4.1 *Cocos nucifera* Coconut

Valérie Hocher, Jean-Luc Verdeil and Bernard Malaurie
IRD/CIRAD Coconut Program, UMR 1098 BEPC, IRD, BP 64501-911 Av. Agropolis, 34394 Montpellier, Cedex 5, France

1. Introduction

1.1. Botany and history

The coconut palm (*Cocos nucifera* L.) is a relatively slow growing woody perennial species. It is the only species in the genus *Cocos*. All forms known to date are diploid (2n = 2x = 32). No closely related species with even partial interfertility has been reported (Bourdeix *et al.*, 2001). The lifespan of a coconut palm can be > 60 years under favourable ecological conditions. Coconuts can grow to a height of approx. 25 m (Ohler, 1999).

Optimum growing conditions for coconut are in the lowland humid tropics at altitudes < 1000 m near coastal areas in sandy, well-drained soils (Persley, 1992); however, coconuts are adaptable to other soil types including coral atolls and soils with moderate salinity (Batugal, 1999). Coconuts are also commonly cultivated several hundred kilometres inland, e.g. surrounding Lakes Victoria, Tanganyika and Malawi in Africa (Lombard, 2001). Coconuts cannot tolerate temperatures < 0°C and ideal growing temperatures range between 24 and 30°C (Woodroof, 1979; Persley, 1992).

Coconuts do not form a tap root, but develop a fasciculated root system, consisting of adventitious roots at the base of the stem, which typically grow laterally to 2–3 m length and 30–120 cm deep and continuously generate adventitious roots (Reynolds, 1988; Persley, 1992). Nutrients and water are absorbed by the rootlets.

The coconut palm 'trunk' is a stem with no true bark, no branches and no cambium. Secondary growth (increased stem diameter) is by secondary enlargement meristem located below the shoot meristem. Growth depends on age, ecotype and edaphic conditions, but is generally between 30 and 100 cm per annum. The stem is surmounted by a crown of approx. 30 compound leaves, which protect the terminal vegetative bud and whose destruction causes the death of the palm. An adult coconut has virtually as many unopened (20–30) as opened leaves. Leaves are produced continuously at approx. 1 month intervals.

The coconut palm is a monoic species. Flowering may begin between 3 and 10 years after planting. Each leaf bears an inflorescence primordium in its axil. The coconut inflorescence is a spadix, which develops within a double sheath referred to as a spathe. When mature, the spadix breaks through the spathe and 30–35 spikelets emerge, each bearing a large number of male flowers (200–300) with one or two female flowers at the base of each spikelet. Flowers are sessile and follow the trinary organization of monocotyledons (Menon and

Pandalai, 1958). Male flowers have three short sepals, three petals, six stamens and one rudimentary pistil. Female flowers are approx. 3 cm in diameter, and are enveloped by small scaly bracteoles enclosing three sepals and three petals, which overlap each other and surround the spherical pistil. The ovary is tricarpous and each carpel has a single ovule. After fertilization, a single ovule develops and the two others abort or degenerate. The inflorescence can be either self- or cross-pollinated (Bourdeix *et al.*, 2001). Pollination is by wind or insects.

The appearance of the fruit (size, shape and colour) varies according to the ecotype (Bourdeix *et al.*, 2001). The coconut is a drupe, whose development requires approx. 1 year. Only 25 to 40% of the female flowers develop into mature nuts and a tree produces < 100 fruits per annum. After fertilization, the husk and shell increase in size and the cavity of the embryo sac enlarges considerably (Menon and Pandalai, 1958). The cavity is filled with a liquid endosperm. After 6 months, the solid endosperm develops as a thin and gelatinous layer against the inner wall of the nut cavity (Ohler, 1999). After 8 months and towards the later stages of ripening, the endosperm becomes hard and white and is surrounded by a hard, brown testa (Ohler, 1984). The immature endosperm is composed of 95% water and < 1% oil, and 50% water and 30–40% oil at maturity (Ohler, 1984). When ripe, the nut generally falls. The seed, which is one of the largest in the plant kingdom, is characterized by lack of dormancy and the time necessary for development from embryo to plantlets (Blake, 1990; Verdeil, 1993).

Four months are generally required for the first leaf to emerge from the husk. A characteristic of coconut zygotic embryos is the substantial development of the haustorium (distal part of the cotyledon) within the nut cavity during germination (Menon and Pandalai, 1958). This organ invades the nut cavity and establishes intimate contact with the endosperm. It enables the hydrolysis of the endosperm and the mobilization of nutrients required for embryo germination. Lipase, protease and saccharase activity have even been detected (Bertrand, 1994). Histological studies have demonstrated digitations in the epidermal layer in contact with the nutrient reserves, and the existence of vascular bundles converging towards the embryonic axis. This villosity displays numerous structural similarities to stomach villi in the digestive system of animals (Verdeil and Hocher, 2002).

Fossil nuts > 15 million years old and very similar to present-day coconuts have been discovered in New Zealand and India (Sauer, 1967, cited by Harries, 1978; De Taffin, 1998); however, the exact geographic origin of this species is uncertain. In all probability, the coconut tree was first cultivated either in India or in South-east Asia. The coconut has attained its highest development in terms of variability and number of local names in South-east Asia.

1.2. Importance

The coconut palm has been referred to as the 'tree of life', because of its importance as a subsistence crop in most tropical areas of the world. It is grown on > 11 million ha, 94% of which are in Asia and the South Pacific (Blake, 1990). World production of coconut has been estimated to be 52,940,408 t (FAOSTAT, 2004). The leading producers are Indonesia and the Philippines (> 13,000,000 t), India (9,500,000 t), Brazil (2,833,910 t), Sri Lanka (1,850,000 t), Thailand (1,400,000 t), Papua New Guinea (570,000 t), Vietnam (920,000 t) and Mexico (959,000 t). Many coconut-producing countries are small islands in the South Pacific and Indian Oceans and the Caribbean region (Daviron, 1995), where coconut can be grown in harsh environments, such as atolls, and can tolerate swampy and water-deficient areas and poor soils. Coconut is an important attribute of the rural economy (Punchihewa, 1999), and is cultivated by many farmers on small landholdings (< 4 ha) often in association with other crops (root crops, vegetables, cacao, etc.) (Barrant, 1978; Reynolds, 1988; Freud and Daviron, 1994). Only 10% of the planted areas constitute commercial plantations. Coconut palm is cultivated mainly for copra (dried endosperm) production, from

which oil is extracted and provides income for smallholders in the tropics and subtropics.

The coconut has been a primary source of food, drink and shelter for millions of people from the earliest days of humankind (Batugal, 1999; Punchihewa, 1999). Coconut farmers are deeply attached to the various products (Punchihewa, 1999), and have contributed to its adaptation to a wide range of environmental conditions. Although significant achievements have been made with respect to the release of high copra-yielding hybrids (Bourdeix *et al.*, 2001), this progress has yet to reach most coconut producers.

The coconut is mainly a subsistence crop, e.g. 70% of the production is consumed locally in Asia. Every part of the plant can be used. Oil from the fresh nuts is used for food preparation in many countries of Asia and the Pacific. The kernel can be oven- or sun-dried to a moisture content of 6% (copra), and can be conserved for months before oil extraction. Coconut water is a very refreshing drink. Endosperm of mature nuts is grated and used in pastries. The woody stem is used as a building material and in joinery. The leaves can serve for local handicrafts and as roofing material. The processed sap provides sugar, syrup and vinegar. The fibres from the husk surrounding the nut can be used to manufacture esparto-type goods. More ecofriendly than rock wool, these fibres can also be used as a substrate for growing plants (Bourdeix *et al.*, 2001).

Plantations were developed throughout the tropics by the end of the 19th century to satisfy the need for coconut oil for industrial uses (Daviron, 1995), including the extraction of glycerine, a component of dynamite. Until the mid-20th century, coconut was the main oil source in the world market. Coconut oil is extracted from the dried endosperm (copra) and, together with oil palm kernel oil, is the only source of short-chain fatty acids (from eight to 14 carbon atoms), and a rich source of lauric acid (~48%) (Persley, 1992). It is used in soap manufacture and in the cosmetic industry (Blake, 1990; Verdeil *et al.*, 1996a). The melting point of coconut oil is 24–27°C and hydrogenation is not required to inhibit rancidness because of its stability; coconut oil is therefore widely used in food products (margarine, confectionery, etc.) (Ohler, 1984). With only 4% of the world oil production, coconut ranks seventh among oil-bearing crops. In the competitive international world oil market, the coconut palm is gradually being replaced by other oil-seed plants such as soya and oil palm (Freud and Daviron, 1994). The coconut palm is therefore reverting to a multipurpose crop, especially for its fruit. Several reasons can explain this gradual decline: (i) low productivity due to old age of coconut plantations (two-thirds of the individuals are > 60 years old) and insufficient replanting; (ii) use of unimproved material and marginal culture practices; (iii) several pests and diseases, e.g. lethal yellowing (LY) and Cadang-Cadang; (iv) production in areas often subjected to natural calamities, e.g. typhoons or volcanic eruptions; and (v) low prices for coconut oil despite its high quality and lower production (Freud and Daviron, 1994). In addition, rapeseed oil, which has been genetically modified to produce oil (Laurical®), with a higher content of lauric acid (37%), has had a significant impact on production. Despite these difficulties and stagnant production for 20 years, coconut oil is still important, and there continues to be demand for lauric oil for the soap industry (Freud and Daviron, 1994). With the assistance of the World Bank, the Philippines has started a replanting programme using improved hybrids, and LY was recently declared a national priority for research in Mexico (Aldaba, 1995; INIFAP, 1998). The CGIAR has even recognized coconut as the oil crop most in need of international research.

1.3. Breeding and genetics

1.3.1. Plant characteristics

Propagation is entirely by seed. Allogamy causes a high degree of variability. The breeding cycle is very long (12 to 16 years), with a low number of seeds produced (100 to 200 seeds/tree per annum) and a large recalcitrant seed that makes exchange and conservation of germplasm extremely difficult. These morphological and biological charac-

teristics impose serious constraints on breeding. There are three groups of coconut palms – Tall (*C. nucifera typica*), Dwarf (*C. nucifera nana*) and hybrids between the two. Tall palms represent the more common type and account for > 95% of coconut production because of their general superiority in copra production (Woodroof, 1979; Persley, 1992). Dwarfs are distinguished mainly by slower growth. They generally produce lower quality copra than Talls and for this reason are often not used for large-scale plantings (Woodroof, 1979). Dwarfs exhibit other features, e.g. preferential autogamy, reduction in organ size, early maturity and rapid fruit production. Because of these last two characters, Dwarfs are very important in breeding programmes (Bourdeix *et al.*, 2001).

1.3.2. Breeding objectives

The diversity of coconut uses ensures that there is no single ideotype. Breeding objectives are particularly complex, and include a tradeoff between food, cultural habits and processing requirements. The highest priority is increased production of copra per hectare (Bourdeix *et al.*, 2001). Other important objectives include precocity, adaptation to certain edaphoclimatic conditions (drought, cold, pH) and resistance to diseases. Several pathogens (see Table 4.1.3), including fungi (*Phytophthora* spp.), trypanosomes (heart rot), nematodes (red ring), viruses (coconut foliar decay (CFDV)), viroids (coconut cadang cadang (CCCVd)) and phytoplasma (LY) cause heavy losses. The genetic improvement of the coconut relies on exploitation of the variability within the species. Coconut breeding began in India in 1916 (Harries, 1978), although major progress was not obtained until the 1960s. Currently, 20 centres throughout the tropics are involved in coconut breeding.

Hybrids can include: Dwarf × Tall, Tall × Tall or Dwarf × Dwarf (Harries, 1991). According to Ohler (1984), breeders and growers prefer the Dwarf × Tall type because of early maturity, ease of production and seed whose quality can be readily controlled. Nevertheless, other hybrid types can also provide certain advantages depending on the cultivation system and use. The breeding programme of the Centre de Coopération Internationale en Recherche Agronomique pour le Développement – Departement Cultures Pérennes (CIRAD-CP) uses reciprocal recurring selection as a starting point. The method involves exploiting ecotype combining ability and basing phenotypic choices on heritable characters (Gascon and de Nucé de Lamothe, 1978) and has been described in detail by de Nucé de Lamothe (1970) and Gascon and de Nucé de Lamothe (1976). Genetic improvement involving hybridization between ecotypes has resulted in a doubling of the outputs within 20 years. The best hybrids can increase profits by 20 to 30% within a generation.

Genetic gain has been assisted by the development of reliable hybrid seed production techniques using assisted pollination (Wuidart and Rognon, 1981). Hybrids are reproduced on a large scale, e.g. 1 ha of seed-bearing trees can produce *c.* 15,000 seeds per annum by assisted pollination (de Nucé de Lamothe and Wuidart, 1992). This method is complex, costly and time consuming (de Nucé de Lamothe and Wuidart, 1992), requiring emasculation of female parents, conditioning and conservation of pollen from male parents and manual or assisted pollination (Wuidart and Rognon, 1981). The cost of a selected seednut can be as much as US$2–4, which is too expensive for smallholders (Verdeil *et al.*, 1998a).

According to Baudouin (1999), the efficiency of breeding can be improved as follows: (i) combining genetically distant genotypes to increase heterosis; (ii) increasing selectable diversity in breeding populations; (iii) using molecular marker and quantitative trait loci (QTLs) to increase selection efficiency using marker-assisted selection (MAS); and (iv) using *in vitro* propagation for rapid dissemination of genetic gain (Verdeil *et al.*, 1995, 1998a).

2. Molecular Genetics

The application of MAS in coconut breeding is urgently needed because desired characters are expressed only after several years of

growth. The use of molecular markers offers certain advantages for identifying cultivars and for determining taxonomic relationships. The studied traits directly reflect variation that occurs within the genome, they are neutral and their expression is independent of the environment (Lebrun and Baudouin, 2002). Their use should increase the efficiency and efficacy of coconut genetic improvement, especially for germplasm management, genotype identification and MAS of important traits. In many species, molecular markers are being used to create genetic linkage maps in order to identify markers linked to specific traits that can form the basis for MAS. Construction of genetic maps would have great benefit for coconut.

2.1. Markers

Initial studies on genetic diversity characterization involved isozymes or polyphenol markers (Carpio, 1982; Canto-Canché *et al.*, 1983; Jay *et al.*, 1989; Fernando and Gajanayake, 1997; Cardeña *et al.*, 1998). The characterization of genetic diversity in coconut germplasm at the DNA level (Ashburner, 1999) has largely replaced these strategies. Various DNA markers have been used to measure coconut genetic diversity: inverse sequence-tagged repeat (ISTR) (Rohde *et al.*, 1995; Duran *et al.*, 1997); randomly amplified polymorphic DNA (RAPD) (Ashburner *et al.*, 1997; Duran *et al.*, 1997; Rodriguez *et al.*, 1997; Wadt *et al.*, 1999); restriction fragment length polymorphism (RFLP) (Lebrun *et al.*, 1998, 1999); amplified fragment length polymorphism (AFLP) (Perera *et al.*, 1998); simple sequence repeat (SSR) (Karp, 1999; Perera *et al.*, 1999; Rivera *et al.*, 1999; Teulat *et al.*, 2000). Two main coconut groups have been identified: Indian and Pacific Ocean. Analysis of DNA polymorphisms has indicated that the Tall and Dwarf types show different degrees of polymorphisms with more polymorphism in Tall types. Using microsatellites, a kit for identifying coconut cultivars is under development in CIRAD and should allow the large-scale application of molecular fingerprinting of coconut (Lebrun and Baudouin, 2002).

2.2. Linkage mapping and QTL analysis

In coconut, the availability of F_1 mapping populations from controlled crosses involving heterozygous parents has allowed linkage mapping of identified polymorphisms as well as the search for QTLs. An initial linkage analysis of the East African Tall (EAT) and Laguna Tall (LAGT) coconut types based entirely on ISTR markers was described by Rohde *et al.* (1999). This work was extended using AFLPs, ISTRs, RAPDs and inter-sample sequence repeats (ISSRs), and allowed the construction of a linkage map of the two parents of the cross involving Malayan Yellow Dwarf (MYD) × LAGT, resulting in 382 identified markers and 16 linkage groups generated for each parent and the identification of QTLs associated with early flowering and yield (Herrán *et al.*, 2000). In addition, QTLs for other traits, including leaf production and girth height, were identified for the same mapping population (Ritter *et al.*, 2000). AFLP and SSR markers have been used to construct a linkage map for a coconut type from the Solomon Islands, the Rennell Island Tall (RIT), which is used in various breeding programmes and as a male parent for commercial hybrids in the Pacific (Lebrun *et al.*, 2001). QTL analysis allowed the identification of loci linked to number of bunches and the number of nuts.

The identification of different QTLs provides the first opportunity for MAS in coconut. The most efficient use of MAS would be to produce parental lines for F_1 hybrid production and to search for LY-resistant hybrids (Cardeña *et al.*, 1999). According to Ashburner (1999), there is still a basic lack of knowledge of the genetics of the species. The large stature, long generation time and low multiplication rate will always hamper breeding. Molecular markers can minimize but not eliminate these problems.

3. Somatic Cell Genetics

3.1. Regeneration

Due to the time required in order to develop improved selections, micropropagation is

essential for distribution of selections that emerge from breeding programmes (Verdeil *et al.*, 1998a). Vegetative multiplication of elite selections is necessary for producing homogeneous planting material and thereby improving plantation productivity. Moreover, *de novo* regeneration of coconut is essential for genetic transformation; however, coconut palm is considered to be one of the most recalcitrant species for *in vitro* culture (Georges and Sherrington, 1984; Hocher *et al.*, 1999).

3.1.1. Somatic embryogenesis

Somatic embryogenesis involving different explant types has been attempted, including apical meristems (Hagedorn, 1990), young roots of mature palms (Justin, 1978), stems and leaves (Pannetier and Buffard-Morel, 1982; Gupta *et al.*, 1984; Raju *et al.*, 1984), zygotic embryos (Bhala-Sarin *et al.*, 1986; Karunaratne and Periyapperuma, 1989; Ueda *et al.*, 1993), inflorescences (Eeuwens, 1978; Branton and Blake, 1984; Sugimura and Salvana, 1989; Verdeil *et al.*, 1989, 1993) and plumules from mature embryos (Hornung, 1995, 1997; Chan *et al.*, 1998).

Induction. The primary explants for embryogenic culture must contain meristematic tissue, which proliferates in the presence of an auxin. Immature leaves and inflorescences are the most useful explants, as the phenotype of the mother tree is already known. Inflorescences are generally preferred because of a simplified protocol and an inflorescence sampling protocol which does not result in death of the tree (Rillo, 1989). Plumules (embryo meristem with the first primordium) have been utilized (Hornung, 1995, 1997), and this pathway can be exploited as a model for developing protocols using other explants and to multiply the progeny from selected parents (Saenz *et al.*, 1999).

Somatic embryogenesis generally occurs indirectly by directive induction; however, there is a single report of direct embryogenesis from leaf explants (Raju *et al.*, 1984), which is unusual since vascular tissue normally produces root primordia (Blake, 1989). Embryogenic cultures are induced from explanted tissues collected from adult coconut palms on various culture media. At the Institut de Recherche pour le Développement (IRD)/CIRAD, the Eeuwens Y3 mineral solution (Eeuwens, 1976) is used with Morel and Wetmore's vitamins (1951), 40 g/l sucrose, 7.5 g/l agar, 2 to 2.5 g/l activated charcoal and 99.55 to 271.5 µM 2,4-dichlorophenoxyacetic acid (2,4-D), due to the variable sensitivity between palms to auxin at pH 4.5–5.8 (Verdeil *et al.*, 1999). Murashige and Skoog medium (1962) (MS) with the addition of sucrose, activated charcoal and auxin is also employed. The cultures are usually incubated in the dark at 27°C (Buffard-Morel *et al.*, 1992; Verdeil *et al.*, 1994). Activated charcoal is necessary to control browning, which is a major constraint of coconut *in vitro* culture (Blake and Eeuwens, 1980, 1981; Pannetier and Buffard-Morel, 1986; Tisserat, 1990). The effect of activated charcoal appears to be due to reversible adsorption of the auxin and its slow and gradual release (Brackpool *et al.*, 1986; Ebert and Taylor, 1990; Ebert *et al.*, 1993; Verdeil *et al.*, 1999). The auxin 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has also been used for induction of nodular calluses from inflorescence explants (Buffard-Morel *et al.*, 1988; Verdeil and Buffard-Morel, 1995). The histology of callus has been studied (Buffard-Morel *et al.*, 1992; Verdeil *et al.*, 1992).

Callus grown on media with a gradually reduced auxin level (Blake, 1990) or with an increase followed by a reduction of auxin (Verdeil *et al.*, 1994) will eventually produce nodular structures (Fig. 4.1.1) that subsequently develop into proembryos (Fig. 4.1.2). Abscisic acid (ABA) appears to affect the formation of coconut proembryos (Samosir *et al.*, 1999b; Fernando and Gamage, 2000). Histological studies of embryogenic cultures indicate that there are two developmental pathways. A multicellular pathway occurs on medium with 2 g/l activated charcoal and 181–362 µM 2,4-D (Buffard-Morel *et al.*, 1992; Verdeil *et al.*, 1992, 1994), but has also been observed on medium containing ABA (Fernando *et al.*, 2003). Embryogenic cultures typically consist of meristematic and proembryonic structures. Initially, cells in the

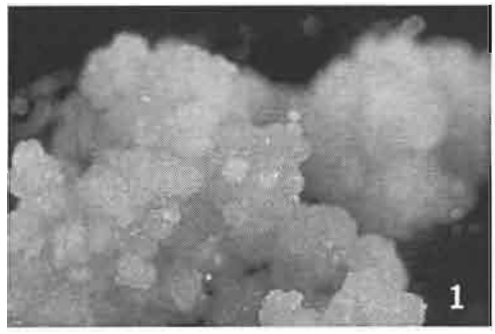

Fig. 4.1.1. Coconut embryogenic culture.

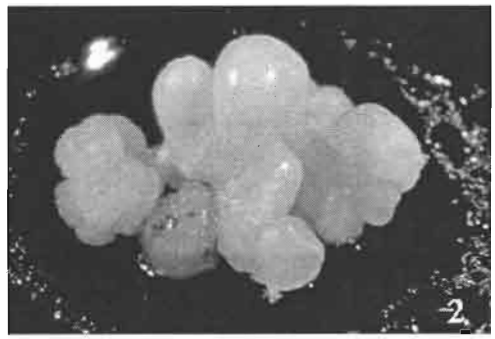

Fig. 4.1.2. Globular stage coconut somatic embryos.

cambium-like zones proliferate, and actively dividing cells give rise to meristematic nodules that develop a protoderm or epidermis. Proembryos develop from proembryonic cells in the periphery; however, if the auxin concentration is too low, anomalous structures, e.g. haustorium only, a root pole, foliar-type somatic embryos, etc., can develop (Branton and Blake, 1983; Brackpool *et al.*, 1986).

Another pathway occurs in the presence of 2–3 g/l charcoal and 362–543 µM 2,4-D, whereby individual embryos develop from single embryogenic cells (Schwendiman *et al.*, 1988; Verdeil *et al.*, 1994). In that case, typical proembryos develop according to the description by Haccius and Phillip (1979). The embryogenic cells have dense cytoplasm, a high nucleo-cytoplasmic ratio, a single and voluminous nucleolus and many starch and protein reserves. They become separated from the culture as a result of cell wall thickening (Lu and Vasil, 1985; Williams and Maheswaran, 1986; Schwendiman *et al.*, 1988). There are deep invaginations of the nuclear envelope, proliferation of dictyosomes and emission of Golgi vesicles, which is directly related to increased cell wall thickness (Verdeil *et al.*, 2001). Seven to 14 days after explanting, callose deposition blocks the plasmodesmata, resulting in physiological isolation. Acquisition of embryogenic competence was linked to the appearance of an outer layer of pectic material (mainly non-methyl-esterified) that entirely coats the embryogenic cells (21 days after explanting) (Verdeil *et al.*, 2001). Specific nutrient requirements have been observed (Dussert *et al.*, 1995a,b; Magnaval *et al.*, 1995, 1997). Tyrosine phosphorylated proteins and tyrosine kinase activity increase under induction conditions (Islas-Flores *et al.*, 2000). A similar observation has been made during coconut zygotic embryo development (Islas-Flores *et al.*, 1998, 2000).

Maintenance. Embryogenic cultures, irrespective of origin, are slow growing and nodular, and proliferation occurs from the peripheral region (Buffard-Morel *et al.*, 1992). Embryogenic cultures are maintained on a proliferation medium based on MS macro- and Nitsch (1969) micro-elements, Morel and Wetmore (1951) vitamins, 40 g/l sucrose, 2 g/l activated charcoal and 7.5 g/l agar. This medium is supplemented with 271.5–362 µM 2,4-D. Cultures are maintained in darkness and subcultured every 2 months.

Maturation. Somatic embryo development is asynchronous and occurs from < 10% of cultures. Regeneration of complete somatic embryos requires lower 2,4-D concentrations (181–271.5 µM) (Fig. 4.1.3). Thidiazuron (TDZ) or 2-isopentenyladenine (2iP) has been utilized effectively to stimulate development (Verdeil *et al.*, 1996b). Somatic embryos are maintained in the dark and subcultured every 2 months until shoot emission. Differentiation of the shoot meristem of the somatic embryo is cytokinin-dependent (Verdeil *et al.*, 1994) and has been corroborated by the increase in isopentenyl forms of cytokinin during early somatic embryo development (Hocher *et al.*, 1998a).

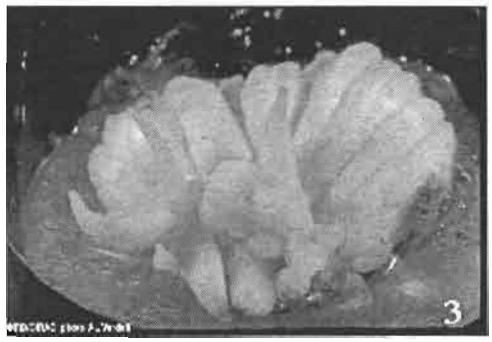

Fig. 4.1.3. Somatic embryos during the maturation phase.

Germination. Germination of the somatic embryos occurs on maturation medium containing benzyladenine (BA). Gibberellic acid (GA_3) can promote somatic embryo germination in the presence of BA (Fig. 4.1.4). Cultures are transferred to the light after the development of two to four leaves. Root induction can be promoted by naphthaleneacetic acid (NAA). Maturation and acclimatization of plantlets are major bottlenecks for regeneration by somatic embryogenesis. Foliar development is very slow and is sometimes associated with leaf chlorosis. The physiological status of *in vitro* shoots has been studied using *in vitro*-germinated zygotic embryos as a model. Different photosynthetic parameters have been studied (Triques *et al.*, 1997a,b): (i) chlorophyll fluorescence to determine photosynthetic efficiency; (ii) activities of phosphoenolpyruvate carboxylase (PEPC) and ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) were determined and the PEPC:RubisCO ratio was used as an indicator of autotrophism; (iii) net photosynthesis rate was estimated through CO_2 exchange measurements; and (iv) chloroplast ultrastructure. A lower rate of net photosynthesis was recorded for *in vitro*-grown plantlets compared with acclimatized palms, possibly due to lower RubisCO activity together with lower chlorophyll content compared to acclimatized plants (Triques *et al.*, 1998). Santamaria *et al.* (1999) demonstrated that sucrose lowered RubisCO activity, while slightly increasing the activity of PEPC. Since PEPC/RubisCO is a measure of plant photoautotrophy (Desjardins, 1995), these

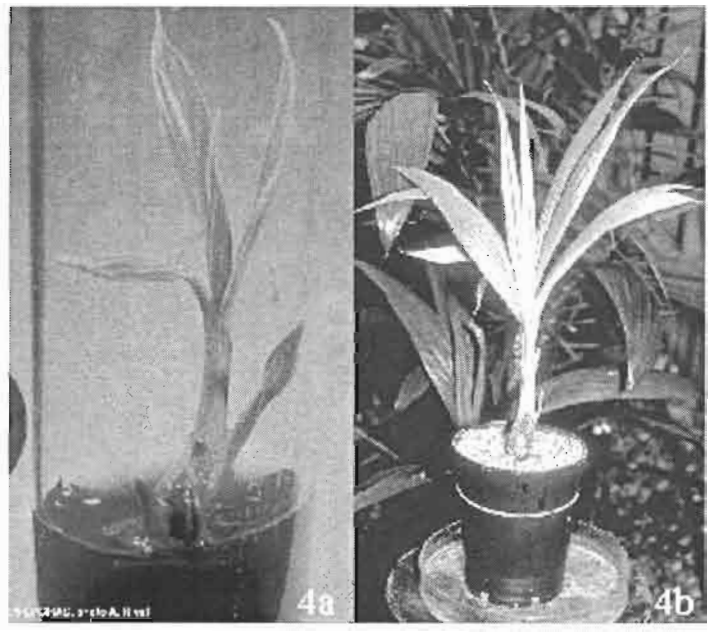

Fig. 4.1.4. Coconut somatic plantlets from the test tube (a) to the greenhouse (b).

results suggest that sucrose inhibits the development of photoautotrophy *in vitro*. They suggested that sucrose might be important in early stages of somatic embryo development; however, continuous growth in sucrose-rich medium in later stages could affect photoautotrophism and also plant performance *ex vitro*.

In vitro-grown plants (derived from zygotic embryos) have reduced capacity to control water loss compared to field-grown plants, due to altered stomatal functioning. Ventilation of the culture containers resulted in an increased capacity of *in vitro*-grown plants to control water loss (Talavera *et al.*, 2001). These results have implications for *in vitro* hardening and acclimatization.

3.1.2. Haploids

Haploidy is of great interest considering the allogamy of numerous coconut varieties and hybrids (Than-Tuyen and De Guzman, 1983). Monfort (1985) and Thanh-Tuyen (1985) reported promising results but no regeneration, and they were unable to recover complete embryos. More recently Griffis and Litz (1997) obtained proembryos from cultured anthers, anther filaments and unfertilized ovary cultures on medium containing diethylstilboestrol; however, no further development was reported.

3.1.3. Protoplast isolation and culture

Haibou and Kovoor (1981) described the isolation of protoplasts from immature inflorescence rachillae and microcallus regeneration from some of them. Unfortunately, a low rate of division was observed in coconut protoplast cultures and no regeneration was reported.

3.2. Conservation

Coconut seeds have no dormancy, causing problems in transporting and storing germplasm (Assy-Bah *et al.*, 1987; Engelmann and Dussert, 2000). Coconut genetic resources are maintained in field collections (Verdeil *et al.*, 1996a) in five countries: Côte d'Ivoire, Indonesia, India, Papua New Guinea and Vanuatu. The Côte d'Ivoire collection is the most important in terms of genotypic diversity, with 24,962 accessions including 53 ecotypes (36 Tall types represented by 20,600 palms and 17 Dwarf types represented by 4200 palms) and 12 inter-ecotype hybrids (Bourdeix *et al.*, 1998; N'Cho *et al.*, 1998). *Ex situ* conservation is costly, and collections are subject to diseases and climatic adversity. The Coconut Genetic Resources Network (COGENT) was created in 1992 with the support of the International Plant Genetic Resources Institute (IPGRI) to bring together 35 producing countries in order to maintain and protect coconut genetic resources (Baudouin *et al.*, 2000; Table 4.1.1). The highest priority is to duplicate field collections *in vitro* as pollen and embryos (Ramanatha Rao and Batugal, 1998) and to facilitate international exchange of germplasm. Short- and medium-term storage *in vitro* is essential for conservation of germplasm that is free of known diseases,

Table 4.1.1. Countries with an international coconut genetic resources database (CGRD). Coconut germplasm collections with passport and characterization data: a French-funded project. Number of accessions per country. (Adapted from Batugal, 1997, 1999; Baudouin *et al.*, 2000.)

Africa	na	Latin America/ Caribbean	na	South Asia	na	South-east Asia	na	South Pacific	na
Benin	4	Brazil	16	Bangladesh	4	Indonesia	156	Fiji	11
Côte d'Ivoire	99	Jamaica	60	India	212	Malaysia	92	Papua New Guinea	57
Tanzania	72	Mexico	20	Pakistan	32	Philippines	224	Vanuatu	66
				Sri Lanka	78	Thailand	52	Western Samoa	9
						Vietnam	31	Solomon Islands	21
Total per region	175		96		326		555		164

na, number of accessions.

and represents the safest method for international exchange of material (Withers and Williams, 1985). It is also a prerequisite for cryogenic storage.

Routine techniques for collecting zygotic embryos have been developed, including field collection, disinfecting and embryo culture (Assy-Bah *et al.*, 1987; Rillo, 1995; Ashburner *et al.*, 1996; Samosir *et al.*, 1999a; Karun, 2001; N'Nan *et al.*, 2002a). Excised embryos can be stored in KCl for up to 14 days before *in vitro* culture (Assy-Bah *et al.*, 1989). Coconut embryo culture was initially developed in the Philippines for embryo rescue of 'Makapuno', a highly valued Philippine mutant genotype (De Guzman and Del Rosario, 1964; Del Rosario, 1998). Karunaratne *et al.* (1991) used coconut embryo culture to measure drought tolerance in Sri Lanka, and were able to screen a large number of genotypes in a short time (2 years). Rillo (1985) used embryo rescue to screen for disease tolerance.

Different protocols for embryo culture have been described (Del Rosario and De Guzman, 1976; Karunaratne *et al.*, 1985; Assy-Bah, 1986; Sossou *et al.*, 1987; Assy-Bah *et al.*, 1989; Rillo and Paloma, 1991; Karun *et al.*, 1993; Ashburner *et al.*, 1996; Rillo, 1999). Low germination and survival rates of plants *ex vitro* indicate that the protocol requires improvement. An international programme coordinated by COGENT has begun to focus on improving *in vitro* culture and acclimatization protocols (Batugal and Engelmann, 1998).

Zygotic embryos can be stored *in vitro* for medium-term periods (6 to 12 months) without loss of germination (Assy-Bah and Engelmann, 1993; M'kumbo, 1995). Development can be suppressed by high levels of sucrose and activated charcoal (Assy-Bah, 1992; Verdeil *et al.*, 1998b). Increased osmolarity and reduction of nutrient concentration can also impede development (Damasco, 2002). None the less, long-term conservation by cryopreservation is essential to reduce the loss of important genetic resources.

Early attempts to cryopreserve coconut embryos by Bajaj (1984) and Chin *et al.* (1989) were not very successful. Assy-Bah and Engelmann (1992a,b) demonstrated that mature coconut embryos could be cryopreserved after 4 h desiccation in a laminar air flow followed by immersion for 11–20 h in a cryoprotectant consisting of 600 g/l glucose and 15% glycerol (Assy-Bah and Engelmann, 1992b). Four coconut varieties (hybrid PB121, Indian Tall, Cameroon Red Dwarf and Rennell Island Tall) were successfully cryopreserved with a germination rate of 10–93%, depending on ecotype. These results were validated with West African Tall (WAT) and MYD (N'Nan, 1997), and later with ten more ecotypes (N'Nan *et al.*, 2003).

Plumules have been cryopreserved by encapsulation/dehydration (N'Nan, 1999; Malaurie and Borges, 2001; Malaurie *et al.*, 2002). Plumules were excised and encapsulated in alginate beads, and exposed to different sucrose concentrations and dehydration periods, resulting in 40–80% survival after cryopreservation. Up to 70% of plumules of some ecotypes germinate normally following cryopreservation (Malaurie and Borges, 2001; N'Nan *et al.*, 2002b; Fig. 4.1.5). Hornung *et al.* (2001) cryopreserved plumules, and attempted to induce embryogenic cultures according to the protocol of Chan *et al.* (1998). Other cryopreservation techniques, e.g. encapsulation, osmoprotection, dehydration and encapsulation, osmoprotection and vitrification (Sakai *et al.*, 2000), have been applied to plumular tissues, and shoot development has been reported (Malaurie *et al.*, 2003).

Hybridization and improved nut production are facilitated by assisted pollination (Wuidart and Rognon, 1981; de Nucé de

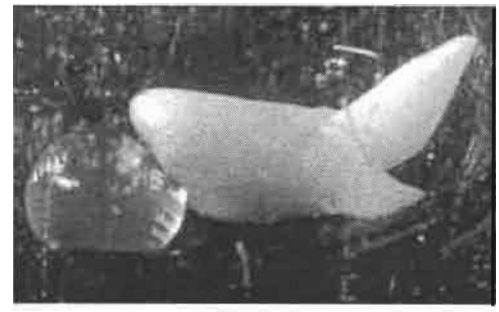

Fig. 4.1.5. Somatic embryo development from dehydrated, encapsulated and frozen plumule.

Lamothe and Wuidart, 1992). According to Towill (1985), palms have long-lived pollen; however, for long-term breeding programmes, extended storage of pollen is essential (Towill and Walters, 2000). Coconut pollen storage was reported by Whitehead (1965) using freeze-drying. Pollen desiccation to 4–5% moisture content over silica gel, followed by storage *in vacuo* in a freezer, does not cause loss of viability for > 6 months (Rognon and de Nucé de Lamothe, 1978). Cryopreservation of pollen is also feasible (Frison *et al.*, 1993; Engelmann, 1999), and recommendations for collecting, conditioning and cryogenic storage of pollen have been reported (Frison *et al.*, 1993).

Technical guidelines for the safe movement of coconut germplasm have been established (Frison *et al.*, 1993; Diekmann, 1997; Baudouin, 1998; Table 4.1.2). Indexing techniques for screening germplasm for known diseases is critical, e.g. CFDV, which causes foliar decay in Vanuatu, CCCVd in the Philippines and LY, a phytoplasma-associated disease, which has caused great devastation in the Caribbean region and more recently in Ghana (Harrison *et al.*, 1999; Rodriguez, 1999). All of these diseases (Table 4.1.3) should be prevented from being transferred outside their current area of distribution (Frison *et al.*, 1993; Diekmann, 1997, 1999; Hanold and Randles, 1997; Dollet, 1999; Hodgson and Randles, 1999; Howard and Harrison, 1999; Jones *et al.*, 1999; Nair *et al.*, 1999). A list of treatments has been proposed for controlling the spread of these diseases in the technical guidelines for the safe movement of coconut germplasm (Table 4.1.4). There are no therapies for eliminating coconut virus, viroid and phytoplasma diseases of coconut. Reverse transcription polymerase chain reaction (RT-PCR) has demonstrated the presence of LY phytoplasma in embryonic tissue, including the plumule (Cordova *et al.*, 2003). Exchange of coconut germplasm by means of zygotic embryos corresponds to the basic Food and Agriculture Organization (FAO)/Inernational Board for Plant Genetic Resources (IBPGR) guidelines for moving coconut germplasm (Diekmann, 1997, 1999; Ramanatha Rao and Batugal, 1998); however, existing indexing protocols do not provide adequate security. *In vitro* collections of coconut germplasm are located in six coconut-producing countries and two European countries (Table 4.1.5).

The establishment of the multi-site International Coconut Genebank (ICG), hosted by India, Indonesia, Papua New Guinea and Côte d'Ivoire for their respective regions, will have the responsibility to conserve and share a maximum of 200 important accessions from South and Southeast Asia, the Pacific region and Africa and Indian Ocean islands, respectively (Table 4.1.6). The accessions maintained in ICG will include: (i) the principal varieties; (ii)

Table 4.1.2. Summary of FAO/IBPGR Technical Guidelines for the Safe Movement of Coconut Germplasm. General recommendation: to move embryo culture or pollen, not nuts. (Adapted from Harrison *et al.*, 1995; Diekmann, 1997; Ramanatha Rao and Batugal, 1998; Dollet *et al.*, 2001a,b.)

Pathogen	Specific recommendation
CFDV	Indexing or exclusion of germplasm from Vanuatu
CCCVd	Indexing or exclusion of germplasm from the Philippines
CtiVd	Indexing or exclusion of germplasm from Guam
Viroid-like sequence	Indexing or exclusion of germplasm that is moved from countries where these sequences are known to occur to countries where they have not yet been reported. Recommendation under revision
LY, phytoplasma Kerala wilt, phytoplasma	Transmission through seed, embryo culture or pollen not reported, but suspicion still exists
Tatipaka disease, phytoplasma Blast, phytoplasma	A nursery disease which does not occur on adult trees

CtiVd, coconut tinangaja viroid.

Table 4.1.3. Causal agent, vector, final disease evolution, geographical distribution of the coconut diseases, and techniques available for indexing (adapted from Frison *et al.*, 1993; Hanold and Randles, 1997; Diekmann, 1999; Dollet, 1999; Hodgson and Randles, 1999; Howard and Harrison, 1999; Jones *et al.*, 1999; Nair *et al.*, 1999; Dollet *et al.*, 2001).

Type of disease	Disease name	Cause	Vector	Final disease evolution	Geographical distribution	Indexing: conventional techniques	Indexing: molecular approach
Viral	Foliar decay	Coconut foliar decay virus (CFDV); icosahedral virus	*Myndus taffini* (Cixiidae) planthopper	In susceptible coconut palms, the crown dies within 6 months to 2 years	Vanuatu, and suspected in other areas	–	Dot-blot hybridization and complementary labelled DNA probe
Viroid	Coconut cadang-cadang	Coconut cadang-cadang viroid (CCCVd); circular single-stranded RNA in a rod-like structure	Field and seed transmission are observed and pollen suspected. Mechanism of transmission remains unknown	8 to 16 years elapse between first symptoms and death of the palm. Some palms die soon, those that continue to develop never flower	Occurs in certain parts of the Philippines	PAGE	MHA. Hybridization analysis with radioactive RNA probes (Northern blotting) ; Rt-PCR
	Coconut tinangaja	Coconut tinangaja viroid (CtiVd); single-stranded circular RNA	Means of natural transmission unknown	Diseased palms decline and die in similar manner to cadang-cadang	Guam	PAGE	Hybridization analysis with radioactive probe
Viroid-like sequences	–	Viroid-like sequence similar to but not identical to CCCVd	Means of natural transmission unknown	–	South Asia to French Polynesia	–	Northern blotting technique with a complementary RNA probe specific to CCCVd
Mollicute	Blast	Mycoplasma-like organism (MLO)	*Recilia mica* Kramer (Jassidae)	–	Africa, and South America and Indonesia for similar symptoms	–	–
	Lethal yellowing (LY)	Phytoplasma	*Myndus crudus* (Cixiidae) planthopper; suspicion over different phloem-feeding insects for LY in Africa	The whole of the crown eventually rots and falls off within 3–6 months of the appearance of the first symptoms. Complete destruction of plantation in Mexico	Africa, Central America and Caribbean	Light or electron microscopy with fluorescent staining (DAPI)	Amplification by PCR of the 16–23S rRNA region of phytoplasma
	Root wilt or Kerala wilt	Mycoplasma-like organism (MLO)	*Stephanistis typica*; *Proutista moesta* (putative vector)	Symptoms appeared only on 30-month-old palms. The disease is not lethal, but significantly reduces production	India (parts of Kerala and Tamil Nadu states)	Light microscopy with fluorescent staining (DAPI)	PCR
	Tatipaca disease	Mycoplasma-like organism (MLO)	Unknown	The disease is not lethal, but significantly reduces production	India (East and West Godavari, Srikakulam and Nellore in Andhra Pradesh)	Light microscopy with fluorescent staining (DAPI)	PCR
	Heartrot disease	Trypanosomatid	Pentatomid bugs from the genus *Lincus*		Surinam, Salvador de Bahia Province, north Honduras, Trinidad, Costa Rica	40 x 10 phase-contrast light microscope	None

DAPI, 4′-6-diamidino-2-phenylindole; MHA, Mueller-Hinton agar; PAGE, polyacrylamide gel electrophoresis; RT-PCR, reverse transcription polymerase chain reaction.

Table 4.1.4. Therapy available against the different coconut diseases (adapted from Frison *et al.*, 1993; Diekmann, 1997).

Disease name	Cause	Therapy
Foliar decay	CFDV	None
Coconut cadang-cadang	CCCVd	None
Coconut tinangaja	CtiVd	None available
	Viroid-like sequence	None
Blast	Phytoplasma, MLO	None
Lethal yellowing (LY)	Phytoplasma, MLO	Tetracycline, but no elimination of the phytoplasma from palms
Root wilt or Kerala wilt	Phytoplasma, MLO	Tetracycline, but no elimination of the phytoplasma from palms
Tatipaca disease	Phytoplasma, MLO	Tetracycline, but no elimination of the phytoplasma from palms

Table 4.1.5. COGENT member countries concerned in international exchange of coconut (*Cocos nucifera* L.) germplasm, and expected COGENT member countries (adapted from Batugal, 1997, 1999).

Africa	Latin America/ Caribbean	South Asia	South-east Asia	South Pacific
Côte d'Ivoire	Brazil	Bangladesh	China	Cook Islands
Ghana	Costa Rica	**India** *	**Indonesia** *	Fiji
Kenya	Cuba	Pakistan	Malaysia	Kiribati
Mozambique	Guyana	Sri Lanka *	Myanmar	**Papua New Guinea** *
Nigeria	Haiti		Philippines *	Solomon Islands
Seychelles	Jamaica		Thailand	Tonga
Tanzania	Mexico *		Vietnam	Vanuatu
	Trinidad-Tobago			Western Samoa
Possible future members				
Comoro	Colombia			Marshall Islands
Madagascar	Dominican Republic			Tuvalu
	Ecuador			
	El Salvador			
	Guatemala			
	Panama			
	Venezuela			

In bold, regional coconut genebank, also called International Coconut Genebank (ICG). * Number of countries with *in vitro* collection (this number reflects more the laboratories involved in tissue culture in coconut, where United Kigdom (Imperial College, Wye) and France (IRD/CIRAD team, Montpellier) have an important and active place).

threatened varieties, and varieties with special traits; (iii) additional diversity discovered during national explorations; and (iv) duplicates of accessions from other regions (Batugal, 1997). In addition, the ICG will undertake field evaluations and share data and germplasm with member countries using safe exchange guidelines as prescribed by FAO and IPGRI (IPGRI, 2000).

4. Conclusions

The coconut palm is a major agricultural species and is an important subsistence crop. Since the mid-20th century, a decline in productivity has occurred worldwide, despite the use of improved planting material and agronomic practices. Biotechnology and its application to coconut can create new oppor-

Table 4.1.6. State of the coconut germplasm present in the host countries of the regional coconut genebank. The state of coconut germplasm present in Vanuatu is given taking account of its interesting diversity despite the great risk of genetic erosion caused by coconut foliar disease (CFD). (Adapted from Baudouin, 1998; N'Cho *et al.*, 1998; Ramanatha Rao and Batugal, 1998.)

Ecotypes	Côte d'Ivoire Ecotype/ Accession	India Ecotype/ Accession	Indonesia Ecotype/ Accession	PNG Ecotype/ Accession	Vanuatu Ecotype/ Accession
Tall	36/20,600	68* + 34**/nc	79/4,337	17/nc	24/2,261
Semi-Tall	–	2 + 0/nc	–	–	–
Dwarf	17/4,200	16 + 12/nc	9/923	6/nc	17/1,085
Hybrids	12/nc	nc	nc	nc	nc
Indigenous		34 Tall/12 Dwarf			
Total accessions	27,962	nc	nc	nc	nc

nc, not communicated; PNG, Papua New Guinea.
*Number of ecotypes collected in different areas outside India; ** number of indigenous Indian ecotypes.

tunities in breeding, cloning, disease control and germplasm exchange/conservation. COGENT/IPGRI encourages and supports collaboration among various national coconut research groups; this is absolutely critical as there are insufficient funds to support the research needs for this crop (Hocher *et al.*, 1998b; Punchihewa, 1999; Rohde *et al.*, 1999). The development of molecular breeding tools, e.g. linkage maps and QTLs, should facilitate MAS for the recovery of hybrids with greater productivity and resistance to diseases (Cardeña *et al.*, 1999). Safe exchange of germplasm can only occur if there are accurate methods for detecting and elimination of diseases.

Cryobanks for zygotic embryos are a reality (N'Nan *et al.*, 2003), and investigations based upon cryopreservation of plumules will have a great impact on storage and management of genetic resources (Hornung *et al.*, 2001; Malaurie *et al.*, 2003).

Somatic embryogenesis is promising as a means for propagating elite material and for genetic manipulation. After several decades of little success, there are now clonally propagated plants in the field (Verdeil *et al.*, 1999). The number of plantlets that have been recovered from somatic embryos remains low and their conversion rate is unacceptable. There is a need to better understand the basic botany and biochemistry of coconut somatic and zygotic embryo development. Studies are under way that would characterize genes that are implicated in the cell cycle regulation of coconut (Sandoval, 2002; Sandoval *et al.*, 2003). Such studies together with genetic transformation (C. Oropeza, personal communication) should provide opportunities for coconut genetic engineering and improvement.

Acknowledgements

This work is supported by IRD and CIRAD-CP. The authors would like to thank the following institutes* for their fruitful collaboration: CNRA (Côte d'Ivoire), CICY and INIFAP (Mexico), PCA (the Philippines), Hanover University (Germany), Imperial College at Wye (United Kingdom), CRI (Sri Lanka), COGENT and IPGRI. Part of the work cited in this chapter was realized with European Community (EC) funding (Contract STD3 ERBTS3*CT940298). We also want to thank the United Nations Educational, Scientific and Cultural Organization (UNESCO) and BRG for their support.

*BRG, Bureau des Ressources Génétiques, Paris, France; CICY, Centro de Investigación Cientifica de Yucatán, Mexico; CNRA, Centre National de Recherche Agronomique, Côte d'Ivoire; CRI, Coconut Research Institute; INIFAP, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Mexico; PCA, Philippines Coconut Authorities.

References

Aldaba, F.R. (1995) Philippines et cocotiers: problèmes et perspectives. *Oléagineux Corps Gras Lipides* 2, 203–205.

Ashburner, G.R. (1999) The application of molecular markers to coconut genetic improvement. In: Oropeza, C., Ashburner, R., Verdeil, J.-L. and Zizumbo, D. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 33–44.

Ashburner, G.R., Faure, M.G., Tomlinson, D.R. and Thompson, W.K. (1996) A guide to the zygotic embryo culture of coconut palms (*Cocos nucifera* L.). *ACIAR Technical Reports* 36, 3–16.

Ashburner, G.R., Thompson, W.K. and Halloran, G.M. (1997) RAPD analysis of South Pacific coconut palm populations. *Crop Science* 37, 992–997.

Assy-Bah, B. (1986) Culture *in vitro* d'embryons zygotiques de cocotier. *Oléagineux* 41, 321–328.

Assy-Bah, B. (1992) Utilisation de la culture *in vitro* d'embryons zygotiques pour la collecte et la conservation des ressources génétiques du cocotier (*Cocos nucifera* L.). PhD thesis, Université Paris VI.

Assy-Bah, B. and Engelmann, F. (1992a) Cryopreservation of immature embryos of coconut (*Cocos nucifera* L.). *Cryo-Letters* 13, 67–74.

Assy-Bah, B. and Engelmann, F. (1992b) Cryopreservation of mature embryos of coconut (*Cocos nucifera* L.) and subsequent regeneration of plantlets. *Cryo-Letters* 13, 117–126.

Assy-Bah, B. and Engelmann, F. (1993) Medium-term conservation of mature embryos of coconut (*Cocos nucifera* L.). *Plant Cell, Tissue and Organ Culture* 33, 19–24.

Assy-Bah, B., Durand-Gasselin, T. and Pannetier, C. (1987) Use of zygotic embryo culture to collect germplasm of coconut (*Cocos nucifera* L.). *Plant Genetic Resources Newsletter* 71, 4–10.

Assy-Bah, B., Durand-Gasselin, T., Engelmann, F. and Pannetier, C. (1989) Culture *in vitro* d'embryons zygotiques de cocotier (*Cocos nucifera* L.) Méthode, révisée et simplifiée, d'obtention de plants de cocotiers transférables au champ. *Oléagineux* 44, 515–523.

Bajaj, Y.P.S. (1984) Induction of growth in frozen embryos of coconut and ovules of Citrus. *Current Science* 53, 1215–1216.

Barrant, C.I. (1978) Coconut intercropped with cocoa. *Philippine Journal of Coconut Studies* 3, 29–34.

Batugal, P.A. (1997) Implications of restricted coconut germplasm movement. In: Diekmann, M. (ed.) *Proceedings of Viroid-like Sequences of Coconut*. ACIAR/IPGRI, Rome, pp. 4–7.

Batugal, P.A. (1999) The role of international cooperation in the development of biotechnology in coconut. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardena, R. and Santamaria, J.M. (eds) *Current advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 19–30.

Batugal, P.A. and Engelmann, F. (eds) (1998) *Coconut Embryo In Vitro Culture*. IPGRI-APO, Serdang, pp. 17–28.

Baudouin, L. (1999) Genetic improvement of coconut palms. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardena, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 45–56.

Baudouin, L. (1998) International movement of coconut cultivars. In: Ramanatha Rao, V. and Batugal, P. (eds) *Proceedings of the COGENT Regional Coconut Genebank Planning Workshop*. IPGRI-APO, Serdang, pp. 34–40.

Baudouin, L., Bourdeix, R., Bonnot, F., Hamelin, C. and Rouzière, A. (2000) COGENT establishes an International Coconut Genetic Resources Database (CGRD). *Cogent Newsletter IPGRI* 3, 1–2.

Bertrand, F. (1994) Extraction de l'huile de cocotier assistée par les enzymes. PhD thesis, Université de Droit, d'Economie et des Sciences d'Aix-Marseille, St Jérôme, France.

Bhala-Sarin, N., Bagga, S., Sopory, S.K. and Guha-Mukherjee, S. (1986) Induction and differentiation of callus from embryos of *Cocos nucifera* L. by IAA conjugates. *Plant Cell Reports* 5, 322–324.

Blake, J. (1989) Coconut tissue culture research at London University's unit for advanced propagation systems, Wye College. In: *COCOTECH XXVI Meeting, Bangkok*. APCC, Bangkok, Thailand, p. 15.

Blake, J. (1990) Coconut (*Cocos nucifera* L.): micropropagation. In: Bajaj, Y.P.S. (ed.) *Biotechnology in Agriculture and Forestry*, Vol. 10. *Legumes and Oilseed Crops I*. Springer-Verlag, Berlin, pp. 538–553.

Blake, J. and Eeuwens, C.J. (1980) Inflorescence tissue as source material for vegetative propagation of coconut palm. In: *Proceedings International Conference on Cocoa and Coconuts, Kuala Lumpur*, pp. 549–556.

Blake, J. and Eeuwens, C.J. (1981) Culture of coconut palm tissues with a view to vegetative propagation. In: Rao, A.N. (ed.) *Tissue Culture of Economically Important Plants*. Committee on Science and Technology in Developing Countries and Asian Network for Biological Sciences, Singapore, pp. 145–148.

Bourdeix, R., N'Cho, Y.P. and Sangare, A. (1998) Coconut breeding in Côte d'Ivoire. In: Batugal, P.A. and Ramanatha Rao, V. (eds) *Coconut Breeding. Papers Presented at a Workshop on Standardization of Coconut Breeding*. IPGRI-APO, Serdang, pp. 101–113.

Bourdeix, R., Baudouin, L., Billotte, N., Labouisse, J.-P. and Noiret, J.-M. (2001) Coconut. In: Charrier, A., Jacquot, M., Hamon, S. and Nicolas, D. (eds) *Tropical Plant Breeding*. Science Publishers, Enfield, pp. 106–127.

Brackpool, A.L., Branton, R.L. and Blake, J. (1986) Regeneration in palms. In: Vasil, I.K. (ed.) *Cell Culture and Somatic Cell Genetics of Plants*, Vol. 3. Academic Press, Orlando, Florida, pp. 207–222.

Branton, R.L. and Blake, J. (1983) Development of organized structures in callus derived from explants of *Cocos nucifera* L. *Annals of Botany* 52, 673–678.

Branton, R.L. and Blake, J. (1984) Clonal propagation of coconut palm. In: Pushparajah, E. and Soon, C.P. (eds) *Cocoa and Coconut: Progress and Outlook*. Incorporated Society of Planters, Kuala Lumpur, pp. 771–780.

Buffard-Morel, J., Verdeil, J.-L. and Pannetier, C. (1988) Vegetative propagation of coconut palm through somatic embryogenesis, obtention of plantlets from leaf explant. In: *8th International Symposium on Biotechnology, Paris*, p. 117 (Abstract).

Buffard-Morel, J., Verdeil, J.L. and Pannetier, C. (1992) Embryogenèse somatique du cocotier (*Cocos nucifera* L.) à partir de tissus foliaires: étude histologique. *Canadian Journal of Botany* 70, 735–741.

Canto-Canché, B., Quintal-Salazar, E. and Villanueva, M. (1983) Biochemical markers of variety in *Cocos nucifera* L. from Yucatan. *Turrialba* 42, 375–381.

Cardeña, R., Oropeza, C. and Zizumbo, D. (1998) Leaf proteins as markers useful in the genetic improvement of coconut palms. *Euphytica* 102, 81–86.

Cardeña, R., Ashburner, G.R. and Oropeza, C. (1999) Prospects for marker-assisted breeding of lethal yellowing-resistant coconuts. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 145–159.

Carpio, C.B. (1982) Biotechnical studies of *Cocos nucifera* L. Kalikasan. *Philippine Journal of Biology* 11, 319–338.

Chan, J.L., Sáenz, L., Talavera, C., Hornung, R., Robert, M. and Oropeza, C. (1998) Regeneration of coconut (*Cocos nucifera* L.) from plumule explants through somatic embryogenesis. *Plant Cell Reports* 17, 515–521.

Chin, H.F., Krishnapillay, B. and Hor, Y.L. (1989) A note on the cryopreservation of embryos from young embryos of coconuts (*Cocos nucifera* var. Mawa). *Pertanika* 12, 183–186.

Cordova, I., Jones, P., Harrison, N.A. and Oropeza, C. (2003) *In situ* PCR detection of phytoplasma DNA embryos from coconut palms with lethal yellowing disease. *Molecular Plant Pathology* 4, 99–108.

Damasco, O. (2002) Utilization of embryo culture technology for germplasm conservation: development of medium-term conservation for coconut zygotic embryos in the Philippines. In: Engelmann, F., Batugal, P.A. and Oliver, J.T. (eds) *Coconut Embryo In Vitro Culture*, Part II. IPGRI-APO, Rome, pp. 67–79.

Daviron, B. (1995) Les pays en développement et le marché international des oléagineux. *Oléagineux Corps Gras Lipides* 2, 191–196.

De Guzman, E.V. and Del Rosario, A.G. (1964) The growth and development of *Cocos nucifera* L. 'Makapuno' embryo *in vitro*. *Philippine Agriculture* 48, 82–94.

Del Rosario, A.G. (1998) Status of research and coconut embryo culture and acclimatization techniques in UPLB. In: Batugal, P. and Engelmann, F. (eds) *Coconut Embryo* In Vitro *Culture*. IPGRI-COGENT, Serdang, pp. 12–16.

Del Rosario, A.G. and De Guzman, E.V. (1976) The growth of coconut Makapuno embryos *in vitro* as affected by mineral composition and sugar level of the medium during liquid and solid culture. *Philippine Journal of Science* 105, 215–222.

de Nucé de Lamothe, M. (1970) Application du principe des croisements interorigines au cocotier. Premiers résultats obtenus en Côte d'Ivoire. *Oléagineux* 25, 207–210.

de Nucé de Lamothe, M. and Wuidart, W. (1992) La production de semences hybrides de cocotier: cas des semences hybrides Nain × Grand. *Oléagineux* 47, 93–96.

Desjardins, Y. (1995) Factors affecting CO_2 fixation in striving to optimize photoautotrophy in micropropagated plantlets. *Plant Tissue Culture and Biotechnology* 1, 13–25.

De Taffin, G. (1998) *The Coconut*. MacMillan Education, London.

Diekmann, M. (ed.) (1997) *Viroid-like Sequences of Coconut*. ACIAR/IPGRI, Rome, 56 pp.

Diekmann, M. (1999) The use of biotechnology for the safe movement of coconut germplasm. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 259–264.

Dollet, M. (1999) Conventional and molecular approaches for detection and diagnosis of plant diseases: application to coconut. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 163–181.

Dollet, M., Sturm, N.C., Ahomadegbe, J.-C. and Campbell, D.A. (2001) Kinetoplast DNA minicircles of phloem-restricted *Phytanonas* associated with wilt diseases of coconut and oil palms have a two-domain structure. Publised by Elsevier Science B.V. on behalf of the Federation of European Microbioloical Societies. Available at http://www.mimg.ucla.edu/faculty/campbell/pdf_files/57.pdf+coconut+gene+cloning&hl=fr&ie=UTF-8

Duran, Y., Rohde, W., Kullaya, A., Goikoetxea, P. and Ritter, E. (1997) Molecular analysis of East African Tall coconut genotypes by DNA marker technology. *Journal of Genetics and Breeding* 51, 279–288.

Dussert, S., Verdeil, J.L., Rival, A., Noirot, M. and Buffard-Morel, J. (1995a) Nutrient uptake and growth of *in vitro* coconut (*Cocos nucifera* L) calluses. *Plant Science* 106, 185–193.

Dussert, S., Verdeil, J.-L. and Buffard-Morel, J. (1995b) Specific nutrient uptake during initiation of somatic embryogenesis in coconut calluses. *Plant Science* 111, 229–236.

Ebert, A. and Taylor, H.F. (1990) Assessment of the changes of 2,4-D dichlorophenoxyacetic acid concentrations in plant tissue culture media in the presence of activated charcoal. *Plant Cell, Tissue and Organ Culture* 20, 165–172.

Ebert, A., Taylor, H.F. and Blake, J. (1993) Changes of 6-benzylaminopurine and 2,4-dichlorophenoxyacetic acid concentrations in plant tissue culture media in the presence of activated charcoal. *Plant Cell, Tissue and Organ Culture* 33, 157–162.

Eeuwens, C.J. (1976) Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut (*Cocos nucifera* L.) and date (*Phoenix dactylifera* L.) palms cultured *in vitro*. *Physiologia Plantarum* 36, 23–28.

Eeuwens, C.J. (1978) Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (*Cocos nucifera*) and date (*Phoenix dactylifera*) palms cultured *in vitro*. *Physiologia Plantarum* 42, 173–178.

Engelmann, F. (1999) Cryopreservation of coconut germplasm. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 289–296.

Engelmann, F. and Dussert, S. (2000) Développement de la cryoconservation pour la conservation des ressources génétiques végétales. *Cahiers d'Études et de Recherches Francophones/Agricultures* 9, 237–244.

FAOSTAT (2004) http://faostat.fao.org

Fernando, S.C. and Gamage, C.K.A. (2000) Abscisic acid induced somatic embryogenesis in immature embryo explants of coconut (*Cocos nucifera* L.). *Plant Science* 151, 193–198.

Fernando, S.C., Verdeil, J.-L., Hocher, V., Weerakoon, L.K. and Hirimburegama, K. (2003) Histological analysis of plant regeneration from plumule explants of *Cocos nucifera* L. *Plant Cell, Tissue and Organ Culture* (in press).

Fernando, W.M.U. and Gajanayake, G. (1997) Patterns of isozymes variations in coconut (*Cocos nucifera* L.) populations used for breeding improved varieties. *Plantation, Recherche, Développement* 4, 256–263.

Freud, C. and Daviron, B. (1994) L'huile de coprah dans le monde, une demande croissante, une production à relancer. *Plantation, Recherche, Développement* 3, 33–36.

Frison, E.A., Putter, C.A.J. and Diekmann, M. (eds) (1993) *FAO/IBPGR Technical Guidelines for the Safe Movement of Coconut Germplasm*. FAO/IBPGR, Rome, 48 pp.

Gascon, J.-P. and de Nucé de Lamothe, M. (1976) L'amélioration du cocotier: méthode et suggestions pour une coopération internationale. *Oléagineux* 31, 479–483.

Gascon, J.-P. and de Nucé de Lamothe, M. (1978) Genetic improvement of the coconut: results and prospects. In: *Proceedings of the International Conference on Cocoa and Coconuts, Kuala Lumpur*, pp. 489–499.

Georges, E.F. and Sherrington, P.D. (eds) (1984) *Plant Propagation by Tissue Culture, Handbook of Directory and Commercial Laboratories*. Exegetics Eversley, London.

Griffis, J.L. and Litz, R.E. (1997) Advances in the *in vitro* morphogenesis of several coconut: (*Cocos nucifera* L.) tissues in Florida. In: *International Cashew and Coconut Conference, Dar es Salaam*, pp. 349–357.

Gupta, P.K., Kendurkar, S.V., Kulkarni, V.M., Shirgurkar, M.V. and Mascarenhas, A.F. (1984) Somatic embryogenesis and plants from zygotic embryos of coconut (*Cocos nucifera* L.) *in vitro*. *Plant Cell Reports* 3, 222–225.

Haccius, B. and Phillip, V.J. (1979) Embryo development in *Cocos nucifera* L.: a critical contribution to a general understanding of palm embryogenesis. *Plant Systematic Evolution* 132, 91–106.

Hagedorn, V. (1990) Untersuchungen zur vegetativen Vermehrung von Kokospalmen (*Cocos nucifera* L.) mit Hiulfe von Gewebekulturen. *Göttingen Beitrage zur Land-und Forstwirtschaft in den Tropen und Subtropen* 52, 188.

Haibou, T.K. and Kovoor, A. (1981) Regeneration of callus from coconut protoplasts. In: Rao, A.N. (ed.) *Proceedings COSTED Symposium on Tissue Culture of Economically Important Plants*. COSTED ANBS, Singapore, pp. 149–151.

Hanold, D. and Randles, J.W. (eds) (1997) Report on ACIAR-funded research on viroids and viruses of coconut palms and other tropical monocotyledons 1985–1993. ACIAR monograph. http://www.aciar.gov.au/viroids/viroids.htm

Harries, H.C. (1978) Evolution, dissemination and classification of *Cocos nucifera* L. *Botanical Reviews* 44, 265–320.

Harries, H.C. (1991) The promise, performance and problems of F1 hybrid coconut. In: Silas, E.G. *et al.* (eds) *National Symposium on Coconut Breeding and Management*. Kerala Agricultural University, Trichur, pp. 39–44.

Harrison, N., Richardson, P.A. and Tsai, J.H. (1995) Detection and diagnosis of lethal yellowing: conventional and molecular techniques. In: Oropeza, C., Howard, F.W. and Ashburner (eds) *Lethal Yellowing: Research and Practical Aspects*. Kluwer, Dordrecht, The Netherlands, pp. 79–91.

Harrison, N., Cordova, I., Richardson, P. and Dibonito, R. (1999) Detection and diagnosis of lethal yellowing. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 183–196.

Herrán, A., Estioko, L., Becker, D., Rodriguez, M.J.B., Rohde, W. and Ritter, E. (2000) Linkage mapping and QTL analysis in coconut (*Cocos nucifera* L.). *Theoretical and Applied Genetics* 101, 292–300.

Hocher, V., Lyakurwa, R., Grosdemange, F., Huet, C. and Verdeil, J.L. (1998a) Changes in levels of cytokinins during the early development of coconut somatic embryos. In: *L'Usine Cellulaire Végétale In Vitro. Congrès IAPTC France, Amiens*, p. 45 (Abstract).

Hocher, V., Verdeil, J.L., Grosdemange, F., Huet, C., Bourdeix, R., N'Cho, Y., Sangare, A., Hornung, R., Jacobsen, H.J., Rillo, E., Oropeza, C. and Hamon, S. (1998b) Collaboration internationale pour la maîtrise de la multiplication végétative *in vitro* du cocotier (*Cocos nucifera* L.). *Cahiers Agricultures* 7, 499–505. http://www.aupelf-uref.org/revues/agri/6.98/not11fm.htm

Hocher, V., Verdeil, J.-L., Rival, A. and Hamon, S. (1999) Application of *in vitro* techniques to the conservation and propagation of coconut palms. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 267–278.

Hodgson, R.A.J. and Randles, J.W. (1999) Detection of coconut cadang-cadang viroid-like sequences. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 227–246.

Hornung, R. (1995) Micropropagation of *Cocos nucifera* L from plumular tissues excised from mature zygotic embryos. *Plantations, Recherche, Développement* 212, 38–41.

Hornung, R. (1997) Recent adavnces in the cloning of coconut. In: Eden-Green, S.J. and Ofori, F. (eds) *Proceeding of an International Workshop on Lethal Yellowing-like Diseases of Coconut*. NRI, Chatham, UK, pp. 119–135.

Hornung, R., Domas, R. and Lynch, P.T. (2001) Cryopreservation of plumular explants of coconut (*Cocos nucifera* L.) to support programmes for mass clonal propagation through somatic embryogenesis. *Cryo-Letters* 22, 211–220.

Howard, F.W. and Harrison, N.A. (1999) Lethal yellowing of palms. http://www.ftld.ufl.edu/lyfacts.htm

INIFAP (1998) *Memorias del Seminario Tecnico. Primera Reunion Nacional de Palma de Coco*. INIFAP, Acapulco.

IPGRI (2000) Editorial promoting sustainable conservation and use of coconut genetic resources. No. 33, December 2003. Available at http://www.ipgri.cgiar.org/regions/apo/newsletters/news33/33edit.htm

Islas-Flores, I., Oropeza, C. and Hernandez-Sotomayor, S.M.T. (1998) Protein phosphorylation during coconut zygotic embryo development. *Plant Physiology* 118, 257–263.

Islas-Flores, I., Chan-Rodriguez, J.L., Oropeza-Salin, C. and Hernandez-Sotomayor, S.M.T. (2000) Occurrence of phosphorylated proteins and kinase activity in coconut tissues cultured *in vitro* in a medium that induces somatic embryogenesis. *Plant Physiology and Biochemistry* 38, 825–836.

Jay, M., Bourdeix, R., Potier, F. and Sanlaville, C. (1989) Premiers résultats de l'étude des polyphénols foliaires du cocotier. *Oléagineux* 44, 151–161.

Jones, P., Tymon, A.M. and Mpunami, A.A. (1999) Detection and diagnosis of African lethal yellowing-like diseases. In: Oropeza, C., Ashburner, G.R., Verdeil, J.L. and Zizumbo, D. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 197–220.

Justin, S.H.F.W. (1978) Vegetative propagation of coconut. In: *Report East Malling Research Station 1977*, pp. 175–176.

Karp, A. (1999) The use of polymorphic microsatellites for assessing genetic diversity in coconut. In: Oropeza, C., Ashburner, R., Verdeil, J.-L. and Zizumbo, D. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 121–129.

Karun, A. (2001) Embryo culture protocol for collection and exchange of coconut germplasm. *Indian Coconut Journal – Cochin* 31, 17–18.

Karun, A., Shivanakan, S., Sahni, K.K. and San, K.V. (1993) Field collection and *in vitro* germination of coconut embryos. *Journal of Plantation Crops* 21, 219–294.

Karunaratne, S. and Periyapperuma, K. (1989) Culture of immature embryos of coconut, *Cocos nucifera* L.: callus proliferation and somatic embryogenesis. *Plant Science* 62, 247–253.

Karunaratne, S., Santha, S. and Kovoor, A. (1991) An *in vitro* assay for drought-tolerant coconut germplasm. *Euphytica* 53, 25–30.

Karunaratne, S., Kurukulaarachi, C. and Gamage, C. (1985) A report of the culture of embryos of the dwarf coconut, *Cocos nucifera* L. var. *nana*, *in vitro*. *Cocos* 3, 1–8.

Lebrun, P. and Baudouin, L. (2002) The development of a microsatellite kit and dedicated software for use with coconuts. *BUROTROP Bulletin* 17, 16–20.

Lebrun, P., N'Cho, Y.P., Seguin, M., Grivet, L. and Baudouin, L. (1998) Genetic diversity in coconut (*Cocos nucifera* L) revealed by restriction fragment length polymorphism (RFLP) markers. *Euphytica* 101, 103–108.

Lebrun, P., Grivet, L. and Baudouin, L. (1999) Use of RFLP markers to study the diversity of the coconut palm. In: Oropeza, C., Ashburner, R., Verdeil, J.-L. and Zizumbo, D. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 73–87.

Lebrun, P., Baudouin, L., Bourdeix, R., Konan, J.-L., Barker, J.H.K., Aldam, C., Herran, A. and Ritter, E. (2001) Construction of a linkage map of the Rennell Island Tall coconut type (*Cocos nucifera* L.) and QTL analysis for yield characters. *Genome* 44, 962–970.

Lombard, K. (2001) Reviewing the coconut (*Cocos nucifera* L.), Tree of Life. PSS5326 Advanced Seed Science, Texas Tech University. http://www.pssc.ttu.edu/agforest/review.htm

Lu, C.Y. and Vasil, I.K. (1985) Histology of somatic embryogenesis in *Panicum maximum* (Guinea grass). *American Journal of Botany* 72, 1908–1913.

Magnaval, C., Noirot, M., Verdeil, J.-L., Blattes, A., Huet, C., Grosdemange, F. and Buffard-Morel, J. (1995) Free amino acid composition of coconut (*Cocos nucifera* L.) calli under somatic embryogenesis induction conditions. *Journal of Plant Physiology* 146, 155–161.

Magnaval, C., Noirot, M., Verdeil, J.-L., Blattes, A., Huet, C., Grosdemange, F., Beulé, T. and Buffard-Morel, J. (1997) Specific nutritional requirements of coconut calli (*Cocos nucifera* L.) during somatic embryogenesis induction. *Journal of Plant Physiology* 150, 719–728.

Malaurie, B. and Borges, M. (2001) Cryopreservation of coconut (*Cocos nucifera* L.) plumules by encapsulation/dehydration. In: *International Workshop on Plant Biotechnology – Plant Breeding and Biotechnology, Ciego de Avila, 16–20*, p. 59 (Abstract).

Malaurie, B., N'Nan, O., Hocher, V., Ilbert, P., Grosdemange, F., Konan Konan, J.-L., Zakra, N. and Verdeil, J.-L. (2002) State of research on culture and cryopreservation of zygotic coconut embryos at IRD/CIRAD (France). In: Engelmann, F., Batugal, P.A. and Oliver, J.T. (eds) *Coconut Embryo In Vitro Culture* Part II. IPGRI-APO, Rome, pp. 146–156.

Malaurie, B., Borges, M. and N'Nan, O. (2003) Are encapsulation/osmoprotection/dehydration and encapsulation/osmoprotection/vitrification techniques suitable for cryopreservation of caulinary meristems of coconut (*Cocos nucifera* L.)? In: *IVth International Workshop on Plant Biotechnology and Sustainable Development*. Universidad de Ciego de Avila, Ciego de Avila, p. 95 (Abstract).

Menon, K.P.V. and Pandalai, K.M. (1958) *The Coconut Palm: a Monograph*. India Press, Bombay.

M'kumbo, O.K.E. (1995) Medium term *in vitro* storage of zygotic coconut embryos. PhD thesis, University of London.

Monfort, S. (1985) Androgenesis of coconuts: embryos from anther culture. *Zeitschrift für Pflanzenzüchtung* 94, 251–254.

Morel, G. and Wetmore, R.M. (1951) Fern callus tissue culture. *American Journal of Botany* 38, 141–143.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and biassays with tobacco tissue cultures. *Physiologia Plantarum* 15, 473–497.

Nair, M.K., Karun, A. and Rajesh, M.K. (1999) Review article – research potentialities of coconut biotechnology. CPCRI, Kasaragod-671 124, India, AgBiotechNet. Vol. 1, June, ABN 019. http://www.agbiotechnet.com/reviews/june99/html/nair.htm

N'Cho, Y.P., Sangare, A. and Bourdeix, R. (1998) Africa (Côte d'Ivoire). In: Ramanatha Rao, V. and Batugal, P. (eds) *Proceedings of the COGENT Regional Coconut Genebank Planning Workshop*. IPGRI-APO, Serdang, pp. 104–111.

Nitsch, J.P. (1969) Experimental androgenesis in *Nicotiana*. *Phytomorphology* 19, 389–404.

N'Nan, O. (1997) *Recherche d'une méthode de déshydratation simple, favorable à la survie et à la régénération des embryons zygotiques matures cryoconservés de cocotier (*Cocos nucifera *L.).* DEA, Université Abidjan, Cocody.

N'Nan, O. (2004) Utilization des biotechnologies comme seconde voie pour les échanges et la conservation des ressources génétiques du cocotier (*Cocos nucifera* L.). PhD thesis, Angers University, 199 pp.

N'Nan, O., Verdeil, J.-L., Hocher, V., Konan, J.-L., Zakra, N. and Malaurie, B. (2002a) Mise au point d'une méthode de cryoconservation d'apex caulinaire de cocotier (*Cocos nucifera* L.). In: El Hadrami, I. (ed.) *Biotechnologies Végétales: Valorisations pour une Agriculture Durable. Proceedings of VIIIèmes Journées Scientifiques du réseau 'Biotechnologies, Amélioration des Plantes et Sécurité Alimentaire' de l'Agence Universitaire de la Francophonie, 7–9 Octobre 2002, Marrakech*. AUF, Paris, pp. 152–153.

N'Nan, O., Verdeil, J.-L., Hocher, V., Konan-Konan, J.-L., Sangaré, A. and Malaurie, B. (2002b) *Collecting Mission of Coconut Albumen Core for the Comparison of Disinfection and Culture Process of Zygotic Embryos in Tropical Conditions*. Short Report, IPGRI/COGENT, Rome, 2 pp.

N'Nan, O., Borges, M., Verdeil, J.-L. and Malaurie, B. (2003) Are cryopreservation of mature zygotic embryos of coconut (*Cocos nucifera* L.) an easiest and safest way to preserve coconut germplasm? In: *IVth International Workshop on Plant Biotechnology and Sustainable Development*. Universidad de Ciego de Avila, Ciego de Avila, p. 96 (Abstract).

Ohler, J.G. (1984) *Coconut, Tree of Life*. FAO, Rome.

Ohler, J.G. (1999) *Modern Coconut Management*. FAO, Rome.

Pannetier, C. and Buffard-Morel, J. (1982) Premiers résultats concernant la production d'embryons somatiques à partir de tissus foliaires de cocotier, *Cocos nucifera*. *Oléagineux* 37, 349–354.

Pannetier, C. and Buffard-Morel, J. (1986) Coconut palm (*Cocos nucifera* L.). In: Bajaj, Y.P.S. (ed.) *Biotechnology in Agriculture and Forestry 1. Trees I*. Springer Verlag, Berlin, pp. 430–450.

Perera, L., Russell, J.R., Provan, J., McNicol, J.W. and Powell, W. (1998) Evaluating genetic relationships between indigenous coconut (*Cocos nucifera* L.) accessions from Sri Lanka by means of AFLP profiling. *Theoretical and Applied Genetics* 96, 545–550.

Perera, L., Russell, J.R., Provan, J. and Powell, W. (1999) Identification and characterisation of microsatellite loci in coconut (*Cocos nucifera* L.) and the analysis of coconut populations in Sri Lanka. *Molecular Ecology* 8, 344–346.

Persley, G.J. (1992) *Replanting the Tree of Life: Towards an International Agenda for Coconut Palm Research*. CAB International, Wallingford, UK, 156 pp.

Punchihewa, P.G. (1999) Current status of the coconut industry. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 3–17.

Raju, C.R., Prakash Kumar, P., Chandramohan, M. and Iyer, R.D. (1984) Coconut plantlets from leaf tissue cultures. *Plantation Crops* 12, 75–81.

Ramanatha Rao, V. and Batugal, P. (eds) (1998) *Proceedings of the COGENT Regional Coconut Genebank Planning Workshop*. IPGRI-APO, Serdang.

Reynolds, S.G. (1988) *Pastures and Cattle Under Coconuts*. FAO, Rome.

Rillo, E.P. (1985) Cadang-Cadang research: *in vitro* mechanical inoculation of different coconut cultivars and hybrids with ccRNA. In: *Annual Report*. Philippine Coconut Authority, p. 93.

Rillo, E.P. (1989) A non-destructive technique for collecting immature inflorescences for tissue culture. *Philippine Journal of Coconut Studies* 14, 16–17.

Rillo, E.P. (1995) *Embryo Culture Of Coconut. A Laboratory Manual*. GTZ Philippine–German Coconut Tissue Project, Legaspi, 42 pp.

Rillo, E.P. (1999) Coconut embryo culture. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 279–288.

Rillo, E.P. and Paloma, M.B.F. (1991) Storage and transport of zygotic embryos of *Cocos nucifera* L. for *in vitro* culture. *Plant Genetic Research Newsletter* 86, 1–4.

Ritter, E., Rodriguez, M.J.B., Herrán, A., Estioko, L., Becker, D. and Rohde, W. (2000) Analysis of quantitative trait loci (QTL) based on linkage maps in coconut (*Cocos nucifera* L.). In: Arencibia, A. (ed.) *Plant Genetic Engineering Towards the Third Millennium*. Elsevier Science, Amsterdam, pp. 42–48.

Rivera, R., Edwards, K.J., Barker, J.H.A., Arnold, G.M., Ayad, G., Hodgkin, T. and Karp, A. (1999) Isolation and characterisation of polymorphic microsatellites in *Cocos nucifera* L. *Genome* 42, 668–675.

Rodriguez, M.J.B. (1999) Detection and diagnosis of coconut cadang-cadang. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 221–226.

Rodriguez, M.J.B., Estioko, L.P., Namia, M.I.T. and Soniego, J.A. (1997) Analysis of genetic diversity by RAPD. *Philippine Journal of Crop Science* 22, 133–135.

Rognon, F. and de Nucé de Lamothe, M. (1978) Récolte et conditionnement du pollen pour la pollinisation des champs semenciers de cocotier. *Oléagineux* 33, 17–23.

Rohde, W., Kullaya, A., Rodriguez, J. and Ritter, E. (1995) Genetic analysis of *Cocos nucifera* L. by PCR amplification of spacer sequences separating a subset of copia-like EcoRI repetitive elements. *Journal of Genetics and Breeding* 49, 179–186.

Rohde, W., Becker, D., Kullaya, A., Rodriguez, M.J.B., Herrán, A. and Ritter, E. (1999) Analysis of coconut germplasm biodiversity by DNA marker technologies and construction of a first genetic linkage map. In: Oropeza, C., Ashburner, R., Verdeil, J.-L. and Zizumbo, D. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 99–120.

Saenz, L., Chan, J.-L., Souza, R., Hornung, R., Rillo, E., Verdeil, J.-L. and Oropeza, C. (1999) Somatic embryogenesis and regeneration in coconut from plumular explants. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 309–319.

Sakai, A., Matsumoto, T., Hirai, D. and Niino, T. (2000) Newly developed encapsulation–dehydration protocol for plant cryopreservation. *Cryo-Letters* 21, 53–62.

Samosir, Y.M.S., Godwin, I.D. and Adkins, S.W. (1999a) A new technique for coconut (*Cocos nucifera* L.) germplasm collection from remote sites: culturability of embryos following low-temperature incubation. *Australian Journal of Botany* 47, 69–75.

Samosir, Y.M.S., Godwin, I.D. and Adkins, S.W. (1999b) The use of osmotically active agents and abscisic acid can optimize the maturation of coconut somatic embryos. In: Oropeza, C., Verdeil, J.-L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 341–354.

Sandoval, A. (2002) Contribution à l'étude du cycle cellulaire au sein de tissus de cocotier (*Cocos nucifera* L.) cultivés *in vitro* et recherche de marqueurs moléculaires de l'activité méristématique. PhD thesis, ENSAM, Montpellier.

Sandoval, A., Hocher, V. and Verdeil, J.-L. (2003) Flow cytometric analysis of the cell cycle in different coconut palm (*Cocos nucifera* L.) tissues cultured *in vitro*. *Plant Cell Reports* 22, 25–31.

Santamaria, J., Talavera, C., Lavergne, D., Trabelsi, S., Verdeil, J.-L., Huet, C., Rival, A., Hamon, S. and Naoto, A. (1999) Effect of medium sucrose on the photosynthetic capacity of coconut vitroplants formed from zygotic embryos. In: Oropeza, C., Verdeil, J.-L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 371–381.

Schwendiman, J., Pannetier, C. and Michaux-Ferriere, N. (1988) Histology of somatic embryogenesis from leaf explants of the oil palm *Elaeis guineensis*. *Annals of Botany* 62, 41–52.

Sossou, J., Karunaratne, S. and Kovoor, A. (1987) Collecting palms: *in vitro* explanting in the field. *Plant Genetic Resources Newsletter* 69, 7–18.

Sugimura, Y. and Salvana, M.J. (1989) Induction and growth of callus derived from rachilla explants of young inflorescences of coconut palm. *Canadian Journal of Botany* 67, 272–274.

Talavera, C.R., Espadas, F.L., Aguilar, M.L., Maust, B.E., Oropeza, C.M. and Santamaria, J.M. (2001) The control of leaf water loss by coconut plants cultured *in vitro* depends on the type of membrane used for ventilation. *Journal of Horticultural Science and Biotechnology* 76, 569–574.

Teulat, B., Aldam, C., Trehin, R., Lebrun, P., Barker, J.H.A., Arnold, G.M., Karp, A., Baudouin, L. and Rognon, F. (2000) An analysis of genetic diversity in coconut (*Cocos nucifera*) populations from across the geographic range using sequence-tagged microsatellites (SSRs) and AFLPs. *Theoretical and Applied Genetics* 100, 764–771.

Thanh-Tuyen, N.T. (1985) Anther culture: its prospects for coconut improvement. *Philippine Journal of Crop Science* 10, 28–36.

Thanh-Tuyen, N.T. and De Guzman, E. (1983) Pollen developmental stages for coconut anther culture. *Philippine Journal of Biology* 12, 135–144.

Tisserat, B. (1990) *Palm Tissue Culture*. USDA, ARS, Pasadena, pp. 55–60.

Towill, L.E. (1985) Low temperature and freeze-/vacuum-drying preservation of pollen. In: Kartha, K.K. (ed.) *Cryopreservation of Plant Cells and Organs*. CRC Press, Boca Raton, Florida, pp. 171–198.

Towill, L.E. and Walters, C. (2000) Cryopreservation of pollen. In: Engelmann, F. and Takagi, H. (eds) *Cryopreservation of Tropical Plant Germplasm. Current Research Progress and Application*. JIRCAS/IPGRI, Rome, pp. 115–129.

Triques, K., Rival, A., Beulé, T., Dussert, S., Hocher, V., Verdeil, J.-L. and Hamon, S. (1997a) Developmental changes in carboxylase activities in *in vitro* cultured coconut zygotic embryos: comparison with corresponding activities in seedlings. *Plant Cell, Tissue and Organ Culture* 49, 227–231.

Triques, K., Rival, A., Beulé, T., Puard, M., Roy, J., Nato, A., Lavergne, D., Havaux, M., Verdeil, J.-L., Sangare, A. and Hamon, S. (1997b) Photosynthetic ability of *in vitro* grown coconut (*Cocos nucifera* L.) plantlets derived from zygotic embryos. *Plant Science* 127, 39–51.

Triques, K., Rival, A., Beulé, T., Morcillo, F., Hocher, V., Verdeil, J.-L. and Hamon, S. (1998) Changes in photosynthetic parameters during *in vitro* growth and acclimatization of coconut (*Cocos nucifera* L.) zygotic embryos. *Acta Horticulturae* 461, 275–280.

Ueda, S., Ceniza, M.S. and Sugimura, Y. (1993) Proliferative response induced from coconut embryo tissues cultured *in vitro*. *Japanese Journal of Tropical Agriculture* 37, 38–41.

Verdeil, J.-L. (1993) Etude de la régénération du cocotier (*Cocos nucifera* L.) par embryogenèse somatique à partir d'explants inflorescentiels. PhD thesis, l'Université Paris VI.

Verdeil, J.-L. and Buffard-Morel, J. (1995) Somatic embryogenesis in coconut (*Cocos nucifera* L.). In: Bajaj, Y.P.S. (ed.) *Biotechnology in Agriculture and Forestry*, Vol. 30. *Somatic Embryogenesis and Synthetic Seed I*. Springer-Verlag, Berlin, pp. 299–317.

Verdeil, J.-L. and Hocher, V. (2002) Digestion and absorption of food in plants: look a plant stomach! *Trends in Plant Science* 7, 280–281.

Verdeil, J.-L., Buffard-Morel, J. and Pannetier, C. (1989) Embryogenèse somatique du cocotier (*Cocos nucifera* L.) à partir de tissus foliaires et inflorescenciels. Bilan des recherches et perspectives. *Oléagineux* 44, 403–411.

Verdeil, J.-L., Huet, C., Grosdemange, F., Rival, A. and Buffard-Morel, J. (1992) Somatic embryogenesis in coconut (*Cocos nucifera* L.): obtention of several ramet clones. *Oléagineux* 47 (7), 465–469.

Verdeil, J.-L., Buffard-Morel, J., Dussert, S., Rival, A., Grosdemange, F., Huet, C. and Pannetier, C. (1993) Coconut clones through somatic embryogenesis. In: Nair, M.K., Khan H.H., Gopalasundaram P. and Bhaskara Rao, E.V.V. (eds) *Advances in Coconut Research and Development*. Kasaragod, pp. 173–179.

Verdeil, J.-L., Huet, C., Grosdemange, F. and Buffard-Morel, J. (1994) Plant regeneration from cultured immature inflorescences of coconut (*Cocos nucifera* L.): evidence for somatic embryogenesis. *Plant Cell Reports* 13, 218–221.

Verdeil, J.-L., Baudoin, L. and N'Cho, Y.P. (1995) Perspectives de la multiplication végétative du cocotier par embryogenèse somatique. *Oléagineux Corps Gras Lipides* 2, 92–97.

Verdeil, J.-L., Assy-Bah, B., Bourdeix, R., N'Cho, Y., Hocher, V., Buffard-Morel, J. and Sangare, A. (1996a) Le cocotier. In: Demarly, Y. and Picard, E. (eds) *Collection Biotechnologies Végétales. Intégration aux plantes tropicales*, Vol. 2. AUPELF UREF CNED, Paris, pp. 125–141.

Verdeil, J.-L., Hocher, V., Huet, C., Grosdemange, F., Sangare, A. and Hamon, S. (1996b) Effect of thidiazuron and 2,4-D on the maturation of coconut somatic embryos. In: *Plant Embryogenesis Workshop, Hamburg*. p. SVI-28.

Verdeil, J.-L., Baudoin, L., Hocher, V., Bourdeix, R., N'Cho, Y.P., Sangare, A., Rillo, E., Oropeza, C. and Hamon, S. (1998a) Quelles applications pour la micropropagation du cocotier (*Cocos nucifera* L.)? *Plantations, Recherche, Développement* 5, 333–342.

Verdeil, J.-L., Hocher, V., Triques, K., Lyakurwa, R., Rival, A., Durand-Gasselin, T., Engelmann, F., Sangare, A. and Hamon, S. (1998b) State of research on coconut embryo culture and acclimatization techniques in the IDEFOR (Côte d'Ivoire) and ORSTOM/CIRAD laboratories (France). In: Batugal,

P.A. and Engelmann, F. (eds) *Coconut Embryo In Vitro Culture*. IPGRI-APO, Serdang, Malaysia, pp. 17–28.

Verdeil, J.-L., Hornung, R., Jacobsen, H.G., Rillo, E., Oropeza, C., Bourdeix, R., N'Cho, Y.P., Hocher, V., Hamon, S. and Sangare, A. (1999) Recent progress on coconut micropropagation through a joined effort involving different countries. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 391–405.

Verdeil, J.-L., Hocher, V., Huet, C., Grosdemange, F., Escoute, J., Ferrière, N. and Nicole, M. (2001) Ultrastructural changes in coconut calli associated with the acquisition of embryogenic competence. *Annals of Botany* 88, 9–18.

Wadt, L.H.O., Sakiyama, N.S., Pereira, M.G., Tupinamba, E.A., Ribeiro, F.E. and Aragao, W.M. (1999) RAPD markers in the genetic diversity study of the coconut palm. In: Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardeña, R. and Santamaria, J.M. (eds) *Current Advances in Coconut Biotechnology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 89–97.

Whitehead, R.A. (1965) Freeze-drying and room temperature storage of coconut pollen. *Economic Botany* 19, 267.

Williams, E.G. and Maheswaran, G. (1986) Somatic embryogenesis: factors influencing co-ordinated behaviour of cells as an embryogenic group. *Annals of Botany* 57, 443–462.

Withers, L.A. and Williams, J.T. (1985) *In vitro Conservation. IBPGR Research Highlights*. International Board for Plant Genetic Resources, Rome.

Woodroof, J.G. (1979) *Coconuts: Production, Processing, Products*. AVI Publishing, Westport, Connecticut, 307 pp.

Wuidart, W. and Rognon, F. (1981) La production de semences de cocotier. *Oléagineux* 36, 131–138.

Hocher Valérie, Verdeil Jean-Luc, Malaurie Bernard (2004)

Cocos nucifera coconut

In : Litz R.E. (ed.) Biotechnology of fruit and nut crops : 4.1. Arecaceae

University of Florida ; s.n. : (USA) ; Cabi Publishing (USA), (29), 90-112. (Biotechnology in Agriculture Series ; 29)