

**REPRODUCTIVE BIOLOGY AND MICROPROPAGATION
OF
SELECTED SPECIES OF *CALAMUS* L. (Arecaceae)**

**Thesis submitted to the University of Calicut
in partial fulfilment of the requirement for the
degree of
Doctor of Philosophy**

**by
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UNIVERSITY OF CALICUT
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April, 2003

'In God I trust'


Dedicated to my beloved parents

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CERTIFICATE

This is to certify that the thesis entitled “**Reproductive Biology and Micropropagation of Selected Species of *Calamus L. (Arecaceae)***” submitted to the University of Calicut by Sulekha. K for the Degree of Doctor of Philosophy in Botany embodies the results of bonafide research work carried out by her under our supervision and guidance, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title or recognition.

Calicut
April 2003.



Dr. C. Renuka
Supervising Guide



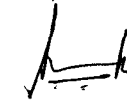
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DECLARATION

I, Sulekha. K, do hereby declare that the work presented in this thesis entitled "Reproductive Biology and Micropropagation of Selected Species of *Calamus* L. (Areaceae)" submitted by me in partial fulfilment for the Ph. D. degree in Botany of the University of Calicut under the supervision of Dr. C. Renuka, Scientist, Kerala Forest Research Institute, Peechi and Dr. Aleyamma Thomas, Professor of Botany, University of Calicut and has not been submitted earlier, either in part or in full, for any degree or diploma of any University.

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Abbreviations

Part I

KFRI	- Kerala Forest Research Institute
DAPI	- 4' 6 – diamidino 2 – phenylindole
CPD	- Critical point drying
SEM	- Scanning electron microscopy
TEM	- Transmission electron microscopy
VCD	- Vinyl cyclohexane dioxide
DER	- Diglycidyl ether of polypropylene glycol
NSA	- Non – eny succinic anhydride
DMAE	- Dimethyl amino ethanol
FAA	- Formalin acetic alcohol
PVP	- Polyvinylpyrrolidone
NTFP	- Non timber forest product
INBAR	- International network for Bamboo and Rattans
PAS	- Periodic acid Schiffs reagent
GA	- Gibberlic acid
TTC	- 2, 3, 5 – triphenyl tetrazolium chloride

Part II

2, 4-D	- 2, 4 – dichlorophenoxyacetic acid
BAP	- 6 - benzylaminopurine
DMRT	- Duncan multiple range test
EDTA	- Ethylene dimethyl tetra acetate
IAA	- Indol – 3 – acetic acid
IBA	- Indol – 3 – butyric acid
KIN	- Kinetin (6 – furfurylaminopurine)
mM	- Micromolar
MS	- Murashige and Skooge (1962) medium
NAA	- α – Naphthalene acetic acid
SH	- Schenk and Hildebrandt (1972) medium
UV	- Ultra violet
WH	- White's (1963) medium
Y3	- Eeuwens (1973) medium

PART-I
REFRODUCTIVE BIOLOGY

INTRODUCTION

1. INTRODUCTION

Rattans are climbing palms exploited for their flexible stems, the canes, which are extensively used in the furniture and handicraft industries. The rattan furniture is considered as a novelty with great demand in the world market. Rattans provide gainful employment to many people in rural and remote areas particularly the tribal people.

Rattan is perhaps the most extensively traded non-timber forest product (NTFP), the value in annual global trade being over US \$ 7 billion (Sastry, 2002). About 95 million people are estimated to be involved in growing, harvesting, processing, trading, and using cane and products. Undoubtedly, furniture is the most popular rattan product. Besides furniture, other products include carpet, walking sticks, umbrella handles, sporting goods, hats, ropes, cordage, matting, basketry, panelling and a host of other utility products. The cultural and socio-economic importance of rattans is therefore enormous. In South East Asia, it is estimated that over 5 million people are involved directly or indirectly in rattan industry. Indonesia accounts for about 90% of trade in raw rattan with an estimated value of US \$ 50 million.

In India, rattans have played a significant role in human activity since time immemorial. It is believed that they have been in use since 5th century B.C., particularly in the making of household articles, furniture, tool handles etc. (Lakshmana, 1993). Apart from their importance as a commodity for the furniture making industries, there are a great many other uses also known to the Indian people since ancient times. It is stated that the root of *Calamus rotang*, in combination with other herbs, is useful for treating snake bites and scorpion stings. Some species of rattans are used in tribal

rituals and festivals in some parts of India (Basu, 1985). The species also play a vital role in enriching the soil by their leaf litter, which adds to the organic content of the soil (Lakshmana, 1993).

Uhl and Dransfield (1987) have placed rattans under a distinct sub family, Calamoideae of Arecaceae (Palmae). With about 600 species under 13 genera, they are mainly distributed in Paleotropics with the maximum species diversity in South East Asia.

In India, rattans are reported to have 60 species under 4 genera viz., *Calamus*, *Daemonorops*, *Korthalsia* and *Plectocomia* (Renuka, 1999). Among the Indian rattans, 42 species are endemic to the country and are distributed in three centres viz., Peninsular India, North Eastern India and Andaman & Nicobar Islands. *Calamus* is the only rattan genus in Peninsular India with a representation of 22 reported species. In the genus *Calamus*, 15 species have been reported from Kerala (Renuka and Bhat, 2002).

The species *Calamus* occurs from almost sea level to 2000 m in ever green, semi-evergreen and moist deciduous forests. Most of the raw material for the local industry is obtained by harvesting wild rattan resources in the natural tropical forests but, since mid -1990's, the dwindling raw material supplies are posing a serious threat to the rattan industry. The thriving international and domestic trade in rattan and rattan products has led to substantial overexploitation of the wild rattan resource in natural forests. The rampant destruction of forests and habitats, and over extraction, that too, before flowering and fruiting has compounded this problem. The economic and social importance of the rattan sector is directly linked to wild rattan resource which is dwindling very fast due to unscientific extraction and lack of management. The rattan industries in Southern India are not getting enough raw materials locally and hence

have to depend on canes from North Eastern India and Andamans. To cope with the increasing global demand for rattan, there is an urgent need for sustainable management of the resources. Conservation and mass multiplication are some of the methods needed to be undertaken urgently to maintain a sustainable resource base for these valuable plants.

For developing effective conservation strategies, knowledge on the reproductive biology such as the time of flowering and fruiting, the availability of seeds, quantity of seeds produced, the rate of germination etc. is essential. Phenological information allows nursery activities to be synchronized with fruit harvest so that large quantities of seeds and subsequently of seedlings can be efficiently handled. Armed with such information, management would, in fact, be able to plan accurately all its planting activities.

A perusal of literature shows that the reproductive biology of rattan is poorly known. There is great variation in flowering and fruiting pattern in rattans. The observations recorded are incomplete in most of the cases. More details on flowering and fruiting are needed to improve our knowledge regarding the reproductive biology of various rattan species especially the commercially important ones (Williams *et al.*, 1995).

Rattans are gaining importance as a plantation crop. When large scale plantations are grown, the use of genetically improved seeds is desirable. In order to produce genetically improved rattans, basic information on floral biology and breeding system are essential.

At present our knowledge on pollination mechanism and fruit set is very limited. Both anemophily and entomophily have been recorded in rattans (Bogh, 1996; Alloysius, 1999; Lee and Jong, 1995 and Renuka *et al.*, 1998).

The time taken for germination and germination percentage also vary widely between and within the species (Generalo, 1977; Manokaran, 1978; Renuka *et al.*, 1998). They found that germination varied from 0.2 percent to 83-89 percent and the period from first to final germination varied from 2 weeks to one year. To understand this phenomenon, knowledge of embryo development is necessary.

Rattan seeds cannot be stored for a long time. The seeds should be put to germination soon after collection. When seeds are collected in large quantities, this becomes difficult, because seeds should be removed of sarcotesta and be cleaned before sowing to get a good germination percentage. Hence seed storage methods suitable for our climatic condition should be developed.

For certain commercially important species, seeds are not available in sufficient quantities. For certain others even though seeds are available germination percentage is very low. If enough seedlings can be produced through micropropagation the scarcity of seeds will not hinder the establishment of large scale plantations. In addition, micropropagation is the most reliable way for producing genetically stable and true – to – type progenies. Eventhough there are reports of successful micropropagation of some rattan species from South East Asia and China (Yusoff *et al.*, 1985; Bingshan and Guangtian, 1996), but not much literature is available on the work in India (Padmanabhan and Ilangovan, 1989; Valsala and Muralidharan, 1999).

The present study was undertaken with a view to shed more light on the reproductive biology and micropropagation of two commercially important species of *Calamus* - *C. hookerianus* and *C. thwaitesii*.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

A perusal of literature shows that even though considerable amount of work has been carried out in the reproductive biology in the family Palmae, not much work has been done in the subfamily Calamoideae.

The literature review has been dealt under two main headings, studies on Palms and studies on Rattans. Under these, the references are arranged in the following order: floral biology; pollination; microsporogenesis; megasporogenesis; fertilization; endosperm; embryo; fruit ; seed viability and germination.

2.1. STUDIES ON PALMS

2.1.1. Floral biology

The flowering and fruiting behaviour of various palms were studied from time to time by Blatter (1926), Juliano (1926), Patel (1938), Mahabale and Chennaveeraiah (1957), Douglas and Bimontoro (1956), Rao (1959a), Murthy and Bavappa (1960), Nair (1962), Mahabale and Biradar (1968), Bavappa and Rao (1970), Thomas *et al.* (1970), Schmid (1970), Tomlinson (1971), Uhl (1972), Tomlinson and Soderhlon (1975), Dransfield (1976, 1978), Murthy (1977), Mogeia (1978), Bullock (1981), Stephen (1981), Basu (1985), Beach (1984), Henderson (1984, 1985), Gonzalez *et al.* (1997), Burquez *et al.* (1987), Scariot *et al.* (1991) and Herrera (1989).

Uhl and Moore (1977) studied the centrifugal stamen initiation in three genera of Phytelephantoid group and found that the staminate flowers possessed 120 to over 900 stamens, the largest number known in palms. DeMason and Tisserat (1980) have

reported the occurrence of apparently bisexual flowers in the date palm, *Phoenix dactylifera*. The male flowers were with expanded carpel which produced parthenocarpic fruit. Histologically these carpels were similar to the carpels of pistillate flowers. Hermaphrodite flowers were also reported in the male inflorescence of *Pseudophoenix* (Read, 1968) and in *Salaca edulis* (Mogea, 1978).

Olesen and Balslev (1990) have studied the floral biology of the Amazonian monoecious palm, *Geonoma macrostachys* in detail.

Multiple inflorescences, i.e., several panicles per leaf axil, occur in a few genera in different major groups of palms (Moore, 1973). Maturation of inflorescences is acropetal within the crown in the majority of palms. In most caryotoid palms, however, the order of maturation is reversed and flowering is basipetal. Monocarpy characterizes several other genera in which all the axillary panicles mature at the same time (Tomlinson, 1977).

Guanchez and Romero (1995) have shown light to the unusual inflorescence of species of *Leopoldinia*. Even though Martius (1824) described *Leopoldinia* as a monoecious genus, the most recent account of the genus (Henderson, 1995) stated that *L. piassaba* may be dioecious (Wessels-Boer, 1988) or monoecious and have unisexual flowers. The plants of *Leopoldinia piassaba* studied by Francisco and Gustavo (1995) bore dimorphic, either staminate or pistillate inflorescence.

Prestoes schultzeana, a monoecious protandrous palm in the understory of forest in Amazonian Ecuador was studied by Ervik and Feil (1997).

The flowering phenology of 27 taxa of *Bactris* in a low land moist forest in the Central Amazon was recorded over a 40 month period by Henderson *et al.* (2000).

Barfod and Uhl (2001) have described the organogenesis of staminate flower clusters and the corresponding pistillate structures of *Aphandra natalia* and compared it with those of the other two genera in the Phytelphantoideae (*Phytelphas aequatorialis* and *P. macrocarpa*).

Miller (2002) has studied the reproductive phenology, fruit production and relationship between vegetative characteristics and fruit production in the palm *Oenocarpus bataua*. It was observed that the palm's biennial fruit production required 10-18 months for an inflorescence bud to mature, and the time from pollination to mature fruits is 10-14 months.

Male flowers of *Asaroa triandra* (Joseph, 1972) consisted of 3 stamens only and this character distinguished this palm from other genera of Arecaceae.

In monoecious palms of *Phychosperma* (Uhl, 1976), *Geonoma macrostachys* (Olesen and Balslev, 1990) and *Prestoea schultzeana* (Ervik and Feil, 1997), flowers are grouped in triads consisting of two lateral staminate and one pistillate flower.

Flowering biology of three *Aiphanes* species which were monoecious, protrandrous and spiny understorey palms was studied by Borchsenius (1993). In all the species studied, the proximal part of the rachilla bore triads of one pistillate and two staminate flowers and distally on the rachillae the triads were substituted by dyads.

2.1.2. Pollination

The reproductive biology of few of the 2, 800 species of palms has been studied in detail, so that we know very little about the modes of reproduction in this predominantly tropical family (Tomlinson, 1990). Though palms were long thought to be largely wind pollinated, their pollination is now known to involve several insect orders (Schmid, 1970; Moore and Uhl, 1982).

Studies on pollination in palms date back from the work of Read (1968, 1975) in *Thrinax parviflora*, *Asterogyne martiana* (Schmid 1970), *Bactris* (Essig, 1971), in *Ptychosperma macarthurii* (Essig, 1977), *Nypa fruticans* (Essig, 1973), in *Butia leiospatha* (Silberbauer, 1973) and *Sabal palmetto* (Brown, 1976).

Faegri and Van der Pyl (1996) stated that the oil palm, in common with most species in the Cocoideae was generally believed to be primarily anemophilous with a number of characteristics typical of wind pollinated species such as an abundant production of pollen, an enlarged stigmatic surface, a reduced perianth, raised flowers and small pollen grains (20-30 μm in diameter) with a smooth and dry surface.

Hartley (1979) reported that oil palm is almost exclusively wind pollinated. Considering the fact that the morphology of the flower presented several features of essentially wind pollinated plants, viz. lack of showy parts, bright colour, sticky and rough surfaced pollen, he concluded that coconut is basically wind-pollinated.

Uhl and Moore (1977) studied pollination in six genera of palms, *Thrinax*, *Phoenix*, *Nypa*, *Ptychosperma*, *Bactris* and *Asterogyne*, and found that the first two exhibited anemophilous and the other four, different entomophilous mode of pollination.

Mogea (1978) has studied the floral nature and insect pollination in *Salacca edulis*. This species is dioecious and the insects found in the inflorescences were mainly *Trigona* sp. (Hymenoptera) and *Rhynchophora palmarum* (Coleoptera).

The phenology of inflorescences and pollination of some rain forest palms in Costa Rica such as *Prestoea decurrens*, *Iriartea gigantea*, *Socratea durissima*, *Cryosophila albida*, *Bactris wendlandiana*, *Bactris longiseta*, *Astrocaryum alatum* and *Welfia georgii* have been described by Bullock (1981). Flowers of both sexes were diurnal and showed maturation of all flowers or organs of one sex throughout the inflorescence before any part of the other sex matured.

Morphology, phenology and insect visitors of *Cryosophila albida* inflorescence were studied by Henderson (1984). His observations, which included inflorescence, temperature, elevation, scent, and protogyny and beetle visitation indicated that this species is cantharophilous.

Inflorescence morphology, development and insect visitors of *Socratea exorrhiza* and *Iriartea ventricosa* were observed by Henderson (1985). Beetle and bee pollination, respectively were found in these species.

Moncur and Watson (1987) have reported the development of both staminate and hermaphrodite flowers on the same inflorescence of a monoecious palm *Salacca zalacca*; Observations suggested that ants were active in pollen transfer.

The observations carried out in the populations of *Chamaerops humilis* (Herrera, 1989) have shown that the species is dioecious and the plant shares traits of both anemophily and entomophily.

Kunth (1904) suggested specifically that several species of *Bactris*, including *B. major* are wind pollinated. But Essig (1971) reported beetle pollination in *B. guineensis* and *B. major*.

Observations made by Beach (1984) on two species of *Bactris*, *B. gasipaes* and *B. perschiana* showed that though, nectar was not produced by the palm, exceptional level of insect activity coupled with the presence of several features found in beetle pollinated species revealed that beetles were the most likely pollinators of the two species.

The review of "Pollination studies in the Palmae" by Henderson (1986) revealed that great diversity occurs in pollination. Three basic syndromes were found common in the family, cantharophily, mellitophily and myophily. Anemophily appeared uncommon and derived. He added that close association between certain beetles and palms may be indicative of ancestral cantharophily.

Since Henderson's review on the literature on palm pollination, a number of studies have been published. These studies have added much more information in many groups (Anderson *et al.*, 1988; Borchsenius, 1993; Burquez *et al.*, 1987; Ervik, 1991; Listabarth, 1992; Olesan and Balslev, 1990; Scariot *et al.*, 1991 and Zona, 1987).

Floral biology and pollination ecology of *Desmoncus polyacanthos* and *D. mitis* were investigated by Listabarth (1994). *Desmoncus*, a monoecious, clustered, climbing palm with pleoanthic type of flowering was found to be insect pollinated.

Ervik and Bernal (1996) reported that in *Prestoea decurrens* halictid bees play the principal role in pollination followed by trigonids bees and small flies.

Listabarth (2001) after studying the pollination of *Hyospathe elegans*, reached to a conclusion that except for beetle pollinated species, many palms lack major anthecological adaptations that apparently promote visitors of a particular taxonomic group of insects.

Structural and cytochemical features of the pistil, pollination and pollen-pistil interaction in the *Tenera* hybrid of African oil palm was investigated by Tandon *et al.* (2001). Pollination was found to be mediated by weevils and wind.

2.1.3. Developmental studies

The development and structure of embryo in monocotyledons have not been received the attention as those in dicots. Most of the systems of classification of angiosperm embryo are based on the embryology of dicots, and naturally try to derive the monocot embryo from the one in dicots.

Much of the work on monocot embryogeny seems to have been done on herbaceous plants, very little on the woody monocots. According to Corner (1966) woody monocots such as *Agave*, *Yucca*, Palms or *Pandanus* are the representatives of the ancient tropical flora, which seem to have deviated from the rest of the monocots and primitive dicots at a very early stage in the evolution of angiosperm.

Swamy (1942) has suggested a working hypothesis on the structure of the monocot embryo and Tomlinson (1960) has described the form of seedling leaves in palms.

The economically important family of palms was long neglected by the embryologists (Haccius and Philip, 1979) and the information on it is very scanty.

The available literature has been reviewed under the following sub heading.

2.1.3.1. *Microsporogenesis*

Santos (1929) and Juliano and Quisumbing (1931) gave an account of the morphology of the male flower of *Cocos nucifera*. They made a study on the anther wall and observed the differentiation of a plate of 4-6 hypodermal cells which functioned as the archesporium. Similar observations were made in many palms by Rao (1955a, 1959a, 1959b). They found that of the 6-8 sub-epidermal layers, the innermost layer functioned as the tapetum. However, the anther wall in *Cocos coronata*, and *C. schizophylla* was 4-5 layered as in most of the other palms such as Arecoid and Sabaloid palms (Rao, 1959a and 1959b). The wall of anther was 5-6 layered in *Hyphaene indica* as recorded by Mahabale and Chennaveeraiah (1957) and in *Caryota* species (Shrike, 1963); 4-6 layered in *Borassus flabellifer*, *Pritchardia*, and *Licuala* (Rao, 1959a, 1955b) and *Livistona chinensis* (Kulkarni and Mahabale, 1974); 4-5 layered in species of *Phoenix* by Mahabale and Biradar (1968); Biradar (1968); Biradar and Mahabale (1969) and 6 layered in *Chamaedorea elegans* (Gonzalez *et al.* 1997).

Binucleate tapetal cells were reported in *Hyphaene indica* (Mahabale and Chennaveeraiah, 1957), *Cocos nucifera* (Rao, 1959b) and in other members of the Arecineae, Cocoineae and Sabalae (Rao, 1959a and 1959b). In *Phoenix* species (Mahabale and Biradar, 1968) the tapetal cells are uninucleate initially, but become binucleate or even more later. Multinucleate tapetal cells are reported in *Cocos plumosa* (Rao 1959b). Tapetum was reported as secretory type in all the palms studied.

Sussenguth (1921) found simultaneous division of the microspore mother cells in *Chamaedorea sartorii*, *C. glaucophylla*, and in *C. karwinskian* and Schnarf (1931) in *C. corollina*, *Areca triandra* and *Caryota* species, Julianao and Quisumbing (1931) in *Cocos nucifera* and Rao (1959a) in *Chrysalidocarpus lutescens*, Mahabale and Chennaveeraiah (1957) in *Hyphaene indica*, Rao (1959a, 1959b) in *Licuala grandis*, *L. Peltata*, *L. spinosa*, *Livistona chinensis* and *L. rotundifolia*, *Trachycarpus* species, *Sabal adansonii*, *S. blackburnians*, *S. palmetto* and *Washingtonia* species, Mahabale and Biradar (1968) in *Phoenix sylvestris*, Shrike and Mahabale (1972) in *Caryota urens* and Robertson (1976a) in *Jubaeopsis caffra*. On the other hand Radermacher (1925) found successive type of cytokinesis in *Nypa fruticans*, and Mahabale and Biradar (1968) in *Phoenix sylvestris*.

The microspore tetrads were usually isobilateral and tetrahedral in palms (Johri *et al.*, 1992). In *Areca catechu* Rao (1955a) observed decussate and isobilateral tetrads in addition to the common tetrahedral arrangement. In *Hyphaene indica*, Mahabale and Chennaveeraiah (1957) reported the occurrence of polyspory as a result of the subsequent division of the members of the tetrad. Rao (1959b) found degeneration of one or more microspores of the tetrad in *Areca catechu*.

In all palms investigated, the pollen grains were shed at 2-celled stage and mature pollen grains were ellipsoidal or nearly spherical, monocolpate, and aporate. In *Cocos nucifera*, the pollen grains were smooth walled, monocolpate oval to elliptical and measured 49-52 μ and are larger than in other species. However Selling (1947) mentioned the rare occurrence of a trichotomosulcate grain in this species. Reticulate thickenings were observed on the exine of *Areca* and *Caryota* (Rao, 1959a). The

average size of pollen grains were reported as 29.5-34.04 μm in *Areca* (Rao, 1959a). The exine of *Hyphaene indica* (Mahabale and Chennaveeraiah, 1957) possessed rounded warts similar to those on the grains of *Borassus flabellifer* (Erdtman, 1952). Electron microscopic study of pollen of *Phoenix* by Tisserat and DeMason (1982) has revealed that the pollen grains were monosulcate, elliptical and had tectate-perforate exines.

The cytoplasm of the vegetative cell in palms was packed with starch and the generative cell was crescent-shaped with tapering ends (Rao 1959a). Similar shape was also reported in members of Asclepiadaceae (Rao and Rao, 1954). *In situ* germination of pollen was observed by Rao (1959a) in *Caryota urens*.

Punt and Wessels (1966) made a palynological study on Geonomoid palms. Pollen morphology with special reference to trends in aperture was studied by Sowunmi (1968). Muller (1970) recorded the presence of fossil pollen grains of *Nypa* from upper cretaceous.

The general morphology of pollen in the Palmae has been briefly described by Wodehouse (1935), Cranwell (1953), Erdtman (1969), Parthasarathy (1970), Zavada (1983) and studied more extensively by Thanikaimoni (1970).

2.1.3.2. Ovary and ovule

In the Palmae, wide variation is reported with respect to ovule structure. Davis (1966) stated that palm ovules were anatropous and bitegmic, while Moore and Uhl (1973) reported that many were in fact tri-tegmic, *ie*, with an aril.

The development of the female flower in coconut has been studied by Juliano (1926) and Patel (1938). The development of the ovule has been traced by Quisumbing and Juliano (1927).

Ovules were reported to be anatropous in *Cocos nucifera* (Juliano and Quisumbing, 1931), *Trachycarpus*, *Caryota*, *Cocos*, different species of *Phoenix*, and *Pritchardia* (Rao, 1959a, 1959b) and *Washingtonia* (Rao, 1959b); hemianatropous in *Sabal* species and *Areca* (Raghavan and Baruah, 1956a; Murthy and Bavappa, 1960; Bavappa, 1966), *Chrysalidocarpus lutescens* (Rao, 1959a), *Licuala* species and *Livistona chinensis* (Rao, 1956), and orthotropous in *Hyphaene indica* (Rao, 1956), *Actinoploesus*, *Bactris* and *Howea* (Rao, 1959b) and nearly orthotropous in *Livistona rotundifolia* (Rao, 1959a).

The ovule was crassinucellate and bitegmic in all the species studied. A well developed endothelium was organized by the radial elongation of the inner layer of the inner integument in *Licuala peltata*, *Pritchardia pacifica*, *Trachycarpus* species, *Washingtonia* species (Rao, 1959 a) and *Cocos plumosa* and *C. schizophylla* (Rao 1959b) and *Livistona chinensis* (Kulkarni and Mhabale, 1974). In *Livistona rotundifolia* the nucellar cells functioned as the nutritive tissue (Rao, 1959a).

Postament formation was reported in *Licuala* species and *Washingtonia* (Rao, 1959a). There was a well developed funicular obturator of radically elongated glandular cells in *Livistona chinensis* and *Elaies guineensis* (Kajale & Ranade, 1952). The development of the chalaza was very prominent in Sabaleae (Rao, 1959a). A hypostase differentiated during post fertilization stages.

2.1.3.3. *Megasporogenesis and female gametophyte*

In 1911, Bauch observed degenerating megaspores in *Cocos nucifera* thus showing it to be monosporic type. It was however reported to be of the Adoxa type by Quisumbing and Juliano (1927) and Juliano (1926) who, thought that all the four megaspore nuclei took part in the maturation of the embryo sac. In *Nypa fruticans* Radermacher (1925) also observed a tetranucleate megaspore cell similar to that of *Cocos*. Supernumerary megaspore mother cells were reported in *Cocos nucifera* (Bauch, 1911) and *Elaeis guineensis* (De Poerck, 1950).

Quisumbing and Juliano (1927) and De Poerck (1950) have reported tetrasporic Adoxa type of embryo sac development in *Elaeis* and *Cocos*. Kajale and Ranade (1953) observed linear, isobilateral, T-and L-shaped tetrad in *Elaeis guineensis*.

Kajale and Ranade (1953) reinvestigated megasporogenesis and revealed all types of megaspore tetrads in *Elaeis guineensis*, where as the work of Reddy and Kulkarni (1989) showed the presence of both linear and 'T' type megaspore tetrads in *Cocos nucifera*.

Rao (1959b) studied megasporogenesis in *Trachycarpus* species, *Sabal adansoni*, *S. blackburniana*, *S. palmetto*, *Washingtonia* species, *Licuala grandis*, *L. spinosa*, *Livistona chinensis* and *L. rotundifolia* and observed linear and 'T'-shaped megaspore tetrads. Linear and 'T' shaped tetrads were also reported in *Caryota mitis* and *Chrysalidocarpus lutescens* (Rao 1959a), *Howea belmoreana*, *Actinophloeus macarthurii*, *Areca catechu*, *Areca concinna* (Rao, 1959b) and in *Caryota urens* (Shrike, 1963).

The polygonum type of embryo sac seemed to be the most common type in the majority of palms investigated. Radermacher (1925) reported it to be so in *Actinophloeus macarthurii*, Swamy (1942) in *Areca catechu*, Rao (1959a and 1959b) in *A. concinna*, *A. triandra* and other species of *Actinophloeus*, *Pritchardia*, *Licuala*, *Livistona*, *Caryota*, *Trachycarpus*, *Washingtonia* and *Sabal* and Robertson (1976c) in *Jubaeopsis caffra*. However, Allium type of embryo sac has been reported by Johnsson (1979) in *Chamaedorea latifolia*, *Borassus flabellifer*, Mahabale and Chennaveeraiah (1957) in *Hyphaene indica* and Radermacher (1925) in *Nypa fruticans*. According to Maheshwari (1955) the embryo sacs in them were not bisporic as was supposed earlier.

Quisumbing and Juliano (1927) reported the presence of Adoxa type in *Cocos nucifera*, which however, on reinvestigation was confirmed to be of the Polygonum type by Kulkarni (1965) thus confirming the earlier observation of degenerating megaspores in this species by Bauch (1911).

In *Elaeis guineensis*, DePoerck (1950) reported the tetrasporic Adoxa type of embryo sac. This species was reinvestigated by Kajale and Ranade (1952) who found four different types of megaspore tetrads, but with a further development into the normal 8 nucleate Polygonum type of embryo sac.

Rao (1959a) studied the embryology, embryo, seed and seed germination in some members belonging to the tribes Arcineae and Coccoineae. In all the species investigated, namely *Caryota urens*, *C. mitis*, *Chrysalidocarpus lutescens*, *Howea belmoreana*, *Actinophloeus macarthurii*, *Areca catechu*, *A. triandra* and *A. coccinea* all belonging to *Arcineae* and *Cocos plumosa*, *C. schizophylla* and *Bactris major* of the Coccoineae, he reported the presence of the normal 8-nucleate embryo sac.

The same normal type of embryo sac has also been reported by Mahabale and Biradar (1968) and Biradar (1968) for *Phoenix sylvestris*, *P. robusta*, *P. pusilla*, *P. acaulis* and *P. reclinata*, Kulkarni and Mahabale (1974) in *Livistona chinensis* and Javalgekar (1979) in *Cocos nucifera* and *Borassus flabellifer*. Thus, though the number of species investigated so far by various workers forms only a fraction of the total number of palm species known today, many species consistently showed the normal 8-nucleate Polygonum type of embryo sac.

The report on the number and structure of the antipodal cells differ considerably. In *Chamaedorea concolor* there are three insignificant ephemeral antipodal cells (Sussenguth, 1921). In *Pinanga moluccana* (Lotscher, 1905) and *Calyptrocalyx* species (Bauch 1911) they were persistent and sometimes became 2-3 nucleate, a condition also reported by Rao (1959b) for *Chrysalidocarpus*. The antipodals are large, persistent and sac-like in *Areca catechu*, *Actinophloeus macarthurii* and *Howea belmoreana* and multinucleate in *Chrysalidocarpus lutescens* (Rao 1959 b).

Schnarf (1931) reported that the mature embryo sac of *Nypa fruiticans* lacks antipodal cells. Occurrence of twin embryo sacs has been reported in *Eleis guineensis* (Kajale and Ranade 1953). Guevara (1961) reported that in *Cocos nucifera* twin embryos were formed due to cleavage polyembryony.

2.1.3.4. Fertilization and endosperm development

In all the palm species studied, the pollen tube traversed through rich protoplasmic cells of the style and entered the ovule porogamously affecting the synergides by its entry into the embryo sac (Rao, 1959a; Mahabale and Biradar, 1968; Biradar, 1968). The

downward movement of pollen tubes was facilitated by the glandular cells of transmitting tissue, locular extension and funicle (Rao, 1956).

Most of the workers have reported the endosperm as free-nuclear in the beginning and later becoming cellular. The endosperm of *Cocos nucifera* showed many special features. In early stages the endosperm was in the form of a liquid syncytium with free polyploid nuclei (Datta, 1955), and fine cytoplasmic particles. Later vesicles appeared around the free nuclei, and they coalesced and a large vacuole appeared in the centre. The walls were laid down in the gelatinous mass around the central cavity (Cutter *et al.*, 1955). Rao (1959 a) had observed similar type of development in *Actinophloeus* and *Areca* species. In *Actinophloeus* species, according to Rao (1959 a), cell wall formation started in the micropylar region at 8-10 celled stage of the embryo, and proceeded to the chalazal region. Rao (1959a, 1959b) reported it to be so in species of *Caryota*, *Chrysalidocarpus*, *Howea*, *Cocos*, *Bactris*, *Licuala*, *Livistona*, *Trachycarpus*, *Washingtonia* and *Sabal* species. Mahabale and Biradar (1968) and Biradar (1968) noticed the same pattern of development in all the species of *Phoenix* they studied, Kulkarni (1965) in all species of *Cocos* he studied and Kulkarni and Mahabale (1974) in *Livistona chinensis*.

The endosperm cells of mature seeds of *Trachycarpus* sp., *Sabal* sp. *Washingtonia* sp. And *Livistona* sp. showed thick pitted walls which stored hemicellulose, starch and proteins in their lumens (Mahabale and Biradar, 1968).

In *Actinophloeus* sp. according to Rao (1959 a) cell wall formation started in the micropylar region at 8-10 celled stage of the embryo and proceeded to the chalazal

region as in members of Ceroxylineae. The endosperm cells near the placental groove were small, squarish or round but those towards the periphery were radially elongated.

Voiget (1888) has reported rumination in *Actinophloeus ambiguous* and *Caryota furfucata*. This has been confirmed by Periasamy (1962). Ruminant endosperm was also observed by Rao (1959b) in species of *Caryota*, *Howea* and *Areca*. Rumination, in species of *Phoenix* was confined only to the placental region (Biradar, 1968).

According to Lang (1943), the endosperm was free-nuclear in *Phoenix dactylifera*. The same pattern of development was also reported in *Areca catechu*, *Chrysalidocarpus lutescens* and in species of *Licuala*, *Livistona*, *Trachycarpus*, *Washingtonia* and *Sabal* (Rao, 1955b; 1959a and 1959b) and later became cellular in all species.

The structural characteristics of the endosperm in two species, *Phoenix dactylifera* and *Washingtonia filifera* has been described by DeMason, *et al.* (1985) and DeMason (1985).

In *Actinophloeus* the endosperm become cellular by the 8-10 celled stage of the embryo (Rao, 1959b). The cell wall formation started when the pro-embryo was in 4-5 celled stage in *Phoenix pussilla* and 8-10 celled in *P. acualis* and *P. reclinata* (Biradar, 1968).

Ruminated endosperm was reported in many palms. In *Livistona grandis* the chalaza becomes hump-like and developed warty protuberances which made the endosperm ruminant (Rao, 1956). In *Licuala* species it divided into islands or pockets by chalazal ruminaiton (Rao, 1959a). Ruminated endosperm was also reported in *Caryota urens*, *Howea belmoriana* and *Areca catechu*. Padmababhan and Regupathy (1981) studied the nature of endosperm in *Bentinckia condapanna*, raising some fundamental

questions regarding the rumination of endosperm. According to them in this species, it was the fruit wall which caused rumination: the pericarp was closely appressed to the seed coat. Ruminated seeds were also reported in *Arikuryoba schizophylla* and *Rhyticocos amara* (Reddy and Kulkarni, 1989).

The endosperm in *Chrysalidocarpus* differed from that of the other members in showing a massive chalazal haustorium (Rao, 1959b).

2.1.3.5. Embryo

Much of the work on monocot embryogeny seems to have been done on herbaceous plants and very little on the woody monocots. According to Corner (1966) woody monocots such as *Agave*, *Yucca*, Palms or *Pandanus* are the representatives of the ancient tropical flora, which seem to have deviated from the rest of the monocots and primitive dicots at a very early stage in the evolution of angiosperms.

The economically important family of palms was long neglected by the embryologists. The scattered references to palm embryology prior to 1931 have been reviewed by Schnarf (1931) who found that several species needed reinvestigation. A short report on the anatomy of the embryo of *Cocos nucifera* was published by Selvaratnam (1952). Ontogenetic sequences of the embryo were not studied and he had compared this embryo with that of the family Gramineae. He derived the embryo of coconut from the latter by the “scutellum and the epiblast extending over the plumule and tending to fuse completely, while the haustorial region extended into the endosperm to increase the surface of absorption”.

The important contribution towards the understanding of the embryology of a number of important members of Palmae was due to the work of Mahabale (1954-1968) and his students at the University of Poona.

In 1954, Deshpande worked on the anatomy and embryology of *Sabal palmetto* and Chennaveeraiah (1955) on the cyto-embryology of *Hyphaene indica*.

The first account of a palm embryo (*Areca catechu*) was given by Rao (1955a). According to him, the 'epiphysal' initial formed from the derivatives of apical cell gave rise to the stem tip, while those of the sub-epiphysal cells contributed to the formation of the cotyledon and the rest of the embryo proper. He reported that during the development of the embryo, there was a marked growth of the cotyledon on one side, resulting in the shifting of the portion of the stem tip towards a lateral position. Further he classified the embryo development as conforming to the Onograd type of Johansen (1950), where the embryo proper is exclusively derived from the products of apical cell. He mentioned that 'so far the formation of an epiphysis in any monocotyledon showing the Onograd type is not known.' Subsequently Rao (1956, 1959a, b) investigated other palm species such as *Licuala grandis*, *Livistona spinosa*, *L. chinensis*, *L. grandis*, *L. rotundifolia*, *Actinophloeus macarthurii*, *Sabal palmetto* and *Pritchardia* and came to the same conclusions.

On the other hand, Guignard (1961), without referring to Rao's publications, (Rao 1959a and 1959b) expressed the opinion that in *Chamaerops humilis* the stem tip is initiated in a lateral position, and that not only apical cell but also some derivatives of basal cell contribute to the embryo proper.

Shrike (1963) gave an account of the embryology in two spp. of *Caryota*. Kulkarni (1965) gave it in species of *Cocos*, such as *C. schizophylla*, *C. plumosa*, *C. yatay* and *C. capitata* and compared it with that of *C. nucifera*. He found the normal, monosporic, 8-nucleate Polygonum type of embryo sac in them and Onagrad type of embryo in all of them. Polyembryony was noticed in all species studied.

According to Mahabale and Biradar (1968), Biradar (1968), Biradar and Mahabale (1969) and Kulkarni and Mahabale (1974), the embryogeny in different species of *Phoenix* and *Livistona chinensis* conforms to that in *Areca catechu* with respect to the epiphysis and the initially terminal stem tip. The development conforms not to the Onagrad but to the Asterad type of Johansen (1950). Further, in contrast to earlier publications, these authors reported that in all species studied, the embryo developed two lateral cotyledonary lobes and one of the cotyledon elongated considerably and formed a massive cylindrical body at maturity. Thus according to them, a mature embryo has single well developed cotyledon, shoot apex, nodal plate, radicle and much reduced hypocotyledonary region.

Surange (1966) studied the embryology in *Licuala* and Mahabale and Biradar (1968) and Biradar (1968) in the genus *Phoenix*. Together they worked on the embryology of *Phoenix sylvestris*, *P. pusilla*, *P. robusta*, *P. acaulis* and *P. reclinata*. In all these species they found the normal Polygonum type of embryo sac and an Asterad type of embryo.

The embryogeny of *Jubaeopsis caffra* was investigated by Robertson (1976c). Without referring to the results of Mahabale (1982) and his co-workers, he described the embryo development in this palm species as belonging to the Asterad type.

Regarding the origin of stem tip, *Jubaeopsis caffra* seemed to resemble *Chamaerops humilis* as described by Guignard (1961).

Haccius and Philip (1979) with a view to assess the validity of these contrasting views on palm embryogenesis in general and *Cocos* in particular. They reported that in *Cocos* (and probably in all other Palms) the embryogenesis showed a number of primitive characters, such as differentiation of the embryo proper from one cell of the pluricellular pro-embryo origin of the single cotyledon from a position lateral to the terminal stem tip, and a tendency to cleavage polyembryony.

Haccius and Philip (1979) reported that in *Cocos nucifera* the zygote divided obliquely or transversely, the capital cell 'ca' divided more or less vertically and 'cb' the basal cell transversely to form a T-shaped tetrad, 'cb' may divide vertically also. A transverse division separated an epiphysis-like cell from the hemispherical daughter cell of 'ca' by a transverse division. Derivatives of the epiphysis-like cell lead to the differentiation of shoot tip and cotyledon and the other daughter cells of 'ca', along with those of 'cb', formed a multicellular suspensor.

Haccius and Philip (1979) emphasize that at the globular proembryo stage, a conspicuous shoot tip differentiates in the exact terminal position, and the cotyledon was lateral in position. Peripheral meristematic activity in the derivatives of the epiphysis-like cell leads to the differentiation of shoot tip and cotyledon.

Guignard (1984) and Robertson (1976c) interpreted the lateral origin of the shoot tip in the embryos of *Chamaerops humilis* and *Jubaeopsis caffra*.

Haccius and Philip (1979) refute the formation of two cotyledonary primordia which was indeed due to an erroneous interpretation of sections cut in an incorrect plane.

Haccius and Philip, 1979 are of opinion that two cotyledons in certain embryos of Palmae (Shriker, 1963; Kulkarni, 1965; Mahabale and Biradar, 1968; Biradar, 1968; Biradar and Mahabale, 1969; Kulkarni and Mahabale, 1974; Javalgeker, 1979) were erroneous conclusions due to examining merely the longisections.

2.1.4. Cytology

Chromosome numbers of palms were generally difficult to ascertain, but counts based on pollen germination provided reliable information (Read, 1963, 1965, 1966). The evidence suggested that in palm the basic haploid number was 18 and that lower numbers were derived from it. Mahabale and Chennaveeraiah (1957) showed that the great majority of pinnately leaved palms have 18 chromosomes while palmately leaved palms have 16. Within the range of genus also the number of chromosomes seems to be fixed, eg, *Cocos*=16, *Caryota*-18, *Borassus*=16, *Licuala*=16, *Phoenix*=18, *Hyphaene*=18 and *Sabal*-18.

Ninan and Ravindranath (1965) reported a naturally occurring haploid embryo in *Cocos nucifera* and suggested that haploid parthenogenesis occurs in Coconut and could possibly be induced. The only known polyploid is a dwarf *Arenga* from Malaya with $n = 32$ (Moore and Uhl, 1973).

2.1.5. Fruit and seed germination

Mahabale (1982) in his book "Palms of India" and Corner (1966) in his work on Natural history of Palms have devoted a complete chapter to palm fruits, seeds and seedlings. According to Corner (1966), various fruit types occur in different groups of palms. In all cocoid and Arecoid palms, drupes are encountered, while in *Phoenix* one seeded berries occur. Most palm fruits developed a pulpy wall at maturity. According to him, the seed coat, the hard endosperm and the small, soft cylindrical embryo were typical features of palm, seeds. He had described germination of seed and seedling structure in *Phoenix dactylifera*, *Nypa fruticans*, *Areca catechu*, *Elaeis guineensis*, *Archontophoenix*, *Ptychosperma* etc. He considered the sucking organ as the cotyledon and the tube-like outgrowth in the remotive type as the stalk of the cotyledon.

Size of the fruit varied from 4.5 mm in diameter as in the species of *Geonoma* to a very large size as in *Lodoicea*, where they may be 50 cm long and weigh 18 kg (Dransfield, 1970).

Although most fruits were smooth, scales occurred in those of all Calamoideae, hairs appeared on those of *Rhapidophyllum* and *Wettinia*, prickles were present on species of *Astrocaryum* and *Bactris*, while warty processes that arose by cracking of the pericarp characterized fruits of one species of *Chelyocarpus* and also of *Itaya johannesteijsmannia*, a few species of *Licuala* and one species of *Pholidocarpus*, *Manicaria*, *Pelagodoxa*, *Sommieria* and all genera of Phytelphantoideae (Dransfield, 1970). Uhl and Dransfield (1987) have reported an unusual specialization in *Lytocaryum* where fruits had a thin mesocarp that splitted into three valves to expose

the endocarp, and in some species of *Astrocaryum* and *Socratea* in which the mesocarp splitted irregularly at the apex and recurved to expose its coloured or white flesh.

Tomlinson (1960) has given a brief account of the germination and their significance as adaptations to habitat. He has reviewed on this point the work of Martius (1824) and Gatin (1906). It deals with the germination and seedling characters in *Chamaerops*, *Phoenix*, *Copernicia*, *Hypphaene*, *Lodoicea*, *Archontophoenix*, *Phytelephas*, *Nypa* etc.

Cook (1916) has suggested that in *Cocos* there developed a suctorial organ which was an extension of the cotyledon which he called by the term 'apocole' which was also recognized as such by Gatin (1906) on the basis of an earlier suggestion by Martius (1824). The latter has found that there are two types of germination in palms. The admotive type, in which elongation of the cotyledon was slight or nil and a cotyledonary ligule was invariably, present and 2) the remotive type in which the elongation of the cotyledon was much marked. Other than the work of Tomlinson (1960), there was some information on the seed behaviour and germination of palm other than rattans (Wyuatt, 1963; Corner, 1966; Foster and Gifford 1974; Ishihata, 1974; Manokaran, 1976; Padmanabhan *et al.*, 1978; Sabariah, 1978; Grout *et al.*, 1983; DeMason, 1985; Alang *et al.*, 1988; Davies and Pritchard, 1998; Muralikrishna *et al.*, 2001).

Germination studies conducted by Basu and Mukherjee (1972) showed that the duration of germination period vary greatly.

In monocotyledons, the cotyledon, or its distal tip, remained inside the seed during early development and was called as haustorium because of its presumed function in absorbing degradation products from the endosperm (Foster and Gifford, 1974).

The palm family, *Arecaceae* is an important family both botanically and horticulturally (Balick 1988; Tomlinson 1990; Uhl and Dransfield 1987). Most species are propagated solely by seed and many species are difficult to germinate. Very little is known about the controls of seed germination of palms or other noncereal monocotyledonous species Kalzowski & Gunn⁽¹⁹⁷²⁾ reported that as a rule, true seed dormancy is absent among the Palmae. In many instances development of the intact embryo after fruit ripeness is continuous and vivipary is not common.

Palm seeds contain abundant, hard, semitransparent, non-starchy endosperm and a small lateral embryo (Martin, 1946). During germination of palm seeds, the whole cotyledon or just the distal portion expanded within the seed and was called a haustorium because of its presumed function in absorbing degradation products from the endosperm (Boyd, 1932; Gatin, 1906; Tomlinson, 1960).

The structural and developmental features of the palm haustorium has been studied only in two species, *Phoenix dactylifera* (DeMason, 1984, 1985; DeMason and Stillman, 1986; DeMason *et al.*, 1989) and *Washingtonia filifera* (DeMason, 1986; DeMason and Stillman, 1986). The outermost layer, epithelium, consisted of tightly packed tubular cells that contain abundant cell organelles. The haustorium stored reserves, such as starch, lipid and phosphate granules (DeMason, 1984, 1985, 1986; DeMason and Stillman, 1986). In addition to absorption, it has been proposed that the palm haustorium secreted some factor that initiates endosperm degradation

(Chandrasekhar and DeMason, 1989, 1990; DeMason *et al.*, 1985, 1989; Lloyd, 1910) or that it secreted hydrolytic enzymes itself (Keusch, 1968; Opute, 1975).

Work of DeMason *et al.* (1991) on *in vitro* experiments with germinating date embryos, hypothesized that diffusible substances from the endosperm were responsible in controlling haustorium initiation and development.

In date palm, haustorium expansion and endosperm digestion were completed within 10 weeks after germination. (DeMason, 1985), but in coconut, these processes took several months (Troll, 1935).

2.1.6. Seed storage

Of the 2600 palm species in the world (Uhl and Dransfield, 1987), only a very small portion have been investigated for observing their germination and storage behaviour (Dickie *et al.*, 1992).

Information on seed storage potential is very limited. In an early review, De Leon (1961) primarily classified palm genera as being either long-lived or orthodox (remain viable for two to three months); intermediate-lived (four to six weeks) or short-lived or recalcitrant (two to three weeks).

Listabarth (2001) observed that in *Hyospathe elegans*, the seeds treated with potassium iodide and calcium oxychloride maintained significantly higher seed germination (98.5%).

2.2. STUDIES ON RATTANS

Rattan has been recognized as one of the most important non-timber products from the forest. Statistics abound to the fact that it is a unique plant with many excellent qualities. However literature also abounds with statements that rattan is a depleting resource, that the natural forests are being depleted of their rattan populations while there has been increasing attention to research rattan in the past decades, there are still many problem and challenges that need to overcome, if the rattan industry were to truly develop (Salleh, 2000).

Most of the rattans found in Asia are found to be dioecious. *Korthalsia* is the only monoecious genus in Asia but all the three genera in Africa (*Oncocalamus*, *Ancistrophyllum*, *Eremospatha*) are monoecious (Dransfield, 1979). *Korthalsia* has bisexual flowers. Little is known about flowering behaviour or of pollination of this genus other than chance observations of insect visitors (Dransfield 1981)

2.2.1. Flowering age

Several authors have reported the age of first flowering of rattan. Manokaran (1985) observed that *Calamus caesius*, *C. manan*, *C. trachycoleus* and *C. filipendulus* started flowering at 5.3, 5.5, 4 and 4.5 years respectively, after planting in the field. *C. rotang* and *C. hookerianus* under plantation in India started flowering from 4th year onwards. (Renuka, 1994). In a large progeny trial of *C. subinermis* in Sabah, Lee and Jong (1995) reported that some of the plants started flowering 3.5 years after seed germination and about 22 months after planting in the field. In Luasong, Sabah,

C. subinermis started flowering 5.3 years after seed germination and about 3.4 years after planting in the field (Alloysius, 1999).

Flowering patterns in tropical rainforests are often unpredictable. Once flowering is observed, fruit maturation can be predicted provided the time it takes between flowering and fruit ripening is known (Beniwal, 1987).

The phenology of rattan is poorly known except for some of the commercial species like *C. manan* and *C. tumidus* (Darus and Rasip, 1989; Manokaran, 1978; Raja-Barizan, 1992).

Mohd.-Zaki and Othman (1998) found that three species of rattan, *C. palustris*, *C. scipionun* and *C. ornatus* flowered in April-May and August-September during the study period, 1995 to 1997. A total of 12-13 months were needed from the emergence of the inflorescence to fruit ripening.

2.2.2. Floral biology

There are two types of flowering in rattans: hapaxanthic and pleoanthic (Dransfield, 1979). All species of *Korthalsia*, *Plectocomia*, *Plectocomiopsis*, *Myrialepis*, *Oncocalamus* and *Ancistrophyllum* are hapaxanthic. All other rattans are pleoanthic except several species of *Daemonorops* (Dransfield, 1979). Rattan inflorescences vary greatly in size and morphological structure. In rattans, as in other species, inflorescence structure is one of the most important characteristic for separating genera (Dransfield, 1992).

In *Korthalsia*, inflorescence axis pierced the leaf sheaths and emerged laterally, branched and produced spreading catkin-like flower-bearing structures. In *Calamus*, inflorescences often ended in a long flagellum (climbing organ) sometimes with a gradual succession of branches, often with discrete distant branches, also called partial inflorescences (Dransfield, 1984).

2.2.2.1. Flowers

Male inflorescences of *Calamus* usually consisted fertile male or staminate flowers. The staminate flowers were symmetrical with three sepals and three petals enclosing six stamens (Uhl and Dransfield, 1987 and Bogh, 1996).

The female inflorescence of *Calamus* had female or pistillate flowers; borne in pairs together with sterile male flowers (acolyte flowers). The pairs were known as diads. The acolyte flower had all the appearance of a fertile male flower except that the anthers were empty (Dransfield, 1984). The pistillate flowers had a tubular calyx which was shallowly 3-lobed (Uhl and Dransfield, 1987).

Chia (2000) has reported the occurrence of andromonoecy in *C. subinermis*. Andromonoecy was observed in all provenances in Sabah, and these were all mature plants producing canes so that the occurrence of hermaphroditic plants was not a juvenile character.

2.2.2.2. Pollination

Reports on the pollination of *Calamus* and other rattans have been based on static observations on the inflorescences during daylight hours and inferences from the

structure of the inflorescences. Beccari (1911) reported the presence of nectaries in two species of *Daemonorops*, a genus closely related to *Calamus*.

Corner (1966) postulated that hapaxanthic rattans (e.g. *Korthalsia*, *Plectocomia* and *Plectocomiopsis*) are mellittophilous. Dransfield (1979) reported trigonids and honeybees visiting male flowers of *Plectocomia* sp. Insect pollination was also suggested by Madulid (1980) in *Plectocomia*, which has extremely fragrant flowers and conspicuous inflorescences.

In most species of *Daemonorops*, *Ceratolobus*, *Calospatha* and *Calamus* of Calamoideae the inflorescences were wholly or partially enclosed by bracts at anthesis, emitted a musty odour and were visited by many beetles which were suggested by Dransfield (1979) to be pollinators. In other members of Calamoideae, the inflorescences were lax and were not enclosed by bracts, and have been reported by Dransfield (1979) to produce a sour odour and to be visited mostly by wasps and flies.

Kiew and Muid (1989) reported visits of *Trigona melina* to the male but not to the female, flowers of *Daemonorops didymophylla*. Uhl and Dransfield (1987) also suggested bees to be the most likely pollinators of many species of *Calamus*, although in some instances they may in fact be pollen thieves.

Lee and Jong (1995) made observations on the visitors to inflorescences of *Calamus subinermis* and *C. caesius* and came to the conclusion that the diurnally active trigonids and other *Hymenoptera* as well as other insect visitors were not thought to play a significant role because pollen release was mostly nocturnal. Pollen dispersed by wind

and collected with sticky suspended slides showed that wind may contribute to some short distance pollination.

Bogh (1996) after studying the pollination biology of four species of *Calamus*, *C. longsetus*, *C. peregrinus*, *C. rudentum* and *Calamus* sp. suggested that the exposed stigmas and anthers were easily accessible to any potential insect visitor and even to the wind. However, species studied suggested an entomophilous pollination syndrome.

Alloysius (1999) opined that wind pollination seemed to be not important in rattans. A study by Lee and Jong (1995) showed that the maximum horizontal distance of *Calamus subinermis* pollen dispersed by wind was 4 km and about 88% of the pollen was dispersed within 3.5 m from the inflorescence. Renuka *et al.* (1998) reported wind pollination in some of the South Indian species of *Calamus*. All the above mentioned researchers observed that during the opening of staminate flowers, nectar was produced and a strong fragrance was emitted.

The role of acolyte flowers in pollination was suggested as a visual attractant and providing nectar for pollination (Lee and Jong, 1995).

2.2.2.3. Fruiting

Manokaran (1985) reported that the supply of *C. manan* fruits varied with the season. From six years experience in seed collection, the author concluded that in Sabah, there are several months in a year when certain commercial species of rattans bear ripe fruits. Several researchers agree that flowering of rattans may be induced by fluctuations in temperature and weather (Manokaran, 1989; Raja-Barizan, 1992).

Burquez and Griffin (1990) stated that generally in natural forests, flowering period or duration varied widely with site, with provenance, from tree to tree within a species, and to some extent from year to year. Renuka (1992) reported that most of the rattan in India started flowering in October-November and fruits were available in April-May. Observations of Alloysius *et al.* (1994) in SAFODA'S rattan plantation revealed that inflorescences of *C. caesius* emerged between June to September every year.

Bogh (1996) investigated the phenology of four species of *Calamus* viz. (*C. longisetus*, *C. perigrinus*, *C. rudentum* and *C. sp.*) in Southern Thailand. Staminate plants flowered continuously for several months, whereas pistillate plants had much more shorter flowering periods.

Alloysius (1997) observed that for *C. caesius*, the period from the emergence of inflorescence to the anthesis stage was about 2 months and for *C. manan* it took about 3 months, where as *C. subinermis* takes 1.5 – 2 months. The period from inflorescence emergence to fruit maturity in *C. manan* has been reported to be 16 months (Darus and Rasip, 1989; Alloysius *et al.*, 1994). Banik and Nabi (1981) have observed the flowering behaviour and seed germination of *Calamus viminalis* var. *fasciculatus* and reported that the species flowers twice in a year and seed germinated 10-18 week after sowing.

Flowering and fruiting behaviour of *C. manan* was studied by Darus and Ghani (1989). Renuka *et al.*, 1998 had undertaken an ecogeographical study to understand the phenotypic variations of different population of *C. thwaitesii*, *C. andamanicus* and *C. palustris* throughout the Western Ghats of Kerala and Andaman and Nicobar islands

and the following time was reported to vary slightly from one place to another. All the species started flowering during July – August (Renuka, 1995).

Mohd Zaki and Othman (1998) have made phenological observations on *C. palustris*, *C. ornatus* and *C. scipionum* for 3 continuous flowering seasons. Morakinyo (1995) has briefly described the taxonomy, morphology and phenology and use of *Calamus deeratus*, *Eremospatha hooker*, *E. laurentii*, *E. macrocarpa*, *E. wendlandiana*, *Laccosperma leave*, *L. opacum*, *L. secundiflorum* and *Oncocalamus manni*.

2.2.3. Seed storage and germination

The seeds of many tropical plants contained high concentration of phenolic compounds and phenolic oxidase enzymes. These compounds were normally compartmentalized within cells. On desiccation, the cell membranes were damaged and phenolic compounds were released. They were oxidised and protein/phenol complexes were formed with a consequent loss of enzyme activity (Loomis and Battaile, 1966).

Roberts (1979) suggested that the death of the recalcitrant seeds occurred rapidly below the critical level of moisture content. This varied from species to species and was normally within the range of 12 to 31 percent.

Generalao (1977) reported that viability of rattan seeds was related to moisture content and dry storage methods were not effective.

Manokaran (1978) studied the percentages and rate of germination of some Malaysian rattans. The study involved 12 species of *Calamus*, 14 species of *Daemonorops*, 3 species of *Korthalsia* and one species each of *Ceratolobus*, *Myrialepis* and

Plectocomiopsis. The results indicated that there were some variations in germination, not only within the genera, but also between different samples of the same species. He concluded that this may be probably due to genetical differences, as well as to different degrees of ripeness of the various seed samples.

The experiments conducted by Guangtian and Huangcan (2000) revealed that the germination percentage of seed of *Daemonorops margaritae* was low and irregular with germination period of 10-12 months. They found that the temperature and moisture content are important for seed storage, which also influence seed viability. They found that the germination rate remained high even after 6 months when seeds were stored at 15^o C with moisture of 55% - 65 %.

Generalao (1977) and Manokaran (1978) found that the germination of various rattans varied from 4 to 41 weeks and seed germination percentage varied from 0.2% to 100%. The variation in germination was not only between species but also between different samples of the same species. Rai (1979) was of the opinion that the germination period varied from 2 weeks to one year based on the species and the maturity of the seed lot.

With a view to establish techniques for seed germination and storage, Generalao (1977), Manokaran (1978, 1983), Mori *et al.* (1980), Mendoza and Generalao (1979), Banik and Nabi (1981), Darus (1983), Alim and Kamaluddin (1985), Aminuddin and Zolipatah (1990), Mohiuddin and Ara (1995) and Siddiqui *et al.* (1996) have studied the behaviour of the seeds of *Calamus* spp.

Manokaran (1978) found that the germination of *Calamus manan* and *C. caesius* varied from 0.2 % to 83 % and 13 % to 89 % respectively. He also found that the period from

the first to the final germination was on an average about 10 weeks for *C. manan* and 11 weeks for *C. caesius* and *C. manan* started to germinate 3 weeks after sowing and final germination occurred between 7 to 8 weeks from the date of first germination.

Mori *et al.* (1980) observed that a complete removal of the sarcotesta was an indispensable pre-treatment for rattan seed germination. His findings on *Calamus manan* suggested that the seeds may not have seed dormancy. He added that the slow rate of germination may be due to the undeveloped seed structure, especially that of the radicle. He observed that the moisture content of the naked seeds must be kept between 45 and 55 percent during the storage period.

Bagaloyos (1987) reported that the viability of four species of rattans namely *Daemonorops mollis*, *Calamus merreillii*, *Calamus ornatus* var. *Philippinensis* and *C. filispadix* declined sharply when the moisture fell below 50 percent.

Mori *et al.* (1980) reported that the rattan fruits stored in closed plastic bags maintained high seed viability for one month at room temperature and for 3 months at room temperature between 10⁰ C and 14⁰ C. He also indicated that naked seeds stored in closed bags at room temperature maintained high seed viability above 50 percent for half a year.

Achmad (1988) concluded his findings that the seeds of *C. manan* germinated between 2-3 weeks after sowing and germination was completed by the ninth week. He also revealed that there was no significant difference in the germination percentage when the seeds of *C. manan* and *C. caesius* were sown in four different germination media. Generalao (1977) also observed that different types of media did not affect germination

percentage of seeds of *C. maximus* and *C. ornatus*. Agmato (1984) stated that the duration from sowing to germination was dependent on the season and time of harvest of the crop and it varied from 90 days to 365 days. He found that the seeds of *C. limuran* germinated within 2 days when the hilar cover was removed. This technique resulted in 97 percent germination. He (1985) detected embryo dormancy in *C. limuran* and found that GA at 1000 ppm concentration was successful in breaking the dormancy.

Renuka (1998) found that in *C. andamanicus* seeds took more than one year to germinate.

Bagalayos (1987) reported that stratification increased the germination to 80 percent in *C. ornatus*.

Aminuddin and Zolipatah (1990) have studied the germination characteristics of *C. manan* and *C. tumidus* under laboratory and nursery conditions. In the laboratory, cumulative germination of *C. manan* was 76 percent over 3-7 week and in the nursery it was 74 percent over 4-11 week. Figures for *C. tumidus* in the nursery were 43 and 6-31 week.

Pritchard and Davies (1998) investigated the fruit and seed characteristics of 28 Calamoid palms from South-East Asia, with a view to improve the conservation and utilisation of rattan species. He reported that germination rates with the seed lots with reasonable germination varied with species.

Biochemical changes during desiccation of *C. rotang* and *C. thwaitesii* have been studied by Girija *et al.* (1998) and their results revealed that hydrolysis of

carbohydrates, degradation of proteins and the accumulation of phenolic substances in seeds during desiccation can contribute to the death of the seeds during desiccation.

Technique for enhancing germination of *C. tenuis* seeds was developed by Baruah (1994). He observed that after the removal of scale and mesocarp, the germination percent could be increased to 90 percent. Effect of different sowing media on the seed germination and seedling growth of *C. tenuis* has been studied by Mohiuddin and Ara (1995) and Siddiqi *et al.* (1996).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. STUDY AREA

The materials for the present study were collected from various sources. The chief sources for the materials were Germplasm collections of rattans at Kerala Forest Research Institute (KFRI), Rattan research plot in Vazhachal Forest Division in Western Ghats, Field research station of KFRI at Veluppadam in Palappilly range of Chalakkudy Forest Division and Rattan seed stands of KFRI at Kachithode in Pattikkad range of Thrissur Forest Division. Materials were also collected from Achenkoil and Arienkavu of Themmala Forest Division.

3.2. MATERIALS

Two commercially important rattans, *Calamus hookerianus* Becc. and *C. thwaitesii* Becc. & Hook. f. were selected for the study.

3.2.1. Description of the species

Calamus hookerianus Becc. (Fig. 1A)

Clustering, moderate sized canes, climbing high into the canopy. Stem to 10 m or more long, with sheaths to 4 cm in diameter, without sheaths to 2.5 cm; internode to 26 cm long. Leaves to 2 m long, ecirrate; sheaths brownish green, densely armed with spines; spines triangular, the largest to 2.5 cm long, wide at the base, interspersed with numerous smaller spines and abundant brown tomentum, mouth of the sheath provided with long papery spines to 18 cm long; knee sometimes present, not conspicuous; ocrea

to 8 cm, falling off easily; flagellum to 5 m long; petioles to 75 cm long, brown or black, triangular; rachis with a single row of spines to 1.5 cm long or with reflexed spines; leaflets regular, the largest to 55 x 2.5 cm. long, acuminate, tips armed with short bristles, narrowly lanceolate, midvein ciliate beneath from centre upwards, lateral veins ciliate above. Inflorescence to 5 m long; primary sheath closely sheathing, armed with small spines; partial inflorescence to 75 cm long, male branching to 3 orders; secondary sheath to 9 cm long, closely sheathing, narrow at base, expanded into a cup at the upper region armed with one or two rows of small spines at the region where expansion begins, rachillae to 1 cm long; involucrophorum sessile; involucre cup-shaped. Fruits 1 x 0.8 cm, subglobose, scales in 18 rows, yellowish brown with a dark brown border. Endosperm ruminant, embryo basal; seedling leaf fan-shaped.

***C. thwaitesii* Becc. & Hook. f (Fig. 1B.)**

Robust, clustering and high climbing canes. Stem to 20 m or more in length, with sheaths to 6 cm in diameter, without sheaths to 3.5 cm, internodes to 45 cm long, sometimes with brown spots. Leaf to 3 m long, ecirrate, sheath yellow, densely armed with spines, spines arising from a raised rim-like surface, the largest 3 x 0.7 cm, flat, smaller spines scattered in between, black with yellow base; knee absent ocrea not conspicuous; flagellum to 9 m long; petiole to 35 cm long, petiole and rachis yellowish, armed with spines; spines arranged in oblique whorls, flat, black; leaflets numerous, variable in arrangement, usually grouped, the longest to 80 x 45 cm, sharply spinulose along the margins and upper side of midvein. Inflorescence to 6 m long; primary sheath upto 10 cm long, splits open distally, densely armed with spines, spines arranged in a semicircular row reflexed; partial inflorescence to about 70 cm long; secondary sheaths

Fig. 1. *Calamus hookerinus* and *C. thwaitesii* – Habit

Fig. 1A. *C. hookerianus* - Habit

Fig. 1B. *C. thwaitesii* - Habit



430

438

tightly sheathing, 3-4 cm long, male rachillae to 8 cm long, flowers distichous, female rachillae to 15 cm long; involucre not stalked; involucre cup-shaped. Fruit 2 x 1.3 cm, ovoid; scales in 12 vertical rows with median grooves, yellow with deep brown margin. Endosperm not ruminant, embryo basal; seedling leaf bifid.

3.3. METHODOLOGY

Frequent visits were made to the study sites to know the flowering season. Regular observations were carried out from the period of initiation of inflorescence to the period of fruit maturation.

3.3.1. Parameters studied in the field

The following parameters were studied during the visit to the plot.

- a) Flowering season
- b) Development of inflorescences/infructescences
- c) Number of inflorescence/infructescences
- d) Number of partial inflorescence
- e) Development of partial inflorescences
- f) Number of flowers per inflorescence
- g) Time and method of anthesis
- h) Insect visitors
- i) Period of stigma receptivity and pollen viability
- j) Size of fruit

- k) Colour of fruit scale
- l) Texture of endosperm
- m) Number of matured fruits.

For recording the time of floral opening and anthesis, observations were made during night hours. In both the species a number of inflorescences were examined. During anthesis these inflorescences were observed for 10-20 minutes, every second or third hour.

These observations were made for inflorescences of both sexes for both species through at least a full day and night. The sequence of flower opening was studied by marking individual rachillae.

3.3.1.1. Pollen viability and stigma receptivity

To study the receptivity of stigma, pollen grains were collected from the male inflorescences undergoing anthesis and kept in a small glass vial. Branches of female inflorescence in which the flowers had started opening were selected and bagged to avoid pollination from other male flowers. The flowers of two rachillae were pollinated with the collected pollen, bagged, and labelled. After two hours the process was repeated in another two sets of rachillae. The pollination was continued for eight hours. Then the time gap was increased and it was continued till the stigma turned brown in colour. The pollinated pistils were fixed in acetic acid-ethyl alcohol in 1:3 ratio for 24 hours, then washed and stored in 70% ethanol. The tissue was processed according to the procedure outlined in Shivanna and Rangaswamy (1993) and observed under the fluorescent microscope.

3.3.2. Parameters studied in the laboratory

Microsporogenesis, megasporogenesis, embryogenesis, seed morphology, viability, vigour of seeds and germination were studied in the laboratory.

3.3.2.1. *Microsporogenesis*

Meiosis in pollen mother cells was studied following the schedule for acetocarmine squash preparations (Shivanna and Rangaswamy, 1993). For anther development, flower buds at different stages of development were fixed in formalin-acetic-alcohol and the customary methods of washing, dehydration, clearing and embedding were followed (Johansen, 1940; Sass, 1958).

Transmission electron microscopic studies

Transmission electron microscopic studies of anthers of both species were conducted at Central Marine Fisheries Research Institute (CMFRI), Kochi to reveal the ultra structure of wall layers and pollen grains (Spurr, 1968). The steps involved in tissue processing for TEM were as follows.

Fixation

Flower buds of staminate flowers of both species were fixed in 3% glutaldehyde in 0.1 M phosphate buffer at pH 7.4 for 4 hrs. The fixed tissue pieces were washed thrice thoroughly in cold 0.1 M Phosphate buffer for 30 min.

Osmication

The tissue pieces were again fixed in 2% osmium tetroxide in 0.1M. Phosphate buffer at pH 7.4 for 2 hrs at 4⁰ C. Osmicated tissue pieces were washed thrice thoroughly in

cold 0.1 M phosphate buffer for 10 min each. The tissue was given two washings in cold distilled water for 30 min each. The washed tissues were kept in 0.5 % uranylacetate solution for overnight and washed in cold distilled water for 5 changes with 10 min.

Dehydration

The osmicated tissues were dehydrated through an ethanolic series of 25, 50, 75, 95 and 100 % for 10 min. Lastly the tissues were dehydrated in 100 % acetone for 2 changes with 30 min, each at room temperature.

Infiltration

Dehydrated tissue pieces were infiltrated in embedding medium. Spurr's low viscosity resin called plastic was used as the embedding medium. It was prepared by the method of Spurr (1968). The composition of the resin was as follows: (a) Vinyl cyclohexane dioxide (VCD) – 10.0 g; (b) Diglycidyl ether of polypropylene glycol (flexibilizer - DER 736) – 6.0 g; (c) non-eny succinic anhydride (hardner –NSA) – 26.0 g; (d) dimethyl amino ethanol (accelerator - DMAE) – 0.4 g. The accelerator was added last after gently mixing the other components. The complete medium was fixed thoroughly by stirring. The infiltration was done in a series of 25 % resin + 75 % acetone; 50 % resin + 50 % acetone for 3 hrs each at room temperature. The tissue was kept in 75 % resin + 25 % acetone for overnight. As the last steps in infiltration, the tissue pieces were kept in 100 % resin for 4 changes each with 2 hrs at room temperature. Continuous mild agitation was given to the tissues during all the steps in filtration using a rotator.

Embedding

The infiltrated tissues were embedded in 100% resin filled in embedding moulds. Specimens were sunk to the bottom of the media. The embedded moulds were polymerised in an oven at 70⁰ C for 24 hrs. The polymerised blocks were ready for ultra thin sectioning.

Sectioning

The tissue blocks were sectioned by an ultra tome (NOVA, LKB). Ultra thin sections were mounted on clean copper grids carefully.

Staining

The mounted grids were first stained for 15 min in 2% aqueous uranyl acetate. The grids were rinsed in double distilled water. The grids were again stained in 4% lead citrate for 5 min. The grids were rinsed in double distilled water and dried under light for one hour. The dried specimen grids were viewed and photographed using a Transmission Electron Microscope (TEM) (JEOL 100S).

Scanning electron microscopic studies

Scanning electron microscopic studies were conducted Sree Chitra Research Institute, Trivandrum to study the external morphology of pollen grains and stigma of both species (Falk, 1980). The steps involved in tissue processing for SEM were as follows.

Fixation

The acetolysed pollen grains and carpels of both the species were fixed in 3% glutaldehyde in 0.1 M phosphate buffer for 15 min. (Nair, 1970).

Dehydration

The fixed tissues were dehydrated through acetone series of 25%, 50%, 75% and 100 % acetone for 10 min each. Finally the tissues were kept in 100 % acetone for 30 min at room temperature.

Critical Point Drying (CPD)

Critical point drying of carpel was done to overcome the damage caused by the passage of a phase boundary through the specimen (Falk, 1980). CPD of dehydrated specimen was done in a critical point drier (H.C.P-2, Hitachi). During CPD, the specimen water was replaced by liquid carbon dioxide at 31⁰ C and 71.8⁰ C atmospheric pressure for 20 min.

Mounting

The dried specimens were mounted on stubs using adhesive tapes. The mounted stubs were coated with gold in a sputter coat (Model E-101-Hitachi). The coated specimens were observed under SEM (S-2400. Hitachi).

3.3.2.2. Megasporogenesis and megagametogenesis

The developing ovaries were fixed in formalin-acetic-alcohol (FAA) for a week and were treated with one percent potassium hydroxide for about 10 minutes at 25 to 40⁰ C, washed in water and stained in cotton blue. The material was pressed under the cover slip and studied under the microscope to observe the megaspore mother cell and further development. Microtome sections were also prepared following the procedure of Johansen (1940).

3.3.2.3. Pollen viability and stigma receptivity

The suitable pollen germination media was studied by dusting pollen grains in different germination media such as sucrose and Breu Baker medium. The maximum percentage of pollen germination was found out by dusting the pollen grains in the germination medium every one hour from the time of anthesis till the next evening.

Pollen viability was studied by dusting pollen samples on a microslide with few drops of 0.5 percent 2, 3, 5 - triphenyltetrazolium chloride (TTC) in 0.5 percent sucrose solution for *C. hookerianus* and 0.3 percent for *C. thwaitesii* and a cover glass was dropped over it. The preparation was incubated in dark under laboratory temperature for 30-60 minutes and observed under a compound microscope.

To study the nuclear state during pollen development, fresh pollen grains before and after anthesis was dusted in DAPI (4', 6-diamidino-2-phenylindole) working solution (Shivanna and Rangaswamy, 1993) in pollen germination medium and lowered a coverslip. The slides were incubated in a humidity chamber for 5 - 10 minutes and observed under the fluorescent microscope.

To study how long the pollen grains remain viable, the grains of both species were collected in tissue paper and they were stored in glass vials after wrapping with wet paper. The vials were kept in the refrigerator at 10⁰ C. They were taken out and germinated periodically.

3.3.2.4. Pollination mechanism

Detailed observations were made on the presence and the behaviour of visiting insects. The insects were collected in polythene bags for identification and examination of

pollen loads. After scrutiny under a dissection microscope, the captured insects were rinsed for pollen. The resulting suspensions were transferred to micro slides and checked for presence of pollen loads.

A strip of transparent adhesive tape was suspended for approximately 24 hours near the pistillate inflorescences of both the species. The strip was observed under microscope for the presence of wind borne pollen deposited on it.

3.3.2.5. *Studies on endosperm*

PAS staining of endosperm tissue was done to study the nature of the endosperm (Berlyn and Miksche, 1976). Method using periodic acid Schiff's (PAS) reagent is as follows.

Staining procedure

The deparaffinated sections were placed in 0.5 % (W/r) periodic solution in distilled water at 24⁰ C for 15 min. and washed thoroughly in running water. Then the slides were stained with Schiff's reagent for 15 min. at 4⁰ C in refrigerator. The sections were rinsed in water and placed in 2% sodium bisulphite for 2 min. and washed in running tap water for 5 min. The slides were dehydrated through alcohol series and cleared in xylene and mounted in DPX.

3.3.2.6. *Embryogenesis*

For embryological studies, developing fruits were collected at an interval of about 4-5 days and this was continued till the fruits were partially matured. Maturation of embryo and endosperm development was studied from fruits collected at an interval of one

week, 15 days and a month till the fruits attained complete maturity and were ready to fall.

The ovary wall in the developing fruits and the outer part of the endosperm in the maturing fruits were carefully trimmed to facilitate proper penetration of the fixative – the formalion acetic alcohol (FAA). Hard seeds were softened with 15 percent hydrofluoric acid in 70 percent ethyl alcohol. The customary methods of washing, dehydration, clearing and embedding were followed (Johansen, 1940; Sass, 1958).

The thickness of the sections generally varied between 6μ and 15μ . Even after softening in hydroflouric acid, usual paraffin method made the material very hard in mature fruits. Hence, in such cases before sectioning, the material in the paraffin block was slightly exposed on one side and soaked in distilled water for a day or two (Johansen, 1940). Haupt's adhesive was used to affix the ribbons to the slide. Staining with Heidenhein's iron-alum hematoxylin (Johansen, 1940) and subsequently with tannic acid and iron chloride (Foster, 1934) proved to be better for staining early developmental stages while safranin-fast green combination was found to be more satisfactory for post-fertilization stages.

Tannin, which was abundant in the material interferred with the smooth working of the paraffin method. This was overcome to some extent by immersing the trimmed material in 5 percent polyvenylpyrrolidine (PVP) for 15 minutes and then washing in distilled water before fixing in FAA. From the trimmed endosperm, the embryo showed tendency to detatch which made a lot of problems throughout the study.

3.3.2.7. Seed morphology

Number of fruits per Kg

The fruits were weighed and fruit number per kg was counted.

Fruit length

The length of the fruit stalk end to the styler end was measured with a vernier calliper and expressed in mm.

Fruit Breadth

The breadth of the fruit was measured at the centre with vernier calliper and expressed in mm.

Fruit weight

The weight of individual fruit was taken on an electronic balance and the mean of 100 fruits taken as the mean weight and expressed in g.

Seed length

The seed length was measured using a vernier calliper and mean value expressed in mm.

Seed breadth

The seed breadth was measured using a vernier calliper and expressed in mm.

Seed thickness

The seed thickness was measured with screw gauge and expressed in mm.

Seed weight

The seed weight of individual seed was taken in an electronic balance and the mean of 100 seeds expressed in mg.

Seed moisture content (ISTA, 1993)

The seed moisture content was estimated in a hot air oven using the low constant temperature at $105^{\circ}\text{C} \pm 2$ for 16 hrs. and expressed as percentage.

Seed longevity

The period upto which the seeds possess viability was assessed by conducting germination test at 2 days interval.

3.3.2.8. Viability and vigour of seeds

The fruits were subjected to the following treatments to study the viability and vigour of seeds.

1. Gibberellic acid – 250 ppm

The sarcotesta and the fleshy mesocarp of the fruits were removed and the seeds were treated in gibberellic acid (250 ppm) for 12 hrs. and were sown in the germination medium.

2. Scarification

The sarcotesta and the fleshy mesocarp of the fruits were removed and the surface of the seeds were cleared using sand and put for germination.

3. Stratification

The cleaned seeds were kept under 5°C and 10°C for 5 days and were sown in the germination medium.

4. Oven drying at 45⁰ C for 4 days

The seeds were kept in an oven for 5 day where the temperature was maintained at 45⁰

C. On the 6th day these seeds were put for germination.

5. Control

Cleaned seeds were sown as such in the germination medium.

To test the viability, tetrazolium test was conducted.

The seeds were cut longitudinally through the embryo and were stained in 1 % 2, 3, 5 – triphenyl tetrazolium chloride in alternate days till the colour of the embryo in the seed remained white indicating the loss of viability.

3.3.2.9. Seed germination

The growth of embryo during germination was studied by trimming the endosperm and scooping the developing embryo.

The seed coat and the endosperm of both the species were trimmed so as to study the behaviour of the distal end of cotyledon at various stages of seed germination.

In all germination experiments, the seeds were sown in plastic trays where the soil consisted of equal amounts of top soil and river sand. The seeds were buried just beneath the soil surface and were kept moist by watering twice daily. The trays were kept under shade to reduce the intensity of light. Observations were carried out weekly, and the seed was considered as germinated when the conical white structure of the embryo appeared above the soil level.

Total phenol

Total phenol content of seeds of both species were estimated at different moisture levels and expressed in mg g⁻¹ dry weight (Bray and Thorpe, 1954).

3.3.2.10. Seed storage

To study an effective method for seed storage, fruits as well as seeds were stored in airtight plastic bags and refrigerator at 5^o C and their viability were assessed periodically by withdrawal of samples for germination. Germination trials were also carried out where the fruits were subdivided into two groups. One group was sown in the usual way with the pericarp and sarcotesta removed while the other was sown with both the sarcotesta and pericarp intact.

OBSERVATION

500

4. OBSERVATION

4.1. FLOWERING SEASON

Five years observation in flowering pattern in both *C. hookerianus* and *C. thwaitesii* showed that the time of initiation of flowering varied slightly with locality and from year to year (Tables 1 and 2).

Table 1. Period of initiation of male inflorescence in both species of *Calamus*

Species	Year	Kachithode, Pattikkad Forest Division	KFRI Campus, Peechi	Rattan Research plot, Vazhachal
Ch	1998	June-July	June-July	June-July
“		June-July	November-December	November-December
Ct		May-June	August	June-July
Ch	1999	February-March	April-May	May-June
“		June-July	July	August
Ct		June-July	July	June-July
“		November-December	-	-
Ch	2000	May-June	April – May	July-August
“		November-December	June-July	-
Ct		June- July	August	May-July
Ch	2001	June-July	April	-
“		December	November-December	May-June
Ct		July-August	July	July-August
Ch	2002	March-April	June-July	February-March
“		November-December	December	June-July
Ct		May-June	-	-
“		August-September	July	May – June

Ch - *C. hookerianus* Ct - *C. thwaitesii*.

Generally male inflorescences are produced twice an year. One season of flowering will be synchronising with that of female inflorescence.

Table 2. Period of initiation of female inflorescence in both species of *Calamus*

Species	Year	Kachithode, Pattikkad Forest Division	KFRI Campus, Peechi	Rattan Research plot, Vazhachal
Ch	1998	July-August	June-July	June- July
Ct	1998	June-July	December – January	November-December
Ch	1999	March – April	June-July	May-June
Ch	1999	May-June	April – May	June - July
Ct	1999	-	-	July-August
Ch	2000	June-July	April-May	May-July
Ct		-	July-August	May-July
Ch	2001	June-July	April	May-June
“		-	July-August	-
Ct		-	June-July	June-July
“		-	May	July-August
Ch	2002	April-May	June-July	-
Ch		-	-	June-July
Ct		March-April	May-June	April-May
Ct		June-July	September	April-May

Ch - *C. hookerianus* Ct - *C. thwaitesii*

It was observed that there were several months in a year in which the two species produced inflorescences and the peak period was between May and August which is relatively a wet period in Kerala. The weather data collected from Kerala Forest Research Institute (KFRI), for the years 1998, 2000 and 2001 showed that the development of inflorescence is triggered by external factors like temperature, humidity and rain fall. A correlation between the climatic factors and initiation of flowering showed that the development of inflorescence in both male and female plants of *C. hookerianus* and *C. thwaitesii* is initiated by high temperature, accelerated by high humidity and heavy rainfall (Table 3.)

Table 3. Corelation of weather fluctuations and initiation of flowering

Month	<i>C. hookerianus</i>			<i>C. thwaitesii</i>			Wind			Temperature			Humidity			Total rain		
	1998	2000	2001	1998	2000	2001	1998	2000	2001	1998	2000	2001	1998	2000	2001	1998	2000	2001
Jan.				♀			1.8	NR	2	17.5	28.0	26.4	71.8	59.0	90.2	0.0	0.0	0.0
Feb.							1.6	NR	0.9	17.4	28.1	27.4	74.5	68.9	93.6	7.8	4.6	26.1
Mar.							1.5	NR	0.8	19.1	29.7	28.0	65.8	67.3	94.3	0.0	0.0	26.3
Apr.		♂♀	♂♀				1.9	NR	0.5	19.5	29.3	28.1	77.7	73.7	97.5	28.2	67.9	271.4
May		♂♀	♂♀	♂	♂♀	♀	2.3	NR	0.6	18.8	29.6	27.9	88.6	72.0	98.8	3.8	117.2	298.8
Jun.	♂♀	♂♀	♂♀	♂♀	♂	♀	5.4	0.3	0.6	16.6	26.1	25.4	96.8	97.9	99.9	1284.1	214.8	987.1
Jul.	♂♀	♂♀	♂♀	♂♀	♂♀	♂♀	4.8	0.6	0.6	16.0	25.5	25.1	97.5	96.5	100.0	1114.4	92.0	423.0
Aug.	♀	♂	♀	♂	♂♀	♂♀	3.1	0.6	0.6	16.3	25.1	25.6	97.8	98.4	100.0	496.8	550.3	287.4
Sep.							3.6	0.6	0.5	15.8	26.1	26.2	97.5	97.8	99.7	441.0	196.6	180.3
Oct.							3.1	0.5	0.5	16.0	25.7	26.3	95.6	98.2	99.0	511.2	162.3	199.2
Nov.	♂	♂	♂	♀			2.2	1.3	0.9	16.1	26.4	26.4	90.1	94.9	99.5	103.0	30.7	149.8
Dec.	♂	♂	♂	♀			3.5	1.8	2.2	15.9	25.2	26.0	86.5	91.0	98.4	50.0	33.9	0.0

♂ - Male ♀ - Female

4.2. DEVELOPMENT OF INFLORESCENCE

The development of inflorescence followed the same pattern in both the species. The first indication of flowering was a slight inflation of the bracts which ensheath the basal part of the partial inflorescence. The development of the partial inflorescence and the rachillae on it were acropetal. It took more than one month to complete the emergence of all partial inflorescences and rachillae. In both species, an average of 4-5 partial inflorescences were produced in a single inflorescence and 3-4 inflorescences were produced in a single plant at one season. The staminate and pistillate inflorescences were long and flagellate in both species studied (Figs 2A-2D).

In the flowering of *C. hookerianus* and *C. thwaitesii*, four stages of development could be recognized. The first was the emergence stage where inflorescence emerged from leaf sheaths and completed development. Second was the pre-anthesis stage, when the inflorescence was fully developed. Third was the anthesis stage, where the male flowers opened releasing pollen and female flowers were receptive to pollen. The final stage was the post-anthesis stage, where the male inflorescence started to dry out and the fertilized ovary started to develop into fruit.

For both species the period from the first to the second stage was more than one month. Small developed fruits could be detected by the naked eye after the third month of emergence of the inflorescences. The size of the fruits increased gradually until the 10th month when most of the fruits reached their maximum size. The free nuclear endosperm turned cellular by the 6th month. The fruits needed two more weeks to complete the ripening stage and the colour of the fruits changed from pale-green to pale-straw green and then to pale straw (yellowish). The time taken from emergence of

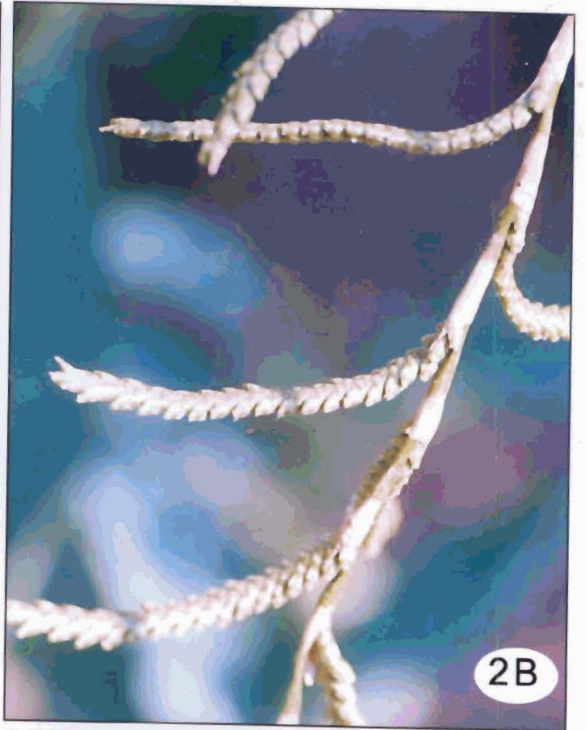
Fig. 2. Inflorescence

Fig. 2A. Male inflorescence of *C. hookerianus*

Fig. 2B. Male inflorescence of *C. thwaitesii*

Fig. 2C. Female inflorescence of *C. hookerianus*

Fig. 2D. Female inflorescence of *C. thwaitesii*



inflorescence to fruit maturity, was about ten months in *C. hookerianus* and 11 months in *C. thwaitesii*.

4.2.1. Male inflorescence

The average length of the male inflorescence in *C. hookerianus* was 579.2 cm and that of *C. thwaitesii* was 684.9 cm. The male flowers were borne on branches of the third order in *C. hookerianus* and on the second order in *C. thwaitesii* (Figs. 3A–3C). The flowers showed two types of arrangements on the rachillae. In few plants the flowers were arranged uniformly on both sides of the rachillae and in others the flowers were on cymose clusters (Figs. 3D & 3E).

4.2.1.1. Male and sterile male flowers

The number of male flowers in both species varied from hundreds to thousands depending on the number of rachilla and the length of the inflorescences. The flowers in *C. hookerianus* were small compared to *C. thwaitesii* and they were closely aggregated on the rachillae. The male flower was symmetrical with three sepals and petals englobing the six widely exposed, shortly epipetalous stamens (Figs. 4A–4D). The tepals had an inconspicuous pale yellow colour. Towards the centre, a minute pistillode was seen. Anthesis commenced about 15 mn before the flowers began to spread their petals.

4.2.1.2. Anthesis

Anthesis commenced from the basal flowers of the partial inflorescence but no sequence of opening was evident as anthesis progressed from proximal part to the distal end of the partial inflorescence (Figs. 5A & 5B). The anthesis of the individual flowers began around 1 AM (both species) and most of the flowers opened by 4 AM, but a few

additional flowers opened until 6.30 AM. The whole process of opening from the splitting of the flower bud to the formation of a fully opened flower took about 15-20 minutes. After the splitting of the bud, the introrse anthers were exposed and dehisced to release the pollen. Large amount of pollen were available which was not sticky. When the flowers opened, it exuded a droplet of nectar at the base. Before opening, the inflorescence emitted a strong, sweet gardenia like scent. This scent was conspicuous through out the night and diminished gradually by day time and greatly at dusk. Following anthesis when most of the pollen had been shed, the flowers shrivelled and fell off. Thus the longevity of the flowers was about 12-18 hours. Anthesis in *C. hookerianus* lasted for 12 days and in *C. thwaitesii* it was found to be more than 15 days.

The behavior of the sterile male flowers on the female inflorescence during anthesis was the same as the fertile males, except that no pollen was produced by the staminodes which bore empty anthers (Figs. 4E & 4F). There was no particular time for the opening of the acolyte flowers. It was observed that in different parts of the same inflorescence few flowers opened before the anthesis of pistillate flowers and in certain other parts they opened after the stigma of pistillate flowers turned black in colour (Figs. 5C & 5D). This irregular opening of acolyte flowers was noticed in both the species.

4.2.2. Female inflorescence

In the female inflorescences of both species, the flowers were borne on second order branching (Figs. 6A & 6B). In both the species studied, the flowers in the female inflorescence were borne in pairs (dyads) with a fertile female flower and a sterile (acolyte) male flower (Figs. 6C & 6D). The total length of the inflorescences kept

Fig. 3. Inflorescence enlarged

Fig. 3A. *C. hookerianus*. Male inflorescence bearing flowers in the third order

Fig. 3B. *C. thwaitesii*. Male inflorescence bearing flowers in the second order

Fig. 3C. *C. thwaitesii*. A part of the inflorescence enlarged.

Fig. 3D. *C. hookerianus*. A part of the inflorescence showing decussate arrangement of flowers

Fig. 3E. *C. hookerianus*. A part of the inflorescence showing flowers in cymose cluster.

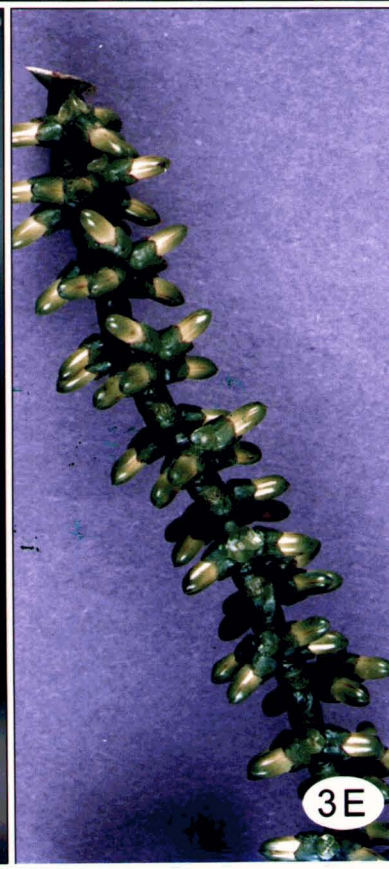


Fig. 4. Flowers – Entire and L.S.

Fig. 4A. *C. hookerianus*. Male flower enlarged

Fig. 4B. L.S. of the male flower

Fig. 4C. *C. thwaitesii*. Male flower enlarged

Fig. 4D. L.S. of the male flower.

Fig. 4E. *C. thwaitesii*. Sterile male flower enlarged

Fig. 4F. L.S. of the male flower showing empty anther.



Fig. 5. Inflorescence showing floral opening

Fig. 5B. *C. thwaitesii*. A part of the male inflorescence showing irregular floral opening.

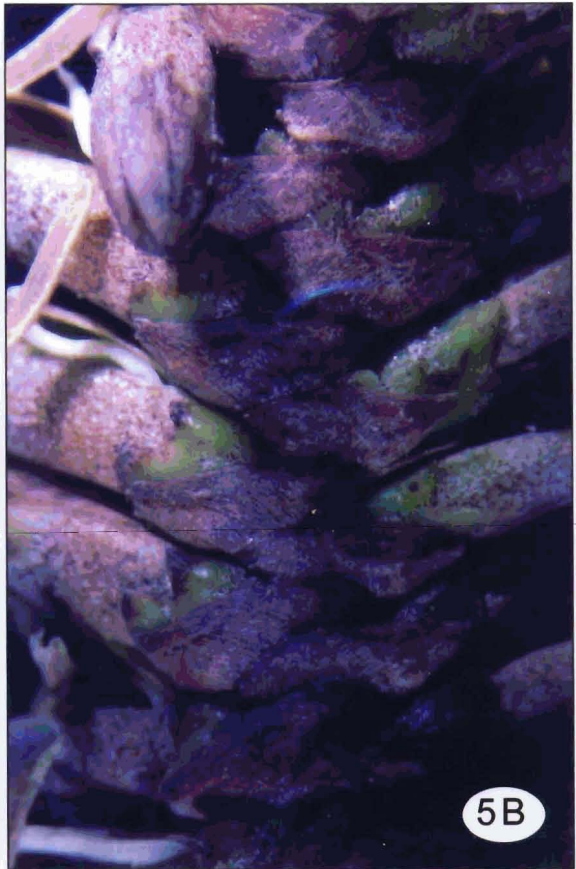
Fig. 5A. *C. hookerianus*. A part of the male inflorescence showing irregular floral opening.

Fig. 5C. *C. hookerianus*. A part of the female inflorescence showing irregular floral opening.

Fig. 5D. *C. thwaitesii*. A part of the female inflorescence showing irregular floral opening in both female and sterile male.



5A



5B



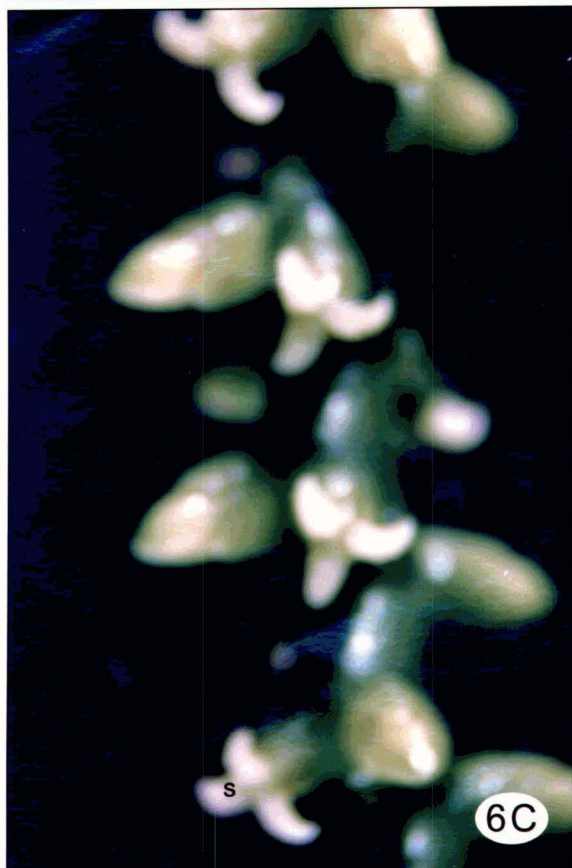
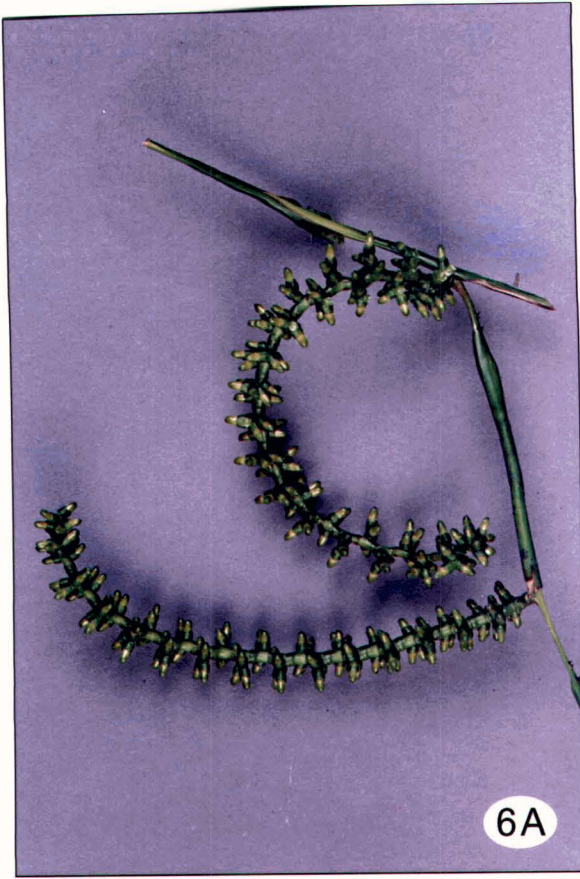
5C



5D

Fig. 6. Inflorescence showing flower arrangement

- Fig. 6B. *C. thwaitesii*. A part of the female inflorescence showing flowers in the second order
- Fig. 6A. *C. hookerianus*. A part of the female inflorescence with flowers in the second order
- Fig. 6C. *C. hookerianus*. A part of the female rachilla showing pistillate and acolyte flowers
- Fig. 6D. *C. thwaitesii*. A part of the female inflorescence with pistillate and acolyte flowers.



under observation ranged from 336.3 cm to 596.3 cm in *C. hookerianus* and 412.8 cm to 693.0 cm in *C. thwaitesii* (Table 4).

Table 4. Length, number of inflorescences and number of partial inflorescences

Plant no	Inflorescences				Number of partial Inflorescences	
	Number		Length (cm)		Ch	Ct
	Ch	Ct	Ch	Ct		
1	1	1	493.0	590.1	4	3
	2	2	454.7	470	6	4
	3	--	386.0	--	4	--
2	1	1	501.0	598.9	5	5
	2	2	478.5	506.0	5	3
	3	3	399.6	435.4	4	4
	4	--	425.4	--	4	--
3	1	1	510.0	536.0	4	3
	2	2	486.5	412.8	5	4
	3	3	556.7	580.1	4	5
	4	--	470.3	565.3	4	--
4	1	1	508.8	491.8	6	2
	2	2	496.4	563.3	6	4
	3	3	413.7	597.0	4	4
5	1	1	496.4	591.0	5	3
	2	2	553.9	503.4	4	3
	3	3	596.3	412.9	3	5
	---	4	--	498.7	--	4
6	1	1	425.0	582.1	6	5
	2	2	465.6	415.8	5	3
	3	3	486.7	486.9	4	4
7	1	1	396.4	532.1	4	4
	2	2	354.6	598.9	6	5
	3	3	432.3	515.2	4	3
	4	4	430.6	636.4	5	5
	--	5	--	656.3	--	4
8	1	1	398.3	513.1	3	4
	2	2	463.9	598.2	4	4
	3	3	475.2	536.4	3	3
	---	4	--	656.3	--	2
9	1	1	502.8	638.4	4	4
	2	2	410.0	518.8	5	3
	3	3	463.0	592.1	3	3
	--	--	--	693.0	--	4
10	1	1	538.6	612.4	4	2
	2	2	364.5	631.5	4	3
	3	3	411.0	590.0	3	3
	4	--	336.3	--	--	--
Average	2.24	2.24	449.47	550.19	4.36	3.65

Ch - *C. hookerianus* Ct - *C. thwaitesii*

taken for their length and rachillas attached to them are presented in Table 5.

Table 5. Length of partial inflorescences and number of rachillae

Plant No.	Inflorescence Number		No. of partial Inflorescences		Length of partial inflorescences (cm)				No. of rachilla			
	Ch	Ct	Ch	Ct	Max.		Min.		Max.		Min.	
					Ch	Ct	Ch	Ct	Ch	Ct	Ch	Ct
1	1	1	4	3	84.00	112.10	53.20	93.00	28	18	18	9
	2	2	6	4	110.00	122.30	66.10	89.40	24	14	16	8
	3	-	4	-	134.00		89.30	-	23	-	12	-
2	1	1	5	5	83.60	86.40	43.80	55.00	22	16	13	10
	2	2	5	3	53.00	78.50	36.10	43.30	18	9	9	4
	3	3	4	4	46.50	93.30	30.60	48.30	16	13	6	8
	4	-	4	-	41.00	-	31.40	-	12	-	9	-
3	1	1	4	3	93.40	78.30	63.20	51.20	21	11	10	8
	2	2	5	4	98.60	65.40	55.40	44.10	19	16	8	7
	3	3	4	5	45.30	48.30	39.80	29.60	13	11	7	6
	4	-	4	-	62.30	-	43.20	-	15	-	7	-
4	1	1	6	2	42.00	95.00	31.20	78.20	21	9	9	5
	2	2	6	4	63.30	86.30	46.30	66.30	16	12	5	6
	3	3	4	4	86.10	56.30	54.80	38.20	17	14	8	7
5	1	1	5	3	98.30	103.40	58.30	89.30	23	18	18	10
	2	2	4	3	91.20	95.60	49.50	68.40	19	12	12	8
	3	3	3	5	89.40	108.30	51.20	79.30	21	17	14	11
	-	4	-	4	-	78.30	-	59.20	14	21	8	13
6	1	1	6	5	87.60	88.10	49.00	49.30	19	14	12	9
	2	2	5	3	90.20	76.40	39.80	39.80	16	18	13	12
	3	3	4	4	89.40	101.30	41.30	76.20	11	11	9	7
7	1	1	4	4	68.60	99.00	38.00	53.20	22	19	17	13
	2	2	6	5	62.00	103.20	31.60	66.30	29	22	14	18
	3	3	4	3	74.40	88.90	29.30	43.20	19	24	13	16
	4	4	5	5	71.30	110.20	27.00	69.00	15	14	10	9
8	1	1	3	4	78.00	68.30	31.00	43.00	14	16	9	11
	2	2	4	4	87.40	74.80	28.60	48.20	18	21	9	14
	3	3	3	3	89.30	79.30	34.30	39.10	23	19	13	8
	-	4	-	2	-	95.80	-	49.20	21	11	10	7
9	1	1	4	4	96.30	108.40	41.20	66.20	31	18	19	11
	2	2	5	3	87.10	103.20	39.10	73.10	20	23	13	10
	3	3	3	3	99.80	99.80	26.30	46.20	17	12	9	8
	-	2	-	4	82.40	102.90	31.00	55.00	21	14	12	9
10	1	1	4	2	78.30	77.40	29.20	38.20	18	23	12	11
	2	2	4	3	69.90	69.30	28.60	28.10	12	17	6	8
	3	3	3	3	54.30	88.60	31.20	31.30	17	12	11	7
	4	-	2	-	66.00	91.40	30.40	52.10	28	15	14	9

Ch - *C. hookerianus* Ct - *C. thwaitesii*.

The length of partial inflorescences ranged between 45.8 cm to 120.5 cm. for *C. hookerianus* and 79.6 cm to 193.9 cm for *C. thwaitesii*.

The average length of rachilla was 6.0 and 8.0 cm with maximum length of 7.9 and 10.0 cm and minimum length of 5.0 and 8.2 cm in both species respectively. An average of 32 and 24 pairs of flowers were available per rachilla in both species respectively.

In *C. hookerianus*, the inflorescence axis of both male and female till anthesis were green in colour. But one month before dehiscence of buds in male inflorescence of *C. thwaitesii*, there was a colour change from green to yellow and later to brown (Fig. 6D).

4.2.2.1. Female flower

Female flowers were borne in pairs with a fertile female flower and sterile male flower (acolyte flowers). The pistillate flower had 3 sepals and petals, the gynoecium was tricarpellate, ellipsoidal, whitish with a pine-apple-like base covered with scales, and with well developed trifold style (Figs. 7A & 7B). Six staminodes surrounded the gynoecium that united into a ring and extended to the top. Anthesis in the female flowers mostly occurred between 1 and 4 AM and senescence and abscission between 6 and 8 PM.

Anthesis in the female flowers resulted in the emergence of receptive stigma which were bright, hyaline and adaxially covered with a liquid film (Figs. 7C & 7D). The loss

of the receptivity was characterized by the disappearance of fragrance and stigmatic liquid and by the colour change of the whitish stigmas to brown and later to reddish black (Figs. 7E & 7F). This stage occurred shortly before dusk. Pistillate flowers did not produce nectar while open.

The longevity of flowers was similar in both species studied and anthesis lasted for 12-15 days in an inflorescence.

4.3. MICROSPOROGENESIS

The mature anthers of *C. hookerianus* and *C. thwaitesii* were composed of four microsporangia (Figs. 8A & 8B). This condition was maintained throughout the lifetime of the anther and was still evident at the time of dehiscence of the anther, although the walls separating the two sporangia in each lobe became crushed, it did not break down completely (Fig. 9F).

Initially the anther comprised the connective and the four lobes of undifferentiated homogeneous cells. Periclinal divisions in the hypodermal cells of the developing anther resulted in the formation of two layers viz., an outer one which constituted the primary parietal layer and an inner layer, the primary sporogenous layer. The latter layer underwent mitotic divisions in both anticlinal and periclinal directions. Thereafter the cells enlarged and their nuclei became conspicuous and these cells constituted the sporogenous tissue.

The cells of the primary parietal layer divided periclinally twice, one of the resulting layers again divided and produced five cell layers between the epidermis of the anther and the sporogenous tissue (Figs. 8C & 8D) Of the four layers, the outermost one

Fig. 7. Female flower and receptivity of stigma

Fig. 7A. *C. thwaitesii*. Female flower enlarged.

Fig. 7B. *C. thwaitesii*. L.S. of female flower

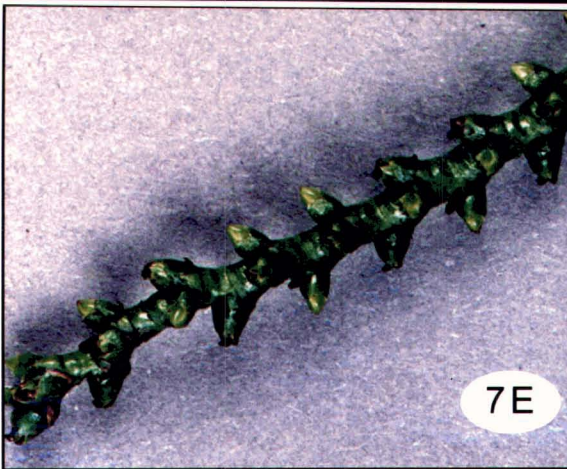
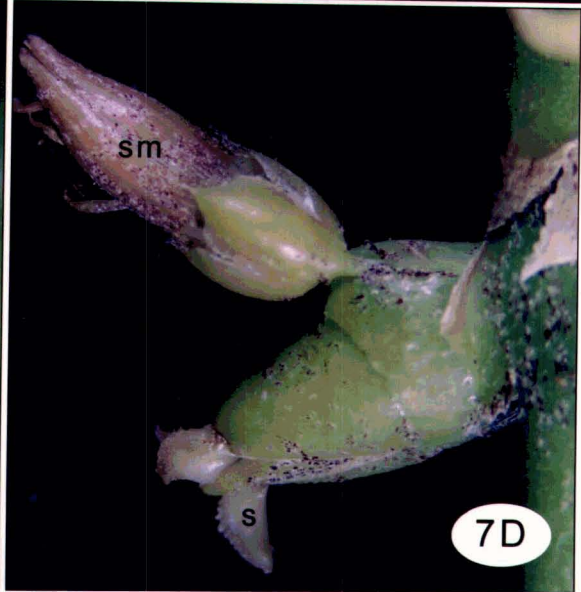
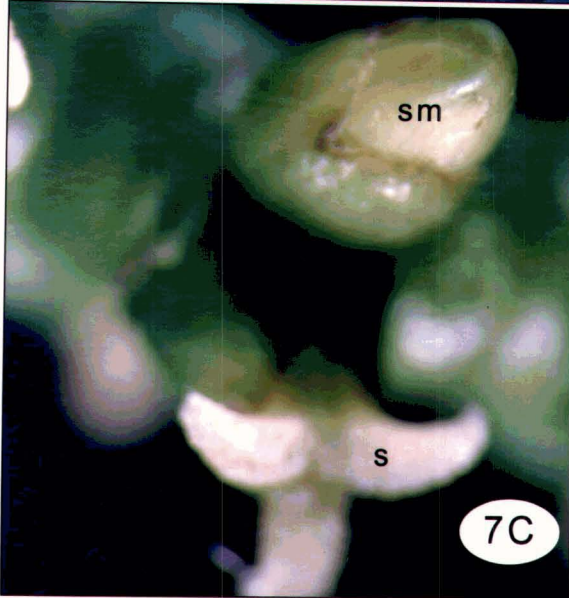
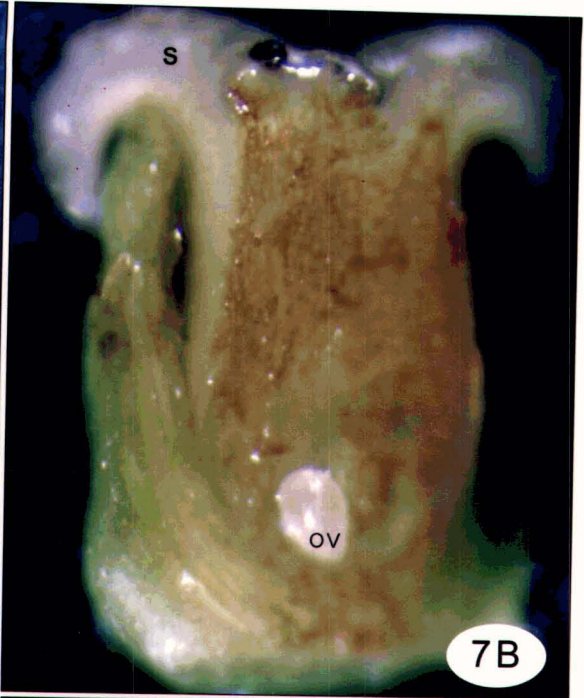
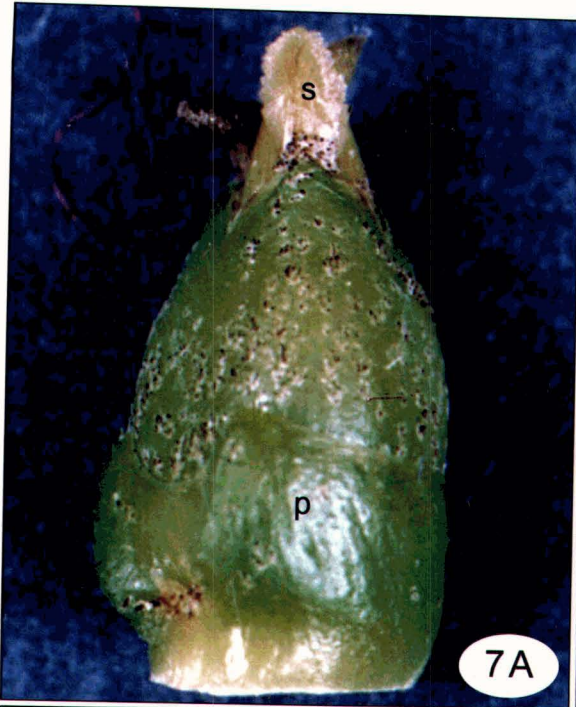
Fig. 7C. *C. hookerianus*. A part of the rachilla with a pistillate flower and acolyte flower bud.

Fig. 7D. *C. thwaitesii*. A part of the rachilla with a pistillate flower and acolyte flower bud.

Fig. 7E. *C. hookerianus*. A part of the rachilla where the stigma has turned black and the acolyte flowers are fallen.

Fig. 7F. *C. thwaitesii*. A part of the rachilla showing the same.

p-perianth, ov-ovary, s-style, sm-strile male



differentiated to form the endothecium; the innermost cell layer i.e., adjacent to the sporogenous tissue developed into the tapetum and the three remaining layers constituted the middle layers of the anther wall. In an immature anther, the sporogenous tissue was completely surrounded by the tapetum (Figs. 8C & 8D)

During the early stages of the ontogeny of the anther, the epidermal cells were more or less isodiametric in shape. During subsequent growth, the surface of the anther increased rapidly and anticlinal divisions occurred in the epidermal cells to keep pace with the increase in volume of the underlying cells. In a mature anther the epidermal cells became stretched and flattened in a tangential plane (Figs. 9A & 9B). The endothelial cells were radially elongated and possessed the characteristic fibrous thickenings. The cells of the middle layers were tangentially elongated. They became crushed and degenerated by the time the anther was ready to dehisce (Figs. 9A, 9B & 9F). The tapetal cells remained uninucleate. They formed a layer around the sporogenous tissue and were initially closely packed against each other with no intercellular spaces between them (Figs. 8C & 8D). This condition was short lived as the middle layers started to degenerate and the circumference of the sporangium increased which made the tapetal cells to become loosely arranged. Even after the formation of the pollen grains, the walls of the tapetal cells remained intact and thus the tapetum was found to be of the glandular or secretory type.

The primary sporogenous cells divided mitotically for a limited number of times and then functioned as the microspore mother cells. The cells of the sporogenous tissue were connected together by plasmodesmata and the cells were more or less polygonal in outline (Figs. 8C & 8D). Their cytoplasm was dense, the nucleus was comparatively

Fig. 8. T.S. of anther

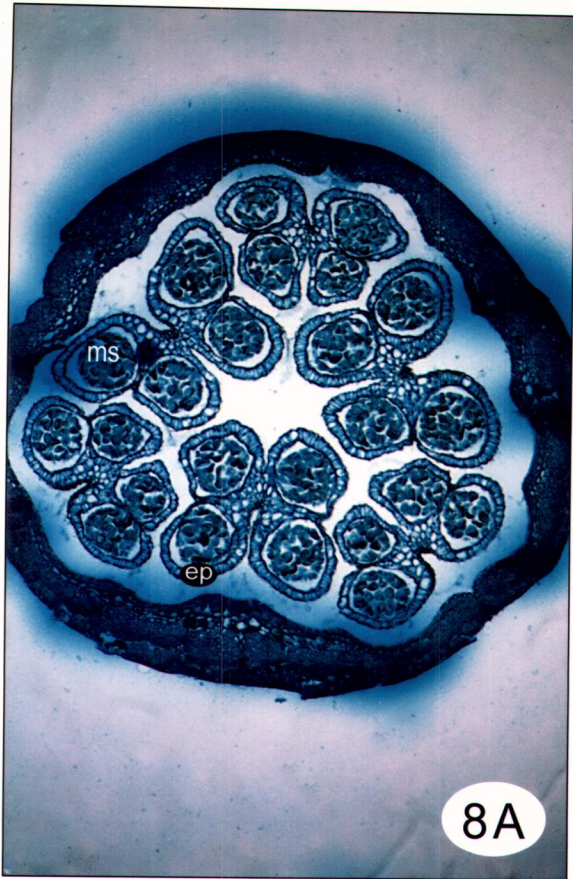
Fig. 8A. *C. hookerianus* T.S. of flower bud showing tetrasporangiate anther $\times 250$

Fig. 8B. *C. thwaitesii*. T.S. of the flower bud showing tetrasporangiate anther $\times 250$

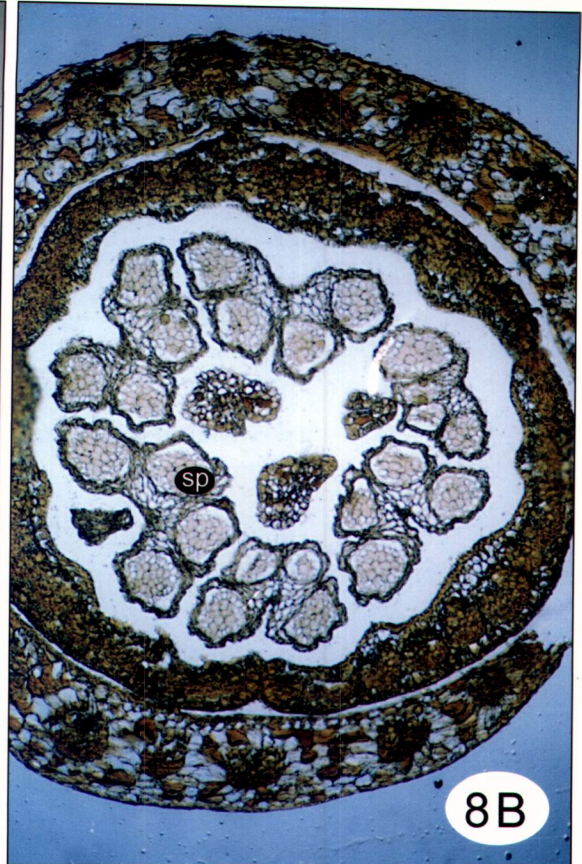
Fig. 8C. *C. hookerianus*. A part of the anther lobe showing wall layers and sporogenous tissue. $\times 400$

Fig. 8D. *C. thwaitesii*. A part of the anther lobe showing wall layers and sporogenous tissue. $\times 400$

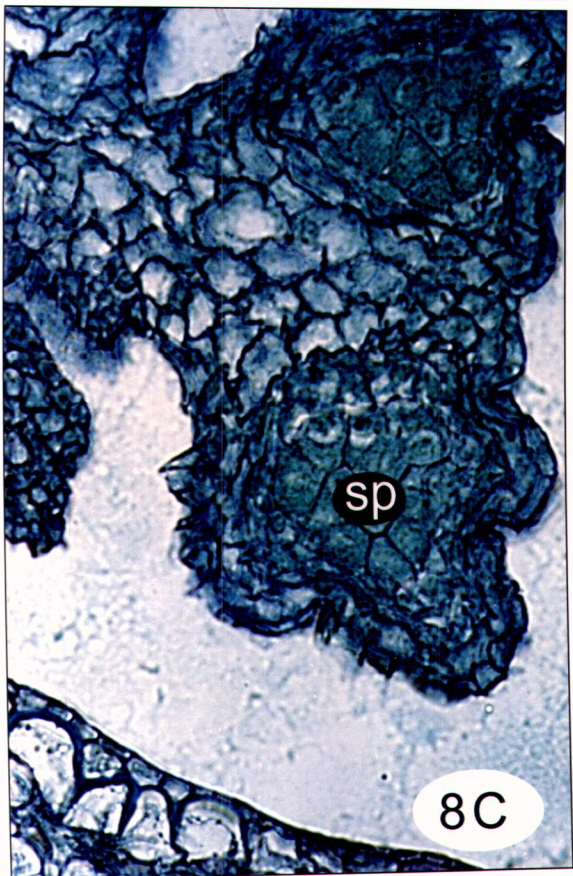
ep. epidermis, ms – microspores, sp - sporogenous tissue,



8A



8B



8C



8D

large with a well developed nuclear membrane. Later on the plasmodesmatal connections were cut off and the cells of the sporogenous tissue separated from one another. In this stage, the microspore mother cells were seen bathed in callus and were seen lying freely in the anther locule.

Initially, the cell walls of the mother cells were thin, but shortly before meiosis, the walls became very much thicker. Cytokinesis of microspore mother cells was of the simultaneous type and a cell wall was formed after the first nuclear division which resulted in the formation of a dyad (Fig. 9C).

The microspore tetrads were isobilateral (Figs. 9D & 9E). At the completion of meiosis the tetrad was surrounded by a thick callose wall. The wall however degenerated soon after and the microspores were released into the anther locule.

Each microspore was initially enclosed in a thin wall which became thicker shortly prior to dehiscence of the anther. The exine was relatively thick and smooth without any spines.

4.3.1. Transmission electron microscopic studies

The middle layers were tangentially elongated and showed the presence of thick walls. Tannin was present in the cells (Fig. 10A). The walls of the tapetal cells were thin walled and were drawn irregularly. The inner walls of the tapetum facing towards the anther locule showed the aggregation of ribosomes and dictyosomes (Fig. 10B). These spherical bodies were then collected in the space between the outer and the inner wall of the tapetum towards the anther cavity and finally they were released to the anther cavity (Figs. 10C & 10D). The sexine (ektexine) portion was differentiated into three

Fig. 9. Microsporogenesis

Fig. 9A. *C. hookerianus*. T.S. of the anther lobe showing epidermis and pollen grains $\times 400$

Fig. 9B. *C. thwaitesii*. T.S. of the anther lobe showing the epidermis and pollen grains $\times 400$

Fig. 9C. *C. hookerianus*. Pollen mother cell undergoing Meiosis - I $\times 800$

Fig. 9D. *C. hookerianus*. Pollen tetrads $\times 800$

Fig. 9E. *C. thwaitesii*. Pollen tetrads $\times 800$

Fig. 9F. *C. thwaitesii*. T.S. of mature anther $\times 400$

ep - epidermis, ms - microspore, t - tannin, mmc - microspore mother cell, dy - dyad.

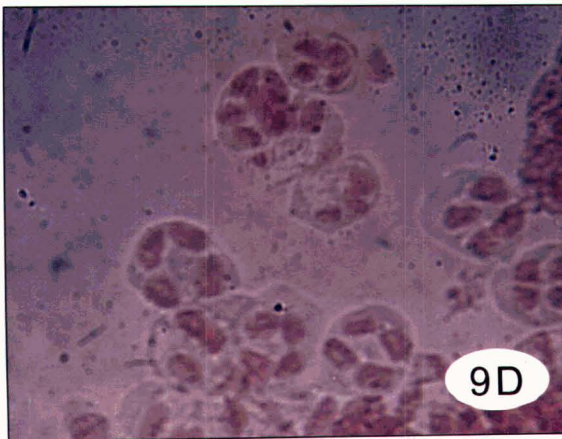
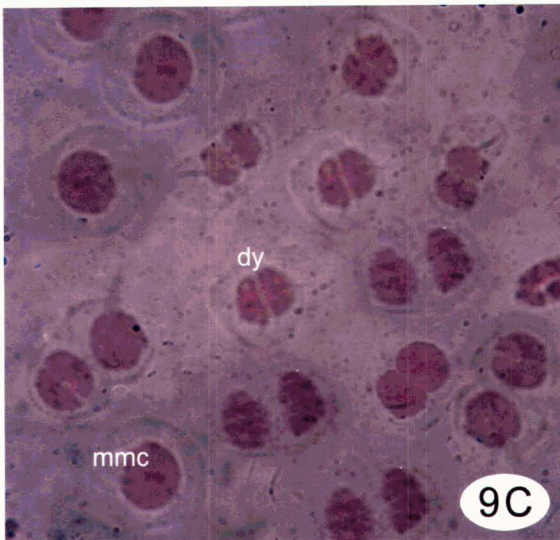
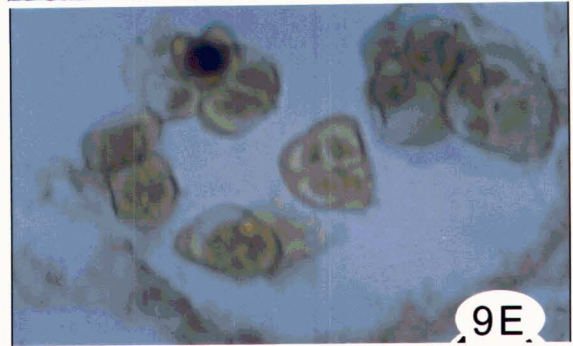
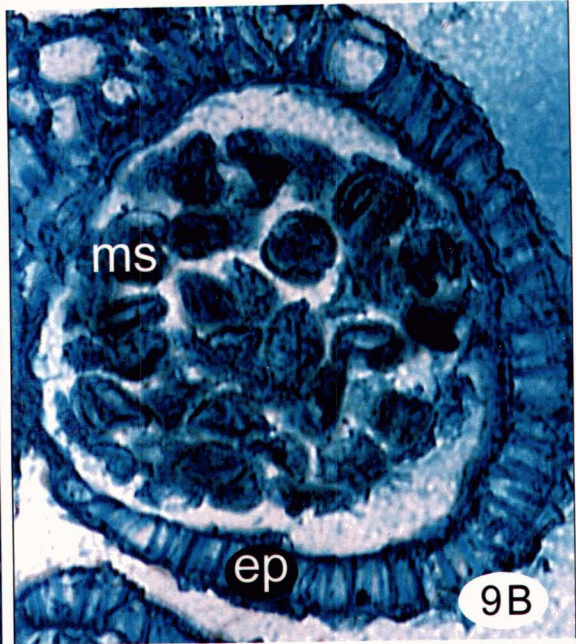
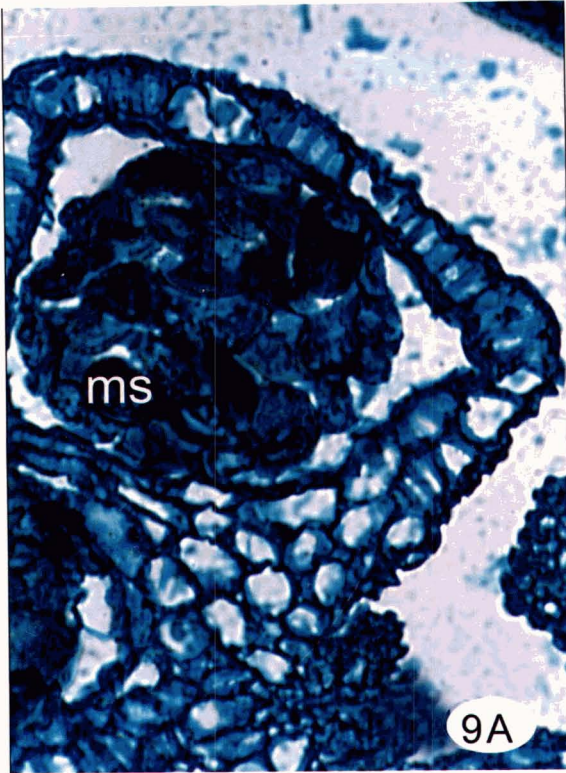


Fig. 10. Transmission electron micrographs of *C. hookerianus* anther

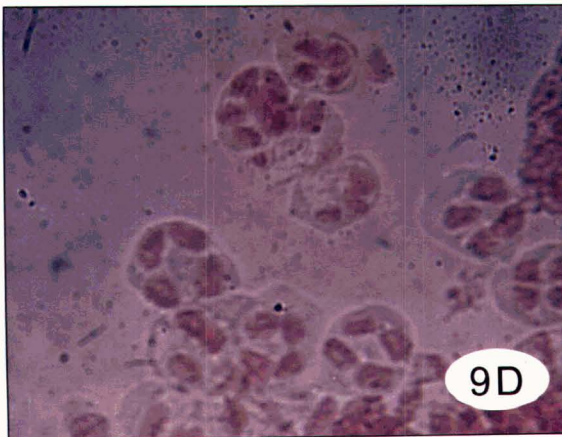
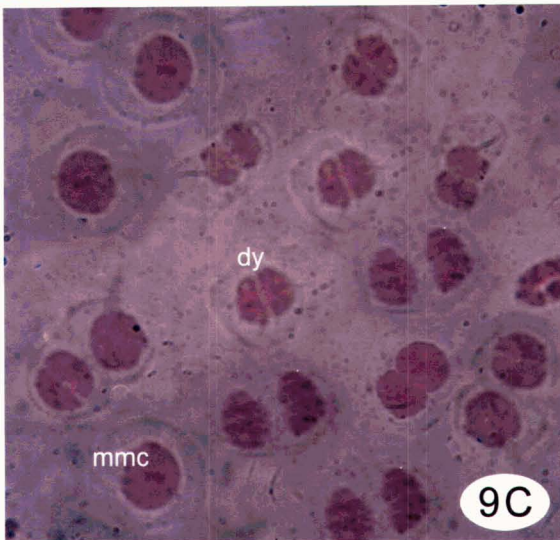
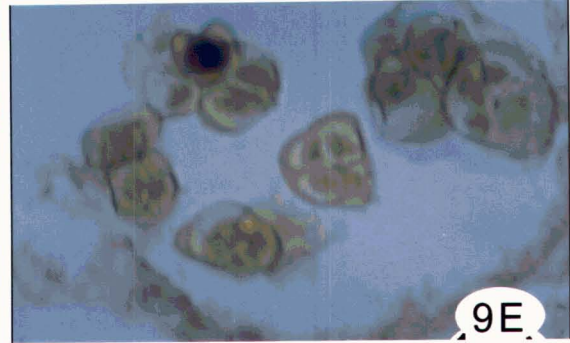
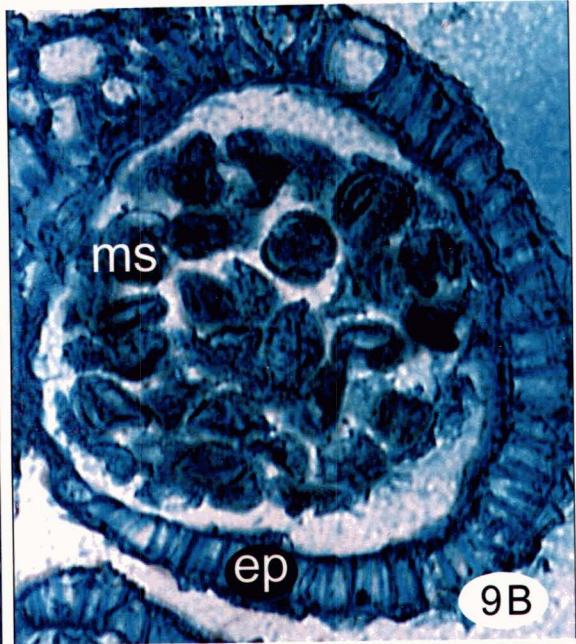
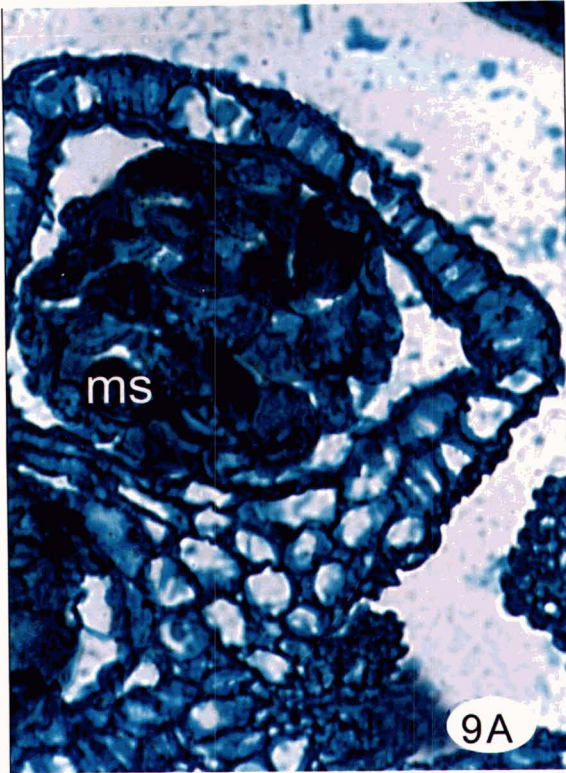
Fig. 10A. Middle layer . x 5000

Fig. 10B. Middle layer and tapetal cell x 6000

Fig. 10C. Tapetum (a) and anther locule showing accumulation of wall materials x 6000

Fig. 10D. Tapetum (a) and part of pollen grain x 4000

MI - middle layer, ta - tapetum, a - anther locule, p - pollen grain.



distinct region; the tectum, columella and a foot layer. The rod like columella formed connections between tectum and the foot layer. The outermost roof of tectum appeared uneven due to projection of reticulum sculptures (Figs. 11C & 11D). Various inclusions like mitochondria, lipid globules, ribosomes and vacuoles could be seen in the cytoplasm indicating that the pollen grains are physiologically active.

The cells of the sporogenous tissue showed prominent nucleus with a nucleolus. The nucleoplasm was rich in chromatin materials. The cytoplasm was rich in cell organelles like mitochondria and ribosomes. The cell walls were thick walled and the inner walls showed an aggregation of cytoplasmic materials (Figs. 11A & 11B). In a still mature anther, the cells possessed more number of cell organelles and lipid bodies.

4.3.2. Scanning electron microscopic studies

Structure of Pollen Grains

Pollen morphology was similar in both the species studied.

Table 6. Morphological characteristics of the pollen

Species	<i>C. hookerianus</i>	<i>C. thwaitesii</i>
Grain length (μm)	39.6	44.5
Grain width (μm)	42.9	43.5
No.of colpi	2	2
Colpi length (μm)	38.8	41
Colpi width (μm)	7	7
Mesocolpia length (μm)	40.7	40.5
Apocolpia width (μm)	15	16.7
Exine thickness (μm)	2	3
Exine sculpturing	Micro reticulate	Micro reticulate

Pollen grains were prolate spheroidal or spherical; equatorial and polar outline elliptic, notched at both side and bicolpate. The grains of *C. hookerianus* measured 39.6 x 42.9 μm ; the colpi were linear or elliptic, measured 38.8 μm long and 7 μm wide at equator

Fig. 11. Transmission electron micrographs of *C. hookerianus* anther.

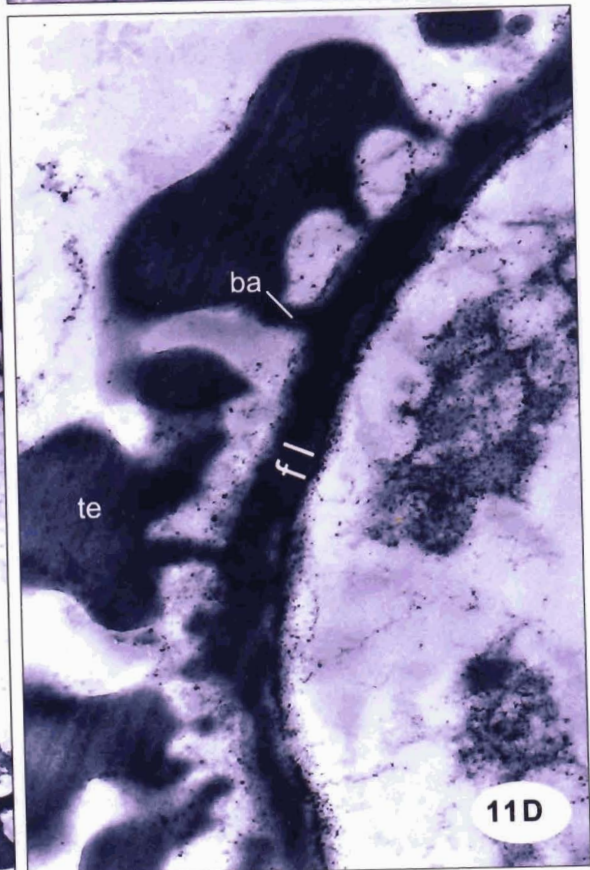
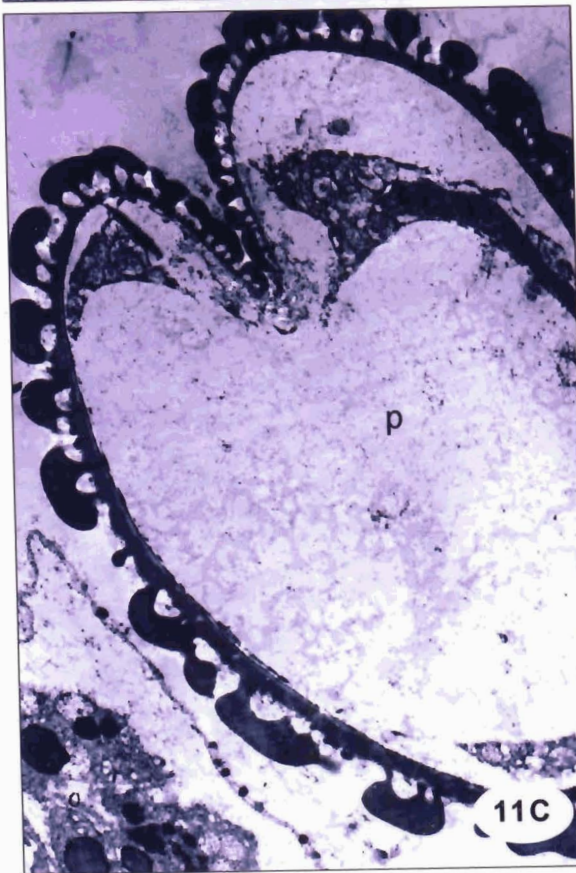
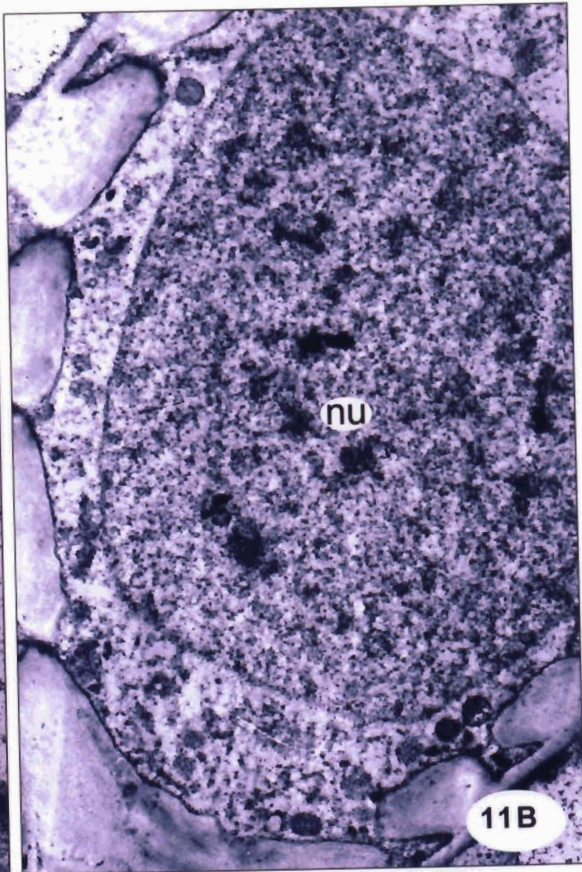
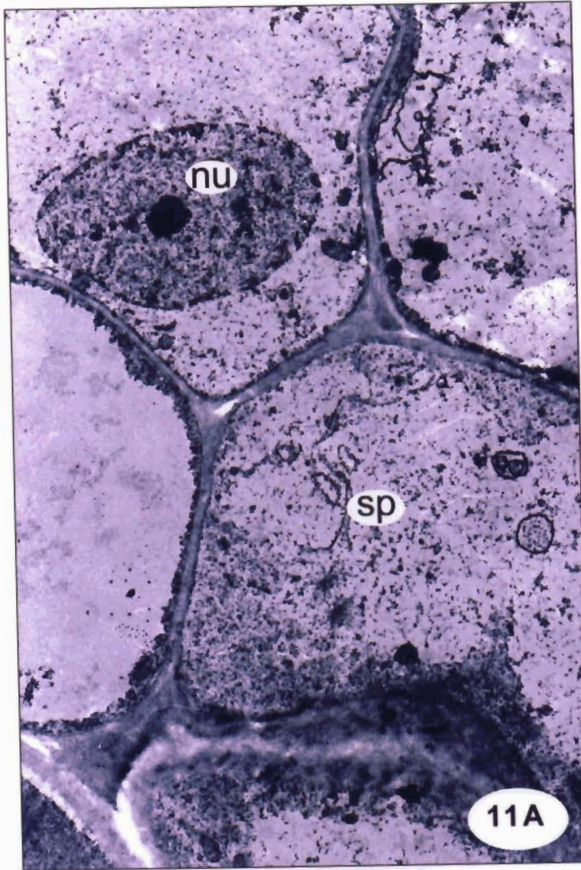
Fig. 11B. Sporogenous cell enlarged $\times 6000$

Fig. 11A. Sporogenous tissue $\times 3500$

Fig. 11C. Pollen grain $\times 4000$

Fig. 11D. Pollen wall enlarged $\times 15000$

sp. Sporogenous tissue, nu - nucleus, p - pollen grain, te - tectum, ba - bacula
f - foot layer.



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sides in *C. hookerianus* and it was 41 μm and 7 μm respectively for *C. thwaitesii* (Figs.12A – 12F and Table 6). Sculpturing was micro reticulate, lumina smaller towards apocolpium and along colpi. Figures 12A to 12D represent scanning micrographs of pollen exhibiting its basal surface. It is clear from the figure that the pollen is some what oval with pointed polar ends and with a prominent stalk like structure which appeared as electron dense. The fine structured analysis of the dorsal side showed a perforated pattern indicating the furrowed tectum. Figure 12F exhibits a magnified view of the perforated tectum of the pollen surface. Each perforation has an average pore size of 0.5 to 0.7 μ . The rich distribution of perforation on the pollen surface and stalked nature are adaptations to biotic means of pollination. As could be seen in the figure 12E, the peripheral part of the pollen has a groove with a raised upper region resembling a “cup and saucer model” with perforation distributed uniformly. All these structural peculiarity of pollen indicated an uneven structure confirming the affinity of pollen towards entomophily.

4.3.3. Anthesis

Shortly prior to dehiscence of the anther, the large centrally placed nucleus of the pollen grain divided mitotically and the vegetative and generative nuclei were formed. The vegetative nucleus remained more or less round and centrally placed while the generative nucleus, which was crescent-shaped was confined to the periphery of the pollen grain (Figs. 13A – 13E). The pollen grains were shed from the anther in 2-celled condition. Germination of pollen grains was initiated one hour after sowing and by this time the vegetative nucleus had degenerated extensively and was only just visible. The

Fig. 12. Scanning electron micrographs of *Calamus* pollen

- Fig 12A. }
Fig 12B. } *C. hookerianus* – lateral and dorsal view
- Fig 12C. }
Fig 12D. } *C. thwaitesii* – dorsal view
- Fig 12E. *C. thwaitesii* Ventral view
- Fig 12F. *C. thwaitesii* - a magnified view of the perforated tectum

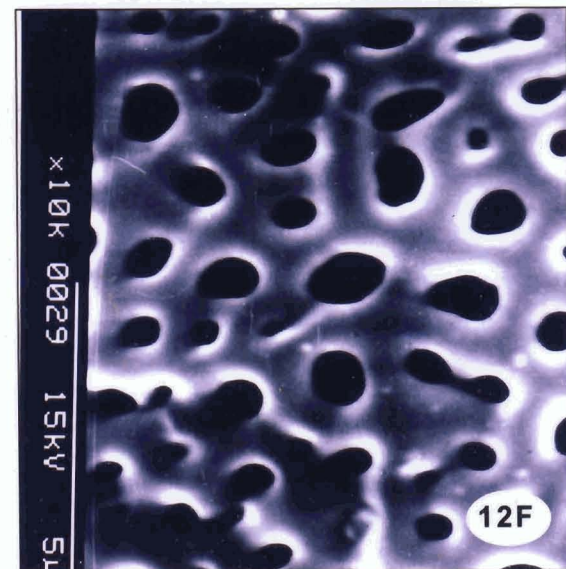
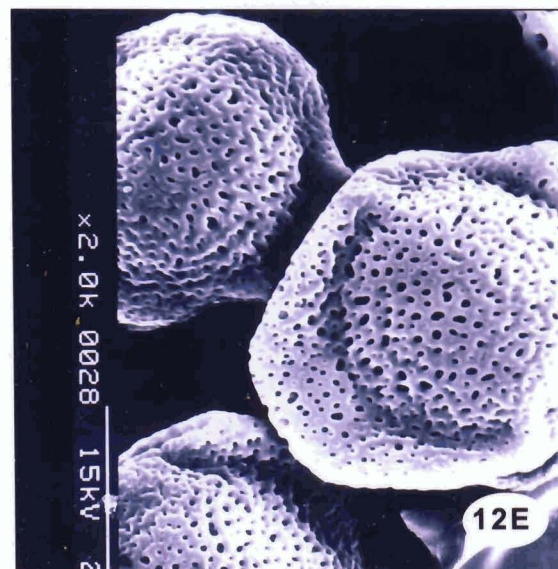
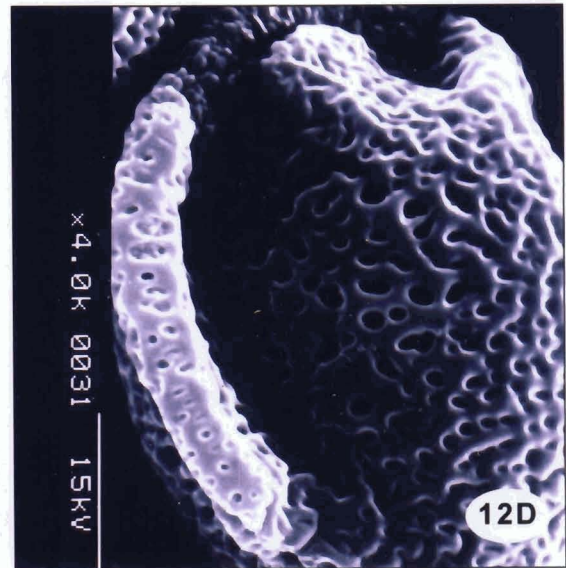
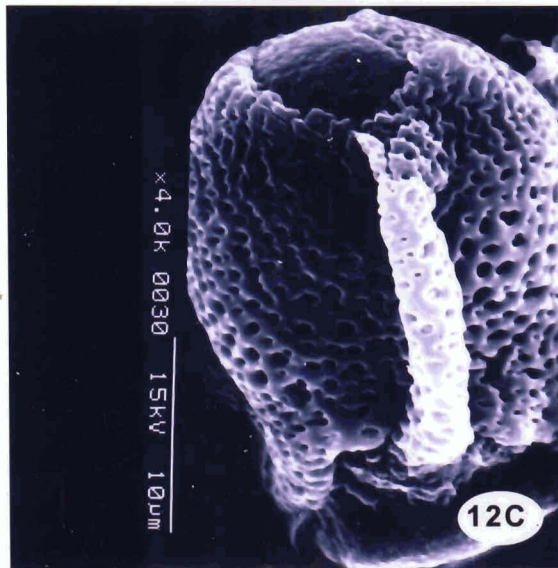
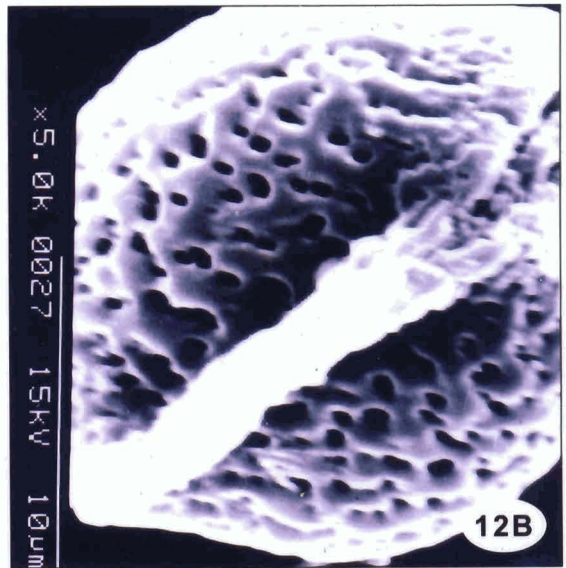
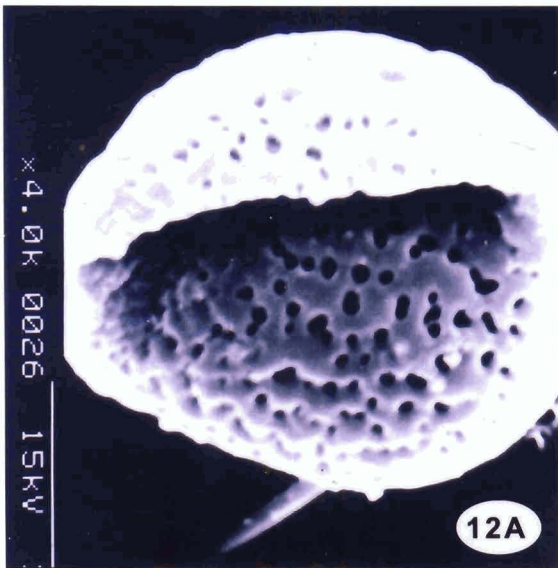


Fig. 13. Pollen grains of *Calamus* observed with fluorescence microscopy.

Fig. 13A. *C. hookerianus*. Pollen before anthesis. $\times 650$

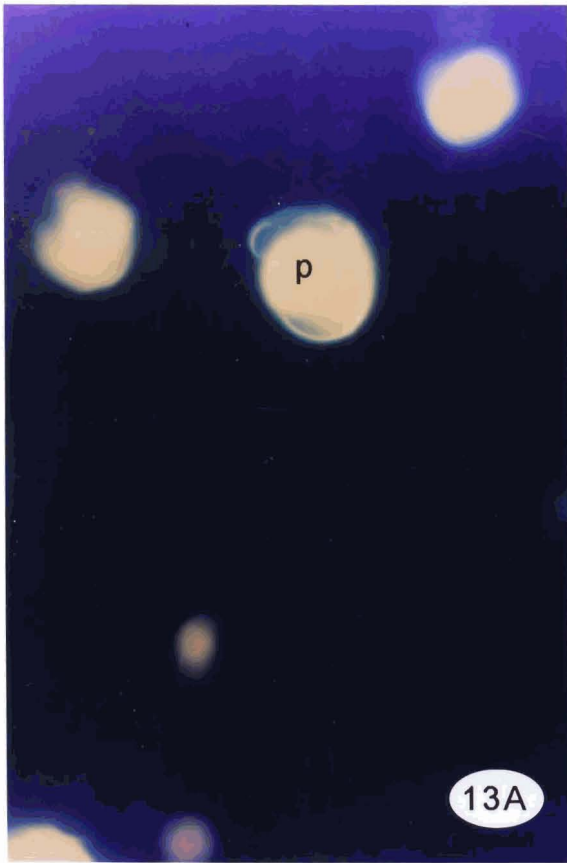
Fig. 13B. *C. thwaitesii*. Pollen before anthesis $\times 650$

Fig. 13C. *C. hookerianus*. 2 celled pollen at anthesis. $\times 650$

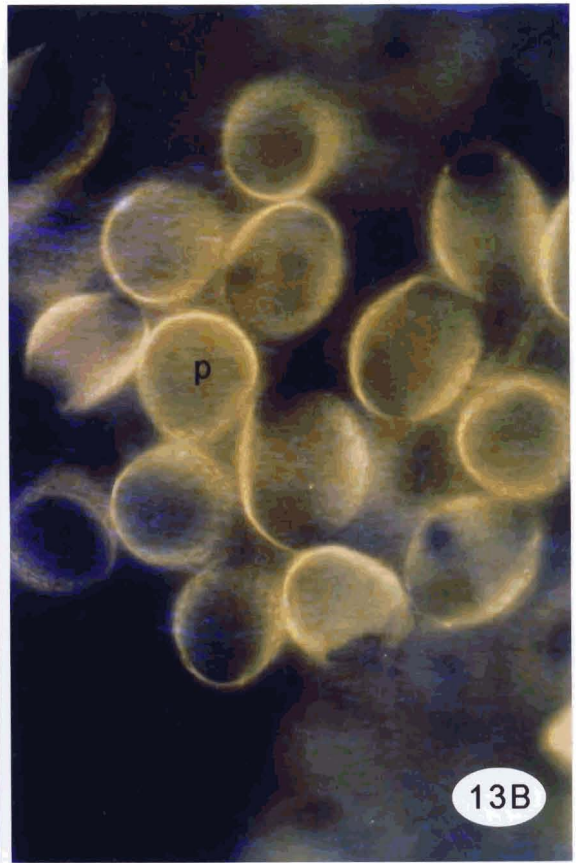
Fig. 13D. *C. thwaitesii*. 2 celled pollen at anthesis. $\times 650$

Fig. 13E. *C. thwaitesii*. Pollen enlarged. $\times 800$

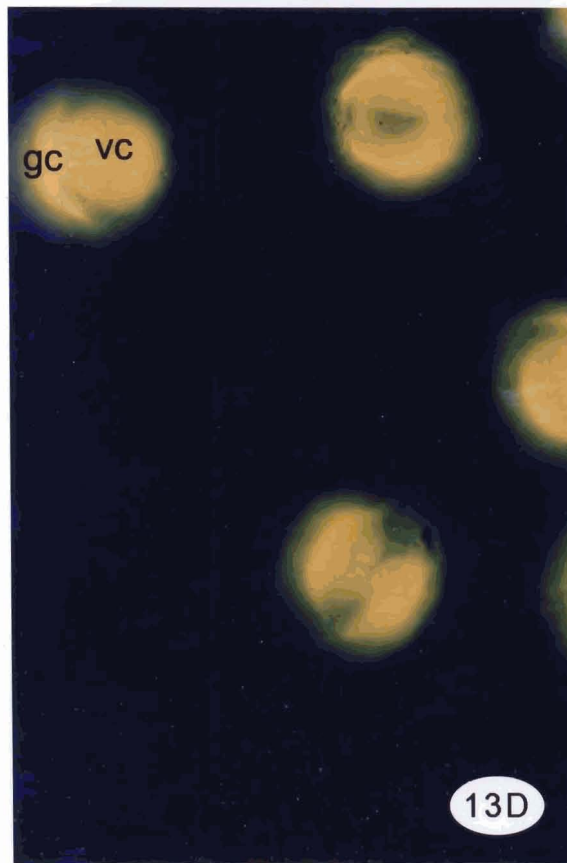
vc – vegetative nucleus, gc – generative cell.



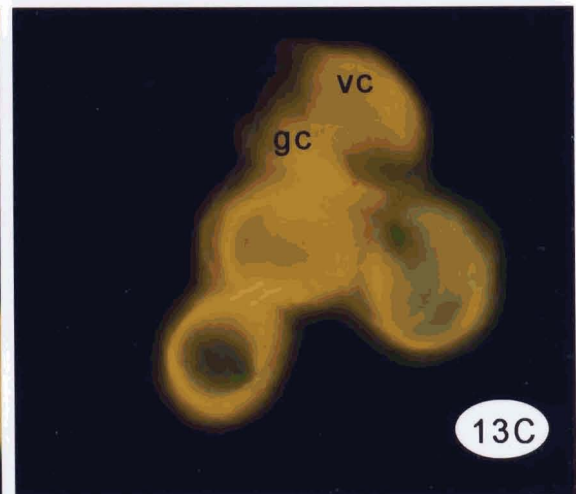
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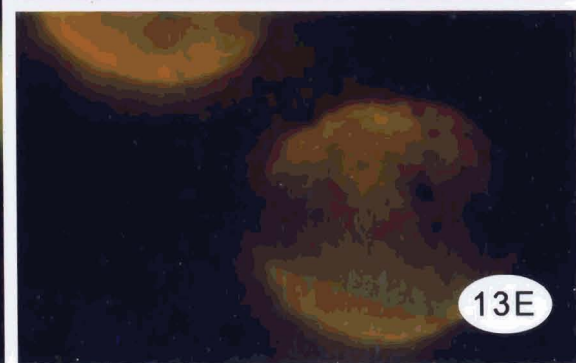
13B



13D



13C



13E

division of generative cell occurred in the pollen tube and formed the male gametophyte (Figs. 17C & 17D).

4.4. OVARY AND OVULE

The ovary comprised of three carpels in both the species. Placentation of the ovules was axial (Fig. 14A). The ovules were with a well developed funicle. In a longitudinal section through the ovary, the ovules were curved downwards by approximately 180° (Figs. 14B – 14D) and they were anatropous.

The ovules were bitegmic and the two integuments were fused together upto a point approximately midway between the chalaza and the micropylar end of the nucellus. The exostome and endostome were in line with each other and the micropyle was straight. The outer integument consisted of six to eight cell layers and dominated the ovule by its massive structure. The inner integument was a much more delicate structure and consisted of four to six wall layers. Tannin was deposited in the cells of the integuments and also in the chalazal region of the ovule (Fig. 14D). During the course of development, two ovules degenerated and only a single ovule developed into seed (Figs. 15A – 15C). The degeneration of the two ovules occurred by the maturation of the embryo sac in the developing ovule (Fig. 15D).

Integumentary tapetum was conspicuous in the ovules of both species. The cells of this layer were palisaded and rich in cytoplasm (Figs. 15E & 15F). During megasporogenesis the cells of this layer elongated radially. They remained uninucleate and divided anticlinally to keep pace with the increasing volume of the developing embryo sac.

Fig. 14. Structure of ovule

Fig. 14A. *C. hookerianus*. T. S. of ovary showing the three ovules. $\times 250$

Fig. 14B. *C. thwaitesii*. L.S. of ovary showing two well developed ovules. $\times 250$

Fig. 14C. *C. hookerianus*. L.S. of ovary showing the degenerating ovule. $\times 250$

Fig. 14D. *C. thwaitesii*. L.S. of the developing ovule $\times 400$

os – ovules scale, es – embryo sac, do – degenerating ovule, ii – inner integument
oi – outer integument, fu – funiculus, nu – nucellus.

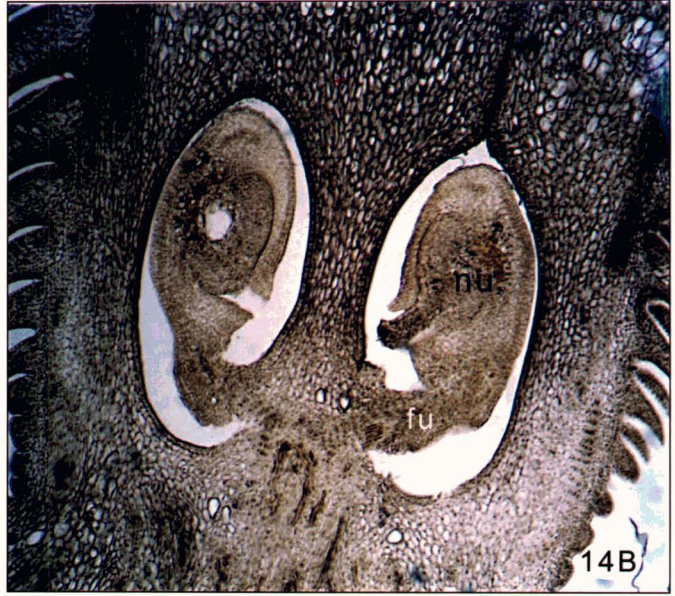
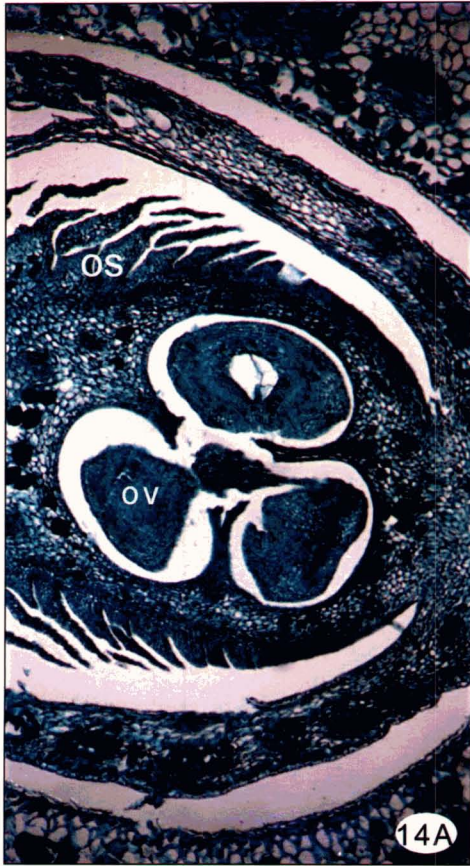


Fig. 15. Structure of ovule

Fig. 15A. *C. hookerianus*. T.S. of ovary showing a completely degenerated, a degenerating and a functional ovule $\times 250$

Fig. 15B. *C. hookerianus*. L.S. of ovary showing detailed ovular structure of the developing ovule. $\times 250$

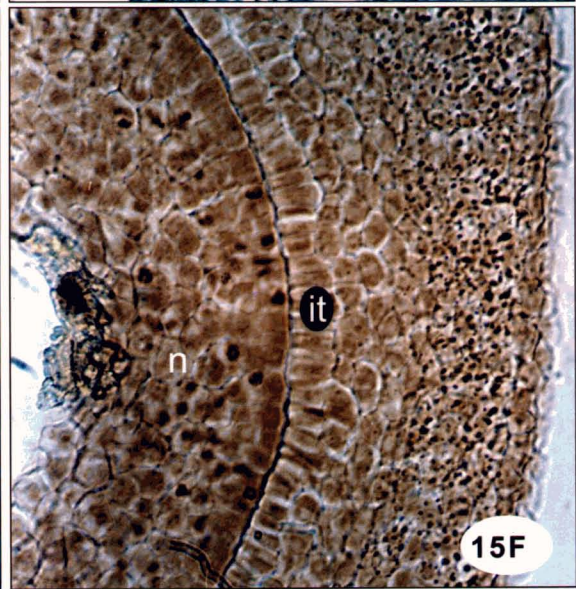
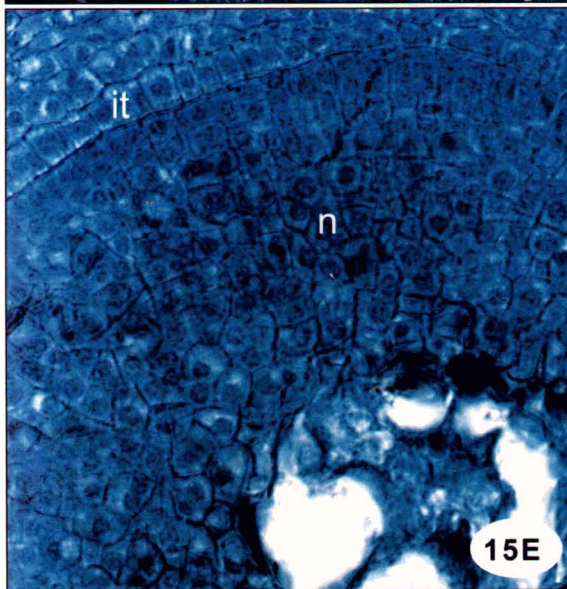
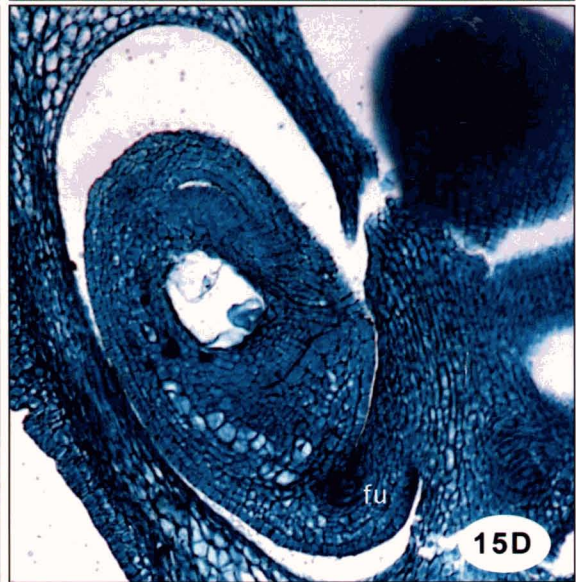
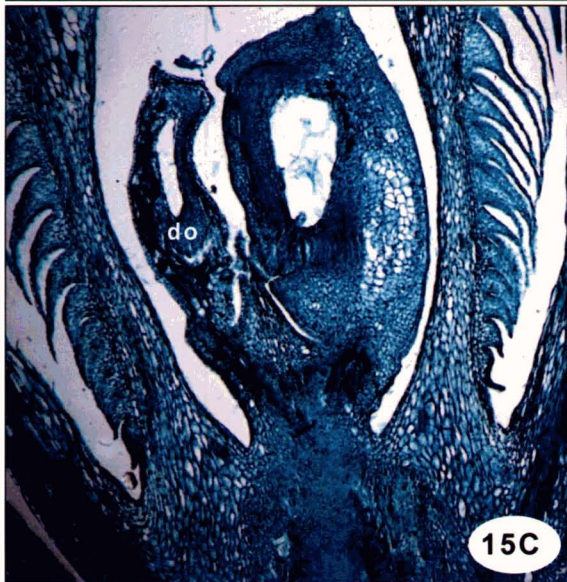
Fig. 15C. *C. thwaitesii*. L.S. of ovary showing a completely degenerated ovule and the developing ovule. $\times 250$

Fig. 15D. *C. thwaitesii*. L.S. of the ovary showing the functional ovule. $\times 250$

Fig. 15E. *C. hookerianus*. L.S. of ovule showing integumentary tapetum. $\times 400$

Fig. 15F. *C. thwaitesii*. L.S. of ovule showing in tegumentary tapelaum $\times 400$

fu - funiculus, it - integumentary tapetum, n - nucellus, o - ovule, do - degenerating ovule.



4.4.1. Megasporogenesis and female gametophyte

A single hypodermal cell of the nucellus differentiated from the rest of the cells and functioned as the archesporium (Fig. 16A). As development of the ovule progressed, the archesporial cell divided periclinally to produce the primary parietal cell and the primary sporogenous cell. The parietal cell divided anticlinally to keep pace with the increase in volume of the nucellus.

The mature megaspore mother cell was relatively large and elongated parallel to the long axis of the nucleus. The mother cell possessed a large nucleus with dense cytoplasm. The megaspore mother cell underwent meiosis and a linear tetrad was formed. The chalazal cell of the tetrad functioned and gave rise to the embryosac with the three micropylar megaspores degenerated and became crushed by the expanding embryosac. The female gametophyte was thus monosporic (Fig.16B).

The functional megaspore enlarged crushing the surrounding nucellar cells. The cytoplasm became highly vacuolated and the nucleus was shifted to the centre of the developing embryosac (Fig.16C). The enlargement of the functional megaspore was followed by three mitotic divisions, resulting in the formation of an 8-nucleate embryosac. The first division of the nucleus was followed by polarisation of daughter nuclei forming micropylar and chalazal nuclei (Fig.16D). In the 4-nucleated and 8-nucleated embryo sac, the nuclei were not aggregated in the polar regions; instead they were scattered in the cytoplasm of the micropylar and chalazal region and in a single section, all the 4 and 8 nuclei could not be located. The mature embryo sac was a 7-celled and an 8-nucleated structure with micropylar egg apparatus consisting of a

The antipodals degenerated even before fertilization. The two synergids were definite cells each with a large nucleus (Fig.16G).

One of the synergids disappeared just prior to fertilization while the other was seen persisting even after fertilization (Fig.16G). The egg cell was smaller than the synergids and was placed back to them and all the three cells of the egg apparatus were rarely be observed in a single longitudinal section.

4.5. METHOD OF POLLINATION

In both the species studied, the male inflorescences during anthesis were predominantly visited by *Drosophila* species (Diptera) honey bees, wasps (Hymenoptera) and ants. Later in the day, hymenopterans became rarer, whereas a few flies were present until sunset. Worker ants (Hymenoptera) were seen crawling during the morning hours in the male and female inflorescences. In the female inflorescence, mainly wasps and *Drosophila* species were observed, but none of these were abundant.

The body of the insect visitors sampled from the pistillate inflorescences did not show any pollen grains. Pollen collected with transparent adhesive tape from near the female inflorescences showed the presence of pollen grains of both the species. The pollen grains were powdery and produced in large quantity in both species. Hence the pollination is anemophilous.

4.6. ULTRA STRUCTURE OF STYLE AND STIGMA

The fine structural analysis of pistil showed that the stigmatic surface contained a lot of lobes which appeared as tubular frills with a groove in the centre. The lobed structures on the ridges of stigma indicated the seating pattern of the stigmatic surface for the insects for pollination (Figs. 18A – 18D). The style was solid type and was composed of parenchymatous cells (Fig. 18E).

4.7. GERMINATION OF POLLEN GRAINS

Pollen grains stained with 2, 3, 5 – tripheny (tetrazolium chloride showed the presence of sterile grains among the fertile grains (Figs. 17A & 17B).

The optimum concentration of sucrose solution for pollen germination was found to be 0.5 percent in *C. hookerianus* and 0.3 percent in *C. thwaitesii*. Pollen germination studied from the time of anthesis upto 18 hrs after floral opening with an interval of 2 hrs showed that maximum germination occurred at 10th hr after anthesis in the case of *C. hookerianus* with a mean of 43.09 and it was in the 2nd hour in *C. thwaitesii* with a mean of 44.28 (Table 7.).

Table 7. Pollen germination at different hours after anthesis

Species	Time interval of pollen collection (hrs)		Percentage of germination (Mean)
<i>C. hookerianus</i>	0*	Concentration of sucrose medium 0.6 (%)	14.2
	2		26.01
	4		20.46
	6		17.61
	8		43.09
	10		31.74
	12		27.06
	14		27.78
	16		23.25
<i>C. thwaitesii</i>	0*	Concentration of sucrose medium 0.3 (%)	23.35
	2		44.28
	4		22.60
	6		22.79
	8		9.43
	10		31.90
	12		29.41
	14		17.46
	16		7.94

* - Just after anthesis

The difference in pollen germination at different intervals is highly significant (P<0.0001).

Fig. 16. Megasporogenesis and megagametogenesis

Fig. 16A. *C. hookerianus*. L.S. of ovary showing megaspore mother cell $\times 400$

Fig. 16B. *C. hookerianus*. L.S. of ovary showing the functional megaspore. $\times 400$

Fig. 16C. *C. hookerianus*. Uninucleate embryosac. $\times 800$

Fig. 16D. *C. hookerianus*. Binucleate embryosac. $\times 800$

Fig. 16E. *C. hookerianus*. A mature embryosac. $\times 800$

Fig. 16F. *C. hookerianus*. A mature embryosac showing secondary nucleus. $\times 800$

Fig. 16G. *C. hookerianus*. Embryosac showing the egg cell and degenerating synergid. $\times 800$

t – tannin, mmc – megaspore mother cell, fm – functional megaspore, nu – nucleus, ea – egg apparatus, sn – secondary nucleus, e – egg, ds – degenerating synergid.

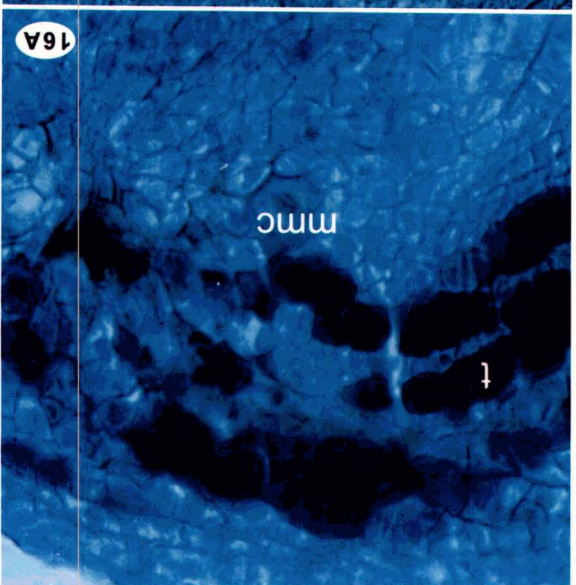
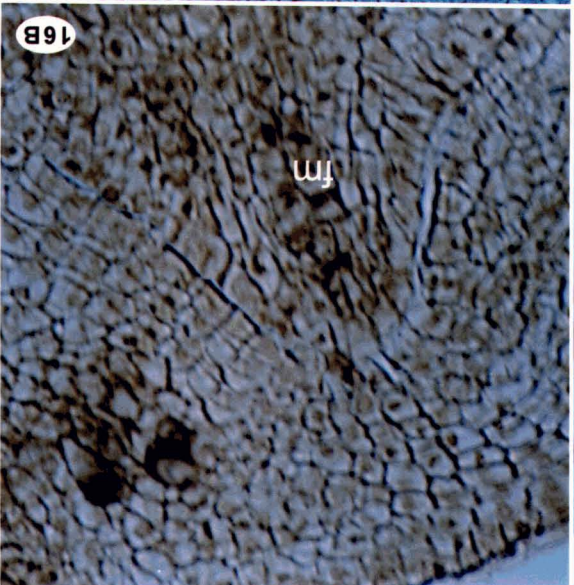
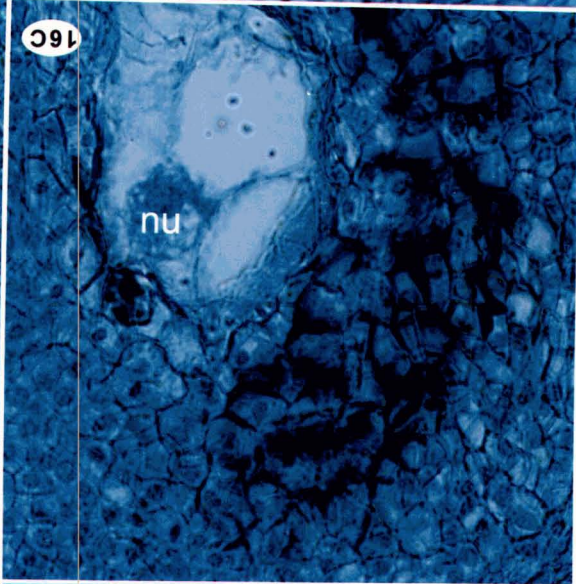
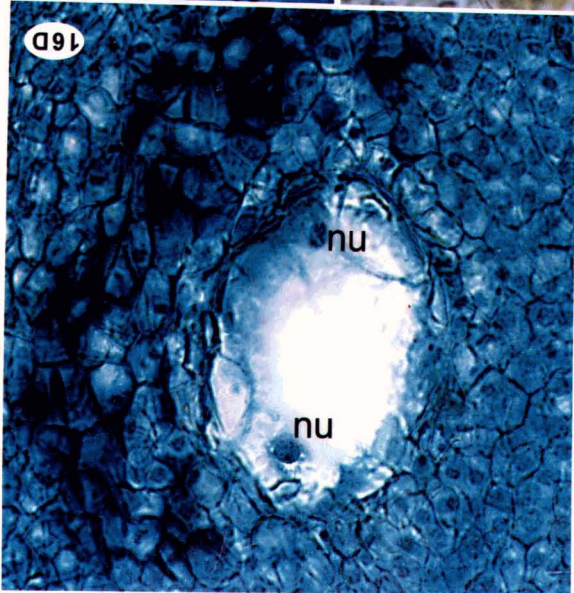
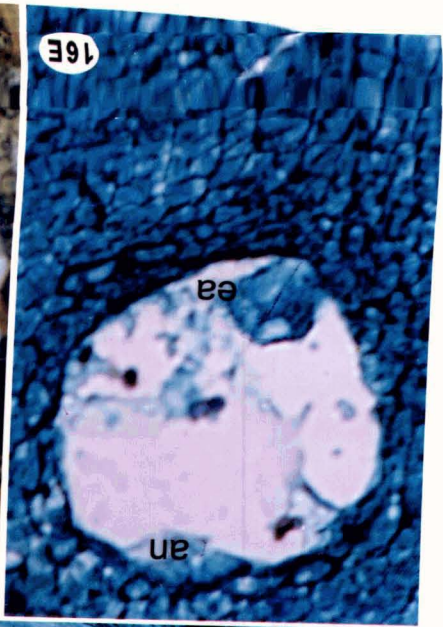
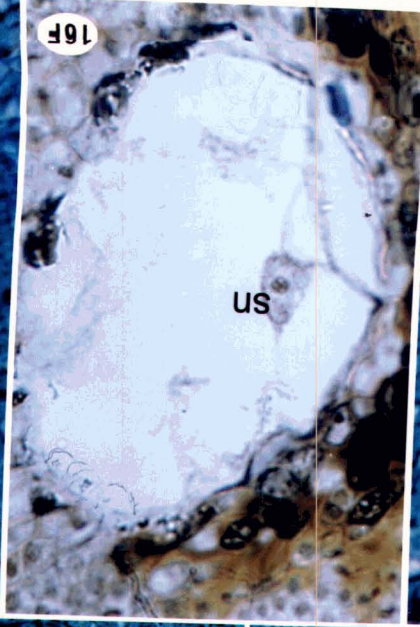
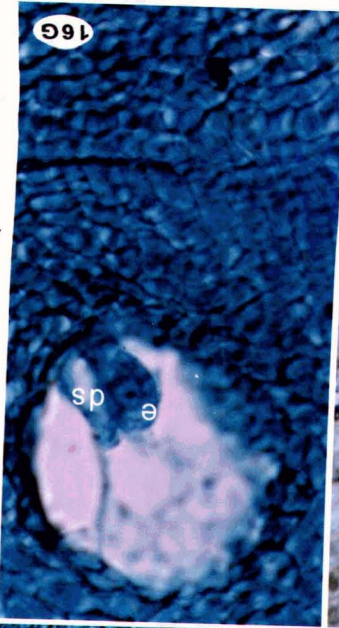


Fig. 17. Pollen viability and germination

Fig. 17A. *C. hookerianus*. Result of tetrazolium test showing sterile pollen grains.
sp.sterile pollen grains. $\times 250$

Fig. 17B. *C. thwaitesii*. Result of tetrazolium test showing sterile pollen grains. $\times 250$

Fig. 17C. *C. hookerianus*. Pollen germination. $\times 800$

Fig. 17D. *C. thwaitesii*. Male a gametophate. $\times 800$

sp – sterile pollen, pt – pollen tube, vn – vegetative nucleus, gn – generative nucleus
s – sperm.

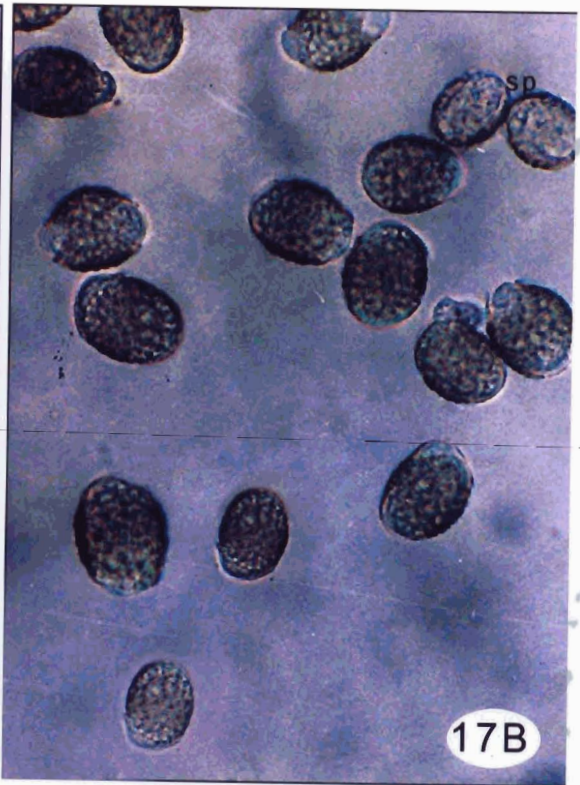
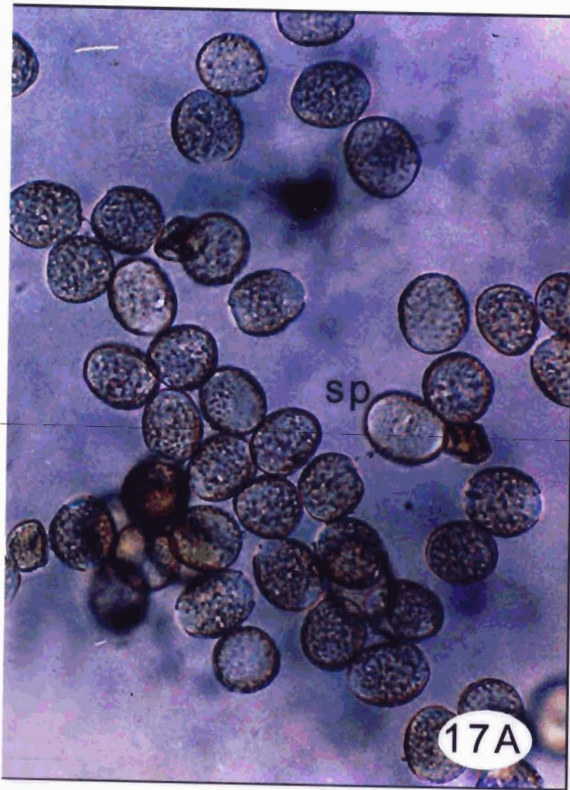


Fig. 18. Scanning electron micrographs of *C. hookerianus* pistil

Fig. 18A. Pistil showing the ovary, style and stigma.

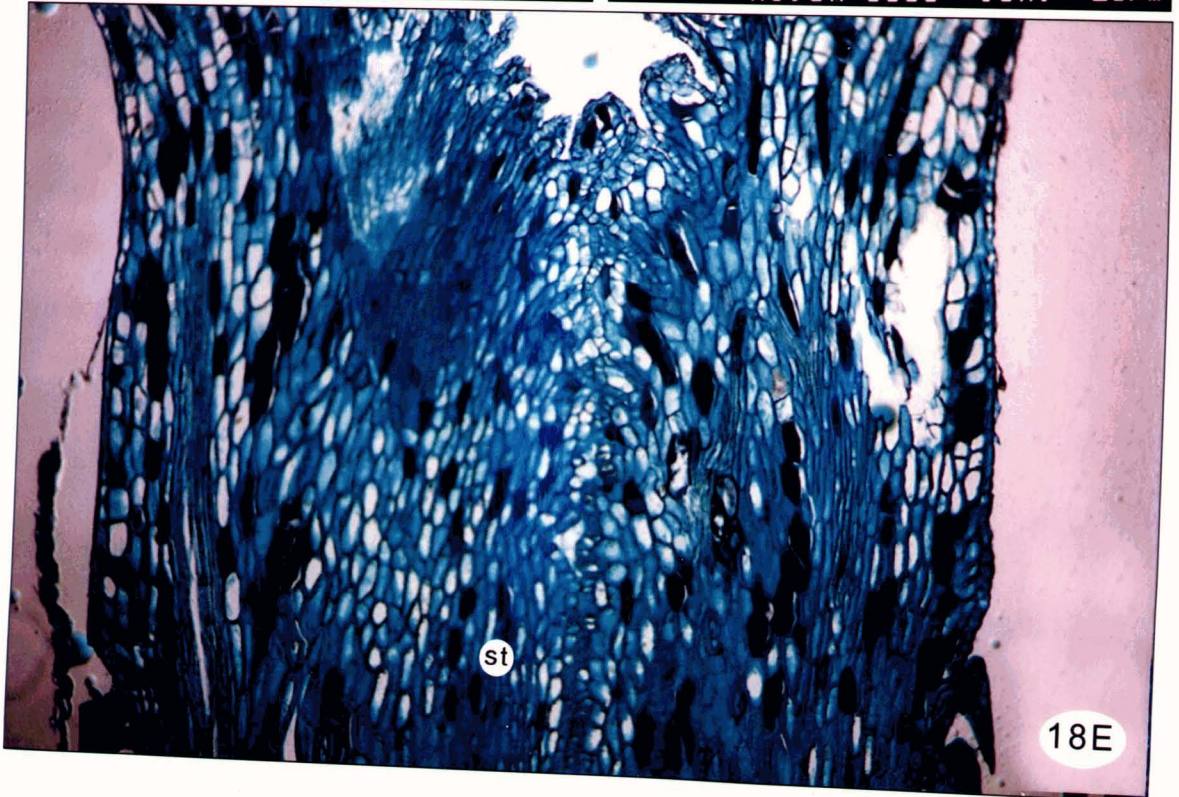
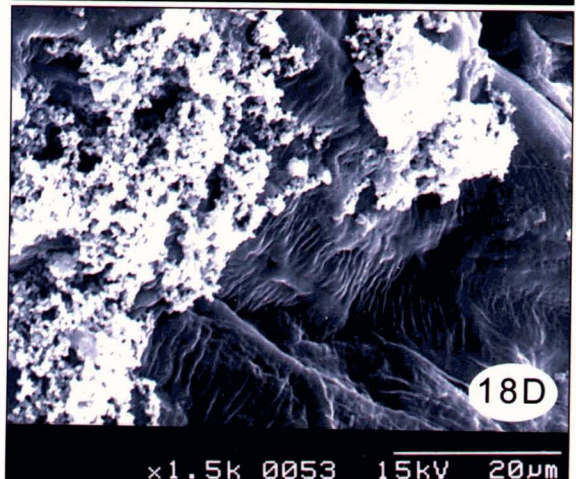
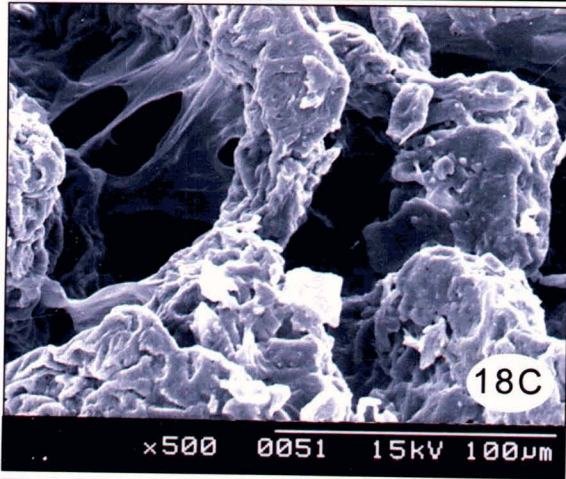
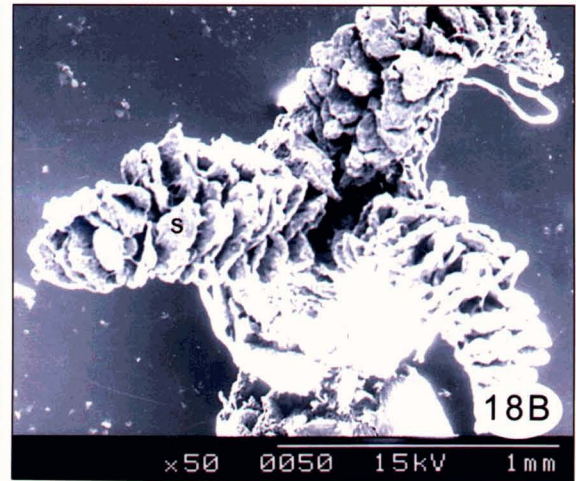
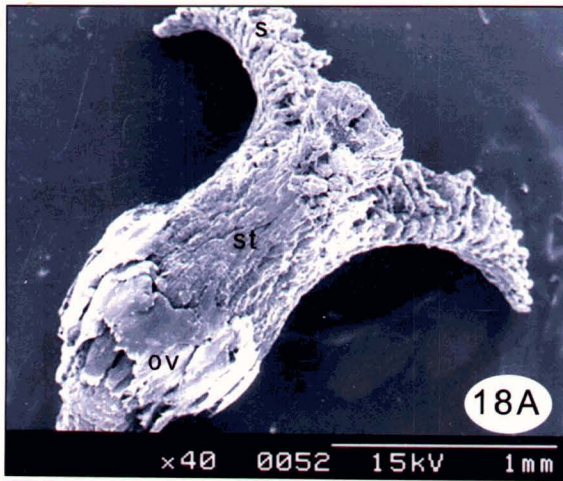
Fig. 18B. The trifold stigma enlarged

Fig. 18C. A part of the stigmatic lobe enlarged.

Fig. 18D. The junction of the stigmatic lobe enlarged.

Fig. 18E. *C. hookerianus*. L.S. of style. $\times 250$

ov – ovary, st – style, s - stigma



It was found that pollen stored in refrigerator at 10⁰ C in packets of tissue paper kept in glass vials after wrapping in wet paper germinated very well upto the third day after the storage.

4.8. ENDOSPERM

The fertilization of the secondary nucleus by one of the male gametes resulted in the formation of the primary endosperm nucleus. This nucleus divided without the formation of a cell wall and consequently the endosperm of both species of *Calamus* was of the nuclear type. The endosperm nuclei became organised in a thin peripheral layer while the central part of the embryo sac was filled with a liquid substance (Figs.19A & 19B). Cellularisation commenced when the embryo was 10-12 celled stage and it progressed in a centripetal fashion.

Endosperm of *C. hookerianus* was ruminant. The rumination was caused by infoldings of the seed coat. The cells of the infoldings contained tannin. The undulation of the wall of the embryo sac appeared even before the endosperm become cellular (Figs.19A).

In a young fruit, the seed cavity was filled with mucilaginous fluid in which numerous amoeboid nuclei were suspended. The embryo at this time consisted of a small group of cells closely pressed to the endothelium at the micropylar end of the embryo sac.

As the cavity enlarged, there was a slow multiplication of the nuclei in the fluid. As the fruit increased in size, many free cells were found suspended in the fluid. While these cells were dispersed in the liquid, the endothelium was composed of a closely packed layer of thin walled cells firmly fused to the inner integument. At this stage, there was

no cellular endosperm attached to the endothelial surface. When the fruit attained a length of approximately 0.7 cm in *C. hookerianus* and 1.2 cm in *C. thwaitesii*, the free nuclei and cells began to settle out of the fluid and attached themselves to the endothelial surface. The first evidence of the cellular aggregation was seen at the lower end of the embryo sac and the deposition of these free nuclei apparently continued until a white tissue was built up over the entire endothelial surface. This tissue by successive cell divisions ultimately developed into a hard endosperm (Figs.19C – 19D).

The micropylar region of the seed was covered by hylar plug ((Fig. 20A). The cell of this plug was rich in tannin globules. The mature endosperm of the seeds showed varying degrees of hardness, the anatomical basis of which was the thickened walls (Fig. 20B). It was found that in both species the walls were extremely thick except in areas of pit fields and consisted of three layers which differed in their staining with PAS reaction, the middle lamella, thickened outer wall and thin inner wall (Fig. 20C).

A characteristic feature noticed during the development of endosperm in both species was that the developing endosperm got adjoined to the dorsal side of the seed because of the inpushing of the placental rumination from the ventral side of the seed. This made the seeds grooved in both the species (Fig. 20D).

4.9. DEVELOPMENT OF EMBRYO

The embryo development was similar in both species of *Calamus* studied. The club-shaped zygote divided transversally to produce a smaller terminal cell and a larger basal cell (Figs.21A – 21B, 22A & 22B). The 2-celled pro-embryo, thus, had a terminal cell 'ca' and a basal cell 'cb' facing the micropyle. The terminal cell divided

longitudinally while the basal cell divided by a transverse wall. However, later divisions were mainly restricted to the products of 'ca', and 'cb' along with few cells of 'ca' contributed mainly to the formation of few celled suspensor. Further division in the products of 'ca' formed a quadrant and then an octant (Figs. 21C – 21F & 22C – 22F).

During the early period of development, one of the daughter cells of 'ca' became obviously more active than the other. Later the pro-embryo went through a globular stage (Figs. 21G – 21H & 22G – 22H). Divisions were more active in the wedge-shaped daughter cell of 'ca' and hence the upper part of the globular pro-embryo became increasingly asymmetrical. Later, a spherical mass of actively dividing cells, the embryo proper was formed and with the addition of more and more cells, this particular group of cells became more or less terminal in position. Thus only one of the upper part of the young embryo contributed to the major part of the embryo proper. The rest of the cells and its derivatives contributed to a cotyledonary sheath. Hence embryogeny in both species corresponded to Onagranad type of Johansen (1950). At the globular stage the embryo stood straight on the suspensor, the cells of which can easily be recognized by their high vaculation (Figs. 23A – 23C & 24A – 24B).

The stem tip was formed exactly at the terminal pole of the embryo. A group of cells with large and weakly staining nuclei showed the characteristic features of young apical meristem cells (Figs. 23D – 23F & 24C – 24D). During further development, the shoot apex came to lie deep within the cotyledonary lobes. The terminally placed shoot apex slowly became shifted to one side of the embryo due to the overgrowth of the cotyledonary sheath.

Fig. 19. Endosperm development and structure.

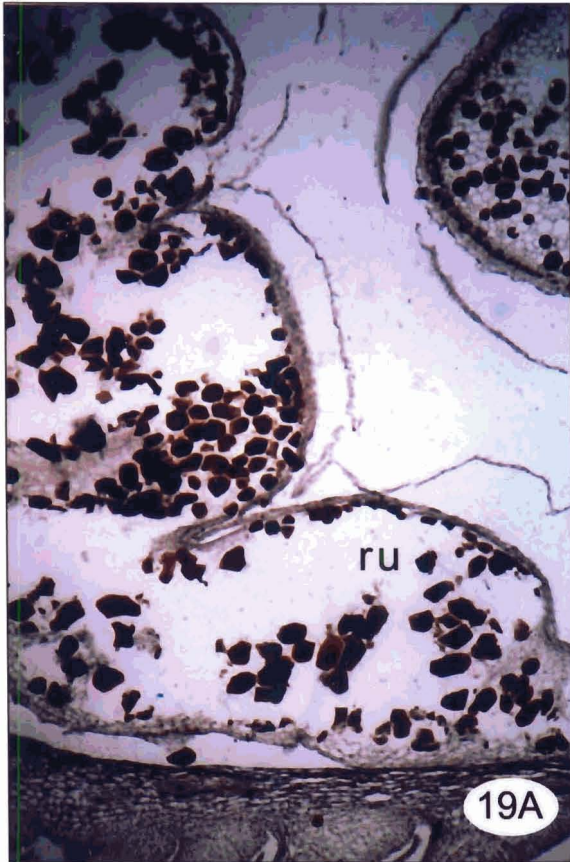
Fig. 19A. *C. hookerianus*. L.S. of young seed showing free nuclear endosperm and ruminations of seed coat. $\times 250$

Fig. 19B. *C. thwaitesii*. L.S. of young seed showing free – nuclear endosperm $\times 250$

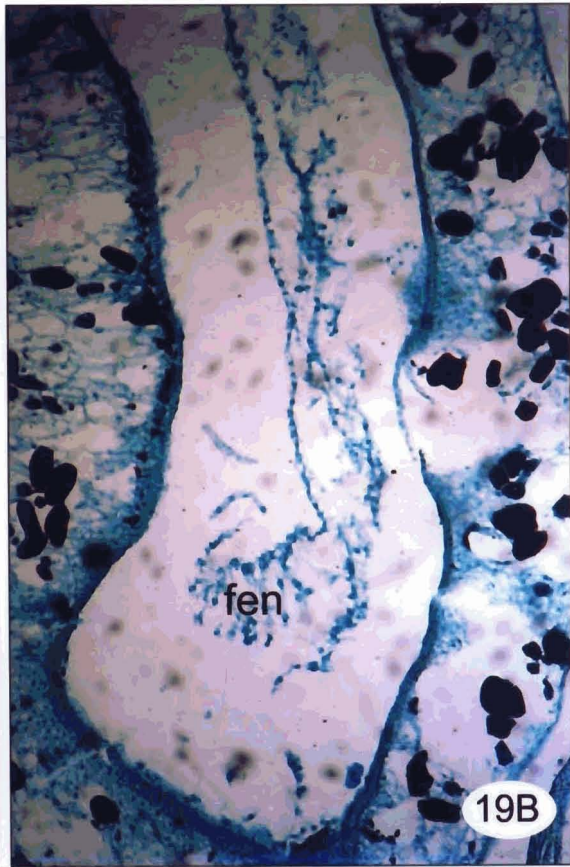
Fig. 19C. *C. hookerianus*. Endosperm of a maturing seed $\times 250$

Fig. 19D. *C. hookerianus*. Endosperm of a mature seed. $\times 250$

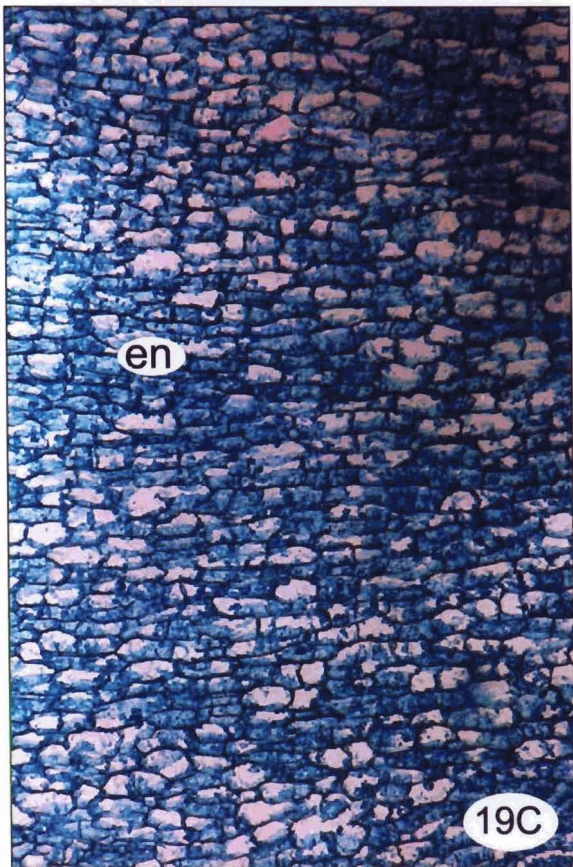
ru – ruminations, fen – free nuclear endosperm, en – endosperm.



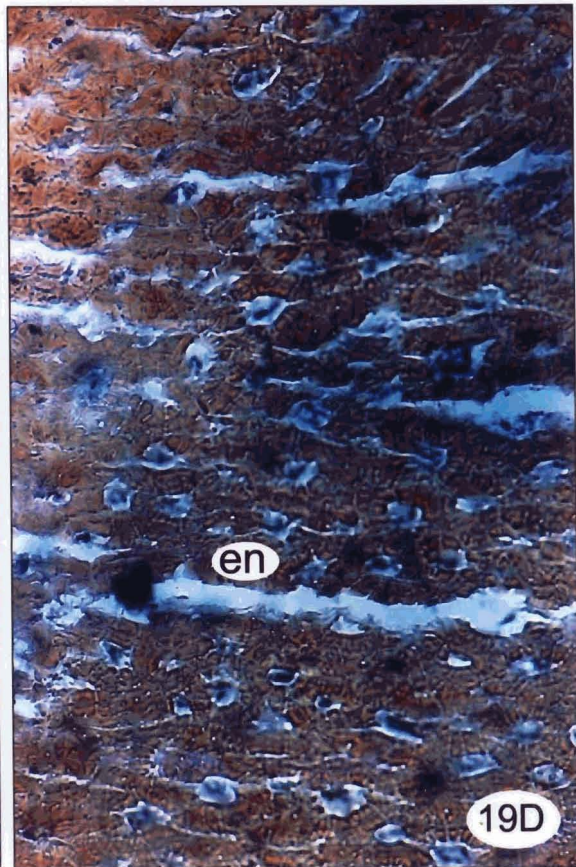
19A



19B



19C



19D

7-226

Fig. 20. Endosperm structure

Fig. 20A. *C. hookerianus*. Scanning electron micrograph of a mature seed

Fig. 20B. *C. hookerianus*. Scanning electron micrograph of mature endosperm.

Fig. 20C. *C. thwaitesii*. Result of PAS staining of mature endosperm. $\times 250$

Fig. 20D. *C. thwaitesii*. L.S. of developing seed showing placental rumination

hp – hylar plug, en – endosperm, em – embryo, pr – placental rumination.

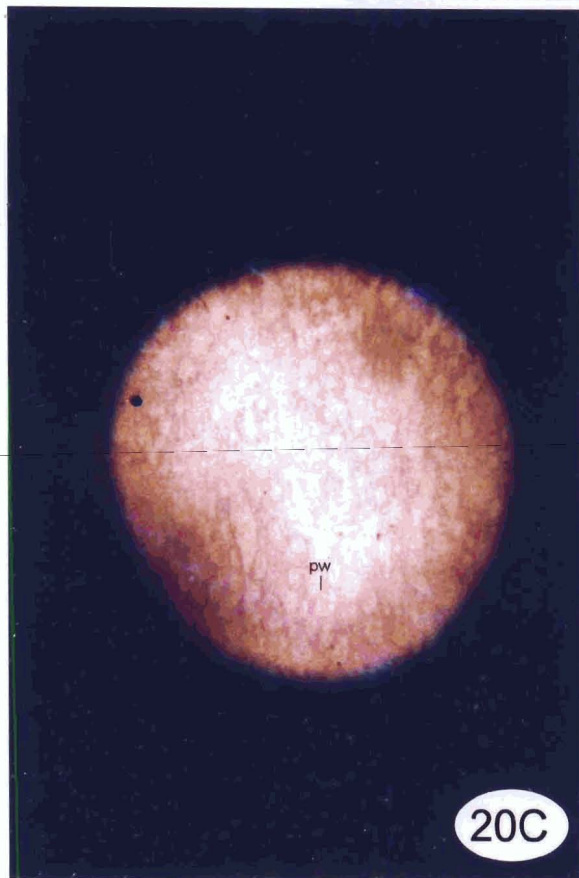
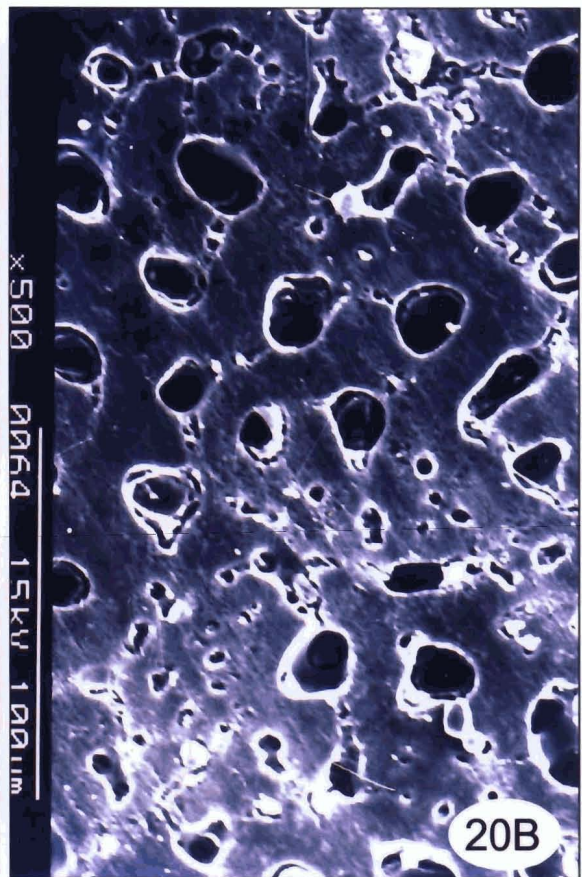
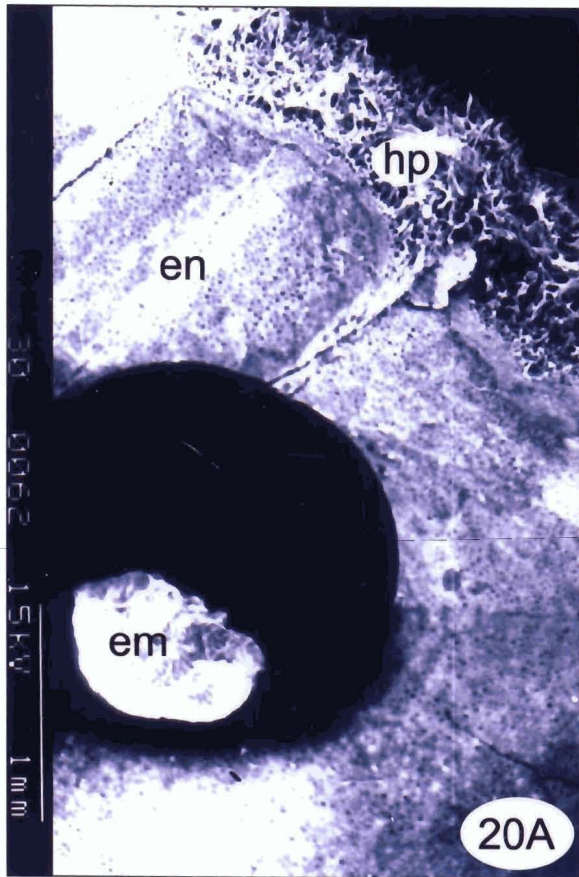


Fig. 21. *C. hookerianus* - Embryogenesis

Fig. 21A. Zygote showing dense cytoplasm and prominent nucleus. $\times 800$

Fig. 21B. 2-celled proembryo. $\times 400$

Fig. 21C. 4-celled proembryo $\times 400$

Fig. 21D. Quadrat stage of proembryo. $\times 400$

Fig. 21E. Young proembryo $\times 400$

Fig. 21F. Octant stage of proembryo $\times 400$

Fig. 21G. Young proembryo $\times 400$

Fig. 21H. Globular stage. $\times 400$

ca - apical cell cb - basal cell

zy - zygote, ca - apical cell, cb - basal cell, - ep - epiphysis like cell.

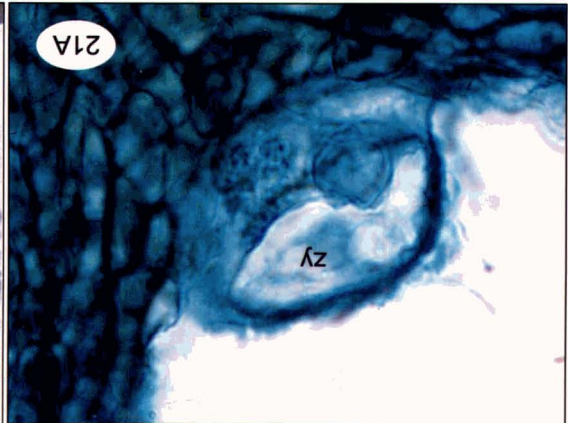
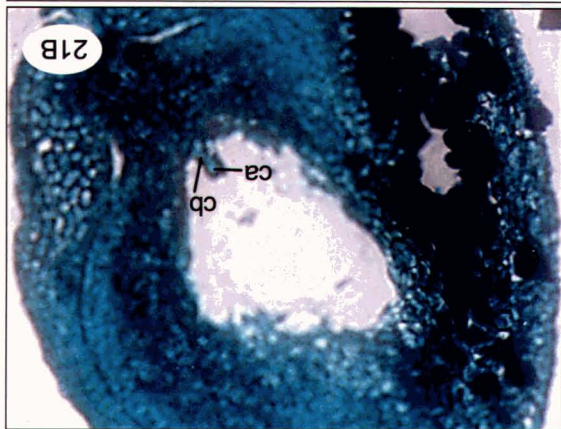
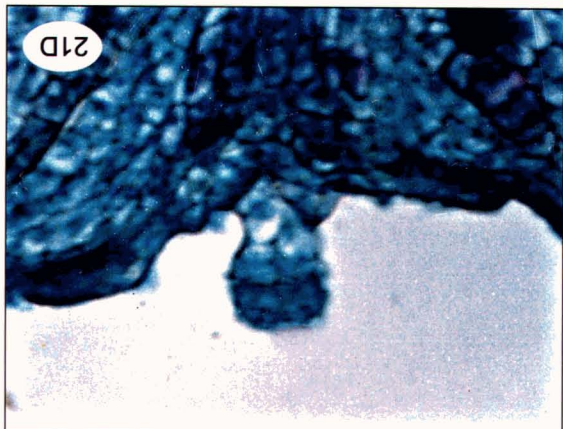
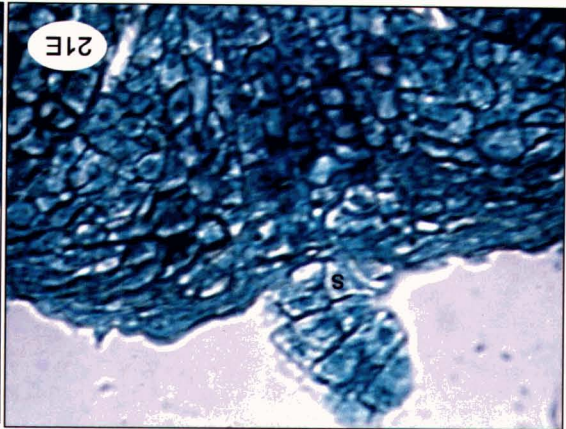
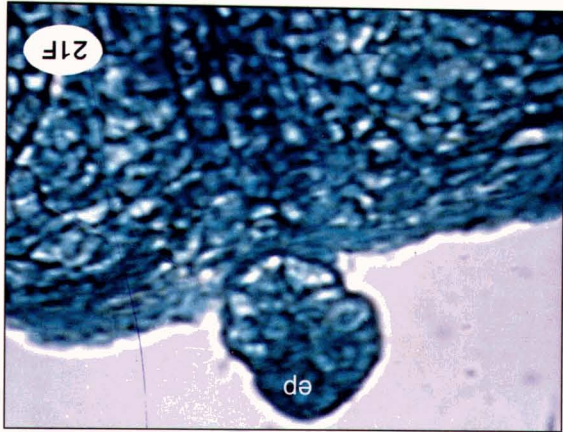
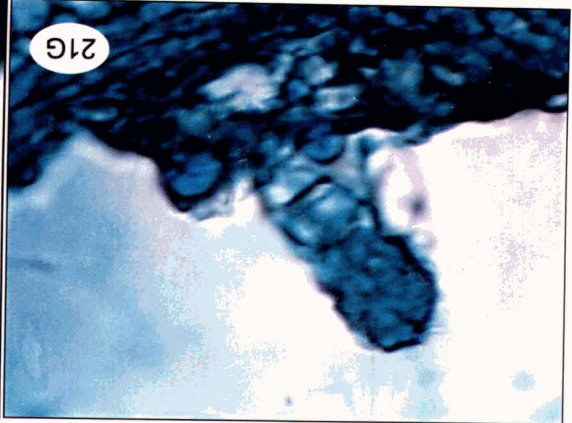
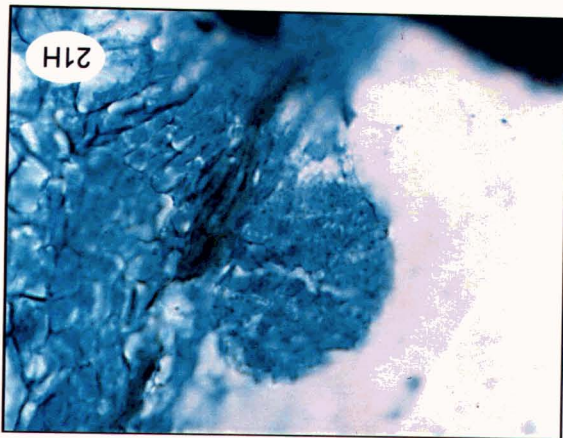


Fig. 22. *C. thwaitesii* - development of proembryo.

Fig. 22A. Club shaped zygote $\times 250$

Fig. 22B. 2 celled proembryo. $\times 250$

Fig. 22C. Proembryo. $\times 400$

Fig. 22D. Proembryo $\times 400$

Fig. 22E. Proembryo $\times 400$

Fig. 22F. Proembryo $\times 400$

Fig. 22G. Proembryo $\times 400$

Fig. 22H. Globular stage $\times 400$

z- zygote, s- suspensor.

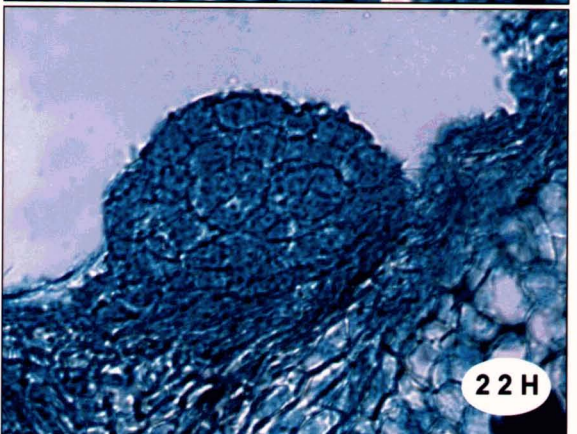
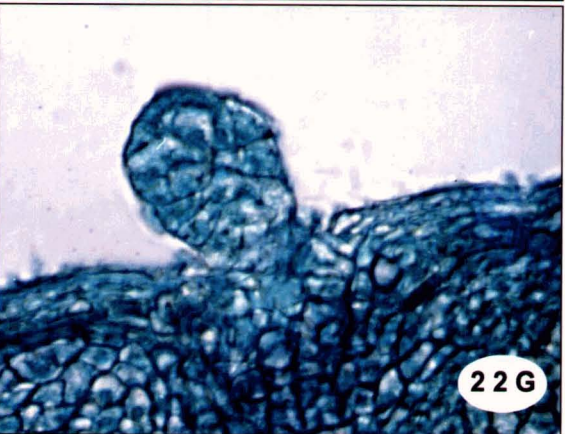
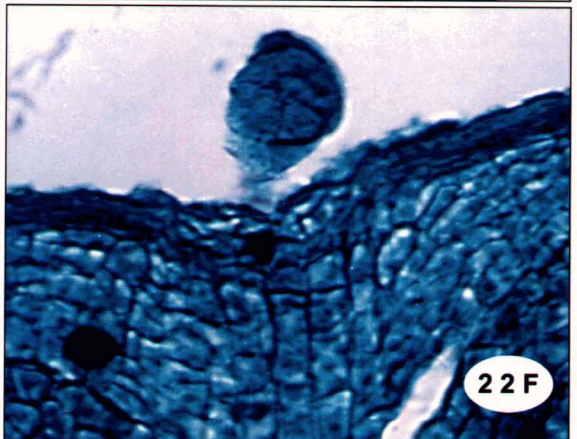
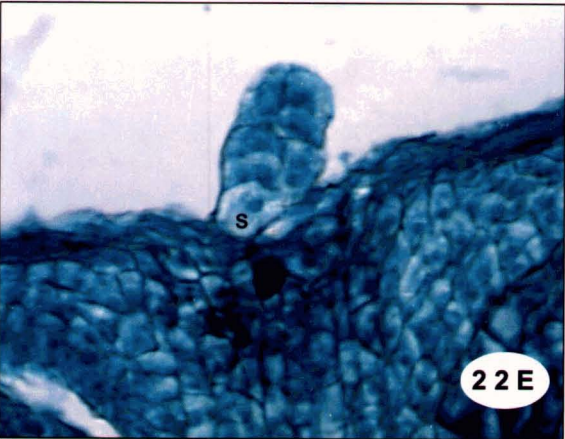
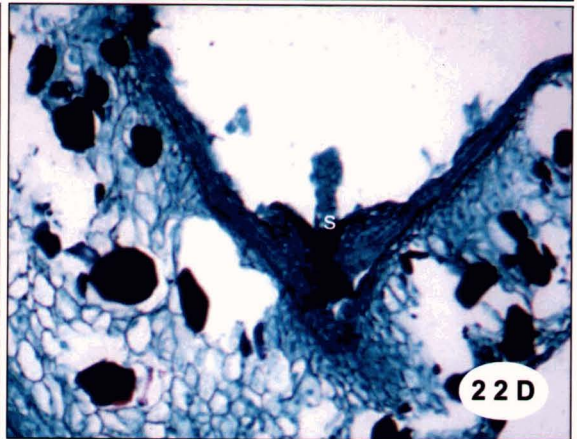
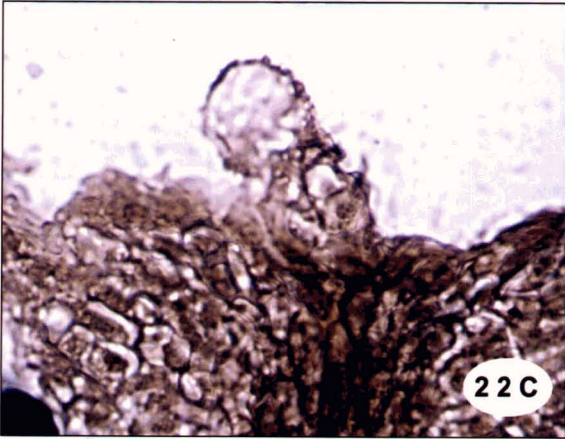
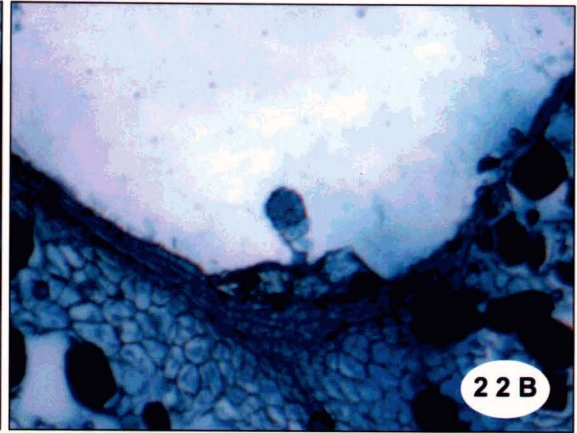
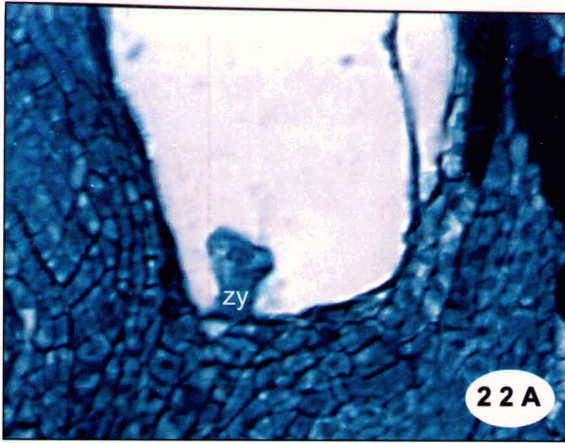


Fig. 23. *C. hookerianus* - Advanced stages of embryo development

Fig. 23A. Proembryo showing embryo proper and suspensor x 400

Fig. 23B. A more advanced embryo. x 250

Fig. 23C. Embryo showing developing cotyledon and suspensor. x 250

Fig. 23D. Embryo showing differentiation of plumule.

Fig. 23E. The sheathing base of cotyledon completely overarched the plumule. x 250

Fig. 23F. The sheathing base of cotyledon has completely overarched the stem tip. x 250

s- suspensor, co - cotyledon, pl - plumule, sh - sheathing base of cotyledon.

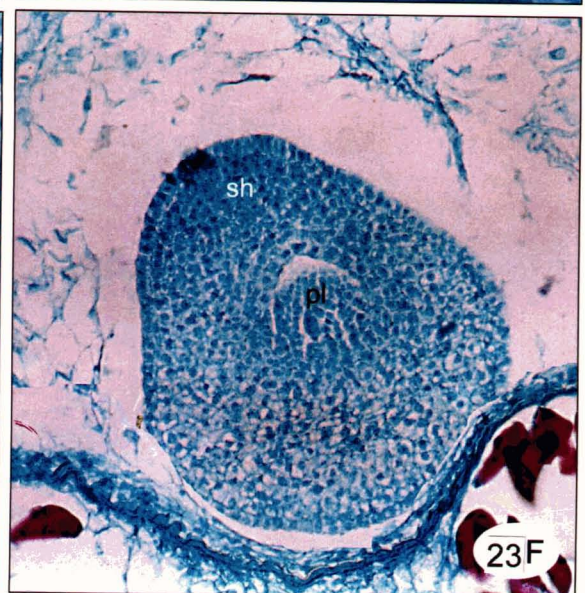
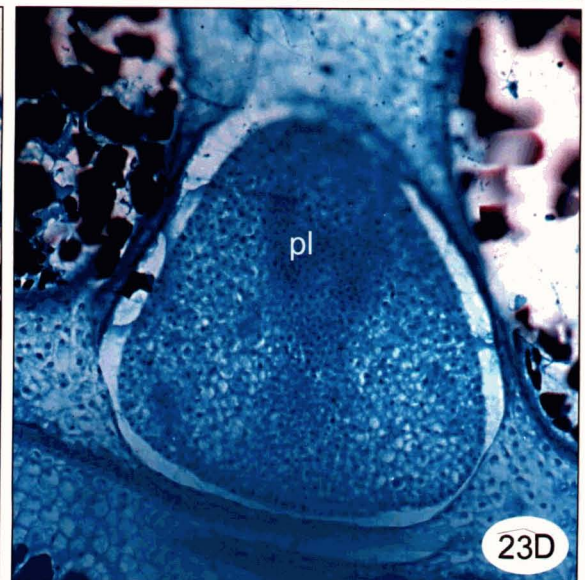
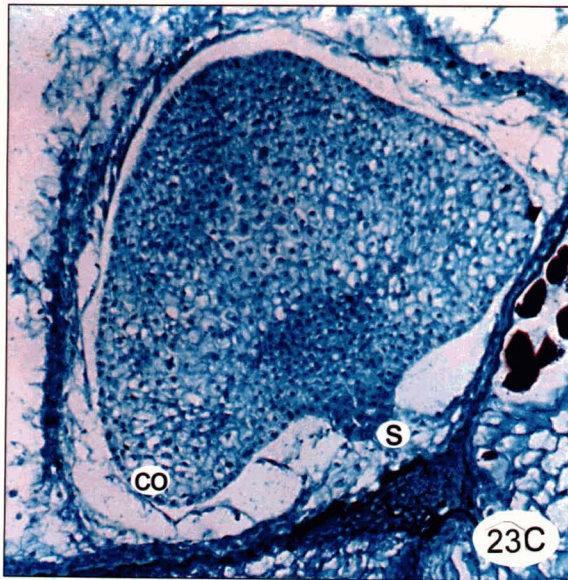
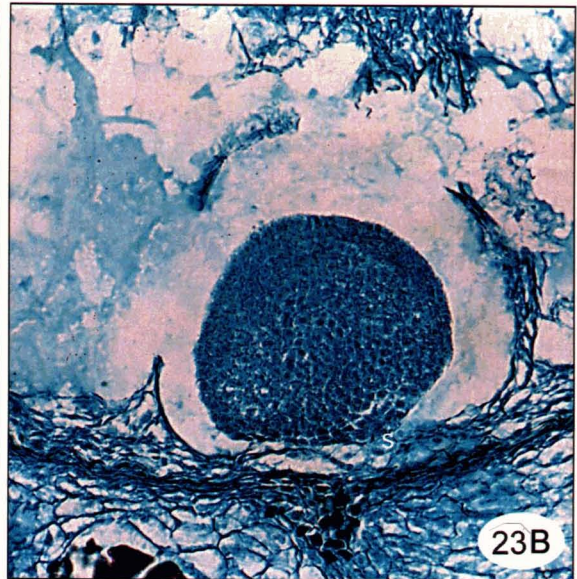
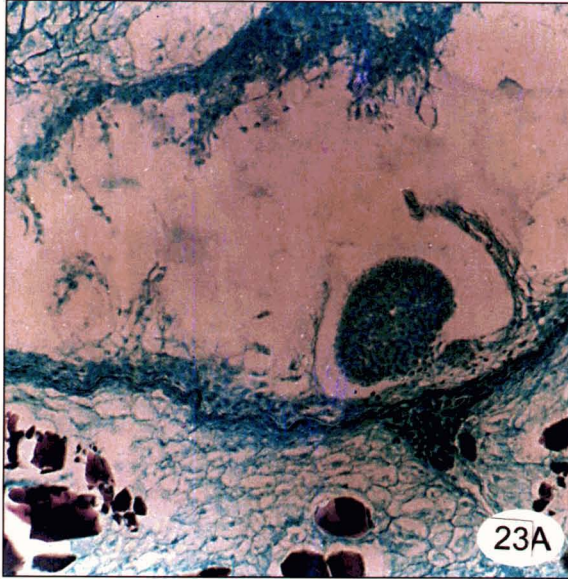


Fig. 24. *C. thwaitesii* - Various stage^s in embryogeny.

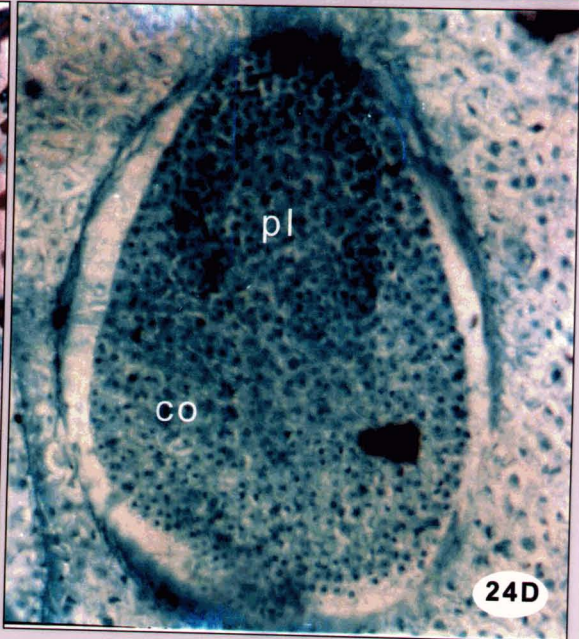
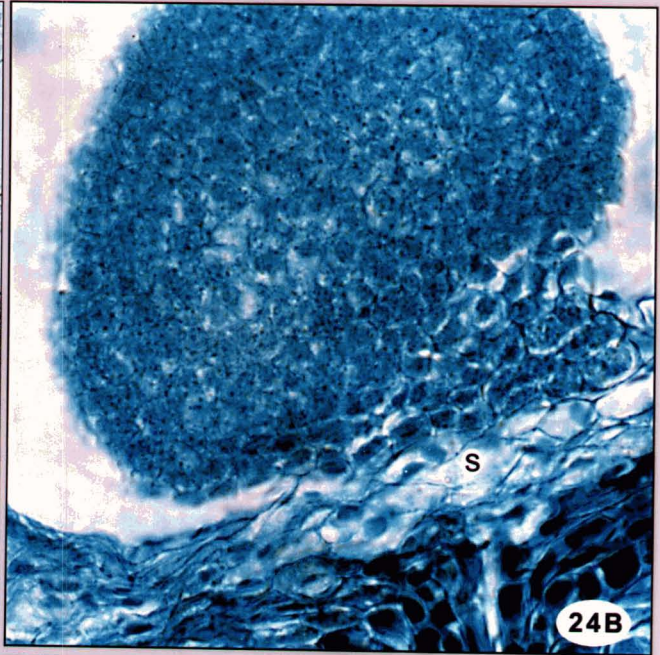
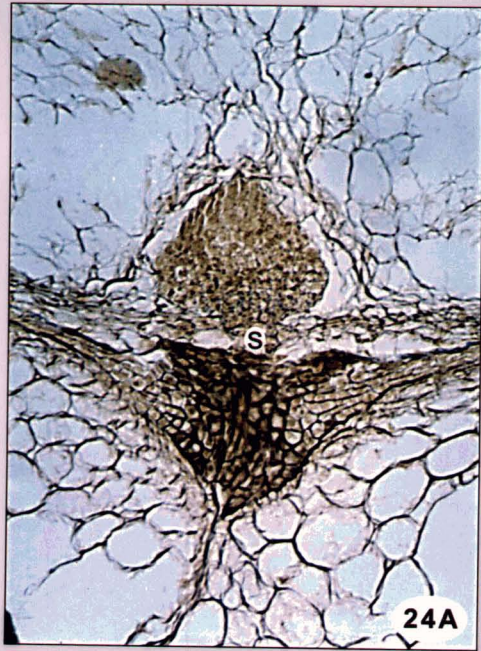
Fig. 24A. A proembryo showing predominant divisional activity. $\times 250$

Fig. 24B. A more advanced stage with actively dividing cells. $\times 350$

Fig. 24C. A more advanced stage showing the differentiation of stem tip. $\times 250$

Fig. 24D. The embryo assuming the characteristic conical shape. $\times 250$

s – suspensor, pl – plumule, co – cotyledon.



In a 3-4 month old fruit, the embryo was still at the globular stage. Embryo development was relatively slow. It took nearly 6-7 months to form 0.44 mm body in *C. hookerianus* and 0.3 mm body in *C. thwaitesii* which remained as such till the fruit matured. Embryo development and endosperm development seemed to go hand in hand. In a mature embryo, the positions of the different organs changed respectively. From the original terminal position facing the center of the embryo sac or seed cavity, the shoot apex turned through an angle of 90° .

The developing root apex, also got shifted through an angle of 90° and in a mature embryo it was in line with shoot apex but on its opposite side (Figs. 25A – 25B & 26A – 26B).

Periclinal divisions in the peripheral cells of the globular pro-embryo resulted in the differentiation of the protoderm and the embryo proper became more and more distinct from the suspensor cells. As the embryo matured, the suspensor cells got stretched side ways against the wall of the embryo sac and in a fully mature embryo the suspensor cells totally got disorganised.

A small group of cells with large and deeply staining nuclei showed the characteristic features of the apical meristem cells. As growth proceeded, this region bulged out and a core of pro-cambial cells below it formed the future hypocotylar central cylinder. Now the whole embryo became asymmetric due to increased activity of the cells on one side. This asymmetry and subepidermal periclinal divisions were the first signs of the developing cotyledonary primordium which initiated as a crescent-shaped mound on one side of the stem tip. The radicle was formed opposite the shoot apex (Figs. 26C &

26D). The terminal end of the cotyledon which enclosed the shoot apex became more elongated and increased in thickness.

4.9.1. Mature embryo

The mature embryo was some what cylindrical in shape and measured 2.2 mm in *C. hookerianus* and 2.0 mm in *C. thwaitesii*, and was embedded in the endosperm at the micropylar end, just below the hylar plug. Internally it showed a much higher degree of differentiation. The bulk of the embryonic tissue was made up of the cotyledon, part of which extended laterally over and enveloped the plumule and was marked off by a slight constriction (Figs.25B & 26B). The region of incomplete fusion of the cotyledon edges near the plumule appeared as a radical slit. The plumule in section showed a central meristematic zone, surrounded by a scale leaf primordium. The cotyledonary portion acted as a sucker. Except at the top, the embryo was flanked on all sides by the endosperm (Figs.25B & 26C).

Lying at an angle slightly less than 48° to the central axis and opposite to the plumule was the radicle, deeply situated and with the apical mass of meristematic cells directed towards the flat expanse of the suspensorial region. The root primordium was not well developed.

4.9.2. Formation of haustorium

During the germination of *Calamus* seed a new tissue, by the progressive enlargement of the basal part of the embryo was formed. This new tissue was termed as 'haustorium' meaning parasitic organ. Within the seed, the haustorium developed steadily. At the initial phase of growth, the haustorium was a spongy, slightly ridged,

Fig. 25. *C. hookerianus* - Mature embryo.

Fig. 25A. A mature embryo showing the shoot apex turning 90° $\times 250$

Fig. 25B. A fully mature embryo. $\times 250$

pl - plumule, co - cotyledon.

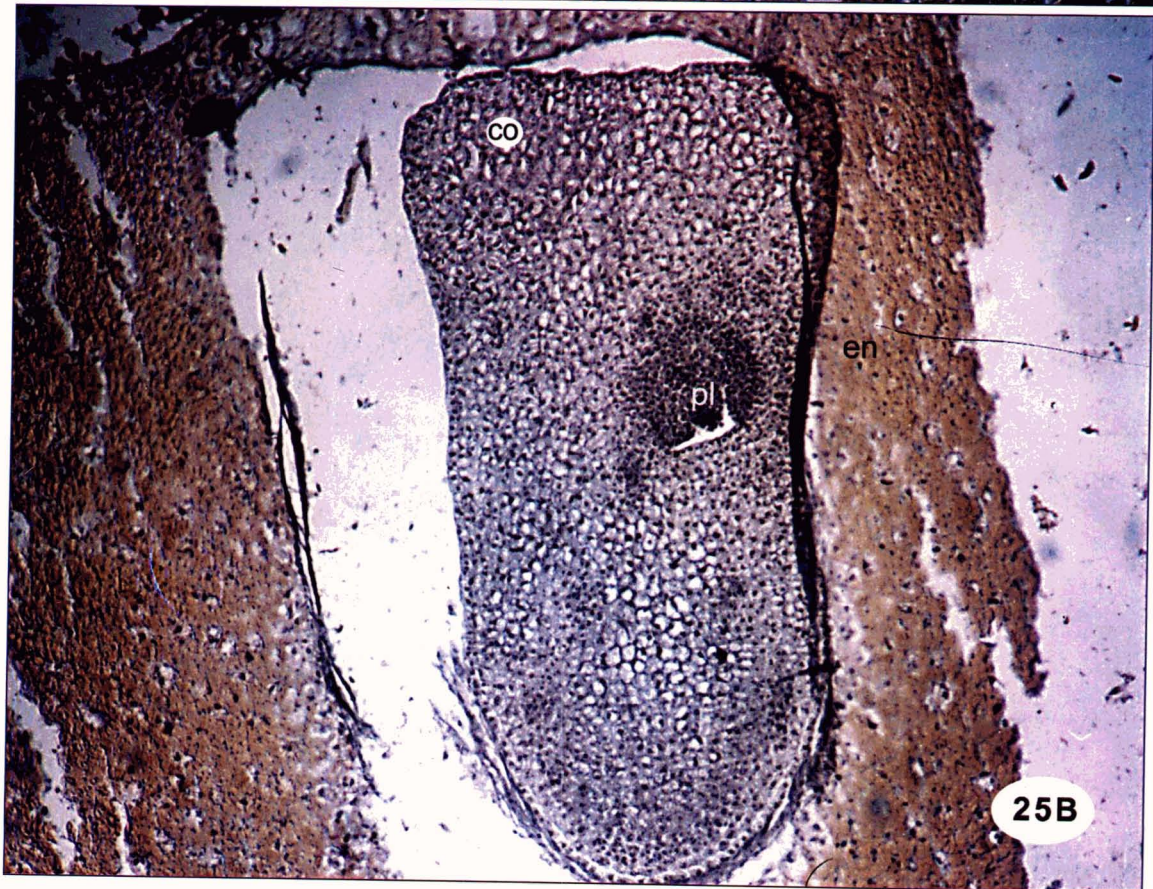
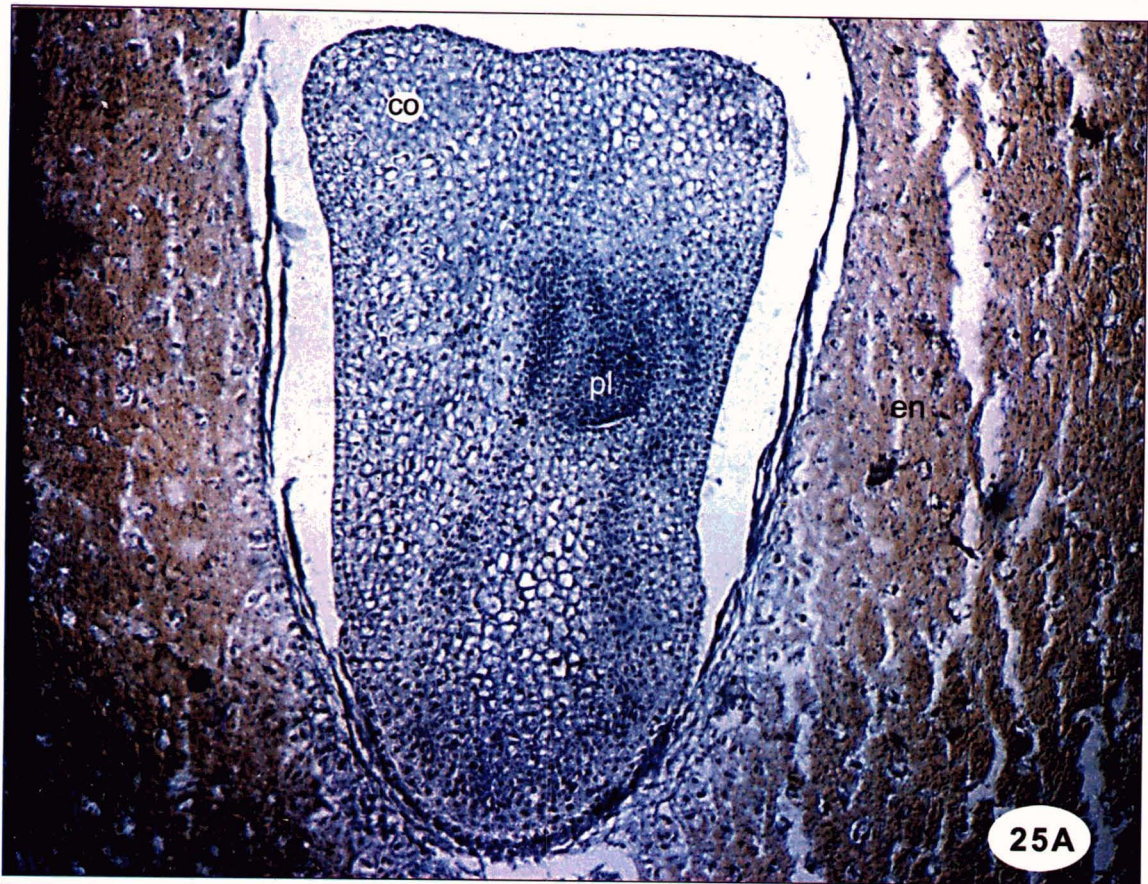


Fig. 26. *C. thwaitesii* - Showing the development of a mature embryo

Fig. 26A. A developing embryo showing the differentiation of plumule and well developed cotyledon. $\times 250$

Fig. 26B. A developing embryo at the time of fruit maturation. $\times 250$

Fig. 26C. A fully mature embryo showing well developed stem tip and radicle. The root apex and shoot apex have turned 90° $\times 250$

Fig. 26D. Embryo dissected from germination bed. $\times 250$

pl - plumule, r - root, co - cotyledon.



pear-shaped structure having rough serrations over the surface (Figs. 27A – 27B). The outer surface was marked by ramifying fissures. As germination proceeded, the enlarged haustorium was found to displace the endosperm almost entirely. The endosperm during this period was progressively disintegrated from the centre to make room for the haustorium. Figs. 28A & 28D clearly shows the enlargement of the haustorium with gradual progress in the germination process. After about one month of the onset of germination the spongy haustorium completely filled the seed cavity (Figs.28B & 28D).

Histologically, the young haustorium consisted of closely arranged round parenchymatous cells with vascular elements in the outer zone. At later stage, the parenchymatous cells of the haustorium became stretched and expanded irregularly (Fig. 27C).

4.10. FRUIT AND SEED

Fruits of both species of *Calamus* consisted of a scaly pericarp and the seed was covered by a fleshy layer of sarcotesta (Figs.29A & 29C). The sarcotesta adhered very tightly to the seed and could be removed by scalification. The fruit of *C. hookerianus* measured 1 x 0.8 cm and was sub-globose with 18 rows of reflexed scales and that of *C. thwaitesii* measured 2 x 1.3 cm and were ovoid with scales in 12 vertical rows. For both species, there was an eye-shaped median groove on the ventral side of the seed (Figs.29B & 29D). The seeds of both species were dorsi-ventrally differentiated. A well developed median groove was present on the ventral side which was filled with tannin cells (Figs.30C & 30D). The small, cylindrical embryo was positioned basally on the

dorsal side of the seed, just below the “hilar plug” (Figs. 30E & 30F). The morphological attributes of fruits and seeds are presented in Table 8.

Table 8. Morphological attributes of fruits and seeds

Sl. No	Character	<i>C. hookerianus</i>	<i>C. thwaitesii</i>
1	Number of fruits/kg	3872	380
2	Fruit length	1 cm	2.0 cm
3	Fruit breadth	0.8 cm	1.3 cm
4	100 fruit weight	45 g	350 g
5	Seed length	0.9 cm	1.8 cm
6	Seed breadth	0.6 cm	1.3 cm
7	Seed thickness	0.2 cm	0.3 cm
8	100 seed weight	12.2 g	97 g
9	Moisture content	31 percent	43 percent
10	Seed longevity	15 days	13 days

Growth of the fruits in both the species followed a sigmoidal pattern from the time of appearance of the flower till fruit matures. During the first 90 days after the opening of the inflorescence, growth was relatively slow. This was followed by a second stage lasting 150 days in which growth was very rapid. In the last stage the rate of growth slowed down dramatically. It was during this final period of fruit development most of the growth of the embryo took place (Figs. 30A & 30B).

4.10.1. Seed structure

The fruits and seeds of the two species studied differed mainly in their size and weight. The fruits of *C. thwaitesii* were larger and the weight of the seeds also was higher. The moisture content of the fruits at the time of collection was 31 percent in *C. hookerianus* while it was 43 percent in *C. thwaitesii*. Seed longevity also varied slightly. The seeds of *C. hookerianus* remained viable for 15 days while that of *C. thwaitesii*, remained viable for 13 days.

Fig. 27. *C. thwaitesii* - Haustorium development.

Fig. 27A. Embryo showing the development of haustorium. $\times 650$

Fig. 27B. Later stage of embryo germination. The haustorium has developed into a spongy spherical structure. $\times 650$

Fig. 27C. Elongated spongy cells with pitted walls. $\times 800$

co - cotyledon, ha - haustorium, e - embryo.

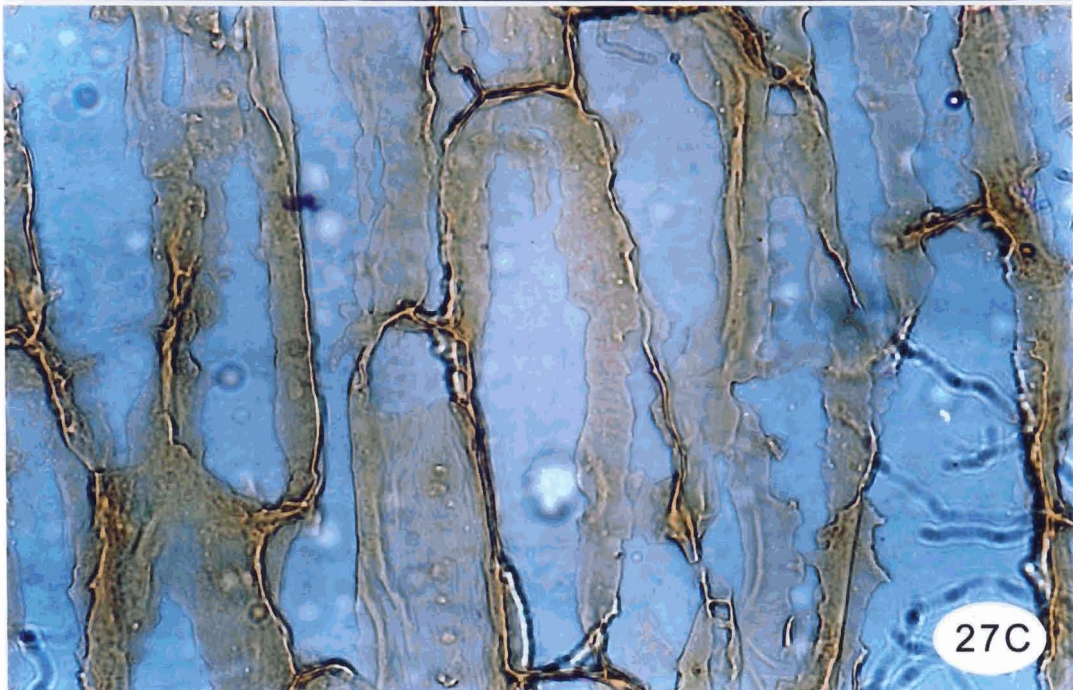
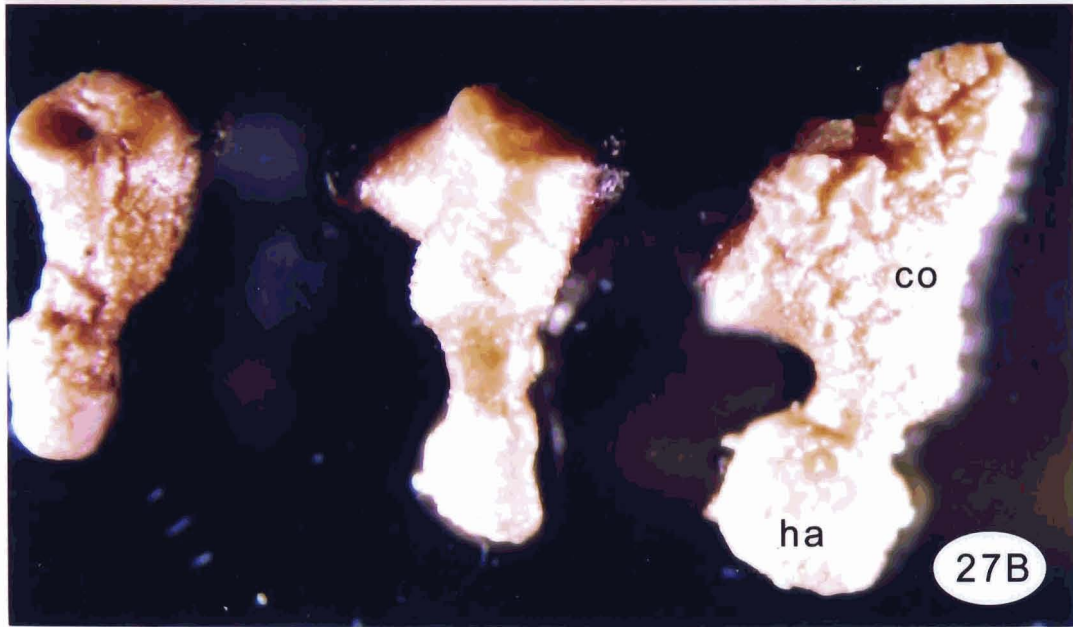
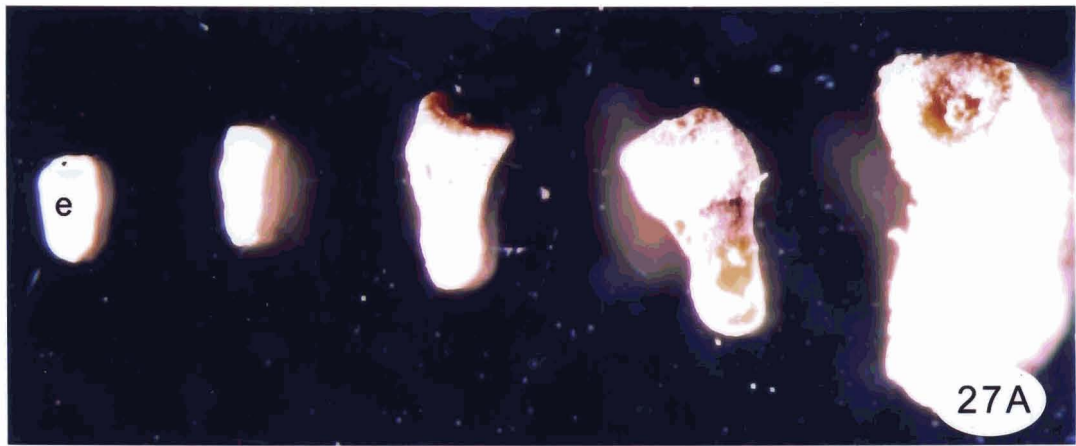


Fig. 28. Haustorial development in *Calamus* seeds

Fig. 28A. The haustorium starts its development before the root and shoot come s out.

Fig. 28B. The haustorium completely digests the endosperm by the time the eophyll develops.

Fig. 28C. The haustorium starts to develop before the root and shoot comes out.

Fig. 28D. The haustorium completely digests the endosperm by the time the eophyll comes out.

ha – haustorium , en – endosperm.



Fig. 29. Ripened fruits

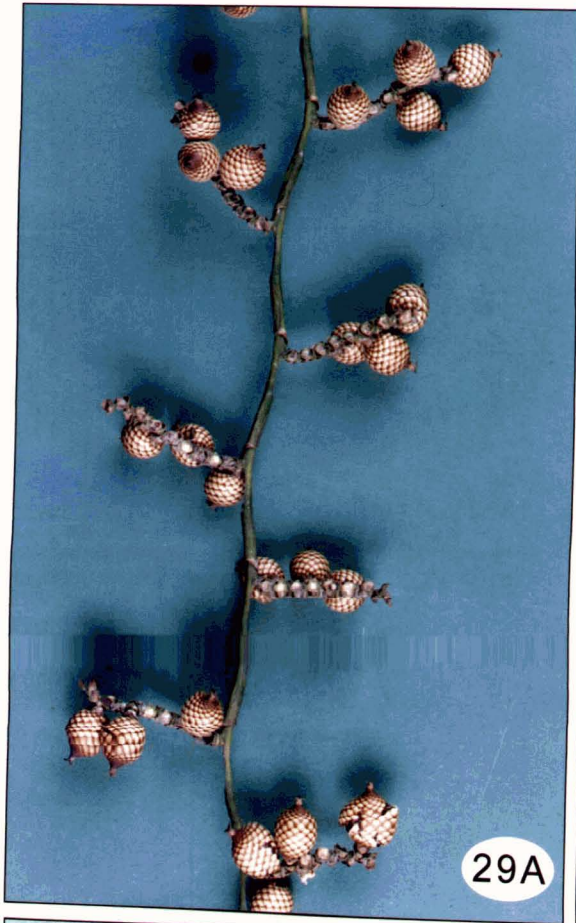
Fig. 29A. *C. hookerianus*. A part of the inflorescence showing ripened fruit.

Fig. 29B. *C. hookerianus*. A ripened fruit with 18 rows of reflexed scales.

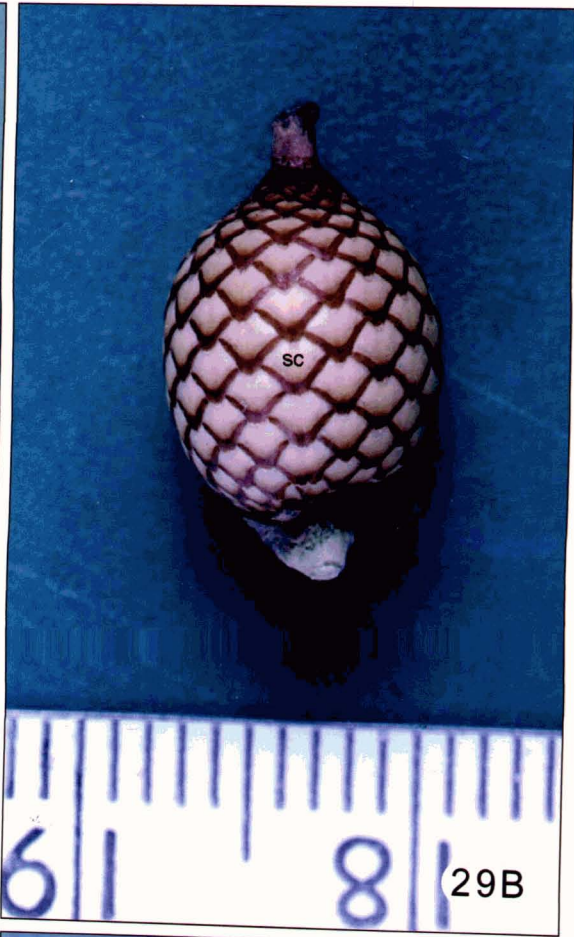
Fig. 29C. *C. thwaitesii*. A rachilla of inflorescence showing ripened fruits.

Fig. 29D. *C. thwaitesii*. A ripened fruit with 12 rows of reflexed scales.

s – scale



29A



29B



29C



29D

Fig. 30. Fruit and seed

Fig. 30A. *C. hookerianus* - various stages in fruit development.

Fig. 30B. *C. thwaitesii* - various stages in fruit development.

Fig. 30C. *C. hookerianus* - dorsal and ventral view of seed. A well develop groove is seen on the entral side.

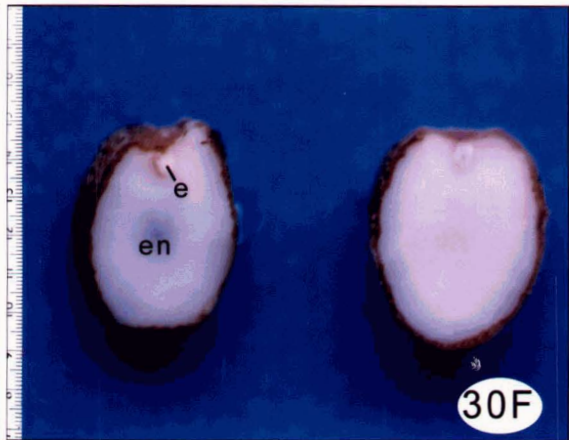
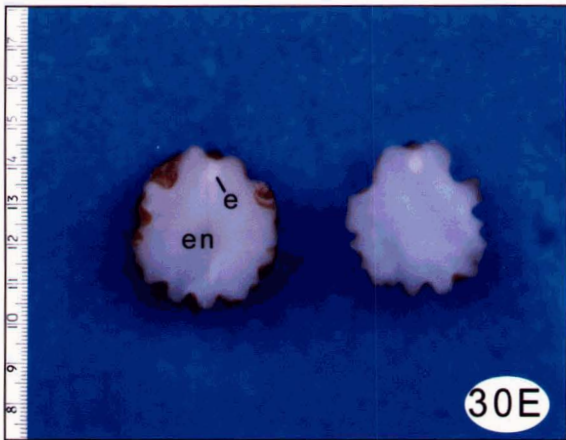
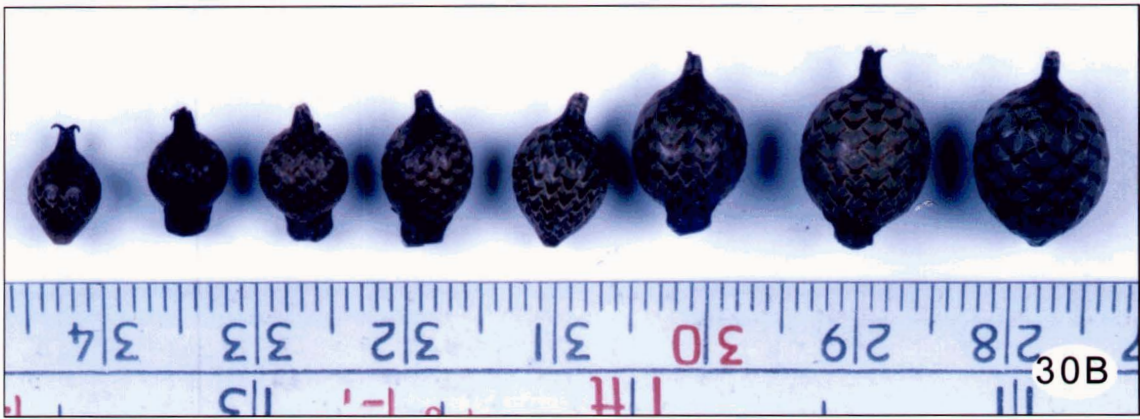
Fig. 30D. *C. thwaitesii* - dorsal and ventral view of seed. The ventral groove is more pronounced.

Fig. 30E. *C. hookerianus* - seed vertically cut to show the basal position of embryo. e – embryo, an – endosperm.

Fig. 30F. *C. thwaitesii*. V.S. of seed to show the basal ovule

e – embryo

en - endosperm



The scanning electronmicrographs of seeds showed a ridged seed coat with a large central groove and the pits on the ridged surfaces were occupied by electron dense structures of tannin deposition (Figs. 31A – 31F).

Seeds of both species were found to be rich in phenolic substances. The content was more in *C. thwaitesii* than in *C. hookerianus*. In both species, the total phenol content of the seeds was found to vary significantly with decrease in moisture level (Table 9.).

Table 9. Total phenol content (mg g⁻¹) of seeds.

Moisture level (%)	Phenol content	
	<i>C. hookerianus</i>	<i>C. thwaitesii</i>
30-40	1.2	3.2
20-30	2.5	4.7
10-20	3.9	6.9
5-10	5.4	9.8

4.10.2. Seed storage

Maturity of seed was found to be an indispensable precondition in seed storage. The fruits stored in the closed plastic bags under room temperature were found to be viable for 3 months for *C. hookerianus* and nearly 2 months for *C. thwaitesii*.

In the storage tests with naked seeds, the seeds stored in the closed bags at room temperature as well as seeds in air tight plastic bags kept in the refrigerator at 5° C, maintained viability for 3 months in both species.

Results of the investigation showed that reduction of the moisture content level of the seeds of both species, adversely affected their viability. In *C. hookerianus* the seeds with 30% moisture content showed 78% germination. The germination percentage declined sharply to 46% when moisture content was reduced to 20%. Further decrease

Fig. 31. Scanning electron micrographs of seed

Fig. 31A. *C. hookerianus*. Ventral side of seed showing groove and ridges

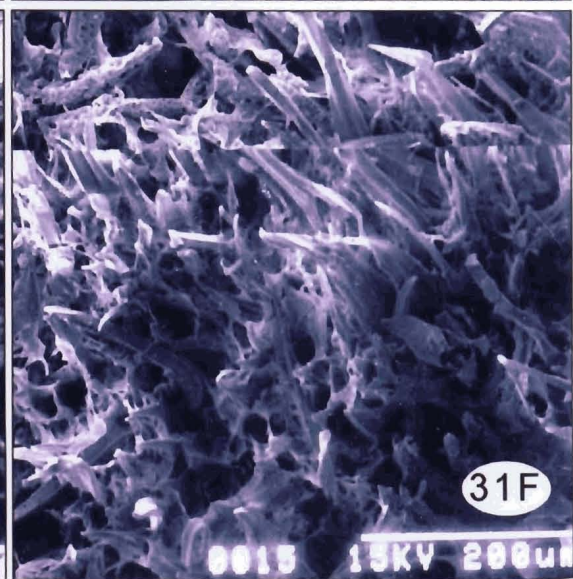
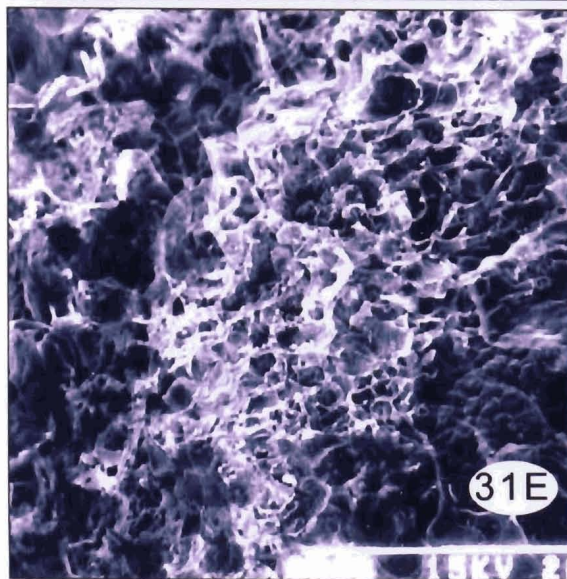
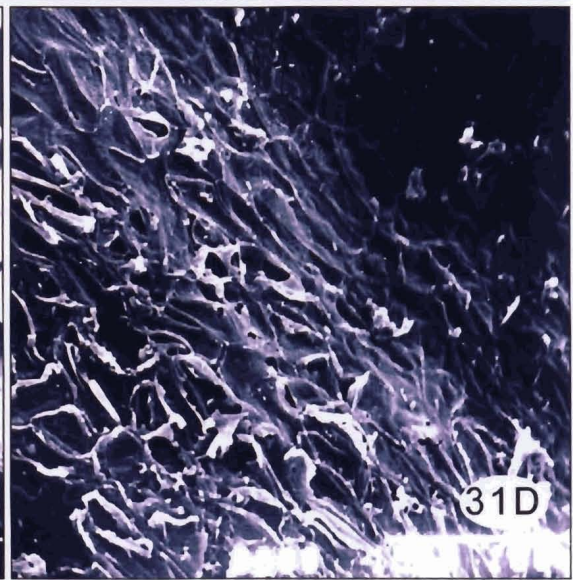
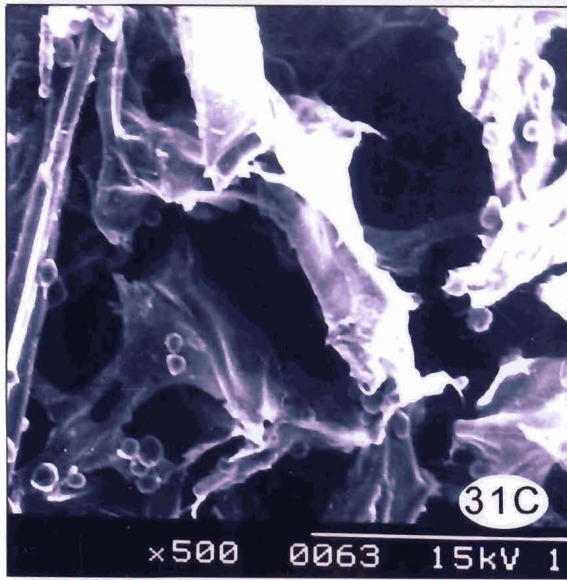
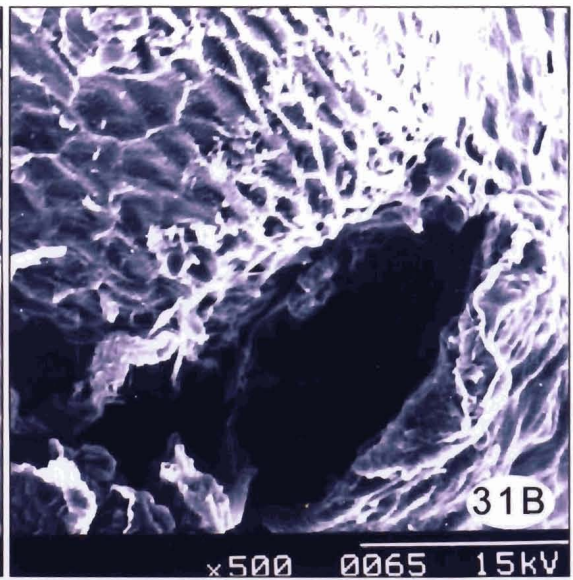
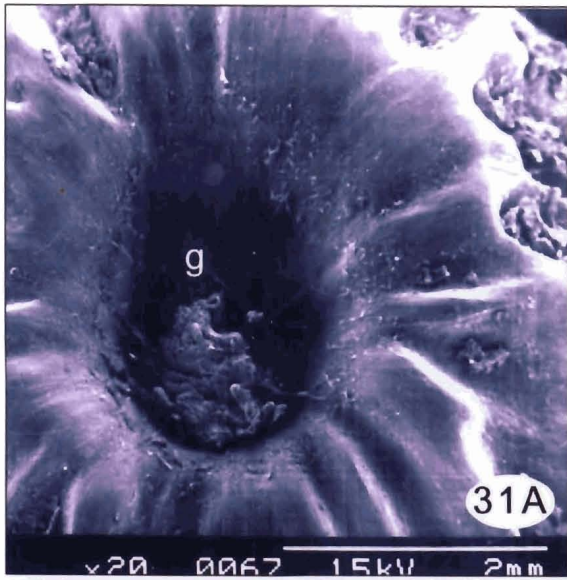
Fig. 31B. *C. hookerianus*. A portion of ventral side

Fig. 31C. *C. hookerianus*. a portion of the dorsal side

Fig. 31D. *C. thwaitesii*. A portion of the dorsal side

Fig. 31E. *C. thwaitesii*. Ventral side towards the centre

Fig. 31F. *C. thwaitesii*. Ventral side towards the margin



of moisture content to 10% resulted in the death of seeds in the seed lot. The seeds took a period of 15 days to reach the critical moisture content.

In *C. thwaitesii* the seeds with an initial moisture content of 40% showed germination of 69%, when moisture content was reduced to 30%, germination was 41%. Further reduction of moisture content level of 20% resulted in 11% germination and later obtained zero germination when moisture content was reduced to 10%. (Table 10.).

Table 10. Viability of seeds at a certain moisture content.

<i>Species</i>	Moisture content (MC) %	Germination %
<i>C. hookerianus</i>	30	78
	20	46
	10	---
<i>C. thwaitesii</i>	40	69
	30	41
	20	11
	10	---

4.10.3. Seed germination

For both species, seeds with the pericarp intact showed a low germination percentage than the group which was sown with the pericarp and sacrotesta removed. In *C. hookerianus*, on the first day after collection, the fruit showed 60 % germination where as in *C. thwaitesii*, it was 65 %. The seeds at the same time showed 75 and 70 % germination in *C. hookerianus* and *C. thwaitesii* respectively. The fruit of *C. hookerianus* lost viability on the 19th day of collection and that of *C. thwaitesii* became nonviable on the 15th day. The naked seeds of *C. hookerianus* and *C. thwaitesii* lost viability on the 12th and 10th day respectively (Tables 11 & 12). A gradual reduction in the weight of the fruit and seed of both species was observed which indicated the loss of water content (Figs. 32 - 35). The tetrazolium test with seeds of

C. hookerianus and *C. thwaitesii* showed a gradual change in the colour of the embryo from deep red to pearl white through an intermediate red colour (Figs.36A – 36F). The correlation between the moisture content and viability indicated that the seeds of both species are recalcitrant.

The mode of seed germination in *C. hookerianus* and *C. thwaitesii* was similar. The germination of seeds was of adjacent ligular type. The first sign of germination was the development of a conical structure formed by a part of the cotyledon which came out through the thin tissue covering the pore (Figs.37A & 37C). This button-like structure carried the radicle and shoot apex from inside the seed. Root emerged first from this tissue through one side of the protrusion after 3-5 days, and later an upright cylindrical outgrowth or ligule was also formed from the tissue. The first eophyll developed after 40 – 50 days (Figs. 37B & 37D).

Table 11. Comparison of fruit and seed weight

Date	Fruit (gm)		Seed (gm)	
	Ch	Ct	Ch	Ct
0	0.3308	3.2104	0.1285	0.9693
2	0.3059	3.0964	0.1185	0.8089
4	0.2695	2.9457	0.1163	0.7445
6	0.2379	2.7860	0.1152	0.7213
8	0.2176	2.6850	0.1135	0.6835
10	0.2131	2.6120	0.1106	Lost viability
12	0.2081	2.5450	Lost viability	
14	0.2053	2.4820		
16	0.2035			
18	0.2032			

Ch - *C. hookerianus* Ct - *C. thwaitesii*

The distal part of the cotyledon remained within the seed in endosperm and was modified as a suctorial organ, the haustorium. Embryos dissected out of the seeds from the germination bed at regular intervals showed that the haustorium was the first organ to develop (Fig.27A & 27B).

Fig. 36. Result of tetrazolium test

Fig. 36A. The embryo retained red colour indicating the viability

Fig. 36B. The embryo turned light red indicating decrease in viability.

Fig. 36C. The embryo remained pearl white indicating the total loss of viability.

Fig. 36D. The embryo remained red in colour indicating the viability.

Fig. 36E. The embryo turned light red indicating decrease in viability.

Fig. 36F. The embryo remained pearl white indicating total loss of viability.

e – embryo

en - endosperm

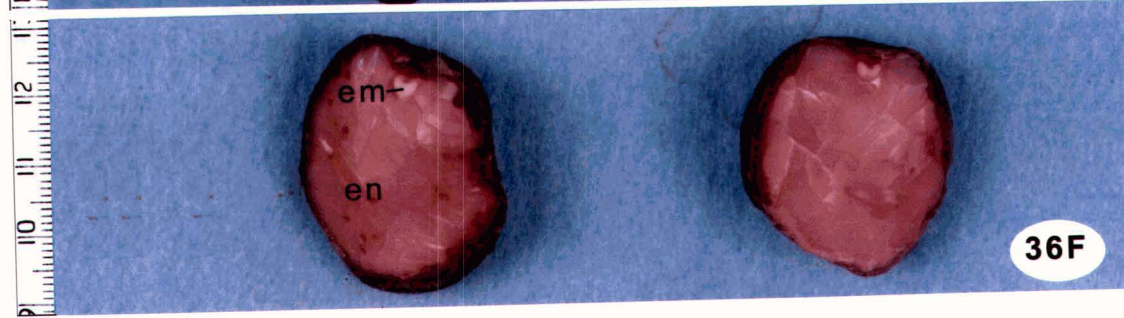
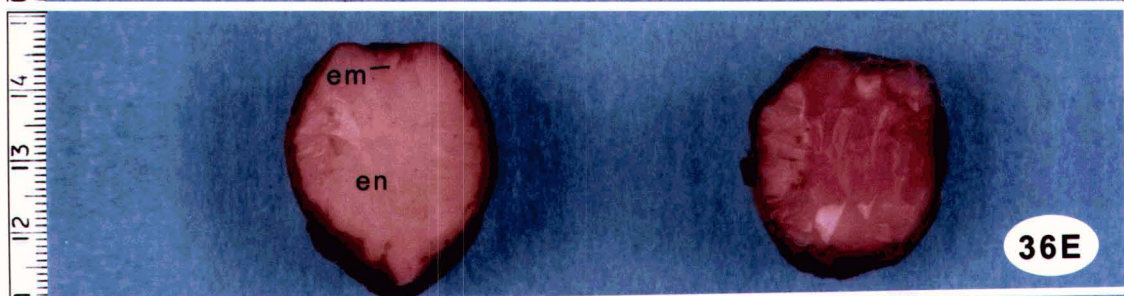
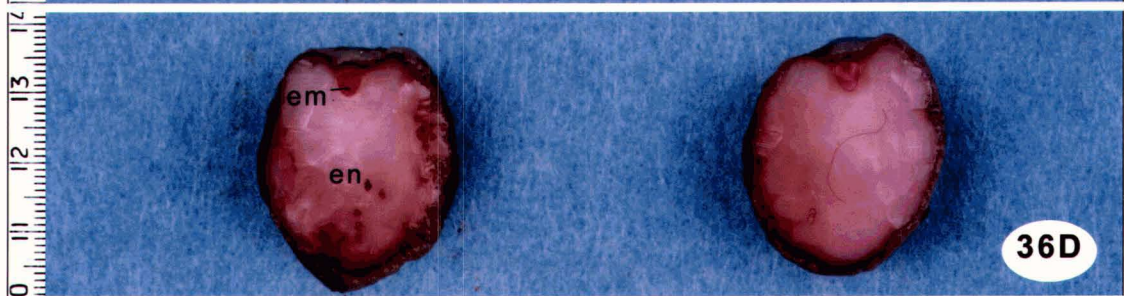
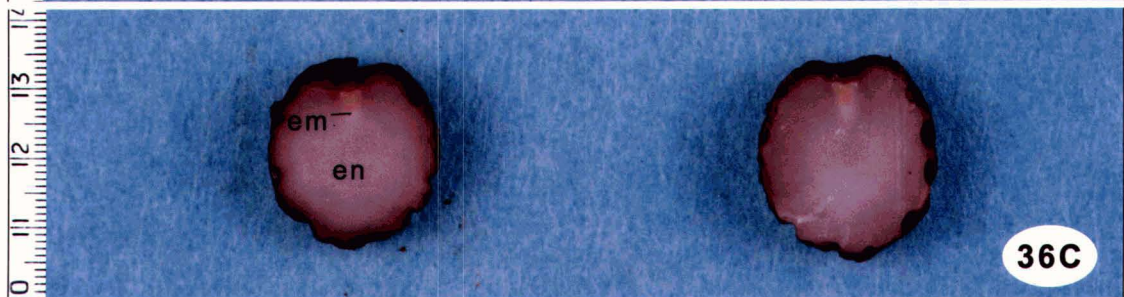
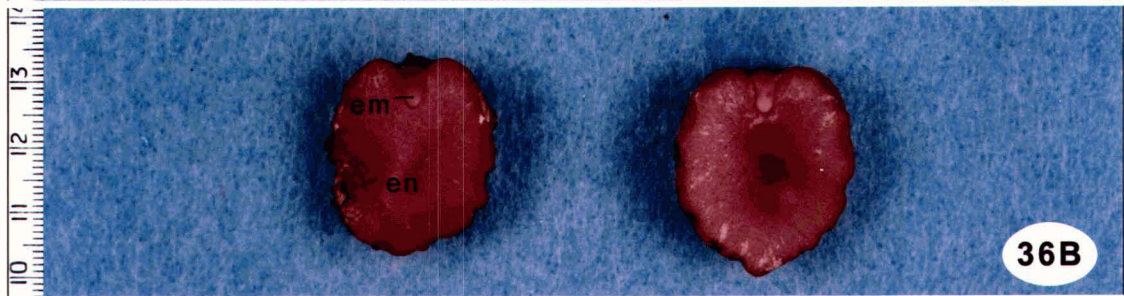
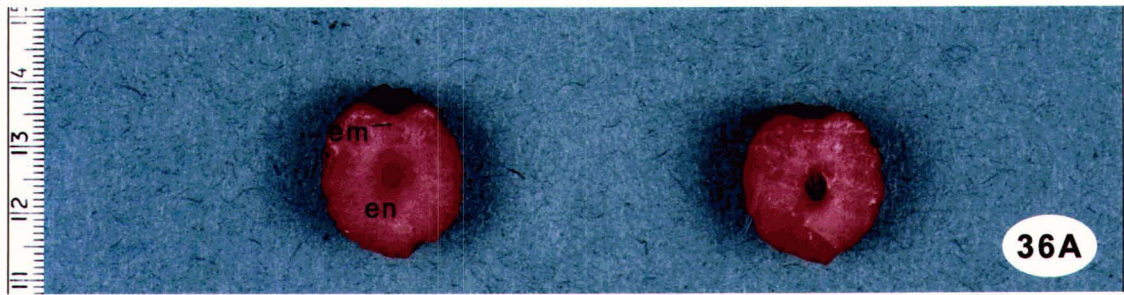


Fig. 37. Seed germination.

Fig. 37A. Different stage in the root and ligule shoot development

Fig. 37B. Different stages in the development of 1st eophyll.

Fig. 37C. Different stage in shoot development of root and shoot apex

Fig. 37E. Development of 1st eophyll.

r - root , cs – cotyledonary sheath.



37A



37B



37C



37D

Table 12. Comparison of germination percentage of fruit and seed

Date	<i>C. hookerianus</i>		<i>C. thwaitesii</i>	
	Fruit Germination (%)	Seed Germination (%)	Fruit Germination (%)	Seed Germination (%)
0	60	75	65	70
2	60	60	60	50
4	50	50	50	40
6	50	40	40	30
8	40	20	40	10
10	40	10	30	
12	30		20	
14	20		10	
16	10			
18	10			

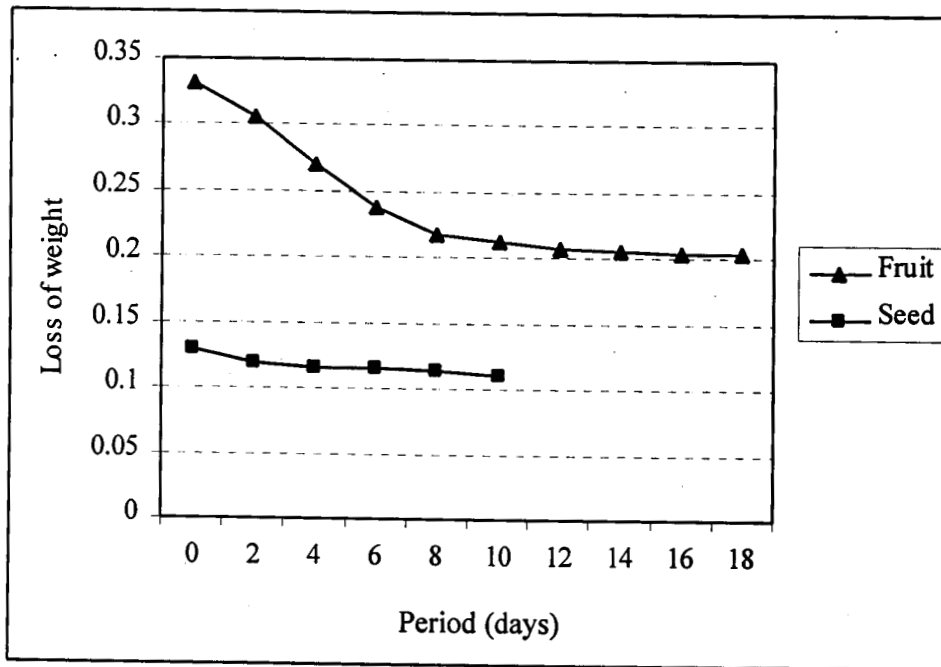


Figure 32. Loss of weight of fruit and seed in *C. hookerianus*

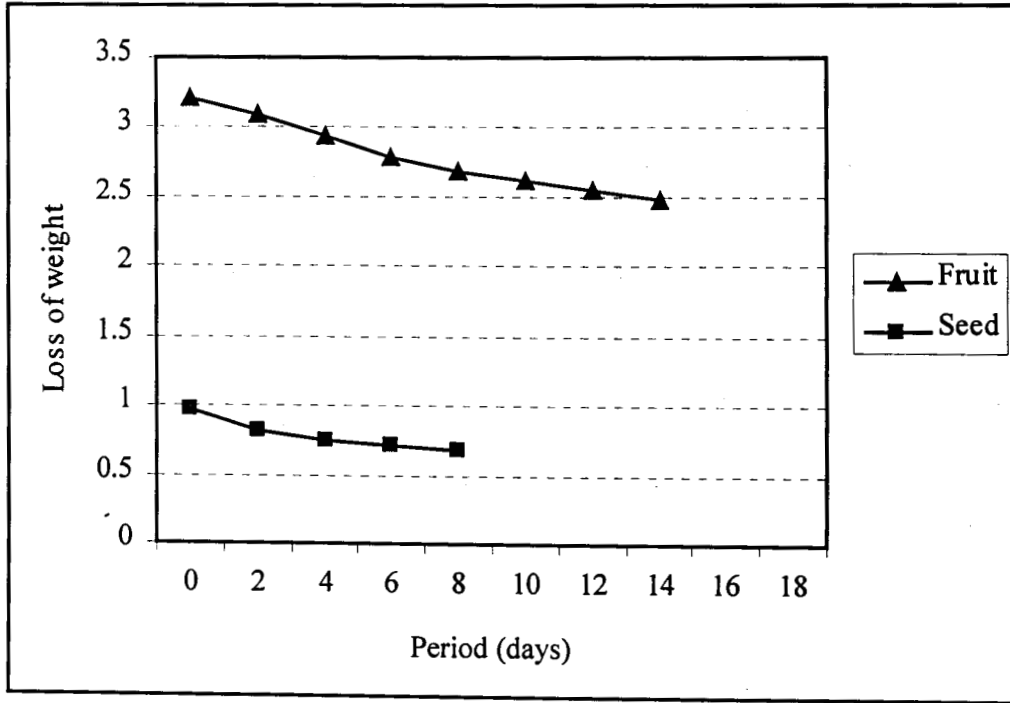


Figure 33. Loss of weight of fruit and seed in *C. thwaitesii*

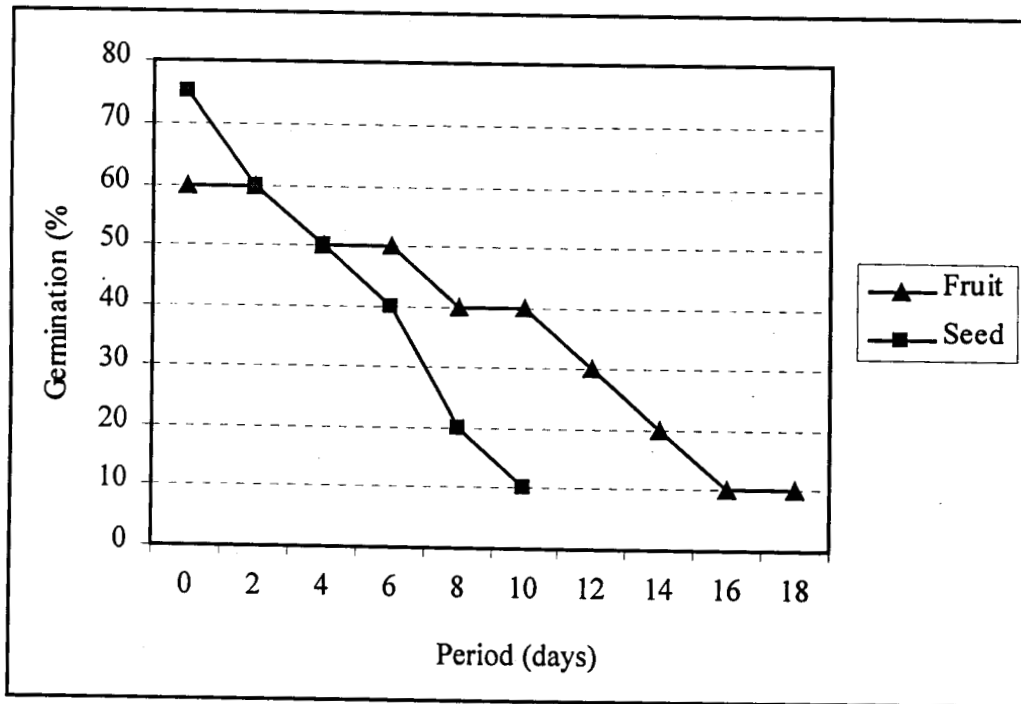


Figure 34. Germination percentage of fruit and seed of *C. hookerianus*

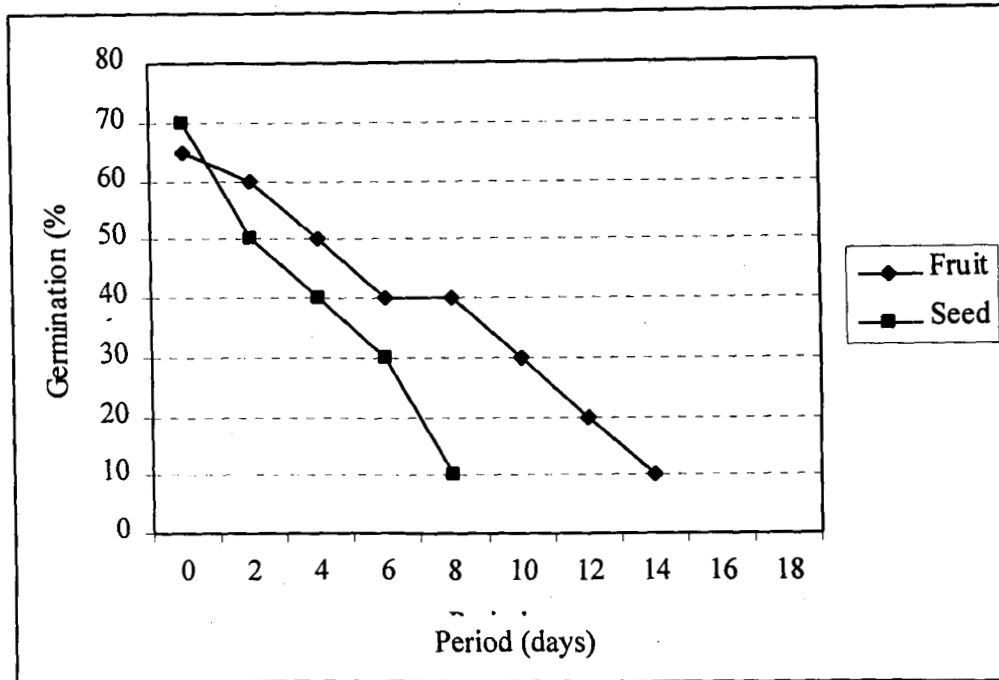


Figure 35. Germination percentage of fruit and seed of *C. thwaitesii*

When moisture reached the embryo it activated the growth of the cotyledon which in turn began to enlarge and digest the endosperm. The onset of enlargement in the haustorium was followed by growth in the lower part of the cotyledonary primordium as a result of which, a portion of the cotyledonary sheath, protruded out as a conical structure pushing out the pore cover as mentioned earlier.

During this period, the other part of the cotyledon enlarged, digested the endosperm and occupied the entire space within the seed, formerly filled by the endosperm. The endosperm was consumed completely by the time the first leaf was expanded.

4.10.3.1. *Effect of seed treatment on germination and seedling vigour*

The effect of different treatments to induce germination revealed that stratification at 10° C was the best. 80 percent germination was obtained in *C. hookerianus* and 76

percent in the case of *C. thwaitesii*. The treatments were also capable of reducing the time taken for germination. The seeds treated with GA (250 ppm) were the first to germinate in both species. Seeds dried in oven failed to germinate (Tables 13 & 14).

Table 13. Effect of seed treatment on germination, shoot length, root length and total seedling length of *C. hookerianus*

Treatment	Germination (%)	Days for germination	Shoot length (cm)	Root length (cm)	Total seedling length (cm)
Control	63	25-48	4.9	4	8.9
GA	59	19-22	6.3	4.8	11.3
Scarification	63	30-43	5.6	4.9	10.5
Stratification at 5 ^o C	68	28-37	5.8	4.2	10
Stratification at 10 ^o C	78	22-49	6.9	5.3	12.2
Oven dried	0	0	0	0	0

Table 14. Effect of seed treatment on the germination, shoot length, root length and total seedling length of *C. thwaitesii*.

Treatment	Germination %	Days for germination	Shoot length (cm)	Root length (cm)	Total seedling length (cm)
Control	50	29-51	5.8	6.4	12.2
GA	56	17-39	6.9	5.2	12.1
Scarification	63	35-48	7.4	6.9	14.3
Stratification at 5 ^o C	59	32-55	7	9.2	16.8
Stratification at 10 ^o C	69	26-47	7.8	9.8	17
Oven dried	0	0	0	0	0

DISCUSSION

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5. DISCUSSION

Reproductive biology, is one of the most exciting areas of plant biology as many of our food crops such as cereals, millets, pulses, vegetables, spices and fruits are plant products derived through the interplay of sexual reproduction. Of all the biological processes that control crop productivity, flowering function and behavior of gametes, embryogenesis and seed development have attracted the attention of biologists. Rattans, one of the important non – timber forest product, have been depleted from the forest areas very rapidly. To raise plantations or for conducting any breeding programme, a knowledge of reproductive biology is essential which in due course will help in the conservation of endangered and threatened rattan plants.

In view of the importance of the biology of reproduction, present attempt has been made to conduct an exhaustive study in this field in two species of rattans, *Calamus hookerianus* and *Calamus thwaitesii*.

5.1. FLOWERING SEASON

Five years observation in flowering pattern in *C. hookerianus* and *C. thwaitesii* has shown that the time of floral initiation varied slightly from year to year. It was observed that there were several months in a year in which the two species produced inflorescences, and the peak period was between May to August which is relatively a wet period in Kerala. It was observed that there was a correlation between the climatic factors and initiation of flowering. The observations made by Alloysius (1997) revealed that *C. caesius* flowered seasonally and each year during the months of June to October. According to Manokaran (1989) the development of the inflorescence of

C. caesius is triggered by external influences such as a change in the weather. According to him, a period of relative dryness before the onset of rains could be the factors which enhance the production of inflorescences in *C. caesius*. The observations made in the present study are also found to be the same as that mentioned above. Phenological observations made on *C. palustris*, *C. scipionum* and *C. ornatus* in Peninsular Malaysia by Abdullah and Jusoh (1995) showed that *C. palustris* flowered in April-May and in August-September, *C. scipionum* in June-July and in December and in *C. ornates* in the East zones, in November-December and in the South zones, in June-July. The studies of Raja-Barizan (1992) agree with that of Manokaran (1989) that flowering of rattans may be triggered by the fluctuations in weather. Burquez and Griffin (1990) stated that in natural forests, flowering vary widely with site, with provenance, from tree to tree within a species and to some extent from year to year. Alloysius (1996) opined that in Sabah (Malaysia) there are several months in a year where certain commercial species of rattans bear ripe fruits.

Henderson *et al.* (1989) observed very obvious patterns in the time of flowering of palms in Central Amazon forests. There seemed to be a definite bias towards rainy season for flowering. On the other hand, some species which occur predominantly in the wet sites, near streams, appeared to flower outside the rainy season.

5.2. DEVELOPMENT OF INFLORESCENCE

The development of inflorescence in both species was found to follow the same pattern. In the flowering of both species, four stages of development could be noticed such as the emergence of inflorescence, the pre-anthesis stage, when the inflorescence was fully developed, the anthesis stage and lastly the post anthesis stage where the fertilized

ovary started to develop into fruits. Alloysius (1996) has also observed the same pattern of inflorescence development in *C. subinermis* and *C. caesius*.

5.2.1. Male inflorescence

In *C. hookerianus* and *C. thwaitesii*, the average length of the male inflorescence was 579.2 cm and 684.9 cm respectively with an average number of 4-5 partial inflorescences. On the otherhand the qualitative measurements made by Alloysius (1997) showed that *C. caesius* and *C. subinermis* developed 2-3 inflorescences per plant with an average length of 130 cm for *C. caesius* and 138.6 cm for *C. subinermis* inflorescence.

In *C. hookerianus*, the male flowers were borne on branches of the third order of the inflorescence and the flowers showed two types of floral arrangement on the rachillae. In the majority of the plants observed, the flowers showed a decussate arrangement whereas in others, they were arranged in cymose clusters. In all the plants of *C. thwaitesii*, the flowers were borne on the second order and showed only decussate arrangement. Alloysius (1996) reported that in most of the species of *Calamus* and *Daemonorops*, the male flowers were borne on branches of the third order.

The male flowers in the two species, as in a typical monocot, were symmetrical with three sepals and petals englobing six widely exposed stamen. Similar floral structure was also observed in other species of *Calamus* by Uhl and Dransfield (1987) and Bogh (1996). Recently Chia (2000) has reported the occurrence of andromonoecy in *C. subinermis*. He opined that andromonoecy was observed in all provenances in Sabah

(Malaysia), and since these were all mature plants producing canes, the occurrence of hermaphrodite flowers was not of a juvenile character.

Occurrence of hermaphrodite flowers was reported in the male inflorescence of *Pseudophoenix* (Read, 1968), *Salacca edulis* (Mogea, 1978) and in *Phoenix dactylifera* (Demason and Tisserat, 1980). Contrary to the occurrence of six stamens in Arecaceae male flowers of *Asaroa triandra* (Joseph, 1972) consisted of three stamens only and this character distinguished this palm from other genera of Arecaceae.

5.2.2. Female inflorescence

In the female inflorescences of both species studied, the flowers were borne on second order of branching. Alloysius (1996) has observed the same pattern of flower arrangement in *C. subinermis*.

The total length of the inflorescence in *C. hookerianus* ranged from 336.3 cm to 656.3 cm and 412.8 cm 693.0 cm in *C. thwaitesii*. Alloysius (1996) has made quantitative measurement of female inflorescence of *C. subinermis* and found that the total length of the inflorescences ranged from 1.1m to 1.5m with a mean of 1.4 m.

The flowers in both species of female inflorescences studied were borne in pairs (dyads) with fertile female flower and a sterile male flower (acolyte flower). The pistillate flowers were also typical of monocots with 3 sepals and petals, 6 staminodes and a tricarpellary gynoecium. Similar structure was reported in different species of *Calamus* by Uhl and Dransfield (1987); Alloysius *et al.* (1994).

In monoecious palms of *Ptychosperma* (Uhl, 1976); *Geonoma macrostachys* (Olesen and Balslev, 1990) and *Prestoea schultzeana* (Ervik and Feil, 1997) flowers are grouped in triads consisting of two lateral staminate and one pistillate flower. Species of *Aiphanes*, which are monoecious, protrandorus and spiny understorey palms, displayed a different pattern of floral arrangement (Borchsenius, 1993). In all the species studied, the proximal part of the rachilla bore triads of one pistillate and two staminate flowers and distally on the rachillae the triads were substituted by dyads.

5.2.3. Anthesis

In both species studied, the anthesis of female flowers commenced from the basal flowers of the rachillae, but no sequence of opening was evident as anthesis progressed from proximal part to the distal end of the inflorescence. Anthesis in *C. hookerianus* lasted for 12 days where as in *C. thwaitesii* it was found to be more than 15 days. It is reported that in *C. caesius*, anthesis lasted for 10-15 days (Alloysius, 1996; Lee and Jong, 1995).

It was observed that anthesis of the individual flowers began around 11 PM in *C. hookerianus* and 1 AM in *C. thwaitesii* and most of the flowers opened by 4 AM, but a few additional flowers opened until 6.30 AM. The whole process of splitting of the male flower bud to the formation of a fully opened flower took about 15-20 minutes. When the male flowers of both species opened, it exuded a drop of nectar. Before opening, the inflorescence emitted a sweet, scent. The longevity of the male flowers of both species was found to be 12-18 hours.

The work of Alloysius (1996) revealed that in *C. subinermis* the process of bud opening of a male flower took about 20 minutes to complete and most of the flowers opened between 6-8 PM. Following anthesis and when most of the pollen had been shed, the flowers shrivelled and fell off between midnight and 6 AM (Lee and Jong, 1995). Observations made by Bogh (1996) on four *Calamus* species in Thailand have revealed that anthesis of male flowers occurred between 10 PM to 6.30 AM. In a similar way to that of *C. hookerianus* and *C. thwaitesii*, few flowers stayed open until 10 AM. He noticed that by noon most of the anthers were empty and the male flowers dropped off by sunset around 6.30 PM. All the above mentioned researchers observed that during the opening of staminate flowers, nectar was produced and a strong fragrance emitted.

It was found in both species that the behaviour of the sterile male flowers on the female inflorescence during anthesis was the same as the fertile males, except that no pollen was produced by the staminodes which bore empty anthers. Similar observations are recorded by Dransfield (1984) and Lee and Jong (1995) in *C. subinermis* and *C. caesius*.

Alloysius (1996) observed the emergence of hyaline receptive trifid stigma during anthesis of female flower which occurred during night hours. The loss of receptivity was indicated by the colour change of the whitish stigmas to brown and later to reddish brown. The same behaviour of female flowers was noticed in the present study also.

5.3. MICROSPOROGENESIS

In both the species studied, the mature anther was tetrasporangiate and the archesporium consisted of 3-4 hypodermal cells. Variations in the number of

hypodermal cells have been reported by various authors in different genera. Juliano and Quisumbing (1931) reported the differentiation of same number of archesporial cells in coconut. Similar observations are made in many palms by Rao (1956, 1959a and 1959b). Mature anther wall consisted of six layers. In addition to other reports, Gonzalez *et al.* (1997) observed the presence of 6 layers in *Chamaedorea elegans*. In the present investigation the middle layers were found to persist till the pollen grains in the anther locule matured and the layers degenerated at a later stage of development. The degeneration of middle layers was reported in all palms studied.

The tapetal cells remained uninucleate in the two species investigated. Similar observations are made in *Cocos nucifera* (Juliano and Quisumbing, 1931), *Borassus flabellifer* (Rao, 1959b) and *Hyphaene indica* (Mahabale and Chennaveeraiah, 1957), while in *Areca* (Rao, 1955a), *Pritchardia* (Rao, 1955b), *Licuala*, *Livistona*, *Sabal* (Rao, 1956) and *Arecastrum romanzoffianum* (Rao, 1959b) are binucleate.

Cytokinesis in the microspore mother cells were successive in both species. Reports show that successive cytokinesis is apparently restricted to *Nypa fruticans* and *Pinanga disticha* (Davis, 1966). With regard to microsporogenesis, simultaneous division of the microspore mother cell occur in the majority of genera and species studied viz., *Chamaedorea*, *Areca triandra*, *Caryota*, *Pteriogospermum* (Schnarf, 1931 and Mahabale and Chennaveeriah, 1957); *Cocos nucifera* (Juliano and Quisumbing, 1931); *Borassus* and *Areca* (Rao, 1955a); *Licuala* and *Livistona*, *Trachycarpus* and *Sabal* (Rao, 1956b); *Hyphaene indica* Mahabale and Chennaveeraiah, 1957); *Caryota*, *Chrysalidocarpus* and *Arecastrum romanzoffianum* (Rao, 1959b).

Sowunmi (1968) found a great variety of exine pattern - punctate, reticulate, negatively reticulate, vermiculate, verrucose, pilate, clavate, spinose and clavate-punctate, the most important and predominant pattern being the reticulate type. The two species studied in the present work revealed that the exine is microreticulate. Thanikaimoni (1970) reported 27 types of pollen grains in Palmae. According to him, in palms, exine is practically absent. His studies revealed that palynologically palms are more affiliated with the Liliales.

The crescent – shaped generative cell with tapering ends observed in the pollen grains at the time of anthesis in the two species of *Calamus* was reported in other members of Palmae and Asclepiadaceae (Rao and Rao, 1954).

5.3.1. Pollen structure

Pollen morphology was similar in both the species studied. Pollen grains were prolate, dicolpate, spheroidal or spherical and equatorial or polar outline elliptical. The grains of *C. hookerianus* measured 39.6 x 42.9 μ m and that of *C. thwaitesii*, measured 44.5 x 43.5 μ m. Sculpturing was microreticulate in both species. The pollen grains of *C. subinermis* and *C. caesius* were round or elliptic and measured 15-25 microns (Lee and Jong, 1995). Bogh (1996) reported the diameter of the pollen grains of *C. manan* as 70-100 microns. He has also studied the pollen grains of *C. peregrinus*, *C. rudentum* and *C. longisetus* and the diameter of the grains was found to be 32-33 μ m; 26—29 μ m and 27 μ m respectively. Sowunmi (1968) opined that there is a wide range in the size of pollen in the Palmae, with the equatorial diameter ranging from 20 μ to 75 μ . In the species studied the pollen grains are comparatively larger than *C. peregrinus*, *C. rudentum*, *C. longisetus*, *C. subinermis* and *C. caesius* but smaller than *C. manan*.

Transmission electron microscopic studies of the pollen wall of both species of *Calamus* revealed that the pollen wall is semitectate and columellate with a well developed foot layer and no endexine was evident. Thanikaimoni (1970) has stated that the pollen of *Calamus* is dicolpate and exine verrucate (semitectate). Dicolpate nature of pollen of *Calamus* has also been reported by Zavada (1983). Other than these two references, there is no report on the ultrastructure of *Calamus* pollen grains. The findings of Zavada (1983) has revealed that the aperture types in the pollen grains of *Korthalsia* and *Plectocomia* are zonosulcate and that of *Calamus* and *Daemonorops* are dicolpate and exine verrucate (semitectate). The studies made by Sowunmi (1968) has revealed that the most prominent aperture type in the Palmae is monocolpate and dicolpate pollen grains occur in several genera in the Lepidocaryoideae sub- family. For both the species studied the pollen grains were dicolpate.

5.4. OVARY AND OVULE

The ovary in the *Calamus* species studied was tricarpellate, trilocular and superior with a single anatropous ovule in each loculus on an axile placenta. Many investigators found anatropous ovules in *Cocos* (Juliano and Quisumbing, 1931), *Caryota* species, *Phoenix* species, *Pritchardia* and *Trachycarpus* (Rao, 1959b). Hemianatropous ovules are reported in *Sabal* species (Raghavan and Baruah, 1956; Murthy and Bavappa, 1960; Bavappa, 1966). Orthotropous ovules are reported in *Hyphaene indica* (Rao, 1957). In all the palms investigated, the ovules were found to be crassinucellate and bitegmic in *Licuala peltata*, *Pritchardia pacifica*, *Trachycarpus* species and *Washingtonia* species. These findings are in accordance to the present investigation. A well developed endothelium, noticed in both the species studied was also reported in

Cocos plumosa (Rao, 1959b) and in *Livistona chinensis* (Kulkarni and Mahabale, 1974).

5.4.1. Megasporogenesis and female gametophyte

The development of the female gametophyte confirmed to the monosporic, eight nucleated, Polygonum type in the *Calamus* species studied.

Three types of megasporogenesis have been described for the Palmae. The Allium or bisporic type (Maheshwari, 1950) occurs in *Chamaedorea latifolia* (Johnsson, 1979 cited by Davis, 1966), *Nipa fruticans* (Radernacher, 1925 cited by Maheshwari, 1950) and *Hyphaena indica* Mahabale and Chennaveeraiah, 1957). The tetrasporic 8-nucleate or Adoxa type (Maheshwari, 1950) is reported by Quisumbing and Juliano (1927) for *Cocos nucifera*. A similar mode of embryo sac development was also described for *Elaeis guineensis* (DePoerck, 1950) as cited by Reddy and Kulkarni (1989). A reinvestigation made by Reddy and Kulkarni (1989) on *Elaeis guineensis* has revealed all type of megaspore tetrads and the polygonum type of embryo sac. The majority of palms appeared to follow third development pattern, viz. the monosporic, 8-nucleate or polygonum type of embryo sac development (Swamy, 1942; Rao, 1955, b; 1956a, b; and Reddi and Kulkarni, 1989).

It is reported to be so in *Actinophloeus macarthurii* (Radermacher, 1925); in *Areca catechu* (Swamy, 1942); in *Areca catechu*, *A. concinna*, *A. triandra*, *Actinophloeus* spp., *Pritchardia*, *Licuala*, *Livistona*, *Caryota urens*, *C. mitis*, *Trachycarpus*, *Washingtonia*, *Cocos plumosa*, *C. schizophylla*, *Bactris major* and *Sabal* (Rao, 1959a,

1959b). Reports are also there on the occurrence of same type of embryo sac in *Phoenix sylvestris*, *P. robusta*, *P. pusilla* (Mahabale and Biradar, 1968 and Biradar, 1968) *P. acualis* and in *Livistona chinensis* (Kulkarni and Mahabale, 1974) and in *Jubaeopsis caffra* (Robertson, 1976b). The occurrence of Allium type of embryo sac is reported in *Chamaedorea* and *Borassus flabellifer* (Johnsson 1979). Same type is also reported in *Hyphaene indica* (Mahabale and Chennaveeraiah, 1957) and in *Nypa fruticans* (Radermacher, 1925). Quisumbing and Juliano (1927) reported the presence of Adoxa type in *Cocos nucifera*, which however on investigation was confirmed to be of the Polygonum type by Kulkarni (1965) thus confirming the earlier observation of degenerating megaspores in this species by Bauch (1911). Likewise, the report on the presence of Adoxa type of embryo sac in *Elaeis guineensis* by De Poerck (1950), on reinvestigation by Kajale and Ranade (1953) confirmed to be of Polygonum type.

The antipodal cells in the species presently investigated showed signs of degeneration even before fertilization. A similar behaviour of antipodal cells in *Chamaedorea concolor* was reported by Sussenguth (1921). In *Pinanga moluccana* (Lotscher, 1905) and *Calyptrocalys* species (Bauch, 1911) persistent antipodals became 2-3 nucleate, a condition also reported by Rao (1956b) for *Chrysalidocarpus*.

5.5. POLLINATION

In both the species studied, anthesis of staminate flowers resulted the release of large quantity of powdery, and non-sticky, yellowish-white pollen grains. The morphology of the flower presented several features of the wind pollinated plants, viz: lack of showy parts, bright colour, sticky and rough surfaced pollen. The production of large quantity

of powdery pollen, a distinct exposure of anthers and stigma to the air designed to scatter the pollen to the wind, the three tongue-like slightly curved stigma well exposed at the time of fertilization, complete separation of sex into staminate and pistillate flowers suggest that the two species are wind pollinated. Eventhough, the inflorescences are visited by *Drosophila* species, honey bees and ants, it seems that they are attracted by the sweet scent and a drop of nectar produced by the male flowers.

At the same time, electron microscopic studies of exine sculpturing and ornamentation of pollen grains can be treated as physical attractants of pollen grains towards biotic pollination. The SEM micrographs support the affinity of pollen towards biotic means of pollination. Thus both species of *Calamus* showed a mixture of primitive and advanced characters. The primitive characters shown are actinomorphic, hypogynous flowers with undifferentiated perianth, successive cytokinesis in pollen mother cells after meiosis, 2- celled pollen grain, anatropous, crassinucellate ovule, polygonum type of embryo sac, nuclear type of endosperm - sometimes ruminant and seed with thick coat. The advanced characters exhibited are unisexual flowers produced in inflorescence, dicolpate pollen grains, axile placentation, fruit a drupe and embryo with single cotyledon.

The present study showed that eventhough the *Calamus* species studied has adaptations to both animophily and entomophily, they prefer anemophily which was proved based on field experiment. In addition, the insect visitors were without any pollen load.

Lee and Jong (1995) after studying pollination in *C. subinermis* and *C. caesius* came to the same conclusion that wind may contribute to some short distance pollination.

Renuka *et al.* (1998) also came to the conclusion that some of the South Indian species

of the *Calamus* are wind pollinated. *Phoenix* and *Chamaerops*, were also considered anemophilous. Wind pollination has been stressed by Eames (1961). Myers (1984) observed that in the staminate flowers of the monoecious *Raphia taedigera*, the flowers were unscented and pollination was considered either by wind or insects. Kunth (1904) reported that the oil palm, like other species of *Cocoideae*, was generally believed to be wind pollinated and the flower showed a number of characteristics typical of wind pollinated species. Only a doubtful role has been given to insects as pollinating agents in *Areca catechu* by Sands (1926) and Raghavan and Baruah (1956a). Jensen (1973) in his classification of plants according to the mode of fertilization of the flower included the palms under the class *Anemophillae*. Kunth (1904) added that this appeared probable in coconut, considering the fact that the morphology of the flower presented several features of the wind pollinated plants such as lack of showy parts, exposed and versatile anthers, trifold stigma and powdery pollen produced in large quantity. The present study shows that even though the *Calamus* species studied has adaptations to both anemophily and entomophily, they prefer anemophily, which was proved based on field experiments. In addition, the insect visitors were without any pollen load.

Corner (1966) opined that hapaxanthic rattans such as *Korthalsia*, *Plectocomia*, and *Plectocomiopsis* are mellittophilous. Similar observations were made by Dransfield (1979) and he reported trigonids and honey bees visiting male flowers of *Plectocomia* species. Madulid (1980) has also reported insect visitation in *Plectocomia* which has extremely fragrant flowers. The presence of musty odor and the attraction of beetles during anthesis of the flowers of *Daemonorops*, *Ceratolobus*, *Calospatha* and *Calamus* made Dransfield (1979) to believe that these species are pollinated by beetles. In other

members of Calamoideae, Dransfield (1979) reported the emittance of a sour odour and the frequent visit of wasps and flies during anthesis.

Uhl and Dransfield (1987) also suggested bees to be the most likely pollinators of many species of *Calamus*. Bogh (1996) perceived that the exposed stigmas and anthers are easily accesible to any insect visitor and to the wind. However, after studying the pollination biology of four species of *Calamus*, *C. longisetus*, *C. peregrinus*, *C. rudentum* and *Calamus* species, he strongly suggested that the most important pollination agents are *Trigona* bees. He sustained his obşervation by adding that the sterile staminate flowers of pistillate inflorescences probably attract pollinators by mimicking functional staminate flowers as well as by providing nectar. Contrary to this, in the present study, it was observed in both species that the opening of sterile male flowers is not in accordance with the opening of female flowers and it may sometimes be prolonged to even after the loss of receptivity of the stigma of female flower, indicating that the sterile male flowers do not play any significant role in pollination.

Alloysius (1999) is of opinion that wind pollination seems not important in rattans. Entomophilous modes of pollination is reported in *Nypa*, *Ptychosperma*, *Bactris* and *Asterogyne* by Uhl and Moore (1977). Essig (1971) reported beetle pollination in *B. major* and followed by him Beach (1984) came to the same conclusion saying *B. gasipaeas* and *B. perschiana* show several features found in beetle pollinated species coupled with exceptional level of insect activity, even though nectar is not produced by this palm. In the review of palm pollination, Henderson (1986) concluded that the palm family possesses a great diversity of pollination modes. Olesan and Balslev (1990) thought the trigonids and Drosophilids may probably be the most important pollinators

in *Geonoma macrostachys*. Insect pollination is also reported in *Desmoncus polycanthos* and *D. mitis* by Listabarth (1994). Studies in *Prestoca decurrens* (Ervik and Bernal, 1996) showed that bees play the principal role in pollination. Listabarth (2001) observed beetles playing a major role in the pollination of *Hyospathe elegans* and he arrived at a conclusion that many palms lack major anthecological adaptations that apparently promote visitors of a particular taxonomic group of insects.

5.6. ENDOSPERM

Endosperm was *ab-initio* free nuclear and later became cellular in both species investigated. Most of the workers have reported the endosperm as free nuclear in the beginning and later becoming cellular in the majority of palms. In a young fruit of *Calamus*, the seed cavity was filled with a mucilaginous fluid in which numerous amoeboid nuclei were suspended. By gradual cellularisation and successive cell divisions this tissue developed into a hard endosperm filling the entire cavity. Datta (1955) reported that in *Cocos nucifera*, in the early stages of fruit development, the endosperm was in the form of a liquid syncytium with free nuclei. Later on, wall formation commenced from the periphery and the gelatinous endosperm gradually became hard which enclosed a large central cavity, (Cutter *et al.*, 1955). The same mode of endosperm development was reported in *Actinophloeus* and *Areca* species (Rao, 1959a). Wall formation commenced from micropylar end to the chalazal region and Rao (1959a, 1959b) reported it to be so in species of *Caryota*, *Chrysalidocarpus*, *Howea*, *Licuala*, *Livistona*, *Trachycarpus*, *Washingtonia* and *Sabal*. Mahabale and Biradar (1968) and Biradar (1968) observed the same pattern of development in all

species of *Phoenix* they studied, Kulakarni, (1965) in species of *Cocos*, Kulkarni and Mahabale, (1974) in *Livistona chinensis* and Robertson, (1976b) in *Jubaeopsis caffra*.

The cells of the mature endosperm of *Calamus* species showed pitted walls and similar observations were made in the endosperm cells of *Trachycarpus*; *Sabal* sp., *Washingtonia* sp. and *Livistona* sp. (Mahabale and Biradar, 1968)

The endosperm of *C. hookerianus* showed rumination, which was formed by inward growth of the inner layers of seed coat. Similar observation is made in many palm species. Voiget (1888) reported rumination in *Actinophloeus ambiguous* and *Caryota urens*. Same observations were made in species of *Caryota*, *Howea*, and *Areca* (Rao, 1959b). Rumination in species of *Phoenix* is confined to the placental region (Biradar, 1968). Padmanabhan and Reghupathy (1981) after studying the nature of rumination in *Bentinckia condapanna*, opined that it is the fruit wall which causes the rumination in the seeds of *Arikuryroba schizophylla* and *Rhyticocos amara*.

5.7. EMBRYOGENESIS

Some of the characteristic embryogenetic features observed in the species studied in the present investigation differ in part from the results of investigations on palm species published so far. The conclusion of Selvaratnam (1952) that the *Cocos* embryo is comparable to that of the family Gramineae and that it may be considered as having been derived from the latter does not seem to be based on any supporting evidence. Findings in this investigation on the early embryogeny, mature embryo and the germinating embryo are contrary to his ideas.

In both the species of *Calamus*, the zygote underwent a transverse division to form an apical cell, 'ca' and a basal cell, 'cb'. One of the derivatives of the apical cell, referred as an epiphysis like cell, by further divisions developed into the terminal stem tip, while those of the sub-epiphysal cells contributed to the formation of the single cotyledon and the rest of the embryo proper. The derivatives of 'cb' contributed to the formation of the suspensor, which became detached from the embryo proper in later stages of development. Table 15 summarises the salient features of the embryogenesis in palms as reported in the literature.

The contribution of 'ca' and 'cb' to the formation of embryo proper in various species of Palmae has been interpreted differently by many authors. The terminal origin of stem tip seems to be widely characteristic of palm embryos. (Rao, 1955a, 1955b, 1959a and 1959b; Mahabale, 1982; Biradar and Mahabale, 1969; Javalgekar, 1979; Haccius and Philip, 1979). Guignard's (1961) and Robertson's (1976c) interpretations of its lateral origin in the embryos of *Chamaerops humilis* and *Jubaeopsis caffra* may be due to difficulty in getting sections through the proper planes because of the smaller size of the embryo compared to similar developmental stages in other members of the Palmae.

The findings that certain embryos in Palmae have two cotyledon primordia (Shrike, 1963; Kulkarni, 1965; Mahabale, 1982; Surange, 1966; Biradar, 1968; Mahabale and Biradar, 1968; Biradar and Mahabale 1969; and Kulkarni and Mahabale, 1974) may be due to an erroneous interpretation of sections cut in an incorrect plane, as observed by Haccius and Philip (1979).

Table 15. The salient features of embryogenesis in palms as reported in literature.

Taxa investigated	Contribution of 'ca' and 'cb' to embryo (Em) and suspensor (S)	Origin of stem tip	Number of cotyledons	References
<i>Areca</i> <i>Pritchardia</i> <i>Actinophloeus</i> <i>Howea</i>	ca-Em cb-S Conagrad type	Terminal from an epiphysis initial	1	Rao, 1955a, 1956, 1959a, b
<i>Phoenix</i> <i>Livistona</i>	Whole of ca Upper part of cb } — Em Lower part of cb-S	Terminal from an epiphysis initial	2	Biradar, 1968 Biradar <i>et al.</i> , 1968 Mahabale and Biradar, 1968 Kulkarni and Mahabale, 1974
<i>Chamaerops</i>		Lateral	1	Guignard, 1961
<i>Jubaeopsis</i>		Lateral	1	Robertson, 1976c
<i>Cocos</i>	One cell of ca-Em Rest of ca + Whole of cb } — S	Terminal but not from an epiphysis initial	1	Haccius and Philip, 1979
<i>Borassus</i>	ca-Em cb- added to S	Terminal	1	Javalgekar, 1979
<i>Cocos</i>	ca-Em cb-S	Terminal	1	Javalgekar, 1979

During the development of the embryo in *Calamus* species, there was a marked growth of the cotyledon on one side resulting in the shifting of the position of the stem tip from the distal part of the embryo towards the basal end. Same mode of cotyledon development has been reported in *Areca catechu* (Rao, 1955a), *Borassus flabellifer* and *Cocos nucifera* (Javalgekar, 1979) and *Cocos nucifera* (Haccius and Philip, 1979). The cotyledon in the monocots has been discussed by a number of workers. In the present investigation, it was observed that the cotyledon originated from a position lateral to the stem tip and developed as a crescent shaped mound expanding around the stem tip, and forming the cotyledonary sheath. Javalgekar (1979) stated that in *Palmae*, where the cotyledon has taken over the function of absorption of stored food and of protection of the plumule, has come to be a complex organ difficult to interpret. The unparalleled

form of the cotyledon that is divided into two parts, one remaining inside the seed and the other coming out during germination itself is quite remarkable.

5.8. FRUIT AND SEED

5.8.1. Assessment of morphological variation

Species variation in the size and weight of rattan fruits has been reported by Wardani (1986). Present studies in the fruit and seed morphology revealed that the fruits and seeds of *C. thwaitesii* were bigger in size and seed weight was also higher than that of *C. hookerianus*.

Studies on seed longevity revealed that bigger the seeds, longer the seed longevity and also more seed moisture content. In the present study the moisture content of *C. hookerianus* was found to be 30 percent and *C. thwaitesii* had 40 percent. Girija *et al.* (1998) had reported the moisture content of *C. rotang* as 33 percent and *C. thwaitesii* as 38 percent.

5.8.2 Change in phenol content associated with desiccation

Rattan seeds were found to be rich in phenolic substance. The content was more in *C. thwaitesii* than in *C. hookerianus*. Wang *et al.* (1997) in *Areca catechu* and Girija *et al.* (1998) in *C. thwaitesii* and *C. rotang* had reported that the total amount of phenolic substance was well correlated with the fruit length and maturity. The result of the present study also indicated a similar trend. The high phenolic content of the seed and its further accumulation during desiccation supported the observation of Loomis and Battaile (1966) who advocated that the high concentration of phenolic substance in the

seeds to be the major cause for the death of recalcitrant seeds. Koslowaska *et al.* (1983) had reported that the oxidized phenolic compound could combine with the essential amino acids which may become toxic.

5.8.3. Seed collection and storage

The present studies confirmed that *Calamus* seeds are best harvested at the just ripe stage of the fruit. At this stage the colour of the pericarp changes to yellow and the pericarp becomes easily detachable. Test of initial seed viability of a sample with triphenyl tetrazolium chloride solution may be helpful.

Pritchard and Davies (1999) also perceive that germination rate is likely to be a function of the quality of seed lot and this may be highly depended on harvesting time and post harvest treatment. They stated that very little information is available on how these parameters affect the germination in rattan. De Leon (1958) pointed out that many failures on germination, attributed to poor practices were really due to low initial viability. This could be a consequence of unripe seeds, faulty storage or of collecting aged desiccated or unhealthy immature fallen fruits from palm bases.

In the present investigation it was found that fruits stored in closed plastic bags and kept in the refrigeration at 10⁰ C maintained viability for 3 months for both species of *Calamus*. Similarly naked seeds of *C. hookerianus* maintained viability for 3 months and that of *C. thwaitesii* for 2 months when stored under the same condition with about 50 percent seed viability. Seeds of both species maintained viability for one month when stored in closed plastic bag at room temperature. Mori *et al.* (1980) indicated that

the naked seeds stored in closed bags maintained high seed viability above 50 percent for half an year.

5.8.4. Seed germination

A complete removal of sarcotesta was found to be a pre-treatment for both species of *Calamus* for a higher percentage of germination Mori *et al.* (1980) and Baruah (1994) perceive that this technique is indispensable for rattan seed germination.

Among the method adopted for improving the germination, seed stratification at 10°C was found to be the best. The positive effect of this technique in improving germination has been earlier reported by Generalao (1977) in *C. maximus* and Bagaloyos (1987) in *C. ornatus*.

Removal of hylar plug was found to enhance germination process in *C. hookerianus* and *C. thwaitesii*. The observations of Bagaloyos (1987) also agree with this finding. They opined that the assessment of viability using various techniques showed that hylar cover removal is more reliable than other methods such as sanding, soaking in H₂O₂, soaking in KNO₃ and tetrazolium staining. Corner (1966) stated that within the family Palmae, there are species such as *Aiphanes erosa* in which germination is accomplished by a wide variety of treatments like soaking in water, heat treatment, hormonal application, scarification or simple placement in polythene bag which provide excellent or satisfactory germination. Result of his investigation also showed that reducing the moisture content level of the seeds of 3 species of *Calamus*, *C. ornatus*, *C. merrillii*, *C. filispadix* and *Daemonorops mollis* adversely affected their viability. This is in accordance to the present findings. In *C. hookerianus* seeds with 30 percent of moisture

content had 78 percent germination and it sharply declined to 46 percent when the moisture content was reduced to 20 percent. But in *C. thwaitesii*, the germination percentage was 69 when the moisture content was 40 percent and germination percentage was reduced to 41 when the moisture content was reduced to 30 percent. The results of the study also indicate the small seeded species had lower moisture content than the large seeded *C. thwaitesii*. Girija *et al.* (1998) has reported similar relationship between moisture content and germination percentage in *C. thwaitesii* and *C. rotang*. The studies related to viability of *Calamus* species have shown that it was related to moisture content and dry storage methods were not effective. This correlation in rattan seeds has also been reported by Generalo (1977) and Pritchard *et al.* (1996). The result of the study by Pritchard and Davies (1998) clearly demonstrates that hydrated storage (at 40 % moisture content) of rattan seeds should be achievable over about 6 months in cool (16°C) but not cold temperature.

Work of Guangtian and Huangcan (2000) revealed that the germination percentage of *Daemonorops margaritae* was low and irregular with the germination period 10-12 months. The germination remained high even after 6 months when seeds were stored at 15°C with moisture content 55% to 65%. The germination period in *C. hookerianus* was found to be 2 to 3 months and that of *C. thwaitesii* it was 3 to 4 months.

The findings of the present study throw light on the fact that rattan seeds do not have seed dormancy. The slow rate of germination may be due to the undeveloped seed structure, especially that of the radicle. The findings of Mori *et al.* (1980) on *C. manan* also corroborated the same conclusion.

PART-II
MICROPROPAGATION

INTRODUCTION

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1. INTRODUCTION

Rattans are the most important forest products in the South East Asian countries and are used principally for making furniture, though several other articles such as baskets are made from it. The properties of rattan worth mentioning are its strength, durability and bending ability, which allows the formation of a wide variety of shapes and enhances the aesthetic value of the canes themselves.

The increase in world demand has led to an overexploitation of this forest resource. The widespread intensive harvesting and the continuous logging operations in tropical forests have resulted in rapid depletion of natural resources of rattans, particularly those of economically important species of *Calamus*. If the depletion of rattan continues in the present rate, the natural rattan resources will almost be totally decimated in few years and we are likely to lose the rattan gene pool necessary for the selection of species with silvicultural potential. Over exploitation of this forest resource has caused not only a shortage of harvestable canes but also of seeds which are the primary means of propagation.

In view of the dwindling rattan supply, many countries have realised the necessity of rattan replanting and have established programmes for rattan cultivation on a plantation scale (Umali-Garcia and Villena-Sanches, 1998 and Rao *et al.*, 1989).

One of the constraints of establishing rattan plantation is the scarcity of seeds. Moreover, the seeds produced cannot be stored for a long time. Besides from heavy extraction of rattan canes in natural forest before flowering and fruiting, wild animals

and members of the upland community utilise fruits of some species as food. As the rattans are dioecious species, harvesting the female plant for the commercial cane would mean an end to its supply of planting materials. Hence conventional seed propagation methods cannot be depended upon for certain species where seed availability is a problem.

In the present scenario, the propagation of rattan by tissue culture method for producing large quantity of seedlings attains importance. The establishment of gene banks, which was one of the strategies recommended by Wong and Manokaran (1985) to ensure conservation of relatively rare rattan species, threatened with extinction, can also be aided by tissue culture. Micro propagation through direct and indirect organogenesis has been proved to be the most reliable way for large scale propagation of genetically stable and true-to-type progenies which help conservation of germplasm.

Meristem tip culture is now commonly used for the rapid propagation of many economically important plant species (Chopra, 1990). One of the most important features of meristem tip culture is that the regenerated plants ensure genetic stability (D, Amato, 1977). Somatic embryogenesis is now recognised as a superior method for *in vitro* propagation as it enables rapid production of large number of complete and uniform plants (Vasil and Vasil, 1972). The artificial seed technology is an exciting and rapidly growing area of research in plant cell and tissue culture (Datta, *et al.*, 1999). Recently, encapsulation techniques for the production of synthetic seeds have gained considerable attention as a novel delivery system of propagules.

In vitro conservation of *Calamus* is an important aspect of research that needs to be intensified. Considering all these facts into account, the present investigation was undertaken with the following objectives:

Micro propagation through shoot tip/collar region and immature embryo.

Callus cultures and indirect organogenesis

Root induction and field transfer

Somatic embryogenesis

Encapsulation of embryoids

Of the above, micro propagation using shoots tips, collar region and immature embryos have been the most widely used method for *in vitro* clonal propagation. Micropropagation techniques are now often preferred to conventional practices of vegetative propagation, because of the advantages like (i) only a small amount of plant tissue is needed as the initial explants for regenerating millions of clones in a year (ii) *In vitro* stocks can be quickly proliferated at any time of the year, within a short period of time (Hu and Wang, 1983). (iii) Technology can be used for storage of valuable germplasm (Chu, 1986).

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Rattans are an important forest produce in the South East Asian countries and are used principally for making furniture. The increase in the world demand has led to an over exploitation of the forest resource. In the present scenario, the propagation of rattan by tissue culture is a potentially useful method for raising large scale plantations especially when the available seeds become limited due to over exploitation.

2.1. STUDIES ON RATTANS

Studies on tissue culture of rattan are much more restricted when compared to other palms. Most of the work done is in the South East Asian countries and India, where rattan grows. A few reports have been published earlier including that of Patena *et al.* (1984); Umali-Garcia (1985); Barba *et al.* (1985); Yusoff (1989) and Dekkers and Rao (1989). which have established that it is possible to propagate rattan through tissue culture.

In early nineties, many rattan species were investigated. Zhuang and Zhou (1991) reported results on *Calamus tetradactylus*, *C. simplicifolius* and *Daemonorops margaritae*. Chen-Zhiying and Fan-Kuan (1995) conducted research on *C. gracilis* and *C. nambariensis* var. *Xishuangbannaensis*. Bingshan (1997) conducted studies on *C. egregius*, *D. jenkinsiana*, *C. dioicus*, *C. rhabadocladus*, *C. guangxiensis*, *C. merrillii* and others; Valsala and Muralidharan (1999) in *C. andamanicus*, *C. pseudotenuis* and *C. thwaitesii*; Goh *et al.* (1999) in *C. manan* and Maitreyee and Rupanarayan (1999) in *C. flagellum*.

Most of the explants used were embryos and collar regions from seedlings germinated *in vitro*. Terminal buds or collar regions from natural seedlings were also used. Of the different types of media, MS medium (Murashige and Skooge, 1962) is commonly used for *Calamus* species. As in other palms the browning of tissues during the early period of incubation was heavy and autocatalytic.

Most of the research papers on *in vitro* culture of rattan species are listed in Table 1 which includes the work done in different Asian countries.

Table 1. Research papers on *in vitro* studies of *Calamus* species

Species	Explant	Results	References
<i>C. manan</i>	Collar region of Seedling	Multiple shoots	Yusoff, 1989
„	Multiple shoots from culture	Rooting (plantlets)	„
„	Embryo	Morphogenic callus	„
„	Morphogenic callus from embryo culture	Shoots	„
„	Shoots from embryo culture	Rooting (plantlets)	„
„	Root and shoot including leaf blade from nursery & aseptically-raised seedlings	White friable callus. Better callusing from aseptically-raised seedlings	Yusoff & Manokaran, 1985
„	Young leaves embryo	No callusing. White friable callus & seedling development	„
<i>Calamus</i> sp. <i>C. discolor</i>	Stem & petiole explants from "cabbage"	Callus induction. MS+4% sucrose identified as best medium	Umali-Garcia, 1985
<i>C. filispadix</i>	„	„	„
<i>C. halconensis</i>	„	„	„
<i>C. merillii</i>	„	„	„
<i>C. microcarpa</i>	„	„	„
<i>C. mindorensis</i>	„	„	„
<i>Cocos nucifera</i> var. <i>philippinensis</i>	„	„	„
<i>C. ramulosis</i>	„	„	„
<i>C. reyesianus</i>	„	„	„
<i>Cocos nucifera</i>	„	„	„

Contd...

Table I. continuing

<i>Daemonorops mollis</i> , <i>D. ochrolepis</i>	”	”	Umali-Garcia, 1985
<i>Cocos nucifera</i>	Seed	Rooting of plantlets	Patena <i>et al.</i> , 1984
<i>C. hookerianus</i>	Leaf primordia	Increase in size of explant	Padmanabhan & Krishnan, 1989
<i>C. thwaitesii</i>	Leaf	Crescent-shaped and wrinkled green outgrowths	”
<i>C. manna</i>	Embryo	Callus & plantlets	Gunawan & Yani, 1986
<i>C. trachycoleus</i>	Embryo	Shoot & root	Dekkers & Rao, 1989
”	Embryo	Callusing of collar region	”
”	Callus from embryo culture	Callusing	”
<i>C. manan</i>	Embryo	Seedling development	Chuthamas <i>et al.</i> , 1989
<i>Cocos nucifera</i>	”	Shoots	Barba <i>et al.</i> , 1985
”	Shoots from culture	Rooting (plantlets)	
<i>Calamus discolor</i>	Stem and petiole explants from tender leaves	Callus induction. MS + 4% sucrose identified as best medium	Umali-Garcia, 1985
<i>C. lilispadix</i>	”	”	”
<i>C. halconensis</i>	”	”	”
<i>C. merrillii</i>	”	”	”
<i>C. microcarpa</i>	”	”	”
<i>C. mindorensis</i>	”	”	”

Contd...

Table 1. continuing

<i>C. ornatus</i> var. <i>philippinensis</i>	”	”	”
<i>C. ramulosus</i>	”	”	”
<i>C. reyesianus</i>	”	”	”
<i>C. manan</i>	Root and shoot including beat blade from nursery and aseptically raised seedlings	While friable callus. Better callusing from aseptically raised seed lings	Yusoff & Manokaran, 1985
<i>C. manan</i>	Young leaves Embryo	No callusing. While friable callus and seedling development	
<i>C. manan</i>	Embryo	Callus and plantlets	Gunawan & Yani 1986
<i>C. ornatus</i>	Embryo	Shoot and root production	Calinawan & Halos, 1988
<i>C. hookerianus</i>	Leaf primordia	Increase in size	Padmanabhan & Krishnan, 1989.
<i>C. manan</i>	Embryo	Seedling	Chuthamas <i>et al.</i> 1989.
<i>C. manan</i>	Collar region of seedling	Multiple shoots	Yusoff, 1989.
”		Rooting plantlets	”
<i>C. manan</i>	Embryo	Morphogenic callus	”
”	Morphogenic callus from embryo culture	shoots	”
”	shoots from embryo culture	Rooting (plantlets)	”
<i>C. trachycoleus</i>	Embryo	Shoot and root	Dekkers & Rao, 1989
”	Embryo	Callusing of collar region	”
”	Callus from embryo culture	Callusing	”

Contd...

Table 1. continuing

<i>C. thwaitesii</i>	Leaf	Crescent-shaped wrinkled green outgrowths	Padmanabhan & Sudhersan, 1989.
<i>C. manan</i>	Collar region of <i>in vitro</i> seedlings	Multiple shoot formation	Yusoff & Ahmad, 1989
<i>C. rotang</i>	Embryo	Seedling development	Padmanabhan & Ilangovan, 1989.
<i>C. yunnaensis</i>	Collar region of seedling germinated <i>in vitro</i>	Multiple shoot formation and plant regeneration	Zhuang and Zhou, 1991.
<i>C. obovoideus</i>	Bud sheaths, leaf segments, petioles and root segments	Plantlet development	
<i>C. thwaitesii</i>	Embryo	Callus formation, anomalous organogenesis	Ramanayak & Kovoov, 1995.
<i>C. caesius</i>	Seedling sucker	Shoot formation	Teo, 1990.
<i>C. gamblei</i>	Embryo	Multiple shoots	Padmanabhan & Ilangovan 1993
<i>C. tetradactylus</i>	”	Multiple shoot formation and out planting	Zhuang, 1993
<i>C. egregius</i>	Terminal bud	Multiple shoot formation and one or two subculture	Bingshan, 1997
<i>C. simplicifolius</i>	Embryo	Multiple shoot formation	Zhuang, 1993
”	”	Outplanting in plantation	Bingshan, 1997.
	Suckers	Shoot development	”
<i>Daemonorops margaritae</i>	Embryo	Multiple shoot formation	Zhuang, 1993.
”	”	Outplanting in plantation	Bingshan, 1997
”	Suckers	Shoot development	”

Contd...

Table 1. continuing

<i>C. egregius</i>	Embryo	Multiple shoot formation, out planting in plantation	„
<i>C. gracillis</i>	Collar region of seedling germinated <i>in vitro</i>	Multiple shoot formation and out planting.	Zhiying & Kuan 1995
<i>C. nambariensis</i> var. <i>xishuangbannaensis</i>	Collar region of seedling germinated <i>in vitro</i>	Multiple shoot formation	„
<i>C. flagellum</i>	Collar region of seedling germinated <i>in vitro</i>	Multiple shoot formation	„
<i>Daemanorops jenkinsiana</i>	Embryo	Multiple shoot formation and out planting.	Bingshan, 1997
<i>C. rhabdocladus</i>	Embryo	Multiple shoot formation	„
<i>C. dioicus</i>	Embryo	Multiple shoot formation and out planting	Bingshan & Guangtian, 1996
	Terminal bud	Multiple shoot formation, but only one or two subcultures	„
<i>C. guangxiensis</i>	Embryo	Inflorescence development, no shoot formation.	„
<i>C. merrilli</i>	Embryo	Multiple shoot formation	„
<i>C. merrillii</i>	Embryo	Multiple shoot formation	Umali-Garcia & Villena-Sanches, 1998
<i>C. manan</i>	Roots and leaves of seedling and mature plants	Shoot production and somatic embryogenesis	Goh, 1998
<i>C. manan</i>	Root tips of <i>in vitro</i> plantlets.	Somatic embryos	„
<i>C. flagellum</i>	Shoot apical meristem	Regeneration of plantlets via. primary callus	Maitreyee, & Rupnarayan, 1999

2.2. STUDIES ON COCONUT

Coconut palm (*Cocos nucifera* L.) is a major agricultural crop in tropical areas. The productivity of coconut needs to be increased substantially to have a chance in competition with other vegetable oils.

Since the coconut palm is generally cross-pollinated and heterozygous, propagation by seeds give rise to great variability in hybrid progenies. *In vitro* vegetative multiplication of individuals with better performance thus remains the only short and medium term hope for the production of homozygous planting material for an improvement in the productivity in plantations.

Efforts are being made since 50's to achieve a commercially viable protocol for micropropagation at several laboratories around the world. Almost every part of the tree has been tried for the culture initiation and the subsequent work. It has always remained as a challenging work for all coconut tissue culturists all over the world to develop a repeatable and commercially viable technology.

Considering the regeneration potential, immature embryos of 1.5 - 3mm size from tender nuts were used for standardising a protocol in many centers. The inflorescence is also being used as a source of explant in coconut. Non destructive sampling of the spadix from coconut palm was described by Blake and Eeuwens (1978) and Rillo (1989). Raju *et al.* (1984) obtained few clonal plantlets via. callus leaf tissues of young coconut seedlings. Seedling tissues from tender leaves, leaf base and leaf sheaths were first cultured in Y3 medium supplemented with various combinations of auxins and

cytokinins. Embryoid development from leaf tissues were reported by Pannetier and Bufford-morel (1982) and Bhaskaran (1985). Branton and Blake (1983), Samosir *et al.* (1997), Fernando and Gamage (2000) and ~~...~~ Karun (2001) reported somatic embryogenesis and plantlet regeneration from tissues of inflorescence.

Kuruvinashetty & Iyer (1980) used different explants like rachilla and anthers, but a repeatable protocol could not be obtained. Successful induction of callus from endosperm was achieved by Rillo (1989).

Severe browning of explants (80%) immediately after inoculation or during the early period of culture was noticed by Nair *et al.* (1999). They stated that the oxidation of poly phenols which leads to the formation of highly toxic quinines is responsible for browning. Ebert^{et al.} (1993) reported the role of activated charcoal on preventing browning with inflorescence as the explant.

Two basic media have generally been used for *in vitro* culture of palms viz., Eeuwens's Y3 medium (1976), Murashige and Skoog's MS medium (1962) Avril *et al.* (1986).

Karun (2001) reported that the growth regulators such as 2, 4-D, NAA, IBA, 2iP, Kinetin and BAP are commonly used. Kuruvinashetty and Iyer (1980); Mascarenhas *et al.* (1988) and Kalamani and Rangasamy (1990) have stressed the advantage of using Y3 media. The efforts made at various research centers are presented in Table 2.

Table 2. Tissue culture studies on coconut palm

Species	Explant	Result	References
<i>Cocos nucifera</i>	Immature rachilla from leaf axils	Callus formation	Kuruvinashetty & Iyer, 1980
”	”	”	”
”	Leaf tissues	Somatic embryos	Pannetier and Buffard-Morel, 1982
”	Immature inflorescence	Somatic embryos	Branton & Blake, 1983
”	Leaf tissues of young seedling	Plantlet formation	Raju <i>et al.</i> , 1984
”	Leaf tissues	Somatic embryos	Bhaskaran, 1985
”	Endosperm	Callus formation	Rillo, 1989
”	Immature inflorescence	Plantlet formation (4.10 μ M) with AC (0.25%)	Mascarenhas <i>et al.</i> , 1988

Contd...

Table 2. continuing

”	Immature embryo	Plantlet development	Assy-Bah <i>et al.</i> , 1987
”	”	Germination and development of embryogenic callus	Karunaratne & Periyapperuma, 1990
”	”	Plantlet formation	Kalamani & Rangasamy, 1990
”	”	Somatic embryos	Karunaratne & Periyapperuma, 1990
”	Immature embryo	Nodular and Knobby callus	Rillo & Ebert, 1993
”	Plumular tissue from embryo	Plantlet regeneration	Hornung, 1995
”	Immature inflorescence	Shoot formation	
”	Immature embryo	Somatic embryos and plantlet formation	Samosir <i>et al.</i> , 1997
”	Plumular tissue of embryo	Somatic embryos and germination	Chang <i>et al.</i> , 1998
”	Immature embryo	Plantlet formation	Fernando & Gamage, 2000

2.3. STUDIES ON OIL PALM

The oil palm (*Elaeis guineensis*) is a major oil producing crop of great economic value. As in the case of coconut, the propagations are exclusively through nuts. Here the production of propagules is more complicated as hybrids alone are used for commercial planting. Hence there is an alternative method for production of planting material. The *in vitro* technique would prove useful in the large scale propagation of the elite palms.

The advantage of producing good clonal population of selected high yielding trees in oil palm has been described by Corley *et al.* (1981). The major work on *in vitro* culture of oil palm was carried out in France, UK, Malaysia and Indonesia.

It has been claimed that through clonal propagation, yield of oil would be increased by as much as 30% over the seedling progeny. Progress in commercialization of clonal propagation suffered a set back when abnormal flower development (mantled) was reported in palms transferred to field by Corley *et al.* (1981).

The first attempt in India to develop a protocol for clonal multiplication of oil palm from tender leaf explants through somatic embryogenesis was by Thomas and Rao (1985) at Baba Atomic Research Centre, Bombay. Raju *et al.* (1989) successfully cultured explants from tender leaf of 6 and 18 months old tenera oil palm seedlings. Rajanaidu *et al.* (1997) reviewed the current status of oil palm clonal production on commercial scale. In addition to leaf explants, immature inflorescences were also found to be responsive for shoot induction. In callus induction, 2, 4-D was found to be superior to NAA by different researchers. Sogeke (1998) opined that 6-benzylandaine

is capable of inducing shoot production upto 70% from embryogenetic callus. Activated charcoal and PVP was found to be effective in minimizing browning of explants. Karun and Sajini (1996) have described an effective protocol for embryo culture. The noteworthy achievements towards the goal till now are summarised below (Table 3).

Table 3. Tissue culture studies on oil palm (*Elaeis guineensis*)

Species	Explants	Results	References
<i>Elaeis guineensis</i>	Leaf	Plantlet formation	Pannetier <i>et al.</i> , 1981
<i>E. guineensis</i> x <i>E. melanococca</i>	„	Embryos	Abee <i>et al.</i> , 1981
<i>E. guineensis</i>	„	Accumulation of fat droplets in the cells just before the development of embryoids	Turnham & Northcote, 1982.
<i>E. guineensis</i> var. <i>pisifera</i>	Embryo and leaf	Plantlet formation via. embryoids	Nwanko & Krikorian, 1983
<i>E. guineensis</i>	Excised embryos	Regrowth when cultured in MS with 0.5 mg/l NAA and 0.1 mg/l Kn	Grout <i>et al.</i> , 1983.
<i>E. guineensis</i>	Leaf	Anti – auxins reduced the inhibitory effects of callus seen in the proximity of embryogenic callus	Hanower & Hanower, 1984.
<i>E. guineensis</i>	Embryo	Somatic embryos	„
<i>E. guineensis</i> var. <i>tenera</i>	Leaf	Somatic embryos	Thomas & Rao, 1985
<i>E. guineensis</i>	Embryo	Platelets from zygotic embryos	Cid, 1987.

Contd...

Table 3. continuing

<i>E. guineensis</i>	Protoplast	Protoplast divided within 48 h and grew into colonies of 20-30 cells within 2 weeks	Sekak <i>et al.</i> , 1987.
<i>E. guineensis</i>	Leaf	Mass production of secondary embryogenesis without any external growth regulator	Dural <i>et al.</i> , 1988.
<i>E. guineensis</i>	Leaf	Calli were formed from perivascular cells. Subsequent proliferation from a cambium like zone	Schwendiman <i>et al.</i> , 1988.
<i>E. guineensis</i>	Leaf	Formation of bipolar and tripolar embryos	Raju <i>et al.</i> , 1989.
<i>E. guineensis</i> var. <i>dura x pisifera</i>	Embryo	Plantlets from somatic embryos	Rodriguez, 1989.
<i>E. guineensis</i>	Embryo	Somatic embryos and plantlet formation	„
<i>E. guineensis</i>	Immature inflorescence	Somatic embryos	Teixeira <i>et al.</i> , 1993.
<i>E. guineensis</i>	Immature inflorescence	Somatic embryos	Teixeira <i>et al.</i> , 1994.
<i>E. guineensis</i>	Immature inflorescence	Somatic embryos and plantlet development	„

Contd...

Table 3. continuing

<i>E. guineensis</i> cv. <i>dura</i> and <i>tenera</i>	Leaf	Plantlet development through both somatic embryogenesis and organogenesis	Karun and Sajini, 1996
<i>E. guineensis</i> cv. <i>dura</i> and <i>tenera</i>	Leaf	Clones were negatively affected by somaclonal variation and exhibited abnormal female flowers	Ginting <i>et al.</i> , 1996.
<i>E. guineensis</i> cv. <i>dura</i> and <i>tenera</i>	Leaf and leaf base	Plantlet regeneration by somatic embryogenesis and organogenesis	Nair, 1997
<i>E. guineensis</i>	Leaf	A very short callus stage and plantlets from somatic embryos	Sogeke, 1998.
<i>E. guineensis</i>	Leaf	Synchronous development of somatic embryos	Tahardi, 1998.
<i>E. guineensis</i>		Glucose induced terminal inflorescence and rigidity in <i>in vitro</i> shoots	Tarmizi, 1999.
<i>E. guineensis</i>	Leaf	Embryoid proliferation and germination stages were low, unpredictable and variable. Fruit matting occurred sporadically when they were grown in fields	Wong <i>et al.</i> , 1999.
<i>E. guineensis</i>	Leaves	Slow generation rate of somatic embryos in suspension cultures were due to the absence of a culinary meristem. Addition of BA during development enhanced shoot production	Bertossi <i>et al.</i> , 1999.

2.4. STUDIES ON DATE PALM

Date palm, *Phoenix dactylifera* is a dioecious fruit tree that is vegetatively propagated through off shoots. However, there are many problems associated with this system. The availability of shoots is limited because of the number produced by each palm tree is low, very erratic and cannot be successfully controlled.

To satisfy the increased demand in international markets, it is the need of the time to develop an alternative method of plant production from selected genotypes. Clonal propagation by tissue culture has the potential to produce plants at a competitive cost and in large numbers needed to meet the demand.

Organogenesis in date palm has a low efficiency due to the minimum number of explants that respond *in vitro*, the long time required for the initiation phase, the low multiplication rate and the strong influence of the variety (Poulain *et al.*, 1979 and Beauchesne, 1982). Generally shoot tips and leafy buds from off shoots were cultured in media containing a high concentration of 2, 4-D for initial callus formation. In most cases, somatic embryos differentiated on solid medium, but Daguin and Letouze (1988) were able to induce embryogenesis in shaken liquid medium. The important achievements in this field are summarised in Table 4.

Table 4. Tissue culture studies on date palm

Species	Explant	Results	References
<i>Phoenix dactylifera</i>	Shoot tips, Embryo and rachilla tissue	Plantlet formation	Tisserat, 1982
<i>P. dactylifera</i>	Embryo	Somatic embryogenesis	Daguin and Letouze, 1988
<i>P. dactylifera</i>	Embryo	Somatic embryogenesis	"
<i>P. dactylifera</i> cv. kabkab and Estamaran	Juvenile leaf and shoot apex	Callus formation	Hervan <i>et al.</i> , 1991
<i>P. dactylifera</i>	Leaf primordium shoot apex, cotyledon and root of seedling	Callus formation and shoot development	"
<i>P. dactylifera</i> cv. deglet Nour	Embryo and seedling parts	Somatic embryogenesis and plantlet formation	Falcone & Marcheschi, 1988
<i>P. sylvestris</i>	Shoot tip	Shoot formation	Omar, 1988
<i>P. sylvestris</i> cv. barhee	Shoot tip and immature inflorescence	Somatic embryogenesis and plantlet formation	Bhaskaran & Smith, 1992
<i>P. sylvestris</i> cv. Bouskri	Leaf base	Plantlet formation	Anjarne & Zaid 1993
<i>P. dactylifera</i> cv. Muscat	Shoot tip	Auxiliary bud formation	Gabr & Tisserat, 1985
<i>P. dactylifera</i>	Embryo	Somatic embryos and plantlets	Daguin and Letouze, 1988
<i>P. dactylifera</i>	Shoot tip	Somatic embryos	Dass <i>et al.</i> , 1989

Contd...

Table 4. continuing

<i>P. dactylifera</i>	Shoot tips and leafy bud fragments	Development of somatic embryos were more in liquid medium shaken at 100 rpm for 2 weeks with out source followed by culture on 3% sucrose	Veramendi and Navarro, 1997
<i>P. dactylifera</i> cv. zaghoul and Samans	Shot tip	cv. zaghoul had a higher shoot and root length than Samani	Belal and Deeb. 1997
<i>P. dactylifera</i> cv. Aguellid	Leaf base	Callus proliferation	Bougerfaoui and Zaid 1993
<i>P. dactylifera</i> var. estamaran and kkbab	Shoot tip, lateral buds and young leaves	Somatic embryogenesis and plantlet formation	Nazeri <i>et al.</i> , 1993
<i>P. dactylifera</i>	Apical bud and auxiliary bud	Somatic embryogenesis and plantlet formation	Veramendi and Navarro, 1997
<i>P. dactylifera</i>	Embryos	Plantlet formation through direct germination	Sarkar, <i>et al.</i> , 1998
<i>P. dactylifera</i> var. <i>canariensis</i>	Shoot meristem	Multiple shoot formation	Rousseau, <i>et al.</i> , 1999
<i>P. dactylifera</i>	Embryo	Somatic embryos and plantlets	Huqng <i>et al.</i> , 1999

2.5. STUDEIS ON OTHER PALMS

In addition to the above mentioned palms, scattered references are there in other members of Arecaceae in which *in vitro* propagation is also found to be successful.

Dias *et al.* (1994) have cultured immature embryos of *Geonoma gamiova* on semisolid medium containing MS salts, vitamins 30g sucrose per litre and 1.5g activated charcoal per litre, 100 g 2, 4-D per litre and 2 mg 2iP (isopentenyladenine) per litre. Embryogenic masses had formed from the epidermal tissue of the mesocotyl and on generation medium plantlets were developed from somatic embryos.

Alang *et al.* (1991) by culturing the immature embryos of Sago palm (*Metroxylon sago*) found that the most effective medium for balanced growth of both shoot and root was MS medium with 0.4 mg thiamine HCl, 100 mg myo-inositol, 30g sucrose and 3g activated charcoal per litre, 10 µm NAA, 1 µm BAP and 7 g agar per litre.

Guerra and Handro (1998) studied the development of somatic embryos and plant regeneration in different organs of *Euterpe edulis*. Embryogenesis was included using a gelled MS medium or Euevens salts supplemented with 2, 4-D (50-100 mg/l).

Excised embryos of *Areca catechu* (cv 5. Kasargodan and Mangako) were cultured on (BB) medium, with permutations and combinations of auxins and cytokinin by Mathew and Philip (2000) and they observed that subsequent to germination of the embryo, meristemoid differentiated from single epidermal cells of the cotyledonary sheath.

Moran (1984) has studied the possibilities of the use of *in vitro* techniques in some palm species of Amazonian Peru including *Bactris gasipaës* and *Jessenia bafava*.

The response of the embryo of *Jubaea chilensis* whose poor seed germination (<2% in nurseries using traditional methods) and slow seedling growth presented problems in Chile was studied by Yuri (1987) by culturing in both MS medium supplemented with NAA and BAP and without any hormones and embryo germination was noticed on solid medium without hormones.

Hyophorbe amaricaulis, an endangered palm which has only a single specimen surviving in Mauritius, that can not be propagated vegetatively or by seeds, was cultured *in vitro* by Douglas (1987). He cultured embryos from 51 fruits and only one germinated and produced a true leaf but its transfer to soil was not achieved.

MATERIALS AND METHODS

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3. MATERIALS AND METHODS

3.1. PLANT MATERIALS

Rattan, one of the most economically important forest produce in the Asian countries has recently received much attention because of the depletion of the resource in natural forests as mentioned in the earlier chapter. The removal of mature plants is also causing scarcity of seeds leading to serious regeneration problems. Taking into account the global economic importance, planting in the natural forest as well as rising of large scale plantation of commercially important species should be resorted to since enough mature seeds are not available for many important species; micropropagation can be used as an alternative method.

Detailed descriptions of explant selection, surface sterilization, medium preparation and culture conditions have been given separately.

3.1.1. Selection of explants

Immature embryos, collar region and terminal bud from seedlings grown in *in vitro* conditions and in the nursery were used as the explants for the present experiments to establish clones.

The immature fruits of *C. hookerianus* were collected from germplasm collection of rattans at Kerala Forest Research Institute, Peechi and rattan seed stands of KFRI at Kachithode in Patikkad Range of Thrissur Forest Division and that of *C. thwaitesii*

from rattan seed stands of KFRI, Kachithode and the rattan research plot in Vazhachal Forest Division in Western Ghats. The shoot tip and collar region of both species were extracted from 6 months to one-year-old seedlings grown at KFRI Peechi.

3.1.2. Extraction and surface sterilization of explants

The immature fruits, 7-8 months old, were collected, washed in running water and peeled off the scaly wall, and treated with a detergent solution, washed in sterile water followed by treatment with 0.1% aqueous mercuric chloride (wt/vol.) solution for 7-10 minutes and there after washed thoroughly in sterile distilled water. The endosperm was sliced carefully without damaging the embryos with a surgical blade. The embryos were taken out carefully using sterilized steel needles. These embryos were washed again in sterilized distilled water and inoculated on different media. Growth responses of embryos to the culture medium were monitored periodically.

3.1.3. Collection of collar region

The collar regions from six months to one year old seedlings of field grown plants and *in vitro* grown seedlings of immature embryos germinated on basal medium were collected as the explants. Five to ten days were required for embryos to germinate under *in vitro* conditions. Seedlings of 3-4 months growth with a height of 5-6 cm were suitable for the collection of collar regions as explants. The radicles were cut off along with one or two outer layers of sheath and the collar with top part of inner sheath was inoculated on culture medium. Contamination rate could be reduced in the collar region obtained from *in vitro* raised seedlings.

3.1.4. Collection of shoot apex

The shoot apex was excised both from nursery seedlings and *in vitro* seedlings. As in other palms, terminal shoot buds of the rattans are also deeply located on the stem tip. The top sheaths wrap up the terminal bud very closely, which makes the location of terminal buds very deep and is difficult to trace out. The location of terminal bud was determined by the dissection of shoot tip and removing all the sheaths and the terminal bud was excised undamaged.

3.1.5. Surface sterilization of collar region and shoot apex

The explants, collar region and shoot apex were washed under running tap water thoroughly followed by a wetting reagent either with Extran or Teepol for varying time followed by washing with double distilled water. Surface disinfections were done with aqueous HgCl₂ (0.1% to 0.5%) (w/v) for 5-7 minutes. The HgCl₂ solution was drained off and finally the explants were rinsed repeatedly with sterile double distilled water till all the traces of the sterilant was removed. The sterilized explants were inoculated aseptically on different culture media.

3.1.6. Prevention of phenolic discolouration of media and explants

After inoculation, the explants exhibited characteristic brown exudation in the medium. This was often seen to originate from the newly cut surface of the explants. This, commonly called as polyphenolic oxidation products, seen associated with tissue culture of woody plants also (Hu and wang, 1983), resulted in browning and death of

the explants as well as decolouration of the culture. In order to prevent this, various pre-treatments of explants were carried out individually or in combination and they are summarised in Table 10.

3.1.7. Preparation of media

For the present culture studies on *Calamus* species both MS, Y3 and SH media were used. Separate stock solutions of macro and micro nutrients, vitamins, amino acids and hormones were prepared according to the methods followed by George and Sherrington (1984). Same media supplemented with different growth regulators were used for callus induction and other *in vitro* establishments. Stock solutions were stored in refrigerator at a temperature of 2-4⁰ C. Only double distilled water was used for the preparations and the necessary media were formulated from basic ingredients. All supplements to the basal medium were added prior to the final adjustment of the volume. The pH of the medium was adjusted to 5.6/5.7 either with 0.1 N sodium hydroxide (NaOH) or in hydrochloric acid (HCl). The culture media were solidified with 0.8 percent agar (BDH; bacteriological grade) for static culture. 3 percent sucrose was used as the carbon source. After adding agar, the media were gently heated by constant stirring and about 25 and 10 ml of molten medium was dispensed into 150 conical flasks (Borosil, India) and culture tubes respectively. The flasks were capped with aluminium foil. The culture tubes were plugged with colon bunks made with non-absorbent cotton wool, wrapped with double layered gauze/aluminium foil or polypropylene caps.

The glasswares, chemicals and the hormones used for the preparation of culture media were as follows:

3.2. GLASSWARES

Only Borosil or Corning glassware were used for the present experimental work. All cultures were carried out either in conical flasks (100 –250 ml) or in culture tubes of different sizes (25 x 200 mm, 25 x 150 mm, and 10 x 180 mm).

All the culture vessels were autoclaved and subjected to 40 percent chromic acid treatments for an overnight. Later they were thoroughly washed using a liquid detergent (Teepol). The cleaned glassware were again rinsed with distilled water, drained off and dried in hot air oven at 50-100⁰ C before proceeding to experiments.

3.3. CHEMICALS

The chemicals used for the preparation of culture media were of high analytical grade, obtained from Sigma chemical company, UAE, E. Merck (India) Ltd., Qualigens (India) and British Drug House (BDH), India.

The composition of different media viz. Murashige and Skooge (1962); Schenk and Hildebrandt (1972) and Y3 and the list of plant growth regulators used in the study are given in the following table (Table 5.).

Table 5. Compositions of culture media (mg/l)

Ingredients	SH	Y3	MS
(NH ₄) NO ₃	2500.00	-	1650.000
KNO ₃	2500.00	2020.00	1900.000
Ca Cl ₂ . 2N ₂ O	200.00	274.00	440.000
Mg SO ₄ . 7H ₂ O	400.00	247.00	370.000
KH ₂ PO ₄	-	-	170.000
NH ₄ H ₂ PO ₄	300.00	-	-
Na H ₂ PO ₄ . H ₂ O	-	312.00	-
KCl	-	1492.00	-
Fe SO ₄ . 7H ₂ O	15.00	13.90	27.800
Na ₂ EDTA 2H ₂ O	20.00	37.30	37.300
Mn SO ₄ . 4H ₂ O	10.00	11.20	22.300
Zn SO ₄ . 7H ₂ O	1.00	7.20	8.600
H ₃ BO ₃	5.00	3.10	6.200
NH ₄ Cl	-	535.00	-
KI	1.00	8.30	0.830
Na ₂ Mo O ₄ . 2 H ₂ O	0.10	0.24	0.250
CuSO ₄ . 5 H ₂ O	0.20	0.25	0.025
CoCl ₂ . 6 H ₂ O	0.10	0.24	0.025
Myoinositol	1000.00	-	100.000
Nicotinic acid	5.00	-	0.500
Pyridoxine HCl	0.50	-	0.500
Thiamine HCl	5.00	-	0.100
Glycine	-	-	2.000
Sucrose	30000.00	30000.00	30000.00

By observing the response in culture in the above media, MS medium (Murashige and Skooge, 1962) was finally selected for further studies. The basal medium was manipulated with different auxins viz. 2, 4 – dichlorophenoxy acetic acid (2, 4-D), Indole-3 acetic acid (IAA), α - naphthalene acetic acid (NAA), Indole –3 butyric acid

(IBA) and cytokinins such as 6-benzyl aminopurine (BAP) and 6-furfuryl aminopurine (KIN) in different concentrations either singly or in combinations.

Table 6. List of plant growth regulators used in the study.

Compound		Chemical Formula	Molecular weight
Auxins	2, 4-D	2-4-dichlorophenoxyacetic acid $C_8 H_6 Cl_2 O_3$	221.00
	NAA	α -naphthalene acetic acid $C_{12} H_{10} NO_2$	186.20
	IAA	Indole-3 acetic acid $C_{10} H_9 NO_2$	176.00
	IBA	Indole-3 butyric acid $C_{12} H_{13} NO_2$	203.20
Cytokinins	BAP	6-benzylaminopurine $C_{12} H_{11} N_5$	225.00
	KIN	Kinetin (6-furfuryl aminopurine) $C_{10} H_9 N_5 O$	

3.4. STERILIZATION OF MEDIA

After adding agar, the media were gently heated by constant stirring and about 25 ml and 10 ml of molten medium was dispensed into 150 ml conical flasks and culture tubes respectively. The culture vials containing the media were sterilized by autoclaving at 1.06 kg/cm² for 20 minutes. After sterilization, the culture tubes were placed in slanting position and cooled which provided greater areas.

3.5. CULTURE PROCEDURES AND ENVIRONMENT

All procedures demanding aseptic conditions were carried out in a clean inoculation chamber (Laminar flow) maintained with filtered air and UV radiation. The work area of laminar air flow chamber was wiped with 70 percent alcohol and all requirements were exposed to UV radiation for 30 minutes, prior to inoculation to avoid

contamination from microbes. All the cultures were maintained in the culture room, where a thermostatically controlled air-conditioner was installed to maintain the temperature at $24 \pm 1^{\circ}$ C. The cultures were incubated under 16/8 h photoperiod from cool white fluorescent tubes (1500-2000 lux). All the responses of explants and the nature of morphogenesis were recorded at regular intervals:

3.6. IMMATURE EMBRYO GERMINATION

The surface sterilised embryos of *C. hookerianus* were planted on nutrient agar media in culture tubes. Growth of embryos was monitored periodically. The following media were tested for normal germination of isolated embryos.

1. Murashige and Skooge (1962) (MS) basal medium
2. Eeuwens (1976) (Y3) basal medium
3. Schenk and Hildebrandt (1972) (SH) basal medium

3.7. CALLUS CULTURE

Callus was induced from immature embryos of both species of *Calamus* on MS medium fortified with different concentrations of auxins and cytokinins. The auxins, 4-D and NAA and cytokinins BAP and KIN were used for the maintenance of callus.

3.7.1. Indirect organogenesis

Primary calli derived from embryo explants on MS medium incorporated with BAP KIN or in BAP and 2, 4-D at different concentrations was used for inducing

morphogenesis. Subsequent subcultures were done at periodic intervals. Observations were recorded visually at regular intervals.

3.7.2. Somatic embryogenesis

Indirect somatic embryogenesis was induced from the callus obtained from immature embryos of *C. thwaitesii* on MS medium supplemented with 2, 4 – D and BAP. The callus thus obtained upon transfer to MS medium with low auxin and cytokinin produced friable callus which upon transfer to hormone free medium developed into embryoids.

3.7.3. Synseeds

Encapsulation studies were conducted using immature embryos of *C. hookerianus* and embryoids derived from callus culture. For encapsulation 1-5 percent solution of sodium alginate was prepared containing the ingredients of MS medium (without CaCl_2). pH was adjusted to 5.8. Various levels of $\text{CaCl}_2 (2\text{H}_2\text{O})$ (25, 50, 75 and 100 mM) were prepared in double distilled water. Both solutions were sterilized.

Under aseptic conditions, the blot dried embryoids were immersed in the encapsulation matrices. With a sterilized pipette of 1.0 cm diameter the alginate solution with an embryoid was drawn and dropped into $\text{CaCl}_2 (2\text{H}_2\text{O})$ solution. The drops with embryoids, set as small white transparent beads were incubated in the $\text{CaCl}_2 (2\text{H}_2\text{O})$ solution for 20 – 40 minutes. After the incubation period, the encapsulated embryoids were retrieved by decanting the $\text{CaCl}_2 (2\text{H}_2\text{O})$ solution and the beads were washed 2 –

3 times with basal MS medium. The encapsulated embryoids were cultured on hormone free MS medium to test germination percentage.

3.8. DIRECT ORGANOGENESIS (MULTIPLE SHOOT FORMATION)

Multiple shoots were induced from shoot apex, collar region and immature embryos cultured on MS medium supplemented with different concentrations and combinations of cytokinins and auxins.

Experimental data like percentage of explants responding for multiple shoot induction, number of shoots induced were recorded at regular intervals for each treatment.

3.9. ACCLIMATIZATION AND TRANSFER OF PLANTLETS TO THE FIELD

The plantlets regenerated through various *in vitro* techniques, were washed thoroughly with distilled water to remove all the traces of adhering agar medium from the roots. The washed plantlets were then transferred to small pots containing sterile sand. The pots were then covered with polythene bag to maintain high humidity and kept under laboratory conditions. The plantlets were watered twice a day. The polythene bags were removed after 20 days. The hardened plantlets were then planted in pots containing garden soil and sand in 1:1 ratio. The *in vitro* grown plantlets exhibited a survival rate of 75 percent in the field with healthy green leaves.

The photographs of culture tubes and flasks were taken using a Pentax K1000/Nikon Camera. and

3.10. STATISTICAL ANALYSIS

Analysis of variance of data obtained on number of shoots was carried out after applying square root transformation. This was followed by mean comparison test viz., Duncan multiple range test (DMRT) Gomez and Gomez (1980).

Data on response frequency was subjected to analysis of variance. This was followed by DMRT

OBSERVATION

115A

4. OBSERVATIONS

In the present experimental studies, an efficient and reproducible protocol for the propagation and conservation of two species of *Calamus*, *C. hookerianus* and *C. thwaitesii* have been established through *in vitro* germination of immature embryos. direct shoot regeneration, callus induction, indirect shoot regeneration, somatic embryogenesis and synseeds.

The following aspects were studied in detail and the observations and results obtained during the present studies are given below.

4.1. CULTURE OF IMMATURE EMBRYOS

Immature embryos of *C. hookerianus* and *C. thwaitesii*, after 6 months of anthesis, were cultured on MS, SH and Y3 basal media supplemented with 3% sucrose, 0.6% agar and 0.2% activated charcoal. The growth responses of both the species in different basal nutrient media and the medium with the supplementation of different concentrations and combinations of auxins and cytokinins were recorded periodically. About 70 - 80 percent of the immature embryos of both the species cultured on different basal media showed germination (Table 7)

Table 7. Percentage of germination of embryos in different basal media

Media	Number of immature embryos inoculated	Percentage of contamination		Percentage of browning		Percentage of germination	
		Ch	Ct	Ch	Ct	Ch	Ct
MS	20	10.0	5.0	10.0	15.0	80	80
SH	20	10.0	15.0	20.0	15.0	70	70
Y3	20	10.0	10.0	15.0	20.0	75	70

Ch - *C. hookerianus* Ct - *C. thwaitesii* (Observations were made after 30 days of culture)

4.1.1. Selection of basal medium

Of the three basal media tested (MS, SH and Y3), the immature embryos inoculated on MS medium germinated into healthy seedlings within 65 days with a high frequency of growth rate of 80 percent. The other two media tested were inferior to MS medium with regard to percentage of germination, growth response and establishment (Fig. 1)

On the basis of the above observations, MS medium was selected for further *in vitro* experiments.

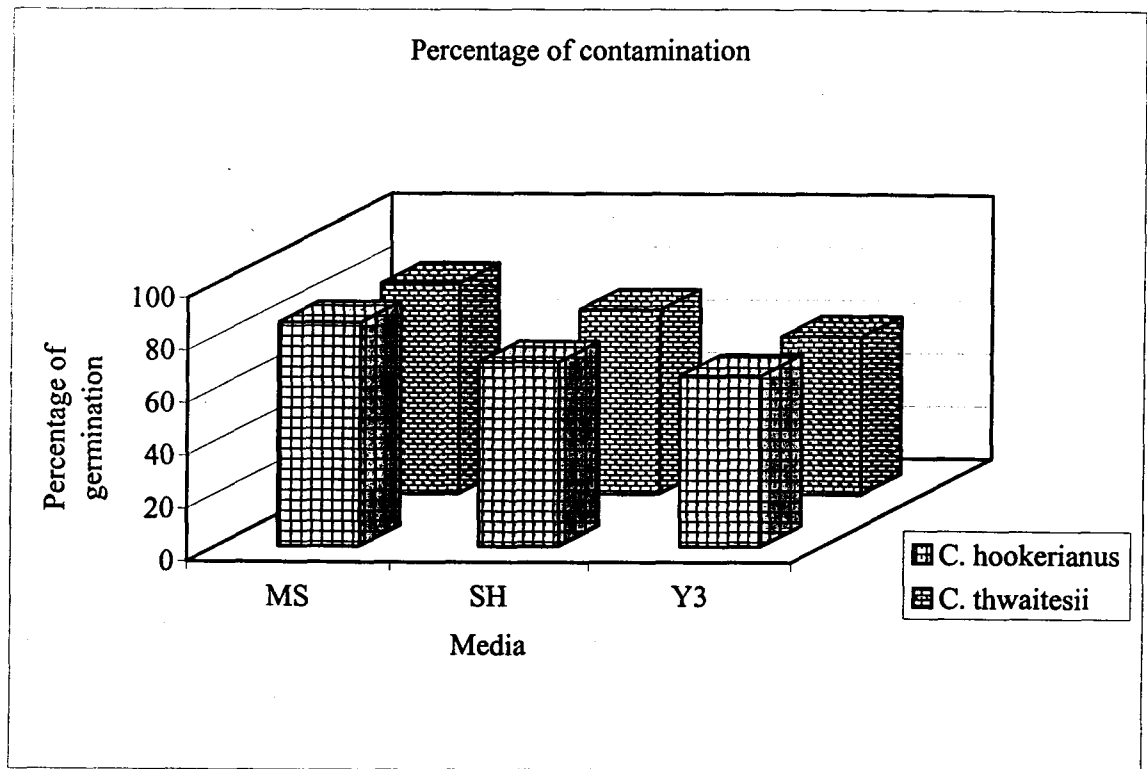


Fig. 1. Establishment frequency of sterilized embryos of *C. hookerianus* and *C. thwaitesii* on different basal media without hormones after 65 days of incubation.

4.1.2. Effect of sucrose concentration on growth of explant

The effective concentration of sucrose for better growth response in *C. hookerianus* and *C. thwaitesii* was studied by using different levels of sucrose (1 - 5%) in MS medium.

Of the different levels of sucrose tested, 3 percent was found to be superior (Fig. 2).

Mature and immature embryos of both species developed into rooted seedlings on MS basal medium within a period of 8 - 10 weeks (Figs. 3 & 4). The responses of the embryos on MS medium without any growth regulator supplement at different periods of culture are presented in Tables 8 and 9.

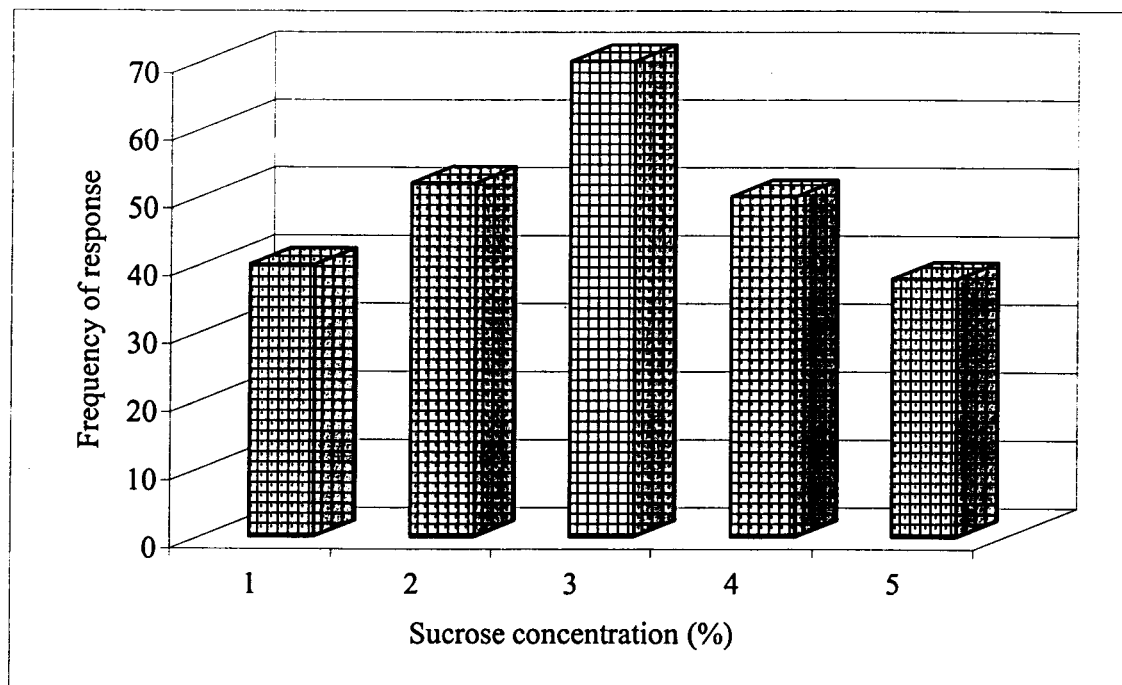


Fig. 2. Response frequency of embryos of both species of *Calamus* on MS medium containing different concentrations of sucrose.

Fig. 3. *C. hookerianus* different stages in immature embryo germination

Fig. 3A A germinating embryo showing root, shoot and haustorium development

Fig. 3B A later stage in embryo germination

Fig. 3C The haustorium has developed into a sac-like structure

Fig. 3D The size of haustorium decreases as the shoot grows

Fig. 3E The eophyll has developed and the size of haustorium gets reduced

ha - haustorium



3A



3B



3C



3D



3E

Fig. 4. *C. thwaitesii* - different stages in embryo germination

Fig 4A A germinating embryo showing root, shoot and haustorium development

Fig 4B A later stage in embryo development. Auxillary buds are seen developing from the collar region

Fig 4C The haustorium has developed into a sac-like structure

Fig 4D A well developed seedling with a spongy haustorium

ha - haustorium

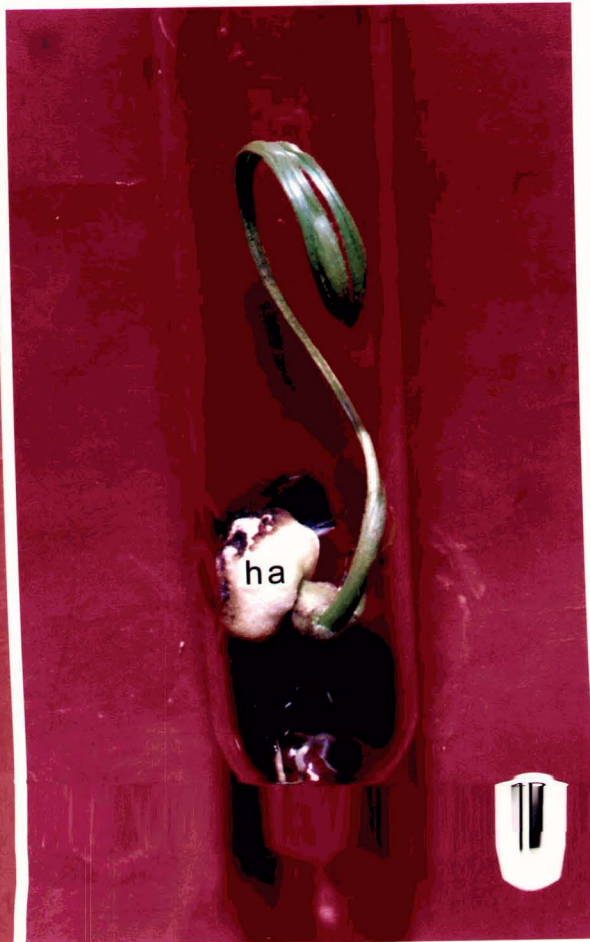
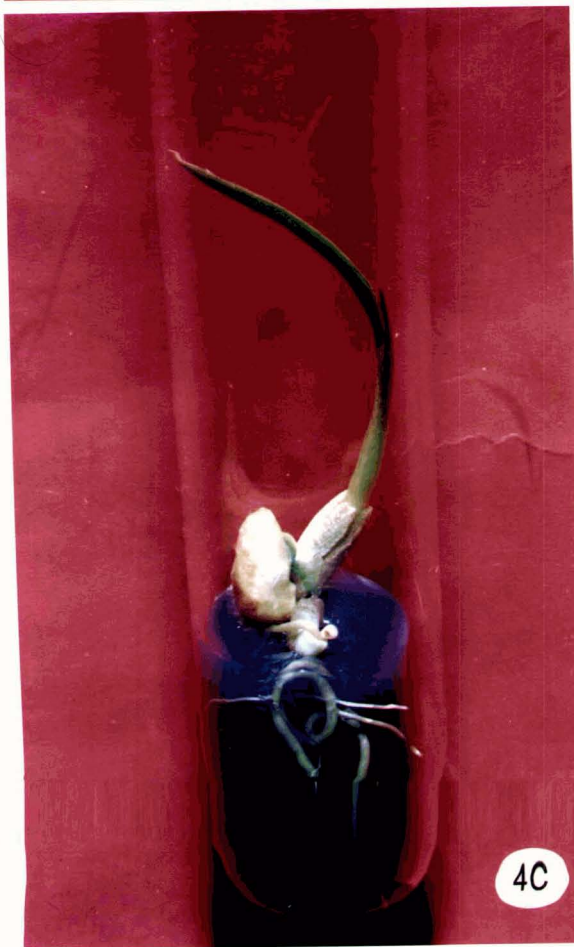
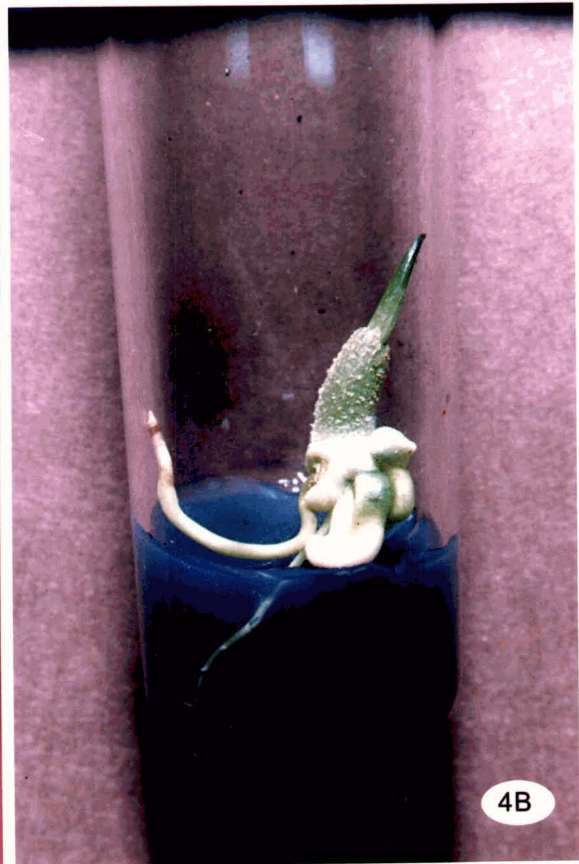


Table 8. Response of *C. hookerianus* embryos at different periods of culture in MS basal medium.

Period	Observation
15 days	White coloured embryos showed initial enlargement about 3 - 4 times of the original size and the elongation of root-shoot axis was more pronounced.
30 days	Green coloured shoot emerged and developed to a length of 1.0 cm and root to a length of 1 - 2 cm. Haustorium started to develop.
45 days	Shoot length increase to 2 - 3 cm and root length to 3 - 4 cm. Size of the haustorium increased.
60 days	The embryo developed into a plantlet, the green shoot having 3 - 4 cm length. Number of shoots increased, each plantlet having more than two roots.

The embryos of both species responded more satisfactorily on MS basal medium than the other two media tested.

Table 9. Response of *C. thwaitesii* embryos at different periods of culture in MS basal medium.

Period	Observation
15 days	White coloured embryos enlarged 2 - 3 times of the original size and a small shoot pole (0.3 cm) and root pole (0.2 cm) emerged out.
30 days	The embryo produced a shoot of an average length of 2 cm and a root of 3.5 cm. Towards the lateral side of the developing embryo, a small protuberance developed.
45 days	Shoot and root developed into 3.5 cm and 4.5 cm respectively. Additional roots also started to develop. The size of the haustorium increased.
60 days	The shoots attained a length of 4.5 cm. Additional roots developed. The haustorium developed into a pale green sac-like structure.

4.2. BROWNING OF TISSUES

As in other palms, the browning of tissues during the early period of incubation was high. Phenolic exudates were abundant suppressing tissue growth, especially when collar regions and shoot tips were cultured. The leachates covered the medium and made it black. As to embryo explants, browning was relatively less. Maintaining cultures in darkness was not effective.

To minimise the browning problem, various methods were tried. Addition of charcoal with the medium controlled browning to some extent. Combined effect of charcoal and polyvinylpyrrolidone (PVP) was found satisfactory (Table. 10). The most effective method was frequent transfer of explants to fresh medium by time interval. During subculture, old media attached to the explants was removed thoroughly to get rid of all exudates.

Table. 10. Effect of pre-treatments for alleviating phenolic oxidation from explants

Explant pre-treatment	Degree of phenolic exudation and medium discolouration		% of survival of explants after 10 days	
	Ch	Ct	Ch	Ct
1. Control (light)	++++	++++	0	0
2. Suspended in antioxidant solution immediately after excision for 5 minutes	++++	++++	4	1
3. Kept under running tap water for 1 hour prior to sterilization and in sterile distilled water for 30 minutes after sterilization	+++	+++	6	4
4. Incorporation of 0.5 mg/l PVP and charcoal in the medium	++	++	20	10
5. A combination of pre-treatment 3 and 4 and frequent transfers to fresh medium	+	+	40	30

+ signs indicate the extent of browning. 20 explants were employed per treatment
Ch - *C. hookerianus*, Ct - *C. thwaitesii*

4.3. CALLUS STUDIES

Regeneration studies were made with the aim of evaluating the efficacy of immature embryos of *C. hookerianus* and *C. thwaitesii* in inducing callus and subsequent regeneration. The frequency of response, morphology of the callus and proliferation were determined by the type of growth regulators, concentrations and combinations of hormones used in the medium (Tables 11 and 12).

For the establishment of callus and subsequent regeneration, the embryos of both the species of *Calamus* were inoculated on MS medium supplemented with different concentrations of auxins (NAA and 2, 4-D) either singly or in combination with cytokinins (BAP or KIN). Effect of different growth regulators on callus induction is as follows:

4.3.1. MS + 2, 4-D

For the induction of callus, embryos were cultured on MS medium fortified with different concentrations of 2, 4-D (0.5 - 3.0 mg/l). Lower concentration of 2, 4-D (0.5 mg/l) was not effective for callus induction in both species. A small amount of callus initiation was observed on embryos of *C. hookerianus* after an incubation period of 30 - 40 days on MS medium containing 1.0 or 2.0 mg/l 2, 4-D and the callus was non-organogenic. The immature embryos of *C. thwaitesii* needed 40 - 50 days for callus development and the response of the callus was similar to that of *C. hookerianus* (Figs. 5A & 5B). However, a higher concentration of 2, 4-D (3.0 mg/l) produced maximum amount of callus, which was also non organogenic in both species.

4.3.2. MS + NAA

MS medium supplemented with varying concentrations of NAA (1.0 - 2.0 mg/l) was not effective to produce callus. The calli obtained was white with spongy texture which upon subculture turned brown and eventually dead in both species (Figs. 5C & 5D). The browning of the tissue was more in *C. thwaitesii* than in *C. hookerianus*.

4.3.3. Effect of auxin – cytokinin combination

To study the combined effect of an auxin and cytokinin, combinations with an auxin, 2, 4-D and cytokinins either BAP or KIN was used for the induction of callus from immature embryos.

4.3.4. MS+2, 4-D + BAP

The combined effect of high auxin and low cytokinin concentration was found to be congenial for callus induction and proliferation in both species of *Calamus*. Even at low concentration of BAP (0.5 mg/l) along with 2, 4-D (3 mg /l) callusing occurred with 70 percent frequency, within a period of 35 - 40 days in *C. hookerianus*; *C. thwaitesii* needed 45 to 55 days to obtain 50 % response frequency.

The callus was creamy in colour and later became friable. The callus showed a high rate of proliferation during subculture.

Combination of 2, 4-D + BAP was found superior to all other combinations tested in both species. The embryos inoculated in medium containing 2, 4-D (3.0 mg/l) and BAP

(0.5 mg/l) produced organogenic white friable callus with high regeneration potential (Figs. 5E – 5F).

Fig. 5. Induction of different types of callus from immature embryos

- Fig 5A Nature of organogenic callus obtained from immature embryos of *C. hookerianus* and *C. thwaitesii*
- Fig 5B *C. hookerianus* embryos cultured on MS medium fortified with 2, 4-D (3.0 mg/l)
- Fig 5C White spongy callus induced on MS medium fortified with NAA (1.0 - 2.0 mg/l)
- Fig 5D *C. thwaitesii* - browning of callus induced on MS + NAA(1.0 - 2.0 mg/l)
- Fig. 5E-5F Organogenic callus induced in *C. hookerianus* and *C. thwaitesii* on MS media supplemented with 2, 4-D (3.0mg/l) and BAP (0.5 mg/l)
- Fig 5G-5H Non organogenic creamy and spongy callus induced in *C. hookerianus* and *C. thwaitesii* MS medium supplemented with 2, 4-D (1.0 - 3.0 mg/l) and KIN (0.5 - 1.5 mg/l)

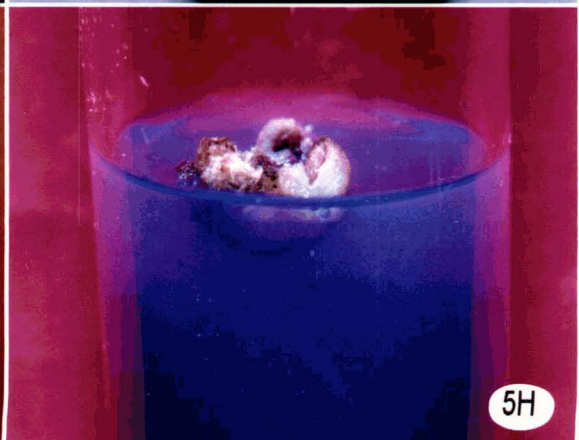
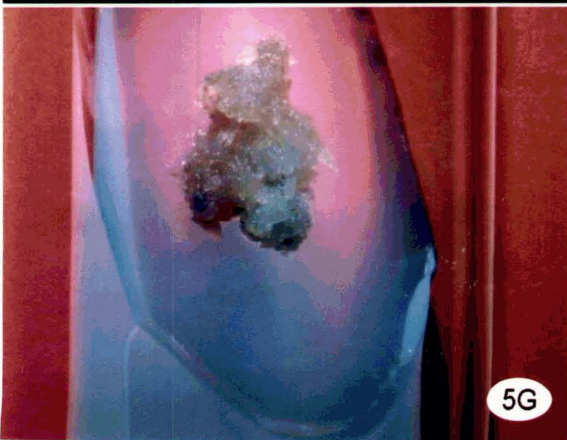
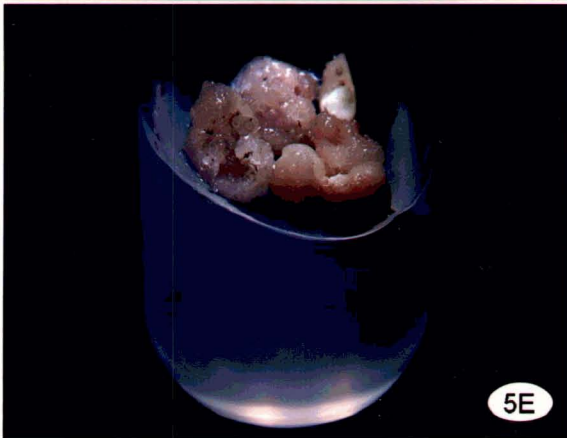
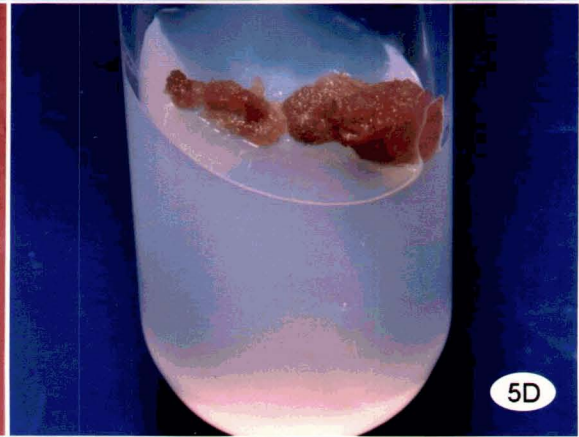
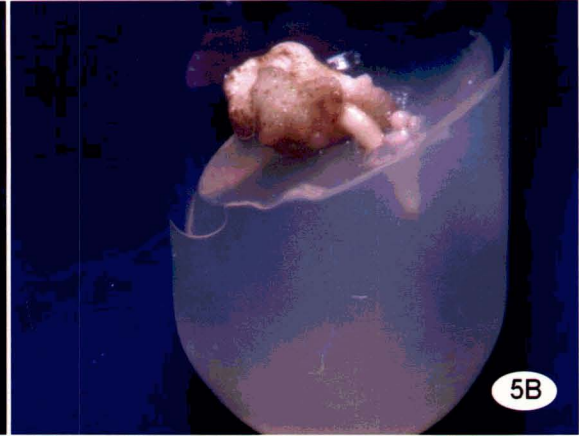


Table 11. Effect of 2, 4-D, NAA, KIN and BAP on induction of callus from immature embryos of *C. hookerianus*

Growth regulators (mg/l)				% of response	Amount of callus	Nature of response	Days taken for response
2, 4-D	NAA	KIN	BAP				
0.5					0	No response	
1.0				30	+	Non-organogenic callus	40 days
2.0				40	++	Non-organogenic callus	30 - 40 days
3.0				60	+++	Non-organogenic callus	30 - 40 days
	1			30	+	White spongy non-organogenic callus	35 days
	2			40	+	White spongy non-organogenic callus	40 - 45 days
0.5			0.1		0	No response	
1.0			0.1	30	+	Non-organogenic callus	35 - 45 days
2.0			0.5	50	+++	Non-organogenic cream coloured callus	30 - 40 days
3.0			0.5	70	++++	Organogenic white friable callus	35-40 days
1.0		0.5		30	+	Creamy and spongy non-organogenic callus	45 - 50 days
2.0		1.0		40	+	Creamy and spongy non-organogenic callus	40 - 45 days
3.0		1.5		50	++	Creamy and spongy non-organogenic callus	40 - 50 days

Data represents an average of 10 replicates

Basal medium MS + 3% Sucrose + 0.8 % Agar. 0= No callus, + = Very slight, ++ = Little, +++ = Moderate, ++++ = Profuse

Table 12. Effect of 2, 4-D, NAA, KIN and BAP on induction of callus from immature embryos of *C.thwaitesii*

Growth regulators (mg/l)				% of response	Amount of callus	Nature of response	Days taken for response
2, 4-D	NAA	KIN	BAP				
0.5				NIL	0	No response	
1.0				20	+	Non-organogenic callus	40 - 50 days
2.0				40	++	,,	,,
3.0				70	+++	,,	,,
	1.0			40	++	White spongy non organogenic callus, callus turned brown	30 - 40 days
	2.0			50	++	,,	,,
0.5			0.1	NIL	0	No response	
1.0			0.1	NIL	0	,,	
2.0			0.5	40	++	Non-organogenic callus	40 - 45 days
3.0			0.5	50	++++	Organogenic callus	45 - 55 days
1.0		0.5		20	+	Creamy non-organogenic callus	40 - 50 days
2.0		1.0		40	++	,,	35 - 45 days
3.0		1.0		40	++	,,	40 - 50 days

Data represents an average of 10 replicates. 0= No callus, += Very slight, ++= Little, +++= Moderate, ++++= Profuse. Basal medium - MS + 3% Sucrose + 0.8 % Agar.,

4.3.5. MS+2, 4-D+KIN

Different concentrations of KIN (0.5 to 1.5 mg/l) in combination with 2, 4-D (1.0 – 3.0 mg/l), were also tested for callus induction. The callus induced was a mixture of slightly creamy, spongy and non-organogenic in *C. hookerianus* (Fig. 5G & 5H). In *C. thwaitesii* the creamy, non-organogenic callus turned brown and died.

4.3.6. Indirect organogenesis

Indirect organogenesis was established by sub culturing the calli obtained on MS medium with 2, 4-D + BAP. To establish indirect organogenesis the primary organogenic calli derived from embryos of both species of *Calamus* were cultured on MS medium supplemented with varying concentrations of auxins and cytokinins.

The calli developed from embryos on 2, 4-D + BAP medium showed regeneration potential when subcultured on BAP alone or in combination with 2, 4-D. BAP at 3.0 mg/l was found effective in shoot multiplication, but the rate of growth was slow. However, a combination of BAP (3.0 mg/l) and 2, 4-D (0.5 mg/l) showed maximum shooting response and all the shoots showed elongation (Table 13. and Fig. 6A).

The response of callus to growth hormones was slightly high in *C. hookerianus* than in *C. thwaitesii*. Browning of callus was more in *C. thwaitesii* than in *C. hookerianus* (Figs. 6B – 6F).

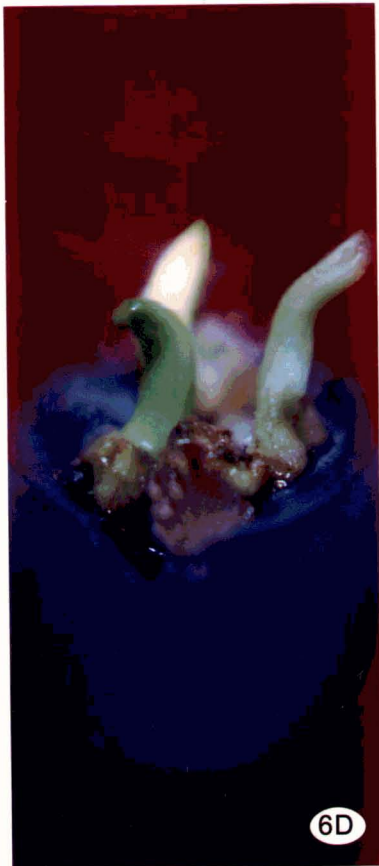
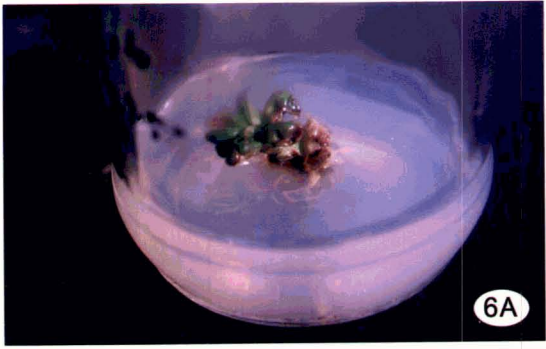
Fig 6 Callus regeneration

Fig 6A *C. hookerianus*-Differentiation of greenish structures on MS medium supplemented with BAP (3.0mg/l) and 2, 4-D(0.5 mg/l)

Fig 6B Multiple shoots from callus on MS supplemented with BAP(3.0mg/l) 2, 4-D(0.5mg/l)

Fig 6C *C. hookerianus* multiple shoots from callus on MS medium supplemented with BAP (3.0mg/l) and 2,4-D (0.5mg/l)

Fig 6D - Fig 6F. *C. thwaitessi*. Multiple shoots from callus on MS medium supplemented with BAP (3.0mg/l) and 2,4-D (0.5mg/l).



The combination of KIN (3.0 – 4.0 mg/l) and 2, 4-D (0.01 – 0.1 mg/l) was not effective in inducing multiple shoots. The combination of KIN (0.5 – 4.0 mg/l) and NAA(0.01 - 0.5 mg/l) was not effective in inducing well developed multiple shoots, even though small greenish shoots differentiated in both species (Tables 14 and 15).

Multiple shoots were subcultured in the same medium for further shoot initiation and multiplication.

Table 13. Effect of various concentrations of BAP and 2, 4-D on organogenesis

Growth hormones (mg/l)		% of response	No. of shoots per culture	Morphological response
BAP	2, 4-D			
0.5	-	Nil	-	No response
1	-	Nil	-	
1.5	-	40	-	Slow callus growth
2	-	60	-	Vigorous with small green globular structures
2.5	-	60	-	”
3	-	70	-	”
3.5	-	70	-	”
4	-	60	-	”
4.5	-	60	-	”
5	-	30	-	Slow callus growth
0.5	0.01	20	-	No response
0.5	0.05	30	-	”
1	0.01	30	-	Slow callus growth developed into a tightly packed clump
1	0.05	40	-	”
1.5			-	
2	0.1	40	-	Slow callus growth
2	0.5	40	-	”
2.5	0.1	50	2	Small green shoot buds
2.5	0.5	60	3	”
3	0.5	80	6	Well developed plantlets
3.5	0.5	70	4	”
4	0.5	60	3	Small green shoot buds

4	0.5	60	3	”
4.5	0.5	40	2	”
5	0.5	40	1	Unorganised mass

Table 14. Effect of various concentrations of KIN and 2, 4-D on organogenesis

Growth hormones (mg/l)		% of response	Morphological response
Kinetin	2, 4-D		
0.5	0.01	Nil	Slow callus growth
1.0	0.01	Nil	”
1.5	0.01	Nil	Small greenish structures
2.0	0.01	20	”
2.5	0.05	30	”
3.0	0.05	40	”
3.5	0.1	50	Slow callus growth with greenish globular structure
4.0	0.1	40	”

Table 15. Effect of various concentrations of KIN and NAA on organogenesis

Growth hormones (mg/l)		% of response	Morphological response
Kinetin	NAA		
0.5	0.01	Nil	No callus growth
0.5	0.05	Nil	”
1.0	0.01	Nil	”
1.0	0.05	5	Slow callus growth
1.5	0.01	10	”
1.5	0.05	10	”
2.0	0.1	20	”
2.0	0.5	20	”
2.5	0.1	40	Small greenish globular structure
2.5	0.5	50	”
3.0	0.5	60	Small greenish shoots
3.5	0.5	40	”
4.0	0.5	30	Greenish globular structures

4.3.7. Somatic embryogenesis

Pale greenish yellow calli developed from the embryos on MS medium fortified with 2, 4-D and BAP were used to establish embryogenic potential on both *C. hookerianus* and *C.thwaitesii*. These calli, as described earlier, were characterised by their highly friable and hydric nature in *C. thwaitesii* and have proved its organogenic potential when subcultured on fresh medium of the same composition. The primary calli and the serially subcultured calli were transferred to different media combinations to examine their potential for somatic embryogenesis as follows.

4.3.8. 2, 4-D + BAP

The primary callus upon transfer to MS medium supplemented with 2, 4-D (2.0mg/l) and BAP (0.1 mg/l) produced massive amount of white friable callus. The potential of somatic embryogenesis was studied using this white friable callus. The ability of the callus to become embryogenic declined gradually with an increase in the concentration of 2, 4-D. The continued presence of 2, 4-D in the medium exerted an inhibitory effect on embryoid formation.

The calli obtained from the embryo explants of *C. hookerianus* after inoculation into a lower auxin-containing medium (0.5 - 0.1 mg/l) did not differentiate embryogenic tissue. The calli consisted of large spongy tissues which later developed into a tightly packed clump. Repeated subculture to media with lower concentrations or free of any hormone failed to respond.

The white friable callus differentiated from the embryos of *C. thwaitesii* when transferred to medium with lower concentration of 2, 4-D (0.5 mg/l), developed nodular structures or friable yellowish calli (Figs. 7A – 7D). By transferring these friable calli to media containing a lower concentration of auxin and auxin free media, at an interval of 3 - 4 weeks, differentiated into globular embryos. When the globular embryos and embryogenic clumps were further transferred to suspension of hormone free ½ MS medium with half strength concentration of sucrose under dark, for a period of 3 - 4 weeks, developed into club-shaped and banana shaped (elongated) stages of embryos and were characterised by shoot and root differentiation (Fig. 7E). Some of the globular embryos showed maturation in the hormone free MS solid medium.

Development of somatic embryos was not synchronous even within the same cluster (Fig. 7E). Splitting up the cluster into respective stages of growth was generally difficult as the individual units ceased to develop further. Throughout subculturing of the calli, its browning and mortality resulted when they were split up. Hence, each cluster was transferred as a whole unit with different stages of maturation.

4.3.9. Encapsulation studies

Encapsulation studies in both species of *Calamus* were carried out using immature embryos and cotyledonary embryoids in *C. thwaitesii*, derived from embryogenic callus cultures. Somatic embryos with distinct shoot and root poles were the most suitable for encapsulation. Embryos that were encapsulated in 3 percent sodium alginate showed high rate of conversion. Synseed conversion frequently decreased with an increase in sodium alginate concentration above 3 percent (Fig. 8) Beads of uniform

Fig 7. Induction of somatic embryogenesis from immature embryos of *C. thwaitesii*

Fig 7A. Proliferation of immature embryo of *C. thwaitesii* on MS medium supplemented with BAP (1.0 mg/l) and 2, 4-D (2.0 mg/l)

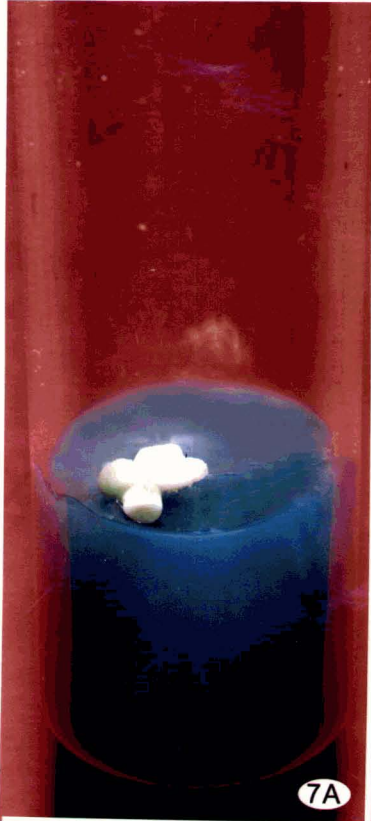
Fig 7B. Embryogenic callus on MS medium supplemented with BAP (0.1 mg/l) and 2, 4-D (1.0 mg/l)

Fig 7C. Callus with high embryogenic potential on MS medium supplemented with BAP (0.1 mg/l) and 2, 4-D (0.5 mg/l)

Fig 7D. } Appearance of white globular and elongated structured on hormone free
Fig 7E. } half MS medium

Fig 7F. Synseed of *C. thwaitesii*

se – somatic embryo



size , shape and correct firmness were obtained when 3 percent sodium alginate was used (Fig. 7F). At higher concentration of sodium alginate (4 - 5%), beads were hard and shapeless.

The concentration of complexing agent $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ also affected the frequency of conversion of encapsulated embryos. Of the different concentrations of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ tested (25, 50, 75 mM), 50 mM was found suitable for the beads with 80% response of conversion (Fig. 9).

The synseeds made in 3 percent sodium alginate exposed to 50 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ started to germinate within 10 days on hormone free MS medium

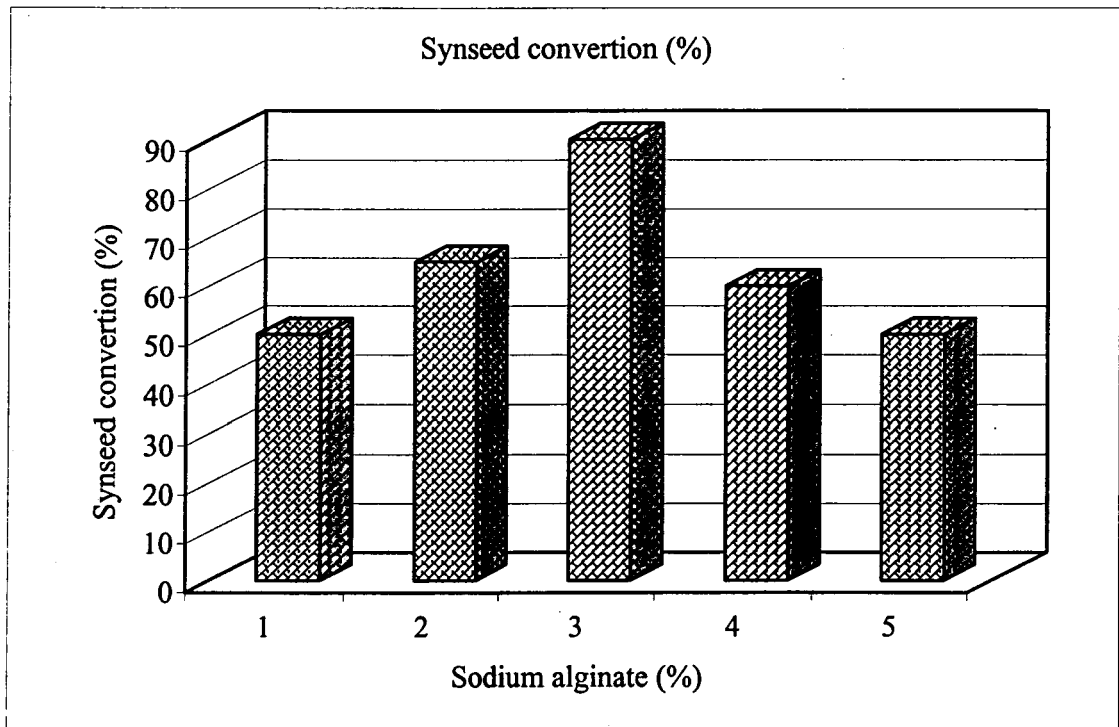


Fig. 8. Effect of sodium alginate in conversion of synseeds of both species of *Calamus*

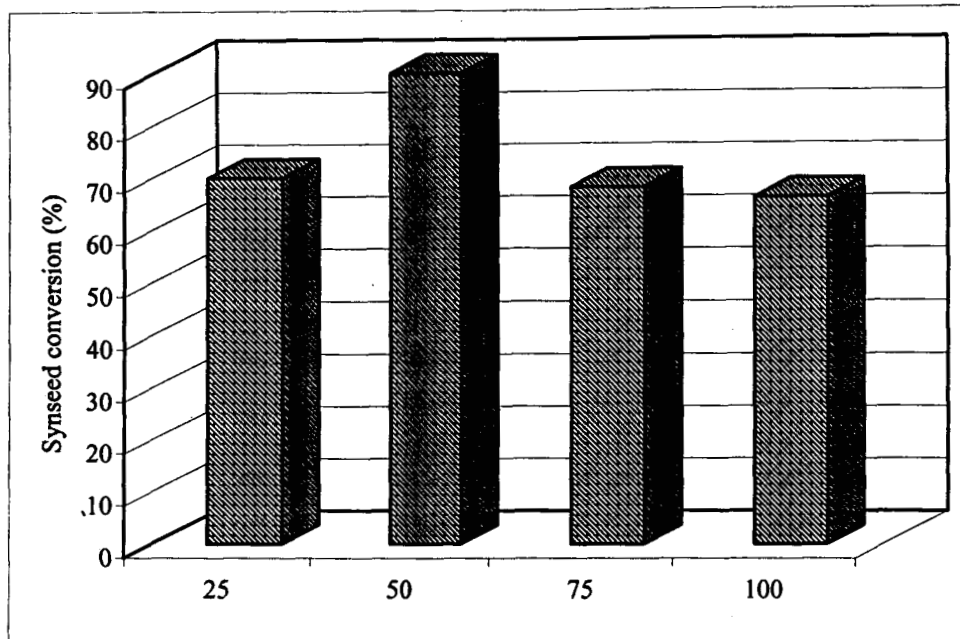


Fig. 9. Effect of calcium chloride in conversion of synseeds of both species of *Calamus*

4.4. DIRECT ORGANOGENESIS

For the induction of direct organogenesis, collar region and shoot apex of the *in vitro* and nursery grown seedlings and immature embryos of both *C. hookerianus* and *C. thwaitesii* were cultured on MS medium fortified with various growth regulators (BAP, KIN, NAA, 2, 4-D, IAA and IBA) either individually or in combinations. Establishment of contamination free cultures was difficult in the case of explants collected from nursery grown seedlings.

4.4.1. Shoot tip culture

In vitro shoot tip explants of *C. hookerianus* and *C. thwaitesii* when cultured on various concentrations of BAP (0.5 – 3.5 mg/l) and 2, 4-D (0.1 – 0.5 mg/l) responded in a

Fig 10 Induction of multiple shoots

Fig 10A. Response of shoot apex of *C. thwaitesii* after 15 days

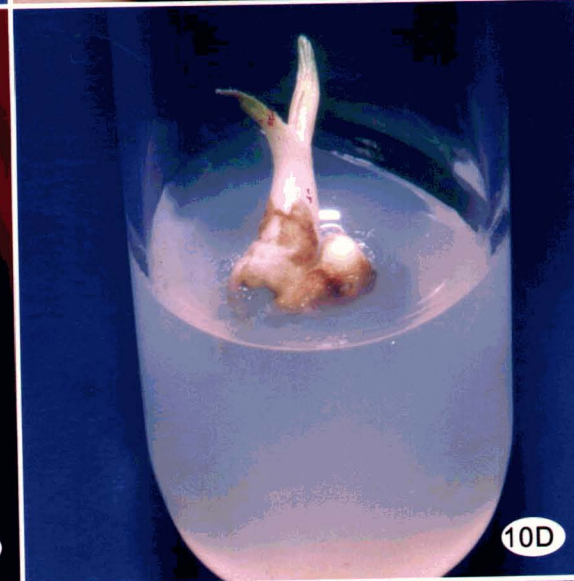
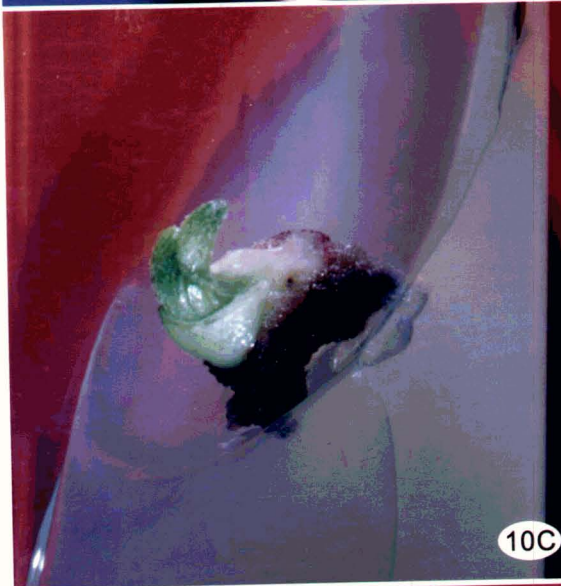
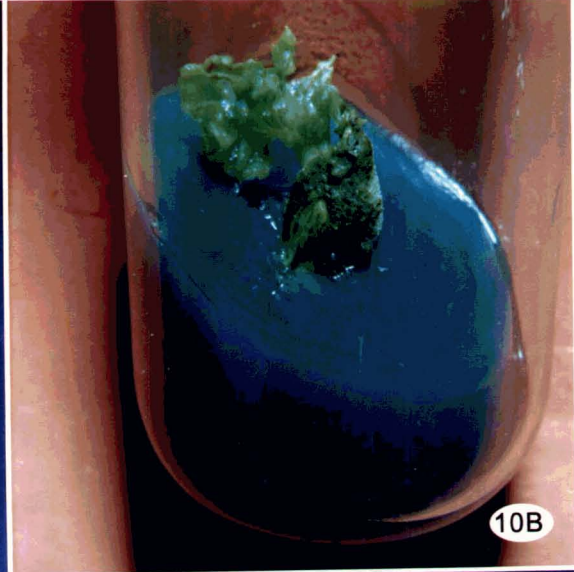
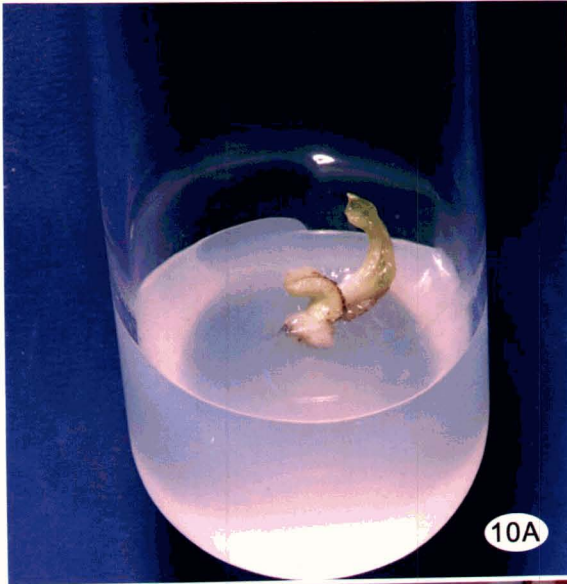
Fig 10B. Induction of multiple shoots in *C. thwaitesii* on MS medium containing BAP (3.5 mg/l) and 2, 4-D (0.1 mg/l)

Fig 10C. Induction of multiple shoots in *C. hookerianus* from collar region

Fig 10D. Plantlet subcultured on same medium for elongation

Fig 10E. *C. thwaitesii* multiple shoots form collar region on medium fortified with BAP (3.5 mg/l) and 2, 4-D (0.1 mg/l)

Fig 10F. Plantlet subcultured on same medium for elongation



and 2, 4-D (0.1 mg/l) concentrations. Lower concentrations of BAP resulted in the enlargement of explants in both species where as higher concentration culminated in the browning of the explant which after a month became dead (Figs. 10A – 10B).

4.4.2. Collar region culture

High rate of contamination and severe browning made a lot of problems in the culturing of collar region from nursery grown seedlings of *C. hookerianus* and *C. thwaitesii*. The collar regions excised from *in vitro* grown seedling of both species responded when inoculated on medium supplemented with BAP (4.0 mg/l) and 2, 4-D (0.1 mg/l). An average of 3 shoot buds initiated in the case of *C. hookerianus*, but the shoot elongation was delayed and it remained stunted. The collar region of *C. thwaitesii* in a different BAP (3.5 mg/l) and 2, 4-D (0.1 mg/l) concentrations produced 2 - 3 shoots and upon subculturing in the same medium developed into shoots with more leaves (Figs. 10C – 10F).

4.4.3. Immature embryo culture

The immature embryos of both species were cultured on MS medium supplemented with auxins and cytokinins and the response of the explant to different combinations are described in the following headlines.

4.4.3.1. MS + BAP

To study the effect of BAP on shoot multiplication, MS medium supplemented with various concentrations of BAP (0.5 mg/l - 5.0 mg/l) was used. Among the various

concentrations of BAP tested, 3.5 mg/l was effective but the number of shoots were less. The shoots were initiated after three weeks of culture. Higher concentrations of BAP (4.0 - 5.0 mg/l) caused few number of shoot initiation which ceased growth in both the species (Figs. 11A – 11B).

4.4.3.2. MS + KIN

The immature embryos of both species of *Calamus* when inoculated in a medium fortified with various concentrations of kinetin (1.0 - 4.0 mg/l) did not produce multiple shoots. The embryos enlarged many times and after a month turned brown and finally ceased growth in both species.

4.4.3.3. MS + BAP + KIN

A combination of BAP + KIN was tested to observe the direct organogenic potential from different explants. Concentration of BAP at 2.0 mg/l and KIN at 1.0 mg/l resulted in the production of shoots but the result was almost the same as that obtained in the medium with BAP alone. Low concentration of BAP (1.0 mg/l) in combination with KIN (1.0 mg/l) was not much effective in inducing multiple shoots.

4.4.3.4. Auxin and cytokinin interaction

More than the absolute concentration of the plant growth regulators, the balance between an auxin and cytokinin is important to initiate growth and differentiation in tissue culture.

4.4.3.5. MS + BAP + 2, 4 - D

MS medium with lower concentrations of BAP (1.0 – 2.0 mg/l) and 2, 4-D (0.1 mg/l) did not influence the development of multiple shoot.

4.4.3.6. Shoot Development

Analysis of variance (ANOVA) indicated that the effect due to BAP turned out significant, while the effect due to 2, 4-D and interaction between BAP and 2, 4-D turned out non-significant (Table 16.). Duncans multiple range test (DMRT) revealed that BAP (3.5 mg/l) and 2, 4-D produced the maximum number of shoots (Table 17).

The mean number of shoots obtained at 3.5 mg/l BAP for *C. hookerianus* and *C. thwaitesii* were 2.85 and 3.90 respectively. (Table 17). The mean number of shoots obtained at 0.5 mg/l 2, 4 -D for *C. hookerianus* and *C. thwaitesii* were 1.5 and 2.0 respectively (Table 18.). The number of shoots produced at 3.5 mg/l BAP and 0.5 mg/l 2, 4-D was higher than that obtained from other concentration levels of BAP and 2, 4-D. Hence the combination of BAP (3.5 mg/l) and 2, 4 -D (0.5 mg/l) was found to be the best when compared to other combinations (Fig. 11C –11F).

Table 16. Analysis of variance of *C. hookerianus* and *C. thwaitesii* on the number of shoots in square root scale.

Source	df	Mean sum of squares		F-ratio	
		Ch	Ct	Ch	Ct
BAP	4	4.6220	8.9700	23.5090**	176.0230**
2, 4-D	1	0.0004	0.0306	0.0020 ^{ns}	0.6010 ^{ns}
BAP& 2, 4-D	4	0.0315	0.0080	1.6020 ^{ns}	0.1570 ^{ns}
Error	90	0.1970	0.0509		
Total	100				

** Significant at p=0.01

Ch - *C. hookerianus* Ct - *C. thwaitesii*

^{ns} Non significant

Table 17. Mean number of shoots corresponding to BAP at different concentrations of *C. hookerianus* and *C. thwaitesii*

BAP (mg/l)	Mean Number of shoots	
	Ch	Ct
0.5	0.00 ^a	0.00 ^a
1.0	0.35 ^a	0.30 ^a
2.0	1.60 ^b	1.70 ^b
3.0	2.50 ^c	3.75 ^c
3.5	2.85 ^c	3.90 ^c

• Values superscribed by the same letter do not differ significantly

Ch - *C. hookerianus* Ct - *C. thwaitesii*

Table 18. Mean numbers of shoots corresponding to 2, 4-D at two concentrations

2, 4-D (mg/l)	Mean	
	Ch	Ct
0.1	1.40	1.88
0.5	1.50	2.00

Ch - *C. hookerianus* Ct - *C. thwaitesii*

4.4.3.7. Response Frequency

ANOVA on response frequency obtained for *C. hookerianus* and *C. thwaitesii* indicated that the effect due to BAP and due to 2, 4-D turned out significant. (Table 19).

Pairwise comparison test showed that for both species 3.5 mg/l BAP differed significantly from all other concentration levels of BAP (Table 20). Similarly it was found that for both species, 0.5 mg/l 2, 4-D level differed significantly from 0.1 mg/l 2, 4-D (Table 21). The mean response frequency at 3.5 mg/l BAP and 0.5 mg/l 2, 4-D had

maximum response frequency when compared to other combinations of BAP and 2, 4-D tested.

Table 19. Analysis of variance on response frequency

Source	Df	Mean of sum of squares		F-Ratio	
		Ch	Ct	Ch	Ct
BAP	4	1350.00	3900.00	48.808**	152.75**
2, 4-D	1	400.00	100.00	14.462**	3.917**
Error	94	27.600	25.532		
Total	100				

**Significant at P=0.01.

Ch - *C. hookerianus*

Ct - *C. thwaitesii*

Table 20. Mean response frequency corresponding to different concentrations of BAP

BAP(mg/l)	Mean response frequency	
	Ch	Ct
0.5	70.00 ^a	40.00 ^a
1.0	70.00 ^a	40.00 ^a
2.0	75.00 ^b	50.00 ^b
3.0	75.00 ^b	65.00 ^b
3.5	90.00 ^c	70.00 ^c

• Values superscribed by the same letter do not differ significantly in each column

Ch - *C. hookerianus* Ct - *C. thwaitesii*

Table 21. Mean response frequency corresponding to two concentrations of 2, 4-D

2, 4-D mg/ l	Mean response frequency	
	Ch	Ct
0.1	74.00 ^a	52.00 ^a
0.5	78.00 ^b	54.00 ^b

• Values superscribed by the two letters do not differ significantly in each column

Ch - *C. hookerianus* Ct - *C. thwaitesii*

4.4.3.8. Effect of BAP+NAA

MS medium with different concentrations of BAP (0.5 – 4.0 mg/l) and NAA (0.01 – 1.0 mg/l) did not influence shoot multiplication. However, callus formation was observed at the base of the explant when NAA concentration was increased above 1.0 mg/l.

4.4.3.9. Effect of KIN+NAA

A combination of KIN and NAA at different levels was also tested to observe the response and induction of shoots. Multiple shoot induction was not noticed and the developed shoots showed a stunted nature and the growth rate was very low when the hormones were supplied with KIN (3.0 mg/l) and NAA (0.5mg/l).

Higher concentration at KIN (4.0 mg/l) and NAA at (1.0 mg/l) showed a reduction in the number of shoots and lower concentration of hormones KIN (2.0 mg/l) + NAA (0.1 mg/l) did not make any response in the callus. In the latter case, the explants showed sign of browning and turned necrotic after a period of 30 days.

In conclusion, it was observed that an enhanced rate of direct shoot multiplication was obtained for both species of *Calamus* with the combination of BAP at 3.5mg/l and 2, 4-D at 0.5 mg/l.

4.5. SUB-CULTURE / ROOT INDUCTION

In vitro multiple shoots developed on MS medium with BAP (3.5 mg/l) and 2, 4-D (0.5 mg/l) were subcultured to the medium for further multiplication. The regenerated shoots could be rooted in MS supplemented with 0.5 mg/l of IBA (Fig. 11G & 11H).

Plantlets with well developed shoots and roots were subjected to hardening and transferred to garden plots (Figs. 12A & 12B) using standard methods described in materials and methods.

The above results indicate that it is possible to establish rattan cultures either by using embryos or tissues from the collar region of seedlings. Complete plantlets can be obtained by this methods. It will be necessary, however, to refine and scale up the procedures, so that tissue culture becomes a viable method of mass-production of the rattans.

Fig. 11. Direct organogenesis from immature embryo explant and root induction

- Fig. 11A.] Formation of green structures from immature embryos of *C. hookerianus*
&
Fig. 11B] on MS medium with BAP.
- Fig. 11C. Development of multiple shoots from immature embryos of *C. thwaitesii*
on MS medium fortified with BAP (3.5mg/l) and 2, 4-D (0.5mg/l)
- Fig. 11 D. Plantlet subcultured on the same medium for elongation
- Fig. 11 E. *C. hookerianus* Induction of root on MS medium supplemented with
(0.5mg/l)
- Fig. 11 F. *C. thwaitesii*- Induction of root on MS medium supplemented with IBA
(0.5mg/l).

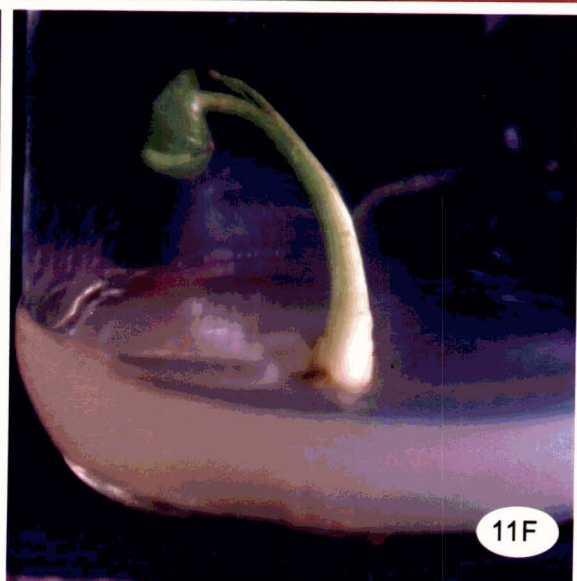
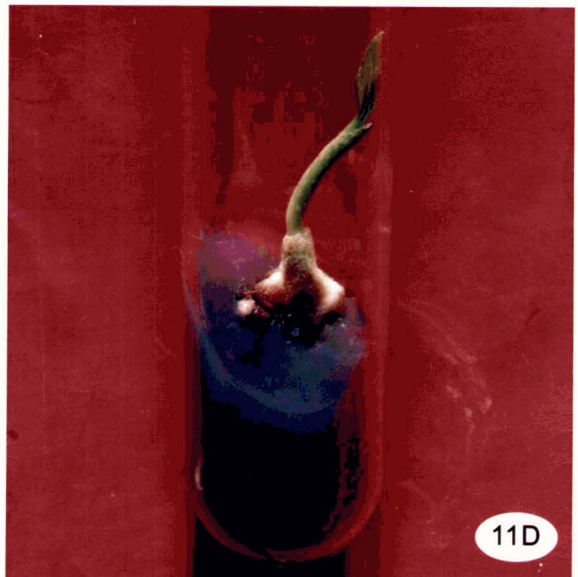
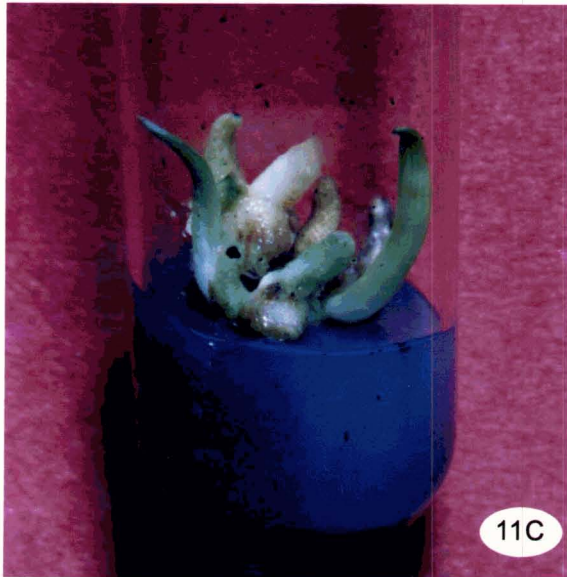


Fig. 12. Hardening of plantlets

Fig. 12A. Hardened plantlets of *C. hookerianus*

Fig. 12B. Hardened plantlets of *C. thwaitesii*



12A



12B

DISCUSSION

21 60

5. DISCUSSION

In the past, germplasm conservation was done through seed storage. For species, where the moisture content of the embryo can be reduced to about 5-7%, long term seed storage at below freezing temperature is possible. The moisture content of rattan seed is usually very high and long term seed storage is not possible (Bingshan *et al.*, 2000). Again, seeds of most of the species may not be available in large quantity from forests because plants were cut before flowering and fruiting.

Tissue culture based mass propagation method could produce large number of seedlings in a short time and can solve the problem of seedling shortage. Therefore, rattan tissue culture became an important area of forestry research. In the present work, different kinds of regeneration have been established on two commercially important species of rattans of Western Ghats viz., *C. hookerianus* and *C. thwaitesii*.

The major problems in palm tissue culture are difficulties in acquiring explants, and microbial contamination free culture, browning of explants due to phenolic exudation, delayed callus induction, low somatic embryogenesis and poor germination of regenerated plantlets (Reynolds, 1982).

Standardisation of a viable protocol for clonal propagation through *in vitro* approaches opens up tremendous possibilities of meeting the requirements for quality planting materials and breaking down productivity barriers.

5.1. EXPLANTS

The choice of explant is pivotal in tissue culture of mature tree species. The explants used in the present studies included embryos from immature fruits, collar region and shoot apex of nursery grown and *in vitro* seedlings. Use of immature embryos as a good source of explant in rattan tissue culture was reported by many workers (Patena *et al.*, 1984; Yusoff *et al.*, 1985; and Yusoff, 1989; Calinawan and Halos, 1988; Padmanabhan and Ilangovan, 1989; Dekkers and Rao 1989; Chuthamas *et al.*, 1989; Goh, 1997; Zhang, 1993; Bingshan, 1997 and Valsala and Muralidharan, 1998). Collar region of seedlings germinated *in vitro* has been used as explant by Chengji and Jiankui (1991); Zhiying and Kuan (1995) and Valsala and Muralidharan (1999). Investigators like Umali-Garcia (1985), Bingshan (1997) and Valsala and Muralidharan (1999) have also used shoot tips as explants.

Nair *et al.* (1999) have stated that the commonly used explants from juvenile coconut palms included immature inflorescences from apices, tender leaves, leaf bases and the leaf sheath. Immature embryos were also used as explants. The use of shoot tip and immature inflorescence have been reported by Bhaskaran and Smith (1992), Veramendi and Navarro (1996 and 1997) in date palm.

In the present study, when embryos were used, the scaly pericarp and fleshy sarcotesta were first removed, leaving the endosperm. The seeds were then subjected to sterilization procedures. The disinfection of all explants were done using 0.5 per cent mercuric chloride solution for 7-10 minutes. Bingshan *et al.* (2000) also sterilised the seeds with 0.1% mercuric chloride solution for 10 to 20 minutes for 3 times.

Padmanabhan and Ilangovan (1989) used one per cent mercuric chloride solution for 10 minutes in their work on embryo culture of *C. rotang*. Yusoff *et al.* (1985) surface sterilised the hard seeds of *C. manan* by first washing them in several rinses of distilled water followed by washing in 20 per cent chlorax solution for 10 minutes. The seeds were washed again in several rinses of sterile distilled water. In contrast, Patena *et al.* (1984) sterilised the newly extracted seeds in hypochloric solution for 30 minutes. Calinawan and Halos (1988), on the other hand, first soaked the seeds in 10 per cent chlorax solution and then washed them with sterile distilled water. The embryos were then excised and soaked in 5 per cent chlorax solution, followed by rinsing in sterile distilled water. Valsala and Muralidharan (1999) reported the use of 0.1% (w/v) mercuric chloride solution for 10 minutes for the surface sterilization of *C. andamanicus*, *C. thwaitesii* and *C. pseudotenius* fruits for effective embryo extraction.

In the present study, the shoot apex and the collar region from nursery grown seedlings were excised after washing with a detergent and after surface sterilization by the method employed for fruits. The outer leaves were removed with a sharp knife, leaving only the shoot meristem block. A similar method has been reported by many investigators. They reported the use of 1% sodium hypochlorite solution containing one drop of Tween 20 per 1000 ml for surface sterilization.

5.2. MEDIA

In the present investigation, in all the experiments the basal medium used was MS medium (Murashige and Skoog, 1962). The use of modified MS basal medium in either solid or liquid form has been reported by Yusoff *et al.* (1985); Yusoff (1989);

Padmanabhan and Ilangovan, (1989); Gunawan and Yani, (1986); Dekkers and Rao. (1989); Chuthamas *et al.* (1989) and Teo, (1990) and Valsala and Muralidharan, (1999). Other media included, de Fossard medium, Hartney and Baker (1980), reported by Calinawan and Halos (1988) and Umali-Garcia (1985), Y3 medium (Eeuwens, 1976); Chuthamas *et al.*, 1989 and Padmanabhan and Ilangovan (1989) who also used SH medium (Sochenk and Hildebrandt, 1972).

The growth regulators commonly used in the present work included BAP, 2, 4-D and IBA. These were found to be superior to KIN, NAA and IAA. Many investigators have reported the preference of BAP and 2, 4-D to other cytokinins and auxins.

5.3. BROWNING AND CONTROLLING

Browning of tissue during the early period of incubation was heavy and autocatalytic. Phenolic exudates were abundant suppressing tissue growth, especially when collar zone and shoot apex explants were cultured. Compared to *C. hookerianus*, browning was more in all explants of *C. thwaitesii* including the immature embryos. As to embryo explant, browning was relatively slight. The browning of embryo explants was limited around its edge. The leachates covered the medium and made it black. A similar observation of browning of explant and medium has made by many workers in *Calamus* and other palms (Patena *et al.*, 1984; Umali-Garcia, 1985; Yusoff, *et al.*, 1985; Gunawan and Yani, 1986; Yusoff, 1989a; Umali-Garcia and Canlas-Mendoza, 1997; Goh *et al.*, 1999; Padmanabhan and Ilangovan, 1989, 1993; Valsala and Muralidharan, 1999; Bingshan, *et al.*, 2000 and Nair *et al.*, 1999).

Charcoal and polyvinylpyrrolidone to the media minimised the degree of browning in the present investigation. The most effective method was frequent timely transfer to fresh medium. Umali-Garcia (1985), Valsala and Muralidharan (1999) and Bingshan *et al.* (2000) have also suggested the use of charcoal and polyvinylpyrrolidone in *Calamus* species. Charcoal was also used by Tisserat (1999), Rillo and Ebrt (1993) and Nair *et al.* (1999) to reduce browning of coconut embryo explants. Branton and Blake (1983) considered that activated charcoal is a necessary additive to prevent chemical killing of cells resulting from high concentrations of 2, 4-D. Gupta *et al.* (1981) with *Tectona grandis* bud cultures utilised hydrogen peroxide, ascorbic acid and soluble and insoluble polyvinylpyrrolidone to reduce blackening. Maintaining cultures in darkness was not effective in the present study. This is in accordance to the observation of Bingshan *et al.* (2000) in different species of *Calamus*.

5.4. CALLUS STUDIES

Regeneration studies made with an objective of evaluating the efficacy of culturing immature embryos of two species of *Calamus* showed that the combined effect of high auxin and low cytokinin was indispensable for callus induction, proliferation and shoot development. The collar zone swelled and formed callus on MS medium supplemented with NAA or 2, 4-D. This is in accordance with the results observed by Dekkers and Rao (1989) in *C. trachycoleus*, Yusoff (1989b) on *C. manan* and Valsala and Muralidharan (1999) on *C. andamanicus*, *C. thwaitesii* and *C. pseudotenius*.

5.4.1. Indirect organogenesis

Embryo culture in rattan has been attempted earlier by many workers (Padmanabhan and Ilangovan 1989; Dekkers and Rao 1989; Yusoff and Ahmd 1989; Chutamas *et al.*, 1989 and Valsala and Muralidharan, 1999). Cream coloured organogenic callus was obtained in the present study by culturing immature embryos in the medium supplemented with 2, 4-D (3.0 mg/l) and BAP (0.5 mg/l). This callus showed high rate of proliferation upon sub-culture. Organogenic callus induced embryos when transferred to a low auxin/high cytokinin containing medium developed, shoot. Umali-Garcia (1985) observed a comparatively faster, or earlier, positive response to callus induction when the salt strength of MS medium was doubled. Calinawan and Halos (1988), using embryos as initial explants, obtained callus after 14 days. These calli when transferred to modified de Fossard medium (Hartney and Baker, 1980) developed small shoots after 4 weeks. Earlier, Yusoff and Manokaran (1985) were able to obtain callus from cultured embryos of *C. manan*. In a subsequent study (Yusoff, 1989) it was found that the initial friable callus became nodular creamy and opaque. Later, green protuberances appeared and developed into shoots.

In the present investigation, it was observed that 2, 4-D can induce callus development in the collar zone even after the shoot expanded normally after embryo germination. A similar observation has been reported by Padmanabhan and Ilangovan (1989) in the embryos of *C. rotang* and Yusoff (1989) in *C. gamblei*. Callusing of the collar zone under the influence of hormone (2, 4-D) appears to be common to many species of *Calamus*. Many other reports in the literature also support a high productivity of the

collar region in palm embryos (Tisserat and DeMason 1980; Sudharsan 1988 and Yusoff 1989).

The present work revealed that 2, 4-D is the most effective auxin for callus induction. A similar observation has been made by Valsala and Muralidharan (1999) and Bingshan *et al.* (2000) in various species of *Calamus*.

5.4.2. Somatic embryogenesis

In vitro somatic embryogenesis potentially offers an alternative form of large scale propagation of plants (Ananthakrishnan *et al.*, 1999). This approach is used for establishing clonal lines from selected mature plants, if the genetic stability of the embryogenic calli can be assured.

The induction of somatic embryogenesis is strongly dependent on the concentrations of auxins and cytokinins used during the culture (Korac and Neskovic 1999 and Rout *et al.*, 2000). In the present experiment, the pale calli developed on medium supplemented with high 2, 4-D and low BAP was found to be the most effective for the induction of embryogenic callus from embryo explants of *C. thwaitesii*. Somatic embryogenesis has also been reported from immature embryos by Muralidharan (1994) in *C. rotang*. Goh *et al.* (1999) scientifically demonstrated the development of somatic embryos in *C. manan* for the first time from root tips.

Most of the protocols for somatic embryogenesis display the use of a strong auxin, 2, 4-D and a cytokinin in the primary culture medium which support both cell proliferation

and induction of embryoids (Cushman, *et al.*, 2000 and Tang, *et al.*, 2000). Ammirato (1983) stated that the induction of callus with embryogenic competence was acquired during the initial period of culture at high concentrations of auxins. Teixeira *et al.*, 1994 perceive that in oil palm addition of 2, 4-D is essential for callus induction and for the maintenance of embryogenic tissues. The findings in the present work is in consonance with the work of authors mentioned above and also with that of Bhaskaran and Smith (1992) in *Phoenix dactylifera*, Veramendi and Navarro (1996) in the same species and Nair *et al.* (1999) in coconut.

5.4.2. Synseed

Synthetic seed technology is designed to combine the advantages of clonal propagation with those of seed propagation (Janeiro *et al.*, 1997). In the present work synseed preparation and its germination was achieved using somatic embryos of *C. thwaitesii*. This can be used as a method for conservation of germplasm. Encapsulation of somatic embryos and their subsequent retrieval of complete plantlets is reported by Muralidharan (1994) in *Calamus rotang*. Ara *et al.* (2000) stated that although various micropropagules have been considered for synthetic seed production, the somatic embryos are largely favoured. The superiority of sodium alginate over other encapsulation matrices has been reported earlier by Redenbaugh *et al.*, (1986) and Bapat and Rao (1988). The high acceptability of sodium alginate may be due to its moderate viscosity, low toxicity and quick gelation (Redenbaugh *et al.*, 1986 and Onishi *et al.*, 1992).

5.5. DIRECT ORGANOGENESIS

The present investigation revealed the coupling effect of cytokinin with low concentration of auxin in inducing more number of shoots than that of using a cytokinin alone. A combination of BAP (3.5 mg/l) and 2, 4-D (0.5 mg/l) was found to be superior to other combinations for shoot development from collar region and immature embryos.

Umali-Garcia and Canlas-Mendoza (1997) reported that immature embryos of *C. merrillii* could be made to initiate multiple shoot proliferation in a medium containing 10 ppm BAP either in the presence of 1 ppm GA3 + NAA or just 250 ml coconut water per litre of MS solution.

Multiple shoot formation from embryos cultured on medium supplemented with high level of cytokinin and low level of auxin has also been reported by Yusoff *et al.* (1989a); Gunawan and Yani (1986), Yusoff (1989) and Valsala and Muralidharan (1999) and Bingshan *et al.* (2000).

5.6. ROOTING AND HARDENING

The regenerated shoots could be rooted in MS supplemented with 0.5-1.0 mg/l of IBA. Valsala and Muralidharan (1999) have reported the use of IBA (0.5-2.0 mg/l) for rooting on *Calamus* species. Bingshan *et al.* (2000) opined that most cultures of *Calamus* required the presence of auxins for efficient root formation. They found NAA as most effective for *C. simplicifolius*, *C. egregius* and *Daemonorops margaritae*. IBA

was effective in *C. yunnanensis*, *C. obovoideus* and *C. gracilis* at concentrations of 0.5 to 2.0 mg/l. Rooting in coconut plantlets is achieved by supplementing the medium with NAA (Ashburner, *et al.*, 1993) or transferring the cultures to liquid medium supplemented with IBA (5 mg/l) and NAA(1 mg/l) (Karun *et al.*, 1999).

SUMMARY

6. SUMMARY

Calamus species commonly called as rattans, are climbing spiny palms with characteristic scaly fruits and are classified under the subfamily Calamoideae of the palm family, Arecaceae (Palmae) (Uhl and Dransfield, '1987). They constitute an integral part of the tropical forest ecosystem and hold great social significance as a source of lively hood for the people residing near the forest areas.

Extensive unscientific harvest, loss of habitat and poor regeneration have resulted in dwindling rattan populations, necessitating an urgent need to conserve the existing rattan genetic resources. However, for effective conservation programme, information on the reproductive biology is very critical. The problem ever faced for making rattan plantation is the low germination rate. This may be due to immaturity of seeds or due to any restraints for germination. Lack of information on time of ripening of fruits for individual species, small number of fruits produced for certain species, a few plants producing fruits in each season due to variation in climatic condition, difficulty in collecting fruits, the dioecious nature of the genus etc. contributed to the problem.

The shortage of seeds were the biggest obstacle for the rattan plantation for certain species. The only alternative to meet this crisis is the tissue culture based mass clonal propagation which can produce large number of seedlings in short time to solve the problem of seedling shortage.

With a view to understand the reproductive biology and the possibilities of micropropagation, the present study is taken up and the result of the research work is summarised below.

Calamus hookerianus and *C. thwaitesii* are clustering, climbing, pleoanthic, dioecious rattans. *C. thwaitesii* has the thickest cane available along the Western Ghats. The inflorescence arises in the axil of the leaf and wears series of tubular bracts which is the characteristic feature of the genus *Calamus*. The male inflorescence consisted of thousands of fertile male flowers arranged in decussate manner on either side of the rachilla. The female inflorescence has flowers arranged in pairs, each pair consisted a fertile female with a sterile male. Five years observation in flowering pattern of two *Calamus* species showed that the time of floral initiation varied slightly with locality and from year to year. *C. hookerianus* and *C. thwaitesii* took about 10 and 11 months respectively from the emergence of inflorescence to fruit maturity.

The male flowers were symmetrical, trimerous with shortly epipetalous stamens. Anthesis of the male flowers began around 1 AM and most of the flowers opened by 4 AM but the process of opening of a few flowers continued till 6.30 AM. During opening the flowers emitted a strong scent and exuded a droplet of nectar. Anthesis in *C. hookerianus* lasted for about 12 days and in *C. thwaitesii* for more than 15 days. The behaviour of the sterile male flowers on the female inflorescence during anthesis was the same as the fertile males except that no pollen was produced by the anthers. The pistillate flowers had a tubular calyx, which was shallowly 3-lobed. The gynoecium was tricarpellate, ellipsoidal, whitish with a pineapple like base covered with scales, and a well developed and fleshy style with trifid stigma. Six staminodes surrounded the gynoecium which united to a ring and extended to the tip. Anthesis in the female flowers mostly occurred between 1 AM and 4 AM and senescence and abscission between 6 PM and 8 PM. At the time of floral opening receptive stigma was white and hyaline and by evening it turned brown and later to reddish black. The longevity of

female flowers was similar in both species studied and lasted for 12 to 15 days. Pistillate flowers did not produce nectar while opening. The electron microscopic studies of exine sculpturing an ornamentation of pollen grains can be treated as physical attractants of pollen grains towards biotic pollination. But the morphology of the flower presented several features of the wind pollinated plants, viz. lack of showy parts, bright colour and sticky and rough surfaced pollen. The production of large quantity of pollen, a distinct exposure of anthers and stigma to the air designed to scatter the pollen to the wind, the three pointed stigmatic teeth standing erect at the stage of fertilization, the complete separation of sex into staminate and pistillate flowers are the other factors that supported wind pollination in both the species studied. Again, transparent adhesive tape suspended near the pistillate flower showed the presence of pollen grains. Thus both species of *Calamus* a mixture of primitive and advanced characters.

To study micro and mega sporogenesis, embryological and endosperm development, male flower buds and developing fruits were collected at regular intervals, depending on the stage of development and they were fixed in formalin-acetic-alcohol (FAA). Hard seeds were softened using 15 percent hydrofluoric acid. The customary methods of washing, dehydration, clearing and embedding were followed (Johansen, 1940).

The anther wall consisted of an epidermis, fibrous endothecium, middle layers and a secretary type of tapetum. Cytokinesis was successive and the tetrads were isobilateral. The pollen grains were 2-celled at the dispersal stage and were bicolpate, spheroidal or spherical measuring 39.6 x 42.9 μm in *C. hookerianus* and 44.5x 42.5 μm in *C. thwaitesii*. Sculpturing of exine was microreticulate.

The ovule was basal, anatropous, bitegmic and crassinucellate. The outer integument was massive and many layered and the inner integument was 4-5 layered. Both integuments formed the micropyle. Tannin was present in the cells of the integument. The differentiation of integumentary tapetum was more pronounced in *C. thwaitesii* than in *C. hookerianus* and the cells were pallisaded .

The archesporial cell was formed from a hypodermal cell. Megaspore tetrad was linear and the chalazal megaspore formed the embryo sac. Mature embryo sac developed in one ovule and the other two ovules got aborted. Embryo sac development was of the monosporic, 8-nucleate, Polygonum type.

Endosperm development was of the free nuclear type. Wall formation at a later stage proceeded from periphery to centre. Endosperm of *C. hookerianus* was ruminant and the ruminations were due to the ingrowths of outer layers of the inner integuments (*Myristica* type).

Embryogeny corresponded to the Onagrad type (Johansen, 1950). Mature embryo measured 2.2 μm in *C. hookerianus* and 2.0 μm in *C. thwaitesii* and the ovule was basal in position. Vegetative point of the embryo was terminal and curved over by cotyledonary sheath. It was placed opposite to the radicle. Entire head part was turned through an angle of 90° in mature embryo.

Fruits in both species were drupes and the size ranged from 0.8-1.0 cm. in length with scales in 18 vertical rows in *C. hookerianus*. The mature fruit of *C. thwaitesii* measured 1.5 – 2.0 cm and the scales were in 12 vertical rows. The seeds of both the species were grooved on the ventral side. Germination of the seeds was of the adjacent

ligular type. During germination, the distal part of the embryo by progressive enlargement developed into a haustorium.

Removal of sarcotesta was found to be an indispensable pre-treatment to improve germination percentage and to shorten the germination period of both the species studied. Seed treatment studies revealed that stratification at 10⁰ C for 4- 5 days was the best method for improving the germination percentage.

Seed desiccation resulted in an increase of phenolic substances and that may be one of the reasons for the death of rattan seeds when dried. Delay in radicle differentiation is one reason for the delay in germination of the seeds.

Maturity of seeds was found to be an important pre-condition in seed germination and storage. Fruit and seeds stored in closed plastic bags or kept in the refrigerator at 10⁰ C maintained viability nearly for 3 months in both species.

Although, the studies undertaken are limited to categorise the storage behaviour of *Calamus* seeds, from the observations, it is elucidated that *Calamus* seeds fall under recalcitrant category.

With rampant destruction of forests and habitats, rattan stock at present is highly depleted. Over extraction, that too before flowering and fruiting, has compounded this problem. The most practical approach to over come this problem is to replenish the destroyed logged – over areas through massive reforestation, enrichment planting and establishment of rattan plantations.

The low percentage of seed germination in certain species, coupled with the long period from sowing to germination have contributed much to the dwindling supply of

planting materials. Hence, in the present study an attempt has been made to develop an efficient protocol for the rapid multiplication through multiple shoot induction, callus regeneration, somatic embryogenesis and conservation through synthetic seeds.

Of the different media (MS, Y3 and SH) tested, MS medium was found to be the most suitable for immature embryo germination as well as for all cultural studies. Among the different sucrose concentrations tested, 3 percent was found to be the most congenial for the successful establishment of the above-mentioned studies.

Callus induction was achieved from immature embryos of *C. hookerianus* and *C. thwaitesii* when cultured on MS medium fortified with 2,4-D and BAP. The callus growth was slow in *C. thwaitesii* because of the browning of the culture. Addition of activated charcoal to the media minimised the degree of browning and maximum shoot regeneration in both species occurred on MS medium supplemented with BAP (3.0 mg/l) and 2, 4-D (0.5 mg/l)

A protocol has been standardised for the induction of somatic embryos in *C. thwaitesii*. The calli obtained from the embryo explant of *C. hookerianus* after inoculation onto a lower auxin containing medium did not differentiate into embryogenic tissue.

The calli induced on MS medium supplemented with 2, 4-D (3.0 mg/l) and BAP (0.5 mg/l) upon transfer to MS medium with reduced levels of 2, 4-D (0.5 mg/l) developed into nodular structures or friable calli. By transferring these friable calli to media devoid of auxin, at an interval of 3-4 weeks, differentiation could be triggered into globular embryos. These globular embryos, when transferred to hormone free MS medium showed the development of germinable somatic embryos.

Synseeds have been prepared by encapsulating the somatic embryos of *C. thwaitesii* and immature embryos of both the species of *Calamus*. Of the different concentrations of calcium chloride of sodium alginate used for encapsulation, 3 percent sodium alginate and 50 mM concentration of calcium chloride was found suitable for the beads with 80 percent response in both species. The synseeds showed signs of germination within 10 days on hormone free MS medium.

Direct shoot multiplication was achieved from immature embryos of *C. hookerianus* and *C. thwaitesii* in MS medium supplemented with various growth regulators. MS medium with cytokinins (BAP or KIN) or in combinations with auxins (2, 4-D or NAA) were tested for multiple shoot induction. Of the different combinations tested an enhanced rate of direct shoot multiplication was obtained for both species of *Calamus* with the combination of BAP at 3.5 mg/l and 2, 4-D at 0.5 mg/l.

The present investigation has resulted towards an understanding of the reproductive biology which will be of great use in organising the seed collection and nursery work in the forestry operations such as establishment of large scale plantations and management practices in addition to the future hybridization and breeding work for establishing elite plants. This study helped in developing a protocol for rapid micropropagation and ex-situ conservation of two commercially important species of *Calamus* – *C. hookerianus* and *C. thwaitesii*.

REFERENCES

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- Abdullah, M.Z. and O. Jusoh. 1995. Phenological observations on *Calamus palustris* Griff., *C. scipionum* Loureiro and *C. ornatus* Blume in Peninsular Malaysia. In: R. Bacilieri and S. Appanah (eds.). Rattan cultivation: Achievements, problems and prospects. An International Consultation of Experts for the Project: Conservation, Genetic Improvement and Silviculture of Rattans in South East Asia, 12-14 May, 1998, Kuala Lumpur, Malaysia. pp. 61-77.
- Achmad, B. 1988. The effect of storage media on germinating ability and moisture content of rattan manan (*C. manan* Miq.) seeds. Buletin Penelitian Kehutanan, 4(2): 15-20.
- Agmato, A. 1984. Rapid germination method for Palasan, *C. merrillii* Becc. seed. Technical paper. 10th Annual FORI Anniversary Research Symposium, FORI Auditorium College, Laguana, pp. 15-19.
- Ahee, J., P. Arthuis, G. Cas, Y. Duval and G. Guenin. 1981. Vegetative propagation of oil palm *in vitro* by somatic embryogenesis. Oleagineux, 36(3): 113-198.
- Alang, Z.C., B. Krishnapillay, S.F. Pilus and T.T. Hesajina. 1991. Embryo culture and nursery establishment of sago palm (*Metroxylon sago* Roxb.) plantlets. Towards greater advancement of the sago industry in the 90s. In: Y.L. Tie and H.S. Kueh (eds.). Proceedings of the fourth international sago symposium, 6-9 August 1990, Kuching, Sarawak, Malaysia. p. 65.
- Alang, Z.C., G.F.J. Moir and L.H. Jones. 1988. Composition, degradation and utilization of endosperm during germination in the oil palm (*Elaeis guineensis* Jacq.). Ann. Bot., 61: 261-268.
- Alim, A and M. Kamaluddin. 1985. Development of nursery procedure of three cane species. Bano – Biggyan – Patrika, 14 (1/2): 26 – 27.

- Alloysius, D. 1997. Reproductive biology of rattans. *In* : A.N. Rao, V.R. Rao (eds.) Rattan : Taxonomy, Ecology, Silviculture, Conservation, Genetic Improvement and Biotechnology. Proceedings of Training courses cum workshops, 14-26 April 1996, Sarawak, Sabah. IPGRI-APO, Serdang, Malaysia. pp. 137-145.
- Alloysius, D. 1999. Reproductive Biology of *Calamus manan* Miq. Abstract of thesis presented to the Senate of University Putra, Malasia in fulfilment of the requirements for the degree of master of science. Malaysia. pp. 12-13.
- Aminuddin, M. and A.R. Zollpatah. 1990. A note on germination characteristics of *Calamus manan* Miq. and *Calamus tumidus* Furtado under laboratory and nursery conditions. *J. Trop. For. Sci.*, 2(3): 260-262.
- Ammirato, P.V. 1983. Embryogenesis. *In*: D.A. Evans, W.R. Sharp, P.V. Ammirato and Y. Yamada (eds.). Hand Book of plant cell culture. Vol. I. Techniques for propagation and breeding. MacMillian, New York. pp. 32-123.
- Ananthakrishnan, G., R. Ravikumar, R.P. Anand, G. Vengadesan and A. Ganapathi. 1999. Induction of somatic embryogenesis from nucellus-derived callus of *Anacardium occidentale* L. *Scientia Hort.*, 79: 91-99.
- Anderson, A.B., W.L. Oval and A. Henderson. 1988. Pollination ecology of a forest dominated palm (*Oribignya phalerata* Mart.) in Northern Brazil. *Biotropica*, 20: 192-205.
- Anjarna, M. and M. Zaid. 1993. The effects of various growth regulator levels on early rooting in date palm (*Phoenix dactylifera* L.) tissues. *Al-Awamia*. 82: 197-210.
- Ara, H., U. Jaiswal and V.S. Jaiswal. 2000. Synthetic seed. Prospects and limitations. *Curr. Sci.*, 78: 1438-1444.

- Ashburner, G.R., W.K. Thomson and J.M. Burch. 1993. Effect of α - Naphthaleneacetic acid and sucrose levels on the development of cultured embryos of coconut. *Pl. Cell. Tiss. Org. Cult.*, 35: 157-163.
- Assy-Bah, B., J. Durand – Gasselin and C. Pannetier. 1987. Use of zygotic embryo culture to collect germplasm of coconut (*Cocos nucifera* L.). *Plant Genetic Resources News letter, FAO/IBPGRI*. pp. 71, 4-10.
- Avril, L.B., L.B. Richard and J. Blake. 1986. Regeneration in palms. *Cell culture and Somatic Cell Genetics of Plants*, 3: 207-222.
- Bagaloyos, A.P. 1987. Rattan seed collection and storage. Rattan Information Centre. Occasional paper, No. 5, Kuala Lumpur, Malaysia. pp. 7-12.
- Balick, M.J. 1988. The palm-trees of life biology, utilization and conservation. *Adv. Econ. Bot.*, 6:1-28.
- Banik, R.L. and M.N. Nabi. 1981. An observation on the flowering time and seed germination in bara bet *Calamus viminalis* var. *fasciculatus* (Griff.) Becc. In: R.L. Banik and M.N. Nabi (eds.). *Proceedings of the 4th and 5th Bangladesh Science Conference, Rajshastri, Bangladesh, 2-5 March, 1980*. pp. 52-56.
- Bapat, V.A. and P.S. Rao. 1988. Sandalwood plantlets from synthetic seeds. *Pl. Cell Rep.*, 7 : 434-436.
- Barba, R.C., L.J. Patena, M.M. Mercado and L. Lorico. 1985. Tissue culture of rattan (*Calamus manillensis* Becc.). Paper presented at the Second Natl. Symp. on Tissue culture of Rattan; 1985. University Pertanian Malaysia. pp. 31-36.
- Barfod, A.S. and N.W. Uhl. 2001. Floral development in *Aphandra* Barfod (Arecaceae). *American J. Bot.*, 88(2): 185-195.

- Baruah, S. 1994. Technique for enhancing germination frequency of rattan seeds. J. Agri. Sci. Soc. of North East India. 7(1): 131-132.
- Basu, S.K. 1985. The present status of rattan palms in India—An overview. In: K.M. Wong and N. Manokaran. Proceedings of the Rattan Seminar, 2-4 Oct. 1984, Kuala Lumpur, Malaysia. pp. 77-90.
- Basu, S.K. and D.P. Mukherjee. 1972. Studies on the germination of palm seeds. Principes, 16 : 136-137.
- *Bauch, K. 1911. Beitrage entwicklungsgeschichte and physiologischen anafonie der palmen blute. Berlin.
- Bavappa, K.V.A. and K.A. Rao. 1970. Floral initiation and abortion in arecanut (*Areca catechu*, L.) Indian J. Agric. Sci., 40: 371-378.
- Bavappa, K.V.A. 1966. Morphological and anatomical studies in *Areca triandra* Roxb., Phytomorphology, 16: 436-443.
- Beach, J.H. 1984. The reproductive biology of the peach or pejibaye palm (*Bactris gosipaes* Kunth) and a wild congener (*B. porschiana* Burret) in the Atlantic lowlands of Costa Rica. Principes, 28 (3): 107-119
- Beccari, O. 1911. Asiatic palms—Lepidocaryeae. Part II. The species of *Daemonorops* Schult. f. Ann. Roy. Bot. Gard., Culcutta. 12 (1): 1-237.
- Beerlyn, G.P. and J.P. Miksche. 1976. Botanical microtechnique and cytochemistry. The Iowa State University Press. Ames, Iowa. 20-80.
- Belal, A.H. and M.D. Deeb-El. 1997. Direct organogenesis of date palm (*Phoenix dactylifera* L.) *in vitro*. Assiut-Jour. Agri. Sci., 28(2): 67-77.

- Beniwal, B.S. 1987. Phenological studies of trees in Arunachal Pradesh. *Indian For.*, 113 (12): 779-791.
- Bertossi, F.A., M. Noirot and Y. Duval. 1999. BA enhances the germination of oil palm somatic embryos derived from embryogenic suspension cultures. *Pl. Cell Tiss. Org. Cult.*, 56(1): 53-57.
- Bhaskaran, S and R.H. Smith 1992. Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* L. *Pl. Cell Rep.*, 12: 22-25.
- Bhaskaran, S. 1985. Tissue culture technology for higher vegetable oil production. *In*: H.C. Srivastava, S. Bhaskaran, B. Vatsya and K.K.G. Menon. (eds.). Oil seed Production Constraints and Opportunities. Oxford and IBH Publications, New Delhi. pp. 537-544.
- Bingshan, Z. 1997. Tissue culture of *Calamus egregaus*. *J. Central South Forestry University*, 17(4): 563-569.
- Bingshan, Z. and Y. Guangtian. 1996. Silviculture, genetic improvement, conservation, utilization and socio economics of rattans. *In*: A.N. Rao and V.R. Rao (eds.). Rattan Taxonomy. ecology, silviculture conservation, genetic improvement and Biotechnology. Proceedings of training courses cum workshops, Sarawak, Sabah, Malaysia, IPGRI. APO, Serdang. pp. 193-198
- Bingshan, Z., X. Hungacan, Y. Yingil, Y. Guangtian and Q. Zhenfei. 2000. Tissue culture for mass propagation and conservation of rattans. *In*: H.C. Cxu, A.N. Rao, B.S. Zeng and G.T. Yini (eds.). Research on Rattan in China. IPGRI, pp. 103-145.
- Biradar, N.V. 1968. Studies on palms. Embryology of *Phoenix pusilla* Gaertn., *P. acaulis* Buch. and *P. reticulata* Jacq. *Proc. Indian. Acad. Sci., Sect. B.* 67: 165-173.

- Biradar, N.V. and T.S. Mahabale. 1969. Studies on palms. Fruits, seeds and seed germination in the genus *Phoenix* L. Proc. Indian Acad. Sci., Sect. B. 70: 55-65.
- Blake, J. and C.J. Eeuwens. 1978. Inflorescence tissue as source material for vegetative propagation of coconut palms. In: Proceedings of International Conference on Cocoa and Coconut. Kuala Lumpur. pp. 549-556.
- Blatter, E. 1926. The palms of British India and Ceylon. Oxford University Press, London. 600p.
- Bogh, A. 1996. The reproductive phenology and pollination biology of four *Calamus* (*Arecaceae*) species in Thailand. Principes, 40: (1) 5-15.
- Borchsenius, F. 1993. Flowering biology and insect visitation of tree palm Ecuadorean *Aiphanes* species. Principes, 37: 139-150.
- Bougerfaoui, M. and A. Zaid. 1993. The effect of ammonium nitrate content of the culture medium on tissue vitrification of date palm (*Phoenix dactylifera* L.) grown *in vitro*. Al Awamia, 82: 177-196.
- Boyd, L. 1932. Monocotyledonous seedlings: Morphological studies in the post seminal development of the embryo. Proc. Bot. Soc., 31: pp. 1-224.
- Branton, R. and J. Blake. 1983. A lovely clone of coconuts. New Scientist, 98: 554-557.
- Bray, G.G. and W.V. Thorpe. 1954. Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal., 1: 27-52.
- Brown, K.E. 1976. Ecological studies of the cabbage palm, *Sabal palmetto* (Walter) Lodd. Principes, 20: 3-11.

- Bullock, S.H. 1981. Notes on the phenology of inflorescences and pollination of some rainforest palms in Costa Rica. *Principes*, 25(3): 101-105.
- Burquez, A., J.K. Sarukhan and A.L. Pedroza. 1987. Floral biology of a primary rain forest palm. *Astrocaryum mexicanum* Liebm. ex Mart., Bot. J. Linn. Soc., 94: 407-419.
- Burquez, I.P. and A.R. Griffin, 1990. Flowering time of *Eucalyptus* species in their natural habitat. Division of Forestry User Series No.1, CSIRO, Division of Forestry, Canberra. pp. 16-17.
- Calinawan, N.M. and S.C. Halos. 1988. Mass propagation of *Calamus ornatus* Blume by tissue culture. *Canopy International*, 14(2): 1-9.
- Chandra Sekhar, K. N. and D.A. DeMason. 1989. Differential activity of acid phosphatases from the endosperm and haustorium of date palm. (*Phoenix dactylifera* L.) seeds. *Canadian J. Bot.*, 67: 1096-1102.
- Chandrasekhar, K. N. and D.A. DeMason. 1990. Identification and immuno cytochemical localization of α -galactosidase in resting and germinating date palm (*Phoenix dactylifera* L.) seeds. *Planta*, 18(12): 53-61.
- Chang, J.L., L. Saenz, C. Talavera, R. Hormung, M. Robert. and P. Orepeza. 1998. Regeneration of coconut (*Cocos nucifera* L.) from plumule explants through somatic embryogenesis. *Pl. Cell Rep.*, 17: 515-521.
- Chengji, Z. and Z. Jiankui 1991. Plant regeneration in tissue culture of rattan. *Acta Botanica Yunnaica*, 13(1): 97-100.
- Chennaveeraiah, M.S. 1955. 'Embryological and anatomical studies on *Hyphaene* Gaertn. - a genus of branched palms (Palmae) with a note on cyto-analysis of palms.' Ph.D thesis submitted to the University of Poona.

- Chen-Zhiying and Fan-Kuan. 1995. Rattan propagation and nursery techniques in Yunnan. *For. Sci. and Tech.*, (2): 24-25.
- Chia, F.R. 2000. The sex ratio of *Calamus subinermis* H. Wendl. planted in a secondary forest and *Acacia* plantation. *J. Trop. For. Sci.*, 12(1): 185-187.
- Chopra, V.L. 1990. Biotechnology for plant breeding. *Sci. Rep.*, 27: 34-38.
- Chu, U.Y.E. 1986. The application of tissue culture to plant improvement and propagation in the ornamental horticulture industry *In: R.H. Zimmermann, R.J. Griesbach, F.A. Hammerschlag, R.H. Lawson and Martinus Nijhoff. (eds.). Tissue culture as a plant production system for horticultural crops. The Netherlands. pp. 15-33.*
- Chuthamas, P., P. Prutpong, I. Vongkaluang and S. Tantiwiwat. 1989. *In vitro* culture of immature embryos of *Calamus manan* Miq. *In: A.N. Rao and Isara Vongkaluang (eds.). Recent Research on Rattans. Faculty of Forestry, Kasetsart University, Thailand and International Development Research Centre, Canada. pp. 144 – 147.*
- Cid, L.P.B. 1987. Regeneration of plants of *Elaeis oleifera* and its hybrid with *Elaeis guineensis* Jacq. by somatic embryogenesis. *Pesquisa-Agro-pecuaria-Brasileira*, 22(1): 109-113.
- Corley, R.H.V., C.Y. Wong, K.C. Wool and L.H. Jones. 1981. Early results from the first oil palm clone trials. *In: E. Pushparajah and P.S. Chew (eds.). Oil palm in agriculture in the eighties. Incorporated Society of Planters, Kuala Lumpur. 173-196.*
- Corner, E.J.H. 1966. *The Natural History of palms. Weidenfield & Nicolsen, London. pp. 35-126.*

- Cranwell, L.M. 1953. New Zealand pollen studies. The monocotyledons - a comparative account. Bull. Auckland Inst. & Museum. 3: 30-120.
- Cushman, J.C., P. Wulan, V. Kuscuoglu and M.D. Spatz. 2000. Efficient-plant regeneration of *Mesembryanthemum crystallinum* L. via. Somatic embryogenesis. Pl. Cell Rep., 19: 459-463.
- Cutter, V.M., K. Wilson and B. Freeman. 1955. Nuclear behaviour and cell formation in the developing endosperm of *Cocos nucifera* L. American J. Bot., 42: 109-115.
- D'Amato, F. 1977. Cytogenetics of differentiation in tissue and cell cultures. In: J. Reinert and Y. P.S. Bajaj (eds.). Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture. Springer-Verlag. Berlin. pp. 343-356.
- *Daguin, F. and R. Letouze. 1988. Regeneration du palmier dattier (*Phoenix dactylifera* L.) par embryogenese somatique amelioration de iefficacite par passague an milieu liquide agite. Fruits, 43: 191-194.
- Darus, H. A. and A.G, Rasip. 1989. Flowering and fruiting in *Calamus manan* Miq. RIC Bull. 8 (1/4): 2.
- Darus, H.A. and A.R. Ghani, 1989. Flowering and fruiting in *Calamus manan* Miq. RIC Bull. 7(¼) :2.
- Darus, H.A. 1983. The effect of sowing media on the germination of *Calamus manan* Miq. and *C. caesius* Blume. Malaysian For., 46(1): 77-80.
- Dass, H.C., R.K. Kaul, S.P. Joshi. and R.R. Bhansati. 1989. *In vitro* regeneration of date palm plantlets. Curr. Sci., 58(1): 22-24.
- Datta, M. 1955. The occurrence and division of free nuclei in the endospermal milk in some Palmae. Trans. Bose Res. Inst., 19: 117-125.
- Datta, K.B. , B. Kanjilal and D.D.Sarkar. 1999. Artificial seed technology : Development of a protocol in *Geodorum densiflorum* (Lam.) Schl.—An endangered orchid. Curr. Sci. 76 : 1142—1145.

- Davies, R.I. and W. Pritchard. 1998. Seed storage and germination of the palms *Hyphaene thebaica* (L.) Mart., *H. petersiana* and *Medernia argun* Mart. Seed Sci. and Technol., 26: 823 – 828.
- Davis, G.L. 1966. Systematic Embryology of Angiosperms. John Wiley and Sons, New York. pp. 16-120
- De Poerck, R.A. 1950. Contribution al 'etude' de Palmier a huile African (*Elaeis guineensis* Jacq.) Ole Aegineux, 5: 627-628.
- Dekkers, A.J. and A.N. Rao. 1989. Some observations on *in vitro* culture of *Calamus trachycoleus* Becc. In: A.N. Rao and A.M. Yusoff (eds.). Proceedings of the Seminar on Tissue Culture of Forest Species. Forest Research Institute Malaysia and International Development Research Centre, Singapore. pp. 63-68.
- DeLeon, N.J. 1958. Viability of palm seeds. Principes, 2 : 96 – 98.
- DeLeon, N.J. 1961. Viability of palm seeds. American Hort. Mag., 40: 131 – 132.
- DeMason, D.A and J.I. Stillman. 1986. Identification of phosphate granules occurring in seedling tissue of two palm species (*Phoenix dactylifera* L. and *Washingtonia filifera* (Lind.) H.A. Wendl.). Planta. 167: 321-329.
- DeMason, D.A. 1984. Growth parameters in the cotyledon of date seedlings. Bot. Gaz., 145: 176-183.
- DeMason, D.A. 1985. Histochemical and ultra structural changes in the haustorium of date (*Phoenix dactylifera* L.). Protoplasma, 126: 168-177.
- DeMason, D.A. 1986. Endosperm structure and storage reserve biochemistry in the palm, *Washingtonia filifera* (Lind.) H.A. Wendl. American J. Bot., 73: 1332-1340.

- DeMason, D.A. and B. Tisserat. 1980. The occurrence and structure of apparently bisexual flowers in the date palm, *Phoenix dactylifera* L. (Arecaceae). Bot. J. Linnean Soc., 81(4): 283-292.
- DeMason, D.A., D. Widney and J.I. Stillman. 1991. *In vitro* and transplantation experiments with germination of date embryos. Canadian J. Bot., 70: 965-975.
- DeMason, D.A., J.I. Stillman and G.S. Ellimore. 1989. Acid phosphatase localization in seedling tissues of the palms, *Phoenix dactylifera* L. and *Washingtonia filifera* (Lind.) H.A. Wendl. and its relevance to controls of germination. Canadian J. Bot., 67: 1103-1110.
- DeMason, D.A., R. Sexton, M. German and J.S.G. Reid. 1985. Structure and biochemistry of endosperm breakdown in date palm (*Phoenix dactylifera* L.) seeds. Protoplasma, 126: 159 – 178.
- Deshpande, J.V. 1954. Studies on the family Sonneratiaceae and its fossil allies and on the anatomy of palms. Ph.D. thesis submitted to the University of Bombay.
- Dias, A.C., M.P. Guerra, A.S Cordaba, E. Kember and M.E.S.P Dematte. 1994. Somatic embryogenesis and plant regeneration in the tissue culture of *Geonoma gamiova*. First International Symposium on ornamental palms, Jaboticabal, SP, Brazil. 25-29 Jan. 1993. Acta-Horticulturae, 360: 167-171.
- Dickie, J.B., M.J. Ballick and I.M. Linington. 1992. Experimental investigations into the feasibility of *ex situ* preservation of palm seeds; an alternative strategy for biological conservation of this economically important plant family. Biodiversity and Conservation, 1: 112–119.
- Douglas, G.C. 1987. Embryo culture of a rare plant, *Hyophorbe amaricaulius* Mart. J. Plant Physiol., 130(1): 73-77.

- Douglas, J. and R.R. Bimontoro. 1956. Identification of the *Corypha* palms which flowered in the Hortus Bogoriensis during 1953 to 1955. *Ann. Bogor.*, 2: 137-148.
- Dransfield, J. 1970. Studies in the Malayan Palms. *Eugeissona* Griff. and *Johannesteijsmannia* H.E. Moore Jr. Ph. D Thesis, Univeristiy of Cambridge.
- Dransfield, J. 1978. Growth forms of rainforest Palms. *In*: P.B. Thomlinson and M.H. Zimmermann (eds.) *Tropical trees as living systems*. Cambridge University Press. New York. pp. 247-268
- Dransfield, J. 1979. A manual of the rattans of the Malay Peninsula. Forest Department, Ministry of Primary Industries, Malay. *For. Rec.*, 29: 270 p.
- Dransfield, J. 1981. The biology of Asiatic rattans in relation to the rattan trade and conservation. *In*: H. Syngé. (ed.). *The Biological aspects of rare plant conservation*. J. Wiley & Sons Ltd. London. pp. 179-186.
- Dransfield, J. 1984. The rattans of Sabah. Sabah Forest Records No.13. Forest Department, Sabah. Sandakan. pp. 31-69.
- Dransfield, J. 1992. Morphological considerations. The structure of rattans. *In*: Wan Razar Wan Mond (ed.). *A guide to the cultivation of rattans*. Malay. *For. Rec.* No.35, FRIM, Kepong, Kuala Lumpur. pp. 11- 25.
- Dural, Y., K. Konan, C. Penetier, and T.D, Gasselin, 1988. Vegetative propagation of oil palm (*Elaeis guinensis* Jacq.) by *in vitro* culture. Strategy and results. *Oleagineaux*, 43(2): 39-47.
- Eames, A.J. 1961. *Morphology of the angiosperm*. McGraw Hill Book Co. Inc. New York. pp. 25-160.

- Ebert, A. F. Taylor and G. Blake. 1993. Changes of 6-bezylaminopurine and 2, 4-D concentration in plant tissue culture media in the presence of activated charcoal. *Pl. Cell. Tiss.*, 33(2): 157-162.
- Eeuwens, C.J. 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature tissues (*Cocos nucifera* L.) and cultured *in vitro*. *Physiol. Plant*, 36: 23-28.
- Erdtman, G. 1969. Hand book of palynology (An introduction to the studies of pollen grains and spores). Munksgaard, Copenhagen. 486p.
- Erdtman.G. 1952. Pollen morphology and Plant taxonomy; Angiosperms. Waltham, Mass. U.S.A.pp. 84- 110.
- Ervik, F. 1991. Notes on the phenology and pollination of the dioecious palm *Mauritia flexuosa* L. and *Aphandra natalia* Balsla and Henderson. *In: W. Brathlott, C.M. Naumanna, K. Schmidt-Loske, and K.L. Schuchmann (eds.). Animal plant interaction in tropical environment. Zool. Forschunginst und Museum Alexander Koenig, Bonn. pp. 38-41.*
- Ervik, F. and J.P. Feil. 1997. Reproductive biology of the monoecious palm, *Prestoea schultesiana* H. E. Moore in Amazonian Ecuador. *Biotropica*, 29(3): 309-317.
- Ervik, F. and R. Bernal. 1996. Floral Biology and Insect visitation of the monoecious palm *Prestoea decurrens* (H.A. Wendl. ex Burret) H.E. Moore on the Pacific Cost of Columbia. *Principes*, 40(2): 86-92.
- Essig, F.B. 1971. Observations on pollination in *Bactris* N.J. Jacq. *Principes*, 15: 20-24, 35.
- Essig, F.B. 1973. Pollination in some new Guinea palms. *Principes*, 17: 75-83.

- Essig, F.B. 1977. A systematic histological study of Palm fruits. The *Ptychosperma* alliance. Systematic Botany, 2: 151 – 168.
- Faegriand, K. and L. Vander Pyl. 1996. The principles of pollination ecology. Pergamon, London. 362p.
- Falcone, A.M and G.L. Marcheschi. 1988. Rivista-di-Agricoltura-Subtropicale-Tropicale, 82(1–2): 379–389.
- Falk, R.H. 1980. Scanning electron microscopy (SEM) INC., AMF O'Hare, Chicago, IL-60666, U.S.A. pp. 79-87.
- Fernando, S.C. and C.K.A. Gamage. 2000. Abscissic acid induced somatic embryogenesis in immature embryo explants of coconut (*Cocos nucifera* L.). Pl. Sci., 151(2): 193-198.
- Fisher, J.B., W.S. Roger and E. Nancy. 1987. The flowering and fruiting of *Corypha umbraculifera* L. in Miami, Florida. Principes, 31(2): 68 – 77.
- Foster, A. 1934. The use of tannic acid and iron choride for staining cell walls in meristematic cells. Stain Tech., 9: 1-92.
- Foster, A. and E.M. Gifford. 1974. Comparative morphology of vascular plants. (2nd ed.) W.H. Freeman. San Francisco. pp. 131-186.
- Francisco, J.G. and A. R. Gustavo, 1995. The flowers and unusual inflorescences of *Leopoldinia* Mart. Principes, 39(3): 152 – 158.
- Gabr, M.F. and B. Tisserat. 1985. Propagating palms *in vitro* with special emphasis on the date palm (*Phoenix dactylifera* L.). Scientia-Horticulturae, 25(3): 255-262.
- *Gatin, C.L. 1906. Recherches sur la germination des palminers. Ann. Sci. Nat. Bot., Scr., 9(3): 191 –314.

- Generalao, M.L. 1977. Effect of pre-treatment media on the germination of Palasan (*Calamus maximus* Becc.) and Limuran (*C. ornatus* Blume) seeds at Pagbilao. *Quezon, Sylvatrop Philipp. For. Res. J.*, 2: 215 – 218.
- George, E.F. and P.D. Sherrington. 1984. Plant propagation by tissue culture : Hand book of Directory of commercial Laboratories. Exegetics Ltd.U.K.
- Ginting, G., C. Mollers. and K. Pamiñ, 1996. Somatic embryogenesis in oil palm for the *in vitro* propagation of elite clones. *J. Penelitian Kelapa Sawit*, 4(1): 1-16.
- Girija, T., P.S. Srinivasan, M. Marzalina, K.C. Khoo, N. Jayanthi, Tsan and B.Krishnapillay. 1998. Metabolic changes associated with desiccation in *Calamus* L. seeds. In: B. Krishnapillay (ed.). "Recalcitrant seeds". Proceedings of the IUFRO - Seed Symposium,, Kuala Lumpur, Malaysia, 12-15 October, 1998. pp 25-28.
- Goh, D. 1997. *In vitro* culture studies on rattan. In: A.N Rao and V.R. Rao (eds.). Rattan Taxonomy, ecology, silviculture, conservation, genetic improvement and biotechnology. Proceedings of training courses cum workshops, Sarawak, Sabah, 14-26, April 1996. IPGRI, Regional Office for Asia. The Pacific and Oceania, Serdang, Malaysia. pp. 173-178.
- Goh, D. 1998. Micropropagation of three *Calamus* species with emphasis on somatic embryogenesis. Rattan cultivation. Achievements, Problems and prospects. In: R. Bacillieri, S. Appanah (eds.). An International consultation of experts for the project: Conservation, genetic improvement and silviculture of rattans in South East Asia, Kuala Lumpur, Malaysia. 12-14 May 1998: pp. 72-77.
- Goh, D., N. Michaud Ferriere, O. Monteuuis and M.C. Bon. 1999. Evidence of somatic embryogenesis from root tip explants of the rattan *Calamus manan* Miq. *Plant*, 35(5): 424-427.
- Gomez, K.A. and K.C. Gomez. 1976. Statistical procedures for agricultural research with emphasis of rice. Los Banos. Philippines, International Rice Research Institute. pp. 207-215.

- Gonzalez, C.E., M.A. Martine, H.J. Quero and G.J. Marquez. 1997. Embryology of *Chamaedorea elegans* Mart. (Arecaceae). In: microsporangium, microsporogenesis and microgametogenesis. *Principes*, 41(3): 131– 137.
- Grout, B.W.W., K. Shelton and H.W. Pritchard. 1983. Orthodox behaviour of oil palm seed and cryopreservation of the excised embryo for genetic conservation. *Ann. Bot.*, 52: 381 – 384.
- Guanchez, F.J. and G.A. Romero. 1995. The flowers and unusual inflorescence of *Leopoldinia* Mart. *Principes*, 39(3): 152-158.
- Guangtian, Y. and X. Huangcan. 2000. Suitable storage conditions for seeds of *Daemonorops margaritae* Becc. In: H.C. CXu, A.N Rao, B.S. Zeng and G.T. Yini (eds.). *Research on Rattans in China*. IPGRI. pp. 35– 38.
- Guerra, M.P. and W. Handro. 1998. Somatic embryogenesis and plant regeneration in different organs of *Euterpe edulis* Mart. (Palmae): Control and Structural features. *J. Plantation Research*, 3(1101): 65-71.
- Guevara, V.F. 1961. The occurrence of twin embryos in *Cocos nucifera* L. *Philippine Agric.* 44: 520-522.
- Guignard, J.L. 1961. Embryogenic des palmiers. *Development de Pembryon chez le Chamaerops humilis* L. *C.R. Acad. Sci. Paris, Ser. D.*, 253. 1834-1836.
- Gunawan, L.W. and S.A. Yani. 1986. *In vitro* propagation of rattan manan (*Calamus manan* Miq.) for agroforestry plantations. In: Gunawan, L.W. and S.A. Yani (eds.) *Abstr. VI Intl. Congress of Pl. Tiss. and Cell cult.* Minne apolis, U.S.A. p 282.
- Gupta, P. K., A. L. Nadgir, A.F. Mascarenhas and V. Jaganathan. 1980. Tissue culture of forest trees: clonal multiplication of *Tectona grandis* L. (Teak) by tissue culture. *Pl. Sci. Lett.*, 17: 259-268.

- Haccius, B and V.J. Philip. 1979. Embryo development in *Cocos nucifera* L. A critical contribution to a general understanding of palm embryogenesis. *Pl. Syst.*, 132: 91– 106.
- Hanower, J. and P. Hanower. 1984. Inhibition and stimulation of *in vitro* embryogenesis in callus tissue derived from leaf explants of oil palm. *Comptes - Rendus-des-Seances-de-l' Academie-des-Sciences, III - Sciences - de-la-vie.* 298(2): 45-48.
- Hartley, C.W.N. 1979. The oil palm (*Elaeis guineensis* Jacq.) Longman. London, New York. pp. 58-110.
- Hartney, V.J. and P.K. Baker. 1980. The vegetative propagation of *Eucalyptus*. Symposium and workshop on Genetic Improvement of Fast Growing Trees, IUFRO. 8: pp. 25 – 30.
- Henderson, A. 1984. Observations on pollination of *Cryosophila albida* H.H. Bart. *Principes*, 28(3): 120 – 126
- Henderson, A. 1985. Pollination of *Socratea exorrhiza* (Mart.) H.A. Wendl. and *Iriartea ventricosa* Mart. *Principes*, 29(2): 64-71.
- Henderson, A. 1986. A review of pollination studies in the Palmae. *The Bot. Rev.*, 52(3): 221-259.
- Henderson, A. 1995. The Palms of the Amazon. Oxford University Press, New York. pp. 24-28.
- Henderson, A., A. Scariot and M.P. Whitaker. 1989. The Ecology of the Palmae in an Amazon Rainforest. *Projecto dinamica biologica de fragmentos florestais, BDFF.* pp. 1-12.

- Henderson, A., B. Fischer, A. Scariot, M.A.W. Pacheo and R. Pardini. 2000. Flowering phenology of a palm community in a central Amazon forest. *Brittonia*, 52(2): 149-159.
- Herrera, J. 1989. On the reproductive biology of the dwarf palm, *Chamaerops humilis* L. in Southern Spain. *Principes*, 33(1): 27- 32.
- Hervan, E.M., A.M. Shakib, M. Modiri, M. Afshari, S. Khoshkam and S. Nazeri, 1991. Study of callus induction from *in vitro* culture of different seed parts of date palm. *Seed and Plant*, 7: 1-2.
- Hornung, R. 1995. Micropropagation of *Cocos nucifera* L. from plumular tissues excised from mature zygotic embryos. *Plantations Research Development*, 2(2): 38-41.
- Hu, C.Y, and P.J. Wang 1983. Meristem shoot tip and bud culture. *In*: D.A. Evans, W.R. Sharp, P.V. Ammirato and Y. Yamada. (eds.). *Handbook of Plant Cell Culture. Vol. I. Techniques for propagation and breeding.* Mac Millan Publ. Co., New York. pp. 177-227.
- Huang, L.T.L, M. Baiocco, B.P. Huy, B. Mezzetti, R. Santilocchi, H.L.T.L. Rosati, H. Baophan and B.P. Huy. 1999. Somatic embryogenesis in Canary Island date palm. *Pl. Cell Tiss. and Org. Cult.*, 56(1): 1-7.
- Ishihata, K. 1974. Studies on the morphology and cultivation of palms. On the germination of seed in ornamental palms. *Bulletin of the Faculty of Agriculture*, No. 24, - Kagoshima. University. pp. 11 – 23.
- ISTA, 1993. International rules for seed testing. *Seed Sci. and Technol*, 2 : 25-30.
- Janeiro, L.B., A. Ballester and A.M. Vieitez. 1997. *In vitro* response of encapsulated somatic embryos of *Camellia* Link. *Pl. Cell Tiss. Org. Cult.*, 51: 119-125.

- Javalgekar, S.R. 1979. Embryogeny of *Borassus* and *Cocos*. Ph.D. thesis submitted to Pune University.
- Jensen, W.A. 1973. Fertilization in flowering plants. *Bioscience*, 23: 21-27.
- Johansen, D.A. 1940. Plant Microtechnique. McGraw Hill Co., New York. pp. 23-110.
- Johansen, D.A. 1950. Plant embryology. Waltham Mass: Chronica Botanica. pp. 35-150.
- Johnsson, B. 1979. On embryosackens untrieckling hos Angiospermerna. Lunds. Univ. Arsskar. 16: pp. 1-86.
- Johri, B.M., K.B. Ambegaokar and P.S. Srivastava. 1992. Comparative Embryology of Angiosperms. Vol. II. Springer, Verlag, Berlin Heidenberg, New York. pp. 710-1012.
- Joseph, J. 1972. *Asraoa triandru* J. Joesph (Arecaceae) A new genus and species of palm from Lohit district, Arunachal Pradesh. *Bull. Bot. Surv. India*. 14(1-4): 144-148.
- Juliano, J.B. 1926. Origin, development and nature of the stony layer of the coconut (*Cocos nucifera* L.) *Philippines J. Sci.*, 30: 187-200.
- Juliano, J.B. and E. Quisumbing. 1931. Morphology of male flower of *Cocos nucifera* L. *Philippines J. Sci.*, 45: 449-58.
- Kajale, L.B. and S.G. Ranade. 1952. Occurrence of four kinds of tetrads in *Elaeis guineensis* Jacq. *Curr. Sci.*, 21: 170.
- Kajale, L.B. and S.G. Ranade. 1953. The embryosac of *Elaeis guineensis* Jacq., A re-investigation. *J. Indian Bot. Sci.*, 32: 101-117

- Kalamani, A. and S.R. Rangasamy. 1990. *In vitro* culturing of coconut embryo. Indian coconut Journal, Cochin, 20(11): 9-10.
- Kalzlowski, T.T and Gunn, C R .1972. Importance and characteristics of seeds. *In*: T.T Kalzlowski (ed.). Seed Biology, Academic Press. New York and London. pp. 1-20.
- Karun, A. 2001. GA3 and ABA mediated effect on maturation and germination of zygotic embryos in coconut (*Cocos nucifera* L.). Indian J. Hort., 58(3): 276-281.
- Karun, A. and K.K. Sajini. 1996. Plantlet regeneration from leaf explants of oil palm seedlings. Curr. Sci., 71(11): 922-926.
- Karun, A., K.K. Sajini and Shivashankar. 1999. Embryo culture – A CPCRI protocol. Indian J. Hort., 56: 348-353.
- Karunaratne, S. and K. Periyapperuma. 1990. Culture of immature embryos of coconut, *Cocos nucifera* L. Callus proliferation and somatic embryogenesis. Cocos., 8: 13-32.
- Keusch, L. 1968. Die Mobilisierung des Reservematerials in Keimenden Dattelsamen. Planta. 78: 321-350.
- Kiew, R. and M. Muid. 1989. Bees and palms in Peninsular Malaysia. Principes, 32(2): 74-77.
- Korac, S.Z. and M. Neskovic. 1999. Induction and development of somatic embryos from spinach (*Spinacia oleracea* L.) leaf segments. Pl. Cell Tiss. Org. Cult., 55: 109-114.
- Kulkarni, A.R. 1965. 'Studies on Palms – genus *Cocos* L. in India'. Ph.D thesis submitted to University of Poona.

- Kulkarni, K.M. and T.S. Mahabale. 1974. Studies on Palms. Embryology of *Livistona chinensis* R. Br. Proc. Indian Acad. Sci., 80: 1-17.
- *Kunth, E.P. 1904. Handbuch der Blütenbiologie. III Band I, Teil, Lipzig, Wilhelm Engelmann. pp. 18-56.
- Kuruvinashetty, M.S. and R.D. Iyer. 1980. Tissue culture studies of coconut palm for its clonal propagation. In: P.S. Rao, M.R. Heble and M.S. Chandha (eds.). Proceedings of National Symposium on Tissue Culture, Genetic Manipulation and Somatic Hybridization of plant cells. BARC, Bombay, India. pp. 184-191.
- Lakshmana, A.C. 1993. Rattans of South India. Evergreen Publishers, Bangalore. 180p.
- Lang, E.M. 1943. Developmental anatomy of the fruit of deglect noor-date. Bot. Gaz., 104: 426- 36
- Lee, Y.F. and K. Jong. 1995. Longevity of *Calamus subinermis* H.A Wendl. and *Calamus caesius* Bl. flowers. Sandakania, No. 6: 1-14.
- Listabarth, C. 1994. Pollination and pollinator breeding in *Desmoncus* Mart. Principes, 38(1): 13 – 23.
- Listabarth, C. 1992. A survey of pollination strategies in *Bactridinae* (Palmae). Bull. Inst. Fr. Etudes Andines 21: 699-714.
- Listabarth, C. 2001. Palm pollination by bees, beetles and flies: Why pollinator taxonomy does not matter. The case of *Hyospathe elegans* Mart. (Arecaceae). Plant Species Biology, 16(2): 165-181.
- Lloyd, F.E. 1910. Development and nutrition of the embryo, seed and carpel in date, *Phoenix dactylifera* L. Ann. Mo. Bot. Gard. 21: 103-164.
- Loomis, W. D and J. Battaile. 1966. Plant pnenetic compounds and the isolation of plant enzymes. Phytochemistry, 5: 423 – 438.

- Lotscher, H. 1905. Über den Bau und die Funktion der Antipoden in der Angiospermen Samenanlage. *Flora, Jena*, 94: 213 – 62
- Madulid, D.A. 1980. Notes on the reproductive biology of rattans. I. Pollination in *Plectocomia* Mart. ex Blume. *Sylvatrop*, 5: 157-160.
- Mahabale, T.S. 1982. Palms of India. Maharashtra Association for the Cultivation of Science, Research Institute, Law College Road, Pune, India. 245p.
- Mahabale, T.S., and N.V. Biradar. 1968. Studies on palms : Embryology of *Phoenix sylvestris* Roxb. *Proc. Indian Acad. Sci.*, 67 B, 77 – 96
- Mahashwari, P. 1950. An introduction to the embryology of Angiosperm. New York : Mc Graw-Hill Book Company. pp. 32-185
- Mahabale, T.S. and M.S. Chennaveeraiah. 1957. Studies on *Hyphaene indica* Becc. Morphology. *Phytomorphology*, 7: 184-194
- Maheshwari, C. 1955. The Occurrence of bisporic embryosacs in angiosperms – A critical review. *Phytomorphology*. 5, 67- 69
- Maitreyee, K. and S.Rupnarayan. 1999. Regeneration through organogenesis in rattan. *Pl. Cell Tiss. Org. Cult.*, 59(3): 219-222.
- Manokaran, N. 1983. Survival and growth of Rotan semambu (*Calamus scipionum* Lour.) seedling at 7 years after planting. *Malay. For.*, 46(1): 81-85.
- Manokaran, N. 1976. Germination and early growth rates of the bertam palm – *Eugeissona* Trist. *Malay. For.*, 39: 83 – 90.
- Manokaran, N. 1978. Germination of fresh seeds of Malaysian rattans. *Malay. For.*, 41(4): 319-324.

- Manokaran, N. 1985. Biological and Ecological considerations pertinent to the silviculture of rattan. *In*: K.M. Wong and N. Manokaran (eds.) Proceedings of the Rattan seminar, Kuala Lumpur. October 2 – 4, 1984. The Rattan Information Centre, FRIM, Kepong. pp. 95 – 105.
- Manokaran, N. 1989. Flowering and fruiting patterns in *Calamus caesius* Bl. *In*: A.N. Rao (ed.). Recent Research on Rattans, Proceedings of the International Rattan Seminar. Nov. 12– 14, 1987. Chiangmai, Thailand. Kasetsart University, Thailand and IDRC Canada. pp. 122 – 129.
- Martin, A.C. 1946. The comparative internal morphology of seeds. *Am. Midl. Nat.*, 36: 513-560.
- Martius, C.F.P. V. 1824. Studies on *Leopoldinia* Mart. *Historia Naturalis Palmarum*, 2: 58-60.
- Mascarenhas, A.F., P.K. Gupta, S. Nair, and S.S. Khuspe. 1988. Studies on Plant Tissue Culture at the National Chemical Laboratory, Pune. *In*: S. Nair and A. Karun (eds.). Proceedings of National Seminar on Plant Tissue Culture. 2-4 March, 1983 C.P.C.R.I., Kasaragod. pp. 198-199.
- Mathew, M.M. and V.J. Philip. 2000. *In vitro* adventitious shoot formation from embryos of *Areca catechu* L. *Phytomorphology*, 50(3-4): 221-227.
- Mendoza, M.P. and M.L. Generalao. 1979. Germination, survival and growth of *C. ornatus* Becc. *Sylvatrop, Philippine For. Res. J.*, 4(2): 87-91.
- Miller, C. 2002. Fruit production of the understoried palm (*Oenocarpus bataua* Mart. sub sp. *bataua*. (Arecaceae)). *Economic Botany*, 56(2): 165-176.
- Mogea, J.P. 1978. Pollination in *Salacca edulis* Reinw. *Principes*, 22(2): 56-63.

- Mohd. Zaki, H.A. and J. Othman. 1998. Phenological observations on *Calamus palustris* Griff., *C. scipionum* Mart. and *Cocos nucifera* L. in Peninsular Malaysia. In: R. Bacillieri and S. Appanah (eds.). Rattan cultivation: Achievements, problems and prospects. An international consultation of experts for the project Conservation, genetic improvement and silviculture of rattans in South East Asia, Kuala Lumpur, Malaysia. 12-14 May 1998: pp. 61-71.
- Mohiuddin, M. and R. Ara. 1995. Effect of different sowing media on the seed germination of *Calamus tenuis* Roxb. Chittagong – University studies – Science, 19(2): 303-305.
- Moncur, M.W and B.J. Watson. 1987. Observations on the floral biology of the monoecious palm of *Salacca zalacca* (J. Gaertn.) Voss ex Vilmorin. Principes, 31(1): 20 – 22.
- Moore , H.E. and N.W. Uhl. 1982. Major trends of evolution in palms . Bot. Rev., 48: 1-69.
- Moore, H.E. Jr. 1973. The major groups of palms and their distribution. Gentes Herbarum, 11(2): 27-141.
- Moore, H.E. Jr. and N. W. Uhl. 1973. The monocotyledons: Their evolution and comparative biology. VI. Palms and the origin and evolution of monocotyledons. Q. Rev. Biol., 48: 414-436.
- Morakinyo, A.B. 1995. Profiles and Pan-African distributions of the rattan species *Calamoideae* recorded in Nigeria. Principes, 39(4): 197-209.
- Moran, R.M.J. 1984. Possibilities of the use of *in vitro* techniques in some palm species of Amazonian Peru. Proceedings of the workshop on under utilized palms of tropical America, Turrialba, Costa Rica, 8-10. August 1983 [Organized by CATIEFAO] 1983. pp. 161-163.

- Mori, T.H., A.R. Zollfatah bin, and C.H. Tan. 1980. Germination and storage of rotan manan (*Calamus manan* Miq.) seeds. *The Malaysian Forester*, 43(1): 44-55.
- Mullar, J. 1970. Palynological evidence on early differentiation of angiosperms. *Biol. Rev.*, 45: 417-450.
- Muralidharan, E.M. 1994. Tissue culture of Indian rattans species. Proceedings of the 2nd Asia-Pacific Conference on Agricultural Biotechnology. March 6-10, 1994. Madras. pp. 148-151.
- Muralikrishna, H., V. Rajagopal and K.V. Kasturi Bai. 2001. Effect of desiccation on viability of recalcitrant arecanut. (*Areca catechu* L.) *J. Plantation Crops*, 29(2): 11-15.
- Murashige, T. and Skooge, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, 15: 473-497.
- Murthy, K.N. 1977. Floral and pollination biology of the betel nut palm, *Areca catechu* L. *J. of Plantation Crops*, 5(1): 35-38.
- Murthy, K.N. and K.V.A. Bavappa. 1960. Floral biology of areca (*Areca catechu* L.). *Arecanut J.*, 11(2): 57-55.
- Myers, R.L. 1984. Growth form, growth characteristics and phenology of *Raphia taedigera* Mart. in Costa Rican Palm Swamps. *Principes*, 28: 64-72.
- Nair, M.K. 1997. Standardizing protocol for plantlet regeneration from seedling explants of oil palm. ICAR. News Science and technology Newsletter, 3(1): 1-2.
- Nair, M.K., A. Karun and M.K. Rajesh. 1999. Research potentialities of coconut biotechnology. *Ag. Biotech. Net.*, 1: pp. 1-8.

- Nair, P.K.K. 1970. Pollen Morphology of Angiosperm. Barner and Boble, Inc. New York. pp. 20-65.
- Nair, R.B. 1962. Spathe in Arecanut, *Arecanut J.*, 13(2): 5-12.
- Nazeri, S., S. Khoshkam, M. Afshart, M. Modiri, A. M. Shakib and E. Majidi. 1993. Somatic embryogenesis in date palm varieties Esamaran and Kabkab. *Seed and Plant*, 8(3-4): 16-20.
- Ninan, C.A. and T. G. Ravindranath. 1965. A naturally occurring haploid embryo in the coconut palm *Cocos nucifera* L. *Caryologia*, 16: 619- 623.
- Nwanko, B.A. and A.D. Krikorian. 1983. Morphogenetic potential of embryo and seedling derived callus of *Elaeis guineensis* Jacq. var. *pisifera*. *Ann. Bot.*, 51(1): 65-76.
- Olesan, J.M. and H. Balslev. 1990. Flower biology and pollinators of the Amazonian monoecious palm, *Geonoma macrostachys* Mart.: A case of Bakerian mimicry. *Principes*, 34: 181-190.
- Omar, M.S. 1988. *In vitro* responses of various date palm explants. *Date palm J.*, 6(2): 371-389.
- Onishi, N. T. Mashika and A. Okamoto. 1992. Cultural system producing encapsulatable units of synthetic seeds in celery. *Acta Hertic.* 319 : 113-118.
- Opute, F.I. 1975. Lipid composition and the role of haustorium in the young seedling of the West African Oil Palm, *Elaeis guineensis* Jacq. *Ann. Bot.*, 39: 1057-1061.
- Padmanabhan, D. and A. Regupathy. 1981. Studies on *Bentinckia condapanna* Berry. The fruit and the seed. *Principes*, 25(4): 172-177.
- Padmanabhan, D. and C. Sudhersan. 1989. Laminoids in leaf cultures of a rattan palm. *In: A.N. Rao and Isara Vongkaluang (eds.). Recent Research on Rattans. Faculty of Forestry, Kasetsart University, Thailand and International Development Research Centre, Canada. pp. 148-151.*

- Padmanabhan, D. and P. Krishnan. 1989. Rattan research and tissue culture in South India. *In*: A.N. Rao and A. M. Yusoff (eds.). Proceedings of the Seminar on Tissue culture of Forest species. Forest Research Institute, Malaysia and International Development Research Centre, Singapore. pp. 50 – 62.
- Padmanabhan, D. and R. Ilangovan. 1989. Studies on embryo culture in *Calamus rotang* L. RIC Bulletin, 8 (1/4): 1, 6-9.
- Padmanabhan, D. and R. Ilangovan. 1993. Surgical induction of multiple shoots in embryo culture of *Calamus gamblei* Becc. RIC Bulletin, 12 (1/2): 8-12.
- Padmanabhan, D., V.S. Pushpa, M. Gunamani and A. Regupathy. 1978. Multiple shoot induction in *Calamus* species, Principes, 22(4): 119-126.
- Pannetier, C. and J. Bufford-Morel. 1982. Premiers resultats concernant production of embryons somatiques a partir de tissue foliaires de cocotier, *Cocos nucifera* L. (First result of somatic embryo production from leaf tissue of coconut). Oleagineux, 37: 349-354.
- Pannetiér, C., P. Arthuis, and D. Lievoux. 1981. Neof ormation of young *Elaeis guineensis* Jacq. plants from primary calli obtained on leaf fragments *in vitro*. Oleagineux, 36(3): 119-122.
- Parthasarathy, M.V. 1970. Fine structure of pollen surface in palms. Principes, 14: 55 – 62.
- Patel, J.S., 1938. The Coconut – A monograph, Govt. Press, India. Madras.
- Patena, L.F., M.M.S. Mercado and R.C. Barba. 1984. Rapid propagation of rattan (*Calamus manillensis* H.A. Wendl.) Philippine J. Crop. Sci., 9: 217-218.
- Periasamy , K. 1962. Morphological and ontogenetic studies in palms . Development of the plicate condition in the palm leaf. Phytomorphology, 12: 54 –56.

- *Poulain, C.R. and G. Beauchesne. 1979. Multiplication vegetative en culture *in vitro* du palmier dattier (*Phoenix dactylifera* L.). *Comptes Rendus des seances de l'Academie d'Agriculture de France*. 65: 1151-1154.
- Pritchard, H.W. and R.I. Davies. 1998. Biodiversity and conservation of rattan seeds. *In: R. Bacilieri and S. Appanah (eds.). Rattan cultivation: Achievements, problems and prospects. An international consultation of experts for the project: Conservation genetic improvement and silviculture of rattans in South East Asia, Kula Lumpur, Malaysia, 12-14 May 1998. pp 51-60.*
- Pritchard, H.W., P.B. Tompsett and K. Manoger. 1996. Development of a thermal time model for the quantification of dormancy loss in *Arculus hippocastarum* seeds. *Seeds Science Research*, 6: 127-135.
- Punt, W. & Wessels J.G. Boer. 1966. A palynological study in Cocoid palms. *Acta. Bot. Neerl.*, 15: 255-265.
- Quisumbing, E. and J.B. Juliano. 1927. Development of the ovule and embryo sac of *Cocos nucifera* L. *Bot. Gaz.*, 84: 279-293
- Radermacher, A. 1925. Die gametophyten von *Nypa fruticans* Wurm. and *Actinophloeus macarthurii*. *Ann. Jard. Bot. Buitenz.*, 35: 1-54
- Raghavan, V. and H.K. Baruah. 1956. On factors influencing fruit set and sterility in Arecanut. *Studies on pollen grains. Arecanut J.*, 7: 48-55.
- Rai, S. N. 1979. Pre-treatment of cane seeds. *Indian For.*, 76: 159-163.
- Raja-Barizan, R. S. 1992. Phenology of rattans. *In: Wan Razalli Wan Mohd. (ed.). A guide to the cultivation of rattan. Malay. For. Rec. No. 35, FRIM, Kepong, Kuala Lumpur. pp. 39- 46.*

- Rajanaidu, N., O. Rohani and B.S. Jalani. 1977. Oil palm clones, current status and prospects for commercial production. *The planter*, 73(853): 163-184.
- Raju, C.R., K.K. Sajini, S.M. Balachandran, K.V. Saji, K.G. Maheshan, P.E. Rajasekharan, L. Geetha and K.V.A. Bavappa. 1989. Clonal multiplication of oil palm (*Elaeis guineensis* Jacq.). *In*: C.R. Raju, and S.M. Balachandran (eds.). Proceedings of the seventh symposium on plantation crops. Coonoor, India. 16-19 October, 17-20.
- Raju, C.R., P.P. Kumar, M. Chandramohan and R.D. Iyer. 1984. Coconut plantlets from leaf tissue cultures. *J. Plantation Crops*, 12: 75-78.
- Ramanayak, S.M.S.D. and A. Kovoov. 1995. Zygotic embryo culture to produce planting stock of the rattan – *Calamus thwaitesii* Becc. Hook. f., Sri. Lanka Forester, 20(1 – 2): 15 – 22.
- Rao, C.V. 1955a. Embryo development in Arecanut. *Nature*, 175: 432-433.
- Rao, C.V. 1955b. Embryological studies in Palmae. 1-111. Proc. 42nd Indian Sci. Cong. Abs. Part 3: 230-232.
- Rao, C.V. 1956. Embryological studies in Palmae. IV – VI. Proc. 43rd Ind. Sci. Congr. Part 63: 225-227.
- Rao, C.V. 1958. Contribution to the embryology of Palmae II. Ceroxylineae, *J. Indian Bot. Soc.*, 38: 46-75.
- Rao, C.V. 1959a. Contribution to the embryology of Palmae I. Sabaleae. *Proc. Nat. Inst. Sci. Ind.*, 25: 143-169.
- Rao, C.V. 1959b. Contributions to the embryology of Palmae II. Ceroxylineae. *J. Indian Bot. Soc.*, 38: 46- 75

- Rao, C.V. and S.R. Rao. 1954. Embryology of *Cryptostegia grandiflora* R. Br. and *Caralluma attenuata* Wt. J. Indian Bot. Soc., 33: 453-472.
- Rao, I.V.R., A.M.Yusoff, A.N. Rao and C.B. Sastry. 1989. Propagation of Bamboo and rattan through tissue culture. IDRC, Canada. pp. 14-16.
- Read, R.W. 1963. Palm chromosomes. Principes, 7(3): 85-88
- Read, R.W. 1965. Palm Chromosomes by Air Mail. Principes, 9(1): 4-10.
- Read, R.W. 1966. New Chromosome counts in Palmae. Principes, 10: 55-61.
- Read, R.W. 1968. A study of *Pseudophoenix* H.A. Wendl. (Palmae). Genetes Herbarum Vol. 100, Fasc. 2, pp. 169-213.
- Read, R.W. 1975. The genus *Thrinax* (Palmae Coryphoideae) Smithson. Contr. Bot., 19: 1-98.
- Reddy, G.N. and A.R. Kulkarni. 1989. Megasporogenesis in *Cocos nucifera* L. – A reinvestigation. Curr. Sci., 58 (3): 156-157.
- Redenbaugh, K., B.D. Pasch, J.W. Nichol, M.E. Kossler, P.R. Viss and K.A. Walker. 1986. Somatic seeds: Encapsulation of asexual plant embryos. Biotechnology, 4: 797 – 801.
- Renuka, C. 1992. Rattans of the Western Ghats- A taxonomic Manual. Kerala Forest Research Institute, Peechi. India . 61p.
- Renuka, C. 1994. Genetic diversity and conservation of rattans. In: V. Ramanatha Rao and A.N Rao (eds.). Bamboo and Rattan Genetic Resources and Use. Proceedings of the first Rattan Biodiversity, Genetic Resoursces and conservation working Group. November 7-9, 1994. Singapore. IPGRI, Rome & INBAR. pp. 39 – 41.

- Renuka, C. 1995. Reproductive biology of rattans. *In*: J.T. Williams, I.V.R. Rao and A.N. Rao (eds.). Genetic enhancement of Bamboo and Rattan. INBAR, Technical Report No. 7, New Delhi. pp. 135-141.
- Renuka, C. 1997. Distribution of rattan resources in India. *In*: A.N. Rao and V. Ramanath Rao(eds.). Rattan–Taxonomy, Ecology, Silviculture, Conservation, Genetic improvement and Biotechnology, Sarawak, Sabah, 14-26 April, 1996, IPGRI, Regional Office for Asia, The Pacific and Oceania, Serdang, Malaysia. pp. 55-64.
- Renuka, C. 1998. Rattans, Bamboos and Reeds. *In*: K. Balakrishnan Thampi, N.M. Nayar and C.S. Nair (eds.). The Natural Resource of India, 1998 W.W.F. India, Trivandrum. pp. 454-459.
- Renuka, C. 1999. Palms of Kerala. Kerala Forest Research Institute, Peechi , Kerala. India. P.72
- Renuka, C. and K.M. Bhat. 2002. Commercial rattans of Kerala. KFRI Handbook No.10, 26p.
- Renuka, C., E.P. Indira and E.M. Muralidharan. 1998. Genetic diversity and conservation of certain species of rattan in Andaman and Nicobar Islands and Southern India. KFRI Research Report, No.157. 25p.
- Reynolds, J.F. 1982. Vegetative propagation of palm trees. *In*: J.M. Bonga and D.J. Durzan (eds.). Tissue culture in Forestry. Martinus Nijhoff, The Hague. pp. 182-207.
- Rillo, E.P. 1989. A non-destructive technique for collecting immature coconut inflorescence for tissue culture. Philippine J. Coconut Studies, 4: 16-17.
- Rillo, E.P. and A.W. Ebert, 1993. *In vitro* regeneration of coconut. Progress report of the Philippines German coconut tissue culture project. Proceedings of International GTZ workshop of plant biotechnology held at Trinidad, Legaspi City, Philippines, 6-11 October, 1993. pp. 84-88.

- Roberts, E.H. 1979. Seed determination and loss of viability. *Adv. Seed Res. Technol. Seeds*, 4: 25-42.
- Robertson, B.L. 1976a. Embryology of *Jubaeopsis caffra* Becc. Microsporangium, microsporogenesis and microgametogenesis. *J. S. African Bot.*, 42(2): 97-108.
- Robertson, B.L. 1976b. Embryology of *Jubaeopsis Caffra* Becc. Megasporangium, megasporogenesis and megagametogenesis. *J.S. African Bot.*, 42(3): 173-184.
- Robertson, B.L. 1976c. Embryology of *Jubaeopsis caffra* Becc. Endosperm and Embryogeny. *J.S. African Bot.*, 42(4): 285-299.
- Rodriguez, A.J.A. 1989. Production of embryoids from immature embryos of oil palm. *Revista-Brasileira-de-Fisiologia-Vegetal*. 1(1): 119-120.
- Rousseau, M., S. Manfort, M. Ferry and M. Caballero Ruano. 1999. *In vitro* vegetative propagation of the Canary Islands Palm (*Phoenix canariensis* Hort.). In: M. Rousseau and S. Manfort (eds.). Proceedings of the Second International Symposium on Ornamental Palms and other Monocots from the Tropics, Tenerife, Spain. 3-6, February 1997. *Acta Horticulturae*. No. 486. pp. 155-158.
- Rout, G.R., S. Samantaray and P. Das. 2000. *In vitro* somatic embryogenesis from callus cultures of *Cephaelis ipecacuanba* Rich. *Scientia Hortic.*, 86: 71-79.
- Sabariah, A. 1978. Pretreatment of *Dialium* (KerANJI) and *Sindora* (sepetir) seeds to promote germination. *Malay. For.*, 41: 26-28.
- Salleh, M.N. 2000. Policy and institutional framework for sustainable development of rattan. In: J. Dransfield, F.O. Tesoro and N. Manokaran. (eds.). *Rattan Current Research issues and prospects for conservation and sustainable development*. Sida, INBAR, FAO, Rome.

- Samosir, Y.M.S., I.D. Godwin, S.W. Adkins and R.A. Drew. 1997. An improved protocol for somatic embryogenesis in coconut (*Cocos nucifera* L.) In: Y.M.S. Samosir and I.D. Godwin (eds.). Proceedings of the international symposium on biotechnology of tropical and subtropical species, Part II, Brisbane, Queensland, Australia. 29 September - 3, October 1997. Acta-Horticulture, 1998. No. 461: pp. 467-474.
- Sands, W.M. 1926. Observations on the betel nut palm (*Areca catechu* L.). Malayan Agric.J., 14: 109-110.
- Santos, J. 1929. Cytological study of *Cocos nucifera* L. Philipp. J. Sci., 37: 417-37.
- Sarkar, M.M., H.A. Moursy and S.A. Bekhet. 1998. *In vitro* propagation of Egyptian date palm: morphogenic responses of immature embryos. Bulletin of Faculty of Agriculture, University of Cairo. 49(22): 203-214.
- Sass, J.E. 1958. Botanical microtechnique. Iowa : Iowa State University Press. 20-185.
- Sastry, C.B. 2002. Rattan in the Twenty-First Century – An outlook. In: J. Dransfield, F.O. Tesoro and N. Manokaran (eds.). Rattan Current Research issues and prospects for conservation and sustainable development, Sida, INBAR, FAO, Rome. pp. 237-244.
- Scariot, A.O., E. Lineras and J.D. Hay. 1991. Reproductive biology of the palm *Acrocomia aculeata* (N.J. Jacq.) Lodd. in central Brazil. Biotropica, 23(1): 12-22.
- Schenk, R.U. and A.C. Hildebrandt. 1972. Medium and techniques for induction of growth of monocotyledons and dicotyledonous plant cell cultures. Canadian J. Bot., 50: 199-204.
- Schmid, R. 1970. Notes on the reproductive biology of *Asterogyne martiana* H.A. Wendl. (Palmae).II.Pollination by syrphid flies. Principes, 14: 39-49.

- Schnarf, K. 1931. Vergleichende Embryologie der Angiosperm gebredur, Brotranger, Berlin.
- Schwendiman, J., C. Pannetier and N. Michaux Ferriere. 1988. Histology of somatic embryogenesis from explants of the oil palm, *Elaeis guineensis* Jacq. Ann. Bot., 62(1): 43-52.
- Sekak, M.A., A.H. Hassan and M.R. Noor. 1987. Research note on oil palm protoplasts culture. FRIM-Bulletin, Palm oil Research Institute, Malaysia. No.14: 1-4.
- Selling, O.H. 1947. Studies in Hawanian pollen statistics. Part II. The pollens of Hawanian Phanerogams. Bemice Paushi Bishop Mus. Spec. Pub., 38: 1-430.
- Selvaratnam, E.M. 1952. Embryo of coconut. Nature, 169: 714-715.
- Shivanna, K.R. and N.S. Rangaswamy. 1993. Pollen Biology - A Laboratory Manual. Narosa Publishing House, New Delhi. 119p.
- Shrike, N.S. 1963. Studies on Palms - *Caryota* L. in India. Ph.D. thesis submitted to the University of Pune.
- Shrike, N.S. and T.S. Mahabale. 1972. Studies on Palms: Embryology of *Caryota urens* L. In: Y.S. Murthy, B.M. Johri, H.Y. Mohan Ram and T.M. Varghese (eds.). Advances in plant morphology (Prof. V. Puri Conn. Vol.) Sarita Prakashan, Meerut, India. pp. 218-232.
- Siddiqui, N.A., R. Ara. and S.R. Merry. 1996. Germination and seedling growth of jali - bet (*Calamus tenuis* Roxb.). Bangladesh J. For. Sci. Pl., 25(1/2): 15-20.
- Silberbauer, G.I. 1973. Blüten and Fruchtbiologie von *Butia leiospathus* Barlett (Arecaceae) Ost. Bot. Z., 121: 171-185.

- Sogeke, A.K. 1998. Stages in the vegetative propagation of oil palm. (*Elaeis guineensis* Jacq.) through tissue culture. Journal of oil palm Research, 10(2): 1-9.
- Sowunmi, M.A. 1968. Pollen morphology in the Palmae, with special reference to trends in aperture development. Rev. Palynol., 7: 45-53.
- Spurr, A.R. 1968. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., 26: 31-43.
- Stephen, H.B. 1981. Notes on the phenology of inflorescences and pollination of some rain forest palms in Costa Rica. Principes, 25(3): 101 – 105.
- Sudharsan, C.1988. Morphological and developmental studies on embryos and seedlings in some palms. Ph.D. Thesis submitted to the Kamaraj University, Madurai.
- Surange, S.R. 1966. Studies on palms *Licuala* Thunb. and *Rhapis* L. in India. Ph.D. thesis submitted to the University of Poona.
- *Sussenguth, K. 1921. Betrage zur frage der systematischen gusschiusses der monokotyl, Beih. Bot. Zbl., 38: 1 – 70.
- Swamy, B.G.L. 1942. Embryological studies in Palmae. A preliminary note on the megasporogenesis in *Areca catechu* L. Curr. Sci., 11, 109.
- Tahardi, J.E. 1998. Somatic embryogenesis from cell suspension culture of oil palm. Proceedings of the Fourth-ETIG workshop on oil palm Improvement through Biotechnology, pp. 27-32.
- Tandon, R., T.N. Manohara, B.H.M. Nijalingappa and K.R. Shivanna. 2001. Pollination and pollen-pistil interaction in oil palm, *Elaeis guineensis* Jacq. Ann. Bot., 87(6): 831-838.

- Tang, H., Z. Ren and G. Krezal. 2000. Somatic embryogenesis and organogenesis from immature embryo cotyledons of three sour cherry cultivars (*Prunus cerasus* L.) *Scientia Hort.*, 83: 109-126.
- Tarmizi, A.H. 1999. Effect of glucose concentration on the formation of terminal inflorescences and rigid shoots in oil palm cultures. *J. Palm Research*, 11(2): 41-45.
- Teixeira, J.B., M.R. Sundahl and E.G. Kirby. 1993. Somatic embryogenesis from immature zygotic embryos of oil palm. *Pl. Cell Tiss. Org. Cult.*, 34 (3): 227-233.
- Teixeira, J.B., M.R. Sundahl, and E.G. Kirby. 1994. Somatic embryogenesis from immature inflorescence of oil palm. *Pl. Cell Rep.*, 13(5): 247-250.
- Teo, C.K.H. 1990. Tissue culture of *Calamus caesioides* Blume, *Biotechnol. Paper* presented at Symposium on Biotechnology for Forest Tree Improvement, March 21-23. 1990. Bogari, Indonesia. pp. 75-79.
- Thanikaimoni, G. 1970. Pollen morphology, classification and phylogeny of palmae. *Adansonia*, Ser. 2, 10(3): 347-365.
- Thomas, R.L., K.W. Chan and S.C. Nag. 1970. Phyllotaxis in the Oil palm : arrangement of the male /female spikelets on the inflorescence stalk. *Ann. Bot.*, 34: 93.
- Thomas, V. and P.S. Rao. 1985. *In vitro* propagation of oil palm (*Elaeis guineensis* Jacq. var. *tenera*) through somatic embryogenesis in leaf-derived callus. *Curr. Sci. India*, 54(4): 184-185.
- Tisserat, B. 1982. Factors involved in the production of plantlets from date palm callus cultures. *Euphytica*, 31: 201-214.
- Tisserat, B. and D.A. De Mason. 1982. A scanning electron microscope study of pollen of *Phoenix* L. (Arecaceae), *J. American Soc. Hort. Sci.*, 107(5): 883 – 887

- Tisserat, B. and D.A. DeMason. 1980. A histological study of development of adventive embryos in organ cultures of *Phoenix dactylifera* L. *Ann. Bot.*, 46: 465 – 472.
- Tomlinson, P.B. 1960. Essays on the morphology of palms 1. Germination and the seedlings. *Principes*, 4: 56-61.
- Tomlinson, P.B. 1971. Flowering in *Metroxylon* (The Sago Palm). *Principes*, 15: 2, 49-62.
- Tomlinson, P.B. 1990. The structural biology of palms. Oxford University Press, Oxford. 477p.
- Tomlinson, P.B. and P.K. Soderholm. 1975. The flowering and fruiting of *Corypha* L. *Principes*, 19: 3, 83-99.
- Troll, W. 1935. Vergleichende Morphologie der höheren Pflanzen. Vol. I, Fasc.I. Gebruder Borntrager, Berlin. pp. 67-98
- Turnham, E. and Northcote, D.H. 1982. The use of acetyl CoA carboxylase activity and changes in wall composition as measures of embryogenesis in tissue cultures of oil palm (*Elaeis guineensis* Jacq.). *Biochemical J.*, 208(2): 323-332.
- Uhl, N.W. 1972. Inflorescence and floral structure in *Nypa fruticans* Wurm. (Palmae). *American J. Bot.*, 59(7): 729 – 743.
- Uhl, N.W. 1976. Developmental studies in *Ptychosperma* (Palmae). 1. The inflorescence and the flower cluster. *American J. Bot.*, 63(1): 82-96.
- Uhl, N.W. and H.E. Moore. 1977. Centrifugal stamen initiation in Phytelephantoid palms. *American J. Bot.*, 64(9): 1152-1161.

- Uhl, N.W. and J. Dransfield. 1987. Genera Palmarum, The L.H. Bailey hortorium and the International palm society, Allen Press, Lawrence, Kansas. 610p
- Umali-Garcia, M. 1985. Tissue culture of some rattan species. *In*: K.M. Wong and N. Manokaran (eds.). Proceedings of the Rattan seminar. The Rattan Information Centre and Forest Research Institute, Malaysia. pp. 23 – 32.
- Umali-Garcia, M. and E. Villena-Sanches. 1998. Tissue culture of Rattan: Progress and Prognosis. *In*: N.K. Torreta and E.H. Belen (eds.). Proceedings of the National Symposium/workshop on Rattan. Philippine Council for Agriculture and International Development Research Centre, The Philippines. pp. 98-102.
- Umali-Garcia, M., and A. Canlas- mendosa. 1997. *In vitro* culture of rattan in the Philippines. *In*: A.N. Rao and V. Ramanatha Rao. (eds.). Progress, Problems and Prospects. Rattan taxonomy, ecology, silviculture, conservation, genetic improvement and biotechnology. Proceedings of the training courses cum workshops, Sarawak, Sabah. 14–26 April, 1996. pp. 179–184.
- Valsala, K. and E.M. Muralidharan. 1998. Plant regeneration from *in vitro* cultures of rattan (*Calamus*) *In*: Damodharan, A.D. (ed.). Proceedings of 10th Kerala Science Congress, Kozhikode. January, 1998. Science and Technology, Trivandrum. pp. 161-163.
- Valsala, K. and E.M. Muralidharan .1999. *In vitro* regeneration in three species of rattan (*Calamus* sp.). *In*: P.B.K. Kishor(ed.). Plant tissue culture and biotechnology- Emerging trends. Proceedings of the symposium held at Hyderabad, India. 29-31. January 1997: pp. 118-122.
- Vasil, I.K. and V. Vasil. 1972. Totipotency and embryogenesis in plant cell and tissue culture. *In vitro*, 8: 117-125.

- Veramendi, J and L. Navarro. 1996. Influence of physical conditions of nutrient medium and sucrose on somatic embryogenesis of date palm. *Pl. Cell Tiss. Org. Cult.*, 45 : 159-164.
- Veramendi, J. and L. Navarro. 1997. Influence of explant sources of adult date palm (*Phoenix dactylifera* L.) on embryogenic callus formation. *J. Horti. Sci.*, 72(5): 665—671.
- *Voiget, A. 1888. Untersuchungen Ueber Bau and Entwicklung von Samen mit ruminiertem Endosperm aus den Familien der Palmen, Myristicacean and Anonacean. *Ann. Jardin Bot. Buitenz*, 7: 150-190.
- Wessels Boer, J.G. 1988. Clave descriptive as de Palms indigenas de Venezuela. *Pittieria* 17: 1-332.
- Williams, J.T., I.V.R. Rao and A.N. Rao. 1995. Genetic enhancement of bamboo and rattan. INBAR. Technical report 7: 159 p.
- Wodehouse, R.I. 1935. Pollen grains: their structure, identification and significance in science and medicine. Mc Graw – Hill, New York. 153p.
- Wong, C.K., W.H. Lee and C.H. Peng. 1997. Content of phenols and alkaloids in *Areca catechu* L. during maturation. *J. Agri. Food.Chem.*, 45 (4): 1185-1188.
- Wong, G., C.C. Tan, A.C. Soh and S.P. Chang. 1999. Clonal propagation of oil palm through tissue culture. *Planter*, 75(878): 221-230.
- Wong, G., C.C. Tan, A.C. Soh and S.P. Chang. 1999. Clonal propagation of oil palm through tissue culture. *Planter*, 75(878): 221-230.
- Wong, K.M., and N. Manokaran. 1985. Proceedings of the Rattan Seminar, 2nd - 4th October 1984., Kuala Lumpur, Malaysia. The Rattan Information Centre, Forest Research Institute, Kepong, Malaysia. p. 247.

- Wyuatt, S. J. 1963. Manual of Malayan silviculture for land forests. Malayan Forest Record No. 23. Forest Development. Kuala Lumpur, Malaysia. pp. 16-32
- Yuri, S. 1987. Propagation of Chilean wine palm (*Jubeaea chilensis* (Molina) Bail.) by means of *in vitro* embryo culture, *Principes*, 31(4): 183 – 186.
- Yusoff, A.M. 1989. Shoot formation in *Calamus manan* Miq. under *in vitro*. In: A.N. Rao & A.M. Yusoff (eds.). Proceedings of the Seminar on Tissue culture of Forest species, Kuala Lumpur, June 15-18, 1987. FRIM/ IDRC, Kepong. pp. 45-49.
- Yusoff, A.M. and D.H. Ahmad. 1989. Production of clustering rotan manan (*Calamus manan* Miq.) stock plants. *J. Trop. For. Sci.*, 1(4): 398–399.
- Yusoff, A.M. and N. Manokaran. 1985. Seed and Vegetative propagation of rattans. In: K.M. Wong and N. Manokaran (eds.). Proceedings of the Rattan Seminar, 2nd - 4th October 1984., Kuala Lumpur, Malaysia. The Rattan Information Centre, Forest Research Institute, Kepong, Malaysia. pp. 13 – 22.
- Yusoff, A.M. and U.Salmiah and K.Paranjothy. 1985. Tissue culture of *Calamus manan* Miq. Paper presented at the 2nd Natl. Plant tissue culture workshop, Serdang, UPM. 15- 17 Oct. 1985. pp. 1- 13.
- Zavadil, M.S. 1983. Comparative morphology of monocot pollen and evolutionary trends of apertures and wall structures. *Bot. Rev.* 49(4) : 331-379
- Zhiying, C. and F. Kuan. 1995. Rattan propagation and nursery in Yunnan. *For. Sci. Tech.*, (2): 22-25.
- Zhuang, C. and J. Zhou. 1991. Plant regeneration in tissue culture of rattan. *Acta Botanica Yunnanaica*, 13(1): 97-100.
- Zhuang, F. 1993. A study on rattan tissue culture. *For. Res.*, 6(5): 486-492.
- Zona, S. 1987. Phenology and pollination biology of *Sabal etonia* in south Eastern Florida. *Principes* 31 : 18—24
- * Original not seen