storage systems do offer a more secure and cost-effective system of conserving vegetatively propagated crops than field conservation, once sufficient research and development has been carried out to suitably refine the technique for a given crop. In addition, material can be rapidly propagated from the genebank when required. However, it must be remembered that there are not enough data on the long-term effects of slow growth, and therefore caution must be used before the large-scale adoption of slow growth methodology to any crop.

Tissue culture methodology exists for all the root and tuber crops of the South Pacific island countries, and in some cases, slow-growth storage is used for conservation of these crops. Slow growth storage of yams has been extensively studied at IITA in Nigeria. Reduction in temperature gave a storage time of 1.5 to 2 years on standard culture medium lacking in growth regulators. The addition of mannitol to the culture medium allowed storage for 13 months. A relatively large yam collection has been maintained in the Ivory Coast on unsupplemented Knops medium; at 28°C subculturing was required at 6 to 12 month intervals depending on the clone. A large number of these accessions were from the West Indies and the Pacific, indicating that the methods are applicable to yam germplasm worldwide (Jackson, 1994). In this laboratory yams are maintained on an unsupplemented Murashige and Skoog medium for approximately eight months between subculture periods.

Cultures of sweet potato are stored in this laboratory at a temperature of 20°C on an unsupplemented Murashige and Skoog medium; this extends the subculture period to six to eight months. However, it has been noted that if the temperature drops below 20°C, the plants are stressed and there is leaf senescence. In contrast, studies at CIP on a range of temperatures for slow growth storage of sweet potato found that 15°C was optimal (Lizarraga *et al.*, 1992). CIP use a growth restriction medium which allows transfer of cultures to be made every 12 months. This laboratory is presently collaborating with IPGRI and tissue culture laboratories in the Caribbean region through CARDI in an assessment of slow growth methods for sweet potato. The use of abscicic acid in the medium can slow down plant growth; this agrees with the work by Jarret *et al.*, (1991) which showed that abscicic acid at a concentration of 10 mg/l was effective in inhibiting growth, but that the effect was genotype-dependent. Although the use of the sugar alcohols, sorbitol and mannitol can

reduce the growth rate of sweet potato, morphological changes have also been observed.

The taro collection in this laboratory is maintained at a temperature of 20°C, allowing subculturing to take place every 4 to 6 months. There are reports in the literature of taro being stored for more than eight years with transfer intervals of approximately three years at 9°C in total darkness (Bessembinder *et al.*, 1993). However, the experiment was only conducted with one clone, and it does not state in the report whether it was *Colocasia esculenta* var. *esculenta* or var. *antiquorum*. Similarly, three species of *Xanthosoma* could be stored in the dark for at least two years at 13°C. (Zandvoort *et al.*, 1994). The problem with these very reduced growth temperatures is the need for a separate room, and the practicalities of maintaining this temperature in the tropics. Some preliminary experiments carried out in this laboratory on slow growth systems of taro have looked at the effect of combining a low temperature environment, reduced light, and osmoticums. To date, mannitol (3%) in combination with a temperature of 20°C significantly suppressed growth. However, when mannitol was used with cultures initiated directly from the field, a phytotoxic effect was observed.

At CIAT cassava accessions are maintained on a slightly modified Murashige and Skoog medium at a temperature of 21°C to 23°C. Under these conditions subculturing takes place every 12 to 18 months, depending on the genotype (Mafla *et al.*, 1996). In this laboratory the cassava accessions used to be maintained at a temperature of 20°C, but this proved to be suboptimal and so the accessions are now maintained at 23°C on an unsupplemented Murashige and Skoog medium. Cultures are transferred every nine months depending on the genotype.

It is generally felt that the full potential of slow growth storage has not been realised. Sufficient research has not been carried out with a wide range of crops, and in many cases where research has been carried out, results have not been published. In addition, there is also the on-going doubt regarding genetic stability. Studies at IITA on sweet potato and cassava did not detect any morphological changes in plants that had been in culture for 6 to 7 years (Ng, 1991). Similarly, analysis of stability of 7 varieties after 10 years of slow growth conservation at CIAT demonstrated complete genotypic stability of all material tested (IPGRI/CIAT, 1994). In contrast, a reversal of corm flesh and cortex colours were observed with a Fijian variety of taro that had been maintained in culture for several years (Jackson, 1994). Again lack of data does not allow realistic assessments to be made, in that there is yet no evidence that the genetic instability of vegetatively propagated crops in tissue culture is any more or less than that which occurs in field genebanks.

CRYOPRESERVATION

Cryopreservation is low temperature storage under liquid nitrogen. At this temperature plant growth stops, and in theory, storage can take place for an indefinite period without any genetic modification. Cryopreserved material also requires very limited space, is protected from contamination, and needs very little maintenance.

Cryopreservation protocols are in the development stage for most crop species. Crop species for which cryopreservation is routinely used across a range of genotypes includes only *Rubus, Pyrus, Corylus, Solanum* spp., and oil palm. The techniques in use and under investigation vary from the older classical techniques, based on freeze-induced dehydration of cells (Kartha, 1985), as well as newer techniques, based on vitrification (Kartha and Engelmann, 1994; Withers and Engelmann, 1995). With the classical techniques, the tissue is initially cooled slowly, at a controlled rate, to approximately –40°C, followed by rapid cooling in liquid nitrogen. One problem in the application of this technique is the need for relatively sophisticated and expensive programmable freezing equipment, thus making the technology difficult, if not impossible for genebanks in the smaller, less industrialised countries.

The newer techniques based on vitrification have been developed over the last ten years. In this context vitrification means the transition of water directly from the water phase to an amorphous phase or glass, avoiding crystalline ice formation (Fahey *et al.*, 1984). All of the methods based on this concept utilise a very rapid freezing process, with tissue samples being plunged directly into liquid nitrogen

once the pretreatment stages have been completed. Consequently, the internal solutes vitrify and deleterious intracellular ice formation is avoided. The recent successes of these more simpler techniques allow cryopreservation to be tested with more crops without the purchase of expensive equipment such as programmable freezers. This also enables cryopreservation to be used in the more basic tissue culture laboratories.

There has been some progress in cryopreservation techniques for the root and tuber crops important to the South Pacific island region. In Japan, at JIRCAS, meristems of taro have been successfully cryopreserved using the vitrification technique; after cryopreservation shoot formation of taro meristems was nearly 100% (Engelmann, pers. comm.). Successful cryopreservation of sweet potato has also been reported using the vitrification technique, although survival and regeneration rates varied widely among genotypes (Towill *et al.*, 1992). Successful cryopreservation of *Dioscorea* spp. has been reported from India using the encapsulation-dehydration technique with survival rates of 71% and regeneration of 20% to 40% from the species tested (Mandal *et al.*, 1996).

Work at CIAT has resulted in the development of an ultra-rapid freezing technique for cassava which is being used to establish a long-term base genebank of cassava clones using liquid nitrogen. Work is continuing to refine the method so that it is applicable to a wider range of clones (Escobar *et al.*, 1992). As with slow growth storage the real potential of cryopreservation has not been realised. Some indication of its economic value can be seen from the following table.

As well as the savings in cost and space there is also increasing evidence that provided the cryopreservation technique used ensures the integrity of the frozen plant material, there is no modification at the phenotypic, biochemical, chromosomal or molecular level attributable to cryopreservation.

WHAT COULD BE THE CONSERVATION STRATEGIES FOR THE MAJOR ROOT AND TUBER CROPS IN THE SOUTH PACIFIC REGION?

This raises the question of what are the criteria that should be considered when evaluating conservation strategies. Security is obviously of major importance otherwise there would not be any concern about the present situation. In addition, cost must also be a significant factor in this age of limited resources. Finally, there is the question of accessibility; decisions have to be made as to which accessions are wanted for immediate use and/or distribution.

Field collections are often used as both active (for distribution and use), and base (for conservation) collections, with no back-up collection (Jarret *et al.*, 1990). Although curators may be very efficient and attempt to minimise losses, material which is not duplicated in another form, or at another location is at risk. Loss of an entire collection is possible for any storage method, so secondary collections are very important.

In vitro storage can be used successfully for medium-term storage, and for some active genebanks. It can be a back-up for a field collection, or the main collection backed up by a second culture collection, or field collection. As *in vitro* collections are not very useful for evaluation and characterisation, this information should be available before any action is taken with an existing field collection. Long-term storage of clonal materials should make use of cryopreservation, if possible, with *in vitro* or field collections for active use. Other important considerations are risk assessment of the field collections from biotic or abiotic factors; if the risk is significant, then *in vitro*

Table 3. Maintenance costs of cassava germplasm under three conservation methodologies (Escobar et al., *1992)*

Method of Conservation	Collection Size No. of accessions	Area utilised in m	Estimated cost p.a. in US$
Field Genebank	5,300	50,000	30,000
In vitro genebank	4,850	50	25,000
Cryopreservation	6,000	1	5,000

storage is a possibility. If distribution is one of the main uses of a collection, and there are difficult quarantine problems with the crops in the collection, then again *in vitro* storage would solve this problem. Field collections may be preferred for genotypes that are known to produce variants in order to facilitate rogueing.

Cryopreservation can provide a secure back-up at little ongoing cost. Initial costs can be high but procedures can be accommodated within any tissue culture facility. This is especially true now that the less complex techniques such as vitrification are available. Cryopreservation is ideal as a base collection as an insurance against loss of field or *in vitro* accessions. It is generally considered that for long-term conservation, cryopreservation is the preferred method, with *in vitro* as the second choice, and field as the third. For accessibility, that is, study and use, field is preferred, with *in vitro* as the second choice.

Very few thorough economic studies have been carried out on the different forms of conservation. The cost of field and *in vitro* storage can be similar especially with smaller collections, but this does not necessarily include a cost for the greater security of the *in vitro* collection. However, as the collections increase in size, *in vitro* becomes the more economical option. The cassava collection in the field at CIAT holding 6,000 accessions costs US$30,000 whereas *in vitro* the same number of accessions cost US$25,000. One would assume that some, if not all of the *in vitro* collection has been pathogen-tested and this would add an extra value to the *in vitro* collection and make it more 'useful' with regards to distribution compared to the field collection. Sweet potato field collections (virus-infected) or *in vitro* (virus-free) are almost identical in cost for 1,000 accessions, about US$28 and US$22 per annum respectively (Jarret *et al.*, 1990). However, the advantages of virus-free material must be remembered, and also that the relatively high cost of the *in vitro* collection could be due to the costs of pathogen testing. In addition, as already stated there is the hidden cost of security. The security of *in vitro* collections as compared to field collections has been shown with Vanuatu taro, in that virtually all of their field collection has been lost, but the selected varieties evaluated as part of a FAO project have been safely maintained in tissue culture. This element of security attached to a tissue culture genebank has been illustrated by a collaborative pilot project between CIAT and IPGRI. In the field genebank one clone was completely lost from the field and 61 clones lost between 1 to 4 replicates, whereas not one clone was lost from the *in vitro* collection (IPGRI/CIAT, 1994). Cryopreservation has not really been costed accurately but estimated costs are US$50–75 for initial storage with small maintenance costs.

For all the crops mentioned tissue culture methods are available and have long been established. In addition, for most of the crops slow growth methodology is also available, and research into practicable crypreservation techniques is ongoing. As tissue culture laboratories are being developed in many of the island countries it is feasible that countries could establish their own *in vitro* collections of material that is either actively being used by the growers and/or is considered of prime importance to that country. Where countries do not have their own tissue culture facilities, arrangements could be made with the regional tissue culture laboratories or where relevant with the International Agricultural Research Centres (IARCs), for example, CIAT for cassava. However ideally for security purposes there is a need to have more than one collection. The choice now lies between maintaining a duplicate collection in the field, or having another *in vitro* collection at another site. Maintaining field collections have proved problematic, and in a sense are of little value unless the material is really being actively used. A field collection of taro in some of the countries such as Papua New Guinea, would be sensible because there is an active breeding programme ongoing in the country, but what of the other crops? A duplicate *in vitro* collection could be maintained at one of the regional centres assuming the country maintains one collection in its own tissue culture facility. If the country has no tissue culture facility then collections could either be maintained at both regional centres, or at a regional centre and an IARC, if appropriate. There is justifiable concern about the ownership of plant genetic resources and so the maintenance of collections outside the country might sound some alarm bells but it would be very simple for countries to enter into some form of contractual agree

ment with the regional genebank so that distribution of their germplasm was to some degree under their control. Furthermore, Material Transfer Agreements have been proposed for use in the regional genebanks and these would work to protect plant genetic resources from exploitation and abuse. There is also the issue of pathogen-tested status; it is likely that much of the germplasm from the island countries would not be pathogen-tested and this would work to stop distribution of the germplasm from outside its conservation centres.

At a recent meeting of the Ministers of Agriculture for the six PACP countries held in Suva, a resolution was endorsed stating '*Conserving genetic diversity is the key to crop performance and thus its neglect could imperil agriculture. Linked to this is the need to protect and utilise plant genetic resources so that there is an equitable sharing of benefits. The Honourable Ministers of Agriculture are urged to put in place both in their countries and through regional cooperation, policies to conserve, protect and best utilise their plant genetic resources*'. This resolution further emphasises the importance of plant genetic resources and the need to establish more effective systems for their conservation and use.

So what should be the immediate strategy if one is genuinely concerned about conservation of the genetic resources of the root and tuber crops. Where countries have tissue culture laboratories material could be initiated into tissue culture, with the technical support of PRAP Project 7 and any other similar projects. Where countries are lacking tissue culture capability, negotiations can begin with the regional genebanks for maintenance of their germplasm; this would involve discussions with quarantine as much of the material would not be pathogen- tested. Where appropriate, the IARCs can also be approached. In the meantime decisions can be made about the field collections; this decision making would have to take into account whether further evaluation and characterisation was needed. Consideration would also have to be given as to whether there was a need for further collecting, so that the diversity of the genome was fully represented in the collection. Further collecting would also necessitate evaluation of characterisation methods and the use of descriptors. However, this is after the continuing existence of presently held accessions of these crops has been secured; if time is taken in considering all the above then more material will be lost.

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Intellectual Property Rights and Plant Germplasm in the South Pacific Region

Mary Taylor[1]

INTRODUCTION

In March 1996 a paper titled 'Intellectual Property Rights and Plant Germplasm in the South Pacific Region' was presented at the PHALPS meeting in the Cook Islands. The same paper was later available as *PRAP Working Paper* 96-3. This paper focussed on the issues surrounding plant genetic resources and intellectual property rights that would provide the information necessary to formulate policies for the protection of the germplasm of this region. This second paper looks at other aspects relating to that area and also discusses the use of Material Transfer Agreements (MTAs) for germplasm exchange both within and outside the region.

INTELLECTUAL PROPERTY RIGHTS

Intellectual property rights became important in the plant kingdom because self-pollinating plants could, by their very nature, be easily copied. With advances in biotechnology, researchers argued that if their 'inventions' could not be protected they had little motivation with which to carry on their work. There are five forms of law covered by intellectual property rights: patents; plant breeders' rights; trademarks; copyrights and trade secrets. The first two are usually considered more applicable to plant genetic resources, and were discussed in the first paper.

Patents

Patents offer protection for 20 years, but are only applicable if certain criteria are satisfied, that is, the product or process must be new, useful and non-obvious. The problems in using patents for plant material, especially 'unimproved' material were discussed in the previous paper, but there is increasing pressure, mainly from the developed countries, such as the US, for this system to be used. There is justifiable concern that the use of patents could reduce access to plant germplasm, and therefore limit research. The implications of patenting have been considered by Powelman, (from the US Agricultural Research Service, ARS, of the Department of Agriculture):

> An examination of 140 important rice accessions done recently indicates that their ancestry can all be traced to 22 introductions in the Southern rice belt and 23 introductions in the Western rice belt. What if the genes of several of these ancestral lines had been unavailable or financially out of reach of the breeders? If you eliminated parents that were themselves released varieties, would we have made such progress? And how expensive would the seed be if growers were paying a royalty for each ancestor?

Although it is generally thought that naturally occurring substances are not easily patentable. The European Patent Convention has argued on the following lines: 'to find a substance freely occurring in nature is also mere discovery and therefore unpatentable. However, if a substance found in nature has first to be isolated from its surroundings and a process for obtaining it is developed, that process is patentable. Moreover, if a substance can be properly characterised either by its structure, by the process by which it is obtained or by other parameters...and it is 'new' in the absolute sense of having no previously recognised existence, then the substance *per se* may be patentable...'(Correa, 1995). This type of argument could be applied to kava. It is possible that the German pharmaceutical companies that are importing kava from the Pacific to make kava anti-stress tablets could patent the active in-

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gredients. What are the implications here for the growers and exporters of kava in the South Pacific?

Gollin (1993) suggests the use of petty patents or the modification of the standard utility patent to be more like petty patents. To qualify for a petty patent, novelty, industrial utility and an inventive step have to be demonstrated. The latter is less demanding than the non-obviousness step of the standard patent. Petty patents give exclusive rights for seven to ten years, but are only limited to the country of application, that is, there is no international protection. Petty patents have been incorporated into national policy in Kenya specifically for traditional medicinal knowledge. It is suggested that the stronger, internationally recognised utility patents could be adapted by maintaining a low standard of inventiveness, thereby providing protection for minor advances.

The patenting of genetic sequences can present many legal problems. A given DNA sequence may exist in materials collected in multiple locations, and therefore it is difficult to trace the origins of a variety so that the 'rights' can be conferred to the 'original' innovator. If a plant is protected but the genome has not been characterised, can a sequence of that genome be used and patented? It is likely that FAO through the IARCs will not protect material made available by them. It could, therefore, be easy to extract genetic material, use it and patent it. However, it is unlikely that one could prove that patent applications involve gene sequences isolated from material originating from one of the IARCs.

The US Plant Patent Act (PPA) grants monopoly protection for 17 years to the 'inventor' of new varieties of asexually propagated plants, primarily nuts, fruits and flowers. It was enacted by US Congress in 1930 to encourage plant breeding. However, a recent report, (1995) by the Rural Advancement Fund International (RAFI), states that breeders have been using the PPA to obtain patents on increasingly minute variations within species; 12 species account for more than 68% of PPA patents. In addition, many of the 'new' varieties awarded patents have actually been traditional or naturally-occurring varieties that have been taken from developing countries. The report describes a recent case of plant theft in which a US breeder was granted a PPA patent for *Banisteriopsis caapi*, a medicinal and hallucinogenic vine, bred and used by indigenous people for centuries in the Amazon. RAFI do make the observation that PPA patents compared to other patents have certain advantages, such as an inexpensive application process, and a relatively simple description requirement. As such PPA patents could be used as a model for developing countries. To conclude, the general consensus regarding patents is that they are too exclusive and too restrictive. In addition they tend to favour the wealthy over the poor; the sophisticated over the inexperienced and the developed over the developing nations.

Plant Breeders' Rights. (PBRs)

This is a more flexible system and more suited to the protection of plant material. Many countries have developed national systems of PBRs, also known as plant variety protection (PVP). The rules that govern PBRs are under an intergovernmental association, the International Union for the Protection of New Varieties of Plants (UPOV). New members joining the UPOV after 31 December 1995 will have to adhere to a new set of rules as laid out by the 1991 UPOV Convention. These state that right holders may prevent the multiplying, selling, exporting and importing of harvested materials, as well as the reproductive materials (only the latter was covered in the previous 1978 UPOV). In addition, with the 1991 UPOV farmers' privilege has to be actively elected by a country. Many consider that the 1991 UPOV reflects pressure from the large biotechnological companies.

Breeders' rights have been adopted by a relatively limited number of countries, mostly industrialised ones. Only a few developing countries have implemented this system, and no-one yet has become a member of the UPOV Convention. However, this situation is likely to change with the implementation of the Trade-Related Aspects of Intellectual Property Rights (TRIPs). TRIPs was established with the Uruguay round of GATT in 1994. It requires all signature states to provide for the protection of plant varieties either by patents or by an effective *sui generis* system. It is generally felt that accession to UPOV will satisfy the 'effective *sui generis* system' requirement. Some countries have used UPOV

to formulate their national plant protection policies, for example, Kenya, Chile, Mexico.

Trade secret

Trade secret is usually regarded as a formula, pattern, device or compilation of information that is used in business, and may confer a competitive advantage. Trade secret can be used in plant breeding by keeping the parents of a hybrid secret, or in the application of plants for therapeutic purposes. Trade secrets do not confer an exclusive right, but the right to prevent acquisition and use by third parties of the protected information in a manner that does not fit in with honest commercial practices. Trade secret law requires that measures have to be taken to maintain secrecy. Trade secrets are difficult to establish, protect and enforce, and they can be criticised because they would obviously prevent full and open exchange of information; an advantage of patents is that they require public disclosure. However, trade secrets can provide some form of protection in countries where patents are unavailable.

Copyright

Copyright law allows the protection of original works of authorship expressed in a tangible medium, but not the underlying ideas. It has generally been used to cover artistic and literary work, but could be a useful model for addressing intellectual property rights associated with biodiversity. It is easy and inexpensive to obtain, and protection can extend to 50 years beyond the life of the author. Copyright law would be useful for the protection of information pertaining to genetic resources. Although it has been suggested that genetically engineered DNA sequences could be protected by copyright, (Kayton, 1982), naturally occurring sequences would not be eligible for this form of protection as they are not a work of authorship.

Trademark

Trademarks can protect the competitive advantage of a company/organisation providing the green product, and the revenues can be returned to the source of the product through licensing and other contractual arrangements. This approach has been effective in providing funds to indigenous people sustainably producing, for example, Brazil nuts and materials for cosmetic products (Gollin, 1993).

Cultural property

It has been suggested that traditional knowledge may be protected as a cultural property under the terms of the Convention on the Means of Prohibiting the Illicit Import, Export and Transfer of Ownership of Cultural Property, administered by UNESCO (Reid *et al.*, 1993).

Expressions of folklore

The UNESCO/WIPO Model Provisions for National Laws for the Protection of Expressions of Folklore against Illicit Exploitation and other Prejudicial Actions have often been suggested as a possible framework for the protection of traditional knowledge. This is a similar protection to that of copyright in that only the expression of the work, and not the underlying ideas are protectable.

Community Intellectual Property Rights (CIPR)

This is a proposal for IPR legislation relating to plant genetic resources providing for both breeders' rights and farmers' rights. For effectiveness, CIPR might need national legislation and international consensus on reciprocality and/or a global database to monitor germplasm movement. As part of this legislation a public defender/ombudsman would be needed to adjudicate CIPRs or redress inequality of bargaining power, for example between countries and multinationals (ten Kate, 1995).

Model Draft Community Intellectual Rights Act

This proposal was formulated by the Third World Network, in the context of TRIPs and the Convention. Their aim was to establish new criteria for claiming patent rights compatible with the practices and cultural values of indigenous peoples. The proposal includes a 'Registry of Innovation' allowing communities to state ideas, to control access to them, and to prevent others from patenting them, in a manner similar to defensive publication (ten Kate, 1995).

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THE MAIN PROBLEM AREAS

Although there has been substantial progress in the area of plant genetic resources and intellectual property rights, certain issues remain in debate. There is the question of whether plant genetic resources, especially those held in *in situ* collections are subject to ownership or to other types of rights. Controlling a physical entity, that is, the plant material is feasible, but control of the genetic information presents a more complex issue. The genetic information is the intangible property of the plant whereas the plant itself is the physical property. The intangible property is the real value of the resource, but protection of that intangible property can offer very complex legal problems.

If a plant is protected, does that mean there is automatic protection of the genome? Or in order for the genome to be protected does it have to be characterised, and specifically protected? If schemes are implemented so that farmers or countries can be compensated for the conservation and continued improvement of landraces, what is the situation when a variety combines a multiplicity of landraces collected in different countries? IR64, an improved rice variety has some 75 parents, and among these are 20 landraces from eight countries (Brush, 1992). How can one assess the contributions of the different countries? Similarly, some confusion could arise with the neurotoxin, homobactrachotoxin, which is found both in such widely different species as a New Guinea bird called a hooded pitohui, and the Amazonian poison-dart frog (Angier, 1992). Plant genetic resources are not governed by national boundaries; there is the obvious example in the South Pacific with the taro variety known as Niue in Western Samoa but existing under other names in Vanuatu, Cook Islands, Fiji. If there is financial recompense for this genetic resource, who can lay claim to it?

There is also the question of farmers' rights. It is fairly argued that commercial varieties are usually obtained through applying breeders' technologies to farmers' germplasm. In that case why should the former obtain returns through PBR or some other form of intellectual property legislation, and the latter not be acknowledged for having provided the germplasm in the first place. Generally speaking this is accepted, but it is the implementation of this 'acknowledgement' that causes much debate. How should the compensation be obtained? How are the original donors of the material identified, especially if the material was donated to an IARC sometime ago? Alternatively is this identification essential, or can any financial compensation be awarded to the country of origin for conservation and/or agricultural improvement? If there is compensation in the form of a fund how should that fund be used?

As the exercise of exclusive rights is very difficult, if not impossible, it has been suggested that farmers' rights could fit in within a copyright system (Correa, 1995). Farmers' rights could be implemented within such a system, either through a multilateral agreement, or through national legislation, on the condition that the correct parties can be identified. The rights would not accrue to the farmers themselves, but to the governments or other organisations representing the farmers' interests. This is similar to the way in which public lending libraries work or the royalty that is imposed when a blank tape is purchased. An example of the implementation of farmers' rights at the national level is contained within a draft law under consideration in India. It is proposed that a 'National Community Gene Fund' is established. The monies from this fund would be used in trust of Indian farmers for collecting, evaluating, upgrading, conserving and utilising genetic variability. Although such proposals are a step forward in acknowledging the role played by farmers on the agricultural stage, it does not solve the problem at the global level. In reality there is no acknowledgement of the 'right' until the corresponding 'obligation' is defined and legally established. It has been suggested that an international taxation system be set up to provide for the international fund, a fund proposed by FAO as a means of distributing the benefits of improved agriculture more fairly. This taxation would be introduced on the sale of patented natural products of plant origin, and on plant varieties protected under patent and/or an effective *sui generis* system. It was further suggested that the fund would be used to: pay royalties to the country donating the genetic resources; compensate host countries offering *in situ* conservation areas to the international community for bio-prospecting; provide additional financial resources for national and international plant genetic resource activi-

ties; encourage the marketing and consumption of products which have originated from traditional-based farming systems. The taxation rate would vary from 7.5% to 30% depending on the country of origin of the plant material. The meeting did acknowledge that the design and implementation of such a system would be difficult, however it was considered essential that some system of payment was necessary. It was also felt that such a system would assist in international collaboration, removing fears in developing countries that their genetic material would be used without adequate recompense.

Another complication is the differences that exist between countries with respect to IPRs. International treaties, such as the Paris Convention, the Berne Convention and the Patent Cooperation Treaty do provide for some reciprocity and consistency in international intellectual property practice. However, it has been stated that no legal adviser in one country may confidently plan a global strategy for intellectual property protection without extensive help from foreign lawyers (Fox, 1991). There is a need to harmonise intellectual property laws on a global basis.

Finally there is the problem of monitoring and enforcement. It has to be admitted that once genetic resources have left a country or an institution, it is not an easy task to guarantee that they are used according to the terms of their supply. The difficulty becomes greater once the material is transferred; no organisation will have the resources to monitor the use of materials by third parties, and beyond. Again the problem of similar or identical resources available from a number of different sources makes it difficult to prove that final products resulted from work on raw materials provided by a particular supplier. In addition, although the idea for the work might have been inspired by the original material, the final product may bear little resemblance to the original. However, certain steps can be taken to monitor agreements made when genetic material is distributed. The keeping of efficient records will enable the donating body to locate at any time who has received what material, and under what conditions. Furthermore access legislation, material transfer agreements can precisely state restrictions on use, and ask for periodic assessment of progress and exchange of results, etc. Independent third parties can exist to settle any disputes.

WHAT ARE THE SOLUTIONS?

Although there are arguments that the implementation of IPRs leads to restricted access to plant germplasm, it is generally accepted that most countries would gain from having in place policies and legislation that allow them to control their plant genetic resources, benefiting from their use when possible. There is evidence from Latin America that growers benefit from better opportunities to collaborate with foreign breeders, when PBR protection in their country proves adequate (Jaffe *et al.*, 1995). Caution must be exercised by developing countries that they do not hamper their agricultural development by restricting access to germplasm, either through making whatever legislation exists too bureaucratic or by having the attitude that the technology required for germplasm improvement can be carried out in their country. In many developing countries the technological capability necessary for such work does not exist, and even the acquisition of equipment and machinery does not necessarily add to national technological capacity It is surely far better to establish sound policies and legislation which will allow a country to gain first from the provision of the germplasm, and secondly from its improvement.

Countries should develop policies and legislation which enable access to germplasm to continue, but at the same time, work to protect these resources, and result in the fair and equitable sharing of the benefits from those resources. Such legislation should cover the need for prior informed consent, access agreements based on mutually agreed terms, requirements for benefit-sharing, and material transfer agreements. Institutions responsible for the exchange of genetic resources need to be aware of how they can be used, so that agreements are formulated which account for any possibility. For example, DNA can be extracted from some herbarium samples, and yet herbarium specimens are often still exchanged or donated freely.

Within the South Pacific region there is a need to give some serious thought to these issues, and to determine whether national or regional policies are relevant. There is a wealth of resources in the region, many with pharmaceuti-

cal value, for example, kava. Very recently ethnobotanical interest has been expressed in taro; it has also been shown that taro flour is a good source of non-gluten flour, and therefore could find an excellent market globally with gluten-intolerant diets. For countries that individually have a great diversity in genetic resources, such as Papua New Guinea, Solomon Islands, national policies would seem to be the logical conclusion. For those smaller countries who have resources in common, a regional policy would seem more appropriate. In reviewing the literature it appears that many countries in formulating national policy and legislation look to the UPOV rules for guidance.

As stated in the previous document, *Working Paper* no. 96-3, countries should also maintain a careful watch on the interplay, and the corresponding results between the TRIPs agreement and the objectives of the Biodiversity Convention. The areas that require clarification have been discussed in both papers, that is, a clear definition of types of genetic material that can be protected, and a re-evaluation of criteria that are appropriate for the protection of local and collective rights over genetic resources. In order to assist countries in establishing internationally acceptable policies and legislation, there should be a clear definition as to what counts as an 'effective *sui generis* system', as required by GATT through TRIPs. Finally there should be consensus on how disputes are to be settled, with the establishment of an independent body for that purpose.

A novel approach to the situation of genetic resources and their potential value has been shown by some countries who have established institutions for technology transfer enabling them to add value to their resources. One such institute is INBio in Costa Rica, which is accumulating the scientific and technological capacity required for biodiversity prospecting and biotechnology development. INBio is involved in a production process that involves screening and characterisation of genetic resources, and where appropriate, moves through the stages of product development. To date INBio has been very successful, for example, in the acquisition of an up-front payment of $1 million, in addition to the promise of royalty payments in the event of commercially valuable discoveries. The question immediately arises as to why this institute has been so successful, and is it possible for other countries to replicate the INBio experience. It is suggested that INBio's excellent results are due to three main reasons: a high degree of international co-operation from the beginning so that links with experts and other sources of support are maintained; a strong base of national competence, especially in institutional management; strong political and legislative support from the government. There is criticism of INBio from some who believe that the advance payment made by Merck is very little considering what Merck stand to gain from the relationship. However, in answer to that criticism it could be argued that it is preferable to receive some payment rather than none at all.

PROTECTING THE REGIONAL GENEBANKS

In the last paper it was suggested that the regional genebanks could protect the germplasm that they are responsible for by developing material transfer agreements (MTAs). These agreements would have to be completed by the recipient of any germplasm originating from the regional genebanks in the South Pacific. As already discussed, MTAs can offer a means which allows plant germplasm to be monitored when it leaves its point of distribution. MTAs can be relatively concise documents, yet provide sufficient flexibility so that they can be used in a number of research and development scenarios. It is these characteristics that make MTAs useful to developing countries who wish to promote research and development with their genetic resources. Such an agreement should set out the mutually agreed terms for access. It has been suggested (ten Kate, 1995) that the following key issues are clarified in such an agreement: rights and responsibilities of the parties, description of identity; ownership of genetic resources exchanged; any payment of royalties, or other forms of compensation if commercialisation occurs; any obligation for the parties to negotiate more specific benefit sharing at a later stage; technology transfer; conditions if material is passed onto a third party; sharing of results and reporting procedures necessary.

CONCLUSIONS

As the paper has indicated the issues surrounding this area remain complex, and in

many cases in a state of flux. Countries have to decide whether it is necessary for them to formulate policies and legislation to govern all aspects of access to plant genetic resources, and whether these are to be national and/or regional.

What can be done now is the establishment of a MTA that will offer some protection to the material that is held, 'in trust', by the regional genebanks. Although legal opinion has yet to be acquired it is generally accepted that MTAs are a sound method of regulating access to plant genetic resources, and are used by many institutions, including the IARCs. It has also been suggested that such 'contracts' work even in the absence of a well-developed system of intellectual property protection. Governments can work with MTAs, agree upon minimum terms of equity in research agreements, and develop a mechanism to verify Prior Informed Consent (Putterman, 1996)

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Tissue Culture Laboratory Management in the Tropics

Mary Taylor[1]

INTRODUCTION

The main problem encountered in managing a tissue culture laboratory in the tropics is contamination. Contaminants are bacteria, fungi, yeasts, etc., which enter cultures by way of the air which can pass into the containers, or through poor sterile techniques. Poor sterile techniques can include the carry-over of micro-organisms on the surface or in the tissues of explants and faulty procedures in the laboratory. However, some endogenous micro-organisms can be extremely difficult to remove regardless of the technique used. As tissue culture medium generally contains optimal levels of nutrients and sugars, it is an ideal medium for the growth of these micro-organisms. Contaminants can overgrow and kill the cultures, or, at least, impair their growth, and consequently, prevention is the only control because there is no satisfactory cure. Growth of micro-organisms can be inhibited by the medium through a high salt concentration, a low sugar level and an acidic pH, but these factors are also likely to inhibit the growth of the plant tissue. Therefore, as the medium provides a limited means of controlling the growth of micro-organisms, other alternatives have to be examined.

CONTAMINATION IN THE TRANSFER ROOM

Contamination can be controlled in this area by consideration of the following:

1. Filtered air
2. Entrance lobby with pressurised air.
3. Easy to clean laboratory with regular cleaning regime in process.
4. Regular monitoring of contaminants.
5. The use of UV lights.
6. Reduced human traffic.
7. Correct techniques.

Filtered air

In some laboratories HEPA filters are fitted as part of the air conditioning unit so that all the air entering the laboratory is filtered in the same way as the air within the laminar airflow cabinet. HEPA filters, (high efficiency particulate air), strain out particles as small as 0.3 microns. Alternatively, some companies, for example, Panasonic, are making air conditioners that are fitted with a form of microbial filter. If either of these two options are not possible, and only a 'normal' air conditioner is in use in this room then the filter must be regularly cleaned. A regular spray of alcohol into the air conditioner can control the extent to which spores might settle and develop within the unit itself.

Entrance lobby with pressurised air

This is recommended so that whenever the door opens the air tends to flow out of the laboratory into the 'dirty' area, rather than the reverse. This can be achieved by having a double door entrance system into the laboratory, but the system is more effective if fans are fitted in the lobby to differentiate the air pressure more. The other advantages of having a lobby-type entrance include the use of 'sticky' mats, and the availability of a place where shoes and other items can be left.

Easy to clean with a regular cleaning regime in progress

The facility should be designed so that it is easy to clean. Floors, walls etc., should be wiped down at least once a week with a bleach

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solution. Brushes, brooms, etc., should never be used as they stir up dust. Any working surfaces should also be cleaned regularly with a bleach solution. The prefilters on the airflow cabinets should be regularly washed, and renewed when necessary. Clutter should be avoided in the laboratory, as clutter can be a good source of fungal spores. Another practice that can be worthwhile is the regular spraying of the room with alcohol.

Regular monitoring of contaminants

This can be achieved simply by placing open petri dishes with bacteriological media in various locations in the laboratory. This simple method will reveal whether there are any danger spots in the working area. If an airflow monitor, which measures the rate of flow of air coming from the HEPA filter is not available petri dishes can also be used for assessing the efficiency of the laminar airflow cabinets.

The use of UV lights

It is often difficult to control contamination in the tropics, especially when the air coming into the laboratory is not filtered, and when there is an extreme wet season. Many laboratories use UV lights strategically placed to try and eliminate/reduce contamination. General consensus from the tissue culture laboratories where UV lights are in use is that they do have a significant effect on contamination levels. Care obviously must be taken in their use as they are damaging to the eyesight but switches can be positioned outside the laboratory so that they can be switched on after leaving the room and switched off before entering.

Reduced human traffic

It is generally agreed that a major source of contamination are the humans that work in the laboratory, therefore access should only be available to tissue culture personnel. Many laboratories have a viewing window into the tissue culture laboratory so that other persons can see into the room without entering. In addition, all personnel working in the laboratory should have appropriate footwear, wear laboratory coats, and if necessary wear headgear, masks and gloves.

Correct techniques

Most of the principles behind the use of correct techniques is common sense, and involves giving major consideration to cleanliness and sterility. If these last two factors are always foremost in the carrying out of any technique, then it will be the correct technique. A few areas where mistakes can be made are: inadequate sterilisation of the explant; poor cleaning of the laminar airflow cabinet; too much clutter in the airflow cabinet; working in front of equipment which is blocking the flow of sterile air from the HEPA filter; inadequate flaming of the instruments; bringing the instruments too close to the front of the airflow cabinet when working; working too far away from the HEPA filter; arranging equipment so that manipulations are difficult. There are others but these are the most common areas where mistakes are made and contamination can be introduced.

CONTAMINATION IN THE GROWTH ROOMS

The factors to consider here are the same as those stated in the previous section, but consideration should also be given to the container. Well sealed containers are not an option in the tropics, they are essential. Old containers with lids with poor seals should be replaced immediately. It is also worth considering that small containers often have lids with better seals than the larger containers. Some laboratories often use parafilm or some form of glad-wrap to enforce the seal around the lid, though this can exacerbate problems if the culture vessels are allowed to cool down after autoclaving in a room where the level of sterility is not high. As the vessels cool down fungal spores collect on the vessel and placing a wrapping around the lid area can provide the perfect microclimate for fungal organisms. It is obviously crucial to remove any contaminated cultures immediately from the growth rooms.

MITES AND THRIPS

Mites are the most frequent and inconvenient pest within tissue culture vessels. They are very small, less than 1 mm, and occur everywhere. They can be carried on dust particles, insects, animals and humans. Once they have

gained entrance to the tissue culture facility, they cause severe contamination problems because of the fungal and bacterial spores carried on their bodies. The easiest way in which mites can be introduced is when tissue culture vessels are received from elsewhere and so it is good practice to spray all incoming vessels with 70% alcohol. If mites are discovered in the growth rooms, then all contaminated cultures and those adjacent to them should be destroyed. The outside of all the remaining cultures should be wiped with 70% ethanol. The room can also be sprayed with miticide, and the appropriate places painted with acaricidal paint.

Thrips are air-borne, and can obviously cause similar contamination problems to mites. If thrips are discovered, then all infested cultures should be destroyed, and any 'non-infected cultures' kept under strict observation. In addition, non-phytotoxic insecticide can be added to the cultures.

GENERAL CONTROL MEASURES

It is difficult to completely eliminate contamination, if not impossible. The task is even more difficult in the tropics, but the extent of the problem can be controlled through the regular practice of careful hygiene. Some of the important measures have already been discussed, such as regular cleaning of filters, monitoring of the laboratory and the laminar airflow cabinet, etc. Another important task is the routine examination of the cultures and the removal of any contaminated ones from the growth rooms. Similarly, cultures should not be allowed to get too old, as dead leaves can be a source of contamination within the culture vessel. The use of a three-stage process in the sterilisation of the instruments in the airflow cabinet has also proved to be effective in the battle against contamination. This three-stage process involves taking the instruments through soapy water, 70% ethanol, and then 90/95% ethanol prior to flaming. The low pH of the soapy water can prevent the transfer of contaminants from one explant to another.

There are other areas where cleanliness can be improved, but with many of these it can depend on the laboratory, the facilities and the crop. Contamination should not be higher than 5%; this is an acceptable level. Of key importance is to remember to act quickly once an increase in contamination levels is noticed, and then the situation will not get out of hand. This reinforces the need to regularly inspect the cultures in the growth room.

Problem Solving

Mary Taylor[1]

INTRODUCTION

If a tissue culture explant is not growing well, it could be due to a number of problems. The two most likely are contamination or incorrect medium formulation. Other problems can be temperature stress, moisture stress, oxygen deficiency, ethylene toxicity and plant volatiles/plasticisers. It can be difficult to identify exactly what is wrong and often the process of elimination is necessary before a solution can be found.

CONTAMINATION

If the former is the problem it will be necessary to carry out a sterility test. Assuming the results of the sterility test to be positive, disinfestation will be necessary either using sterilising agents and/or antibiotics. Contaminated plants can be very difficult to clean up, and so it is often easier to discard contaminated plants and reinitiate from stock plants.

Detection of internal contamination in stock material

To detect internal contamination, explants are placed in 1/2 strength liquid Murashige and Skoog medium (1962) containing 256 mg of peptone and 88 mg of yeast extract at a pH of 6.7. Contamination will look like cloudiness or flocculent growth in the medium. If no contamination shows after one week, then the material can be multiplied. If recontamination shows, then new explants should be initiated into tissue culture.

Detection of internal contaminants in tissue culture material

If tissue culture plants are suspected of contamination they can be streaked on petri plates containing a bacterial detecting medium (Viss 523 medium). This medium contains 10 g/l sucrose, 4 g/l yeast extract, 2 g/l potassium dihydrogen phosphate, 0.15 g/l magnesium sulphate and 8 g/l agar with a pH of 6.9 prior to autoclaving. The base of each explant is streaked on the plate before planting in 16 mm tubes containing 5 ml of multiplication medium. The plates are incubated for 48 hours at 24°C. Plantlets showing contamination on the plates are discarded.

Initiating clean material into tissue culture

The ideal system to use in tissue culture to prevent any problems at a later date with contamination is to initiate from clean material. The first step is to use 1/2 strength liquid MS with peptone and yeast extract as described above. If the medium turns cloudy those explants are discarded. All clear tube plants are then cultured on Viss 523 medium. Those explants that are clean after this step can then go on to be multiplied.

INCORRECT MEDIUM FORMULATION

The obvious solution to this problem is to remake the culture medium and transfer the cultures to new medium. However, it is possible to identify specific nutrient deficiencies and toxicities. The following list indicates what symptoms are associated with certain nutrients.

Deficiency symptoms

Nitrogen: Leaves generally yellowish in colour with older leaves more severely affected; these may turn yellow and abscise. Leaves tend to be stunted with reduced internode and stem diameter.

Phosphorus: Overall stunting of leaves and shoot growth. Later the older leaves may drop off without turning yellow. Root development is reduced.

[1]PRAP/IRETA Tissue Culture Unit, USP, Private Bag, Apia, Samoa.

Potassium: Stunted growth. Tip and marginal yellowing, browning and necrosis of older, yellow leaves.

Calcium: Young leaves are distorted. Later the edges of the leaves turn yellow and brown. Discoloured spots on the leaves may coalesce into large necrotic blotches.

Magnesium: Shows initially on lower, older leaves. Chlorosis of the older leaves begins in the interveinal areas and progresses to necrosis. Overall growth is stunted.

Sulphur: Slight interveinal chlorosis of the young leaves. This may progress to an overall light yellow-green coloration of the entire new growth.

Iron: Main veins remain green but interveinal chlorosis of the young leaves occurs. If this deficiency continues, the newly formed leaves may remain very small and eventually become pale yellow or almost white.

Iron deficiency might not be due to an actual shortage of iron, but interference in uptake caused by poor aeration, high soluble salt levels, excessively high or low temperatures, high concentrations of managanese, zinc or phosphorus.

Copper: The first symptoms are distorted young leaves with yellow tips that later become necrotic. The growing point dies, and short stunted lateral shoots develop.

Zinc: The symptoms are almost identical to those caused by copper deficiency except that with zinc deficiency the lateral shoots that develop after the meristem dies are severely stunted.

Boron: Necrosis of the growing point occurs followed by development of lateral shoots with eventual death of the growing point. Further development of lateral shoots occur, i.e. a witches broom type of symptom.

Molybdenum: The symptoms resemble those of moisture stress, namely browning and necrosis of the tips and edges of the leaves.

Toxicity symptoms

Nitrogen: Stunted growth and dark green foliage. Osmotic stress also occurs resulting in marginal necrosis of the leaves.

Phosphorus: Interference in the availability of copper, iron, zinc and calcium may occur.

Potassium and Sulphur: A number of symptoms result when either of these two nutrients is present at too high a concentration; chlorosis and marginal leaf necrosis; root loss; wilting of soft succulent shoots.

Calcium and Magnesium: Excessive levels of either of these two nutrients can cause adverse effects on the availability and uptake of other essential mineral elements, e.g. iron and phosphorus.

Manganese: Can get small black spots in the interveinal areas. Interveinal chlorosis of the young leaves can also occur because a balance between iron and managanese is needed for normal growth.

Iron: May cause deficiency symptoms of copper, manganese, or zinc because of the interrelationships between these nutrients and iron.

Copper: This often resulted in leaf abscission followed by excessive leaf drop.

Boron: If levels only rise slightly above normal, marginal browning and necrosis of older leaves occurs. Boron toxicity symptoms may be confused with magnesium or calcium deficiency symptoms.

Zinc: Initially there is the appearance of light green, transparent, water-soaked areas along the veins of the leaves. Later, the rest of the leaf turns yellow, then brown. Affected areas abscise irregularly. Foliage drops only after the leaves have turned completely brown.

OTHER PROBLEMS

Temperature and moisture stress

With both these problems marginal necrosis of stems and leaves occur. One side effect of increased tempertures is to exacerbate any problem there might be with endogenous bacterial contamination to the extent that the tissue culture plant dies.

Oxygen deficiency

The symptoms of oxygen deficiency are yellowing of the main veins followed by an interveinal necrosis of young foliage, and a major drop of the more mature leaves.

Ethylene toxicity

The symptoms of ethylene toxicity are curling of the youngest leaves, and yellowing along

the principal veins of the older leaves. Stem elongation is also slowed. There is some decrease in apical dominance, often resulting in the growth of more than the normal number of axillary buds. The concentration of ethylene is said to increase if flaming of the container and the lid takes place during subculturing and prior to closing the lid.

Plant volatiles/plasticisers

Paints containing xylene, naphthalene and mineral spirit cause necrosis and distortion, and lower leaves readily abscise. There has been some evidence recently that some forms of plastic containers can emit oestrogen-like substances; these substances affect animal cells in their growth and development, and so it is possible that they also affect plant cells.

REFERENCES

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Appendices

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TISSUE CULTURE MEDIA

ANTHURIUM MEDIA (INGREDIENTS FOR 1 LITRE OF MEDIUM)

A. Stage 1 medium (coconut water medium)

Ammonium nitrate	0.825 g
Potassium nitrate	0.95 g
Magnesium sulphate	0.185 g
Potassium phosphate	0.085 g
Calcium chloride	0.220 g
NaFeEDTA	0.037 g
Boric acid	6.2 mg
Manganese sulphate	22.3 mg
Zinc chloride	3.93 mg
Potassium iodide	0.83 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Cobaltous chloride	0.025 mg
Myo-inositol	0.100 g
Deproteinised coconut water (Boiled & filtered)	150 ml
Benzyladenine (BA)	9 mg
Adenine sulphate	0.080 g
Biotin	0.25 mg
Glycine	1.00 mg
Nicotinic acid	0.50 mg
Pyridoxine-HCl	1.00 mg
Thiamine-HCl	0.50 mg
Gelrite	2 g
Sucrose	30 g
pH	5.7

B. Stage 1 medium (INRA, Antibes, France)

Ammonium nitrate	1.65 g
Potassium nitrate	1.9 g
Magnesium sulphate	0.37 g
Potassium phosphate	0.17 g
Calcium chloride	0.44 g
NaFeEDTA	0.037 g
Boric acid	6.2 mg
Manganese sulphate	22.3 mg
Zinc chloride	3.93 mg
Potassium iodide	0.83 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Cobaltous chloride	0.025 mg
Myo-inositol	0.100 g
Benzyladenine	0.200 mg
Gibberellic acid	2.00 mg
Indoleacetic acid	0.100 mg
Biotin	0.25 mg
Glycine	1.00 mg
Nicotinic acid	0.50 mg
Pyridoxine-HCl	1.00 mg
Thiamine-HCl	0.50 mg
Gelrite	2 g
Sucrose	30 g
pH	5.9

C. Multiplication Medium (Nitsch & Nitsch modified medium)

Ammonium nitrate	0.72 g
Potassium nitrate	0.95 g
Calcium chloride	0.166 g
Potassium phosphate	0.068 g
Magnesium sulphate	0.185 g
NaFeEDTA	0.037 g
Myo-inositol	0.100 g
Boric acid	10 mg
Manganese sulphate	25 mg
Zinc sulphate	10 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Benzyladenine	0.5 mgBiotin 0.05 mg
Glycine	2 mg
Nicotinic acid	5 mg
Pyridoxine-HCl	0.5 mg

Thiamine-HCl	0.5 mg
Folic acid	5 mg
Sucrose	20 g
Gelrite	2 g
pH	5.9

D. Growing Medium – Nitsch & Nitsch N69 Modified Medium (used when cultures have acquired a good multiplication rate)

Ammonium nitrate	0.72 g
Potassium nitrate	0.95 g
Calcium chloride	0.166 g
Potassium phosphate	0.068 g
Magnesium sulphate	0.185 g
NaFeEDTA	0.037 g
Myo-inositol	0.100 g
Boric acid	10 mg
Manganese sulphate	25 mg
Zinc sulphate	10 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Biotin	0.05 mg
Glycine	2 mg
Nicotinic acid	5 mg
Pyridoxine-HCl	0.5 mg
Thiamine-HCl	0.5 mg
Folic acid	5 mg
Gelrite	2 g
Sucrose	20 g
pH 5.9	

E. Rooting Medium (MS modified by Pierik)

Ammonium nitrate	0.825 g
Potassium nitrate	0.475 g
Magnesium sulphate	0.093 g
Potassium phosphate	0.043 g
Calcium chloride	0.110 g
NaFeEDTA	0.025 g
Myo-inositol	0.100 g
Boric acid	6.2 mg
Manganese sulphate	22.3 mg
Zinc chloride	3.93 mg
Potassium iodide	0.83 mg
Sodium molybdate	0.25 mg
Copper sulphate	0.025 mg
Cobaltous chloride	0.025 mg
Indoleacetic acid	0.75 mg
Biotin	0.05 mg
Glycine	2 mg
Nicotinic acid	5 mg
Pyridoxine-HCl	0.5 mg
Thiamine-HCl	0.5 mg
Folic acid	5 mg
Gelrite	2 g
Glucose	30 g
pH	5.9

F. Weaning Off soil Mix

Fine river gravels (2mm diameter)	2 volumes
Coarse river sand (0.5-1.0mm diameter)	1 volume
Peat moss	3 volumes
Aliette	0.4 g/l of mix
Thiram	0.25 g/l of mix

YAM MEDIUM

Ammonium nitrate	1.65 g
Potassium nitrate	1.9 g
Magnesium sulphate	0.3706 g
Potassium phosphate	0.17 g
Calcium chloride	0.4398 g
NaFeEDTA	0.0367 g
Magnesium sulphate	0.3706 g
Boric acid	6.20 mg
Cobaltous chloride	0.025 mg
Copper sulphate	0.025 mg
Potassium iodide	0.83 mg
Manganese sulphate	22.30 mg
Sodium molybdenate	0.25 mg
Zinc sulphate	8.60 mg
Myo-inositol	1.0 mg
Calcium pantothenate	1.0 mg
Nicotinic acid	1.0 mg
Thiamine-HCl	1.0 mg
Biotin	0.1 mg
Agar	6 g
Sucrose	30 g
pH	5.7

The Pacific Regional Agricultural Programme, Phase II

The overall objective of PRAP II is to contribute to an increase in staple food and export crops production. By improving food security and increasing agricultural incomes, the Programme will contribute to an improvement in the quality of life for the rural population.

The fact that the necessary agricultural research is best implemented within the framework of regional co-operation was the catalyst for the first phase of PRAP. The second phase will, while continuing with the improvement of selected crops, place particular emphasis on improved conditions of dissemination, agricultural information and application of agricultural techniques for the farmers in the eight PACP countries: Fiji, Kiribati, Papua New Guinea, Solomon Islands, Tonga, Tuvalu, Vanuatu and Western Samoa.

The main results of PRAP II are expected to be (by component):

1. Farming systems in low lands: Appropriate agricultural research with emphasis on agroforestry-based food cropping systems in the PACP non-atoll countries will be facilitated and implemented.

2. Production and dissemination of improved coconut cultivars: The production potential of coconut and its adaptability to different agro-climatic conditions will be increased.

3. Seed and planting materials: The availability, suitability and quality of vegetable seed will be improved.

4. Selection, trial and dissemination of sweet potato cultivars: A great number of improved sweet potato cultivars will be tested and supplied to national agricultural research systems (NARS) for extension to farmers.

5. Taro beetle control: Environmentally acceptable control measures will be identified using natural enemies as well as cultural and chemical means.

6. Atoll farming systems: Farmers in the atolls will be shown how to apply the recommended sustainable farming system practices on their farms.

7. Provision of tissue culture services for the region: Tissue culture techniques will be well integrated into all NARS and the supply of tissue culture materials to PRAP states will be secured.

8. Regional biometric service: Comprehensive and solidly-based field data will be produced, collected and analysed, and the results clearly presented.

9. Agricultural information support: Relevant agricultural information will be made available and its use increased.

10. Programme coordination: Effective and transparent overall co-ordination of PRAP II will be implemented.

11. Agricultural rural development: An efficient methodology for improving linkages for agrotechnology development and transfer in PACP countries will be elaborated and demonstrated.

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