VIABILITY OF *Hopea parviflora* SEEDS WITH REFERENCE TO TEMPERATURE, MEDIUM OF STORAGE AND MICROENCAPSULATION TECHNIQUES

BY

SUNIL KUMAR K. K.

THESIS

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Faculty of Agriculture
Kerala Agricultural University

Department of Silviculture and Agroforestry
COLLEGE OF FORESTRY
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DECLARATION

I hereby declare that the thesis entitled "Viability of *Hopea parviflora* seeds with reference to temperature, medium of storage and micro encapsulation techniques" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society

Vellanikkara
30 3 1996

SUNIL KUMAR, K.K.

Dr. K. Sudhakara
Associate Professor
Department of Silviculture
and Agroforestry

College of Forestry
Kerala Agricultural University
Vellanikkara, Thrissur

CERTIFICATE

Certified that this thesis, entitled "**Viability of *Hopea parviflora* seeds with reference to temperature, medium of storage and micro encapsulation techniques**" is a record of research work done independently by **Sri. Sunil Kumar, K.K.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him

Vellanikkara
30 3 1996

Dr. K. Sudhakara
Chairman
Advisory Committee

CERTIFICATE

We, the undersigned members of the Advisory Committee of Sri. Sunil Kumar, K.K., a candidate for the degree of Master of Science in Forestry, agree that the thesis entitled **"Viability of *Hopea parviflora* seeds with reference to temperature, medium of storage and micro encapsulation techniques"** may be submitted by **Sri. Sunil Kumar, K.K.,** in partial fulfilment of the requirement for the degree

Dr. K. Sudhakara
Associate Professor
Dept of Silviculture and Agroforestry
College of Forestry
Vellanikkara, Thrissur
(Chairman)

Dr. B. Mohankumar
Associate Professor and Head
Dept of Silviculture
and Agroforestry
College of Forestry
Vellamkkara, Thrissur
(Member)

Dr. K.K. Seethalakshmi
Scientist
Div of Plant Physiology
Kerala Forest Research Institute
Peechi

Dr. R. Keshavachandran
Associate Professor
Dept of Plantation Crops
College of Horticulture
Vellanikkara, Thrissur
(Member)

External Examiner

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Introduction

INTRODUCTION

Seeds are broadly classified into two categories viz orthodox and recalcitrant to describe their storage behaviour (Roberts 1973) Orthodox seeds are shed from the parent plant at low moisture content after undergoing maturation drying They can be further dried to moisture contents below five per cent without damage In this dehydrated state the seeds can resist the changes of the environment, and unless dormant will resume full metabolic activity, growth and development when conditions favourable to germination are provided (Roberts and King, 1980) Due to these properties they can be stored for long periods

On the other hand, recalcitrant seeds do not undergo maturation drying and are shed at relatively high moisture contents Such seeds are highly susceptible to desiccation injury and are killed if the moisture content is reduced below some relatively high critical value (Roberts and King 1980) They cannot be stored for long by conventional storage methods Hanson (1984) suggested that it is more accurate and meaningful to call orthodox seeds as desiccation tolerant and recalcitrant seeds as desiccation sensitive As the basic difference between orthodox and recalcitrant seeds resides on their water relations Berjak *et al* (1990) described them as poikilohydrous and homoiohydrous respectively

Storage of economically important recalcitrant seeds especially that of tree species is an important problem *Hopea parviflora* Bedd is one of the most economically important recalcitrant species coming under the family Dipterocarpaceae (Troup, 1921) The seeds of *H parviflora* loose their viability within 7 10 days under natural conditions, when the seed moisture content reduces below a high critical value

Since recalcitrant seeds are desiccation sensitive storing the seeds under moist conditions is suggested to avoid desiccation injuries (Chin, 1988) But this has only been successful for short term storage Furthermore, storing seeds with high moisture content at sub ambient or ambient temperatures will lead to fungal contamination and germination of the seeds while being kept for storage Storing the seeds under low temperature has been suggested to prevent fungal contamination and germination during storage (King and Roberts 1980) But most of the recalcitrant seeds are sensitive to temperature below 15°C (Hor *et al* 1984) Treating the seeds with fungicides before storage has also been suggested to prevent fungal contamination (King and Roberts, 1980) Still the period of viability is very short under higher temperatures

In the past, different methods including storing recalcitrant seeds in moist conditions sealed containers perforated polythene bags etc have been tried at different

temperatures but most of them were unsuccessful for long term storage Short viability of the *H parviflora* seeds has hindered the setting up of species trials, enrichment and plantation programmes though this species possesses superior wood qualities These forestry programmes involving *Hopea parviflora* will be possible only if the viability and longevity of their seeds are increased by devising suitable storage techniques

The present study was conducted during 1994-'95 in College of Forestry, Vellanikkara with the following objectives

1 To standardise most suitable storage temperature for *Hopea parviflora* seeds

2 To study the effect of sand and neem cake as a storage medium in mud pot containers for extending the storage life of *Hopea parviflora* seeds

3 To investigate the possibility of micro encapsulation of zygotic embryo (synthetic seed) as a storage method of *Hopea parviflora* seeds

Review of Literature

REVIEW OF LITERATURE

It is becoming increasingly evident that world plant genetic resources are being drastically reduced through loss of old cultivars and replacement of primitive land-races by new genetically uniform cultivars (King and Roberts, 1980) Seed storage is the easiest and least expensive method to arrest this trend (Harrington, 1970) Unfortunately, seeds of most of the economically important tree species in the tropics are recalcitrant in nature and cannot be stored for long periods Their requirement for a high moisture content reduces their storability since they can only be stored in wet medium to avoid dehydration injury and at warm temperature because chilling injury is common to these type of seeds (Chin and Roberts, 1980, Come and Corbineau, 1992) Major factors responsible for the short longevity of these seeds include desiccation sensitivity, chilling injury, microbial contamination and germination during storage (King and Roberts, 1980)

2.1 Desiccation sensitivity

Seeds of many tropical and sub tropical trees are high in moisture content at the time of shedding and cannot withstand desiccation (Roberts *et al* 1984) Farrant *et al* (1988) proposed

a continuum of recalcitrant seed type mainly based on the sensitivity towards water loss and low temperature Variation in desiccation sensitivity between species has been reported in many genera such as *Dipterocarpus* (Tompsett, 1984), Acer (Olsen and Gabrial, 1974) and in *Araucaria* (Tompsett, 1984) According to King and Roberts (1980), variation in the moisture content at fully imbibed state is the main reason for apparent variation in susceptibility to desiccation in different species

The gradations in the degree of recalcitrance may be associated with a corresponding variation in the relative quantity and type of carbohydrate (Teichman and Vanwyk, 1994), late embryosenesis abundant proteins (Farrant *et al* 1992a), rate of dehydration (Berjak *et al*, 1989, King and Roberts, 1980) and the developmental status of the seed (Berjak *et al*, 1992)

2 1 1 Seed development and desiccation tolerance

During seed development, three broad stages are generally recognized (Raghavan, 1986) The first stage is characterised by fertilization, cell division and histo-differentiation of all major tissues During the second stage, a massive accumulation of storage proteins, lipids and starch occurs followed by the third stage in which the reserve deposition

and desiccation culminates The occurrence and the extent of drying varies among species and embryo enters a state of quiescence when metabolism is nil or not measurable (Lynch and Clegg, 1986)

Before seeds experience the severe water loss, they acquire the ability to withstand it (Leprince *et al*, 1994) Many seeds undergo a fast transition from desiccation intolerance to tolerance approximately midway through development and preceding or coinciding with reserve deposition (Kermode and Bewley, 1989, Hong and Ellis, 1992) Upon germination, desiccation tolerance is maintained for several hours after the onset of imbibition Prior to radicle emergence, seeds can withstand extreme drying, but as germination progresses, drying is highly damaging and ultimately lethal (Leprince *et al*, 1990b)

Maturation drying is considered to be necessary for the completion of the life cycle of orthodox seeds The metabolically quiescent state enables seed survival during storage and ensures ability to withstand unfavourable environmental conditions Germination is initiated only when water becomes available and other environmental conditions are also favourable In contrast, recalcitrant seeds do not experience a developmental maturation drying (Leprince *et al* 1994) and seeds are naturally shed on moist soil and the

events occurring during development facilitate immediate germination In other words, when ripe orthodox seeds fall from a plant, they have a moisture content less than that necessary for germination where as recalcitrant seeds are usually fully imbibed and capable of immediate germination

2.1 2 Desiccation induced membrane damage

Ultrastructural studies of tissue after desiccation have revealed that cellular membranes are one of the primary sites of injury Leprince *et al* (1994) described dehydration injuries primarily on the basis of alterations in membrane structural integrity, function and physico-chemical properties An irreversible collapse of membrane is reported in many cases (Dasgupta *et al* 1982, Sergent *et al*, 1981) and leakage of various cytoplasmic solutions is accepted as the early indicator of desiccation induced damage Desiccation induced changes result in the loss of membrane integrity As a result more ions will be leached out Impact of desiccation is mainly manifested in the transition of lipid phase of membranes from liquid crystaline to gel state (Berjak *et al*, 1994) There are many reports characterising the positive correlation between the rate and extent of cytoplasmic leakage and desiccation sensitivity (Espindola *et al*, 1994, Senaratna and Mckersie, 1983, 1986)

2.1 3 Role of plant growth regulators

Farrant *et al* (1993b) described the role of plant growth regulators in different stages of seed development for both desiccation tolerant and intolerant species Though embyogenesis and histo differentiation appears to be influenced by plant grwoth regulators (PGR) in much the same way as it occurs in orthodox species, control of reserve accumulation and subsequent utilization appears to be different in recalcitrant seeds

Although all classes of PGRs have been implicated to some extent in reserve accumulation in various orthodox seeds, it is widely believed that ABA plays a prominent role especially in the accumulation of proteins (Kermode, 1990) In these seeds net reserve utilization subsequent to the decline in ABA is prevented by low water status consequent to drying or the metabolically quiescent nature while in recalcitrant seeds as they remain metabolically active, reserve utilization will be followed by germination

In *Avicennia marina* seeds, only pericarp contains high level of ABA which continue to increase till pre-mature embryos become fully germinable At this stage pericarp of the seed is sloughed off to complete the process of germination (Farrant *et al* 1993b) The influence of ABA could be overcome by sloughing off the external structures preventing

germination as in the case of *A marina* or declining the ABA concentrations as occurs in *Theobroma cacao* and *Quercus* spp (Farrant *et al* 1993a) Other possibilities include leaching of ABA or decrease in the sensitivity of the embryo to ABA (Kermode, 1990), which are common in desiccation tolerant seeds

2.1 4 Role of sugar in desiccation tolerance

Sugars are involved in the mechanisms of resistance of seeds to desiccation (Koster and Leopold, 1988) mainly through protein and lipid stabilization and enhancement of glass formation (Leprince *et al* 1994 Leopold *et al*, 1994) There is considerable variation among sugars in their capability to act as protective agents Trehalose is reported to be best protective agent, followed by disaccharides (sucrose, maltose etc) and then monosaccharides (Crowe *et al*, 1987) In addition, disaccharides considered to favour more glass formation (Leopold *et al* 1994) Koster and Leopold (1988) suggested that the ratio of oligosaccharides of the raffinose family to sucrose is relevant to the desiccation tolerance mechanism of seeds Oligosaccharides of raffinose family prevent sucrose crystalization during dehydration allowing glassy state of the cytoplasm to occur (Koster and Leopold 1988, Leopold and Vertucci, 1986)

The water replacement hypothesis suggests that during dehydration, sugars replace water on macro molecular surfaces, thus enabling stabilization of membranes in desiccated state Existence of such a system is suggested to be the main reason for desiccation tolerance (Koster and Leopold, 1988, Leopold and Vertucci, 1986) in orthodox seeds Furthermore Koster (1991) has suggested that the levels of oligosaccharides characteristic of desiccation tolerant phase of orthodox seeds are more likely to facilitate vitrification at ambient temperature, which might occur only at sub zero temperature in desiccation sensitive seeds

2 1 5 Role of late embryonic abundant (LEA) proteins

Studies on a variety of desiccation tolerant seeds have shown that a set of dehydration or ABA inducible hydrophilic proteins (LEA dehydrin like proteins) is produced and accumulates during the late stages of development (Blackman *et al*, 1991, Bradford and Chandler, 1992) Some of these proteins have been implicated in the mechanism of seed desiccation tolerance (Kermode, 1990) or in protection against dehydration damage in seedlings (Gomez *et al*, 1988), possibly by binding macromolecular structures (Dure *et al* 1989) The maturation of orthodox seeds is characterized by the accumulation of LEA proteins while desiccation sensitive seeds like *Avicennia marina* lack such proteins (Farrant *et al*, 1992a),

supporting the hypothesis of Bradford and Chandler (1992) that lack of such proteins might be an inherent feature of desiccation sensitive tissues Farrant *et al* (1992a) suggested that the lack of LEA proteins in embryos of *A marina* during their development might be due to either the absence of relevant genes or the loss of competence to transcribe them

2 1 6 Drying rates

One of the main reason for apparent variation in susceptibility to drying injury is the variation shown by individual species in the moisture content at fully imbibed condition (King and Roberts, 1980) According to them sometimes this susceptibility may also be due to slow drying in combination with orthodox behaviour Even though Hunter (1959) has shown that drying rates have little effect on loss of viability of cocoa, Farrant *et al*, (1988) proposed an interesting model in which he explained that rate of drying is of considerable importance

2.2 Chilling injury

In nature many trees and shrubs can survive freezing temperatures, but some are prone to injury Most of the recalcitrant seeds belonging to timber, plantation crops and fruit species grow in, and are adapted to a warm and tropical forest habitat (King and Roberts, 1980) Thus it is not

surprising that they do not tolerate freezing temperatures although the failure of the seeds of some species to survive at 15°C is hard to understand (Chin, 1988)

Chilling injury may be exhibited as a loss of viability or reduced growth during germination at favourable temperatures (Simon, 1979, Wolk and Herner, 1982) Many species, especially those of tropical and sub-tropical origin, suffer injury when exposed to temperatures above the freezing point of tissue but below 15°C (Bedi and Basra, 1993)

Among the recalcitrant seeds there are varying degrees of tolerance to low temperature The temperate species are more tolerant to low temperature (Chin, 1988) Seeds of *Quercus* spp for example can germinate at 2°C after 8 months in cold storage whereas the seeds of many tropical species are killed on exposure to sub-ambient temperature or suffer chilling injury (Boroughs and Hunter, 1963, Chin, 1975, Sasaki, 1976, Tang and Tamari, 1973, Tompsett, 1992) Hence the potential advantages associated with the seed storage at sub-ambient temperatures cannot be utilized in the case of recalcitrant seeds (King and Roberts, 1980)

The most marked feature of the effect of temperature on the storage of dipterocarp seeds is the incidence of chilling damage (Tompsett, 1992) Tang (1971) found that *Shorea curtisii* could be fatally damaged by exposure to only 16 hours of 4°C

indicating extreme susceptibility to chilling temperatures Seeds of *Shorea roxburghii* and *Hopea hainanensis* lose viability rapidly when stored at 2 to 5°C (Purohit *et al*, 1982, Tompsett, 1985) while latter retained seed viability for more than 6 months when stored at 15 to 20°C (Song *et al*, 1984) Sasaki (1980) and Yap (1981) proposed classification of dipterocarps based on the chilling sensitivity

The literature on chilling injury in seeds covers both orthodox and recalcitrant seeds Succeptability of orthodox seeds, if not dried to a sufficiently low moisture content to freezing temperatures has been reported very early (Becquerel, 1925, Lipman and Lewis, 1934) Stanwood (1984) stated that there is a high moisture freezing limit (HMFL) which is the threshold that if exceeded will result in a decrease in viability of a seed sample during liquid nitrogen cooling This threshold is normally a relatively narrow range of seed moisture content within a species, but it can vary between species The cause of seed death in these cases is similar to that of recalcitrant seeds with high moisture contents when frozen Freezing damage in moist seeds is presumably associated with the formation of ice crystals, and usually occurs at moisture contents above 14-20 per cent (Roberts, 1972)

Studies on cocoa seeds have shown that the fall in viability with declining temperature can be extremely abrupt

Possible reasons are explained by Boroughs and Hunter (1963) Lyons-Raison hypothesis (Lyons, 1973) states that the primary cause of chilling injury is due to the physical response of the membrane lipids to low temperature

The membrane lipid fluidity of recalcitrant seeds declines at sub ambient temperature which results in rapid membrane disruption (Wolfe, 1978) On the contrary, the lipids of chilling-resistant species do not show these changes until much lower temperature are reached Protein denaturation at low temperature is also suggested to be the factor causing chilling damages (Simon *et al*, 1976)

2.3 Microbial contamination

Microbial contamination of stored seeds is a well recognized problem (Christensen, 1972) It is generally considered that at seed moisture contents in excess of 10 to 13 per cent fungal invasion can either rapidly destroy seed viability (Harrington, 1963) or atleast hasten seed death (Roberts, 1972) Since recalcitrant seeds should be stored in a moist condition, microbial contamination could be an important constraint to the conservation of recalcitrant seeds Microbial growth can be reduced to some extent by lowering the temperature of the storage environment (King and Roberts, 1980) But this is also inappropriate to

recalcitrant seeds since they are susceptible to chilling injury

Jensen (1971) emphasised the need to protect moist dipterocarp seeds against fungal attack There are reports which reveal the beneficial effects of fungicides in improving the storage life of seeds (Bergh, 1975, Tamari, 1976, Yap 1981)

2.4 Germination during storage

A problem associated with storage of recalcitrant seeds is the germination during storage and has been reported by a number of workers (King and Roberts, 1980, Tompsett, 1992) Recalcitrant seed sheds usually at fully imbibed state and is capable of immediate germination Tompsett (1992) stated that if desiccation and mechanical damage to the radicle are avoided, viable seedlings can still be produced by a high proportion of pre-germinated seeds

For majority of the recalcitrant seeds the simplest method to control pre-germination is to reduce the storage temperature to a permissible limit which will not cause chilling injuries (King and Roberts, 1980) This also facilitates reduction of microbial growth and physiological deterioration Maury-Lechon *et al* (1981) recommended drying dipterocarp seeds to half the original moisture content on

collection to prevent pre-germination in storage Many attempts were made to prevent germination during storage by using natural germination inhibitors (Chin, 1975, Swarbrick 1965) artificial chemical inhibitors (Barton, 1965, Goldbach, 1978), and by using light (Villiers, 1974, Wessen and Wareing, 1969) But most of them were unsuccessful

A number of scientists have suggested that the germination of recalcitrant seeds during storage could be reduced if they were harvested before attaining full maturity (Pyke *et al*, 1934, Tompsett, 1992) However the literature also suggests that there is no real benefit in harvesting immature seeds because any slight reduction in germination is generally accompanied by reduced and irregular germination, the resultant seeds are often prone to genetic deformities (Barton, 1965, Bitters, 1977)

2.5 Storage methods

King and Robers (1980) suggested that any storage method for recalcitrant seed should give emphasis on preventing desiccation, chilling injury, germination during storage, microbial contamination and maintaining adequate oxygen supply A number of different storage methods have been tried (Jensen, 1971, Sasaki, 1976, Song *et al*, 1984, Tamari, 1976, Tang and Tamari, 1973, and Tompsett, 1985) But none of them can be applicable for long term storage

As recalcitrant seeds are desiccation sensitive the obvious answer would be to store them in moist media, but this has only been successful for short term storage (Chin, 1988) There has been many attempts to increase the viability of recalcitrant seeds by using different media for storage Use of several moisture retaining media including damp peat, saw dust and charcoal were reported to be successful for storage of cocoa (Evans, 1953, Thompson, 1950)

Song *et al* (1984) achieved excellent results with coconut dust medium for storage of *Hopea hainanensis* over a period of one year Recently, storage in perlite has achieved good results, especially for *Parashorea smythiesu* seed (Tompsett, unpublished)

Attempts have been made to store seeds within the fruit or in fruit juice but these have been generally unsuccessful as has been shown with cocoa (Pyke *et al*, 1934) Storage of cocoa seeds in different concentrations of aqueous solutions of polyethylene glycol '6000' at different temperature were also tried (Sakhibun, 1981) but found to be unsuccessful in enhancing seed longevity

Enhancing the viability period of neem seeds using moist sand as the medium of storage has been reported by Ponnuswamy *et al* (1991) Neem seeds stored in earthen pots buried upto the neck level in 20-25 per cent moist sand bed retained

62 per cent viable seeds at the end of three months However germination during storage and fungal growth has been reported as major problems of all moist storage methods

Tompsett (1983) stressed the importance of oxygen availability in imbibed storage High quality seeds of *Hopea hainanensis* stored at 18°C in a slowly ventilated atmosphere (Oxygen content, 10 13%) with a moisture content controlled to 35-38 per cent retained a germination potential of more than 80 per cent after storage for more than one year (Song *et al* 1984) Viability declined when moisture content was reduced below 35 per cent for *Shorea roxburghu* and below 40 per cent for *Shorea robusta* (Tompsett, 1985)

Use of sealed polyethylene bags is generally not preferred for storing recalcitrant seeds because ventilation is needed to remove toxic gases and to prevent anoxia, increasing oxygen concentration above atmospheric levels does not however, improve storage life (Tompsett, 1987) Neem seeds stored in sealed polyethylene bags exhibited rapid deterioration in germination capacity irrespective of the storage temperature (Maithani *et al*, 1989), while seeds stored in perforated polyethylene bags at 15°C retained seed viability for longer period

Currently partial drying plus application of fungicide is reported to be most promising method for storage of recalcitrant seeds Tang and Tamaii (1973) became successful in enhancing longevity of *Hopea helferi* and *Hopea odorata* by storing them in polythene bags at about 15°C after air drying for few hours and treating with fungicide Similarly enhancement of seed longevity by storing partially dried seeds after fungicide application in perforated polybags has been reported by Chin (1988) and Hor *et al* (1984)

According to Roberts *et al* (1984), the most promising method is the storage in liquid nitrogen and many of the current difficulties in maintaining the viability of stored recalcitrant seed could be overcome by this method But so far there has been no success with truly recalcitrant seeds However there are number of reports that hydrated plant cells can survive immersion in liquid nitrogen provided both cooling and thawing rates are ultra rapid (Luyet, 1937, Sakai, 1966a) So adopting cryogenic techniques similar to those found to be successful in the preservation of other biological materials, the low temperature storage of recalcitrant seeds or embryos may be practicable

2.6 Sythetic seeds

The use of somatic embryos to produce synthetic seeds was first proposed by Murashige (1978) Synthetic seed is a novel analog to botanic seed, capable of development to an entire plant body which comprises of meristematic tissue, encapsulated free from botanical accessory structures in a hydrated gel capsule (Redenbaugh, 1986a) The gel act as a synthetic seed coat and protects embryo from injuries

It is now common to encapsulate somatic embryos in alginate salt (Bapat and Rao, 1988, Gray, 1987) Literature revealed that during the production of artificial seeds, usage of $CaCl_2$ concentration beyond 75 mM adversely affected shoot emergence (Ahuja *et al* 1989) Best results in terms of bead quality and per cent shoot emergence were obtained when the beads were formed using a combination of 5 per cent sodium alginate solution with 75 mM $CaCl_2$ $2H_2O$, while studies conducted in encapsulation of zygotic embryo of cocoa *(Theobroma cacao)* showed that a combination of 4 per cent sodium alginate and 75 mM $CaCl_2$ $2H_2O$ are appropriate (Nagaraj, 1994)

It has been frequently observed that simply encapsulated embryo could not emerge from the gel bead even if the embryo had excellent quality Possible causes for this inhibition of conversion are unsuitable elasticity and/or strength of the gel bead (Onishi *et al* 1994) A novel self breaking or self

splitting gel bead has been developed to overcome the above mentioned difficulty (Sakamoto *et al*, 1992)

2 6 1 Storage and germination

The most important requirement for a synthetic seed to be used for clonal mass propagation of plants is high and uniform conversion under practical sowing situation such as nursery bed in a green house or in the field (Onishi *et al*, 1994) In an experiment conducted in College of Forestry, Vellanikkara, encapsulated zygotic embryo of cocoa were stored at 4°C on dry cotton and wet cotton and was found that synthetic seeds stored on wet cotton retained viability for longer period (Nagaraj, 1994) Seventy one per cent of synthetic seeds stored on wet cotton regenerated to complete plantlet after 25 days while synthetic seeds stored on dry cotton showed a gradual decline in regeneration after 15 days of storage and only 50 per cent germination was obtained after 25 days of storage

Gupta and Darzan (1987) encapsulated somatic embryos of loblolly pine in alginate But conversion of embryo into seedlings was not achieved after storing at low temperature (4°C) for four months

However successful development of plantlets were reported by Redenbaugh *et al* (1986) with encapsulated alfalfa and celery

somatic embryos using sodium alginate This technique was successfully applied for the encapsulation of axillery and apical shoot buds of *Valeriana wallichii* and *Picorrhiza kurroa* (Ahuja *et al*, 1989)

Encapsulation of protocorm like bodies (plbs) obtained by culturing shoot apices of *Dendrobium wardianum* increased plant regeneration as well as storage period (Sharma *et al*, 1992) Increase in storage period of somatic embryos by encapsulation is also reported in *Asparagus cooperi* where the frequency of conversion of artificial seeds to plants was affected by the concentration of $CaCl_2$ and commercial sources of sodium alginate and nutrient medium (Ghosh and Sen, 1994)

Ganapathi *et al* (1992) encapsulated shoot tips isolated from multiple shoot cultures of banana in 3 per cent sodium alginate The encapsulated shoot tips recorded 100 per cent regeneration in White's medium, and also were successfully established in soil Successful regeneration of artificial seeds in MS medium + 3 ppm 2,4-D + 0 5 ppm kinetin was also reported in *Dendrocalamus strictus* (Mukunthakumar and Mathur, 1992) The germination frequency was 96 per cent and 45 per cent in *in vitro* and in soil respectively

Twenty days old green protocorms cultured from seeds of *Spathoglottis plicata* were encapsulated in sodium alginate (Singh and

Singh, 1991) and found that they could be stored for upto six months at 4°C with little loss of viability Encapsulated protocoms regenerated to complete plantlets in Vacin and Went modified medium, while non-encapsulated protocorms stored at same temperature showed no viability after 30 days of storage

Artificial seeds can also be directly sown to artificial media like vermiculture, agar, soil, filter paper, green house mix etc under *in vitro* conditions But sowing of beads directly into soil under *in vivo* conditions generally resulted in failure of germination To solve this problem Mathur *et al* (1989) working in *Valeriana wallichii* suggested removal of sucrose from liquid media and incorporation of antimicrobial agents like Chloramphenicol or Bavistin in the encapsulation medium Bapat and Rao (1990) have also reported the beneficial effect of adding fungicides in the encapsulation medium

2 6 2 Desiccation tolerance

The use of somatic embryos from cell culture systems in the clonal propagation of plants would be greatly facilitated if the somatic embryos could be dried and stored in a dormant state in a similar way to true seeds (Senaratna *et al* 1990) Application of ABA, heat shock, high sucrose concentration (McKersie *et al* 1990), water or nutrient stresses (McKersie *et al*, 1989), applied to the embryoids at the cotyledonary

stage of development resulted in acquiring desiccation tolerance The embryoids could be subsequently air dried slowly (over 7 days) or rapidly (over 1 day) to moisture contents of less than 15 per cent and retained viability upto 8 months (McKersie *et al* 1989)

The rate of drying also affects survival of somatic embryos Senaratna *et al* (1989) reported that slow drying (1 2 g H_2O/g/day over six days) gave higher and more consistent embryoid survival, compared to fast drying (6 g H_2O/g/day over one day) Desiccation tolerance was also induced by exposing somatic embryos to sub lethal levels of low temperature However, some of the stress pre-treatments had other deleterious effects on embryoid maturation and seedling vigour after inhibition Treating the embryos with ABA for ten days before encapsulation is also reported to be enhancing germination even when the embryo is dehydrated to five per cent moisture content (Liu *et al*, 1992)

Anandarajah and McKersie (1990) investigated the effect of sucrose concentration in the maturation medium in combination with a heat shock treatment at 36°C to improve the vigour of seedlings grown from dry somatic embryos of *Medicago sativa* It was found that with 3 per cent sucrose in the maturation medium, the somatic embryos germinated precociously and were unable to survive desiccation At higher sucrose

concentration germination was delayed and after 13 days on 6 per cent sucrose medium, the somatic embryos tolerated drying to 12 per cent moisture content without exposure to exogenous ABA Heat shock, which presumably stimulated endogenous ABA synthesis, improved the desiccation tolerance of somatic embryos if applied prior to 27 days after sieving

Materials and Methods

MATERIALS AND METHODS

The present investigation on improving storage techniques of the recalcitrant seeds of *Hopea parviflora* by selecting different media of storage, different temperatures and embryo encapsulation (synthetic seeds) techniques was carried out in the College of Forestry, Vellanikkara

3.1 Materials

3 1 1 Seed source

Seeds were collected from the *Hopea parviflora* stand in the Kerala Forest Research Institute, subcentre, located at Karimpuzha, Nilambur Taluk of Malappuram District This stand was established in 1920's Eventhough establishment of plantation was a failure, it became successful through natural regeneration when adequate protection was given Other tree species found in the area are *Swietenia macrophylla, Vateria indica, Xylia xylocarpa Terminalia paniculata* etc The area is located at 11°17'N and 76°4'E and enjoys warm humid tropical climate with mean annual temperature of 18°C to 30°C Mean annual precipitation of this area is 2400 mm

3 1 2 Media

River sand and powdered neem cake were used as media for seed storage Alginate was used for embryo encapsulation and basic tissue culture medium (MS) was used for studying their viability The technical composition of MS media is given in Table 1 Dry cotton and wet cotton were used for storing the synthetic seeds

3.2 Methods

3 2 1 Seed collection

An area of 6 m x 6 m was selected from the *Hopea parviflora* stand Sheets of nylon nets were spread and tied to the sample plots above the ground surface to avoid the seeds falling on the ground and seeds were collected at an interval stretching maximum of 48 h Site was visited at weekly intervals and the following observations were recorded

1 Date of onset of flowering
2 Date of fruit set
3 Date of fruit shedding
4 Important insects attacking the seeds

Seeds were collected from the site during May-June 1993 and 1994 and brought to the College of Forestry on the same day for conducting the experiment

Table 1 Chemical composition of Murashige and Skoog medium

Compound	Quantity (mg l^{-1})
INORGANIC	
Ammonium nitrate	1650 0
Boric acid	6 2
Calcium chloride - 2 hydrate	440 0
Calcium nitrate 4 hydrate	0 0
Cobalt chloride - 6 hydrate	0 025
Copper sulphate 5 hydrate	0 025
Ferrous sulphate - 7 hydrate	27 8
Manganese sulphate 1 hydrate	22 3
Magnesium sulphate 7 hydrate	370 0
Na_2 EDTA 2 hydrate	37 3
Potassium dihydrogen phosphate	170 0
Potassium iodide	0 83
Potassium nitrate	1900 0
Potassium sulphate	0 0
Sodium molybdate - 2 hydrate	0 25
Zinc sulphate 7 hydrate	8 6
ORGANIC	
Inositol	100 0
Nicotinic acid	0 5
Thiaine Hcl	0 1
Pyridoxine Hcl	0 5
Glycine	2 0
OTHERS	
Sucrose (in per cent w/v)	3 0
Agar (in per cent w/v)	0 7

1/2MS denotes half the amounts of the inorganic constituents per litre

3 2 2 Seed characteristics

Thousand seed weight, moisture content and germination percentage of *Hopea parviflora* seeds immediately after natural shedding were determined The seed components (seed coat, cotyledon and embryo) were separated and individual moisture content were also determined

3 2 3 Effect of storage temperature on seed viability

Naturally shed seeds were collected from nylon nets during last week of May and brought to the College after removing the litter, and insect attacked seeds On the same day homogenous samples of dewinged seeds were kept in perforated polythene bags (50 seeds per polythene bags) at three different temperatures viz , 10°C, 20°C and room temperature (29 ± 1°C) BOD incubator was used to store the seeds at 10°C and 20°C R H was maintained at 100 per cent by keeping beakers filled with distilled water in each tray inside the incubator For comparing the effect of storage on dewinged seeds as against winged seeds, four replicates of winged seeds were also stored at room temperature Samples were taken at weekly intervals and moisture content, leachate conductivity and germination parameters were determined till the complete loss of seed viability or for two months whichever was earlier Initial seed moisture content, leachate conductivity and germination parameters were also recorded

before seed storage The treatments were set up in Completely Randomised Block Design

3 2 3 1 Germination methods

Seeds were kept for germination on a double layer of filter paper (ordinary) inside petri dishes having a diameter of 9 cm The petri dishes were covered and kept for germination at room temperature (29 ± 1°C) The filter paper was moistened with distilled water daily

Germination was observed daily.

A seed was considered as having germinated when the radicle reached about one centimetre length and the green hypocotyl became visible All germinated seeds were collected and removed at every assessment to prevent double counting At the end, cumulative germination percentage was calculated for each treatment Vigour parameters were also calculated using Germination Value (GV), Mean Daily Germination (MDG) and Peak Value (PV) (Czabator, 1962)

3 2 3 2 Seed moisture content

Low constant temperature oven method (ISTA, 1985) was used to determine the moisture content After determining the initial weight, seeds were oven dried at a constant temperature of 103 ± 2°C for 17 hours At the end of this

period, they were removed from the oven and allowed to cool for 30 40 minutes and then reweighed

Moisture content of seed coat, cotyledon and embryo were also determined separately

Moisture content was determined on wet weight basis,

$$\text{Moisture content (\%)} = \frac{\text{Original wt.- oven dry wt.}}{\text{Original wt}} \times 100$$

3 2 3 3 Leachate conductivity measurement

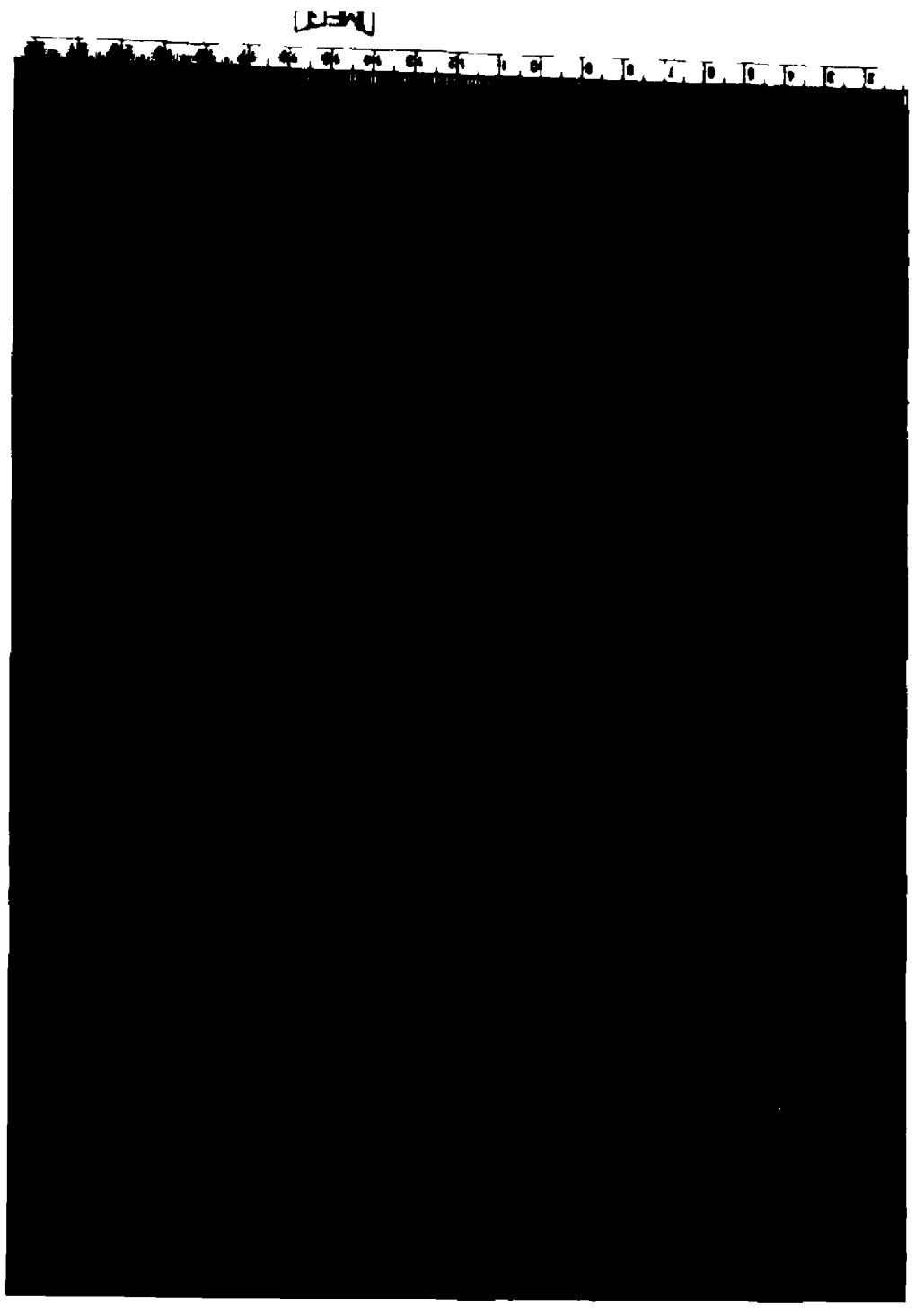
Leachate conductivity measurement is a quick test to know seed deterioration Ten seeds were immersed in 50 ml of distilled water overnight and leachate conductivity was measured using a conductivity meter (Elico, CM 180)

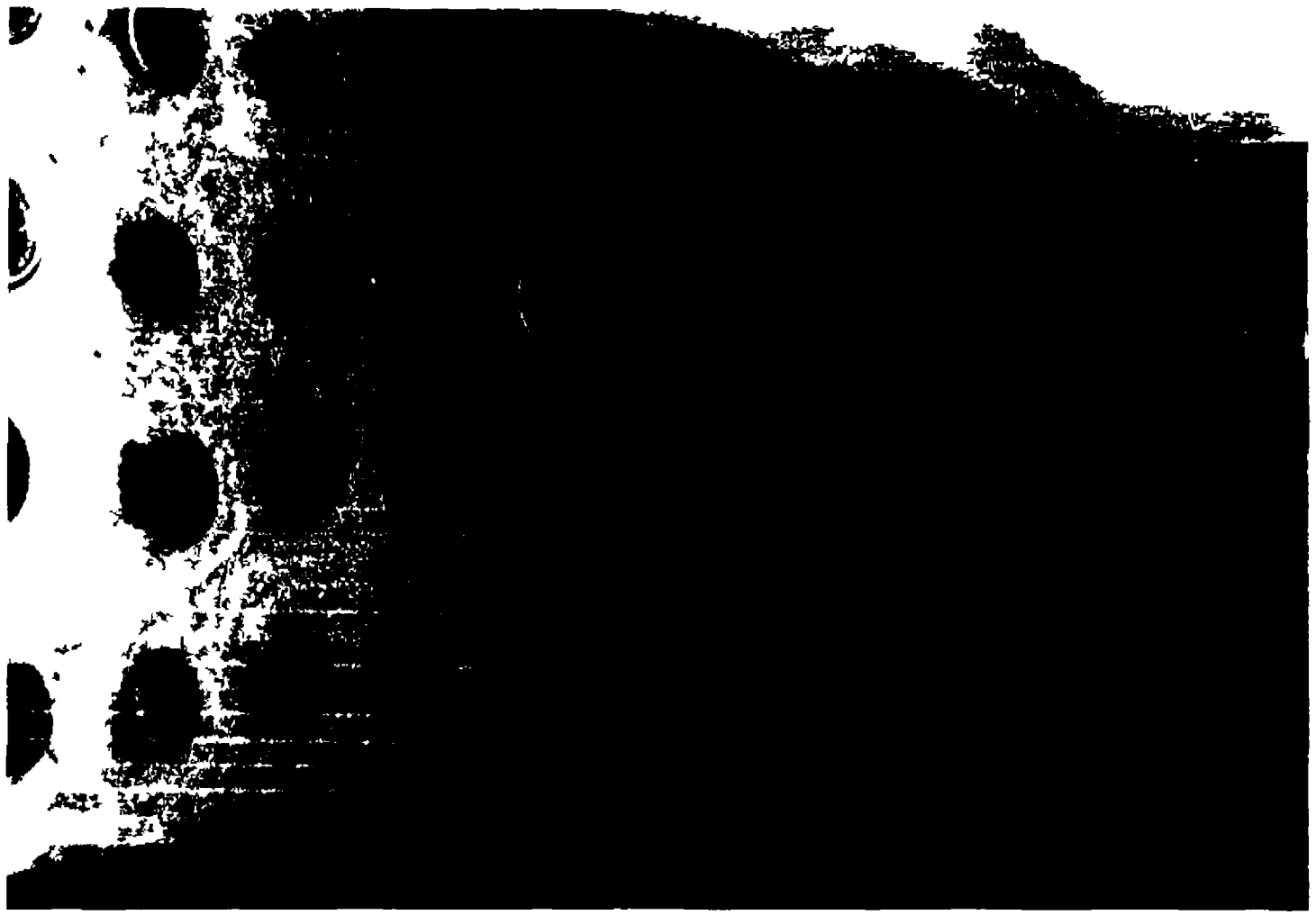
3 2 4 Effect of medium of storage on seed viability

Homogenous samples of fifty dewinged seeds were transferred to earthern pots of 600 ml capacity along with sand (2 06 per cent moisture content) or neem cake (11 08 per cent moisture content) as medium of storage for two months The pots were buried upto neck level in 20-23 per cent moist sand bed under shade (Plate 1) The mouth of the pot was kept open The sand bed was moistened once in every two days A similar number of dewinged seed lots stored in mud pots without any medium served as the control The treatments were

Plate 1 Mud pots immersed in sand bed

Plate 2 Seeds harvested manually just before natural shedding





set up in Completely Randomised Block Design At weekly interval, four pots (replications) belonging to each treatment were drawn and moisture content, leachate conductivity and germination parameters were determined Initial moisture content, leachate conductivity and germination parameters were determined prior to storage The moisture content of sand and neem cake were also determined at weekly intervals

3 2 5 Effect of storage temperature and fungicide on seed viability

Seeds were harvested before natural seed shedding by shaking the branches gently and collected immediately (Plate 2) The seeds along with their wings were immersed in one per cent emisan (methoxy ethyl mercuric chloride) for 20 minutes and air dried to bring the moisture content to around 37 per cent Quadriplicate samples of fifty homogenous seeds along with their wings were kept in perforated polythene bags (50 seeds per pot) under two temperatures viz , 10°C and room temperature (29 ± 1°C) Seeds kept as control was not soaked in water for the same period since immersion of seed in water favour quick germination Seeds were taken out at weekly intervals and moisture percentage and germination parameters were recorded upto 40 days Initial moisture content and germination percentage (at seed collection), experiment set moisture content and experiment set germination percentage were recorded prior to storage

3 2 6 Standardisation of micro-encapsulation techniques

3 2 6 1 Preparation of the propagule

Since the selected mother trees are grown under open conditions, extreme hygiene was maintained while collecting seeds to avoid culture contamination Seeds were first washed in running tap water using a detergent After detaching the wings, seeds were blot dried, transferred to 0 1 per cent $HgCl_2$ for sterilization and moved to laminar air flow cabinet Seeds were removed from the chemical after 5 minutes and rinsed with sterilized distilled water to remove traces of sterilant sticking to the surface Seeds were split open using a sharp razor blade and the embryonic axis carefully excised All materials used were sterilized prior to work and the entire work was done under aseptic condition

3 2 6 2 Encapsulation media

The best combination of sodium alginate and calcium chloride standardised for cocoa (Nagaraj, 1994) was used for the production of synthetic seeds of *Hopea parviflora* Sodium alginate slurry was prepared by adding 40 g of sodium alginate (Sd fine, 40105) to one litre of distilled water and heating slowly with constant stirring Calcium chloride (2 mM) solution was prepared by adding 11 026 g of $CaCl_2$ $2H_2O$ to one litre of distilled water

The above two solutions were sterilized in an autoclave at 15 psi pressure and 121°C temperature for 20 minutes Fresh solutions of sodium alginate and calcium chloride were used for encapsulation whenever required

3 2 6 3 Encapsulation of the propagule

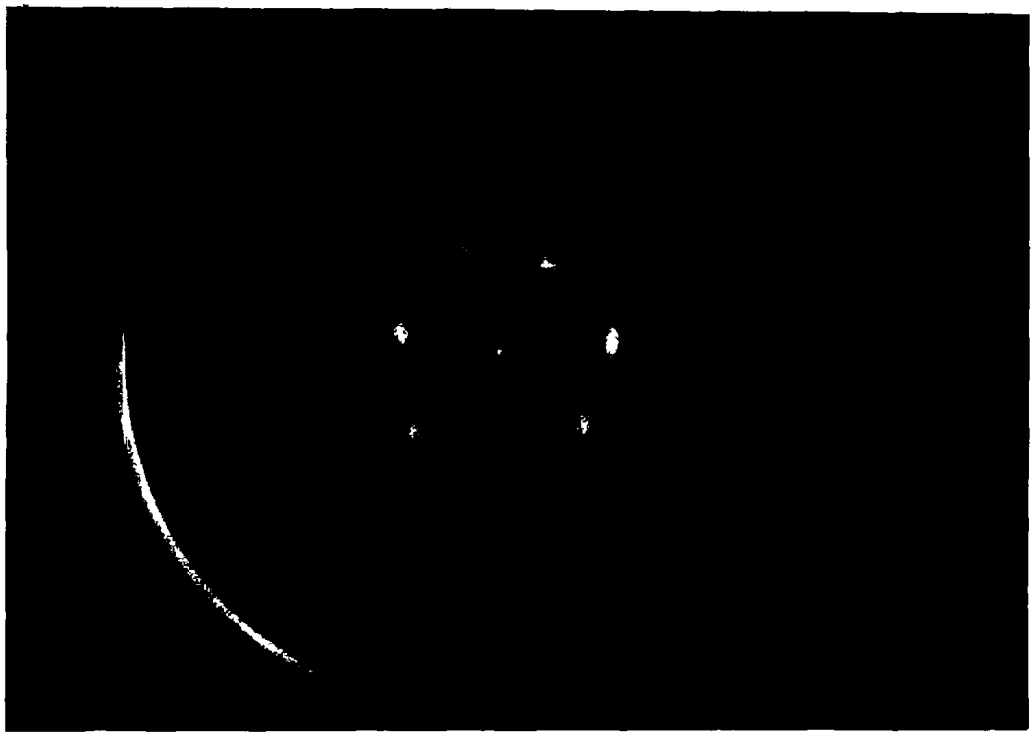
The excised embryos were dropped into sodium alginate solution and stirred well Each of this embryo was then carefully sucked using a glass tube having an inner diameter of 4 mm The sucked gel coated embryos were then dropped into calcium chloride solution for complexation The encapsulated embryo (synthetic seeds) (Plate 3) were allowed to stay in the calcium chloride solution with intermittent stirring for 25-30 minutes and then transferred to a petri plate with sterilized distilled water to remove excess calcium ions All the process were done under aseptic conditions

3 2 6 4 Storage of synthetic seeds

Twenty synthetic seeds each were placed on dry sterilized cotton kept at the bottom of twelve Erlenmeyer flasks (500 l capacity) Mouth of the Erlenmeyer flasks were plugged with sterilized cotton Four flasks each were stored at 4°C, 10°C and at room temperature (27±2°C) In order to study the influence of wet cotton and dry cotton as storage media, sterilized, dry cotton was placed at the bottom of four Erlenmeyer flask and soaked with 10 ml of MS medium Twenty

Plate 3 Synthetic seeds of *Hopea parviflora*

Plate 4 Germination of *Hopea parviflora* seeds while being kept for storage in sand



freshly prepared synthetic seeds were placed on the wet cotton medium contained in each Erlenmeyer flask and stored at 10°C for different durations The temperature was selected as 10°C because germination of synthetic seeds placed on dry cotton was found to be best at this temperature Only one temperature treatment was used due to scarcity of seeds

3 2 6 5 Effect of ABA on the viability of stored synthetic seeds

In order to study the effect of ABA on the storage life of synthetic seeds, dewinged seeds were soaked overnight in different concentrations of ABA viz , 0, 1, 2 and 3 ppm Afterwards, embryos were excised and synthetic seeds prepared under aseptic conditions as described under section 3 2 6 4 Immediately after encapsulation they were stored on dry cotton medium under 4°C as described in section 3 2 6 5

3 2 6 6 Culture media

Standard procedures (Gamborg and Shyluk, 1981) were followed for the preparation of the media Stock solutions of major and minor nutrients were prepared by dissolving the required quantity of the chemical in distilled water and were stored in amber coloured bottles under refrigerated conditions The stock solutions of nutrients were prepared freshly every four weeks and that of vitamins, amino acids and growth regulators every week

Specific quantities of the stock solutions of the chemicals were pipetted out into a 1000 ml beaker previously rinsed with distilled water Sucrose and inositol were added fresh and dissolved Required quantities of growth regulators and other supplements were also added and the solution was made upto the required volume pH of the solution was adjusted to the range 5 6 to 5 8 (using 1 N NaOH or 1 N HCl) Agar was then added to the medium and the final volume made upto 1000 ml

The solution was then boiled for melting the agar by keeping in a microwave oven 20 ml each of the melted media was poured hot to the oven dried culture tubes (15 x 2 5 cm), which were previously washed rinsed in distilled water and dried The tubes with the medium were then tightly plugged with cotton plugs and autoclaved After sterilization, the culture tubes were stored in culture room maintained at a temperature of 27 $\pm$ 2°C for further use

3 2 6 7 Testing of viability

The effect of storage of synthetic seeds was studied by testing their viability at weekly intervals At weekly intervals four synthetic seeds were taken from each flask and studied for their germination percentage, number of days taken for initiation of germination and number of days taken for sprout production Transfer of synthetic seeds to and from

the Erlenmeyer flask was done inside the laminar air flow cabinet and all the operations were done under aseptic conditions

The influence of encapsulating the excised embryonic axis in sodium alginate was studied by comparing the germination of excised embryonic axis with or without encapsulation Similarly the effect of cotton and agar as a germination substrate was studied in the case of excised embryonic axis The effect of concentration of germination of synthetic seeds was also studied using either MS medium or 1/2 MS medium

3.2 6.8 Inoculation and culturing of synthetic seeds

To inoculate the synthetic seeds in the culture medium, the cotton plug of the culture tube was removed and the neck was first flamed over a gas burner kept in the chamber After transferring the synthetic seed to the medium, the neck of the culture tube was again flamed and the cotton plug replaced All the inoculation procedures were carried out under aseptic conditions

The cultures were incubated in a culture room provided with cool white fluorescent lamps to give a light intensity of 2000 lux for 16 hours light period The temperature was maintained at 27 ± 2°C

3.2 7 Statistical analysis

All the observations recorded were statistically analysed following the methods suggested by Panse and Sukhatme (1978) Arcsin transformed data were used wherever found necessary

Results

RESULTS

The results of the study on the viability of *Hopea parviflora* seeds with reference to temperature, medium of storage and microencapsulation techniques are presented in this chapter

4.1 Flowering and fruiting

The onset of flowering and fruit set in *H parviflora* during the first year of study (1993) was during the first week of April and May, respectively After fruit set they attained maturity within 15 20 days and shed by the end of third week of May However, the periodicity of these events was altered to some extent during the second year (1994) wherein trees flowered at the end of March and fruit shedding started during the last week of May It was observed that if there is intense rainfall at the time of fruits reaching maturity, most of the seeds exhibited vivipary and the wings of the fruits retained their green colour (data not shown) Dominant trees of the stand were the major flowering trees Another striking observation was that most of the trees which flowered during the first year did not flower during the second year showing the phenomenon of irregular bearing

Two important insects attacking the *Hopea parviflora* seeds identified are

1 *Cocotrypes carpophagus* (weevil)

2 *Eurytoma curculionum* (seed chalcid)

4.2 Seed characteristics

Seed characteristics of *Hopea parviflora* are given in Table 2 Mean fresh weight of 100 seeds was 33 98 g *H parviflora* shed the seeds naturally at around a moisture content of 40 8 per cent (on wet weight basis) which had a germination percentage of 95 per cent Seeds harvested by gentle shaking of the branches before the natural shedding had comparatively higher moisture content (41 7%) and had a germination percentage of 98

Moisture content of different components of the seed shed naturally viz , embryo, cotyledon and seed coat were also determined separately (Table 2) They were 68 5 per cent, 41 7 per cent and 18 2 per cent, respectively

Table 2 Characteristics of *Hopea parviflora* seeds shed naturally or by manual shedding

	Seed shed naturally				Seeds shed manually	
	1000 seed weight (g)	Moisture content (%)			Germination percentage	Seed moisture content	Germination percentage
		Seed	Seed coat	Cotyledon	Embryo			
Mean	339.80	40.80	18.20	41.70	68.50	95.00	41.70	98.00
SD ±	1.89	0.22	0.29	1.04	0.88	3.83	6.37	1.63
CV(%)	5.59	0.53	1.62	2.50	1.29	4.03	15.57	1.66

4.3 Effect of storage temperature and storage period on moisture content, leachate conductivity and germination parameters

4 3 1 Moisture content

The effect of storage temperature and storage period on the moisture percentage (wet weight basis) of dewinged seeds of *Hopea parviflora* is shown in Table 3 and Fig 1 Storage temperature did not significantly affect the moisture percentage of dewinged seeds However, storage period significantly affected (P<0 01) the seed moisture content The interaction effect of storage temperature and storage period was also found to be significant Compared to fresh seed which had 29 48 per cent moisture content, at the end of eight weeks of storage the moisture content significantly decreased by 37 per cent on an average to 18 5 per cent

4 3.2 Leachate conductivity

Variation in leachate conductivity of dewinged seeds of *H parviflora* seeds as affected by storage temperature and storage period is shown in Table 4 and Fig 2 Leachate conductivity was significantly affected by storage temperature (P<0 01) and storage period (P<0 05) while interaction effect of storage temperature and storage period was not significant The leachate conductivity increased more than three times when the seeds were stored for one week However a significant

Table 3 Moisture content of dewinged *Hopea parviflora* seeds asaffected by storage temperature and storage period

Storage temperature (°C)	Storage period (weeks) 0	1	2	3	4	5	8
10	29 48	26 72	22 59	19 47	19 91	18 53	17 62
20	29 48	23 83	23 32	22 77	22 96	20 62	19 00
29(±)	29 48	23 11	21 10	23 53	22 19	20 76	18 73

	Storage temperature	Storage period	Interaction
CD (0 05)	NS	1 66	2 88
SEM(±)	0 416	0 588	1 02

Table 4 Leachate conductivity of dewinged *Hopea parviflora* seeds as affected by storage temperature and storage period

Storage temperature (°C)	Storage period (weeks) 0	1	2	4	5	6	7
10	0 055	0 145	0 089	0 084	0 076	0 073	0 066
20	0 055	0 167	0 099	0 100	0 077	0 068	0 065
29±1	0 055	0 186	0 096	0 111	0 080	0 065	0 065

	Storage temperature	Storage period	Interaction
CD (0 05)	0 026	0 036	NS
SEM(±)	0 009	0 013	0 001

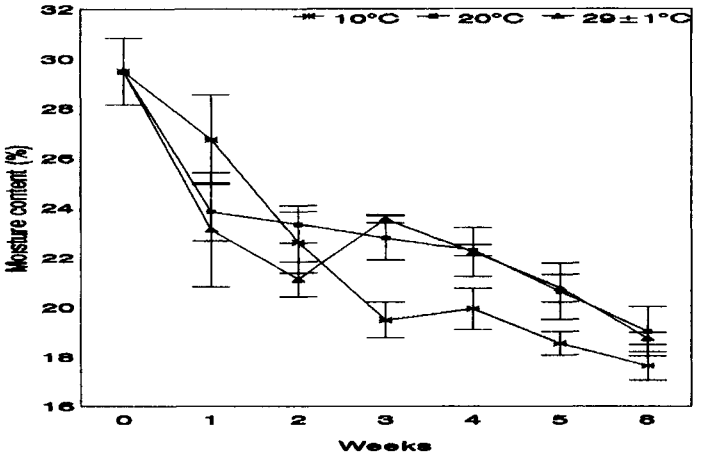


Fig. 1 Moisture content of dewinged **Hopea parviflora** seeds as affected by storage temperature and storage period

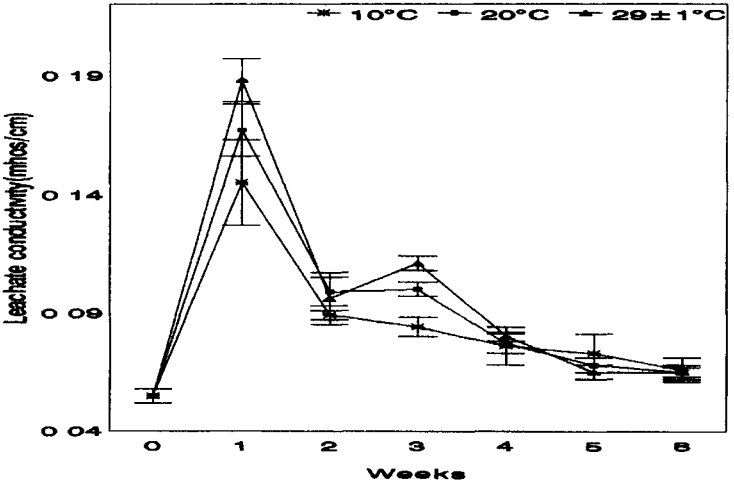


Fig. 2 Leachate conductivity of dewinged **Hopea parviflora** seeds as affected by storage temperature and storage period

reduction was observed at the end of two weeks of storage and subsequently it remained steady

4 3 3 Germination parameters

A highly significant (P<0 01) reduction in the cumulative germination percentage of dewinged seeds of *Hopea parviflora* was seen with the increase in duration of storage (Table 5 and Fig 3) Germination percentage of hopea seeds was 88 per cent soon after collection which declined to 21 per cent at the end of first week of storage and by fifth week of storage it was 3 5 per cent only Storage temperature did not have any influence on the cumulative germination percentage Interaction effect of storage temperature and storage period was also not significant

Mean Daily Germination percentage (MDG) also showed similar trends (Table 6 and Fig 3) Compared to the MDG of 14 66 soon after collection, a drastic reduction was observed at the end of first week of storage itself At the end of fifth week of storage, MDG was observed to be 0 6 only Effect of storage temperature and the interaction between storage period and storage temperature were not significant

Peak Value (PV) of germination of dewinged seeds of *H parviflora* also significantly reduced (P<0 01) with the storage period (Table 7 and Fig 3) However, the main effect of

Table 5 Cumulative germination percentage of dewinged seeds of *Hopea parviflora* as affected by storage temperature and storage period

Storage temperature (°C)	Storage period (weeks)				
	0	1	2	3	4	5
10	88	24 00	15 00	4 00	6 0	2 0
20	88	15 00	11 00	5 00	13 0	2 0
29±1	88	25 00	19 00	10 00	7 0	5 0

	Storage temperature	Storage period	Interaction
CD (0 05)	NS	5 80	NS
SEM ±	1 59	2 05	3 55

Table 7 Peak value of germination of dewinged seeds of *Hopea parviflora* as affected by storage temperature and storage period

Storage temperature (°C)	Storage period (weeks)					
	0	1	2	3	4	5
10	29 00	5 00	3 27	1 08	3 0	1 00
20	29 00	3 08	2 19	1 25	4 0	0 50
29±1	29 00	6 00	4 15	4 33	3 0	0 83

	Storage temperature	Storage period	Interaction
CD (0 05)	NS	1 78	NS
SEM ±	0 49	0 63	1 08

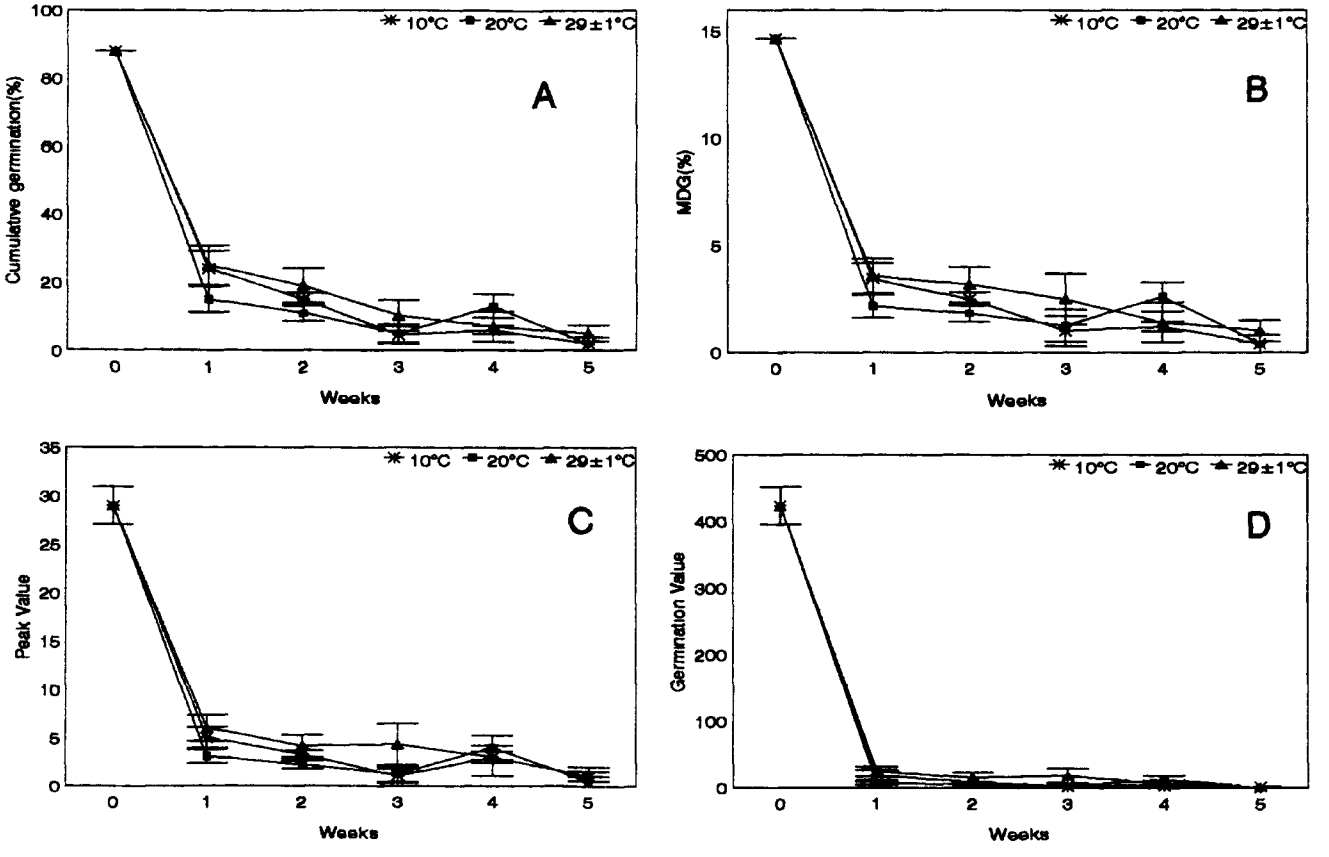


Fig.3 Germination parameters of dewinged **Hopea parviflora** seeds as affected by storage temperature and storage period

A. Cumulative germination (%)
B. Mean daily germination (%)
C. Peak value of germination
D. Germination value

storage temperature and interaction between storage period and storage temperature were not found to be having any significant effect Compared to the initial PV of *H paniflora* seed germination before storage (29) on an average six fold reduction in PV was observed within a period of one week Subsequently gradual reduction in PV was observed with the storage period irrespective of storage temperature

Germination Value (GV) of dewinged seeds of *H paniflora* was significantly affected by storage temperature ($P<0.05$) and storage period ($P<0.01$) However the interaction between the main effects of storage period and storage temperature was not significant (Table 8 and Fig 3) Compared to GV of 425 33, soon after collection, on an average it reduced drastically to 17 17 after one week of storage It was further reduced with the storage period and reached to 0 98 after storage for five weeks

4.4 Effect of dewinging the seeds on moisture content, leachate conductivity and germination parameters

4 4 1 Moisture content

Variation in moisture content (wet weight basis) of winged and dewinged seeds of *H paniflora* during storage is represented in Table 9 and Fig 4 Generally winged seeds retained significantly higher moisture content throughout the

Table 8 Germination value of dewinged seeds of *Hopea parviflora* as affected by storage temperature and storage period

Storage temperature (°C)	Storage period (weeks)					
	0	1	2	3	4	5
10	425 33	19 14	8 30	2 58	3 60	1 60
20	425 33	7 67	4 48	3 25	12 80	0 80
29±1	425 33	24 72	15 87	18 33	7 20	0 55

	Storage temperature	Storage period	Interaction
CD (0 05)	6 13	7 92	NS
SEM ±	1 98	2 80	4 85

Table 9 Moisture content of *Hopea parviflora* seeds at different storage period as affected by dewinging

Treatment	Storage period (weeks)						
	0	1	2	3	4	5	8
Winged	29 48	27 70	26 97	26 35	25 91	24 03	22 46
Dewinged	29 48	23 11	21 10	23 53	22 19	20 76	18 73
CD (0 05)		NS	3 21	2 26	2 94	NS	1 97
SEM(±)		1 66	0 88	0 62	0 81	1 9	0 54

Table 10 Leachate conductivity of *Hopea parviflora* seeds at different storage period as affected by dewinging

Treatment	Storage period (weeks)						
	0	1	2	4	5	6	7
Winged	0 055	0 095	0 097	0 106	0 078	0 077	0 079
Dewinged	0 058	0 188	0 096	0 111	0 080	0 065	0 065
CD (0 05)	-	0 022	NS	NS	NS	NS	NS
SEM(±)		0 006	0 007	0 003	0 003	0 00	0 003

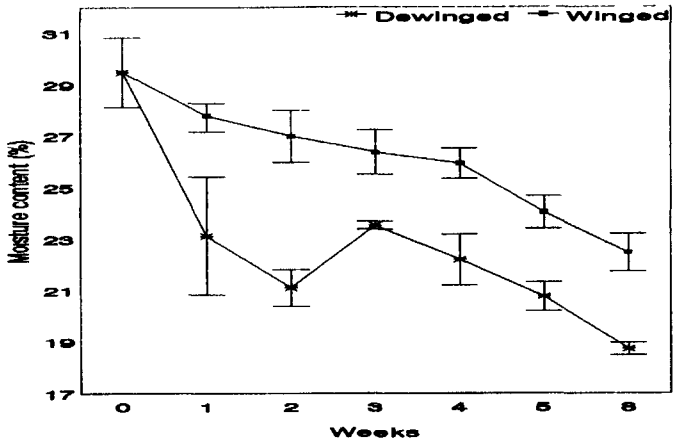


Fig.4 Moisture content of **Hopea parviflora** seeds at different storage period as affected by dewinging

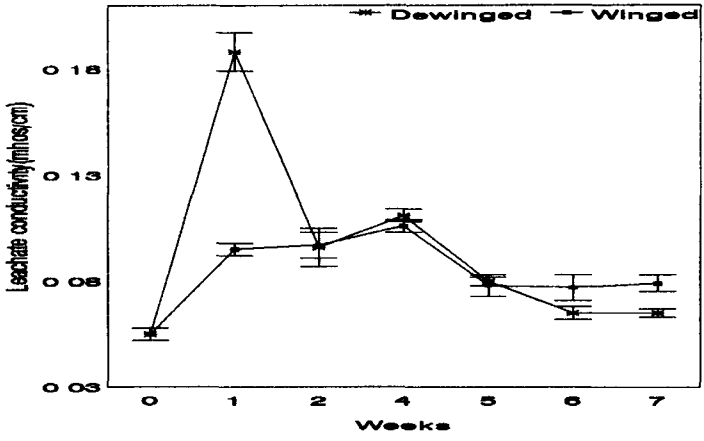


Fig.5 Leachate conductivity of **Hopea parviflora** seeds at different storage period as affected by dewinging

storage period Irrespective of keeping winged or dewinged seeds, a decline in seed moisture content was observed with the storage period Seed moisture content before storage was 29 48 per cent and after 8 weeks of storage it was significantly reduced to 22 46 per cent and 18 73 per cent in the case of winged and dewinged seeds respectively

4 4 2 Leachate conductivity

Variation in leachate conductivity of winged and dewinged seeds of *H parviflora* during storage is represented in Table 10 and Fig 5 On an average more amount of leachate was produced by dewinged seeds during storage Compared to the fresh seeds, which had a leachate conductivity of 0 055 mhos/cm more than three fold increase (0 188 mhos/cm) was observed in leachate conductivity of dewinged seeds after one week of storage and was significantly different ($P<0$ 01) from winged seeds However, the leachate conductivity of winged and dewinged seeds was almost similar afterwards upto the end of six weeks of storage

4 4 3 Germination parameters

Dewinging the seeds did not have any significant effect on the germination percentage compared to winged seeds (Table 11 and Fig 6) On an average irrespective of storing the seeds in winged or dewinged condition, a drastic and

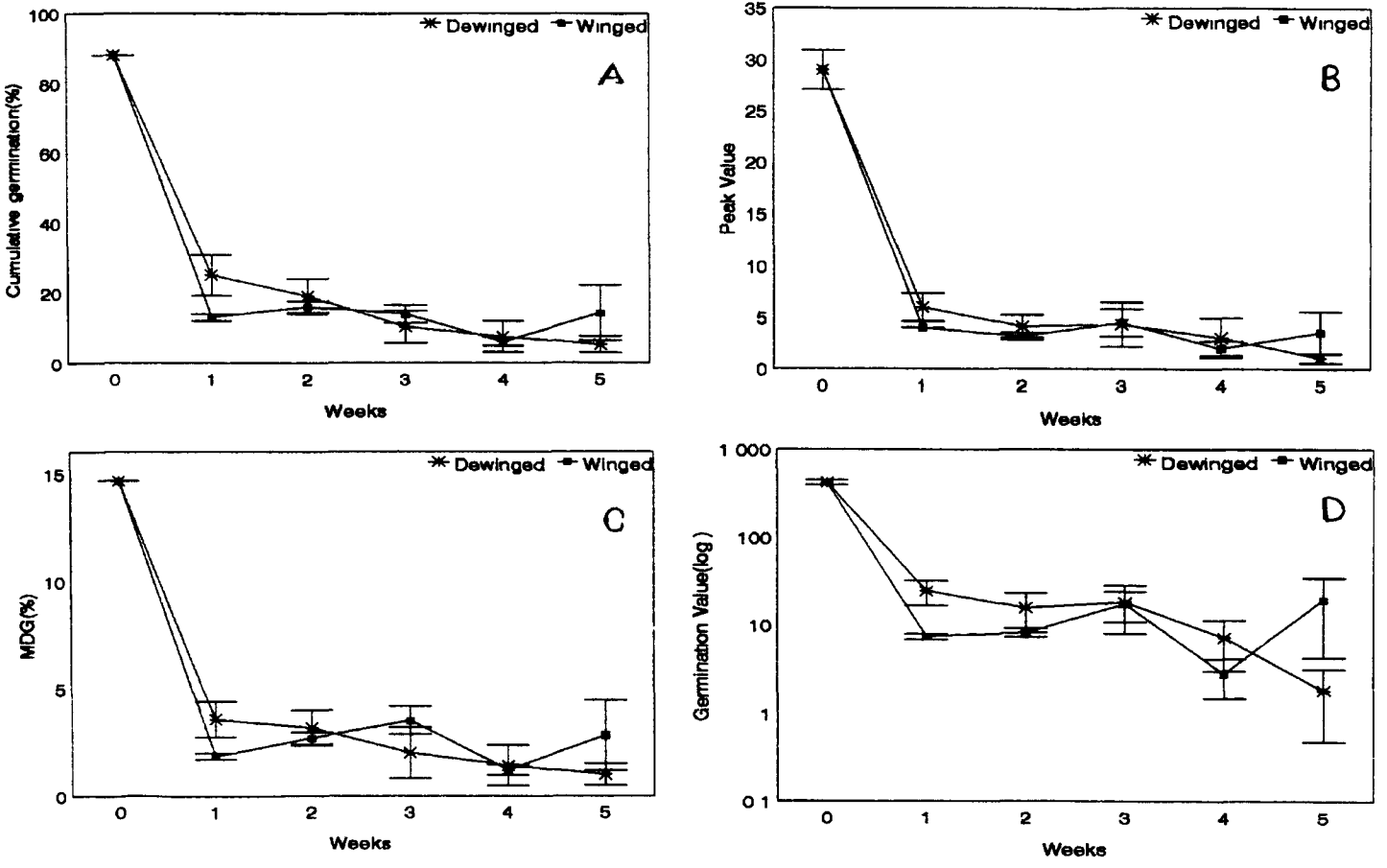


Fig. 6 Germination parameters of **Hopea parviflora** seeds at different storage period as affected by dewinging

A. Cumulative germination (%)
B. Mean Daily Germination (%)
C. Peak value of germination
D. Germination value

significant reduction in germination percentage was observed after one week of storage Compared to the initial germination percentage of 88, at the end of four weeks of storage, this declined by 92 per cent to 6 75 per cent Interaction effects were not significant

The MDG of winged and dewinged seeds of *H parviflora* seeds as affected by storage period is shown in Table 12 and Fig 6 Storing the seeds for different durations either as winged or dewinged did not have significant influence on MDG of hopea seeds Compared to the MDG of seeds before storage (14 66) on an average 84 per cent reduction in MDG was observed after five weeks of seed storage This reduction was drastic during the first week of storage itself

Similar trends were observed in PV of germination also (Table 13 and Fig 6) Significant difference in PV of germination was not observed when seeds were stored either in winged or dewinged state Eventhough storage period was also not significant, compared to the initial PV (29), PV of germination after one week storage was drastically declined in both cases PV of germination was higher in the case of dewinged seeds after one week storage On the contrary winged seeds retained higher PV after five weeks of storage Interaction effects were also not significant

Table 12 Mean Daily Germination percentage of *Hopea parviflora* seeds at different storage period as affected by dewinging

Treatment	Storage period (weeks)					
	0	1	2	3	4	5
Winged	14 66	1 85	2 67	3 5	1 20	2 80
Dewinged	14 66	3 57	3 17	2 5	1 40	1 00

	Dewinging	Storage period	Interaction
CD (0 05)	NS	NS	NS
SEM(±)	0 376	0 596	0 17

Table 13 Peak value of germination percentage of *Hopea parviflora* seeds at different storage period as affected by dewinging

Treatment	Storage period (weeks)					
	0	1	2	3	4	5
Winged	29	4 00	3 17	4 5	2 0	3 5
Dewinged	29	6 0	4 15	4 33	3 0	1 05

	Dewinging	Storage period	Interaction
CD (0 05)	NS	NS	NS
SEM(±)	0 599	0 947	1 92

GV also showed similar trends (Table 14 and Fig 6) Irrespective of storing seeds in winged or dewinged condition, significant reduction (96 per cent) in GV was observed after one week of storage itself Dewinged seeds retained comparatively higher GV after one week of storage while winged seeds showed higher GV after 5 weeks of storage However, keeping seeds in winged or dewinged condition did not affect GV significantly The effect of storage period and interaction were also not significant

4.5 Effect of storage media on moisture content, leachate conductivity and germination parameters

4 5 1 Moisture content

Variation in moisture content of *H parviflora* seeds as affected by media of storage and storage period is shown in Table 15 and Fig 7 Moisture content was significantly affected ($P<0.01$) by the storage medium Compared to the fresh seeds which had a moisture content of 29 12 per cent, an increase in moisture content was observed in the case of seeds stored in sand and that kept in mud pot without any medium with increasing storage period However the moisture content of seeds stored in neem cake was significantly reduced to 19 12 per cent within one week which remained more or less steady with subsequent period of storage

Table 14 Germination value of *Hopea parviflora* seeds at different storage period as affected by dewinging

Treatment	Storage period (weeks)					
	0	1	2	3	4	5
Winged	245 33	7 43	8 33	17 5	2 8	19 6
Dewinged	425 33	24 72	15 87	18 33	7 2	1 80

	Dewinging	Storage period	Interaction
CD (0 05)	NS	NS	NS
SEM(±)	3 237	5 119	28 08

Table 15 Moisture content of *Hopea parviflora* seeds as affected by medium of storage and storage period

Storage media	Storage period (weeks)			
	0	1	2	3
Sand	29 12	42 50	47 36	Nil
Neem cake	29 12	19 15	24 36	21 62
Control	29 12	31 94	39 97	36 70
CD (0 05)		10 48	15 50	NS
SEM(±)		2 33	3 47	0 85

Table 16 Leachate conductivity of *Hopea parviflora* seeds as affected by medium of storage and storage period

Storage media	Storage period (weeks)			
	0	1	2	3
Sand	0 048	0 254	0 163	0 146
Neem cake	0 048	0 147	0 107	0 130
Control	0 048	0 081	0 112	0 088
CD (0 05)		0 19	0 003	0 001
SEM(±)		0 03	0 007	0 004

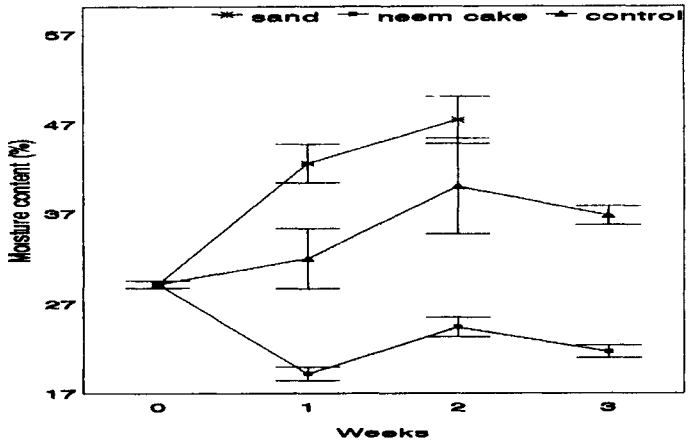


Fig. 7 Moisture content of **Hopea parviflora** seeds as affected by medium of storage and storage period

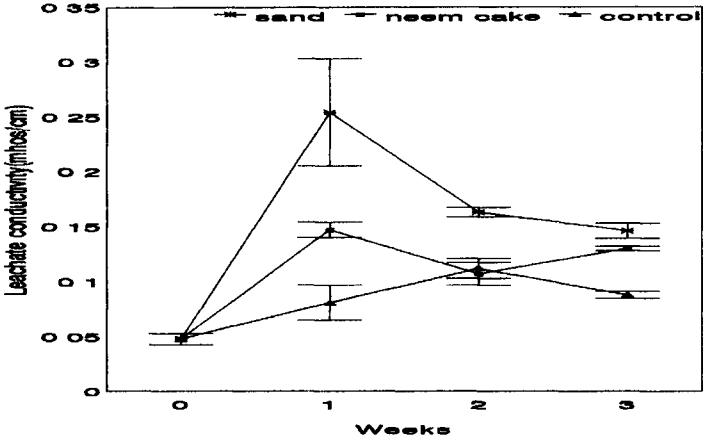


Fig. 8 Leachate conductivity of **Hopea parviflora** seeds as affected by medium of storage and storage period

4 5 2 Leachate conductivity

Significant difference in (P<0 01) leachate conductivity of *H parviflora* was observed due to the storage media (Table 16 and Fig 8) During first week of storage the leachate conductivity of seeds stored in sand was increased to the tune of more than five times while it was increased by more than three times in the case of seeds stored in neem cake as compared to the fresh seeds Seeds stored without any medium produced comparatively less amount of leachate

4 5 3 Germination parameters

Variation in cumulative germination percentage of *H parviflora* seeds as affected by media of storage at different time interval is shown in Table 17 and Fig 9 Significant difference (P<0 01) in germination percentage due to the medium of storage was observed during the storage period Compared to the fresh seeds, which had a germination percentage of 87 per cent a drastic reduction was observed after one week of storage in all cases However, germination percentage was reduced almost by 50 per cent when seeds were stored in sand or kept as such in mud pots for one week Germination percentage of seeds stored in neem cake was reduced tremendously by 92 per cent, compared to the fresh seed This reduced germination per cent was more or less maintained upto three weeks of storage Interestingly enough

Table 17 Cumulative germinations percentage of *Hopea parviflora* seeds as affected by medium of storage and storage period

Storage media	Storage period (weeks)			
	0	1	2	3
Sand	87	41	Nil	Nil
Neem cake	87	7	3	7
Control	87	47	15	Nil
CD (0 05)		20 03	8 7	
SEM(±)		6 62	2 51	

Table 18 Mean Daily Germination percentage *Hopea parviflora* seeds as affected by medium of storage and storage period

Storage media	Storage period (weeks)			
	0	1	2	3
Sand	14 5	6 83	Nil	Nil
Neem cake	14 5	1 17	5 88	1 17
Control	14 5	7 84	0 52	Nil
CD (0 05)		3 34	1 24	
SEM(±)		1 04	0 36	

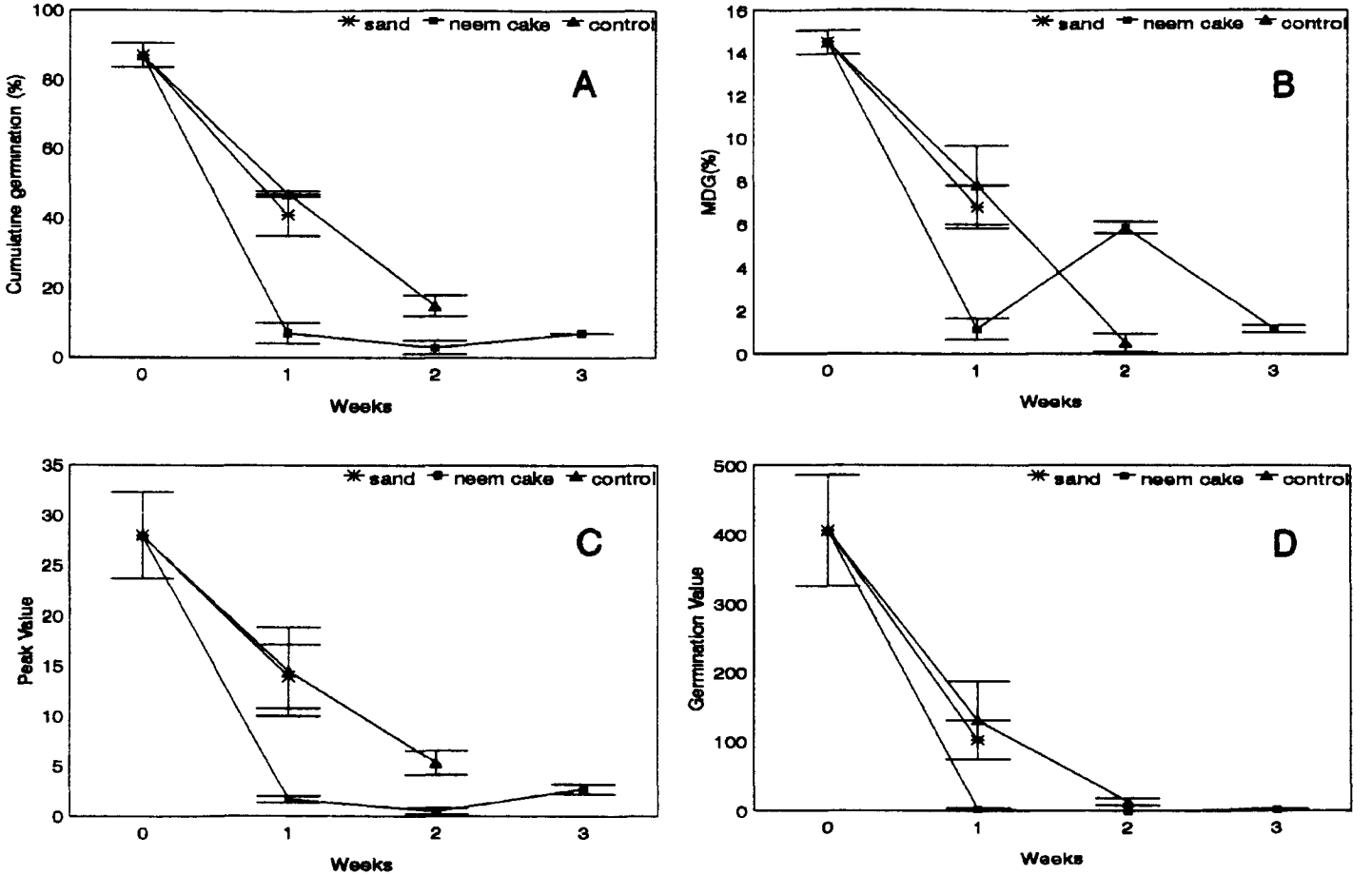


Fig. 9 Germination parameters of **Hopea parviflora** seeds as affected by medium of storage and storage period

A. Cumulative germination (%)
B. Mean Daily Germination (%)
C. Peak value of germination
D. Germination value

24 per cent and 49 per cent of the seeds had germinated within the pot itself while being kept for storage in mudpots without any medium and sand (Plate 4) respectively, during the second week Rest of the seeds stored in sand failed to germinate completely This trend was maintained throughout the storage In the case of seeds stored without any medium, germination percentage at the end of second week of storage was 15 Interestingly 46 and 36 per cent of seeds had germinated during second and third week respectively, while being kept in storage At the end of third week of storage, seeds had lost their viability completely Compared to other media none of the seeds kept in neem cake germinated while undergoing storage treatments

Effect of storage medium on the MDG of *H parviflora* seeds as affected by medium of storage is shown in Table 18 Fig 9 Medium of storage significantly affected ($P<0.01$) the MDG of seeds Compared to the MDG of fresh seeds (14 5), the MDG of stored seeds in all the three cases declined significantly after one week of storage in which that of neem cake was most prominent (1 17) In general MDG was decreased with the storage period

Similar trends were observed in the case of PV of germination also (Table 19 and Fig 9) After one week of storage, PV of seeds stored in sand and kept as control reduced by more than 48 per cent while that stored in neem

Table 19 Peak value of germination of *Hopea parviflora* seeds as affected by medium of storage and storage period

Storage media	Storage period (weeks)			
	0	1	2	3
Sand	28 0	13 92	Nil	Nil
Neem cake	28 0	1 67	0 48	2 67
Control	28 0	14 42	5 33	Nil
CD (0 05)		10 42	3 07	
SEM(±)		3 25	0 88	

Table 20 Germination value of *Hopea parviflora* seeds as affected by medium of storage and storage period

Storage media	Storage period (weeks)			
	0	1	2	3
Sand	406	102 78	Nil	Nil
Neem cake	406	2 45	0 4	2 89
Control	406	131 28	12 96	Nil
CD (0 05)		NS	11 65	
SEM(±)		40 24	3 36	

cake declined by 93 per cent when compared to the PV of fresh seeds In general PV reduced with storage period

GV also followed similar trends (Table 20 and Fig 9) GV was significantly not different with the storage media after one week of storage However, when compared to the GV of fresh seeds more than 67 per cent reduction was observed in the case of seeds stored in sand and kept without any medium while GV of seeds stored in neem cake declined by 99 per cent just after one week of storage After two weeks of storage, GV of seeds stored in neem cake was significantly less than seeds stored without any media Generally GV reduced with storage period

4.6 Effect of temperature and fungicide on moisture content and germination parameters

4 6 1 Effect of temperature on moisture content

Variation in moisture content of *Hopea parviflora* seeds treated with fungicide as affected by temperature of storage is shown in Fig 10 On an average significant reduction ($P<0.01$) in moisture content was observed due to storage at higher temperature Compared to the initial moisture content of 37 1 per cent before storage the moisture content of seeds stored at room temperature reduced to 32 82 per cent after one week of storage itself which was also found to be significantly ($P<0.01$) declining with the storage period

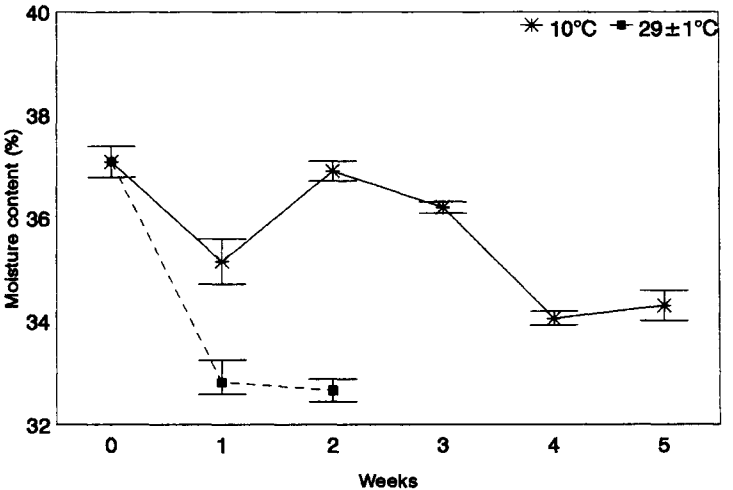


Fig 10 Moisture content of ***Hopea parviflora*** *seeds treated with fungicide as affected by storage temperature and storage period*

However, in the case of seeds stored at 10°C the moisture content was reduced only to 35 16 per cent

4 6 2 Effect of temperature on germination parameters

Effect of storage temperature on germination parameters of *H parviflora* seeds treated with fungicide is given in Table 21 Significant reduction (P<0 01) in germination percentage, MDG, PV and GV was observed due to storage at room temperature for one week Significant increase in all the vigour parameters (MDG, PV & GV) was observed in the case of seeds stored at 10°C for one week, but germination percentage was not significantly affected

Data pertaining to the effect of storage period on germination parameters of fungicide treated *H parviflora* seeds stored at 10°C is given in Table 22 Significant difference was observed in cumulative germination per cent, MDG, PV and GV with the storage period Germination percentage remained more or less steady upto 21 days storage However, significant reduction in germination percentage was observed when stored for 40 days

Compared to the fresh seeds significant increase in MDV, PV and GV value was observed during the initial stage of seed storage and they attained the maximum value after 21 days of

Table 21 Germination parameters of *Hopea parviflora* seeds treated with fungicide as affected by storage temperature after one week

Storage temperature (°C)	Germination parameters			
	Cumulative germination (%)	Mean daily germination (%)	Peak value of germination	Germination value
10	89	44 5	76 00	34 00
29±1	51	6 38	10 5	67 17
Initial germination parameters	95	23 75	27 25	646 92
CD (0 05)	7 46	2 94	44 25	538 84
SEM ±	2 33	0 92	2 63	168 44

Table 22 Germination parameters of *Hopea parviflora* seeds treated with fungicide and stored at 10°C for different storage period

Parameters	Storage period (days)						CD (0 05)	SEM±
	0	7	15	21	28	40		
Cumulative germination per cent	95 75	89 00	95 00	98 00	94 00	87 00	4 82	1 23
Mean daily germination	23 93	44 50	31 67	49 00	23 50	17 40	3 12	0 79
Peak value	27 33	76 00	51 00	95 00	74 00	24 00	9 51	2 42
Germination value	654 14	3400	1616	4656	1743	417 4	571 4	145 50

storage However, all the parameters reduced significantly when stored for 40 days

4 6 3 Effect of fungicide on moisture content

Variation in moisture content of *H parviflora* seeds stored at room temperature as affected by fungicide treatment is represented in Fig 11 In general seed treated with fungicide retained higher moisture content during storage Moisture content of seeds without fungicide treatment was reduced significantly ($P<0.01$) after one week itself The main effect of storage period and the interaction between fungicide treatment and storage period was not significant

4 6 4 Effect of fungicide on germination parameters

Effect of fungicide treatment on germination parameters of *H parviflora* seeds stored at room temperature is given in Table 23 When compared to the fresh seeds all the germination parameters declined at the end of one week storage at room temperature irrespective of fungicide treatment MDG and GV were significantly affected when seeds were stored for one week at room temperature All the vigour paramters were lowest when the seeds were stored for one week with fungicide treatment compared to no fungicide treatments

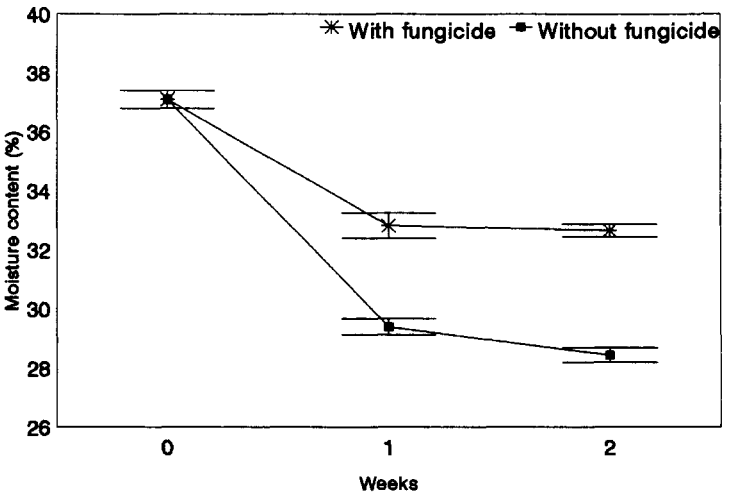


Fig 11 Moisture content of ***Hopea parviflora*** *seeds as affected by fungicide and storage period*

Table 23 Germination parameters of *Hopea parviflora* seeds stored at room temperature for one week as affected by fungicide treatment

Treatment	Germination parameters			
	Cumulative germination (%)	Mean daily germination (%)	Peak value of germination	Germination value
Seed stored at 29±1°C with fungicide treatment	51	6 38	10 50	67 17
Seeds stored at 29±1°C without fungicide	49	8 17	13 67	110 89
Initial germination parameters	95	23 75	27 25	646 92
CD (0 05)	NS	1 18	NS	28 13
SEM ±	2 24	0 34	1 05	8 13

4.7 Standardisation of microencapsulation techniques

4 7 1 Growth of excised embryo

Effect of different substrates on the germination of excised embryonic axis immediately after excision is given in the Table 24 MS medium along with cotton as the substrate was found to be better for the growth of embryonic axes Eighty three per cent of embryonic axis germinated when they were cultured in MS medium with cotton as the substrate which took an average of three days for radicle initiation Four out of sixteen embryos sprouted within twenty days However, only 67 per cent of embryonic axis germinated when agar was used as the substrate and they took an average of four days to initiate germination In addition only two out of sixteen embryos produced sprouts in 57 days (Plate 5) Root development was absent in all cases

4 7 2 Effect of different concentrations of MS medium on the germination characteristics of synthetic seeds

Data pertaining to the effect of different concentrations of MS medium on the germination characteristics of synthetic seeds of *Hopea parviflora* is given in Table 25 Compared to the 1/2 MS medium, culturing synthetic seeds in MS medium gave slightly higher germination percentage Number of days taken for radicle initiation was also less in this case Two synthetic seeds out of sixteen inoculated produced sprouts

Table 24 Effect of different substrates on the germination characteristics of embryonic axis of *Hopea parviflora*

Substrate	Germination percentage	Number of days for radicle initiation	Number of days to produce sprout
Cotton substrate with MS basal media	83	3	20
MS semi solid media	67	4	57

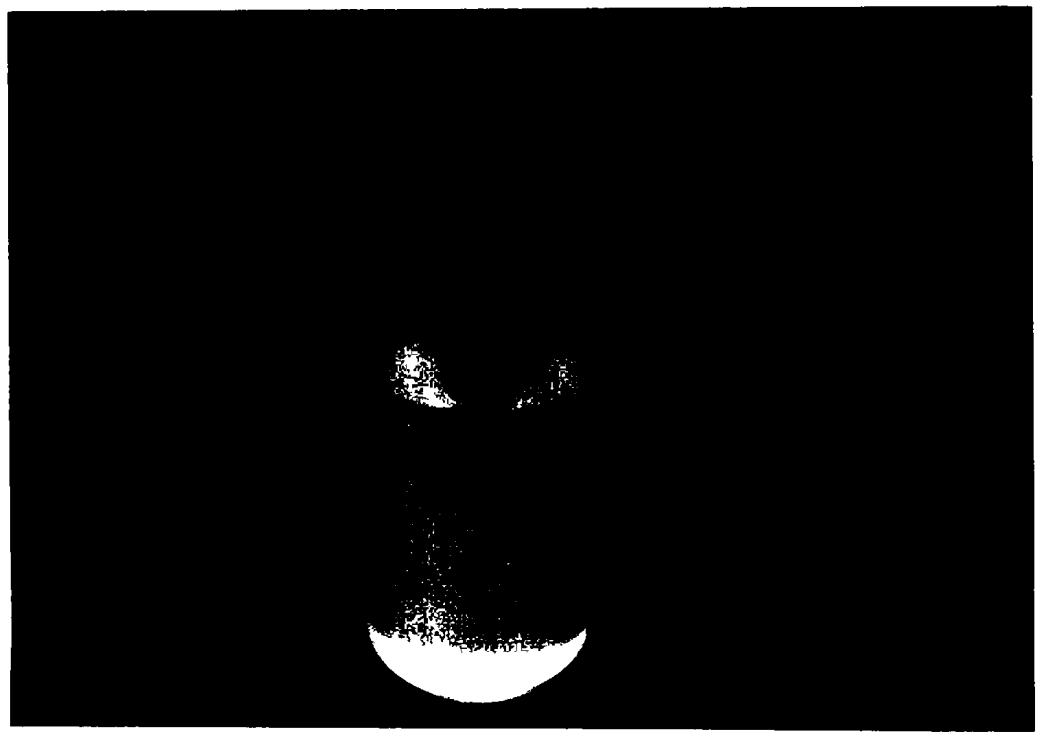
Table 25 Effect of different concentrations of media on the germination characteristics of synthetic seeds of *Hopea parviflora* immediately after encapsulation

Media	Germination percentage	Number of days for radicle initiation	Number of days to produce sprout
MS semi solid media	83	6	21
1/2 MS semi solid media	82	7	Absent

Table 26 Germination percentage of synthetic seeds of *Hopea parviflora* stored in dry cotton as affected by storage temperature and storage period

Storage temperature (°C)	Storage period (weeks)				
	0	1	2	3	4
4	83	83	88	75	75
10	83	100	100	88	88
27±2	83	28	Nil	Nil	Nil

Plate 5 Sprout produced by excised embryonic axis



within twenty one days where as none of the synthetic seeds inoculated in 1/2 MS medium sprouted

4 7 3 Effect of storage temperature on the germination characteristics of synthetic seeds

4 7 3 1 Germination percentage

The germination percentage of synthetic seeds stored in dry cotton as affected by storage temperature and storage period is given in the Table 26 When the synthetic seeds were stored for one week at room temperature, a tremendous decline in germination percentage was observed This decline was almost three times compared to initial germination By second week of storage synthetic seeds were completely desiccated and shrivelled and did not germinate at all On the contrary, synthetic seeds stored at 10°C gave 100 per cent germination at the end of first and second week of storage A slight decline in germination percentage was observed at the end of third week of storage No further decline could be noticed at the end of fourth week of storage Nevertheless, compared to the initial germination percentage this was slightly higher When the synthetic seeds were stored at 4°C, germination percentage remained steady at the end of first week of storage compared to initial germination percentage Eventhough a slight increase in germination percentage was observed at the end of second week of storage, it declined at the end of three weeks of storage

4 7 3 2 Initiation of germination

Variation in initiation of germination of synthetic seeds of *Hopea parviflora* as affected by storage temperature and storage period is given in the Table 27 It was observed that in general stored synthetic seeds took lesser number of days to initiate germination compared to that of synthetic seeds immediately after encapsulation But the days needed to initiate germination increased with the storage period upto the end of three weeks of storage and slightly reduced after fourth week of storage Among the different temperatures at which dry cotton storage of synthetic seeds was employed storage at 10°C was better (Plate 6 and 7) Synthetic seeds stored at 4°C and 10°C took an average of 3 to 6 days and 3 to 4 days respectively to initiate germination during the storage period But synthetic seeds stored under room temperature took 5 days to initiate germination after one week None of the synthetic seeds produced sprouts and they had dried within one week after germination

4 7 4 Effect of storage medium on the germination characteristics of synthetic seeds

4 7 4 1 Germination percentage

Data pertaining to the germination percentage of synthetic seeds of *H parviflora* stored at 10°C as affected by storage media and storage period is given in Table 28 The

Plate 6 Initiation of germination of synthetic seeds of *Hopea parviflora*

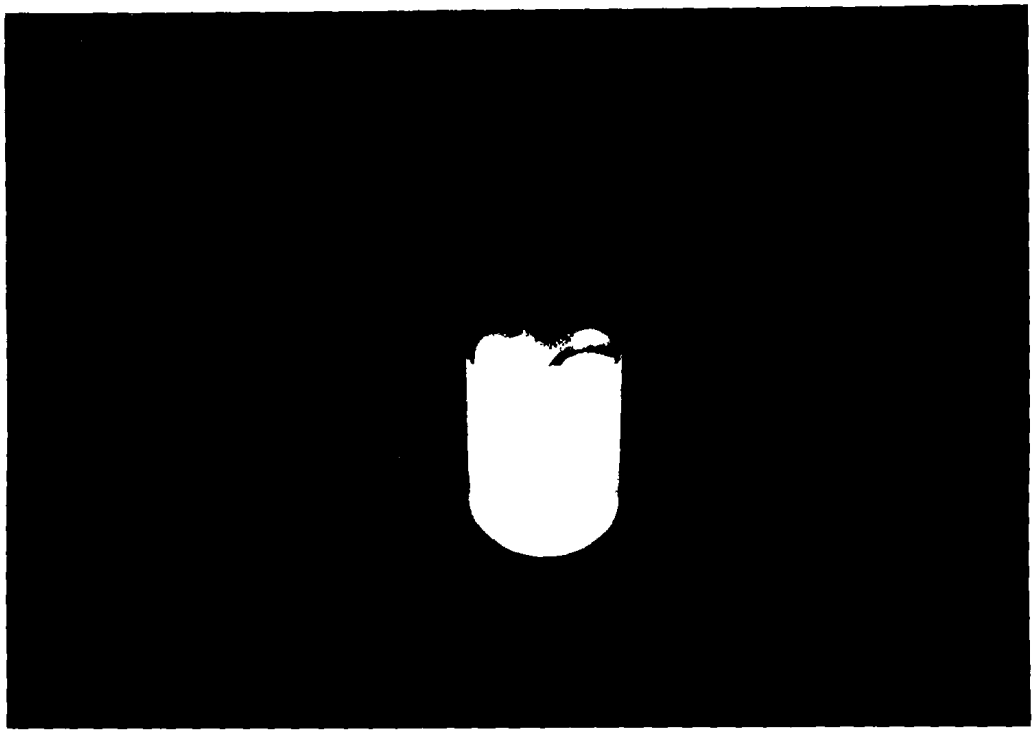
Plate 7 Initiation of sprout by synthetic seeds stored at 10°C

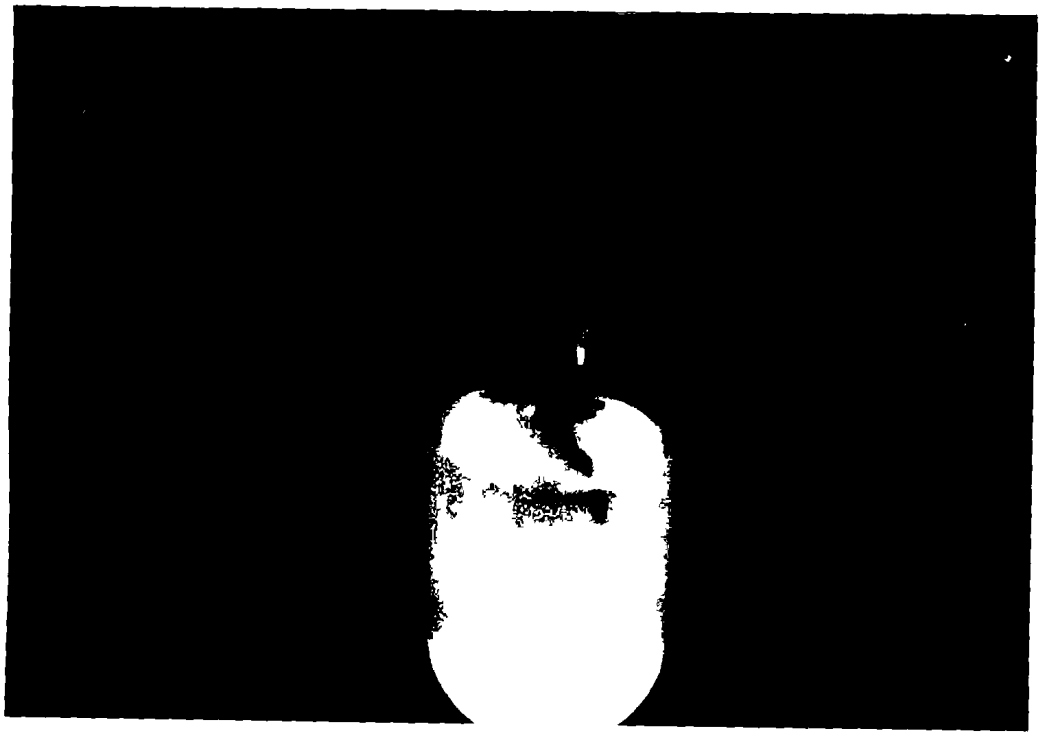
Table 27 Number of days taken for initiation of germination by synthetic seeds of *Hopea parviflora* stored in dry cotton as affected by storage temperature and storage period

Storage temperature (°C)	Storage period (weeks)				
	0	1	2	3	4
4	6	3	5	6	5
10	6	3	3	4	6
27±2	6	5	-	-	-

Table 28 Germination percentage of synthetic seeds of *Hopea parviflora* stored at 10°C as affected by storage medium and storage period

Storage medium	Storage period (weeks)				
	0	1	2	3	4
Dry cotton	83	100	100	88	88
Wet cotton	83	100	100	100	88





germination percentage of synthetic seeds stored for different periods was not influenced by the medium of storage considerably Compared to the germination percentage of synthetic seeds before storage (83 per cent) germination percentage of synthetic seeds stored on both media was increased to 100 per cent after one week of storage and was kept steady upto the end of two weeks of storage

Germination percentage of synthetic seeds stored on dry cotton was reduced to 88 per cent after three weeks of storage and maintained as such upto the end of four weeks However, germination percentage of synthetic seed stored on wet cotton was reduced after four weeks of storage to 88 per cent

4 7 4 2 Initiation of germination

Effect of medium of storage on the initiation of germination of synthetic seeds of *Hopea parviflora* at different storage intervals is given in the Table 29 As in the case of germination percentage, initiation of germination was also not affected by the storage media However, compared to the initiation of germination of synthetic seeds immediately after encapsulation, stored synthetic seeds germinated quickly Synthetic seeds stored in dry cotton took 3 to 4 days while that stored on wet cotton took 2 to 3 days for germination

Table 29 Number of days taken for initiation of germination by synthetic seeds of *Hopea parviflora* stored at 10°C as affected by storage media and storage period

Storage media	Storage period (weeks)				
	0	1	2	3	4
Dry cotton	6	3	3	4	3
Wet cotton	6	2	2	3	3

Table 30 Germination percentage of synthetic seeds of *Hopea parviflora* as affected by different concentrations of ABA and storage period

Concentration of ABA	Storage period (weeks)				
	0	1	2	3	4
		(MS medium only)			(MS+BA 1 ppm+ Kinetin 1 ppm)
1 ppm	100	83	66	dead	
2 ppm	100	83	83	50	83
3 ppm	60	50	33	33	83
Control	83	83	88	75	75

4 7 5 Effect of different concentrations of ABA on the germination characteristics of synthetic seeds

4 7 5 1 Germination percentage

Variation in germination percentage of synthetic seeds of *Hopea parviflora* as affected by different concentrations of ABA at different storage intervals is given in Table 30 Soaking the dewinged seeds in 1 ppm ABA solution overnight resulted in 100 per cent germination of intact seed or of synthetic seed soon after encapsulation of the embryonic axis A gradual but steady decline in germination percentage was observed at the end of first and second week of storage of these synthetic seeds at 4°C and by the third week of storage they failed to germinate completely

When the dewinged seeds were soaked in 2 ppm ABA solution, 100 per cent germination of intact seed or of synthetic seeds of the excised embryonic axis was obtained soon after encapsulation When these synthetic seeds were stored, germination declined slightly to 83 pei cent at the end of first and second week There was further decline in germination percentage at the end of third week of storage However, when the germination medium was modified by incorporating 1 ppm each of BA and kinetin at the end of four weeks of storage, germination percentage increased to 83 per cent

When the dewinged seeds were soaked in 3 ppm ABA solution, germination percentage of intact seeds declined to 70 per cent and that of synthetic seeds to 60 per cent soon after encapsulation Surprisingly, eventhough soaking the dewinged seeds in 3 ppm ABA solution gave rise to lower germination percentage of synthetic seeds upto third week of storage, when the growth medium was modified by incorporating 1 ppm each of BA and kinetin at the end of fourth week of storage, germination percentage comparable to that of 2 ppm treated seeds were obtained Another striking observation was the tendency for the synthetic seeds treated previously with 1 ppm or 2 ppm ABA to gradually change from its almost spherical shape to an elongated torpedo shape while being in storage It is also worth mentioning that when the dewinged seeds were treated with 1 ppm or 2 ppm ABA, their embryonic axis soon after excision gave rise to 100 per cent germination and those treated with 3 ppm ABA gave rise to 70 per cent germination

4 7 5 2 Initiation of germination

Variation in number of days taken to initiate germination by synthetic seeds of *H parviflora* as affected by different concentrations of ABA and storage period is given in Table 31 Drastic difference was not observed in number of days taken by synthetic seeds to initiate germination which was previously treated with different concentrations of ABA Synthetic seeds

Table 31 Number of days taken for initiation of germination by synthetic seeds of *Hopea parviflora* as affected by different concentrations of ABA and storage period

Concentration of ABA	Storage period (weeks)				
	0	1	2	3	4
		(MS medium only)			(MS+BA 1 ppm+ Kinetin 1 ppm)
1 ppm	2	3	4	dead	
2 ppm	2	3	4	6	3
3 ppm	3	4	5	7	5

previously treated with 1 ppm and 2 ppm ABA took two days to initiate germination, whereas those treated with 3 ppm ABA took three days, immediately after encapsulation

The number of days taken by synthetic seeds to initiate germination increased gradually with the storage period irrespective of the ABA concentration At the end of three weeks of storage synthetic seeds previously treated with 2 ppm and 3 ppm ABA took six and seven days respectively for germination But synthetic seeds previously treated with 1 ppm ABA were completely dried after third week of storage and not germinated However, when the growth medium was modified by incorporating 1 ppm each of BA and kinetin, the number of days taken by synthetic seeds treated previously with 2 ppm and 3 pm ABA to initiate germination was reduced to 3 and 5 days respectively Interestingly, all the dewinged seeds soaked in different concentrations of ABA had germinated after one day itself

Discussion

DISCUSSION

The present investigation was conducted to study the effect of temperature, storage medium, fungicide and microencapsulation of zygotic embryo on the storage behaviour of *Hopea parviflora* seeds The results obtained are discussed in this chapter

5.1 Flowering and fruiting

The results showed that *Hopea parviflora* flowered during the first week of April and fruits ripened by the end of May The delayed shedding of fruits during the second year (1994) compared to the first year (1993) may be due to the earlier onset of rainfall resulting in delayed maturity The earlier onset of monsoon might have lowered the atmospheric temperature there by increasing the number of days to attain maturity of fruits (Longman and Jenik, 1987) The reason for the dominant trees to be the major flower/fruit bearers may be the greater availability of sunlight compared to the rest of the trees (Longman and Janik, 1987) Troup (1921) observed that in *H parviflora* intensive seeding occurs once in four to six years and such years are followed by one or two years of comparative sterility and same number of moderate seed years Eventhough, present study was conducted only for two years,

earlier observations (Sudhakara, Personal Communication) has also shown similar trends

5.2 Seed characteristics

As in the case of other recalcitrant seeds, *H parviflora* seeds are shed at a high moisture content (41 per cent on wet weight basis) and 95 per cent of the seeds had germinated at this time The 1000 seed weight observed was 339 8 g Chin (1988) reported that the initial moisture content of recalcitrant seeds may range 30 to 70 per cent (on wet weight basis) and 1000 seed weight often exceed 500 g due to the large seed size and high moistuie content The comparatively lower 1000 seed weight of *H parviflora* seeds may be due to the smaller size of the seeds

Among the seed components embryonic axis showed relatively high moisture content than seed coat or cotyledon Similar high moisture content of embryonic axis is also reported in other recalcitrant seeds viz , *Avicenia marina* 63 per cent (Berjak *et al* 1984), *Azadiracta indica* 91 per cent (Maithani *et al* 1989) and *Artrocarpus heterophyllus* 68 per cent (Fu *et al* 1993)

5.3 Effect of storage temperature on seed viability

The seeds used for determination of seed characteristics (3 2 2) were collected as and when they were shed from the trees whereas, seeds used for studying the effects of storage temperature (3 2 3) and storage medium (3 2 4) on seed viability were collected from the nylon net at an interval stretching to a maximum of 48 hours, as a result seeds might have undergone some amount of dehydration This may be the reason for the lower moisture content of the seeds used for studying the effect of storage temperature and storage media compared to those used for determination of seed characteristics

Results revealed that eventhough the seed moisture content before storage was comparatively lower than that of immediately after natural shedding, they had shown a high germination percentage At this partially dehydrated state seeds may exhibit mild water stress and this can upset the relative rates of individual reactions associated with germination (Vertucii, 1993) However, the membrane disruption would not have reached upto a level to cause viability loss, as evidenced from the low leachate production by the seeds before storage So when the seeds were re-hydrated at this stage to study germination, the membrane repair mechanisms might have been activated The comparatively high germination percentage of seeds before storage may be explained based on

this activation of repair mechanisms Similar observations were reported in *Hopea hainanensis* also (Song *et al* 1986) wherein desiccation to 31 per cent moisture content disturbed the ribosomes and endoplasmic reticulum which were reversed on rehydration

It was observed that when the dewinged seeds were stored at different temperatures (10°C, 20°C and 29±1°C) germination percentage and vigour parameters rapidly declined after first week itself irrespective of storage temperature This rapid decline in germination percentage and vigour parameters after one week of storage could be ascribed to the dehydration damages The continued dehydration during storage and injury caused by dewinging might have resulted in quick loss of seed moisture content and hence germination percentage Farrant *et al* (1989 1993a) suggested that the survival of seeds is a function of the extent to which germination of embryos within the seed has progressed Seeds might have already initiated germination associated events before storage and hence they became more desiccation sensitive with the progress of these events during storage

After a rapid decline in germination percentage at the end of first week of storage a progressive decrease in germination percentage was observed with the storage period irrespective of storage temperature This may be due to the progressive cellular deterioration occurred due to the water

stress with the increasing storage period The deteriorative changes in the long run resulted due to the water stress was reported by Pammenter *et al* (1994) The possible processes responsible for the deterioration of stored seeds are reduced rates of protein synthesis, increased proteolysis and variable effects on the catabolic activity of different enzymes (Pammenter *et al* 1994)

The progressive decrease in germination percentage of stored seeds may also be due to the removal of structured water which might have resulted in the disruption of metabolic pathways (Farrant *et al* 1988) Immediately after seedfall retention of relatively high proportion of water as bulk water has been reported in recalcitrant seeds (Farrant *et al* 1988) As germination associated events proceed, more and more pathways are initiated and more amount of water will have a structure imposed on it Seeds dehydrated further during storage might have resulted in the removal of structured water which ultimately lead to disruption of metabolic pathways

The rapid decline in viability of *Hopea parviflora* seeds after one week of storage may be associated with the cell membrane damage as shown by the high electrolyte leakage The rapid increase in leachate conductivity during first week of storage may be due to the loss of membrane semipermeability Membrane purturbations are often cited as a consequence of dehydration

(Leopold and Vertucci, 1986) and the apparent loss of semipermeability may be a primary result of desiccation or one aspect of general loss of metabolic capability of the seed (Tompsett, 1992)

5.4 Effect of storage media on seed viability

Results indicated that the use of either sand or dry neem cake as a storage medium is not appropriate for the seeds of *Hopea parviflora* The rapid decline in germination percentage of the seeds stored in dry neem cake may be due to the dehydration damages The initial moisture content of the seed indicates that they are already in a partially dehydrated condition Storage of these seeds in neem cake resulted in the occurrence of a steep gradient in moisture content between seed and the storage media, neem cake (see Table 32 for moisture content of the storage media) The steep gradient in moisture content existing between the seed and the storage medium may be the major reason for quick desiccation of seeds Dehydration could result in severe membrane damages (Leopold and Vertucci 1986 Tompsett, 1992) and ultimately lead to loss of viability The high leachate production by the seeds stored in neem cake confirms the membrane disruption caused by dehydration

As recalcitrant seeds are desiccation sensitive, storage in moist condition is recommended for increasing seed

Table 32 Moisture content (dry weight basis) of the storage media as affected by storage period

Media	Storage period (weeks)				
	0	1	2	3	4
Sand	2 06	8 02	8 97	10 02	12 7
Neem cake	10 27	16 69	20 15	38 12	45 42

longevity (Chin, 1988) However it was found that when seeds were stored in sand, germination progressed in storage condition itself and at the end of second week of storage germination was completed However, successful storage of neem seeds in moist sand was reported upto 3 months (Ponnuswami *et al* 1991) The maintenance of constant seed moisture content (30 8 per cent to 30 1 per cent) throughout the storage period was the actual reason behind this success In the present study, moisture content of the seed was increased with the storage period and this may be due to the increase in moisture content of the storage medium (sand) with the storage period (Table 32) The increase in seed moisture content due to its storage in moist medium may be the main reason for higher germination of seeds while being kept in storage The problem of germination during storage was also encountered earlier in many recalcitrant seeds when seeds were stored above 40 per cent moisture content (Tompsett, 1985) So moist storage of *Hopea parviflora* seeds is not useful for long term seed storage The present study also showed that moisture content of the seeds kept in storage act more as an indicator of seed germination, rather than leachate conductivity serving as an indicator of seed deterioration

5.5 Effect of temperature and fungicide on seed viability

Results indicated that when the seeds collected manually before natural shedding were stored at 10°C, after partial drying and fungicide treatment, they retained high germination percentage for a considerably long period The importance of collecting seeds before their natural shedding is well recognised (Panochit *et al* 1984, 1986) Collection and partial drying of the seeds well before the initiation of any germination related events is reported to impart desiccation tolerance upto a certain extent (Farrant *et al*, 1988)

Storage temperature and seed moisture content during storage are the major factors influencing viability of hopea seeds *Hopea parviflora* seeds stored at 41 per cent moisture content under 21°C was reported to retain 45 per cent germination after 41 days (Tompsett, unpublished) Similarly *Hopea hainanensis* seeds retained high viability upto one year when stored at 36 per cent moisture content under 15 20°C (Song *et al* 1984) In the present study also a high seed germination of 87 per cent was observed after 40 days of storage when seed moisture content was maintained about 34 to 37 per cent under 10°C

Eventhough storage temperature below 15°C were reported to be lethal for most of the recalcitrant seeds *Hopea parviflora*

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Eventhough storage temperature below 15°C were reported to be lethal for most of the recalcitrant seeds *Hopea parviflora*

seeds were not sensitive to similar low temperature at higher moisture content The retention of high germination percentage and vigour parameters by hopea seeds during storage at 10°C may be due to the advantages of both low temperature and sub imbibed storage All the metabolic reactions including seed respiration is too low under low temperature (King and Roberts, 1980) So storage of seeds at low temperature with high moisture content might not have imposed any immediate dehydration induced water stress

However a decline in germination percentage and vigour parameters was observed in the case of *H parviflora* seeds at room temperature This may be due to the higher rate of dehydration as indicated by the decline in moisture content Kovach and Bradford (1992) showed that seed desiccation is related to the temperature at which seeds were dehydrated At higher temperature seed looses moisture quickly because of the existence of a steep gradient in moisture content of the seed and the storage environment Quick dehydration and loss of viability under ambient temperature was reported by various workers (Purohit *et al* 1982, Sasaki, 1976, Hor *et al*, 1984)

Results show that germination percentage is not affected by fungicide treatment whereas higher moisture content was observed in the case of seeds treated with fungicide after one week of storage The retention of higher moisture by seeds

treated with fungicide may be due to the immersion of seeds in fungicide solution prepared in water before storage

Fungal contamination one of the major problem when attempting storage of recalcitrant seeds under high moisture content above 15°C is reported by various workers (Hor *et al* 1984 Roberts 1973) So treating the seeds with fungicides before storage was suggested by King and Roberts (1980) In the present study, when hopea seeds were treated with Emisan fungicide (1% w/v) it was found to be very effective in preventing fungal contamination both at 10°C and room temperature during storage Effectiveness of fungicide in preventing fungal contamination during seed storage is well documented (King and Roberts, 1982, Teng, 1978, Yap, 1981)

5.6 Germination characteristics of synthetic seeds

Results of the present study indicated that synthetic seeds produced by encapsulation of excised embryos retained their viability to a great extent and was dependent on storage temperature Synthetic seeds stored at 10°C retained 88 percentage of viability after four weeks of storage This is against an initial viability of 83 per cent exhibited by fresh synthetic seeds Similar high germination percentage of synthetic seeds was also reported in cocoa when they were stored at 10°C (Nagaraj, 1994)

Synthetic seeds stored at 4°C showed a slight reduction in germination percentage after three weeks of storage, which remained as such upto the end of four weeks The reduced germination percentage may be associated with the delayed initiation of germination showing over all decline in vigour The reduction in germination percentage indicates that low temperature around and below 4°C will cause damages to synthetic seeds in the long run Such low temperature dehydration has been reported to cause rapid decline in viability of excised embryos of *Zizania palustris* (Berjak *et al* 1994) Synthetic seeds stored at room temperature are quickly dehydrated and lost their viability after two weeks of storage itself

Significant difference in germination percentage of synthetic seeds stored due to storage on either wet cotton or dry cotton was not observed when they were stored for four weeks at 10°C However, after three weeks of storage the germination per cent of synthetic seeds stored on dry cotton decreased slightly This may be due to the water stress resulted because of the germination associated changes like extensive vacuolation and increase in cell size occurring during storage which implies an additional water requirement (Farrant *et al* 1986) Nagaraj (1994) also observed similar phenomenon in the case of synthetic seeds of cocoa

Additional supply of moisture to the embryo by the wet cotton avoided occurrence of any water stress upto three weeks of storage But this storage condition was not enough to maintain the same germination percentage of synthetic seed for longer periods The slight reduction in germination percentage of synthetic seeds stored on wet cotton after four weeks might be due to the desiccation of the wet cotton medium

Germination percentage of synthetic seeds derived from intact seeds treated with 1 ppm ABA declined rapidly after the end of two weeks and were completely dead by the third week of storage Since intact seeds were immersed in 1 ppm ABA solution, the water content of the embryo before encapsulation may be high and storage after encapsulation at low temperature might have caused damages to the embryo Recalcitrant seeds are generally sensitive to low temperature storage at higher moisture content (Hendry, 1993)

When intact seeds were treated with higher (2 and 3 ppm) concentrations of ABA, synthetic seeds obtained from them showed tolerance to low temperature and retained higher germination percentage The importance of ABA application for imparting desiccation tolerance during storage of somatic embryos is well recognised (Senaratna *et al*, 1989, 1990) The effect of ABA at higher concentrations was observed to be slightly inhibitory with the storage period Increasing delay in initiation of germination indicates this phenomenon

However, this inhibitory action of ABA was nullified by incorporating cytokinin to the growth medium and hence synthetic seeds have shown a high germination percentage Based on this data, it is not possible to choose an appropriate concentration of ABA for extending the storage period of synthetic seeds Effect of different concentrations of ABA for extending storage life of synthetic seeds has to be studied in detail

The present study has indicated that the stage and moisture content at which seeds are stored are important factors that determine the viability of stored seeds Storing seeds below 30 per cent moisture content either under different temperatures or in different media did not enhance the seed longevity whereas the seeds collected before the initiation of any germination related events retained higher germination percentage and seed vigour when they were dried to 37 per cent moisture content and stored under low temperature Successful production of synthetic seed was achieved with high germination percentage in comparable to that of fresh intact seeds The failure in further development of plantlets from synthetic seed may be due to the problems associated with storage or growth conditions Occurrence of germination associated events during storage indicates the necessity of making the embryonic axis dormant for long term storage Dormancy and, desiccation tolerance can be imparted to the embryos by treating them with appropriate concentration of ABA

or high sucrose concentrations (Anandarajah and McKersie 1990, Liu *et al* 1992) So the production of synthetic seeds by the method adopted in this study can be used for exploiting the possibilities of long term storage of synthetic seeds but the storage techniques and growth conditions has to be perfected

Summary

SUMMARY

The present investigations were undertaken at the College of Forestry, Vellanikkara to study the effect of temperature, storage medium fungicide and microencapsulation of zygotic embryo on the storage behaviour of *Hopea parviflora* seeds The salient findings of the studies are summarised below

1 Seeds shed naturally and collected from the nylon net with a gap of 48 h had undergone some amount of desiccation as evidenced by the lower moisture content of 29 5 per cent compared to the moisture content of 41 per cent and 41 8 per cent in the case of seeds collected immediately after natural shedding and seeds shed through manual shaking, respectively However, the germination percentage of the seeds collected within 2 days after natural shedding was not significantly affected thereby showing that membrane damages during this short period were low and reversible upon rehydration

2 Storage of dewinged seeds with an initial moisture content of 29 5 per cent lead to rapid loss of viability and vigour parameters irrespective of storage temperature at the end of storage for one week Occurrence of severe membrane damage at this time was indicated by high leachate production Compared to the sharp reduction in

the germination percentage at the end of storage for one week, subsequent reduction in germination percentage was gradual

3 Dewinging the seeds resulted in significant decline in moisture content and increase in leachate conductivity upto the end of second week compared to the winged seeds However, the germination percentage was not significantly affected

4 Rapid decline in germination percentage and vigour parameters were observed due to desiccation when the seeds were stored in dry neemcake medium

5 Storage of seeds in moist sand increased seed moisture content and seeds started germination while being kept for storage from the second week onwards

6 *Hopea parviflora* seeds were successfully stored under low temperature (10°C) upto 40 days without significant reduction in viability Storage of seeds at this temperature maintained seed moisture content around 37-35 per cent

7 Compared to 1/2 MS medium, MS medium gave better results with respect to germination percentage and sprout production of synthetic seeds of *Hopea parviflora*

8 Synthetic seeds of *Hopea parviflora* using zygotic embryo were produced successfully with high germination percentage in comparable to that of fresh intact seeds

9 Synthetic seeds stored on dry cotton maintained high germination percentage upto one month at 10°C and 4°C whereas those stored on dry cotton at room temperature lost viability completely by second week

10 Significant difference in germination percentage either due to wet cotton or dry cotton as storage media for the storage of synthetic seeds was not observed upto the end of one month

11 Synthetic seeds derived from intact seeds treated with 1 ppm ABA lost viability quickly at 4°C, whereas those derived from intact seeds treated with higher concentrations of ABA retained greater germination percentage for alonger period

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VIABILITY OF *Hopea parviflora* SEEDS WITH REFERENCE TO TEMPERATURE, MEDIUM OF STORAGE AND MICROENCAPSULATION TECHNIQUES

BY

SUNIL KUMAR K. K.

ABSTRACT OF A THESIS

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Faculty of Agriculture
Kerala Agricultural University

Department of Silviculture and Agroforestry
COLLEGE OF FORESTRY
Vellanikkara, Thrissur-680 654

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ABSTRACT

A detailed study was conducted at College of Forestry Kerala Agricultural University Vellanikkara, Thrissur, Kerala during 1994 95 to study the effect of temperature, storage medium, fungicide and microencapsulation of zygotic embryo (synthetic seed) on the storage behaviour of *Hopea parviflora* seeds Storing the dewinged seeds with a moisture content below 30 per cent resulted in rapid decline in seed viability due to dehydration injuries irrespective of storage temperature Sand and neemcake was inappropriate as a storage medium because sand favoured early germination of the seeds in storage condition itself and neemcake caused severe desiccation injuries Storing fungicide treated winged seeds collected just before natural seedsheding, at 10°C retained high germination percentage upto 40 days Synthetic seeds were also successfully stored upto 1 month at 10°C without significant reduction in germination percentage Two ppm and three ppm ABA was observed to be helpful for maintaining higher germination percentage of synthetic seeds during storage