CONSERVATION STRATEGY FOR *Hopea parviflora* Bedd SPECIES THROUGH STORAGE OF SEEDS USING CRYOPRESERVATION TECHNIQUES

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Conservation strategy for *Hopea parviflora* Bedd species through storage of seeds using cryopreservation techniques"** is a bonafide research work done by me during thee 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 University or Society

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To My Beloved Ones

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Introduction

Introduction

Based on storage behaviour seeds are classified into orthodox and recalcitrant groups (Roberts 1973) Berjak *et al* (1990) described them as poikilohydrous and homoiohydrous respectively More recently a third category called intermediate has been identified between the orthodox and recalcitrant groups (Ellis *et al* 1990a) Orthodox seeds can be stored satisfactorily *ex situ* over a long term in appropriate environments The maintenance of viability of seeds of species with intermediate and recalcitrant nature is problematic Recalcitrant seeds are damaged by dehydration, may be chilling sensitive and generally cannot be stored effectively for useful periods (Roberts 1973 Chin and Roberts 1980) Seeds with intermediate storage behaviour are relatively desiccation tolerant but will not withstand removal of water to levels as low as orthodox seeds Such seeds particularly if they are of tropical origin, may also be chilling sensitive even in dehydrated stage (Ellis *et al* 1990 Hong and Ellis 1996)

Recalcitrant seeds do not undergo maturation drying and are shed at relatively high moisture contents Their moisture content can be in a wide range of 30 to 80 per cent on a wet mass basis They are desiccation sensitive both before and after shedding and have very limited post harvest life spans even in the hydrated condition They are killed if moisture content is reduced below some relatively high critical value (King and Roberts 1980) and hence can t be stored for long by conventional storage methods Pammenter *et al* (1994) reported that recalcitrant seed species would, sooner or later die under storage conditions that do not permit any significant degree of water loss

Recalcitrant seeds cannot be dried without damage and so they cannot conform to the viability equation which describes relations between longevity and air dry seed storage environments (Roberts 1973) When fresh recalcitrant seeds begin to dry viability is first slightly reduced as moisture is lost, but then begins to be reduced considerably at a certain moisture content termed the critical moisture content (King and Roberts 1979 1980) or lowest safe moisture content (Tompsett, 1984) If drying continues further, viability is eventually reduced to zero

Majority of the economically important trees species found in the tropics have recalcitrant seeds storage of which is one of the major problems *Hopea parviflora* Bedd is one of the most economically important recalcitrant species coming under the family Dipterocarpaceae which lose their viability within 7 to 10 days under natural conditions when the seed moisture content reduces below a high critical value (Troup 1921) However, Sunilkumar and Sudhakara (1998) while studying on the effect of temperature media and fungicide on the storage behaviour of *Hopea parviflora* seeds found that seeds of *Hopea parviflora* seeds could maintain their viability up to a period of 14 days The physiological basis of seed recalcitrance is as yet unknown Hypothesis suggesting possible causes have been proposed (Roberts 1973 Flood and Sinclair 1981) but the end result is that intact recalcitrant seeds cannot be stored for long periods of time Thus if the seed crop fails nurseries will be unable to draw upon a storage reserve of seeds in order to meet the demands of growers

Storing seeds with high moisture content at sub-ambient or ambient temperatures will lead to fungal contamination and germination of the seeds while being in storage Storing the seeds under low temperature has been suggested to prevent fungal

contamination and germination during storage (King and Roberts 1980) Most of the recalcitrant seeds are sensitive to temperatures below 15^{0}C (Hor *et al* 1984)

The degree of sensitivity of recalcitrant seeds to desiccation varies with physiological maturity of the seed (Pammenter *et al* 1991) Immature embryos might be more adaptable to manipulation than mature embryos or mature embryonic axes Immature embryos might be highly embryogenic than mature embryos (Pence 1991) There is evidence in the literature on orthodox seeds that the immature seeds of certain species may exhibit dormancy or require after ripening e g *Brassica japonica* Sieb (Tokumasu, 1970) and *Agrostis gigantea* Roth (Williams 1973) A number of workers have suggested that the germination of recalcitrant seeds during storage could be reduced if they were harvested before attaining full maturity e g in cocoa (Pyke *et al* 1934) in coffee (Veen van de 1934) and in citrus seed (Patt, 1953)

Recalcitrant seeds remain metabolic throughout development to shedding and continue to accumulate dry mass They will either entrain germinative metabolism at the shedding water content or under certain conditions (Berjak *et al*, 1989 Finch Savage *et al* 1993) will continue with pregermination development. Recalcitrant seeds are characterized by desiccation sensitivity although there is a developmental stage at which most species are relatively at their most desiccation tolerant level This appears to coincide with the lowest water content attained (Finch Savage 1996) Recalcitrant seeds will sooner or later die under storage conditions that do not permit any significant degree of water loss (Pammenter *et al* 1994)

Hong and Ellis (1996) suggested that removal of seed covering structures might help to promote speedy germination This may be because hard seed coats serve as protective mechanisms which can exclude oxygen and/or water from the embryonic tissue

thus extending seed life Cohen (1958) and Mumford and Grout (1979) suggested that the germination of lemon seed (*Citrus limon* L) was improved when the testa was removed and they produced evidence to prove that the testa of lemon seeds has a marked deleterious effect on the seed s response to desiccation Recalcitrant nature may be also due to their seed coat and storage tissue characteristics (King and Roberts, 1980)

Fu *et al* (1990) suggested that embryonic axes have higher desiccation tolerance than intact recalcitrant seeds Because of its organized small structure and its ability to produce a whole plant from the meristematic tissues, embryonic axes are preferred There are many reports on the advantage of rapid drying over slow drying which would help surpass desiccation injuries if any inflicted due to the loss of structural water from the cells Several scientists have proposed rapid drying as one of the methods to reduce desiccation injury Hunter (1959) found that a rate of drying of 5 % loss in moisture content per day is apparently no more deleterious than a rate of 1 9 % per day and the viability of seed dried to 16 7 % moisture content was the same for both treatments It has been found that the more rapidly dehydration can be achieved the lower is the water content to which seeds or axes can be dried, without damage accumulation that culminates in viability loss This is particularly the case when excised axes are dried (Normah *et al* 1986 Berjak *et al* 1990) The rate of drying also depends on the amount of seed (particularly the depth of the layer of seeds) the circulation of dry air within the drying cabinet and the species (Hong and Ellis 1996) Bonner (1996) suggested that the rate of desiccation could influence the expression of recalcitrant behaviour in seeds

Fu *et al* (1993) reported that drying excised embryonic axes by silica gel or an aseptic air current allowed excised embryonic axes to survive desiccation to a lower value than that achieved by the vacuum method

According to Roberts *et al* (1984) the most promising method of germplasm conservation for recalcitrant seeds is storage in liquid nitrogen Bajaj (1985) also suggested that the germplasm of recalcitrant seeds could possibly be conserved through the cryopreservation of their excised embryos For seeds to survive at very low temperature they must be dried prior to freezing If seeds have sufficiently low moisture content, they will not suffer injury even at 196^0C (Stanwood and Bass 1978) Normah *et al* (1986) found that at moisture contents between 14 per cent and 20 per cent, 20-60 per cent of the excised embryos of rubber survived cryopreservation for 24 hours and formed seedlings when cultured *in vitro* Cryopreservation of somatic and zygotic embryos has been reported to be successful for many species, which show orthodox, intermediate and recalcitrant seed storage behaviour (Engelmann *et al* 1995b) For successful cryopreservation, excised embryos from recalcitrant seeds must survive desiccation below the threshold freezable moisture content (Hor *et al* 1990) below which value there is no freezable water for ice formation by cooling to ultra low temperatures However very few true recalcitrant seeds can be dried and stored in liquid nitrogen and that the desiccation of embryonic axes should be investigated in detail prior to the works on cryopreservation As per Vertucci *et al* (1991) a survival rate of 90 % following freezing to -70^0C and storage at that temperature for 6 months was achieved for embryonic axes excised from desiccation sensitive seeds of *Landolphia kirkii* these had been very rapidly dehydrated to the optimal moisture level prior to freezing

Various storage methods *viz* storing in moist conditions sealed containers perforated polythene bags burying in soil *etc* have been tried at different temperatures to find out an ideal storage method for long term storage of recalcitrant seeds but in vain Similarly species trials and plantation programmes involving *Hopea parviflora* were

often at doldrums owing to its recalcitrant seed storage behaviour Suitable storage techniques have to be devised to achieve long term storage of these seeds without any loss of their viability and longevity to make forestry programmes involving *Hopea parviflora* a success

The present study was carried out during 1998 2000 in the College of Forestry Vellanikkara with the following objectives

1 To find out the best desiccation protocol as a pre treatment for the purpose of storage of *Hopea parviflora* seeds using cryopreservation techniques

2 To determine the ideal maturity level of the seeds of *Hopea parviflora* for the purpose of storage using cryopreservation.

Review of Literature

Review of Literature

The importance of seed storage has been recognized ever since man began to domesticate plants The duration of successful storage depends upon both the objectives and the species concerned Seed longevity varies greatly among species Not all species seeds respond to the environment before and during storage in the same way Roberts (1973) defined two categories of seed storage behaviour orthodox and recalcitrant More recently a third category intermediate between the orthodox and recalcitrant categories has been identified (Ellis *et al* 1990) Seeds of species with orthodox seed behaviour can be maintained satisfactorily *ex situ* over the long term in appropriate environments and short term storage is usually the best that can be achieved with seeds which shows recalcitrant seed storage behaviour Medium term storage is feasible for seeds of species with intermediate seed storage behaviour provided the storage environment is well defined and well controlled Recalcitrant seeds are usually short lived and very sensitive to desiccation and low temperatures making it very difficult to store them on a long term basis (Chin *et al* 1991) Seeds of most of the economically important tree species in the tropics are recalcitrant in nature and cannot be stored for long periods A current view is that hydrated recalcitrant seeds are metabolically active and undergo germination associated changes in storage Some of these changes such as extensive vacuolation and increase in cell size imply a requirement for water additional to that present in the seed on shedding (Pammenter *et al* 1994) The quick loss of viability may also be due to chilling damage microbial contamination and germination

during storage (King and Roberts 1980) Mature seeds survive desiccation to low moisture contents at least to 2 6 % depending on the species Above this value there is a negative logarithmic relation between seed moisture content and longevity (Ellis and Roberts 1980a, 1980b)

2 1 Desiccation sensitivity

Recalcitrant seeds are those that undergo little or no maturation drying and remain desiccation sensitive both during development and after they are shed In reality however the situation is far more complex than this perception implies because of the wide range of variability among recalcitrant seeds of different species (Berjak and Pammenter 1997) Such seeds are shed in a hydrated condition but the water content can be anywhere in a wide range Shedding water content is partially species characteristic depending on the degree of dehydration that occurs late during seed development this goes hand in hand with the degree of desiccation tolerance developed by individual species (Finch Savage 1996) Desiccation tolerance in recalcitrant seeds increases during seed development on the mother plant, however unlike orthodox seeds maturation drying to low moisture contents does not occur (Hong and Ellis 1990) Fresh recalcitrant seeds have high levels of moisture contents at maturity/shedding between, for example 36 % for rubber (Chin *et al* 1981) and 90 % for choyote (*Sechium edule*)(Ellis 1991) When acorns of *Quercus nigra* L were dried at three different rates and two temperatures there were losses of both germination and moisture content as desiccation increased and critical moisture content of 10 15 % was identified by Bonner (1996)

There are marked differences in the rate at which water will be lost from seeds of various species under the same dehydrating conditions (Farrant *et al* 1989) There are other factors too that influence the post harvest responses of recalcitrant seeds e g developmental status (Berjak *et al* 1993 Finch Savage and Blake 1994) and chilling sensitivity (Berjak and Pammenter 1997) Farrant *et al* (1988) proposed a continuum of recalcitrant seed behaviour from species that are highly desiccation and probably also chilling sensitive to those that will tolerate drying to the lowest water contents still commensurate with recalcitrant seed behaviour and will also tolerate relatively low temperatures The idea of an extended continuum of seed behaviour from the most desiccation tolerant of orthodox species to the recalcitrant species that are most sensitive to even slight water loss embodies many properties of seeds and their responses (Berjak and Pammenter 1994 1997)

Recalcitrant seeds are sufficiently hydrated at shedding and germination commences without any additional water (Berjak *et al* 1989 Farrant *et al* 1988) Desiccation sensitivity of recalcitrant seeds is intimately associated with their persistent state of metabolic activity

Generally the axes of recalcitrant seeds are at considerably higher water content than are the cotyledons (Berjak *et al* 1989 Maithani *et al* 1989 Fu *et al* 1993) Finch Savage *et al* (1992a) have demonstrated that for *Quercus robur* there is a higher proportion of matrix bound water in the cotyledons which may underlie the greater desiccation sensitivity of the cotyledons relative to the axes *Hopea parviflora* seeds were successfully stored under low temperature (10^0C) upto 40 days without significant

reduction in viability Seeds stored at this temperature maintained seed moisture content of about 37 35 per cent (Sunil kumar and Sudhakara, 1998)

Methods of drying seeds using silica gel have been described by Hanson (1985) Dry (newly regenerated) silica gel is in equilibrium with about 5 per cent relative humidity at 20^{0}C The first change of the colour of the indicator (diminishing intensity of blue) occurs when the silica gel moistens to equilibrium with about 12 13 per cent relative humidity By the time it has become pale blue it is in equilibrium with about 49 per cent relative humidity (and thus very moist) 100 g of regenerated silica gel (in equilibrium with about 5 per cent relative humidity) can absorb about 7 g of moisture from seeds until the first colour change is apparent (in equilibrium with 13 5 per cent relative humidity) At this point the silica gel should be regenerated. It should be done by drying in an oven maintained at about 130^{0}C for 3-4 hours It should then be stored in a sealed container overnight to cool to ambient temperature before being used to dry the seeds (Hong and Ellis 1996)

Drying excised embryonic axes by silica gel or an aseptic air current allowed excised embryonic axes to survive desiccation to a lower value than that achieved by the vacuum method (Fu *et al* 1993) For example although the vacuum drying method provided more rapid drying, no excised embryonic axes of *Artocarpus heterophyllus* survived desiccation to 44 per cent moisture content, while the excised embryonic axes dried with an aseptic air flow and silica gel tolerated desiccation to 26 per cent and 16 per cent moisture content respectively (Fu *et al* 1993)

2 1 1 Seed development and levels of desiccation tolerance

For both recalcitrant and orthodox seeds the relative level of desiccation tolerance changes through out development so that embryos become more tolerant as

they mature and less tolerant as they germinate (Adams *et al* 1983 Berjak *et al* 1989) However only orthodox seeds achieve considerable tolerance of desiccation. The acquisition of tolerance is presumably developmentally controlled (Kermode 1990 Bewley and Oliver 1992) Galau *et al* (1991) divided post differentiation embryo genesis into five stages based on the appearance of molecular markers (1) maturation (2) post vascular separation (PVS) (3) pre desiccation (4) desiccation and (5) quiescence They suggested that desiccation tolerance is acquired during the PVS stage

Studies of the effect of premature harvest on seed vigour and viability suggest that maximum desiccation tolerance is achieved only upon the successful completion of the first three stages of embryogenesis and the rapid completion of the fourth stage Complete maturation is required for *Acer plantanoides* to survive complete desiccation (Hong and Ellis 1992a) Recalcitrance appears to be a product of either an abbreviated PVS stage (Progression toward germination process following abscission)(Berjak *et al* 1990 Farrant *et al* 1985) or an early termination of development (Finch savage 1992b)

Hong and Ellis (1996) suggested that removal of seed covering structures filing or chipping seeds with a scalpel or nicking with a needle might help to promote germination during prolonged tests Cohen (1958) and Mumford and Grout (1979) suggested that the germination of lemon seed (Citrus limon L) was improved when the testa was removed and they produced evidence to prove that the testa of lemon seeds as a marked deleterious effect on the seed s response to desiccation

At the early stages of development, embryos are extremely sensitive to dehydration stress (Rogerson and Matthews 1977) There is however little information

regarding how much water is actually required, but sufficient quantities to allow cell division are certainly necessary According to Myers *et al* (1992) water potentials greater than 1 6 MPa are required

During the maturation phase the embryo accumulates dry matter and become germinable Coincident with these changes tolerance to low water potentials increases In orthodox seeds there is a transition at a particular stage in the developmental pathway from a relatively desiccation intolerant to a tolerant state (Kermode 1990) This transition can be prematurely induced or prolonged by the environmental or chemical manipulations (Blackman *et al* 1991) Once induced, the immature embryo can become fairly tolerant of desiccation within a few days (Galau *et al* 1991)

Recalcitrant seeds may become increasingly tolerant of drying, as maturation proceeds (Berjak *et al* 1992 Finch Savage 1992b) they remain hydrated and metabolically active through out development (Berjak *et al*, 1992 Farrant *et al* 1992) Recalcitrant seeds appear to initiate germination related metabolism shortly after shedding (Farrant *et al* 1988 Berjak *et al* 1989) and in *Avicennia marina* 10 to 15 days before shedding (Farrant *et al* 1993a) As germination events progress the seeds become increasingly sensitive to drying (Farrant *et al* 1986)

2 1 2 Desiccation, metabolic stresses and membrane damage

Metabolism will continue in recalcitrant seeds even after shedding when water is loosing slowly but at some stage when the seeds are still at relatively high water contents metabolism will become unbalanced or out of phase as a result of internal water stresses (Senaratna and Mc Kersie 1986) As water is removed from the cell the concentration of the solutes is increased, and eventually the fluidity of the aqueous

medium declines These changes affect the metabolic status of the cell The changes in metabolic activity are believed to occur at specific moisture levels (Leopold and Vertucci 1989) Below a moisture level of about –1 5 MPa, tissues no longer grow and expand (Mc Intyre 1987) and protein and nucleic acid synthesis patterns change (Dell Aquila and Spada, 1992) This slight desiccation may induce production of protectants (Close and Chandler 1990) Greater levels of desiccation can result in metabolic imbalances At about 0 45g H_2O/g dm or about –3MPa (Dell Aquilla, 1992) protein synthesis ceases and repair processes become inoperative (Dhindsa and Cleland, 1975 Dell Aquila, 1992) Respiratory activity continues until tissues are dried below about 0 25g/g or –11MPa (Leopold and Vertucci, 1989 Vertucci 1989) At moisture levels between 3 and –11 MPa (about 0 45 to 0 25g H_2O/gdm) catabolic activities continue unabated and processes utilizing the high energy intermediates are impaired (Leopold and Vertucci 1989)

Dehydration injuries occur primarily on the basis of alterations in membrane structural integrity function and physico-chemical properties Sun *et al* (1994) found that the glossy state of membranes is not sufficient for desiccation tolerance where as the ability of membranes to retain the liquid crystalline phase is correlated with desiccation tolerance Poulsen and Eriksen (1992) in a study using excised embryonic axes of recalcitrant *Quercus robur* acorns proved that critical water potential for the initiation of damage was –5 MPa Salmen *et al* (1994) studying *Araucaria augustifolia* found that dehydration induced deterioration of cell membranes as evidenced by a high increase in leakage of solutes Dehydration also resulted in the damage of the nuclei, which was not repaired upon rehydration During desiccation respiratory activity was decreased and

however O_2 uptake could not be correlated as an indication of germination ability Desiccation also resulted in a rapid decrease in the ability for protein synthesis

2 1.3 Role of plant growth regulators

The plant growth regulator abscisic acid (ABA) appears to play an important role in the development of desiccation tolerance ABA has also been implicated in the response of vegetative tissues to water stress (Bewley and Oliver 1992) In general ABA level increases during the late stages of development of orthodox seeds and declines only during maturation drying (King 1982 Kermode 1990) Exogenous application of ABA induces desiccation tolerance to developing embryos (Bartels *et al* 1988) and prolongs the desiccation tolerant phase in mature embryos (Blackman *et al* 1991) Application of fluridone an inhibitor of ABA synthesis results in embryos with viviparous characteristics (Oishi and Bewley 1992) Mature embryos of recalcitrant seeds of *Avicennia marina Theobroma cacao* or *Quercus robur* have low levels of ABA (Farrant *et al* 1993a Pence 1991) In addition, double mutants of *Arabidopsis* and corn that are both lacking in and insensitive to ABA produce desiccation sensitive seeds (Koornneef *et al* 1989)

ABA may act as a signal transducer for the transcription of protectants (Dure *et al* 1989) Exogenous application of ABA to seedlings or immature embryos induces mRNAs for proteins associated with water stress (Kermode 1990) Lin Tsan Piao *et al* (1995) found that developing embryos and mature seeds of *Persia thunbergii* have properties common to many recalcitrant seeds with seeds being sensitive to desiccation at all stages having a prominent ABA peak, little proline lacking oligosacharides and specifically little dormancy and a moderate rate of respiration of mature seeds As per

Farrant *et al* (1993b) with the exception of ABA embryonic contents of PGRs were relatively high at seed shedding consistent with rapid germination of the highly recalcitrant seed of *Avicennia marina* ABA contents in the *Avicennia marina* embryo were low during reserve accumulation which could be related to desiccation sensitivity of seeds of *Avicennia marina* but concentrations in the pericarp are high throughout this developmental stage ABA in the pericarp could not act to prevent precocious germination This imposes a constraint upon germination until these outer coverings are sloughed or otherwise removed Pattern of protein synthesized remains qualitatively similar throughout seed development in *Avicennia marina* and no LEA proteins are produced This suggests both that seedling establishment is independent of maturation proteins and that absence of LEAs and desiccation sensitivity might be related (Farrant *et al*, 1993b)

Recalcitrant seeds are sufficiently hydrated at shedding and germination commences without any additional water (Berjak *et al* 1989 Farrant *et al* 1988) Desiccation sensitivity of recalcitrant seeds is intimately associated with their persistent state of metabolic activity A further trait of recalcitrant seed species is that all will, sooner or later die under storage conditions that do not permit any significant degree of water loss (Pammenter *et al* 1994)

As per Farrant *et al* (1992) the embryos of *Avicennia marina* do not appear to produce LEA proteins supporting the suggestion that production of such proteins may facilitate desiccation tolerance Desiccation tolerance as per them is a complex phenomenon, possibly requiring the interaction of several biochemical processes An important role for soluble sugars in desiccation tolerance is confirmed, as well as their

relevance to membrane phase changes The presence of soluble sugars does not adequately explain the nature of desiccation tolerance in these seeds (Sun *et al* 1994)

2 1 4 Role of Late Embryogenesis Abundant (LEA) Proteins

ABA has been associated with survival of water stress and also induces the production of dehydrine like proteins (Galau *et al* 1986 Kermode 1990) Farrant *et al* (1993b) described the role of plant growth regulators in different stages of seed development for both desiccation tolerant and intolerant species

A particular set of proteins termed LEAs (Late Embryogenesis Abundant) has been implicated in the acquisition of tolerance to drying in developing seeds (Galau *et al* 1986 Bewly and Oliver 1992) The presence of these proteins has been associated with high contents of ABA (Kermode 1990) and ABA can induce their production also (Galau *et al* 1986) Walters *et al* (1997b) have suggested that LEA proteins occurring in temperate recalcitrant seeds may play a role in their survival during over wintering

The physical nature of these proteins together with situations under which they are expressed has led to the suggestion that they function in the survival of water stress by acting as protectants (Close *et al* 1989) and/ or by stabilizing the sub cellular structures in the dry state (Close *et al* 1993)

Indirect evidences for the role of these proteins in the mechanism of desiccation tolerance would be their absence from recalcitrant seeds However dehydrin like proteins have been shown to be present in the recalcitrant seeds of temperate species *Zizania palustris* (Bradford and Chandler 1992) To date there are

no reports of any species where dehydrin like or LEA proteins have not been detected in mature seeds

Farrant *et al* (1996) found the presence of dehydrin like proteins in the axes of mature recalcitrant seeds of a variety of species from a range of habitats These proteins appeared to be absent from the mangroves and fresh seeds of *Barringtonia racemosa* which are tropical wetland species

2 1 5 Role of sugar in desiccation tolerance

The possible role of non reducing sugars in relation to desiccation tolerance in seeds is a much debated subject The accumulation of non reducing sugars has been implicated in acquisition and maintenance of the orthodox seeds generally in two major ways These are in terms of the Water Replacement Hypothesis (i e the replacement of water by sucrose to maintain lipid head group spacing so preventing gel state transformation)(Clegg 1986 Crowe *et al* 1992) and vitrification otherwise referred to as glassy state formation (Koster and Leopold 1988)

Orthodox seeds maturation is accompanied by the accumulation of non reducing oligosaccharides which coincides with the reduction of monosaccharides In other words maintenance of the desiccated state is associated with high levels of sucrose and other oligosaccharides While evidence for the replacement of membrane associated water is equivocal for orthodox seeds (i e the replacement of water by sucrose to maintain lipid head group spacing so preventing gel state transformation) that for the formation of intracellular glasses (i e vitrification) are more convincing Promotion of the metastable glassy state occurs at low water contents when sucrose and certain oligosaccharides or

galactosyl cyclitols form high viscosity amorphous super saturated solutions (Obendrof 1997)

Walters *et al* (1997b) have suggested a quite different role for sugars viz that a significant proportion of the sugars might be tightly associated with LEAs and that these complexes act to control and optimize the rate of water loss during dehydration of orthodox seeds It should be noted however that this should not obviate the participation of either the LEAs or the sugars in the maintenance of orthodox seeds viability in the desiccated state

While some of the few recalcitrant seeds species that have been assayed for sucrose and other oligosaccharides do produce these compounds (Berjak *et al* 1989 Farrant *et al* 1993a) glass formation will occur only at water contents well below the lethal limit. When recalcitrant seeds are dehydrated under ambient conditions they loss viability at relatively higher water contents in the region of 0 8g(or more) water per g dry mass (Berjak *et al* 1989 1992) Similarly if water replacement by sugars is an operative phenomenon in orthodox seeds this too would occur only at water contents $\leq$0 3g/g dry material (Hoekstra and van Roekel 1988) which is well below the lethal limit for recalcitrant seeds It is possible that the significance of sugars and LEAs in embryo of recalcitrant seeds lies in the modulation of the drying rate by complex formation

2 1 6 Effect of drying rate on viability of seeds

In experimental manipulation of recalcitrant seeds or excised axes, the rate of dehydration must be equated with the time taken for the material to pass through a series of declining water contents It has been found that the more rapidly dehydration can be

achieved, the lower is the water content to which seeds or axes can be dried, without damage accumulation that culminates in viability loss This is particularly the case when excised axes are dried (Normah *et al* 1986 Berjak *et al* 1990) Far from actually being desiccation tolerant, axes from recalcitrant seeds will survive only for very short periods (hours to a day or two) at the lowest water contents attainable without immediate deleterious effects (Walters *et al* 1997a) Similar effects of drying rate on whole seeds are usually less marked and generally are harder to attain, because their size often prevents the achievement of suitably rapid dehydration However the ability to achieve lower water contents while retaining viability has been recorded for *Avicennia marina* (Farrant *et al* 1985)

Pammenter *et al* (1997) studied the effect of drying rate on whole seeds of *Ekebergia capensis* Burying it in silica gel after removal of the endocarp performed rapid drying It was found that slowly-dried seeds lose viability at water contents of ≤ 1 25 g water per g dry material while those that were dehydrated rapidly showed unimpaired vigour and full germinability at a water content of 0 7g g^{-1} Seeds dried at an intermediate rate retained viability to the intermediate water content level of c 1 0 g g^{-1} Electron microscopical observations suggested that different deleterious mechanisms brought about intracellular damage depending on the drying rate When rapidly dried there will be a water content at which material that is desiccation sensitive will sustain injury This is usually near the range where only structure associated (non freezable) water remains (Pammenter *et al* 1991 1993) Damage occurring at relatively low water contents is defined as desiccation damage *sensu stricto* (Walters *et al* 1997a) and coincides with the perturbation of the non freezable water Desiccation tolerant material

on the other hand can withstand the removal of a considerable proportion of this water (Pammenter *et al* 1991)

In contrast to desiccation damage *sensu stricto* slowly dried, desiccation sensitive material is held to sustain damage as a result of aqueous based, degradative reactions that are the result of unbalanced metabolism (Pammenter *et al* 1991 1997) The severity of metabolic damage will increase in inverse proportion to the drying rate with viability loss occurring at increasingly high water contents (Pammenter *et al* 1997)

2.2 Chilling sensitivity

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 a failure of seeds of some species to survive at 15^0C is hard to understand (Chin 1988) Some tropical recalcitrant seeds are damaged by chilling injury at temperatures of 10 15^0C and below The longevity of recalcitrant seeds is generally short, particularly for species adapted to tropical environments typically from a few weeks to a few months (King and Roberts 1979 1980) However the longevity of seeds of species adapted to temperate environments can be maintained for much longer periods

A study on the hydrated seeds of *Azadirachta indica* indicated that in response to chilling, a decline in viability was accompanied by ultra structural degeneration mitochondria and plastids in axis cells lost internal organization and vacuoles generally collapsed. Areas of advanced degradation also occurred comprising cells in which the plasmalemma was discontinuous and vesiculated (Berjak *et al* 1995b)

Two key enzymes of glycolysis, phospho fructokinase and pyruvate kinase have also been identified as being cold labile (Guy 1990) The vacuolar collapse reported for cold-exposed *Azadirachta indica* cells might have been a consequence of dismantling of cytoskeleton in response to chilling (Berjak *et al* 1995 Raison and Orr 1990) This would affect glycolysis in view of the structural association between key glycolytic enzymes and actin microfilaments (Masters 1984) Maintenance of intracellular spatial organization includes the existence of multi enzyme particles (Hrazdina and Jensen 1992) such as those of glycolysis (Masters 1984) If some key enzymes of both the glycolytic pathway and the TCA cycle become impaired, then out of phase metabolism must be likely (Lyons and Breidenbach, 1990) As a consequence free radical activity might escalate to proportions where considerable damage could accumulate if enzymatic and anti oxidant scavenging systems operate inefficiently (Senaratna and McKersie 1986) As a result, membrane lipids would be adversely affected

As per Murata and Nishida (1990) membrane lipids might well be a primary intracellular site of chilling injury and Raison and Orr (1990) have suggested the phase change from the liquid crystalline to gel state to be likely Sharom *et al* (1994) have demonstrated on tomato fruit that chilling in fact induces phase changes in cellular membranes

Boroughs and Hunter (1963) found an extremely abrupt fall in viability with declining temperature on cocoa seeds The primary cause of chilling injury may be due to the physical response of the membrane lipids to low temperature (Lyons 1973) Species of tropical and subtropical origin suffer chilling injury when exposed to temperatures above freezing point of tissue but below 15^0C (Bedi and Basra, 1993)

Chilling injury may be exhibited as a loss of viability or reduced growth during germination at favourable temperatures (Wolk and Herner 1982)

Incidence of chilling damage is the most marked feature of the effect of temperature on the storage of dipterocarp seeds (Tompsett, 1992) Tang (1971) found that *Shorea curtissi* would be fatally damaged by exposure to only 16 hours of 4^0C indicating extreme susceptibility to chilling temperature Sasaki (1980) and Yap (1981) proposed classification of dipterocarps based on the chilling sensitivity

2.3 Recalcitrant seed storage

Recalcitrant seeds cannot be dried without damage and so they cannot conform to the viability equation which describes relations between longevity and air dry seed storage environments (Roberts 1973) When fresh recalcitrant seeds begin to dry viability is first slightly reduced as moisture is lost but then begins to be reduced considerably at a certain moisture content termed the critical moisture content (King and Roberts 1979 1980) or lowest safe moisture content" (Tompsett, 1984) If drying continues further viability is eventually reduced to zero Moist storage for recalcitrant seeds should be moisture content levels between the lowest safe moisture content" and the fully imbibed level at the coolest temperature which is not damaging to seed viability

Critical moisture content levels vary greatly among species and even among cultivars and seed lots (King and Roberts 1979 Chin, 1988) They may also vary with the method of drying (Farrant *et al* 1985) Protective mechanisms such as hard seed coats can exclude oxygen and/or water from the embryonic tissue thus extending seed life

There is no satisfactory method for maintaining the viability of recalcitrant seeds on a long term basis owing to the fact that they cannot be dried They cannot be stored at sub zero temperatures because they would get killed by freezing injury resulting from ice formation The longevity of recalcitrant seeds is generally short, particularly for species adapted to tropical environments typically from a few weeks to a few months (King and Roberts 1979 1980)

As per Bonner ((1990) recalcitrant seeds can be subdivided into those of tropical origin, and those adapted to temperate climates (temperate latitudes or high altitudes in the tropics) the latter can be stored at cooler temperatures and for longer Germination during storage is a problem identified with storage of recalcitrant seeds as they shed usually at fully imbibed state and is capable of immediate germination in moist ecosystems to which they belong (King and Robrets 1980 Tompsett 1992) Any storage method for recalcitrant seed should give emphasis on preventing desiccation, chilling injury germination during storage microbial contamination and maintaining adequate oxygen supply (King and Roberts 1980)

Depending on the species hydrated, intact recalcitrant seeds can be stored only for periods from days to months (Chin and Roberts 1980) Storage lifespan can be prolonged by various manipulations but the effective extension of longevity is still not useful for long term conservation of the germplasm One of the major difficulties even in the short term is that the high RH conditions necessary to prolong storage life span of the seeds are also conducive to the proliferation of the micro-organisms especially as chilling is precluded in many instances (Berjak, 1995) As the vigour of the wet stored recalcitrant seeds declines as a result of inherent changes (Pammenter *et al* 1994) it has

been suggested that natural defence mechanisms fail facilitating fungal invasion of the debilitated seed tissues Short of *in situ* conservation, and minimal growth storage of seedlings or *in vitro* cultures the only option for conservation of germplasm, and thus the biological diversity of species with recalcitrant seeds is cryo-storage (Berjak *et al* 1995b)

The optimum moisture level for storage represents a compromise between slowing aging reactions and preventing lethal ice formation by drying and retaining the structural integrity of cellular constituents by supplying sufficient structural water For desiccation sensitive tissues there may not be a moisture content and temperature combination at which aging reactions are sufficiently slowed, and lethal freezing injury and desiccation damage are prevented (Vertucci 1993)

The longevity of recalcitrant seeds is maximal when stored fully or almost fully imbibed when oxygen is freely available However germination is either prevented or reduced to a very slow rate In other words treatments are akin to slow growth treatments in tissue culture It is therefore easier to store recalcitrant species with dormant seeds than those with non-dormant seeds under such conditions because seeds of the latter tend to germinate during storage Low temperatures can reduce the rates of both seed deterioration and germination provided that they remain above the value which results in chilling damage or the lower value at which ice crystallization occurs (Hong and Ellis, 1996)

With normal harvesting and storage procedures it is practically impossible to harvest seed free of fungi Over 50 species of fungi have been isolated from agricultural seed The optimum temperature for fungal invasion is about 30^0C but certain species

grow well at 145^{0}C and slow growth of others has been observed at 5^{0}C(Christensen, 1957) It is rather doubtful that deterioration due to fungal invasion would occur at moisture contents of 10 per cent or below As per Harrington (1963) at seed moisture contents in excess of 10 to 13 per cent fungal invasion can rapidly diminish seed viability Since recalcitrant seeds should be stored in a moist condition, microbial contamination could be an important constraint to reckon with in 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 in the case of recalcitrant seeds being chilling sensitive this may not be applicable

2 4 Cryopreservation

Storage in liquid nitrogen seems to be the most promising method and many of the current difficulties in maintaining the viability of stored recalcitrant seed could be overcome by this method (Roberts *et al* 1984) Dry orthodox seeds at 2 18 % moisture content often survive exposure to liquid nitrogen A quick method for identifying orthodox seeds which has been used to some extent, is the determination of survival following short duration exposures to liquid nitrogen (from 1 hour to 6 days) However this method is not always reliable since both moisture content and the rates of cooling and rewarming need to be optimized for survival in each species separately (Hong and Elhs 1996) However such quick tests cannot distinguish between orthodox and intermediate seeds

In vitro techniques have a clear role in conserving specific genotypes such as recalcitrant seeds These involve the use of conventional micro propagation, restricted growth techniques and cryopreservation Although these techniques have been used

primarily with herbaceous species increasing attention is being given to woody species (Blakesley *et al*, 1996)

Cryostorage of recalcitrant germplasm involves the maintenance of zygotic embryonic axes explants of various kinds or somatic embryoides in liquid nitrogen (196^{0}C) or liquid nitrogen vapour (at c 150^{0}C) At such temperatures low energy levels should preclude molecular movement and thus reactions although events such as free radical generation and macromolecule damage by ionizing radiation cannot be eliminated (Grout *et al* 1983) While it may seem enigmatic to suggest that freezing chilling sensitive material will succeed there are distinct differences between chilling and freezing that allows this It is essential in the first instance that the material is rapidly dehydrated to a range of water contents that obviates both dehydration damage and the lethal injury that results from ice crystal formation This can be achieved by a combination of flash drying (or other relatively rapid means of lowering the water content) and very rapid freezing (Berjak *et al* 1995b) Additionally the specimen to be frozen must be as small as possible and, when necessary cryoprotectants or other appropriate pre treatments are required Nearly 100 % survivals of zygotic axes from a variety of recalcitrant seed species as well as of somatic embryoids have been reported by Berjak *et al* (1995b)

Cryopreservation of somatic and zygotic embryos has been reported to be successful for many species, which show orthodox. intermediate and recalcitrant seed storage behaviour (Engelmann *et al* 1995) For successful cryopreservation excised embryos from recalcitrant seeds must survive desiccation below the threshold freezable

moisture content (Hor *et al* 1990) below which value there is no freezable water for ice formation by cooling to ultra low temperatures

As per Vertucci *et al* (1991) a survival rate of 90 % following freezing to 70^0C and storage at that temperature for 6 months was achieved for embryonic axes excised from desiccation sensitive seeds of *Landolphia kirkii* These had been very rapidly dehydrated to the optimal moisture level prior to freezing

A preferred technique of cryopreservation requires optimizing the moisture level of embryonic axes so that there is minimal damage by desiccation or freezing (Grout *et al* 1983 Normah *et al* 1986) This method has been demonstrated to work well for seeds which have minimally recalcitrant characteristics and can be dried to moisture contents as low as 10 % At this moisture content, water freezing and melting transitions are not detected in seed tissues (Vertucci, 1989)

Though storage of seeds like *Theobroma cacao Hevea brasiliensis Mangifera indica and Juglans sps Thea sinensis Cinamommum zeylanicum etc* can t be preserved under ordinary conditions for long periods due to degeneration of the embryos (Roberts and King 1980) In such cases where seeds are short lived the germplasm could possibly be conserved through the cryopreservation of excised embryos or their segments In wide scale hybridization programmes especially those dealing with intergeneric crosses which are incompatible due to degeneration or abortion of the embryos possibly they can be dissected out at immature stages and cryopreserved There are a number of reports on the cryobiology of zygotic embryos of rice (Bajaj 1981) wheat (Bajaj 1984) barley mustard (Withers 1982) and coconut (Dougall and Wetherell, 1974) Although entire plants could be regenerated from the retrieved embryos in the first four cases only callus

was obtained with coconut In all these experiments quick freezing followed by thawing at 35 to 40^{0}C was employed, however their ability varied considerably

Cryopreservation techniques (e g liquid nitrogen LN2 at 196^{0}C) provide the potential for indefinite preservation by reducing metabolism to such a low level (Ashwood Smith and Farrant, 1980) that all biochemical processes are significantly reduced and biological deterioration virtually stopped

The longevity of seeds or the maintenance of seed viability is a balance between extrinsic and intrinsic deleterious factors and repair or protective mechanisms Deleterious factors may include depletion of essential metabolites denaturation of macromolecules accumulation of toxic metabolites attack by microorganisms and insects and effects of ionizing radiation (Osborn 1977 1980)

Liquid Nitrogen (LN2) storage appears to be both practical and desirable for long –term preservation of numerous kinds of seed. The utilization of LN2 as a storage medium is predicted on the capability of seeds to survive LN2 exposure without significant damage to viability Seeds fall in to three general categories with regard to exposure to LN2 temperatures (Kartha, 1985) They are (1) Desiccation tolerant LN2 tolerant seeds (comprises most common agricultural and horticultural species, with which considerable success had been there in cooling such seeds to LN2 temperatures and re warming them to ambient (20^{0}C) without loss of viability as m *Allium cepa* (Harrison and Carpenter 1977) (2) Desiccation tolerant LN2 sensitive seed (consists of seeds of many fruit and nut crops such as *Prunus sp Juglans sp Corylus sp* and *Coffea sp* Most can be dried to moisture contents less than 10 % but cannot withstand storage temperatures lower than –40^{0}C Seeds fall in to this category are also noted for their short storage life

of usually less than 5 years) and (3) Desiccation sensitive LN2 sensitive seed (these are most difficult to preserve and listed by Chin and Roberts (1980) Several attempts have been made to develop long term preservation techniques for desiccation sensitive seeds with minimal success (Merryman and Williams 1980)

2 4 1 Cryoprotectants

DMSO has been extensively used and proved to be an excellent cryorpotectant, for animal as well as for plant cultures As efficient cryoprotectant should (1) have low molecular weight, (2) be easily miscible with the solvent, (3) be non toxic even at low concentration, (4) be easily washed from the cells and (5) permeate rapidly in to the system The long term freeze storage of germplasm requires special caution with regard to the choice and concentration of the cryoprotectant It should not bring about any genetic aberrations in the form of chromosome breakage or mutations

Many of the cryoprotectively effective substances especially those that penetrate the cell only slowly or not at all display osmotic character i e they withdraw water from and plasmolyze the cells The accompanying shrinkage of both the cell contents and plasma membrane particularly the danger of irreversible shrinkage has often called for a cautious rate of addition of cryoprotective compounds to avoid an osmotic shocking effect and a damaging excess of pressure across the cell membrane (Kartha, 1982)

Because of the toxic effects the cryoprotective additives may have on growth (Dougall and Wetherell 1974) it often appears desirable to dilute out or remove the cryoprotective compounds after the cells are frozen and thawed In achieving this the rate of post thaw dilution (deplasmolysis) has also been assigned a critical role in cell survival (Towill and Mazur 1976)

Cryoprotective compounds are being used in both structural and viability studies Cells that have been treated with cryoprotectants are frozen for the purpose of preserving ultra structural detail are not viable after the cells are thawed (Farrant *et al* 1977) Cells cryoprotected and frozen in order to preserve viability are for the most part not typical in their ultrastructural appearance (Withers and Davy 1978) The interactions of cryoprotective compounds with themselves and with cells are not well understood (Moiseyev *et al* 1982 Franks 1977) Cells are often cooled before they are cryoprotected as part of the prefreezing protocol Plant tissues that are susceptible to low temperatures may show changes in cell structure following only brief exposures to lowered temperatures (Patterson *et al* 1979) Chilled but unfrozen cells undergo reversible as well as irreversible structural changes (Niki *et al* 1979) The presence of cryoprotectants results in apparent cellular disorganization and this does not always lead to cell death Cryoprotectants are indeed, often necessary when frozen to 196^0C thawed, and recovered even though there are exceptions (Nei 1978 Sakai and Otsuka, 1967) The effects of cyoprotectants on the structure of plant cells when treated for preservation of viability have not been extensively studied (Zavala and Finkle 1980 1981) Two basic approaches have been used in ultrastructural investigations (1) to fix cells in the presence of cryoprotectants or (2) to freeze cells according to normal cryogenic routine and to freeze substitute them with the coyprotectant *in situ* Such studies can provide clues as to how cryoprotectants function to promote viable freezing

Cryoprotective additives are used in the freezing procedure most often as single compounds and lists of such compounds have been compiled for plant and animal tissues (Sakai and Yashida, 1968 Karow 1969) Of these compounds DMSO (Dimethyl

sulfoxide) has dominated the plant literature with glycerol or sucrose appearing prominently in other experiments DMSO fulfills all the criteria of being an ideal cryoprotectant also

After freezing in LN2 in the presence of protective compounds higher plants have in several cases given rise to a whole plant after thawing in contrast with freezing without any cryoprotectant, a treatment that would usually kill the tissue The compounds considered as cryoprotectants and usually used just prior to freezing have often been found useful in the 5 to 20 % range (Withers 1980) Yet as little as 0 3 % DMSO has been reported to give maximal protection to conidia of *Neurospora crassa* (Barnhart and Terry 1979)

For the somatic embryos and embryogenic suspensions of carrot, DMSO in the range of 5 to 10 % has been used (Dougall and Wetherell, 1974 Bajaj 1976) Withers (1982) claims 15 % DMSO to be optimal for *Hordeum* embryos Dimethyl sulfoxamine has been reported as a successful cryoprotectant for *Chlamydomonas* (Gresshorf 1977)

2 4 2 Slow freezing

Method of freezing plant meristems slowly is based on the physicochemical events occurring during freezing Mazur (1969) has identified the factors to which a cell is subjected during freezing and thawing With progressive temperature reduction, the cell and its external medium initially supercool, followed by ice formation in the external milieu In the case of plant cells the cell wall and the plasma membrane act as barriers and prevent the ice from seeding the cell interior at temperatures above ca 10^0C and thus the cell remains unfrozen but supercooled As the temperature is further lowered an increasing fraction of extra cellular solution is converted in to ice resulting in the

concentration of extra cellular solutes Since the cell remains supercooled and its aqueous vapour pressure exceeds that of frozen exterior the cell equilibrates by loss of water to external ice (dehydration) Slowly cooled cells reach equilibrium with the external ice efflux of water and will remain shrunken, provided the cell is sufficiently permeable to water In such cases intracellular ice formation considered to be one of the most important factors responsible for causing freezing injury will not occur This phenomenon is utilized in devising slow freezing cryopreservation techniques with meristems (Kartha, 1985)

According to Farrant (1980) an intermediate rate of cooling generally protects the cells from freezing injury The cells are generally cooled at optimum rates to 30 or -40^{0}C by which time all the freezable water from the cells has escaped to become external ice and a subsequent drop in temperature to that of liquid nitrogen has very little adverse effect. Therefore it should be theoretically possible to reduce the water content of the cells by osmotic agents prior to freezing and subsequently increasing the post freezing survival The intermediate cooling rates used for cryopreservation of plant cells are in the range of 0 5 to 2^{0}C/min which is often referred to as slow cooling Slow freezing has been extended with success to a number of different types of plant material such as meristems, somatic embryos and clonal plantlets (Withers 1979)

2 4 1 Rapid freezing

The cooling rates imposed during rapid freezing of plant meristems are of the order of several hundred degrees per minute and often there exists no control on the rate at which the cells are frozen During rapid lowering of temperature in contrast to freezing by regulated slow cooling the cells do not have time to equilibrate with the

external ice or the vapour pressure deficit by efflux of cellular water The cells attain equilibrium by intracellular freezing which is lethal to biological specimens Therefore rapid freezing techniques may not be advisable for cryopreservation of meristems

The rapid freezing method is based on the mechanism of Luyet (1937) which suggests that viability may be maintained by preventing the growth of intracellular ice crystals formed during rapid cooling by rapidly passing the tissue through the temperature zone in which lethal ice crystal growth occurs (Seibert and Wetherbee 1977) In most of the examples of rapid freezing the viability of the cryopreserved meristems is not very high and it has been demonstrated that better survival rates could be obtained by slow freezing as was the case with potato (Towill 1981) strawberry and cassava (Kartha *et al* 1980) Moreover rapid freezing has resulted in complete loss of viability of pea meristems and a very low rate of survival of strawberry meristems (Kartha *et al* 1979 1980) The advantage of using rapid freezing technique mainly lies in the simplicity of the operation

Materials and methods

Materials and Methods

The present investigation on improving storage techniques of the seeds of *Hopea parviflora* by selecting different maturity levels desiccation techniques and cryopreservation methods were carried out at the College of Forestry Vellanikkara

3 1 Materials

3 1 1 Seed source

Seeds for the study were collected from the *Hopea parviflora* stand at the Kerala Forest Research Institute Subcentre located at Karimpuzha, Nilambur Taluk of Malappuram District This stand was established in 1920 s Though establishment of the plantation was a failure a robust stand was formed as a result of profuse natural regeneration and adequate protection given to it Other tree species found in the area are *Swietenia macrophylla Xylia xylocarpa Vateria indica Terminalia tomentosa* etc The area is located at $11^0$17 N and $76^0$4 E and enjoys a warm humid tropical climate with mean annual temperature of 18^0C to 30^0C Mean annual precipitation of this area is 2400 mm

3 2 Methods

3 2 1 Seed collection

An area of 6 m x 6 m was selected from the *Hopea parviflora* stand The site was visited at weekly intervals and observations recorded on the date of onset of flowering date of fruit set and date of fruit shedding were made

Inflorescences were observed daily and upon anthesis they were tagged Seeds were collected at weekly intervals from second week after anthesis onwards from

the site during June July 1998 and 2000 (1999 was not a seed year at all) and brought to the College of Forestry on the same day for conducting the experiment

3 2 2 Seed characteristics

Diameter of seeds after 2nd 3rd 4th 5th 6th and 7th weeks at each week s maturity were determined by taking samples of 25 seeds each using a vernier caliper The seeds were also kept for germination in petri plates double layered with filter paper and moistened on a daily basis for which homogenous samples (25seeds each per sample) were used with four replications Seeds at 2nd 3rd 4th and 5th weeks after anthesis failed to germinate altogether Therefore it was decided to exclude seeds/seeds without seed coat/embryonic axes at 2nd 3rd 4th and 5th weeks after anthesis and to carry out the experiments using seeds of 6th and 7th weeks after anthesis Six weeks after anthesis the fruits were mature and started shedding At seven weeks after anthesis the fruits were shed more or less completely

3 2 3 Preparation of the propagule

Extreme hygiene was maintained while collecting seeds to avoid culture contamination Seeds were first washed clean in running tap water After dewinging seeds were blot dried, transferred to 0 1 per cent HgCl solution for sterilization and moved to laminar airflow cabinet Seeds were removed from the chemical after 5 minutes and rinsed with sterilized distilled water to remove traces of the sterilant if any sticking to the surface Sterile surgical blade was used for preparing seeds without seed coat and for extracting embryonic axes from the seed All materials used were sterilized prior to work and the entire work was done under aseptic conditions

Table 1 Solutions to maintain different relative humidities

Relative humidities	Salts	Concentration	
100 %		100ml	distilled water
85 3 %	KCl	100ml	saturated solutions
75 6 %	NaCl	100ml	-- do----
46 3 %	Ca $(NO_3)_2$ $4H_2O$	100ml	—do----
30 %	KOH	42 3 g/100ml	distilled water
20 %	KOH	47 0 g/100ml	distilled water

3.2 4 Maintenance of relative humidities

Different relative humidities were maintained in desiccators of uniform size by pouring 100 ml each of the solutions as shown in Table 1 Distilled water (100 ml) was used to maintain 100 per cent relative humidity The propagules were kept in sterilized petri plates inside the desiccators and covered with the lid after coating the edges with petroleum jelly to keep the desiccators airtight

3.2 5 Effect of desiccation on seed viability

On the same day of collection homogenous samples of dewinged seeds seeds without seed coat and embryonic axes were pretreated in desiccators set at relative humidities 100 % 85 3 % 75 6 % 46 6 % 30 % and 20 % each with time durations 3 hr 6 hr 12 hr and 24 hr For vacuum treatment, the test materials kept in petri dishes were placed inside sealed vacuum desiccator and vacuum was created by using vacuum pump The whole work was done under sterile conditions inside a laminar flow Similarly for dry air treatments the test materials were kept in U tube which was connected to another U tube filled with dry silica gel through which sterile air was blown using vacuum pump for prescribed durations The whole experiment was conducted under sterile environment inside a laminar flow Relative humidities 30 and 20 per cent and vacuum and dry air treatments were intended to effect rapid drying to the propagules

Germination characteristics of the test material were studied at the end of the treatment Moisture content and leachate conductivity of these test materials were also determined The experiments were conducted using four replications having samples of ten seeds and seeds without seed coat and four embryonic axes per replication Moisture

content and leachate conductivity were also determined using ten seeds/seeds without seed coat and four embryonic axes per replication Due to the limited availability of seeds moisture content and leachate conductivity were determined with one replication

Initial moisture content leachate conductivity and germination parameters were also recorded before the treatments The treatments were set up in Completely Randomized Block Design

3 2 6 Germination methods

Seeds/ seeds without seed coat were germinated on a double layer of filter paper (Borosil No 1) inside petri dishes having a diameter of 9 cm The petri dishes were covered and kept for germination at room temperature (29 ± 1^{0}C) The filter paper was moistened with distilled water on daily basis The embryonic axes were inoculated into ½ MS media in test tubes and 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 ^{0}C Germination was observed daily

Seed and seed without seed coat was considered to have germinated when the radicle reached about one centimeter length and the green hypocotyls 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 7 Culture media

Regeneration potential of the embryonic axis was studied using ½ MS medium The technical composition of MS medium is given in Table 2

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 bottle in refrigerators While the nutrient stock solutions were prepared afresh every four weeks that of vitamins amino acids and growth regulators were prepared every week

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

The solutions for MS and Recovery media were then boiled for melting the agar 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 media solidified 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± 2^0C for further use

The cryoprotection medium is neither boiled nor added with agar like the other two media. This is poured to propagules after sterilizing it under laminar flow in three increments over 45 minutes The composition of the cryoprotection media is given in Table 3

Table 2 Chemical composition of Murashige and Skoog medium

Compound	Quantity (mg/l)
INORGANIC	
Ammonium nitrate	1650 0
Boric acid	6 2
Calcium chloride 2 hydrate	440 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_2EDTA 2 hydrate	37 3
Potassium dihydrogen phosphate	170 0
Potassium iodide	0 83
Potassium nitrate	1900 0
Sodium molybdate 2 hydrate	0 25
Zinc sulphate 7 hydrate	8 6
ORGANIC	
Inositol	100 0
Nicotinic acid	0 5
Thiamine 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

½ MS denotes half the amounts of the inorganic constituents per litre

Table 3 Cryoprotection medium (I + II)

I

1 Basal MS
2 Sucrose 3 % (Control)
3 pH 5 6

II

1 Basal MS
2 Sucrose 1M (Control)
3 pH 5 6
4 DMSO 20 %

Table 4 Recovery medium

Salts	Concentration
1 Basal MS	
2 Casein hydrolysate	1 g/l
3 Coconut water (De proteinized)	10 %
4 ABA	10 mm
5 NAA	3 ppm
6 Charcoal (Activated)	0 05 %
7 Agar	0 8 %
8 Sucrose	3 %
9 pH	5 6

The viability of seeds seeds without seed coat and embryonic axis after cryopreservation was tested using recovery medium, the composition of which is given in Table 4

3 2 8 Seed moisture content

Low constant temperature oven method (ISTA 1985) was used to determine the moisture content After determining the initial weight, seeds/seeds without seed coat and embryonic axes were oven dried at a constant temperature of 103 ± 2^{0}C for 17 hours At the end of the period they were removed from the oven and allowed to cool for 30-40 minutes and then reweighed

Moisture content was determined on wet weight basis

$$\text{Moisture content (\%)} = \frac{\text{Original weight} - \text{Oven dry weight}}{\text{Original weight}} \times 100$$

3.2 9 Leachate conductivity measurement

Leachate conductivity measurement is a quick test to know seed deterioration Ten seeds /seeds without seed coat/embryonic axes were immersed in 50 ml of distilled water overnight and leachate conductivity was measured using a conductivity meter (Elico CM 180)

3 2 10 Cryopreservation techniques

3 2 10 1 Direct plunging of propagules in liquid nitrogen

The germination studies conducted on the test materials after subjecting them to different relative humidities showed that subjecting the propagules to different relative humidities for 24 hours duration by and large was deleterious Therefore this 24 hour pre treatment was excluded for the cryopreservation studies For the same reason in the

case of embryonic axes, relative humidities of 30 and 20 percent and time durations of 12 and 24 hours were excluded for cryopreservation studies

The vacuum and dry air treatments adversely affected germination percentage along with severe culture contamination therefore these treatments were not included for cryopreservation studies

After subjectmg the seeds/seeds without seed coat/embryonic axes to different desiccation treatments as mentioned above they were placed in cryovials of 1 2 ml capacity and then kept inside canisters and quickly plunged by hand into liquid nitrogen contained in 30 litre cryocans filled with liquid nitrogen At the end of 24 hour duration these materials were removed from liquid nitrogen and were rapidly thawed in a water bath at 37^0C for 30 minutes Afterwards their regeneration potential was studied as described in section 3 2 6 Due to limited availability owing to poor seed year 3 seeds/seeds without seed coat/embryonic axes were used with 3 replications for the study

3 2 10 2 Slow freezing of propagules

Seeds/seeds without seed coat /embryonic axes subjected to the best desiccation treatments as described in the previous section, were selected for slow freezing as in the case of direct plunging Here also three propagules /sample were used with four replications

As a pre treatment before subjecting to slow freezing the propagules after the said levels of desiccation were treated in a liquid cryo protection medium for 45 minutes at room temperature For this treatment, the propagules after various levels of desiccation were first added to medium consisting of MS salts and 3 % sucrose at a pH of 5 6 Over a period of 45 minutes an equal volume of MS medium with 1 m sucrose and 20 %

DMSO was added in 3 increments This will give a final concentration of 0 5 m sucrose and 10 % DMSO

After the pre treatment of the propagules they were transferred aseptically to 1 2 ml cryo- vials filled with cryoprotection medium and slow freezing was accomplished in two stage freezing protocol using the Programmable Biofreezer model PTC 1000C of Apex Instruments Calcutta The freezing was accomplished using the freezing programme PTC 1000C version 1 22 released on 14 04 1998 supplied by M/s Apex Instruments Calcutta Initially the propagules were cooled to 4^0C from room temperature at a rate of 5^0C per minute Now there was a pause in which the propagules were kept at 4^0C for 30 minutes and then they were cooled down to 40^0C at a rate of $0\,4^0$C per minute After keeping the propagules for 30 minutes at -40^0C in the Biofreezer they were immediately plunged into liquid nitrogen contained in 30 litre cryocans At the end of 24 hours the propagules were thawed in warm water bath at 37^0C for 30 minutes The thawed seeds/seeds without seed coat were inoculated on to the recovery medium contained in a petri dish and embryonic axes were inoculated into test tubes containing the recovery medium Control propagules were placed directly on the recovery medium without freezing The entire propagules were incubated in a culture room at 26^0C with a 16 8 hour light dark cycle

3 2 11 Statistical analysis

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

Results

Results

The result of the study on the viability of *Hopea parviflora* seeds seeds without seed coat and embryonic axes collected after six and seven weeks after anthesis as effected by different treatments are presented in this chapter

4 1 Flowering and fruiting

The onset of flowering and fruit set in *Hopea parviflora* trees during the first year of study (1998) was in the first week of June and July respectively After anthesis they took six weeks to attain maturity and started shedding during the mid July with the commencement of the southwest monsoon It was also observed that mature seeds exhibited vivipary by and large with the onset of intense downpour associated with the monsoon The flowering was rare during 1999 in the whole stand and hence rest of the work could not be accomplished during that year The periodicity of flowering and fruiting during 2000 was almost the same as in 1998

4 2 Seed characteristics

Fruits of *Hopea parviflora* at different weeks after anthesis are shown in Plate 1 Diameter variations of seeds at different maturity levels are shown in Fig 1 The diameter of the seeds ranged from 1 9 mm to 3 0 mm at two weeks after anthesis with a mean 2 3 mm which increased to 6 1 mm at seven weeks after anthesis showing an increase of 160 per cent

From second week onwards after anthesis seeds were collected at weekly intervals and kept for germination in four replications Homogenous samples of 25 seeds each were kept in a petri plate with double layered filter paper and moistened daily to

a One week after anthesis

c Three weeks after anthesis

b Two weeks after anthes s

d Four weeks after anthes s

e Five weeks after anthesis

g Seven weeks after anthesis

f Six weeks after anthesis

Plate 1 Fruits of *Hopea parviflora* at different weeks after anthesis

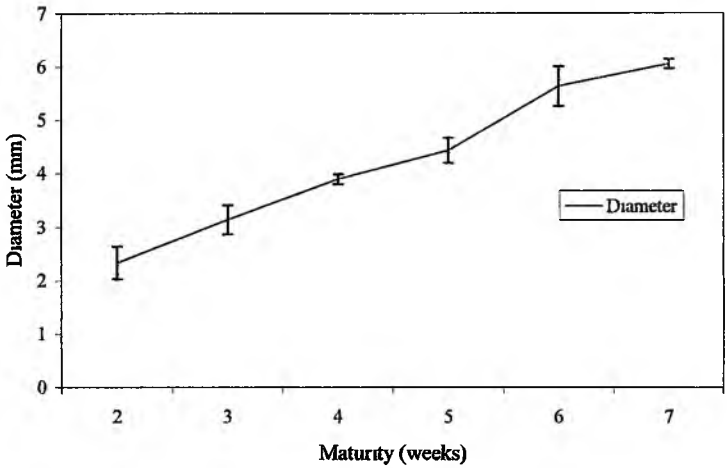


Fig 1 Diameter (mm) of seeds at different maturity levels

observe germination It has been found that up to fifth weeks after anthesis seeds failed to germinate altogether There fore the experiments were conducted with seeds seeds without seed coat and embryonic axes collected at six and seven weeks after anthesis

4 3 Moisture content, leachate conductivity and germination parameters of intact seeds of *Hopea parviflora* as affected by different relative humidities and time

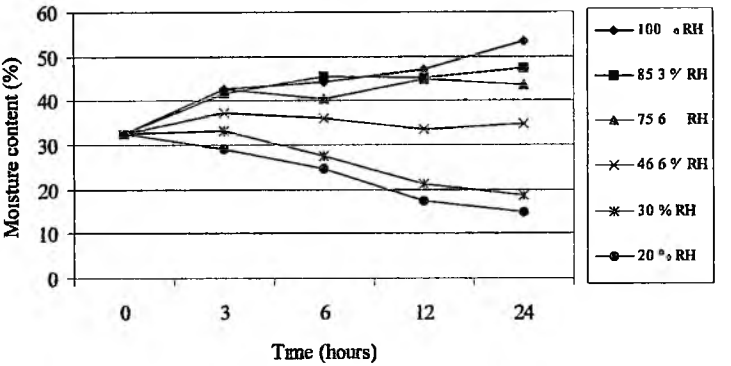
4 3 1 Moisture content

4 3 1 1 Six weeks after anthesis

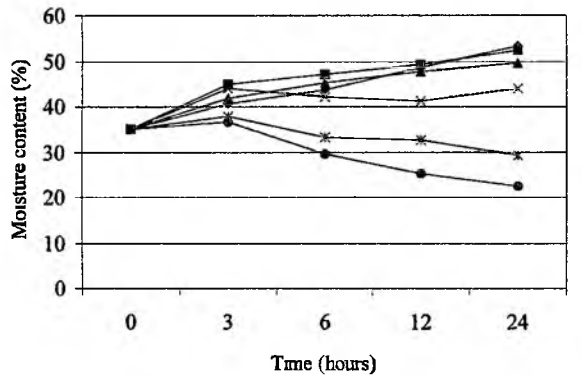
The initial moisture content of the seeds collected at six weeks after anthesis was 32 6 per cent The data pertaining to this is as shown in Fig 2a. When the seeds were kept in dessicators having relative humidities 100 85 3 and 75 6 per cent, the moisture content increased with increasing duration The greatest increase being in 100 per cent relative humidity In general there was no increase in the moisture content of the seeds when kept at 46 6 per cent relative humidity for different durations On the other hand, moisture content decreased by 42 6 per cent when the seeds were rapidly dried at 30 per cent relative humidity for 24 hours and by 54 6 per cent at 20 per cent relative humidity for 24 hours

4 3 1 2 Seven weeks after anthesis

The initial moisture content of the seeds collected at seven weeks after anthesis was 34 9 per cent The data pertaining to this is as shown in Fig 2b When the seeds were kept in desiccators having relative humidities 100 85 3 75 6 and 46 6 per cent, the moisture content increased with increasing duration The greatest increase was in 100 per cent relative humidity followed by 85 3 per cent relative humidity Moisture content decreased by 15 9 per cent when the seeds were rapidly dried at 30 per cent relative



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 2 Moisture content (%) of intact seeds of *Hopea parviflora* at six and seven weeks after anthesis as affected by different relative humidities and time

humidity for 24 hours and by 35 9 per cent in 20 per cent relative humidity after 24 hours

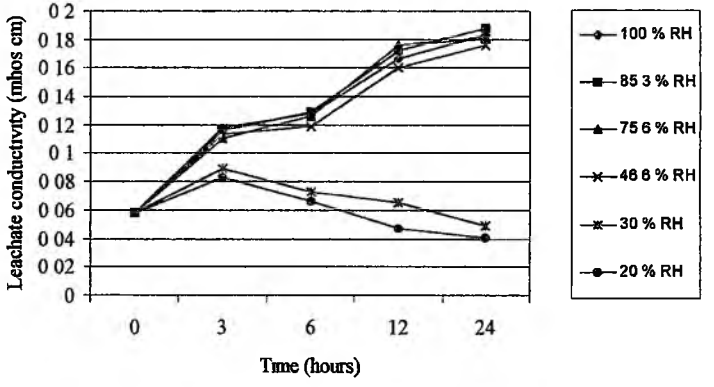
4.3 2 Leachate conductivity

4.3 2 1 Six weeks after anthesis

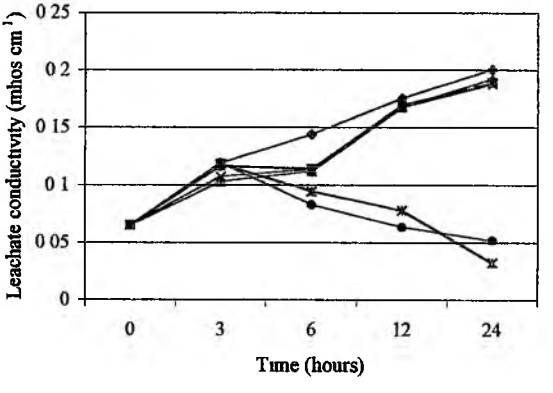
The initial leachate conductivity of intact seeds collected at six weeks after anthesis was 0 058 mhos/cm The data pertaining to this is as shown in Fig 3a This increased tremendously by 103 5 per cent to 94 8 per cent when the seeds were kept for 3 hours at relative humidities ranging from 100 to 46 6 per cent When the duration was increased to 24 hours at 100 to 46 6 per cent relative humidity the leachate conductivity increased by 215 5 to 203 5 per cent On the other hand, when the seeds were rapidly dried at 30 and 20 per cent relative humidities for 3 hours the leachate conductivity increased by 53 5 and 43 1 per cent respectively and when the duration was increased beyond 3 hours a gradual reduction in leachate conductivity was observed and after 24 hours it was less than the control

4 3 2 2 Seven weeks after anthesis

The initial leachate conductivity of seeds collected at seven weeks after anthesis was 0 065 mhos/cm The data pertaining to this is as shown in Fig 3b The leachate conductivity increased by 83 1 per cent to 209 2 per cent after 3 hour and 24 hour in the case of 100 per cent relative humidity Even though leachate conductivity increased by about 82 per cent when the seeds were rapidly dried at 30 and 20 per cent relative humidities for 3 hours a gradual decrease was observed afterwards and at the end of 24 hours it was less than the control



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 3 Leachate conductivity (mhos cm ^{1}) of intact seeds of *Hopea parviflora* at six and seven weeks after anthesis as affected by different relative humidities and time

4 3.3 Germination parameters

4 3 3 1 Cumulative germination percentage

4 3.3 1 1 Six weeks after anthesis

Significant difference in germination percentage of intact seeds collected at 6 week after anthesis was observed when kept at different relative humidities for different durations Interaction effect was also significant ($P < 0\ 05$) The data pertaining to this is given in Table 5 When compared to the initial germination percentage (93 8) keeping for 24 hours at all relative humidities significantly affected the germination percentage in general The lowest value was however noticed when rapidly dried at 30 per cent relative humidity after 12 hours (80 0)

4 3 3 1 2 Seven weeks after anthesis

Keeping the seeds at different relative humidities did not significantly after the germination percentage of intact seeds ($P < 0\ 01$) The data pertaining to this is given in Table 6 However time was found to be significant Keeping for 12 hours and beyond significantly affected the germination percentage Compared to the control keeping for 24 hours resulted in 9 3 per cent reduction in germination percentage in 20 pert cent relative humidity Interaction between relative humidity and time were found to have no significant effect

4 3 3 2 Mean daily germination

4.3 3 2 1 Six weeks after anthesis

Significant difference in mean daily germination percentage was observed due to relative humidity ($P < 0\ 01$) The data pertaining to this is given in Table 7 Time and interaction between relative humidity and time were found to be nonsignificant Keeping

Table 5 Cumulative germination percentage of six week old intact seeds of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	93 75	93 75	93 75	93 75	93 75	93 75	93 75
3	93 75	91 88	93 75	90 94	93 75	93 75	92 97
6	93 75	86 88	93 75	93 75	89 38	93 75	91 88
12	92 81	90 00	85 94	90 94	80 00	92 81	88 75
24	88 44	75 00	85 00	87 50	88 44	80 00	84 06
Mean	92 50	87 50	90 44	89 13	89 06	90 81	

	Time	Relative humidity	Interaction
CD (0 05)	1 826	2 003	4 477
SEM (±)	0 932	1 022	2 284

Table 6 Cumulative germination percentage of seven week old intact seeds of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	93 75	93 75	93 75	93 75	93 75	93 75	93 75
3	93 75	93 75	93 75	93 75	93 75	93 75	93 75
6	93 75	93 75	93 75	92 81	93 75	93 75	92 59
12	93 75	93 75	92 81	91 88	91 87	90 94	92 50
24	90 94	90 00	87 50	88 44	85 00	85 00	87 81
Mean	93 19	93 00	92 31	92 13	91 63	91 44	

	Time	Relative humidity	Interaction
CD (0 01)	1 175	NS	NS
SEM (±)	0 456	0 499	1 118

Table 7 Mean daily germination of six week old intact seeds of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	8 75	8 75	8 75	8 75	8 75	8 75	8 75
3	4 35	12 73	8 33	7 50	5 00	6.25	7 36
6	15 00	11 45	8 25	6 05	6 65	5 00	8 73
12	16 25	7 48	8 75	7 50	5 40	6 65	8 67
24	11 68	5 08	7 30	7 50	6 45	6 80	7 47
Mean	11 21	9 10	8 28	6 71	5 95	6 69	

	Time	Relative humidity	Interaction
CD (0 01)	NS	2 336	NS
SEM (±)	0 828	0 907	2 028

Table 8 Mean daily germination of seven week old intact seeds of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	27 08	27 08	27 08	27 08	27 08	27 08	27 08
3	15 88	18 35	21 25	22 50	23 75	21 68	20 57
6	17 53	19 18	24 58	22 93	27 90	23 75	22 64
12	17 53	16 70	17 85	16 60	19 00	16 18	17 31
24	15 73	13 95	13 53	15 75	16 70	15 58	15 20
Mean	18 75	19 05	20 86	20 97	22 89	20 85	

	Time	Relative humidity	Interaction
CD (0 01)	1 816	1 991	NS
SEM (±)	0 705	0 773	1 727

of intact seeds at relative humidities less than 46 6 per cent significantly reduced the mean daily germination

4 3 3.2.2 Seven weeks after anthesis

Even though keeping the seeds at different relative humidity is significantly affected the mean daily germination, no definite trend could be observed The data pertaining to this is given in Table 8 Different durations significantly reduced the mean daily germination compared to the control At the end of 24 hours mean daily germination had decreased by 43 9 per cent

4 3 3.3 Peak value

4.3 3.3 1 Six weeks after anthesis

Significant effect was observed in peak value of germination of intact seeds collected at six weeks after anthesis due to relative humidity and the interaction between relative humidity and time ($P < 0\ 05$) The data pertaining to this is given in Table 9 Time did not have significant effect Peak value of intact seeds was not affected much when kept at 100 per cent relative humidity But due to 85 3 75 6 30 and 20 per cent relative humidities the peak value was significantly affected when kept for 24 hours

4 3 3 3 2 Seven weeks after anthesis

Significant effects were observed in the peak value of intact seeds at seven weeks after anthesis due to relative humidity and time ($P < 0\ 05$) The data pertaining to this is given in Table 10 But the interaction effect was non significant In the case of relative humidity no definite trend could be observed Among the durations 12 hr and 24 hr were found to have more significant impact compared to the initial value

Table 9 Peak value of germination of six week old intact seeds of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	19 50	19 50	19 50	19 50	19 50	19 50	19 50
3	15 33	25 00	19 95	19 80	18 35	20 00	19 74
6	20 63	16 56	16 40	18 55	16 15	18 35	17 77
12	29 18	14 28	14 80	17 25	12 85	16 48	17 47
24	26 25	9 74	13 58	17 30	17 30	13 00	16 19
Mean	22 11	17 01	16 85	20 08	16 93	17 47	

	Time	Relative humidity	Interaction
CD (0 05)	NS	2 675	5 982
SEM (±)	1 246	1 365	3 052

Table 10 Peak value of germination of seven week old intact seeds of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	27 93	27 93	27 93	27 93	27 93	27 93	27 93
3	21 38	19 18	22 93	22 93	24 18	22 10	22 11
6	20 75	19 80	27 50	27 00	29 78	25 00	24 97
12	18 55	17 85	18 68	18 05	20 25	20 00	18 93
24	15 83	15 15	15 10	16 08	18 13	15 58	15 98
Mean	20 89	19 98	22 43	22 40	24 05	22 16	

	Time	Relative humidity	Interaction
CD (0 05)	1 462	1 601	NS
SEM (±)	0 746	0 817	1 827

4 3.3 4 Germination value

4.3 3 4 1 Six weeks after anthesis

Significant differences in germination value of intact seeds collected at six weeks after anthesis were observed due to relative humidity and the interaction between relative humidity and time ($P < 0.01$) The data pertaining to this is given in Table 11 The main effect of time did not have any significant influence on the germination value In general keeping at 100 and 46 6 per cent relative humidity did not have any significant influence However 85 3 75 6 30 and 20 per cent relative humidities significantly affected the germination value when the seeds were kept for 24 hours In general germination value decreased with decreasing relative humidity and increasing duration of pre treatment

4.3 3 4.2 Seven weeks after anthesis

Significant difference in germination value of intact seeds collected at seven weeks after anthesis was observed due to time ($P < 0.01$) The data pertaining to this is given in Table 12 The main effect of relative humidity and the interaction between relative humidity and time was found to be non significant When compared to the initial value 12 and 24 hour durations were found to have significantly reduced the germination value

4 4 Moisture content, leachate conductivity and germination parameters of seeds without seed coat of *Hopea parviflora* as affected by different relative humidities and time

4 4 1 Moisture content

4 4 1 1 Six weeks after anthesis

The initial moisture content of seeds without seed coat collected at six weeks after anthesis was 36 8 per cent The data pertaining to this is as shown in Fig 4a When they

Table 11 Germination value of six week old intact seeds of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

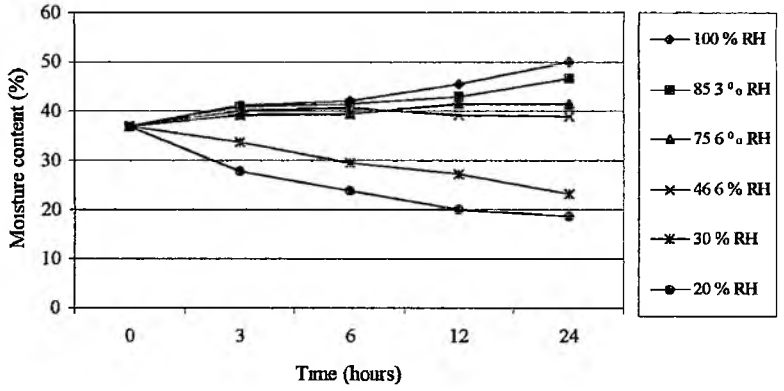
Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	375 15	375 15	375 15	375 15	375 15	375 15	375 15
3	215 13	435 63	357 00	379 76	322 95	369 72	346 70
6	448 36	201 31	235 99	282 41	264 95	322 95	292 66
12	644 72	190 13	193 47	270 60	168 04	264 95	288 65
24	514 75	80 00	174 92	271 11	271 11	168 04	246 66
Mean	439 62	256 44	267 31	282 37	279 35	300 16	

	Time	Relative humidity	Interaction
CD (0 01)	NS	79 501	177 772
SEM (±)	28 173	30 862	69 011

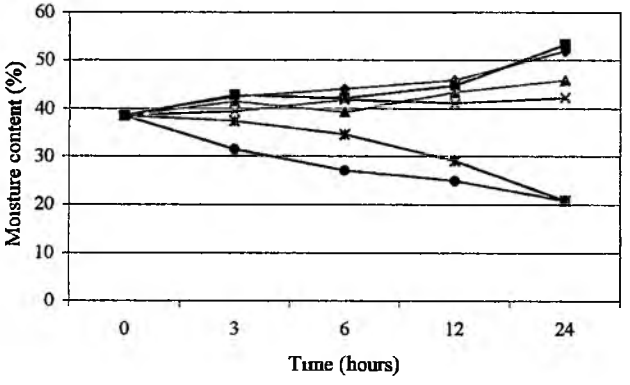
Table 12 Germination value of seven week old intact seeds of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	767 22	767 22	767 22	767 22	767 22	767 22	767 22
3	339 53	353 22	489 75	523 13	613 82	492 85	468 72
6	367 15	382 22	698 22	637 65	854 45	600 00	589 95
12	326 29	298 10	334 22	300 50	387 00	328 98	329 18
24	255 39	212 08	204 17	256 62	312 59	822 72	343 93
Mean	411 12	402 57	498 72	497 02	587 02	602 35	

	Time	Relative humidity	Interaction
CD (0 01)	143 277	NS	NS
SEM (±)	55 620	60 929	136 241



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 4 Moisture content (%) of seeds without seed coat of *Hopea parviflora* at six and seven weeks after anthesis as affected by different relative humidities and time

were kept in desiccators having relative humidities 100 85 3 and 75 6 per cent the moisture content increased with increasing durations The greatest increase was noticed in 100 per cent relative humidity In general, there was not much increase in moisture content when kept at 46 6 per cent relative humidity for different durations On the other hand, moisture content was decreased by 37 per cent when stored at 30 per cent relative humidity for 24 hours It was further decreased by 45 4 and 49 8 per cent when rapidly dried for 12 and 24 hour at 30 and 20 per cent relative humidities

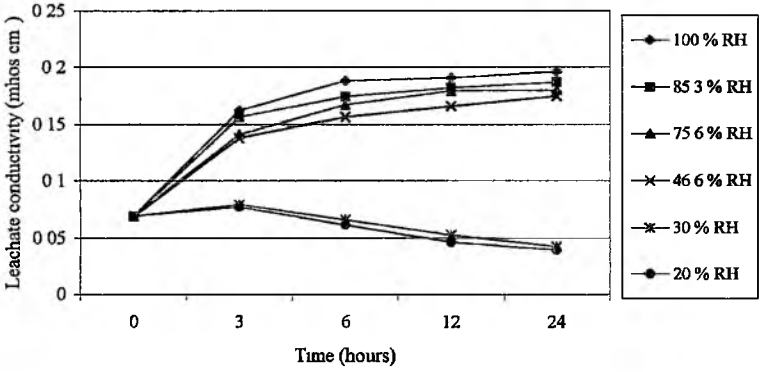
4 4 1 2 Seven weeks after anthesis

The initial moisture content of seeds without seed coat collected at seven weeks after anthesis was 38 41 per cent The data pertaining to this is as shown in Fig 4b In general, the moisture content registered a steady increase due to 100 85 3 and 75 6 per cent relative humdities with increase in time duration and the higher value was noticed at 85 3 per cent relative humidity at 24 hour duration There was not much difference noticed when kept at 46 6 per cent relative humidity for different durations On the other hand a decrease of 45 7 per cent was noticed in the cases of 30 and 20 per cent relative humidities after 24 hour duration

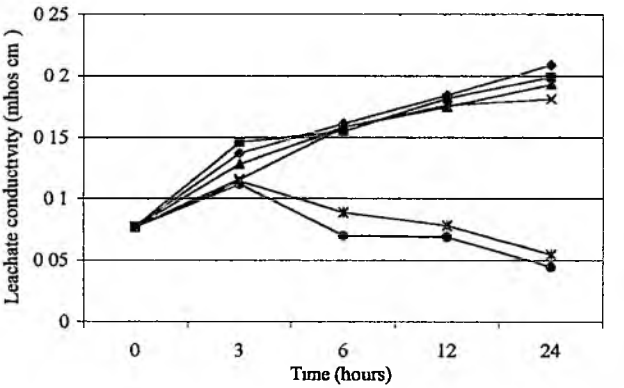
4 4.2 Leachate conductivity

4 4 2 1 Six weeks after anthesis

The initial leachate conductivity of seeds without seed coat at six weeks after anthesis was 0 069 mhos/cm The data pertaining to this is as shown in Fig 5a The leachate conductivity increased tremendously by 134 8 to 100 per cent when they were kept for 3 hours at relative humidities ranging from 100 to 46 6 per cent When the duration was increased to 24 hours at 100 to 46 6 per cent relative humidity the leachate



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 5 Leachate conductivity (mhos cm ^{1}) of seeds without seed coat of *Hopea parviflora* at six and seven weeks after anthesis as affected by different relative humidities and time

conductivity increased further by 184 1 to 153 6 On the other hand when the seeds without seed coat were rapidly dried at 30 and 20 per cent relative humidities for 3 hours the leachate conductivity increased by 14 5 and 11 6 per cent respectively and a decrease to 39 1 and 43 5 per cent were observed in 30 and 20 per cent relative humidities respectively after 24 hour duration

4 4 2 2 Seven weeks after anthesis

The leachate conductivity of seeds without seed coat collected at seven weeks after anthesis was 0 077 mhos/cm The data pertaining to this is as shown in Fig 5b The leachate conductivity increased by 77 9 to 50 7 per cent when they were kept for 3 hours at relative humidity ranging from 100 to 46 6 per cent When the duration was increased to 24 hours at 100 to 44 6 per cent relative humidities the leachate conductivity increased by 171 4 to 135 1 per cent On the other hand when the seeds without seed coat were rapidly dried at 30 and 20 per cent relative humidities for 3 hours the leachate conductivity increased by 48 1 and 45 5 per cent respectively and a decrease to 40 to 41 6 was observed after 24 hours

4 4.3 Germination parameters

4 4 3 1 Cumulative germination percentage

4 4.3 1 1 Six weeds after anthesis

Significant differences in cumulative germination percentage of seeds without seed coat collected at six weeks after anthesis were observed due to relative humidity and time ($P < 0\ 05$) The data pertaining to this is given in Table 13 But their interaction was found to be non significant However no definite trend could be observed in the

Table 13 Cumulative germination percentage of six week old seeds without seed coat of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	93 75	93 75	93 75	93 75	93 75	93 75	93 75
3	93 75	93 75	93 75	93 75	93 75	93 75	93 75
6	92 81	93 75	93 75	93 75	91 88	93 75	93 28
12	90 00	92 38	90 94	90 94	90 00	91 88	91 02
24	88 44	88 44	85 00	85 00	82 50	85 00	85 73
Mean	91 75	92 41	91 44	91 44	90 38	91 63	

	Time	Relative humidity	Interaction
CD (0 05)	1 076	1 179	NS
SEM (±)	0 549	0 602	1 347

Table 14 Cumulative germination percentage of seven week old seeds without seed coat of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	93 75	93 75	93 75	93 75	93 75	93 75	93 75
3	93 75	93 75	93 75	93 75	93 75	93 75	93 75
6	93 75	93 75	93 75	93 75	93 75	93 75	93 75
12	93 75	93 75	93 75	93 75	92 81	90 94	93 13
24	93 75	93 75	87 50	92 81	92 81	91 88	92 08
Mean	93 75	93 75	92 50	93 56	93 38	92 81	

	Time	Relative humidity	Interaction
CD (0 01)	0 634	0 696	1 556
SEM (±)	0 246	0 270	0 604

case of relative humidities Keeping for 24 hours significantly reduced the germination percentage compared to the control

4 4 3 1.2 Seven weeks after anthesis

Significant differences in cumulative germination percentage of seeds without seed coat collected at seven weeks after anthesis were observed due to relative humidity time and their interaction ($P < 0.01$) The data pertaining to this is given in Table 14 In general, keeping at 100 85 3 75 6 46 6 and 30 per cent relative humidities for different durations did not have any significant effect However 12 and 24 hour durations of 20 per cent relative humidity was found to have a significant effect

4 4 3 2 Mean daily germination

4 4.3 2 1 Six weeks after anthesis

Significant differences in mean daily germination percentage of seeds without seed coat collected at six weeks after anthesis were observed due to relative humidity and time ($P < 0.01$) The data pertaining to this is given in Table 15 But the interaction between them was found to be non significant However the main effect of time was found to be significant at all levels when compared to the initial value In the case of relative humidity no definite trend could be observed

4 4.3 2 2 Seven weeks after anthesis

Significant differences in mean daily germination percentage of seeds without seed coat collected at seven weeks after anthesis were observed due to relative humidity time and interaction between then ($P < 0.05$) The data pertaining to this is given in Table 16 In general a decreasing trend was observed in all relative humidity levels with time duration It registered a decrease of 29 2 per cent and 45 8 per cent after 3 hour

Table 15 Mean daily germination of six week old seeds without seed coat of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	41 65	41 65	41 65	41 65	41 65	41 65	41 65
3	25 83	29 15	25 83	29 15	31 25	37 08	28 88
6	21 88	25 83	23 75	37 40	23 75	41 65	29 04
12	21 38	21 38	35 00	31 65	25 95	29 78	27 52
24	19 25	19 00	25 35	20 88	20 18	26 48	21 85
Mean	26 00	27 40	30 32	32 15	26 86	34 33	

	Time	Relative humidity	Interaction
CD (0 01)	3 820	4 183	NS
SEM (±)	1 483	1 624	3 632

Table 16 Mean daily germination of seven week old seeds without seed coat of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	50 00	50 00	50 00	50 00	50 00	50 00	50 00
3	35 40	41 65	35 40	27 08	31 23	27 08	32 97
6	33 30	37 48	37 48	35 40	27 08	29 15	33 31
12	37 48	29 15	27 08	27 08	26 45	23 13	28 39
24	27 08	29 15	19 63	24 38	23 13	27 70	25 18
Mean	36 65	37 49	39 92	32 76	31 56	31 40	

	Time	Relative humidity	Interaction
CD (0 05)	2 168	2 376	5 309
SEM (±)	1 106	1 212	2 709

duration in 100 and 20 per cent relative humidities respectively After 24 hours of duration the corresponding decreases were 45 8 and 44 6 per cent respectively

4 4 3 3 Peak value

4 4 3.3 1 Six weeks after anthesis

Significant differences were observed in peak value due to relative humidity time and their interaction ($P < 0.01$) The data pertaining to this is given in Table 17 A general decreasing trend was registered in all relative humidities with time durations The peak value was significantly reduced after 24 hour duration in all relative humidities However 3 and 6 hour durations were not having any significant influence in any of the relative humidities

4 4.3.3 2 Seven weeks after anthesis

Significant differences were observed in peak value due to relative humidity time and the interaction between the two ($P < 0.05$) The data pertaining to this is given in Table 18 The peak values of seeds without seed coat were not significantly affected at 100 85 3 75 6 46 6 and 30 per cent relative humidities when pre treated for 3 6 and 12 hours But it was significantly influenced due to rapid drying at 20 per cent relative humidity at all time durations and also at 24 hour duration in all relative humidities

4 4 3 4 Germination value

4 4 3 4 1 Six weeks after anthesis

Significant differences were observed in germination value of seeds without seed coat collected at six weeks after anthesis due to relative humidity and time The data pertaining to this is given in Table 19 But their interaction was found to be non significant However the main effect of time was found to be significant in 12 and 24 hour durations when compared to the initial value and relative humidities 75 6 46 6 and

Table 17 Peak value of germination of six week old seeds without seed coat of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	44 58	44 58	44 58	44 58	44 58	44 58	44 58
3	25 83	31 25	28 95	28 33	26 25	46 25	31 14
6	24 18	31 68	32 93	40 83	30 63	53 75	35 66
12	21 88	22 43	42 50	30 00	21 10	32 70	29 43
24	20 63	20 83	25 85	24 38	22 50	27 73	23 65
Mean	27 42	30 15	34 96	33 62	30 21	41 00	

	Time	Relative humidity	Interaction
CD (0 01)	3 823	4 186	9 361
SEM (±)	1 484	1 625	3 634

Table 18 Peak value of germination of seven week old seeds without seed coat of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	52 50	52 50	52 50	52 50	52 50	52 50	52 50
3	35 83	48 75	37 08	27 49	33 33	27 91	35 06
6	33 30	48 75	39 15	44 17	33 33	30 83	38 25
12	37 48	31 24	32 08	29 16	34 58	26 88	31 90
24	28 33	29 15	24 17	32 50	23 75	30 82	28 12
Mean	37 49	42 08	36 99	37 16	35 50	33 79	

	Time	Relative humidity	Interaction
CD (0 05)	2 995	3 281	7 336
SEM (±)	1 528	1 674	3 743

Table 19 Germination value of six week old seeds without seed coat of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	1901 85	1901 85	1901 85	1901 85	1901 85	1901 85	1901 85
3	689 72	933 35	764 72	908 82	656 25	1524 63	912 92
6	531 44	851 88	785 63	1582 38	807 00	2332 63	1148 42
12	469 69	486 94	1575 00	877 41	607 84	1078 41	849 21
24	406 25	394 85	671 45	487 50	464 72	745 51	528 38
Mean	799 79	913 73	1139 73	1152 59	887 45	1516 60	

	Time	Relative humidity	Interaction
CD (0 01)	264 978	290 269	NS
SEM (±)	102 864	112 682	251 964

Table 20 Germination value of seven week old seeds without seed coat of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	2625 00	2625 00	2625 00	2625 00	2625 00	2625 00	2625 00
3	1346 32	2082 63	1422 72	756 41	1057 85	766 85	1238 80
6	1303 70	1957 38	1568 22	1707 69	902 22	912 35	1391 93
12	2107 10	922 79	870 97	798 10	914 72	620 31	1039 00
24	777 22	865 70	475 19	790 63	551 56	799 97	710 04
Mean	1631 87	1690 70	1392 42	1335 56	1210 27	1144 90	

	Time	Relative humidity	Interaction
CD (0 05)	203 338	222 746	498 073
SEM (±)	103 744	113 646	254 119

20 per cent were found to be significant compared to the initial value when the main effect of relative humidity is concerned

4 4.3 4.2 Seven weeks after anthesis

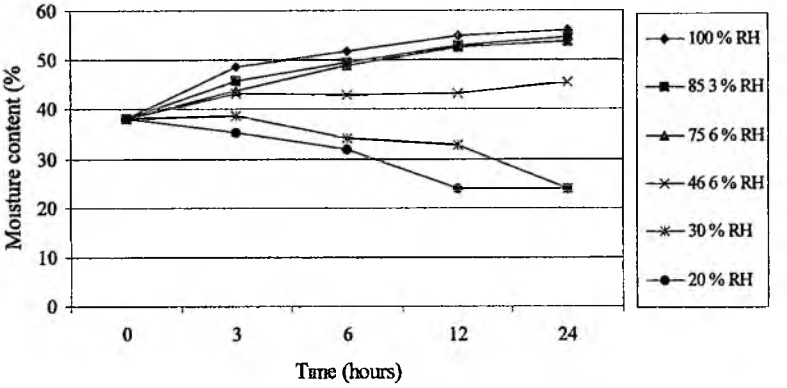
Significant differences were observed in seeds without seed coat collected at seven weeks after anthesis due to relative humidity time and the interaction between them ($P < 0.05$) The data pertaining to this is given in Table 20 It was decreased by 48 7 and 70 8 per cent in the case of 100 and 20 per cent relative humidities after pre treated for 3 hour duration It was further decreased by 70 4 and 69 5 per cent after 24 hours in 100 and 20 per cent relative humidity respectively In general a decreasing trend was noticed as evident in the cases of 46 6 30 and 20 per cent relative humidities with time duration

4 5 Moisture content, leachate conductivity and germination parameters of embryonic axes of *Hopea parviflora* seeds as affected by different relative humidities and time

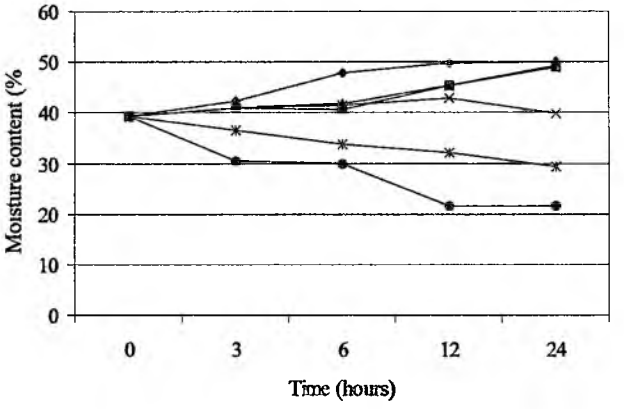
4 5 1 Moisture content

4 5 1 1 Six weeks after anthesis

The initial moisture content of the embryonic axes was 38 10 per cent. The data pertaining to this is as shown in Fig 6a When the embryonic axes were kept in desiccators having relative humidities 100 85 3 and 75 6 per cent, the moisture content increased with increasing duration The greatest increase was observed in 100 per cent relative humidity after 24 hours In general there was not much increase in moisture content of embryonic axes kept at 46 6 per cent relative humidity for different durations On the other hand 37 per cent reduction in moisture content was observed when the



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 6 Moisture content (%) of embryonic axes of *Hopea parviflora* at six and seven weeks after anthesis as affected by different relative humidities and time

embryonic axes were rapidly dried at 30 and 20 per cent relative humidity for 24 and 12 hours respectively

4 5 1 2 Seven weeks after anthesis

The initial moisture content of embryonic axes at seven weeks after anthesis was 39 36 per cent The data pertaining to this is shown in Fig 6b The moisture content of embryonic axes increased with increasing duration but it was not much affected at 46 6 and 30 per cent On the other hand, moisture content decreased by 45 per cent due to rapid drying at 20 per cent relative humidity for 12 hours and beyond

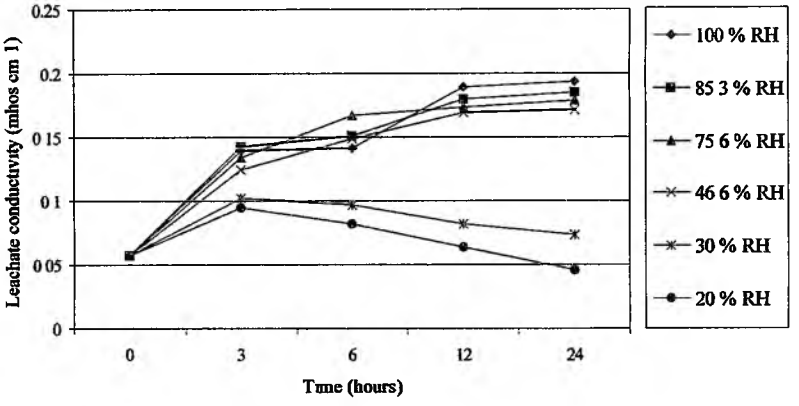
4.5 2 Leachate conductivity

4 5 2 1 Six weeks after anthesis

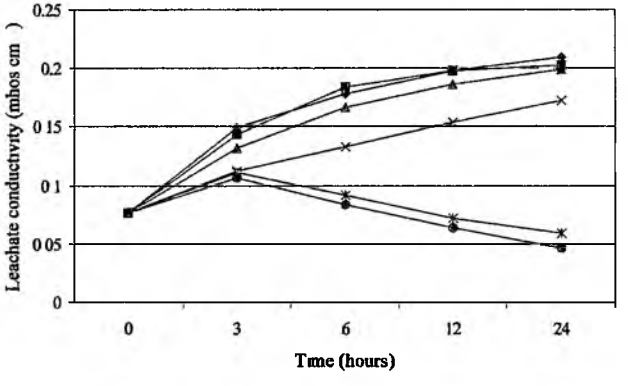
The initial leachate conductivity of embryonic axes at six weeks after anthesis was 0 057 mhos/cm The data pertaining to this is shown in Fig 7a The leachate conductivity increased by 143 to 119 per cent when they were stored for 3 hours at relative humidities ranging from 100 to 46 6 per cent When the duration of pre treatment was increased to 24 hours at 100 to 46 6 per cent relative humidity the leachate conductivity increased by 240 per cent and 200 per cent respectively On the other hand, when the embryonic axes were rapidly dried at 30 and 20 per cent relative humidities for 3 hours the leachate conductivity increased by 79 and 67 per cent respectively When the period of pre treatment was increased to 24 hours at 20 per cent relative humidity the leachate conductivity decreased by 19 per cent

4 5 2.2 Seven weeks after anthesis

The initial leachate conductivity of embryonic axes after seven weeks of anthesis was 0 076 mhos/cm The data pertaining to this is shown in Fig 7b It increased to



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 7 Leachate conductivity (mhos cm ^{1}) of embryonic axes of *Hopea parviflora* at six and seven weeks after anthesis as affected by different relative humidities and time

96 1 to 47 4 per cent when kept at 100 to 46 6 per cent relative humidity for 3 hours When the duration of pre treatment was increased to 24 hours at 100 to 46 6 per cent relative humidity the leachate conductivity increased to 175 and 126 per cent respectively On the other hand, when the embryonic axes were rapidly dried at 30 and 20 per cent relative humidities for 3 hours the leachate conductivity increased by 46 and 39 per cent respectively Beyond this leachate conductivity declined gradually and by 24 hours it was less than the original leachate conductivity

4 5.3 Germination parameters

4 5 3 1 Cumulative germination percentage

4 5 3 1 1 Six weeks after anthesis

Significant differences in cumulative germination percentage was observed due to relative humidity time and their interaction, ($P < 0.01$) The data pertaining to this is given in Table 21 The initial germination percentage was 84 4 Embryonic axis is highly sensitive to pre treatment as evidenced from the significant reduction in the germination percentage when kept at different relative humidities even for 3 hours When stored for 24 hours at relative humidities ranging from 100 to 46 6 per cent, the germination percentage decreased to 68 5 to 75 9 per cent However rapid drying at 30 and 20 per cent relative humidity drastically reduced the germination percentage at the end of 3 hour duration and when kept for 24 hours the germination percentage was reduced by 87 8 per cent in both cases

4 5.3 1.2 Seven weeks after anthesis

Significant difference in cumulative germination percentage was observed due to relative humidity time and the interaction between the two ($P < 0.01$) The data pertaining to this is given in Table 22 Initial germination percentage recorded was 79 7

Table 21 Cumulative germination percentage of six week old embryonic axes of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	84 38	84 38	84 38	84 38	84 38	84 38	88 38
3	68 75	68 75	68 75	62 50	37 50	20 31	54 43
6	62 50	68 75	68 75	56.25	20 31	15 63	48 70
12	43 75	43 75	37 50	31 25	15 63	10 94	30 47
24	26 56	20 31	20 31	20 31	10 94	10 94	18 23
Mean	57 19	57 19	55 94	50 94	33 75	28 44	

	Time	Relative humidity	Interaction
CD (0 01)	6 525	7 148	15 982
SEM (±)	2 533	2 775	6 204

Table 22 Cumulative germination percentage of seven week old embryonic axes of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	79 69	79 69	79 69	79 69	79 69	79 69	79 69
3	78 13	79 69	67 19	68 75	43 75	15 63	58 85
6	75 00	73 44	67 19	75 00	26 56	15 63	55 47
12	50 00	50 00	50 00	37 50	15 63	10 94	35 68
24	26 56	32 81	31 25	15 63	10 94	10 94	21 35
Mean	61 88	63 13	59 06	55 31	35 31	26 56	

	Time	Relative humidity	Interaction
CD (0 01)	7 709	8 444	18 879
SEM (±)	2 992	3 278	7 329

Considerable reduction in germination percentage was noticed when embryonic axes were kept for 12 and 24 hour duration at all levels of relative humidities The germination percentage was reduced to 66 7 and 86 3 per cent when kept at 100 and 30 per cent relative humidities for 24 hour duration Relative humidities 30 and 20 per cent registered minimum values for all time duration The lowest steady values were noticed after 12 hours at 20 per cent relative humidity and for 24 hour duration at 30 and 20 per cent relative humidities

4 5 3 2 Mean daily germination

4 5 3.2 1 Six weeks after anthesis

Significant differences in mean daily germination were observed due to relative humidity time and their interaction The data pertaining to this is given in Table 23 Initial mean daily germination recorded was 12 9 In the case of relative humidities 100 85 3 and 75 6 per cent, up to 6 hour duration there was not much difference A considerable reduction was noticed due to rapid drying at 30 and 20 per cent relative humidities for all time durations Minimum values were recorded for 12 and 24 hour durations at all relative humidity levels A decrease by 93 per cent was observed at 20 per cent relative humidity after 12 and 24 hour durations

4 5 3.2.2 Seven weeks after anthesis

A similar trend was noticed here also Relative humidity time and the interaction between them were found to have significant effect on mean daily germination ($P < 0.01$) The data pertaining to this is given in Table 24 Pre treatment at relative humidities of 100 to 46 6 per cent for up to 6 hours did not have any significant influence on the mean daily germination Only when the duration was increased to 12 hours and beyond, significant reduction in the mean daily germination was observed the magnitude of

Table 23 Mean daily germination of six week old embryonic axes of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	12 93	12 93	12 93	12 93	12 93	12 93	12 93
3	11 45	11 42	10 68	8 90	6 15	3 15	8 63
6	10 38	10 55	10 55	10 55	3 15	2 10	7 88
12	7 20	7 28	5 85	4 78	1 80	0 90	4 64
24	3 50	2 70	2 73	3 40	1 05	0 90	2 38
Mean	9 09	8 98	8 55	8 11	5 02	4 00	

	Time	Relative humidity	Interaction
CD (0 01)	0 948	1 038	2 321
SEM (±)	0 368	0 403	0 901

Table 24 Mean daily germination of seven week old embryonic axes of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	11 58	11 58	11 58	11 58	11 58	11 58	11 58
3	15 00	13 10	9 95	10 10	6 25	2 10	9 42
6	12 68	11 90	10 08	12 05	3 73	1 95	8 73
12	8 53	7 88	8 43	6 78	1 95	0 90	5 74
24	4 80	5 40	5 13	2 10	1 05	0 90	3 23
Mean	10 52	9 97	9 03	8 52	4 91	3 49	

	Time	Relative humidity	Interaction
CD (0 01)	1 337	1 466	3 277
SEM (±)	0 519	0 569	1 272

reduction increasing with decreasing relative humidity A drastic decline in values was observed due to rapid drying at 30 and 20 per cent relative humidities at all time durations Pre treatment at 30 and 20 per cent relative humidities for different durations significantly reduced the mean daily germination A decrease of 86 per cent was observed for 12 hour duration and beyond at 20 per cent relative humidity and 24 hour duration at 30 and 20 per cent relative humidities

4 5 3.3 Peak value

4 5 3.3 1 Six weeks after anthesis

Significant differences were observed in peak value of embryonic axes due to relative humidity time and their interaction ($P < 0.01$) The data pertaining to this is given in Table 25 The initial value obtained was 12 9 There was not much change noticed for 3 and 6 hour duration in relative humidities ranging from 100 to 46 6 per cent In all other cases a drastically decreasing trend was noticed with the duration Relative humidities 30 and 20 per cent affected the peak value significantly for all durations A decrease of 93 per cent was recorded for 12 and 24 hour duration when rapidly dried at 20 per cent relative humidity

4 5.3.3 2 Seven weeks after anthesis

Significant differences in peak value of embryonic axes at seven weeks after anthesis were observed due to relative humidity and time ($P < 0.01$) The data pertaining to this is given in Table 26 But the interaction between relative humidity and time was not significant Compared to the initial value rapid drying at relative humidities 30 and 20 per cent found to have significantly reduced the peak value Similarly in the case of main effect of time 12 and 24 hour durations were found to have significant effects

Table 25 Peak value of germination of six week old embryonic axes of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration.

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	12 93	12 93	12 93	12 93	12 93	12 93	12 93
3	11 45	11 42	10 68	8 90	6 15	3 15	8 63
6	10 38	10 55	10 55	10 55	3 15	2 10	7 88
12	7 20	7 28	5 98	4 78	1 80	0 90	4 65
24	3 68	2 70	2 73	3 40	3 55	0 90	2 83
Mean	9 13	8 98	8 57	8 11	5 49	4 00	

	Time	Relative humidity	Interaction
CD (0 01)	1 036	1 136	2 537
SEM (±)	0 402	0 441	0 985

Table 26 Peak value of germination of seven week old embryonic axes of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	11 58	11 58	11 58	11 58	11 58	11 58	11 58
3	15 00	13 10	9 95	10 10	6 25	2 10	9 42
6	12 68	11 90	10 08	12 05	3 73	1 95	8 73
12	8 53	7 88	8 43	6 78	1 95	0 90	5 74
24	4 80	5 40	5 13	7 30	1 05	0 90	4 10
Mean	10 52	9 97	9 03	9 56	4 91	3 49	

	Time	Relative humidity	Interaction
CD (0 01)	1 747	1 914	NS
SEM (±)	0 678	0 743	1 662

4 5 3 4 Germination value

4.5 3 4 1 Six weeks after anthesis

Significant differences were observed in germination value due to relative humidity time and the interaction between relative humidity and time (P <0 01) The data pertaining to this is given in Table 27 The initial germination value obtained was 167 60 A general decreasing trend was observed in all relative humidities for different time durations A decrease of 20 to 89 per cent was observed for 3 and 24 hour duration in 100 per cent relative humidity itself A drastic decline was noticed when rapidly dried at 30 and 20 per cent relative humidities for different duration with maximum decrease of 98 per cent recorded at 20 per cent relative humidity for 12 and 24 hours

4 5 3 4.2 Seven weeks after anthesis

An almost similar trend was observed here also The data pertaining to this is given in Table 28 Relative humidity time and the interation between the two were found to have significant influence on germination value The initial value observed was 136 3 A decreasing trend was observed in all relative humidities with different durations A drastic reduction could be seen for 12 and 24 hours and also in 30 and 20 per cent relative humidities The maximum decrease was 97 6 percent as evidenced at 20 per cent relative humidity for 12 and 24 hour durations

Table 27 Germination value of six week old embryonic axes of *Hopea parviflora* Bedd as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	167 60	167 60	167 60	167 60	167 60	167 60	167 60
3	134 41	131 75	117 44	82 45	40 49	13 23	86 63
6	109 91	113 53	113 53	113 53	13 23	8 82	78 76
12	56 04	56 08	36 49	24 66	6 48	3 24	30 50
24	17 64	9 72	10 05	15 74	4 41	3 24	10 13
Mean	97 12	95 74	89 02	80 80	49 13	39 23	

	Time	Relative humidity	Interaction
CD (0 01)	13 029	14 274	31 914
SEM (±)	5 058	5 541	12 389

Table 28 Germination value of seven week old embryonic axes of *Hopea parviflora* Bedd. as affected by different levels of relative humidities for different time duration

Time (hrs)	Relative humidity (%)						
	100	85 3	75 6	46 6	30	20	Mean
0	136 28	136 28	136 28	136 28	136 28	136 28	136 28
3	243 20	176 47	112 07	103 09	47 71	8 82	115 22
6	163 00	146 47	105 29	145 81	20 25	7 65	98 08
12	79 76	67 48	72 05	50 10	7 65	3 24	47 71
24	35 66	40 70	27 67	30 66	4 41	3 24	23 72
Mean	113 58	113 48	90 67	93 19	43 26	31 85	

	Time	Relative humidity	Interaction
CD (0 01)	24 366	26 695	59 691
SEM (±)	9 459	10 363	23 172

4 6 Moisture content, leachate conductivity and germination parameters of intact seeds of *Hopea parviflora* as affected by vacuum and dry air for different durations

4 6 1 Moisture content

4 6 1 1 Six weeks after anthesis

The initial moisture content of intact seeds collected six weeks after anthesis was 32 6 per cent The data pertaining to this is shown in Fig 8a There was not much influence on moisture content by vacuum treatment in general However a considerable reduction in moisture content was observed due to rapid drying by dry air for different durations A decrease of 1 8 and 12 per cent was found due to rapid drying by dry air for 15 minutes and 60 minutes duration respectively

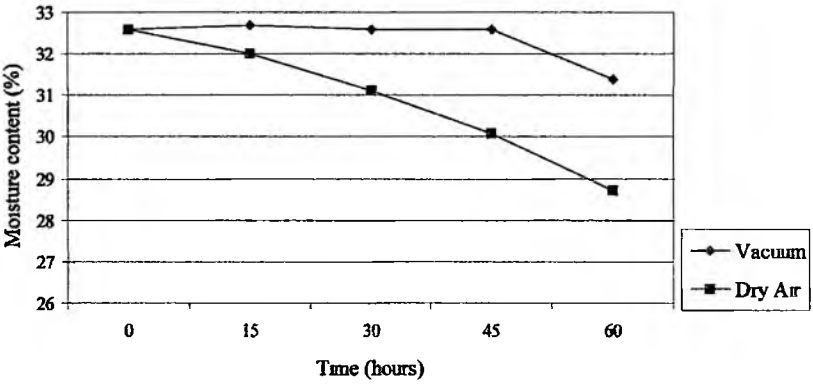
4 6 1 2 Seven weeks after anthesis

The initial moisture content observed was 34 9 per cent The data pertaining to this is as shown in Fig 8b A slight increase in moisture content could be seen in vacuum with the duration However a decreasing trend is evident due to rapid drying by dry air and the moisture content was reduced by 29 8 per cent due to rapid drying by dry air for 60 minutes duration

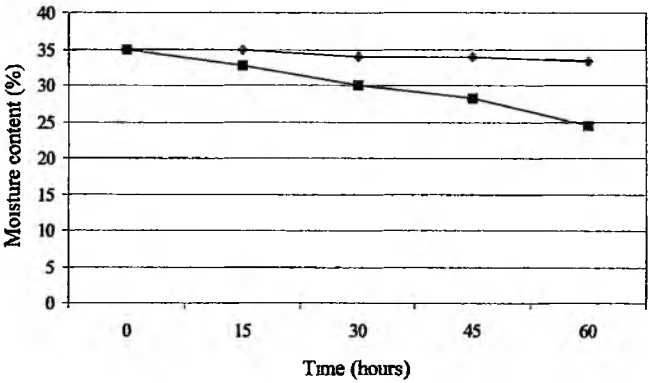
4 6.2 Leachate conductivity

4 6 2 1 Six weeks after anthesis

The initial leachate conductivity recorded was 0 058 mhos/cm The data pertaining to this is shown in Fig 9a Vacuum did not have any significant influence on leachate conductivity for different durations There was a slight decreasing trend in leachate conductivity due to rapid drying by dry air and a decrease by 22 4 per cent was observed for 60 minutes duration

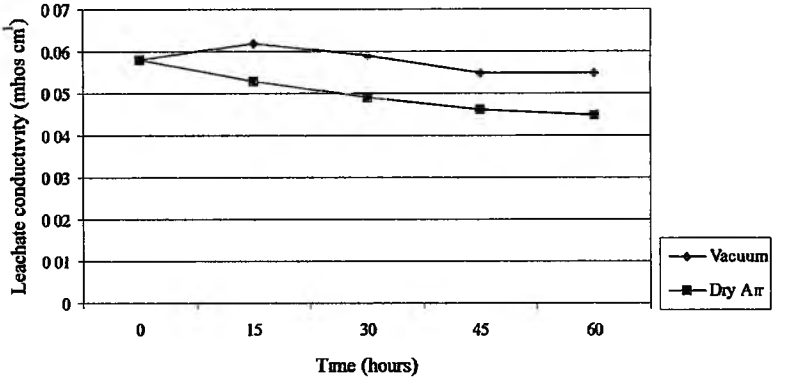


a) Six weeks after anthesis

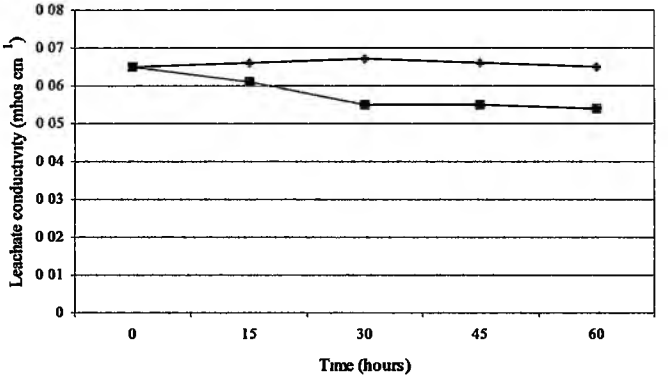


b) Seven weeks after anthesis

Fig 8 Moisture content (%) of intact seeds of *Hopea parviflora* at six and seven weeks after anthesis as affected by vacuum dry air and time



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 9 Leachate conductivity (mhos cm $^{-1}$) of intact seeds of *Hopea parviflora* at six and seven weeks after anthesis as affected by vacuum dry air and time

4 6 2 2 Seven weeks after anthesis

The initial leachate conductivity noticed here was 0 065 The data pertaining to this is shown in Fig 9b There was not much variation in leachate conductivity due to vacuum for any duration A decreasing trend could be observed due to rapid drying by dry air with duration and a decrease by 16 9 per cent was noticed for 60 minutes

4 6.3 Germination parameters

4 6 3 1 Cumulative germination percentage

4 6 3 1 1 Six weeks after anthesis

Significant differences were observed due to vacuum and dry air time and their interaction ($P < 0.01$) The data pertaining to this is given in Table 29 The initial value recorded was 93 8 per cent A decreasing trend could be observed due to rapid drying by vacuum and dry air for different durations

4 6.3 1 2 Seven weeks after anthesis

Non significant influence due to vacuum and dry air time or their interaction was noticed in cumulative germination percentage of intact seeds collected at seven weeks after anthesis The data pertaining to this is given in Table 30 There was not much variation noticed due to rapid drying by vacuum and dry air for any of the durations

4 6 3 2 Mean daily germination

4 6 3 2 1 Six weeks after anthesis

Significant difference was observed due to time only ($P < 0.01$) where as vacuum and dry air and interaction with time was proved to be non significant The data pertaining to this is given in Table 31 A decreasing trend was observed due to rapid drying at both vacuum and dry air with different durations However all the time durations were found to have significantly influenced the mean daily germination

Table 29 Cumulative germination percentage of six week old intact seeds of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	93 75	92 81	90 00	90 00	87 50	90 81
Dry air	93 75	92 81	90 94	90 00	87 50	73 50
Mean	93 75	92 81	90 47	90 00	87 50	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	2 389	1 513	3 383
SEM (±)	0 869	0 550	1 230

Table 30 Cumulative germination percentage of seven week old intact seeds of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	93 75	93 75	92 81	91 88	91 88	92 31
Dry air	93 75	93 75	92 81	93 75	91 86	93 19
Mean	93 75	93 75	92 81	92 81	91 87	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	NS	NS	NS
SEM (±)	0 514	0 325	0 726

Table 31 Mean daily germination of six week old intact seeds of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	19 18	10 75	5 63	6 25	9 00	10 16
Dry air	19 18	7 93	5 40	8 95	7 73	9 84
Mean	19 18	9 34	5 51	7 60	8 36	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	2 536	NS	NS
SEM (±)	0 922	0 583	1 304

Table 32 Mean daily germination of seven week old intact seeds of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	27 08	21 25	22 00	20 25	18 18	21 75
Dry air	27 08	17 53	17 23	19 00	18 25	19 82
Mean	27 08	19 39	19 61	19 63	18 21	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	1 991	1 259	NS
SEM (±)	0 975	0 617	1 380

4 6.3 2 2 Seven weeks after anthesis

Significant differences were observed due to vacuum and dry air and time (P <0 05) The data pertaining to this is given in Table 32 But the interaction between them was found to be non significant A decreasing trend could be observed due to rapid drying at vacuum and dry air for different durations Initial value noticed was 27 1 Different durations of time were found to have a significant impact on mean daily germination both in vacuum and dry air

4 6 3 3 Peak value

4 6.3.3 1 Six weeks after anthesis

Significant difference was observed on peak value due to time only where as vacuum and dry air and the interaction between them were found to be non significant. The data pertaining to this is given in Table 33 However a decreasing trend is observed due to rapid drying at both vacuum and dry air The initial value noticed was 19 2 and a decrease of 53 1 and 59 7 per cent was observed due to 60 minutes duration in both vacuum and dry air

4 6 3 3 2 Seven weeks after anthesis

Significant differences were observed in peak value due to vacuum and dry air and time (P <0 01) whereas the interaction between them was found to be non significant The data pertaining to this is given in Table 34 The initial value recorded was 27 9 A decreasing trend is more clearly observed due to rapid drying at dry air with different durations All durations of time as well as vacuum and dry air treatments were found to have significantly influenced the peak value

Table 33 Peak value of germination of six week old intact seeds of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	19 50	16 48	13 13	14 33	14 93	15 67
Dry air	19 50	19 50	14 30	16 30	14 18	16 76
Mean	19 50	17 99	13 71	15 31	14 55	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	0 841	0 531	1 188
SEM (±)	0 412	0 260	0 582

Table 34 Peak value of germination of seven week old intact seeds of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	27 93	22 50	22 50	22 38	19 50	22 90
Dry air	27 93	19 33	18 05	19 00	18 50	20 56
Mean	27 93	20 91	20 28	20 69	19 00	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	2 492	1 576	NS
SEM (±)	0 906	0 573	1 282

4 6 3 4 Germination value

4 6.3 4 1 Six weeks after anthesis

Significant differences were observed on germination value due to vacuum and dry air time and the interaction between them (P <0 05) The data pertaining to this is given in Table 35 The initial germination value recorded was 375 2 A drastic decline was observed both due to rapid drying by vacuum and dry air for different durations A decrease of 14 and 46 per cent was noticed due to dry air for 15 minutes and 60 minutes duration

4 6 3 4 2 Seven weeks after anthesis

Significant differences were observed in germination value due to vacuum and dry air treatment and time (P <0 05) The data pertaining to this is given in Table 36 But the interaction between them was proved to be non significant The initial germination value recorded was 767 2 A decreasing trend for different durations could be observed due to rapid drying at both vacuum and dry air Different durations of time as well as vacuum and dry air treatments were observed to have significantly influenced the germination value

4 7 Moisture content, leachate conductivity and germination parameters of seeds without seed coat of *Hopea parviflora* as affected by vacuum and dry air for different durations

4 7 1 Moisture content

4 7 1 1 Six weeks after anthesis

The initial moisture content noticed was 36 8 per cent The data pertaining to this is as shown in Fig 10a There was not much variation in moisture content for different

Table 35 Germination value of six week old intact seeds of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

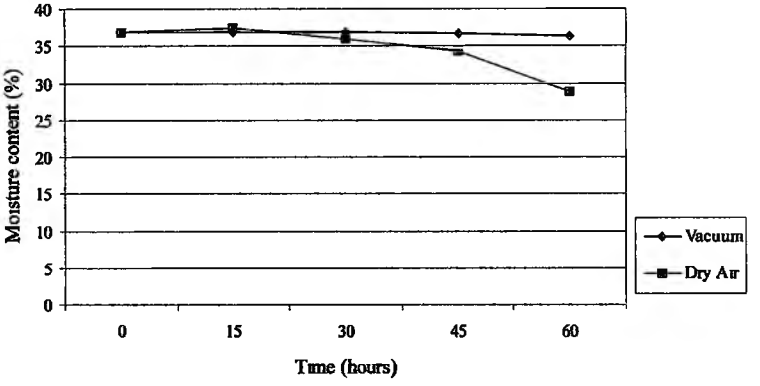
Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	375 15	258 26	181 42	184 79	201 81	240 29
Dry air	375 15	323 15	197 35	235 73	202 17	266 71
Mean	375 15	290 70	189 38	210 26	201 99	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	27 308	17 271	38 620
SEM (±)	13 373	8 458	18 913

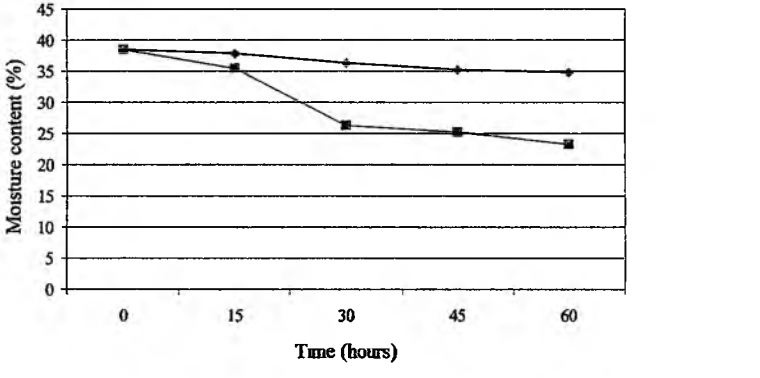
Table 36 Germination value of seven week old intact seeds of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	767 22	481 25	502 50	462 25	354 50	513 55
Dry air	767 22	341 95	307 29	350 30	350 15	423 38
Mean	767 22	411 60	404 89	406 28	352 33	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	92 329	58 381	NS
SEM (±)	45 215	28 590	63 944



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 10 Moisture content (%) of seeds without seed coat of *Hopea parviflora* at six and seven weeks after anthesis as affected by vacuum dry air and time

duration in vacuum but it was slightly reduced in the case of dry air A decrease of 1 8 to 12 per cent was observed for 15 and 60 minutes due to dry air treatment.

4 7 1 2 Seven weeks after anthesis

A similar trend was noticed in this case also The data pertaining to this is as shown in Fig 10b There was not much difference observed due to vacuum treatment The initial value observed was 38 4 A decrease of 7 9 and 39 3 per cent was noticed after 15 and 60 minutes in the case of dry air

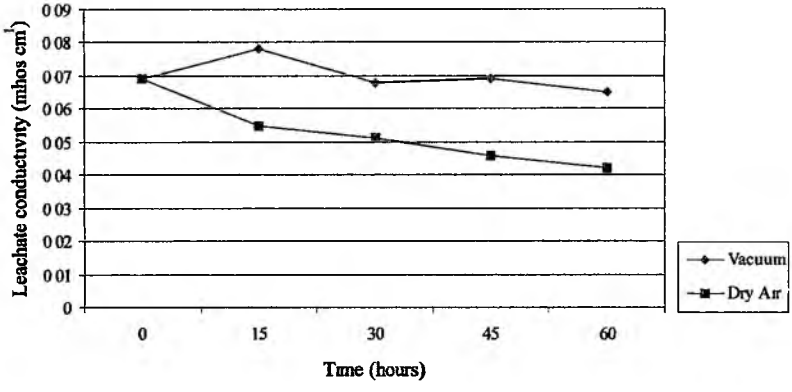
4 7 2 Leachate conductivity

4 7 2 1 Six weeks after anthesis

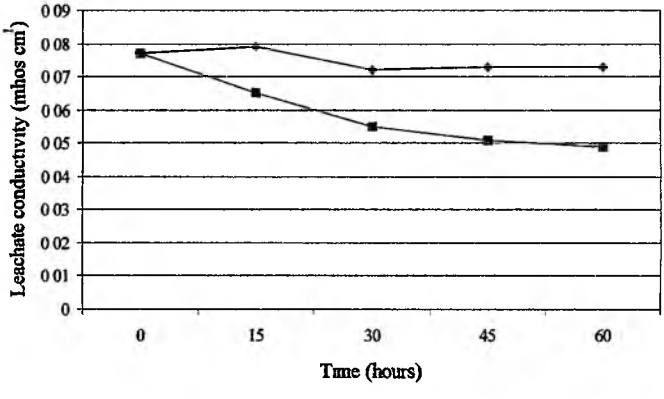
The initial leachate conductivity observed was 0 069 mhos/cm The data pertaining to this is as shown in Fig 11a. There was not much variation in leachate conductivity due to vacuum but due to rapid drying at dry air a decreasing trend was noticed with duration of time A decrease of 20 and 39 per cent was observed after 15 and 60 minutes in the case of dry air

4 7 2 2 Seven weeks after anthesis

The initial leachate conductivity observed was 0 077 The data pertaining to this is as shown in Fig 11b Not much variation in leachate conductivity was observed due to vacuum for different durations whereas it registered a decreasing trend in dry air A decrease of 15 6 and 36 4 per cent was noticed for 15 and 60 minutes in the case of dry air



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 11 Leachate conductivity (mhos cm $^{-1}$) of seeds without seed coat of *Hopea parviflora* at six and seven weeks after anthesis as affected by vacuum dry air and time

4 7 3 Germination parameters

4 7.3 1 Cumulative germination percentage

4 7.3 1 1 Six weeks after anthesis

Significant differences were observed in germination percentage due to time and interaction between vacuum and dry air and time (P <0 05) The data pertaining to this is given in Table 37 The initial value observed was 93 8 per cent Not much variation was noticed due to vacuum whereas a decreasing trend was clearly seen due to rapid drying at dry air It was decreased to 1 0 and 9 3 percentages after 45 and 60 minutes duration in dry air

4 7.3 1 2 Seven weeks after anthesis

No significant difference was observed in germination percentage due to vacuum and dry air time or their interaction The data pertaining to this is given in Table 38 There was not much variation in germination percentage due to rapid drying at vacuum or dry air treatments for different durations

4 7.3 2 Mean daily germination

4 7.3.2 1 Six weeks after anthesis

Significant difference was observed in mean daily germination due to time only (P <0 01) The data pertaining to this is given in Table 39 Vacuum and dry air and their interaction with time were proved to be non significant A slight decreasing trend could be observed due to vacuum and dry air for different durations Different durations of time were found to have significantly influenced the mean daily germination due to rapid drying at both vacuum and dry air treatments

Table 37 Cumulative germination percentage of six week old seeds without seed coat of *Hopea parviflora Bedd* as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	93 75	93 75	93 75	92 81	90 94	93 00
Dry air	93 75	93 75	92 81	92 81	85 00	91 63
Mean	93 75	93 75	93 28	92 81	87 97	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	1 570	NS	2 224
SEM (±)	0 769	0 487	1 089

Table 38 Cumulative germination percentage of seven week old seeds without seedcoat of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	93 75	93 75	93 75	93 75	92 81	93 56
Dry air	93 75	93 75	93 75	93 75	93 75	93 75
Mean	93 75	93 75	93 75	93 75	93 28	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	NS	NS	NS
SEM (±)	0 209	0 133	0 297

Table 39 Mean daily germination of six week old seeds without seed coat of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	41 65	33 30	35 40	28 33	21 88	32 11
Dry air	41 65	31 23	30 40	28 33	23 80	31 08
Mean	41 65	32 26	32 90	28 33	22 84	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	6 146	NS	NS
SEM (±)	2 235	1 414	3 161

Table 40 Mean daily germination of seven week old seeds without seed coat of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (mm)					
	0	15	30	45	60	Mean
Vacuum	50 00	37 48	37 48	27 08	36 65	37 74
Dry air	50 00	33 30	27 08	29 15	29 15	32 90
Mean	50 00	35 39	32 28	28 11	32 90	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	4 398	2 781	NS
SEM (±)	2 154	1 362	3 046

4 7 3.2 2 Seven weeks after anthesis

Significant differences could be observed in mean daily germination due to vacuum and dry air and time ($P < 0.05$) whereas the interaction between them was found to be non significant The data pertaining to this is given in Table 40 A decreasing trend was noticed due to vacuum and dry air for different durations All durations of time as well as vacuum and dry air treatment were found to significantly influence the mean daily germination

4 7 3 3 Peak value

4 7.3.3 1 Six weeks after anthesis

Significant differences were observed in peak value due to time and interaction between vacuum and dry air and time ($P < 0.05$) The data pertaining to this is given in Table 41 The initial value noticed was 44 6 A decreasing trend was observed with duration due to vacuum and dry air A decrease of 15 9 to 44 4 per cent was observed due to rapid drying by dry air after 15 and 60 minutes duration

4 7 3.3 2 Seven weeks after anthesis

Significant differences were observed in the peak value due to vacuum and dry air time and the interaction between them ($P < 0.05$) The data pertaining to this is given in Table 42 The initial value recorded was 52 5 A decreasing trend is more evident due to dry air for different durations A decrease of 40 and 41 per cent was observed after 15 and 60 minutes duration due to rapid drying by dry air

4 7 3 4 Germination value

4 7 3 4 1 Six weeks after anthesis

Significant difference in germination value was observed due to time ($P < 0.01$) whereas vacuum and dry air and the interaction between vacuum and dry air and time

Table 41 Peak value of germination of six week old seeds without seed coat of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	44 58	41 25	43 75	28 33	24 60	36 50
Dry air	44 58	37 50	30 40	30 00	24 80	33 46
Mean	44 58	39 38	37 08	29 16	24 70	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	3 794	NS	5 366
SEM (±)	1 858	1 175	2 628

Table 42 Peak value of germination of seven week old seeds without seedcoat of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	52 50	42 50	43 75	29 99	33 74	40 50
Dry air	52 50	36 23	27 93	30 40	30 83	35 58
Mean	52 50	39 36	35 84	30 20	32 28	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	3 567	2 256	5 044
SEM (±)	1 747	1 105	2 470

were found to be non significant. The data pertaining to this is given in Table 43 All durations of time was found to have significantly influenced the germination value due to rapid drying at both vacuum and dry air

4 7.3 4 2 Seven weeks after anthesis

Significant differences were observed due to vacuum and dry air and time (P <0 05) The data pertaining to this is given in Table 44 But the interaction between them was found to be non significant The initial value recorded was 2625 Different durations of time as well as vacuum and dry air was found to have significantly influenced the germination value

4 8 Moisture content, leachate conductivity and germination percentage of embryonic axes of *Hopea parviflora* seeds as affected by vacuum and dry air for different durations

4 8 1 Moisture content

4.8 1 1 Six weeks after anthesis

The initial moisture content observed was 38 1 per cent The data pertaining to this is as shown in Fig 12a There was not much variation in moisture content due to vacuum for different durations but it registered a decreasing trend with duration in dry air There was a decrease of 3 7 to 24 7 per cent for 15 and 60 minutes duration due to rapid drying at dry air

4.8 1 2 Seven weeks after anthesis

The initial value observed was 39 4 per cent The data pertaining to this is as shown in Fig 12b No difference in moisture content could be observed for different durations in the case of vacuum but there was a considerable decrease as evidenced in the

Table 43 Germination value of six week old seeds without seed coat of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

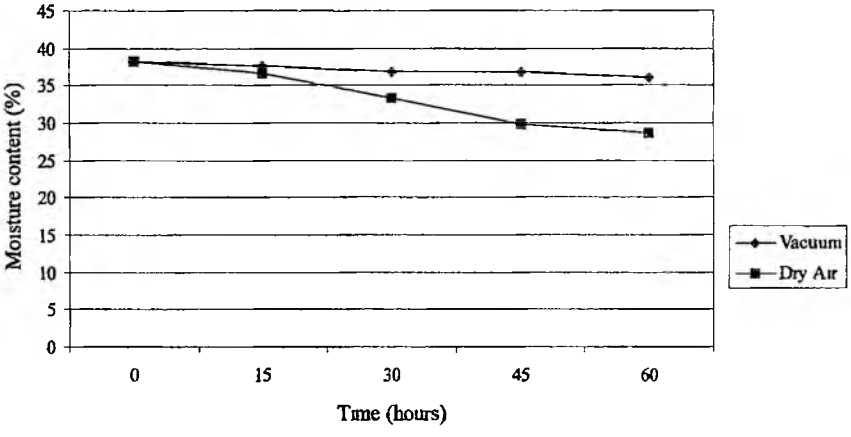
Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	1901 85	1373 63	1593 00	814 72	539 44	1244 53
Dry air	1901 85	1186 50	935 70	856 60	609 79	1098 09
Mean	1901 85	1280 06	1264 35	835 60	574 61	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	410 308	NS	NS
SEM (±)	149 203	94 364	211 005

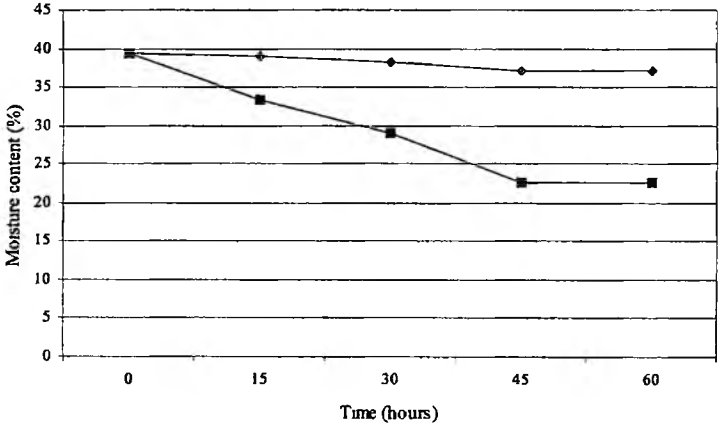
Table 44 Germination value of seven week old seeds without seed coat of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	2625 00	1624 00	1665 63	818 91	1035 29	1553 76
Dry air	2625 00	1206 29	770 56	898 20	912 35	1282 48
Mean	2625 00	1415 15	1218 09	858 55	973 82	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	243 845	154 22	NS
SEM (±)	119 415	75 524	168 879



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 12 Moisture content (%) of embryonic axes of *Hopea parviflora* at six and seven weeks after anthesis as affected by vacuum, dry air and time

case of dry air with different durations A decrease of 15 5 to 42 6 per cent was observed after 15 and 60 minutes duration due to rapid drying by dry air

4 8 2 Leachate conductivity

4 8 2 1 Six weeks after fruit set

The initial value observed was 0 057 mhos/cm The data pertaining to this is shown in Fig 13a A slight decreasing trend was observed due to vacuum and dry air with different durations A decrease of 3 5 and 14 per cent was observed after 15 and 60 minutes duration due to rapid drying at dry air

4 8 2 2 Seven weeks after anthesis

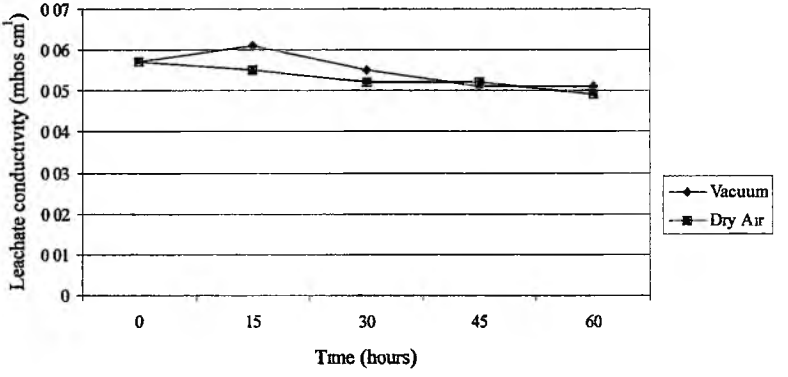
The initial value observed was 0 076 mhos/cm The data pertaining to this is shown in Fig 13b There was no considerable variation in leachate conductivity due to vacuum in any of the durations A decreasing trend was evident by 9 2 and 30 3 per cent decrease in leachate conductivity due to rapid drying at dry air after 15 and 60 minutes duration

4 8 3 Germination parameters

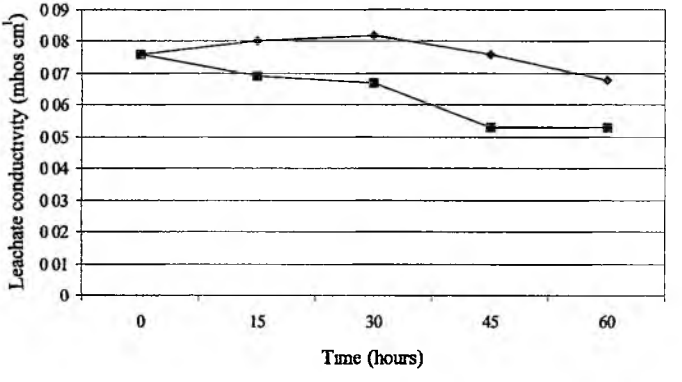
4 8 3 1 Cumulative germination percentage

4 8 3 1 1 Six weeks after anthesis

Significant differences were observed in germination percentage due to vacuum and dry air and time ($P < 0.01$) The data pertaining to this is given in Table 45 However the interaction between them was not found to be significant The initial value noticed was 84 4 per cent A decreasing trend could be seen due to rapid drying at vacuum and dry air for different durations Different durations of dry air treatment were found to influence the germination percentage significantly



a) Six weeks after anthesis



b) Seven weeks after anthesis

Fig 13 Leachate conductivity (mhos cm ^{1}) of embryonic axes of *Hopea parviflora* at six and seven weeks after anthesis as affected by vacuum, dry air and time

Table 45 Cumulative germination percentage of six week old embryonic axes of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	84 38	56 25	37 50	20 31	15 63	42 81
Dry air	84 38	31 25	15 63	10 94	6 25	29 69
Mean	84 38	43 75	26 56	15 63	10 94	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	10 497	6 639	NS
SEM (±)	3 817	2 414	5 397

Table 46 Cumulative germination percentage of seven week old embryonic axes of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	79 69	50 00	26 56	10 94	10 94	35 63
Dry air	79 69	26 56	15 63	10 94	6 25	27 81
Mean	79 69	38 28	21 09	10 94	8 59	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	12 367	NS	NS
SEM (±)	4 497	2 844	6 359

4.8.3 1.2 Seven weeks after anthesis

Significant difference was observed on the germination percentage due to time (P <0 01) The data pertaining to this is given in Table 46 Vacuum and dry air and their interaction with time were not found to be significant Duration of 45 minutes had a significant influence in germination percentage due to vacuum and dry air Beyond 45 minutes no difference was noticed due to vacuum However the dry air registered the lowest value of 6 3 after 60 minutes duration

4 8.3.2 Mean daily germination

4 8.3 2 1 Six weeks after anthesis

Significant differences were observed due to vacuum dry air and time (P <0 01) whereas the interaction between them was found to be non significant The data pertaining to this is given in Table 47 The initial value recorded was 12 9 There was a drastic reduction in mean daily germination due to rapid drying at vacuum and dry air with different durations The decrease was from 26 7 to 86 per cent due to vacuum for 15 and 60 minutes durations and 61 5 to 99 9 per cent due to dry air for 15 and 60 minutes respectively

4 8.3 2 2 Seven weeks after anthesis

Significant differences were observed in mean daily germination due to time (P <0 01) The data pertaining to this is given in Table 48 Vacuum and dry air and interaction between vacuum and dry air and time did not have any significant influence The initial value observed was 11 6 and a drastic reduction was observed due to vacuum and dry air for different durations A decrease from 34 5 to 90 9 per cent was observed after 15 and 60 minutes in vacuum where as the decrease was from 64 8 to 100 per cent in dry air after 15 and 60 minutes respectively

Table 47 Mean daily germination of six week old embryonic axes of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	12 93	9 48	6 15	3 20	1 80	6 71
Dry air	12 93	4 98	1 95	1 25	0 01	4 22
Mean	12 93	7 23	4 05	2 23	0 90	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	1 177	1 125	NS
SEM (±)	0 646	0 409	0 914

Table 48 Mean daily germination of seven week old embryonic axes of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	11 58	7 58	3 73	1 05	1 05	5 00
Dry air	11 58	4 08	2 10	0 90	0 00	3 73
Mean	11 58	5 83	2 91	0 98	0 53	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	2 148	NS	NS
SEM (±)	0 781	0 494	1 104

4 8.3.3 Peak value

4 8.3.3 1 Six weeks after anthesis

Significant differences were observed due to vacuum and dry air and time (P <0 01) The data pertaining to this is given in Table 49 But the interaction between them was found to be non significant A drastic decline could be observed here also due to rapid drying at both vacuum and dry air for different durations A decrease of 26 7 to 86 per cent was observed in vacuum and 61 5 to 99 9 per cent in dry air for 15 minutes and 60 minutes respectively

4 8.3.3.2 Seven weeks after anthesis

Significant difference in peak value was observed due to time (P <0 01) whereas vacuum and dry air and their interaction with time was found to be non significant The data pertaining to this is given in Table 50 The initial value recorded was 11 6 It was drastically reduced with different durations due to rapid drying at vacuum and dry air A decrease of 64 8 to 100 per cent was observed due to dry air after 15 and 60 minutes duration.

4 8.3 4 Germination value

4 8.3 4 1 Six weeks after anthesis

Significant differences in germination value was observed due to vacuum and dry air time and interaction between them The data pertaining to this is given in Table 51 The initial value recorded was 167 6 A drastic decline was observed due to rapid drying at vacuum and dry air for different time durations Due to vacuum a decrease of 44 to 96 per cent was observed for 15 and 60 minutes respectively where as due to dry air the corresponding decrease was from 84 to 99 9 per cent

Table 49 Peak value of germination of six week old embryonic axes of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	12 93	9 48	6 15	3 20	1 80	6 71
Dry air	12 93	4 98	1 95	1 25	0 01	4 22
Mean	12 93	7 23	4 05	2 23	0 90	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	1 777	1 125	NS
SEM (±)	0 646	0 409	0 914

Table 50 Peak value of germination of seven week old embryonic axes of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	11 58	7 58	3 73	1 05	10 05	5 00
Dry air	11 58	4 08	2 10	0 90	0 00	3 73
Mean	11 58	5 83	2 91	0 98	0 53	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	2 148	NS	NS
SEM (±)	0 781	0 494	1 104

Table 51 Germination value of six week old embryonic axes of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	167 60	93 89	40 49	13 90	6 48	64 47
Dry air	167 60	26 50	7 65	6 25	0 01	41 60
Mean	167 60	60 20	24 07	10 08	3 24	

	Time	Vacuum & Dry air	Interaction
CD (0 05)	15 170	9 595	21 455
SEM (±)	7 429	4 699	10 507

Table 52 Germination value of seven week old embryonic axes of *Hopea parviflora* Bedd as affected by vacuum and dry air for different time duration

Treatment	Time (min)					
	0	15	30	45	60	Mean
Vacuum	136 28	62 86	2025	4 41	4 41	45 64
Dry air	136 28	23 26	8 82	3 24	0 01	34 32
Mean	136 28	43 06	14 54	3 83	2 21	

	Time	Vacuum & Dry air	Interaction
CD (0 01)	25 036	NS	NS
SEM (±)	9 104	5 758	12 875

4 8.3 4.2 Seven weeks after anthesis

Significant difference in germination value was observed due to time ($P < 0.01$) The data pertaining to this is given in Table 52 Vacuum and dry air and their interaction with time was proved to be non significant A drastic reduction could be noticed due to rapid drying at vacuum as well as dry air for different durations A decrease from 53 9 to 96 8 per cent could be noticed after 15 and 60 minutes duration due to vacuum where as in the case of dry air the decrease was from 82 9 to 99 9 per cent for 15 and 60 minutes durations respectively

The embryos were inoculated on to nutrient rich ½ MS medium and in embryo cultures a high degree of culture contamination was observed due to vacuum and dry air treatment.

4 9 Germination parameters as affected by cryopreservation

Cryopreservation studies were carried out using intact seeds seeds without seed coat and embryonic axes of *Hopea parviflora* collected at six and seven weeks after anthesis after subjecting them to different relative humidities and time durations Both direct plunging of the propagules into liquid nitrogen and slow freezing them using a programmable biofreezer were tried The propagules were pretreated with DMSO (Di Methyl Sulfoxide) prior to subjecting them to slow freezing

After cryopreservation and thawing the propagules were inoculated on to the recovery medium to study their regeneration potential But seeds seeds without seed coat and embryonic axes of *Hopea parviflora* failed to regenerate after cryopreservation However the seeds and seeds without seed coat retained their colour for two to three days after which they turned brown, showing no signs of viability

Discussion

Discussion

The present investigation was conducted to find out the best desiccation protocol by applying different levels of relative humidity vacuum and dry air for different durations for the purpose storage of *Hopea parviflora* Bedd seeds using cryopreservation techniques The results obtained are discussed in this chapter

5 1 Flowering and fruiting

The results showed that *Hopea parviflora* trees flowered during the last month of May and the fruits ripened by the end of June The year 1999 was comparatively a poor seed year in the whole stand Troup (1921) observed that in *Hopea 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 The periodicity of flowering and fruiting was almost the same in both years of study Sunilkumar and Sudhakara (1998) however noticed difference in the periodicity of flowering in *Hopea parviflora* trees

5 2 Seed characteristics

Diameter of the seeds increased each week after anthesis and largest diameter was observed in seven week old seeds after anthesis Only those seeds collected at six weeks after anthesis and beyond germinated This shows that physiological maturity is attained in *Hopea parviflora* seeds between fifth and sixth weeks after anthesis Further study is required to find out the physiological maturity and harvest maturity in *Hopea parviflora* seeds Berjak *et al* (1992) observed that in seeds of *Landolphia kirkii*

as it matured the embryonic axis became larger almost doubling in weight from the immature (nongerminable) to the fully mature readily germinable stage

5.3 Effect of Relative humidities, Vacuum and Dry air for different durations on germination parameters of seeds, seeds without seed coat and embryonic axes of *Hopea parviflora* Bedd at six and seven weeks after anthesis

The initial moisture content of seeds collected at six and seven weeks after anthesis was 32 6 and 34 9 per cent Sunilkumar and Sudhakara (1998) observed 29 6 per cent moisture content in seeds of *Hopea parviflora* after natural shedding In the case of seeds without seed coat and embryonic axes the moisture content ranged from 36 8 to 38 4 per cent and 38 1 to 39 4 per cent respectively at six and seven weeks after anthesis Higher moisture content of embryonic axes was also reported in other recalcitrant seeds *viz Avicennia marina* 63 per cent (Berjak *et al* 1984) *Azdirachta indica* 91 per cent (Maithani *et al* 1989) and *Artocarpus heterophyllus* 68 per cent (Fu *et al* 1993) Sunilkumar and Sudhakara (1998) also reported in *Hopea parviflora* that among the seed components the embryo has the highest moisture percentage Chin (1988) reported that the initial moisture content of recalcitrant seeds might range from 30 to 70 per cent (on wet weight basis)

When the seeds/seeds without seed coat/ embryonic axes were kept at relative humidities ranging from 100 to 46 6 percent for periods up to 24 hours their moisture content increased but a decrease was observed when kept at 30 per cent relative humidity for different durations The decreasing trend was further magnified at 20 per cent relative humidity This shows that equilibrium moisture content of *Hopea parviflora* propagules lies between 75 6 to 30 per cent relative humidities Further studies are required to find

out exactly the equilibrium moisture content of different components of *Hopea parviflora* seeds at different maturity levels Seed moisture content has been considered the most important factor controlling physiological reactions in seeds (Fang and Moore 1998) They also reported that seed moisture content increased with increase in relative humidity and decreased with increase in time but relative humidity had the greater influence Critical moisture content levels vary with the method of drying (Farrant *et al* 1985 Pritchard, 1991) The values of the lowest safe moisture content vary between extremes of 23 per cent for cocoa (*Theobroma cacao*) (Mumford and Brett 1982) and 61 5 per cent for *Avicennia marina* (Farrant *et al* 1986) Critical moisture levels have been postulated for germination metabolism germination and continued embryogenesis and the cessation of growth and cell division (Palit, 1987 Mc Intyre 1987 and Adams and Rinne 1980) In the present study rapid drying did not have much deleterious effect on the germination parameters of seeds/seeds without seed coat There are many reports on the advantage of rapid drying over slow drying which would help surpass desiccation injuries if any inflicted due to the loss of structural water from the cells Bonner (1996) suggested that the rate of desiccation could influence the expression of recalcitrant behaviour in seeds But in the case of embryonic axes reduction in moisture content as evidenced from the results might have had a deleterious effect on the germination parameters especially when they were subjected to rapid drying Lin Tsan Piao *et al* (1995) reported that *Michelia compressa* seeds could survive desiccation to moisture content as low as 10 per cent (fresh weight basis)

In the case of vacuum and dry air treatment the trend was similar as in the case of relative humidities for different durations But in vacuum not much variation in

moisture content was observed in seeds or seeds without seed coat collected at six and seven weeks after anthesis compared to the initial value But in dry air the moisture content registered a decreasing trend with increase in duration of time The minimum values were recorded after 60 minutes duration in all the cases In the light of the good germination percentage obtained, it may be presumed that the desiccation *per se* did not have any deleterious effects on seed/seed without seed coat in this study Several scientists have proposed rapid drying as one of the methods to reduce desiccation injury The rate of drying also depends on the amount of seed (particularly the depth of the layer of seeds) the circulation of dry air within the drying cabinet and the species (Hong and Ellis 1996) This indicates that either there would not have been any ill effects due to desiccation or the repair mechanisms or aqueous reactions might have been reactivated on rehydration after partial dehydration to nullify membrane damages inflicted Chandel *et al* (1995) reported that in recalcitrant seeds of tea (*Camellia sinensis* L) cocoa (*Theobroma cacao* L) and jackfruit (*Artocarpus heterophyllus* L) fully mature seeds survived desiccation to 24 35 and 31 per cent moisture content respectively

Even though embryonic axes at both weeks maturity also followed suit in terms of moisture content, the germination percentage was deleteriously affected by both vacuum and dry air treatments As in the case of relative humidities it may be presumed that germinative mechanisms might have been upset as the embryos are much more delicate and sensitive to desiccation as against seeds and seeds without seed coat Recalcitrant seed species will sooner or later die under storage conditions that do not permit any significant degree of water loss (Pammenter *et al* 1994) Paucity of oxygen might have also been resulted due to the vacuum suction and it may have a major bearing on the loss

of viability of embryonic axes which are actively respiring entities coupled with the considerably low water loss due to vacuum treatment When the seeds of *Shorea almon S robosta* and *S roxburghii* were stored above 40 per cent moisture content, problem in germination was encountered (Tompsett, 1985) The exact causes of recalcitrant seed death and its relationship with moisture content are not fully understood Chin *et al* (1989) stated that seed death could be due either to the moisture content falling below a certain critical value or simply a general physiological deterioration with time and that a number of factors may contribute to viability loss

Keeping in different relative humidities resulted in higher leachate conductivity in the case of seeds/seeds without seed coat/embryonic axes harvested at seven weeks after anthesis compared to those harvested at sixth week The leachate conductivity of the propagules harvested at both six and seven weeks after anthesis registered a sharp increase when kept at different relative humidities for 3 hours and it increased gradually afterwards Leachate conductivity slightly decreased when rapidly dried at 30 and 20 per cent relative humidities with duration of time This also shows that loss of membrane semi permeability was minimum in the case of seeds/seeds without seed coat when kept at low relative humidities for different durations Hence it may be presumed that the membrane disruption, if at all happened, would not have reached upto a level to cause viability loss as evidenced from the low leachate conductivity of the seeds/seeds without seed coat and comparatively good germination percentage obtained in the case of seeds/seeds without seeds coat Bonner (1996) reported that leachate conductivity was not at all a sensitive indicator of loss of seed viability The presence of soluble sugars does not adequately explain the nature of desiccation tolerance in recalcitrant seeds (Sun *et al* 1994) Hence the initial increase in leachate conductivity in relative humidities from

100 to 46 6 per cent i e before effecting significant desiccation may not be connected to loss of viability at all

Germination parameters of the embryonic axes are significantly reduced after different levels of relative humidities for diferent duration When the seeds/seeds without seed coat were rehydrated at this stage to study germination the membrane repair mechanisms might have been fully activated to ward off deleterious effects if any inflicted by the water stress due to desiccation Vertucci *et al* (1994a) reported that detrimental effects during storage under dry conditions were not initially evident, but became more apparent with time So it may be assumed here that cellular membrane disruption might have occurred with increasing duration of time in these relative humidity levels in embryonic axis unlike in the case of seeds/seeds without seed coat. Progressive deterioration of cell which is irreversible on moisturization as in the cases of seeds/seeds without seed coat might not have taken place in the case of embryos This may be behind the low germination percentage of embryonic axis as against the comparatively good germination percentage obtained in the case of seeds/ seeds without seeds coat Song *et al* (1984) reported in *Hopea hainanensis* that desiccation upto 31 per cent moisture content disturbed the ribosomes and endoplasmic reticulam that were reversed on hydration

The leachate conductivity of seeds collected at six and seven weeks after anthesis was not changed much due to both vacuum and dry air treatments However there was an increase in leachate conductivity due to vacuum for 15 minutes duration This is true in the case of seeds without seed coat and embryonic axis also Studying the response to drying of recalcitrant seeds of *Quercus nigra* L Bonner (1996) reported that leachate

conductivity was not at all a sensitive indicator of loss of seed viability As per the present study also moisture content of the seeds/ seeds without seed coat/embryonic axes act as an indicator of seed germination rather than leachate conductivity The inverse relation between leachate conductivity and viability has proven useful with a number of agricultural and tree species (Perry 1981 Bonner and Agmata Paliwal 1992) but all were orthodox in storage behaviour Desiccation tolerance as per them is a complex phenomenon, possibly requiring the interaction of several biochemical processes The presence of soluble sugars does not adequately explain the nature of desiccation tolerance in recalcitrant seeds (Sun *et al* 1994)

Germination percentage of seeds and seeds without seed coat collected at six and seven weeks after anthesis was 93 8 But germination percentage of embryonic axes at six weeks maturity was 84 4 while that of embryonic axes at seven weeks maturity was 79 7 Keeping in different relative humidities for durations up to six hour did not seem to have any influence on germination percentage of seeds/seeds without seed coat collected at six or seven weeks after anthesis by and large However 12 and 24 hours durations slightly reduced the germination percentage

In the case of embryonic axes slight decrease was observed in germination percentage when kept at relative humidities ranging from 100 to 46 6 per cent for 3 and 6 hour duration Embryonic axes are delicate structure and more sensitive to desiccation than other propagules The decrease was tremendous beyond this At partially dehydrated state seeds may exhibit mild water stress upsetting the relative rate of individual reactions associated with germination (Vertucci 1993) However the membrane disruption would not have been so drastic as to cause severe viability loss The propagules might have been subjected to rapid drying while being kept at low levels

of relative humidities like 30 and 20 percent. There are several reports that rapid drying will not reduce germination parameters even though moisture percentage is reduced and several scientists have proposed rapid drying as one of the methods to reduce desiccation injury It has been found that the more rapidly dehydration can be achieved, the lower is the water content to which seeds or axes can be dried, without damage accumulation that culminates in viability loss This is particularly the case when excised axes are dried (Normah *et al* 1986 Berjak *et al* 1990) The rate of drying also depends on the amount of seed (particularly the depth of the layer of seeds) the circulation of dry air within the drying cabinet and the species (Hong and Ellis 1996) Bonner (1996) suggested that the rate of desiccation could influence the expression of recalcitrant behaviour in seeds Hunter (1959) found that a rate of drying of 5% loss in moisture content per day is apparently no more deleterious than a rate of 1 9% per day and the viability of seed dried to 16 7% moisture content was the same for both treatments Delay in drying or slow drying (i e at a comparatively high relative humidity) together with high temperature (above 25^0C) will tend to reduce viability considerably in orthodox seeds particularly oily seeds Because embryos from recalcitrant seeds are always metabolically active and never achieve the competence for complete desiccation this type of damage probably occurs during dehydration unless drying is extremely rapid (Pammenter *et al* 1991) Bonner (1996) suggested that the rate of desiccation could influence the expression of recalcitrant behaviour in seeds

In the case of embryonic axes rapid decline in germination percentage might have been caused by the progressive cellular deterioration when kept at decreasing relative humidity levels with increasing duration The deteriorative changes due to water stress have been reported by Pammenter *et al* (1994) the possible process being reduced

rates of protein synthesis increased proteolysis and variable effects on the catabolic activity of different enzymes Removal of structural water from the cells in embryos might have happened on dehydration to a greater magnitude than that happened in seeds/seeds without seed coat This might have paved way for minimum values as shown in germinative parameters of embryonic axes at seven weeks after anthesis especially at 12 and 24 hours duration at all relative humidities Drying embryos may result in the interruption of metabolism, which becomes lethal possibly because of the build up of high energy intermediates of metabolism that have toxic effects (McKersie *et al* 1988 Leprince *et al* 1992) The result obtained in the case of embryonic axes may bank on this finding also

Due to vacuum and dry air no significant variation in germination percentage was observed for different durations in seeds and seeds without seed coat at six and seven weeks maturity In general the lowest germination percentages obtained here was higher compared to that obtained due to rapid drying at 20 and 30 per cent relative humidities for 24 hour duration It was also reported by Leopold and Vertucci (1989) that when water is added to the desiccated seed to obtain the second physiological level aqueous reactions are facilitated as documented by enhanced enzyme activity with increasing water content.

Decrease in germination percentage was more evident in the case of embryonic axes of both weeks of maturity The decrease was higher in the case of vacuum treatment for different durations By using the silica gel and aseptic air current method the moisture content of axes of jackfruit seeds was reduced to a lower safe level and critical moisture content (16% and 26%) than that achieved by vacuum method (Fu *et al* 1993)

In the case of embryonic axes it may be presumed that germinative mechanisms might have been upset irreversibly as observed in the case of diminished germinative parameters compared to the seeds/ seeds without seed coat The greater decrease in moisture content attained after subjecting the propagules to dry air treatment in general may have a bearing on the comparative reduction in germination percentage as evidenced from the result obtained No clear trend could be observed in the case of leachate conductivity which could be correlated to loss of viability Fang and Moore (1998) also suggested that seed moisture content is the most important factor controlling physiological reactions in seeds and moisture content changes with relative humidity and time Moisture content is presumed to be more relevant in this study than leachate conductivity in terms of viability Sunilkumar and Sudhakara (1998) found that moisture content of seeds of *Hopea parviflora* acts more as an indicator of seed germination rather than leachate conductivity This is binding upon the present study also

High level of culture contamination was seen in the cultures with embryonic axes after treating in vacuum and dry air Fungal contamination one of the major problems when attempting storage of recalcitrant seeds under high moisture content above 15°C has been 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 vacuum has been created using vacuum pump and dry air was passed through the propagules kept in U tube Dry air from a laminar flow was blown over the propagules using a rubber tube But it had also passed through the large and heavy vacuum pump difficult to be kept inside a laminar flow It might have lead to the high contamination level even though the propagules and the instruments were sterilized prior

to the experiment Contamination in embryonic axes cultures was on a higher degree This may be due to nutrient rich media (½ MS) used for the inoculation of the embryos

In terms of vigour parameters of propagules after being kept at different relative humidities trend was similar to the germination percentage Mean daily germination of seeds/seeds without seed coat of seven weeks after anthesis was generally higher than that of six weeks maturity The lowest values were observed in the case of embryonic axes and also the values were almost on par in both weeks maturity Peak values of germination of intact seeds at six and seven weeks after anthesis were 19 5 and 27 9 respectively while that of seeds without seed coat and embryonic axes recorded values like 44 6 to 52 5 and 12 9 to 11 6 respectively In general highest peak values of germination were observed in seeds without seed coat at both weeks and the lowest in embryonic axes Drying/desiccating to extremely low water contents may shorten seed longevity (Walters and Engels 1998) More over the embryonic axes are more delicate structures and are sensitive to desiccation This may be the reason for the lowest peak values of germination recorded in the case of embryonic axes rapidly dried at 30 and 20 per cent relative humidities for maximum durations of time

Germination value of intact seeds of *Hopea parviflora* collected at six and seven weeks after anthesis were 375 2 and 762 2 respectively In the case of seeds without seed coat, the values were 1910 9 and 2625 and in the case of embryonic axes they were 167 6 and 136 3 respectively Higher values were recorded in the case of seeds without seed coat and lower in the case of embryonic axes in both weeks experiments Hong and Ellis (1996) suggested that removal of seed covering structures filing or chipping seeds with a scalpel or nicking with a needle might help to promote germination during

prolonged tests Cohen (1958) and Mumford and Grout (1979) suggested that the germination of lemon seed (Citrus *limon* L) was improved when the testa was removed and they produced evidence to prove that the testa of lemon seeds has a marked deleterious effect on the seed s response to desiccation

In general a slight decrease in vigour parameters were observed when the propagules were kept at 100 to 46 6 per cent relative humidities for 3 and 6 hour durations in both weeks study Beyond this the decrease was drastic Vertucci and Roos (1990) and Vertucci *et al* (1994a) reported that detrimental effects during storage under dry conditions were not initially evident, but became more apparent with time In other words, the seeds aged more rapidly under extremely dry conditions This may also be a probable reason for the decrease in vigour parameters

In vacuum and dry air treatment, trends were similar in vigour parameters like mean daily germination peak value and germination value to that obtained in the case of germination percentage in both weeks study Nearly stable values were recorded in vacuum and dry air for different durations in seeds and seeds without seed coat at six and seven weeks after anthesis but the decrease was more evident in the case of embryonic axis especially in the case of vacuum treatment for different durations

Seeds/seeds without seed coat collected at seven weeks after anthesis registered higher germination parameters by and large compared to that obtained at six weeks after anthesis in the whole study But the trend was not much evident in the case of embryonic axes Reports are available about higher germination vigour parameters in the case of seeds which are more mature Complete maturation is required for *Acer plantanoides* to survive complete desiccation (Hong and Ellis 1992a) Recalcitrant seeds may become increasingly tolerant of drying as maturation proceeds (Berjak *et al* 1992

Finch Savage 1992b) even if they remain hydrated and metabolically active throughout development (Berjak *et al* 1992) The performance of the propagules of seventh week over that of sixth week even though of lesser magnitude may bank on this finding Recalcitrant seeds appear to initiate germination related metabolism shortly after shedding (Farrant *et al* 1988 Betrjak *et al* 1989) Sunilkumar and Sudhakara (1998) also observed vivipary in *Hopea parviflora* on natural shedding which coincides with the monsoon showers At the early stages of development embryos are extremely sensitive to dehydration stress (Rogerson and Matthews 1977)

5 4 Germination parameters as affected by Cryopreservation techniques

Successful cryopreservation requires the optimization of numerous variables including the size of the specimen the correct type and concentration of cryoprotectant sample water content and rate of freezing and thawing (Mycock *et al* 1995) There are many reports on the advantage of rapid drying over slow drying which would help surpass desiccation injuries if any inflicted due to the loss of structural water from the cells Several scientists have proposed rapid drying as one of the methods to reduce desiccation injury Bonner (1996) suggested that the rate of desiccation could influence the expression of recalcitrant behaviour in seeds So rapid drying and ultra rapid drying cab be used as a pre treatment for the cryopreservation techniques Results showed that the seeds/seeds without seed coat/embryonic axes of *Hopea parviflora* at six and seven weeks after anthesis failed miserably to regenerate following cryopreservation techniques Areas of advanced degradation also occurred comprising cells in which the plasmalemma was discontinuous and vesiculated As per Murata and Nishida (1990) membrane lipids might well be a primary intracellular site of injury and Raison and Orr (1990) have suggested the phase change from liquid crystalline to gel state to be likely

Bedi and Basra (1993) found that species of tropical and subtropical origin suffer chilling injury when exposed to temperatures above freezing point of tissue but below 15°C Chilling injury may be exhibited as a loss of viability or reduced growth during germination at favourable temperatures (Wolk and Herner 1982)

Cryopreservation techniques provide the potential for indefinite preservation by reducing metabolism to such a low level (Ashwood Smith and Farrant 1980) that all biochemical processes are significantly reduced and biological deterioration virtually stopped The longevity of seeds or the maintenance of seed viability is a balance between extrinsic and intrinsic deleterious factors and repair or protective mechanisms Deleterious factors may include depletion of essential metabolites denaturation of macromolecules accumulation of toxic metabolites attack by microorganisms and insects and effects of ionizing radiation (Osborn 1977 1980)

In the present study the propagules especially seeds and seeds without seed coat at six and seven weeks after anthesis survived various levels of desiccation for different durations but they altogether failed to overcome cryopreservation both in terms of direct plunging and slow freezing techniques Chandel *et al* (1995) studied the desiccation and freezing sensitivity in recalcitrant seeds of tea (*Camellia sinensis* L) cocoa (*Theobroma cacao* L) and jackfruit (*Artocarpus heterophyllus* L) Fully mature seeds of tea cocoa and jack fruit survived desiccation to 24 35 and 31 per cent moisture content respectively but at these moisture levels seeds were not able to tolerate freezing in liquid nitrogen This finding is having a bearing on the result obtained in the present study Biochemical investigations on fully mature axes after freezing showed that the decline in viability with moisture level was associated with increased leachate

conductivity lipid peroxidation products and/or soluble carbohydrates (Chandel *et al* 1995) Seeds/ seeds without seed coat were able to retain their green colour for two to three days after cryopreservation there exists a ray of hope in achieving success in long term storage of *Hopea parviflora* seeds using this highly promising treatment But very soon the whole tissues turned dark brown due to oxidation of phenolics released from the cells In this context use of antioxidants during thawing and regeneration stage assumes significance The type and concentration of cryoprotectant and freezing rate are also of great significance While in general a fixed concentration of 5 to 10 per cent DMSO appears to be optimal for the tissue survival at low temperatures greater concentrations may be necessary for the maintenance of large structures to alter the concentration of cryoprotectant the temperature is reduced (Chin and Roberts 1980) Different protocols may be formulated and tried for cryopreservation studies to overcome chilling injury Different types of propagules of *Hopea parviflora* may also be tried Low relative humidities and different rates of drying under extreme ascept c conditions should be tried as drying rate is having a pivotal role in enabling the tissues to stand reduced moisture contents and subsequent shocks due to water stress

Summary

Summary

The present investigations were undertaken at the College of Forestry Vellanikkara to study the conservation strategy for *Hopea parviflora* Bedd species through storage of seeds using cryopreservation The salient findings of the studies are summarized below

1 The size of seeds was observed to be largest at seven weeks after anthesis which coincides with the natural shedding of seeds

2 Seeds collected at six and seven weeks after anthesis only were found to germinate easily under laboratory conditions This proves that the physiological maturity of *Hopea parviflora* seeds lie between five and six weeks

3 Moisture content of the embryonic axes was higher compared to seeds and seeds without seed coat

4 Moisture content of the propagules was generally increased when kept at relative humidities from 100 to 46 6 per cent for different durations up to 24 hours whereas it was considerably decreased when rapidly dried at 30 and 20 per cent relative humidities for different durations Moisture content was not reduced much in vacuum treatment whereas it was decreased due to dry air for different durations

5 The leachate conductivity of the propagules collected at seven weeks after anthesis was found to be higher than that collected at sixth week Leachate conductivity of the propagules registered a drastic increase when kept at different relative humidity levels for three hours and thereafter the increase was gradual But when rapidly dried at 30 and 20 per cent relative humidities it finally decreased

Leachate conductivity of the propagules did not show any clear trend either due to vacuum or dry air treatments and it can poorly be correlated to loss of viability

6 Germination parameters of the seeds and seeds without seed coat was not affected much when kept at different relative humidity levels for different durations in both weeks study but that of the embryonic axes was significantly reduced when rapidly dried at 30 and 20 per cent relative humidities for different durations Germination parameters of seeds and seeds without seed coat were not reduced much in any of the treatments due to both vacuum and dry air but drastic decline was observed in germinative parameters of embryonic axes Subjecting the propagules to different durations of dry air treatment amounted to rapid drying Paucity of oxygen may have also resulted due to the vacuum suction and may have a bearing on the loss of viability of embryonic axes which are actively respiring entities

7 High degree of culture contamination was observed due to both vacuum and dry air treatments for different durations. Size and weight of the vacuum pump were the stumbling blocks in effecting complete sterilization

8 After the cryopreservation studies i e both direct plunging and slow freezing techniques the propagules invariably failed to regenerate Chilling injury of the propagules on exposure to liquid nitrogen might have led to the loss of viability However the seeds and seeds without seed coat retained their green colour for two to three days before turning to dark brown Implicit in this is that different rates of drying different protocols of cryopreservation, concentration of cryoprotectant and freezing rate might help to achieve success in the long run

9 Reduction in moisture content observed due to different treatments could be correlated to the loss of viability where as the leachate conductivity was found to be a poor indicator Desiccating the seeds and seeds without seed coat by different relative humidities for different durations did not reduce the germination parameters by and large However germination parameters of embryonic axes were declined drastically due to rapid drying at 30 and 20 per cent relative humidities In the case of vacuum and dry air treatments also germination parameters of the seeds and seeds without seed coat was not deleteriously affected where as that of embryonic axes was truncated to a greater extent despite having high culture contamination Different rates of drying, different protocols of cryopreservation concentration of cryoprotectant and freezing rate may be tried to achieve success in the case of cryopreservation

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CONSERVATION STRATEGY FOR *Hopea parviflora* Bedd SPECIES THROUGH STORAGE OF SEEDS USING CRYOPRESERVATION TECHNIQUES

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ABSTRACT OF THE THESIS

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Abstract

A detailed study was conducted at College of Forestry Kerala Agricultural University Vellanikkara, Thrissur Kerala during 1998 2000 to standardize the conservation strategy for *Hopea parviflora* Bedd species through storage of seeds using cryopreservation Diameter of seeds collected at seven weeks after anthesis was found to be higher than that at sixth week Physiological maturity of the propagules is attained between five and six weeks after anthesis The propagules were subjected to different relative humidities vacuum and dry air for different durations as a treatment for cryopreservation studies In 100 to 46 6 per cent relative humidities the moisture content and the leachate conductivity of the propagules were found to increase with duration compared to the initial value Equilibrium moisture content of *Hopea parviflora* propagules was found to lie between 75 6 to 30 per cent relative humidities Germination parameters of seed and seed without seed coat were not decreased significantly due to desiccation by relative humidities vacuum or dry air but that of embryonic axes was considerably reduced as rapid drying might have been effected due to 30 and 20 per cent relative humidities and also due to vacuum and dry air with duration High culture contamination was observed in vacuum and dry air treatments After cryopreservation techniques the propagules invariably failed to regenerate but could retain green colour for two to three days