Accepted Manuscript

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PII: S0981-9428(16)30423-5

DOI: 10.1016/j.plaphy.2016.11.003

Reference: PLAPHY 4716

To appear in: *Plant Physiology and Biochemistry*

Received Date: 18 April 2016

Revised Date: 1 November 2016

Accepted Date: 3 November 2016

Please cite this article as: Q.T. Nguyen, H.D.D. Bandupriya, M. Foale, S.W. Adkins, Biology, propagation and utilization of elite coconut varieties (makapuno and aromatics), *Plant Physiology et Biochemistry* (2016), doi: 10.1016/j.plaphy.2016.11.003.

Review

Biology, propagation and utilization of elite coconut varieties (makapuno and aromatics)

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Abstract

Coconut farming is not only a vital agricultural industry for all tropical countries possessing humid coasts and lowlands, but is also a robust income provider for millions of smallholder farmers worldwide. However, due to its longevity, the security of production of this crop suffers significantly from episodes of natural disasters, including cyclone and tsunami, devastating pest and disease outbreaks, while also affected by price competition for the principal products, especially the oil. In order to reduce these pressures, high-value coconut varieties (makapuno and aromatics) have been introduced in some regions, on a limited scale, but with positive outcomes. Even though these two varieties produce fruit with delicious solid or flavoursome liquid endosperm, their distinct biochemical and cellular features unfortunately prevent their *in situ* germination. In fact, embryo rescue and culture have been developed historically to nurture the embryo under *in vitro* conditions, enabling effective propagation. In an attempt to provide a comprehensive review featuring these elite coconut varieties, this paper firstly introduces their food values and nutritional qualities, and then discusses the present knowledge of their biology and genetics. Further possibilities for coconut in general are also highlighted, through the use of advanced tissue culture techniques and efficient seedling management for sustainable production of these highly distinct and commercially attractive varieties of coconut.

Keywords: Aromatic · Biology · Coconut · Genetics · Makapuno· Micropropagation

Contents

1. Introduction

Being one of the most important palm crops, coconut (*Cocos nucifera* L.) provides direct food and vital revenue for millions of farmers across tropical and subtropical regions of the world. The palm is well-known for the diversity of its products, particularly within the realms of food, drink, structural material and energy supply. In fact, coconut oil remains the dominant commercial product from the global production base of 12 Mha, including 3 Mt traded around the world annually [1]. There is a wide range of industrial and edible products derived from the oil, as well as its desiccated kernel, coconut water, nectar, sugar, charcoal, fibre and coco peat produced in many locations where there has been investment in processing technology. However, producers are facing a number of agro-economic challenges including the declining productivity of ageing palms – as the main stimulus to undertake plantings was many decades ago. Also there is the falling price of the traditional products, and intense price competition from highly productive and mechanisable crops such as oil palm (*Elaeis guineensis* Jacq.) [2]. In addition, the field cultivation of coconut in some regions has been seriously threatened by a wide variety of biotic and abiotic factors, including lethal diseases and pests, and natural calamities [3]. The major era of establishment of coconut plantations for export of copra and oil was the period of its very high price, between the years 1900 and 1930. Due to the decline of the price paid for oil and copra from the 1960s until the present It is reported that at least one half of the 12 Mha of plantations needs regeneration through replanting [4, 5]. According to a recent report from the Asia and Pacific Coconut Community, ideally around 700 million palms should be replanted each year in the next two decades [5].

The coconut palm is also potentially vulnerable to acute insect and microbial bio-hazards specific to diverse locations world-wide, such as the Lethal Yellowing phytoplasma, *Phytophthora* bud-rot fungus and the *Brontispa* leaf beetle [6]. The capacity provided by cloning technology, to multiply material from resistant or tolerant populations, would provide a means to achieve protection [3]. Hence, there is an urgent need to foster alternative coconut products with higher economic value, which can help sustain the viability of the smallholder and the entire coconut industry. In this context, elite coconut types with unique attractive endosperm properties are potential candidates. There are two widely acknowledged varieties: those with a tasty jelly-like endosperm and those with flavoursome aromatic water [2]. Attention in this review will be focussed on these two valuable elite mutant forms of coconut fruit, because these are particularly valuable at the farm gate, thereby offering the farmer a boost to income wherever there is demand for these forms. Success achieved in cloning these elite genotypes would contribute directly to multiplication of main-stream genotypes from which all the products mentioned above can be derived.

The variety with fruit containing a jelly-like endosperm is known as ‘makapuno’ (or macapuno), a word derived from a Filipino term meaning ‘tends to fullness’ [7]. This variety was first identified in the Philippines and communicated by Gonzalez [8] who described the fruit as one where the cavity becomes completely or partially filled with white, gelatinous endosperm. It was also found that not every fruit from a makapuno-bearing palm had these attributes [9]. Interestingly, there are many similar varieties found in other countries with different local names: Dua Sap (Vietnam); Dikiri Pol (Sri Lanka); Kopyor (Indonesia); Maphrao Kathi (Thailand); Dahi Nariyel (Myanmar); Thairu Thengai (India); Dong Kathy (Cambodia); and Niu Garuk (Papua New Guinea) (Fig. 1A). The delicious makapuno endosperm can be consumed directly, or used to make a wide array of foods, or as a flavouring agent in ice-cream and pastries [10].

The second elite variety, that has refreshing fragrant, flavoursome water, is commonly known as the ‘aromatic’ coconut. This kind of fruit is highly regarded in many coconut growing countries, particularly in the Southeast Asia region, where it is consumed as a fresh drink. Despite the growing demand for aromatic coconut, supplies from the existing palm populations are somewhat limited. The cultivation of both varieties of palm is hindered by their inability

to naturally germinate. This is believed to be due to natural mutation in the formation of the endosperm, in which both biochemical and physical properties fail to nurture the embryo through the early stages of germination [11]. A combination of a short fruit storage life and a wide geographic dispersal of the unique parent palms has created a situation whereby these two elite coconut varieties have become highly valuable [12]. For example, an aromatic fruit in Thailand is worth roughly double the price of a normal fruit, and the makapuno in the Philippines is priced up to 10 times higher than an ordinary coconut [2].

In the past, much effort has been put into understanding the cause of low-germinability in the elite coconut varieties. The early cytological studies showed that an abnormally high frequency of small, polyploid cells were found in the makapuno endosperm [13]. The multiplication of these highly disorganized, unsystematically shaped microcells by irregular cytokinesis [14] led to the uncontrolled proliferation of the endosperm tissue. The resulting endosperm tissue was therefore unable to nurture the development of the zygotic embryo haustorium during germination. In the early 1980s, biochemical research provided further insight into the distinctive properties of the makapuno endosperm. Deficiencies of the enzymes α-D-galactosidase and β-mannosidase were discovered, and found to be associated with a unique accumulation of galactomannan. This was thought to prevent the mobilisation of other reserved polysaccharides, thereby preventing germination [15, 16]. In addition, higher activity of β-mannanase was found in the makapuno endosperm than in normal endosperm, which led to the galactomannan being less viscous [17]. It is believed that the changes in enzyme balance noted in the makapuno endosperm [15, 16] may have resulted from misregulation of genes encoding for those enzymes [11]. Recently, molecular approaches have been undertaken to determine the differences in the genetic make-up of the endosperm in normal and makapuno varieties [18].

To a lesser extent, the aromatic variety has also been researched, but only for the last 20 years. The work has focused on maintaining and improving the flavour of this variety's liquid endosperm. In order to reduce changes in quality during post-harvest containment, appropriate packaging of fruit, together with pasteurization and cold storage, have all been studied in detail [19]. Gentle burning of the dehusked fruit, which helps to trigger the production of additional volatiles in the liquid endosperm, has also been used to enhance water flavour [20]. In Thailand, the production of aromatic fruit has already played a major economic role in stimulating the coconut industry in that country [21]. Attempts to identify the genes involved in the aromatic trait, using DNA-based markers, are underway [22, 23], and hopefully will lead to an understanding of this unique characteristic in the near future.

As the zygotic embryo from either of the two elite coconut varieties is unlikely to germinate in nature, a conventional farming approach for establishing these varieties is not effective, and the slow dilution of the elite fruit trait, through cross-pollination, results in the loss of its production. In order to achieve the highest frequency of elite fruit production through pollination from similar palms, the planting of these varieties needs to be undertaken in isolated compact stands. There has been a considerable research effort put into the development of techniques to rescue and nurture the elite embryos using *in vitro* techniques. Through embryo rescue and culture, elite coconut embryos can now be routinely saved, preserved in aseptic culture, and subsequently manipulated in order to produce viable plantlets. Fifty years have passed since the first attempt to undertake *in vitro* culture of makapuno embryos [24], and since then a succession of improvements has been made to the *in vitro* culture technique [2]. To a lesser extent, effort has also been made to boost seedling growth using improved nursery techniques [25, 26]. However, mass planting of these elite varieties on a large commercial scale is still to be achieved.

This review aims to highlight the efforts that have been made to understand the unique biochemical and cytological properties, and genetic make-up of these two elite varieties, makapuno and aromatic. A detailed summary is also

presented about how tissue culture and related biotechnological approaches have been used in the past to propagate these highly distinctive and commercially attractive coconut varieties.

2. Uses and nutritional values

Elite coconuts (makapuno and aromatic-varieties) have been in great demand not only because of their rarity but also for their palatability and dietary value for the consumer (Fig. 1F). The deliciousness of the jelly-like makapuno endosperm can be enjoyed, either when consumed fresh or when processed into a wide range of attractive products [7]. The makapuno endosperm has attracted attention as the feed-stock for a number of food products, due to its 'buttery' texture, together with a mild nutty taste and pleasant aroma [10, 27]. In the Philippines, the jelly is a popular ingredient in ice-cream, as well as in syrups, jams and other delicacies. Unsurprisingly, the availability of these products in distant markets is relatively low due to the limited fruit supply, the short fruit storage life and inadequate homemade processing facilities [7]. In order to maintain quality, harvested fruit should be wrapped in oxygen transmission-rated bags and then kept at 5°C during shipping [12]. This approach can help extend the shelf-life of the fresh makapuno endosperm up to about 6 weeks, enabling a standard international delivery. In fact, this approach of wrapping and cooling has been widely used to conserve normal coconut fruits\ for shipping to long-distance markets such as Europe and the USA.

The makapuno endosperm has higher protein and moisture contents than normal fruit [28]. The major amino acids are glutamic acid, arginine and aspartic acid [10] while in normal coconut lysine is more abundant. In contrast, it has been shown that the lipid content of the makapuno endosperm is much lower than in normal endosperm, even though the fatty acid profiles are alike. This special attribute makes makapuno a favoured choice in the food processing industry, being less susceptible to rancidity [12]. It has also been shown that the attractive taste is due to a moderately high content of simple sugars (such as sucrose, glucose and fructose), as well as to high levels of citric and malic acids. It also has a higher content of nutritionally beneficial minerals, especially potassium [10].

The aromatic endosperm provides a refreshing drink, with a pleasant taste and fragrance, due to the presence of natural volatile components, particularly δ-lactones [19]. A wide range of δ-lactones, primarily saturated δ-octalactone and δ-decalactone, are known to contribute to the fragrance and flavour of normal coconut endosperm [29, 30] and may be expected to be part of the fragrance of aromatic coconuts. The aromatic strength and sweetness of the aromatic liquid endosperm have been shown to increase as the fruit matures [31]. According to a recent study, the main volatile compounds found in both the liquid and solid endosperm of the aromatic coconut are 2-methyl-1-butanol acetate, nonane, and butylated hydroxytoluene [32]. However, the fragrance diminishes significantly only a few days after the fruit is harvested, and especially after dehusking. Similar to makapuno, appropriate packaging is essential in the transport of aromatic coconuts. Jangchud, Puchakawimol and Jangchud [19] have shown that the vacuum-packing method can help to double the storage life of burnt coconuts as compared to unwrapped ones. This approach also reduces microorganism contamination and water quality loss. Recently, efforts have been made to provide a practical technique for opening of the aromatic fruit for easier consumption. In this approach the shell is partially cracked by boiling after creating groove lines using a laser engraver [33].

Overall, the popularity of elite coconut varieties is guaranteed, as they have a wide-ranging nutritional value and attractive taste that is building a market that makes them highly valuable for large-scale production. Undoubtedly the growers who invest in the planting of an elite coconut variety could achieve a significantly increased income, but building up supply will depend upon the availability of healthy seedlings [2, 34].

3. Makapuno

3.1. Botanical descriptions

The first published botanical description of makapuno-bearing palms by Zuniga [35] was based on fruit taken from a Laguna Tall palm found in Laguna Province, in the Philippines. This coconut variety had a white viscous and jelly-like endosperm with little or no water, while the firmness of the inner endosperm was much reduced as compared to the outer layer, which was firmly attached to the hard shell. On the other hand, apart from the extraordinary characteristic of the makapuno endosperm, there were no other marked morphological differences in the palms (including crown, trunk, root, inflorescence, fruit size, shape and colour) as compared to normal ones [36]. Likewise, weight and volume of the dehusked makapuno nuts were indistinguishable from normal [27]. Shaking or tapping the fruit has become the only way at harvest time to distinguish makapuno from normal fruit, and this can actually be done while the fruit is still on the palm [2, 8, 37]. Each makapuno-bearing palm can produce approximately 6.8 to 12.2 bunches of fruit per year, in the Bicol region of the Philippines, with fruit set ranging from *c.* 15 to 49% of the female flowers produced, with makapuno fruit incidence in these palms ranging from *c.* 3 to 18% [38].

3.2. Non-germinability of makapuno fruit

The makapuno embryo appears to be morphologically normal, but fails to germinate due to biochemical and physical features of the extraordinary endosperm that is unable to support its germination. A study by Zuniga [35] indicated that, from the same spadix, the makapuno embryo was generally larger than the normal embryo and kept developing within the fruit without germinating, as long as the endosperm remained intact. The non-germinability of the makapuno embryo was believed due to the failure of the haustorium to develop, thereby losing the connection between the viable embryo and abnormal endosperm [13]. The consequent absence of energy-converting enzymes, which are normally used to break down and mobilise reserve lipids and polysaccharides during the germination process [16], explains the failure. The lack of the α-D-galactosidase enzyme, leading to the reduced breakdown of galactomannans, was shown to be the outcome of misregulation of genetic transcription [11, 39].

3.3. Cytology and biochemistry of the makapuno endosperm

The makapuno embryo was found to contain the diploid chromosome number (2n=32), and the majority (78.4%) of the endosperm cells showed nuclei with the expected triploid chromosome number (3n = 48). These cells were of regular size during the early stages of development [37]; however, there was a notable increase in the frequency of abnormalities (e.g. cells of 6n, 9n, 12n, 24n, and 48n) with increasing endosperm maturity [40]. The production of numerous polyploid cells, and the increasing incidence of abnormalities in the older makapuno endosperm, prevented further cell division, but promoted an altered mechanism of cell division - the so-called ‘amitosis’ [13, 40]. It was found in these studies that the makapuno endosperm, with a high frequency of polyploid cells (54.2%) showed the lowest oil concentration (58.5%). In contrast, the Laccadive Tall variety, with only 34.9% of polyploid cells, produced the highest oil concentration (72.2%). In fact, the inverse relationship between oil concentration and ploidy level was commonly observed in many dwarf varieties [40]. Significant differences between makapuno and normal coconut have been observed in the make-up of the water-soluble and alkali-extractable endosperm fractions [41]. In this study the analysis of the hydrolysis products from the two fruit types showed a consistent difference in the kind and amount of sugars present. The study also indicated that the amount of pectin and hemicellulose in the endosperm cell walls of the makapuno fruit were higher than in the normal coconut fruit.

In another study, it was discovered that there were two major cell types found in the makapuno endosperm [14]. Other than the normal cells, with a regular shape, there were microcells which were smaller and had a highly disorganized shape. These microcells were found to have originated from the irregular cell division of normal cells, or from amitosis as mentioned above. By using a differential staining procedure, Sebastian, Mujer and Mendoza [42] showed that the makapuno microcells possessed a loose cell wall structure, had indistinguishable cell borders, irregular size, and were often elongated, which markedly reduced their intercellular adhesion, leading to the unique texture of the endosperm. This microcellular state was proven to be unique to makapuno endosperm, as it was irreproducible in carrot [*Daucus carota* (L.) Crantz] callus tissues cultured on a medium supplemented with makapuno endosperm extract [43]. The over-proliferation of microcells directly contributed to the viscous component, and the high concentration of galactomannans [15]. The viscous component of the endosperm was shown to contain a high level of polysaccharides (14.4% - mainly galactomannans) as compared to normal fruit (7.5%). The viscosity could be reduced when makapuno endosperm was mixed with a crude extract made from mature, normal endosperm, suggesting that the absence or inactivity of factors found in the makapuno endosperm could be overcome. [44]. This study also showed that, when compared to normal fruit endosperm, peroxidases in makapuno endosperm had a higher activity during the early stages of development and a lower activity in the maturing stages. In this study, the changes in peroxidase activity increased the biosynthesis of indole-3-acetic acid which possibly triggered the over-proliferation of microcells in the makapuno endosperm (Fig. 2). More importantly, the acute deficiency of α-D-galactosidase (8,300-fold lower as compared with normal fruit endosperm), and the decreased activity of β-mannosidase in makapuno endosperm, might inhibit the degradation and mobilisation of certain reserve polysaccharides. Loss of this energy source would then lead to the failure of embryo germination [17, 45].

3.4. Genetics of the makapuno trait

It has been suggested that expression of the makapuno endosperm trait is due to a triploid, homozygous single recessive gene (mmm) trait, with the makapuno-bearing palm being heterozygous (Mm) with respect to this trait [9]. Zuniga [35] observed that reciprocal crosses between makapuno-bearing (Mm) and non-bearing (MM) palms produced all normal fruit, reinforcing the recessive gene hypothesis. Theoretically, the occurrence of a homozygous triploid (mmm) should be 1:8 (12.5%), given an equal yield of this gene in pollen and in ovary cells. In a further study by Zuniga [36] it was reported that self-pollination of makapuno-bearing palms led to fruit being produced in the ratio of 1:3.8 (21%), makapuno to normal ratio, while that of inter-crosses between makapuno-bearing palm gave 1:4.7 (17%). In fact, the yield of ma kapuno fruit varied drastically (from 2 to 17%) with uncontrolled pollination [38]. These observations, however, differed from the expected outcome from a single recessive gene. It is also worth noting that the percentage of fruit set ranged from 54 to 69% in all trials involving controlled pollination. It appears that expression of the makapuno trait may not be regulated by a single gene, but combined with one or more genetic alterations. It has been recently proposed that alterations in the transcription of genes encoding for the enzymes involved in galactomannan metabolism might create such a behaviour [11].

Over the past few years molecular analyses have been employed to understand better the mechanism(s) underpinning the formation of the makapuno endosperm. The characterization of a coconut β-mannanase encoding gene, which participates in the metabolism of galactomannan, has been achieved by Nguyen, Diaz and Nguyen [46]. In this study, eight genes involved in the galactomannan biosynthesis were successfully cloned and sequenced. They had a high similarity to their counterparts in rice (*Oryza sativa* L.). However, further investigation may now be needed to look at the expression pathway of these genes, as their functions may still be different. In another attempt to identify the

genes responsible for the formation of the makapuno endosperm, de la Cruz et al. [18] isolated a gene encoding for the protein actin, which could then be used as an internal reference marker to aid the evaluation of gene expression in normal and makapuno endosperm tissue at 5 to 8 months of fruit age. In addition, candidate genes involved in regulation of glycolysis, galactomannan degradation, alcoholic fermentation, fatty acid biosynthesis, cytokinin biosynthesis, polyamine synthesis and cell cycle regulation, have all been identified [47]. These genes showed a relatively high similarity (68 to 98%) to corresponding genes from other species. In this study, no difference was found among the 13 tested cDNAs and their associated protein sequences. The issue of gene control of makapuno endosperm production therefore has yet to be resolved. Further studies are required.

Interestingly, a number of coconut mutations similar to makapuno have also been found in countries other than the Philippines [10, 25]. Even though these mutations have been found to have slight differences in taste, and the occupation of the fruit cavity by jelly-like endosperm is similar, direct comparison has yet to be done. Noticeably, a makapuno-like variety (the Kopyor) has recently been found in a Dwarf variety, found around Surakarta (also known as Solo) in Indonesia. This is highly unusual because so far makapuno-bearing palms have been reported solely in Tall varieties. These observations therefore require further investigation, in order to achieve a better understanding of the genetic mechanisms associated with the makapuno endosperm trait.

4. Aromatics

4.1. General background and genetic diversity

The aromatic coconut variety has recently emerged as a highly profitable commodity in a number of coconut producing countries, because of its unique appeal as a refreshing drink. These countries include Vietnam (Dua Dua); Malaysia (Pandan Najikeram); China; the Philippines; and Thailand (Maphrao Num Hom) where it has become an increasingly important export [48]. This variety has been strategically promoted as a new commodity by the agricultural authorities of Vietnam [25], Malaysia [49] and China [50] [25, 49, 50]. Research has attempted to improve the propagation efficiency of aromatic coconut through genetic selection (e.g. introduction of ‘Wenye 4’ cultivar in Hainan, China) and tissue culture (e.g. embryo culture of ‘Dua Dua’ cultivar in Mekong delta, Vietnam). Interestingly, it is only the weakly aromatic coconut fruit that can germinate (at a reduced rate), while strongly aromatic fruit are unable to germinate in nature [2]. Hence, there is still a need for an improved propagation method, as well as for a method to enhance fruit storage life, in order to cope with the growing demand from distant markets for this attractive fruit.

In an attempt to investigate the genetic diversity of the aromatic coconut varieties, research has been undertaken in a range of Dwarf accessions, including the ‘Aromatic Green Dwarf’ [22]. This study revealed a genetic dissimilarity between the aromatic green Dwarf and other accessions tested, based on hierarchical cluster analysis. It was also shown that the genetic variation within the aromatic varieties was relatively low, with a gene diversity index of 0.067, while that of other accessions was at least three times higher. However, the research was conducted solely on Philippines Dwarfs and might not hold true for aromatic varieties in other regions. It is also important to identify genes involved in this distinctive trait, as well as learning how they function. Further research should therefore be focused on assessing the genetic relationship between and within aromatic varieties, using genome-wide markers, thereby enabling breeding for unique fragrance and flavour to be achieved.

4.2. Quality improvement

The developmental stage at which the aromatic fruit is harvested is an extremely important factor regulating the fragrance and flavour of the liquid endosperm. It has been determined that the liquid endosperm of an 8-month-old

aromatic fruit, which has reached a maximum sugar content (7.2%), and a very low level of acidity, together with an intense fragrance, is the most suitable time for harvest if the fruit are to be used for fresh consumption [31]. However, the pH of the aromatic water and endosperm starts to decrease dramatically in just a few weeks after harvest, leading to an unsatisfactory reduction in fruit quality [19]. In order to extend the life of these desirable characteristics of fragrance and flavour, fruit can be gently roasted in a wood fire, so to generate an internal temperature of *c.* 70 °C within the fruit (monitored by an inside thermocouple) for 40 minutes. Such a treatment helps stop: the decline in endosperm pH; the development of sourness; the loss of transparency of the liquid endosperm; and the loss of total soluble solids; thus retaining fragrance and flavour [20]. In addition, the fragrance can be intensified by burning, since the volatile profiles of the liquid endosperm were found to be enhanced after burning [51]. Apart from increasing the level of pre-existing aromatic compounds (2-methyl-1-butanol acetate, nonane, and butylated hydroxytoluene), gas chromatography-mass spectrometry analyses revealed that new volatiles, including hexatriacontane, 2, 4, 6-trimethyl-decane and 2, 4-bis (1,1-dimethylethyl) phenol, were produced in both liquid and solid endosperm of the burnt fruit [32]. Further investigation should now be undertaken to determine the exact chemicals that are linked to fragrance and flavour, enabling identification of biochemical pathways involved in the formation of this special kind of fruit.

4.3. Utilisation

Storage life of the aromatic coconut, once burnt, is often less than 2 weeks, which strongly limits market access. It is therefore essential to provide alternative protection in order to extend the shelf-life of the fruit. It has been demonstrated that the burnt fruit, when vacuum-packed in a nylon-polyethylene double-layer bag, maintained a satisfactory quality for up to 28 days [19]. This double-layer bag method was superior to unwrapped and polyvinylchloride wrapped fruit in terms of avoiding bacterial contamination, preventing fruit weight decline, and keeping undesirable chemical changes to a minimum.

Pasteurisation of the whole dehusked fruit at 95 °C for 100 s is another approach that has been shown to suppress microbial growth during storage of the aromatic coconut [52]. It has also been shown that the stage of fruit maturity at the time of harvest is a critical factor affecting the quality of pasteurised fruit. The technique of pasteurisation, followed by low temperature storage (*c.* 4 to 5 °C), was shown to work best on fruit harvested 33 weeks after pollination. However, recent research has shown that thermal pasteurisation generates unwanted compounds, including aldehydes, which are perceivably unpleasant at low levels, and possibly become toxic if the treatment is prolonged [53]. Recent findings indicate that high-pressure CO_2 pasteurization reduces both the presence of aldehydes and the production of undesirable medium- and short-chain alcohols. This form of pasteurization has been shown to better maintain the existing array of volatile compounds when compared with the original product.

To provide an accurate identification of maturity of aromatic coconut fruit without opening them, image processing technology has been developed [54, 55]. This identification process is based on mathematical correlations between the fruit's morphology and the percentage of white pixels formed on the image. Three major categories of aromatic fruit have been identified, representing the thickness observed in the inner solid endosperm tissue (thin endosperm indicating an aromatic fruit), *viz.* (i) a single thin endosperm layer (ii) moderately thick endosperm layer or (iii) thick endosperm layer. The use of image processing technology is a field-applicable method and can be used to monitor the thickness of the solid endosperm at the time of harvest. The precision of the technique is significantly improved under natural lighting conditions. It was shown that the classifying power of this system could achieve an accuracy of at least 77% in identifying aromatic fruit [55].

5. Propagation of elite coconut varieties

5.1. Planting management

As the makapuno trait relates to the expression of a recessive gene or genes, out-crossing between makapuno fruit-bearing palms and normal ones should be avoided if the highest proportion of makapuno fruit is to be produced [12]. In fact, the few makapuno plantations that are in existence have been established in isolated regions, away from normal coconut plantations, to prevent cross-pollination with those normal palms. Tahardi [56] reported that makapuno fruit can be produced up to a rate of 92% from 8-year-old embryo-cultured makapuno palms when cultivated separately from normal ones. This result supports the view that the makapuno genotype is preserved through *in vitro* embryo culture. However, the cultivation of the embryo culture-produced seedlings cannot guarantee the production of a true-to-type palm since a makapuno-bearing palm is presumably heterozygous [3]. Recent research using DNA-based markers has developed a way to identify planting material that is carrying the makapuno trait, by assessing visually its genetic make-up in the young [23] and mature palms (53). Morphological differences in the thickness of makapuno endosperm have been used to access the genetic diversity of embryo-cultured makapuno palms, and also to distinguish this variety from normal [57]. Twelve variants, grouped into six clusters, were characterised with respect to fruit attributes (weight, equatorial diameter, and elongated shape), and leaf attributes (number of green leaves, petiole length). In contrast, very little research has been done on trying to find some way of identifying aromatic coconuts seedlings. There is a need to explore the occurrence of this trait and the underlying genetic mechanism. In addition to that, creation and propagation of new elite varieties using marker-assisted breeding approaches could be worthwhile.

5.2. Embryo culture

An attempt to tissue culture the makapuno embryo was first undertaken more than five decades ago [58], and was achieved two years later when De Guzman and Del Rosario [24] made use of a basal medium formulated by White [59] and Nitsch [60]. Coconut water, a good source of energy, with added vitamins and mineral nutrients, was also used at 25% (v/v) in this study, to enhance the germination rate. It was shown that the shoot developed well in the above medium, but root growth was constrained [61]. Further improvements in the approach have been made since the 1960's, including an improved surface sterilisation protocol [62], taking into account: the age of the embryo at the time of culture [63]; an improved embryo culture medium [64]; and procedures to promote rooting and seedling acclimatisation [26].

In the most widely used protocol, the embryo is extracted within a small section of solid endosperm (so-called cylindrical plug or core) using a cork borer [65, 66]. Comparisons of age showed the 11-month-old makapuno embryo to be the most suitable for *in vitro* growth [63]. Surface sterilization is usually undertaken in two steps. The first step is to surface sterilize the cylindrical plug in the field with a strong commercial bleach (*c.* 5% sodium hypochlorite) and triple rinse in sterile water. Once transported to the laboratory and into the laminar flow cabinet, the embryos are isolated and surface sterilized with lighter bleach (*c.* 0.5% sodium hypochlorite) and 70% ethanol followed by at least a triple rinse with sterile water.

An embryo culture procedure often consists of four stages: (i) embryo germination; (ii) shoot formation; (iii) root formation; and (iv) acclimatization (summarized in Table 1). Even though many well-known basal media, including White [59], Nitsch [60] and Murashige and Skoog [67] have been tried, the Y3 medium [68] is the most beneficial, being now most commonly used for makapuno embryo culture [62, 66]. This medium provides a greater quantity of micro-elements for tissue growth, including cobalt, copper and particularly iodine. A high level of sucrose (8%) has been shown to be the most beneficial for embryo germination and helps to create a desirable shoot-to-root ratio [69].

This high concentration of sucrose is thought to increase metabolic rate and also act as an osmotic agent, promoting germination. The first step of the embryo culture procedure is undertaken in a liquid phase, and sucrose can be replaced with dextrose which acts as the energy source [70]. Activated charcoal (0.20 to 0.25% w/v) is commonly added to the medium to prevent tissue browning [25, 66, 71] (Fig. 1D). Other studies have shown that the germination rate can be improved by the addition of gibberellic acid to the liquid medium [72]. Maintaining the germinated embryo in liquid culture can improve rooting [72]; however, a subsequent study has indicated that keeping makapuno embryos in a liquid medium for more than 6 weeks is detrimental to their shoot growth [73]. Autoclaved coconut water and a high concentration of indole acetic acid (IAA) have been shown to be inhibitory to root development, while other IAA-like plant growth regulators, such as naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA), were found to significantly enhance root formation [66, 74]. During subculture in this second liquid medium, high light intensity was shown to adversely affect growth, especially of the roots (Guzman 1974).

In the early 2000s, the technique has been introduced to several developing countries including Vietnam, Indonesia, Fiji, and PNG under internationally-funded programs, and was used to successfully establish a number of pilot plantations of elite coconuts in Vietnam (Fig. 1E). By using this improved protocol the average time taken for plantlets to reach the three-leaf stage and be ready for deflasking was approximately 9 months. This might be longer if younger than 9-month-old embryos were cultured [75]. To speed up the time to deflasking and to improve the efficiency of establishment in soil, a photoautotrophic chamber enriched with CO_2 (1,600 µmol mol^{-1}) to promote seedling growth has been developed [26].

It is interesting to note that the embryo culture technique developed for makapuno has been successfully used for other makapuno varieties as well as the aromatic coconut [25]. Success has been reported for the makapuno-like 'Dahi Nariyel' in the Andaman Islands (India) and Kopyor in Indonesia [76]. In the latter study, excised embryos were first cultured on White's [77] basal medium [20] to promote their germination, then rooting was achieved using a solid MS [67] medium supplemented with 10 mg L^{-1} IAA, 0.5 mg L^{-1} indole-3-butyric acid (IBA), 6% dextrose and 0.5% activated charcoal. It was shown that this medium combination produced balanced growth of shoot and roots.

Very recently, a new technique has been used to divide the kopyor embryo longitudinally, then to culture both halves on a plant growth regulator-added medium, thereby successfully producing two kopyor plantlets on most occasions [78]. However, the application of this technique is highly reliant on cutting the embryo correctly, and consequently is not suitable for producing plantlets on a large scale. In addition, an innovative embryo transplantation technique, in which a zygotic embryo from a non-germinable fruit is aseptically transferred to a viable fruit, could be an alternative to embryo culture [79]. With this approach, non-viable fruits might be rescued and nurtured without using any artificial culture medium, providing a much less expensive outcome.

5.3. Somatic embryogenesis

As it is possible to convert undifferentiated cells of many species into embryogenic structures, and for these structures to subsequently develop into plantlets, somatic embryogenesis (SE) has become a prospective approach for the mass production of makapuno seedlings [80]. Little research on SE has been conducted with this elite variety, due to the scarcity of explant supply as well as the low efficiency of the somatic embryogenesis protocols. However, it is worth noting that during the past three decades many efforts have been undertaken to optimise the procedure for rapid multiplication of the normal coconut genotype via SE (Reviewed in [2]). Successful and consistent plantlet production of normal coconut through SE has been achieved using plumule [81], unfertilized ovary [82], and immature inflorescences [83] as the explant. Among these potential explants, the plumule is ideal for multiplication of makapuno

through SE. Somatic tissues are not suitable as a certain portion of makapuno-bearing palms are heterozygous (Mm) with respect to this trait. However, somatic tissues can be successfully utilised only if the donor makapuno-bearing plants are raised using embryo culture technology. Certain measures such as the introduction of a callus multiplication phase [81] and prior selection of amenable palms for SE have been identified for maximising the plant production through SE. Such inventions can be taken into consideration when developing a protocol for micropropagation of the makapuno coconut. Moreover, advanced techniques, such as cell suspension culture and semi-submerged cultivation, should be explored in order to improve the efficiency of SE. Progresses of SE in the normal coconut will hopefully then provide important information for rapid multiplication of elite varieties.

It is now evident that advances in molecular analysis of gene expression have provided a better understanding of somatic embryogenesis in the normal coconut. By following the expression of particular genes during the embryogenesis process, the early selection of the appropriate cell culture lines most likely to undergo SE can be achieved. A range of marker genes have been discovered, including *CnSERK*, *CnANT*, *CnCDKA* [84-86] that can indicate those cell culture lines that are most likely to undergo SE. In addition, DNA-based markers can also be used to assess the extent of somaclonal variation within the SE-derived plantlets using Random Amplification of Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) or Amplified Fragment Length Polymorphism (AFLP) analysis. These techniques are also expected to be helpful for the optimisation of true clonal propagation of elite coconut varieties.

6. Conclusion and future prospects

As the economic demand for elite coconut varieties has grown rapidly in recent years, there is now an urgent need to provide a substantial quantity of new seedlings for planting where producers are able to achieve a boost in income with them. The embryo culture technique successfully converts otherwise non-germinable embryos to plants, thereby enabling the planting of elite coconut varieties. Its use is already underway on a moderate scale in the Philippines, and to a lesser scale in Indonesia, Thailand and Vietnam. Even though little practical success has been achieved so far, through SE clonal propagation, this approach in reality has great potential for the mass production of uniform elite coconut seedlings. It is also helpful for the mass production of seedlings of genotypes possessing specific resistance on tolerance to otherwise highly destructive bio-hazards. Further optimisation should be undertaken to exploit the full capacity of this method, particularly testing other somatic tissues as explants (e.g. the immature inflorescence). In addition, some innovative techniques such as embryo transplantation and marker-assisted breeding should be considered. With the recent worthwhile increase in genetic information for the identification of marker genes, molecular screening and identification of candidate cultivars can now be undertaken far more efficiently than in the past. Hybridization of known coconut varieties, such as between aromatics and Malayan Gold (Orange) Dwarf, is currently underway to improve fruit production and harvest index. Apart from increasing the production of such improved planting material, rigorous quality maintenance of products should also be considered. Since the shelf life at the market of these fruit types controls their viable shipping time, limitation of their market reach remains an issue which hampers prediction of their full market potential. Improvements in relevant food processing techniques will further expand the opportunity for these uniquely interesting coconut varieties to reach markets beyond the tropics.

Conflicts of interest

The authors declare no financial or other conflicts of interest

Acknowledgement

The authors would like to thank the Australian Agency for International Development (AusAID) for a scholarship awarded to Quang Thien Nguyen. We also acknowledge the independent reviews from Ms. Cristeta Cueto (Albay Research Center, Philippine Coconut Authority) and Dr Roland Bourdeix (Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement, CIRAD, Montpellier, France).

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Figure 1. Steps in the embryo culture of the makapuno - jelly-like endosperm coconut mutant. (A) Halved fruit showing different levels of manifestation of the makapuno trait . (B) Aseptic preparation of endosperm plugs taken from makapuno fruit for embryo culture. (C) Development of an embryo-cultured makapuno plantlet after 3 months of culture in medium supplemented with activated charcoal. (D) A makapuno seedling raised by embryo-culture after 12 months transferrin the field. (E) A small plantation of embryo-cultured Makapuno palms in Trang Bang Province, Vietnam. (F) Makapuno (Dua Sap) and Aromatic (Dua Dua) specialty coconut fruit with catchy display in Ben Tre Province, Vietnam.

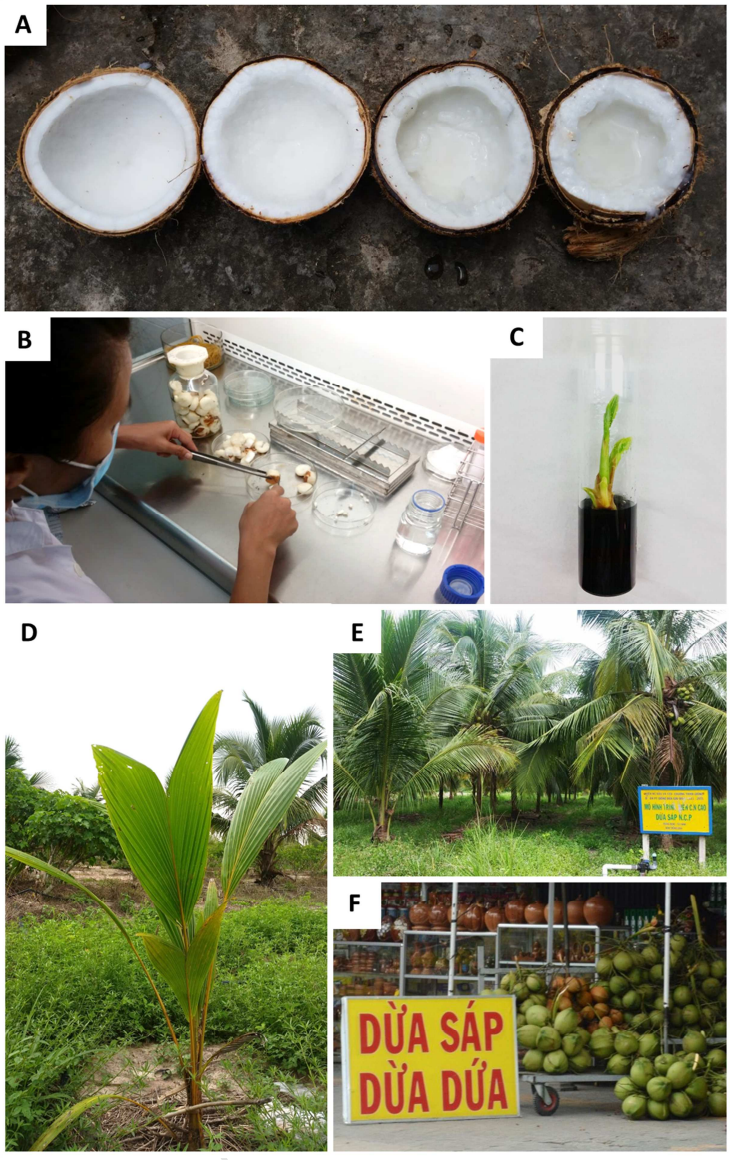

Figure 2. Schematic diagram showing the altered biochemical pathways in the Makapuno endosperm. Firstly, the increase in β-mannanase leads to an abundance of the breakdown products of gallactomannans (including mannobiose, mannotriose, and galactomannobiose). However, the acute lack of α-D-galactosidase and β-mannosidase significantly hinders the production of the simple sugars (galactose and mannose) which are normally used as an energy source during the germination process. Secondly, the higher activity of tryptophan aminotransferase, together with the decrease in peroxidase and IAA oxidase activity, triggers the abnormally high biosynthesis of indole-3-acetic acid (IAA). The elevated levels of IAA and partially the degradation products of gallactomannans contribute to the lower viscosity and jelly-like characteristics of the makapuno endosperm. *Increases in substrate availability or enzyme activity are shown in red; and decreases are shown in blue*.

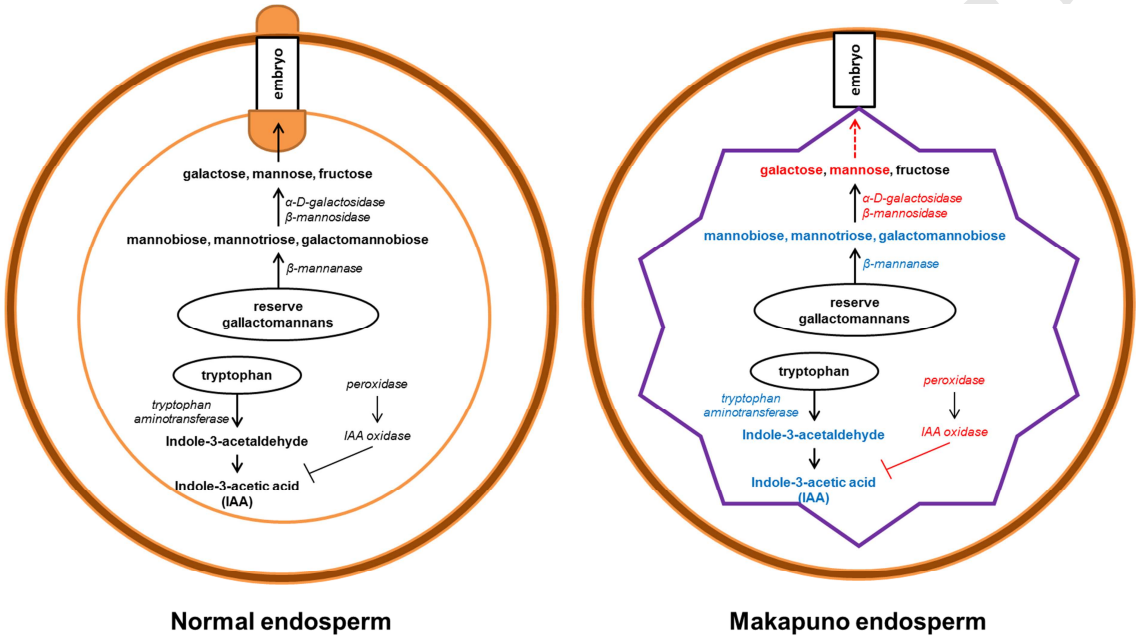

Table 1. Attempts and suggestions in embryo culture for the makapuno variety.

Stage	Medium composition	Incubation conditions	Yield	Reference
Embryo germination	White (1943) medium + 1.2% (w/v) agar + 25% (v/v) CW	Dark; room temperature	50-70%	De Guzman et al. 1964 [24]
	White (1943) medium + Nitsch (1951) vitamins + CW + GA + IBA	Dark; 28-30°C	n/a	Ventura et al. 1966 [61]
	White (1943) medium + 1.2% (w/v) agar + 10 mg L^{-1} GA	Dark; 27-29 °C	88%	De Guzman 1969 [72]
	Y3 (Eeuwens 1976) medium + 6-8% (w/v) sucrose + 0.25% (w/v) AC	Dark; 28-30 °C	50-60%	Rillo 1998 [62]
	Y3 (Eeuwens 1976) medium + 7% (w/v) sucrose + 0.25% (w/v) AC + 0.7% (w/v) agar	Dark; 28-30 °C	up to 100%	Carandang et al. 2002 [71]
Shoot formation	Y3 (Eeuwens 1976) medium + 4% (w/v) sucrose + 0.25% (w/v) AC + 0.4% (w/v) agar + 10 mg L^{-1} BAP	Light; 28-30 °C	97%	Carandang et al. 2002 [71]
Root formation	White (1943) medium + 10 mg L^{-1} GA	Light; 27-29 °C	72%	De Guzman 1969 [72]
	White (1943) medium + 8 % sucrose + 10 mg L^{-1} IAA	Light; 27-29 °C	auxilary roots	De Guzman et al. 1970 [69]
	Y3 (Eeuwens 1976) medium + 3-4.5% (w/v) sucrose + 0.25% (w/v) AC + 0.4% (w/v) agar + 7-10 mg L^{-1} NAA/IBA	Light; 28-30 °C	60-80%	Rillo et al. 2002 [66]
	Y3 (Eeuwens 1976) medium + 4% (w/v) sucrose + 0.25% (w/v) AC + 0.4% (w/v) agar + 17 mg L^{-1} NAA	Light; 28-30 °C	86%	Carandang et al. 2002 [71]
Acclimatization	Dipping roots in 2.5 g/l Benlate before planting	Screenhouse condition	95%	Carandang et al. 2002 [71]
	Y3 (Eeuwens 1976) medium + 3 to 4.5% (w/v) sucrose + 0.1% (w/v) activated charcoal	CO2 enrichment system (1,600 µmol/mol CO2) 14:10 h Light:dark (90 µmol m-2 s-1)	up to 100%	Samosir et al. 2014 [26]

Highlights

- Providing the first comprehensive review on elite coconut varieties for global stakeholders
- Addressing the uniqueness and commercial potential in booming market worldwide
- Summarising the biological insights and distinctive genetics of these elite varieties
- Presenting possibilities for mass propagation of these elite varieties

Contribution

QTN studied the literature, constructed the outline and wrote the manuscript. SWA and MF contributed to the structure of the manuscript and corrected the writing style. HBBD contributed to the 'Somatic Embryogenesis' section.