Clonal Propagation of Coconut: Prospects and Limitations

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ABSTRACT

Coconut (*Cocos nucifera* L.) is the most important palm in the tropics. Introduction of new varieties and hybrids with superior qualities is essential for crop improvement. However, achieving this goal by conventional means is very slow. Tissue culture is the only tool available for speeding up the process. This paper summarizes the current status, limiting factors in developing a viable clonal propagation method for coconut, possible measures to overcome limitations and future directions. The research shows that clonal propagation of coconut is possible through somatic embryogenesis. However, some major constraints that need to be overcome are heterogeneous responses due to the influence of explant maturity and genotype, undefined culture conditions possibly caused by activated charcoal influencing the hormonal composition of the culture medium, production of highly heterogeneous and compact calloids due to partial de-differentiation of cells of explants, poor efficiency in plant regeneration due to marked influence of genotype and variable culture conditions and slow growth of tissue-cultured plants under *in vitro* and early *ex vitro* conditions. Selection of the most responsive explant, standardization of activated charcoal used and repeated multiplication of embryogenic cell masses are some of the measures currently applied to overcome the limitations. Research towards selection of mother palms amenable to tissue culture, use of AGPs to increase the number of responsive mother palms and genetic manipulation of elite palms with regenerable genes are seen as the future directions in clonal propagation of coconut.

Key words: *Coconut, clona propagation, AGP (Arabinogalatan proteeins)*

INTRODUCTION

Coconut palm, the "Tree of Life", is an important oil crop in the tropics. There is a gradual decline in coconut production over the years due to constraints such as, maintaining senile plantations with low productivity, declined soil fertility, use of unimproved planting material, marginal cultural practices, spread of pests and diseases and increasing competition from other oil crops. However, coconut is still economically important for Sri Lanka and the industrial demand for lauric oil is high. Therefore, introduction of new varieties and hybrids with superior qualities is essential for the improvement of the industry. Conventional breeding programmes for genetic improvement of coconut are slow due to limitations attributed to the bulkiness, perennial nature of the palm and its biology. Thus the development of a reliable clonal propagation method of coconut to assist breeder is exciting. Although research leading to a method of clonal propagation in coconut was initiated in many countries in the early 1970's, an economically viable protocol has not yet been developed.

This paper summarizes the Sri Lankan experience on the current progress, limiting factors and possible measures to overcome the limitations and future directions towards the development of a viable clonal propagation method for coconut.

Current status of coconut clonal propagation

Since the inception of research in coconut tissue culture at Wye College, UK in the early 1970's, the response of various somatic tissues (tender leaf, immature inflorescence, root and shoot) and zygotic tissues (immature and mature zygotic embryos and plumule) to *in vitro* culture has been reported in many other countries worldwide (summarized in Fernando, 2001). These indicated the possibility of cloning coconut through several steps i.e., callogenesis, somatic embryogenesis, embryo maturation and germination. The regenerated plants, though low in number, were suitable for field establishment. Up to now, hundred coconut palms regenerated in the Tissue Culture Division of the Coconut Research Institute of Sri Lanka have been field established. These palms at different stages of development including some of which are already bearing nuts. A study using microsatellite markers showed that these clones are genetically stable with field performance comparable to seed-derived palms (Fernando *et al.*, 2004).

Factors limiting the development of a clonal propagation protocol for coconut

Callogenesis

Heterogeneous response of explants

The embryogenic potential of coconut leaf explants is very low (< 10 %). Only explants derived from young leaves of 10-20 cm length from 12-24 month-old seedlings responded. Furthermore, the embryogenic potential of these explants was of short duration. This limits the use of coconut leaf for clonal propagation (Karunaratne *et al.*, 1991).

Immature inflorescence is a promising explant as it bears numerous meristematic points. The embryogenic potential depends on the maturity stage of the explant. However, the lack of a marker greatly limits the ability to select the correct maturity stage. The highest callusing reported so far is 30 % (Vidhanaarachchi and Weerako on, 1997).

Zygotic tissues such as immature zygotic embryo and plumule (excised from mature zygotic embryo) hold potential for micropropagation. Unlike many somatic tissues (inflorescence and leaf tissues), the response of zygotic tissues is more consistent. However, the callusing frequency depends on embryo developmental stage. Under optimum conditions, callusing is usually above 75 % in immature zygotic embryo (Diyasena, 1998) and 60 % in plumule explants (Weerakoon, 2007).

Recent research unveiled the high potential of unfertilized ovary as an initial explant for cloning. The highest frequency of callusing reported from unfertilized ovary is 76 % (Perera *et al.*, 2007).

Somatic tissues are the ideal explants for clonal propagation as the performance of mother palm is known. However, the response of these tissues (except unfertilized ovaries) is generally poor. Moreover, there is a marked difference among explants collected from different mother palms in terms of callus initiation. This might be due to differences in genotype, explant maturity or mother palm age.

Effect of activated charcoal

Activated charcoal is an essential component in coconut tissue culture medium. It has strong adsorptive properties and its beneficial effects are attributed to the adsorption of phenols and other growth inhibitory substances. However, a major disadvantage of using activated charcoal is that it also can adsorb plant growth regulators (hormones and vitamins) and some micro elements (Cu and Zn) (Pan and Staden, 1998). This creates undefined culture conditions which could lead to variable tissue response and non-reproducible results. Different types/ brands of charcoal have different adsorptive capacities based on their origin, age, storage conditions and method of activation. This has a significant effect on callus development even from zygotic tissues that give high yields. A charcoal-free medium was developed with the addition of polyvinyl pyralidon (20 g L^{-1}) or ascorbic acid (1100 μM) to culture medium in place of charcoal that gave rise to 60 % callus in immature zygotic embryo explants. However, the embryogenic potential of these calli was low (Diyasena, 1998). The low embryogenic potential of coconut calli developed in charcoal-free medium was further confirmed by Adkins *et al.* (1999).

Quality and quantity of callus initiated

Coconut tissues generally produce highly heterogeneous compact callus in relatively small quantities. Establishment of cell suspensions is a useful tool for rapid multiplication of callus but it requires friable callus. However, despite the various treatments applied, no friable callus was obtained (Fernando, 2001).

Somatic embryogenesis and plant regeneration

Generally, lowering in auxin levels and addition of cytokinin to the medium induced somatic embryogenesis in coconut (Chan *et al.*, 1998). However, we found that application of abscisic acid (ABA) induced somatic embryogenesis (Fernando and Gamage, 2000). The plant regeneration efficiency was further improved by increasing agar and addition of polyethyleneglycol and $AgNO_3$ in combination with ABA. But, the plant regeneration was low at 10 % (Weerakoon, 2004). However, calli derived from unfertilized ovary showed an increased somatic embryo induction when they were transferred to a medium with lower level of auxin and then to a hormone-free medium (Perera *et al.*, 2007).

Comparison of different treatments revealed that callus from certain plumules/ immature zygotic embryos regenerated plants irrespective of the treatment. Thus it is necessary to study the genotype effect further to decide on the vital factor for obtaining positive results. This phenomenon complicates the improvement of the medium that is essential for induction and expression of somatic embryogenesis at a higher frequency.

Slow growth of regenerated plants

Growth of clonal plants *in vitro* and early *ex vitro* is very slow. When the growth of regenerated clones was compared with embryo-cultured plants, clonal plants were seen to be smaller at the time of transplanting and took a longer time to reach the stage required for field planting (Fernando *et al.*, 2004).

Possible ways to overcome limitations

Selection of the most suitable explant

Based on our studies, the unfertilized ovary seems to be the most suitable explant for clonal propagation of elite coconut palms. Further research to determine the effect of genotype and plant maturity stage needs to be carried out.

Source of activated charcoal

Activated coconut shell charcoal (ACSC), manufactured locally by Haycarb PLC was identified for incorporation in the coconut tissue culture medium. Its use is economically better over imported charcoal. Its suitability was confirmed by culturing immature zygotic embryos and plumules and also by testing free 2,4-dichlorophenoxyacetic acid available in the medium by HPLC analysis. The use of this source of activated charcoal continuously under controlled conditions of moisture level is important to achieve consistent results in callogenesis and somatic embryogenesis (Fernando, 2008).

Multiplication of embryogenic callus, somatic embryos and clonal plants

The report by Perez-Nunez *et al* (2006) on embryogenic callus multiplication by subdividing callus and repeated subculturing is an efficient method for increasing callus quantity. Application of this method for callus multiplication showed an average multiplication rate of five-fold and a variation of the rate with genotype (Weerakoon, 2007).

Secondary somatic embryogenesis is a phenomenon where new somatic embryos are initiated from primary somatic embryos. Induction of secondary somatic embryogenesis will also lead to higher multiplication rates of regenerated plants. Use of explants from *in vitro*-raised plants (re-culturing) may also be useful in obtaining higher multiplication rates. This will overcome the difficulties related to repeated collection of explants from mother plants.

Use of arabinogalactan proteins (AGPs) in culture medium

Cultured cells secret metabolites to the culture medium. The secretions can stimulate *in vitro* development of cultured tissues. Among these secretions, AGPs are important as they are reported to induce/ promote somatic embryogenesis in non-responsive/ low-responsive tissues (Egertsdotter and Arnold, 1995; Letarte *et al.*, 2006). The type and quantity of AGPs are cell type specific. Furthermore, the presence of specific AGPs in specific quantities is essential for successful somatic embryogenesis. If AGPs are not secreted, an external supply to the medium is an alternative (Kreuger and van Holst, 1993). It is thus possible that AGPs may have potential to induce somatic embryogenesis in highly recalcitrant species like coconut. This has not been applied in any palm. Studies are in progress to assess the effect of externally applied AGPs on initiation of embryogenic callus and somatic embryos. Conditioned media i.e. culture media in which tissues have been cultured for sometimes, carrot seed extract, coconut endosperm and Arabic gum are among the sources of AGPs under investigation.

Selection of mother palms more amenable to tissue culture

Many tissue culture protocols have shown strong genotype dependence. Work done over the years in coconut tissue culture confirms that it applies to coconut as well (Weerakoon, 2007). It has been observed that callus originated from individual embryos/ embryos collected from open pollinated nuts of individual palms could regenerate plants at a high frequency. Therefore, selection of most responsive such mother palms among elite palms to be cloned would increase the efficiency and consistency of clonal propagation protocol.

Presently, selection of such palms is only possible by culturing of explants collected from a large number of elite mother palms and selecting the most responsive palms. As this is a long-term, laborious and costly process, development of molecular markers is an alternative. Molecular markers in oil palm for selection of responsive palms for clonal propagation have been developed (Ong-Abdullah and Eng, 2007).

Studies on gene transformation are a new avenue as genes related to *in vitro* regeneration in certain crops have been identified (Boutilier *et al.*, 2002). Genetic transformation of coconut using such genes could lead to a more efficient *in vitro* plant regeneration system.

CONCLUSIONS

The importance of developing a clonal propagation method for coconut is now well accepted. The difficulties encountered in realizing it have also been identified. At present, the unfertilized ovary appears to be the most suitable explant due to a relatively high regeneration potential and application in clonal propagation of adult elite palms. Efforts to standardize callusing medium and multiplication of embryogenic callus have given encouraging results.

Further research towards selection of mother palms amenable to tissue culture, use of AGPs to increase the number of responsive mother palms and genetic manipulation of elite palms with genes related to *in vitro* regeneration will ultimately pave the way to a successful clonal propagation of coconut.

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