THE POTENTIAL FOR USING *IN VITRO* TECHNIQUES FOR GERMPLASM COLLECTION

International Board for Plant Genetic Resources

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INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES

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INTRODUCTION

1. Following a decision by the IBPGR at its tenth meeting in February 1983 to consider novel collecting techniques for vegetatively propagated species and material such as coconut which presents practical problems, the Chairman of the In Vitro Committee and the Executive Secretary of the IBPGR were asked to organize a sub-committee which would meet and discuss the scientific implications.

2. The sub-committee was convened and met at the School of Agriculture of the University of Nottingham, UK, 14-16 September, 1983. The arrangements were made by Dr. Lyndsey A. Withers, Technical Secretary of the In Vitro Committee who works at that Institute. Professor E.A.L. De Langhe was Chairman. The following members of the In Vitro Committee attended: Drs. Roca, Scowcroft, Williams and Withers and in addition the membership of the sub-committee included Mr. J.B. Allen, Dr. J.M. Noiret and Dr. G. Staritsky. The names and addresses of participants are shown in Appendix I.

3. The Agenda of the meeting, as adopted, is shown in Appendix II.

4. Participants were welcomed by Professor De Lange and Dr. Williams who outlined the wish of the Board to maintain the leadership role in the scientific discussion of the applicability of in vitro techniques to genetic conservation and the wish to see carefully planned international programmes on the tropical crops of global priority. These had, in essence, already been agreed by the relevant IBPGR Working Groups but major practical constraints hinder their development, but least the methodology of collecting. The specific mandate of the sub-committee was to advise on how collecting techniques can be improved and to identify areas where research and development could lead to acceleration and improvement of work in the tropics and sub-tropics.

5. The sub-committee agreed that discussions in the past had been overly concerned with the need for genetic stability. It is now widely recognized that the eukaryotic genome has a tremendous amount of dynamic flux, that this phenomenon is not confined to the in vitro situation, and that stresses induce mechanisms which release or reveal variability. Some genotypes are more static than others. These relatively new ideas emerging from population genetic studies have not yet become absorbed in the thinking of crop evolutionists. More work is needed: the in vitro workers need more points of reference (vide the first report of the In Vitro Committee [AGP:IBPGR/82/84] and the need for the proper biochemical characterization of cultures) and more information is needed on instability of clones in field conditions.

6. There was a consensus that breeders dealing with clonal materials will, sooner or later, require the parts of the genepools represented by wild species. The IBPGR should be aware of a possible long-term harmful result if too much emphasis during the collecting of clonal crops is laid on cultivars. Long-term planning of field work through ecogeographical survey is supported. Too many collecting missions have spent too short a time in the field and samples have been collected without a clear rationale. Practical problems in collecting contribute to difficulties in systematic, thorough exploration.

REPORT

7. A number of crops were discussed in detail and related presentations were made by participants (see Agenda). The following points and conclusions emerged:

8. (i) Cassava. At present, Centro Internacional de Agricultura Tropical (CIAT), jointly with IBPGR, is gathering together clones of Manihot esculenta from Latin American countries. Materials are collected as stakes, taken to selected national programmes, meristem cultured and thereafter transferred to the World Collection at CIAT. There are now over 3,000 clones, of which over 1,500 are maintained as in vitro cultures. The collection of wild species is rather slow for several reasons, not least being erratic seed production if any, and the success of regeneration from stakes. The work, especially from remote locations, could be greatly accelerated if in vitro collecting techniques could be applied. However, the applicability of the culture media normally used for the cultigens to the wild species requires urgent attention. In view of quarantine needs, plants cannot be moved easily from country to country. In vitro methods have already assisted here and should be utilized further.

9. (ii) Sweet potato. The global programme is still in its infancy. There should not be over-emphasis on the building up of extremely large collections of cultivars (due to outcrossing) and emphasis should be put immediately on wild species. From the point of view of collecting, there is a need to develop in vitro techniques. In some species which do not tuberize, vines have to be collected and from the quarantine point of view in vitro techniques would be most useful.

10. (iii) Aroids. In view of current practise, the loss of material in field genebanks shows the urgent need is the applicability of in vitro techniques for diverse genotypes to maintain collections. There is less need for in vitro collecting techniques except for aerial material; corms and cormels survive well but contamination affects the continued well-being of plants established in collections. In vitro techniques should be used for maintenance.

11. (iv) Yam. Not enough information is available at present on the response of all of the different species to in vitro maintenance. Hence, collecting should follow the traditional methods until there is a wider scientific base to examine.

12. (v) Banana and Plantain. Material of wild species remains uncollected in Southeast Asia. African plantains are being collected and plans are being made to collect the high altitude cultivars of East Africa. Risks of devastation by various diseases require that screening for resistance, particularly in all varieties for local food consumption, be carried out. There is a need to coordinate and strengthen collecting for and maintenance in current field genebanks including Davao, Ekona and Jamaica. In vitro techniques are recognized as providing a means of fast multiplication of genotypes although genetic stability must be monitored. Associated methods for pathogen eradication and disease indexing should also be developed. A world collection housing ca. 1,000 clones would meet genetic diversity requirements including synthetic diploids of value in breeding for disease resistance. Techniques for in vitro storage are under development. Collecting is currently carried out by digging up and transplanting suckers. These propagules are of a size which makes simple collection in vitro problematic. It is anticipated that the most practical approach will be to

transfer material in an early stage to in vitro culture in a laboratory in the country of collection. Additional areas for attention in the future include a study of seed germination and sterility, and embryo culture. The potential of cell culture techniques in breeding is recognized.

13. (vi) Coconut. Cloning is of immense importance to propagate good planting materials, especially F1 hybrids. Transportation of pollen is already in use to facilitate breeding between parents in geographically distant locations. Conservation of pollen by cryopreservation is strongly recommended for attention. The development of in vitro methods for cloning via culture of somatic tissues is progressing. The process of proliferation appears to be by somatic embryogenesis and various tissues may be used as inocula. A programme for cryopreservation of somatic embryos of oilpalm is underway in France under the direction of CNRS. Its greatest utility is envisaged in conservation of clones of breeding material. Experience with oilpalm should aid development of similar methodology for coconut. Several institutes in Southeast Asia and South Asia are tackling collecting and conservation in field genebanks, but this work should be expanded if it is to meet international conservation requirements. Collecting is conducted by harvesting nuts (ca. 100 per population) for germination in nursery plantations. The main practical problem is the bulk of the collected material. Thus there may be potential for excising zygotic embryos at a location in the same country. Attention to the development of in vitro methods for storage of zygotic embryos is required. For future collecting missions, it is strongly recommended that surveying first be carried out. (Attention is drawn to paragraph 5).

14. (vii) Cacao. Current breeding material is based on a large number of accessions (>2,500 seedlings) derived from a very small number of wild trees (ca. 50) collected some 40 years ago. The basis of collection was strictly acquisition of material resistant to witches' broom, and therefore inadequate in a general sense. Some of this material is still under-evaluated and under-used. In view of the clear potential for further useful, widely-based collecting and threats to virgin forest in the Amazon Basin, the London Cocoa Trade Amazon Project (LCTAP) was initiated to collect material in Ecuador, in collaboration with Instituto Nacional de Investigaciones Agropecurias (INIAP). Other collecting activity is underway but some areas remain under-explored, e.g. in Peru and Colombia (and outside the Amazon basin in Venezuela and the Guianas). Collected material consists of seeds and budsticks. Seeds are germinated at a local field station and established in nursery plantations. Patch grafting onto seedlings is carried out for budsticks. Despite high success rates in establishing the latter material at the field station, transfer to intermediate quarantine is problematic. Loss of material at this stage represents a serious waste of collecting effort. Facilities developing in Barbados may help to overcome this problem. Whilst field collecting is not technically difficult, practical constraints may limit the duration of expeditions and the quality of material collected. In vitro methods may overcome these problems and also offer alternative ways of transferring material to intermediate quarantine and onwards. Preliminary experimentation at the University of Nottingham, with support by the IBPGR, has shown that it should be possible to modify methods for in vitro culture. The modifications are such that material may potentially be collected and transferred directly to culture with the minimum of manipulation and avoiding use of complex equipment and culture media. Further development is required to accommodate ecological, seasonal and physiological variables. It is recommended that this work continue in parallel with experimental implementation at actual collecting sites. In vitro methods may in future be applicable to floral structures, immature embryos and vegetative tissues such as leaves, thus broadening the scope of the collector. This example may serve as a model for other in vitro collecting work.

RECOMMENDATIONS

Priority areas for technical development in the crops under discussion

15. The following is a summary of points taken from paragraphs 8-14:

(i) Cassava. In vitro methods should aid collecting especially from remote locations, and should be utilized further to aid distribution of germplasm. This will require improvement of culture methods for wild species.

(ii) Sweet potato. In vitro methods for collecting wild species should be developed. These would also help to overcome quarantine problems experienced in distributing germplasm.

(iii) Aroids. In vitro techniques have their greatest utility in maintaining collections.

(iv) Yam. No action should be taken until more work has been done on the general in vitro culture of yam.

(v) Banana and Plantain. In vitro methods for collecting should be developed. Other areas for urgent attention are the solution of problems associated with seed viability and the development of methods for pathogen eradication and disease indexing.

(vi) Coconut. Methods should be developed for cryopreserving pollen and in vitro collecting of zygotic embryos from nuts.

(vii) Cacao. Work on the development of in vitro methods for collecting should be continued and implementation at collecting sites attempted.

Collecting and conserving genetic diversity - General points

16. The sub-committee, after looking at the report of the first meeting of the committee as endorsed by the IBPGR, agreed that from the point of view of the IBPGR there is probably an additional category of genebank to be considered: an "evolutionary genebank" which would be maintaining materials in a continual state of genetic flux. It would conserve genes which have been collected and allow them to recombine and segregate and could be very important for perennial species.

17. The sub-committee wished to reiterate that collecting should aim to conserve all alleles which occur in a population with a frequency of >5%. These, in effect, constitute the useful variability, and if a gene allele is a very rare mutation, there is neither hope of nor necessity to conserve it. The aim of collecting is one copy of each common allele in the target population (and the common genes may be distributed in three ways: widespread, sporadic and localized in the populations). However, with vegetatively propagated species where mating systems are often mixed, the population structures not known and there are overlapping generations, there has not been any mathematical treatment developed for sampling. Nonetheless, the following are broad generalizations:

(i) Number of samples per site: for a spectrum of allelic relationships ranging from strong overdominance, i.e. balancing selection, to maintenance of alleles at low frequency by mutation and/or selection, a random sample of 50 plants (100 gametes) will contain at least one copy of each common allele with 95% certainty.

(ii) Number and distribution of sites: evidence indicates that patterns of genetic differentiation result from and are strongly correlated with environmental heterogeneity. Thus, pre-collecting surveys (in the field - see paragraph 5) and use of decoded satellite pictures incorporating data from remote sensing and aerial surveys are essential. The IBPGR is

urged strongly to address this for the tropical areas, and in particular for crops such as cacao, coconut, tropical fruits, root crops within the tropical rain forest belts, etc. Effective pre-collecting surveying, planning and data acquisition can only lead to better collecting. In relation to sampling (see above) this is most important because any allele which is common will ipso facto be collected. It is the sporadic and localized alleles which need capture and which will only be so captured by proper sampling for representative variability.

In vitro collecting techniques

18. The sub-committee noted that two broad approaches may be taken to in vitro collecting, depending upon the crop in question:

(i) Direct transfer to in vitro culture, with all of the necessary manipulations being carried out at the collecting site.

(ii) Transfer of material collected by conventional methods to in vitro culture at a laboratory in the same country.

The latter is less technically demanding and does not introduce any truly new methods, whereas the former requires a complete appraisal of the in vitro culture initiation process and at the same time offers the greatest potential for improving the efficiency of germplasm collecting.

19. In considering the development of in vitro collecting techniques, the following points should be addressed for each crop:

(i) The conventional collecting methods:

(a) Their practical limitations.

(b) Their biological limitations.

(ii) The status of general in vitro methods for the crop.

(iii) The relationship between these in vitro methods and conventional collecting methods.

(iv) The requirements for translating in vitro methods into workable collecting methods:

(a) Further development of inadequate in vitro culture methods.

(b) Extension of these methods to handle appropriate collected material.

(c) Technical modifications relevant to the collecting environment and subsequent handling of the collected material.

(d) Cooperation and training needed to implement in vitro collecting.

(e) Interactions of in vitro collecting and germplasm storage by conventional or in vitro methods.

20. Research attention should be directed at two main areas which must also represent the biological criteria of success for collecting in vitro:

(i) To minimize microbial contamination. This will involve:

(a) Technical aspects of environmental hygiene and handling of the collected material to exclude contaminants; and

(b) Treatment of the collected material with sterilants and the addition of antimicrobial compounds to the culture medium.

(ii) To maintain physiological condition and morphogenic capacity in the collected material.

It is acknowledged that the two main aspects are not entirely separable in technical terms in that there will be strong interactions between them, and that requirements will vary from crop to crop.

21. The sub-committee identified three main types of explant which are likely to be involved in collecting germplasm by in vitro methods:

(i) Zygotic embryos dissected from collected seeds.

(ii) Pre-existing buds (e.g. axillary buds on stems or storage organs).

(iii) Other somatic material (e.g. leaf, stem, root and similar tissues which will yield adventitious [de novo] buds/embryos in culture).

22. In view of the likely differences in culture requirements of the three types of explant, the sub-committee recommended that projects should be initiated involving a chosen crop or crops for each. These are listed below. The subjects for examination were selected with attention being given to the importance of including woody and non-woody material, taxonomic diversity and present availability of culture systems.

Explant	Crop
Zygotic embryo	Coconut
Pre-existing buds	Cassava Sweet Potato
Other somatic material	Cacao Potato

23. The above work is expected to cost ca. US$300,000 over a 24-month period. However, the sub-committee is confident that the results will provide a framework for making widely applicable technical recommendations. Further it is noted that the proposed research programme is exceptional in that it addresses major scientific initiatives within the context of an unusually high proportion of priority crops.

24. The sub-committee wished to record its strong interest in the future development and application of culture systems involving somatic material such as leaf discs capable of regeneration by de novo embryogenesis. Collecting such material could overcome seasonal effects in the availability of suitable germplasm, and the resultant cultures could be particularly suitable for conservation by cryopreservation and for rapid propagation of uniform material for agricultural production.

25. Finally, the sub-committee emphasized the great advantages which could accrue in relation to the acquisition, conservation, exchange (including passage through quarantine) and utilization of germplasm through adoption of the revolutionary approaches presented in this report.

APPENDIX I

PARTICIPANTS

Members of Sub-Committee

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APPENDIX II

Agenda

1. Chairman's welcome

2. Adoption of agenda

3. Discussion of specific crops (with presentation by persons indicated)

   (i) Cassava and Sweet Potato (W.M. Roca)
   (ii) Aroids and Yam (G. Staritsky)
   (iii) Banana and Plantain (E.A.L. De Langhe)
   (iv) Coconut (J.M. Noiret)
   (v) Cocoa (J.B. Allen, L.A. Withers)

5. Discussion of *in vitro* collecting methods

6. Discussion of Report and agreement on Recommendations