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INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES

IBPGR ADVISOPY COMMITTEE ON *IN VITRO* STORAGE

Report of the Third Meeting

Held in Valencia, Spain

4-6 June, 1986

IBPGR Headquarters

Rome, November 1986

The International Board for Plant Genetic Resources (IBPGR) is an autonomous international scientific organization under the aegis of the Consultative Group on International Agricultural Research (CGIAR). The IBPGR was established by the CGIAR in 1974. The basic function of the IBPGR is to promote and coordinate an international network of genetic resources centres to further the collection, conservation, documentation, evaluation and use of plant germplasm and thereby contribute to raising the standard of living and welfare of people throughout the world. Financial support for the core programme is provided by the Governments of Australia, Austria, Belgium, Canada, China, Denmark, France, Federal Republic of Germany, India, Italy, Japan, the Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom, and the USA as well as the World Bank. FAO of the United Nations provides the Headquarters.

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INTRODUCTION

1. The third meeting of the IBPGR Advisory Committee on *In Vitro* Storage was held 4 to 6 June, 1986, at Valencia, Spain. Dr. L. Navarro, Director, Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, kindly hosted the meeting. The following members attended: Prof. E.A.L. De Langhe, (Chairman), Dr. J. Moyer, Prof. A.N. Rao, Dr. O. Reuveni, Dr. W.M. Roca, Prof. J.T. Williams and Dr. L.A. Withers (Technical Secretary). Dr. L. Navarro, Dr. K-L. Tao (IBPGR Staff) and Dr. A. Ballester (Instituto de Investigaciones Agrobiologicase de Galicia), Dr. N. Duran-Vila (IVIA) and Ms. M.L. Marin (IVIA) also participated. Dr. W.R. Scowcroft and Prof. Y. Yamada were unable to attend. Addresses of participants are shown in Appendix I.

REPORT

Review of past and ongoing IBPGR in vitro research

2. Profs. de Langhe and Williams provided a brief review of research conducted to date on *in vitro* conservation which derived from previous proposals of the Committee. Noteworthy among these were:

(i) *In vitro* collecting techniques are being developed for cacao, cassava and coconut.

(ii) Techniques for the initiation and maintenance in slow growth of cultures of *Musa*, sweet potato, *Colocasia* and *Xanthosoma* have progressed.

(iii) Techniques for the *in vitro* propagation and culture of *Citrus* cacao and sugarcane have also been explored.

(iv) Attempts to cryopreserve cassava, sweet potato, *Colocasia*, *Xanthosoma*, *Musa*, cacao and *Citrus* have met with varying degrees of success, most progress to date being made with cassava.

(v) A limited amount of work has been carried out on disease indexing of sweet potato and potato. It is noted that a considerable amount of information is already available on disease indexing for the latter.

(vi) Isozyme analysis for the characterization of materials has been explored for cacao, Colocasia and Musa; in the case of Musa this relates to the stability of in vitro propagated material.

3. A detailed report was received on the IBPGR In Vitro Conservation data bases. A summary is provided on Appendix II and the Committee noted with satisfaction that the data bases are now being used widely and commended the personnel involved (Ms. S.K. Wheelans and Dr. L.A. Withers). Some remarkable trends in scientific work world-wide were noticed, in particular:

(i) There is a notable increase in the degree to which cultures are used as a vehicle for exchange of germplasm, albeit most frequently for research rather than conservation purposes.

(ii) There is an increasing level of interest in the use of slow growth storage for medium-term maintenance of a wide range of in vitro material.

(iii) There is a most disappointing level of research activity on the cryopreservation of cultures. Much of the ongoing research still relates clearly to cells - especially those derived from species which are of little interest for crop genetic conservation.

(iv) Despite an increasing awareness for the need for characterizing cultures in order to monitor stability, this has rarely been translated into practical terms particularly for stored materials. Although more characterization is being carried out in current work cytological checks still greatly outnumber the instances of biochemical analysis.

4. The data base is increasing in size both with respect to the survey of ongoing research (including much unpublished material), and literature related

to in vitro conservation. The Committee agreed that the data base fulfils a most needed function and should be continued over the coming years so that IBPGR may continue to readily and freely provide information. In this respect it was thought that all attempts should be made to raise the level of awareness of the data base among scientists, for example by the printing of a small brochure to be made available at scientific meetings and congresses.

Status reports commissioned by the Committee

5. Reports have already been published and distributed on genetic variability in tissue culture [1/], the potential use of in vitro storage for temperate fruits [2/] and isozyme analysis [3/]. In addition, a report on the potential applications of in vitro culture in Allium species had been circulated to the Committee and to the IBPGR Allium Working Group and constructive comments received. The Committee felt that IBPGR should only be involved in developing in vitro culture techniques for the wild genepool of species which tend to be sterile and not for either cultivars of garlic since there is little breeding activity or shallots which are of minor food interest worldwide. For clonal material there is a very clear need for disease indexing, eradication of diseases and the maintenance of cultivars in a clean state, but this was felt not to be the responsibility of IBPGR in the case of garlic and shallots. The Allium report [4/] is available in mimeographed form from IBPGR Headquarters.

6. Prof. A.N. Rao presented a detailed report on the state of in vitro work in Indonesia, Malaysia, Philippines, Singapore and Thailand and the Committee thanked him for his efforts. The report covered expertise, facilities and research interests. The Committee noted that most facilities needed institutional building as a priority and that IBPGR should reassess the situation in the future in regard to possible collaborative efforts. IBPGR now felt able to consider, in the short-term, the in vitro culture of the

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1/ Scowcroft, W.R. 1984. Genetic Variability in Tissue Culture: Impact on Germplasm Conservation and Utilization. IBPGR.

2/ Stushnoff, C. and Fear, C. 1985. The Potential Use of In Vitro Storage for Temperate Fruit Germplasm. IBPGR.

3/ Simpson, M.J.A. and Withers, L.A. 1986. Characterization Using Isozyme Electrophoresis: A Guide to the Literature. IBPGR.

4/ Altman, A. and Rabinowitz, H.T. 1985. Actual need and possible techniques for in vitro storage of Allium genepool. Internal report B5/159. IBPGR.

southeast Asian collection of Musa local cultivars, subject to INIBAP (International Network for the Improvement of Banana and Plantain) and IBPGR consultations with the Philippines (see also para 23).

7. In relation to characterization of cultures, biochemical techniques are clearly applicable and the status report on isozyme analysis illustrates the value of careful scientific monitoring. It was felt that it is not appropriate to try to develop a practical manual for the use of curators of germplasm collections because each crop requires very specific work and much of the research related to individual crops has yet to be carried out. Hence, it would be very misleading for the IBPGR to promulgate the notion that a quick and easy finger printing method is universally applicable.

8. The Committee emphasized the need to encourage the use of systems of genetic markers in conservation work and noted that molecular techniques are rapidly being developed which interface with in vitro work and complement (but do not replace) both morphological and biochemical characterization procedures. The Committee agreed to commission a status report covering these new molecular approaches and that the opinion of several experts should be sought to ensure comprehensive coverage.

9. The Committee considered in depth the problems related to the need for disease indexing and the degree to which it is carried out and noted that although the basic technology is available, it has been adapted only to a few specific cases. The reasons for this deficiency are that often the disease is of unknown etiology and thus the new technologies cannot be used. Developmental studies may be needed to know when the indexing should be conducted and it may also be necessary to modify standard techniques for specific hosts. The case of Musa was cited as a striking example in which gaps in disease indexing present problems. Movement of primitive germplasm (including numerous diploid and triploid cultivars from Southeast Asia and the Pacific), throughout the world is hampered, if not totally blocked, by the existence of Bunchy Top Virus and the lack of an appropriate indexing method for in vitro materials. The Committee asked Dr. J.W. Moyer to convene a specialist Subcommittee to address the strategies which will aid IBPGR's work in these areas. The terms of reference of the Subcommittee are to:

(i) Develop a basic philosophy for moving germplasm of high priority crops (especially primitive germplasm), giving particular

attention to problems of disease indexing and movement through quarantine, where bottlenecks are often encountered;

(ii) Link that philosophy with genetic resources activities from collecting in the field to conservation (particularly in vitro storage); and

(iii) Identify areas which need research (e.g. etiology, developmental work, neutralization of interfering host substances).

It was agreed that the Subcommittee members must be involved in the advance preparation of reports which will enable the Subcommittee to reach clear decisions and produce a status report for IBPGR.

Practical manuals

10. The IBPGR-sponsored research on sweet potato had resulted in a draft manual for curators and others on in vitro culture and disease indexing. This has been circulated to scientists active in relevant areas, especially at Centro Internacional de la Papa (CIP), Peru, International Institute of Tropical Agriculture (IITA), Nigeria, and Asian Vegetable Research and Development Center (AVRDC), Taiwan, China and useful comments received.

11. The Committee agreed that IBPGR should issue a series of manuals entitled "Practical Manuals for Handling Crop Germplasm In Vitro" and that the draft sweet potato manual should be finalized including an input from the IBPGR data base to form the first in the series. Subsequent manuals will deal with Musa, cassava (in association with Centro Internacional de Agricultura Tropical CIAT<, Colombia), taro and other aroids.

12. Following a suggestion from the IBPGR Advisory Committee on Seed Storage, the Committee discussed those in vitro techniques which could readily be used by seed physiologists to deal with problem material such as immature seeds collected in the field, damaged seeds, recalcitrant seeds and small samples of rare material. In general, seed genebank curators appear unaware of the value of suitable in vitro techniques which are "low-tech". As a consequence, the Technical Secretary was asked to coordinate inputs to a manual.

In vitro genebanks

Model genebank

13. Following the Subcommittee's report on designing in vitro genebanks [1/], on the recommendation of the IBPGR Programme Committee, IBPGR has agreed to establish for a 3-year period a model in vitro active genebank to test out the details and to identify and thereafter resolve any constraints which might arise. IBPGR has approached CIAT to determine whether a joint IBPGR-CIAT model in vitro active genebank can be established for cassava. CIAT has agreed to this and negotiations will be finalized shortly. The Committee welcomed this initiative.

Documentation systems for in vitro genebanks

14. The Technical Secretary had kindly started to acquire information on approaches to computer documentation of in vitro collections. Available information included a publication of the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia (which promises to provide an important input), the current systems in use at CIAT and American Tissue Culture Collection (ATCC), USA. Discussions were scheduled to take place with the Genetic Resources Information Network (GRIN) of the United States Department of Agriculture (USDA) and the IBPGR in August 1986 and a format agreed upon prior to discussions with computer technologists. It was felt essential that these developments must interact with the establishment and testing of the IBPGR-CIAT model genebank.

15. Specific technical items for inclusion in the documentation system would relate to the kinetics and descriptor states of material in slow growth, with careful definition of those in vitro phenotype descriptor states. These items are necessary for the efficient management of facilities and development of appropriate, secure storage procedures.

16. Inherent in the documentation systems design is the Committee's

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1/ IBPGR. 1986. Design, Planning and Operation of In Vitro Genebanks: Report of a Subcommittee of the IBPGR Advisory Committee on In Vitro Storage. IPBGR.

endorsement of the Subcommittee's emphasis on careful maintenance of family lines of cultures.

Areas for further development and application

Cell culture techniques - the genetic conservation/utilization interface

17. The Committee acknowledged the dramatic progress being made in the development of techniques based upon cell/protoplast technology which present novel ways of utilizing plant genetic resources. The potential interest was recognized in storing sequences of genes e.g. in bacterial cloning systems, and in storing cell cultures *per se* in contrast to the organized systems generally considered to be preferable from the standpoint of genetic stability. In view of the importance of somatic cell genetics as a means of improving clonal crops in particular, the Committee agreed to keep a watching brief on developments in this area.

18. In view of the observations in para. 17 the relative merits of organized and unorganized culture systems were discussed, especially in the context of the occurrence of somaclonal variation and techniques available for storage of *in vitro* cultures. It was noted that to date the choice of shoot-tips/meristems for cryopreservation, whilst sound on present knowledge of the incidence of somaclonal variation in explants of different origin, may in fact exacerbate problems of stability when the stresses of cryopreservation are introduced. Thus, a potentially "low-risk" explant may present a high risk in storage. Accordingly it was felt that future work should also examine medium to low-risk systems (such as somatic embryos/embryoids) that may present a low *net* risk in cryopreservation. The choice of organized systems such as shoots for slow growth was reaffirmed, although it was recognized that in many of the crops under consideration, morphological considerations would prevent the use of the "ideal" explant (a pre-existing meristem) for *any* approach to storage.

Tropical and arid zone forages

19. IBPGR-funded work on priority forage grasses of the tropics and sub-tropics had led International Livestock Center for Africa (ILCA) to consider the need to develop *in vitro* work on *Brachiaria*, *Digitaria*, *Cynodon* because no seed is formed or seeds are not viable. In addition, seed handling

of many species is difficult due to dormancy, outcrossing and the need to maintain pure lines e.g. Aristida, Cynodon, Echinochloa, Eleusine, Oryzopsis and Panicum. Suitable in vitro techniques are lacking for most of these species; however, cooperative ILCA-CIAT work has developed culture techniques for Brachiaria.

20. The Committee agreed that IBPGR should fund some strategic research in this area and requested the Director to consider a tripartite project based at ILCA (Ethiopia), with back-stopping of the Welsh Plant Breeding Station (UK), where much basic in vitro work has been developed for temperate species, and CIAT (Colombia) which can assist in the developmental work through visits to ILCA. It was also agreed that the in vitro collecting technique should be applied to plantlets of such materials. Prof. Rao also offered facilities for testing collecting techniques for tropical forage species. In addition, it was suggested that IBPGR should explore such work for the collection of arid zone species which are heavily grazed and that Ben-Gurion University, Israel, might be able to initiate basic work in this area.

Citrus relatives

21. IBPGR has initiated field work in Southeast Asia to study the primary genepool of the Auranticideae related to orange, lemon and lime. Most of the species occurring in the jungle are widely scattered and produce fruits only sporadically. The taxonomy of the species is also in need of revision. The Committee proposed that:

(i) Cuttings could be collected in vitro and shipped to Instituto Valenciano de Investigaciones Agrarias (IVIA) where there are good quarantine facilities and some disease indexing can be done in culture tubes;

(ii) Flushes generated from cuttings could be used for characterization by analysis of isozymes as well as total proteins and flavanones; and

(iii) Immature seeds or embryos could be saved through an in vitro technique as used at IVIA. The Director of IVIA offered his full cooperation to IBPGR to assist in this research on adapting the in vitro collecting techniques and on specific diversity.

Allium

22. As noted in para. 5, there is an urgent need to develop culture techniques for wild species of the genepool. Further, in vitro techniques may be used to store disease-free material in a condition free of the risks of reinfection. A study of the genetic diversity of the wild genepool, in the first instance of garlic, utilizing biochemical techniques is urgently needed.

Banana

23. Since slow growth methods are now available for the culture of Musa and since in vitro propagation is now the norm, IBPGR had commissioned a status report to summarize all IBPGR - and otherwise - supported research in order to identify gaps. In this way, a combination of banana field genebanks - as designated by IBPGR - and new in vitro work could lead to a viable international network for this crop. A shortened version of the report, prepared by Dr. R.L. Jarret is provided in Appendix III. Members of the Committee agreed to investigate potential sites for in vitro genebanks and to keep Prof. De Langhe informed in his capacity as Director of INIBAP.

Temperate fruits

24. The Committee was informed that IBPGR has experienced major constraints in existing quarantine facilities; these are unlikely to be resolved in the foreseeable future. It was felt that IBPGR should attempt as soon as possible the use of the in vitro collecting techniques, e.g. for Prunus and that this should interface with in vitro propagation and storage (involving buds and other tissues). IBPGR is asked to develop a project in relation to its large collecting programme in the Mediterranean area.

Cryopreservation

25. The Committee, having noted the low level of research interest in the area of cryopreservation (para. 3), stressed that the centres of expertise where research could be satisfactorily commissioned were extremely few in number. Nonetheless it is urgent that cryopreservation research be accelerated for banana and cassava in particular, in addition to the ongoing IBPGR supported work on aroids and sweet potato; other species will need

examination in the future. The attention of the IBPGR was drawn to the need to identify centres of expertise which are well equipped with a critical mass of scientists and capacity to help in much-needed strategic research in the area of cryopreservation.

Stability

26. The continued need for futher information on the stability of germplasm stored *in vitro* was emphasized by the Committee. Appropriate research should be both basic, utilizing model systems possessing good markers, and applied, directed towards crops of interest (such as the study of *Musa* noted in para. 2).

Other items

27. The Committee thanked Dr. Navarro for his great help during the meeting and looked forward to close collaboration with his institute.

APPENDIX I

List of Participants

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APPENDIX II

IBPGR In Vitro Conservation Data Bases - A Report

L.A. Withers [1] and S.K. Wheelans [2]

The survey of *in vitro* research from which a major part of the data bases are constructed is now in its third phase. (Phases 1 and 2 were initiated in 1980 and 1983, respectively). Summary data on the survey data base contents are given in Table 1 below. The surveys are supplemented by

	Phase 1 1980	Phase 2 1983	Phase 3 1985
Contacts	--746-------- [3]		1950
Respondents	--309-------		574
Percentage response	----------41--------		29 [4]
Entries received	569	545	1334
Countries represented	46	52	67 [5]
Genera covered (approx.)	182	165	330 [6]
Species covered (approx.)	325	317	648
Entries recording:			
Storage under normal growth conditions	297	361	888
Storage under slow growth	139	132	355
Storage by cryopreservation	39	24	60
Characterization of stored material (all methods)	0	25	43
Characterization of *in vitro* propagated plants by:			
Cytological analysis	59	49	210
Biochemical analysis (e.g. isozymes)	6	31	74
Disease indexing	- [7]	87	295
In vitro exchange of germplasm	67	117	309

1/ University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough LE12 5RD, UK

2/ School of Agriculture, University of Aberdeen, UK

3/ Data were amalgamated for first 2 phases.

4/ The 1985 survey included a much higher number of speculative contacts (i.e. other than scientists known to be working on species or techniques of interest). Accordingly, the reduced percentage response is not considered to reflect a waning of genuine interest in the survey.

5/ A total of 71 countries are represented by the 3 phases.

6/ The 1985 survey accepted data on all genera and species. Thus, the increase recorded is not considered to reflect wider use of techniques.

7/ Disease indexing was not included in the Phase 1 questionnaire.

bibliographic data bases holding literature references on general aspects on *in vitro* culture, propagation and storage, and specialist topics including temperate fruits, *Allium* and isozyme analysis.

The survey data bases have been searched in response to enquiries from scientists. The 1980 survey has been subjected to 94 searches of which 29 (31%) were on behalf of IBPGR collaborators. Corresponding figures for the 1983 survey are 120 searches with 49 (38%) for IBPGR collaborators. All entries to the 1985 survey data bases have been computerized and only a very small number of queries referred back to contributors are outstanding. The data base was made available for searching in a preliminary form in early 1986. The 30 searches (13, i.e. 43% for IBPGR collaborators) carried out on the incomplete data base are now to be repeated in order to provide the requesting scientists with more up-to-date information. Prominent among the search topics are the priority crops of IBPGR, *in vitro* storage and characterization. Only 5 (2%) of the searches requested have failed to find relevant information in the data bases. It is concluded that the survey is successfully meeting a genuine need for information among IBPGR collaborators and the wider scientific community.

APPENDIX III

In Vitro Propagation and Genetic Conservation of Bananas and Plantains

R.L. Jarret [1/]

I. IN VITRO SYSTEMS

A number of progress reports have been reviewed. These described IBPGR-funded research related to the development of rapid propagation systems for *Musa*. The summarized results of some of these have been published (Banerjee and De Langhe, 1985; Banerjee *et al.*, 1986b). These studies are reviewed in relation to other literature on *in vitro* manipulations with *Musa*.

SHOOT-TIP CULTURE

Explant preparation

(i) Shoot-tips (meristem plus 1-4 leaf primordia) may be harvested from dormant buds, eyes, peepers, sword suckers, or corms of various sizes (Banerjee and De Langhe, 1985; Bower and Fraser, 1982; Jarret *et al.*, 1985b; Vuylsteke and De Langhe, 1985). Explant survival from all is approximately 100%. Due to greater ease of handling, dormant eyes, buds, peepers, and small sword suckers are generally preferred.

(ii) Plant materials are washed to remove adhering soil.

(iii) Outer leaves, leaf bases and corm tissue are trimmed away until the diameter at the leaf base is 1-2 cm. (Care must be taken to avoid cutting through the apex during this procedure.)

1/ Current address: United States Department of Agriculture, Agricultural Research Service, Regional Plant Introduction Station, Experiment, Georgia 30212, USA

(iv) The more or less cubical piece of tissue containing the shoot-tip is surface sterilized by immersion in 1.5% CaOCl for 15-30 secs. following a 95% alcohol dip for 10-15 secs. (Banerjee and De Langhe, 1985; Vuylsteke and De Langhe, 1985). Commercial laundry bleach containing sodium hypochlorite is equally effective at a concentration of about 10% (v/v) - approximately 0.5% NaOCl (Cronauer and Krikorian, 1984b; Jarret et al., 1985b). Damasco and Barba (1984) utilized full strength bleach (5.25% NaOCl) for 45 mins. However, such lengthy sterilization is unnecessary. Berg and Bustamante (1974) excised meristems in the absence of a sterilization procedure.

(v) In a laminar air-flow hood, and with the aid of a stereo microscope, additional corm and leaf tissue is removed until the apical dome and leaf primordia are visible.

(vi) The apical dome and 1-4 leaf primordia are excised and transferred directly to culture medium. It should be remembered that the size of the explant affects both the hormonal requirements for its survival (Doreswamy et al., 1983) and its regenerative potential (Vuylsteke and De Langhe, 1985). Sterilization of the apex after excision (Cronauer and Krikorian, 1984a) is not necessary.

(vii) The cultures are incubated at 27°-30°C under a 12-16 h. photoperiod with 1000-2000 lux illumination. Contaminated cultures are removed as they are observed.

(viii) Over a period of several weeks, the shoot-tips become green and expand. They may then be transferred to rooting medium or propagated further as described in the following sections.

For routine culture initiation, the procedure outlined above, without modification, works well with a broad range of Musa clones and species (Table 1).

Culture medium and environment

Several basal medium formulations have been examined for their ability to sustain growth and development of excised meristems and shoot-tips. Ma and Shii (1972) cultured 'Cavendish' shoot-tips on the medium of Smith and Murashige (1970). Subsequently, Berg and Bustamante (1974) cultured 'Cavendish' apical meristems on Knudson's (1946) medium. All studies since that time have utilized the basal medium of Murashige and Skoog (1962), or modifications of it, supplemented with 2-4% sucrose and solidified with 0.45-0.8% agar. Vitamins are routinely included although no data yet presented suggest that they are required. The most frequently included vitamins are nicotinic acid, pyridoxine.HCl and thiamine.HCl. Glycine and i-inositol are also included. The inclusion of undefined organic additives such as coconut water, casein hydrolysate or yeast extract is neither necessary nor desirable. Semi-solid medium is preferred by most investigators. Several investigators routinely include antioxidants such as ascorbic acid or cysteine in the culture medium. The long-term effectiveness of antioxidants on inhibition of explant browning is questionable. The preferred method for controlling medium discoloration and tissue oxidation is by reculture to fresh medium.

Although hormones are not essential for explant survival (Berg and Bustamante, 1974), the inclusion of auxin and cytokinin is generally required (Banerjee and De Langhe, 1985; Doreswamy et al., 1983; Jarret et al., 1985b; Vuylsteke and De Langhe, 1985). The basal medium defined in Table 2, supplemented with 0.1 mg/l indole-3-acetic acid (IAA) and 0.2 mg/l N6-benzyladenine (BA), will support the growth and occasionally the proliferation of shoot-tips excised from a diverse range of Musa germplasm (Banerjee and De Langhe, 1985; Vuylsteke and De Langhe, 1985).

Multiplication of the propagule

Established shoot-tip cultures are utilized as a source of explants for further proliferation. Alternatively, proliferating shoot-tip cultures may be initiated directly by placing the freshly excised shoot-tip onto basal medium supplemented with a greater concentration of cytokinin than that used for culture initiation. Either procedure is satisfactory.

Table 1. List of *Musa* cultivars and species which have been successfully propagated *in vitro*

Taxon	Name	BA (mg/l)	Number of shoots per culture	Ref.[1/]
M. acuminata				
AA cultivars				
subsp. *microcarpa*	Inarnibal	10	2	1
	V. cohol	10	1	1
subsp. *banksii*	Pamotion	10	1	1
subsp. *errans*	Manang	10	1	1
subsp. *zebrina*	Morado	10	2	1
	Moradong Putl	10	1	1
AA fertile diploids				
subsp. *burmannica*		3	3	5
subsp. *malaccensis*		3	2	5
subsp. *truncata*		3	4	5
AAA cultivars				
	Gros Michel	3	4	5
	Dwarf Cavendish	2.25	35	7
	Robusta	2.25	14	1
	Giant Cavendish	no data	no data	2
	Pisang masak hijau	5	no data	4
	Ambon	10	3	3
	Ouhoy	10	1	3
	Tanggung	10	1	3
AAAA cultivars				
	Calypso	10	1	3

Table 1. List of *Musa* cultivars and species which have been successfully propagated *in vitro* (Continued)

Taxon	Name	IIA (mg/l)	Number of shoots per culture	Ref.[1/]
M. acuminata × *M. balbisiana* hybrids				
AAB cultivars				
	Silk	2.25	29	7
	Prata	2.25	16	7
	Asamiensa	2.25	33	7
	Agbagba	2.25	29	7
	Ntanga	2.25	25	7
	Mysore	3	3	5
	Dominco	3	2	5
	Horse plantain	3	4	5
	Horn plantain	3	3	5
	Latundan	10	6	3
	Inangel	10	1	3
	Gelamay senora	10	4	3
	Radja	10	1	3
ABB cultivars				
	Cardaba	5	16	5
	Pelipita	5	9	2
M. balbisiana				
BB fertile diploid		3	1	5
BBB cultivars	Butuhan	10	1	3
Other *Musa* species				
M. velutina		3	1	5
M. textilis		10	8	6

1/ 1 = Banerjee and De Langhe, 1985
2 = Cronauer and Krikorian, 1984a
3 = Damasco and Barba, 1984
4 = De Guzman *et al.*, 1980
5 = Jarret *et al.*, 1985b (and unpub. data)
6 = Manto and Tepper, 1983
7 = Vuylsteke and De Langhe, 1985

Table 2. Basal medium for culture of *Musa* shoot-tips

Media constituent	Concentration (mg/l)
Inorganic salts	MS 1/
I-inositol	100.0
Thiamine.HCl	0.4
Nictonic acid	0.5
Pyridoxine.HCl	0.5
Glycine	2.0
Sucrose	30,000
Agar	7,000

1/ Murashige and Skoog, 1962

Stimulation of axillary branching/adventitious bud formation is routinely accomplished by placing of an intact or fragmented shoot apex onto basal medium supplemented with 0.2-10.0 mg/l of cytokinin. Auxin may also be included. The concentration of exogenous cytokinin appears to be the principal factor affecting the rate of proliferation. BA is the cytokinin of choice and is more effective than kinetin (Cronauer and Krikorian, 1984a) or zeatin (Vuylsteke and De Langhe, 1985). Wounding the meristem is a technique commonly employed to reduce or eliminate apical dominance, thereby allowing for axillary bud development. Ma and Shii (1972) decapitated shoot apices of 'Cavendish' bananas whereas Vessey and Rivera (1981) made a series of cuts across the meristematic dome. Later investigators have followed the procedure first described by De Guzman *et al*. (1980) who split the shoot-tip longitudinally through its apex and cultured individual pieces (Cronauer and Krikorian, 1984b; Damasco and Barba, 1984; Doreswamy *et al*., 1983; Jarret *et al*., 1985b). No data are currently available by which the effectiveness of this technique may be evaluated in comparison with non-wounded shoot-tips. However Doreswamy *et al*., (1983) noted that quartering shoot apices did not stimulate further axillary branching, and recent reports have described the stimulation of axillary branching from cultured intact shoot-tips (Banerjee and De Langhe, 1985; Vuylsteke and De Langhe, 1985). Further consideration of different proliferation systems is given below (*Budding systems in Musa shoot-tip cultures*).

Root initiation during the multiplication process should be avoided if possible since this adversely affects the rate of propagation (Cronauer and Krikorian, 1985a).

Preparation for re-establishment of plants in soil

Rooting is easily induced when individual shoots are transferred to basal medium alone (Cronauer and Krikorian, 1985a; Damasco and Barba, 1984; Jarret et al., 1985b; Sandoval, 1985). However, addition of auxin to the basal medium may stimulate further root initiation. Berg and Bustamante (1974) found that napthaleneacetic acid (NAA) at 1.0 mg/l stimulated rooting of 'Cavendish' shoots. Indole-3-butyric acid (IBA) is also very effective over a range of concentrations (Banerjee and De Langhe, 1985; Cronauer and Krikorian, 1984a; 1984b; Doreswamy et al., 1983; Vuylsteke and De Langhe, 1985). Cronauer and Krikorian (1984a; 1984b) reported no difference in the root-inducing effects of IAA, NAA or IBA in the presence of 0.025% activated charcoal. Basal medium supplemented with 1.0 mg/l IBA or NAA is suitable for rapid root initiation and its inclusion is recommended. When rooting individual shoots, care should be taken to ensure that they are free of developing secondary axillary buds, the occurrence of which inhibits root formation and shoot development (Jarret et al., 1985b).

Transfer to soil is readily accomplished when an adequate root system has developed. A period of high humidity prior to movement to the field may also be beneficial.

Budding systems in Musa shoot-tip cultures

Two types of budding are frequently observed in cultures of Musa shoot-tips. The first of these is apparently the development of axillary buds which might be expected to be genetically stable. A second type of budding as described by, for example, Vuylsteke and De Langhe (1985) and Banerjee et al. (1986b) involves the development of bulbil-like structures which in some cultures continue to develop into a whitish tissue. This is covered on its surface with numerous minute meristems. A similar budding behaviour occurs in shoot-tip cultures derived from floral apices (Jarret, unpub.). These minute meristems have been observed by numerous investigators and have been suggested to be adventitious in origin. Their occurrence is apparently a function of the propagation rate of the culture in which it occurs. The propagation rate is, in turn, a function of the cytokinin concentration in the culture medium. It is not possible, at this time, to state unequivocally that genetic variation among plants derived from these apparently adventitious buds will be

greater than among those derived from developed axillary buds or meristems. This information should be forthcoming in the near future as several field studies are now in progress (Cronauer and Krikorian, 1985b; Reuveni et al., 1985).

Using 'Bluggoe' as an example, Banerjee et al. (1986b) studied the shoot formation process in Musa shoot-tip cultures. In the presence of 1 u M IAA and 1 u M BA, shoot development, but not proliferation (for most cultivars) was observed. Entire rooted plantlets were readily recovered and transferred to soil. However, in the presence of 10 u M BA, multiple buds were produced. The proliferation rate was found to be a function of the genomic constitution of the cultivar; that is, dosage effects associated with the B genome were observed. Elevated levels of BA (10 u M) resulted in the de novo formation of meristems at the base of the central leaf primordia. These were arranged in a semi-circular pattern. Interestingly, this pattern of bud formation is similar to that described for the in vivo stimulation of adventitious bud formation in plantain (Devos, 1985).

In some cultures, white bulb-like structures bearing minute meristems over their surface were observed. Adventitious bud formation across the surface of the meristem was studied histologically. Although a truly complete anatomical investigation of the origins of these adventitious shoots prior to stimulation by elevated levels of BA was not conducted, it appears that many of the shoots which arise from subcultured shoot-tips of cooking bananas and plantains are adventitious and that they may arise through one or more patterns of development. Histological examination of the origin of shoot buds in proliferating cultures of the AAA group was not conducted, although it was later suggested (Banerjee et al., 1986b) that their origin is also adventitious.

ADDITIONAL IN VITRO RESEARCH

Somatic embryogenesis

Srinivasa Rao et al. (1982) reported the occurrence of "proembryo-like" structures in callus derived from floral axes cultured in the presence of 2,4,5-trichloracetic acid (2,4,5-T). In this instance, only rhizogenesis was subsequently observed. Cronauer and Krikorian (1984c), utilizing a similar

culture medium supplemented with 2,4,5-T for leaf base and blade explants, reported the occurrence of somatic embryos from callus in liquid media. An anatomical investigation of these structures was not conducted, and only root elongation was observed. Jarret et al. (1985a), suggested that the mature structures observed by Cronauer and Krikorian (1984c) were roots and not somatic embryos. This opinion was validated by Bakry and Rossignol (1985). However, it seems likely that in both instances (Cronauer and Krikorian, 1984c; Jarret et al., 1985a), embryogenically competent callus cultures had been initiated and that subsequent embryo development was then inhibited as a result of a hormonal or other nutrient deficiency in the culture medium. Banerjee et al. (1986a) obtained proembryos when thin layers of meristematic tissue excised from proliferating shoot-tip cultures were placed in liquid MS supplemented with 2,4,5-T. These proembryos continued to develop into bipolar structures when transferred to MS medium containing BA (0.25-2.5 mg/l) and NAA (0.02 mg/l). The somatic embryos showed some resemblance to zygotic embryos. However, germination was not observed.

Culture of floral apices

Ma et al. (1978) were the first to report on the induced reversion of cultured floral apices to vegetative structures. This technique has been further refined by Cronauer and Krikorian (1985a) and Bakry et al. (1985). Jarret (unpub. data) has shown that reversion is readily accomplished by culturing the floral apex (2-4 mm in length) in liquid MS medium supplemented with BA (1-10 mg/l). Reculture to identical medium 3-4 weeks after culture initiation stimulates additional growth and proliferation. 8 weeks after culture initiation, shoot development is generally well advanced and the developing shoots may be excised for rooting or divided and recultured. Techniques for further proliferation, rooting and establishment are identical to those used for multiplication using shoot-tips as an original explant source. This technique appears to be applicable over a wide range of genotypes (Bakry et al., 1985; Jarret, unpub. data).

Cell cultures, callus cultures and protoplasts

Various investigators including Mohan Ram and Steward (1964), Tongdee and Boon-Long (1973), De Guzman et al. (1980), Srinivasa Rao et al. (1982), Brasil (1982), Cronauer and Krikorian (1984c; 1985b), Jarret et al. (1985a;

1985b), Vuylsteke and De Langhe (1985), Banerjee and De Langhe (1985), and Banerjee et al. (1986a) have reported the induction of callus from tissues of bananas and plantains. In several instances, organ formation from sub-cultured callus has been observed (Cronauer and Krikorian, 1984c; Srinivasa Rao et al., 1982; Jarret et al., 1985a; Banerjee et al., 1986b). Difficulty in the maintenance of proliferating callus cultures has repeatedly been alluded to in the literature (Cronauer and Krikorian, 1985b). A morphogenically competent cell suspension has not yet been reported.

Isolation of protoplasts from Musa clones has been reported by Bakry (1984) and more recently by Cronauer and Krikorian (1985b). However, neither cell division nor organ formation has been achieved. In view of the difficulties associated with regeneration from monocot protoplast systems, (see below: SUMMARY AND CONCLUSIONS; rapid advances in this area should not be expected).

Embryo culture

Both Cox et al. (1960) and Rowe and Richardson (1975) have reported the successful in vitro culture ("embryo rescue") of zygotic embryos from M. balbisiana and polyploid acuminata. Maturation and germination of cultured immature embryos of various diploid subspecies of acuminata, M. balbisiana, M. velutina, M. textilis and M. ornata have been observed when these are placed on hormone-free MS medium containing 2% sucrose (Jarret, unpub. data).

SUMMARY AND CONCLUSIONS

The literature pertaining to shoot-tip culture of Musa clones and species indicates that members of this genus, especially acuminata and acuminata × balbisiana hybrids, are amenable to in vitro shoot-tip propagation techniques. To date, all tested genotypes have responded favourably, although significant variation in the multiplication rates achieved for most clones has been observed. This is because extensive efforts have not been made to define the optimum hormonal concentrations for individual clones. The techniques and methods described by Vuylsteke and De Langhe (1985) adequately explain the propagation procedure as it applies to a wide range of Musa germplasm.

Progress in the development of additional in vitro techniques for

propagation that might also be used in genetic improvement or storage has lagged far behind that achieved for shoot-tip culture propagation.

The development of cell culture/protoplast culture techniques has not proceeded as rapidly as hoped. In addition, there is some question at this time as to the occurrence of plantlet regeneration via isolated plant protoplasts from any monocot tissue culture system (with the exception of rice), despite claims to this fact. Therefore, rapid progress in this area is not to be expected. Certainly, the development of a protoplast system capable of regeneration would be a large step towards the use of the techniques of microinjection, protoplast fusion, transformation, and germplasm utilization. For example, the possibility of fusing protoplasts collected from or derived from parthenocarpic diploid acuminata clones with protoplasts isolated from anther-derived or microspore-derived haploids of M. balbisiana would result in the direct production of plantain cultivars.

The literature suggests that the development of embryogenically competent callus cultures will be a reality in the near future. This assumption is based principally upon the articles of Cronauer and Krikorian (1984c), Jarret et al. (1985a) and Banerjee et al. (1986a). However, considerable additional research will probably be necessary in order to ensure reproducibility and normal germination of somatic embryos. The development of embryogenically competent callus cultures is important as it relates to the use of somatic embryos for plant propagation, germplasm conservation and the production of non-chimeric somaclonal variants.

II. IN VITRO GERMPLASM STORAGE AND GENETIC STABILITY

Compelling reasons justifying the need to develop in vitro techniques for the conservation of Musa germplasm (and a variety of tropical perennials) have been presented by De Langhe (1984) and will not be repeated here. Also, numerous and extensive reviews on the use of in vitro slow growth and cryopreservation techniques for germplasm conservation are now available. Obviously, the ability to conserve germplasm in vitro has great implications for exchange of germplasm (Roca et al., 1979) pending its certification as pathogen-free (Chiarappa and Karpati, 1984).

FORMS OF THE CONSERVED GENOME

There are, at the present time, several trains of thought pertaining to the conservation of germplasm in vitro. These can arbitrarily be divided as follows:

1. The conservation of genes,
2. The conservation of genomes, and
3. The conservation of genotypes.

They are differentiated as follows:

1. The conservation of genes - that is the maintenance of a fragmented genome or parts thereof, for example, gene libraries and total genomic libraries. This form of preservation refers to the conservation of the information carried in genes but not the coordination of this information (Peacock, 1984).

2. The conservation of genomes - this is a rather more stringent definition of germplasm conservation and refers to the preservation of more or less intact and coordinated information systems. This would include, for example, the conservation of cell or protoplast cultures which are incapable of regeneration and within which some reorganization of the genome has already occurred or may occur. Applications for this type of germplasm conservation include, for example, preservation of variant cell lines (Withers, 1984).

3. The conservation of genotypes - the is is the most conservative definition of germplasm conservation and refers to the preservation of the intact genome in a manner which allows for its recovery/expression in the form of a whole plant. That is, for example, germplasm storage via normal and limited growth shoot-tip culture, cryopreservation of meristems etc. Obviously, in this type of preservation system some risk of genetic variation, albeit limited in some cases, will undoubtedly arise.

THE DIPLOID GENEPOOL

In relation to the conservation of Musa germplasm, it seems likely that conservation measures such as preservation of germplasm as total genome libraries is probably not justified at this time, as great (though diminishing) genetic diversity undoubtedly exists in the fertile diploid progenitor species whose seeds are orthodox and thus may be conserved via conventional techniques. It seems likely that the range of variation for any given agriculturally important character will be found to be greater in the wild diploids than in the cultivated clones. It is well known that these species (acuminata and balbisiana) and the various acuminata subspecies are all interfertile. If the conservation of genetic diversity is the chief objective, then collection and maintenance of these diploids must be a priority objective in the immediate future. In addition, the fact that these materials are fertile and, therefore, lend themselves to classical genetic studies, should inspire many more scientists to investigate and conduct fundamental and applied crop improvement orientated research with Musa acuminata and the interspecific hybrids. Dissemination of seed will make these materials readily available. Such an approach is clearly not possible at the present time when working with triploids due to the extensive land area needed for seed production (Rowe and Richardson, 1975). In almost all crop species, the conservation of wild relatives has always been a priority issue. This must be accelerated for Musa.

In terms of the conservation of triploid Musa germplasm (clones), it seems desirable, at this time, to press for the development of techniques for the preservation of specific genotypes (see below).

IN VITRO GENETIC STABILITY

A relatively high rate of variation is commonly associated with the propagation of Musa clones via shoot-tip culture. This is emphasized in a report submitted to the IBPGR (Reuveni et al., 1985). Although there are as yet no published data, numerous personal communications suggest that the mutation rate appears to vary between 4 and 14% for phenotypic characteristics observed in the field and considered to be genotypically controlled. The most frequently observed variants are those which affect plant height and pigmentation. In vitro propagated plants tend to show various juvenile

characteristics (Hwang et al., 1984). In at least one instance, variation in plant pigmentation and fruit bunch characteristics was the result of the unmasking of chimerism in the parent plant. Unfortunately there are no data as yet on the effects of medium constituents on the production of mutant plantlets. However, a review of the literature suggests that two pathways of shoot production occur in Musa shoot-tip cultures and that regulation of these pathways may profoundly effect the genetic fidelity of the resultant plantlets. These are discussed in detail above (Budding systems in Musa shoot-tip cultures).

Any form of adventitious regeneration is best avoided and stimulation of axillary branching in cultured Musa shoot-tips is favoured as a means of propagation for in vitro germplasm preservation. Instability in these materials is generally less than that encountered in disorganized cell and tissue cultures. However, variation in cultured meristems, either as a result of chimerism in the stock plant or as a result of de novo variation via undetermined genetic mechanisms (somaclonal variation) may give rise to mutant types.

GERMPLASM CONSERVATION IN VITRO: SUCCESS TO DATE

Significant data on the feasibility of cryopreservation of Musa shoot-tips are not yet available. Investigations in this area are only just beginning.

Few published studies exist by which the potential for in vitro medium-term germplasm storage of Musa might be evaluated. Nevertheless, there is reason to be optimistic about this approach (Banerjee and De Langhe, 1985; Jarret et al., 1986).

Banerjee and De Langhe (1985) maintained proliferating shoot-tip cultures of various Musa clones at 15°C for over 1 year. These cultures were maintained in the presence of 10 u M BA. Jarret et al. (1986) have also reported on the medium-term low temperature storage of Musa shoot-tip cultures on liquid media in the absence of growth regulators. Storage of these cultures for over 1 year is also feasible.

For some crops, storage in slow growth periods in excess of 5 years is already a reality. It is believed that by manipulation of various chemical

and environmental variables, the storage life of Musa shoot-tip cultures may considerably be prolonged. From the viewpoint of genetic fidelity, storage of shoot-tips in the absence of high levels of BA seems desirable in order that adventitious shoot formation be limited.

CONCLUSIONS

Conservation of clones, other than in field genebanks, will require additional research emphasis in the development of techniques to prolong the period of time between reculture. Ideally, medium-term storage might be used to maintain shoot-tip cultures for 5 to 10 years without reculture. A host of techniques is available for supporting culture growth at a slow rate. These include osmotic adjustments to the culture media, reduced light intensity, the addition of chemical growth retardants, and low pressure storage, among others. These have not yet been evaluated fully with Musa, but progress with other species is promising. The lack of data on progress in the development of cryopreservation techniques for Musa organs and cells should not deter continued efforts to develop these longer-term conservation techniques.

The use of morphological and isozyme markers to examine genetic variation in plants derived from the various budding systems described by Banerjee et al., (1986b) may help to identify the preferred in vitro propagation and conservation method. In addition, the growing out of populations of plants for field observation should also help to identify which budding system more closely conserves the donor genome.

It seems likely that in the near future, techniques for the stimulation of somatic embryogenesis with the subsequent recovery of whole plants will be a reality. These techniques need development in as much as they may have a substantial impact on propagation, conservation and, possibly, the genetic improvement of Musa.

III. OTHER POINTS

1. Some consideration must be given to the development of virus indexing techniques for Musa. This is important in the context of meristem culture for international exchange of plant germplasm which is essentially meaningless unless this germplasm be pathogen free.

Several sophisticated techniques are now available for the detection of single and double stranded of RNA viruses using a molecular biological approach.

2. A variety of electrophoretic systems have now been developed and utilized to study enzyme polymorphisms in the genus Musa (Jarret and Litz, 1986a; 1986b). Studies to date have been conducted only at the whole plant level. These techniques are probably applicable to analysis of small plantlets, regenerated from in vitro cultured shoot-tips and possibly the proliferating shoot-tip cultures themselves. However, before these techniques are utilized in this manner, an extensive evaluation of the effects of the culture process itself on regulation of the expression of individual isozymes must be conducted.

3. With a look towards the future, molecular markers could be developed for use with the genus Musa. These markers can be utilized to estimate genetic diversity in vivo and culture stability in vitro. For instance, there could be the creation of a restriction endonuclease cleavage site linkage map for Musa (using the fertile diploids). These cleavage sites may then be correlated with isozyme markers. The creation of such a linkage map may then be used to compare estimates of genetic diversity/culture stability using both of these techniques. In addition, the creation of a linkage map should yield significant data on the structure and evolution of the Musa genome.

4. The collection of seed fertile Musa acuminata subspecies, M. balbisiana, and other related taxa remains a priority.

References

Bakry, F. 1984. Choix du materiel a utiliser pour l'isolement de protoplastes de bananier (Musa sp.), Musacees. Fruits, 39:449-452.

Bakry, F., F. Lavarde Guignard, L. Rossignol and Y. Demarly. 1985. Développement de pousses vegetatives a partir de la culture in vitro d'explants inflorescentiels de Bananiers (Musa sp., Musacees) Fruits, 40:459-465.

Bakry, F. and L. Rossignol. 1985. Analyse des capacites de callogenese et d'organogenese obtenues a partir de differents tissus de Bananiers (Musa sp., Musacees). Fruits, 40:697-708.

Banerjee, N. and E. De Langhe. 1985. A tissue culture method for rapid clonal propagation and storage under minimal growth conditions of Musa (banana and plantain). Plant Cell Rpts., 4:351-354

Banerjee, N., J. Schoofs, S. Hollevoet, F. Dumortier and E. De Langhe. 1986a. Aspects and prospects of somatic embryogenesis in Musa, ABB, cv. Bluggoe. Proc. Conf. In Vitro Problems Related to Horticultural Plant Species, Gembloux (in press).

Banerjee, N., D. Vuylsteke and E.A. De Langhe. 1986b. Meristem tip culture of Musa: Histomorphological studies of shoot bud proliferation. In, L.A. Withers and P.G. Alderson (eds.) Plant Tissue Culture and Its Agricultural Applications. Butterworth, UK. pp.139-147.

Berg, L.A. and M. Bustamante. 1974. Heat treatment and meristem culture for the production of virus-free bananas. Phytopathology, 64:320-322.

Bowen, J.P. and C. Fraser. 1982. Shoot-tip culture of Williams banana. Subtropica, 3:13-16.

Brasil, O.G. 1982. Tissue culture from banana fruit (Musa acuminata AAA). Growth and some respiratory enzyme property from banana fruit callus. In, A. Fujiwara (ed.) Plant Tissue Culture (1982). Maruzen Co., Tokyo. p.79.

Chiarappa, L. and J.F. Karpati 1984. Plant quarantine and genetic resources. In: J.H.W. Holden and J.T. Williams (eds.) Crop Genetic Resources: Conservation and Evaluation. George Allen and Unwin, London. pp. 158-162.

Cox, E.A., G. Stotsky and R.D. Goos. 1960. In vitro culture of M. balbisiana embryos. Nature, 185:403-404.

Cronauer, S. and A.D. Krikorian. 1984a. Multiplication of Musa from excised stem tips. Ann. Bot., 53:321-328.

Cronauer, S. and A.D. Krikorian. 1984b. Rapid multiplication of bananas and plantains by in vitro shoot-tip culture. HortSci., 19:234-235.

Cronauer, S. and A.D. Krikorian. 1984c. Somatic embryos from cultivated tissues of triploid plantains (Musa 'ABB'), Plant Cell Rpts., 2:289-291.

Cronauer, S. and A.D. Krikorian. 1985a. Aseptic multiplication of banana from excised floral apices HortSci., 20:770-771.

Cronauer, S. and A.D. Krikorian. 1985b. Banana (Musa spp.). In, Y.P.S. Bajaj (ed.) Biotechnology in Agriculture and Forestry. I. Trees. I. Springer-Verlag, Berlin. pp. 233-252.

Damasco, O.P. and R.C. Barba. 1984. In vitro culture of Saba (Musa sp. cv. Saba BBB). Phil. Agr., 67:351-358.

De Guzman, E.V., A.C. Decena and E.M. Ubalde. 1980. Plantlet regeneration from unirradiated and irradiated banana shoot-tip tissues cultured in vitro. Phil. Agr., 63:140-146.

De Langhe, E. 1984. The role of in vitro techniques in germplasm conservation. In, J.H.W. Holden and J.T. Williams (eds.) Crop Genetic Resources: Conservation and Evaluation. George Allen and Unwin, London. pp. 131-137

Devos, P. 1985. The location of lateral buds of banana (Musa sp.) clarified by the discovery of two new types of adventitious buds within the AAB plantain group. Fruits, 40:471-474.

Doreswamy, R., N.K. Srinivasa Rao and E.K. Chacko. 1983. Tissue-culture propagation of banana. Scient. Hortic., 18:247-252.

Hwang, S.C., C.L. Chen, J.C. Lin and H.L. Lin. 1984. Cultivation of banana using plantlets from meristem culture. HortSci., 19:231-233.

Jarret, R.L., J.B. Fisher and R.E. Litz. 1985a. Organ formation in Musa tissue cultures. J. Plant Physiol., 121:123-130.

Jarret, R.L., W. Rodriguez and R. Fernandez. 1985b. Evaluation, tissue culture propagation and dissemination of 'Saba' and ' Pelipita' plantains in Costa Rica. Scient. Hortic., 25:137-147.

Jarret, R.L. and R.E. Litz. 1986a. Enzyme polymorphisms as genetic markers in bananas and plantains. Euphytica (In press).

Jarret, R.L. and R.E. Litz. 1986b. Enzyme polymorphisms in Musa acuminata Colla. J. Hered. (in press).

Jarret, R.L., R. Fernandez and S. Salazar. 1986. In vitro germplasm storage at CATIE. Pl. Genet. Resources Newsl. (in press).

Knudson, L. 1946. A new nutrient solution for the germination of orchid seed. Bull. Amer. Orch. Soc., 15:214-217.

Ma, S.S. and C.T. Shii. 1972. In vitro formation of adventitious buds in banana shoot apex following decapitation. Chinese J. Hort. Sci., 18:135-142.

Ma, S.S., C.T. Shii and S.O. Wang. 1978. Regeneration of banana plants from shoot-tips and inflorescence section in vitro. XXth Int. Soc. Hort.Sci., Sydney, Abstract 1369

Mante, S. and H.B. Tepper. 1983. Propagation of Musa textillis NEE plants from apical meristem slices in vitro. Plant Cell Tiss. Org. Cult., 2:151-159.

Mohan Ram, H.Y. and F.C. Steward. 1964. The induction of growth in explanted tissue of the banana fruit. Can. J. Bot., 42:1559-1579.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15:473-497.

Peacock, W.J. 1984. The impact of molecular biology on genetic resources. In, J.H.W. Holden and J.T. Williams (eds.) Crop Genetic Resources: Conservation and Evaluation, George Allen and Unwin, London. pp.268-276.

Reuveni, O., Y. Israeli, H. Degani and Y. Eshdat. 1985. Genetic variability in banana plants multiplied via in vitro techniques. IBPGR Internal Report/85/9 (mimeographed).

Roca, W.M., J.E. Bryan and M.R. Roca. 1979. Tissue culture for international transfer of potato genetic resources. Amer. Potato J., 56:1-11.

Rowe, P.R. and D.L. Richardson. 1975. Breeding bananas for disease resistance, fruit quality and yield. Trop. Agr. Res. Serv. SIATSA, La Lima, Honduras. Bull., 2:1-144.

Sandoval, J.A. 1985. Micropropagación de Musaceas. Asbana, 9:21-23.

Smith, R.H. and T. Murashige. 1970. In vitro development of the isolated shoot apical meristem of angiosperms. Amer. J. Bot., 57:562-568.

Srinivasa Rao, N.K., E.C. Chacko, R. Doreswamy and S. Narayanswamy. 1982. Induction of growth in explanted inflorescence axis of banana. Curr. Sci., 51:666-667.

Tongdee, S.C. and S. Boon-Long. 1973. Proliferation of banana fruit tissues grown in vitro. Thai. J. Agric. Sci., 6:29-33.

Vessey, J.C. and J.A. Rivera. 1981. Meristem culture of bananas. Turrialba, 31:162-163.

Vuylsteke, D. and E. De Langhe. 1985. Feasibility of in vitro propagation of bananas and plantains. Trop. Agric. (Trin.), 62:323-328.

Withers, L.A. 1984. Freeze preservation of cells. In, I. Vasil (ed.) Cell Culture and Somatic Cell Genetics of Plants, Vol. 1. Academic Press, Orlando. pp.608-620.