PR-6193 E -A8

Coconut Research Institute of Sri Lanka

Advisory Circular No A 8

APPLICATION OF TISSUE CULTURE TECHNOLOGY FOR IMPROVEMENT OF COCONUT

Introduction

The coconut industry plays a vital role in sustaining the national economy and food security of the people. The stagnation of coconut production is a serious threat to the sustainability and economic viability of the coconut industry. Introduction of new, higher yielding coconut varieties and superior hybrids remains essential for crop improvement. Unfortunately, production of new coconut varieties by conventional breeding methods is slow mainly due to the constraints linked to the palm's biological characteristics: high heterozygosity, very long breeding cycle and lack of a vegetative propagation method. The ability to propagate high yielding, disease-resistant varieties rapidly is critical to break the present yield barrier and to retain its status as an important food source. It has now become necessary to harness modern biotechnologies to achieve this goal. Thus biotechnology has been identified as an important area in the research programme of the Coconut Research Institute (CRI). One area under focus is clonal propagation of coconut through tissue culture technology.

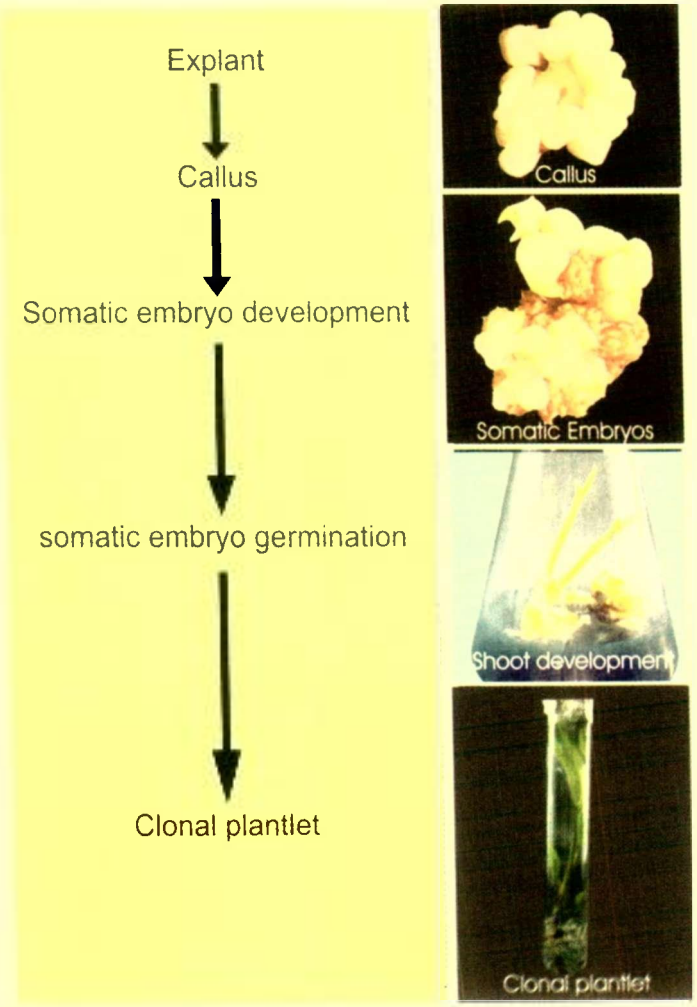
Clonal propagation of coconut through tissue culture

Tissue culture is a technique by which small pieces of plant tissues are cultured aseptically in specially formulated nutrient media, under controlled environmental conditions. The resulting plants are usually identical to the mother plant and referred to as a clone. This is a valuable tool for plant breeders to speed up the breeding and multiplication of promising genotypes when conventional breeding methods take more than a decade. Conventional propagation of coconut by the use of seednuts induces a high degree of palm-to-palm variation in yield and other selected characters. The advantages to be gained by eliminating this variability are immense, and therefore vegetative propagation of coconut by means of tissue culture technique is highly desirable. This would allow significant yield increases by propagation of high yielding individuals. In the same way, palms exhibiting resistance to certain diseases and tolerance to adverse growing conditions could be propagated. Furthermore, clonal propagation would mean a more rapid availability of breeding results to the growers.

Cloning coconut has been addressed in a number of research centers worldwide. However, coconut has remained highly recalcitrant to propagation through tissue culture and is still propagated exclusively through seeds. Hence it requires intensive research in order to perfect clonal propagation methodology.

The tissue culture laboratory at the CRI was established in 1983 with the aim of developing a reliable clonal propagation method for coconut. Various tissues including tender leaf, immature inflorescence, shoot tip, immature embryo and plumule (obtained from the mature embryo) have been used in clonal propagation research. Inflorescence and leaf tissues are ideal sources of explants for clonal propagation since the performance of the mother palm is known. However, immature embryo and plumule (embryo meristem + first leaves) explants have shown a better response to *in vitro* conditions than somatic tissues. These zygotic tissues could only produce clones of palms with unknown performance. However, they could be used as model systems to generate further knowledge for refining protocols developed using other explants. Moreover, they could be useful in cloning selected parents (e.g. disease resistant palms) that are available in low numbers.

As a result of extensive research done over the years, it is now possible to regenerate plants from above tissues. These plants, apparently have come through a process called "somatic embryogenesis", that involves a sequence of steps. The first step in the process is the production of callus (an undifferentiated mass of cells) from the *in vitro* cultured tissue. Then the callus cells redifferentiate into somatic embryos. These somatic embryos later develop into complete plantlets (Picture 1) . These plants are then transferred to the external environment for acclimatization. This is the process of bringing the plants out of culture and placing in free-living conditions where they must photosynthesize and overcome challenges from microbes.



Picture 1: Steps involved in coconut plant regeneration

During the past decade, different phases of plant regeneration protocol (callogenesis, somatic embryo initiation, somatic embryo maturation and their conversion to plantlets) have been improved. The knowledge gained by recent studies on histological, hormonal, nutritional and physiological aspects of plant regeneration has contributed immensely in achieving this success.

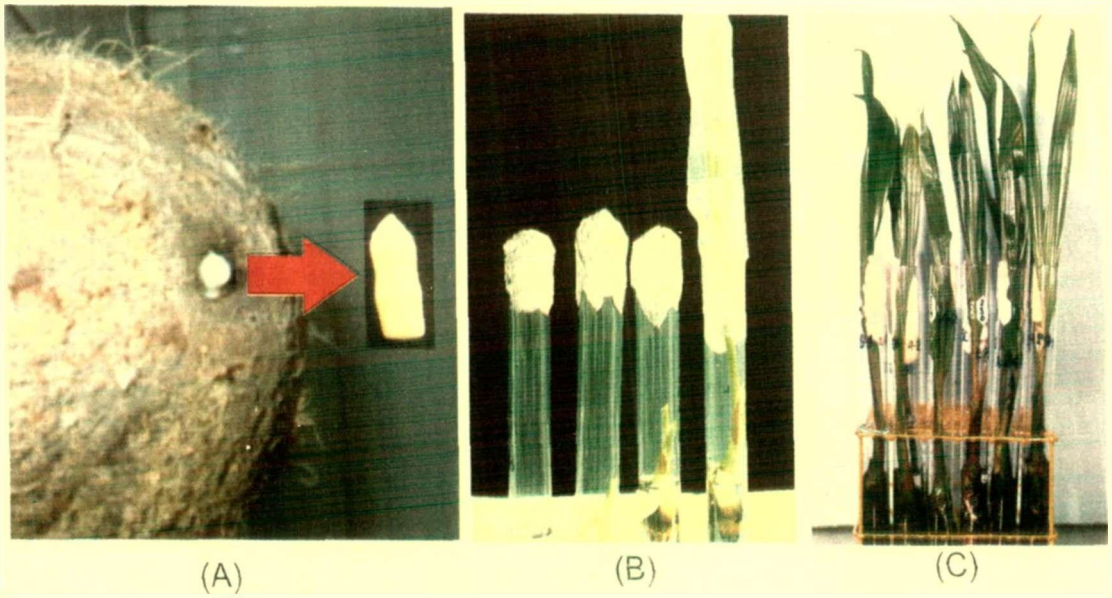
Even though plant regeneration from various tissues is possible, the number of clonal plants obtained still remains low and the technique needs to be perfected. One major problem is that many of the embryogenic structures that are developed from callus are abnormal. Shoot meristems occur only occasionally but leaves and roots are frequently seen. An important requirement is to control the balance of development between shoot, root and cotyledon type tissues. Slow growth and low survival rate of clonal plants after transplanting are other handicaps that need to be resolved through further research efforts.

In spite of all these constraints, significant progress has been achieved in regard to field establishment of tissue-cultured coconut plants. The first batch of tissue-cultured coconut plants were field planted in 1999 and since then nearly 100 clonal coconut plants have been successfully established in the field. The growth of these plants is monitored regularly and no abnormalities in vegetative growth or nut characters were observed in these palms. Analysis of clonally propagated material to ensure genetic integrity is also of utmost importance. Thus molecular markers were used for testing genetic fidelity of clonal coconut plants that have already been established in the field. Thirty-one tissue-cultured coconut plants of 7 clones were analyzed and no variations were observed within a single clone.

It is clear from the limited success in cloning coconut that there is a need for better understanding of the process of somatic embryogenesis. At present, CRI scientists work in collaboration with several research groups worldwide to gain more knowledge on fundamental aspects of somatic embryogenesis and plant regeneration. It is hoped that these research efforts will provide valuable clues to overcome problems associated with plant regeneration and ultimately lead to the development of an efficient protocol for cloning coconut.

Mature zygotic embryo culture

Besides clonal propagation, work has been done on the development of embryo culture technology mainly to facilitate collection, exchange and conservation of coconut germplasm.The earliest attempts at coconut tissue culture were in fact those of embryo culture. In this technique, the embryos are removed from mature nuts and after surface sterilization, they are cultured in test tubes containing a nutrient medium. The culture medium provides all the necessary ingredients for the embryos to germinate and develop into complete plants. Within 6-8 months of culture, these *in vitro*-raised plants could be transferred to soil. After a few months of hardening, they could be planted in the field (Picture 2 and 3).



(A) (B) (C)

Picture 2: Invitro growth of coconut embryos

A: Coconut embryo

B: Germination and subsequent growth of coconut embryo in an artificial growth medium

C: Fully-grown embryo-cultured coconut plants ready for transplanting in soil

(A) (B) (C)

Picture 3: Acclimatization (Hardening) of embryo-cultured coconut plants

A: Embryo-cultured coconut plants just after transplanting in soil

B: Coconut plants at different stages of acclimatization

C: Fully-grown embryo-cultured coconut plants ready for field planting

There is now considerable interest in embryo culture as a tool for safe germplasm exchange as well as for genetic conservation. Furthermore, it facilitates rescuing embryos of non-germinating and economically important types of coconut such as Dikiri. Apart from above mentioned applications, zygotic embryo culture would provide a model system which could be applied to improve conditions used for growth and acclimatization of clonal plants obtained through tissue culture.

In general, embryo culture is not difficult to perform and there are a number of laboratories, actively engaged in coconut embryo culture research. However, techniques used for *in vitro* culture of embryos and *ex vitro* hardening of resulting plants have to be optimized in order to achieve maximum recovery of embryos for field establishment. Hence, an international research program was launched by COGENT (Coconut Genetic Resources Network), to refine the coconut embryo culture and acclimatization technology. As a result of this two-year project (from 1999- 2000), which involved 13 laboratories in 11 countries, an upgraded embryo culture protocol with increased efficiency was developed. The project also strengthened research collaborations among the member countries.

The limited availability of genetic material for the breeders is one of the reasons that hinder the progress in crop improvement. As a result of the initiatives taken by COGENT recently, germplasm material are now made available to interested coconut producing countries. Exchange of coconut germplasm is hampered by the large size of the nut, lack of dormancy and phytosanitory difficulties. Embryo culture provides a solution to these problems as germplasm can be exchanged in the form of excised embryos. The safe movement of germplasm is assured through this technology as the risk of contamination is minimal.

Under the germplasm exchange program, embryos from 24 varieties of coconut (4 varieties from India, 10 varieties each from Papua New Guinea and Ivory Coast) were brought to CRI, over the past 2 years. Embryos were initially cultured in sterile water and after bringing them to the tissue culture laboratory at CRI, they were transferred to the standard growth medium. Some of the plants raised from these embryos have already been established in the field and the rest of the plants will be transferred to the field in due course. Thus exchange of exotic coconut germplasm using embryo culture technology has paved the way to strengthen the future-breeding programmes.

In vitro techniques have also been developed to complement the genetic conservation efforts launched by the breeders. Appropriate culture media have been developed to allow short and medium-term preservation of mature embryos. Once cultured in these media, the embryos are subjected to a slow growth phase and they can be recovered later by transferring to germination medium. Investigations are underway to develop an efficient cryopreservation technique to facilitate long-term conservation of coconut.

Another useful application of embryo culture technology is propagation of dikiri coconut. Dikiri coconuts are characterized by the soft, jelly-like kernel that almost fills the interior of the nut. The milk in these nuts is thick and viscous instead of watery. Normally, only 2-20% dikiri nuts could be harvested from an ordinary dikiri-bearing palm and these nuts do not germinate under normal conditions due to the abnormal kernel. A coconut cultivar with similar kernel characters, termed Makapuno, is found in the Philippines. The Makapuno is an expensive delicacy and its planting material is highly priced in the Philippines.

In Sri Lanka, dikiri-bearing palms are rare and they are mainly confined to Weligama area in the Matara District. In this area, there is a high demand for dikiri nuts as they are used for making a variety of sweets. A dikiri nut can be sold in the market for about 80 rupees and due to the economic benefits, the farmers are keen to propagate and build up a population of dikiri plants.

The embryo culture technology can be used successfully to rescue dikiri embryos and through this technique, plants having a yield potential of nearly 100% dikiri nuts could be produced. At the CRI, embryo culture technology is being used successfully to raise dikiri plants and the long-term objective is to mass propagate dikiri plants through this technique. This could have an impact on poverty alleviation by way of improving the smallholder's income. Nearly 100 *in vitro*-raised dikiri plants have been established in Bandirippuwa Estate, Lunuwila. Over the past few years, a considerable number of embryo-cultured dikiri plants have been distributed among farmers. The CRI also aims to establish demonstration farms with *in vitro*-raised dikiri plants in several regions of the country.