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Revealing the potential of elite coconut types through tissue culture

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Abstract

Low income, smallholder coconut farmers have been facing difficulties for decades due to the falling price of copra, their traditional cash product from coconut. These farmers are now looking towards new, higher value products from coconut to make their industry viable over the longer term. There are a number of elite coconut types that have either a soft, jelly-like endosperm or a flavoursome, aromatic drinking water. They have a high commercial value in the confectionary and ice cream industries (soft endosperm types) or the soft drink market (aromatic types). The soft endosperm types ('makapuno') are naturally occurring mutants which cannot germinate in nature as their endosperm cannot support the germination of the embryo. They are known from a number of countries and are called makapuno (the Philippines, Vietnam and Thailand), kopyor (Indonesia), garuk (Papua New Guinea) or dikiri pol (Sri Lanka), depending on their origin. For propagation, the embryo has to be removed from the fruit and grown in vitro to produce a seedling. The aromatic types are also thought to be naturally occurring mutants which can only germinate in nature at a very low rate. They are known from a number of countries including Thailand and Vietnam. For propagation, the same kind of embryo culture procedure has to be used.

The first attempt at the embryo culture of these mutant coconut types was by De Guzman in the Philippines in the early 1960s (for the makapuno coconut). Subsequent studies have led to the commercialisation of a technique so it is now possible to mass produce seedlings of the Filipino makapuno. Other countries are now attempting to use the same or modified embryo culture techniques to develop their own soft-endosperm or aromatic coconut production industries. The common problems these projects encounter are the low rate of conversion of the isolated embryos to plantlets and the duration of the protocol, which can be as long as 1 year. These issues ultimately lead to high production costs for the elite seedlings, well above that possible for subsistence farmers. However, a recent collaborative project funded by the Australian Centre for International Agricultural Research (ACIAR) has made some impressive improvements to the standard embryo culture protocol. These improvements include the use of a CO_2-enrichment step within a photoautotrophic culture system and the application of plant regulators NAA or IBA to promote seedling rooting. By using this protocol on the embryos of normal coconuts the in-vitro stage can be reduced from 10–12 months to 3–4 months and the success rate in transferring embryos to seedlings in the field can be improved from about 50% to 100%. The new improved protocol now needs to be refined and applied to the different mutant coconut types that are found around the world.

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Introduction

Despite the huge potential of coconut (*Cocos nucifera*) as a tropical crop, producers are suffering from low productivity and the low price for copra, the main traditional product from coconut. Most of the trees that are presently harvested are old specimens of unimproved, low-yielding varieties. In some countries financially unsound coconut palms are being replaced with other crops such as oil palm *(Elaeis guineensis*). Thus, there is a need to revitalise the coconut industry, particularly in the countries where coconut plays an important role in the local economy. In the rebuilding of this industry it is imperative that other, high-value products from coconuts be developed and used to replace the traditional copra-based industry. Fortunately there are several elite coconut types that produce high-value fruit. Such types have fruit with a tasty, jelly-like endosperm or a refreshing, flavoursome aromatic water.

The coconut types that have the jelly-like endosperm are widely known as the 'makapuno-types'. The name makapuno comes from the Filipino word 'makapuno' which means 'almost full'. Such coconut types were first identified in the Philippines more than 40 years ago and similar types are now known to exist in several other countries, for example kopyor (Indonesia), dikiri (Sri Lanka), garuk (Papua New Guinea) and makapuno (Thailand and Vietnam). Makapuno-type fruit are used in the production of flavoursome foods and can be priced up to 10 times higher than ordinary, hard kernel fruits. Their value can be even higher on special occasions like Christmas and Idul Fitri when they are purchased in larger numbers and given as gifts.

Aromatic-type coconuts have a distinctive flavoursome liquid endosperm (water) and solid kernel which can give a refreshing effect when consumed. This coconut type is popular in Thailand and recent interest has grown in Vietnam. The nuts are sold fresh in local as well as export markets and can fetch prices up to five times those for standard fruits.

The future potential for both makapuno- and aromatic-type coconuts, however, still needs to be developed and this is hampered by the lack of planting stock availability. The makapuno-type coconut cannot germinate in nature because of the inability of its soft endosperm to nourish its own embryo. While the aromatic-type coconut can germinate in nature, they do so at a very low rate. In addition, there is a belief in Vietnam that those nuts that are able to germinate have only a weak aromatic flavor. To produce planting stock of both elite types, an embryo culture technique is needed to rescue the embryos and take them to full seedlings using an in-vitro procedure.

The successful embryo culture of the makapuno-type coconut was first reported in the 1960s (De Guzman and Del Rosario 1964). Since then the technology has been transferred to a number of tissue culture laboratories in the Philippines, particularly at the Philippine Coconut Authority (PCA), and in Indonesia for the kopyor variety. The coconut embryo culture technique is also used for other purposes, such as germplasm collection, and therefore research on this technique has also been conducted in other countries including Papua New Guinea, Vietnam, France and Australia. This paper aims to highlight recent developments in embryo culture technology as well as discuss future applications of the technique to the large-scale production of elite coconut seedlings.

The potential and challenge of elite coconut types

Despite the high commercial value of the elite coconut types (makapuno and aromatic), their potential has not yet been fully realised. There are only about 31,000 makapuno-bearing palms (out of 300 million coconut trees) in the Philippines (Rillo and Rillo 2001). The demand for their fruit is increasing as the makapuno food and drink industries grow. In 2003 the export of products from Filipino makapuno coconuts reached 1,200 million t, which was about 44% more than that exported in the previous year (Rillo 2005). A survey in the Philippines in 1999 revealed that the gap in supply to meet demand was about 4 million kg/year of makapuno endosperm, equivalent to about 8 million makapuno fruit (Rillo 2005) or at least 120,000 trees.

In Indonesia there has been a growing interest in the local makapuno-type coconut, the kopyor. To date there has been no comprehensive study undertaken on the number of kopyor palms present in Indonesia or what the local demand for this fruit is. Nevertheless, in 2004 a total of 378 ha (equivalent to 47,000 trees) of kopyor were reported to be growing in Pati district, East Java (Syariefa 2005), and some kopyor production had been reported from the Lampung and Madura districts for many years. The

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present Indonesian production of kopyor fruit is for the local market or is sent to the cities of Jakarta or Surabaya for fresh consumption. In the cities the endosperm is also used for ice cream manufacture.

In Thailand about 2,000 makapuno trees are known to have been planted in the 1970s from embryos imported from the Philippines (Sarian 2001). A recent survey in Vietnam has shown the existence of 237 makapuno- and 70 aromatic-type coconut palms in smallholder fields near Ho Chi Ming City (Lien 2005); however, large scale production is not possible from so few trees. In other countries such as Sri Lanka and Papua New Guinea there have been no reports of the large-scale planting of their makapuno- or aromatic-types.

As mentioned above, makapuno-type coconut fruit are popular for the manufacture of various foods including ice cream, cakes, pastries and syrup. Other potential markets are within the pharmaceutical and microchip industries as the makapuno-type fruit is rich in galactumanan, a form of cellulose that is used in the creation of materials used in encapsulation procedures.

Since the embryos from the makapuno-type fruit cannot germinate in situ*,* farmers cannot produce new palms. The embryos, however, can be rescued through tissue culture and nurtured to produce seedlings. The planting of these seedlings in close proximity (and away from normal palms to reduce pollen contamination) will give rise to palms producing a high proportion of makapuno fruit. A success rate of up to 95% has been achieved in the Philippines (Rillo 2005).

Mass propagation of makapuno seedlings is yet to be achieved, although some tissue culture laboratories have started producing seedlings for commercial purposes in the Philippines and discussions are underway to do something similar in Vietnam.

The current process of embryo culture for makapuno- and aromatic-type coconuts is long and tedious, making the production costs for seedlings very high. However, recent research funded by ACIAR has made some significant improvements to this protocol, which should now be applied to the commercial production of makapuno.

An embryo culture technique for elite coconut types

Coconut embryo culture was first attempted in the early 1960s at the University of the Philippines, Los Banos (UPLB). This early work was able to successfully culture makapuno embryos and grow them into mature palms at a low success rate (De Guzman and Del Rosario 1964). From this early start, the Philippines has now developed an industry based on the tissue culture of makapuno coconut. In addition to a number of private and university laboratories, six PCA laboratories are now providing tissue-cultured plantlets to farmers (Rillo 2005). Other countries are also showing an interest in this procedure, including Vietnam, Indonesia and Thailand. For the makapuno industry to establish elsewhere, a more widely applicable embryo culture protocol is needed. With the help of ACIAR, a recent project may have developed such a technique.

Today there are a number of methods described in the literature for undertaking coconut embryo culture. However, in most cases, these protocols have been developed for local coconut types and have little applicability to other kinds grown elsewhere. The need for a uniform, highly efficient embryo culture protocol is indicated by the low rates of success achieved when using these methods in other areas of the world. Rates of success can be as low as zero (Ashburner et al. 1994) but can reach 80% (Engelmann and Batugal 2002) in the hands of an expert. A common problem encountered by many is the low rate of conversion of plantlets established under in-vitro compared to ex-vitro conditions. This transfer step is always going to be difficult as most seedlings grown in vitro have a poorly developed root system and a low photosynthetic capacity (Triques et al. 1998), making establishment in soil very difficult. In addition, these seedlings are highly susceptible to infection when first transferred to soil and take a very long time to develop further, with the whole process of embryo culture taking up to 12 months to complete.

Thus, there is a need to develop a more widely applicable and reliable embryo culture technique that can be applied to a wide range of germplasm types and used by inexperienced technicians. Such a technique would have great applicability for commercial production of the makapuno- and aromatic-type seedlings as well as for germplasm collection. Through a collaborative research program linking several countries, Coconut Genetic Resources Network (COGENT) has investigated various forms of the embryo culture technique (Batugal 1998; Engelmann and Batugal 2002), and have proposed a new 'hybrid embryo culture' protocol (Engelmann and Batugal 2002; Rillo et al. 2002) which is being

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used as a platform onto which further improvements are being proposed.

At the present time major improvements to this protocol are being considered, some of which were identified and discussed at the most recent review of the ACIAR funded coconut project held in Manado, Indonesia, 10–14 October 2005. This new information includes the following observations.

At the Cocoa and Coconut Institute (CCI) it has been found that mature embryos (aged 10–11 months after fertilisation) are the best ones to be used for embryo culture. However, younger embryos can also be used if mature ones cannot be found or nuts cannot be aged correctly, for example, when collecting germplasm in remote areas. In terms of culturing costs, about 20% savings can be made if an embryo-culling step is included to remove unresponsive embryos 6 weeks after the start of the culture period.

An attempt to improve germination in cultured embryos was undertaken at UPLB. However, the application of spermidine, silver thiosulphate or polyethylene glycol, to the culture medium did not increase the germination percentage. In another experiment it was found that the collective acclimatisation method applied to new ex-vitro seedlings, using a wooden box covered with a transparent plastic sheet, produced better quality seedlings than was possible using the older plastic bag method of the hybrid protocol.

At the Indonesian Coconut and Other Palmae Research Institute (ICOPRI) coconut seedlings treated with auxins (plant growth regulators, either NAA or IBA) grew better in vitro. Their ex-vitro survival rate was also much greater than was achieved with the hybrid protocol method, which does not use auxins. The amount of auxin needed was determined by the method of application. For plantlets that were dipped once into the auxin, 200 mM NAA was needed, while prolonged application needed only 100 mM NAA. The benefit of adding NAA and/or IBA to the culture media is thought to be the promotion of root initiation. At the Oil Plant Institute of Vietnam (OPI) good in-vitro and ex-vitro growth were possible when using 0.5 mg/L of the auxin IBA. When combined with 0.5 mg/L NAA, not only was the embryo germination percentage increased but ex-vitro survival was also improved.

At Albay Research Centre (PCA), it was found that seedling growth could be improved and the rate of seedling establishment increased by reducing the medium carbohydrate load (from 45 to 25 g/L) at the last seedling subculture step. Coconut dust and fibre has been shown to be a superior potting mixture when compared to soil and sand for growing seedlings ex vitro. It has been also shown that 3-month-old seedlings previously grown in vitro can survive at higher rates ex vitro when maintained with a Hoagland mineral solution. This shortens the in-vitro stage and therefore reduces the cost of seedling production.

Work undertaken at the University of Queensland (UQ), Australia, has shown that enriching the gaseous environment around the in-vitro culture with CO_2 produced much more vigorous seedlings than using the hybrid embryo protocol. This vigour could be achieved at a much lower sucrose level (5 g/L compared to 45 g/L in the hybrid embryo protocol) or without sucrose at all (Samosir and Adkins 2005a). This approach has significantly improved the success rate of transferring the in-vitro plantlets to ex-vitro conditions (about 50–100%), as well as shortening the time in vitro, from the normal 12 months down to 4 months.

Another important improvement coming out of the work at UQ involves an embryo transplantation step. If this improvement were to be fully incorporated into the protocol, the need to undertake any aspect of in-vitro culture would be bypassed. In this improvement embryos are aseptically removed from the donor nut and transferred to their proposed destination. At the recipient laboratory a surrogate fruit is prepared to receive the isolated embryo or the endosperm plug carrying the embryo. The surrogate nut, with the transplanted embryo, is then allowed to germinate naturally under protected environmental conditions. The first batch of embryo-transplanted coconut seedlings using this technique has now been established in soil at UQ (Samosir and Adkins 2005b).

Commercial production of elite coconut seedlings

So far it has taken about 4 decades to transfer the technology of coconut embryo culture from the research laboratory to commercial production of makapuno seedlings. Currently, the Philippines is the leading country in the production of the makapuno-type seedlings. To date there are 11 tissue culture laboratories (six PCA-based, two private, two within the Department of Agriculture and one university-based) working on the production of makapuno seedlings using embryo culture. Each PCA laboratory has at least a 1 ha demonstration plot used to produce

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embryos for their work. The price of a single seedling (300–1000 pesos), however, is still too expensive for most farmers. Nevertheless, nearly 100 ha have been planted with makapuno-type coconuts obtained from in-vitro culture.

Some tissue culture laboratories have been developing embryo culture of kopyor coconut in Indonesia, including Indonesian Coconut and Other Palmae Research Institute (ICOPRI), University of Gajahmada and the Indonesian Biotechnology Research Institute for Estate Crops (IBRIEC). But only the latter laboratory is producing seedlings for commercial purposes and this is still at a low level. The ready-to-field plant seedlings are sold at US$10 each. A new tissue culture laboratory at Jombang town has been established with technical assistance from ICOPRI.

There has been no production of seedlings of elite coconut types for commercial purposes in Thailand, Sri Lanka, Vietnam and Papua New Guinea. Nevertheless, OPI Vietnam has been able to produce seedlings of aromatic-type coconuts for planting of about 4.5 ha, which could later act as the source of embryos for mass production of seedlings.

Future work direction

It has been shown that the embryo culture technique can reveal the potential of elite coconut types, particularly makapuno which cannot germinate in nature. However, the demand for embryo-cultured seedlings of these elite types is increasing. The problem is that they are too expensive and not widely available to farmers. Thus, there is a need to lower the cost of seedling production and speed up the production rate. The new protocol, developed during the ACIAR funded project, still requires further development, particularly in its modification for elite types and how it can be scaled up for more production.

The number of embryos available for culturing is limited, and often it is difficult to meet the needs of the nearby laboratory. In the future the program should include the establishment of seed gardens of elite coconut types near the tissue culture laboratory to act as the main source of embryos. In addition, the garden may also be a home for the germplasm collection of elite coconut types. To date very little work has been done in the selection and breeding of such types. There is a potential to combine the two traits to form 'aromatic–makapuno' nuts.

Despite the high demand for both aromatic and makapuno coconuts and the availability of the embryo culture technique in the public domain (not patented), private industry has not shown a great interest in exploiting the potential of elite coconuts so far. The bad image of coconut oil, although wrongly claimed, in advanced countries may have contributed to this slow uptake. In addition, the decreasing coconut industry in general may also have caused the private sector to 'wait-and-see' on the makapuno and aromatic coconut business. Respective governments therefore should provide incentives and develop programs to enhance the elite coconut industry in their countries.

References

Ashburner, G.R., Faure, M.G., Franz, P.R., Tomlinson, D.R., Pulo, P., Burch, J.M. and Thompson, W.K. 1994. Coconut embryo culture for remote locations. In 'Coconut improvement in the South Pacific: proceedings of a workshop', 10–12 November 1993, Taveuni, Fiji. ACIAR: Canberra.

Batugal, P. 1998. COGENT and 1997 activities; workshop objectives and expected outputs. Pp. 1–5 in 'Coconut embryo in vitro culture, papers presented at a workshop on embryo culture', ed. by P. Batugal and F. Engelmann, 27–31 October 1997, Banao, Guinobatan, Albay, Philippines. IPGRI-APO: Serdang, Malaysia.

De Guzman, E.V. and Del Rosario, A.G. 1964. The growth and development of *Cocos nucifera* 'Makapuno' embryos in vitro. Philippine Agriculture 48, 83–94.

Engelmann, F. and Batugal, P. 2002. Background on the development and implementation of the coconut embryo in vitro culture project. Pp. 1–4 in 'Coconut embryo in vitro culture: Part II', ed. by F. Engelman, P. Batugal and J. Oliver. IPGRI-APO: Serdang, Malaysia.

Lien, V.T.M. 2005. A survey of production and consumption of elite coconut varieties in Vietnam. Oil Plant Institute: Ho Chi Minh City.

Rillo, E.P. 2005. The successful mass production of the Makapuno coconut by embryo culture in the Philippines. Pp. 16–30 in 'Proceedings of the International Conference of the Coconut Research Institute of Sri Lanka – Part 2 (Contributed Papers)', ed. by T.S.G. Peiris and C.S. Ranasinghe. The Coconut Research Institute of Sri Lanka: Luniwila.

Rillo, E.P., Cueto, C.A., Medes, W.R. and Areza-Ubaldo, M.B. 2002. Development of an improved embryo culture protocol for coconut in the Philippines. Pp. 41–66 in 'Coconut embryo in vitro culture: Part II, ed. by F. Engelmann, P. Batugal and J. Oliver. IPGRI-APO: Serdang, Malaysia.

From: Adkins, S.W., Foale, M. and Samosir, Y.M.S. (eds) 2006. Coconut revival—new possibilities for the 'tree of life'. Proceedings of the International Coconut Forum held in Cairns, Australia, 22–24 November 2005. ACIAR Proceedings No. 125.

Rillo, A.R. and Rillo, E.P. 2001. Survey of Makapuno population. Philippine Journal of Coconut Studies 26, 1–26.

Samosir, Y.M.S. and Adkins, S.W. 2005a. Coconut embryo culture: CO_2-enrichment and root aeration for improved seedling establishment. Pp. 92–102 in 'Proceedings of the International Conference of the Coconut Research Institute of Sri Lanka – Part 2 (Contributed Papers)', ed. by T.S.G Peiris and C.S. Ranasinghe. The Coconut Research Institute of Sri Lanka: Luniwila.

Samosir, Y.M.S. and Adkins, S.W. 2005b. Embryo transplantation and *ex-vitro* germination for germplasm exchange and the production of high value, endosperm mutant coconuts. Pp. 77–91 in 'Proceedings of the International Conference of the Coconut Research Institute of Sri Lanka – Part 2 (Contributed Papers)', ed. by T.S.G. Peiris and C.S. Ranasinghe. The Coconut Research Institute of Sri Lanka: Luniwila.

Sarian, Z.B. 2001. Thailand report: Visits to Makapuno Island, gardens, nurseries and markets. Agriculture Monthly Magazine, September.

Syariefa, E. 2005. Bisnis kopyor 100%. Trubus 432, 35, 11–15.

Triques, K., Rival, A., Beule, T., Morcillo, F., Hocher, V., Verdeil, J.L., Hamon, S. and Drew, R.A. 1998. Changes in photosynthetic parameters during in vitro growth and subsequent acclimatization of coconut (*Cocos nucifera*) zygotic embryos. Acta Horticulturae 461, 275–282.