In vitro culture of embryos of areca nut (*Areca catechu* L)

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ABSTRACT

INTRODUCTION. In order to attempt to shorten the time for developing planting material of *Areca catechu*, usually issued from seed germination, in vitro culture of embryos was tried successfully. This work is the first demonstration of in vitro culture and plantlet development from embryo cultures of areca nut. **MATERIALS AND METHODS**. Mature zygotic embryos excised from green fruits of areca nut were cultured on a medium comprising of Knop's salt solution and Murashige and Skoog's minor elements, iron, vitamins, activated charcoal and NAA (1 mg/l) with different concentrations of sucrose (0.5–10.0%). **RESULTS AND DISCUSSION**. Sucrose (6%) in the presence of NAA and charcoal enhanced the embryo germination and almost 95% of the embryos developed into quality plantlets. This efficient embryo culture technique offers an easy and safe material for exchange and transportation of valuable germplasm, satisfying most phytosanitary requirements for germplasm transfer. Additionally, embryo culture can be used to study nutrition and metabolism of the embryos at various stages of development, effects of various phytohormones and environmental conditions on zygotic embryogenesis.

KEYWORDS
Areca catechu, in vitro culture, plant propagation.

Culture in vitro d'embryons de noix de bétel (*Areca catechu* L).

RÉSUMÉ

INTRODUCTION. Pour tenter de raccourcir le temps d'obtention du matériel de plantation utilisé pour la mise en place d'arbres d'*Areca catechu*, habituellement issus de la germination de graines, la technique de culture in vitro d'embryons a été testée avec succès. Ce travail est le premier à rapporter de telles expérimentations permettant d'observer le développement de plantules à partir de la culture d'embryons de noix de cola. **MATÉRIEL ET MÉTHODES**. Des embryons matures, d'origine zygotique, excisés de fruits verts de noix de cola, ont été cultivés sur un milieu de base constitué d'une solution contenant les sels de Knop, les oligoéléments de Murashige et Skoog, du fer, des vitamines, du charbon actif et de l'acide naphtalène acétique (ANA : 1 mg/l) ; en outre, différentes concentrations de saccharose allant de 0,5 à 10,0 % ont été testées. **RÉSULTATS ET DISCUSSION**. L'addition dans le milieu de base de saccharose à la concentration de 6 %, mis en présence d'ANA et de charbon actif, a amélioré la germination des embryons dont presque 95 % ont donné des plantules de qualité. Cette technique efficace de culture d'embryons permet de produire facilement du matériel sûr pour l'échange et le transfert de germplasm sélectionné qui satisfait alors à la plupart des exigences phytosanitaires requises pour de telles actions. De plus, la culture d'embryons peut être utilisée pour étudier la nutrition et le métabolisme des embryons à différents stades de développement, ainsi que les effets de diverses phytohormones et des conditions de l'environnement sur l'embryogenèse zygotique.

MOTS CLÉS
Areca catechu, culture in vitro, multiplication des plantes.

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introduction

Areca catechu (Palmaceae) is cultivated for the production of betelnut which is consumed as a masticatory nut in the tropics. Areca nut wrapped in betel leaf with cardamom, clove, saffron, nutmeg, mace and tobacco and lime acts as a stimulant. The seed in powdered form is used as a vermifuge for dogs and goats (RAO, 1994). The nut is also used in dyeing and tanning. Areca nut trees grow in a wide range of tropical and subtropical soils, although moist lowlands and moist valleys are ideally suited for its cultivation. It is propagated by seed which takes about 3–4 months for germination after harvesting and 8–10 month–old seedlings are transplanted in the field.

Embryo culture is generally used for producing successfully plants from in vitro cultured embryos in cases of weak embryos, obtaining haploid plants and shortening the breeding cycle (SHARMA et al, 1996). Considerable work has been done in the embryo culture of coconut, date palm and oil palm (de GUZMANN and Del ROSARIO, 1964; RABECHAULT et al, 1970; NWANKO and KRIKORIAN, 1983; ZAID and TISSERAT, 1984; RAO and GANAPATHI, 1993). MOURA and CARNEIRO (1992) reported the culture of immature embryos of *Howea forsteriana* Becc, an indoor ornamental palm, mainly to shorten the time required to develop the plant. In areca nut, much time is needed for developing transplantable seedlings from seeds and, hence, there is a need to shorten this time for developing planting material. Here is the first report of successful development of plantlets from cultured excised zygotic embryos.

materials and methods

Green fruits of *Areca catechu* L were collected from areca nut gardens in January, 1995. Entire seeds were surface–sterilized in 0.1% $HgCl_2$ for 5 min and then rinsed three times in sterile distilled water. Embryos (4 mm in length) were excised aseptically using a scalpel to break open the endosperm. As the fruits were still green, the endosperm was not too tough and could easily be dissected with the scalpel. The embryos were excised carefully without injury and cultured on a nutrient medium which consisted of Knop's salt solution [250 mg/l KNO_3, 1 000 mg/l Ca $(NO_3)_2$, 250 mg/l $MgSO_4$ $7H_2O$, 250 mg/l KH_2PO_4] supplemented with MURASHIGE and SKOOG's (1962) minor elements, iron and vitamins. The medium was supplemeneted with different levels of sucrose (0.5–10% w/v) and activated charcoal (0.1% w/v) and 1 mg/l naphthalene acetic acid (NAA). The pH was adjusted to 5.8 before adding agar (0.8% w/v). The embryo cultures were maintained under 16 h light period at a temperature of 25 ± 2 °C. For each treatment, 24 embryos were cultured.

results and discussion

The excised embryos cultured on Knop's salt solution with MS minor, iron, vitamins supplemented with 1 mg/l NAA, 0.1% activated charcoal and 2% sucrose turned green and began to enlarge within 4 weeks. On the contrary, embryos cultured on medium with sucrose, but without any hormones and activated charcoal, did not enlarge and develop. Germination of embryos started with initial swelling and slight elongation. Then the haustorium enlarged considerably, which was lighter in colour than the rest of the embryo. The basal part of the embryo with the coleoptile and the radicle, initially cream coloured, turned green and increased in size followed by the elongation of the coleoptile. The first root appeared after 6–8 weeks. Germination of embryos to form transplantable plantlets took nearly 6 months (figure 1). In order to minimize the time required for the better development of plantlet, embryos were cultured on medium supplemented with various levels of sucrose (figure 2).

Low levels (0.5, 1.0%) of sucrose showed slow shoot growth (2–4 cm in length) with scanty root development. On the other hand, 3% sucrose resulted in balanced development of root (3 roots/plantlet) and shoot (6 cm). The embryos germinated rapidly and formed seedling-quality plants on media

supplemented with 6% sucrose. On this media, the plantlets obtained were deep green in colour with a good shoot (9 cm) and root system (3-4 roots/plantlet). Transplantable plantlets could be obtained within 5 months time. High levels of sucrose (10%) retarded the development of root and shoot formation (figure 2).

The growth response of embryos of *Areca catechu* was dependent on the percentage of sucrose in the nutrient medium (figure 2), and the presence of charcoal and NAA was found to be essential. In coconut embryo cultures, the response of shoot and root development was highly dependent on both the concentration of sucrose and NAA and period and timing of exposure to NAA (ASHBURNER et al, 1993). In the present study, higher levels of sucrose had a negative effect on shoot elongation. Such a response and an interaction with NAA have also been reported earlier in coconut (de GUZMANN et al, 1971; del ROSARIO and de GUZMAN 1976; KARUNARATNE et al, 1985); however, our results indicate that sucrose (6%) in the presence of NAA and charcoal enhanced the embryo germination and almost 95% of the embryos developed into plantlets.

As compared to seed nuts, embryo culture in areca nut offers an easier and safer material for exchange and transportation of valuable germplasm, satisfying most phytosanitary requirements for germplasm transfer. Also, high yielding elite clones can be propagated using embryo culture within 6–8 months as compared to normal conventional seed propagation which requires 8–10 months. Additionally, embryo culture can be used as an excellent system to study nutrition and metabolism of the embryos at various stages of development, effects of various phytohormones and environmental conditions on zygotic embryogenesis (BRIDGEN, 1994).

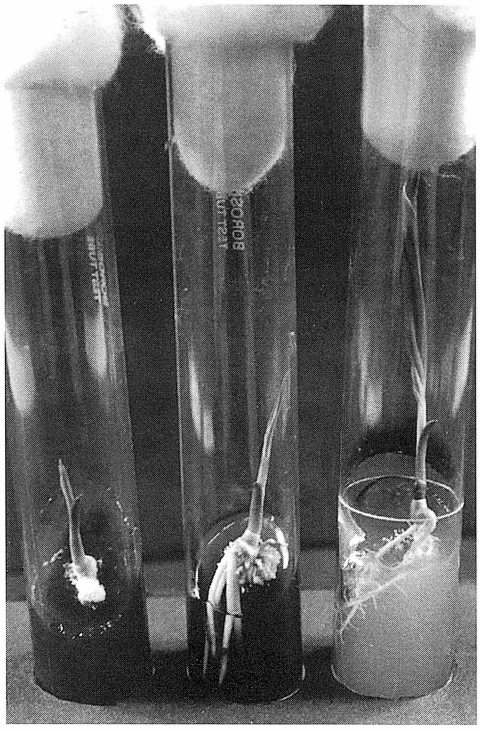

Figure 1
Stages during the development of embryo culture of areca nut on Knop's salts with MS minor, iron, vitamins and 1 mg/l NAA and 0.1% charcoal.

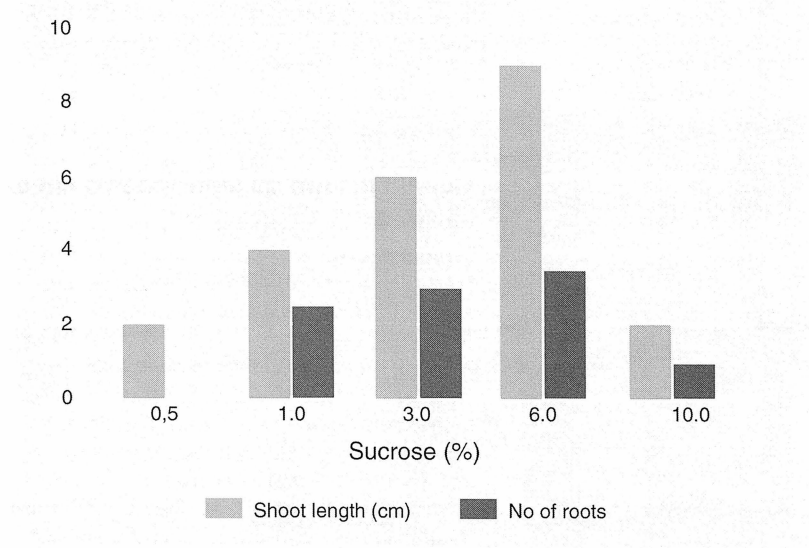

Figure 2
Effect of different levels of sucrose on embryo culture of areca nut (average of 24 embryos per treatment).

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Cultivo in vitro de embriones de nuez de *Areca catechu* L.

RESUMEN

INTRODUCCIÓN. Para intentar abreviar el tiempo de obtención del material de plantación utilizado para plantar los árboles de *Areca catechu*, que normalmente se producen a partir de la germinación de semillas, se ha probado con éxito la técnica de cultivo in vitro de embriones. Este trabajo es el primero que relata estos experimentos que permiten observar el desarrollo de plantones a partir del cultivo de embriones de nuez de cola. **MATERIAL Y MÉTODOS.** En frutos verdes de nuez de cola, se tomaron mediante escisión unos embriones maduros de origen zigótico que fueron cultivados en un medio base constituido por una solución que contenía las sales de Knop, los oligoelementos de Murashige y Skoog, hierro, vitaminas, carbón activo y ácido naftaleno acético (ANA : 1 mg/l) ; además, se han realizado pruebas con diferentes concentraciones de sacarosa que iban desde 0,5 hasta 10,0 %. **RESULTADOS Y DISCUSIÓN.** El añadido de sacarosa en el medio de base con una concentración del 6 %, y en presencia del ANA y del carbón activo, mejoró la germinación de los embriones ya que un 95 % de los mismos produjeron plantones de calidad. Esta eficaz técnica de cultivo de embriones permite producir fácilmente un material seguro para el intercambio y la transferencia de germoplasma seleccionado, que cumple con la mayor parte de las exigencias fitosanitarias requeridas para tales acciones. Ademas de esto, el cultivo de embriones puede utilizarse para estudiar la nutrición y el metabolismo de los embriones en diferentes periodos de desarrollo, así como los efectos de distintas fitohormonas y de las condiciones del medio ambiente en la embriogénesis zigótica.

PALABRAS CLAVES

Areca catechu, cultivo in vitro, propagación de plantas.