Background on the development and implementation of the coconut embryo *in vitro* culture project

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Introduction

The efficient use of coconut genetic resources has been hampered by difficulties in collecting and exchanging germplasm. Within the plant kingdom, coconut is the species with the largest seeds. Moreover, it has no dormancy period and germination immediately follows maturation of the seeds. These characteristics drastically limit the amount of material that can be gathered during collecting missions. Germplasm exchange is even made more difficult due to the high risk of introducing pests and diseases as well as the high cost of transporting whole nuts.

With coconut, *in vitro* techniques have been used by various groups to address these problems (Engelmann 1998). Simple and efficient *in vitro* protocols have been developed for collecting coconut germplasm in the field, which involve extracting the embryos from the nuts and inoculating them *in vitro*. *In vitro* techniques have also been used in some instances for the international exchange of coconut germplasm in the form of excised embryos inoculated *in vitro*. Preliminary experiments have also shown that medium-term conservation of coconut embryos is feasible *in vitro*, as well as their long-term conservation through cryopreservation (using liquid nitrogen at −196°C).

Using *in vitro* techniques for collecting, exchanging and conserving coconut germplasm requires efficient protocols for *in vitro* germination of embryos, development of embryos into whole plantlets, acclimatization to *in vivo* conditions and further development into seedlings which can be outplanted in the field. This becomes especially significant with the establishment of the multi-site International Coconut Genebank or ICG (Rao and Batugal 1998) and the implementation of the various international breeding and testing programmes coordinated by the International Coconut Genetic Resources Network (COGENT), which rely heavily on *in vitro* techniques for collecting and exchanging germplasm.

The coconut embryo *in vitro* culture project

In vitro culture protocols have been developed by various coconut research institutes worldwide (Batugal and Engelmann 1998). However, a survey conducted by the International Plant Genetic Resources Institute (IPGRI) in 1996 revealed the relatively low overall efficiency of existing protocols (Engelmann 1998). It was therefore decided to organize a workshop to assess more precisely the status of coconut *in vitro* culture and to upgrade and standardize the protocols, so that coconut researchers could use them with better efficiencies (Batugal 1998). The first International Workshop on Coconut Embryo Culture held at PCA in Albay, Philippines on October 1997 documented and précised the information generated from the initial survey. Results of the first workshop revealed large discrepancies between the performances of the existing *in vitro* culture protocols, with only 14 to 55% of inoculated embryos developing

into plantlets *in vivo*. These differences were also strongly accentuated by the experience of the research teams utilizing these protocols. An important discovery from the analysis of the existing protocols was that the main bottleneck was not the acclimatization of plantlets, as initially thought, but the low efficiency of embryo germination and plantlet development *in vitro*. Findings also showed that these protocols differed greatly in the culture conditions, the composition and sequence of media employed, the developmental stage selected for weaning of plantlets and the number and type of coconut varieties tested, which were limited.

Based on these findings, two main objectives were identified for the UK Department for International Development (DFID)-funded research project established during the first workshop:

- Improve the maturation and germination rates of embryos and their development into whole plantlets; and
- Determine and select the most efficient *in vitro* culture protocol and test it with a large number of varieties.

The research activities implemented by the project participants focused on the following:

1. Improvement of maturation and germination rate of embryos by determining the effects of growth regulators (ABA, GA_3), osmoticums (PEG, mannitol) and amino acids (proline).

2. Improvement of plantlet development.
   - 2.1 Anatomical and physiological studies of plantlets
     - Histology of root and leaf development
     - Functionality assessed by measuring root absorption capacity and leaf transpiration, as well as photosynthetic capacity (photosynthetic rate)
   - 2.2 Study of atmospheric composition in culture vessels
     - Changes in CO_2, O_2 and ethylene in culture vessels in relation with plantlet development, monitored and modified if necessary
   - 2.3 Effect of CO_2 enrichment on development and acclimatization of plantlets
     - Effect of CO_2 enrichment at the end of *in vitro* culture period or immediately after transfer of plantlets *in vivo*
   - 2.4 Effect of photoperiod on germination of embryos
     - Effect of different lighting regimes on the germination of embryos
   - 2.5 Effect of rooting substrates
     - Efficiency of rooting substrates (e.g. vermiculite, coconut fibre) compared with the standard protocols

3. Comparison of the efficiency of four selected protocols (CPCRI, India; PCA-ARC and UPLB, Philippines; and IRD-CIRAD, France) and experimentation with different varieties (MYD and RT as common control; additional varieties in the screening experiments selected by each participating institute).

4. Medium-term conservation of embryos using *in vitro* storage.

A rapid survey of the reports submitted by project participants at the time of completion of the first phase of the project, which are all published in these Proceedings, allow some preliminary observations of the results achieved. *In vitro* culture of coconut embryos and production of plantlets are now operational in all participating laboratories. Success rates have also significantly improved, with 31 to 80% of inoculated embryos developing into plantlets *in vivo*. A large diversity of coconut

germplasm has been employed since the tissue culture protocols have been tested with over 20 varieties. These tests have revealed a very strong genotypic effect in response to *in vitro* culture. Although the overall performances of the *in vitro* culture technique have greatly improved, an optimal protocol has not been idenitified due to the high variability of the responses obtained in different laboratories.

Additional experiments have provided other important information. It has been demonstrated that GA_3 has a positive effect on the germination of embryos and on the further development of plantlets. The position of the embryo on the medium also seems to be extremely important as embryos placed with the plumule directed upwards displayed the highest germination percentages. Finally, the "hybrid protocol " proposed by one participating laboratory, which combines the most efficient steps of the four protocols tested, seems to hold good promises for further increasing the performances of coconut embryo *in vitro* culture.

Conclusion

During 2nd International Coconut *In Vitro* Culture Workshop held in Merida, Mexico, results obtained were extensively analyzed and discussed with the aim of identifying the most important topics to be further studied and refined in the planned second phase of the project. It is our hope that through the successful implementation of this global project, the upgraded and standardized embryo *in vitro* culture technology will rapidly make a significant impact on the establishment of the multi-site ICGs and on the development of breeding and testing programmes by facilitating the conservation and exchange of coconut germplasm.

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Table 1. Summary of results obtained in eight laboratories with *in vitro* culture of coconut embryos, before and after the implementation of the DFID/CFC-funded project.

Country/ Laboratory	Before project									After project								
	Initial number of embryos	Germinated *in vitro* (%)		Recovered *in vitro*		Recovered in nursery		Recovered after field transplanting		Initial number of embryos	Germinated *in vitro*		Recovered *in vitro*		Recovered in nursery		Recovered after field transplanting	
		No.	%	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	No.	%
Sri Lanka	2000	1140	57	920	46	740	37	629	31	561	389	69	254	45	158/ 300	53	na	na
Philippines (PCA)	2085	1161	56	770	37	770	37	770	37	4287	3258	76	2079	48	na	na	na	na
Vietnam na		na	na	na	na	na	na	na	na	na	450	373	83	309	69	na	na	na
Mexico	300	247	83	107	36	87	29	na	na	741	504	68	na	na	na	na	na	na
Cuba	200	147	74	59	29	0	0	0	0	240	134	56	48	20	na	na	na	na
Indonesia	54	na	na	8	15	na	na	na	na	847	625	74	324	38	159	49	na	na
India	1677	1239	74	na	na	na	na	215/ 409	52	960	835	87	605	63	521	54	na	na
Philippines (IPB)	na	na	na	na	na	na	na	na	na	256	150	59	49	19	22	9	na	na

Note: Figures indicated in the Table are based on all the data provided by participants in the Summary Tables included in their respective contributions.
na: Data not available at the time of publication.

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