REPRODUCTIVE BIOLOGY AND HALF-SIB FAMILY PERFORMANCE OF *DYSOXYLUM MALABARICUM* BEDD. : AN IMPORTANT THREATENED TIMBER SPECIES

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Thesis submitted to the
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IN

FOREST BIOLOGY AND TREE IMPROVEMENT

By

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CERTIFICATE

This is to certify that the thesis entitled **"REPRODUCTIVE BIOLOGY AND HALF-SIB FAMILY PERFORMANCE OF *Dysoxylum malabaricum* Bedd.: AN IMPORTANT THREATENED TIMBER SPECIES"** submitted by **Mr. Manjunath, L.,** for the degree of **MASTER OF SCIENCE (FORESTRY) in FOREST BIOLOGY AND TREE IMPROVEMENT,** to the University of Agricultural Sciences, Dharwad is a record of research work carried out by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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Introduction

I. INTRODUCTION

India apart from being known for it's ancient civilization and deep-rooted tradition is also known for its rich diversity, both cultural as well as biological. Current level of assessment puts our country's total count of flora at more than 40,000 species and that of fauna at more than 89,000 species. This has fetched India a well-deserved place in the top ranking mega biodiversity countries of the world. Nearly one third of the floral species are endemic to our country. The current count of flowering plant species in India, estimated at 17,500 represents approximately 7 per cent of the global count (Ravikumar and Ved, 2000).

The Western Ghats of India, which run parallel to the west coast of peninsular region, form chain of hills, about 1600 km long, has been identified as one of the 25 biodiversity hot spots recognized in the world (Myers *et al.*, 2000). It comprises of various types of tropical forests ranging from wet-evergreen to dry deciduous. Though it occupies only about 5 per cent of the India's land, it constitutes about 4000 or 27 per cent of the countries total plant species (Ramesh *et al.*, 1991). Nearly 63 per cent of the tree species of the low and medium elevation evergreen forests of this region are endemic. Among the 60 endemic genera described from these hills, 49 are monotypic, suggesting its uniqueness. As many as 260 plants species have been newly described from these regions in the past 20 years. This high level of diversity and endemism in the Western Ghats has conferred on them the status as one of the biodiversity 'hot spots' of the world (Nayar, 1996).

Uttara Kannada district of Karnataka, situated in the central Western Ghats is having the largest percentage (75.86%; FSI 2001) of forest cover in the state. Ecologically and biogeographically also this district is important as the northern most limit of the distribution of major evergreen forest formations and many endemic tree species (Pascal, 1988).

The rich flora of the Western Ghats is characterized by presence of many economically important plants, which find use as timber, medicine, fodder, food or other minor use. *Dysoxylum malabaricum* Bedd. (family Meliaceae) is an important timber yielding tree species, endemic to the Western Ghats, highly valued for its lustrous, sweet-scented, durable timber (Anon, 1952) and it is also regarded as useful medicinal plant in the Ayurvedic medicine (Kirtikar and Basu, 1991). It is a large evergreen tree growing up to 40 m and distributed in the evergreen forests of the Western Ghats from Uttara Kannada southwards up to Kerala. It tends to be gregarious and is frequently found in association with *Artocarpus hirsutus* (Troup, 1986).

While there is something to feel proud about our rich biodiversity, over the decades it has been recklessly harvested leading to several of the medicinal plant species becoming threatened and at the verge of extinction in the current day world. The causes of such threats are numerous, the prominent being heavy biotic pressure, degradation and fragmentation of wild habitats, and unsustainable harvesting. About 1000 medicinal plant species have become threatened in India (Ravikumar and Ved, 2000). *Dysoxylum malabaricum* has been illegally harvested over the years for its valuable timber resulting in its population decreased to certain fragmented pockets at Uttara Kannada, Kodagu and Kerala (Ramesh and Pascal, 1997). Based on

reports of its rarity, it has been listed as **endangered-globally** in terms of its conservation status by the Foundation for Revitalization of Local Health Traditions (Ravikumar and Ved, 2000). Endemic species confined to narrow geographic range, high habitat specificity and low population density, exhibit the most vulnerable form of rarity (Robinowitz, 1981) and needs vigorous protection of its few remaining habitats (Varghese and Menon, 1999). This requires basic information regarding its population status, breeding system, ecological requirements and the available genetic variability (Vasudeva *et al.,* 2001). Hence there is an immediate need to assess the population status of this species. Appropriate scientific measures, based on thorough knowledge of its biology, ecology and genetic base have to be taken to ensure its conservation (Fig. 1.1).

Studying how the species is surviving naturally, *i.e.,* its reproductive biology is the initial step in this regard and a prerequisite for its genetic improvement and domestication (Vasudeva *et al.,* 2001). There is an urgent need to study its breeding system and seed biology to standardize methods of propagation for *ex situ* conservation. Progeny testing is the most rigorous breeding methods being practiced for improvement of forest trees (Kedarnath, 1986) as it helps in understanding degree of genetic influence on various characters. Vegetative propagation serves as a primary method to conserve superior genotypes and multiply them in nurseries. Dogra (1981) has outlined the genetic potential of many Indian trees and felt that improvement programs should be based on indigenous species than exotics. But in many of our local and important tree species like *D. malabaricum* and *Artocarpus hirsutus etc.,*

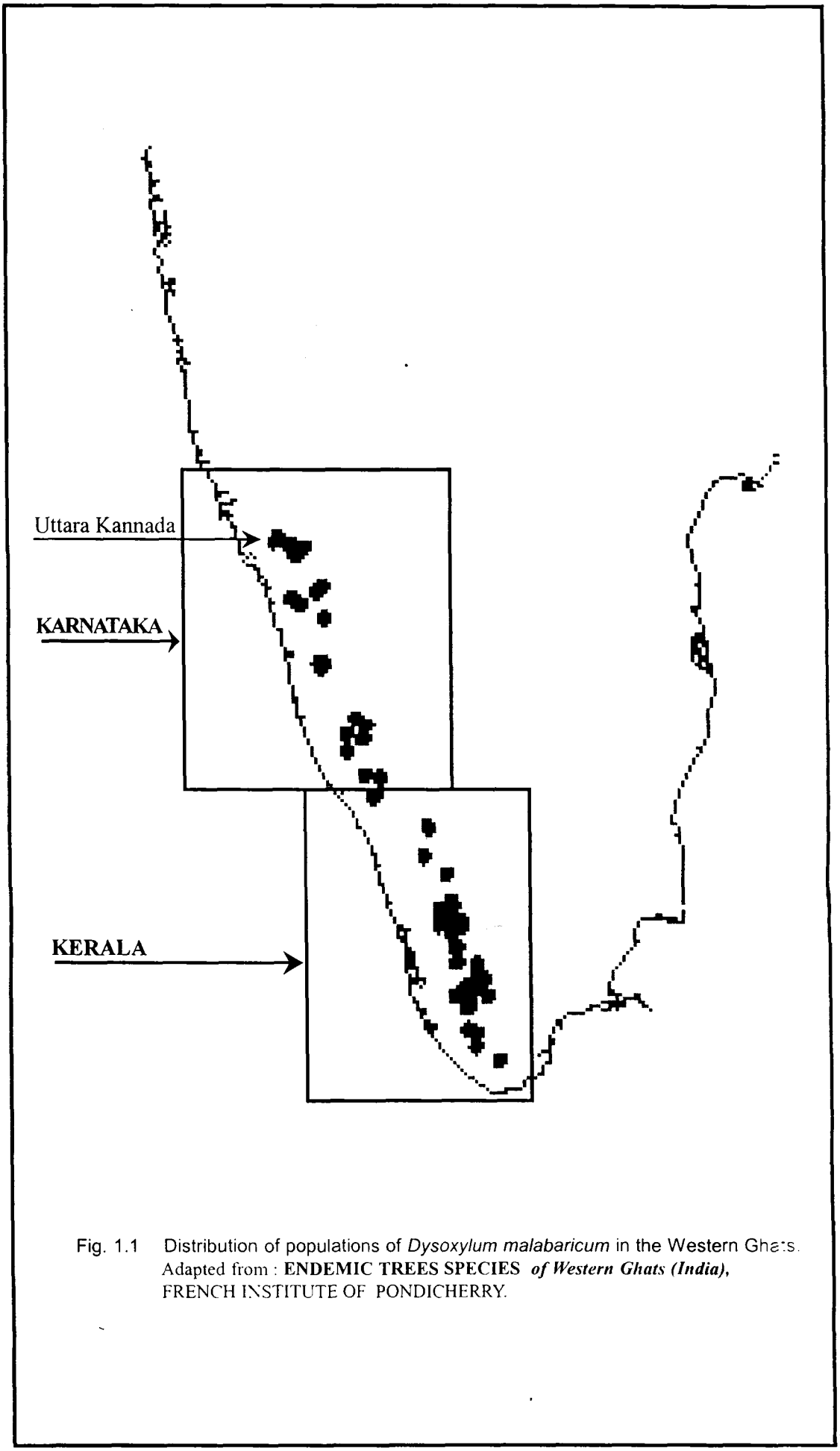

Fig. 1.1 Distribution of populations of *Dysoxylum malabaricum* in the Western Ghats. Adapted from : **ENDEMIC TREES SPECIES** ***of Western Ghats (India),*** FRENCH INSTITUTE OF PONDICHERRY.

selection and genetic improvement is still in its infancy stage, and needs to be undertaken with immediate urgency.

In this background the present study was taken with the following objectives:

1. To understand a few aspects of breeding system and seed biology of *Dysoxylum malabaricum.*
2. To assess the level and degree of genetic variation for seed germination and early seedling vigour among the half-sib families of *Dysoxylum malabaricum.*
3. To standardize a vegetative propagation technique for *Dysoxylum malabaricum.*

Review of Literature

II. REVIEW OF LITERATURE

Literature pertaining to biology and importance of *Dysoxylum malabaricum* is presented in this chapter. A few studies related to reproductive phenology, breeding systems, standardization of seed pre-germination treatment and protocols of vegetative propagation with respect to other forest tree species are also reviewed.

2.1 Nomenclature, botany and distribution of *Dysoxylum malabaricum*

2.1.1 Nomenclature and botany

Dysoxylum malabaricum Bedd. Syn. *D. glandulosm* Talb. belongs to family Meliaceae (Anon, 1952). The genus *Dysoxylum* has about 40 species distributed in tropical Asia, Malay Archipelago and a few in Australia and New Zealand. Hooker (1872) has described about fourteen of its species in his monumental book *Flora of British India*. Six of its species occur in Northern and Eastern Bengal, two in Burma, four in Andaman Islands and three in South India. The three species of *Dysoxylum* occurring in the Western Ghats are *Dysoxylum malabaricum, Dysoxylum beddomei* that are strictly endemic, while *D. binectariferum* also occurs in the north east of India. In Karnataka *Dysoxylum malabaricum* is commonly called "*Bili devadari*" or "*Bili agilu*" and its trade name is "White cedar" (Saldanha, 1996). *D. malabaricum and D. binectariferum,* are difficult to distinguish due to their similar morphological characters. Some of the characters by which they can be distinguished are listed in the Table 2.1.

The *Dysoxylum malabaricum* is a very large evergreen tree growing up to 40 m height and 3.5 m girth. It has undivided straight bole, dense crown with compound leaves, measuring up to 35 cm, and has 4-5 pairs of leaf lets,

Table 2.1 Distinguishing characters between *Dysoxylum malabaricum* and *D. binectariferum.*

Sl. No	Characters	***D. malabaricum* Bedd.**	***D. binectariferum Roxb.***
1.	Synonym	*D. glandulosm* Talb.	*D. macrocarpum* Bedd.
2.	Common name	Bili devadari	Kadu gandha
3.	Leaf rachis	35 cm long	15 cm long
4.	Leaf lets	About 4-5 pairs sub-opposite	About 5-9 alternate
5.	Calyx	Cleft halfway short	Cup shaped, urceolate, sub entire
6.	Flower	tetra-merous	tetra-merous, obsoletely tomentose
7.	Petals	Linear-oblong	Oblong
8.	Fruit	Verricose, longitudinally lined, nearly glabrous, bright yellow when ripe	Reddish, obovoid, some what pyriform
9.	Seed	Bluntly 3 sided, attached by their whole inner face to the central placenta, testa reddish brown, cotyledons green.	Polished, dark purple, exarillate, cotyledons green.

which are 10-23 cm long, sub-opposite, ovate or sub elliptic. It has 9-20 pairs of lateral nerves, which are prominent beneath. The bark is pale grey, rough, fissured, covered with white warty lenticels, exfoliating into large flakes. The wood is yellowish or light orange in colour (Plate 2.1). Flowers are fragrant, white to greenish yellow in colour, bisexual borne in auxiliary panicles. Fruits are pear-shaped, 5-7 cm in diameter, blight yellow when ripe, containing 3-4 seeds with reddish testa and orange tegmen enclosing green cotyledons (Ravikumar and Ved, 2000).

2.1.2 Distribution

Dysoxylum malabaricum is an endemic evergreen tree species restricted to evergreen forests of the Western Ghats from North Karnataka, Southwards Coorg, Anamalai and Travancore up to 850 m elevation (Saldana, 1996 and Troup, 1986). In Karnataka, it is present in Coorg, Mysore, Shimoga, Uttara Kannada and Chikmangalur districts. Over the years due to its illegal harvest its population has decreased to certain fragmented pockets of Uttara Kannada, Kodagu and Kerala (Ramesh and Pascal, 1997).

2.2 Uses of *Dysoxylum malabaricum*

2.2.*1 Importance of* D. malabaricum *as a timber species.*

Dysoxylum malabaricum is highly valued for its lustrous, sweet-scented and durable timber, which is little liable to termite attack. The sapwood is yellowish white and the heartwood brownish grey with yellow tint turning darker on exposure to air. It saws and works easily, takes good polish and finishes to a smooth surface. Its properties are comparable with that of teak. It is a high class timber used for making furniture, railway carriages,

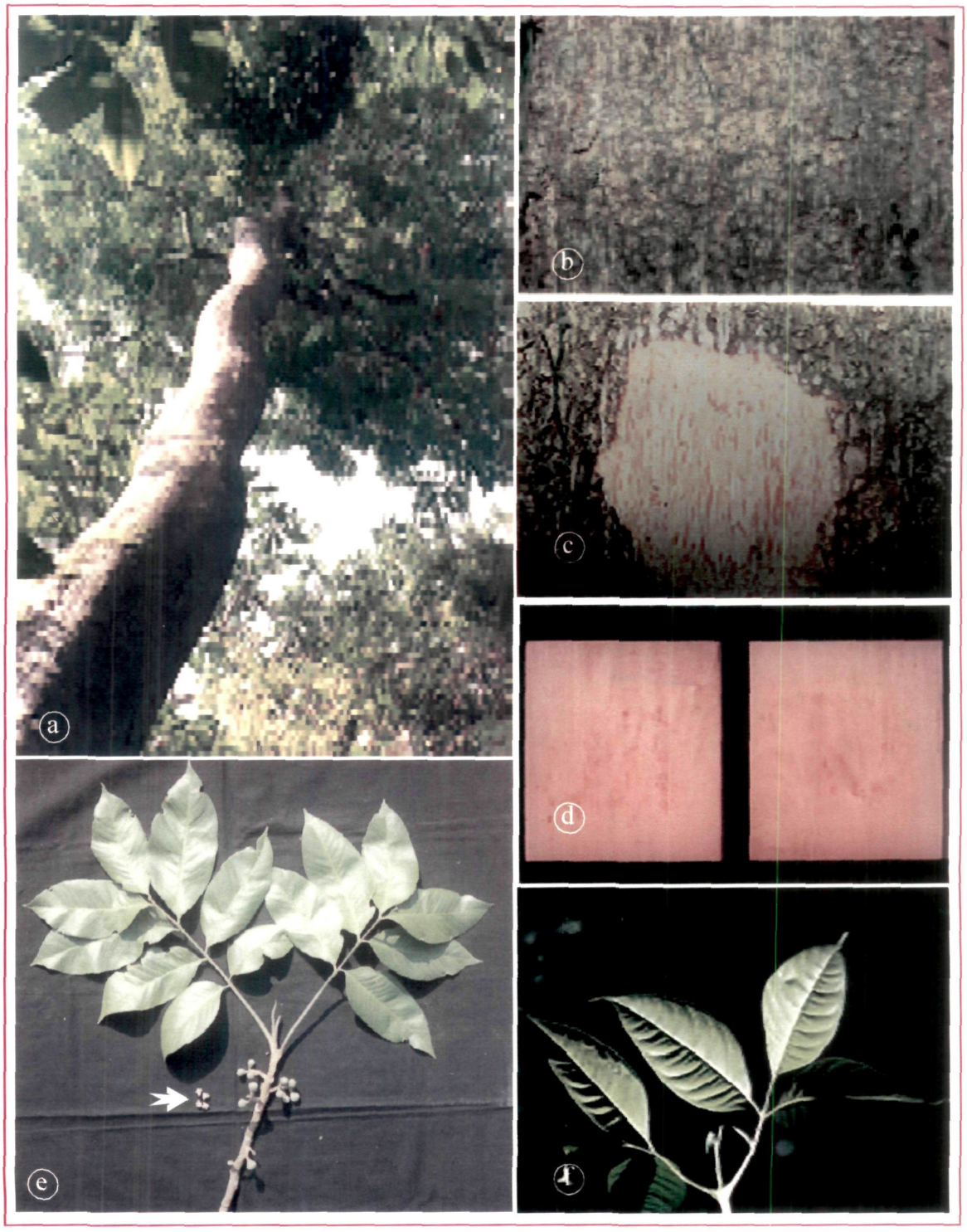

Plate : 2.1

a) Tree with fruit bearing canopy.
b) Close-up of bark showing prominent lenticels.
c) Whitish sapwood.
d) Grain pattern of the timber (Yellowish markings indicate oil oozing out of freshly cut surface).
e) Twing bearing freshly set fruits; arrow mark indicate flowers.
f) Prominent veins of the leaves.

building construction materials, plywood, cigar boxes, cooperage and casks for transporting oil as it does not discolour the oil or allow to percolate (Anon, 1952).

2.2.2 Medicinal importance of D. malabaricum

The tree is used in making Ayurvedic medicine. A decoction of the wood is said to be useful for rheumatism. The wood oil is used for ear and eye disease (Kirtikar and Basu, 1991).

2.3 Conservation status of *Dysoxylum malabaricum*

Due to its valuable timber white cedar is harvested from natural habitats illegally and its population has decreased to a great extent. Foundation for Revitalization of Local Health Tradition (FRLHT), based on Conservation Assessment and Management Plan (CAMP) workshop for categorization of threatened medicinal plants conducted from 1995 onwards, has prepared a list of 112 threatened medicinal plants of South India. *D. malabaricum* is listed as "endangered-globally" in terms of its conservation status based on population reduction in the form of decline in area of occupancy, extent of occurrence and or quality of habitat (A1c) and the actual or potential levels of exploitation (A1d; Ravikumar and Ved, 2000).

2.4 Reproductive biology

Reproductive biology is an important interdisciplinary area of plant science, which helps in understanding the evolutionary dynamics of a species, the range of genetic variability, controlled by reproductive system, which in turn controls the adaptive changes (Simmonds, 1962). It helps in exploitation of the economic potential of the species and also developing effective conservation strategy. Contrary to the situation in other countries, interest in

this field is dwindling in India. This is very disappointing particularly in the light of India harboring a rich flora, distributed over a wide range of habitats. Many of these are threatened and require development of an effective conservation strategy especially for a large number of endemic species. There is also lack of data on the economic potential of a majority of our endemic species. Studies on reproductive biology of special group of plants such as, endangered species, endemic species, species of medicinal and other economic importance has been identified as the priority area (Shivanna and Parveen, 1994). *D. malabaricum* come under all these groups and requires immediate attention on study of its reproductive biology.

2.4.1 Phenology

The time of appearance of floral buds, anthesis, fruit development and fruit fall during the reproductive phase of a plant is known as reproductive phenology. Norman (1989) studied phenology of 101 tropical woody species and observed four distinct categories of species *viz.*, flowering throughout the year (23.8 %), once a year (36.6 %) twice a year (35.6 %) and thrice a year (4.0 %). Species flowering once a year were in bloom for the longest period (5.8 months), followed by species flowering twice a year (4 months) and those flowering thrice a year (1.9 month). In a study on phenology of 37 woody angiosperms, it was found that in majority of the species leaf fall occurred during December-January, leaf bud formation and flower bud formation during February-March, anthesis during March-May and fruiting during May-August (Ansari and Bhadola, 1989).

Bisht *et al.*, (1986) made a comparative study on phenology of evergreen and deciduous trees in central Himalayas. They observed that deciduous trees flowering and fruit formation peaked in March, a month earlier then evergreen trees. Fruit maturation and seed dispersal occurred from June to September and was ahead by one month in deciduous species.

In Walnut orchard (*Juglans regia)* at Slovenia it was observed that early leafing genotypes were tall, with dense branching and large trunk diameter. The leafing character was also well correlated with the appearance of the first male flowers (Solar *et al.*, 2001). Leaf flushing and flowering of *Betula pendula, Prunus padua* and *Sorbus aucuparia* was found to occur earlier with increase in mean annual air temperature, which also prolonged the vegetative phase (Medvedovic and Milkovic, 2000).

Clonal variation for phenology and its associated low fruit production was studied in a 19 year old teak Clonal Seed Orchard. It was observed that there was significant inter-clonal difference for all phenological phase initiation. The overlap index indicated that clones of different provenance showed asynchronous flowering. Clones that initiated leaf flushing early and possessed longer peak flowering duration tend to produce high number of fruits per inflorescence (Rajesh and Vasudeva, 2002).

2.4.2 Breeding system

All aspects of expression of sex in plants that effect the relative genetic contribution to the next generation of individuals with in a species are known as breeding system. Most of the studies on breeding system in forest tree species have revealed that they are predominantly out-crossing. Jindal *et al.*,

(1985) opined that the knowledge of phenology and breeding system is a pre-requisite in any improvement programme.

Victor *et al.*, (2000) observed sexual systems in tropical plant species and concluded that they showed 3 types of breeding systems *viz.*, xenogamy (pollination between two flowers borne on two separate plants), facultative xenogamy and facultative autogamy, the second being the most dominant. Facultative xenogamy and autogamy, allow both a high or low degree of fitness to the environment through self-pollination and the necessary flexibility through xenogamy required for the development of new genotypes. The xenogamous breeding system seemingly is an adaptive change for ensuring the expansive distribution in more diverse and marginally located habitats.

Borges *et al.*, (1997) studied sexual system in *Bridelia retusa* a monoecious tree species at Maharastra and found that it exhibited temporal dioecy a system of sex expression in which there is little or no overlap between the production of male and female flowers within an individual tree.

Sindhu and Ananath (1996) studied breeding system in *Santalum album* and showed that sandal is predominantly out breeding species though its flower structure is designed for self-pollination.

Jindal *et al.*, (1985) studied breeding system of *Tecomella undulata* a small timber tree at Jodhpur and observed that fruit set varied from 0.64 per cent for selfing and 3.94 per cent for cross pollination indicating that the species is predominately out-crossing.

Pant *et al.*, (1997) studied breeding system in *Grewia optiva* an important agroforestry tree at Solan. The fruit set percentage by artificial

pollination was 77.27 per cent compared to 40.0 per cent in self-pollination indicating that the species is predominantly out crossing.

Madhuca indica, which flowers in the month of March to May had bisexual flowers and produced abundant pollen, which remained viable for 5-6 days. But the fruit set occurred only in cross-pollinated flowers (Kuruvilla, 1989). In *Gliricidia sepium* also, the fruits were produced only after cross-pollination, indicating that majority of the forest tree species are xenogamous (Kiill, 2001).

2.5 Seed biology

To achieve success in any plantation programme, a good start is essential from the germination stage. In order that a seed germinates, it must be placed under appropriate environmental conditions. Seeds of many tree species do not germinate even under favorable conditions, which may be due to the presence of hard seed coat or some germination inhibitors. Germination problem especially in endangered species is a curse to its own survival. *D. malabaricum* being one among them has very less recruitment in wild. In the light of its population getting declined, the species requires detailed study of its seed biology to establish problems involved in its germination and device methods to ensure its survival in perpetuity.

As there are no studies on these aspect on *D. malabaricum* a review of studies focusing on seed germination of other forest tree species is presented in Table 2.2.

2.6 Half-sib family structure and estimation of heritability in forestry

Use of genetically superior seeds is widely recognized as the best means of raising healthy plantation to ensure rapid growth and good quality

Table 2.2 Summary of review on seed pre-germination treatment in different tree species

Sl. No.	Species	Pretreatment	Germi-nation (%)	Reference
1.	*Albizia lebbeck*	Hot water treatment with 150 ppm IAA	81.5	Hanumantha *et al.*, 2002
2.	*Azadirachta indica*	2% dihydrogen phosphate for 24 hrs	84.0	Kumaran, *et al.*, 1995
3.	*Ptreocarpus. santalinus*	40% Hcl soaking for 24 hrs	75.8	Kalimuthu and Lakshamanan, 1995.
4.	*Pterocarpus marsupium*	40% Hcl soaking for 24 hrs	84.5	Kalimuthu and Lakshamanan, 1995.
5.	*Cassia fistula*	Sulphuric acid for 10 min.	70.0	Srimathi *et al.*, 2002
6.	*Adenanthera povonina*	Manual scarification	55.0	Pranab and Thapliyal, 1999
7.	*Gleditsia assamica*	Manual scarfication	67.0	Pranab and Thapliyal, 1999
8.	*Tectona grandis*	6 min hot water at 500C + 10 min air cooling + 6 min hot water + 10 min air cooling + 6min hot water	60.5	Saini *et al.*, 1999
9.	*Hardwickia binata*	Water soaking + pretreatment with ethrel 200 ppm	78.0	Masilamani and Vadivelu, 1997.
10.	*Terminalia chebula*	Cold water soaking 24hurs + cow dung stratification for 5 week	77.0	Bhardwaj and Chakraborthy, 1984
11.	*Terminalia catappa*	24 hrs water soaking	80.0	Prins *et al.*, 1994
12.	*Carissa carandas*	GA 3 at 25 ppm for 24 hrs	67.0	Banker, 1987
13.	*Garcinia indica*	GA3 250 ppm	90.0	Mathew and Geroge, 1995
14.	*Bauhinia purpurea*	GA at 500 ppm for 120 min	96.1	Mathew and Sivakumar, 2002.
15.	*Ulmus wallichiana*	Soaked in GA3 100 ppm	71.0	Massodi and Massodi, 2000
16.	*Diospyros melanoxylon*	IAA 200 ppm	43.4	Sivakumar *et al.*, 2002.

timber. Seeds collected from phenotypically superior trees, need to be evaluated for their genetic superiority. It has been observed that all phenotypically superior trees do not produce better progenies (Pitcher, 1982). Progeny testing is the best approach to isolate good genotype rather than merely the good phenotypes (Kedarnath, 1986).

In an experiment on teak progeny trial of different age groups, Sharma *et al.*, (1996) concluded that the estimates of genetic parameters obtained at the early age do not change much at later stage. In a recent study conducted at Tropical Forest Research Institute, Jabalpur, Madhya Pradesh; Gera *et al.*, (2001) assessed variability for plant height, collar diameter and survival per cent in 40 teak clones of three and half years age. Plant height and collar diameter gave comparable values for genotypic and phenotypic variation and coefficient of variation indicating that the parameters are under genetic control. Both these growth attributes were found to be highly heritable (H^2= 85.5% and 74.02% respectively) when compared to survival percent (H^2 = 23.07%). Considerable amount of genetic variation for economically important traits such as height, diameter and basal area has been reported (Mathew, 2001). This provides ample scope for family selection, which ensures elimination of inferior families.

2.6.1 Progeny evaluation at seedling stage

Seedling selection and progeny testing are the two rigorous breeding methods being practiced for the improvement of forest trees (Kedarnath, 1986; Chaturvedi, 1986). Nursery selection of half-sib families has been recommend by many workers (Neinstadet, 1981; Sidhu 1993). Progeny evaluation at seedling stage aims to derive preliminary information for

developing juvenile-mature correlation for traits of economic importance. It is also useful in eliminating poor progenies from field testing. Progeny tests with characteristics of high juvenile-mature correlation can be designed for relatively few years. This will go a long way in reducing the cost associated with carrying out the test and also effect the genetic improvement at a relatively faster rate. However, assessment of late expressing traits such as stem form and wood quality requires a minimum of half rotation age.

Dhillon *et. al.,* (2000) evaluated progeny performance in thirty provenances of Shisham (*Dalbergia sissoo)* for seven seedling traits. The genotypic and phenotypic correlation co-efficient between different pairs of characters did not give a comprehensive picture of association between them. Path analysis, however, revealed that seedling height, weight of leaves and height up to first branch contributed more directly to the collar diameter.

Srivastav *et. al.,* (1997) found strong genetic association of seedling characters during nursery and plant characters during field progeny trait of *Terminalia arjuna* and *T. tomentosa.* They recommended selection of seedlings with higher leaf length, lesser breadth and greater height for increasing leaf productivity of plantations.

Kondas *et al.,* (1973) and Banick (1980) reported different types of half-sib seedlings segregation into grassy, grass erect and very erect in *Bambusa arundinaceae* and *B. glaucesecens.* Of the four types of segregation, erect type has shown faster growth rate and more vigour.

Tan (1998) carried out progeny testing of *Heava brasiliensis* in nursery for early selection. Nursery yield, girth, height and branching habit were used as the main characters in selecting progeny for cloning.

Pitcher (1982) evaluated progeny performance of 42 open pollinated families of Black cherry (*Prunus serotina*) at age three in a nursery and then at age 12 years in a plantation. Family mean height at age three was well correlated with height at 12 years (r=0.67). Significant family height correlation between the two different ages suggests that nursery performance could be used as an indicator of subsequent field performance and restrict the size of progeny test. However, he cautioned the risk of losing some desirable genotypes.

2.7 Vegetative propagation

2.7.1 Importance

The vegetative propagation involves plant regeneration by means of its vegetative parts. It circumvents seed generation and thereby, ensures genetically uniform plantlet population identical to donor (mother) tree. The vegetative propagation constitutes a very important component of the "tree improvement programme" and helps achieve the following objectives:

- Conservation of selected superior genotypes *i.e.*, establishment of germplasm banks.
- Establishment of clonal seed orchard.
- Clonal multiplication of superior genotypes for clonal forestry, facilitating quick genetic gains.
- Curtailing 'long seedling cycle' and 'size' of woody perennials for undertaking breeding work.

- Capturing physiological status such as early flowering and fruit set for quick economic returns (Ansari and Sarkar, 1997).

2.7.2 Studies on effect of plant growth regulator in rooting of cuttings

It is almost evident that the growth regulators play a significant role in controlling rooting ability. Auxins have a marked influence on root formation of stem cuttings. The most widely used auxins are Indole acetic acid (IAA), Indole butyric acid (IBA) and Naphthalene acetic acid (NAA). However, there are no studies on their effect in *D. malabaricum*. A summary of the studies focusing on vegetative propagation of forest tree species is presented in the Table 2.3. It is evident from the table that the most used growth regulator for successful vegetative propagation is IBA in either prolong dip method at low growth regulator concentration or quick dip method for few minuets using higher concentration of growth regulator.

Table 2.3 Summary of review on vegetative propagation in forest tree species

Sl. No.	Species	Cuttings	Treatment	Reference
1.	*Santalum album*	Two node cuttings	IBA 400, treated by powder dip method	Anon, 2000.
2.	*Tectona grandis*	Nodal cutting 15-20 cm long and 1-1.5 cm diameter	IBA 2000/100 for 24 hrs or 50 seconds respectively	Anon, 2000.
3.	*Messua ferrea*	Stem cuttings	IBA 200 for 24 hrs	Thomas, 2001.
4.	*Anogeissus latifolia*	Branch cuttings of 20 cm long	IBA 200 ppm, for 24 hours	Nautiyal, *et al.*, 1992.
5.	*Grewia optiva*	Stem cutting 20 cm long	IBA 250 ppm for 24 hours	Kamlesh *et al.*, 1995.
6	*Eucalyptus tereticornis*	Half leaf of cuttings	IBA 4000 ppm powder dip method	Anon, 2000
7.	*Acacia nilotica*	Branch cutting 20 cm long and 12-15 mm thickness	IBA 200 ppm for 24 hrs	Gurumurti *et al.*, 1994.
8.	*Acer oblongum*	10-15 cm long cutting	IBA 5000 ppm for 10 seconds	Bhardwaj and Mishra, 1998.
9.	*Pongamia pinnata*	25 cm long cuttings	IBA 200 ppm for 24 hrs	Palaniswamy and Pramod Kumar, 1997.
10.	*Azadirachta indica*	Branch cutting 24 cm long, 1-15 cm diameter	1000 ppm IBA for 30 sec.	Palaniswamy and Pramod Kumar, 2001.
11.	*Acacia mangium*	Single nodal cuttings	IBA 2000 ppm	Ashok kumar, 2000.
12.	*Melia azedirachta*	Branch cutting	IBA 50 ppm	Gupta *et al.*. 1989.
13.	*Woodfordia fruticosa*	Cutting of 18-20 cm long and 1-1.5 cm diameter	IBA 200 ppm for 24 hrs	Bahuguna *et al.*, 1988.
14.	*Ochreinauclea missionis*	Soft cutting	IBA 1000 ppm, for 30 sec.	Jose *et al.*. 1995.
15.	*Taxus baccata*	3-4 node stem cuttings	IBA 1000 ppm for 2-3 sec.	Rakesh, 2003.

Material and Methods

III. MATERIAL AND METHODS

3.1 Study area

The present study was carried out at Sirsi Forest Division (15' 44' to 14' 40' N and 74' 53' to 74'42') of Uttara Kannada district in Karnataka. About 72 per cent of the geographical area of the district is covered by forest and it has one of the largest forest covers in Karnataka. The district forms the northern end of the Central Western Ghats and is ecologically and bio-geographically important as the northern most limit of the distribution of major evergreen forest formations and many evergreen endemic tree species including *Dysoxylum malabaricum.*

3.2 Study site

Dysoxylum malabaricum occurs only in the evergreen forest types. A good population of the species was found in the evergreen forests (*Kan*) near *Kalli* and *Navanagere* hamlets in the Banavasi forest range (13° 4' 45.1" N and 77° 34' 45.3" E) and were the focal populations of the study. The site is located 20 km south-east of Sirsi towards Banavasi. The vegetation of the study site is evergreen forest type associated with species such as, *Saraca asoca, Hydnocarpus indicus, Artocarpus heterophyllus, A. hirsutus, A. lakoocha, Syzygium cumini* and *Dipterocarpus indicus.*

The area falls under tropical monsoon climate, and rainfall occurs by south-west monsoon during June to September. The region receives an annual average rainfall of 2500 mm with a minimum of 2100 mm and a maximum of 3500 mm. The mean annual temperature varies from 13 to 37° C (Table 3.1).

Table 3.1 Meteorological data for the period from June 2002 to July 2003 at the Agrometry Advisory Service (AAS) unit, College of Forestry, Sirsi.

Month and Year	Temperature (°C)		Rainfall (mm)	Number of rainy days
	Mean of minimum	Mean of maximum		
June-02	21.9	29.8	412.5	19
July-02	21	28	261.7	27
Aug-02	21	27	665.4	25
Sept-02	20	30	70.6	10
Oct-02	21	31	270.6	15
Nov- 02	16	31	0	0
Dec-02	13	32	0	0
Jan-03	15	32	0	0
Feb-03	16	35	0	0
March-03	19	37	10.3	2
April-03	21.2	37.4	46.6	5
May-03	22	36	12.6	2
June-03	22	30	542.6	20
July-03	22	27	536.3	28

3.3 Reproductive phenology

Phenological observations were recorded on naturally occurring tagged trees (n = 20) at the study site. The observations were recorded throughout the year once in a fortnight on the following phenophases:

a. Flower bud initiation

b. Flowering and anthesis

c. Initiation of fruit, development and retention

d. Dehiscence and fruit fall

These phenophases were scored as given in the Table 3.2.

3.3.1 Seed dispersal

Observations on activities of seed dispersers were recorded in two periods within a day coinciding with the peak fruit maturation stage (from 25th to 31st July, 2003). One observation from 7.30 AM to 10.30 AM in the morning hours and other during the evening from 3.00 PM to 5.30 PM for one week to identify the seed dispersal agents. High fruit bearing trees at the seed dehiscing stage were selected for the observations.

3.4 Reproductive success

3.4.1 Flowers and fruits per inflorescence

At least 25 inflorescence collected randomly per tree were studied to determine the number of flowers per inflorescence and the per cent fruit set.

3.4.2 Seed set

The number of seeds per fruit at immature stage (when the diameter of the fruit was around 1 cm) and at mature stage (when the fruits turned yellow) was studied to assess fruit abortion over the developing period. At least 10 fruits per tree were considered for this study.

Table 3.2 Scores assigned to different phenophases in *D. malabaricum*

Item	Score
Flowering phenology	
Flower less	0
Bud initiation	1
Less than 50 % flowers are bloomed	2
More than 50 % flowers are bloomed	3
Fruiting phenology	
No fruit	0
Just set	1
Immature (small size)	2
Mature (fully grown - brownish)	3
Dehisce and fallen	4

3.4.3 Fruiting intensity

All the adult trees (n=110) found in the study site were marked and were categorized into classes of fruiting intensity by visually counting all the fruits as given below:

Category	**Number of fruits per tree**
Very high	More than 150
High	100-150
Medium	50-100
Very low	Less than 10
No fruit	0

3.4.4 Variation between trees for fruit and seed parameters

Fruit diameter, seed width, seed length, seed thickness and weight were recorded for a minimum of 25 seeds for each of ten trees.

3.5 Seed biology

3.5.1 Seed weight and density

Seed parameters such as 100 seed weight and seed density were recorded using bulk seed collection. Hundred-seed weight was measured using electronic balance and seed density was measured on volume basis as given below:

$$\text{Specific gravity} = \frac{\text{Weight of 100 seeds in grams}}{\text{Volume of water (ml) replaced by seed sample}}$$

3.5.2 Seed viability

The seed of *Dysoxylum malabaricum* being recalcitrant, looses its viability soon after its dispersal. After removing the pulpy seed coat, the

seeds were sown in sand with 10 replications of 25 seeds each, to ascertain its viability.

3.5.3 Seed longevity and critical moisture content

Sixty seeds were stored in each of the two airtight desiccators containing silica gel, which absorbs moisture and reduces the seed moisture content. A sample of twenty seeds was drawn at 5, 7, 10, 14 and 19 days after storing. About 15 seeds were soaked in water for 24 h and the germination was assessed using paper- towel method. The remaining 5 seeds were used to determine seed moisture content in oven following its drying for 48 h at 85° C or till constant weight was obtained.

3.6 Growth performance of half-sib families

Open pollinated seeds from ten isolated trees, which did not have overlapping canopy were colleted from the study site. The progenies of each of the mother tree were considered as half-sib and were raised in root trainers, filled with sand, separately. After about 60 days of sowing, they were transplanted from root-trainers to the poly-bags.

3.6.1 Shifting of seedlings

Sand, soil and FYM were taken in the ratio 1:1:1 and mixed well before filling the polythene bags of size 30 x 20 cm. The bags were provided with sufficient number of holes at the bottom to facilitate drainage. The bags were then filled upto about 5 cm below the surface and kept ready for potting the seedlings. Seedlings were transplanted to polythene bags after two months of their growth in the root trainers. These progenies were maintained in a glass house till one year. The labeled plants were randomly assigned to the positions within the glass house. The positions of the plants were regularly

interchanged to reduce the possible edge effect and/or effect of differential light intensities within the glass house.

3.6.2 Watering

The seedlings were watered once in two days using a rose-can.

3.6.3 Plant protection

After care of the seedlings in the nursery included, besides daily irrigation, weeding at regular intervals and their protection against defoliators and scale insect. Weeding was done at regular intervals. Soil drenching of Bavistin was made @ 1g/litre for all the polythene bags. Melathion was dusted on the leaf after the notice of defoliators. To reduce the infestation of scale insect, Monocrotophos was sprayed @ 1 ml/litre immediately after the notice of the pest.

3.6.4 Biometric and biomass observations

At the end of the experiment (one year), measurement of parameters such as height to first branch, internodal length, shoot length, root length, number of lateral roots, number of leaves, leaf area, fresh and dry weight of shoot and root were taken on five randomly selected seedlings from each family. At the end of the study the polythene bags were carefully split opened using a penknife and the seedlings were taken out with the root system intact. The soil sticking onto the roots were carefully removed by repeated washing in water. The seedlings were then spread out for a brief period of drying and the following observations were recorded.

3.6.4.1 Height to first branch

The height from the collar region to the first branch was measured using a measuring scale in centimeters.

3.6.4.2 Internodal length

The length of two internodes next to the first branch was measured and mean was taken as the internodal length.

3.6.4.3 Seedling height

The seedlings were spread on the table and the length of the shoot was measured from the tip of the growing point to the collar region using a measuring scale and the mean height in centimeters worked out.

3.6.4.4 Collar diameter

The collar diameter of the seedlings was measured using vernier calipers. As the stem is angular (and not round) the broader end was taken to measure the collar diameter.

3.6.4.5 Taproot length

The length of the taproot was measured from the collar region to the tip of the main taproot using a measuring tape. In the case of root coiling a thread was run along the root and then it was measured using scale. The mean length recorded in centimeters.

3.6.4.6 Number of lateral roots

The number of secondary lateral roots was counted for each seedling and the mean number was worked out.

3.6.4.7 Dry matter production per seedling

After counting, the leaves were first detached from the shoot and the fresh weight noted. The stem was detached from the taproot at the collar region and fresh weight of both stem and root were recorded. The leaves, stem and root were then kept separately in a hot air oven maintained at 80° C for 72 hours and dried to constant weight. The weight was then determined

using an electronic balance. The dry weight of stem, leaves and root were added together to obtain the total dry weight of a single seedling.

3.6.4.8 Leaf area per seedling

Leaf area was worked out based on both dry weight and fresh weight basis. Leaf area was measured on randomly selected 50 leaves from all families using leaf area meter and then the leaves were completely dried to get the dry weight of each leaf separately. A regression line fitting leaf area and dry weight and fresh weight was computed using MS Excel software as follows:

$Y = 190.83 x + 6.4669$ (dry weight basis)

$Y = 46.559 x + 6.6372$ (fresh weight basis)

Where Y = Leaf area in cm^2

x = dry weight of leaf in grams.

Using this relation, leaf area on individual seedling basis was worked out.

3.6.4.9 Seedling quality indices

The seedling quality indices such as sturdiness quotient (SQ) and quality index (QI) were determined using the following formulae.

$$SQ = \frac{H}{D}$$ (Thomson, 1985)

Where, H = Final shoot height (cm)

D = Final stem diameter (mm)

$$QI = \frac{TDW}{[SQ + (SDW/RDW)]}$$ (Dickson *et. al.*, 1960)

Where, TDW = Final total seedling dry weight (g)

SDW = Final shoot dry weight (g)

RDW = Final root dry weight (g)

3.7 Statistical analysis

The data relating to each character observed were analysed by analysis of various techniques using MSTAT programme on PC.

3.7.1 Estimation of genetic parameters

In order to estimate the genetic parameters the data were subjected to the ANOVA to divide the variability into genetic and environmental components. The following model was adopted:

Sl. No.	Source	Degree of freedom	Expected mean squares
1.	Between family (n)	(n-1)	$\sigma^2 + r\sigma^2_{hf}$
2.	Within family (r)	(r – 1)	$\sigma^2 + n\sigma^2_r$
3.	Error	(n-1) (r-1)	σ^2

Where n = Number of families

r = Number of progenies within a family.

σ^2 = Sum of squares due to error

σ^2_{hf} = Mean sum of square due to half-sib family (interpreted as genotypic variance)

σ^2_r = Mean sum of square due to male effect

V_E = Environmental variance computed as σ^2/r

Narrow sense heritability (h^2)

$$h^2 = \frac{V_A}{V_P} = \frac{V_A}{V_A+V_E}$$

Where, V_A = Additive variance

V_P = Phenotypic variance

Phenotypic coefficient of variation (PCV)

It is the measure of total variation existing in a particular character and was calculated as suggested by Burton and Devane (1953).

$$PCV\ (\%) = \frac{\sqrt{\sigma^2 P}}{X} \times 100$$

Where, X = Population mean for each trait

$\sigma^2 p$ = Phenotypic variance

Genotypic co-efficient of variation (GCV)

A measure of total genetic variability existing in a particular character and was calculated by using the formula as suggested by Burton and Devane (1953)

$$GCV\ (\%) = \frac{\sqrt{\sigma^2 C}}{X} \times 100$$

Where, X = Population mean for each trait

$\sigma^2 C$ = Genotypic variance

3.8 Vegetative propagation

Two experiments were conducted to standardize vegetative propagation technique using stem-cuttings. In both the CRD experiments the treatments were replicated three times, and the five cuttings were used per replication. Sand and coir pith in the ratio of 1:1 was used as rooting media. The whole experiment was conducted in a mist chamber in the month of June. For both the experiments, semi-hard cuttings of 15-20 cm length were used. The cuttings were treated with plant growth regulator at different concentrations as well as in their combination.

In the experiment-I the different treatments were as follows:

T1 - IBA 100 ppm

T2 - IBA 300 ppm

T3 - IBA 500 ppm

T4 - COU 100 ppm

T5 - Coumarin 300 ppm

T6 - Coumarin 500 ppm

T7 - IBA 100 ppm + Coumarin 100 ppm

T8 - IBA 300 ppm + Coumarin 300 ppm

T9 - IBA 500 ppm + Coumarin 300 ppm

T10 - Control

The cuttings were treated by prolonged dipping method for 24 hours.

In the experiment-II the treatments were as follows.

Ta - IBA 100 ppm

Tb - IBA 2000 ppm

Tc - IBA 3000 ppm

Td - Coumarin 1000 ppm

Te - Coumarin 2000 ppm

Tf - Coumarin 3000 ppm

Tg - IBA 1000 ppm + Coumarin 1000 ppm

Th - IBA 2000 ppm + Coumarin 2000 ppm

Ti - IBA 3000 ppm + Coumarin 3000 ppm

Tj - Control

The cuttings were treated by powder dip method where in the plant growth regulator was mixed with talc powder and treated to the base of the cutting for one minute.

3.8.1 Pre-treatment

The cuttings were given a slant cut at the base and the whole of the cutting was immersed in 0.1 per cent Bavistin for 6 hours to avoid fungal attack. Later cuttings were planted in trays containing rooting media and observed for sprouting and rooting.

The following observations were recorded.

3.8.2 Sprouting per cent

It was calculated using the formula

$$\text{Sprouting per cent} = \frac{\text{Number of sprouted cuttings}}{\text{Total number of cuttings}} \times 100$$

3.8.3 Rooting per cent

It was calculated using the formula

$$\text{Rooting per cent} = \frac{\text{Number of rooted cuttings}}{\text{Total number of cuttings}} \times 100$$

3.8.4 Mean number of roots, root length and number of leaves.

The above parameters will be calculated for every treatment separately.

Experimental Results

IV. EXPERIMENTAL RESULTS

4.1 Description of the flower, fruit and the seed

4.1.1 Description of flowers

The bisexual flowers of *Dysoxylum malabaricum* are white to greenish-yellow in colour and posses a mild attractive fragrance. The pedicel of flower is 1.35 mm in length and flowers are arranged in axillary-racemiform panicles. The average length of flowers is around 6.55 mm. Calyx is extremely short, finely pubescent outside and lobed deeply. Lobes are ovate and acute. There are four petals, which actually look like sepals. The androecium is fused together to form a cup like structure with an average diameter of 4.5 mm (plate 4.1). Anthers are sessile and 8 in number, alternate with the crenatures and attached at top third portion of the flower. Ovary densely pubescent tapering into 4 celled style with a maximum of eight ovules. Stigma is capitate and 4 lobes with an average length of 6.5 mm. A small quantity of nectar is present at the cup shaped tube, which attracts small insects such as thrips. Prominently no major pollinators such as honeybees were observed to visit the flowers.

4.1.2 Description of fruit and the seed

Fruit in *D. malabaricum* is borne on the old wood of the branch inside the canopy. They have a diameter of 5 to 8 cm, pyriform, verricose, and bright yellow in colour when ripe. It contains about 1 to 4 seeds, which are bluntly trigonous. The seed coat is pulpy and rich in fat. Testa of the seed coat is reddish brown and tegmen is orange in colour removal of which exposes the green cotyledons (Plate 4.1).

4.2 Reproductive phenology

The phenogram of various life history events of *D. malabaricum* is shown in the Fig 4.1. *D. malabaricum* flowers during the dry season of the year. The flower buds appear during mid January and it takes about 25 days to develop and reach the flowering stage. Flowering started during 2nd week of February and was extremely narrow. Most of the trees completed their blooming stage within 10 days and hence many were escaped from being observed for more than once. Soon after a week of seizure of the anthesis the immature fruits appeared during 1st of March. The immature fruits were green in colour and take nearly 3 ½ months to attain maturity, till 15th of June. The green fruits turned bright yellow during the maturation phase. The ripe fruits appeared in first week of June and dehiscent mature fruits were present until the last week of August. The mature fruit fall started from mid of June and it continued till the end of August (Plate 4.1).

The stage of fruit maturation and fruit fall clearly coincided with the rainfall season. The seeds being recalcitrant in nature get the much needed moist condition for germination. During this time the young seedlings are very sensitive to bright sunshine. The seeds get a brief period of 2 ½ to 3 ½ months for their establishment, which may vary with the rainy season. The late matured and dispersed seeds get lesser time for establishment and hence vulnerable for mortality. The peak fruit fall was the last week of July.

4.3 Seed dispersal

Three major bird species were found to visit and probe the matured fruits of *malabaricum*. Prominent among them is the Malabar Grey Hornbill (*Ocyceros griseus*) which made 92.5 per cent of the total visitations by all the

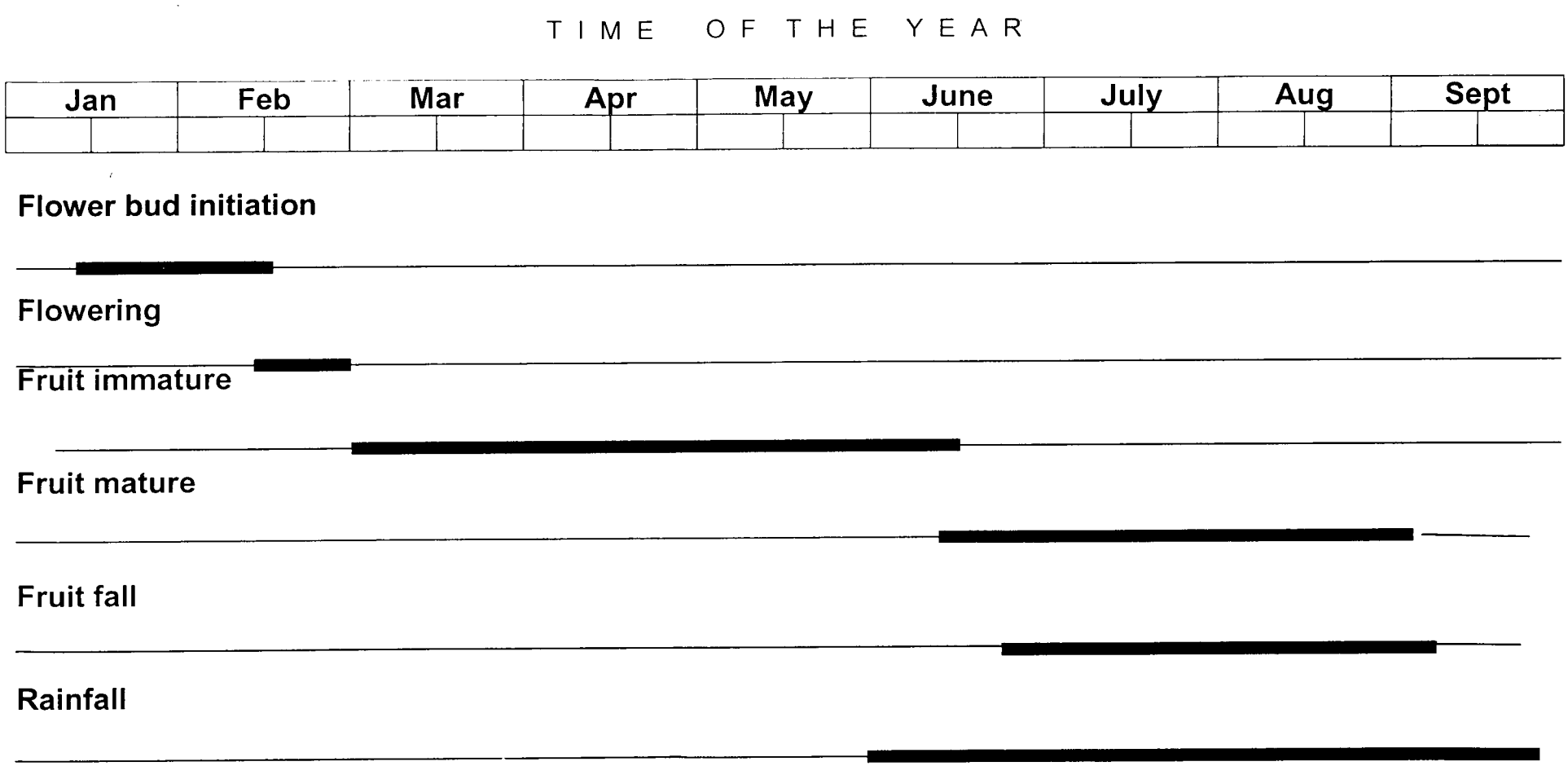

Figure 4.1 Phenogram showing various reproductive phenophases of *Dysoxylum malabaricum* in Sirsi forest division

Plate : 4.1

a) Flower bud initiation.
b) Flowers dropped from the tree.
c) Micro photograph of a single flower (arrow mark indicate petals).
d) Immature fruits.
e) Matured and dehiscing fruits exposing seeds (stage at which hornbills pick the seeds).
f) Fruit rinds and seeds under the canopy.
g) Close up of fruit, seed and seed without testa.

birds (Fig 4.2) when compared to Hill Myna (*Gracula indica*) which made 6.2 per cent visits and a fraction of the visits were made by Yellow-footed Green Pigeons (*Treron phoenicoptera;* Plate 4.2) Among 149 instances of visitation by Malabar Grey Hornbills, 69 per cent of the times they were found to pick at least one seed.

During fruit dehiscing phase, fruit splits into four parts exposing the reddish brown seeds. Hornbills, which had their nests in the nearby trees and bushes, approached the canopy of *D. malabaricum* trees in flocks of 15 birds, mostly during 8.15 – 9.30 AM. The birds first enter the canopy and sit on the branch. Then they hop on it and come near the fruits. Then it picks the seed with its large beak and swallow by lifting its neck. Then they search for seeds in the same fruit, and if found, it will swallow. If not, they move towards other fruits. They swallow around 1-2 seeds at a time. Immediately after they swallow the seeds, they fly away from the tree and sit on nearby trees. After about 10 minutes, they regurgitate the cotyledons devoid of seed coat. These regurgitated seeds devoid of pulpy seed coat were not attacked by the fungus on the forest floor, enhancing probabilities of germination. The Malabar Grey Hornbills, in addition to dispersal of the seeds gives an effective seed pre-treatment and aid in germination.

Even though hill mynas and pigeons were also found to approach the fruit they were not able to effectively pick and swallow the big seeds due to their small beak and gape. Snails and millipedes were also found to feed on the seed coat and aid in its germination (Plate 4.2).

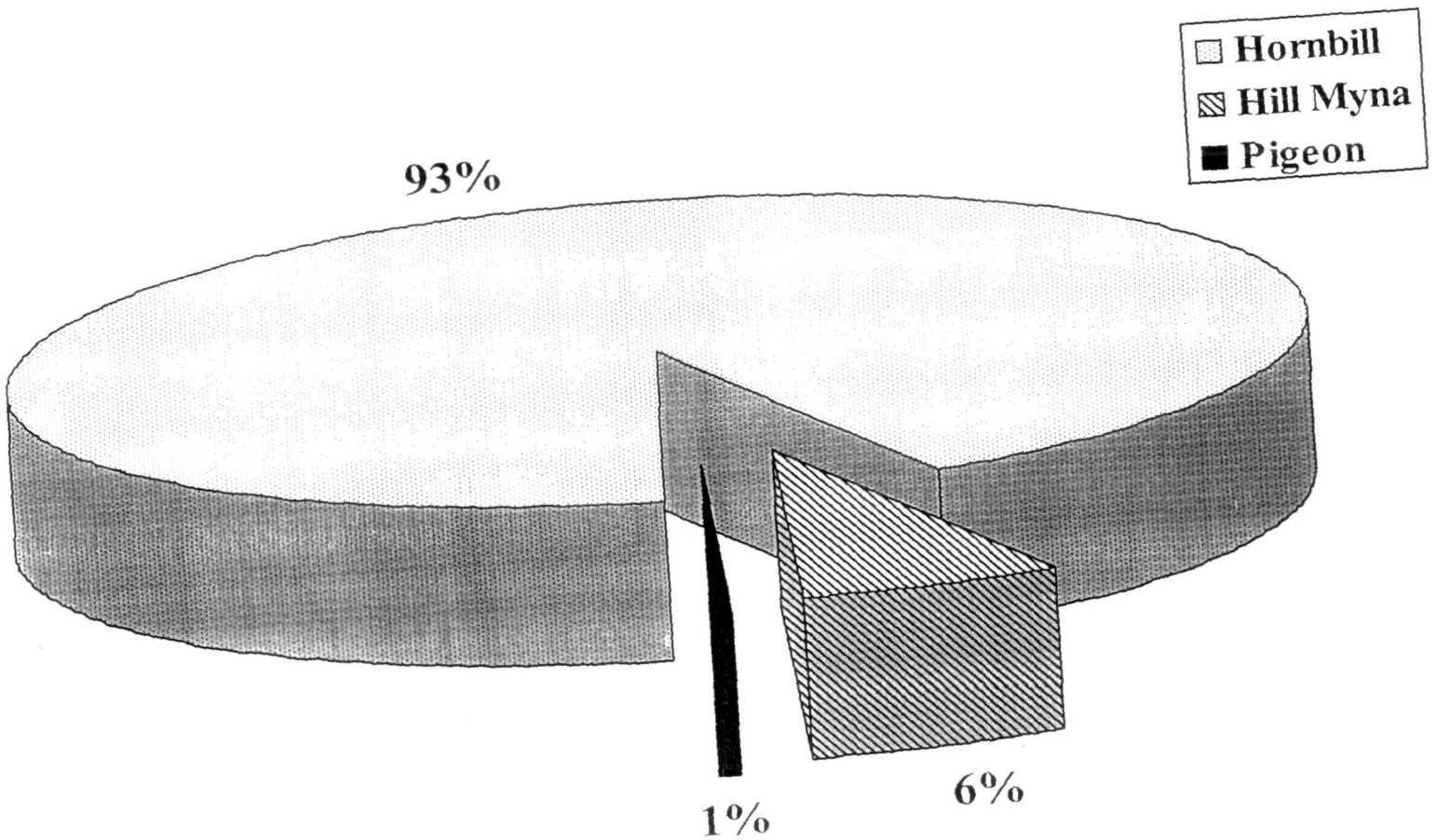

Fig 4.2 Per cent visitation by different birds to *Dysoxylum malabaricum (total number of birds n= 160)*

Plate : 4.2

a) Malbar Grey Hornbill perching on the canopy.
b) Malbar Grey Hornbill *(Ocyceros griseus)*
c) Yellow-footed Green Pigeon *(Treron phoenicoptera)*
d) Hill myna *(Gracula indica)*
e)-f) A land Snail and a millipede predating on fat rich seed testa of *D.malabaricum.*
g) Epigeal germination of *D.malabaricum.*
h) An young recruit.

4.4 Reproductive success

4.4.1 Flowers per Inflorescence

The number of flowers per inflorescence ranged from 3 to 38 with a mean of 15.

4.4.2 Per cent fruit set

Observation on percent fruit set was restricted to trees, which had high fruiting intensity class. The average fruit set per inflorescence was 23.9 per cent, which ranges from 6.6 to 45.71 percent, however, this may be an over estimation of the population level fruit set per cent.

4.4.3 Comparison between Initial and final seed set

The distribution of number of seeds per fruit during initial stage and final stage of fruit maturation is depicted in Fig. 4.3. The two distributions differed significant (K.S. test $D_{max} = 0.3$ $p<0.01$). In the early stage, a large proportion of fruits possessed four seeds. Interestingly during late maturation stage, a large number of fruits consisted of only 2 seeds.

4.4.4 Intensity of fruiting

About 110 trees were scored during the peak fruiting season to categorize them into classes of fruiting intensity. Trees bearing about 150 fruits or more, where categorized as 'very high group'. Similarly the trees with 100-150 fruit per tree were grouped into 'high group. Those with 50 to 100 and 10 to 50 fruits per tree were categorized into 'medium group' and 'low group', respectively. The trees with less than 10 fruits were category as 'very-low fruiting intensity'. The distribution of these trees into different classes is shown in the Table 4.1.

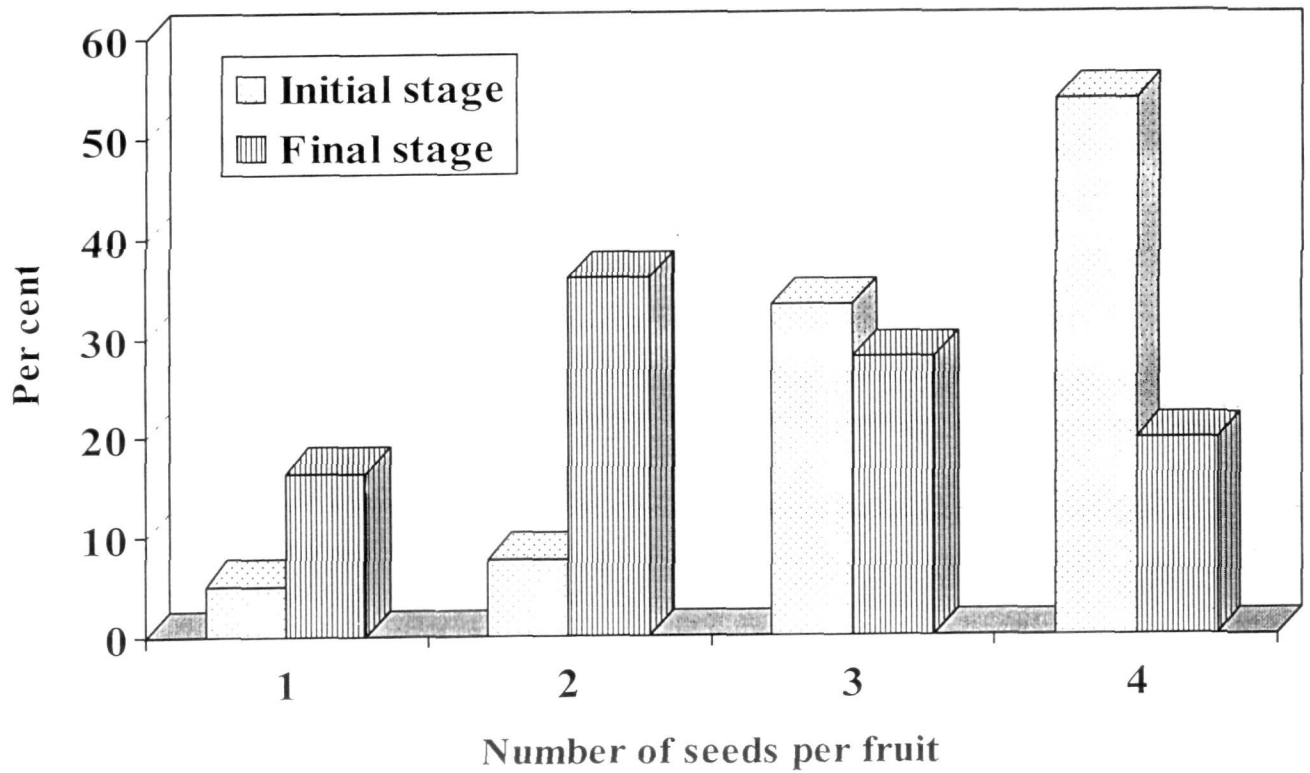

Fig 4.3 Distribution of number of seeds per fruit in initial (n=35) and final stage (n=61) of fruit maturation in *Dysoxylum malabaricum* ($D_{max} = 0.342$)

Table 4.1 Fruiting intensity among *D. malabaricum* trees

Category	Number of fruits per tree	Percent of trees (%)
Very high	> 150	3.6
High	100-150	9.0
Medium	50-100	17.27
Low	10-50	12.7
Very low	< 10	9.0
No fruiting	0	48

Forty-eight per cent trees did not bear fruit. Among the fruiting trees, the 'medium fruiting group constituted the largest group (17.27%), followed by 'low fruiting intensity' group (12.7%). About 9.0 per cent and 3.6 per cent of the trees showed 'high' and 'very high' fruiting intensity, respectively (Fig. 4.4).

4.5 Seed biology

The seeds of *D. malabaricum* are recalcitrant in nature and lose their viability with reduction in seed moisture content.

4.5.1 Test weight (100 seeds weight)

Average weight of 100 seeds without seed coat was 515 g with a range of 493 to 537 g. Hence, one kg of seeds contains about 200 seeds.

4.5.2 Seed density

Average seed density was 1.154 g cc^{-1}, which ranges from 1.073 to 1.189 g cc^{-1}.

4.5.3 Moisture content of fresh seeds

The freshly fallen seeds were used to determine moisture content of seeds. Average moisture content of fresh seeds was about 54.78 per cent, and ranged from 52.5 to 57.0 per cent.

4.5.4 Germination

Dysoxylum malabaricum shows an epigeal type of germination, where the cotyledons emerge out of the soil along with the plumule (Plate 4.2). On an average it takes about 12 days for radicle emergence and 27 days for plumule emergence. The cotyledons are green in colour and attached to the seedlings for over 6 months under laboratory conditions.

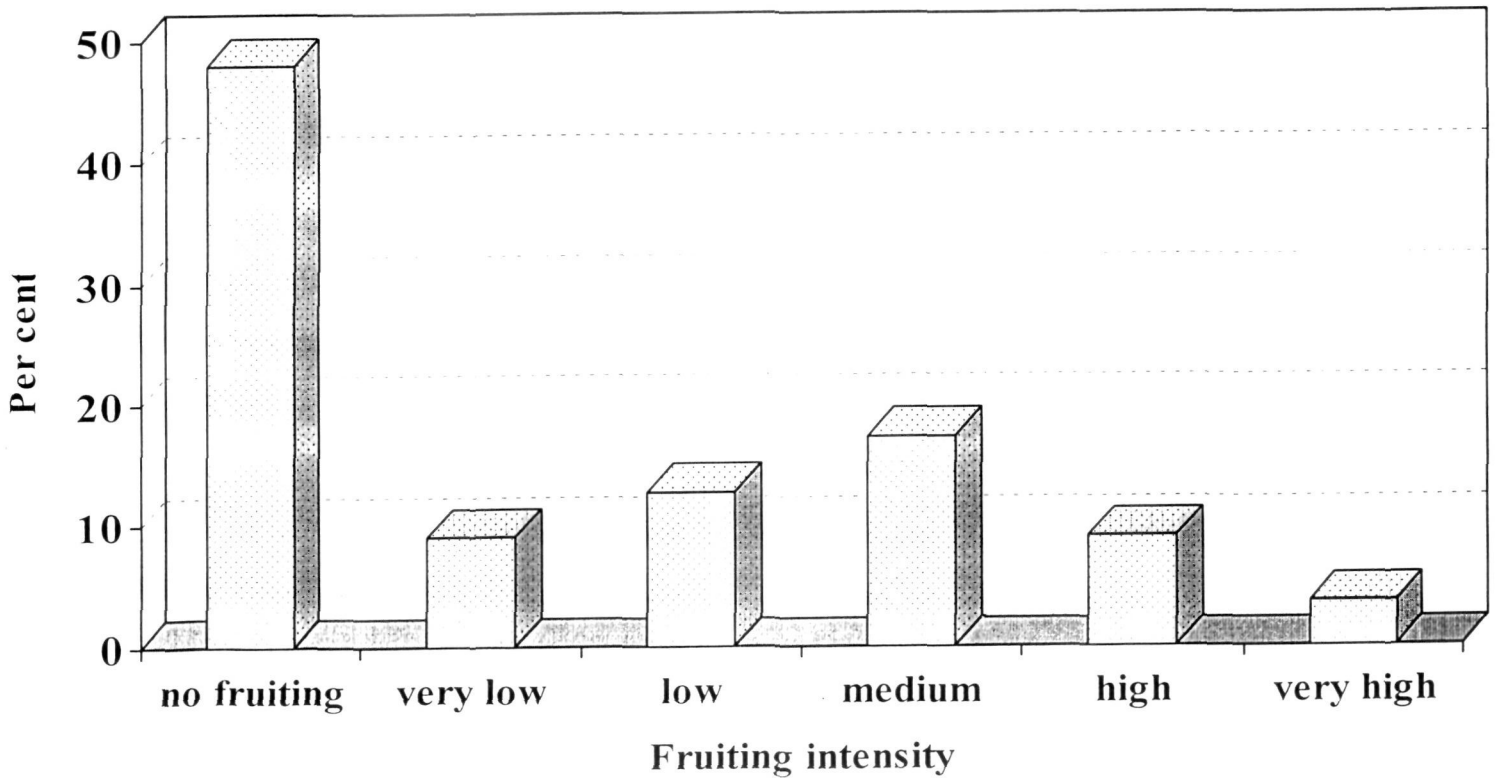

Fig 4.4 Distribution of trees into classes of fruiting intensity in *Dysoxylum malabaricum (number of trees considered =110)*

Each species requires its own characteristic set of conditions for successful germination. During the field observations, it was noticed that seeds dispersed along with seed coat were infected with saprophytic fungi than those that were dispersed as naked cotyledons **(Plate 4.3)**. In order to test whether retention of seed coat on the seed hinders the germination, a simple experiment was conducted. Twenty seeds each with and without seed coat were sown on a sterile sand medium and were watered regularly. Only 10 per cent of seeds with seed coat recorded germination after 60 days, while 100 per cent germination was observed in seeds sown without seed coat (Fig. 4.5).

4.5.5 Seed pest

The seeds were infested by fruit fly larva (unidentified, Family: Diptera), which are white in colour and feed on the cotyledons and embryo and make them non-viable **(Plate 4.3)**.

4.5.6. Variation among fruit and seed parameters

The variation among ten trees for fruit and seed parameters is given in the Table 4.2. The fruit had a mean diameter of 5.45 cm and the seeds had a mean of length of 3.04 cm, width of 2.00 cm, thickness of 1.41 cm and a weight of 5.56 g.

4.5.7 Effect of storage on seed moisture and germination

Seeds stored under zero relative humidity using silica gel in a desiccator did not loose moisture content quickly. The moisture content was around 50 per cent till ten days of storage. Consequently, the germination was also high (100%). However, after 14 days of storage, the moisture content reduced to 45 per cent and germination also reduced to 66 per cent (Table

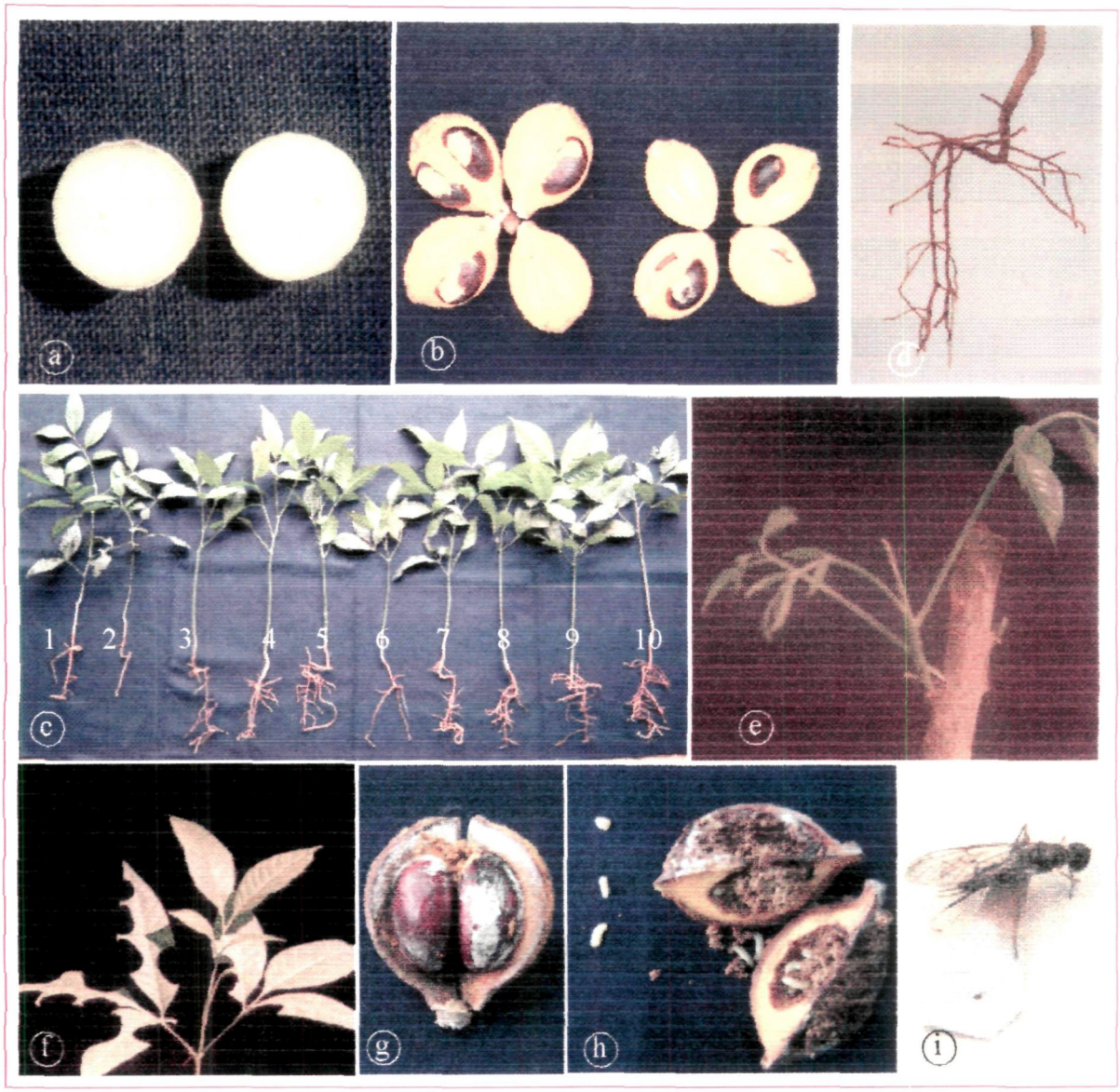

Plate : 4.3

a) Initial seed abortion in *D. malabaricum.*

b) Final seed abortion.

c) Variation for root and shoot characteristics among ten half-sib families.

d) A close-up of root system

e) A stem cutting showing good sprouting.

f) Seedling predated by grasshopper.

g) Fruit infected with fungus.

h) - i) Fruit infected with larva; adult stage

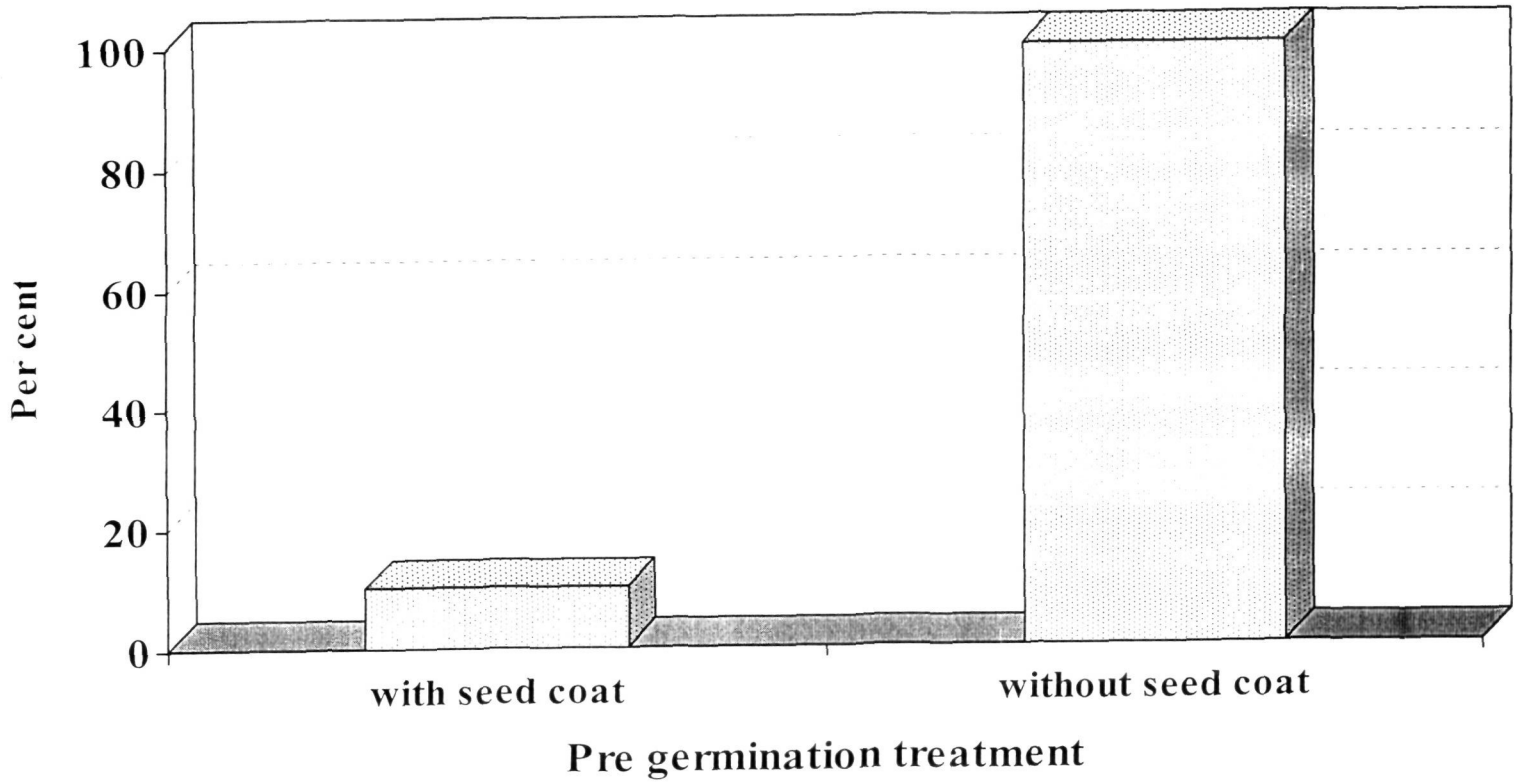

Fig 4.5 Effect of seed coat removal on per cent germination in *Dysoxylum malabaricum (number of seeds=40)*

Table 4.2 Variation for fruit and seed parameters among ten trees (n=200).

Parameter	Range		Mean ± SD
	Minimum	Maximum	
Fruit Diameter (cm)	4.73	6.55	5.45 ± 0.52
Seed length (cm)	2.63	3.38	3.04 ± 0.24
Seed width (cm)	1.85	2.18	2.00 ± 0.11
Seed thickness (cm)	1.30	1.52	1.41 ± 0.08
Seed weight (g)	4.62	6.97	5.56 ± 0.89

4.3). At 19 days of storage, the moisture content dropped to 30 per cent and none of the seeds germinated at this moisture content (Fig 4.6 & 4.7).

4.6 Variation for germination and initial growth of half-sib families

The variation for seed weight, number of days for radicle and plumule emergence, root and shoot length after two months of sowing is presented in the Table 4.4. There was a significant difference among the families for seed weight, number of days to plumule emergence, root length and shoot length. Number of days required for radicle emergence did not show significant difference. However, highest coefficient of variation was 38.31 per cent observed for radicle emergence.

4.6.1 Seed weight

Seed weight showed significant differences among half-sib families of *D malabaricum*. Considering the mean values of all the families, the seed weight was highest (7.22 g) in family 2 and it was lowest in family 6 (4.34 g).

4.6.2 Days for radicle emergence

On an average about 12 days were required for radicle emergence in all families. However, the differences among families were not statistically significant.

4.6.3 Days for plumule emergence

On an average about 27 days were required for plumule emergence, which ranged between 23.47 (family 4) and 32.47 days (Family 9).

4.6.4 Shoot length

There was a moderate variation among the families (CV= 22.49 %) for shoot length. Family 10 had the highest shoot length of 15.57 cm and family 1

Table 4.3 Effect of storage on seed moisture content and germination in *D. malabaricum* (n=20 per treatment).

Sl. No.	Days in storage	Seed moisture content X ± SD	Germination (%)
1	5	48.79 ± 1.69	100
2	7	52.84 ± 3.98	100
3	10	53.11 ± 2.20	88
4	14	45.38 ± 5.16	66
5	19	30.84 ± 3.12	0

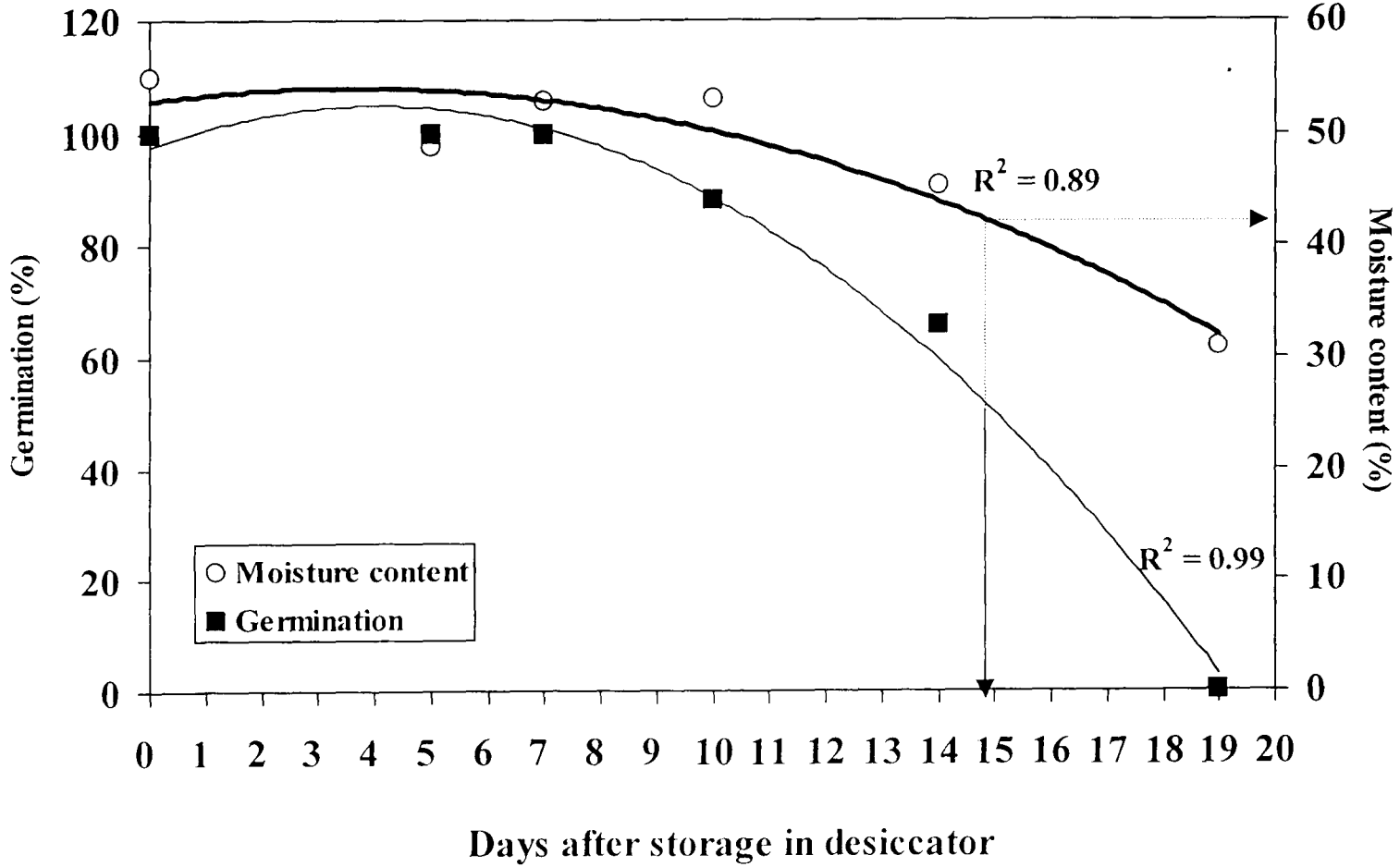

Fig 4.6 Effect of storage on seed moisture content and germination in *Dysoxylum malabaricum*
(the arrows indicate the days required for 50 % reduction in germination and the corresponding moisture content)

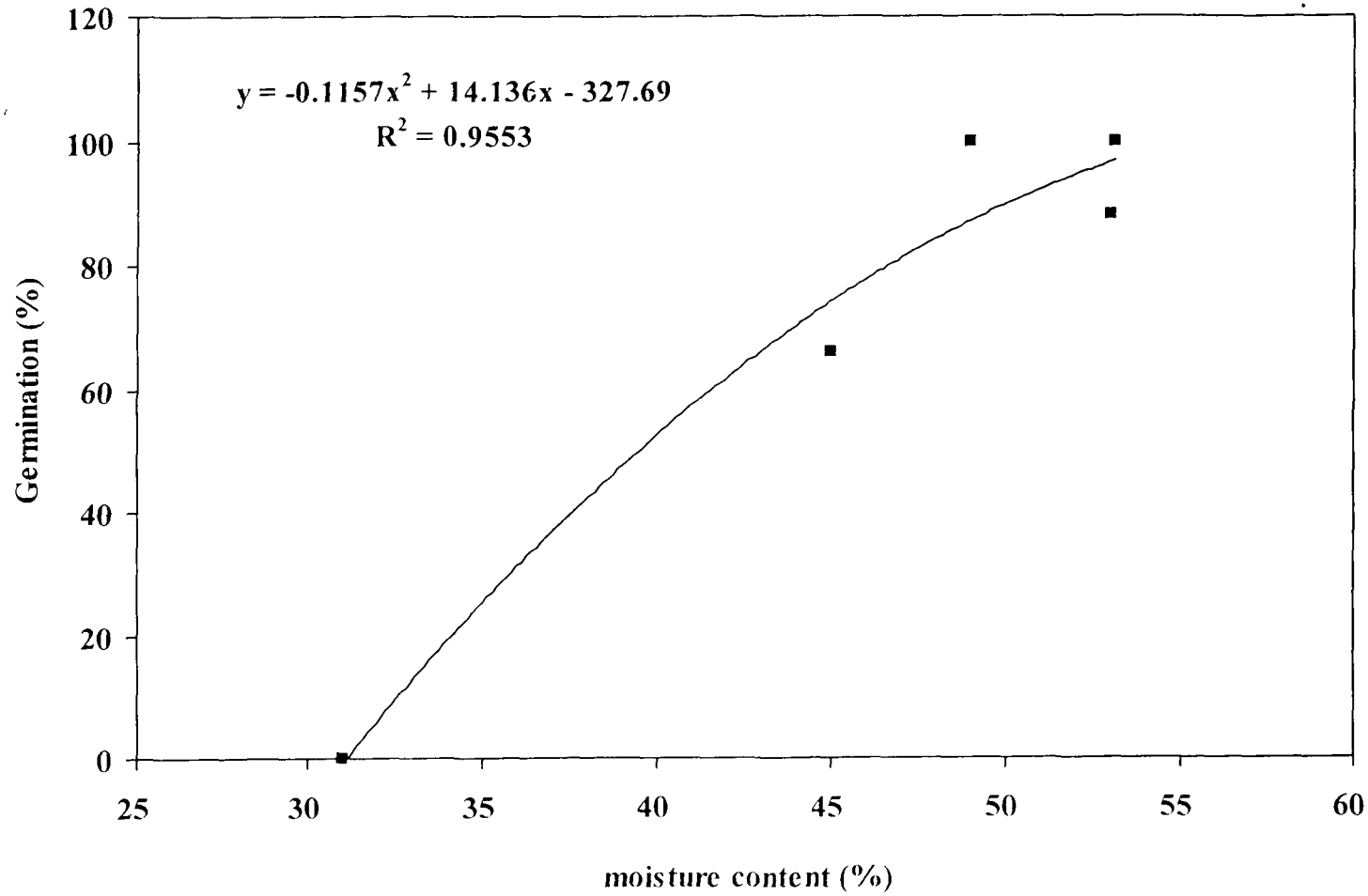

Fig 4.7 Relation between seed moisture content and germination percentage in *Dysoxylum malabaricum*

Table 4.4 Variation for seed germination and initial growth of seedlings belonging to different half-sib families of *D. malabaricum*

Family Number	Seed Weight (g)	Days for radicle emergence	Days for plumule emergence	Root length after 60 days	Shoot length after 60 days
1.	5.57	12.93	26.73	13.21	10.57
2.	7.22	13.10	27.80	14.60	11.80
3.	5.96	12.33	27.60	15.30	12.67
4.	6.00	10.60	23.47	16.70	13.83
5.	4.50	12.60	28.27	15.10	14.17
6.	4.34	13.00	23.80	13.96	14.67
7.	4.78	11.47	28.10	13.50	13.37
8.	4.80	09.73	27.30	14.07	15.00
9.	6.22	10.60	32.50	13.40	12.90
10.	5.37	10.53	28.33	14.03	15.57
Grand Mean	5.44	11.7	27.38	14.38	13.45
F ratio*	**8.58**	**1.16**	**2.39**	**2.95**	**3.82**
P-level	**<0.01**	**0.05**	**0.05**	**<0.01**	**<0.01**
CD at 0.05 %	**0.84**	**3.24**	**4.54**	**1.73**	**2.19**
CV (%)	**21.49**	**38.31**	**22.97**	**16.72**	**22.49**

*** Degrees of freedom (df) at 9 and 126**

had the lowest shoot length of 10.57 cm. On an average the family has a shoot length of 13.45 cm (Plate 4.3).

4.6.5 Taproot length

Mean root length of 14.36 cm was observed among families of *D malabaricum.* Family 4 had the highest mean root length of 16.7 cm with family 1 having the lowest root length of 13.21 cm.

4.7 Variation for biometric characteristics among half-sib families

The vigour characteristics among the 10 families for height to first branch, internodal length, shoot height, collar diameter, number of leaves, leaf area, root length, number of lateral roots and sturdiness quotient after one year of growth are presented in the Table 4.5.

Among all the parameters mentioned above, height to first branch, shoot height, number of lateral roots and Sturdiness Quotient (SQ) showed significant differences among families. Number of lateral roots had the highest variation (CV= 39 %) and collar diameter recorded least CV of 15.74 per cent. The biometric observations on randomly selected five seedlings from each of the 10 families are presented in the following paragraphs.

4.7.1 Height to first branch

Mean height to first branch was 13.1 cm recorded among 10 families of *D. malabaricum.* Family 9 recorded the highest mean value of 15.94 and family 1, the lowest of 10.1 cm.

4.7.2 Internodal length

There was moderate variation (CV = 26.75 %) among the families for internodal length and it did not vary significantly. Considering the mean value

Table 4.5 Variation for biometric characters of seedlings belonging to different half-sib families of *D. malabaricum*

Family Number	Height to first branch (cm)	Inter nodal length (cm)	Shoot height (cm)	Collar diameter (cm)	Number of Leaves	Leaf area (cm^2)	Root length (cm)	Number of lateral roots	SQ
1.	10.10	2.63	19.60	0.30	4.6	253.47	29.48	7.40	6.66
2.	10.48	2.13	18.72	0.33	5.4	214.81	29.00	9.80	5.75
3.	11.96	2.68	21.62	0.33	4.2	263.05	31.52	20.40	6.59
4.	12.70	2.72	21.12	0.33	5.2	296.37	37.90	17.20	6.50
5.	12.40	2.99	20.72	0.31	4.8	277.63	36.80	15.80	6.70
6.	14.36	2.21	20.40	0.27	4.6	253.09	34.80	14.00	7.56
7.	13.12	2.45	20.60	0.32	5.2	354.54	32.10	23.20	6.51
8.	14.32	1.80	19.60	0.25	4.0	183.10	25.90	13.40	8.05
9.	15.94	2.92	25.88	0.33	5.8	334.80	31.40	20.80	7.91
10.	15.60	2.90	24.14	0.34	5.4	372.63	32.70	23.40	7.51
Grand mean	13.10	2.54	21.31	0.31	4.92	280.40	32.16	16.54	6.97
F ratio*	**3.40**	**1.64**	**2.39**	**1.76**	**1.43**	**1.90**	**1.07**	**3.49**	**2.49**
P-level	**<0.01**	**0.14**	**0.03**	**0.11**	**0.21**	**0.08**	**0.4**	**<0.01**	**0.05**
CD at 0.05 %	**3.08**	**0.87**	**4.36**	**0.06**	**1.38**	**125.46**	**10.1**	**8.42**	**1.35**
CV (%)	**18.31**	**26.75**	**15.94**	**15.74**	**21.88**	**34.89**	**24.48**	**39.71**	**15.16**

*** Degrees of freedom (df) at 9 and 36** **SQ= Sturdiness quotient**

of the families, family 5 (2.99 cm) had the highest internodal length, where as family 8 (1.8 cm) had the lowest internodal length.

4.7.3 Shoot height

The shoot height ranged from 18.72 cm (family 2) to 25.88 cm (family 1) with a mean value of 21.34 cm.

4.7.4 Collar diameter

Families did not differ significantly for seedling collar diameter. The collar diameter ranged from 0.25 cm (family 8) to 0.34 cm (family 10) with an average of 0.311 cm. Comparatively a low CV of 15.74 per cent was observed.

4.7.5 Number of leaves

The variation among families did not show statistical significant difference among families for number of leaves produced per seedling after one year of growth. Considering the mean values, family 9 had the highest number of leaves (5.8), while family 8 exhibited the least number (4).

4.7.6 Leaf area

Leaf area did not vary significantly among families. Coefficient of variation was high (34.89 %) in leaf area. Considering the mean values of the families, family 10 had the highest leaf area of 372.63 cm^2, followed by family 7 (354.54 cm^2) and family 8 had the lowest leaf area of 183 cm^2. Average leaf area recorded in one year old seedling of *D. malabaricum* was 280.349 cm^2.

4.7.7 Taproot length

Length of taproot did not show significant variation among 10 half-sib families. A mean taproot length of 32.16 cm was recorded among families,

which ranges from 29.0 (family 2) to 37.9 cm (family 4). Taproot length observed a moderate CV of 24.48 per cent.

4.7.8 Number of lateral roots

Number of lateral roots varied significantly among families. Mean number of lateral roots was 16.54, which ranges form 7.4 (family 1) to 23.4 (family 10). The variation among families was relatively high (CV = 39.71 %).

4.7.9 Sturdiness quotient

The sturdiness quotient varied significantly among families, but had low variation (CV = 15.16 %). Considering the mean values, family 8 recorded the highest value of 8.05 and family 2 registered the lowest of 5.75. Average sturdiness Quotient of 6.97 was recorded in *D. malabaricum* at the age of one year.

4.8 Variation for biomass characteristics among half-sib families

The results obtained on the biomass characteristics of seedlings of different families are presented in the Table 4.6. Highest co-efficient of variation was 37.28 pre cent observed in dry root weight, followed by Quality Index (36.83 %). The lowest co-efficient of variation of 25.56 per cent was recorded in shoot : root ratio.

4.8.1 Fresh weight of shoot

The families did not vary significantly for fresh shoot weight. However, co-efficient of variation was found to be highest (33.77%) in this trait. Family 9 had the highest mean of 8.19 g, where as family 8 had the lowest mean value of 3.94 g

Table 4.6 Variation for biomass characters of seedlings belonging to different half-sib families of *D. malabaricum*

Family Number	Fresh root weight (g)	Fresh shoot weight (g)	Dry root weight (g)	Dry shoot weight (g)	Shoot:Root ratio	QI
1.	2.35	5.56	0.67	1.69	2.52	0.25
2.	2.42	5.22	0.72	1.50	2.14	0.30
3.	3.27	6.10	1.02	1.92	1.98	0.35
4.	3.71	7.13	1.14	2.18	1.94	0.40
5.	2.90	5.98	0.95	1.95	2.15	0.33
6.	2.54	5.70	0.74	1.76	2.45	0.26
7.	3.16	7.74	0.95	2.39	2.50	0.37
8.	1.90	3.94	0.58	1.27	2.01	0.19
9.	3.19	8.19	1.06	2.44	2.36	0.34
10.	3.73	7.94	1.33	2.72	2.18	0.44
rand Mean	2.92	6.34	0.92	1.98	2.22	0.34
F ratio *	**2.25**	**2.06**	**2.38**	**2.19**	**0.74**	**2.00**
P-level	**0.04**	**0.06**	**0.03**	**0.04**	**0.66**	**0.06**
CD at 0.05 %	**1.154**	**2.75**	**0.44**	**0.88**	**0.73**	**0.15**
CV (%)	**30.84**	**33.77**	**37.28**	**34.45**	**25.56**	**36.83**

*** Degrees of freedom (df) at 9 and 36 QI= Quality index**

4.8.2 Dry weight of shoot

Shoot dry weight was statistically significant among families (Table 4.6), which ranged between 2.72 (family 10) and 1.27 (family 8)

4.8.3 Dry weight of root

Significant variation was also observed for this trait. The highest family mean value was 1.33g attained by family 10 and the minimum was 0.67g recorded by family 1.

4.8.4 Shoot : root ratio

There was no significant difference among families for shoot : root ratio. Based on mean value, highest shoot : root ratio of 2.519 was observed in family 1, followed by family 7 (2.5) and it was least in family 4 (1.94). This suggests that the distribution of biomass to different above and below ground parts does not vary among families.

4.8.5 Quality Index (QI)

Even though the variation for quality index was high (36.83 %), the families did not vary significantly. Family 4 had the highest QI of 0.397 and family 8 had the lowest value of 0.188.

4.9 Estimation of genetic variance, heritability and other genetic parameters

Estimation of genetic variance, heritability and other genetic parameters (such as phenotypic co-efficient of variation and genotypic co-efficient of variation) for seed weight, germination, biometric and biomass characteristics are presented in Table 4.7.

The phenotypic co-efficient of variation as well as genotypic co-efficient of variation was highest for number of lateral roots (33.16 %, 28.0 %,

Table 4.7 Estimation of genetic variance, heritability and other genetic parameters for *D. malabaricum*

Variable	σ_G^2	σ_P^2	h^2	PCV (%)	GCV (%)
Seed weight	0.69	0.78	0.88	16.23	15.27
PlumuleS emergence	3.68	6.32	0.58	9.18	7.01
Root length	0.75	1.13	0.66	7.41	6.02
Shoot length	1.72	2.33	0.74	11.35	9.75
Height first branch	2.76	3.91	0.71	15.10	12.68
Number of lateral root	21.45	30.08	0.71	33.16	28.00
Shoot length	3.28	5.54	0.58	11.03	8.42
Fresh root weight	0.20	0.36	0.55	20.69	15.42
Fresh stem weight	0.07	0.11	0.63	22.28	17.64
Fresh leaf weight	0.54	1.2	0.46	22.25	15.06
Dry root weight	0.03	0.06	0.58	25.74	19.59
Total shoot dry weight	0.11	0.20	0.54	22.81	16.82
Total dry weight	0.27	0.45	0.60	23.28	18.03

respectively) and lowest for root length (9.18 %, 7.01 % respectively). Heritability values on family means ranged from 0.46 (fresh leaf weight) to 0.88 (seed weight). The shoot height, height to first branch and number of lateral roots showed higher heritability values of 0.74, 0.71 and 0.713, respectively.

4.10 Association studies

Correlation analysis for seed weight and days for germination was non significant (Table 4.8). Seed weight was negatively correlated with days for radicle emergence, suggesting that bigger seeds take more number of days for radicle emergence. The root length was positively correlated with seed weight and significant, indicating that bigger size seed give longer root leading to better establishment in field.

.4.11 Vegetative propagation

Data on per cent sprouting of stem cuttings in different plant regulator treatments under prolonged liquid-dip method as well as quick powder dip method are presented in the Table 4.9 and Table 4.10, respectively. Sprouting was obtained in most of the treatments even though its percentage was low. The sprouting percentage was relatively higher in the experiment - II (Quick powder dip method), when compared to experiment - I (cuttings were treated by prolong soaking for 24 h). The highest sprouting of 20 per cent was obtained in IBA 2000 ppm, (Plate 4.3) followed by the IBA 3000 ppm. The control also had a sprouting of 19 per cent. However, in both the experiments no rooting was obtained in any of the treatments.

Table 4.8 Association among seed mass and germination parameters in *D. malabaricum* (n=200).

Characters	Pearsons correlation Co-efficient	Significance
Days for radicle emergence	-0.072	ns
Days for plumule emergence	0.109	ns
Root length	0.225	*
Shoot length	- 0.186	*

* Significant at P-Level = < 0.05 % ns = Non significance

Table 4.9 Sprouting percentages among the stem cuttings of *D. malabaricum* subject to 24 h dipping in different plant growth regulators.

Sl. No.	Treatment	Sprouting (%)
1.	T1 – IBA 100	2.2
2.	T2 – IBA 300	2.2
3.	T3 – IBA 500	4.3
4.	T4 – COU 100	0
5.	T5 – COU 300	0
6.	T6 – COU 500	4.4
7.	T7 – IBA 100 + COU 100	0
8.	T8 – IBA 300 + COU 300	0
9.	T9 – IBA 500 + COU 500	2.2
10.	T10 – Control	0

Table 4.10 Sprouting percentages among the stem cuttings of *D. malabaricum* subject to powder dip method in different plant growth regulators.

Sl. No.	Treatment	Sprouting (%)
1.	T1 – IBA 1000	11.0
2.	T2 – IBA 2000	20.0
3.	T3 – IBA 3000	19.1
4.	T4 – COU 1000	4.4
5.	T5 – COU 2000	4.4
6.	T6 – COU 3000	2.2
7.	T7 – IBA 1000 + COU 1000	11.0
8.	T8 – IBA 2000 + COU 2000	4.4
9.	T9 – IBA 3000 + COU 3000	13.0
10.	T10 – Control	19.1

Discussion

V. DISCUSSION

Dysoxylum malabaricum Bedd. (Meliaceae) is a large top-canopy evergreen tree distributed only in the evergreen forests of the Western Ghats from Uttara Kannada southwards up to Travancore (Troup, 1986). It is a high-quality timber yielding species valued for its lustrous and cedar scented timber whose price is comparable with that of teak in the present day market. Its wood also has medicinal properties and used in Ayurvedic medicine to cure rheumatism as well as disorders of eyes and ears. For its valuable timber it has been harvested both legally and illegally from its natural habitat, resulting in reduction of its population to few small fragmented pockets in Uttara Kannada, Kodagu districts of Karnataka (Ramesh and Pascal, 1997). Foundation for Revitalization of Local Health Tradition (FRLHT), which prepared a list of 112 threatened medicinal plants of South India, has assigned 'endangered-globally' threat status on to it (Ravikumar and Ved, 2000). In spite of this, hardly any effort has been made to conserve its genetic resources. As on today, there are no established plantations of this species.

Developing any conservation strategy requires basic information regarding its breeding system, reproductive phenology, silvicultural requirements and its mutualistic association with other components of the ecosystem. Economic exploitation of the species by domestication will help in meeting the demand and reduce the pressure on natural populations. This requires genetic improvement of the species, which has the highest potential of all forestry practices for increasing the volume per unit area and unit time (Zobel and Talbert, 1984). To achieve this, basic data regarding the amount

of variation present in the natural population and genetic basis of this variation for economical traits is required. Early selection at seedling stage for vigour and growth habit and developing juvenile-mature correlation for traits of economic importance is the quickest way by which this can be achieved. Except for exotic tree species and a few plantation species, genetic potential of many indigenous trees such as *D. malabaricum* are untapped and needs urgent steps to improve them.

With this background the present study was carried out with the following objectives:

1. To understand a few aspects of breeding system and seed biology of *Dysoxylum malabaricum.*
2. To assess the level and degree of genetic variation for germination and early seedling vigour among the half-sib families of *Dysoxylum malabaricum.*
3. To standardize a vegetative propagation technique for *Dysoxylum malabaricum.*

The results obtained in the present investigation are discussed in these chapters under the following headings.

5.1 Reproductive phenology of *D. malabaricum.*

5.2 Floral and pollination biology.

5.3 Fruiting and seed dispersal.

5.4 Reproductive fitness.

5.5 Half-sib family structure and heritability.

5.6 Vegetative propagation.

5.7 The conservation concerns and future line of work.

5.1 Reproductive phenology of *D. malabaricum*

5.1.1 Flowering phenology

Flowering of tree crops is a highly complex process involving many developmental stages. Trees must interact with the environmental factors at all times of the year, and flowering is often closely related to seasonal climate change (Sedgley and Griffin, 1989). The first stage in the flowering process is floral induction, when the vegetative meristem becomes programmed to change into a reproductive meristem. There have been relatively few studies on floral induction in tree crops, with the exception of apple. Tree crops vary enormously in the timing of floral initiation in relation to anthesis. In general the period between initiation and flowering is correlated with the growth habit of the tree, which is in turn governed by the climatic range of the species. The flower bud initiation in *D. malabaricum* started one month before flowering during January (Fig. 4.1). Flowering is essentially a growth process, however, the nature of the stimulus required to trigger this process is not completely understood. Most tropical and sub tropical tree species have a very short period between floral initiation and anthesis (Sedgley and Griffin, 1989). Flowering period in *D. malabaricum* was short for only around ten days during second week of February, coinciding with the dry season. This is surprisingly a very narrow window of time for blooming of an evergreen tree species.

5.1.2 Fruiting phenology

In temperate regions the seeds and fruits of most tree species ripe and shed during the autumn or early winter (Kreugman *et al.*, 1974). This is a natural consequence of the necessity to flower and develop fruit during the

restricted period of the year when temperatures are favourable for growth. However fruiting phenology among tropical trees, as reviewed by Howe and Small Wood (1982), ranges from extremely seasonal among forests with a distinct dry season to aseasonal among forests with rainfall throughout the year.

Since fruiting is but one step in the continuum of the plant's life cycle it is difficult to demonstrate that such variation is a direct adaptive response, rather than an inevitable developmental consequence of variation in flowering time or of selection for optimal seed germination and establishment. However, the weight of evidence suggests that adaptation to dispersal opportunities is not uncommon. The wind-dispersed fruits tend to be produced during the dry season among deciduous forests of Western Costa Rica (Frankie *et al.,* 1974), when conditions are most suitable for desiccation and flight while vertebrate-dispersal fruits tend to be produced during the wetter months. These forests, therefore, provide a year-round food resource and have evolved a number of obligate frugivores. *D. malabaricum* is a typical species dependent highly on a large bird for its dispersal (Fig 4.2). Seeds are recalcitrant in nature and its fruit maturity coincides with rainy season (*i.e.* during June to September), ensuring safe germination and establishment (Fig 4.1). Although the flowering is having highly narrow time window (10 days), the fruit maturation seems to be spread over a larger time period (2 ½ - 3 ½ months). It would be interesting to understand how this is achieved.

In the temperate deciduous forests of the eastern U.S.A. most plants with fleshy fruits ripe during the peak of the bird migrations during autumn (Thompson and Willson, 1979; Stiles, 1980). Majority of autumn fruiting

species have a high nutrient value and are taken in large numbers by migratory birds, but are also vulnerable to invasion by microorganisms. *D. malabaricum* ripen during rainy season and has high nutrient rich seed coat and acts as a food resource for frugivores (Plate 4.1).

5.2 Floral and pollination biology

The pollination process always requires an intervention of a vector to effect pollen transfer. Across the whole gymnosperm and angiosperm flora, wide ranges of vectors are implicated in pollination (Faegri and Van der pijl, 1979). Among forest tree species, wind and insects are the most significant. Wind is a vector for pollen for many north temperate angiosperm tree species, while the remainder especially tropical and subtropical angiosperm trees are pollinated by insects, birds and mammals (Sedgley and Griffin, 1989). In an evergreen forest, which is the habitat of *D malabaricum,* pollen grain movement by wind is highly restricted due to the dense canopy, hence wind pollination may not be important. The role of a biotic pollinator to carryout pollination is most essential.

The identification of effective pollinator is possible only by direct observation in its natural habitat, which is difficult for a huge tree like *D. malabaricum* which bears small flowers only once in a season, that also for a small time window (10 days). Effective pollination requires the establishment of some relationship between the plant and its pollinators. The vector must receive sufficient benefits from visiting the flowers so that, visitation becomes a regular part of its life activity. The benefit is generally food, or a suitable breeding site.

The general syndrome of biotic pollination is that the flowers produce a primary attractant or reward and some means of making its existence known by the secondary attractant like fragrance. The *D. malabaricum* flower produces a small quantity of nectar as primary attractant and fragrance as secondary attractant. This indicates that the *D. malabaricum* flowers have insect attracting mechanisms and depend on them for pollination. The size of the flower, spatial arrangement of pistil and stamens, accessibility of nectar and inflorescence structure influence the plant-pollinator interaction (Wyatt, 1981). Since visiting animals show a corresponding variation in their sizes, sensory perception, feeding behaviour and energy requirements, certain general relationships exist between bloosom architecture and pollinator types (Faegri and Van der Pijl, 1979).

In general the more exposed and accessible the nectar and pollen the greater is the variety of flower visitors. A more specialized zygomorphic flower with a deep corolla enclosing the nectar such as members of Leguminoceae and Verbenaceae is indicative of a more specialized pollination syndrome because the visitor must harvest the flower in a very precise manner and be of a suitable size to contact both stigma and anthers in the process (Sedgely and Griffin, 1989). In *D. malabaricum,* the flowers are small, the anther tube is deep with a small opening suggesting the presence of a more specialized pollinator (Plate 4.1). The style of the gynoecium is long with the stigma nearer to the opening of the corolla, and the anthers are situated below the stigma. The architecture of the flower is such that an insect entering the flower to feed on the nectar has to first make contact with stigma and in the course if carrying pollen grains of other flowers may result in

pollination. The anthers being located just below the stigma, an insect moving in, to feed, or out after feeding on the nectar, has to make contact with the anthers due to the presence of a small corolla opening, and most of which is covered by the stigma. In the process it carries the pollen grains out of the flower and help in cross-pollination when it visits another flower. The flowers of *D. malabaricum* being small (6.6 mm) should also have a pollinator small in size like thrips or small bees. Thrips as a pollinator has been well documented for Asteraceae, Solanaceae and Fabaceae families. It is considered as a pure pollinator because unlike bees and butterflies, they carry pollen grains of only one species (Ananthakrishnan, 1993). Thrips generally have around 10 –15 days of life-cycle which is akin to the blooming period. In fact adult and developing thrips were identified in a few flowers of *D. malabaricum*.

5.3 Fruiting and seed dispersal

5.3.1 Seed dispersal

While a few plants are equipped with autonomous mechanisms for ejecting seeds from fruits and so dispersing them (Fahn and Werker, 1972), the vast majority of dispersal events involve an interaction between the plant and a physical or biotic element of its environment. *D. malabaricum* is distributed only in the evergreen forests where the conditions are more mesic. Hence it has to relay on animal dispersal agents, which are mainly birds and mammals because, other types of animals are of very minor significance for tree-crop species (Sedgley and Griffin, 1989). The fruits of *D. malabaricum* are borne on the old wood inside the canopy and hence it requires a more specialized dispersal agent, which can expend energy in entering the crown to harvest the attached fruit. On a worldwide basis birds are by far the most

significant of the animal agents of dispersal for tree-crop fruits and seeds. Birds have a strong visual sense but a weak sense of smell. Given these attributes, diasporas, which provide an attractive food source for birds, exhibit some or all of the following characters (Van der pijl, 1982):

(i) an attractive edible part;

(ii) some protection against premature predation (greens/acidity)

(iii) an inner protection of the seed against digestion (such as a bitter kernel or toxic endocarp);

(iv) a signaling colour at maturity;

(v) no smell (although not an impediment if present);

(vi) no closed, hard rind on the fruit and

(vii) seeds of hard fruits exposed or dangling.

Most of the above mentioned characters are being exhibited by the *D. malabaricum* to attract birds. The fruit turns from green to orange at maturity; bears fat rich reddish brown edible seed coat; absence of hard rind and smell; seeds are hard enough to prevent digestion and the seeds will be exposed during the dehiscing phase of the fruit. The results from field observation have revealed that 93 per cent of birds visiting the species is Malabar Grey Hornbills and in 62 per cent of the visits made by them, they were observed to pick at least one seed of *D. malabaricum* (Plate 4.2). Snow (1981) further distinguished between plants, which are adapted to specialized and unspecialized or opportunist frugivores. The former have high-quality fruits, which are rich in fats and proteins, and are typically large with few large seeds and generally of sub-tropical or tropical origin, which is true in case of *D. malabaricum*. Hornbills are predominately frugivorous and their breeding

cycles are synchorous with food productivity of the forest (*i.e,* fruiting phenology; Khannan, 1994). Functionally, they have been described as keystone mutualists as they play an important role in the dispersal of many rare rainforest tree species (Kinnaird, 1998). The seeds of *D. malabaricum*, which are regurgitated by hornbills, will be devoid of seed coat and have a higher germination rate and are not attacked by fungi at the forest floor. Observation on a few freshly regurgitated seeds reveled that they were hot to touch and showed acidic litmus test. Hence the association between *D. malabaricum* and hornbills is a classical example of a co-evolved system of mutual benefit to the plant and animals. Perhaps this is the first report of Malabar Grey Hornbills predation on the seed coat of *D. malabaricum.* Even though Hill myna and Green pigeon visited the *D. malabaricum* tree they were not able to effectively pick and disperse the seeds due to their small beak and neck and big size of the seeds.

Snails and millipedes were also found to feed on the seed coat of *D. malabaricum*, which were dropped on to the ground and aid in its germination without damaging it (Plate 4.2). Even though their role is relatively minor, they are also closely associated with the ecology and life cycle of *D. malabaricum.* Perhaps this is first evidence suggesting that land snails help in germination of an endangered tree. Identification and understanding of such mutualistic associations is very important when conservation strategies are to be developed for *D. malabaricum.* Sustenance of association with the pollinator (Thrips) and seed dispersal agent (Malabar Grey Hornbill) will be prominent among them. Malabar Grey Hornbills are globally threatened (Krys and Ber,

2000) and adequate steps to conserve their habitats will go a long way in conserving *D. malabaricum.*

5.3.2 Seed biology

Dysoxylum malabaricum produces a large sized recalcitrant seed often typical of a species restricted to wet-evergreen forests and which readily germinates without even sowing. However, it is difficult to increase its longevity. To increase its storability the moisture content has to be reduced slightly to prevent it from germination but without killing it. The seeds, which were stored under silica gel for ten days showed reduction in germination from 100 to 66 per cent, when the moisture content reduced from around 50 per cent to 45 per cent (Fig. 4.6). In the initial days there was slight reduction in moisture content, which later increased. This is mainly due to the time required for stabilization of the experiment, which was non-significant. At 30 per cent seed moisture content, the germination reduced to zero. Hence the critical seed moisture content required maintaining good germination is around 45 - 48 per cent. However, a fine tuned experiment needs to be conducted to confirm this aspect.

The dehiscence of fruits of *D. malabaricum* coincides with the rainy season, as it provides the much-needed moisture for its germination and initial establishment. However, seeds that are dispersed latter in the monsoon are more vulnerable to mortality. The seed mass of *D. malabaricum* is high suggesting that initial seedling growth is dependent on stored energy for a longer duration. This may be because in an evergreen forest, sunlight available for photosynthesis at the forest floor is very less. The cotyledons

are also green in colour and help in efficiently trapping the less available diffused sunlight at the forest floor.

5.4 Reproductive fitness

All the physiological and morphological reproductive attributes have evolved as components of the diverse armory of methods by which plants control their breeding system and hence the genetic structure of populations (Richards, 1986). Any modification of these traits through disturbance, management practices or plant breeding is therefore likely to have genetic consequence, which must be understood if we are to ensure the long term conservation, management and improvement of tree crops (Sedgley and Graffin, 1989).

Tropical trees are strongly out crossed and gene flow in most species is extensive. In many species there is considerable genetic variation within population and substantial differentiation among populations. If natural populations are disturbed, then the selective advantage of genes controlling different aspects of reproductive biology may change radically. The reduction in the forested area as well as fragmentation of habitats, as a result of anthropogenic activity, will alter the pattern of gene flow. The magnitude of its effect depends upon pollen and seed vectors involved in gene exchange (Bawa, 1993). All the available evidence show that pollinator abundance and diversity decline with fragmentation. This may result in reduced pollination and seed set. For both pollinators and plants, presence of specialized mutualistic partner will increase their risk of local extinction in fragments (Beverly and Krik, 1993). In *D. malabaricum* the fruit set is low, which may be due to pollinator-limitation, as a result of fragmentation of its habitat. The

results have revealed that embryo abortion occurs both at initial as well as later stages (Plate4.3). This indicates that considerable amount of inbreeding occurs in these fragmented populations, may be as a result of reduced availability of pollinators, lack of synchrony among breeding individuals and may be due to a possible genetic bottleneck of populations. Consequently the reproductive success at the population level may be reduced.

5.5 Half-sib family structure and heritability

Selection of individual trees on the basis of phenotype alone is often incomplete, as the influence of environment and genotype cannot be separated. Progeny testing is the most vigorous breeding method being practiced for improvement of forest trees, since it helps in evaluation of genetic worthiness of selected trees (Kedarnath 1986, Chaturvedi, 1986 and Jagannadha Rao, 1992). Progeny testing also helps in understanding degree of genetic influence on various characters. Early vigour and growth habits of the seedlings have been considered as the two important criteria for seedling selection (Mathew, 2001). Family selection is a method widely used in breeding programs dealing with segregating materials. Family selection ensures elimination of inferior families and inferior individuals within a family.

Even though *D. malabaricum* is highly valued for its timber, no concerted efforts have been done on its improvement. There are no data available on the variability of its vigour traits and genetic basis of these variations among progenies of different families. These are the basic information required while carrying out any improvement programmes in this species. As there are no plus trees or clones available in this species, the present investigation was carried out with seeds (half-sib) collected from ten

trees at the study site, to assess the family variation for germination, early vigour, biometric and biomass characteristics, to understand the genetic basis of these variation among progenies.

In *D. malabaricum* the overall germination was high (98 to100 %) and significant variation was observed among families for days to germinate after sowing (Table 4.4). The heritability values for seed weight is quite high (h^2 = 0.884) and it has a GCV of 15.27 per cent indicating that selection can yield good results (Table 4.7). The root length was positively correlated with seed weight (r = 0.225). Hence seeds of higher weight should be preferred to produce better quality seedlings. However, these generalizations need to be confirmed utilizing families derived from several populations.

5.5.1 Seedling growth parameters

Early selection is an important aspect in tree improvement, which requires information on seedling growth and vigour. Biometric characters such as, height to first branch, shoot height, number of lateral roots and sturdiness quotient showed significant differences at family level (Table 4.5). This suggests that there is good variation among the population for vigour parameters and selection would give better results (Plate 4.3).

Mathew (2001) studied variation for early vigour among progenies of teak clones and found that the families with *per se* better performance also had a better General Combining Ability (GCA) effect for height, collar diameter, tap root length and number of leaves. It means good *per se* performers are also good general combiners. Thus, parents may be selected on the basis of their progeny performance, which is a good indicator of their combining ability. Since superior alleles are distributed among these parents

with fixable components of variation (additive variance), crosses with these parents are expected to throw out good segregants in the following generation. In the present study family 9 and 10 showed the highest values for height to first branch which determines the clear bole length in the future crop. They also had better *per se* performance in majority of the vigour characteristics and can be used for immediate planting programme.

5.5.2 Biomass characteristics

Hazara and Tripathi (1986) reported that biomass production is a function of the photosynthetically active radiation falling on the leaves. As optimal leaf mass levels increase, biomass production would substantially increase. In *D malabaricum,* quality Index of one year old seedling, which is a measure of seedling vigour showed highest value of 0.44 in family 10, followed by family 4 (0.39) and family 7 (0.37). Family 10 obtained the highest values for most of the biomass characteristics. A high co-efficient of variation (36.83%) was observed with respect to QI. Among all the biomass parameters, root dry weight and total dry weight had high genotypic co-efficient of variation (19.6% and 18% respectively) suggesting that selection for dry matter production may lead to better results.

5.6 Vegetative propagation

Vegetative propagation is the primary method of reproduction for several of the forestry species for commercial production in a majority of nurseries. Rooting of stem cuttings is one of the important means of vegetative propagation which is generally practiced in forestry to obtain the plants of desirable genetic constitution of economic importance and multiplication of species and sometimes in bringing out flowering much earlier

from seedling also to conserve the species which posses the problem of natural regeneration in their habitat.

Even though *D. malabaricum* can be easily propagated through seeds, it sets fruits only once in a season and they cannot be stored for more than two weeks, as they are recalcitrant in nature. Hence vegetative propagation will help in its multiplication all through the year and also conserve superior genotypes. Rooting of cuttings could not be achieved with any treatment. A similar result was also arrived at in a recently concluded independent study conducted at Tropical Botanical Gardens and Research Institute, Kerela (Personal communication). In the present study, a maximum sprouting of 20 per cent was obtained in IBA 2000 (plate 4.3). The stem cuttings treated by powder dip method fared better than prolonged soaking for 24 hours. This is mainly due to the rotting of cuttings due to prolong soaking. The highest concentration used to treat semi-hard cuttings in the present study was 3000 ppm and at this concentration no rooting was observed. Hence to get better results higher concentration of hormone ranging between 5000-10000 ppm should be used by powder dip method at different season. Rather than semi-hard cuttings, softwood cuttings can also be tried.

5.7 The conservation concern and future line of work

- Fragmentation of habitat and illegal felling of *D. malabaricum* has resulted in reduction of its genetic variability. Hence distinct genotypes from other provenances have to be introduced into a potential population to increase its genetic base and conserve its gene pool following forest Gene Bank Concept.
- The germination and dispersal of *D. malabaricum* is highly dependent on Malabar Grey Hornbills. Hence the maintenance of a healthy population of

hornbills and protection of its habitat is equally important if we have to conserve *D. malabaricum.*

- Conservation of Elite genotypes needs to be taken up on a war footing.
- Clear identification of its population over its geographic range and assessment of its status. Provenance trial is required to identify the best provenances, and superior families.
- Assessment of genetic variation among its population using molecular techniques has to be taken up to identify populations with high genetic variability and conserve them as Gene Sanctuaries.
- Vegetative and micro propagation techniques have to be standardized to multiply and conserve superior genotypes.
- *D. malabaricum* has good growth rate in the initial years which slows down in the later years. Hence standardization of nursery techniques to obtain vigorous growth in the initial growth stage only is required.

Summary

VI. SUMMARY

Dysoxylum malabaricum Bedd. (Meliaceae) is a large evergreen tree endemic to the Western Ghats and distributed only in the evergreen forests up to an elevation of 850 m. It is highly valued for its lustrous, cedar-scented, durable timber, which is used in making furniture cigar boxes, cooperage *etc.* Its wood is also used in Ayurvedic preparations to treat rheumatism and disorders of eyes and ears. However, it has been illegally harvested from its natural habitat mainly for its valuable timber resulting in the reduction of population sizes to few fragmented pockets in Uttara Kannada, Kodagu and parts of Kerala. Hence, it has been assigned a threat status of 'endangered-globally' by the Foundation for Revitalization of Local Health Tradition (FRLHT).

Despite its importance, no concerted studies have been taken up on this species. There is absolutely no information regarding its reproductive biology, which is very important when conservation strategies have to be developed. Hence, the present study was undertaken to study a few aspects of breeding system, seed biology, to understand the genetic variation among families and also to standardize a vegetative propagation technique, as these are prerequisite for its genetic improvement, domestication and conservation.

D. malabaricum flowered during the second fortnight of February in the dry season and had a short time window of about ten days. The flowers were whitish green in colour, bearing nectar and fragrant. These bisexual flowers were about 6.6 mm in length and require the aid of a pollinator like thrips for its cross-pollination. The immature green fruits appeared in the first week of March, one week after initiation of anthesis. They develop for a period of

about 3 ½ to 4 months and turned to orange colour during maturation stage at the beginning of June. The fruit fall initiated during mid June and continued till the end of August. Peak fruit fall occurred during first week of July. The fruit fall period clearly coincided with the rainy season, which provides the much needed moisture for seed germination and establishment.

The seeds are recalcitrant in nature bearing a pulpy, fat rich seed coat, which will readily attract infection by fungus on the forest floor leading its the decay. Malabar Grey Hornbill was the predominant dispersal agent, which feed on the seed coat and regurgitate the cotyledons. The cotyledons regurgitated by the hornbills were relatively free from fungus infection and had a higher probability of germination, suggesting that both hornbill and *D malabaricum* have co-evolved. Seeds showed 100 per cent germination when the seed coat was removed artificially. It has an epigeal type of germination with green cotyledons coming out of the soil, photosynthesize and nourish the young seedling. The seeds stored under silica gel, a dehydrating agent, showed 100 per cent germination up to 48 per cent seed moisture content. However germination which reduced to 66 per cent at 45 per cent moisture content, suggesting that the critical moisture content at which the seeds can be stored is between 45 – 48 per cent

At the population level, the reproductive success was very low as suggested by a large number of non-fruiting bearing trees (which constituted nearly 50 %) and poor fruiting intensity. It is hypothesized that considerable selfing may be going on due to lack of pollinator activity, habitat fragmentation and genetic bottleneck. However, further studies are required to ascertain this aspect.

Considerable variation was observed for seed weight, growth and biomass characteristics among half-sib families. Family 10 had the highest shoot length and family 4 had the highest root length. Seed weight was positively correlated with initial root length (r = 0.225) indicating that larger seeds will give better root system leading to their better establishment in the field. The heritability for seed weight was also high (h^2 = 0.884), suggesting that selection will help in producing better quality seedlings.

Among biometric parameters, height to first branch, shoot height, number of lateral roots and sturdiness quotient showed significant differences among families. For all these parameters family 9 and 10 showed better *per se* performance and can be used immediately for planting programme. Considering biomass characteristics, shoot dry weight, root dry weight and Quality Index were highest in family 10 followed by family 4. A high co-efficient of variation (36.83%) was observed with respect to QI suggesting that selection would improve seedling quality.

Vegetative propagation of *D. malabaricum* did not succeed due to lack of rooting among semi-hard cuttings. Although sprouting was observed in most of the treatments, the overall sprouting was very less. Hence to obtain rooting, higher concentration of growth regulators ranging between 5000 - 10000 ppm should be tried in different season. Powder dip method should be preferred over prolong socking as the later leads to rotting of cuttings. Softwood cutting can also be tried to get better result.

The present study has revealed the basic information regarding the reproductive biology of *D. malabaricum*, based on which appropriate measures have to be taken to establish a plantation of this species. In addition to that, its natural habitats, which are under high biotic pressure, have to be protected immediately.

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*** Original not seen**

Reproductive biology and half-sib family performance of *Dysoxylum malabaricum* Bedd. : an important threatened timber species

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ABSTRACT

Dysoxylum malabaricum Bedd. (Meliaceae) popularly known as 'white cedar' is an economically important endangered species prized, for its sweet-scented white colored timber. Natural populations of this endemic tree have been drastically reduced due to its illegal harvest through out the Western Ghats. The present study was undertaken to understand a few aspects of its breeding system, seed biology, extent of genetic variation among half-sib families for early vigour traits as well as to standardize a vegetative propagation protocol to aid its genetic improvement and conservation.

Under Sirsi conditions whitish, bisexual, thrips-pollinated flowers *D. malabaricum* appear during the second fortnight of February for a short period of 10 days. The immature green fruits appear in the first week of March and turned bright orange after maturation during June. The fruit fall coincided with the rainy season such that seed germination and establishment is facilitated. Hence seed collection can be done during months of June to August. Malabar Grey Hornbill (*Ocyceros griseus*) feeds on the fruits of this species and regurgitates the kernel after digesting the outer fat-rich pulpy seed coat. The 'near-threatened' Malabar Grey Hornbills form the most effective seed disperser of *D. malabaricum* seeds and the association between these two species seems to be tightly co-evolved.

The natural fruit set per cent of *D. malabaricum* is poor (23.9 %). The seeds (average weight of 5.56 g) are recalcitrant with critical moisture content of 45 per cent. If seeds are stored at 45 per cent moisture content under aseptic conditions, their shelf-life could be extended. Half-sib families varied significantly with respect to seed mass, emergence percentage and root/shoot length. Vigour traits such as plant height, root length and number of lateral roots showed large phenotypic/genotypic coefficient of variation and recorded moderately higher heritability (h^2 = 0.74, 0.66, 0.71 respectively), suggesting that early selection can be made on these traits. Though the per cent sprouting of stem cuttings treated with powder form IBA at 2000 ppm was better (20 %), the stem cuttings did not initiate rooting.