significant for the almond. Application will depend upon the type of culture system in use. For example, the mass production of own-rooted almond cultivars would be particularly significant in various European production areas where almond is typically grown on seedling almond rootstocks. On the other hand, where the almond root system is not particularly adapted, as in irrigated sections of California, the selection and mass propagation of other clonal rootstocks would have much significance.

The commercial distribution of the vigorous clonal rootstock GF 677 in Europe indicates the potentiality of this procedure. The maintenance and distribution of virus-tested, true-to-type source materials in conjunction with nursery production has particular value providing methods for monitoring genetic identity come into use. Maintaining germplasm stocks in a controlled environment in vitro has promise for long-term storage. Similarly, the control of the potential for noninfectious BF might be achieved in propagation source maintenance in the case of susceptible cultivars as Nonpareil, where the development of the BF phenotype is associated with increased temperature. The reported success in micropropagating the Ferragnes almond cultivar needs to be extended to other cultivars and germplasm, since vegetative propagation has been particularly difficult with almond.

Callus and suspension cultures are relatively easy to produce in almond. Besides their application to a wide range of physiological and genetic studies, the potentiality for embryogenesis presents a potential mass propagation technique. With the reported success of protoplast culture in almond and the re-establishment of callus, the prospect of the biotechnical applications in cell selection, gene transfer, etc., will become possible. Cell and tissue culture would have most immediate application in the production of disease-, and insect-resistant clonal rootstocks.

13. Coconut Palm (*Cocos nucifera* L.)

C. Pannetier[1] and J. Buffard-Morel[2]

1 Introduction

1.1 Importance of the Culture of Coconut

Coconut palm (*Cocos nucifera*) has been shown to be present in most areas of the intertropical zone since prehistorical times. This is the only specimen of the *Cocos* genus, and is now cultivated on nearly 10 million hectares in 80 countries.

While its possibilities (water, pulp, oil, fibres, wood) have been exploited since early times by the local populations, it has become increasingly important for industry and commerce. It plays a considerable economic and social role,

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Table 1. World production of oils (in million tons). (*Oil World*)

	1976–1977	1980–1981
Soya	9.4	13.4
Oil palm	3.4	4.9
Sunflower	3.4	4.8
Colza	2.8	4.0
Cotton	2.8	3.2
Coconut	2.7	3.0
Peanut	2.7	2.3
Olive tree	1.5	2.0
Fish	1.0	1.1
Other	17.4	18.3
World total	47.1	57.0

since in most of the countries where it is cultivated it employs and sustains an important part of the population.

The products of coconut are numerous and diversified. According to estimations, 50% of the 35 million tons of nuts produced in 1980 are consumed locally as fresh fruit. The other 50% are transformed into copra and oil, corresponding to 3 million tons in 1980. This makes coconut the 6th source of oil for human consumption (Table 1). The fruit itself offers various possibilities (Fig. 1). Besides the nut, the other parts of the tree are utilized to different ends. The stem is used as building wood, as an aggregate with cement for building material, and for the preparation of particle boards. The leaf is a good basic material for making numerous articles such as shoes, hats, mats; it is also used for covering roofs or shading nurseries.

The roots have interesting qualities for medical uses. All parts of the coconut can be useful for man and in many countries it has been named "tree of life" (for a detailed review of the different productions of coconut, see Thampan 1981).

The geographical origin of the coconut is still controversial. Botanical and ethnological data as well as arguments based on edaphic or climatic limitations are numerous and sometimes contradictory (Child 1974, Harries 1978). Two theories can be retained, one of an American origin (South or Central America), the other of an origin located in the Indonesian, Malaysian, Melanesian area. At present, the latter seems the most widely accepted. However, Asia is the main producing area, with 85% of the world copra production. The first three producing countries are the Philippines, Indonesia, and India (Table 2). The areas under cultivation are estimated at 3.5 million ha in the Philippines, 2.5 million ha in Indonesia, and 1.1 million ha in India (FAO Production Year Book 1981).

Yields are low, less than 700 kg of copra ha^{-1} $year^{-1}$, although progress in breeding during the last 20 years has allowed the production of hybrid seeds with yield potential of 4 to 5 t ha^{-1} $year^{-1}$, under good ecological conditions, ranking coconut among the oleaginous plants having the highest yields per hectare. The aging of trees, the absence of appropriate cultural practices (for example, it is estimated that only a few thousand hectares in the world are correctly fertilized),

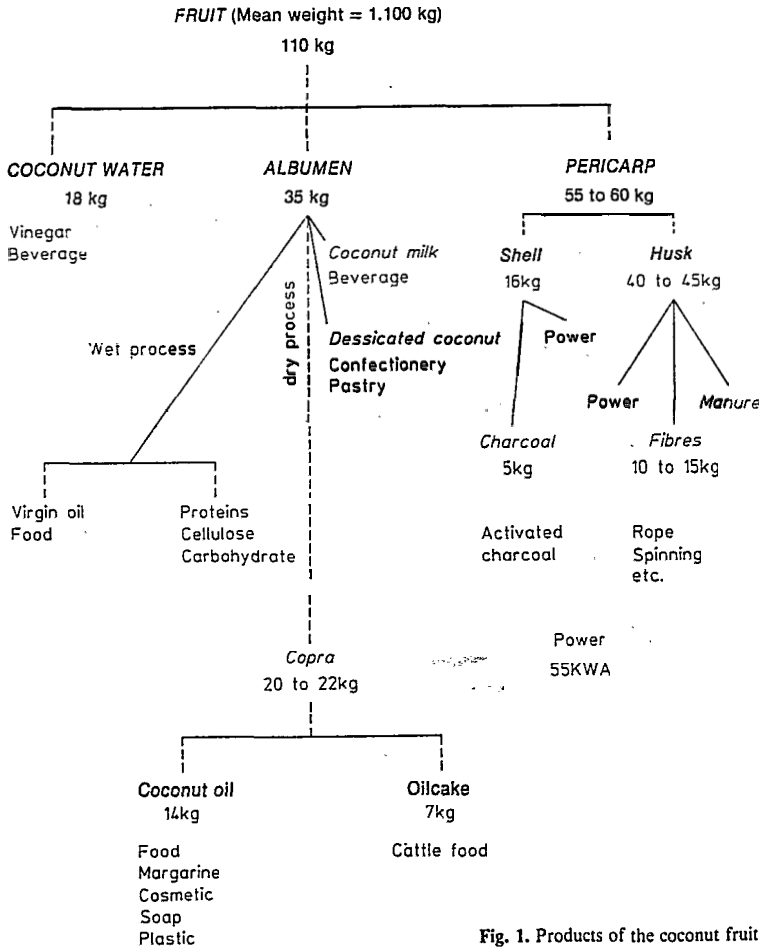

Fig. 1. Products of the coconut fruit

the lack of quality plant material for replanting are the main causes of these low yields.

1.2 Classical Methods of Improvement and Seed Production

We shall present mainly the general improvement scheme employed by IRHO (Institut de Recherches pour les Huiles et Oleagineux) (Gascon and de Nuce de Lamothe 1976, 1978, de Nuce de Lamothe and Rognon 1975, de Nuce de La-

Table 2. Main producing countries in 1981 (in millon tons). (*FAO Production Year Book*, 1981)

	Nut	Copra
Africa	1,514	175
America (Central and South)	2,073	222
Asia	30,803	4,335
Philippines	11,050	2,275
Indonesia	10,800	1,254
India	4,500	370
Sri Lanka	1,716	123
Malaysia	1,270	208
Thailand	900	52
Oceania	2,274	322
World total	36,665	5,054

mothe et al. 1980). In this global approach, increasing the yield is the main objective, but quality criteria are also considered (content and composition of the oil, protein content, and composition), as well as adaptability to ecological conditions and resistance to diseases. Some important biological information must be given for a good understanding of the choice of the improvement method.

1.2.1 Main Biological Characteristics

The coconut is a monoecious perennial monocotyledon. The usual classification distinguishes the tall coconut varieties which form the majority of the existing coconut fields and the dwarf varieties which are usually rare. The tall are generally protandry allogamous trees, their growth is fast and their improductive stage rather long (about 7 years). They usually produce a moderate number of rather big nuts. On the contrary, the dwarfs have a slow vegetative development, they are generally autogamous, rather precocious (3 to 4 years of nonproductive phase) and produce large numbers of rather small nuts. Male and female flowers are scattered separately on the same inflorescence and reproduction is only sexual. The propagation rate is low (100 to 200 nuts per year) and is still further reduced (20 nuts per year) when controlled pollination with pollination bags is used, in order to obtain progenies of identified parentage. The span of one generation is 12 to 16 years, according to ecotype.

1.2.2 Improvement Method

The selection scheme used by IRHO and adopted more or less fully by the breeders working on coconut is based on research for good combining abilities between ecotypes in comparative trials, associated with phenotypical choices for the heritable characteristics. The ecotypes present a low genetic variability and a relatively high average inbreeding rate. The programme for the improvement of coconut can be summarized as follows:

1. Investigations and trials are carried out to gather a maximum of genetic variability.
2. Hybrids between different ecotypes are produced and tested in comparative trials (inter-hybrid selection). The best hybrids are reproduced for seed production.
3. Crosses between the ecotypes which have given the best hybrids are realized, so as to induce recombinations and allow a new cycle of improvement.
4. Simultaneously, an improvement of the best hybrids is looked for, choosing within both parental ecotypes the individuals which lead to the best progeny (intra-hybrid selection).

To carry out the improvement programme, particular techniques are needed (conservation of pollen and directed pollination), which are now fully mastered. Likewise, the seed production must use constraining techniques to guarantee the quality of the plant material produced. For this seed production, the best hybrids detected in the improvement programme are reproduced on a large scale.

At present, this reproduction is relatively easy because of the use of dwarf ecotypes, which are autogamous, as mother trees. To reproduce a good A × B hybrid of which A is a dwarf ecotype, a seed garden of A is planted and the best individuals of B are used as pollinators. The seed garden must be isolated from all undesirable pollen. This method is relatively expensive and demands very strict controls (emasculation, conservation of pollen, pollination) as well as a good organization.

1.3 Significance of Tissue Culture Techniques

The classical methods of coconut breeding are limited by a certain number of constraints. The length of the cycle is important (12 to 16 years including the necessary observation time) and thus reduces the number of generations observable by one scientist. The propagation coefficient is low. Moreover, the considerable volume of the nut, and its often fast germination make storage and transport of seed difficult and expensive. Finally, the heterozygosity of the tall varieties induces an important degree of heterogeneity in hybrid progenies.

1.3.1 Vegetative Propagation

In coconut, vegetative propagation would allow significant yield increases by propagating high-yielding individuals. In the same way, it is obviously interesting to propagate individuals presenting resistance to certain diseases, or a particular adaptability to certain conditions of culture. Vegetative propagation would also allow the more rapid release of breeding results. Spreading a good hybrid through a seed garden takes a long time because the results of the comparative trials must be known before planting it. Then one must wait for 4 more years in the case of the dwarfs to exploit it. If the seed garden were constituted of tall, the delay would be even longer. Finally, the breeding programme might include the propagation of certain particularly interesting genitors. Thus vegetative propagation would be most helpful.

At present the coconut is only sexually propagated because of some morphological and physiological peculiarities; it has only one stem and does not produce any shoot. Axillary buds only give inflorescences and the only vegetative bud is the apex, which builds the whole tree. Therefore, the traditional methods of vegetative propagation (cutting, grafting) are of no use. Ramification phenomena have been observed sometimes, as well as transformation of inflorescences, spikes, or even flowers into vegetative buds (T. A. Davis 1969a, b). Though attempts at rooting these vegetative shoots have been successful (Sudasrip et al. 1978), the problem of reversion towards fructification is still unanswered and no routine method of propagation has been developed so far. Trials of aerial layering were conducted on the crown, but although this technique envisages the rejuvenation of particularly interesting coconuts, it cannot lead to their multiplication. The development of several off-shoots has been obtained by splitting the growing point on young seedlings (T. A. Davis 1969b). Trials carried out on very young seedlings obtained by germination have shown that the neomeristem formation was of adventive origin (Balaga 1975). However, the results of these experiments cannot be directly exploited for propagating elite palms. The determinism of the transformation of inflorescences or flowers into vegetative shoots is hardly known and few attempts to induce such phenomena have been undertaken.

Thus the only possibilities of vegetative propagation are through tissue culture techniques. Studies concerning their utilization have already been developed in the United Kingdom, India, Indonesia, Sri Lanka, the Philippines, the United States, and France. Considering the morphological peculiarities of the coconut, tissue culture seems to be the most suitable technique, perhaps even more than for other crops.

1.3.2 Embryo Culture

Important work has been done in the laboratory of de Guzman in the Philippines on this subject, and in particular on the in vitro germination of Macapuno embryos. In some areas of the Philippines a particular type of tall variety called macapuno, is found on one tree a certain proportion of nuts have an endosperm which remains tender and jelly-like. This endosperm represents a very appreciated delicacy. However, the embryos of these nuts do not germinate naturally, the endosperm rots before the future seedling can develop. The macapuno characteristic might be under the control of a single mendelian recessive factor (Zuniga 1953, Torres 1937). The aim of the in vitro culture of the macapuno embryo is thus to obtain the development of individuals which would yield a high percentage of nuts with a semi-liquid endosperm.

Moreover the in vitro production of young plantlets from excised embryos might be a tool for phytopathological studies. Since coconut seeds are particularly cumbersome, the in vitro culture of embryos can yield in a limited space a large number of individuals for use in different trials and the study of host-parasite relationships. It also offers the possibility of carrying out in vitro studies on some pathogenic agents on living material. The in vitro development of coconut em-

bryos has been studied by Fisher and Tsai (1978) with the aim of investigating the transmission of Lethal Yellowing disease.

Finally, with respect to conservation of germplasm, the embryo culture can offer the possibility of simplifying the storage and transport of a material which is naturally bulky (Bajaj 1983c).

1.3.3 Culture of Gametic Cells

The in vitro production of haploids is of great interest in the case of the coconut, since the length of its cycle and its heterogeneity make the production of inbred lines difficult and expensive. The haploids, and subsequently homozygous plants, would allow the fixation of certain characteristics. The individuals would then be propagated vegetatively. In this field of in vitro haploid production, promising results concerning the use of anthers and induction of embryos from pollen grains have recently been obtained (Thanh Tuyen and de Guzman 1983).

2 In Vitro Studies

2.1 General Account and Bibliographic Summary

In the Philippines, de Guzman carried out the first important work on the in vitro culture of coconut embryos, particularly the development of excised macapuno embryos, and obtained complete seedlings (Table 3). Various groups have carried out studies on embryos of different varieties. In vitro development studies were undertaken in order to determine the influence of endosperm as a complement in culture media (Cutter and Wilson 1954), or to develop a method to test the vigour of certain hybrids (Abraham and Thomas 1962). In the latter case, a good development of the aerial system was obtained, but the root development was rudimentary. Iyer (1982) pointed out that complete plants were obtained and reported attempts to transfer them to soil. In the ORSTOM laboratory, embryos from a Malayan Yellow Dwarf × West African Tall hybrid were cultured, complete seedlings were obtained, and they were successfully transferred to soil (Ahee and Guenin, unpublished results).

With a view to vegetative propagation, work on two genera of palms such as *Howeia forsteriana* Becc (commonly known as Kentia) and *Chamaedora costaricana* (Reynolds 1978) can be mentioned, but the majority of the studies have been made on the oil palm and date plam. The oil palm shows the same morphological characteristics as the coconut, namely a single stem, axillary buds forming inflorescences, and a single vegetative bud. The development of plantlets was obtained from different types of explants such as root tips (Corley et al. 1977) or leaflet fragments (Rabechault and Martin 1976, Pannetier et al. 1981). These methods are used to propagate high-yielding adult trees and the progress of research (Noiret 1981) is such that now the large-scale application of the process is under way. In both cases, it is through somatic embryogenesis from callus. Numerous groups have been interested in tissue culture as far as the date palm is concerned (Ammar and Benbadis 1977, Reuveni et al. 1972, Schroeder

Table 3. Coconut tissue culture: main results

Explant	Response	Reference
Somatic tissue		
Stem explants	Proliferation but no callus subculture	Apavatjrut and Blake (1977)
Root explants	Callus formation and subculture	Fulford et al. (1981)
Leaf explants	Callus formation and embryoids neoformation	Pannetier and Buffard-Morel (1982a, b)
Inflorescence explants	Surface callusing Callus formation and multiplication by subculture	Eeuwens (1976) Blake and Eeuwens (1982)
Endosperm explants	Callus formation and subculture	Fisher and Tsai (1978)
Excised seedling apices	Growth of the vegetative apex and plantlet formation	Blake and Eeuwens (1982)
Embryos in vitro cultured	Calluses Nodular protocorm-like proliferation with occasional scale leaves	D'Souza (1982)
Haploid tissue		
Anthers	Torpedo embryos	Thanh Tuyen and de Guzman (1983)
	Multicellular pollen and embryo-like nodules	Monfort (1985)
Exised embryos		
Macapuno variety	Complete seedlings	de Guzman et al. (1971) del Rosario and de Guzman (1976)
West Coast Tall variety	Complete seedlings	Iyer (1982)
Malayan Yellow Dwarf × West Africa Tall hybrid	Complete seedlings	Ahee and Guenin (pers. comm.)

1970, Poulain et al. 1979, Tisserat 1979a, b, Reynolds 1982, Reynolds and Murashige 1979, Sharma et al. 1980). Although some publications referred to the emergence of buds (Ammar and Benbadis 1977, Rhiss et al. 1979, Poulain et al. 1979), the histological studies have shown that micropropagation of the date palm was made through embryogenesis (Reynolds and Murashige 1979, Tisserat and de Mason 1980). Somatic embryos were also observed in *Howeia forsteriana* and *Chamaedora costaricana* (Reynolds and Murashige 1979).

The morphology and propagation capacity of the four palm trees mentioned above are different in that *Elaeis guineensis* and *Howeia forsteriana* never produce off-shoots, *Phoenix dactylifera* shows a small number of them, and *Chaemaedora costaricana* develops numerous off-shoots. However, the available data have shown their capacity for somatic embryogenesis from tissues cultured

in vitro. Production of somatic embryoids seems to be the most promising line in vegetative micropropagation of the coconut, as will be seen later.

Two main methods were used for in vitro propagation of the coconut; they differ in their objectives and consequently in the explants used. They are, on the one hand, the production of callus along with an attempt to the neoformation of buds or somatic embryos from fragments of stems, leaves, roots or inflorescences and on the other, attempts to reverse young flower meristems to a vegetative growth.

Briefly, it can be mentioned that calli were obtained from different types of organs (see Table 3) and that it was often difficult to maintain these in subculture (Eeuwens 1976, 1978, Apavatjrut and Blake 1977). Attempts at inducing organogenesis from them or more directly from original explants led most often to the root development. Neoformation of buds has never been mentioned and if structures likely to develop into plantlets were observed, these were either protocorm-like bodies (de Guzman et al. 1978) from embryo tissues cultured in vitro, or embryoids from explants of young leaves from young seedlings or adult trees (Pannetier and Buffard-Morel 1982a, b).

Recently, Thanh-Tuyen and de Guzman (1983) have reported the induction of pollen embryos (at a stage termed Torpedo by the authors) from anther cultures. In France, studies have been undertaken on anther and ovule culture. Multicellular pollen were observed, together with a few nodules showing the morphological characteristics of embryos (Monfort 1985).

2.2 Tissue Culture with a View to Vegetative Propagation

2.2.1 Explants

The propagation of individuals with interesting characteristics requires the use of material from adult trees without damaging the donor plant. However, in order to determine the different behaviours in vitro, excised embryos have sometimes been used as original plant material (de Guzman et al. 1978, Noerhadi and Toruan 1975, Noerhadi 1979, D'Souza 1982, Jagadeesan and Padmanabhan 1982). With respect to organs and organ fragments, the different types of explants have been used. Roots, leaves and inflorescences can be sampled without damaging the donor tree.

Roots were studied by Fulford's group at East Malling Research Station in England. As far as disinfection is concerned, root fragments raise serious problems. For example, in the case of oil palm root tissues, it was necessary to use mercuric chloride (Wooi et al. 1982). However, trials conducted on this plant in the IRHO laboratory at La Mé in Ivory Coast showed that once apices of tertiary roots were cleaned, about 30 to 40% of sterile fragments could be obtained using sodium hypochlorite. Moreover, the field-sampling of roots from the adult tree must be made carefully in order to be sure that the samplings correspond to the tree selected for cloning. Different authors (Eeuwens 1976, 1978, Eeuwens and Blake 1977, de Guzman and del Rosario 1979, Noerhadi 1979) have used the tissues from young inflorescences which have the advantage of producing sterile material since they are enclosed in spathes. It is not necessary to use any disinfectant. Generally, spathes are washed and removed, then explants are sampled under aseptic conditions. The outer spathe was washed satisfactorily with dilute sodium hypochlorite (6%). Moreover, inflorescence explants are abundant, since there are over 3000 flower meristems per inflorescence (Eeuwens and Blake 1977).

If young inflorescence is used (Eeuwens 1976, 1978, Suryowinoto et al. 1979, Noerhadi 1979, Blake and Eeuwens 1978), it seems particularly interesting to try to induce the development of flower meristems into vegetative shoots with a view to bypassing the callus stage (Eeuwens and Blake 1977, de Guzman and del Rosario 1979, Blake and Eeuwens 1978, 1982, Iyer 1982). It must be pointed out that very young inflorescences are more likely to induce such reversions. However, the minimum age of inflorescences to be sampled from the adult tree without damaging the apex (so that the tree can continue to develop) is determined. Thus, it was possible to observe that the youngest available inflorescence is found at the F-1 leaf axil on a Malayan Yellow Dwarf × West African Tall hybrid, and is less than 5 cm long (the largest leaf on the spear is defined as F-0). The physiological age of the explant plays a decisive role in the in vitro behaviour. Therefore, from the beginning of the studies, it is essential to use the type of explant which could be the original material for the micropropagation of selected adult trees.

In the ORSTOM laboratory, we have used mainly foliar tissues which are leaflet fragments obtained from young non-chlorophyllous leaves at the stage of elongation. No disinfection is necessary with this type of material, which is very clean, being inside the spear and protected by the petiole bases of the older leaves. Moreover, it is abundant, and it is possible to obtain thousands of explants from one adult tree. It can be transported without any damage and a sample composed of the basal part of the spear was sent by air from the IRHO station at Port Bovat in Ivory Coast. The sample is taken some 30 cm above the apex so that the latter remains intact, and the tree can continue its growth.

Apavatjrut and Blake (1977) described the use of pieces of stem taken from the subapical tissues. These tissues were obtained from adult trees of the Malayan Dwarf variety, and cut into discs. Finally, a more recent study by Blake and Eeuwens (1982) reported the use of excised seedling apices which contained the vegetative apex, a small amount of stem tissue and about five young leaves.

2.2.2 Explant Behaviour and Induction of Callus

The early studies on the formation of callus were aimed mainly at determining the culture conditions which would allow explants to survive and grow. Problems of browning were reported in the first in vitro cultures, however, the publications available give few details about their intensity, location in the tissues, their emergence and consequences. Eeuwens (1976) made a detailed study of the influence of mineral media on the explants of inflorescence rachillae fragments, or stem and leaf base fragments. Observations referred to the increase in the fresh weight of tissues during a maximum of 6 weeks in culture. This culture period is, however, relatively short. These studies emphasized the favourable influence of a specially developed mineral solution (macro- and micro-elements) as compared to

media of MS, Heller (1953) and White (1943). The results showed that the ratio between ammonium and nitrate had a considerable influence, and they could demonstrate a requirement for both a reduced form of inorganic nitrogen and high concentration of nitrate. The presence of both the ammonium ion and the nitrate ion in the culture medium also had a favourable influence on the stem explants sampled in the subapical tissues of adult trees (Apavatjrut and Blake 1977). There was a considerable increase in growth when the iodine concentration was ten times higher than in Murashige and Skoog medium. Eeuwens suggested that this requirement for a mineral-concentrated and iodine-rich solution could feature an adaptation to a natural habitat characterized by soils subjected to the influence of sea water or at least to sprays. However, root explants did not seem to have the same type of requirements, since their growth was not affected by the removal of the iodine ion from culture media (Justin and Fulford 1980). The influence of organic nutrition and hormones on fragments of young inflorescence rachillae was also studied by Eeuwens (1978). This study referred to the influence of the different sources of organic nitrogen (individual amino acids, casein hydrolysate, mixture of L amino acids), carbohydrates, auxins, cytokinins and gibberellins. Results showed that growth substances such as auxins (2,4-D and NAA) stimulated growth only at rather low concentrations (10^{-7} M) and that their presence in the culture medium was inhibitory and led to the death of tissues at concentrations of 10^{-6} M. Among the cytokinins used at the concentration of 10^{-6} M, BAP, SD 8339 and zeatin were the most effective. Apart from the growth of explants, different types of proliferation could be obtained. Cell divisions which led to the formation of a surface callus occurred in the upper part of stem, leaf base or inflorescence explants (Eeuwens 1976). However, the increase in the fresh weight of explants would result mainly from the growth of the original tissue (Eeuwens 1978). Proliferations were more intense in stem explants and they led to the formation of bigger callus-masses (Apavatjrut and Blake 1977). The development of these proliferations is promoted by temperature (31 °C), the presence of coconut milk (15%) and the nitrogen source (NH_4Cl 8 mM and NO_3Na 8 mM). In this case, the first divisions could also be observed on the surface of the explant which occurred throughout the explants. The authors identified different cellular types in these calli and certain cells resembled proembryos, arising from the division of a large cell. In these studies (Eeuwens 1976, 1978, Apavatjrut and Blake 1977), the attempts to subculture callus were not successful. Following subsequent trials, callus obtained from seedling cores or pieces of inflorescence rachillae have been maintained in subcultures (Blake and Eeuwens 1982). The presence or absence of activated charcoal and the auxin concentration had an influence on the formation of these calli. The auxin of choice for callus formation is 2,4-D as compared to IAA, NAA and IBA. Similarly, the authors showed that the multiplication of callus could be achieved under a range of conditions, but it was always improved by the presence of activated charcoal. With repeated subcultures, callus underwent structural changes, suggesting that they were potentially organogenetic (Blake and Eeuwens 1982). Both rapid-growing friable callus from roots, and slow-growing compact form were obtained with different level of cytokinin (Fulford et al. 1981). Growth was stimulated by auxins (NAA 1 mg l^{-1} or 2,4-D 0.02 mg l^{-1}).

Proliferations occurring in the cotyledonary sheath were obtained from excised macapuno embryos in the presence of 2,4-D (de Guzman et al. 1978). They were maintained in subculture and led to two types of growth behaviour. The first, nodular type would be similar to orchid protocorms. The second type consisted of tissue lumps characterized by smooth surface and a spongy texture which could grow into callus. Therefore, de Guzman distinguished two possible types of organogenesis: direct regeneration through protocorm-like structures, and indirect regeneration from callus. D'Souza (1982) cultured excised embryos from the Tiptur Tall variety and observed that callus was obtained from the cotyledonary sheath. These calli, which could be composed of protocorm-like outgrowths, emerged when embryos were transferred to a medium containing 5 to 7.5 mg l^{-1} ANA.

In our laboratory, studies were carried out mainly from explants of young leaves which were excised without damaging the apex. The individuals used were IRHO hybrids PB 121 (Malayan Yellow Dwarf × West African Tall). Explants were composed of fragments of leaflets. They remained healthy, showed no browning and grew even in a simple medium devoid of growth substances. Slight proliferations of scar type were formed in the damaged tissues (cuts or wounds) (Fig. 2A). Eeuwens' mineral medium (1976) seemed to promote the formation of these scar proliferations (Pannetier and Buffard-Morel 1982b). The presence of growth substances, particularly auxins with a view to the formation of callus, led to browning, which was all the more considerable as the auxin concentration (2,4-D or TCPP) was high. Activated charcoal was used to avoid this phenomenon. Then it was possible to observe that the auxin concentration available for tissues was lower by at least a factor of 100 than that of a medium devoid of charcoal. Moreover, the same observation was made by Fulford et al. (1981) in his studies on the culture of root explants. Using Eeuwens mineral medium supplemented with Morel and Wetmore vitamins (Morel and Wetmore 1951), 30 g l^{-1} saccharose, 2,4-D at different concentrations, activated charcoal and agar, Pannetier and Buffard-Morel (1982b) obtained tissue proliferations on explants. First, there were scar proliferations whose growth was limited (Fig. 2A) and internal ones which led to the formation of nodular calluses (Fig. 2B). These proliferations were obtained from both nursery plants and 5-year-old trees or even adult trees. The percentages of explants with nodular callus amounted to 50% in young individuals and to about 30% in adult trees. These nodular calli were most often small-sized and emerged on the lower face of the leaf blade (Fig. 2B). One month after their emergence, they were numerous, and well organized and sometimes joined together (Fig. 2E). In certain cases, they had a more diffuse appearance (Fig. 2F). Once they were isolated from the explants, they could be maintained in culture media containing 2,4-D for several months. The first histological examinations showed that they emerged at the level of the vascular bundle. Calli at earlier stages of development were composed of clumps with small-sized cells with high nucleocytoplasmic ratio (Fig. 2C, D). A number of observations on their histological structure in the subsequent development and their behaviour towards different amounts of auxin led us to think that at least a number of these nodular calli could correspond to pseudo-roots. When they were isolated and cultured on media devoid of auxins, they often gave rise to roots.

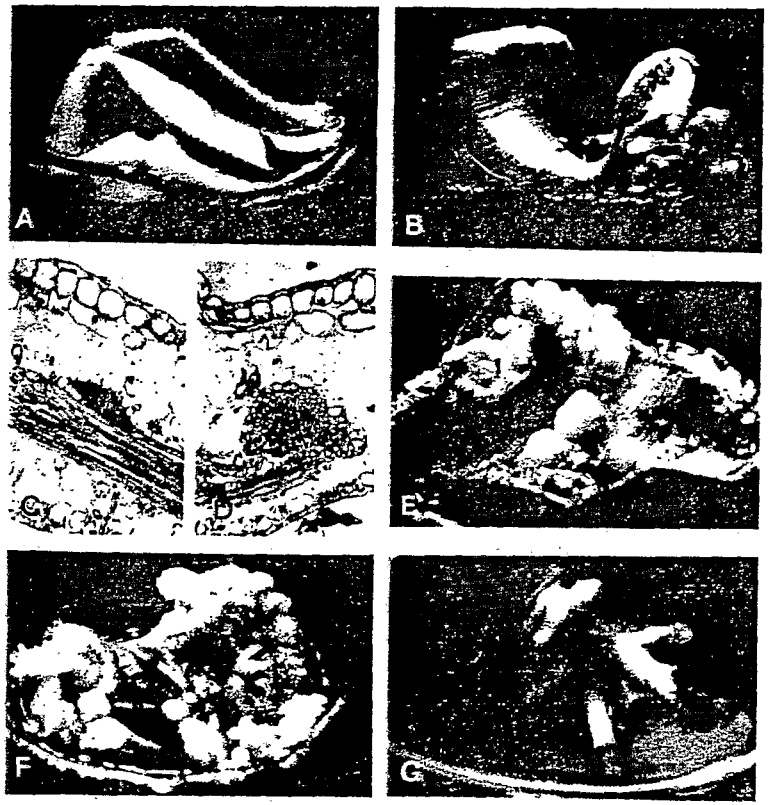

Fig. 2A–G. A Leaflet explant with scar-type proliferations; B Appearance of nodular callus at the lower face of the explant; C, D Sections of explants: C Appearance of first dividing cells; D More advanced stage, meristematic clump near a vascular bundle; E Nodular callus after about 80 days' culturing; F Callus exhibiting a diffuse aspect; G Development of roots directly on the leaf explant

Moreover, roots were obtained directly on explants when they were cultured in the presence of low amounts of auxin, or in an auxin-free medium (Fig. 2G). A detailed study conducted by Hunault (1978, 1979a, b, c, d) had shown that the treatment of monocotyledon explants by strong growth substances such as 2,4-D led to an intense neoformation of primary meristems which were most often of root type. The latter were united and they branched out erratically into nodulous or granular colonies (Hunault 1978, 1979a–d). These formations have been designated organoids by Hunault.

Various studies on the behaviour of coconut explants with respect to the culture medium showed that they were particularly sensitive to auxins. Eeuwens

(1978) pointed out that the auxin concentrations which were commonly used in other palm species (oil palm or date palm) were lethal in coconut tissues. Activated charcoal was used by a number of workers (de Guzman et al. 1978, Fulford et al. 1981, Blake and Eeuwens 1982, Pannetier and Buffard-Morel 1982a, b). Apart from this sensitivity to auxins, one of the roles of charcoal could consist in absorbing these substances and in reducing the amount of free auxin. Of the auxins used, 2,4-D was the most effective in producing callus.

In short, two main types of proliferation were obtained. In the early studies, proliferations appeared only on the surface and their growth was rather limited on fragments of young leaves and inflorescences, and they could not be subcultured (Eeuwens 1978, Apavatjrut and Blake 1977). In subsequent studies, callus grew faster on embryo tissues, inflorescence fragments or pieces of young leaflets, and they could be subcultured (de Guzman et al. 1978, Blake and Eeuwens 1982, Pannetier and Buffard-Morel 1982a, b).

2.2.3 Organogenesis

Root initiation was most often observed from coconut tissues. Eeuwens showed that explants of inflorescence spikes initiated roots after 6 weeks in in vitro culture when they were transferred from a medium with rather low levels of auxin (NAA $2.5\ 10^{-7}$ M) to a medium containing levels of auxin ten times higher. Their frequency of occurrence increased with the NAA concentration, while on the contrary, cytokinins did not seem to have any influence on this phenomenon (Eeuwens 1978). Isolated roots from explants have continued to grow and produced laterals when they were placed in a static liquid medium in which the NAA concentration was reduced. Fulford et al. (1981) succeeded in producing callus from root explants, and the callus gave rise to roots in the absence of auxin. In our laboratory, the explants of young leaves which were cultured on media devoid of activated charcoal but in the presence of low levels of auxins (2,4-D or TCPP) gave rise to roots (Fig. 2G). When maintained in culture, the latter could produce lateral roots. Similarly, callus transferred to media devoid of auxins or in the presence of NAA grew very often into roots. Callus obtained from embryo tissues cultured in vitro could, under certain conditions, grow into roots with pneumatophores (D'Souza 1982). De Guzman et al. (1978) pointed out that root-like structures developed from excised embryos cultured in the presence of 2,4-D. Therefore, the development of more or less typical roots seemed to be a rather general pattern for coconut tissues cultured in vitro. The explants which gave rise to these root neoformations were numerous, and they came from different individuals (embryos, seedlings, or adult trees). The auxin concentration seemed to be the most determining factor. The presence of NAA in culture media seemed to promote the root neoformation from explants or even callus. It could be mentioned again that callus from leaf tissues would represent most frequently pseudo-roots which stopped elongation in the presence of 2,4-D.

Results on the regeneration of buds or embryoids are more fragmentary. The histological study of proliferations obtained from different types of explants showed promising structures. Proliferations in which some cells appeared to resemble pro-embryos or embryoids were obtained from stem explants by Apavat-

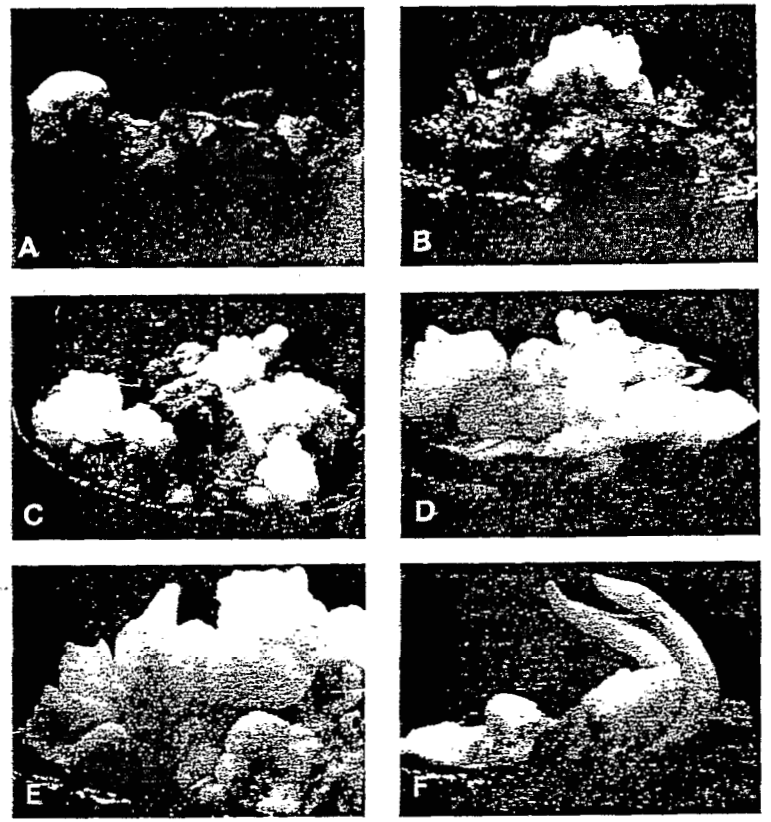

Fig. 3A–F. **A** Young embryoid on isolated callus; **B** Small mass composed of several embryoids; **C** Granular culture made up of very young coalescent embryoids; **D** Culture of embryoids in multiplication; **E** Part of a culture of embryoids in multiplication; **F** Development of embryoids

jrut and Blake (1977). Proliferations of the cotyledonary sheath of in vitro embryos could be transformed after repeated subcultures into nodular structures where shoot primordia or embryoids could be observed (de Guzman et al. 1978). The authors compared these nodules to protocorms, and scale leaves were observed in one of these cultures. Finger-like projections developed from endosperm callus (Fisher and Tsai 1978); although these structures resembled embryoids, they did not show any vascular formation or organized meristem through their histological study. Cultures of tertiary root callus which had been maintained for several months became friable, and led to the formation of meristematic nodules (Justin and Fulford 1980). Although no regeneration was

achieved, Blake and Eeuwens (1982) pointed out that callus on inflorescence explants underwent a morphological change in their structure, suggesting that they were potentially organogenetic. In our laboratory, nodular callus from leaf explants was isolated. Some of these structures gave rise to structures which showed morphological characteristics similar to those of somatic embryos. They appeared in the form of small white nodules, a pearly luster suggested the presence of an epidermis (Fig. 3A, B). Histological studies (Pannetier and Buffard-Morel 1982a, b) revealed a vascularization, an epidermis and an organized meristematic zone. However, these embryoids did not form simple structures and grew quickly into complex masses composed of various united embryoids (Fig. 3D, E). As soon as they appeared on the callus, there was a phenomenon of multiplication. Although leaf-like structures could be observed and some of the embryoids showed a promising development (Fig. 3F), no complete plantlet was obtained. Embryoid-like structures could be obtained from three types of material taken from the Malayan Yellow Dwarf × West African Tall hybrid, namely nursery plants, 5-year-old trees, and adult trees. The latter had been selected because of their productive potential.

2.2.4 Culture of Flower Meristems

The purpose of this culture was to obtain a development of flower meristems into vegetative shoots, thus bypassing the callus stage. This culture has been the second line of research concerning the coconut micropropagation. The age of inflorescences, and therefore the differentiation of flower meristems, played a decisive role. Young inflorescences such as inner spathe 2 to 20 cm long (Eeuwens 1976, 1978, Eeuwens and Blake 1977, Blake and Eeuwens 1978) and outer spathe 3.5 to 22 cm long (de Guzman and del Rosario 1979) were used. As a result of screening studies, Iyer (1982) found that the spathe from the first open leaf after the central spindle was the ideal stage. In our laboratory, preliminary experiments were conducted with inflorescences from 2 to 30 cm long (outer spathe). The inflorescence itself was much smaller than the outer spathe: in fact, a 16-cm-long outer spathe corresponded to a 4-cm-long inflorescence. Using fragments of rachis bearing several flower meristems, different types of development of these meristems were identified (Blake and Eeuwens 1978, 1982): flowering type where floral parts could be recognized, spike-like projections and "shootlet" type with an active growing point laying down leaves or bracts. Iyer (1982) also obtained atypical developments, and observed especially the formation of a columnar structure full of scale leaves. In both cases, cytokinins: BAP and gibberellin (Blake and Eeuwens 1982), BAP and dimethyl allyl amino purine riboside (Iyer 1982) played a considerable role. A study of the influence of BAP concentration on the frequency of occurrence of meristems showed that the optimal level would be 5×10^{-6} M (Eeuwens and Blake 1977). Different types of vegetative shoots with short-scale or elongated enrolled leaves were obtained in the presence of 2,4-D by de Guzman and del Rosario (1979), who emphasized that these differentiations were unstable in that a reversion from a vegetative to a flowering state had been observed in several cultures. Moreover, several shoots could be obtained from a single meristem, which could be significant with a view to vegetative

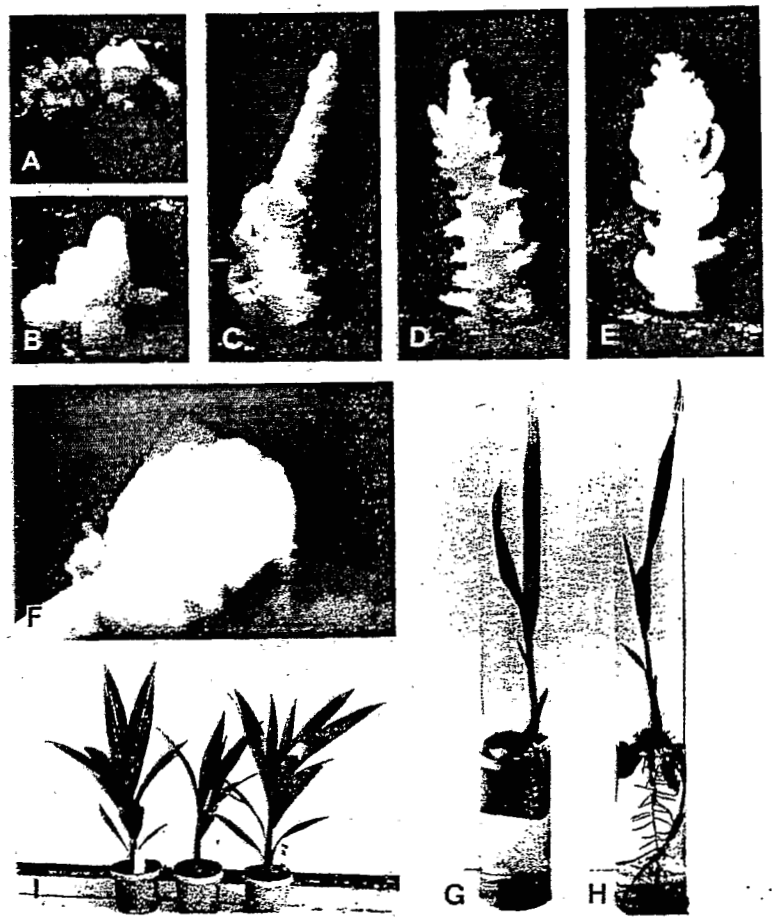

Fig. 4A–I. **A, B** Development of young floral buds; in **B** note the development of stamens; **C, D, E** Different types of shoots obtained by atypical development of floral meristems; **F** Embryo-like nodule emerging from an anther. (Photograph given by Monfort); **G, H** In vitro germination of excised embryos: **G** Seedling with a rudimentary main root; **H** Complete seedling showing a sufficient development to be transferred in soil; **I** Seedlings from embryos germinated in vitro, established in soil

propagation (de Guzman and del Rosario 1979, del Rosario and de Guzman 1982).

In our laboratory, we conducted a preliminary experiment on inflorescence explants taken from a Malayan Yellow Dwarf × West African Tall hybrid. Explants were composed of segments of rachillae or complete rachillae as related to the size of the inflorescence, and a few flower buds were also isolated and cultured. A flower-like development (Fig. 4A, B) was observed in the older inflorescences (15–28 cm long). On the contrary, a number of flower meristems from younger inflorescences yielded shoots which showed different morphological characteristics (size of shoots, number and size of leaf-like structures). These shoots could be isolated and cultured (Fig. 4C–F). These results were obtained on the basal medium of Eeuwens (1976) supplemented with Morel and Wetmore vitamins (Morel and Wetmore 1951), sucrose and phytohormones (2,4-D and BAP) at different concentrations and activated charcoal. Rooting of shoots which were obtained through the atypical development of flower meristems was achieved (Eeuwens and Blake 1977, Blake and Eeuwens 1978) by a high auxin treatment. However, shoots could not be maintained when roots were formed.

Although the development of shoots designated as vegetative by some authors could be induced, a complete reversion from a flowering to a vegetative state has not been obtained.

2.3 Embryo Culture

The early studies on the culture of coconut embryos were aimed at determining the influence of endosperm placed in the culture media (Cutter and Wilson 1954). Results showed that the liquid endosperm extracted from immature nuts (in which solid endosperm was only several layers thick) and subsequently filtered, had a stimulatory influence on the growth of excised embryos. On the contrary, the liquid or solid endosperm of mature nuts inhibited this growth. Unfortunately, these studies had been made on embryos whose age and origin were not known. Afterwards, embryos from mature nuts were cultured by Abraham and Thomas (1962) in order to develop a method likely to test the vigour of some hybrids. Within 6 months, they grew into a seedling whose first leaf was fully developed. However, the root system remained rudimentary. The results of the culture media confirmed the favourable influence exerted by the liquid endosperm of immature nuts. Fisher and Tsai (1978) also carried out studies on the culture of excised embryos of two varieties, namely Jamaican Tall and Green Malayan Dwarf. The development of complete seedlings was obtained and varied considerably with the culture conditions. The development of a young seedling with a good aerial system was obtained by Iyer (1982) within 18 weeks. The root development was improved by decapitating the main root. When seedlings were transplanted to soil, the haustorium portion was infected, resulting in a high mortality of the seedlings.

All the studies mentioned here were aimed at determining the culture conditions which would allow leaves and roots to develop. Although the different authors made use of various media, one could point out that successive media were often used, and promoted firstly the development of the shoot, and secondly the

formation of a vigorous root system. Noerhadi and Toruan (1975) made use of Heller's medium (Heller 1953) and the Murashige and Skoog medium supplemented with IAA. Fisher and Tsai (1978) used a liquid MS medium enriched with coconut milk and various auxins (IAA, IBA, 2ip), and followed by an agar medium supplemented with activated charcoal. On the contrary, embryos were placed by Iyer (1981) first on an agar medium enriched with activated charcoal, and then on a liquid medium with a view to rooting. In our laboratory, studies have been made on the culture of excised embryos of the Malayan Yellow Dwarf × West African Tall hybrid (PB 121 created by IRHO). A comparison was made between different mineral media which showed that the Saunders and Burkholder medium (Saunders and Burkholder 1948) was favourable for the development of embryos (Ahee and Guenin unpublished). After 4 or 6 months in culture, about 30% of the embryos showed a well-developed aerial part. Although a spontaneous rooting occurred, it remained rudimentary and limited to a main root (Fig. 4G). Lateral roots could develop through an auxin treatment. After 8 months in culture, complete seedlings were obtained and their root system was vigorous. Fig. 4H shows one of these seedlings about to be transferred to soil. This return to usual culture conditions has been successfully achieved in greenhouses (Fig. 4I).

The purpose of the work on the macapuno embryo culture was to show that they could develop in vitro, while they did not germinate on the nut (de Guzman and del Rosario 1964). Although these in vitro embryos did not develop as a whole plant, the shoot growth was observed. Similarly, Ventura et al. (1966) observed that, after 4 months in culture, the first leaf emerged. In both cases, the root system was rudimentary, however, de Guzman (1970) found that transfer of the embryos from solid to liquid medium enhanced the root system growth. The addition of gibberellic acid (3×10^{-5} M) to the solid medium as a pretreatment had a favourable effect (de Guzman 1970). Balaga and de Guzman (1971) related that a better root development was obtained through a liquid medium than a solid medium. Although coconut milk had a favourable effect on growth when used in the first medium, it inhibited the root development (de Guzman 1970, Balaga and de Guzman 1971). These authors observed that the inhibitory effect of coconut milk varied with the type of mineral medium used (Balaga and de Guzman 1971). The addition of a high level of sugar (8% dextrose) to the culture medium led to an important root development which had not unfavourable effect on the leaf growth (de Guzman et al. 1971). Finally, del Rosario and de Guzman (1976) reported that the best root and shoot growth was obtained when the embryoids were transferred from a liquid medium with a rather poor mineral concentration (modified White's basal medium) supplemented with 8% dextrose to a solid MS medium containing 4% sugar. The role of sugar had been studied as an osmotic and metabolic agent. These authors later pointed out (del Rosario and de Guzman 1982) that trees obtained by this method of culture gave very high yields of macapuno nuts.

2.4 Haploid Production

Studies on the in vitro production of haploids are much less numerous than those on the embryo culture or vegetative propagation. Anthers cultured at uninucleate microspore stage on a medium containing Eeuwens' mineral elements supplemented with 6% sucrose, 2,4-D, kinetin and activated charcoal showed multicellular pollen within the exine. This result was obtained after pretreatment at 4 °C in the dark (Iyer 1982). Recently, Thanh-Tuyen and de Guzman (1983) reported pollen embryos from anther cultures and designated them as globular, heart-shaped, torpedo. The complex culture medium used is composed of Blayde's elements (Blaydes 1966) supplemented with Bourgin and Nitsch's vitamins (1967), EDTA iron (Murashige and Skoog 1962), inositol, coconut water as well as 2,4-D and NAA. No callus formation from pollen or anther tissues was observed. These results are promising, as it is known that direct embryogenesis from pollen grains affects genetic stability to a lesser extent. In France, similar studies have recently been made and the formation of multicellular pollen and the emergence of a few nodules whose morphological characteristics were similar to embryos have been observed (Fig. 4F and Monfort, personal communication). The studies conducted in the Philippines (Thanh-Tuyen and de Guzman 1983) made use of inflorescences from the Laguna Tall variety. The studies conducted in France made use of two types of hybrids: a Malayan Yellow Dwarf × West African Tall hybrid (IRHO PB 121) and a West African Tall × Rennel Tall hybrid.

2.5 Protoplast Culture

Eeuwens and Blake (1977) pointed out that protoplasts could be liberated from the coconut callus and the viability of these protoplasts is estimated at 50%. The isolation of protoplasts from inflorescence tissues has also been described (Haibou 1981, Haibou and Kovoor 1982). According to these authors, the main problem for keeping protoplasts is the presence of numerous raphides. A technique avoiding agitation during the enzymatic treatment permitted the protoplasts to be maintained. They were then separated from the raphides by a quick sedimentation. The regeneration of the wall and divisions that could lead to the formation of microcallus were obtained (Haibou and Kovoor 1982).

3 Conclusions

One of the main objectives of the use of tissue culture has been to find a method of vegetative propagation, since the coconut morphology does not allow its propagation by classical techniques. Various tissues used by the different research groups are: inflorescences, roots, young leaves, embryo tissues. We have obtained callus formation from individuals of various ages: nursery plantlets, young trees and adult trees. About ten different adult trees, chosen for their yield capacity, have been used and callus has been obtained on all these trees.

Moreover, embryoids have been neoformed from callus within a minimum of 6 months after their being cultured. The present development of these somatic embryos is such that we can reasonably hope to obtain plantlets in a relatively short time.

Using a method of plantlet neoformation via callus could bring some problems of variability. This is why studies aimed at a direct formation of vegetative buds from flower meristems have been carried out by various workers. However, even if shoots without the typical characteristics of flowers have been obtained, the results do not seem, as far as we know, as positive as could be expected. Blake and Eeuwens, who have carried out studies on the flower meristem culture, concluded in 1981 that the most promising results concerning coconut micropropagation have been obtained on embryogenesis from callus. It is still necessary to intensify the research in this area, but we think that in the near future the application of in vitro culture on a large scale for coconut propagation can be envisaged, as is the case now for the oil palm.

Besides vegetative propagation, results obtained on coconut tissue culture allow envisaging certain applications: (1) in vitro haploid individuals, offer the possibility of fixing useful characters in a quicker way than through classical selection methods; (2) The culture of excised embryos of macapuno nuts has led to develop individuals presenting a high percentage of nuts with the desirable endosperm; (3) The culture of excised embryos of different varieties or hybrids can lead to applications in exchange and conservation of germplasm (Bajaj 1983c).

Considering the economical importance of coconut, we think that in vitro research will be intensified in the coming years. Advanced techniques such as protoplast culture are already being studied. Moreover, from a more fundamental point of view, research will supply information that will complete those obtained on other palms and would lead to a better understanding of the phenomena of regeneration of these menocotylodons.

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