PHILIPPINE-GERMAN PROJECT ON COCONUT TISSUE CULTURE - FIRST RESULTS[1]

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The Philippine-German Project on Coconut Tissue Culture is focusing on technology development and transfer of an asexual propagation system for coconut. Based on an Agreement on Technical Cooperation between the governments of the Republic of the Philippines and the Federal Republic of Germany, the project is jointly implemented by the Philippine Coconut Authority and the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH and was started in February 1989 at Albay Research Center (ARC), Banao, Guinobatan, Albay. In 1981 GTZ began funding a British team working on the cloning technique for coconuts under the leadership of Dr. J. Blake at Wye College, University of London, UK. Since 1989, this British team has worked in close cooperation with the tissue culture team at ARC to enhance the development of the cloning technique. In this paper first results obtained by the tissue culture team at ARC are reported. The culture of zygotic embryos is being further improved to facilitate germplasm exchange and disease screening. Embryo rescue of the high-value Makapuno coconut aims at developing a nontraditional coconut industry, especially for small coconut farmers. For clonal propagation immature embryos, leaves, and inflorescences are used.

INTRODUCTION

Conventional propagation of coconut palm is at present carried out entirely by the use of seednuts. This results in a high degree of variation of the yield in the plantations. Therefore, vegetative propagation by means of tissue culture techniques is highly desirable allowing significant yield increases by propagation of high-yielding individuals. In the same way individuals presenting resistance to certain diseases and tolerance to adverse growing conditions could be propagated. Moreover, clonal propagation would mean a more rapid availability of breeding results to the farmers.

CULTURE OF ZYGOTIC EMBRYOS

The culture of zygotic embryos is being further intensified with the objective of optimizing the factors for successful growth and the subsequent transfer to *ex vitro* conditions. Rillo and Paloma (1990) reported that coconut embryos germinate and grow best on Eeuwens' formulation (Y3) with or without 0.25% activated charcoal. This protocol has reduced to almost half the duration of culture *in vitro* using White's medium (de Guzman and del Rosario 1964). Assy Bah (1986) reported the use of Murashige and Skoog macro salts and Morel and Wetmore vitamins. Two milligrams per liter naphthylacetic acid was added to induce root development of the seedlings. Recently, Ashburner (1991) evaluated the effect of solid and liquid phase of the basal medium for coconut embryo culture. A 5-wk duration in the initial liquid phase resulted in the greatest shoot and root development. The results obtained with zygotic embryos could prove useful for the successful establishment of clonal plantlets.

Germplasm Exchange and Screening for Disease Resistance

Embryo culture of coconut can circumvent the problems of storage and transport caused by the weight and bulk of the nuts and the absence of dormancy. This is especially important if international germplasm exchange is envisioned for coconut improvement in breeding programs or for disease studies. Rillo and Paloma (1991) have developed a technique that is now routinely used for collecting large numbers of embryos for tissue culture purposes. The aptness of this technique for international exchange of coconut germplasm has been proven.

A technique to inoculate grown coconut embryos with the *cadang-cadang* viroid has been developed as an aid to screening coconut varieties against this disease (Rillo and Paloma 1989).

Embryo Rescue

Embryo rescue of Makapuno is of special economic importance, as this high-value coconut mutant cannot germinate naturally due to the abnormal condition of the endosperm in the nut. *In vitro* culture of Makapuno

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[1] Paper presented in the Second International Symposium on Coconut Research and Development held at the Central Plantation Crops Reserach Institute, Kasaragod, Kerala, India on Nov. 26-29, 1991.

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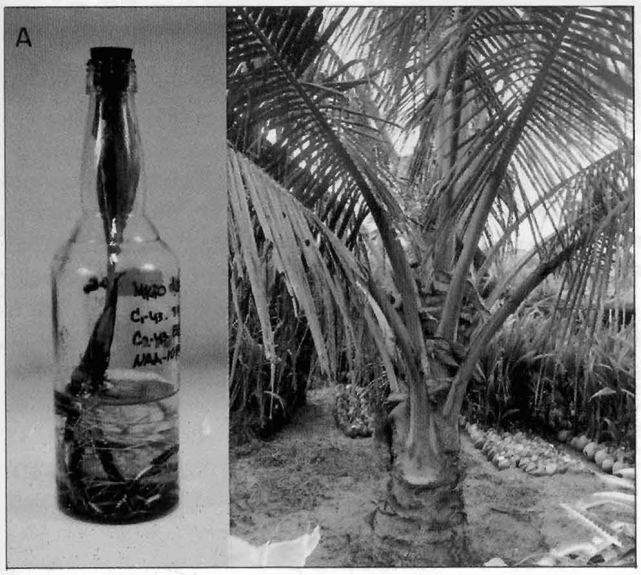

FIGURE 1: a. Makapuno plantlet ready for transfer to *ex vitro* conditions; b. Four-year-old flowering Makapuno palm grown *in vitro*.

(Fig. 1), developed by de Guzman in the early 1960s, has been greatly improved (Rillo and Paloma 1989) allowing an economically feasible commercialization of the technology.

There are now 150 *in vitro* grown Makapuno palms planted at ARC for future research regarding the field performance of these elite palms. In the near future Makapuno seedlings could be made available to coconut farmers at an affordable price. Since the Philippines is recognized to have the comparative advantage on the embryo culture technology and processing of Makapuno coconuts, it is envisioned that Makapuno farming could be developed as a nontraditional coconut industry. This is more imperative especially now that coconut production areas are getting smaller due to fast urban development and increased coconut timber utilization.

TYPES OF EXPLANTS UNDER INVESTIGATION FOR CLONAL PROPAGATION

Immature Embryos

The production of callus on embryo tissue could yield plants through somatic embryogenesis. Karunaratne and Periyapperuma (1989) obtained embryogenic callus tissues from the culture of 6- to 7-mo-old embryos. Part of these callus tissues produced globular embryos which germinated and produced shoots. However, regeneration of plantlets has not yet been achieved.

Immature embryos excised from about 7-mo-old 'Catigan' nuts are cultured on Blake's medium (Ebert and Taylor 1990) supplemented with high 2,4-dichlorophenoxyacetic acid (2,4-D) levels. Good callus formation is being obtained. Further refinement of the media composition is under way to optimize the production of embryogenic callus.

Immature Leaves

Leaf explants comprise fragments of juvenile, non-chlorophyllous leaves, still surrounded by the petiole bases of the older leaves.

Two groups reported successful regeneration of coconut palm from leaf explants. Raju et al. (1984) suggested that a callus stage may not be necessary for regeneration in coconut, but that direct embryogenesis in cultured leaf tissue could lead to the formation of clonal plantlets. Direct embryogenesis from leaf explants, but without successful regeneration, has also been reported by Gupta et al. (1984). In contrast, Verdeil et al. (1989) obtained their first clonal plantlet from leaf explants after undergoing callogenesis and embryogenesis.

At ARC, experiments for calloid initiation are under way using leaf tissues from *in vitro* maintained plantlets at the 2-leaf stage and from greenhouse maintained seedlings at the stage of five expanded leaves. The basic medium, as well as the levels of the added growth regulators, still needs to be refined.

Immature Inflorenscences

The nondestructive collection of immature inflorescences has been successfully demonstrated (Rillo 1989). After collection from the donor palms the inflorescences with outer spathe lengths of 70-150 mm are dissected under aseptic conditions and the central part of the rachillae cut transversely into 1-2 mm thick segments (Fig. 2). For calloid induction the establishment of the explants on a medium with high 2,4-D levels and activated charcoal has proved successful. Continued subculturing on the same medium results in the proliferation of nodular calloid (Fig. 3b). Changing the balance of the growth regulators, especially lowering the 2,4-D concentration in the medium, is associated with embryoid production and germination (Fig. 3b, 4a). As early as 6 mo after initial culture, shoot development from embryoids can occur (Fig. 4b).

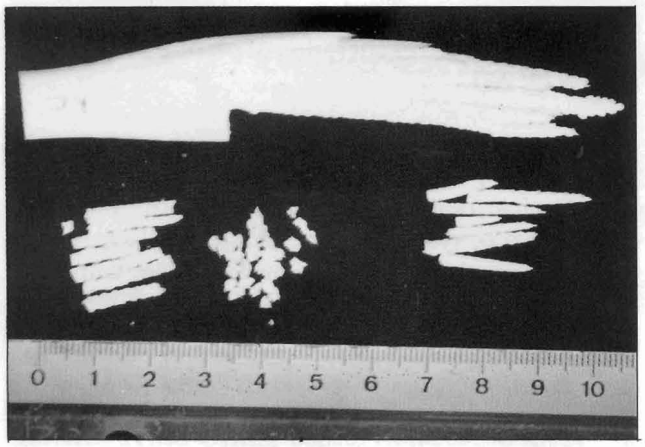

FIGURE 2: Rachillae from immature inflorescence cut transversely into 1-2 mm thick segments.

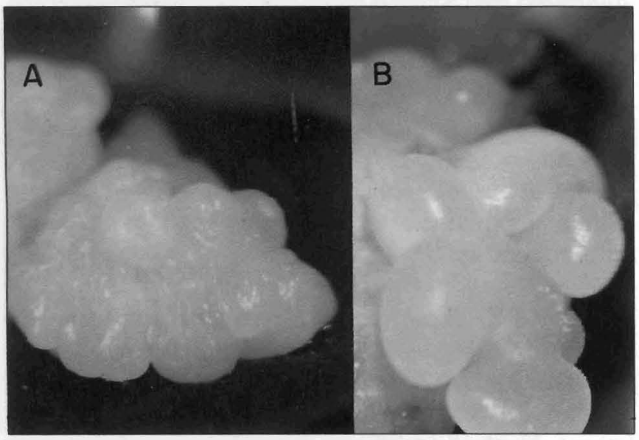

FIGURE 3: a. Proliferating nodular calloids; b. Detail of embryoids, approx. 4 months after initial culture.

FIGURE 4: a. Embryoid formation and germination, approx. 5 months after initial culture; b. A cluster of germinating embryoids, approx. 7 months after initial culture.

SUMMARY AND CONCLUSION

Encouraging results have been obtained during the first phase of the Philippine-German Project on Coconut Tissue Culture which started in February 1989. The technique for embryo rescue of Makapuno has been greatly improved allowing higher success rate in *ex vitro* transplanting and distribution of seedlings to the farmers in the near future. In the field of clonal propagation of coconut good calloid formation is routinely obtained with immature embryos and inflorescence explants. With inflorescence tissues, the obtained calloids develop further into embryoids upon lowering of the 2,4-D level. Shoot development, as well as root formation from embryoids, has recently been obtained. Successful regeneration of coconut can be expected in the near future.

ACKNOWLEDGMENT

The authors gratefully acknowledge the impressive contribution of Mrs. Ingrid Ebert to the Project.

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