PHYSIOLOGICAL AND GENETIC DIVERSITY STUDIES ON REGENERATION OF *SANTALUM ALBUM* L.

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CERTIFICATE

This is to certify that the thesis entitled, **"PHYSIOLOGICAL AND GENETIC DIVERSITY STUDIES ON REGENERATION OF *SANTALUM ALBUM* L."** embodies the result of original work carried out by Ms. Ramya. R (Reg. No. 2701), under our guidance and supervision. No part of this thesis has previously formed the basis for the award of any degree, diploma, associateship or other similar titles of this or other institutes or any University or Society.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

µM	:	micro molar
AFLP	:	Amplified Fragment Length Polymorphism
ANOVA	:	Analysis of Variance
bp	:	base pair
cc	:	cubic centimeter
Chl *a*	:	Chlorophyll *a*
CTAB	:	Cetyl Trimethyl Ammonium Bromide
DBH	:	Diameter at Breast Height
DNA	:	Deoxyribo Nucleic Acid
dNTP	:	deoxyribo Nucleotide Tri-Phosphate
DQI	:	Dickson Quality Index
EDTA	:	Ethylene Diamine Tetra Acetate
ELISA	:	Enzyme Linked Immunosorbant Assay
ETR	:	Electron Transport Rate
F_v	:	variable fluorescence
F_m	:	maximum fluorescence
F_o	:	minimal fluorescence
GIS	:	Geographic Information System
ha	:	hectare
HWE	:	Hardy-Weinberg Equilibrium
ICFRE	:	Indian Council of Forestry Research and Education
IGS	:	Intergenic Spacer
ISSR	:	Inter Simple Sequence Repeat
ITS	:	Internal Transcribed Spacer
IUCN	:	International Union for Conservation of Nature and Natural Resources
KFRI	:	Kerala Forest Research Institute
kg	:	kilogram
m	:	meter
M	:	Molar
mg	:	milligram

ml	:	millilitre
mM	:	milli molar
mm	:	millimeter
ms	:	millisecond
ng	:	nanogram
nM	:	nano molar
nrDNA	:	nuclear ribosomal DNA
NTSYS	:	Numerical Taxonomy system
^{o}C	:	degree celsius
PAR	:	Photosynthetically Active Radiation
PCR	:	Polymerase Chain Reaction
PDA	:	Potato Dextrose Agar
PI	:	Performance Index
PPB	:	Percentage of Polymorphic Bands
PS II	:	Photo System II
PTC	:	Peltier Thermal Cycler
PVPP	:	Poly Vinyl Poly Pyrrolidone
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restriction Fragment Length Polymorphism
RNase	:	Ribo Nuclease
rpm	:	revolutions per minute
rRNA	:	ribosomal Ribo Nucleic Acid
s	:	second
SAHN	:	Sequential Agglomerative Hierarchical and Nested Clustering
SE	:	Standard Error
spp	:	species
SPSS	:	Statistical Package for Social Service
SQ	:	Sturdiness Quotient
SSR	:	Simple Sequence Repeat
TBE	:	Tris Borate EDTA
UPGMA	:	Unweighted Pair Group Method with Arithmetic Averages
UV	:	Ultra Violet

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1. SANDAL AND ITS IMPORTANCE

Sandal, *Santalum album* L. is intimately associated with Indian culture and heritage and is acclaimed as the most precious and valuable among Indian forest trees. Sandalwood trees are the source of highly priced and fragrant heartwood, the 'East Indian Sandalwood' which on steam distillation yields on an average 5-7% oil (Mc Kinnell, 1990) of high perfumery value. The fixative property and tenacious aroma of the oil are due to alpha and beta santalols-the major odoriferous sesquiterpenic components constituting 90 per cent of the oil (Shankaranarayana *et al*., 1998). The heartwood is commercially important; the close grains make it highly amenable for carving and fabricating exquisite handicraft items that are fragrant, elegant and largely self protected from termites and wood borers (Srinivasan *et al*., 1992). Heartwood of sandal is estimated to fetch up to Rs. 3.7 million/ton and wood oil Rs. 70,000-100,000/kg in the international market (Gairola *et al*., 2007). Sandalwood oil is in great demand for manufacture of perfumes, cosmetics, soaps and in ayurvedic and allopathic systems of medicine (Srinivasan *et al*., 1992; Tandon, 1995).

S. album is an evergreen partial root parasite attaining 10-15 m height and 1-2 m girth at full maturity when it reaches the age of 60-80 years (Ghosh *et al*., 1985, Jain *et al*., 1999). The tree flourishes well from sea level up to 1200 m altitude in regions with different soil types and varying climatic conditions and an annual precipitation of 600-1600 mm. Bark is reddish brown to dark brown in colour, smooth in young trees, becoming rough with deep vertical fissures as the tree matures. Leaves are opposite, decussate, flowers unscented, straw-yellow coloured turning to deep purplish brown on maturation and occurs in axillary or terminal cymose panicles. Flowering generally occurs twice a year from March to May and September to December. The fruit is single-seeded succulent drupe, purplish black when mature (Srinivasan *et al*., 1992). Sandal is a highly polymorphic species (Kulkarni, 1995). Morphological studies have shown that the trees vary significantly in leaf length and width, colour of the heartwood and in oil content (Kushalappa, 1983; Bagchi and Veerendra, 1985; Kulkarni, 1995). A study of

the anatomical characteristics viz; xylem cell diameter, epidermal thickness, cortex width and number of vascular bundles have shown that there are genotypic differences in sandal populations (Veerendra and Bagchi, 1986).

Natural regeneration occurs by means of seeds. Seeds are usually dispersed by birds and normally take 4 to 8 weeks to germinate (Venkatesan, 1995). Seeds have a post drop dormancy period up to 2 months due to their impermeable outer covering and retain their viability for 6 to 12 months. Germination is hastened by soaking seeds in 0.05 per cent gibberellic acid. Soaked seeds are sown in germination trays filled with vermiculite or with sieved sand and soil in 1:2 ratio. The germination media in trays must be treated with nematicide and fungicide, periodically as a prophylactic measure.

Artificial regeneration is achieved by dibbling seeds in pits, sowing on mounds and trenching around mother trees for wounding the roots for inducing root sucker production. Planting nursery-raised, vegetatively multiplied and tissue culture-raised seedlings are also carried out (Rai and Kulkarni, 1986). Vegetative propagation is achieved through stem cuttings, grafting, air layering or through root suckers; but rooting of stem cuttings has been achieved only in 15-20 per cent of cuttings (Rao and Srimathi, 1976; Uniyal *et al*., 1985; Balasundaran, 1998; Sanjaya *et al*., 1998). Micropropagation through axillary shoot proliferation, somatic embryogenesis and adventitious shoot induction has also been reported (Bapat *et al*, 1990; Bapat and Rao, 1999; Gairola *et al*., 2007).

A growing sandal tree under natural conditions can put up an increment of 1 kg of heartwood/year and a girth of one cm/year (Venkatesan, 1980; Rai, 1990). The heartwood and oil content vary with locality and from tree to tree, and increase with girth and age of the tree. Oil percentage is higher in the heartwood of root as compared to stem and hence the tree is invariably harvested by uprooting. Global demand for sandalwood is about 5000-6000 tons/year and that of oil is 100 tons/year (Joshi and Arun Kumar, 2007). Out of this, nearly 70-80 per cent is met from Karnataka (Rao *et al*., 2007). Market trend indicates that sandal heartwood prices have increased from Rs. 365/ton in 1900 to Rs. 6.5 lakhs/ton in 1999-2000 and to Rs. 37 lakhs/ton in 2007. India has suffered a substantial decline in sandalwood production from 3176 tons/year during

1960-65 to 1500 tons/year in 1997-98, and to 500 tons/year in 2007 (Jain *et al*., 2003; Gairola *et al*, 2007). Oil production has also declined from 60 tons/year during 1981-1994 to 40-50 tons/year during 1999-2000 (Ananthapadmanabha, 2000).

1.2. DISTRIBUTION OF SANDAL

The genus *Santalum* is tropical in distribution, between latitude 30° North and 40° South, from India in the West to Juan Fernandez Islands in the East and from Hawaiian Archipelago in the North to New Zealand in the South (George, 1984; Srinivasan *et al*., 1992). The economically important and widely distributed genus *Santalum* comprises of 16 species (Shea *et al*., 1998; Ansari *et al*., 2007) which are xylem-tapping root hemiparasites belonging to family Santalaceae. *Santalum album* L., due to its religious significance and high santalol content is acclaimed as the most economically important of all *Santalum* species (Srinivasan *et al*., 1992; Radomiljac *et al*., 1998b) and is the sole species yielding high quality wood and oil (Jeeva *et al*., 1998). Sandal grows in a wide range of temperature and soil types in tropical and sub-tropical areas of India, Sri Lanka and Indonesia (Mc Comb and Jones, 1998). Four *Santalum* species are native to Western Australia viz. *S. spicatum* (R. Br.) A. DC., *S. acuminatum* (R. Br.) A. DC., *S. murrayanum* (Mitchell) C. Gardn. and *S. lanceolatum* R. Br. (Shea *et al*., 1998). Of all the species, *S. album* has the highest oil content (5-7%) while *S. spicatum* (2%) and *S. lanceolatum* (3-5%) yield poorly scented and low quality oil (Mc Kinnell, 1990 and Applegate *et al*., 1990).

S. album is indigenous to peninsular India and is naturally distributed over 9600 km^2 (Srinivasan *et al*., 1992; Radomiljac *et al*., 1998b) from Kerala in the South to Uttar Pradesh in the North, in regions with varying ecoclimatic conditions and edaphic factors (Jain *et al*., 1998). In Kerala, Sandal is spread over 15 km^2 mainly in Marayur in Idukki district, Wayanad district and Thenmalai in Kollam district (Srimathi *et al*., 1995). Over 90 per cent of sandal is distributed in Karnataka (5245 km^2) and Tamil Nadu (3045 km^2), rest in Andhra Pradesh (175 km^2), Orissa (35 km^2), Madhya Pradesh (33 km^2) and Maharashtra (84 km^2) (Jeeva *et al*., 1998).

1.3. STUDIES CONDUCTED ON SANDAL

Institute of Wood Science and Technology (IWST) has identified 9 potential sandalwood provenances across the country on the basis of population density, phenotypic characters, latitude and longitude. These provenances are Bangalore, Tangali and Mandagadde from Karnataka, Chitteri and Javadi hills from Tamil Nadu, Marayur from Kerala, Koraput from Orissa, Seoni from Madhya Pradesh and Horesly Hills (Chittoor East) from Andhra Pradesh (Jain *et al*., 1998).

In sandal, most of the earlier work was focused on sandal spike disease, its causes and control. Thomas and Balasundaran (1999) have developed Sandal Spike Phytoplasma Purification Kit and Immuno Detection Kit for purifying sandal spike phytoplasma and detecting the pathogen in sandal. They used PCR-RFLP technique to confirm the identity of sandal spike phytoplasma as belonging to group I of the eleven major phytoplasma groups. Indirect ELISA tests had also been developed for early detection of spike disease before symptom expression (Thomas and Balasundaran, 2001).

Genetic variation of sandal had been a subject of study by a few investigators. Brand (1994) investigated the genetic diversity in 10 West Timor and two Indian populations of sandal using 23 enzyme systems and found that West Timor and Indian populations are genetically well separated. The genetic diversity among Indian populations and provenances have been documented through isozyme and RAPD studies (Angadi *et al*., 2003; Sashidhara *et al*., 2003; Suma and Balasundaran, 2003, 2004; Rao *et al*., 2007). The diversity was large among provenances probably because of their geographic isolation but it was very low within provenances probably due to inbreeding caused by genetic erosion.

1.4. PROBLEMS FACING SANDAL GROWTH

1.4.1. Lack of regeneration

Depletion of sandal resources can be attributed to several factors, both natural and anthropogenic. Hurdles to natural regeneration of sandal in forests include recurrent annual fires in natural sandal areas, lopping of trees for fodder, excessive grazing, hacking, encroachments and spread of sandal spike disease (Venkatesan and Srimathi, 1981).

While these factors hinder regeneration in forest areas and diminish the growing stock, the situation is accelerated by human activities of chronic over-exploitation and illicit felling.

1.4.2. Low seed setting in seed stands

Sandal is predominantly an out breeding species (Sindhuveerendra and Ananthapadmanabha, 1996). The seeds are produced twice a year and are capable of self regeneration (Srinivasan *et al*., 1992). Seeds from various seed sources exhibit significant variation morphologically and physiologically. Presence of genotypic barriers for embryo development has resulted in lower percentage of mature fruits in spite of good percentage of fruit initiation (Sindhuveerendra *et al*., 1999). The tendency for out breeding in sandal is reinforced by asynchronous flowering, insect pollination, heterostyly and self-incompatibility (Kulkarni and Muniyamma, 1998).

Alternatively, *S. album* regenerates asexually by means of root suckers. Root suckers generally originate as shoot primordia from injured or severed roots and are confined to an area around the mother tree. Studies have shown that natural stands of sandal in Sri Lanka have spread clonally by prolific root suckering (Tennakoon *et al*., 2000). Recruitment by root suckering has been observed to be the sole method of increase in population size in seven remnant populations of *S. lanceolatum* in Victoria (Trueman *et al*., 2001). Though the trees flowered, little or no fruit production occurred within these small populations. Allozyme and RAPD analysis of the populations showed that sexual reproduction had not been contributing to recruitment. Each population appeared to exist as a clone composed of numerous ramets of a single genotype formed through asexual reproduction by root suckering. Lack of seedling recruitment in remnant populations of *S. lanceolatum* was partly explained by pollen-pistil incompatibility and pistil dysfunction which is expected in a cross pollinated species. Clonality may also be due to the past management practices and/or disturbances triggering asexual reproduction.

A parallel situation has happened in one of the seed stands in Marayur. Two seed stands had been established in Nachivayal Reserve II during 1980-1981. Recently it has been reported that expected quantity of seeds are unavailable from these seed stands. Trees were seen growing in groups or clusters. Most of the sandal trees within the clusters flowered profusely, but seed setting was extremely poor. As sandal is a cross

pollinated plant, fruit setting and seed formation will take place usually when pollination and fertilization take place between genetically unrelated genotypes. Failure to develop mature seeds may occur due to unsuccessful pollination and fertilization, pathogenic infection of developing fruits, premature flower and fruit fall, etc. But such major problems were not observed. Since root sucker induction had been adopted as a method of sandal regeneration for the last few decades in Marayur and the seed stand in particular, we investigated the genetic profile of trees in samples plots in order to find out whether clonality existed in the seed stand.

1.4.3. Low seed germination and seedling mortality

Seeds are obtained by removing the fleshy portion of the fruit. Seeds vary in their size and shape. Under normal conditions they retain their viability upto six months and then gradually diminish. Fresh seeds exhibit dormancy for 2 months (Sreenivasan *et al*., 1992). Pathogen infection, rabbit and rodent menace, grazing by animals, high atmospheric temperature and excessive moisture content are the factors which considerably reduce the survival of seedlings (Bapat and Rao, 1998). Sandalwood seedlings and grafted plants are prone to attack from insect pests and diseases, which take a heavy toll and sometimes wipe off the whole stock. Damping off, vascular wilt, web blight, stem infection and seedling blight caused by *Fusarium oxysporum*, *Phytophthora, Pythium* and *Rhizoctonia* are the most serious diseases that cause severe damage and economic loss in nurseries (Remadevi *et al*., 2005; Ramalaksmi and Rathore, 2007). Among these *F. oxysporum* is the most serious pathogen which affects seedlings in nurseries as well as in natural forests.

Seedling mortality caused by *F. oxysporum* affects sandal at various stages causing pre-emergence damping off, collar rot, stem and root infection. The disease has been reported from Karnataka and Tamil Nadu (Remadevi *et al*., 2005) and throughout Kerala including high altitude areas such as Marayur. *F. oxysporum*, a common soil borne fungus with a worldwide distribution, has no known sexual stage. This organism probably causes more economic damage in nurseries than any other plant pathogen. *Fusarium* has been shown to constitute as much as 80-90 per cent of the total fungal microflora in the rhizosphere of several agricultural crops and is responsible for wilt and cortical rot

diseases of more than 100 economically important host plants (Swifty, 2002). Wide variability exists in the pathogenicity of the fungal isolates from different geographical regions. In *Fusarium* species, various formae speciales (f. sp) and physiological races are known. Though, sexual stage of the fungus has not been reported so far, the isolates show wide variability and this variability of the pathogen has been considered as the cause of its adaptability and virulence. The genetic diversity of *F. oxysporum* which may be the reason for its wide spread occurrence and virulence has not been studied so far. Information on the variability of the fungus is necessary for planning its management through the application of appropriate fungicide and also for prophylactic treatment.

1.4.4. Shade requirement

In the early stages of seedling development, sandal derives nutrition from the relatively large seed reserves and later, the formation of host attachment through root connection becomes critical for seedling survival and growth (Barrett and Fox, 1997). The duration of dependency on the kernel as the food source needs to be studied. Sandal is a sciophile in the seedling stage, becomes more heliophilic once established. Full sun light is detrimental to the survival of sandal germinants and they need to be grown in greenhouse, shade house or in a shady area. So the level of shade is important for seedling growth. Early growth of sandal after seed germination is found to be better in the shades of bushes and clumps of vegetation (Rao, 1942; Fox *et al*, 1994b). Fox *et al.* (1994b) have shown that some shade is beneficial for the first three years; the length of time for optimum benefit has not yet been elucidated.

1.4.5. Host plant requirement

Sandalwood, being a hemi-root parasitic tree, root connection with a host species is required for nutrition to young plants as well as to the adult trees (Rao, 1942; Fox *et al*., 1994a; Fox *et al*., 1994b). Additionally, host plants can also provide shade required for the healthy growth of seedlings. Sandal benefits from a primary host at the nursery stage and from a secondary host (long term) in the field (Srinivasan *et al*., 1992; Barrett and Fox, 1995; Fox *et al*., 1996; Radomiljac *et al*., 1998a; Fox, 2000). Early nursery growth is found to be more rapid and secure when grown with a pot host which functions both

in plant nutrition and water relations of sandal and reduces out-planting stress when transferred to the field (Fox, 2000). Poor growth without a primary host even after application of supplementary nutrition has revealed that a pot host is important for healthy and robust development of sandal. So also successful, establishment of sandal seedlings in plantations requires the utilisation of appropriate pot host (Radomiljac *et al*., 1998b; Luong, 2002). This feature has contributed to low regeneration, both naturally and artificially (Radomiljac, 1994).

1.5. OBJECTIVES OF THE PRESENT STUDY

Deterioration of natural sandal populations due to illicit felling, encroachments and diseases have an adverse effect on genetic diversity of the species. The loss of genetic diversity has aggravated during recent years due to extensive logging and changing land-use patterns and poor natural regeneration of sandal. The consequent genetic erosion is of serious concern affecting tree improvement programme in sandal. Conservation as well as mass propagation are the two strategies to be given due importance. To initiate any conservation programme precise knowledge of the factors influencing regeneration and survival of the species is essential. Hence the major objectives of the present study are:

(i) Investigation on the impact of host plant root connection on seedling growth and survival.

(ii) Investigation on the impact of shade on photosynthetically active radiation (PAR) and chlorophyll content.

(iii) Investigation on high seedling mortality caused by fungal infection - identification of the fungus causing the disease and genetic diversity of the fungal pathogen using RAPD marker.

(iv) Investigation on low seed setting in Marayur seed stand - ISSR marker study of sandal seed stand for estimating genetic diversity.

The thesis is organised into six chapters, Introduction, Review of Literature and four chapters dealing with the four major objectives. This is followed by a chapter on summary and conclusions and a common chapter for Reference.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. SANDAL AND ITS IMPORTANCE

Sandal has been intimately associated with human civilisation since time immemorial. Extensive studies have indicated that *S. album* is the sole species yielding high quality sandalwood and oil. A few other species of *Santalum* and four other genera also yield fairly scented wood oil, but the quality of *S. album* oil and wood is superior (Srinivasan *et al*., 1992). Sandalwood (heartwood) is immune to termite attack and wood borers; it is one of the finest woods for carving and ranks only next to ivory. Its smoothness, uniform fibers, straight close grains and knots lend it to intricate workmanship (Dey, 2001). The sapwood and sometimes the mixed woods are used for manufacturing joss sticks. Sandal is highly valued for its fragrant heartwood which yields oil preferred for perfumeries, cosmetics and medicines (Sanjaya *et al*., 1998). The oil is present in the heartwood and roots, and hence the tree is invariably harvested by uprooting for oil extraction (Bapat and Rao, 1992). Indian sandalwood oil has a characteristic sweet, woody odour for which it is widely employed in the fragrance industry, more particularly in the higher-priced perfumes. It has excellent blending properties and the presence of a large proportion of high-boiling constituents in the oil (about 90 per cent Santalols) makes it valuable as a fixative for other fragrances. In India, it is used for the manufacture of traditional attars such as rose attar; the delicate floral oils are distilled directly into sandalwood oil. Sandalwood oil has antipyretic, antiseptic, antiscabetic, and diuretic properties. It is also effective in the treatment of bronchitis, cystitis, and dysuria (Gairola *et al*., 2007).

2.2. MAJOR PROBLEMS FACING SANDAL

Most of the existing sandal populations are not dense. They are devoid of large girth class trees due to illicit felling, hacking, forest fire and encroachments (Parthiban *et al*., 1998). The sandal area is declining drastically due to over exploitation, poor seed germination, poor regeneration and failure of artificial regeneration (Jeeva *et al*., 1998). Though seed germination is generally profuse, population density is poor due to abiotic

and biotic interferences. In view of the high price it fetches, live sandal trees in their endemic habitats have been ruthlessly felled and removed by smugglers. These activities have selectively removed trees possessing large dimensions and quality heartwood, resulting in narrowing of the gene pool leaving population of trees with mostly sapwood. The magnitude of illicit removals has been so intense that sandal has now been enlisted as a 'vulnerable' species by IUCN (IUCN, 2010). Extensive extraction of heartwood has severely decimated the natural stands of the tree in the forests and has rendered many populations fragmented (Nageswara Rao *et al*., 2007). Since much of the sandal wealth and natural sandal bearing areas have been lost, the remaining sandal trees are to be protected effectively and natural sandal bearing areas are to be preserved (Swaminathan *et al*., 1998).

In the early stages of seedling development sandal derives nutrition from the relatively large seed reserves and later, the formation of host attachment becomes critical for seedling survival and growth (Barrett and Fox, 1997). The selection of appropriate hosts is vital to ensure successful sandal plantation establishment (Radomiljac, 2000). Failure of regeneration efforts is one of the main causes of sandal depletion. Failure of artificial regeneration is due to poor understanding of the host-parasite relationship and edaphic factors (Surendran *et al*., 1998). The hemiparasitic nature of sandal is not fully understood and silvicultural techniques to establish it are not fully known.

Spike disease is the most wide-spread and destructive disease of the species which has attracted world wide attention. Infected trees die within one to two years after the appearance of disease symptoms which is characterised by extreme reduction in the size of leaves and internodes accompanied by stiffening of the leaves. In advanced stage, owing to the progressive reduction in leaf size and internodes, the whole shoot looks like a 'spike inflorescence'. Spiked plants do not bear any flowers or fruits; occasionally, only phylloid or abortive flowers are developed. The disease is caused by non-culturable phytoplasmas which are pleomorphic and fragile organisms multiplying within the sieve tubes (phloem) of leaves, petioles, stem and root causing symptoms as yellowing of leaf, little leaf and phyllody. The pathogen is around 0.4 to 1.0 µm in diameter, has a cell membrane, ribosomes and DNA. Because of the absence of cell

wall, the mycoplasmas are pleomorphic and can pass through pores as small as 220 nm (Thomas and Balasundaran, 2001).

Sandal reserves in Marayur Forest Division of Kerala were considered to be free from spike disease till Ghosh *et al*. (1985) reported the widespread occurence of the disease in the sandal reserve 51. It was found that a large number of trees were affected with typical spike disease. Staining techniques had been developed for the detection of sandal spike phytoplasma. Sandal Spike Phytoplasma Purification Kit and Immuno Detection Kit were used for purifying sandal spike phytoplasma and detecting the pathogen in sandal. For PCR detection of phytoplasma, oligonucleotide primers specific to the conserved region of 16S rRNA gene were used to amplify a 558 bp sequence of the phytoplasma. Four DNA fragments were obtained when the PCR products obtained after 20 cycles of amplification were subjected to restriction fragment length polymorphism analysis (RFLP) with *Alu*I restriction endonuclease. This technique confirmed that sandal spike phytoplasma belonged to group I of the eleven major phytoplasma groups (Thomas and Balasundaran, 1999). Indirect ELISA tests have helped in the early detection of spike disease (Thomas and Balasundaran, 2001).

Sandalwood seedlings and grafted plants face problems from insect pests and diseases, which take a heavy toll and sometimes the whole stock is wiped off. *Fusarium oxysporum*, *Rhizoctonia*, *Phytophthora* spp. and *Pythium* spp. cause serious damping off and wilting of sandal seedlings (Remadevi *et al*., 2005). Mortality of seedlings due to pre-emergence and post-emergence damping off, root rot, and wilting of older seedlings were recorded. *Asochyta santali*, *Macrophomina phaseoli*, *Asterina congesta* and *Sphaecelomia santali* are the most common fungi causing leaf spot disease (Sreenivasan *et al*., 1992).

More than 150 insects are known to occur on *S. album,* but only a few have been recorded as serious pests of economic importance. Sandal seedlings in nursery are attacked by defoliators and sap suckers. Defoliators such as *Cryptothelea cramerii* Westwood cuts off the young seedlings at ground level. A weevil, *Sympiezomias cretaceous* Faust feeds on the leaves. Nymphs of *Holochora albida* Kirby, *Letana inflata* Brunner and *Teratodes monticollis* Gray gnaw tender shoots of sandalwood

seedlings. Bagworms *Acanthopsyche moorei* Heyl and *Pteroma plagiophleps* Hampson defoliate young sandalwood seedlings giving a burnt appearance. Two species of coccids *Saisettia coffeae* and *Saisettia nigra* cause wilting and yellowing of leaves of sandal seedlings in nursery. Two coccids *Pulvinaria psidii* Mask and *Pulvinaria maxima* Green infest sandal seedlings causing premature leaf fall (Remadevi *et al*., 2005). Lac insect *Tachardia lacca* Kerr has been observed on nursery plants and seedlings along with severe attack on mature sandal trees.

2.2.1. Lack of sandal regeneration

In India, though sandal is distributed all over the country, nearly 90 per cent of its natural production is in Karnataka and Tamil Nadu and to some extent in Kerala and Andhra Pradesh. Until 2002, state governments had monopoly control over all sandal resources including those on private land. But, this monopoly has neither deterred illegal and indiscriminate felling by smugglers and poachers nor has it helped conserve the species in its natural habitat (Viswanath *et al*., 2007). Sandalwood smuggling is the major problem in all the states where sandal grows (Rao *et al*., 1999). Most of the sandal populations are devoid of larger girth class trees due to selective illicit felling and other biotic interferences. Owing to such dysgenic selection perpetrated by the smugglers, the existing population comprises mostly of inferior trees causing genetic erosion (Venkatesan, 1995; Parthiban *et al*., 1998). Uma Shankar *et al*. (2000) reported decline in the genetic diversity of natural population of sandal due to indiscriminate extraction. Jeeva *et al*. (1998) also reported drastic decline due to over exploitation, poor seed germination, and failure of natural and artificial regeneration. Extensive extraction of heartwood has severely decimated the natural stands in the forests and rendered many populations fragmented (Rao *et al*., 2007).

2.2.2. Loss of genetic diversity

Genetic diversity is a necessary pre-requisite for long term survival and adaptability (Young *et al*., 2000; Bahuguna, 2007). The most serious consequence of depletion of genetic diversity is genetic erosion followed by extinction of species (Kemp *et al*., 1993). Hence, the magnitude and distribution of population genetic diversity are the

most fundamental piece of information required for proper genetic management of species (Brown, 1978). Genetic diversity in sandal is imperilled owing to the wanton felling perpetrated by smugglers and also due to the destruction caused by spike disease (Muthana, 1995).

Reduction in sandal wealth and its genetic erosion is a global phenomenon. Reduction in *S. album* population in West Timor, the second largest population after India has been attributed to low viability (low germination %) of sandalwood seeds due to inbreeding depression (Setiadi and Komar, 2001). Assessment on the genetic structure and clonality within five southernmost populations of *S. lanceolatum* at Victoria in Australia using allozymes and RAPD analyses has shown that asexual reproduction by root suckering alone was responsible for the increase in the population size (Trueman *et al*., 2001). Natural regeneration of *S. spicatum* has been reported to be poor in Western and Southern Australia due to habitat fragmentation, parasitic nature of sandal, grazing and poor seed dispersal (Murphy and Garkaklis, 2003).

2.2.3. Light/shade requirement at early seedling stage

Among the main environmental factors, light is perhaps the most influential factor involved in the survival, growth and reproduction of tropical species. Light responses usually provoke physiological alterations, which are determinants for CO_2 assimilation and optimization of gas exchange (Sands, 1995). Light availability is the primary limiting factor of seedling growth, as demonstrated in several experiments carried out after controlling nutrients and water supply, but with light availability (Kitajima, 1992a; Kitajima, 1996). Despite the necessity of light for autotrophic organisms, no plant is capable of using 100 per cent of maximum solar irradiation for photosynthesis (Demmig *et al*., 1997). Environments that are either too much shaded or under full sunlight can inhibit the photosynthetic process (Zhang *et al*., 2003). When irradiance exceeds the normal level that can be used for photochemistry, protective mechanisms have to be used to dissipate excess excitation energy to avoid damage to plant tissues. Too much of light causes damage to the photosynthetic apparatus by means of photoinhibition. Hence, the optimum amount of shade required for the healthy growth of sandal seedlings need to be evaluated.

2.2.4. Absence of appropriate host

Intriguingly, despite the large host range of the majority of parasitic plants, many of them also show high level of host preference. Impact of hosts on parasite communities not only depend on what is parasitised but also when parasitism occurs. Being a hemi-parasite the silvicultural requirements of sandal are unique and there is no adequate understanding of the same. Its regeneration and establishment has been problematic because of the poor knowledge of host-parasite relationships (Surendran *et al*., 1998). So, an understanding of the complementary and competitive influence of the host plant on sandal is necessary for growing sandal successfully. Proper selection of host species to give maximum benefit to sandal is critical for establishing economically viable sandal plantations (Radomiljac *et al*., 1999).

2.2.5. Fungal disease and seedling mortality

Fungal pathogens are also unique among plant pathogens in being able to breach the plant surface directly, utilizing a number of biochemical and morphogenetic mechanisms in order to penetrate host tissues. For this reason, their control is of great significance to the agricultural and forest economy (Talbot, 1998). As suggested by the theory of adaptive polymorphism (Hunter and Fraser, 1990), genetic diversity is likely to enhance the ability of a species to survive in a wider variety of environmental conditions. Studying the mechanisms of pathogenicity and extent of genetic variation in plant pathogens is therefore critical for the future control of fungal disease.

2.2.6. Clonality and inbreeding in seed stand

Seed stands are highly relevant since they assure immediate availability of adequate quantities of seeds. Knowledge of genetic variation within and between the seed stands is crucial for adopting proper seed management in the seed production areas and in tree improvement programmes (Jagadish *et al*., 2007; Sreekanth, 2009). Two seed stands had been established in Nachivayal reserve now coming under Marayur Sandal Division during 1980-81. But, recently it has been reported that expected quantity of seeds are unavailable from these seed stands. Most of the sandal trees flower profusely, but they

do not set seeds. As sandal is a cross pollinated plant, fruit setting and seed formation will take place usually if pollination and fertilization take place between genetically unrelated genotypes. Lack of seeds has been reported from *Santalum yasi* trees in Fiji (Jiko, 2000). Trueman *et al*. (2001) observed lack of fruit and seed production in remnant populations of *S. lanceolatum* in Victoria due to clonality. Though, failure to develop mature seeds may occur due to unsuccessful pollination and fertilization, pathogenic infection of developing fruits, premature flower and fruit fall, etc., such major problems did not come to our notice. Hence the genetic diversity of sandal seed stand was analysed using ISSR marker.

Fragmented populations can also exhibit disturbed pollination as a result of restricted pollinator movement, inadequate flowering for pollinator attraction and insufficient quantity of pollen or incompatible pollen (Byers, 1995; Oostermeijer *et al*., 1996). Poor fruit set has been observed in *S. lanceolatum* resulting in poor pollen deposition due to limited pollinator visitation (Cindy *et al*., 2000). Sexual reproductive failure in remnant self-incompatible populations poses a threat to persistence (Holsinger and Vitt, 1997) and small populations may be particularly susceptible to catastrophic events or losses of habitat quality (Cropper, 1993; Oostermeijer *et al*., 1996).

2.3. ECOPHYSIOLOGY OF HOST-PARASITE RELATIONSHIP

Parasitic plants are a diverse group accounting for 1 per cent of angiosperm species (about 4000 species) within 270 genera and more than 20 families. They are common in many natural and seminatural ecosystems from tropical rain forests to the high Artic (Musselman and Press, 1998; Nickrent and Duff, 1996). They occur in many life forms, including annual and perennial herbs (e.g. *Rhinanthus* spp. and *Bartsia* spp.), vines (e.g. *Cuscuta* spp. and *Cassytha* spp.), shrubs (e.g. *Olax* spp. and mistletoes) and trees (sandalwoods) (Malcolm and Gareth, 2005). Parasitism is a successful mode of life in many flowering plants (Knutson, 1983; Musselman and Press, 1998) and it has evolved on at least seven separate occasions.

Generally, parasitism reduces host productivity and/or reproductive effort, as has been extensively documented for both root parasites and shoot parasites (Malcolm and

Gareth, 2005). The acquisition of host resources can exert strong effects on host growth, allometry, reproduction and physiology which lead to changes in competitive balances between host and nonhost species and therefore affect community structure, vegetative zonation and population dynamics (Press *et al.*, 1999).

2.3.1. Parasitism

The major characteristic of these plants is that during a part of their life cycle, they depend on the host plant for some or all of their nutrients. Two types of parasitic relationships exist: holoparasitic (obligate parasites) and hemi-parasitic (facultative parasites). Holoparasites are totally dependent on their hosts for nutrition, since they possess no chlorophyll or capacity to assimilate carbon and inorganic nitrogen. Hemiparasites such as sandal are not totally nutritionally dependent on their hosts, since they possess chlorophyll, but require water, minerals and physical support from their host at varying levels depending upon the species. They acquire some or all of their water, carbon and nutrients via the vascular tissue of the host's roots or shoots. They access their host resources through a key organ called the haustorium, which provides a physical as well as a physiological bridge between the parasite and the host, directing the host's resources to the parasite and functioning at multiple stages in the parasitism (Kuijt, 1969). A broad diversity is found in the internal structure of the haustoria belonging to different parasitic plant species. The morphology of the haustorium is directly related to access host resources through either direct vascular continuity, interfacial parenchyma, or a combination of both. Futhermore, there is variability in the extent to which different nutrients and solutes are obtained by parasitic plants (Pate *et al.*, 1990b).

2.3.2. Host preference of parasites

Intriguingly, despite the large host range of the majority of parasitic plants, many also show high levels of host preference. Impact of hosts on parasite communities not only depend on what is parasitised but also when parasitism occurs. Studies have shown that both root and shoot parasites often prefer hosts with a high nitrogen content, such as legumes (Matthies, 1997; Radomiljac *et al.*, 1999), or hosts that have readily

accessible vascular systems (Kelly *et al*., 1988) and/or lower defence capacity (Cameron, 2004). Hosts may also be preferred if they are available as a resource for longer period for e.g. a preference for woody perennials over herbaceous annuals (Kelly *et al*., 1988) or if they have ready access to limiting resources for e.g. a preference for deep rooted hosts with access to the water table during drought (Pate *et al*., 1990a). *Cuscuta* shows greater biomass and reproduction within patches of preferred/good hosts (Kelly *et al*., 1988; Kelly, 1990). Host preference may also depend on the diversity of potential hosts available; mistletoes of Loranthaceae show a low host preference in heterogeneous tropical rain forests and high host preference in less diverse temperate forests.

2.3.3. Host influence on parasites

Greater abundance and/or performance of preferred hosts will enhance the performance (growth and reproduction) of the parasite. Root hemi-parasites are particularly common in grassland eco-systems because grasses are often preferred hosts, having abundant root systems (i.e. easy to locate) and fine roots that are easy to penetrate. The uptake of host alkaloids by root-hemiparasitic Orobanchaceae has been well documented and reductions in herbivory or herbivore performance when feeding on the alkaloid acquiring parasites have been observed (Marko and Stermitz, 1997). Loveys *et al*. (2001) observed that fruits of the root hemiparasite, *Santalum acuminatum* (the quandong) contained a natural insecticidal compound acquired from neighbouring *Melia azadirachta* hosts. The uptake of such compounds from the host was proposed to be beneficial because a bioassay using the apple moth (*Epiphyas postvittana*) showed that its larvae suffered high mortality on feeding on fruits of *S. acuminatum* growing near *Melia* hosts. This also explains the observation of commercial growers that *Santalum* growing near *Melia* have fruits that suffer less insect attack. In addition to such direct benefits, the parasite may also gain indirect benefits from uptake of secondary metabolites. In the case of *Castilleja endivisa*, Adler (2000) observed that the root hemiparasite not only gained from reduced herbivory by insect larvae when acquiring alkaloids from 'bitter' lupine hosts, but the reduced herbivory of floral parts increased the visitation by humming bird pollinators.

2.3.4. *Santalum* spp. and their host plants

Just as in the case of other root hemiparasites, sandalwood is partly dependent on host species for water and nutrients, with leguminous hosts being generally better sources of nitrogen than other species (Tennakoon *et al*. 1997). Host selection is the single most important silvicultural parameter influencing *S. album* plantation establishment (Radomiljac, 1994). Studies on xylem transfer of organic solutes between *Santalum acuminatum* (R. Br.) A. DC. and their respective host plants have shown that substantial differences in amino acid, sugar and organic acid composition of the xylem stream of the parasite occurs when associated with different host species. Such differences have been used alongside parasite growth data to explain why some hosts are markedly superior to others in terms of overall benefit to the parasite (Radomiljac *et al*., 1998a). Moreover studies by Tennakoon and Pate (1998) on the biological and physiological aspects of the woody root hemi-parasite *S. acuminatum* and its common hosts have shown that haustoria of *S. acuminatum* function as major sites of synthesis and export of proline, and might therefore play an important role in osmotic adjustment of *S. acuminatum* in acquiring water from hosts under differing levels of stress. In *S. spicatum*, lack of certain nutrients, or insufficient uptake via host attachments was associated with increased leaf fall (Fox and Barrett, 1994).

Radomiljac (1994) has found pot host species *Alternanthera nana* and *Sesbania formosa* to be best as hosts to *S. album* in plantation establishment at Ord River Irrigation area, Western Australia. Successful haustorial formation is the key to the survival of the individuals of the ecologically important plant *S. album* (Tennakoon and Cameron, 2006). Anatomically, the haustorium of *S. album* resembles that of *Olax* (Pate *et al*., 1990b; and Tennakoon and Pate, 1997) and *Osyris* (Niranjana and Shivamurthy, 1987) in possessing an endophyte which spreads laterally around the surface of the host xylem. The primary host *Cajanus cajan* as an effective species for nourishing sandal in nursery has been reported by many authors like Subbarao *et al*. (1990) and Taide *et al*. (1994). But Annapurna *et al*. (2007) has reported that *C. cajan* as a traditional primary host has several disadvantages, such as its fast growth, high level of competiton with sandal seedlings for light and nutrients, susceptibility to fungal attacks, insect pests and also the requirement for intensive management.

The selection of appropriate pot host species is critical to ensure high levels of *S. album* field survival and growth. Like all autophytes, *S. album* posseses leaf chlorophyll and is able to synthesise carbohydrates. Generally, parasitic plants are smaller in size than their hosts, such as the herbaceous root parasite *Rhinanthus serotinus*. However, *S. album* has a large tree habit and, therefore the host is not the sole source for amino acids and carbohydrates (Radomiljac, 1994). So also a suitable sowing regime is required. If sown too early, pot host species will overcrowd the pot. Conversely, if sown too late, insufficient time for haustorial connection before field establishment will result in *S. album* suffering outplanting stress.

2.4. SHADE REQUIREMENT OF SEEDLINGS

The capacity of a plant to use and dissipate light energy is a function of both genotype and environmental conditions. Most plants have the ability to acclimatise to a specific light environment. Excess light can affect plant growth and reduce field productivity as a result of photoinhibition (Kitao *et al*., 2000a; Goncalves *et al*., 2001; Marenco *et al*., 2001; Kull, 2002). Apart from a group of pioneer species (shade intolerant species), the majority of tropical forest tree species are shade tolerant. However, survival and growth of seedlings in shade varies widely and continuously among tropical tree species closely reflecting light requirements of varying intensities as the seedlings grow and establish in forest soil. In *S. album*, though earlier works (Fox and Barrett, 1994) show that some level of shade is beneficial for first three years, the length of time for optimum benefit has not been elucidated.

2.4.1. Seed reserves and seedling development

The seedling phase is uniquely different from the later stages of plant life in terms of dependency on maternally-derived resources and rapid developmental changes in morphology and allocation patterns. In a strict physiological sense, a plant developed from a seed is a seedling as long as it depends on seed reserves (Fenner, 1987). Initially after radicle emergence, a developing seedling acquires all necessary resources from seed reserves and its growth rate is independent of external resource availability. This is

the stage of complete dependency on seed reserves. Then, following the development of organs necessary for autotrophy, such as photosynthetic cotyledons or leaves for acquisition of energy and roots for acquisition of mineral nutrients, a seedling starts uptake of externally available resources. During this transitional stage, a seedling utilizes both internal (seed-derived) and external sources with increased dependency on the latter, until the former becomes of negligible importance (Kitajima, 1996).

Ecophysiology of cotyledons deserves special attention because of the high diversity of morphology, degree of exposure, position and associated functions of cotyledons found among tropical tree species (Kitajima, 1992b). Before seed germination, cotyledons absorb resources from the endosperm and the mother plant (Murray, 1985). During and after germination, cotyledons transfer reserve materials (lipids, carbohydrates, mineral nutrients) to developing shoot and roots (Ernst, 1988). Cotyledons of some species serve strictly as organs to store and transfer seed reserves throughout their lifespan, while cotyledons of other species develop a second function, photosynthetic carbon assimilation (Kitajima, 1992b). Completely storage cotyledons are globoid, remain partially or completely in the seed coat (cryptocotylar), and are typically positioned at or below the ground level (hypogeal). Photosynthetic cotyledons are free of seed coat (phanerocotylar), thin and paper-like (papyraceous), and raised above ground (epigeal). Physiological function of cotyledons is of great importance in determining growth responses of seedlings to light environment.

Early seedling development is the process by which seed tissues rich in reserves are transformed into seedling tissues. Different mineral nutrients in seeds are exhausted at different rates (Brookes *et al*., 1980). In various temperate herbaceous species, nitrogen is the first among mineral nutrients to become insufficient in supply from seed reserves (Fenner and Lee, 1989). Nitrogen, most of which is stored in the form of storage protein in seeds, is used for synthesis of various enzymes necessary during seedling development, including those of early photosynthetic organs, such as photosynthetic cotyledons or the first leaves. Little is known about utilization of other mineral nutrients in seeds of tropical tree species. Seedlings of temperate *Quercus* species depend on cotyledon reserves rather than on soil for phosphorus and potassium for the first year (Brookes *et al*., 1980).

The duration of seed reserve dependancy is an important aspect of the ecology of seedling establishment (Fenner, 1987; Kitajima and Fenner, 2000). Initially, seedlings are completely dependent on seed reserves for all resources except water, but they gradually become dependent on the external supply of resources acquired by shoots (light energy fixed carbon-based compounds) and roots (mineral nutrients) (Krigel, 1967; Fenner, 1986). The duration of strict seed dependency for a given resource may vary among species in relation to four characteristics; seed size (total seed mass); seed quality (concentration of the focal resource); major function of cotyledons (whether cotyledons serve as photosynthetic or storage organs of seed reserves after germination); and inherent rate of seedling growth and development.

2.4.2. Chlorophyll fluorescence

Photosynthesis is considered as the most fundamental biological process. Photons are absorbed by molecules of antenna complex and the excitation energy produced is transferred to reaction centres of the photosystems. The energy drives primary photo-chemical reactions that initiate the photosynthetic energy conversion in photochemical and biochemical pathway. The excess energy can be dissipated as heat or it can be re-emitted as light-chlorophyll fluorescence. These three processes occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the other two. Hence, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained (Maxwell and Johnson, 2000). Fluorescence is the energy predominantly emitted (3-5% absorbed energy) from chlorophyll complexes of Photosystem II (PSII) (Govindjee, 1995). In recent years, the technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiological studies. No investigation into the photosynthetic performance of plants under field conditions seems complete without fluorescence data.

The first significant realization of the relationship between primary reactions of photosynthesis and Chl *a* fluorescence came from Kautsky and Hirsh (1931). They were the first to report that, upon illumination of a dark adapted sample, the Chl *a* fluorescence emission is not constant but exhibits a fast rise to a maximum followed by a decline to reach finally, in a range of some minutes to a steady level. They further

showed that the declining phase of the fluorescence transient is correlated with an increase in the CO_2 assimilation. Changes in the yield of chlorophyll fluorescence were first recorded by Kautsky *et al*. (1960). There is a fundamental relationship between the quantum yield of fluorescence and photochemical energy conversion. Fluorescence can be used to measure the efficiency of PSII photochemistry, acclimatisation of plants to different microenvironments and to understand the effects of low and high temperatures. It also gives insight into the ability of a plant to tolerate environmental stresses and to the extent to which those stresses have damaged the photosynthetic apparatus (Maxwell and Johnson, 2000).

Chlorophyll *a* fluorescence at 20 s, 2 ms, and 30 ms and the time required to achieve maximum fluorescence are termed as the O, J, I and P step, respectively. Based on the analysis of how the data from the J-I-P fluorescence transient can be processed, a test has been developed called the "JIP - test" after the steps of the transient. This test can be used as a tool for the rapid screening of many samples providing adequate information on the structure, conformation and function of their photosynthetic apparatus (Strasser and Strasser, 1995; Strasser *et al*., 1996). Many investigators have used F_o (minimal fluorescence), F_v (variable fluorescence) and F_m (maximum fluorescence) parameters of chlorophyll *a* fluorescence for testing.

2.4.3. F_v/F_m

The ratio of variable fluorescence to maximal fluorescence (F_v/F_m) of dark-adapted leaves is used commonly to assess the relative state of PSII. F_v/F_m is used frequently as an expression of photoinhibition which is typically associated with, chlorophyll degradation (Griffin *et al*., 2004). Normally, the measurement of pigments from chloroplasts and analysis of chlorophyll fluorescence are used as stress indicators of high irradiance in plants (Stancanto *et al*., 2002). Synthesis and degradation of chlorophyll occurs naturally in the presence of light. Nonetheless, the excess of light can cause greater degradation and reduction in the levels of total chlorophyll. Additionally, excess of light can cause decrease of photosynthetic capacity and lead to occurrence of photoinhibition (Kitao *et al*., 2000b). Shade grown plants often have relatively large antenna complexes for maximum light capture (Lambers *et al*., 1998).

When exposed to high irradiance, the energy absorbed by these relatively large light-capturing complexes turn to be detrimental to the plant if not efficiently dissipated. Even plants that have been grown under high light conditions can experience supraoptimal irradiance. When this occurs, photoinhibition, or a decrease in quantum efficiency of photosystem II (PSII) becomes significant. It is in this context that studies on chlorophyll fluorescence become important. Chlorophyll fluorescence is a useful physiological test because it is non invasive, non destructive and rapid (Vidaver *et al*., 1989). Chlorophyll fluorescence is an indicator of photosynthetic performance of plants and has been increasingly used to understand both the mechanism of photosynthesis and the factors affecting it (Maxwell and Johnson, 2000).

A study with four Virginia Piedmont tree species showed that ratio of variable fluorescence to maximal fluorescence (F_v/F_m) increased with shade, suggesting that quantum yield increased, thereby allowing more efficient energy transfer from chlorophyll to PSII (Groninger *et al*., 1996). Khan *et al*. (2000) studied the effects of shade on morphology, chlorophyll concentration, and chlorophyll fluorescence of four Pacific Northwest Conifer species and found that photochemical efficiency of all four species was lower under higher irradiation. Studies by Goncalves *et al*. (2005) on growth, photosynthesis and stress indicators in young *Aniba rosaeodora* Ducke plants under different light intensities showed that the photosynthetic activity of the species may be limited when grown in shaded environments or environments with high light intensity, either due to insufficient light intensity in the shaded environment or due to photoinhibition as a consequence of excess light in the open environment. Ishida *et al*. (1999) studied the diurnal changes in the leaf gas exchange and chlorophyll fluorescence of two dipterocarps, *Shorea leprosula* (a high light requiring species) and *Neobalanocarpus heimii* (a low light requiring species), and a pioneer tree species (*Macaranga gigantea*) growing in open and gap sites. Data obtained provide evidence to the hypothesis that ecophysiological characteristics link with plant's regeneration behaviour and successional status. In studies with white spruce (*Picea glauca* (Moench) Voss) seedlings, Vidaver *et al*. (1989) suggested that chlorophyll fluorescence provide useful information about photosynthetic responses to environmental stresses such as freezing temperatures and moisture stress. Studies were conducted to evaluate the growth performance of *Adathoda beddomei* under different shade levels (Neerakal *et al*., 2005).

2.4.4. Photoinhibition

When leaves are exposed to more light than that can be utilised through the process of photosynthesis, PSII function can be affected in a stress condition called photoinhibition. The value 0.8 for F_v/F_m ratio is considered as the threshold value for photoinhibition by Liittge *et al*. (1998). Pronounced altitudinal variation in photochemical efficiency of PS II (F_v/F_m) was observed in five indigenous fodder species namely *Quercus leucotrichophora* A. Camus (temperate), *Celtis australis* Linn (sub-tropical), *Grewia optiva* Drummond (sub-tropical), *Bauhinia purpurea* Linn (tropical) and *Melia azedarach* Linn (tropical). The F_v/F_m ratio ranged from 0.39-0.87 and two species were found to be photosynthetically most active. This study provided the basis of screening physiologically active and fast growing tree species at different altitudes. Furthermore, studies on chlorophyll fluorescence gave fairly good idea on productivity and suitability of species for cultivation under a given area (Husen *et al*., 2004). Effect of industrial pollution on Scot pine needles was detected by measurement of fluorescence parameters (Pukacki, 2000). Studies on chlorophyll fluorescence parameters in populations of two legume trees *Stryphnodendron adstringens* (Mart.). *coville* (Mimosoideae) and *Cassia ferruginea* (Schrad.) Schrad. ex DC (Caesalpinoideae) have revealed fluorescence measurements to be an efficient technique to investigate the photosynthetic performance when grown under a non-stressful environment also (Filho *et al*., 2004). In *C. ferruginea* plants, Fv/Fm value of 0.783 had been observed. However, it was observed under low light level and so was not due to photoinhibition (Filho *et al*., 2004).

Studies on photosynthesis, chlorophyll fluorescence and carbohydrate content of *Illicium* taxa grown under varied irradiance showed that three of the eleven taxa experienced less photoinhibition than the other and maintained greater photochemical efficiency of absorbed light (Griffin *et al*., 2004). Chlorophyll fluorescence transients have been used as an indirect method to distinguish between submergence tolerant and susceptible rice cultivars (Sarkar *et al*., 2004). Niu *et al*. (2004) studied gas exchange and chlorophyll fluorescence response to simulated rainfall in *Hedysarum fruticosum* var. *mongolicum* and found that PSII activity was really impaired by water stress and could recover to the normal status only when the water stress disappeared.

The quantitative relationship between chlorophyll fluorescence and the efficiency of photosynthetic energy conversion opens new ways of application in plant breeding,

development of new varieties and evaluation of plant productivity. Slapakauskas and Ruzgas (2005) estimated fluorescence level of newly developed winter wheat varieties. In studies of forest decline and more generally in studies of effects of air pollution on plants and photosynthesis, chlorophyll fluorescence has become an important tool, not only in screening the effects but also in elucidating the nature of damage to photosynthesis (Nordenkampf and Oquist, 1993). Chlorophyll fluorescence measurements can help in determining the health status of a plant before and after disease symptoms appear (Santos *et al*., 2000), especially for early detection of fungal infection (Wagner *et al*., 2006).

2.5. GENETIC DIVERSITY OF TREES

Forest and forest products are renewable resources and contribute substantially to economic development. They play a major role in enhancing the quality of the environment. Forests preserve the genetic diversity of living resources, which is necessary to sustain and improve agricultural and forestry production; forest is the raw material for scientific and industrial innovation. Tropical forests are characterised by a great diversity of tree species and this range of variation provides the basis for selection and improvement of forest products (Kemp *et al*., 1993). The extent of forest in India is 75 million ha which works out to be 19.27 per cent of our geographical area; and this is far below the requirement of 33 per cent (ICFRE, 2000).

Genetic diversity is essential for both the long-term stability and short-term productivity of forest ecosystems. The amount of genetic variation within a species and its distribution within and among populations provides clues to the factors that govern the maintenance of variation, inbreeding and gene flow. Genetic diversity is required for maintaining evolutionary potential in a changing environment, resist pests and avoid the negative consequences of inbreeding (Newton *et al*., 1993; Bawa and Dayanandan, 1998).

Characterization of genetic diversity is a prerequisite for exploitation of genetic resources for plant improvement. Morphological characterization is often faced with the problems of low penetrance and heritability. Molecular markers are highly heritable, are available in high numbers, and often exhibit enough polymorphism to dicriminate closely related individuals. Knowing the distribution of diversity within and among

populations of a species is important for conservation because it provides useful guidelines for the preservation of genetic diversity within the species as a whole. If a large proportion of the diversity resides among populations, then more populations must be conserved than if each population contains much of the species-level diversity (Fransico *et al.*, 2000).

Population genetics is the quantitative study of the amount and distribution of genetic variation in populations, and the dynamics of the underlying genetic processes. Description of population genetic structure and its dynamics is based on the analysis of allele and genotype frequencies of simple traits whose transmission follow Mendelian rules of inheritance (Frankel, 1983). The estimate of allele frequencies at a locus from knowledge of genotype frequencies is forthright under the assumptions of the Hardy-Weinberg principle. The Hardy-Weinberg principle provides the foundation for all population genetic investigations. The principle states that in a large random-mating population with non-overlapping generations, the allele and genotype frequencies will remain constant from generation to generation, when there is no mutation, migration and natural selection which can alter the frequencies. A population is said to be in Hardy-Weinberg equilibrium (HWE) when frequencies of three genotypes viz., homozygous dominant (AA), heterozygous (Aa) and homozygous recessive (aa) at a diallelic locus (dominant and recessive) are p^2, 2pq and q^2 respectively such that $p^2+2pq+q^2=1$, where the allele frequencies p (A) and q (a) can be calculated from genotype frequencies (Yeh, 2000).

To characterize population genetic structure, the following parameters that describe and quantify the genetic and geographic variation patterns are usually investigated viz. polymorphisms (P) to describe what proportion of gene loci are variable, average number of alleles per locus (A), average heterozygosity (h) to describe what proportion of all gene loci are heterozygous and the level of among population differention G_{ST} (Brown, 1989). Forest trees have been exposed to various geographical disturbances and extreme life history characteristics such as long cycle, greater opportunity for accumulation of mutations and exposure to stresses. Thus, to develop and implement effective genetic improvement and conservation strategies in forest trees, it is necessary to integrate the information drawn from the above mentioned population genetic diversity parameters.

2.5.1. Methods of genetic diversity study

The use of genetic markers in plant breeding dates back to the beginning of the century when, in peas, Bateson and Punnett (1905) indicated the possibility of genetic linkage, between genes controlling flower petal colour and shape of the pollen grain. Prior to 1960's, markers used in plant genetics and breeding were those derived from genes controlling discrete phenotypes of easy visual identification such as dwarfism, chlorophyll deficiencies, flower and seed and their morphology. But these morphological markers are limited in number, expressed only at the whole plant level, greatly influenced by environment and exhibit only a low per cent of polymorphism. This picture began to change in the 1960's with the development of molecular markers based on isozyme polymorphism (Lewontin and Hubby, 1966) which continued to provide simple and inexpensive method of obtaining genetic information in tree species also (Grattapaglia *et al*., 1992). However, isozymes detect only a fewer number of loci and only a limited subset of isozyme loci can be assayed across the life cycle stages for most tree species.

The advent of molecular techniques based on the analysis of DNA polymorphisms radically expanded the frontier area of this study as it mitigated the limitations of morphological and biochemical markers both in terms of numbers available and their genetic properties. With the development and application of molecular markers over the last 20 years, we now know more about the genetic structure of forest tree species and the spatial and temporal dynamics of genetic processes, such as mating and gene flow, than ever before. These molecular markers include Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSRs), Amplified Fragment Length Polymorphism (AFLP), microsatellites or Simple Sequence Repeats (SSRs), Restriction Fragment Length Polymorphism (RFLP) and Single Nucleotide Polymorphisms (SNPs).

2.5.2. Randomly Amplified Polymorphic DNA

In Randomly Amplified Polymorphic DNA marker (RAPD), genomic DNA samples are amplified by applying PCR using randomly constructed oligonucleotides as primers. Each RAPD cycle consists of three steps, DNA denaturation (90-94ºC), primer annealing (30-36ºC) and primer extension (72ºC). The template DNA is denatured by subjecting to temperature between 90-94ºC. In annealing step, temperature is rapidly

reduced to 30-36ºC allowing the hybridization of each primer to their complementary regions. The extension step involves the addition of nucleotides using target sequences as templates resulting in the synthesis of a new copy of target sequence in each cycle. The cycle is generally repeated for 20-45 cycles and the amplification process follows geometric progression (Welsch and Mc Clelland, 1990). RAPD is being successfully used to differentiate species, varieties, cultivars, and clones in many crop plants.

RAPD has been successfully applied in differentiating between varieties and clones of *Camellia sinensis* and in evaluating the genetic diversity among elite tea (*Camellia sinensis* var. *sinensis*) accessions (Kaundun *et al*., 2000). The technique has been used to study genetic diversity in many plant genera such as mahoganies (Chalmers *et al*., 1994), *Eucalyptus* (Keil and Griffin, 1994), mango (Schnell *et al*., 1995), *Populus* spp. (Castiglione *et al*., 1993), oil palm (Shah *et al*., 1994), Norway spruce (Scheepers *et al*., 1997), *Cacao* (Whitkus *et al*.,1998), *Amaranthus* (Chan and Sun, 1997), cotton (Iqbal *et al*., 1997) and brassicas (Jain *et al*., 1994). They have also been used to tag genes of agronomic importance (Michelmore *et al*., 1991; Hormaza *et al*., 1994) and to develop genetic maps in Norway Spruce (Binelli and Bucci, 1994) and *Populus* spp. (Bradshaw *et al*., 1994). RAPD markers can be effectively used to determine the specificity in plant-pathogen interaction and to identify markers linked to a resistant gene of interest within a short time (Naqvi *et al*., 1995; Kuginuki *et al*., 1997).

2.5.3. Inter Simple Sequence Repeats

Since 1994, a new molecular technique called Inter Simple Sequence Repeat (ISSR) has become available (Zietkiewicz *et al*., 1994). ISSRs are semiarbitrary markers amplified by PCR in the presence of one primer complimentary to a target microsatellite. ISSR markers use short microsatellite motif containing primers anchored at the 3′ or 5′end by two to four arbitrary degenerate nucleotides to amplify the DNA sequence lying between two microsatellite regions (Zietkiewicz *et al*., 1994). PCR-ISSR amplification does not require prior genome sequence information (Bornet *et al*., 2002b). Each band corresponds to a DNA sequence delimited by two inverted microsatellites. ISSR was first used by Zietkiewicz *et al*. (1994) to differentiate between closely related individuals. ISSRs have been used to study genetic diversity in plants

such as corn (Kantety *et al*., 1995), potato (Bornet *et al*., 2002a; Provan *et al*., 1996; Prevost and Wilkinson, 1999) and rice (Blair *et al*., 1999). They are widely used to investigate clonal diversity and population genetic structure (Tani *et al*., 1998; Esselman *et al*., 1999; Rossetto *et al*., 1999). Extremely high variability and high "mapping density" as compared with RFLP and RAPD data make these new microsatellite-based molecular markers ideal for producing genetic maps of individual species (Nagaoka and Ogihara, 1997). Moreover they can be used for systematic, molecular, ecological, evolutionary and crop improvement studies at the population level and among closely related species (Tsumura *et al*., 1996; Hollingsworth *et al*., 1998; Esselman *et al*., 1999).

ISSR markers have been successfully used for varietal identification and assessment of genetic relationships in many plant species (Narayanan *et al*., 2007). They are widely used in population studies because of their highly variable nature; less investment in time, money and labor than other methods (Wolfe and Liston, 1998; Harris, 1999) and the fact that they exhibit Mendelian inheritance (Gupta *et al*., 1994; Tsumura *et al*., 1996). They have been instrumental in determining variability and correcting misidentifications in large germplasm collections (Fang et al., 1997; Gilbert et al., 1999; Lanham and Brennan, 1999, Charters and Wilkinson, 2000). They have also been used to determine the genetic diversity of species of conservation concern (Esselman *et al*., 1999; Camacho and Liston, 2001; Mc Glaughlin et al., 2002; Smith and Bateman, 2002).

ISSRs have been successful in distinguishing subspecies of *Plantago major* L. (Plantaginaceae), a cosmopolitan species (Wolfe and Morgan- Richards, 1998) and in determining the levels of genetic variation between sympatric species of *Alnus* (Betulaceae) in Italy (King and Ferris, 2000). They have been useful for cultivar identification in numerous plant species, including Rice (Joshi *et al*., 2000), Apple (Goulao and Oliveira, 2001), Strawberry (Arnau *et al*., 2003) and assessment of genetic variations in plants such as Citrus (Fang and Roose, 1997), *Viola pubescens* (Culley and Wolfe, 2000), Potato (Prevost and Wilkinson, 1999) and *Oryza* (Qian and Hong, 2001).

ISSR markers are thought to be particularly useful for study of closely related individuals which exhibit low levels of polymorphism (Zietkiewicz *et al*., 1994) and have been applied as a very useful alternative to fingerprinting and genetic analysis in fruit crops including Citrus (*Citrus* L. spp) (Fang and Roose, 1997; Fang *et al*., 1997),

Grape (*Vitis vinifera* L.) (Moreno *et al*., 1998), Gooseberry (*Ribes* L.) (Lanham and Brennan, 1999) and Plum (*Prunus* L. spp) (Goulao and Oliveira, 2001). Cervera *et al*. (1998), Moreno *et al*. (1998) and Sensi *et al*. (1996) reported discrimination of grape clones using ISSR assays. ISSR profiling has been used as a powerful method for molecular characterisation of *Leucadendron* varieties (Pharmawati *et al*., 2005) and has proved to be a potentially useful tool for the identification of strawberry varieties (Arnau *et al*., 2003). They have been used to screen markers for ascertaining sex in *Carica papaya* (Parasnis *et al*., 1999), *Humulus lupulus* (Danilova and Karlov, 2006) and *Cycas circinalis* (Gangopadhyay *et al*., 2007). Genetic relations between various coffee species and determination of the family ties between coffee hybrids (Paulo *et al*., 2003) was carried out using ISSR marker analysis. In the same way the technique was effective for the study of genetic variation in *Changium smyrnioides* (Apiaceae) (Ying-Xiong *et al*., 2004) and inter and intraspecies variation of genus *Penstemon*. ISSRs have been used in studies of cultivated species to produce genetic linkage maps (Kojima *et al*., 1998; Cekic *et al*., 2001) and to determine the relatedness of lines of agriculturally important species. In a comparitive study of RAPD markers, ISSR markers, and allozymes designed to assess clonal diversity in *Calamogrostis porteri* spp. *insperata*, Esselman *et* al. (1999) found that ISSR markers detected more variation than did RAPD and allozymes.

2.5.4. Genetic diversity of sandal

RAPD markers had been used to assess genetic variation of 54 sandalwood genotypes distributed in India (Shashidhara *et al*., 2003). They concluded that the sandalwood germplasm from India constituted a broad genetic base. Angadi *et al*. (2003) assessed genetic distance between 8 different provenances of sandal using isozyme markers and found that all the sandal provenances are genetically well separated. Using 23 enzyme systems, Brand (1994) investigated the levels of genetic diversity in 10 West Timor and two Indian sandal populations and found that West Timor and Indian populations are genetically well separated. Suma and Balasundaran (2003; 2004) have assessed the level of genetic variation within and between 11 southern Indian *S. album* populations using isoenzymes and RAPD markers. Studies on genetic diversity and seedling survival of eight Indian provenances (Suma and Balasundaran, 2007) have shown that Marayur provenance has better genetic diversity; Marayur provenance also showed better

adaptability not only in Marayur but also in low altitude areas of Kerala than other provenances. Using allozyme markers, Nageswara Rao *et al.* (2007) analyzed the genetic diversity of 19 sandal populations distributed over different parts of Peninsular India.

2.6. SANDAL SEEDLING MORTALITY AND LOW GERMINATION PERCENTAGE : GENETIC DIVERSITY OF *FUSARIUM*

2.6.1. The genus *Fusarium*

Fungi are responsible for serious plant diseases worldwide. Fungi are characterised by a greater complexity and diversity of form than other microbial pathogens. This diversity is highlighted by the extraordinary host range observed by some pathogens and the extreme pathogenic specialization of others. The genus *Fusarium* contains many species that attack a large number of crops, causing seed rots, root rots, foot rots, stalk rots, wilts, yellow and ear and kernel rots. The genus was first described by Link (1809) and later classified within Fungi Imperfecti in the class Hyphomycetes. The sexual or perithecial stage belongs to the order Hypocreales of subdivision Ascomycotina (Prasad *et al*., 2007). This variable fungus, composed of many pathogenic species and strains, lives in the soil and attacks all cultivated crops and many wild plants. The fungus is capable of attacking all parts of the plant. Serious loss may result, especially on susceptible cultivars when weather conditions are favourable for disease development (George *et al*., 1992). *Fusarium* spp. penetrates roots principally through wounds made by nematodes. The fungus grows through the water-conducting tissues, producing toxins that kill the cells, stunt the plant, and yellow the leaves. Often the plant wilts and dies from lack of water. Symptoms usually appear only on one side of the stem and progress upward until the foliage is killed and the stem dies. The water conducting tissues just under the bark turn brown; the discolouration is visible in cross-sections of stems near the base of infected plants (George *et al*., 1992).

The taxonomy of *Fusarium* spp. is confusing and various classification systems have been proposed. Diagnosis of *Fusarium* at the species level is based on conventional methods, which include the description of colonies on appropriate media (texture, colour and pigment) and microscopic description of conidiogenous cells and

conidia. Species identification by morphological traits is problematic because characteristics like mycelia pigmentation, formation, shape and size of conidia are unstable and highly dependent on composition of media and environmental conditions. Phenotypic variation is abundant and much expertise is required to distinguish between closely related species and to recognize variation within species. Random PCR approaches are being increasingly used to generate molecular markers, which are useful for taxonomy and for characterizing fungal populations.

Fusarium is a large cosmopolitan genus of imperfect fungi and is of primary interest because numerous species are important plant pathogens, produce a wide range of secondary metabolites, and/or cause opportunistic mycoses in humans (Singh *et al*., 2006). *Fusarium oxysporum* Schlechtend. Fr. is the sole species of the section Elegans which comprises fungal strains which are morphologically and physiologically similar (Woo *et al*., 1998). This group of cosmopolitan, soil borne, filamentous fungi are economically important because many members are the causal agents of vascular wilt or root rot diseases in diverse agricultural and ornamental crops throughout the world. *F. oxysporum* is anamorphic and comprises non-pathogenic strains also which asymptomatically colonize plant roots or grow saprophytically, as well as pathogenic strains which colonize the xylem, causing diseases (Booth, 1971; Nelson *et al*., 1981). The plant host specificity of the pathogen denotes a specialized form of the fungus: the formae speciales, and the strains of these different formae speciales are not morphologically distinguishable (Synder and Hansen, 1940). More than 120 different formae speciales have been described for *F. oxysporum* (Hawksworth *et al*., 1995), although some fungal strains have been found to have a broad host range and may attack closely related plant species as well as unrelated genera (Kistler, 1997). *F. oxysporum* is a cosmopolitan fungal pathogen responsible for wilt and cortical rot diseases of more than 100 economically important plant hosts (Booth, 1971).

Studies on fungal pathogens in natural environments often require simultaneous analysis of their broad taxonomic range. The laborious nature of microscopic analysis for identification is the driving force behind developing methods such as molecular verification of identified morphotypes by means of PCR-RFLP or such other molecular markers (Horton and Bruns, 2001). Before the advent of DNA markers, pathologists used a

range of phenotypic characters to characterize fungal diversity. Many characters such as colony appearance, growth rate, spore size, spore colour etc. are still used extensively in epidemiological studies; but, they are under polygenic control and hence are of limited value in inheritance studies. DNA-based technologies are used in taxonomic studies since more markers can be scored simultaneously than with other systems. Application of a range of DNA-based techniques have revolutionized the discipline of fungal systematics and provided tools for discrimination of closely related fungi (Mills *et al.*, 1998).

Historically, RFLPs were used initially as markers (Raeder *et al.*, 1989) and then RAPDs. More recently, they have been augmented and partly superseded by AFLPs and SSRs, though they can all be used together. PCR based methods for the rapid detection and identification of *Candida* species include Restriction Fragment Length Polymorphism (RFLP) analysis, PCR-with species-specific probes and Random Amplification of Polymorphic DNA (RAPD) analysis (Fujita *et al.*, 2001). PCR with fungus-specific primers, targeting the conserved sequences of 5.8S and 28S ribosomal DNAs (rDNAs) as well as those of 18S and 28 S rDNAs, results in the respective amplification of the species-specific internal transcribed spaces (ITS) regions which vary in amplicon length and sequence according to the species (Gardes *et al.*, 1991; Henry *et al.*, 2000).

2.6.2. Diversity of fungal isolates and adaptability

Accurate and rapid identification of pathogens is necessary for appropriate management of plant diseases. Genetic characterization of pathogenic variants of plant pathogens prevalent in an area is required for efficient management of the disease and to improvise methods to increase plant productivity. The ability of fungal pathogens to generate extensive genetic diversity is likely to be responsible for the great adaptability which allows them to colonize in a wide range of habitats (Sullivan and Coleman, 1998). So, studying mechanisms of pathogenicity and sources of genetic variation in plant pathogens is critical for the control of fungal disease (Talbot, 1998).

2.6.3. Method of study of *Fusarium* diversity

Random Amplified Polymorphic DNA (RAPD) assay has been used extensively to explore fungal populations at species, intraspecific, race and strain levels. PCR-RAPD

has been applied widely to study the genetic relatedness of various fungal species such as *Armillaria* genets (Smith *et al*., 1994), *Aspergillus niger* (Magnegneau *et al*., 1993), *Colletotrichum* spp. (Mills *et al*., 1992; Fabre *et al*., 1995), *Gibberella fujikuroi* and *Fusarium* (Voigt *et al*., 1995), *Metarhizium anisopliae* (Fegan *et al*., 1993; Bidochka *et al*.,1994), *Puccinia striiformis* (Chen *et al*., 1993) and *Rhizoctonia solani* (Duncan *et al*., 1993). The technique has ben used to identify and discriminate between pathotypes of *Fusarium oxysporum* f. sp. *ciceris* (Kelly *et al*., 1994) and *Leptosphaeria maculans* (Goodwin and Annis, 1991); races of *Fusarium solani* f. sp. *vasinfectum* (Assigbetse *et al*., 1994), *F. oxysporum* f. sp. *pisi* (Grajal-Martin *et al*., 1993) and for variety differentiation in *Hirsutella longicolla* (Strongman and Mackay, 1993). Arbitrary primers (10-mers) have been used to detect genetic diversity in *Colletrotrichum acutatum*, *C. gloeosporioides* and *C. fragariae*. RAPD analysis revealed higher levels of polymorphisms, and in some cases, individual strains were recognized, making RAPD useful for strain identification for commercially important species such as *Trichoderma reesei* and *T. harzianum*. RAPDs have been used to assess the diversity at the intra and interspecific level in the genera *Colletotrichum* and *Trichoderma*. Linkage maps already exist for several plant pathogens such as *Magnaporthe grisea* (Farnian and Leong, 1995), *Cladosporium fulvum* (Arnau *et al*., 1994), and *Giberella fujikuroi* (Desjardins *et al*., 1996).

Studies on molecular variability in *F. oxysporum* are numerous, and vary depending upon the objectives and desired applications. PCR-RFLP analyses of nuclear rDNA and RAPD analysis have been used to determine molecular variability of *F. oxysporum*. Repetitive DNA sequences detected by RFLP and southern analyses have been most frequently used in taxonomic investigations among various *Fusarium* species. A combination of RFLPs and RAPD analysis along with pathogenicity and vegetative compatibility test was used to characterize isolates of *F. oxysporum* f. sp. *phaseoli*, the causal agent of bean yellows or wilt disease of *Phaseolus vulgaris* from various geographic origins (Woo *et al*., 1998). RAPD analysis was found to be useful in distinguishing non-pathogenic *F. oxysporum* isolates from pathogenic isolates of *F. oxysporum* f. sp. *dianthi* (Manulis *et al*., 1994; Kalc Wright *et al*., 1996). *F. oxysporum* f. sp. *vasinfectum* (Assigbetse *et al*., 1994). *F. oxysporum* f. sp. *ciceris* (Kelly *et al*., 1994) and *F. oxysporum* f. sp. *albedinis* (Fernandez and Tantaoui, 1994; Tantaoui *et al*., 1996).

RAPD analysis has been used effectively to distinguish between species of *Fusarium*. The results indicate that RAPD analysis can be effectively employed as a reliable DNA fingerprinting technique to study the spread of the pathogen (Amy *et al*., 1997). RAPD was used to determine the genetic variability among 15 isolates of *F. graminearum* collected from different regions of India. Large genetic variation was detected at the DNA levels which indicated the ability of a pathogen to adapt to different life-cycle conditions (Saharan *et al*., 2007). Gherbawy *et al*. (1999) used RAPD technique to analyse different formae speciales of *F. oxysporum*. Moller *et al*. (1999) studied fungal populations of *F. moniliforme* and *F. subglutinans* using RAPD technique. Gherbawy *et al*. (2002) used RAPD technique for identifying *Fusarium subglutinans*, *F. proliferatum* and *F. verticillioides* strains isolated from maize in Austria. Pasquali *et al*. (2003) characterized isolates of *F. oxysporum* pathogenic to *Argyranthemum frutescens* L. using RAPD technique. Genetic variability in pea wilt pathogen *F. oxysporum* f. sp. *pisi* in north-western Himalayas was studied using RAPD and protein (native protein and esterase isozyme) markers (Sharma *et al*., 2006). Genetic diversity of *F. oxysporum* isolates from cucumber was studied using RAPD by Vakalounakis and Fragkiadakis (1999).

F. oxysporum has received considerable attention from plant pathologists over the past 80 years because of its ability to cause vascular wilt or root rot diseases on a wide range of plants. Nijs *et al*. (1997) studied variation in RAPD patterns within *Fusarium* species from cereals from various parts of the Netherlands. Moller *et al*. (1999) studied fungal populations of *F. moniliforme* and *F. subglutinans* using RAPD technique. Genetic diversity and recombination within populations of *F. pseudograminearum* from Western Canada was investigated using restriction digestion of nuclear ribosomal DNA (nrDNA) using 3 restriction enzymes (*EcoRI*, *Hae III* and *Pst I*), and ISSR markers. The study revealed a substantially high genetic diversity within populations of *F. pseudograminearum* recovered from infected wheat seeds in the provinces of Alberta and Saskatchewan in Western Canada (Mishra *et al*., 2006).

CHAPTER 3

AUTOTROPHIC AND PARASITIC PHASE OF SANDAL SEEDLING GROWTH

3. AUTOTROPHIC AND PARASITIC PHASE OF SANDAL SEEDLING GROWTH

3.1. INTRODUCTION

The seedling phase is unique for the later stages of plant life in terms of dependency on maternally-derived resources and rapid developmental changes in morphology and resource allocation patterns. Early seedling development is the process by which seed tissues rich in reserves are transformed to seedling tissue. In a strict physiological sense, a plant developed from a seed is a seedling as long as it depends on seed reserves (Fenner, 1987). Initially after radicle emergence, a developing seedling acquires all the necessary resources from seed reserves (cotyledons/endosperm) and its growth rate is independent of external resource availability. This is the stage of complete dependency on seed reserves. Then, following development of organs needed for autotrophy, as the photosynthetic cotyledons or leaves for acquisition of energy and roots for acquisition of mineral nutrients, a seedling starts to uptake externally available resources (Kitajima, 1996).

Before seed germination, cotyledons absorb resources from the endosperm and the mother plant. During and after germination, cotyledons transfer reserve materials (lipids, carbohydrates and mineral nutrients) into developing shoot and roots. Initially, seedlings are completely dependent on seed reserves, but they gradually become dependent on the external supply of resources acquired by shoots (light energy fixed in carbon-based compounds) and roots (mineral nutrients). As seed resources become exhausted, growth of the seedling becomes significantly slower (Kitajima, 2002). This indicates the end of seed reserve dependency. At this point of time, the seedling requires an external supply of resources for further growth. Therefore, the duration of seed reserve dependency is an important aspect of the ecology of seedling establishment (Fenner, 1987; Kitajima and Fenner, 2000).

The period upto which seedlings can grow by utilizing reserves varies with the species (Kitajima, 1996). Knowledge of such a phase of autotrophic nutrition is important in nursery management as seedlings grown by sowing seeds in sterile or

nutrient deficient medium needs to be pricked out before seed reserves are fully exhausted. The objective of this study was to understand the seed reserve (cotyledonary) dependency of *Santalum album* L. seedlings.

3.1.1. Host dependency of sandal plants

Parasitic plants are taxonomically diverse group of angiosperms that rely partially or completely on hosts for carbon, nutrients and water, which they acquire by attaching to host roots or shoots using specialised structures known as haustoria and by penetrating host xylem and/or forming close connections with the phloem. The site of attachment to the host and the presence or absence of functional chloroplasts defines the parasite further as being either hemiparasitic or holoparasitic, respectively (Malcom and Gareth, 2005).

Sandal is a hemi root parasite. In the early stages of *S. album* development, nutrition is derived from the relatively large seed reserves and presumably also the soil via the plant's own roots. Later, the formation of host attachment becomes critical for survival (Barrett and Fox, 1997). Nagaveni and Srimathi (1985) have noted that 70 per cent of seedlings of *S. album*, in the presence of potential hosts, initiate haustoria within 30 days of germination, and 90 per cent within one year. They further suggested that *S. album* could survive without hosts for a period of 2 to 3 years, but with poor survival and growth.

For the establishment of sandal in plantations, healthy nursery seedlings with a pot-host is essential. Earlier, sandal hosts were classified into three categories by Radomiljac and Mc Comb (1998) as pot-hosts, intermediate hosts and long-term hosts. The pot host functions both in nutrition and water relations of *S. album*, and it reduces outplanting stress when transferred to the field. The intermediate host acts as a 'bridging-agent' between the pot-host and long-term host. Stimulating early growth may be an important determining factor in the future heartwood production of *S. album*.

Studies have reported that *S. album* benefits from a primary host at the nursery stage and a secondary host (long term) in the field (Srinivasan *et al*., 1992; Surata,

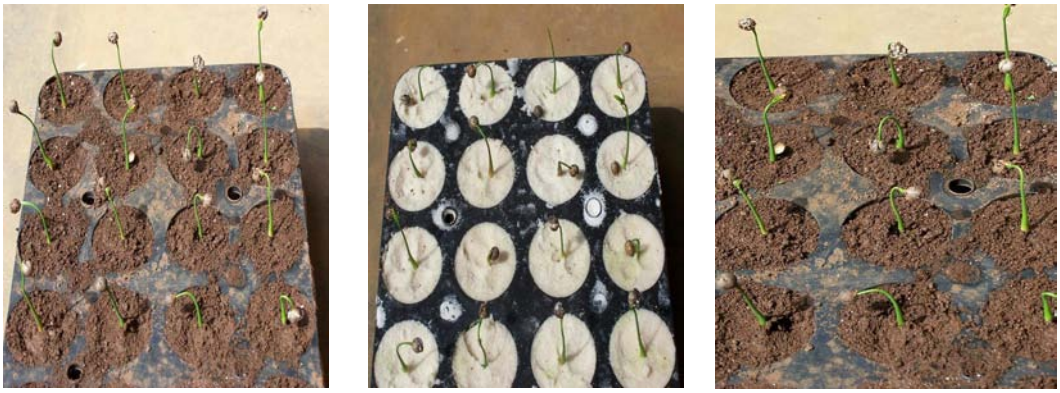
1992; Barrett and Fox, 1995; Fox *et al*., 1996; Radomiljac, 1998; Fox, 2000). Sandal depends on nutrients derived from its hosts for growth and development. Hosts are suggested as the source of Ca, Fe, N, P, K, Mg, Cu and Zn for sandal (Barrett and Fox, 1997). Raising plantable quality of sandal seedlings under nursery conditions has been found to be difficult since efficient hosts which enhance the growth of sandal need be supplied. Tennakoon *et al*. (2000) found high chlorophyll content in parasitized *S. album* (with host) seedlings as compared to control (without host). Though chlorophyll fluorescence technique has been applied to assess the nursery seedling quality in *Pseudotsuga menziesii* (Franco) (Fisker *et al*., 1995) and also to study the effects of shade on four pacific Northwest conifer species (Khan *et al*., 2000), it has not yet been used so far to study host-sandal interactions. Moreover, measurement of chlorophyll fluorescence provides a very good criteria to assess the seedling performance.

Many studies have been conducted earlier on host association of sandal and its growth enhancement. There are many plants which have not been reported to be haustorised by sandal though luxuriant growth of sandal in association with them is observed under nursery conditions. However, no report is available describing physiological basis supporting growth enhancement. In view of these facts, an experiment was conducted with a few selected annuals and perennials to examine the host preferences of sandal seedlings under nursery conditions through physiological measurements and chlorophyll estimation of sandal.

3.2. MATERIALS AND METHODS

3.2.1. Autotrophic phase of sandal seedlings

Sandal seedlings emerging out of seeds with cotyledons intact were pricked out to root trainers of 150cc capacity containing sterile quartz sand, vermiculite and soil (Fig 3.1). Each category had 48 seedlings. Seedlings in sterile quartz sand and vermiculite were maintained under irrigation using distilled water, whereas the seedlings in soil were irrigated using tap water. Conditions of cotyledons, leaves and seedlings as a whole were noted daily for 230 days (Table 3.1.).



In soil In quartz sand In vermiculite

Figure 3.1: Sandal seedlings grown in different potting media in root trainers

3.2.2. Host dependency of sandal plants

Sandalwood seeds were sown in germination trays. After germination, seedlings were transferred to polybags containing potting medium comprising a 2:1:1 mixture of soil:sand:farm yard manure. Seedlings were maintained in nursery conditions under 75 per cent shade for about 8 months and later were transferred to full sunlight. When sandal seedlings were about 4-month-old, seedlings of host plants were planted in each polybag and growth of sandal seedlings with host was monitored for about a year for annuals and about two years for perennials. Perennials were transplanted to earthen pots within a year. Sandal seedlings were grown with annuals such as *Vigna unguiculata* (L.) Walp. (Fabaceae), *Ocimum sanctum* L. (Verbenaceae), *Ageratum conyzoides* L. (Asteraceae), *Phyllanthus niruri* L. (Euphorbiaceae), *Mikania micrantha* Kunth ex. H. B. K. (Asteraceae), *Ludwigia hyssopifolia* (G Don) Exell (Onagraceae), *Scoparia dulcis* L. (Scrophulariaceae) and *Vernonia cinerea* (L.) Lem. (Asteraceae) and perennials such as *Pongamia pinnata* (L.) Pierre (Fabaceae) and *Lantana camara* L. (Verbenaceae) (Fig. 3.9.-3.27.). These species were selected as hosts since sandal seedlings showed better growth with them under nursery conditions when compared with sandal seedlings growing without host. From our earlier observations it was noticed that sandal seedlings haustorise *Vigna ungiculata* as early as 30 days of planting. But we had provided the hosts only after 4 months since they were found to outgrow and compete with sandal seedlings for resources. Sandal plants grown with different hosts showed different growth responses. The polybags were hand weeded as and when necessary.

At the end of the experiment (one year for annuals and two years for perennials) both the set of plants were destructively harvested and measurements of height, collar diameter, total biomass, shoot and root fresh weight, number of haustorial connections, chlorophyll fluorescence and chlorophyll contents of sandal seedlings, and total biomass, shoot and root fresh weight of host plants were recorded. In addition, dry weights of sandal seedlings were measured after partitioning them into shoots and roots, and oven dried at 75°C for 48 hours.

The quality of the seedlings was determined using the following criteria:

- Sturdiness Quotient (SQ)
- Dickson Quality Index (DQI)
- Performance Index (PI) using JIP test

3.2.3. Sturdiness Quotient

This is a seedling quality index that can be measured by a simple and non-destructive method. SQ compares height (cm) over root collar diameter (mm). A small quotient indicates a sturdy plant with a higher expected chance of survival.

SQ = H/D (Thompson, 1986)

where,

H= Final shoot height (cm)

D= Final collar diameter (mm)

SQ has been used to assess height to stem diameter ratio, which tends to increase with shade and is considered as an important morphological adaptation to shade (Wang *et al*., 1994).

3.2.4. Dickson Quality Index

The shoot:root ratio has been conventionally used as an important measure for seedling survival. It relates the transpiring area (shoot) to the water absorbing area (root). It can also evaluate the carbon allocation above and below the ground. It is measured by determining the root and shoot dry weights. However, this ratio has been modified by bringing in the SQ component also into it, resulting in the Dickson Quality Index.

DQI= TDW/ ([H/D] + [SDW/RDW]) (Dickson *et al.*, 1960)

where,

TDW= Final total seedling dry weight (g)

H/D = Sturdiness Quotient (SQ)

SDW= Final shoot dry weight (g)

RDW= Final root dry weight (g)

A higher value for the index is an indication of a healthy seedling.

3.2.5. Physiological measurements

Chlorophyll fluorescence was repeatedly measured at regular intervals on a subsample of seedlings with a Plant Efficiency Analyzer (Handy PEA, Hansatech Instruments Ltd., Norfolk, UK). Chlorophyll fluorescence measurement can yield valuable data on the performance of the photosynthetic apparatus, especially the PS II. The chlorophyll fluorescence transients were induced by light intensity of 1500 µmol m^{-2} s^{-1} and recorded from 10 µs to 1 second. All measurements were made on attached leaves which were fully dark adapted for 10 to 20 minutes using leaf clips supplied with the instrument. Measurements were taken between 09.00 and 12.00 hours. The data collected from the various treatments were downloaded to a computer and analysed for the J-I-P test using BIOLYZER program (Rodriguez, R. M. Bioenergetic Laboratory, University of Geneva; available at www.unige.ch/sciences/ biologie/bioen). Chlorophyll *a* fluorescence at 20 s, 2 ms, and 30 ms and the time required to achieve maximum fluorescence are termed as the O, J, I and P step, respectively. J-I-P test involves the analysis of the transient fluorescent curves given by the chlorophyll when subjected to high light intensity. The J-I-P test refers to the analysis of structural integrity of chloroplasts and function based on the fluorescence emission data. From the induction of fluorescence of fast kinetics, the initial fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence ($F_v=F_m-F_o$) and photochemical efficiency of PS II (F_v/F_m) were obtained.

From the data collected during the initial second, a large number of parameters were available for further analysis of physiological performance. Out of these,

Performance Index (PI) was selected. PI is a synthesized parameter, taking into account several other parameters and is a very useful term providing information concerning 'relative vitality' (Handy PEA, Hansatech Instruments Ltd., Norfolk, UK).

PI = ABS /CS x RC/ ABS x TR/DI x ET/dQA/dt (Strasser *et al*., 1999) where,

ABS= Photon flux absorbed by chlorophyll *a*

CS= Cross sectional area of the leaf

RC= Number of reaction centres

TR= Trapping flux of energy

DI= Dissipated photon flux

ET= Energy flux corresponding to electron transport

dQA/dt= Flux of excitons trapped per reaction center

3.2.6. Chlorophyll content estimation

For the estimation of chlorophyll content, fresh leaves were collected from host plants showing higher F_v/F_m values. 250 mg of leaf sample was homogenised with 5 ml of 80 per cent acetone (pre-chilled). The homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was transferred to a 100 ml conical flask. The procedure was repeated until the residue was colourless. The final volume was made upto 25 ml with 80 per cent acetone. The absorbance of the solution was read at 645 and 663 nm against the blank solvent (80 per cent acetone) using a spectrophotometer (Genova MK3, UK). Using the absorption coefficient, the amount of chlorophyll was calculated based on the protocol of Sadasivam and Manickam (1992). Chlorophyll content of sandal plants and hosts were analysed and compared using the formulae.

mg chlorophyll *a* / g tissue = 12.7 (A663) - 2.69 (A645) x V / 1000 x W

mg chlorophyll *b*/g tissue = 22.9 (A645) - 4.68 (A663) x V/1000 x W and

mg chlorophyll / g tissue = 20.2 (A645) + 8.02 (A663) x V / 1000 x W

where,

A= absorbance at specific wavelengths

V= final volume of chlorophyll extract in 80 per cent acetone and

W= fresh weight of the extracted tissue (g)

3.3. RESULTS

3.3.1. Autotrophic phase of sandal seedlings

Complete withering of cotyledons in quartz sand occurred within 26 days of radicle emergence (Table 3.1.). Hence, sandal seedlings need to be transferred to polybags containing a 1:2:1 mixture of sand:soil:farm yard manure within 26 days after germination for proper growth and development.

Table 3.1. Autotrophic phase of nutrition during developmental stages of sandal seedling after germination

Part of the seedling	Condition of the seedling part	Potting media used		
		Soil	Quartz sand	Vermiculite
Endosperm/seed	Colour:			
	Creamish brown	0-6 days	0-4 days	4-6 days
	Brown	6-8 days	4-7 days	6-8 days
	Complete fall off	8-25 days	8-25 days	8-20 days
Cotyledon	Colour:			
	Green	0-2 days	0-2 days	0-4 days
	Yellowish green	4-6 days	4-8 days	4-7 days
	Light Brown	14-24 days	14-16 days	7-19 days
	Complete fall off	24-27 days	19-26 days	19-34 days
Leaf	Emergence	6-20 days	4-25 days	6-20 days
	Initiation of leaf fall	220 days	210 days	220 days
Whole seedling	Healthy	0-61 days	0-69 days	0-82 days
	Wilting started	210-220 days	220-230 days	220-230 days

3.3.2. Host dependency of sandal plants

On the basis of initial growth of sandal, eight annual and two perennial host species were screened for better sandal seedling growth (Fig 3.2.; Table 3.2.; Table 3.3.). Data for annuals were analysed using one way ANOVA (SPSS 16 software). All the morphological parameters were found to be significantly different for sandal plants grown with different annual hosts (Table 3.4.). However, the suitability of host could

not be judged based on any single growth parameter of sandal. Among the annual host plants better performance for height, sturdiness quotient (SQ), SDW/RDW, Dickson Quality Index (DQI) and haustorial numbers were shown by *V. unguiculata*, *S. dulcis*, *M. micrantha*, *V. cinerea* and *O. sanctum*. Among these, *V. unguiculata* showed better performance for height, SQ, DQI and number of haustoria. The perennial species *Pongamia* and *Lantana* were found to give good support to sandal seedling growth (Table 3.3.). The results of the growth data on height, collar diameter, total fresh weight, total dry weight and shoot dry weight:root dry weight of sandal plants grown with annual hosts (Table 3.2.) revealed that *V. unguiculata* had given good support for sandal as host. Sandalwood plants with *V. unguiculata* was significantly distinct in growth. *Vigna* being an annual, can function as a superior primary host of sandal. With the perennial host species, sandal plants with *Pongamia* showed better performance for height, collar diameter, total fresh weight, total dry weight, DQI and number of haustoria (Table 3.3.). *Pongamia* was found to be the best long term host, being a perennial tree species.

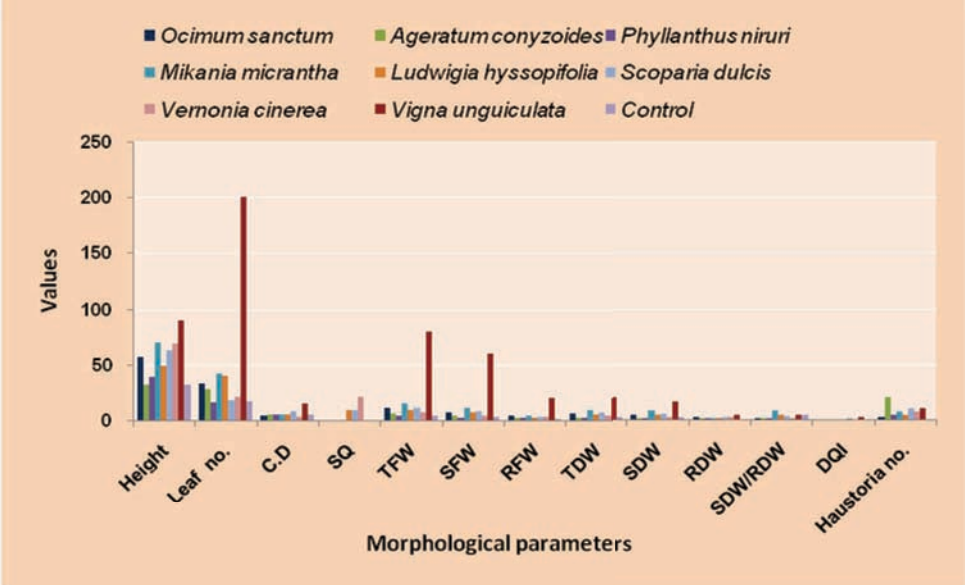


Figure 3.2. Final growth parameters of sandal plants with different annual hosts

Height = Shoot height (cm); C.D = Collar diameter (mm); SQ = Sturdiness Quotient= Height (cm) / Collar diameter (mm); TFW = Total Fresh Weight (g); SFW = Shoot Fresh Weight (g); RFW = Root Fresh Weight (g); TDW = Total Dry Weight (g); SDW = Shoot Dry Weight (g); RDW = Root Dry Weight (g); SDW/ RDW = Shoot Dry Weight / Root Dry Weight; DQI= Dickson Quality Index

Table 3.2. Final growth parameters of sandal plants with annual hosts (mean ± SE)

Sl. No.	Host species grown with sandal	Height (cm)	Leaf No.	C.D (mm)	SQ	TFW	SFW	RFW	TDW	SDW	RDW	SDW/ RDW	DQI	No.of haustoria
1	*Ocimum sanctum*	56.6 ± 6.99	33 ± 4.2	4.29 ± 0.11	13.18 ± 1.72	11.5 ± 2.98	7.5 ± 2.01	4 ± 1.03	6 ± 1.54	4 ± 1	2.5 ± 0.57	1.5 ± 0.29	0.4 ± 0.08	2 ± 0.25
2	*Ageratum conyzoides*	32.3 ± 3.52	28 ± 2.73	5.48 ± 0.27	5.89 ± 0.97	6 ± 0.88	4 ± 0.33	2 ± 0.58	2 ± 0.67	1 ± 0.67	1 ± 0.33	1 ± 0.17	0.29 ± 0.02	20 ± 3.52
3	*Phyllanthus niruri*	38.5 ± 2.97	16 ± 0.58	5.11 ± 0.24	12.37 ± 0.69	4 ± 0.33	2 ± 0.33	2 ± 0.33	2 ± 0.67	1 ± 0.33	1 ± 0.33	1 ± 0.33	0.15 ± 0.02	4 ± 0.58
4	*Mikania micrantha*	70 ± 6.21	42 ± 2.4	5.34 ± 0.24	13.10 ± 0.52	15 ± 2.03	11 ± 1.45	4 ± 0.67	9 ± 1.53	8 ± 0.33	1 ± 0.33	8 ± 2.28	0.34 ± 0.07	7 ± 0.58
5	*Ludwigia hyssopifolia*	48.9 ± 3.49	40 ± 2.73	5.18 ± 0.38	9.44 ± 0.2	9 ± 0.58	7 ± 0.67	2 ± 0.33	5 ± 0.33	4 ± 0.58	1 ± 0.58	4 ± 1	0.37 ± 0.02	4 ± 0.88
6	*Scoparia dulcis*	62.8 ± 4.14	18 ± 1.2	7.43 ± 0.58	8.45 ± 0.14	11 ± 0.88	8 ± 1	3 ± 0.33	7 ± 0.58	5 ± 0.58	2 ± 0.33	2.5 ± 0.44	0.64 ± 0.04	10 ± 1.53
7	*Vernonia cinerea*	69.2 ± 11.5	21 ± 11.5	3.34 ± 0.46	20.71 ± 3.41	7 ± 0.88	4 ± 0.58	3 ± 0.33	4 ± 0.58	2 ± 0.33	2 ± 0.33	1 ± 0.33	0.18 ± 0.03	7 ± 1.86
8	*Vigna unguiculata*	90 ± 5	200 ± 15	15 ± 1.5	6 ± 0.33	80 ± 5	60 ± 5	20 ± 1	20 ± 1	16 ± 1	4 ± 0.5	4 ± 0.6	2 ± 0.11	10 ± 1
	Control (sandal alone)	31.8 ± 1.8	17 ± 1	5.47 ± 0.59	5.8 ± 0.39	4 ± 0.5	3 ± 0.5	1 ± 0.5	2.5 ± 0.53	2 ± 0.45	0.5 ± 0.08	4 ± 0.43	0.25 ± 0.05	0

Height = Shoot height (cm); C.D = Collar diameter (mm); SQ = Sturdiness Quotient= Height (cm) / Collar diameter (mm); TFW = Total Fresh Weight (g); SFW = Shoot Fresh Weight (g); RFW = Root Fresh Weight (g); TDW = Total Dry Weight (g); SDW = Shoot Dry Weight (g); RDW = Root Dry Weight (g); DQI= Dickson Quality Index

Table 3.3. Final growth parameters of sandal plants with perennial hosts (mean ± SE)

Sl. No.	Host species grown with sandal	Height (cm)	Leaf No.	C.D (mm)	SQ	TFW	SFW	RFW	TDW	SDW	RDW	$\frac{SDW}{RDW}$	DQI	No. of haustoria
1	*Lantana camara*	87 ± 15.36	36 ± 12.35	6.31 ± 0.59	13.78 ± 1.22	38 ± 11.37	22 ± 1.52	16 ± 10.02	10 ± 1.53	8 ± 2.03	2 ± 0.67	4.75 ± 1.38	0.54 ± 0.05	40 ± 2.89
2	*Pongamia pinnata*	116 ± 4	210 ± 15	10 ± 0.75	11.6 ± 0.55	184 ± 12	129 ± 9.5	55 ± 2.5	92 ± 5	55 ± 3.5	29 ± 2.5	1.89 ± 0.24	6.81 ± 0.50	156 ± 23

Height = Shoot height (cm); C.D = Collar diameter (mm); SQ = Sturdiness Quotient= Height (cm) / Collar diameter (mm); TFW = Total Fresh Weight (g); SFW = Shoot Fresh Weight (g); RFW = Root Fresh Weight (g); TDW = Total Dry Weight (g); SDW = Shoot Dry Weight (g); RDW = Root Dry Weight (g); DQI= Dickson Quality Index

Table 3.4. ANOVA - Final growth parameters of sandal plants with annual hosts

		Sum of Squares	df	Mean Square	Calculated F value	Table F value
Height	Between Groups	6966.30	7	995.19	8.10*	2.66
	Within Groups	1965.48	16	122.84		
	Total	8931.79	23			
CD	Between Groups	153.21	7	21.89	35.13*	2.66
	Within Groups	9.97	16	.62		
	Total	163.18	23			
SQ	Between Groups	199.51	7	28.50	3.93*	2.66
	Within Groups	116.12	16	7.26		
	Total	315.63	23			
TFW	Between Groups	8457.89	7	1208.27	97.38*	2.66
	Within Groups	198.52	16	12.41		
	Total	8656.41	23			
SFW	Between Groups	4699.39	7	671.34	87.08*	2.66
	Within Groups	123.35	16	7.710		
	Total	4822.74	23			
RFW	Between Groups	611.92	7	87.42	63.34*	2.66
	Within Groups	22.08	16	1.38		
	Total	634.00	23			
TDW	Between Groups	451.62	7	64.52	19.62*	2.66
	Within Groups	52.62	16	3.29		
	Total	504.25	23			
SDW	Between Groups	342.07	7	48.87	39.74*	2.66
	Within Groups	19.68	16	1.23		
	Total	361.75	23			
RDW	Between Groups	37.07	7	5.29	2.85*	2.66
	Within Groups	29.71	16	1.86		
	Total	66.78	23			
SDW/RDW	Between Groups	15.09	7	2.16	0.83	2.66
	Within Groups	41.57	16	2.59		
	Total	56.67	23			
DQI	Between Groups	4.96	7	.71	77.46*	2.66
	Within Groups	.15	16	.01		
	Total	5.11	23			
Haustoria	Between Groups	305.21	7	43.60	5.78*	2.66
	Within Groups	120.75	16	7.55		
	Total	425.96	23			

* significant at 5 % level

Among the annual host species evaluated, *V. unguiculata* registered higher root biomass (18 g) and root:shoot ratio (1.11) (Table 3.5. & 3.7.; Fig 3.3.). Among the perennial host species evaluated *P. pinnata* registered higher root biomass (1122 g) and root:shoot ratio (1.63) (Table 3.6.). DQI was highest for sandal with *V. unguiculata* (2). DQI of sandal without any host was 0.25 (Table 3.2.; Fig 3.4.). Among sandal with perennial hosts DQI was highest for sandal with *Pongamia* (6.81) (Table 3.3.).

Table 3.5. Evaluation of annual host species' growth based on root:shoot ratio (mean ± SE)

Sl. No.	Annual host species grown with sandal	Mean Fresh weight		Root:Shoot ratio
		Shoot (g)	Root (g)	
1	*Ocimum sanctum*	6 ± 0.58	1 ± 0.88	0.17 ± 0.09
2	*Ageratum conyzoides*	4 ± 0.71	0.7 ± 0.23	0.18 ± 0.02
3	*Phyllanthus niruri*	3 ±1.45	2 ± 0.58	0.67 ± 0.09
4	*Mikania micrantha*	12 ± 1.15	11 ± 0.58	0.92 ± 0.08
5	*Ludwigia hyssopifolia*	11 ± 0.88	2 ± 0.33	0.18 ± 0.04
6	*Scoparia dulcis*	8 ± 0.58	3 ± 0.88	0.38 ± 0.08
7	*Vernonia cinerea*	5 ± 0.58	1 ± 0.33	0.2 ± 0.09
8	*Vigna unguiculata*	20 ± 2.5	18 ± 2	1.11 ± 0.04

Table 3.6. Evaluation of perennial host species' growth based on root:shoot ratio (mean ± SE)

Sl. No.	Perennial host species grown with sandal	Mean Fresh weight		Root:Shoot ratio
		Shoot (g)	Root (g)	
1	*Lantana camara*	84 ± 2	87 ± 2.5	1.04 ± 0.01
2	*Pongamia pinnata*	687 ± 116	1122 ± 67.5	1.63 ± 0.27

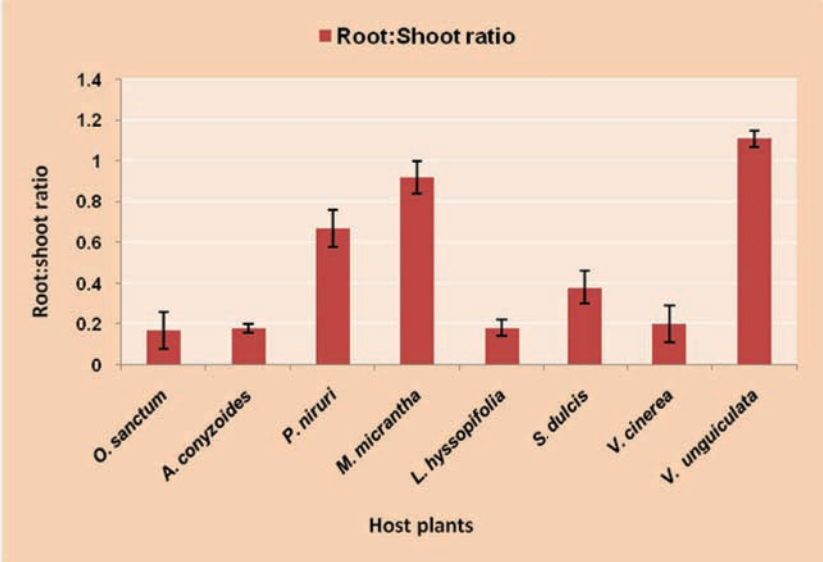


Figure 3.3. Chart showing annual host species' evaluation based on their root:shoot ratio

Table 3.7. ANOVA - Evaluation of annual host species based on root fresh weight, shoot fresh weight and root:shoot ratio

		Sum of Squares	df	Mean Square	Calculated F value	Table F value
RFW	Between Groups	1054.82	7	150.69	98.30*	2.71
	Within Groups	22.99	15	1.53		
	Total	1077.82	22			
SFW	Between Groups	610.78	7	87.25	28.56*	2.71
	Within Groups	45.83	15	3.06		
	Total	656.61	22			
RFW/SFW	Between Groups	1.66	7	.24	13.63*	2.71
	Within Groups	.26	15	.02		
	Total	1.92	22			

RFW = Root fresh weight; SFW = Shoot fresh weight

* significant at 5 % level

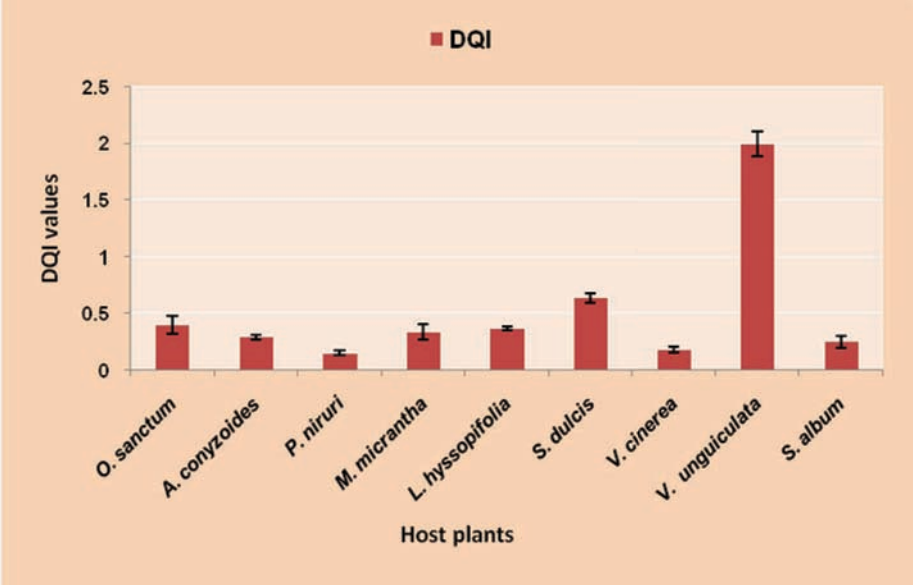


Figure 3.4. DQI of sandal in association with annual hosts

F_v/F_m ratio [$F_v=F_m–F_o$; where F_v = variable fluorescence, F_o = initial fluorescence and F_m = maximum fluorescence] is linearly correlated with the quantum yield of net photosynthesis and indicates the intrinsic (maximum) photosynthetic efficiency of photosystem II. Dark adapted values of F_v/F_m are used as a sensitive indicator of plant photosynthetic performance, with the optimum values around 0.8 measured for most plant species. Changes in F_v/F_m and F_o are still accepted and widely used as reliable diagnostic indicators of photoinhibition (Filho *et al*., 2004). F_v/F_m values were evaluated for sandal growing well with best annual hosts (Table 3.8.; Fig 3.5. & Fig 3.6.). F_v/F_m values were found to be higher for sandal growing with annuals *V. unguiculata* (0.75) and *O. sanctum* (0.67). F_v/F_m values were found to be highest for sandal growing with perennials *P. pinnata* (0.82) followed by *L. camara* (0.76) (Table 3.9.), F_v/F_m values of sandal growing without host was 0.29 (Table 3.8.).

Table 3.8. Fv/Fm of sandal grown along with best annual hosts (mean ± SE)

Host plant name	F_v/F_m of sandal with annual host
O. sanctum	0.67 ± 0.03
V. unguiculata	0.75 ± 0.01
S. album without host (control)	0.29 ± 0.03

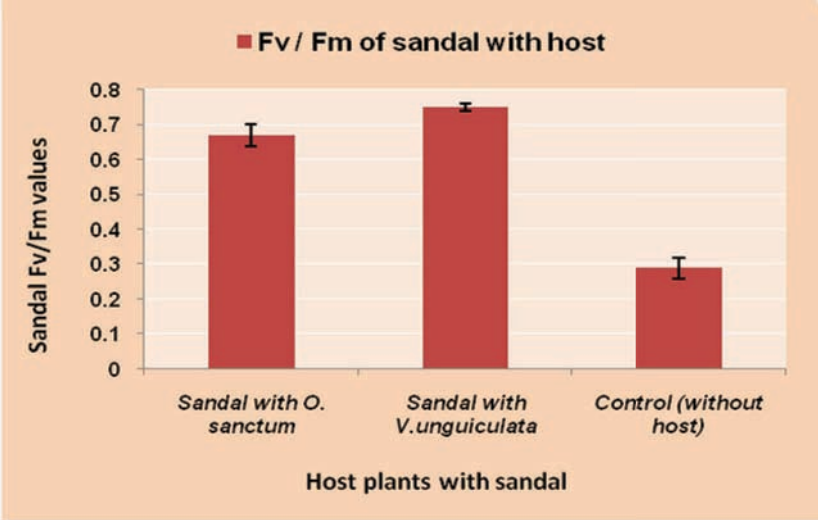


Figure 3.5. F_v/F_m of sandal raised along with annual hosts

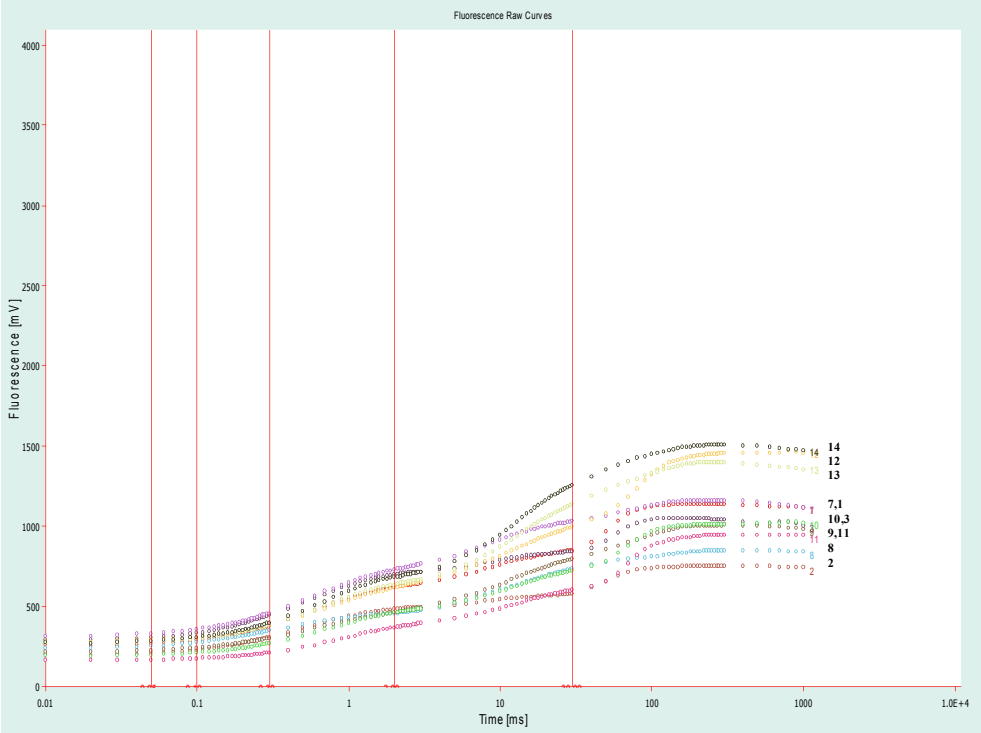


Figure 3.6. Typical fluorescent transients obtained from sandal plants grown with annual and perennial hosts

Curves No. 1-3 : Sandal seedlings with *Lantana camara* as host

Curves No. 7-9 : Sandal seedlings with *Vigna unguiculata* as host

Curves No. 10-12 : Sandal seedlings with *Pongamia pinnata* as host

Curves No. 13-14 : Sandal seedlings with *Ocimum sanctum* as host

Table 3.9. Fv/Fm of sandal grown along with best perennial hosts (mean ± SE)

Host plant name	F_v/F_m of sandal with perennial host
L. camara	0.76 ± 0.01
P. pinnata	0.82 ± 0.02

Performance Index values were highest for sandal seedlings growing with annual hosts *O. sanctum* (61.65) and *V. unguiculata* (27.49) than the values for sandal without host (control) (Table 3.10.; Fig 3.7.). Performance Index values were higher for sandal seedlings growing with perennial host *P. pinnata* (90.84) (Table 3.11.).

Table 3.10. Performance Index of sandal with and without annual host plants (mean ± SE)

Sandal with host	Performance Index
Sandal with *V. unguiculata*	27.49 ± 11.13
Sandal with *O. sanctum*	61.65 ± 2.64
Sandal without host (control)	2.49 ± 1.66

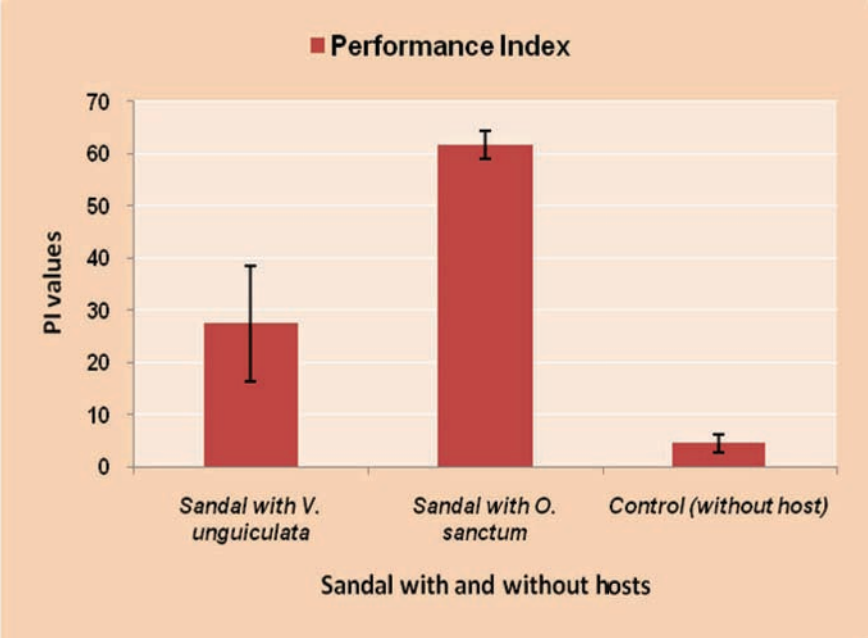


Figure 3.7. Performance Index of sandal grown with best annual host plants

Table 3.11. Performance Index of sandal with perennial host plants (mean ± SE)

Sandal with host	Performance Index
Sandal with *L. camara*	17.83 ± 5.84
Sandal with *P. pinnata*	90.84 ± 19.16

3.3.3. Chlorophyll content estimation

Chlorophyll content of sandal leaf was also significantly influenced by the host plant association (Table 3.12.; Fig 3.8.). Sandal seedlings with annual host *Vigna unguiculata* showed the maximum chlorophyll content (17.61) followed by *Ocimum sanctum* (10.55). Sandal seedlings with perennial host *P. pinnata* showed the maximum chlorophyll content (14.86) followed by *L. camara* (8.62) (Table 3.13). Sandal seedlings without hosts showed reduced chlorophyll content (4.49).

Table 3.12. Chlorophyll content of sandal leaves grown with two annual host plants (mean ± SE)

Host plant	Chlorophyll *a* (mg/g tissue)	Chlorophyll *b* (mg/g tissue)	Total chlorophyll (mg/g tissue)
Ocimum sanctum	6.32 ± 0.06	4.23 ± 0.09	10.55 ± 0.08
Vigna unguiculata	9.21 ± 0.07	8.40 ± 0.08	17.61 ± 0.12
Without host (control)	2.22 ± 0.07	2.27 ± 0.07	4.49 ± 0.08

Table 3.13. Chlorophyll content of sandal leaves grown with two perennial host plants (mean ± SE)

Host plant	Chlorophyll *a* (mg/g tissue)	Chlorophyll *b* (mg/g tissue)	Total chlorophyll (mg/g tissue)
Lantana camara	4.89 ± 0.10	3.73 ± 0.16	8.62 ± 0.26
Pongamia pinnata	8.59 ± 0.14	6.27 ± 0.05	14.86 ± 0.13

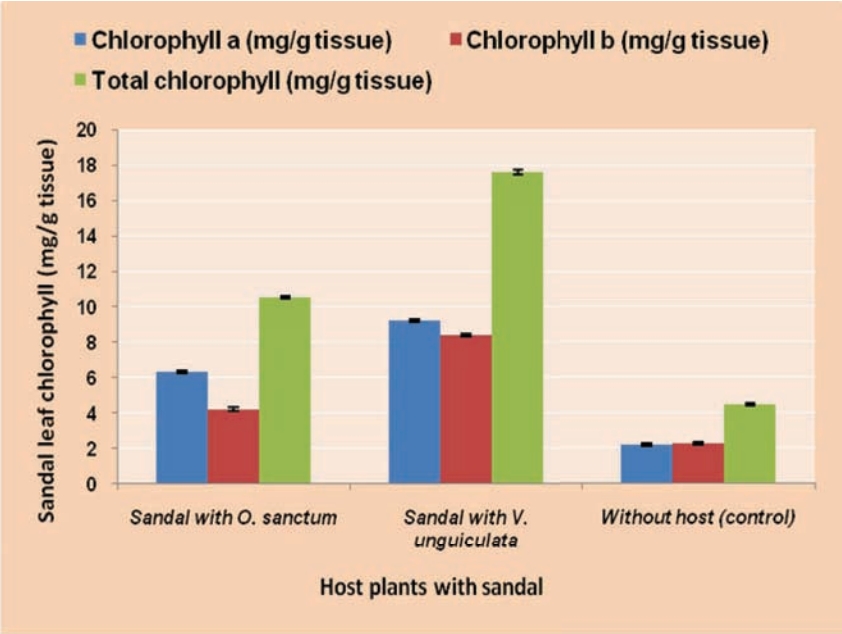


Figure 3.8. Chlorophyll content analysis of sandal leaves grown along with two annual host plants

Number of haustorial connections had direct correlation with the growth parameters. Haustorial connections were found to be higher with the best annual host (*Vigna*-10 ± 1.53) and best perennial hosts (*Pongamia*- 156 ± 23) and (*Lantana*- 40 ± 2.8). Sandal seedlings grow vigorously and suffer less mortality when more number of haustorial connections with the host plants develop, resulting in increased absorbing surface; hence higher amount of nutrient absorption becomes possible. Sandalwood plants growing without host connections showed poor growth and vitality. Root growth appeared stunted with no haustoria. Mortality of sandal plants was highest in control (no host plant) as well as with poor hosts compared with plants having *Vigna unguiculata* and *Pongamia pinnata* as hosts.

Annual herbaceous plants such as *Ocimum sanctum, Ageratum conyzoides*, *Phyllanthus niruri*, *Ludwigia hyssopifolia*, *Scoparia dulcis* and *Vernonia cinerea* were observed to be growing along with sandal seedlings in nursery without overgrowing sandal. *Mikania micrantha* is a perennial weed. Sandal seedlings were seen to haustorise these plants (Figs. 3.9-3.27). Growth rate of sandal seedling varied with each association. The number and size of haustorial connections also varied.

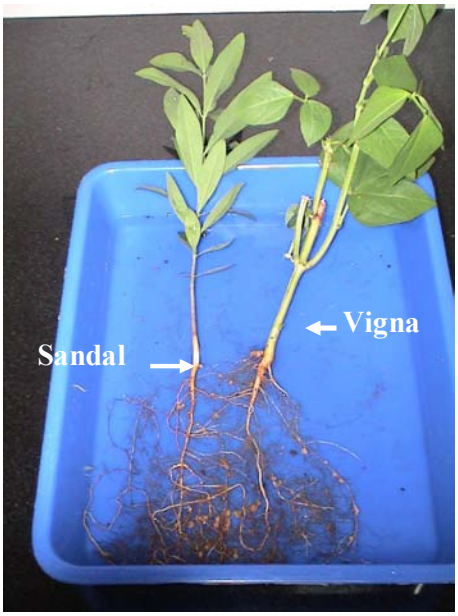


Figure 3.9: Sandal with *V. unguiculata*

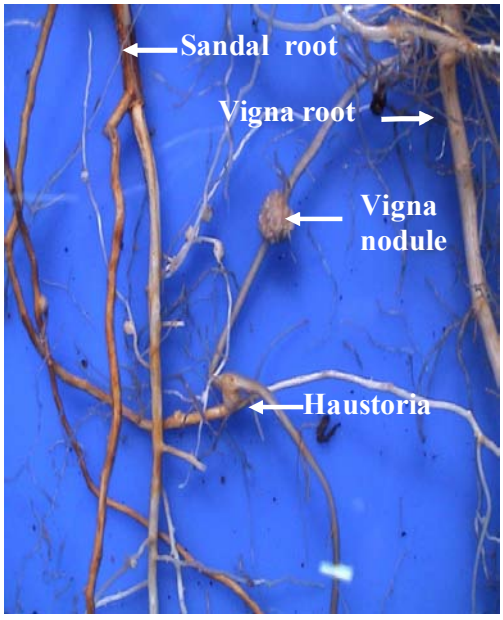


Figure 3.10: Roots of sandal and *V. unguiculata* showing haustorial connection

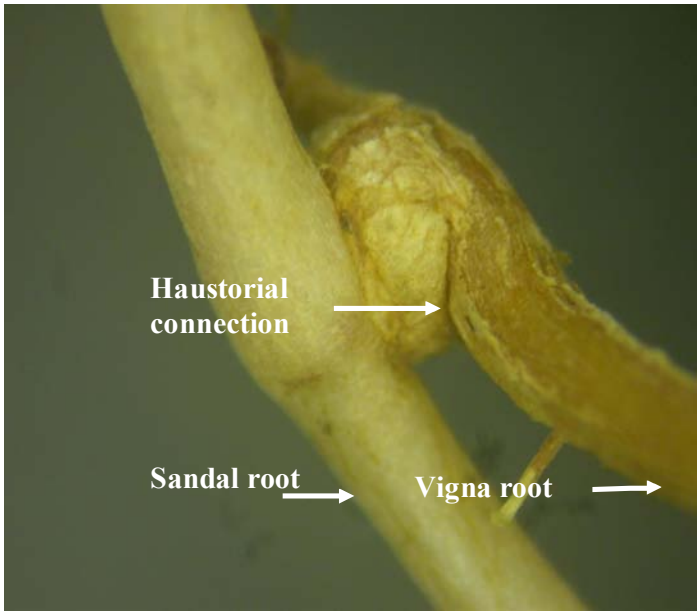


Figure 3.11: Young sandal haustorium establishing connection with host root

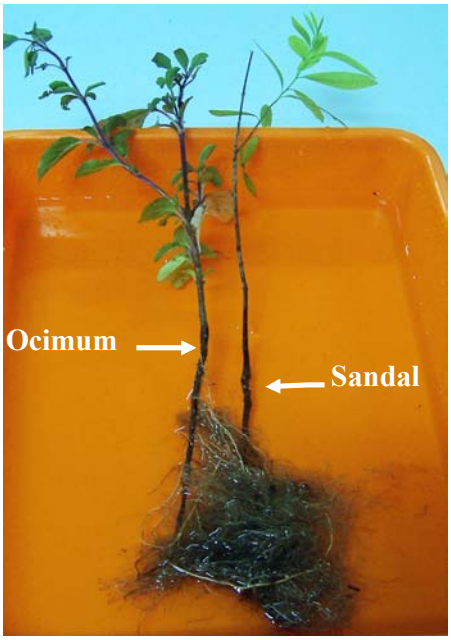


Figure 3.12: Sandal with *O. sanctum*

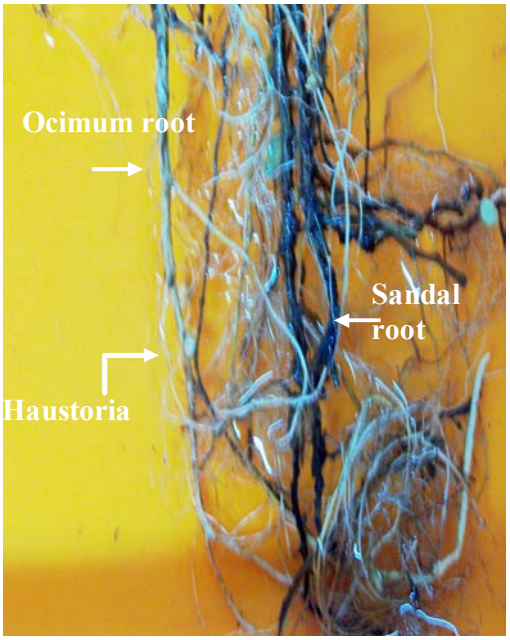


Figure 3.13: Roots of sandal and *O. sanctum* showing haustorial connection

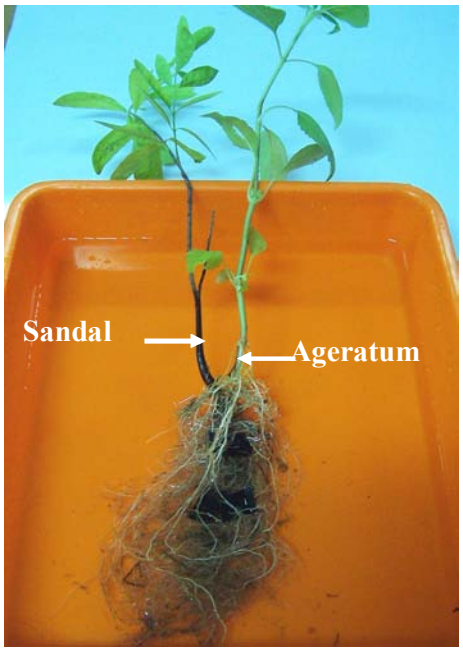


Figure 3.14: Sandal with *A. conyzoides*

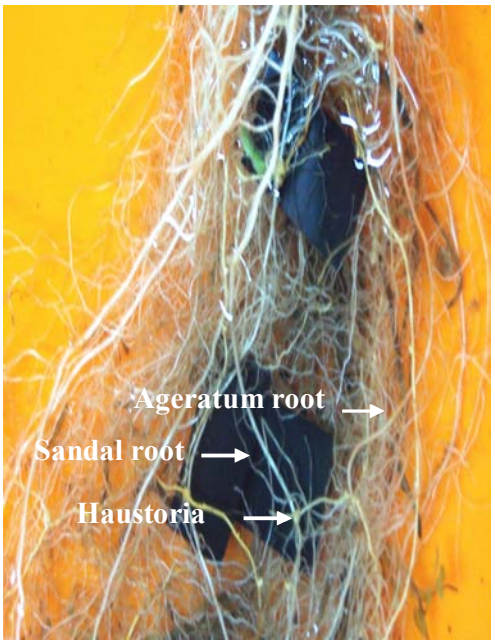


Figure 3.15: Roots of sandal and *A. conyzoides* showing haustorial connection

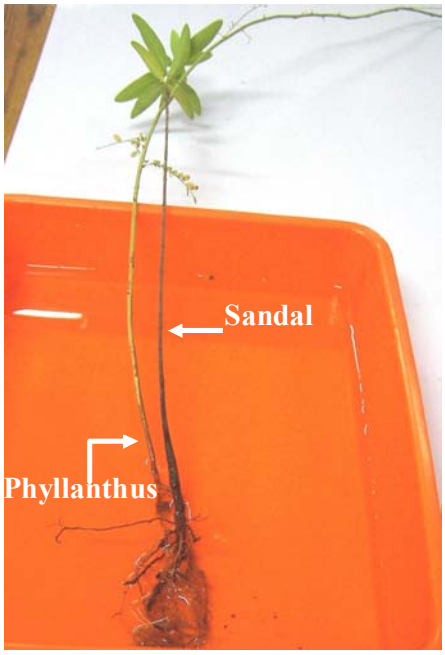


Figure 3.16: Sandal with *P. niruri*

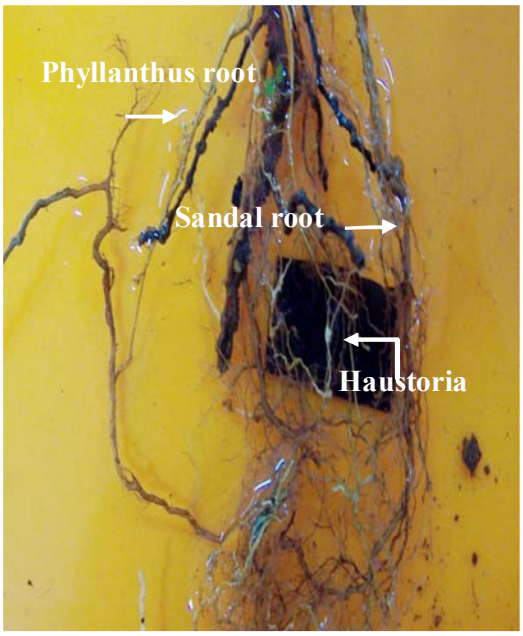


Figure 3.17: Roots of sandal and *P. niruri* showing haustorial connection

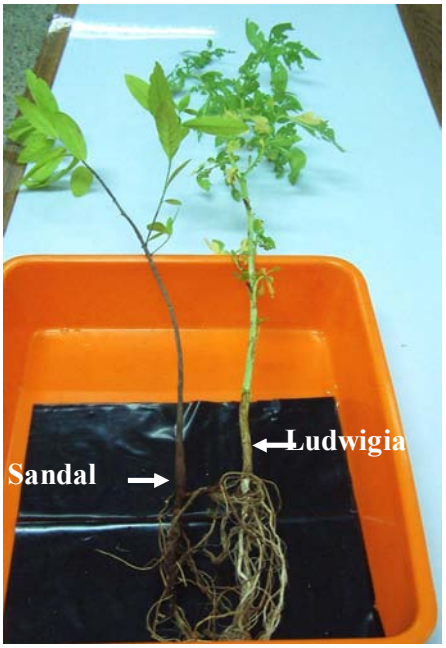


Figure 3.18: Sandal with *L. hyssopifolia*

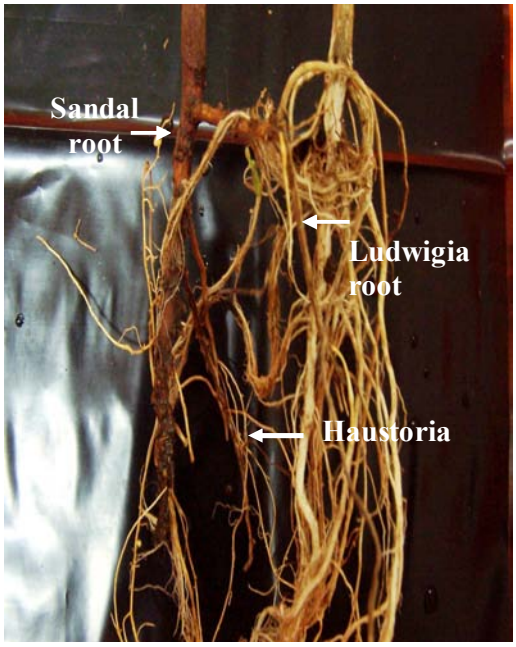


Figure 3.19: Roots of sandal and *L. hyssopifolia* showing haustorial connection

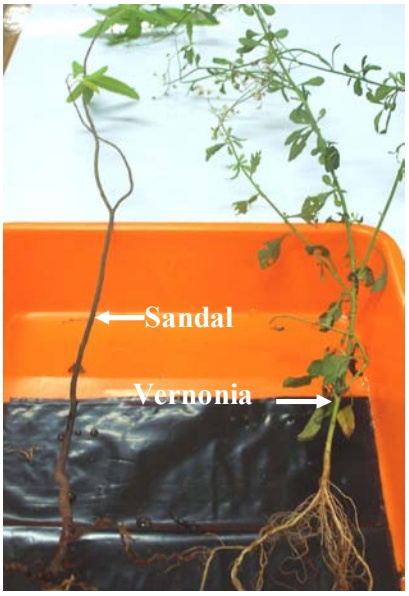


Figure 3.20: Sandal with *V. cinerea*

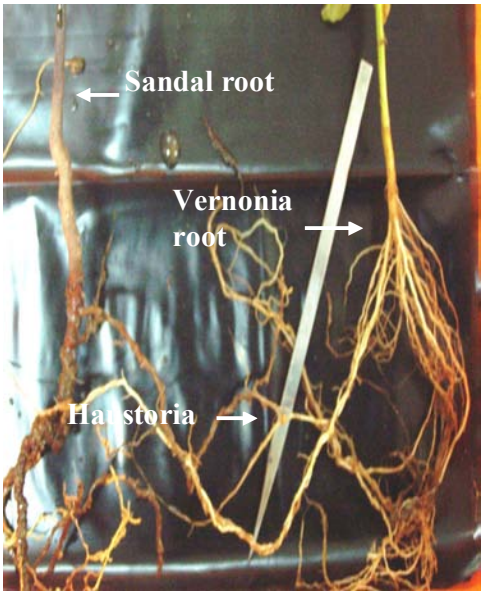


Figure 3.21: Roots of sandal and *V. cinerea* showing haustorial connection

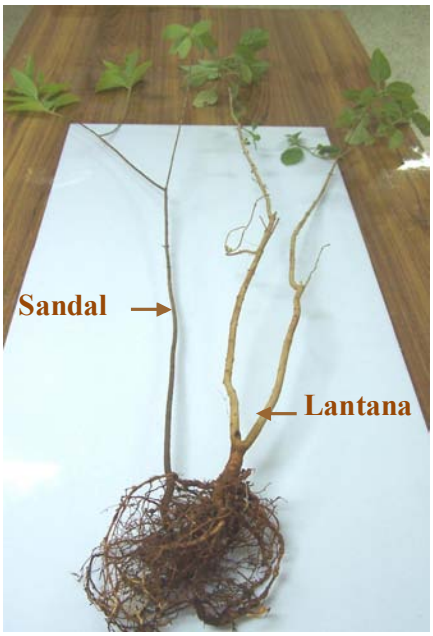


Figure 3.22: Sandal with *L. camara*

Figure 3.23: Roots of sandal and *L. camara* showing haustorial connection

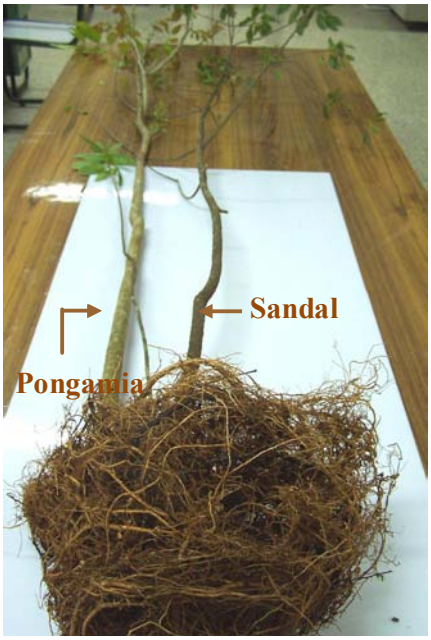


Figure 3.24: Sandal with *P. pinnata*

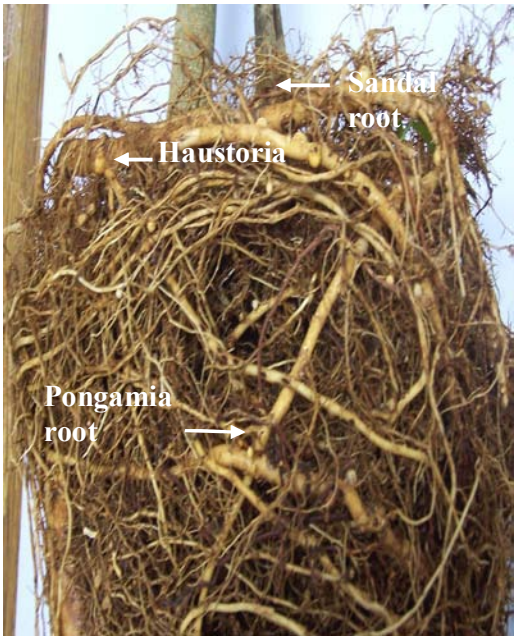


Figure 3.25: Roots of sandal and *P. pinnata* showing haustorial connection

Figure 3.26: Sandal without host plant showing unhealthy growth

Figure 3.27: Stunted root growth of sandal

3.4. DISCUSSION

The requirement of a suitable host plant for successful establishment of sandal had been widely recognised. Host plants were considered as good, medium and poor hosts depending on growth, biomass and number of haustoria produced by sandal when associated with different hosts (Ananthapadmanabha *et al*. 1984; 1988). Host plants were further categorised into pot hosts, intermediate hosts and long term hosts (Fox *et al*., 1990). Considerable variation existed between pot hosts in increasing sandal survival and field growth (Radomiljac, 1998). Hence, selection of appropriate pot hosts is critical to ensure successful establishment of sandal plantation. However, many of the conclusions drawn by earlier workers were not based on sound experimental results. In this chapter we have examined the comparative merits of ten potential host plants.

The study has shown that host plants need be provided within 3-4 months after transfer of sandal seedlings from mother bed to polybags. In the absence of a host, the chlorophyll content will be drastically reduced, resulting in very low photosynthetic efficiency and poor seedling growth. Earlier studies have shown that sandal seedlings without a host did not show significant difference in growth up to 3-4 months as compared to seedlings with hosts. This might be due to the fact that sandal could draw nutrients by its root system and maintain normal seedling growth up to 3 months (Barrett and Fox, 1997). Annapurna *et al*. (2006) has reported that requirement of host and its growth stage play a significant role in the growth of *S. album* seedlings in root trainers. They reported that hosts when supplied immediately after transplantation to root trainers resulted in better growth of sandal seedling in terms of height, collar diameter, total dry weight and quality indices as compared to hosts provided after 1-5 months.

Earlier studies by Radomiljac (2000) on the influence of pot host species, seedling age and supplementary nutrition on *S. album* plantation establishment in Western Australia has concluded that selection of suitable pot host species depended on a multitude of parameters and its use increased the level of *S. album* survival and growth in the field. The selection of the best compatible host to support maximum growth while minimizing competition for nutrients, water and light was very important for quality seedling production of sandal. The pot host species must be compatible with sandal seedlings' nursery and field cultural regimes and an understanding of pot host growth

and vigour also is necessary. Consequently, a suitable pot host 'pricking in' regime is required. If sown too early, the pot host will compete with *S. album* within the pot. Conversely, if sown too late, insufficient time for adequate haustorial connections before the field establishment will result in outplanting stress to the sandal seedling. Radomiljac and Mc Comb (1998) had observed differences in root:shoot ratio in their study and suggested that partitioning of resources changes from root to shoot once effective haustorial connections have been established. So the parasite will be very vulnerable to water and nutrient deficits if the host plants are to die. Earlier studies have also concluded that the requirement of host and the stage of host introduction played a significant role in the growth of *Santalum album* (Barrett and Fox, 1997). In our study we have observed increased height, collar diameter, fresh weight, DQI, F_v/F_m ratio, PI and chlorophyll content for sandal seedlings grown with hosts when compared with sandal seedlings without host (control).

So also, selection of proper hosts is important for growth and survival of sandal seedlings. *Cajanus cajan* had been reported to be a suitable primary host (Rai, 1990; Srinivasan *et al*., 1992; Srimathi *et al*., 1995). Being a leguminous host, it supported the growth of sandal at the nursery stage and at initial establishment in the field. However, the rapid growth of *C. cajan* necessitated frequent pruning. So also it was prone to fungal diseases and insect attacks, which necessitated intensive prophylactic and control measures. Moreover, the high genetic variability of *C. cajan* also resulted in non-uniform growth of *S. album* seedlings which adversely affect sandal seedling growth (Annapurna *et al*., 2006). Among the leguminous and non-leguminous species examined by others, *Alternanthera* spp (Surata, 1992) and *Alternanthera nana* (Radomiljac, 1998) significantly improved seedling growth of *S. album* in terms of height and collar diameter. Similarly *A. sessilis* was proved as the best host for *S. austrocaledonicum* (Bule and Daruhi, 1990). Ananthapadmanabha *et al*. (1988), Rai (1990) and Taide *et al*. (1994) had observed *Casuarina equisetfolia* to be the best pot host in large size containers. In our study *Vigna ungiculata,* was found to be the best primary host. The present study has confirmed that legumes do have an advantage over non legumes as hosts for sandal. *Vigna unguiculata* served as the best primary host during the early stages of sandal seedling development till they were field transplanted. *Pongamia pinnata,* a leguminous tree species was found best suited as a long term host.

Moreover, leguminous hosts supported maximum chlorophyll content in sandal as compared to non-leguminous host. Haustorial size was also found to be bigger with leguminous hosts. Large haustoria ensure better nutrient absorption and growth of sandalwood seedlings. Annapurna *et al*. (2006) found *Mimosa pudica,* another herbaceous leguminous plant as the best primary host for *S. album* seedling growth in root trainers in terms of height, collar diameter, total dry weight, root:shoot ratio, root surface area and quality index at nursery stage.

The growth performance of a seedling is determined by the plant's physiological activity. In the present study, chlorophyll fluorescence and chlorophyll content measurements were used to test the best host combinations for sandal. We have used the chlorophyll fluorescence parameters such as F_v/F_m values and Performance Index values to assess the performance of sandal seedlings with four host plants under nursery conditions. Performance Index (PI) values were found to be higher for sandal seedlings growing with *Ocimum* (61.65) as host followed by *Vigna* (27.49). But, DQI values (Table 3.2), F_v/F_m values (Table 3.8) and chlorophyll content (Table 3.12) were higher when *Vigna* was the host plant when compared to *Ocimum* as host. Moreover, *Vigna,* an annual leguminous species could grow well under shade along with sandal seedlings whereas *Ocimum* required sunlight for its growth. So, *V. unguiculata* was chosen to be the best annual pot host for sandal. Likewise, sandal plants showed higher PI, DQI and F_v/F_m values, and chlorophyll content when *P. pinnata* was the host plant indicating its superiority over *L. camara* as a field host plant.

Studies conducted by Radomiljac *et al*. (1998a) on xylem transfer of organic solutes in *S. album* grown in association with leguminous and non-leguminous hosts have shown that legumes over perform eucalypts as hosts for sandal. When grown in association with N_2 fixing hosts, *Santalum* shoot growth was markedly greater than when grown without a host. This difference has been directly attributed to the substantial benefit in terms of nitrogen gain accruing from the intake of xylem solutes from a well nodulated legume host. The beneficial effect on sandal would be increased foliar Nitrogen concentrations, leaf chlorophyll contents and leaf photosysnthesis and eventually large increase in leaf area. Rai (1990) and Taide *et al*. (1994) had earlier reported *Casuarina equisetifolia* L. (Casuarinaceae), a nitrogen fixing tree with large root nodules to be a good host, although in their experiments several non-nitrogen

fixing species were found to be better hosts than the leguminous species such as *Albizia lebbeck* (L.) Benth., *Acacia auriculiformis* Cunn. Ex. Benth., *Leucaena leucocephala* (Lam.). De Wit (all Mimosaceae) and *Cassia fistula* (Caesalpinaceae). There are other reports of root hemi-parasites performing particularly poor on non-leguminous hosts, such as *Olax phyllanthi* (Labill) R. Br. attached to *Amaranthus caudatus* L. and *Portulaca oleraceae* L. (Tennakoon and Pate, 1996).

3.6. CONCLUSION

The present study has concluded that plantable quality of *S. album* seedling can be produced in polybags within 6-8 months by providing *Vigna unguiculata* as the primary (pot) host among the eight potential annual host plants tested. On field transplantation *Pongamia pinnata* can be supplied as the long term host. This protocol is useful for raising sandal seedlings under nursery conditions and subsequent field transplantation.

CHAPTER 4

EFFECTS OF SHADE ON MORPHOLOGY, CHLOROPHYLL CONCENTRATION AND CHLOROPHYLL FLUORESCENCE OF SANDAL SEEDLINGS

4. EFFECTS OF SHADE ON MORPHOLOGY, CHLOROPHYLL CONCENTRATION AND CHLOROPHYLL FLUORESCENCE OF SANDAL SEEDLINGS

4.1. INTRODUCTION

Sandalwood resource in India is currently threatened because of illicit felling, smuggling, forest fire, grazing and to some extent spike disease. Due to these factors there has been a drastic reduction in the production of sandalwood. Solutions suggested to prevent the loss of sandal wealth are not only the adoption of effective conservation measures and prevention of uncontrolled exploitation, but also implementation of programs of reintroduction of this species in the ecosystem. For the species introduction to be successful, it is necessary to obtain sufficient information about its ecophysiological characteristics.

For achieving successful propagation, a better understanding of the seedling's morphological and physiological requirements under nursery conditions are necessary. Among the major environmental factors, light is perhaps the most influential factor involved in the survival, growth and reproduction of any tree species. Light responses usually evoke physiological alterations, which are determinants for CO_2 assimilation and optimization of gas exchange. Environments that are either shaded or under full sunlight can inhibit photosynthetic processes. Excess of light causes degradation and reduction in the levels of total chlorophyll and reduction in field productivity as a result of photoinhibition (Francisco *et al*., 2005). Therefore the optimum light required for each species need be evaluated for their healthy growth.

Quality seedlings should be used for raising successful plantations. In the past, seedling quality was evaluated primarily on the basis of morphological characteristics such as height, collar diameter and shoot:root ratio. In recent years, physiological characterization has been used more in quality assessment. Chlorophyll fluorescence is a useful physiological test because it is noninvasive and rapid (Vidaver *et al*., 1989), and provides information on the qualitative and quantitative changes in photosynthesis. It also facilitates analysis of chlorophyll fluorescence of intact live leaves (Slapakauskas and Ruzgas, 2005).

Chlorophyll fluorescence of a predarkened leaf exhibits a characteristic temporal pattern (Kautsky and Hirsh, 1931). Chlorophyll fluorescence rises rapidly from a ground state (F_o) when all the electron acceptors are fully oxidised, to a maximum level (F_m), when electron acceptors are fully reduced and unable to accept and transfer electrons. Chlorophyll fluorescence then decreases slowly to a steady state (F_s) as photochemistry and CO_2 assimilation increases (Krause and Weis, 1991). F_v/F_m (where $F_v=F_m-F_o$) is a parameter commonly known as the maximum quantum yield of primary photochemistry or maximal relative electron transport rate (ETR) of Photosystem II (Waldhoff *et al*, 2002). It is frequently used as an expression of photoinhibition which decreases with photoinhibition (Critchley, 1998; Kitao *et al*., 2000a), is linearly correlated with the quantum yield of net photosynthesis (Krause and Somersalo, 1989) and is a good measure of seedling vigour.

Normally, the measurement of the pigments from the chloroplasts and the analysis of fluorescence from chlorophyll *a* are used as stress indicators of high irradiance in plants (Francisco *et al*., 2005). Chlorophyll fluorescence is also used as a diagnostic tool to study environmental stress (Binder and Fielder, 1996; Balasimha and Namboothiri, 1996), genotypic variation (Janssen *et al*., 1995), species specific diurnal changes (Joshi, 1995), early diagnosis of stress (Pukacki and Modrzynski, 1998), responses to shade (Khan *et al*., 2000), screening of physiologically active and fast growing trees at different altitudes (Husen *et al*., 2004) and leaf photosynthetic performance (Slapakauskas and Ruzgas, 2005).

The objective of this study was to evaluate the effects of shade on morphology, chlorophyll concentration and chlorophyll fluorescence of sandal seedlings under nursery conditions.

4.2. MATERIALS AND METHODS

4.2.1. Plant material

Seeds were sown in vermiculite. During the second month after germination the seedlings were transplanted to individual polybags containing 2:1:1 mixture of soil:sand:farm yard manure (FYM). There were four treatments as given below, each with 25 seedlings.

(i) Seedlings grown under full sunlight
(ii) Seedlings grown under 25 per cent shade
(iii) Seedlings grown under 50 per cent shade
(iv) Seedlings grown under 75 per cent shade

4.2.2. Shade treatments

Seedlings were grown under full sunlight and under shade nets that provided approximately 25 per cent, 50 per cent and 75 per cent shade. Shade net samples obtained from market were check-measured for Photosynthetically Active Radiation (PAR) penetration using a Quantum sensor (Li-Cor, Nebraska, USA). The shade given by these nets was then quantified and graded according to their light interception. These shade nets were fabricated on metallic frames so that the height of the enclosures ranged from 1.8 to 2 m from the ground (Fig 4.1). Accordingly, the four treatments provided full sunlight (no shade net), and 25 per cent, 50 per cent and 75 per cent shade. Seedlings were watered regularly, and polybags hand-weeded as and when necessary. No fertilizer was applied because the potting medium was considered to have adequate fertility.

Figure 4.1: Sandal seedlings grown under shade net enclosures

4.2.3. Morphological measurements

Measurements were made at regular intervals on height, collar diameter, number of leaves and leaf area of seedlings grown under each shade percentage (Fig. 4.2 & 4.3). At the end of the study (i.e. eight months after germination), each seedling was harvested and measurements made on height, number of leaves, leaf area, collar diameter, total biomass, shoot biomass and root biomass. In addition, dry weights of shoot and root were recorded after partitioning the seedlings into shoots and roots and oven drying at 75°C for 48 hours.

Figure 4.2: Sandal seedlings under different shade intensity

Figure 4.3: Sandal seedlings under 100 % sunlight

The quality of the seedlings was determined using SQ, DQI and PI as discussed in chapter on host relationships of sandal seedlings (Chapter 3). Chlorophyll *a*, chlorophyll *b* and total chlorophyll concentrations were measured on leaves of randomly selected seedlings from each treatment following the protocol given below.

For the estimation of chlorophyll content, fresh leaves were collected from each group; 250 mg of leaf sample was weighed and homogenised with 5 ml of 80 per cent acetone (pre-chilled). The homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was transferred to a 100 ml conical flask. The procedure was repeated until the residue was colourless. The final volume was made upto 25 ml with 80 per

cent acetone. The absorbance of the solution was read at 645 and 663 nm against blank solvent (80 per cent acetone) using a spectrophotometer (Genova MK3, UK). Using the absorption coefficient, the amount of chlorophyll was calculated based on the protocol of Sadasivam and Manickam (1992). Chlorophyll content of sandal plants from each treatment were analysed and compared using the formulae.

mg chlorophyll *a* / g tissue = 12.7 (A663) - 2.69 (A645) x V/1000 x W

mg chlorophyll *b* / g tissue = 22.9 (A645) - 4.68 (A663) x V/1000 x W

mg chlorophyll / g tissue = 20.2 (A645) + 8.02 (A663) x V/1000 x W

where,

A= Absorbance at specific wavelengths

V= Final volume of chlorophyll extract in 80 per cent acetone

W= Fresh weight of the tissue extracted (g)

4.2.4. Experimental design and statistical analysis

For the experiment a Completely Randomised Block Design with 4 treatments and 25 replicates was accepted. Measurements of morphological characters were made on all the seedlings. Four seedlings of each treatment were randomly selected for chlorophyll fluorescence and chlorophyll concentration measurements. The data were analysed using one way ANOVA (SPSS 16 software).

4.3. RESULTS

The height of the seedlings did not differ much among the treatments during the initial months. But, none of the seedlings exposed to full sunlight survived beyond 6 months from the start of the experiment (Table 4.1 and Figure 4.4). Significant difference in seedling growth and morphological characteristics among the four treatments was observed at the 3rd (February) and 4th month (March) of observation and at the 6th month (May) among the surviving three treatments (Table 4.1, 4.2, 4.3 and Fig 4.4).

The final mean height of the seedlings ranged between 10.8 cm-12.78 cm, shoot fresh weight ranged between 2.79-3.17g, shoot dry weight ranged between 2.16-2.56g, root fresh weight ranged between 1.72-2.08g, root dry weight ranged between 1.26-1.54g and shoot:root dry weight ranged between 1.61-1.74g among the treatments. The

shoot:root dry weight was lowest under 75 per cent shade followed by 25 per cent shade and 50 per cent shade. SQ value of sandal seedlings ranged between 5.75-7.11 and DQI value ranged between 0.35-0.47 among the treatments. DQI was highest under 25 per cent shade followed by 50 per cent shade and 75 per cent shade. Performance Index (PI) value was highest for 75 per cent shade treatment (2.59) followed by 25 per cent shade treatment (1.5) and 50 per cent shade treatment (1.06) (Table 4.3, 4.4 and Fig 4.5). Except collar diameter (CD), Sturdiness Quotient (SQ) and Performance Index (PI), all other parameters showed significant difference between the treatments (P=0.05).

Table 4.1. Height (cm) of sandal seedlings under four shade treatments monitored for six months from two months after germination (mean ± SE)

Shade %	Dec	Jan	Feb	Mar	Apr	May
0	10.2 ± 0.41	10.6 ± 0.48	10.9 ± 0.48	11.5 ± 0.53		
25	10.25 ± 0.06	10.3 ± 0.36	11.1 ± 0.36	11.3 ± 0.52	11.57 ± 0.55	12.78 ± 0.45
50	10.1 ± 0.32	10.3 ± 0.25	10.4 ± 0.31	10.5 ± 0.31	10.5 ± 0.32	10.8 ± 0.33
75	10.25 ± 0.28	10.3 ± 0.35	10.5 ± 0.35	10.58 ± 0.32	10.7 ± 0.38	11.5 ± 0.38

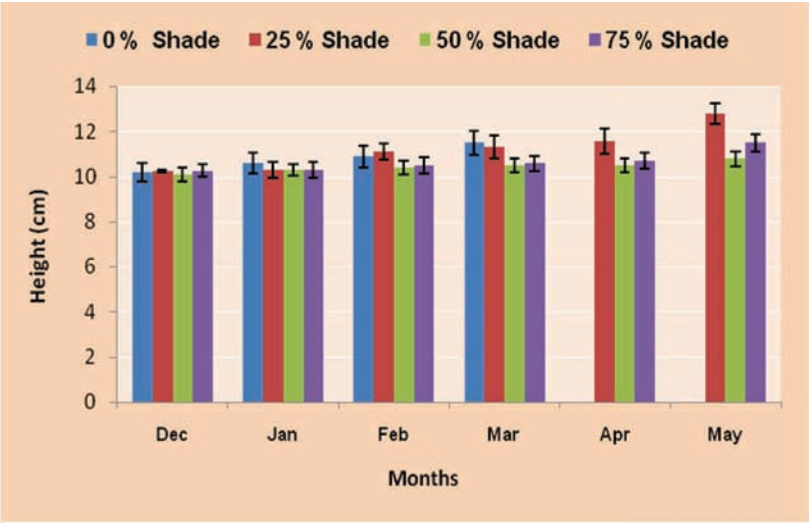


Figure 4.4: Height of sandal seedlings under four shade treatments monitored for six months from two months after germination

Table 4.2. ANOVA - Height of sandal seedlings under four shade treatments monitored for six months from two months after germination

		Sum of Squares	df	Mean Square	F	Table F value at 5%
December	Between Groups	1.639	3	.546	.183	2.76
	Within Groups	250.485	84	2.982		
	Total	252.123	87			
January	Between Groups	29.393	3	9.798	2.715	2.76
	Within Groups	303.115	84	3.609		
	Total	332.509	87			
February	Between Groups	32.796	3	10.932	2.957*	2.76
	Within Groups	310.522	84	3.697		
	Total	343.318	87			
March	Between Groups	48.961	3	16.320	3.452*	2.76
	Within Groups	397.083	84	4.727		
	Total	446.044	87			
April	Between Groups	25.342	2	12.671	2.699	3.15
	Within Groups	295.757	63	4.695		
	Total	321.099	65			
May	Between Groups	59.413	2	29.707	7.955*	3.15
	Within Groups	235.268	63	3.734		
	Total	294.681	65			

* significant at 5 % level

Table 4.3. Final morphological characteristics of sandal seedlings under three shade treatments (mean ± SE)

% Shade	Height	CD	TFW	SFW	SDW	RFW	RDW	SDW/ RDW	SQ	DQI	PI
25	12.78 ± 0.45	1.8 ± 0.07	5.33 ± 0.13	3.17 ± 0.11	2.56 ± 0.11	2.08 ± 0.07	1.54 ± 0.07	1.66 ± 0.11	7.11 ± 0.37	0.47 ± 0.02	1.5 ± 0.21
50	10.81 ± 0.33	1.88 ± 0.06	4.56 ± 0.14	2.79 ± 0.08	2.19 ± 0.08	1.72 ± 0.08	1.26 ± 0.07	1.74 ± 0.36	5.75 ± 0.48	0.37 ± 0.08	1.06 ± 0.48
75	11.05 ± 0.38	1.68 ± 0.11	4.66 ± 0.10	2.83 ± 0.07	2.16 ± 0.06	1.84 ± 0.08	1.34 ± 0.06	1.61 ± 0.20	6.57 ± 0.47	0.35 ± 0.02	2.59 ± 0.66

Height = Shoot height (cm); CD = Collar diameter (mm); TFW = Total Fresh Weight (g); SFW = Shoot Fresh Weight (g); SDW = Shoot Dry Weight (g); RFW = Root Fresh Weight (g); RDW = Root Dry Weight (g); SDW/RDW = Shoot Dry Weight/ Root Dry Weight; SQ = Sturdiness Quotient; DQI = Dickson Quality Index; PI = Performance Index

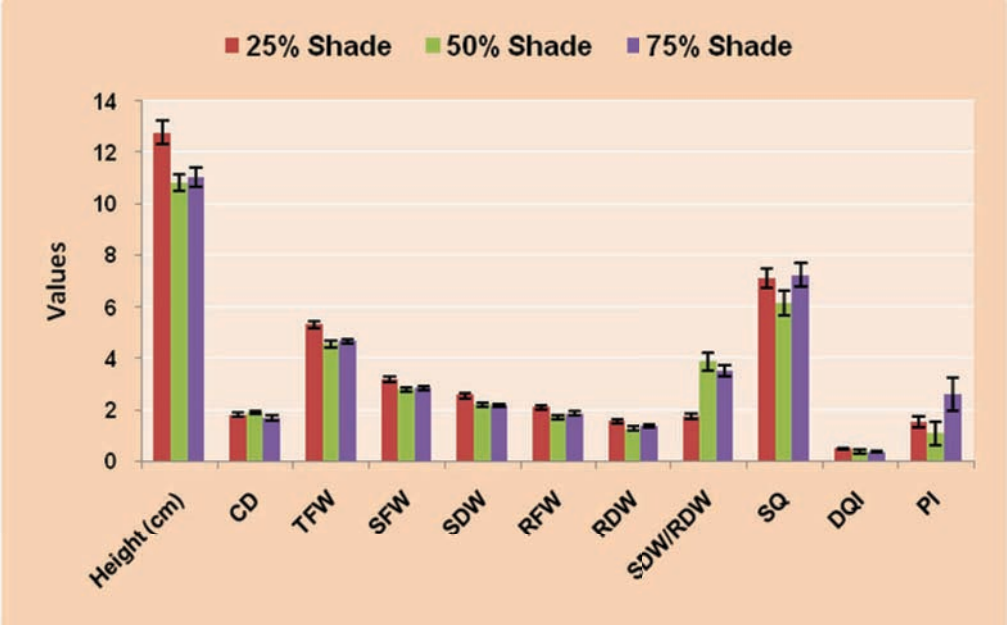


Figure 4.5: Final morphological characteristics of sandal seedlings under three shade treatments

Table 4.4. ANOVA - Final morphological characteristics of sandal seedlings under three shade treatments

Variable	Degrees of freedom	Calculated F value	Table F value
Height	2,69	7.424*	3.15
Collar diameter	2,69	1.652	3.15
Total Fresh Weight	2,69	10.890*	3.15
Shoot Fresh Weight	2,69	5.012*	3.15
Shoot Dry Weight	2,69	5.825*	3.15
Root Fresh Weight	2,69	5.309*	3.15
Root Dry Weight	2,69	4.163*	3.15
Shoot Fresh Weight / Root Fresh Weight	2,69	14.836*	3.15
Sturdiness Quotient	2,69	1.858	3.15
Dickson Quality Index	2,69	13.678*	3.15
Performance Index	2,10	0.157	4.10

* significant at 5 % level

Seedlings grown under zero per cent shade (full sunlight) displayed stress symptoms; chlorosis was especially noticeable compared to other treatments. By the end of the fourth month, about 92 per cent of the seedlings kept exposed to full sunlight wilted and within a month complete mortality was recorded (Table 4.5 and Fig 4.6).

Table 4.5. Mortality percentage of sandal seedlings under four shade treatments monitored for six months from two months after germination

Shade %	% mortality					
	Dec	Jan	Feb	Mar	Apr	May
0	0	4	4	4	92	100
25	0	4	8	8	8	12
50	0	0	0	0	0	0
75	0	0	0	0	0	0

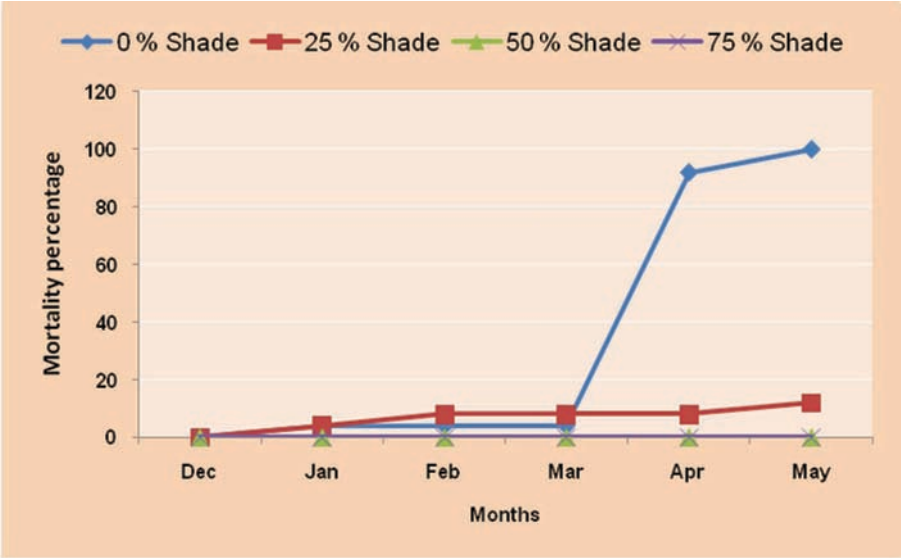


Figure 4.6. Percentage mortality of sandal seedlings under four shade treatments monitored for six months from two months after germination

Figure 4.7 gives the normalization plot of the typical fluorescent transients obtained from the three shade treatments.

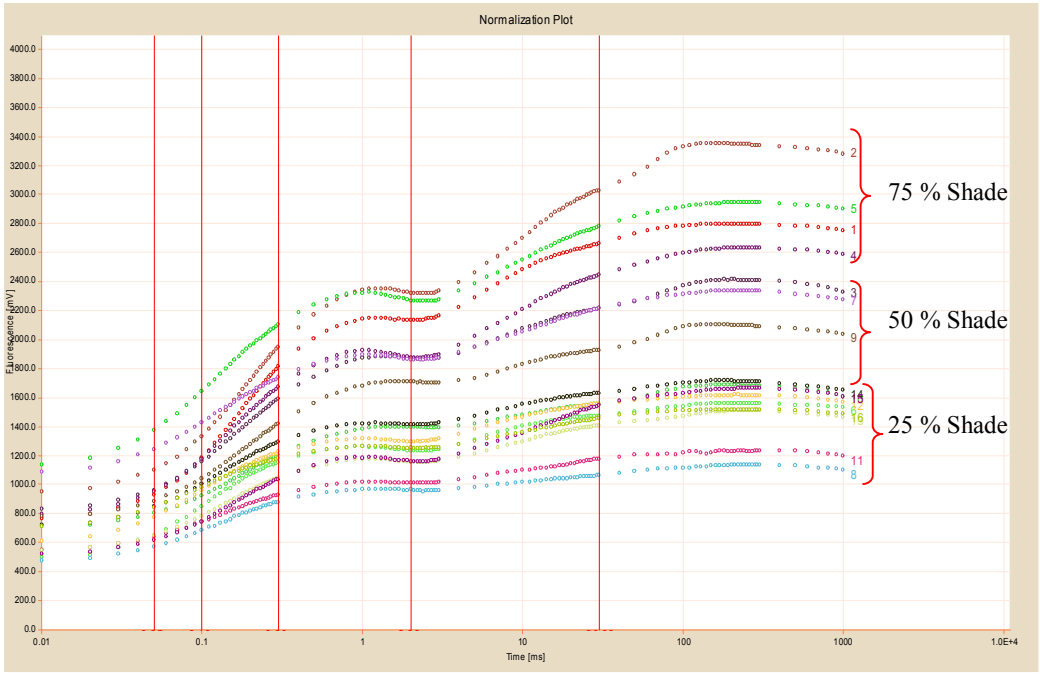


Figure 4.7: Typical fluorescent transients obtained from three different shade treatments in sandal seedlings at 7th month

Curves No. 1-5 : Seedlings under 75% shade
Curves No. 6-10 : Seedlings under 50% shade
Curves No. 12-16 : Seedlings under 25% shade

The effect of shade treatment on chlorophyll fluorescence in sandal is shown in table 4.6. as F_v/F_m ratio during the five months of observation. There was significant difference in F_v/F_m values between the four different shade treatments during the months of January and February for sandal seedlings (Table 4.7 and Fig. 4.8). The highest fluorescence was shown by seedlings subjected to 75 % shade.

Table 4.6. Effect of shade on chlorophyll fluorescence (F_v/F_m) of four shade treatments monitored for five months from three months after germination

% Shade	Jan	Feb	Mar	Apr	May
0	0.34 ± 0.07	0.41 ± 0.04	0.53 ± 0.04	Plants did not survive	Plants did not survive
25	0.58 ± 0.04	0.58 ± 0.03	0.57 ± 0.03	0.60 ± 0.02	0.62 ± 0.03
50	0.61 ± 0.01	0.62 ± 0.02	0.63 ± 0.06	0.61 ± 0.04	0.59 ± 0.01
75	0.67 ± 0.03	0.65 ± 0.02	0.58 ± 0.03	0.58 ± 0.04	0.61 ± 0.05

Table 4.7. ANOVA-Variation in F_v/F_m under four shade treatments monitored for five months from three months after germination

		Sum of Squares	df	Mean Square	Calculated F value	Table F value
January	Between Groups	.251	3	.084	10.511*	3.49
	Within Groups	.096	12	.008		
	Total	.347	15			
February	Between Groups	.135	3	.045	11.409*	3.49
	Within Groups	.047	12	.004		
	Total	.182	15			
March	Between Groups	.021	3	.007	.904	3.49
	Within Groups	.091	12	.008		
	Total	.112	15			
April	Between Groups	.006	2	.003	.273	4.26
	Within Groups	.094	9	.010		
	Total	.100	11			
May	Between Groups	.005	2	.002	.269	4.26
	Within Groups	.076	9	.008		
	Total	.081	11			

* significant at 5 % level

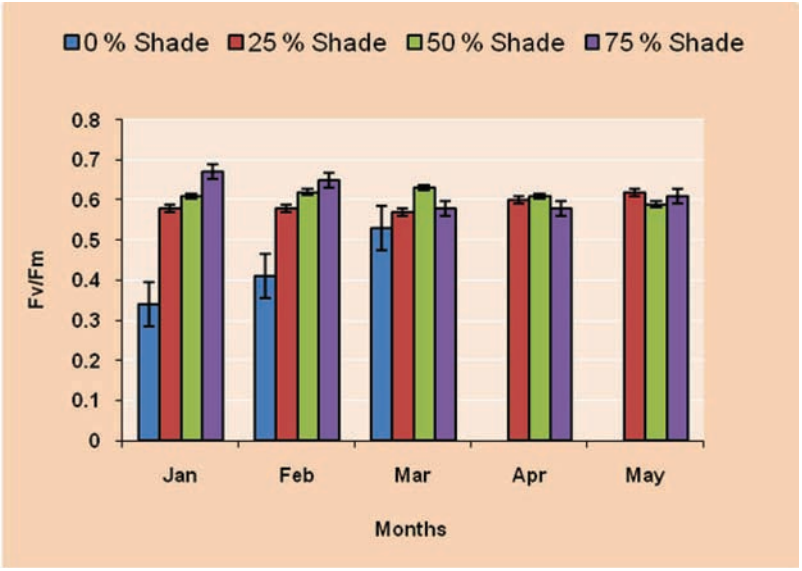


Figure 4.8: Variation in F_v/F_m under four shade treatments monitored for five months from three months after germination

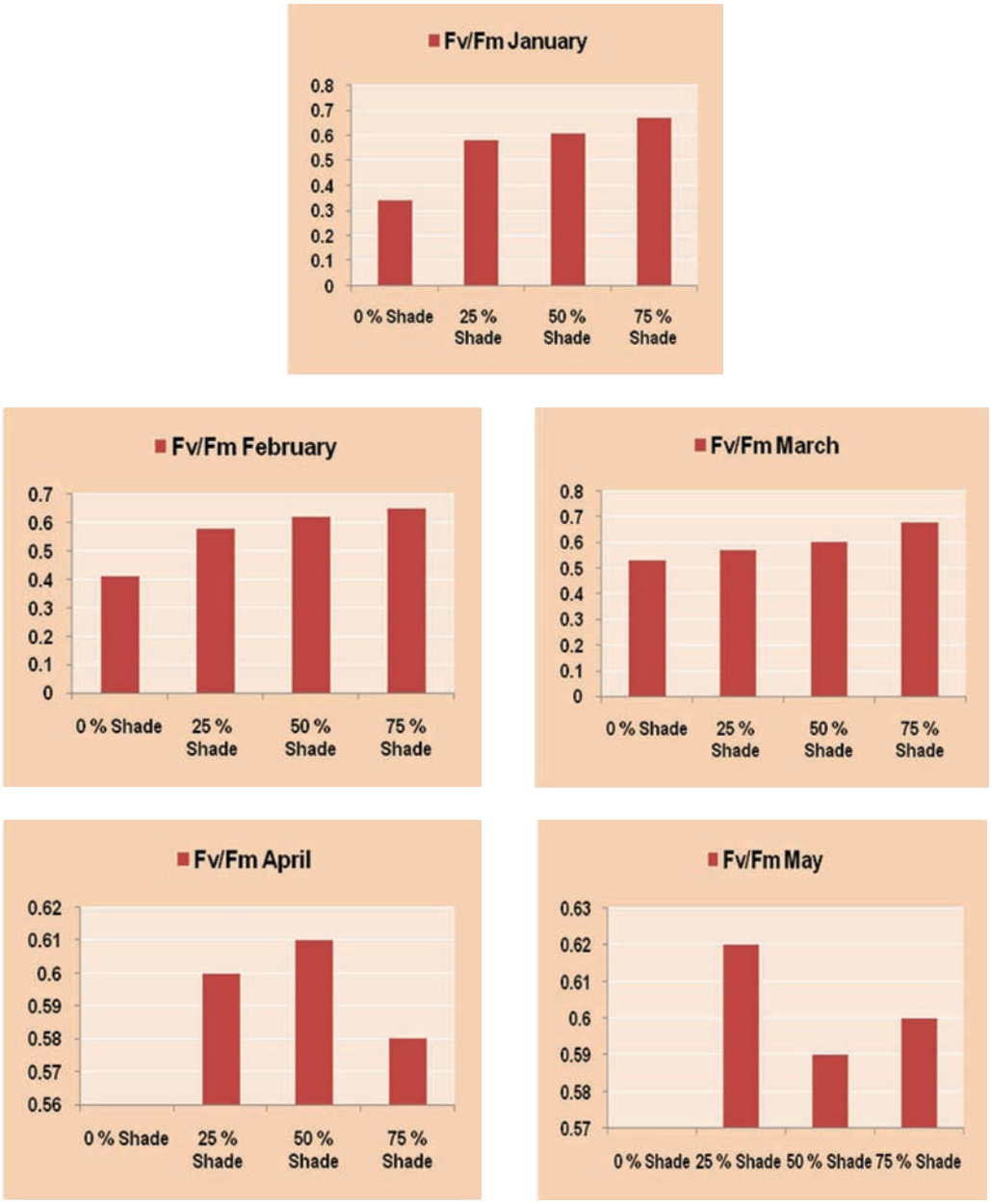


Figure 4.9: Monthly variation of F_v/F_m monitored for five months from three months after germination

There was significant difference in Performance Index (PI) between the four shade treatments during January and February and not during subsequent months (Table 4.8 & 4.9; Fig 4.10). The PI was highest for seedlings raised under 75 per cent shade during fourth and fifth month growth. Analysis of final morphological characteristics and chlorophyll fluoresecence data indicated that the sandal seedlings performed well under 75 per cent shade. Performance Index values also support this observation.

Table 4.8. Performance Index (PI) of sandal seedlings under four shade treatments monitored for five months from three months after germination

% Shade	Jan	Feb	Mar	Apr	May
0	0.30 ± 0.18	0.35 ± 0.20	0.36 ± 0.15	Plants did not survive	Plants did not survive
25	1.61 ± 0.31	1.47 ± 0.42	1.51 ± 0.05	1.74 ± 0.37	1.5 ± 0.21
50	1.37 ± 0.32	2.13 ± 0.24	1.63 ± 0.57	1.41 ± 0.19	1.07 ± 0.45
75	3.45 ± 0.61	2.21 ± 0.39	1.33 ± 0.55	1.78 ± 0.23	2.6 ± 0.67

Table 4.9. ANOVA - Variation in PI under four shade treatments monitored for five months from three months after germination

		Sum of Squares	df	Mean Square	F	Table F value
January	Between Groups	11.158	3	3.719	4.802*	3.41
	Within Groups	10.068	13	.774		
	Total	21.226	16			
February	Between Groups	7.501	3	2.500	5.224*	3.41
	Within Groups	6.222	13	.479		
	Total	13.723	16			
March	Between Groups	3.445	3	1.148	1.479	3.41
	Within Groups	10.090	13	.776		
	Total	13.534	16			
April	Between Groups	.132	2	.066	.207	4.10
	Within Groups	3.177	10	.318		
	Total	3.308	12			
May	Between Groups	.374	2	.187	.157	4.10
	Within Groups	11.874	10	1.187		
	Total	12.248	12			

* significant at 5 % level

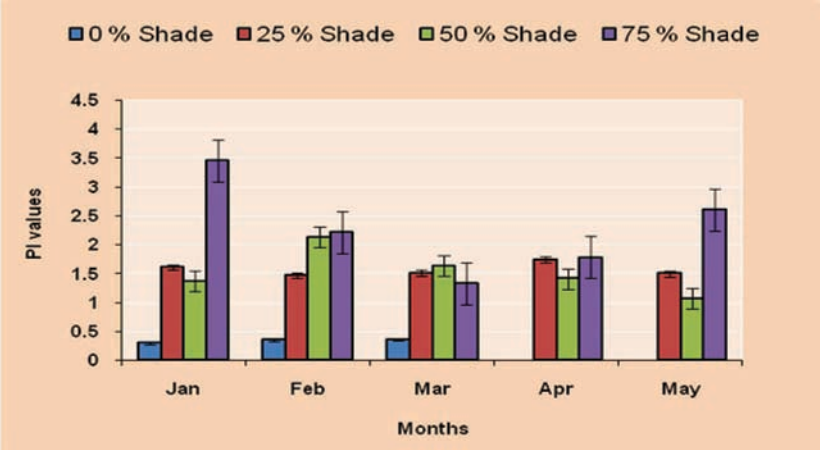


Figure 4.10: Variation in PI under four shade treatments monitored for five months from three months after germination

Chlorophyll *a*, chlorophyll *b* and total chlorophyll per g leaf tissue was highest for seedlings raised under 50 per cent shade (Table 4.10; Figure 4.11) when tested during the sixth month.

Table 4.10. Effect of shade on total chlorophyll content of sandal seedlings under four shade treatments

% Shade	Chlorophyll *a* mg/g tissue	Chlorophyll *b* mg/g tissue	Total chlorophyll mg/g tissue
0	3.12 ± 0.04	2.43 ± 0.05	5.55 ± 0.05
25	2.06 ± 0.04	1.57 ± 0.04	3.63 ± 0.08
50	3.45 ± 0.06	3.42 ± 0.06	6.87 ± 0.11
75	1.92 ± 0.04	1.57 ± 0.03	3.49 ± 0.05

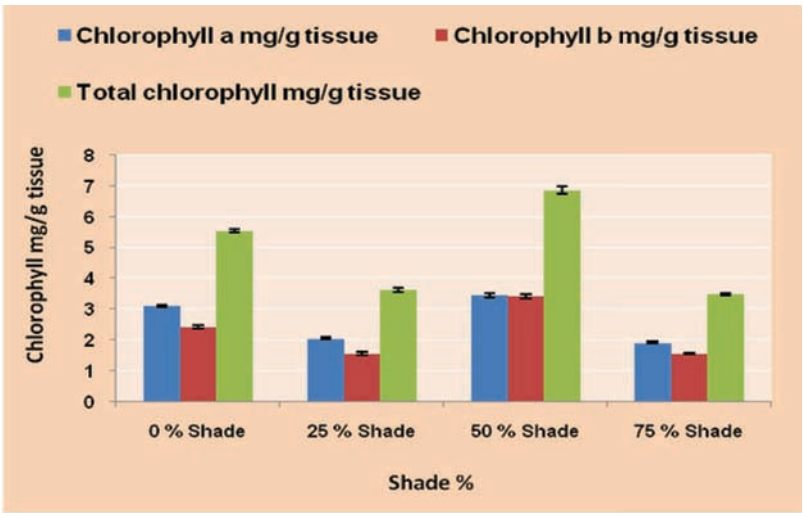


Figure 4.11: Chlorophyll content variation under four different shade treatments

4.4. DISCUSSION

Seedling quality is a concept, widely used in forestry, which has received considerable attention in Europe and the U.S. It is of prime importance since the afforestation programmes cannot receive the same attention as of individual fruit trees in an orchard or ornamental plants. Once planted, the seedlings have to survive the harsh environmental conditions. Many studies have shown that field survival and productivity are related to the quality of seedlings used (McTague and Tiius, 1996). As most of the morphological parameters can be measured easily, they are used for determining seedling quality (Thompson, 1986).

Many seedling quality parameters such as shoot dry weight:root dry weight ratio, have been conventionally used for assessing the quality of seedlings used for planting. According to Thompson (1986), as the planting site becomes more arid, the optimum seedling height for survival is lesser. Larsen *et al*. (1986), Tuttle *et al*. (1987), Wilder-Ayers and Tolliver (1987), and Mexal and South (1991) reported a negative correlation between seedling survival and seedling height. Mexal and Landis (1990) has reported that shorter, stockier seedlings are preferred for arid sites whereas taller seedlings for sites where vegetative competition or animal damage is severe. Seedling root collar diameter also is generally accepted to be better than height as a positively correlated morphological measure of seedling survival and growth (Thompson, 1986; Mexal and Landis, 1990). Root biomass is often correlated with seedling diameter; but shoot height, collar diameter and shoot biomass have been found to be better predictors of field survival.

Sturdiness quotient (SQ) is a simple and non-destructive method of determining the sturdiness of the plant based on a relation between the height and collar diameter of the seedlings. Based on investigations conducted earlier, it has been specified that for seedlings of temperate species the SQ should not be more than 6 for a good seedling. This value cannot be taken as the sole criterion for comparison with tropical plants, although it is generally agreed that if SQ increases, the seedlings will be less sturdy. Unfortunately in India this criterion has not been used to determine the seedling quality. Hence, it is difficult to pinpoint a particular SQ value for optimum seedling quality. According to Ritchie (1984), the final test of a forest tree seedling is its performance after out planting. It is important to study the survival of different species with different

SQ values in different locations under varying climatic conditions, especially for tropical species. The SQ values for sandal seedlings grown under different shade treatments in our study ranged from 5.75-7.11. SQ was lowest (5.75) for seedlings under 50 per cent shade, followed by 75 per cent shade and 25 per cent shade.

The shoot dry weight:root dry weight ratio is another important measure indicative of seedling survival. It relates the transpiring area (shoot) to the water absorbing area (root). Usually a seedling which shows 1:1 to 1:2 shoot:root ratio is considered as healthy. However, in this ratio the height of the plant or collar diameter, which are important factors to be considered in determining the seedling quality, are not taken into consideration. Hence, Dickson Quality Index (DQI) is a more refined value which takes into account both these parameters and so also the total biomass of the seedling. This index is very informative in determining the quality of the seedlings, although destructive sampling is required. In the present study DQI of sandal seedlings ranged from 0.35 to 0.47.

The growth performance of a seedling is basically determined by the physiological activity of the plant. There are very few physiological criteria that can be practically used to test the quality of the seedlings. Measurements of chlorophyll fluorescence have been found to be useful as an indicator for stress identification in plants. However, it has now been realized that there are several other derived parameters such as Performance Index that are much more sensitive in determining the performance of the photosystem in plants. In our study, F_v/F_m ratio ranged between 0.34 and 0.67. F_v/F_m values were almost similar under 50 per cent and 75 per cent shade. Lower F_v/F_m values were observed for sandal seedlings grown under full sunlight. It might be due to impairment of PSII function due to photoinhibition. Seedlings grown under full sunlight exhibited extreme stress symptoms as chlorosis and wilting; 100 per cent mortality was recorded within six months of start of the experiment. Performance Index (PI) value was greater for sandal seedlings under 75 per cent shade (2.6) in comparison with other treatments. Studies on *Tectona grandis* also had shown a steady increase in PI as shade increased upto five months from January to May (Chacko *et al.*, 2002).

Generally, the level of chlorophyll *a* and *b* tends to decrease with the increase in light intensity. Studies on four Pacific Northwest conifer species have shown that the concentration of chlorophyll *a* increased as the level of shade increased (Khan *et al.*,

2000). In our study on sandal seedlings under different shade treatments, chlorophyll *a* and *b* concentration and total chlorophyll content were found to be higher under 50 per cent shade. Though higher chlorophyll content was initially observed in seedlings under full sunlight complete mortality was recorded within six months of the start of the experiment.

Our results indicate that the JIP-test parameters are a good indication of the performance of the photosynthetic apparatus. The visual symptoms are sometimes deceptive or difficult to be used to assess the performance of the seedlings in the nursery. Whereas, the JIP-test, which is rather simple to apply and non-invasive, is very helpful in picking the most suitable treatment from a group of plants under different shade treatments. When the Performance Index given by the JIP-test is applied along with the morphological indices, SQ and DQI, it is possible to make meaningful assessments of the seedling quality.

4.5. CONCLUSION

Evaluating seedling response to shade is of utmost importance in a successful regeneration programme. Shade-grown seedlings can be considered as more efficient than sun grown seedlings in utilising low levels of light because of a much lowered light compensation point. Studies on vegetative competition has shown that a high ratio of height to diameter in seedlings can result in a subsequent decline in growth, leading to suppression and death (Khan *et al.*, 2000).

Sandal seedlings are sciophilic in the early phase, becoming more heliophilic once established. Data from our studies indicate that 50 per cent-75 per cent shade is optimal for growth of sandal seedlings up to one year. Results indicate that the photosynthetic activity of sandal seedlings may be limited due to photoinhibition when grown in open environments with high light intensity. Extremes of light intensity can cause mortality of the seedlings as observed in our study.

CHAPTER 5

GENETIC DIVERSITY OF *FUSARIUM OXYSPORUM* Schlechtend Fr ISOLATES CAUSING SANDAL SEEDLING WILT

5. GENETIC DIVERSITY OF *FUSARIUM OXYSPORUM* Schlechtend Fr ISOLATES CAUSING SANDAL SEEDLING WILT

5.1. INTRODUCTION

Sandalwood seedlings are affected by fungal diseases and insect pests, which take a heavy toll in nurseries and sometimes, the whole stock is wiped off. *Fusarium oxysporum*, *Rhizoctonia solani*, *Phytophthora* spp. and *Pythium* spp. cause serious damping-off and wilting of sandal seedlings. Mortality of seedlings due to pre-emergence and post-emergence damping off, root rot, collar rot, stem infection and wilting of older seedlings have been recorded (Remadevi *et al*., 2005). *Asochyta santali*, *Macrophomina phaseoli*, *Asterina congesta* and *Sphaecelomia santali* are the most common fungi causing leaf spot disease.

5.1.1. *Fusarium* wilt of sandal

Fusarium oxysporum Schlechtend Fr is the most common and most virulent fungal pathogen infecting sandal seedlings causing pre-emergence decay, vascular wilt, root rot and wilting of older seedlings (Sreenivasan *et al*., 1992). Remadevi *et al*. (2005) have reported high incidence of damping off and seedling wilt due to *Fusarium oxysporum* in sandal nurseries in Karnataka and Tamil Nadu which wiped out the entire nursery stock. The *F. oxysporum* isolates from infected tissues were grown on PDA medium and inoculated to healthy seedlings under controlled conditions. Wilt symptoms and chlorosis developed 7-10 days after fungus inoculation. Pathogen was reisolated from diseased plants proving Koch's postulates.

5.1.2. Variability and spore formation in *Fusarium*

Fungi belonging to the genus *Fusarium* contain many species that attack a large number of crops, causing seed rot, root rot, foot rot, stalk rot, wilt, yellows, and ear and kernel rots. *Fusarium* species are present in both temperate and tropical climatic regions of the world. There is wide variability in the pathogenicity of the fungal isolates, and in many *Fusarium* spp. various formae speciales (f. sp) and physiological races are known. The genus *Fusarium* composed of many pathogenic species and strains, lives in the soil and attacks cultivated crops and many wild plants. Serious loss may result, especially on

susceptible cultivars when weather conditions are favourable for disease development and spread (George *et al*., 1992). *Fusarium* spp. produce three kinds of spores - microconidia which are colourless, single celled, spherical spores, about 6-15 µm in length and 3-5 µm in diameter, the sickle shaped, colourless macroconidia, having three to five cross walls and average length of 30-50 µm and a diameter of 2-5 µm and the smooth, spherical single cells about 10 µm in diameter called chlamydospores produced on older mycelium. All three spore types are produced in soil or on infected plants. After infected plants die, the fungus and its spores are returned to soil, where they may persist indefinitely (George *et al*.,1992).

5.1.3. Genetic variability study of pathogenic fungi

Traditional markers used to study variability in plant pathogens are based on cultural characteristics, morphological features and biochemical capabilities of the pathogen besides infection on differential hosts. However, these markers are influenced by host age, inoculum quality and environmental conditions. Moreover, these techniques are time-consuming and laborious. Hence, recently, molecular markers are used for studying genetic variability in plant pathogens. PCR-based DNA fingerprinting, particularly with short oligonucleotide primers, has been used by various researchers for the analysis of genetic variation in plant pathogens. Molecular markers, apart from elucidation of genetic variability, can also be used to study evolution and monitoring movement of pathogen population over time and space (Sharma *et al*., 2006). PCR methods have several advantages over those of RFLP analysis since very little DNA is needed, many samples can quickly be screened and numerous experiments are possible within a short time. RAPD offers a promising, versatile and informative PCR based tool to detect genetic variation within populations of plant pathogens (Saharan *et al*., 2007).

5.1.4. Genetic variability of *F. oxysporum*

F. oxysporum is the sole species of the section Elegans which comprises pathogenic and non-pathogenic strains. Plant pathogenic forms cause wilt disease and are grouped into formae speciales based on their host range; some are further subdivided into pathogenic races. Molecular markers have been used to characterize genetic diversity of different isolates of *F. oxysporum*, formae-speciales (Alves-Santos *et al*., 2002) and

races within form species (Kelly *et al*., 1994, 1998; Balmas *et al*., 2005; Liu *et al*., 2006). *F. oxysporum* causes extensive disease problems such as a vascular wilt on a variety of crops worldwide (Singh *et al*., 2006). Genetic diversity studies using RAPD markers were carried out on *F. oxysporum* isolates from Cucumber (Vakalounakis and Fragkiadakis, 1999), Chickpea (Singh *et al*., 2006; Honnareddy and Dubey, 2006), Carnation (Manulis *et al*., 1994) and isolates from three agroclimatically different regions of north-western Himalayas (Sharma *et al*., 2006), causing vascular wilt on *Erythroxylum coca* var. *coca* in coca-growing regions of Huallaga Valley in Peru (Amy *et al*., 1997) and causing wilt of lentil plants in North-west Algeria (Belabid *et al*., 2004). A detailed review on this topic is provided in Review of Literature (Chapter 2).

5.1.5. Objective of the present study

F. oxysporum, a common soil borne fungus with a worldwide distribution, has no known sexual stage. This organism probably causes more economic damage than any other plant pathogen, and has been shown to constitute as much as 80-90 per cent of the total fungal microflora in the rhizosphere of several agricultural crops; it is responsible for wilt and cortical rot diseases of more than 100 economically important host plants (Swifty, 2002). No previous studies have been done on the genetic variability of *F. oxysporum* which cause serious damping off and wilting of sandal seedlings taking a heavy toll and wiping off the whole nursery stock. The objective of our study was to evaluate the genetic diversity of *F. oxysporum* causing heavy mortality to *S. album* seedlings under nursery and forest conditions.

5.2. MATERIALS AND METHODS

5.2.1. Fungal isolates

Fusarium oxysporum isolates were obtained from infected root, stem and collar region from germinating seedlings to 3-month-old seedlings collected from sandal nursery at KFRI, Peechi, Thrissur campus, and from sandal nursery and sandal regeneration experimental plot in Nachivayal sandal reserve in Marayur Sandal Division, Idukki District, Kerala (Figure 5.1). Seeds were sown in polybags filled with soil, sand and farm yard manure in the ratio 2:1:1 at KFRI nursery and the seedlings

were examined at various stages of growth for fungal infection. Out of the 1720 germinated seedlings 336 (19.5%) died due to serious infection of *F. oxysporum* during a period of nine months at KFRI, Peechi. The infected portions were gently washed first in tap water and then in sterile water, cut into small pieces of about 2-5 mm, and were transferred to sterile petriplates. These pieces were then surface sterilized with 0.1 per cent $HgCl_2$ solution for 15 seconds. The tissue sections were immediately transferred to Petri dishes containing sterile distilled water and washed repeatedly to remove traces of the surface sterilant. The surface sterilised tissues were inoculated to sterile petriplates containing Potato Dextrose Agar (PDA) medium amended with 30mg/l of Streptomycin sulphate to prevent bacterial growth.

After 4-5 days, fungal colonies growing on PDA medium in petriplates were streaked onto fresh PDA plates for obtaining pure cultures. The new plates were then incubated at 25 ± 1ºC and examined for cultural characteristics and for conidial morphology. All the isolates were identified as *F. oxysporum* based on the characters described by Booth (1971). Pure cultures were obtained from diseased sandal seedlings from KFRI nursery at Peechi, Thrissur District, from diseased seedlings from sandal nursery raised at Marayur and the seedlings growing on forest floor in KFRI sandal regeneration experimental plot at Marayur. We had isolated 48 isolates from Peechi and 54 isolates from Marayur. Three isolates were randomly selected based on cultural variations from Peechi isolates (*Fo*.P1, *Fo*.P2 and *Fo*.P3) and three from Marayur isolates (*Fo*.M1, *Fo*. M2 and *Fo*.M3) for genetic diversity studies using RAPD markers (Fig 5.2). Isolates subcultured onto PDA slants were stored at 4ºC for further experiments. The composition of PDA medium is provided below.

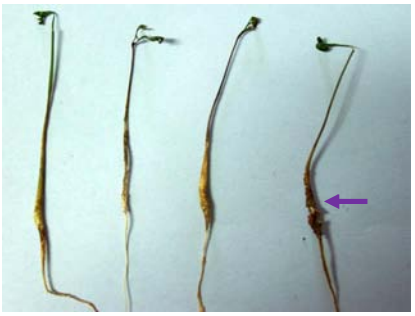
Peeled Potato-250g
Dextrose-20g
Agar-15g
Distilled Water-1000ml
pH-6.8

Potato was peeled into thin chips, boiled in 500 ml of water and extract collected. To the extract, the weighed quantity of dextrose was added. Agar was melted in the other half of water and mixed with potato-dextrose solution and the volume was made up to a litre before sterilising.

5.2.2. Total genomic DNA extraction and purification

Genomic DNA was extracted from mycelial tissue following slightly modified method of Doyle and Doyle (1990). For DNA extraction, fungal mycelium scrapped out from individual cultures grown on PDA medium for 2-3 days were used. The mycelium was homogenated in CTAB buffer containing 100 mM Tris pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 1 per cent PVPP and an additional 2 M NaCl (200 µl). To the fine homogenate, RNase (20 µl) and proteinase (4 µl) were added and incubated at 60ºC for 45 minutes. Samples were then extracted in an equal volume of chloroform:Isoamyl alcohol (24:1) followed by centrifugation at 10,000 rpm for 10 minutes. The supernatant was transferred to an eppendorf tube, equal volume of chloroform added, shaken and again centrifuged at 10,000 rpm for 10 minutes. To the supernatant, double the volume of ice-cold ethanol (100%) was added. After incubation at -20ºC for 12 hours, DNA was precipitated by centrifugation at 10,000 rpm for 10 minutes. The supernatant was discarded. The DNA pellet was resuspended in ethanol (80%) and centrifuged twice at 8,000 rpm for 10 minutes. The air dried pellet was dissolved in 100 µl of double distilled water and checked for purity on agarose gel.

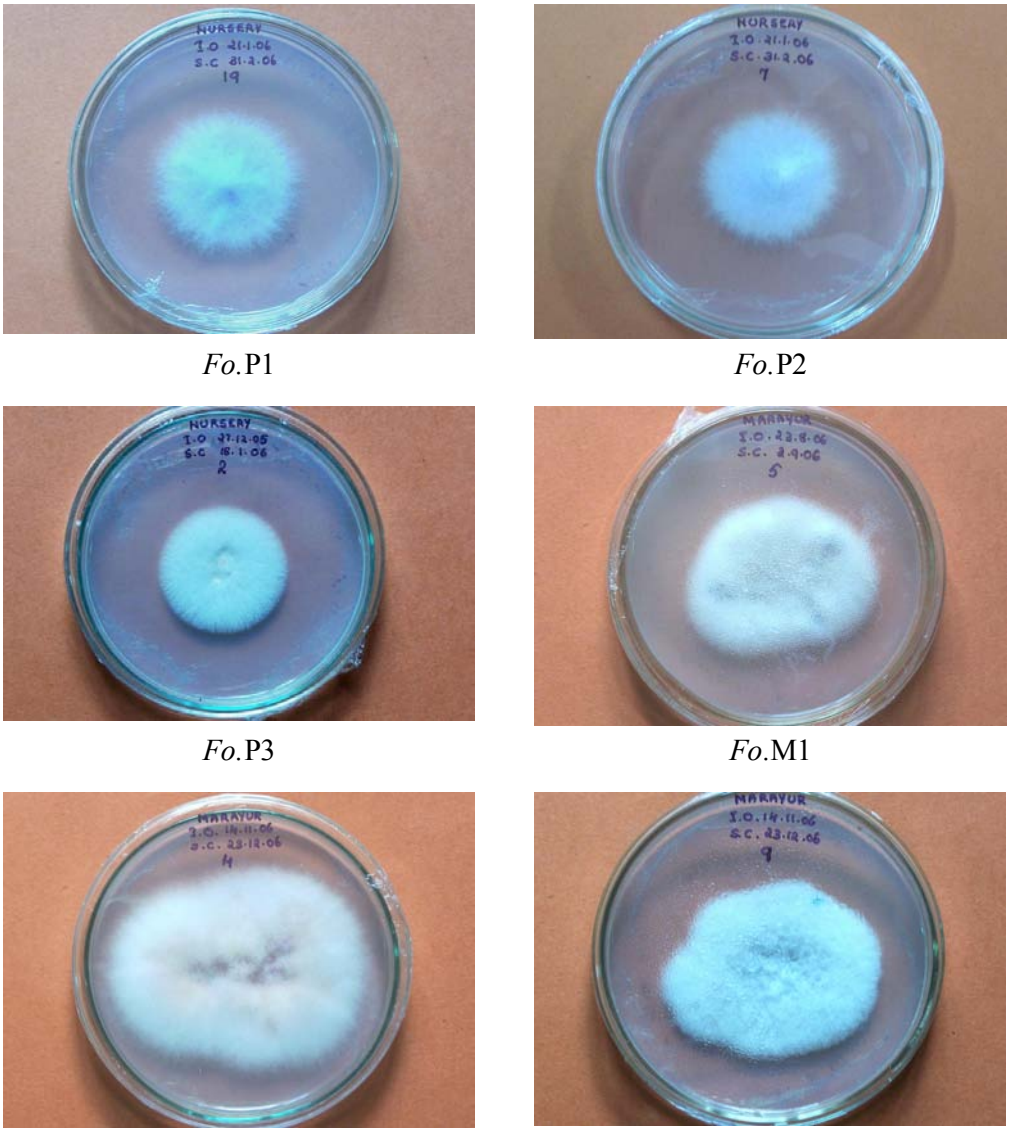
Infection on seedling stem



Infection at collar region

Infection at collar region

Figure 5.1. Sandal seedlings showing typical symptoms of wilt caused by *F. oxysporum*



Fo.P1 *Fo*.P2

Fo.P3 *Fo*.M1

Fo.M2 *Fo*.M3

Figure 5.2. *F. oxysporum* isolates selected for RAPD analysis
Fo.P1, *Fo*.P2 and *Fo*.P3: Isolates from Peechi, Thrissur
Fo.M1, *Fo*. M2 and *Fo*.M3: Isolates from Marayur

5.2.3. RAPD primers and PCR amplification

Twenty four RAPD primers were initially screened for *F. oxysporum* DNA amplification and production of clear, sharp and repeatable amplicons. Of these, 9 primers viz., OPB-01, OPB-04, OPB-07, OPB-10, OPB-11, OPB-14, OPB-15 of kit OPB and OPD-18 and OPD-19 of kit OPD which produced repeatable and unambiguous amplicons on agarose gel were selected for RAPD analysis of the 6

isolates of *F. oxysporum* (Table 5.1). RAPD-PCR amplifications were carried out in a final volume of 25 µl consisting of 1.5 mM $MgCl_2$, 200 µM of dNTPs, 5 µM of primer, 0.125 µl (0.375 U) of Taq DNA Polymerase (Bangalore Genei, Bangalore, India), 2.5 µl of 10X reaction buffer (500 mM KCl, 15 mM $MgCl_2$, 100 mM Tris-HCl-pH 8.0) and approximately 2 µl (30-50 ng) of template DNA. PCR amplifications were performed in PTC-100 Thermal cycler (MJ Research, WaterTown, MA, USA) under the following conditions: Initial denaturation at 94°C for 5 minutes; 30 cycles of PCR, each of 94°C for 1 min (denaturation), 37ºC for 1 min (primer annealing) and 72°C for 2 minutes (strand synthesis and extension) and a final extension of 72°C for 5 minutes. PCR products were stored at -80°C. The amplification products were separated by electrophoresis on 1.5 per cent agarose gel in 1X TBE buffer. After running for approximately 90 minutes at 75V, the gel was stained with 0.05 per cent ethidium bromide and viewed on a UV trans-illuminator. The DNA profile was photographed using Gel Doc System DP-CF-011 (Vilber Lourmat, France) and transferred to a computer.

Table 5.1. Primers used for RAPD analysis and their sequences

Primer code	Primer sequence (5´-3´)
OPB-01	GTT TCG CTCC
OPB-04	GGA CTG GAGT
OPB-07	GGT GAC GCAG
OPB-10	CTG CTG GGAC
OPB-11	GTA GAC CCGT
OPB-14	TCC GCT CTGG
OPB-15	GGA GGG TGTT
OPD-18	GAG AGC CAAC
OPD-19	CTG GGG ACTT

5.2.4. Data analysis

The DNA fragment size was estimated by comparing with DNA size markers (100bp DNA ladder, Bangalore Genei) run on the same gel and DNA fragment size estimated using the software Kodak Digital Science Electrophoresis Documentation and Analysis System 120 (Kodak, USA). Amplified loci were scored for presence (1) or

absence (0) of bands. Only data from intensely stained, unambiguous, clear bands were included in the analysis. The per cent of polymorphic loci (polymorphic bands per total bands), gene diversity index (h) (Nei, 1973) and Nei's genetic distance indices were calculated using POPGENE version 1.31 (Yeh *et al.*, 1999). Pair-wise comparison of the isolates based on the presence or absence of unique or shared fragments were used to generate a similarity matrix based on the Simple Matching (SM) coefficient (Sokal and Michener, 1958) using the NTSYSpc (Numerical Taxonomy and Multivariate Analysis System for personal computers) software, version 2.1(Rohlf, 2000). From the similarity matrix, a sequential, agglomerative, hierarchical, and nested (SAHN) cluster analysis was performed and dendrogram constructed with Unweighted Pair Group Method with Arithmetic Averages (UPGMA) algorithm using NTSYSpc (Rohlf, 2000) package to determine the grouping of isolates.

5.3. RESULTS

The study was designed to characterize and determine the degree of genetic variation in six different cultures of *F. oxysporum* isolated from wilt affected seedlings from sandal nursery and forests of Marayur and KFRI Central nursery Peechi. Fifty four isolates were obtained from sandal nursery and sandal forest of Marayur and forty eight isolates from Peechi. Out of these, 3 isolates each selected at random from each locality (total 6) were used for RAPD analysis. Seven primers from OPB series viz. OPB 1, OPB 4, OPB 7, OPB 10, OPB 11, OPB 14, OPB 15 and two primers from OPD series viz. OPD 18 and OPD 19 were chosen based on the number and reproducibility of amplified fragments out of 20 primers tested. The PCR amplified RAPD products were run on 1.5 per cent agarose gel, stained using 0.05 per cent ethidium bromide and screened for presence or absence of bands (Fig. 5.3, 5.4 and 5.5). The size of the amplified fragments ranged between 150-1500 base pairs. Only sharp, clear and unambiguous bands were enumerated. The numbers of amplicons generated by each primer are given in table 5.2. Assuming that each RAPD product represented a single locus, 98.39 per cent of the loci were found to show polymorphism (122 out of 124).

The estimation of gene diversity index (h) (Nei, 1973) showed that a few of the loci were found genetically identical; for the rest of the loci, gene diversity ranged from 0.27 to 0.50 and the mean gene diversity was 0.37.

Genetic distance coefficients (Nei, 1978) (Table 5.3) showed that, *F. oxysporum* isolate *Fo.*M2 and *Fo.*M3 from Marayur were the genetically nearer ones (D=0.2985) and isolate *Fo.*P1 from KFRI nursery and isolate *Fo.*M2 from Marayur were the genetically distant ones (D=0.9701). The UPGMA dendrogram (NTSYSpc, Rohlf, 2000) constructed based on the genetic similarity coefficients revealed the genetic relatedness of the six isolates of *F. oxysporum.* The gene diversity, genetic distance coefficients between pair of isolates and dendrogram obtained from cluster analysis (UPGMA dendrogram) revealed a great deal of heterogeneity among the isolates. The isolates formed two distinctly major clusters (Fig. 5.6) at the similarity coefficient value of 0.46. The first major cluster separated into two sub-clusters at similarity value of 0.56. The first sub-cluster consisted of isolates from KFRI nursery, Peechi (*Fo.*P1, *Fo.*P2) and the second sub-cluster consisted of 4 isolates. Two isolates *Fo.*M1 and *Fo.*M3 which originated from Marayur showed similarity at 0.78 while the isolate *Fo.*M2 from Marayur joined with the cluster at similarity coefficient 0.70. One isolate from KFRI nursery, *Fo.*P3 joined separately to the second sub-cluster at 0.57.

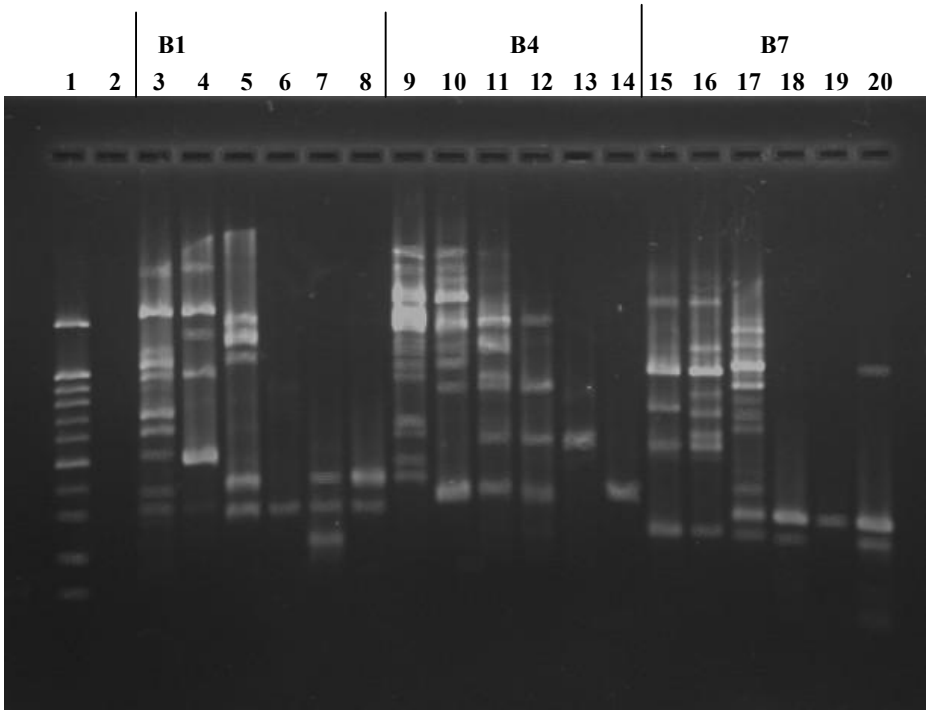


Figure 5.3: RAPD profile of six isolates of *F. oxysporum* obtained using primers B1, B4 and B7. Lane 1: DNA marker (100 bp + 1.5 kb DNA ladder, BIOENZYME, USA); lane 2: negative control, lane 3-8: DNA profile using primer B1; lane 9-14: DNA profile using primer B4; lane 15-20: DNA profile using primer B7.

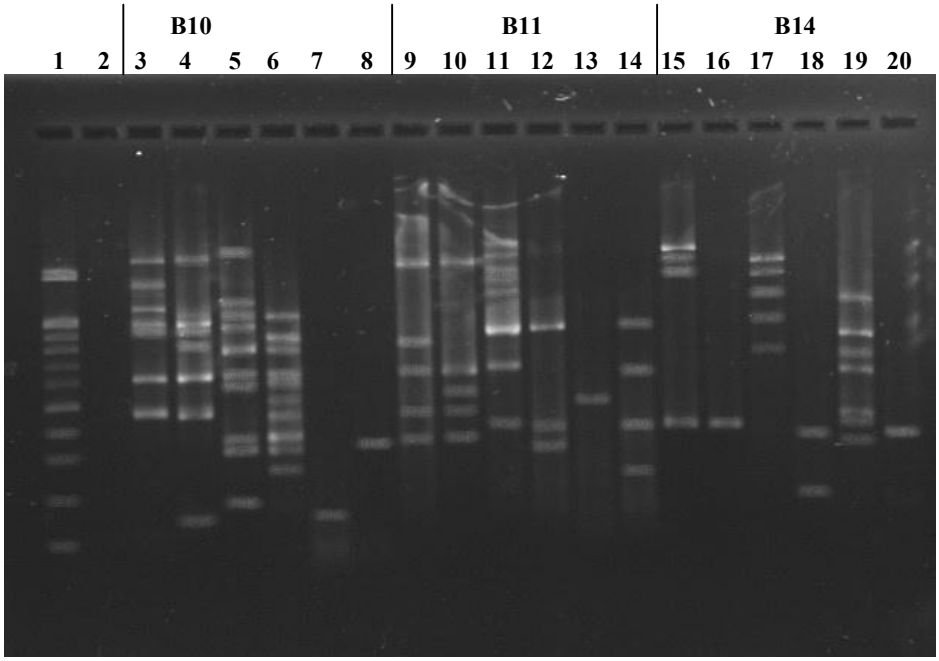


Figure 5.4: RAPD profile of six isolates of *F. oxysporum* obtained using primers B10, B11 and B14. Lane 1: DNA marker (100 bp + 1.5 kb DNA ladder, BIOENZYME, USA); lane 2: negative control, lane 3-8: DNA profile using primer B10; lane 9-14: DNA profile using primer B11; lane 15-20: DNA profile using primer B14.

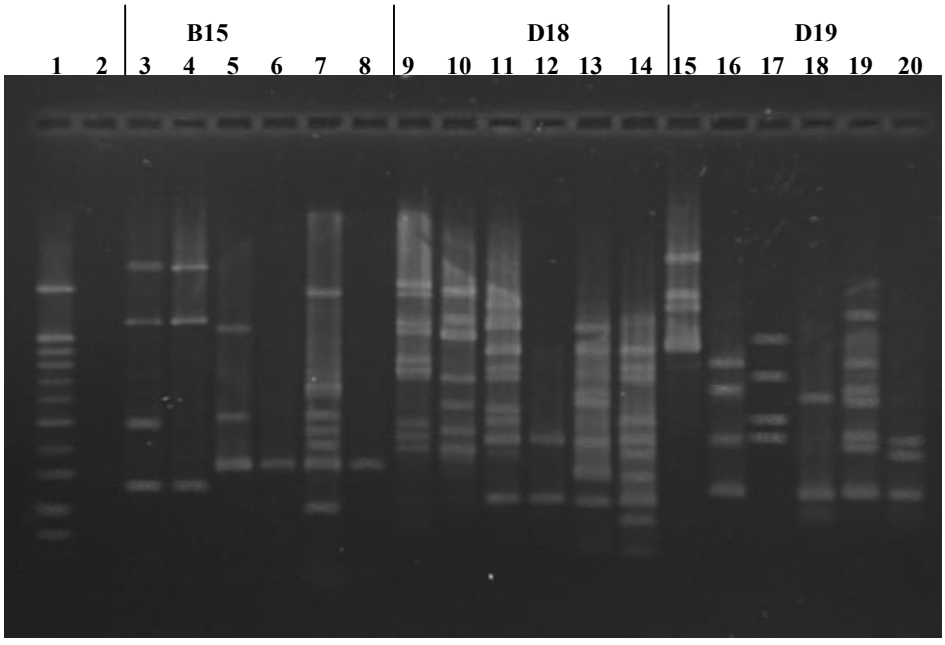


Figure 5.5: RAPD profile of six isolates of *F. oxysporum* obtained using primers B15, D18 and D19. Lane 1: DNA marker (100 bp + 1.5 kb DNA ladder, BIOENZYME, USA); lane 2: negative control, lane 3-8: DNA profile using primer B15; lane 9-14: DNA profile using primer D18; lane 15-20: DNA profile using primer D19.

Table 5.2. Number of RAPD products from six isolates of *F. oxysporum*

F. oxysporum		Primer and Number of Amplicon produced								
Sl. No.	Isolates	OPB 1	OPB 4	OPB 7	OPB10	OPB11	OPB14	OPB15	OPD18	OPD19
1	*Fo.*P1	10	11	6	6	5	4	4	7	4
2	*Fo.*P2	5	8	8	7	5	1	3	7	4
3	*Fo.*P3	5	5	10	10	7	5	3	11	4
4	*Fo.*M1	1	4	2	9	3	2	1	2	2
5	*Fo.*M2	3	1	1	1	1	7	7	7	8
6	*Fo.*M3	2	1	3	1	4	1	1	9	3
	Total	26	30	30	34	25	20	19	43	25

Table 5.3. Nei's (1978) genetic distance coefficients from six different *F. oxysporum* isolates

F. oxysporum Isolates	*Fo.*P1	*Fo.*P2	*Fo.*P3	*Fo.*M1	*Fo.*M2	*Fo.*M3
*Fo.*P1	****					
*Fo.*P2	0.4258	****				
*Fo.*P3	0.9285	0.6306	****			
*Fo.*M1	0.8129	0.6306	0.5436	****		
*Fo.*M2	0.9701	0.8313	0.7259	0.4636	****	
*Fo.*M3	0.7427	0.6614	0.4895	0.2559	0.2985	****

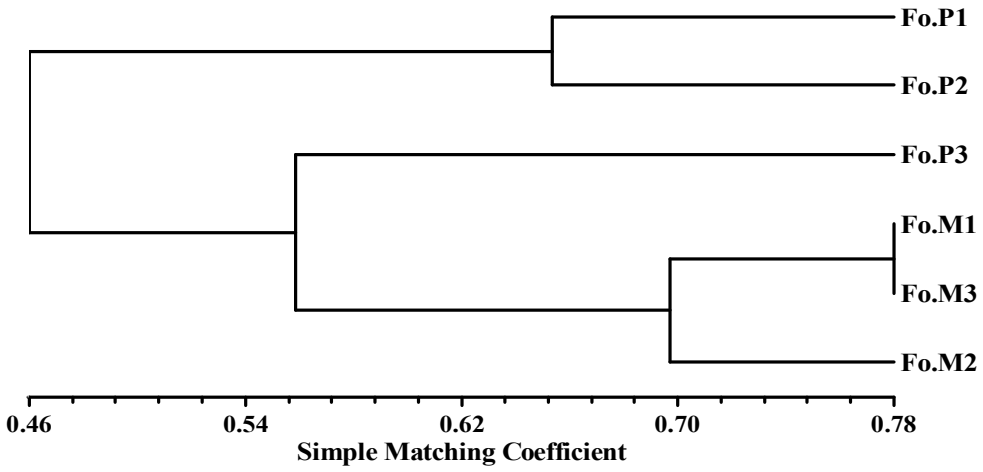


Figure 5.6. UPGMA Dendrogram based on Simple Matching Coefficients

5.4. DISCUSSION

RAPD provides a rapid and efficient means of comparing the overall genetic diversity among different pathogen isolates and also for its molecular characterization (Goodwin and Annis, 1991). Because of these advantages, RAPD markers have been widely used to study genetic variations within and between fungal populations (Crowhurst *et al*., 1991; Guthrie *et al*., 1992). In the present study, molecular characterization of *F. oxysporum* isolates responsible for wilting of sandal seedlings collected from two different localities, KFRI nursery at Peechi, Thrissur and sandal nursery and sandal forest at Marayur in Idukki district, was carried out using RAPD markers. The genetic similarity of the isolates was also assessed from the RAPD data generated.

The various genetic diversity measures viz. percentage of genetic polymorphism and gene diversity index (h) were determined in a group of six *F. oxysporum* isolates. Majority (122 out of 124) of the loci were found to be polymorphic across the six isolates. Similar level of genetic polymorphism was also observed in *F. oxysporum* isolates from Cucumber (97 out of 107) (Vakalounakis and Fragkiadakis, 1999) and in *F. oxysporum* f. sp. *ciceris* isolates causing chickpea wilt (122 out of 135) (Honnareddy and Dubey, 2006).

A gene diversity value ranging from 0.4 to 0.52 was reported by Alfonso *et al*. (2000) in *Botrytis cinerea* and 0.03 to 0.42 in *S. homoeocarpa* isolates (Hsiang and Mahuku, 1999), while a relatively low amount of gene diversity was reported in *Erysiphe graminis* ranging from 0.15-0.23 (Mc Dermott and Mc Donald, 1993). The gene diversity values observed in the present study (ranging from 0.27 to 0.50) thus exhibited a significant level of genetic divergence or differentiation. Genetic drift and low level of inter population gene flow might be the reason for the high level of gene divergence (Hartl and Clark, 1989).

The genetic distance coefficients ranged between 0.2985 to 0.9701. The UPGMA dendrogram revealed the genetic relatedness of the six isolates. The most genetically similar ones were collected from KFRI nursery while the genetically distant ones were collected from KFRI nursery and Marayur. The two locations are geographically far apart, a distance of approximately 175 km. Generally, organisms originating from same

geographical area show genetic similarity. In the present study *F. oxysporum* isolates from Marayur have shown genetic similarity among themselves (lesser genetic distance) and they have aligned into one cluster. But, instead of aligning all the three isolates from Peechi nursery into one cluster, one of them *Fo.*P3 joined with the Marayur isolates (Fig 5.6). The sandal seeds used for raising nursery at Peechi had originated from Marayur forests. Hence, it might be possible that the seeds collected from the forest floor at Marayur might have been contaminated with the pathogen and reached Peechi as the contaminant along with the seeds or probably carried with the soil sticking to the seeds.

5.5. CONCLUSION

F. oxysporum, the fungal pathogen responsible for causing various types of seedling wilt in a wide range of economically important plants, is having a world-wide distribution. *Fusarium* wilt has very high occurrence in sandal seedling nurseries and is known to cause heavy seedling mortality. It is therefore important to have knowledge on the biology and diversity of the pathogen, as it possesses a wide host range.

By using RAPD technique, it is revealed that there is a wide range of genetic variation among the *Fusarium* genotypes. The large genetic variation detected at the DNA level indicates the ability of the pathogen to adapt to different eco-climatic conditions. The pathogen has the potential to be highly successful in infecting sandal seedlings at different growth stages. Since, the pathogen is prevalent in the soils of Marayur, the seeds originating from Marayur will be contaminated with the fungus. Hence, a prophylactic treatment of sandal seeds, preferably with a systemic fungicide is very important. Since the pathogen infects at different growth stages of the seedlings and the infected plant usually perishes, prophylactic fungicidal treatment has to be given at various stages of seedling growth. Studies combining morphological characters from traditional techniques with those from molecular techniques are expected to enhance our knowledge of the complex biodiversity of *F. oxysporum* in soil.

CHAPTER 6

STUDIES ON GENETIC DIVERSITY OF SANDAL IN SEED STAND

6. STUDIES ON GENETIC DIVERSITY OF SANDAL IN SEED STAND

6.1. INTRODUCTION

Genetic variation is an important conservation factor, providing the necessary resilience to allow populations to respond to environmental pressures. It provides a given species adaptability to respond to environmental changes, natural evolution, and survival in the long run (Sheng *et al*., 2005). Isolated and island populations tend to experience reduced genetic variability for a variety of reasons such as small population size, founder effect, and low gene flow from neighboring populations (Frankham, 1997). Knowledge of genetic variation between and within populations plays a significant role in the formulation of appropriate management strategies directed towards their conservation (Lynch and Milligan, 1994). *S. album* is a 'vulnerable' species (IUCN, 2010) and hence it is of paramount importance to evaluate and maintain genetic variability within and among populations because populations consisting of genetically uniform individuals will be vulnerable to major climatic changes, and pest and diseases outbreaks (Aradhya and Philips, 1993). Indiscriminate felling of sandal due to its commercial importance has resulted in depletion of the valuable bioresource. In order to redress this problem, organized efforts of conservation, tree improvement and plantation establishment are needed, which requires high quality planting material. Kulkarni (1995) attributed the wide variation in seed output and reproductive capability in *S. album* to phenological variations with respect to tree's flowering.

6.1.1. Establishment of seed stands

Tree improvement is initiated with population survey, documenting variability within the species and identifying the variability pattern in economically or commercially important traits (Kumar and Joshi, 2007). In sandal, tree improvement began in 1980s and was mainly aimed at evolving trees that can yield more heartwood and oil, can resistant spike disease, attack of heartwood borers and other pests and diseases. As part of this programme, with the intention of clonal propagation and seed

collection, candidate plus trees showing (i) fast growth indicated by higher DBH, clear bole, height, good crown, and pest and disease resistance (ii) higher heartwood and oil content and (iii) fragrance and heartwood colour were selected in Karnataka, Tamil Nadu, Andhra Pradesh and Kerala. Besides this programme, for collecting high quality seeds, seed stands had been located at Anchalpatty (Marayur, Kerala), Chitteri and Javadis (Tamil Nadu), and Arabithittu and Thindlu (Karnataka) (Sindhuveerendra and Ananthapadmanabha, 1998). In Anchalpatty, located at Nachivayal sandal reserve in Marayur, the seed stand was established in 5.3 ha in order to collect seeds for raising sandal plantations and for augmenting sandal population in reserve forests.

Seed stands are seed production areas (SPAs) of suitable provenance which have grown from seeds of a reasonably large number of unrelated trees and managed specifically for seed production. In order to meet the annual planting requirement, SPAs are usually developed by culling inferior trees from even-aged high quality plantations and retaining sufficient number of superior and healthy trees with more than average seed production. Since, cutting and removal of live sandal trees were prohibited by rule, seed production areas (SPAs) of sandal were selected from natural stands of the provenances for use in afforestation programmes. Quality nursery stock raised from source-identified, improved seeds are expected to ensure increased plantations productivity.

6.1.2. Poor seed setting in seed stand

Seed stands are highly relevant since they assure an immediate supply of adequate quantities of quality seeds. But, recently it has been reported that the expected quantities of seeds are unavailable from the seed stand at Marayur. Earlier studies by Kulkarni and Muniyamma (1998) on flowering, fruit development and fruit set at five selected sites in natural sandal forests at Bangalore, Coimbatore, Tirupattur, Salem and Tanjavur Forest Divisions showed that only a small proportion of flowers eventually mature to fruits; this may be due to lack of effective pollination and fertilization, pollen sterility and incompatibility, and immature fall of flowers and fruits. Shepherd (1992) reported that *S. album* is a facultatively selfing and preferentially outcrossing species. The tendency for outbreeding in sandal is reinforced by asynchronous flowering, insect pollination, heterostyly, and self-incompatibility.

6.1.3. Importance of genetic diversity in seed stand

Kulkarni and Muniyamma (1998) reported that in sandal, the high amount of flower and fruit abortion had been correlated with self incompatibility. Moreover, the estimation of natural selfing had showed the presence of high percentage of abnormality in the half-sib seedling populations with lower fitness value indicating the deleterious effects of inbreeding (Kulkarni, 1995). Hence, knowledge of genetic variation within and between the SPAs is crucial for adopting proper seed management in the seed production areas and in tree improvement programmes (Jagadish *et al*., 2007; Sreekanth, 2009).

6.1.4. Clonality in sandal forests and its effect on seed production

Natural stands of *S. album* in Sri lanka has shown that it spread clonally by prolific root suckering (Tennakoon *et al*., 2000). Trueman *et al*. (2001) assessed the genetic structure and level of clonality within five southernmost populations of *S. lanceolatum* at Victoria using allozyme and RAPD markers and found that each of the remnant populations existed as a single unique clone, recruiting individual plants via root suckers. Examination of pollination and fruit set indicated that little or no sexual reproduction was occurring in the remnants, due to pollen sterility in one population and self-incompatibility or pistil dysfunction in the others. Both, allozymes and RAPD studies could not detect genetic variation within populations, suggesting that each population consisted of single genotype. This resulted in inbreeding and extremely poor seed setting.

6.1.5. Tools to study genetic diversity in natural forest and seed stands

In conventional tree breeding programmes, genetic variation and heritability are studied through progeny trial and evaluating the performance of the progenies. Because of long generation period of trees, a successful completion of progeny trial takes a long period. Molecular markers provide a powerful method to generate a large amount of information on genetic diversity and phylogenetic relationships in the germplasm within a short period of time. Some of the widely used PCR based DNA markers for studying genetic diversity are RAPDs (Williams *et al*., 1990), SSRs or microsatellites (Tautz, 1989), ISSRs (Zietkiewicz *et al*., 1994) and AFLPs (Vos *et al*., 1995). Each marker technique has its own advantages and disadvantages. A detailed review of molecular markers are provided in the chapter on Review of Literature (para 2.5.1 - 2.5.3).

6.1.6. ISSR marker as a tool to study genetic diversity

PCR-ISSR which uses the occurrence of SSRs in the plant genome, has also been used successfully to characterize genetic diversity within and among plant populations (Qian *et al*., 2001). They have proved to be efficient in population genetic studies, especially in detecting clonal diversity and fingerprinting closely related individuals (Zietkiewicz *et al*., 1994; Wolfe and Liston, 1998; Esselman *et al*., 1999). ISSR markers are much more effective in genotype identification because the amplification is based on SSRs, which are extremely variable in eukaryotes. A detailed review of ISSR markers are provided in para 2.5.3. of Review of Literature.

6.1.7. Objective of the present study

One of the reasons for poor sandal regeneration in natural forests of Marayur is high seedling mortality. This demands large quantity of seeds for sowing in new localities as well as in sandal forests where practically very little regeneration takes place. Lack of availability of good quality seeds have been reported from forest ranges. But, recently it had been reported that expected quantity of seeds are unavailable from the seed stands established in Nachivayal reserve during 1980-81. Most of the sandal trees flower profusely, but they do not set seeds. Sandal, being a cross pollinated plant, fruit setting and seed formation take place usually if pollination and fertilization occurs between genetically unrelated genotypes. Though, failure to develop mature seeds may occur due to pathogenic infection of developing fruits, premature flower and fruit fall, etc., such major problems did not come to our notice. Hence, our objective was to assess the level of clonality and genetic diversity of sandal trees which may be causing inbreeding in sample plots in the seed stand at Marayur using ISSR markers.

6.2. MATERIALS AND METHODS

6.2.1. Plant material

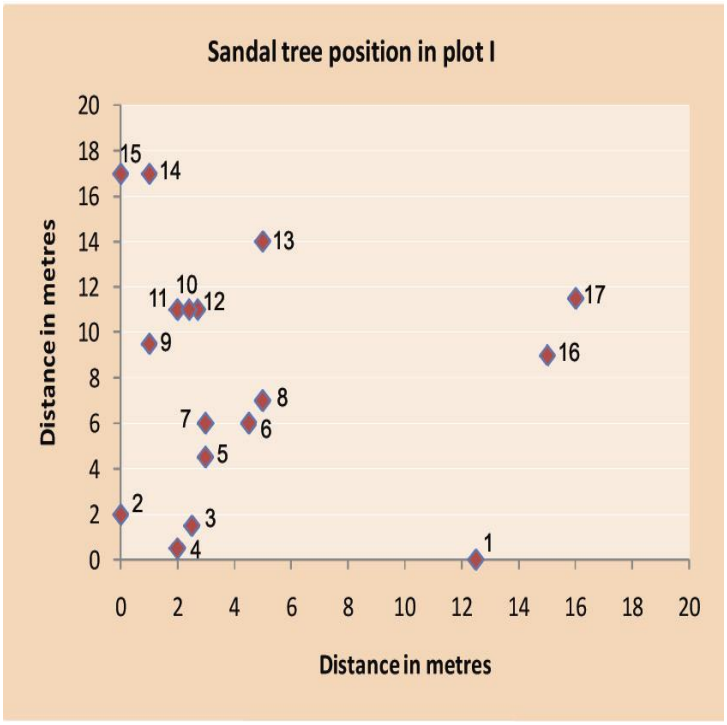
The sandal seeds for the study were located in the seed stand established in the Nachivayal II sandal reserve at Anchalpatty, Marayur. The sketch of the seed stand was drawn on a graph paper and then divided into 20m x 20m plots. Two plots of 20m x 20m size with sufficient number of trees were randomly selected for the study. There

were 17 sandal trees in the first plot and 17 trees in the second plot (Fig 6.1. a & b). The positions of the trees in the sample plots were plotted on a graph paper and a chart showing their position are provided (Fig. 6.2. a & b). The girth at breast height (GBH) measurements of the selected trees are provided in table 6.1. Leaf samples were collected from all the trees in both the plots for DNA extraction.

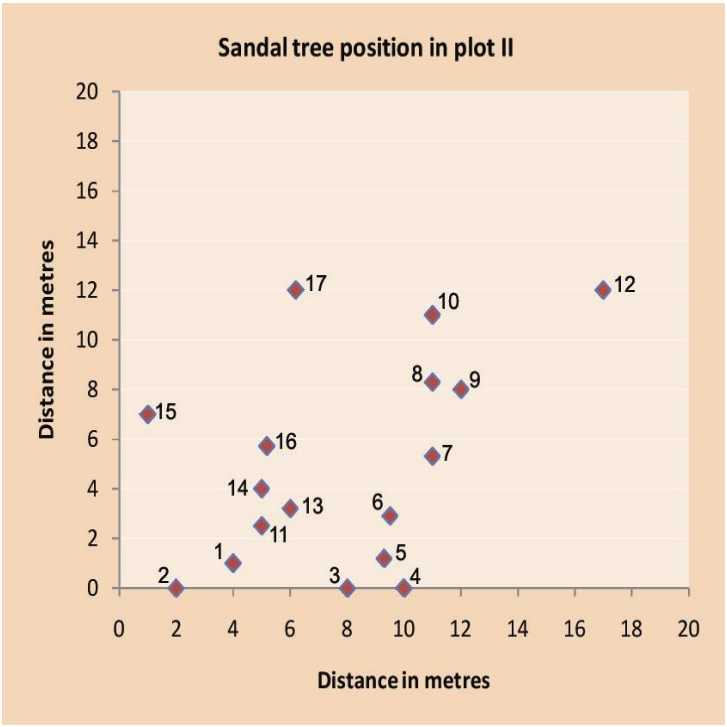
a

b

Figure 6. 1. a & b: Sandal trees in clusters in the experimental plots in Marayur seed stand



a



b

Figure 6. 2. a & b: Sandal tree positions in the experimental plots in Marayur seed stand

Table 6.1. Girth of sandal trees growing in the experimental plots

Experimental plot I		Experimental plot II	
Tree No.	GBH (cm)	Tree No.	GBH (cm)
1	45	1	44
2	52	2	44
3	42	3	55
4	33	4	40
5	20	5	63
6	29	6	30
7	79	7	39
8	59	8	51
9	31	9	49
10	68	10	48
11	51	11	47
12	34	12	52
13	41	13	55
14	36	14	39
15	41	15	50
16	52	16	20
17	47	17	55

6.2.2. Genomic DNA extraction

Genomic DNA was extracted from approximately 250 mg of fresh leaf sample using GeNei™ spin plant genomic DNA preparation kit (Bangalore Genei). The kit contained (for 20 purifications) Lysis buffer (15 ml), Wash buffer (20 ml), Precipitation solution (5 ml), RNase (lyophilized) 5 mg, Proteinase K (lyophilized) 1.5 mg, Elution buffer (6 ml), Spin columns (20 Nos.), tissue grinder (20 Nos.) and 2 ml collection tubes (20 Nos.). Leaf tissue was ground in a sterilized mortar and pestle and 750 µl of lysis buffer was added followed by 5 µl of Proteinase K. The sample was incubated at 65°C in a water bath for 15 minutes with mild shaking and then centrifuged at 12,000 rpm for 10 minutes at room temperature. The supernatant containing genomic DNA was transferred to a fresh tube, 10 µl of RNase was added and incubated at room temperature for 15 minutes. 200 µl of precipitation solution was added to the above sample and the tube incubated in ice for 5 minutes. It was then followed by centrifugation at 12,000 rpm for 10 minutes. To the clear supernatant, equal volume of ethanol (100%) was added, mixed and loaded onto the column provided in the kit. Centrifugation was carried out at 10,000 rpm for 20 seconds. The eluate was discarded after each spin. The column was washed with 500 µl of wash buffer twice by centrifugation at 10,000 rpm for two minutes discarding the

eluate each time. A final spin was given at 10,000 rpm for two minutes. Finally, the column was incubated at 65°C for five minutes. DNA was eluted with 150 µl of preheated elution buffer (65°C) by spinning the column at 10,000 rpm for two minutes.

6.2.3. ISSR primers and PCR amplification

ISSR primers with sequence $(AG)_8CA$(UBC-836), $(GA)_8TC$(UBC-841), $(AG)_8T$(UBC-807), $(CA)_8G$(UBC-818), $(CA)_8AG$(UBC-848) and $(CT)_8GC$(UBC-844) were selected from the primer sequences provided by University of British Columbia (UBC) (Vancover, Canada) and from research papers dealing with ISSR markers (Nagaoka and Ogihara, 1997; Bahulikar *et al*., 2004; Hou *et al*., 2005; Dje *et al*., 2006). The six primers were screened initially for obtaining maximum polymorphic bands with clarity and reproducibility. Accordingly, three ISSR primers which showed clear and consistent bands were finally selected (Table 6.2.). The oligos (primers) were supplied by Bangalore Genei (Bangalore). PCR reactions were carried out in a final volume of 25 µl consisting of 1.5 mM $MgCl_2$, 100 µM of dNTPs, 100 pM of primer, 3 U of Taq DNA polymerase, 10X reaction buffer and approximately 2 µl (20-40 ng) of template DNA. PCR amplifications were performed in PTC-100 Thermal Cycler (MJ Research, Watertown, MA, USA) under the following conditions: initial denaturation at 95°C for 5 minutes; 30 cycles, each of 95°C for 30 seconds, 58°C (annealing temperature for each primer changed suitably) for 45 seconds, 72°C for 2 minutes and finally 95°C for 5 minutes. PCR products were then stored at -80°C. The amplification products were separated by electrophoresis on 1.5 per cent agarose gel in 1X TBE buffer. After running for approximately 90 minutes at 75V, the gel was stained with 0.05 per cent ethidium bromide, viewed on a UV trans-illuminator and photographed using Gel Doc System DP-CF-011 (Vilber Lourmat, France) and transferred to a computer. Scoring of the bands for presence (1) or absence (0) was performed by visual analysis of the gel photographs. Molecular size of the fragments was estimated using the software Kodak Digital Science Electrophoresis Documentation and Analysis System 120 (Kodak, USA), comparing the fragment size with 100 bp DNA ladder.

Table 6.2. Primers used for the study and size range of amplicons generated from *S. album* DNA

Primer	Sequence(5´-3´)	Size range of amplicons
UBC-836	AGAGAGAGAGAGAGAGCA	250-1250
UBC-841	GAGAGAGAGAGAGAGATC	250-1250
UBC-807	AGAGAGAGAGAGAGAGT	350-1250

6.2.4. Data analysis

Amplified fragments were scored for presence (1) and absence (0) of bands. Data from intensely stained, unambiguous, clear bands were included in the analysis. The data matrices were entered into POPGENE version 1.31 software and genotype comparisons were made (Yeh *et al*., 1999). Percentage of polymorphic loci and gene diversity index (h) (Nei, 1973) was calculated using POPGENE version 1.31. Indices of genetic similarity were calculated using Dice genetic similarity coefficient (Dice, 1945) using NTSYSpc software in order to estimate the genetic relationship between trees. From the similarity matrix, a sequential, agglomerative, hierarchical, and nested (SAHN) cluster analysis was performed and dendrogram constructed using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) algorithm, computed using NTSYSpc (Numerical Taxonomy and Multivariate Analysis System for personal computers) software, version 2.1 (Rohlf, 2000).

6.3. RESULTS

The study was designed to determine the level of clonality and genetic diversity of sandal trees using ISSR analysis in two sample plots selected in the seed stand at Marayur. Three primers (viz. UBC-836, UBC-841 and UBC-807) were chosen out of 6 primers tested for maximum number of amplicons having clarity, sharpness and consistency. Leaf DNA samples of 17 sandal trees from each plot were screened for genetic variation using the three selected primers. The resulting ISSR products (Figs.6.3-6.8) were screened for presence or absence of bands. The molecular products ranged from 250-1250 base pairs. Assuming that each ISSR product represented a single locus, 11of the 25 loci were found to show polymorphism in plot I and 12 of 25 loci were found to show polymorphism in plot II. The per cent of polymorphism was 44 and 48 respectively in plot I and plot II. The estimation of gene diversity index (h) (Nei, 1973) showed that many of the loci were found to have similar values and the mean diversity for plot I was 0.1478 and for plot II it was 0.17.

A close look at the Dice similarity coefficient values showed that the values between pairs of trees in plot I ranged from 0.73 to 1 (Table 6.3). The similarity coefficients between tree numbers 2 and 3, 5 and 6 and 16 and 17 were one, indicating

that trees in each pairs were genotypically identical; in other words, they were clones. It could be possible that one of the two trees from each pair might have originated as root sucker from the root of the other tree. It was further observed that similarity coefficients between trees 2 and 5, 2 and 6, 2 and 7, 2 and 8, 3 and 5, 3 and 6, 3 and 8, 5 and 7, 5 and 8, 6 and 7, 6 and 8 and 7 and 8, were 0.95 or above, which revealed that trees of each pair were genetically almost identical. The dendrogram (Fig.6.9) further showed that the tree numbers 2, 3, 5, 6, 7 and 8 were genetically closely related each other by virtue of their grouping in one sub cluster at the similarity coefficient vale of 0.95. In the seed stand also, these trees were clustered together as evident from fig.6.2a. Besides those trees, tree numbers 9 and 10, and 14 and 15 also showed similar clustering both in the dendrogram (Fig.6.9) and in the seed stand (Fig.6.2a). In plot II, the Dice similarity coefficient values ranged from 0.78 to 1 (Table 6.4). Trees 14 and 16 showed complete genotypic identity (Dice similarity coefficient = 1). The similarity coefficient values for tree numbers, 1 and 11, 1 and 14, 1 and 16, 7 and 11, 7 and 14, 7 and 16, 11 and 14 and 11 and 16 were 0.95 or above indicating that the genotype of the paired trees were almost identical. The dendrogram (Fig.6.10) also showed that these five trees formed a small sub cluster. In the seed stand also these five trees were closely located forming cluster of trees as evident from the Figure 6.2b.

The dendrogram generated using the Dice similarity coefficients (NTSYSpc, Rohlf, 2000) revealed the genetic relatedness of the 17 *S. album* trees in plot I and II (Fig. 6.9 & Fig. 6.10). Half of the trees sampled showed high genetic similarity ($\geq$ 0.95) indicating the fact that they were either clones and probably had originated as root sucker or they were of highly inbred seed origin. The 17 genotypes of sandal trees from plot I (Fig. 6.9), first separated to two major clusters at genetic similarity coefficient value of 0.84. Cluster I consisted of twelve genotypes and rest of the genotypes were grouped in cluster II. The 17 genotypes of sandal trees from plot II (Fig. 6.10) also first separated into two clusters at genetic similarity coefficient value of 0.84. Sixteen genotypes were grouped into one large cluster while a lone tree, tree No. 8 formed the other cluster. The large cluster separated into two sub-clusters at Dice similarity coefficient value of 0.87. Both the sub-clusters consisted of eight trees.

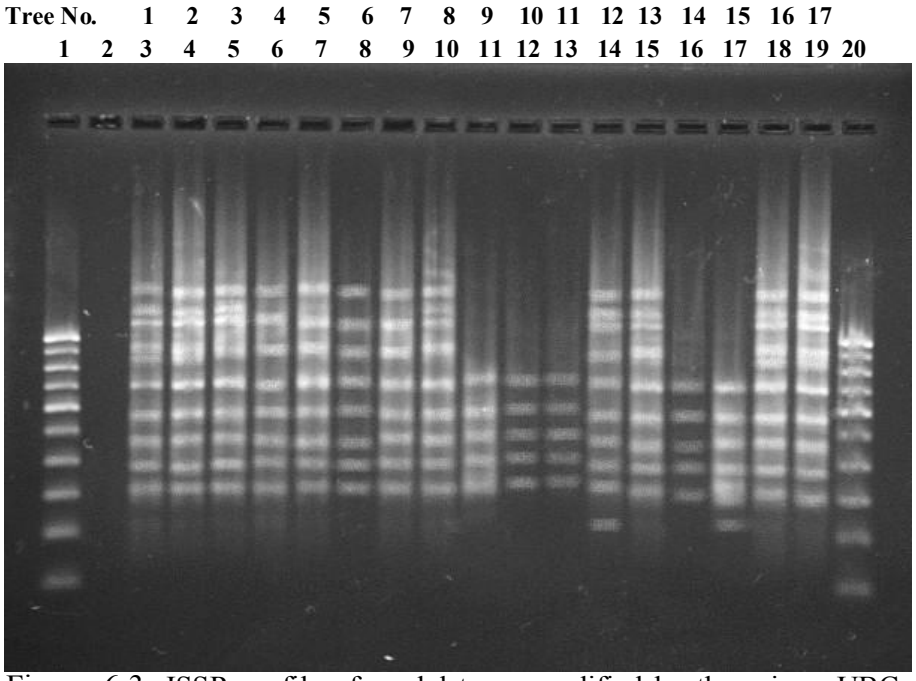


Figure 6.3: ISSR profile of sandal trees amplified by the primer UBC-836. Lane 1 and 20: DNA marker (100 bp ladder); lane 2: negative control; lanes 3-19: DNA profiles of 1-17 trees of plot I.

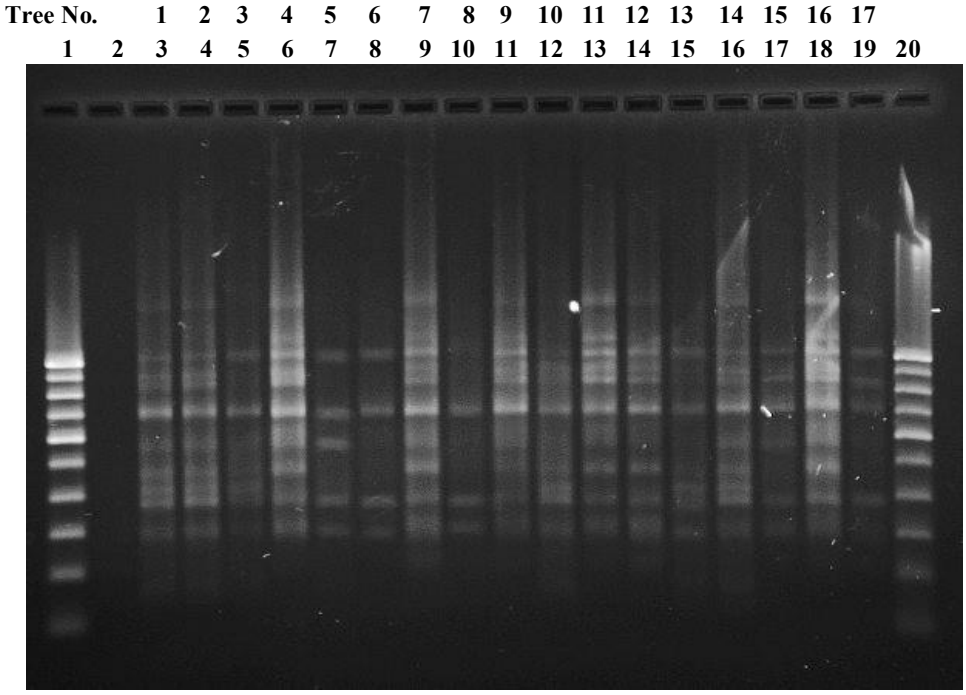


Figure 6.4: ISSR profile of sandal trees amplified by the primer UBC-836. Lane 1 and 20: DNA marker (100 bp ladder); lane 2: negative control; lanes 3-19: DNA profiles of 1-17 trees of plot II.

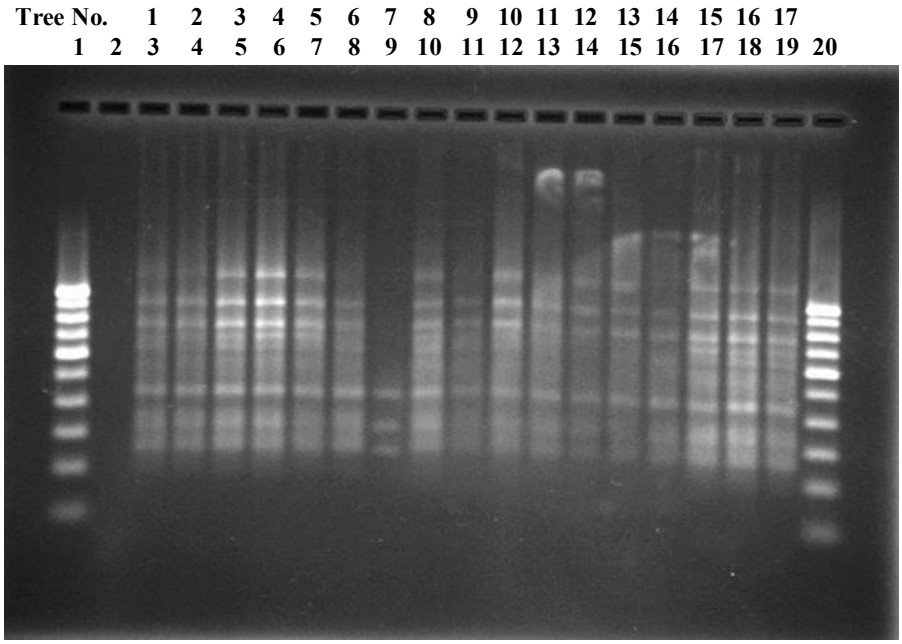


Figure 6.5: ISSR profile of sandal trees amplified by the primer UBC-841. Lane 1 and 20: DNA marker (100 bp ladder); lane 2: negative control; lanes 3-19: DNA profiles of 1-17 trees of plot I.

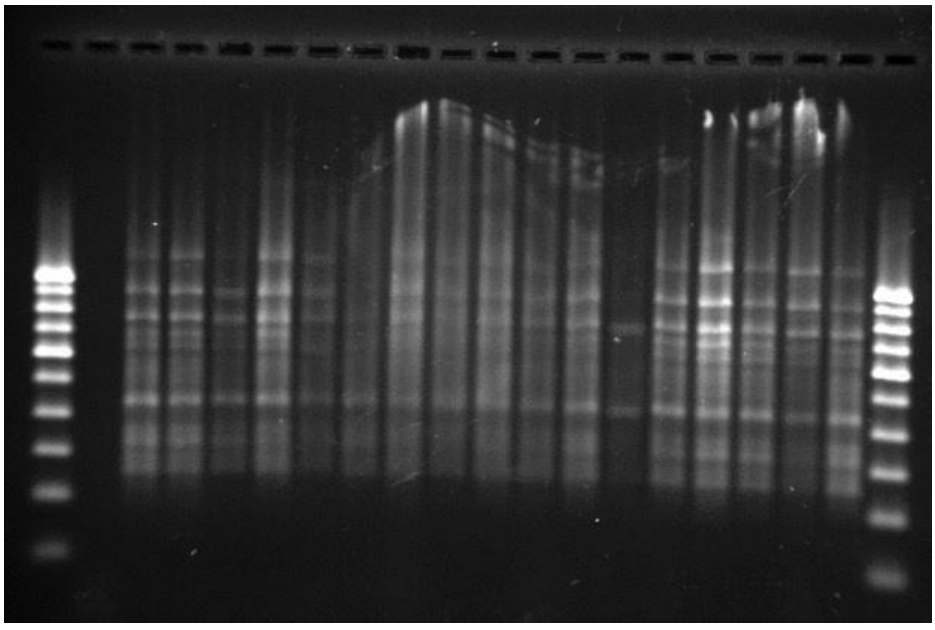


Figure 6.6: ISSR profile of sandal trees amplified by the primer UBC-841. Lane 1 and 20: DNA marker (100 bp ladder); lane 2: negative control; lanes 3-19: DNA profiles of 1-17 trees of plot II.

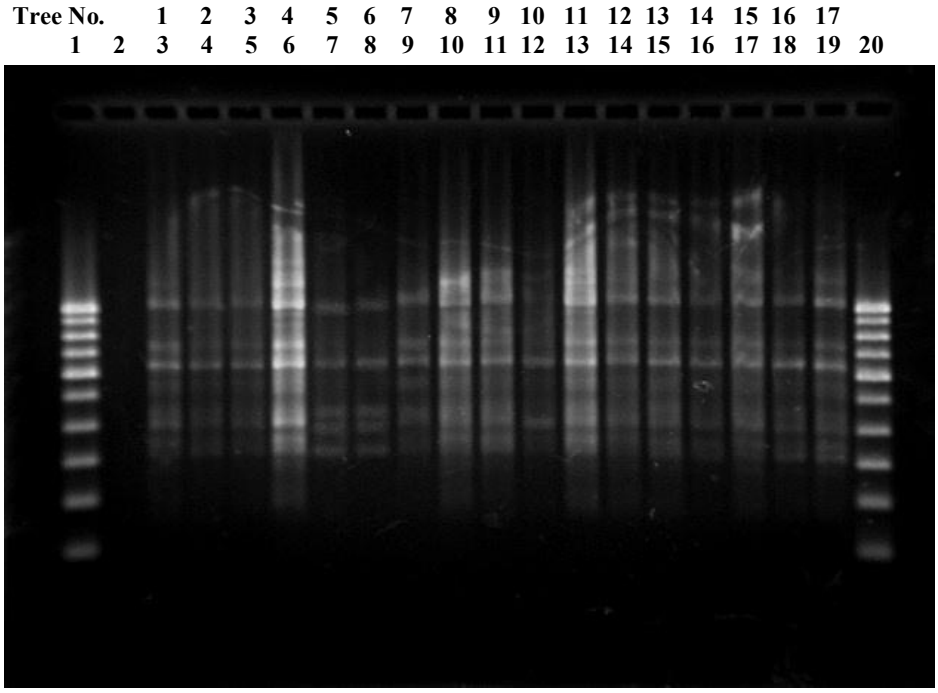


Figure 6.7: ISSR profile of sandal trees amplified by the primer UBC-807. Lane 1 and 20: DNA marker (100 bp ladder); lane 2: negative control; lanes 3-19: DNA profiles of 1-17 trees of plot I.

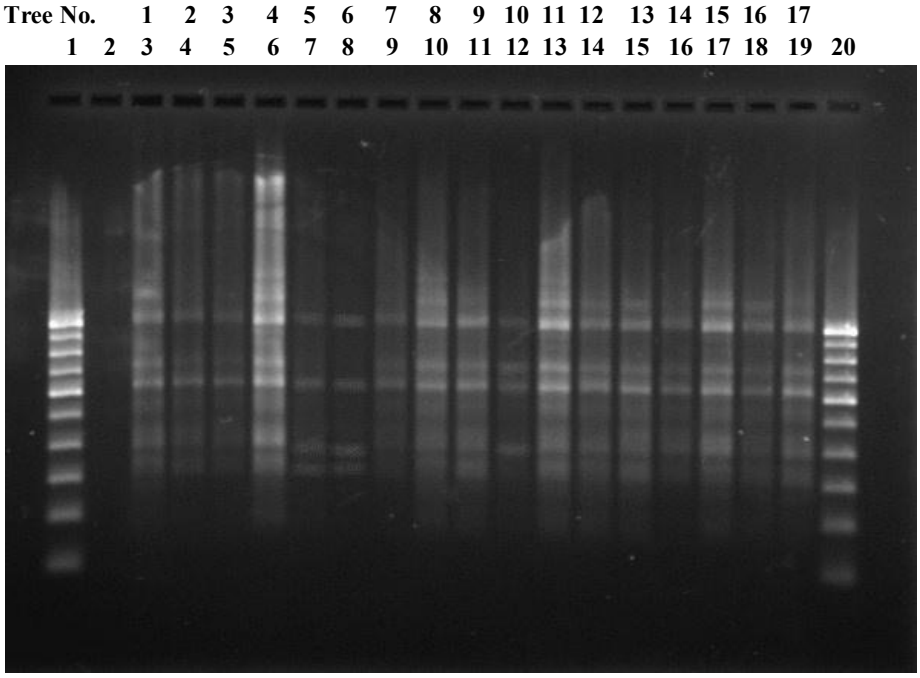


Figure 6.8: ISSR profile of sandal trees amplified by the primer UBC-807. Lane 1 and 20: DNA marker (100 bp ladder); lane 2: negative control; lanes 3-19: DNA profiles of 1-17 trees of plot II.

Table 6.3: Dice genetic similarity coefficients for 17 sandal trees in plot I

Tree No.		Tree Numbers															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1.00																
2	0.91	1.00															
3	0.91	1.00	1.00														
4	0.91	0.94	0.94	1.00													
5	0.89	0.97	0.97	0.92	1.00												
6	0.89	0.97	0.97	0.92	1.00	1.00											
7	0.86	0.95	0.94	0.94	0.97	0.97	1.00										
8	0.86	0.95	0.95	0.89	0.98	0.98	0.95	1.00									
9	0.87	0.85	0.85	0.77	0.82	0.82	0.79	0.80	1.00								
10	0.83	0.81	0.81	0.73	0.79	0.79	0.75	0.76	0.96	1.00							
11	0.80	0.85	0.85	0.84	0.82	0.82	0.84	0.80	0.93	0.80	1.00						
12	0.88	0.92	0.92	0.91	0.89	0.89	0.92	0.87	0.81	0.77	0.88	1.00					
13	0.91	0.95	0.95	0.89	0.97	0.97	0.95	0.95	0.85	0.81	0.84	0.92	1.00				
14	0.88	0.91	0.91	0.85	0.89	0.89	0.86	0.86	0.93	0.89	0.93	0.88	0.91	1.00			
15	0.85	0.89	0.89	0.82	0.86	0.86	0.83	0.84	0.90	0.87	0.90	0.91	0.89	0.97	1.00		
16	0.83	0.92	0.92	0.86	0.95	0.95	0.92	0.98	0.82	0.79	0.82	0.84	0.92	0.83	0.81	1.00	
17	0.83	0.92	0.92	0.86	0.95	0.95	0.92	0.98	0.82	0.79	0.82	0.84	0.92	0.83	0.81	1.00	1.00

Table 6.4. Dice genetic similarity coefficients for 17 sandal trees in plot II

Tree		Tree Numbers															
No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1.00																
2	0.93	1.00															
3	0.82	0.89	1.00														
4	0.90	0.82	0.81	1.00													
5	0.87	0.89	0.82	0.81	1.00												
6	0.84	0.86	0.91	0.78	0.91	1.00											
7	0.93	0.90	0.84	0.93	0.89	0.81	1.00										
8	0.84	0.86	0.85	0.83	0.85	0.81	0.81	1.00									
9	0.95	0.92	0.81	0.90	0.86	0.78	0.93	0.89	1.00								
10	0.87	0.94	0.88	0.81	0.88	0.85	0.89	0.85	0.92	1.00							
11	0.95	0.88	0.82	0.95	0.87	0.79	0.98	0.84	0.95	0.87	1.00						
12	0.90	0.82	0.81	0.90	0.81	0.78	0.93	0.78	0.90	0.86	0.95	1.00					
13	0.93	0.89	0.89	0.87	0.89	0.91	0.85	0.91	0.87	0.83	0.88	0.82	1.00				
14	0.98	0.90	0.85	0.93	0.85	0.82	0.95	0.82	0.93	0.85	0.98	0.93	0.90	1.00			
15	0.93	0.90	0.89	0.88	0.89	0.86	0.90	0.86	0.88	0.84	0.93	0.88	0.95	0.95	1.00		
16	0.98	0.90	0.85	0.93	0.85	0.82	0.95	0.82	0.93	0.85	0.98	0.93	0.90	1.00	0.95	1.00	
17	0.93	0.95	0.89	0.82	0.94	0.91	0.90	0.86	0.87	0.89	0.88	0.82	0.95	0.90	0.95	0.90	1.00

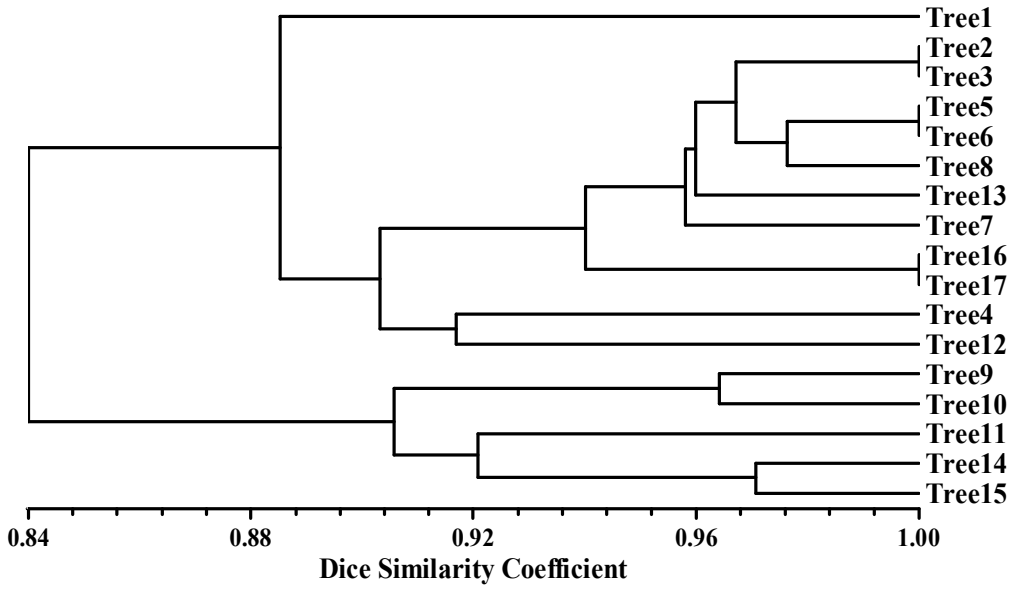


Figure 6.9. UPGMA dendrogram based on Dice genetic similarity coefficients of seventeen sandal trees of plot I

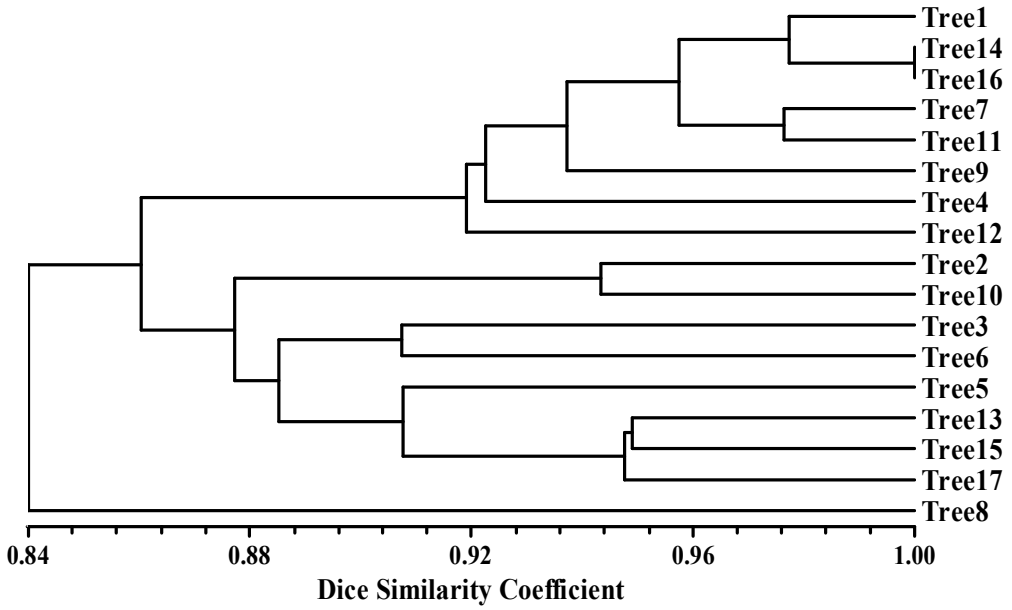


Figure 6.10: UPGMA dendrogram based on Dice genetic similarity coefficients of seventeen sandal trees of plot II

6.4. DISCUSSION

Sandal trees growing in Nachivayal sandal reserve, especially in seed stand at Anchalpatty were not producing sufficient seeds. Being a cross pollinated plant, pollination and fertilization between genetically similar trees may cause inbreeding, resulting in poor seed setting, failure to produce viable seeds or poor performance of seedlings from inbred seeds. In several places of Nachivayal seed stand of Marayur Forest Division, sandal trees were growing in clusters with individual trees of a cluster located very close to one another. Such prominent conglomeration of trees of different sizes prompted us to investigate whether the trees around a larger tree are clones which might have arisen as root suckers from the roots of the mother tree. In order to investigate the genetic similarity of the trees, two sample plots were selected at random in the seed stand, each of size 20m x 20m with 17 trees in each plot. The trees in the plots were subjected to DNA profiling using ISSR markers. The ISSR study showed complete identity of DNA profiles of 3 pairs of trees in plot I and one pair of tree in plot II. This indicated that individual trees of each pair could be clones which might have arisen as root suckers from either of the trees of each pair (Figs 6.3-6.10). Amplicons from such pairs of trees were monomorphic. Moreover, in plot I, three clusters of trees, one cluster comprising seven trees and the other two comprising two trees each showed similarity coefficients of more than 0.95 among individuals of each cluster indicating very high levels of genetic similarity. Similarly, in plot II, five trees (tree numbers 1, 14, 16, 7 and 11) which showed similarity coefficient values of 0.95 or above were among a cluster of trees. The percentage of polymorphism (P) was 44 in plot I and 48 in plot II. The mean genetic diversity (h) was 0.1478 in plot I and 0.17 in plot II. Low percentage of polymorphism and low genetic diversity indicated very high prevalence of inbreeding in the seed stand which could be the major reason for low production of seeds.

ISSR marker techniques have been widely applied to assess genetic diversity and clonality in several economically important crops and fruit plants as part of experiments connected with germplasm conservation since they provide a large amount of data within a short time. ISSR markers also provide a clear portrayal of relationships among closely related congeneric species (Gang *et al*., 2002). Hence, we opted for this method in order to assess the level of clonality of sandal trees in the sample plots at Marayur

seed stand. Furthermore, evidences have shown that ISSRs can generate even more information than RAPD markers, and, therefore, show great potential in studies on clonal diversity and genotype identification (Ang Li and Song Ge, 2001).

Similar low per cent level of polymorphic loci and gene diversity was observed in *Rhododendron fortunei* (P=48.23%, h=0.182) by Jin *et al*. (2006) and in studies on endangered species *Camellia nitidissima* Chi (P=42.44%, h= 0.098) (Bin *et al*., 2005), and at population level in the endangered species *Sinocalycanthus chinensis* (P=23.65%, h=0.084) (Jin and Li, 2007) and *Emmenopterys henryi* Oliv. (P=22.56%, h=0.071) (Li and Jin, 2008).

Inbreeding depression reduces fitness and vigor in terms of survival, growth and fertility through increased homozygosity of deleterious recessive alleles in a normally out breeding population. One of the reasons for poor seed germination and poor seedling recruitment observed in the experimental plot at Marayur might be due to inbreeding depression, since many of the trees have been observed to be clones. Pollen transfer and seed dispersal determine a plant's reproductive success, range expansion, and population genetic structure. In this case, *S. album,* being a cross-pollinated species might have failed to produce viable seeds due to cross-pollination between genetically similar trees or clones. ISSR marker technique is an ideal tool for genotype characterization and assessment of genetic similarity of clones and genetically closely related trees.

6.5. CONCLUSION

The ISSR marker study done in the Anchalpatty seed stand of Marayur Division has shown clonality and very high genetic similarity among the sandal trees. The clonality is presumed to be caused as a result of sandal regeneration through root suckers. Since natural regeneration through seedlings was poor in seed stand because of slow growth and very high mortality, forest department had been carrying out regeneration through root sucker induction for the last few decades. Even though, mortality due to drought occurred among the plantlets arising from root suckers also, this practice continued because root suckers were generally fast growing during the initial stages. Since root suckers arise through vegetative reproduction from root of a

mother tree, there will be complete genetic identity among such trees or in other words they are clones. In the present study, besides clonality, a large number of trees showed very high genetic similarity (>0.95) probably because of very high rate of inbreeding among the sandal trees in the Anchalpatty seed stand. Low percentage of polymorphism, low genetic diversity and clonality indicated very high chance of inbreeding which could be the major reason for poor seed production in the seed stand. Hence, the practice of root sucker induction by trenching around sandal trees in forests especially seed stands has to be discontinued because the technique increases clonality which in turn reduces genetic diversity in the seed stand.

CHAPTER 7

SUMMARY

7. SUMMARY

S. album L. is the source of highly priced and fragrant heartwood which on steam distillation yields on an average 5-7 per cent oil of high perfumery value. Global demand for sandalwood is about 5000-6000 tons/year and that of oil is 100 tons/year. Heartwood of sandal is estimated to fetch up to Rs. 3.7 million/ton and wood oil Rs.70,000-100,000/kg in the international market. Sandal heartwood prices have increased from Rs. 365/ton in 1900 to Rs. 6.5 lakhs/ton in 1999-2000 and to Rs. 37 lakhs/ton in 2007. Substantial decline in sandalwood production has occurred from 3176 tons/year during 1960-‘65 to 1500 tons/year in 1997-98, and to 500 tons/year in 2007.

Depletion of sandal resources is attributed to several factors, both natural and anthropogenic. Low seed setting, poor seed germination, seedling mortality, lack of haustorial connection with host plant roots, recurrent annual fires in natural sandal forests, lopping of trees for fodder, excessive grazing, hacking, encroachments, seedling diseases and spread of sandal spike disease are the major problems facing sandal. While these factors hinder sandal regeneration in forest areas, the situation is accelerated by human activities of chronic over-exploitation and illicit felling.

Deterioration of natural sandal populations due to illicit felling, encroachments and diseases has an adverse effect on genetic diversity of the species. The loss of genetic diversity has aggravated during recent years due to extensive logging, changing land-use patterns and poor natural regeneration. The consequent genetic erosion is of serious concern affecting tree improvement programme in sandal. Conservation as well as mass propagation are the two strategies to be given due importance. To initiate any conservation programme, precise knowledge of the factors influencing regeneration and survival of the species is essential. Hence, the present study was undertaken with the objective of investigating the autotrophic and parasitic phase of sandal seedlings growth, the effects of shade on morphology, chlorophyll concentration and chlorophyll fluorescence of sandal seedlings, genetic diversity in sandal seed stands using ISSR markers, and the diversity of fungal isolates causing sandal seedling wilt using RAPD markers. All these factors directly influence regeneration and survival of sandal seedlings in natural forests and plantations.

7.1. AUTOTROPHIC AND PARASITIC PHASE OF SANDAL SEEDLING GROWTH

Knowledge of autotrophic phase of nutrition is important in sandal nursery management as seedlings grown by sowing seeds in nutrient deficient medium needs to be pricked out before seed reserves are fully exhausted. The objective of our study was to evaluate the seed reserve (cotyledonary) dependency of *S. album* seedlings. Sandal seedlings emerging out of seeds with cotyledons intact were pricked out to root trainers of 150cc containing sterile quartz sand, vermiculite and soil. Conditions of cotyledons, leaves and seedlings as a whole were noted daily for 230 days. Complete withering of cotyledons in quartz sand occurred within 26 days of radicle emergence. Hence, it was concluded that sandal seedlings need to be transferred to polybags containing potting mixture within 26 days after germination for proper growth and development.

Though, many studies had been conducted earlier on host association of sandal and its growth enhancement, there are several plants which have not been reported to be haustorised by sandal. Moreover, no report is available describing physiological basis of growth enhancement. In view of these facts, an experiment was conducted with selected annuals and perennials to examine the host preferences of sandal seedlings under nursery conditions through physiological measurements and chlorophyll estimation of sandal leaves. Four-month-old sandal seedlings were planted with seedlings of host plants, and growth of sandal seedlings with hosts was monitored for a year for annuals and about two years for perennials.

Eight annual and two perennial host species were selected based on screening for better initial sandal seedling growth. Annual host species were *Vigna unguiculata* (L.) Walp. (Fabaceae), *Ocimum sanctum* L. (Verbenaceae), *Ageratum conyzoides* L. (Asteraceae), *Phyllanthus niruri* L. (Euphorbiaceae), *Mikania micrantha* Kunth ex. H. B. K. (Asteraceae), *Ludwigia hyssopifolia* (G Don) Exell (Onagraceae), *Scoparia dulcis* L. (Scrophulariaceae) and *Vernonia cinerea* (L.) Lem. (Asteraceae) and perennial host species were *Pongamia pinnata* (L.) Pierre (Fabaceae) and *Lantana camara* L. (Verbenaceae). At the end of the experiment, measurements of height, collar diameter, total biomass, shoot and root fresh weight and dry weight, number of haustorial connections, chlorophyll fluorescence and chlorophyll contents were recorded. The quality of the seedlings was determined using Sturdiness Quotient (SQ), Dickson Quality Index (DQI) and Performance Index (PI) using JIP test. Chlorophyll

fluorescence was measured at regular intervals on a subsample of seedlings with a Plant Efficiency Analyzer (Handy PEA, Hansatech Instruments Ltd., Norfolk, UK). Data were analysed using one way ANOVA.

All the morphological parameters were found to be significantly different for sandal plants grown with different hosts. Performance Index values were higher for sandal seedlings growing with *O. sanctum* (61.65) and *V. unguiculata* (27.49). DQI values, F_v/F_m values and chlorophyll content for sandal were higher when *V. unguiculata* was the host plant than for sandal with *O. sanctum*. Moreover, since *V. unguiculata,* a leguminous annual, can grow under shade along with sandal seedlings, *V. unguiculata* was chosen to be a better primary host for sandal. Among the perennial host species, sandal plants with *P. pinnata* showed better performance for height, collar diameter, total fresh weight, total dry weight, DQI, PI, F_v/F_m values, chlorophyll content and number of haustoria. *P. pinnata* was found to be the best long term host, being a perennial tree species.

7.2. EFFECTS OF SHADE ON MORPHOLOGY, CHLOROPHYLL CONCENTRATION AND CHLOROPHYLL FLUORESCENCE OF SANDAL SEEDLINGS

To evaluate the influence of shade on morphology, chlorophyll concentration, and chlorophyll fluorescence, sandal seedlings were grown under full sunlight and under shade nets that provided approximately 25 per cent, 50 per cent and 75 per cent shade. At the end of the study, each seedling was harvested and height, number of leaves, leaf area, collar diameter, total biomass, shoot biomass and root biomass were measured. The quality of the seedlings was determined using SQ, DQI and PI. Chlorophyll content was measured on leaves from each treatment and the data were analysed using one way ANOVA.

The results indicated that sandal seedlings performed well under 75 per cent shade upto one year. Total chlorophyll concentrations were found to be higher in 50 per cent shade. F_v/F_m ratio ranged between 0.34 and 0.67. F_v/F_m values were almost similar under 50 per cent and 75 per cent shade. Lower F_v/F_m values were observed for sandal seedlings grown under full sunlight. Performance Index (PI) value was greater for sandal seedlings under 75 per cent shade (2.6) in comparison with other treatments.

Sandal seedlings are sciophilic in the early phase becoming more heliophilic once established. Data from our studies indicated that 50 - 75 per cent shade is optimal for

growth of sandal seedlings up to one year after germination. The measurements were conducted for six months starting from two months after germination. Exposure to full sunlight proved to be detrimental to sandal seedlings. Sandal seedlings exposed to sunlight exhibited extreme stress symptoms such as chlorosis and wilting, and 100 per cent mortality was recorded within six months of the start of the experiment.

7.3. GENETIC DIVERSITY OF *FUSARIUM OXYSPORUM* Schlechtend Fr ISOLATES CAUSING SANDAL SEEDLING WILT

Fusarium oxysporum Schlechtend Fr is the most common and most virulent fungal pathogen infecting sandal seedlings causing pre-emergence decay, vascular wilt, root rot and wilting of older seedlings. The objective of our study was to evaluate the degree of genetic variation in six different cultures of *F. oxysporum* isolated from wilt-affected sandal seedlings of sandal nursery located at Marayur in Idukki district and Peechi in Thrissur district of Kerala state. *F. oxysporum* isolates were obtained from infected root, stem and collar region from germinating seedlings to 3-month-old seedlings collected from sandal nursery at KFRI, Peechi, Thrissur campus, and from sandal nursery and sandal regeneration experimental plot in Nachivayal II sandal reserve forest in Marayur Sandal Division, Idukki District. Out of 102 isolates obtained from the two different localities, three isolates were randomly selected from Peechi isolates (*Fo*.P1, *F*o.P2 and *Fo*.P3) based on cultural variations and three from Marayur isolates (*Fo*.M1, *Fo*.M2 and *Fo*.M3) for genetic diversity studies using RAPD markers.

Genomic DNA was extracted from mycelia following slightly modified method of Doyle and Doyle (1990). Seven primers from OPB series viz. OPB 1, OPB 4, OPB 7, OPB 10, OPB 11, OPB 14, OPB 15 and two primers from OPD series viz. OPD 18 and OPD 19 were chosen based on the number and reproducibility of amplified fragments out of 20 primers tested. RAPD analysis showed that 98.39 per cent of the loci were polymorphic (122 out of 124). The gene diversity indices (h) showed that a few of the loci were genetically identical; for the rest of the loci, gene diversity ranged from 0.27 to 0.50 and the mean gene diversity was 0.37. Genetic distance coefficients showed that, *F. oxysporum* isolate *Fo*.M2 and *Fo*.M3 from Marayur were the genetically nearer ones (D=0.2985) and isolate *Fo*.P1 from KFRI nursery and isolate *Fo*.M2 from Marayur were the genetically distant ones (D=0.9701). The UPGMA dendrogram revealed the

genetic relatedness of the six isolates of *F. oxysporum*. The gene diversity, genetic distance coefficients between pair of isolates and dendrogram obtained from cluster analysis (UPGMA dendrogram) revealed a great deal of heterogeneity among the isolates.

The most genetically similar ones were collected from KFRI nursery while the genetically distant ones were collected from KFRI nursery and Marayur. The two locations are geographically far apart, a distance of approximately 175 km. Generally, organisms originating from same geographical area show genetic similarity. In the present study *F. oxysporum* isolates from Marayur have shown genetic similarity among themselves (lesser genetic distance) and they have aligned into one cluster. But, instead of clustering together all the three isolates from Peechi nursery, one of them *Fo*.P3 clustered with the Marayur isolates. For raising seedlings at Peechi, seeds were brought from Marayur. Hence, the isolate *Fo*.P3 might have reached Peechi as a seed contaminant while collecting fallen seeds from forest floor at Marayur.

By using RAPD technique, it was revealed that there existed a wide range of genetic variation among the *F. oxysporum* genotypes. The large genetic variation detected at the DNA level indicated the ability of the pathogen to adapt to different eco-climatic conditions. The pathogen would be highly successful in infecting sandal seedlings at different stages of the seedlings as revealed by several studies. Hence, prophylactic treatment of sandal seeds, preferably with a systemic fungicide is suggested.

7.4. STUDIES ON GENETIC DIVERSITY OF SANDAL IN SEED STAND

S. album is a 'vulnerable' species and hence it is of paramount importance to evaluate and maintain genetic variability within and among populations because populations consisting of genetically uniform individuals will be vulnerable to major climatic changes, and pest and disease outbreaks. One of the reasons for poor sandal regeneration in natural forests is high seedling mortality. This demands large quantity of seeds for sowing in new localities as well as in sandal forests where practically very little regeneration takes place. Recently it has been reported that expected quantity of seeds are unavailable from the seed stands established during 1980-81 at Anchalpatty in Nachivayal reserve, Marayur Sandal Division in Idukki District, Kerala State. Most of the sandal trees flower profusely, but they do not set seeds. Sandal being a cross pollinated plant, fruit setting and seed formation takes place usually if pollination and

fertilization occurs between genetically unrelated genotypes. Though, failure to develop mature seeds may occur due to unsuccessful pollination and fertilization, pathogenic infection of developing fruits, premature flower and fruit fall, etc., such major problems did not come to our notice. Hence, we assessed the level of clonality and genetic diversity of sandal trees in sample plots from seed stand at Marayur using ISSR markers. Two plots, each of 20m x 20m size with 17 trees in each plot were randomly selected for the study.

Three primers (viz. UBC-836, UBC-841 and UBC-807) were chosen out of 6 primers tested for maximum number of amplicons having clarity, sharpness and consistency. Leaf DNA samples of the 17 sandal trees from each plot were screened for genetic variation using the three selected primers. The resulting PCR amplified products were screened for presence or absence of bands. The per cent of polymorphism was 44 and 48 respectively in plot I and plot II. The mean gene diversity index values were 0.1478 for plot I and 0.17 for plot II which were poor for a cross pollinated tree such as sandal.

Dice similarity coefficient values calculated using the software NTSYSpc ver 2.1 ranged from 0.73 to 1 in plot I and 0.78 to 1 in plot II. Half of the total number of trees sampled showed very high genetic similarity (0.95-1) among each pair of trees. This suggested that one of the two trees from each pair might have originated as root sucker from the other. The dendrogram generated using the Dice similarity coefficients also revealed the genetic relatedness of the 17 *S. album* trees within in each plot. The 17 genotypes of sandal trees from plot I first separated to two major clusters at the genetic similarity coefficient value of 0.84. Cluster I consisted of twelve genotypes and rest of the genotypes were grouped in cluster II. The 17 genotypes of sandal trees from plot II first separated into a large cluster comprising 16 genotypes and another one with only one genotype at the genetic similarity coefficient value of 0.84. The large cluster consisted of two subclusters, each with eight genotypes.

7.5. CONCLUSIONS

It can be concluded from the present study that sandal seedlings need to be transferred to polybags containing a potting mixture within 26 days after germination for proper growth and development. Host plants need to be provided within 3-4 months after transfer of sandal seedlings to polybags. Plantable quality of seedling can be produced in

polybags by providing *V. unguiculata* as the primary (pot) host. On field transplantation *Pongamia pinnata* can be supplied as the long term host. This protocol is useful for raising sandal seedlings under nursery conditions and subsequent field transplantation.

Sandal seedlings are sciophilic in the early phase, becoming more heliophilic once established. Data from studies on the effect of shade on morphology, chlorophyll concentration and chlorophyll fluorescence of sandal seedlings indicated that 50-75 per cent shade is optimal for growth of sandal seedlings up to one year. Results indicated that the photosynthetic activity of sandal seedlings would be limited when grown in environments with high light intensity due to photoinhibition. Extremes of light intensity can cause mortality of the seedlings as observed in the present study.

Fusarium wilt causes heavy seedling mortality in sandal seedling nurseries and natural forests. Information on the biology and genetic diversity of the pathogen is extremely important, as the pathogen possessed a wide host range. By using RAPD technique, it was revealed that there existed a wide range of genetic variation among the *F. oxysporum* genotypes. The large genetic variation detected at the DNA level indicated the ability of the pathogen to adapt to different eco-climatic conditions. Since, the pathogen was prevalent in the soils of Marayur, a major source of good quality sandal seeds, the seeds originating from Marayur could be contaminated with the fungus. Hence, a prophylactic treatment of sandal seeds, preferably with a systemic fungicide is very important. Since the infected plant usually perishes, prophylactic fungicidal treatment has to be given at various stages of seedling growth.

Sandal trees growing in Nachivayal sandal reserve, especially in seed stand were not producing expected quantity of seeds. Being a cross pollinated plant, pollination and fertilization between genetically similar trees may cause inbreeding resulting in failure to produce viable seeds or poor performance of inbred seeds. The ISSR marker study showed identity of DNA profiles of sandal trees indicating that such trees could be clones which might have arisen from root suckers. Low percentage of polymorphism and low genetic diversity showed very high chance of inbreeding in the seed stand which could be the major reason for poor seed production in the seed stands. Hence, the practice of root sucker induction by trenching around sandal trees in forests especially seed stands has to be discontinued because the technique increases clonality which in turn reduces genetic diversity in the seed stand.

REFERENCES

REFERENCES

Adler, L. C. 2000. Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. *American Naturalist*. **156**: 92-99.

Alfonso, C., Raposo, R. and Melgarejo, P. 2000. Genetic diversity in *Botrytis cinerea* populations on vegetable crops in greenhouse in South-eastern Spain. *Plant Pathology*. **49**: 243-251.

Alves Santos, F. M., Cordeiro-Rodrigues, L., Sayagues, J. M., Martin-Dominguez, R., Garcia-Benavides, P., Crespo, M. C. and Eslava, A. P. 2002. Pathogenicity and race characterization of *Fusarium oxysporum* f. sp. *phaseoli* isolates from Spain and Greece. *Plant Pathologist*. **51**: 605-611.

Amy. J. N., Karol. S. E., Arevalo, E. G., Lee. C. D. and Bryan. A. B. 1997. Genetic characterization by RAPD analysis of isolates of *Fusarium oxysporum* f. sp. *erythroxyli* associated with an emerging epidemic in Peru. *Phytopathology*. **87**: 1220-1225.

Ananthapadmanabha, H. S. 2000. Sandalwood and its marketing trend. *My Forest*. **36**: 147-151.

Ananthapadmanabha, H. S., Nagaveni, H. C., and Rai, S. N. 1988. Influence of host plants in growth of sandal. *My Forest*. **24**: 154-160.

Ananthapadmanabha, H. S., Rangaswamy, C. R., Sarma, C. R., Nagaveni, H. C., Jain, S. H., and Krishnappa, H. P.1984. Host requirement of sandal. *Indian Forester*. **84**: 264-268.

Ang Li and Song Ge. 2001. Genetic variation and clonal diversity of *Psammochloa villosa* (Poaceae) detected by ISSR markers. *Annals of Botany*. **87**: 585-590.

Angadi, V. G. S., Jain, S. H. and Shankaranarayana, K. H. 2003. Genetic diversity between sandal populations of different provenances in India. *Sandalwood Research Newsletter*. **18**: 4-5.

Annapurna, D., Rathore, T. S. and Geetha. J. 2006. Modern nursery practices in the production of quality seedlings of Indian sandalwood (*Santalum album* L.) - stage of host requirement and screening of primary host species. *Journal of Sustainable Forestry*. **22**: 33-55.

Annapurna, D., Rathore, T. S. and Geeta. J. 2007. Integrated nursery practices for mass production of quality planting stock of *Santalum album* L. In Proceedings of the National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp. 67-73.

Ansari, S. A., Narayanan, C. and Kumar, P. 2007. Molecular marker analysis of sandalwood. In Proceedings of the National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp 78-81.

Applegate, G. B., Chamberlain, J., Dahuri, G., Feigelson, J. L., Hamilton, L., Mc Kinnell, F. H., Neil, P. E., Rai, S. N., Rodehn, B., Statham, P. C. and Stemmermann, L. 1990. Sandalwood in the Pacific. A state of the knowledge synthesis and summary from the April 1990 symposium. In Proceedings of the symposium on Sandalwood in the Pacific, 9-11 April, 1990, Honolulu, Hawaii. USDA Forest Service Tech. Rep. PSW 122. pp 1-11.

Aradhya, A. M. and Philips, V. D. 1993. Genetic variability in fourteen provenances of Eucalyptus species in Hawaii. *Silvae Genetica*. **42**: 9-15.

Arnau, G., Lallemand, J. and Bourgoiu, M. 2003. Fast and reliable strawberry cultivar identification using inter simple sequence repeat (ISSR) amplification. *Euphytica*. **129**: 69-79.

Arnau, J., Housego, A. P. and Oliver, R. P. 1994. The use of RAPD markers in the genetic analysis of the plant pathogenic fungus *Cladosporium fulvum*. *Current Genetics*. **25**: 438-444.

Assigbetse, K. B., Fernandez, D., Dubois, M. P. and Geigern, J. P. 1994. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. *Phytopathology*. **84**: 622-626.

Bagchi, J. K. and Veerendra, H. C. S. 1985. Study on intra-tree and inter-tree variations in leaves of *Santalum album*. *My Forest*. **21**: 33-39.

Bahuguna, A. 2007. Conservation genetics: A management tool. *Annals of Forestry*. **15**: 159-200.

Bahulikar, R. A., Lagu, M. D., Kulkarni, B. G., Pandit, S. S., Suresh, H. S, Rao, M. K. V., Ranjekar, P. K. and Gupta, V. S. 2004. Genetic diversity among spatially isolated populations of *Eurya nitida* Korth. (Theaceae) based on Inter-Simple Sequence Repeats. *Current Science*. **86**: 824-831.

Balasimha, D and Namboothiri, C. G. N. 1996. Chlorophyll fluorescence of Cocoa in relation to drought tolerance. *Plant Physiology and Biochemistry*. **23**: 64-66.

Balasundaran, M. 1998. A method for clonal propagation of sandal. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No. 84. ACIAR, Canberra, Australia. pp. 126-129.

Balmas, V. S. B., Primo, P., Di Rau, D., Marcello, A. and Migheli, Q. 2005. Molecular characterization of vegetative compatibility groups in *Fusarium oxysporum* f. sp. *radicis-lycopersici* and f.sp. *lycopersici* by random amplification of polymorphic DNA and microsatellite-primed PCR. *European Journal of Plant Pathology*. **111**: 1-8.

Bapat, V. A. and Rao, P. S. 1998. Biotechnological approaches for propagation of Sandal (*Santalum album* L.). In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 42-44.

Bapat, V. A. and Rao, P. S. 1999. Somatic embryogenesis in *Santalum album* L. *Sandalwood Research Newsletter*. **8**: 4.

Bapat, V. A., Fulzele, D. P., Heble, M. R. and Rao. P. S. 1990. Production of sandalwood somatic embryos in bioreactors. *Current Science*. **59**: 746-748.

Barrett, D. R. and Fox, J. E. D. 1995. A protocol suitable for raising seedlings of *S. album* in Timor. In *Sandalwood seed nursery and Plantation Technology*. Edited by L. Gyerum, J. E. D. Fox, L. Ehrhart. Field Document. FAO, Suva, Fiji. pp. 163-164

Barrett, D. R. and Fox, J. E. D. 1997. *Santalum album*: Kernel composition, morphological and nutrient characteristics of pre-parasitic seedlings under various nutrient regimes. *Annals of Botany*. **79**: 59-66.

*Bateson, W. and Punnett, R. C. 1905. Experimental studies in the physiology of heredity. In *Reports in the Evolution Committee of the Royal Society*. Harrison and Sons. London. UK. **2**: 1-131.

Bawa, K. and Dayanandan, S. 1998. Global climatic change and tropical forest genetic resources. *Climatic change*. **39**: 373-385.

Belabid, L., Baum, M., Fortas, Z., Bouznad, Z. and Eujayi. I. 2004. Pathogenic and genetic characterization of Algerian isolates of *Fusarium oxysporum* f. sp. *lentis* by RAPD and AFLP analysis. *African Journal of Biotechnology*. **3**: 25-31.

Bidochka, M. J., Mc Donald, M. A., St. Legu, R. J. and Roberts, D.W. 1994. Differentiation of species and strains of entomopathogenic fungi by Random Amplification of Polymorphic DNA (RAPD). *Current Genetics*. **25**: 107-113.

Bin, X. Y., Tang, S. Q. and Zhou, J. Y. 2005. ISSR analysis on genetic diversity of *Camellia nitidissima* Chi (Theaceae) in China. *Journal Wuhan Botanical Research*. **23**: 20-26.

Binder, W. D. and Fielder, P. 1996. Chlorophyll fluorescence as an indicator of forest hardiness in white spruce seedlings from different latitudes. *New Forests*. **11**: 233-253.

Binelli, G. and Bucci, G. 1994. A genetic linkage map of *Picea abies* KARST, based on RAPD markers, as a tool in population genetics. *Theoretical and Applied Genetics*. **88**: 283-288.

Blair, M., Panaud, O. and Mc Couch, S. R. 1999. Inter-simple sequenece repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*. **98**: 780-792.

Booth, C. 1971. *The Genus Fusarium*. Commonwealth Mycological Institute, Kew, UK.

Bornet, B. C., Muller, F. P. and Branchard, M. 2002a. Highly informative nature of inter simple sequence repeat (ISSR) sequences amplified using tri- and tetra-nucleotide primers from DNA of cauliflower (*Brassica oleraceae* var 'botrytus' L). *Genome*. **45**: 890-896.

Bornet, B., Goraguer, F., Joly, G. and Branchard, M. 2002b. Genetic diversity in European and Argentinian cultivated potatoes (*Solanum tuberosum* subsp. *tuberosum*) detected by inter-simple sequence repeats (ISSRs). *Genome*. **45**: 481-484.

Bradshaw, H. D. JR., Villar, M., Watson, B. D., Otto, K. G., Stewart, S. and Stettler, R. F. 1994. Molecular genetics of growth and development of *Populus*. A genetic linkage map of hybrid poplar composed of RFLP, STS and RAPD markers. *Theoretical and Applied Genetics*. **89**: 167-178.

Brand, J. E. 1994. Genotypic variation in *Santalum album*. *Sandalwood Research Newsletter*. **2**: 2-4.

Brookes, P. D., Wingston, D. L. and Bourne, W. F. 1980. The dependence of *Quercus robur* and *Q. petraea* seedlings on cotyledon potassium, magnesium, calcium and phosphorus during the first year of growth. *Forestry*. **53**: 167-177.

Brown, A. H. D. 1978. Isozymes, plant population genetic structure and genetic conservation. *Theoretical and applied Genetics*. **52**: 145-157.

Brown, P. T. H. 1989. DNA methylation in plant and its role in tissue culture. *Genome*. **31**: 717-729.

Bule, L. and Daruhi, G. 1990. Status of the sandalwood resources in Vanuatu. In *Proceedings of a symposium on Sandalwood in the Pacific*. Edited by L. Hamilton, and C. E. Conrad. Honolulu, Hawaii. Pacific Southwest Research Station, USDA Forest Service, Berkeley. pp. 46-60.

Byers, D. L. 1995. Pollen quantity and quality as explanations for low seed set in small populations exemplified by Eupatorium (Asteraceae). *American Journal of Botany*. **82**: 1000-1006.

Camacho, F. J. and Liston, A. 2001. Population structure and genetic diversity of *Botrychium pumicola* (Ophioglossaceae) based on Inter-simple sequence repeats (ISSR). *American Journal of Botany*. **88**: 1065-1070.

Cameron, D. D. 2004. Role for differential host resistance to the hemiparasitic angiosperm, *Rhinanthus minor* (L.) in determining the structure of host plant communities. Ph.D. thesis. University of Aberdeen, U.K.

Castiglione, S., Wang, G., Damiani, G., Bandi, C., Bisoffi, S. and Sala, F. 1993. RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. *Theoretical and Applied Genetics*. **87**: 54-59.

Cekic, C., Battey, N. H. and Wilkinson, M. J. 2001. The potential of ISSR-PCR primer pair combinations for genetic linkage analysis using the seasonal flowering locus in *Fragaria* as a model. *Theoretical and Applied Genetics*. **103**: 540-546.

Cervera, M. T., Cabezas, J. A., Sancha, J. C., Martinez-de-Toda, F. and Martinez-Zapater, J. M. 1998. Application of AFLPs to the characterization of grapevine *Vitis vinifera* L. genetic resources: A case study with accessions from Rioja (Spain). *Theoretical and Applied Genetics*. **97**: 51-59.

Chacko, K. C., Pandalai, R. C., Balasundaran, M., Mohanan, C., Varma, R. V., Jose Kallarackal., Sujatha, M. P. and Kumaraswamy, S. 2002. Standardization of root trainer technology for *Tectona grandis*, *Eucalyptus tereticornis*, *E. grandis*, *E. globulus*, *E. camaldulensis*, *Acacia auriculiformis*, *A. mangium* and *Paraserianthes falcataria*. KFRI Research Report No. 229. Kerala Forest Research Institute, Peechi, Thrissur. pp. 97-128.

Chalmers, K. J., Newton, A. C., Waugh, R., Wilson, J. and Powell, W. 1994. Evaluation of the extent of genetic variation in mahoganies (Meliaceae) using RAPD markers. *Theoretical and Applied Genetics*. **89**: 504-508.

Chan, K. F. and Sun, M. 1997. Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*. *Theoretical and Applied Genetics*. **95**: 865-873.

Charters, Y. M. and Wilkinson, M. J. 2000. The use of self-pollinated progenies as 'in-groups' for the genetic characterization of cocoa germplasm. *Theoretical and Applied Genetics*. **100**: 160-166.

Chen, X., Line, R. F. and Leung, H. 1993. Relationship between virulence, variation and DNA polymorphism in *Puccinia striiformis*. *Phytopathology*. **83**: 1489-1497.

Cindy, L. W., Elizabeth. A. J., Yvonne. J. F., Stephen. J. T. and Helen. M. W. 2000. Clonality and sexual reproductive failure in remnant populations of *Santalum lanceolatum* (Santalaceae). *Biological Conservation*. **96**: 45-54.

Critchley, C. 1998. Photoinhibition. In *Photosynthesis : A comprehensive treatise*. Edited by A. S. Raghavendra. Cambridge University Press. Cambridge, United Kingdom. pp. 264-272.

Cropper, S. C. 1993. Management of Endangered Plants. CSIRO Publications. Melbourne.

Crowhurst, R. N., Hawthorne, E. H., Rikkerink, E. H. A. and Templeton, M. D. 1991. Differentiation of *Fusarium solani* f. sp. *cucurbitae* races 1 and 2 by random amplification of polymorphic DNA. *Current Genetics*. **20**: 391-396.

Culley, T. M. and Wolfe, A. D. 2000. Population genetic structure of the cleistogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity*. **86**: 545-556.

Danilova, T. V. and Karlov, G. I. 2006. Applications of Inter Simple Sequence Repeat (ISSR) polymorphism for detection of sex-specific molecular markers in hop (*Humulus lupulus* L.). *Euphytica*. **151**: 15-21.

Demmig, A. B., Adams, W. W., III. and Grace, S. C. 1997. Physiology of light tolerance in plants. *Horticultural Review*. **18**: 215-246.

Desjardins, A. E., Plattner, R. D. and Proctor, R. H. 1996. Linkage among genes responsible for fuminosin biosynthesis in *Giberrella fujikuroi* mating population. *Applied and Environmental Microbiology*. **62**: 2571-2576.

Dey, S. 2001. Mass cloning of *Santalum album* L. through somatic embryogenesis: scale up in bioreactor. *Sandalwood Research Newsletter*. **13**: 1-3.

*Dice, L. R. 1945. Measures of the amount of ecological association between species. *Ecology*. **26**: 297-302.

*Dickson, A., Leaf, A. L. and Hosner, J. F. 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. *Forest Chronicle*. **36**: 10-13.

Dje, Y., Tahi, G. C., Zoro, B. I. A., Malice, M., Baudoin, J. P. and Bertin. P. 2006. Optimization of ISSR marker for African edible-seeded Cucurbitaceae species' genetic diversity analysis. *African Journal of Biotechnology*. **5**: 83-87.

Doyle, J. J. and Doyle, J. L. 1990. Isolation of DNA from fresh plant tissue. *Focus*. **12**: 13-15.

Duncan, S., Barton, J. E. and O'Brieu, P. A. 1993. Analysis of variation in isolates of *Rhizoctonia solani* by random amplified polymorphic DNA assay. *Mycological Research*. **97**: 1075-1082.

Ernst, W. H. O. 1988. Seed and Seedling ecology of *Bachystegia spiciformis*, a predominant tree component in Miombo woodlands in South Central Africa. *Forest Ecology and Management*. **25**: 195-210.

Esselman, E, J., Jianqiang, L., Crawford, D, J., Winduss, J, L. and Wolfe, A, D. 1999. Clonal diversity in the rare *Calamogrostis porteri* ssp. *Insperata* (Poaceae): Comparitive results for allozymes and random amplified polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers. *Molecular Ecology*. **8**: 443-451.

Fabre, J. V., Julien, J., Parisof, D. and Dron, M. 1995. Analysis of diverse isolates of *Colletotrichum lindemuthianum* infecting common bean using molecular markers. *Mycological Research*. **99**: 429-435.

Fang, D. Q. and Roose, M. L. 1997. Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theoretical and Applied Genetics*. **95**: 408-417.

Fang, D, Q., Roose, M, L., Kiueger R, R. and Federici, C, T. 1997. Fingerprinting trifoliate orange germplasm accession with accession with isozymes, RFLP's and inter-simple sequence repeat markers. *Theoretical and Applied Genetics*. **95**: 211-219.

Farnian, M. L. and Leong, S. A. 1995. Genetic and physical mapping of telomeres in the rice blast fungus, *Magnaporthe grisea*. *Genetics*. **140**: 479-492.

Fegan, M., Manners, J. M., Maclean, D. J., Irwin, J. A. M., Samuels, K. D. Z., Holdom, D. G. and Li, D. P. 1993. Random amplified polymorphic DNA markers reveal a high degree of genetic diversity in the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae*. *Journal of General Microbiology*. **139**: 2075-2081.

Fenner, M. 1986. A bioassay to determine the limiting minerals for seeds from nutrient deprived *Senecio vulgaris* plants. *Journal of Ecology*. **74**: 497-505.

Fenner, M. 1987. Seedlings. *New Phytologist*. **106**: 35-47.

Fenner, M. and Lee, W. G. 1989. Growth of seedlings of pasture grasses and legumes deprived of single mineral nutrients. *Journal of Applied Ecology*. **26**: 223-232.

Fernandez, D. and Tantaoui, A. 1994. Random Amplified Polymorphic DNA (RAPD) analysis: a tool for rapid characterization of *Fusarium oxysporum* f. sp. *albedinis* isolates. *Phylopathologia Mediterranea*. **33**: 223-229.

Filho, J. P. L., Goulart, M. F. and Lovato, M. B. 2004. Chlorophyll fluorescence parameters in populations of two legume trees. *Stryphnodendron adstringens* (Mart.) Coville (Mimosoideae) and *Cassia ferruginea* (Shrad.) Shrad. ex DC (Caesalpinoideae). *Revista Brasileira de Botanica*. **27**: 527-532.

Fisker, S. E., Rose, R., and Hause, D. L. 1995. Chlorophyll fluorescence as a measure of cold hardiness and freezing stress in 1+1 Douglas-fir seedlings. *Forest Science*. **41**: 564-575.

Fox, J. E. D. 2000. Sandalwood : The royal tree. *The Biologist*. **47**: 31-34.

Fox, J. E. D. and Barrett, D. R. 1994. Silvicultural characteristics associated with the ecology and parasitic habit of sandalwood. In papers presented at the Sandalwood workshop held at Noumea, New Caledonia, August 1-12, 1994. Australian Centre for International Agricultural Research (ACIAR). CIRAD/FAO. pp. 122-125.

Fox, J. E. D., Barrett, D. R., Surata, K. I. and Markum. E. 1994b. Protocol for establishment of *Santalum album* in Timor, Indonesia. In papers presented at the Sandalwood workshop held at Noumea, New Caledonia, August 1-12, 1994. Australian Centre for International Agricultural Research (ACIAR). CIRAD/FAO. pp. 134-148.

Fox, J. E. D., Doronila, A. I. and Barrett, D. R. 1994a. The selection of superior pot hosts for maximum nursery growth in *Santalum album*. In papers presented at the Sandalwood workshop held at Noumea, New Caledonia, August 1-12, 1994. Australian Centre for International Agricultural Research (ACIAR). CIRAD/FAO. pp. 1-11.

Fox, J. E. D., Doronila, A. I., Barrett, D. R. and Surata, K. 1996. *Desmanthus virgatus* (L.) Willd., an efficient intermediate host for the parasitic tree species *Santalum album* L. Timor. Indonesia. *Journal of Sustainable Forestry*. **3**: 13-23.

Fox, J. E. D., Surata, I. K. and Sutarjo, S. 1990. Nursery potting mixture for sandal in Timor. *Mulga Research Centre Journal*. **10**: 38-44.

Francisco, D. C. G. J., Caranhas, D. S. B. D., Ulysses, M. D. S. JR., Andreia, V. F., Paulo, D. T. B. S. and Marcos, S. B. 2005. Growth, photosynthesis and stress indicators in young rosewood plants (*Aniba rosaeodora* Ducke) under different light intensities. *Brazilian Journal of Plant Physiology*. **17**: 325-334.

Frankel, O. H. 1983. The place of management in conservation. In *Genetics and Conservation - A reference for managing wild animals and plant populations*. Edited by C. M. Schonewald-Cox., S. M. Chambers., B. Mac Bryde., and L. Thomas. Benjamin/Cummings, Mento Park, CA, USA. pp. 1-14.

Frankham, R. 1997. Do Island populations have less genetic variation than mainland populations?. *Heredity*. **78**: 311-327.

Fransico, O. J., Arnoldo S. G., Seung. C. K. and Daniel J. C. 2000. Plant genetic diversity in the Canary Islands: A conservation perspective. *American Journal of Botany*. **87**: 909-919.

Fujita, S. I., Senda, Y., Nakaguchi, S. and Hashimoto, T. 2001. Multiplex PCR using Internal Transcribed Spacer 1 and 2 regions for rapid detection and identification of yeast strains. *Journal of Clinical Microbiology*. **39**: 3617-3622.

Gairola, S., Ravi Kumar, G. and Aggarwal, P. 2007. Status of production and marketing of Sandalwood (*Santalum album* L.) In Proceedings of the National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp. 1-8.

Gang, H., Dong-Hee, L., June, S. L. and Nam, S. L. 2002. A study of taxonomical relationships among species of Korean *Allium* sect. *sacculiferum* (Alliaceae) and related species using Inter-simple Sequence Repeat (ISSR) markers. *Botanical Bulletin of Academia Sinica*. **43**: 63-68.

Gangopadhyay, G., Roy, S. K., Ghose, K., Poddar, R., Bandhopadhyay, T., Basu, D. and Mukherjee, K. K. 2007. Sex detection of *Carica papaya* and *Cycas circinalis* in pre-flowering stage by ISSR and RAPD. *Current Science*. **92**: 524-526.

Gardes, M., White, T. J., Fortin, J. A., Bruns, T. D. and Taylor, T. W. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. *Canadian Journal of Botany*. **69**: 180-190.

George, A. S. 1984. *Santalum*. In Flora of Australia. Vol. 22. Australian Govt. Publishing Service, Canberra. pp. 61-66.

George B. L., Campbell, L. C. and Leon T. L. 1992. *Introduction to Plant Diseases. Identification and Management*. Van Nostrand Reinhold, New York. pp. 178-181.

Gherbawy, Y. A. M. H. and Abdel-Zaher, H. M. A. 1999. Isolation of fungi from tomato rhizosphere and evolution of the effect of cellulase enzymes of *Nectria haematococca* and *Pythium ultimum* var. *ultimum*. *Czech Mycology*. **51**: 2-3.

Gherbawy, Y. A. M. H., Adler, A. and Prillingar, H. 2002. Genotypic identification of *Fusarium subglutinans*, *F. proliferatum* and *F. verticillioides* strains isolated from maize in Austria. *Mycobiology*. **30**: 139-145.

Ghosh, S. K., Balasundaran, M. and Ali, M. I. M. 1985. Studies on spike disease of Sandal. KFRI Research Report. No 37. Kerala Forest Research Institute, Peechi. Thrissur. pp. 56.

Gilbert, J. E., Lewis, R. V., Wilkinson, M. J. and Caligare, P. D. S. 1999. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. *Theoretical and Applied Genetics*. **98**: 1125-1131.

Goncalves, J. F. C., Barneto, D. C. S., Santos Junior, V. M., Fernandes, A. V. Sampaio, P. T. B. and Buckeridge, M. S. 2005. Growth, photosynthesis and stress indicators in young rosewood plants (*Aniba rosaeodora* Ducke) under different light intensities. *Brazilian Journal of Plant Physiology*. **17**: 325-334.

Goncalves, J. F. C., Marenco, R. A. and Vieira, G. 2001. Concentration of photosynthetic pigments and chlorophyll fluorescence of mahogany and tonka bean under two light environments. *Brazilian Journal of Plant Physiology*. **13**: 149-157.

Goodwin, P. H. and Annis, S. L. 1991. Rapid identification of genetic variation and pathotype of *Leptosphaeria maculans* by random amplified polymorphic DNA assay. *Applied and Environmental Microbiology*. **57**: 2482-2486.

Goulao, L. and Oliveira, C. M. 2001. Molecular characterization of cultivars of apple (*Malus X domestica* Borkh) using microsatellite (SSR and ISSR) markers. *Euphytica*. **122**: 81-89.

Govindjee, R. 1995. Sixty-three years since Kautsky: Chlorophyll a fluorescence. *Australian Journal of Plant Physiology*. **22**: 131-160.

Grajal-Martin, M. J., Simon, C. J. and Muehlbauer, F. J. 1993. Use of Random Amplified Polymorphic DNA (RAPD) to characterize Race 2 of *Fusarium oxysporum* f. sp. *pisi*. *Phytopathology*. **83**: 612-614.

Grattapaglia, D., O'Mally, D. M. and Dvovak, W. 1992. Multiple applications of RAPD markers to genetic analysis of *Eucalyptus* sp. In Proceedings of the IUFRO International Conference on Breeding Tropical Trees, Section 2.02.08. Columbia. pp.436-450.

Griffin, J. J., Ranney, T. G. and Pharr, D. M. 2004. Photosynthesis, chlorophyll fluorescence, and carbohydrate content of *Illicium* taxa grown under varied irradiance. *Journal of American Society of Horticultural Science*. **129**: 46-53.

Groninger, J. W., Seiler, J. R., Peterson, J. A. and Kreh, R. E. 1996. Growth and photosynthetic responses of four Virginia Piedmont tree species to shade. *Tree Physiology*. **16**: 773-778.

Gupta, M., Chyi, Y. S., Romero-Severson, J. and Owen, J. L. 1994. Amplification of DNA markers from evolutionary diverse genome using single primers of simple sequence repeats. *Theoretical and Applied Genetics*. **89**: 998-1006.

Guthrie, P. A., Magill, C. W., Frederiksen, R. A. and Odvody, G. N. 1992. Random Amplified Polymorphic DNA markers: a system for identifying and differentiating isolates *Colletotrichum graminicola*. *Phytopathology*. **82**: 832-835.

Harris, J. 1999. RAPDs in systematic - a useful methodology. In *Molecular Systematics and plant evolution*. Edited by P. M. Hollingsworth, R. M. Bateman, and R. J. Gornall. Taylor and Francis, London, UK. pp. 221-228.

Hartl, D. L. and Clark, A. G. 1989. *Principles of Population Genetics*. Sunderland, MA, USA: Sinauer Associates, Inc. pp. 226-234.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C. and Pegler, D. N. 1995. Ainsworth and Bisby's Dictionary of the Fungi. 8th edn. CAB International, Wallingford, Oxon.

Henry, T., Iwen, P. C. and Hinrichs, S. H. 2000. Identification of *Aspergillus* species using Internal Transcribed Spacer regions 1 and 2. *Journal of Clinical Microbiology*. **38**: 1510-1515.

Hollingsworth, M. L., Hollingsworth, P. M., Jenkins, G. I., Bailey, J. P. and Ferris, C. 1998. The use of molecular markers to study patterns of genotypic diversity in some invasive alien *Fallopia* spp. (Polygonaceae). *Molecular Ecology*. **7**: 1681-1691.

Holsinger, K. E. and Vitt, P. 1997. The future of conservation biology: What's a geneticist to do?. In *The Ecological Basis of Conservation: Heterogeneity, Ecosystems and Biodiversity*. Edited by S. T. A. Pickett, R. S. Ostfeld, M. Shachak, and G. E. Likens. Chapman & Hall, New York. pp. 202-216.

Honnareddy, N. and Dubey, S. C. 2006. Pathogenic and molecular characterization of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *Current Science*. **91**: 661-666.

Hormaza, J. J., Dollo, L. and Polito, V. S. 1994. Identification of a RAPD marker linked to sex determination in *Pistacia vera* using bulked segregant analysis. *Theoretical and Applied Genetics*. **89**: 9-13.

Horton, T. R. and Bruns, T. D. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black box. *Molecular Ecology*. **10**: 1855-1871.

Hou, Y. C., Yan, Z. H., Wei, M. Y. and Zheng, Y. L. 2005. Genetic diversity in barley from West China based on RAPD and ISSR analysis. *Barley Genetic Newsletter*. **35**: 9-22.

Hsiang, T. and Mahuku, G. S. 1999. Genetic variation within and between local populations of *Sclerotinia homeocarpa*. *Plant Pathology*. **48**: 83-94.

Hunter, P. R. and Fraser, C. A. M. 1990. Application of the theory of adaptive polymorphism to the ecology and epidemiology of pathogenic yeasts. *Applied and Environmental Microbiology*. **15**: 39-54.

Husen, A., Khali, R. and Nautiyal, S. 2004. Attitudinal variation in chlorophyll fluorescence/photosynthetic efficiency in seedlings of some indigenous fodder species. *Indian Forester*. **130**: 89-94.

ICFRE, 2000. *Forest Statistics- India, Directorate of Statistics*. M/S Valley Offset Printers, Dehra Dun. pp. 308.

Iqbal, M. J., Aziz, N., Saeed, N. A., Zafar, Y. and Malik, K. A. 1997. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. *Theoretical and Applied Genetics*. **94**: 139-144.

Ishida, A., Nakano, T., Matsumoto, Y., Sakoda, M. and Ang, L. H. 1999. Diurnal changes in leaf gas exchange and chlorophyll fluorescence in tropical tree species with contrasting light requirements. *Ecological Research*. **14**: 77-88.

IUCN. 2010. 2010 *IUCN Red list of threatened species*. (www.iucnredlist.org).

Jagadish, M. R., Ahmed, S. M., Madhu, K. S., Viswanath, S. and Rathore, T. S. 2007. Effect of seed source and collection time in *Santalum album* L. on germination parameters. In Proceedings of the National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp. 47-51.

Jain, A., Bhatia, S., Banga, S. S., Prakash, S. and Lakshmikumaran, M. 1994. Potential use of Random Amplified Polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. *Theoretical and Applied Genetics*. **88**: 116-122.

Jain, S. H, Angadi, V. G., Rajeevalochan, A. N., Sankaranarayana, K. H., Theagarajan, K. S. and Rangaswamy, C. R. 1998. Identification of provenances of sandal in India for genetic conservation. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No. 84, ACIAR, Canberra, Australia. pp. 117-122.

Jain, S. H., Angadi, V. G. and Shankaranarayana, K. H. 2003. Edaphic, environmental and genetic factors associated with growth and adaptability of Sandal (*Santalum album* L.) in provenances. *Sandalwood Research Newsletter*. **17**: 6-7.

Jain, S. H., Angadi, V. G., Ravikumar, G., Theagarajan, K. S. and Shankaranarayana, K. H. 1999. Studies on cultivation and chemical utilisation of Sandal (*Santalum album* L.) *Fafai Journal*. pp. 49-53.

Janssen L. H. J., Oeveren, V. P. R., Hassett, V. and Kuiper, P. J. C. 1995. Genotypic variation in chlorophyll fluorescence parameters, photosynthesis and growth of tomato grown at low temperature and low irradiance. *Photosynthetica*. **31**: 301-314.

Jeeva, V., Saravanan, S., Devaraj. P. and Lakshmidevi, R. 1998. Malady and remedy of sandal cultivation in farmlands and private lands - An overview. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 16.

Jiko, L. R. 2000. Status and current interest in sandalwood in Fiji. *Sandalwood Research Newsletter*. **10**: 1-3.

Jin, Z. X. and Li, J. M. 2007. ISSR analysis on genetic diversity of endangered relic shrub *Sinocalycanthus chinensis*. *Chinese Journal of Applied Ecology*. **18**: 247-253.

Jin, Z. X., Li, J. M. and Gu, Q. P. 2006. Genetic diversity in the natural populations of *Rhododendron fortunei* revealed by ISSR molecular markers. *Acta Horticulturae Sinica*. **33**: 1263-1267.

Joshi, G. and Arun Kumar. A. N. 2007. Standardization of optimum conditions for storage of *Santalum album* L. seeds for *ex situ* germplasm conservation. In Proceedings of the National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp. 52-54.

Joshi, S. C. 1995. Species specific diurnal changes in chlorophyll fluorescence in tropical deciduous and evergreen plants growing in the field during summer. *Photosynthetica*. **31:** 549-557.

Joshi, S. P., Gupta, V. S., Aggarwal, R. K., Ranjekar, P. K. and Brar, D. S. 2000. Genetic diversity and phyolgenetic relationship as revealed by Inter Simple Sequence Repeat (ISSR) polymorphism in the genus *Oryza*. *Theoretical and Applied Genetics*. **100**: 1311-1320.

Kalc Wright, G. F., Guest, D. I., Wimalajeeva, D. L. S. and Van. H. R. 1996. Characterization of *Fusarium oxysporum* isolated from carnation in Australia based on pathogenicity, vegetative compatibility and random amplified polymorphic DNA (RAPD) assay. *European Journal of Plant Pathology*. **102**: 451-457.

Kantety, R. V., Zeng, X. P., Bennetzen, J. L. and Zehr, B. E. 1995. Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Molecular Breeding*. **1**: 365-373.

Kaundun, S. S., Alexander Zhyvoloup and Toung G. P. 2000. Evaluation of the genetic diversity among elite tea (*Camellia sinensis* var. *sinensis*) accessions using RAPD markers. *Euphytica*. **115**: 7-16.

*Kautsky, H. and Hirsh, A. 1931. Neue Versuche zur Kohlensaureassimilation. *Naturwissenschaften*. **19**: 964.

Kautsky, H., Appel, W. and Amann, H. 1960. Chlorophyll fluorescenzund Kohlensaureassimilation. *Biochenische Zeitschriff*. **322**: 277-292.

Keil, M. and Griffin, A. R. 1994. Use of Random Amplified Polymorphic DNA (RAPD) markers in the discrimination and verification of genotypes in *Eucalyptus*. *Theoretical and Applied Genetics*. **89**: 442-450.

Kelly, A., Alcala-Jimenez, A. R., Bainbridge, B. W., Heale, J. B., Perez-Artes, E. and Jimenez-Diaz, R. M. 1994. Use of genetic fingerprinting and random amplified polymorphic DNA to characterise pathotypes of *Fusarium oxysporum* f. sp. *ciceris* infecting chickpea. *Phytopathology*. **84**: 1293-1298.

Kelly, A. G., Bainbridge, B. W., Heale, J. B., Perez-Artes, E. and Jimenez-Dfaz, R. M. 1998. Planta-polymerase-chain reaction detection of the wilt inducing pathotype of *Fusarium oxysporum* f.sp. *ciceris* in chickpea (*Cicer arietinum* L.). *Physiology and Molecular Plant Pathology*. **52**: 397-409.

Kelly, C. K. 1990. Plant Foraging: a marginal value model and coiling response in *Cuscuta subinclusa*. *Ecology*. **71**: 1916-1925.

Kelly, C. K., Venable, D. L. and Zimmerer, K. 1988. Host specialization in *Cuscuta costaricensis*: An assessment of host use relative to host availability. *Oikos*. **53**: 315-320.

Kemp, R. H., Namkoong, G. and Wadsworth, F. H. 1993. The nature of forest genetic resources. In *Conservation of genetic resources in tropical forest management - Principles and concepts*. FAO Forestry paper 107. Rome. pp. 5-14.

Khan, S. R., Rose, R., Haase, D. L. and Sabin, T. E. 2000. Effects of shade on morphology, chlorophyll concentration, and chlorophyll fluorescence of four Pacific Northwest Conifer Species. *New Forests*. **19**: 171-186.

King, R. A. and Ferris, C. 2000. Chloroplast DNA and nuclear DNA variation in the sympatric older species *Alnus cordata* (Lois.) Duby and *A. Glutinosa* (L.) Gaertm. *Biological Journal of the Linnean Society*. **70**: 147-160.

Kistler, H. C. 1997. Genetic diversity in the plant pathogenic fungus *Fusarium oxysporum*. *Phytopathology*. **87**: 474-479.

Kitajima, K. 1992a. The importance of cotyledon functional morphology and patterns of Seed Reserve Utilization for the Physiological Ecology of Neotropical Tree Seedlings. Ph.D. Thesis. University of Illinois, Urbana.

Kitajima, K. 1992b. Relationship between photosynthesis and thickness of cotyledons for tropical tree species. *Functional Ecology*. **6**: 582-584.

Kitajima, K. 1996. Ecophysiology of Tropical Tree Seedlings. In *Tropical Forest Plant Ecophysiology*. Edited by Stephen S. Mulkey, Robin L. Chazdon and Alan P. Smith. Chapman & Hall. International Thomson Publishing Company. pp. 559-592.

Kitajima, K. 2002. Do shade tolerant tropical tree seedlings depend longer on seed reserves? Functional growth analysis of three Bignoniaceae species. *Functional Ecology*. **16**: 433-444.

Kitajima, K. and Fenner, M. 2000. Seedling regeneration ecology. In *Seeds: Ecology of Regeneration in Plant Communities*. Edited by M. Fenner. CAB International, Wallingford, U.K. pp. 331-360.

Kitao, M., Lei, T. T., Koike, T., Tobita, H. and Maruyama, Y. 2000a. Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. *Plant Cell & Environment.* **23**: 81-89.

Kitao, M., Lei, T. T., Koike, T., Tobita, H., Maruyama, Y., Matsumoto, Y. and Ang, L. H. 2000b. Temperature response and photoinhibition investigated by chlorophyll fluorescence measurements for four distinct species of dipterocarp trees. *Physiologia Plantarum*. **109**: 284-290.

Knutson, D. M. 1983. Physiology of misttetoe parasitism and disease responses in the host. In *The Biology of mistletoes*. Edited by M. Calder and P. Bernhardt. Academic Press, Sydney, Australia. pp. 295-316.

Kojima, T., Nagaoka, T., Noda, K. and Ogihara, Y. 1998. Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. *Theoretical and Applied Genetics*. **96**: 37-45.

Krause, G. H. and Somersalo, S. 1989. Fluorescence as a tool in photosynthesis research. Applications in studies of photo inhibition, cold acclimation, and freezing stress. *Philosophical Transactions of the Royal Society B: Biological Sciences London*. **323**: 281-293.

Krause, G. H. and Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: The basics. *Annual Review of Plant Physiology and Plant Molecular Biology*. **42**: 313-349.

Krigel, I. 1967. The Early requirement for plant nutrients by sub-terranean clover seedlings (*Trifolium subterraneum*). *Australian Journal of Agricultural Research*. **18**: 879-886.

Kuginuki, Y., Ajisaka, H., Yui, M., Yoshikawa, H., Hida, K. and Hirai, M. 1997. RAPD markers linked to a club root resistance locus in *Brassica rapa*. L. *Euphytica*. **98**: 149-154.

Kuijt, J. 1969. *The Biology of Parasitic Flowering Plants*. Berkeley, CA, USA: University of California Press.

Kulkarni, H. D. 1995. Studies on variations and tree improvement aspects of sandal (*Santalum album* L.). Ph.D thesis submitted to University of Mysore, Manasagangotri, Mysore. pp. 197-198.

Kulkarni, H. D. and Muniyamma, M. 1998. Floral biology and breeding systems in sandal. *Santalum album* L. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No. 84. ACIAR, Canberra, Australia. pp. 135-146.

Kull, O. 2002. Acclimation of photosynthesis in canopies: models and limitations. *Oecologia*. **133**: 267-269.

Kumar, A. A. N. and Joshi, G. 2007. Tree Improvement in Sandalwood-Retrospect and Prospect. In Proceedings of the National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp. 32-37.

Kushalappa, K. A. 1983. Sandal as a species for social forestry. *My Forest*. **19**: 185-187.

Lambers, H., Chapin, III, F. S. and Pons, T. L. 1998. *Plant Physiological Ecology*. Springer-Verlag, New York, U. S. A.

Lanham, P. G. and Brennan, R. M. 1999. Genetic characterization of gooseberry (*Ribes grossularis* subgenus Grossularia) germplasm using RAPD, ISSR and AFLP markers. *Journal of Horticultural Science and Biotechnology*. **74**: 361-366.

Larsen, H. S., South, D. B. and Boyer, J. M. 1986. Root growth potential, seedling morphology and bud dormancy correlate with survival of Loblolly pine seedlings planted in December in Alabama. *Tree Physiology*. **1**: 253-263.

*Lewontin, R. C. and Hubby, J. 1966. A molecular approach to the study of genetic heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics*. **54**: 595-609.

Li, J. M. and Jin, Z. X. 2008. Genetic structure of endangered *Emmenopterys henryi* Oliv. based on ISSR polymorphism and implications for its conservation. *Genetica*. **133**: 227-234.

Liittge, U., Haridasan, M., Fernandes, G. W., Mattos, E. A., Trimborn, P., Franco, A. C., Caldas, L. and Ziegler, H. 1998. Photosynthesis of mistletoes in relation to their hosts at various sites in Tropical Brazil. *Trees*. **12**: 167-174.

*Link, H. F. 1809. *Observations in Ordines Plantarum Naturales*, Mag. Res. Natureforschung Freunde. Berlin.

Liu, J., Chen, X., Wang, B., He, Z., Peng, A. and Cai, M. 2006. Race identification and SCAR markers development for the pathogen of banana Panama disease *Fusarium oxysporum* f. sp. *cubense*. *Acta Phytopathologica Sinica*. **36**: 28-34.

Loveys, B. R., Tyerman, B. D. and Loveys, B. R. 2001. Transfer of photosynthate and naturally occurring insecticidal compounds from host plants to the root hemiparasitie *Santalum acuminatum* (Santalaceae). *Australian Journal of Botany*. **49**: 9-16.

Luong, T. M. 2002. Competitive effects within and between *Santalum album* and pot host *Alternanathera dendata*. *Sandalwood Research Newsletter*. **16**: 4-7.

Lynch, M. and Milligan, B. G. 1994. Analysis of population genetic structure with RAPD markers. *Molecular Ecology*. **3**: 91-99.

Magnegneau, B., Debets, F. and Hoekstra, R. F. 1993. Genetic variability and relatedness in the complex group of block *Aspergilli* based on random amplification of polymorphic DNA. *Current Genetics*. **23**: 323-329.

Malcolm, C. P. and Gareth. K. P. 2005. Impacts of parasitic plants on natural communities. *New Phytologist*. **166**: 737-751.

Manulis, S., Kogan, N., Reuven, M. and Ben-Yephet, Y. 1994. Use of the RAPD technique for identification of *Fusarium oxysporum* f. sp. *dianthi* from Carnation. *Phytopathology*. **84**: 98-101.

Marenco, R. A., Goncalves, J. F. C. and Vieira, G. 2001. Leaf gas exchange and carbohydrate in tropical trees differing in successional status in two light environments in Central Amazonia. *Tree Physiology*. **21**: 1311-1318.

Marko, M. D. and Stermitz, F. R. 1997. Transfer of alkaloids from *Delphinium* to *Castilleja* via root parasitism. Norditerpenoid alkaloid analysis by electrospray mass spectrometry. *Biochemical systematics and Ecology*. **25**: 279-285.

Matthies, D. 1997. Parasite-host interaction in *Castillega* and *Orthocarpus*. *Canadian Journal of Botany*. **751**: 1252-1260.

Maxwell, K. and Johnson, G. N. 2000. Chlorophyll fluorescence -a practical guide. *Journal of Experimental Botany*. **51**: 659-668.

Mc Comb, J. A. and Jones, M. G. K. 1998. Interspecific hybridization between *Santalum album* and *Santalum spicatum*. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 36-41.

Mc Dermott, J. M. and Mc Donald, B. A. 1993. Gene flow in plant pathosystems. *Annual Revision of Plant Pathology*. **31**: 353-373.

Mc Glaughlin, M., Karoly, K. and Kaye, T. 2002. Genetic variation and its relationship to population size in reintroduced populations of pink sand verbena, *Abronia umbellata* subsp. *breviflora* (Nyctaginaceae). *Conservation Genetics*. **20**: 411-420.

Mc Kinnell, F. H. 1990. Status of management and silviculture research on Sandalwood in Western Australia and Indonesia. In Proceedings of the Symposium on Sandalwood in the Pacific. Edited by L. Hamilton, and C. E. Conrad. U.S. Department of Agriculture, General Technical Report. PSW - 122, Hawaii. pp. 19-25.

Mc Tague, J. P. and Tiius, R.W. 1996. Forest site weather on field survival of Ponderosa pine. *Tree Planters' Notes*. **47**: 16-23.

Mexal, J. G. and Landis, T. D. 1990. Target seedling concepts: height and diameter. In Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations. August 1-17, 1990. Edited by R. Rose, S. J. Campbell, and T. D. Landis. General Technical Report. RM - 200. Fort Collins, CO: USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. pp. 17-35.

Mexal, J. G. and South, D. B. 1991. Bareroot seedling culture. In Forest regeneration manual. Edited by M. L. Duryea, and P. M. Dougherty. Kluwer Academic Publishers. pp. 89-115.

Michelmore, R. W., Paran, I. and Kesseli, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of National Academy of Sciences. U.S.A*. **88**: 9828-9832.

Mills, P. R., Sreenivasaprasad, S. and Brown, A. E. 1992. Detection and differentiation of *Colletotrichum gloeosporioides* isolates using PCR. *FEMS Microbiology Letters*. **98**: 137-144.

Mills, P. R., Sreenivasaprasad, S. and Muthumeenakshi, S. 1998. Assessing diversity in *Colletotrichum* and *Trichoderma* species using molecular markers. In *Molecular Variability of Fungal Pathogens*. Edited by P. Bridge, Y. Couteaudier and J. Clarkson. CAB International, UK. pp. 105-120.

Mishra, P. K., Tewari, J. P., Clear, R. M. and Turkington, T. K. 2006. Genetic diversity and recombination within populations of *Fusarium pseudograminearum* from Western Canada. *International Microbiology*. **9**: 65-68.

Moller, E. M., Chelkouski, T. and Geiger, H. H. 1999. Species - specific PCR assays for the fungal pathogens *Fusarium moniliforme* and *Fusarium subglutinans* and their application to diagnose maize ear rot disease. *Journal of Phytopathology*. **147**: 497-508.

Moreno, S., Martin, J. P. and Ortiz, J. M. 1998. Inter Simple Sequence Repeats PCR for characterization of closely related grapevine germplasm. *Euphytica*. **110**: 117-125.

Murphy, M. and Garkaklis, M. 2003. Hopping into a bright future-The Woylie Sandalwood Story. *Sandalwood Research Newsletter*. **18**: 6-7.

Murray, D. R. 1985. *Seed Physiology*. *Vol.2, Germination and Reserve Mobilization*. Academic Press, Sydney.

Musselman, L. J. and Press, M. C. 1998. Mistletoes as parasites: host specificity and speciation. *Trends in Ecology and Evolution*. **13**: 101-105.

Muthana, M. A. 1995. Spike disease of sandal (*Santalum album*). *Indian Forester*. **81**: 500-508.

Nagaoka, T. and Ogihara, Y. 1997. Applicability of inter simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theoretical and Applied Genetics*. **94**: 597-602.

Nagaveni, H. C. and Srimathi, R. A. 1985. A note on haustorialess sandal plants. *Indian Forester*. **111**: 161-162.

Nageswara Rao, M. K. N., Ganeshaiah, K. N. and Uma Shaanker, R. 2007. Assessing threats and mapping sandal resources to identify genetic 'hot-spot' for in situ conservation in peninsular India. *Conservation Genetics*. **8**: 925-935.

Naqvi, N. I., Bonman, J. M., Mackill, D. J., Nelson, R. J. and Chattoo, B. B. 1995. Identification of RAPD markers linked to major blast resistant gene in rice. *Molecular Breeding*. **1**: 341-348.

Narayanan, C., Wali, S. A., Shukla, R. K., Mandal, A. K. and Ansari, S. A. 2007. RAPD and ISSR markers for molecular characterization of teak (*Tectona grandis*) plus trees. *Journal of Tropical Forest Science.* **19**: 218-225.

Neerakal, I., Rajagopal, R. and Vijayakumar, K. R. 2005. Influence of inanimate shade on growth of rooted adalodakam (*Adhatoda beddomei* C.B. Clarke) cuttings. *Journal of Tropical Agriculture*. **43**: 91-93.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings National Academy of Sciences. **70**: 3321-3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*. **89**: 583-590.

Nelson, P. E., Toussoun, T. A. and Cook, R. J. 1981. *Fusarium*: Diseases, Biology and Taxonomy. The Pennsylvania State University Press, University Park.

Newton, A. C., Baker, P., Ramnarine, S., Mesen, J. F. and Leakey, R. B. 1993. The Mahogany shoot borer: prospects for control. *Forest Ecology and Management*. **57**: 301-328.

Nickrent, D. L. and Duff, R. J. 1996. Molecular studies of parasitic plants using ribosomal RNA. In Advances in Parasitic Plant Research. pp. 28-52.

Nijs, M., Larsen, J. S., Gams, W., Rombous, F. M., Wernars, K., Thrane, U. and Notermans, S. H. W. 1997. Variations in random amplified polymorphic DNA patterns and secondary metabolite profiles within *Fusarium* species from cereals from various parts of the Netherlands. *Food Microbiology*. **14**: 449-457.

Niranjana, R. and Shivamurthy, G. R. 1987. Graniferous tracheary elements in the haustorium of *Osyris arborea* Wall (Santalaceae). *Annals of Botany*. **59**: 237-243.

Niu, S. L., Li, K. H., Jiang, G. M., Gao, L. M., Li, Y. G., Peng, Y. and Liu, M. Z. 2004. Gas exchange and chlorophyll fluorescence response to stimulated rainfall in *Hedysarum fruticosum* var. *mangolicum*. *Photosynthetica*. **42**: 1-6.

Nordenkampf, B. H. R. and Oquist, G. 1993. Chlorophyll fluorescence as a tool in photosynthesis research. In *Photosynthesis and production in a changing Environment*. *A field and Laboratory Manual*. Edited by D. O. Hall, J. M. O. Scurloch, H. R. Bolhar-Nordenkampf, R. C. Leegood and S. P. Long, Chapman & Hall. pp. 193-206.

Oostermeijer, J. G. B., Berholz, A. and Poschold, P. 1996. Genealogical aspects of fragmented plant populations: a review. In *Species Survival in Fragmented Landscapes*. Edited by Settele., C. Margules, P. Poschold, and K. Henle. Kluwer Academic Publishers. Dordrow. pp. 93-101.

Parasnis, A. S., Ramakrishna, W., Chowdari, K. V., Gupta, V. S. and Ranjekar, P. K. 1999. Microsatellite (GATA)n reveals sex specific differences in Papaya. *Theoretical and Applied Genetics*. **99**: 1047-1052.

Parthiban, K T., Surendran, C., Murugesh, M. and Bhuvaneswaran, C. 1998. In-vitro strategies for the mass multiplication of Sandal. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No. 84. ACIAR, Canberra, Australia. pp. 74-78.

Pasquali, M., Acquandro, A., Balmas, V., Mighali, Q., Garibaldi, A. and Gullino, M. L. 2003. RAPD characterization of *Fusarium oxysporum* isolates pathogenic on *Argyranthemum frutescens*. L. *Journal of Phytopathology*. **151**: 30-35.

Pate, J. S., Davidson, N. J., Kuo, J. and Milburn, J. A. 1990a. Water relations of the root hemiparasite *Olax phyllanthi* (Labill). R. Br. (Olacaceae) and its multiple hosts. *Oecologia*. **84**: 186-193.

Pate, J. S., Pate, S. R., Kuo, J. and Davidson, N. J. 1990b. Growth, resource allocation and haustorial biology of the root hemiparasite *Olax phyllanthi* (Olaceae). *Annals of Botany*. **65**: 437-449.

Paulo, M., Ruas, C., Ruas, F., Leandro, R., Valdemar, P., Carvalho, A. E. and Tumoro, S. 2003. Genetic relationship in *Coffea* species and parentage determination of interspecific hybrids using ISSR. *Genetics and Molecular Biology*. **26**: 519-523.

Pharmawati, M., Yan, G. and Finnegan, P. M. 2005. Molecular variation and fingerprinting of *Leucadendron* cultivars (Proteaceae) by ISSR markers. *Annals of Botany*. **95**: 1163-1170.

Prasad, R. D., Sharma, T. R. and Prameela Devi, T. 2007. Molecular variability and Detection of *Fusarium* species by PCR based RAPD, ISSR and ITS-RFLP Analysis. *Journal of Mycology and Plant Pathology*. **37**: 311-318.

Press, M. C., Scholes, J. D. and Watling, J. R. 1999. Parasitic plants: Physiological and ecological interactions with their hosts. In *Physiological Plant Ecology*. Edited by M. C. Press, J. D. Scholes and M. G. Barker Oxford, U.K. Blackwell. Science, pp. 175-197.

Prevost, A. and Wilkinson, M. J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics*. **98**: 107-112.

Provan, J., Powell, W. and Waugh, R. 1996. Analysis of cultivated potato (*Solanum tuberosum*) using inter microsatellite amplification. *Genome*. **39**: 767-769.

Pukacki, P. M. 2000. Effects of sulphur, fluoride, and heavy metal pollution on the chlorophyll fluorescence of Scots pine (*Pinus sylvestris* L.) needles. *Dendrobiology*. **45**: 83-88.

Pukacki, P. M. and Modrzynski, J. 1998. The influence of ultraviolet-B radiation on the growth, pigment production and chlorophyll fluorescence of Norway spruce seedlings. *Acta Physiologiae Plantarum*. **20**: 245-250.

Qian, W., Ge, S. and Hong, D. Y. 2001. Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. *Theoretical and Applied Genetics*. **102**: 440-449.

Radomiljac, A. M. 1994. The influence of pot host species, seedling age and nursery nutrition on *Santalum album* Linn. Plantation establishment. Ord River Irrigation Area, Western Australia. In papers presented at the Sandalwood workshop held at Noumea, New Caledonia, August 1-12, 1994. Australian Centre for International Agricultural Research (ACIAR). CIRAD/FAO. pp. 60-78.

Radomiljac, A. M. 1998. The influence of pot host species, seedling age and supplementary nursery nutrition on *Santalum album* Linn. (Indian sandalwood) plantation establishment within the Ord River Irrigation Area, Western Australia. *Forest Ecology and Management*. **102**: 193-201.

Radomiljac. A. M. 2000. The influence of pot host species, seedling age and supplementary nutrition on *S. album* plantation establishment in Western Australia. *Sandalwood Research Newsletter*. **9**: 4-5.

Radomiljac, A. M. and Mc Comb, J. A. 1998. Nitrogen-fixing and non-nitrogen fixing woody host influences on the growth of the root hemi-parasite *Santalum album* L. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 54-57.

Radomiljac, A. M., Mc Comb, J. A. and Pate, J. S. 1999. Gas exchange and water relations of the root hemiparasite *Santalum album* L. in association with legume and non-legume hosts. *Annals of Botany*. **83**: 215-224.

Radomiljac, A. M., Mc Comb, J. A., Pate, J. S. and Tennakoon, K. U. 1998a. Xylem transfer of organic solutes in *Santalum album* L. (Indian sandalwood) in association with legume and non-legume hosts. *Annals of Botany*. **82**: 675-682.

Radomiljac, A. M., Shea, S. R., Mc Kinnell, F. H. and Mc Comb, J. A. 1998b. An overview of sandalwood and the development of sandal in farm forestry in Western Australia. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 9-15.

Raeder, U., Thompson, W. and Broda, P. 1989. RFLP based map of Phanerochaete *Chrysosporium* ME446: lignin peroxidase genes occur in clusters. *Molecular Microbiology*. **3**: 911-918.

Rai, S. N. 1990. Status and cultivation of sandalwood in India. In Proceedings of a Symposium on Sandalwood in the Pacific. Edited by L. Hamilton, and C. E. Conrad. Honolulu, Hawaii. Pacific Southwest Research Station, USDA Forest Service, Berkeley. pp. 66-71.

Rai, S. N. and Kulkarni, H. D. 1986. Sandalwood plantations. In *Plantations Crops Vol I. Opportunities and constraints*. Edited by H. C. Srivastava, Bharatendu Vatsya, and K. K. G. Menon. Oxford and IBH Publishing Co., New Delhi. pp. 295-300.

Ramalakshmi, B. S. and Rathore, T. S. 2007. Role of *Trichoderma viride* and *T. harzianum* in the bio-control of plant pathogens of sandalwood in nursery. In Abstracts of the National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp. 26.

Rao, L. N. 1942. Studies in the Santalaceae. *Annals of Botany*. **6**: 151-175.

Rao, N. M., Padmini. S., Ganeshaiah, K. N. and Uma Shaanker, R. 1999. Sandal genetic resources of South India: threats and conservation approaches. In National Symposium on Role of Plant Tissue Culture in Biodiversity Conservation and Economic Development. Kosi- Katarmal, Almora, UP. pp. 63.

Rao, N. M., Ravikanth, G., Ganeshaiah, K. N., Rathore, T. S. and Uma Shaanker, R. 2007. Assessing threats and identifying the ecological niche of sandal resources to identify 'hot-spots' for *in situ* conservation in Southern India. In Proceedings of the National Seminar on Conservation, Improvement, Cultivation and Management of

Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp. 23-31.

Rao, P. S. and Srimathi, R. A. 1976. Vegetative propagation of sandal (*Santalum album* L.) *Current Science*. **46**: 276-277.

Remadevi, O. K., Nagaveni, H. C. and Muthukrishnan, R. 2005. Pests and diseases of sandalwood plants in nurseries and their management. In Diseases and Insects in Forest Nurseries - Proceedings of the 5th Meeting of IUFRO Working Party S7. 03. 04, May 6-8, 2003, Peechi, Kerala, India. Edited by A. Lilja, J. R. Sutherland, M. Poter. and C. Mohanan. Working papers of the Finnish Forest Research Institute, Finland. **11**: 69-75.

Ritchie, G. 1984. Assessing seedling quality. In *Forest Nursery manual: production of bare-root seedlings*. Edited by M. L. Duryea and T. D. Landis. The Hague: Martinus Nijhoff / Dr.Junk, W. Oregon State University, Forest Research Laboratory, Corvallis. pp. 243-259.

Rohlf, J. F. 2000. NTSYSpc. Numerical Taxonomy and Multivariate Analysis System. Version 2.1. Exeter Software, Setauket. New York.

Rossetto, M., Jezierski, G., Hopper, S, D. and Dixon, K, W.1999. Conservation genetics and clonality in two critically endangered eucalypts from the highly endemic south-western Australian flora. *Biological Conservation*. **88**: 321-331.

Sadasivam, S. and Manickam, A. 1992. Biochemical methods for Agricultural Sciences. Wiley Eastern Limited and TNAU, Coimbatore. pp. 184.

Saharan, M. S., Naef, A., Kumar, J. and Tiwari, R. 2007. Characterization of variability among isolates of *Fusarium graminearum* associated with head scab of wheat using DNA markers. *Current Science*. **92**: 230-235.

Sands, P. J. 1995. Modeling canopy production. II. From single leaf photosynthetic parameters to daily canopy photosynthesis. *Australian Journal of Plant Physiology*. **22**: 603-614.

Sanjaya, Ananthapadmanabha, H. S. and Ravishankar Rai, V. 1998. In vitro shoot multiplication from the mature tree of *Santalum album* L. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No. 84. ACIAR, Canberra, Australia. pp. 60–65.

Santos, L., Lucio, J., Odair, J., Carneiro, M. L. and Alberto, C. 2000. Symptomless infection of banana and maize by endophytic fungi impairs photosynthetic efficiency. *New Phytologist*. **147**: 609-615.

Sarkar, R. K., Panda, D., Rao, D. N. and Sharma, S. G. 2004. Chlorophyll fluorescence parameters as indicators of submergence tolerance in rice. IRRN. 29, 1. *Crop Management and Physiology*. **1**: 66-68.

Scheepers, D., Eloy, M. C. and Briquet, M. 1997. Use of RAPD patterns for clone verification and in studying provenance relationships in Norway Spruce (*Picea abies*). *Theoretical and Applied Genetics*. **94**: 480-485.

Schnell, R. J., Ronning, C. M. and Knight, J. R. 1995. Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers. *Theoretical and Applied Genetics*. **90**: 269-274.

Sensi, E., Vignani, R., Rohde, W. and Biricolti, S. 1996. Characterization of genetic biodiversity with *Vitis vinifera* L. Sangiovese and Colorino genotypes by AFLP and ISTR DNA marker technology. *Vitis*. **35**: 183-188.

Setiadi, D. and Komar, T. E. 2001. Current Sandalwood Seed Source in Timor Island. *Sandalwood Research Newsletter*. **13**: 1-3.

Shah, F. H., Rashid, O., Simons, A. J. and Dunsdon, A. 1994. The utility of RAPD markers for the determination of genetic variation in oil palm (*Elaeis guinensis*). *Theoretical and Applied Genetics*. **89**: 713-718.

Shankaranarayana, K. H., Ravikumar, G., Rangaswamy, R. and Theagarajan, K. S. 1998. Sandalwood, HESP and ESPO oils from the heartwood of *Santalum album* L. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 89-92.

Sharma, P., Sharma, K. D., Sharma, R. and Plaha, P. 2006. Genetic variability in pea wilt pathogen *Fusarium oxysporum* f. sp. *pisi* in north-western Himalayas. *Indian Journal of Biotechnology*. **5**: 298-302.

Shashidhara, G., Hema, M. V., Koshy, B. and Farooqi, A. A. 2003. Assessment of genetic diversity and identification of core collection in sandalwood germplasm using RAPDs. *Journal of Horticultural Science and Biotechnology*. **78**: 528-536.

Shea, S. R., Radomiljac, A. M., Brand, J. and Jones, P. 1998. An overview of sandalwood and the development of sandal in farm forestry in Western Australia. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 9-15.

Sheng. Y., Weihong. Zheng., Kequan Pei. and Keping Ma. 2005. Genetic variation within and among populations of a dominant desert tree *Haloxylon ammodendron* (Amaranthaceae) in China. *Annals of Botany*. **96**: 245-252.

Shepherd, D. P. 1992. The breeding systems of the Western Australian Sandalwood, *Santalum spicatum* and the Indian sandalwood, *Santalum album*. Biology project, Curtin University of Technology, Perth, WA.6001. Australia.

Sindhuveerendra, H. C. and Ananthapadmanabha, H. S. 1996. The breeding system in sandal (*Santalum album* L.). *Silvae Genetica*. **45**: 188-190.

Sindhuveerendra, H. C. and Ananthapadmanabha, H. S. 1998. A Further Tree Improvement Strategy in Sandal (*Santalum album* L.). In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 135-146.

Sindhuveerendra, H. C., Ramalakshmi, R. and Mallesha, B. B. 1999. Variation in seed characteristics in provenances of sandal (*Santalum album* L.). *Indian Forester*. **125**: 308-312.

Singh, B. P., Saikia, R., Yadav, M., Singh, R., Chauhan, V. S. and Dilip. K. Arora. 2006. Molecular characterization of *Fusarium oxysporum* f. sp. *ciceri*. causing wilt of chickpea. *African Journal of Biotechnology*. **5**: 497-502.

Slapakauskas, V. and Ruzgas, V. 2005. Chlorophyll fluorescence characteristics of different winter wheat varieties. (*Triticum aestivum* L.). *Agronomy Research*. **3**: 203-209.

Smith, J. F. and Bateman, T. A. 2002. Genetic differentiation of rare and common varieties of *Eriogonum shockleyi* (Polygonaceae) in Idaho using ISSR variability. *Western North American Naturalist*. **62**: 316-326.

Smith, M. L., Bruhn, J. N. and Anderson, J. B. 1994. Relatedness and spatial distribution of *Armillaria* genets infecting red pine seedlings. *Phytopathology*. **84**: 822-829.

*Snyder, W. C. and Hansen, H. N. 1940. The species concept in *Fusarium*. *American Journal of Botany*. **27**: 64-67.

*Sokal, R. R. and Michener, C. D. 1958. A statistical method for evaluating systematic relationships. *University of Kansas Scientific Bulletin*. **38**: 1409-1438.

Sreekanth, P. M. 2009. Population genetic structuring and gene flow estimates in *Tectona grandis* L.f. using AFLP markers. Ph. D. Thesis submitted to Forest Research Institute, Dehradun, Uttaranchal. pp. 78-79.

Srimathi, R. A., Kulkarni, H. D. and Venkatesan, K. R. 1995. In *Recent Advances in Research and Management of Santalum album* L. *in India*. Edited by R. A. Srimathi, H. D. Kulkarni, and K. R. Venkatesan. Associated Publishing Co., New Delhi. pp. 416.

Srinivasan, V. V., Sivaramakrishna, V. R., Rangaswamy, C. R., Ananthapadmanabha, H. S. and Shankaranarayana, K. H. 1992. *Sandal (Santalum album* L.). Indian Council of Forestry Research and Education, Dehradun. pp. 233.

Stancato, G. C., Mazzafera, P. and Buckeridge, M. S. 2002. Carbon assimilation and partitioning in epiphyte Orchid *Cattleya forbesii* Lindl. x *Laelia tenebrosa* Rolfe under extreme levels of irradiance. *Revista Brasileira de Botanica*. **25**: 229-235.

Strasser, B. J. and Strasser, R. J. 1995. Measuring fast fluorescence transients to address environmental questions. The JIP test. In *Photosynthesis: from light to biosphere*. Edited by P. Mathis. Kluwer Academic. The Netherlands. **5**: 977-980.

Strasser, R. J., Eggenberg, P. and Strasser, B. J. 1996. How to work without stress but fluorescence. *Bulletin of Royal Society of Sciences of Liege*. **65**: 330-349.

Strasser, R. J., Srivastava, A. and Tsimilli-Michael. 1999. Screening the vitality and photosynthetic activity of plants by fluorescent transient. In *Crop improvement for food security*. Edited by R. K. Behl, M. S. Punia, and B. P. S. Lather. SSARM, Hissar, India. pp. 72-115.

Strongman, D. B. and Mackay, R. M. 1993. Discrimination between *Hirsutella longicolla* var. *longicolla* and *Hirsutella cornuta* using random amplified polymorphic DNA fingerprinting. *Mycologia*. **85**:65-70.

Subba Rao, N. S., Yadav, D., Padmanabha, A., Nagaveni, C. S., Singh, S. and Kavimandan, S. K. 1990. Nodule haustoria and microbial features of *Cajanus* and *Pongamia pinnata* parasitized by sandal (sandalwood). *Plant and Soil*. **128**: 249-256.

Sullivan, D. and Coleman, D. 1998. Variability of Human Pathogenic Fungi. In *Molecular Variability of Fungal Pathogens*. Edited by P. Bridge, Y. Couteaudier and J. Clarkson. CAB International, UK. pp. 219-220.

Suma, T. B. and Balasundaran. M. 2003. Isozyme variation in five provenances of *Santalum album* in India. *Australian Journal of Botany*. **51**: 243-249.

Suma, T. B. and Balasundaran. M. 2004. Genetic diversity of eight *Santalum album* provenances of India based on RAPD analysis. *Indian Journal of Genetics and Plant Breeding*. **64**: 167-168.

Suma, T. B. and Balasundaran. M. 2007. Variation in genetic diversity and seedling survival of sandal provenances. In Proceedings of the National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp. 40-46.

Surata, K. 1992. Effect of host plants on growth of sandalwood (*Santalum album*) seedlings. *Santalum*. **9**: 1-10.

Surendran, C., Parthiban, K. L., Bhuvaneshwaram, C. and Murugesh, M. 1998. Silvicultural strategies for augmentation of sandal regeneration. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 69-73.

Swaminathan, M. H., Hosmath, B. J. and Mallesha, B. B. 1998. The status of Sandalwood in India. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No. 84. ACIAR, Canberra, Australia. pp. 3-8.

Swifty, C. E. 2002. Vegetative compatibility groups of *Fusarium oxysporum* f.sp. *cepae* from Onion in Colarado. *Plant Disease*. **86**: 606-610.

Taide, Y. B., Babu, J. C. and Abraham, C. C. 1994. Influence of host species on the initial growth of sandal (*Santalum album* L.). *Indian Journal of Forestry*. **17**: 288-292.

Talbot, N. J. 1998. Molecular variability of fungal pathogens: using the Rice blast fungus as a case study. In *Molecular Variability of Fungal Pathogens*. Edited by P. Bridge, Y. Couteaudier and J. Clarkson. CAB International, UK. pp. 1-18.

Tandon, J. N. 1995. Sandalwood oil industry. In *Recent Advances in Research and Management of Santalum album* L. *in India*. Edited by R. A. Srimathi, H. D. Kulkarni, and K. R. Venkatesan. Associated Publishing Company, New Delhi. pp. 186-193.

Tani, N., Tomaru, N., Tsumura, Y., Araki, M. and Ohba, K. 1998. Genetic structure within a Japanese stone pine (*Pinus pumila* Ragel) population on Mt.Aiko-dake in Central Honshu, Japan. *Journal of Plant Research*. **111**: 7-15.

Tantaoui, A., Ouinten, M., Geiger, J. P. and Fernandez, D. 1996. Characterization of a single clonal lineage of *F. oxysporum* f. sp. *albedinis* causing Bayoud disease of date palm in Morocco. *Phytopathology*. **86**: 787-792.

Tautz, D.1989. Hypervariabilty of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*. **17**: 6463-6471.

Tennakoon, K. U., Ekanayake, S. P. and Etampawala, E. R. L. B. 2000. An overview of *Santalum album* research in Sri Lanka. *Sandalwood Research Newsletter*. **11**: 1-4.

Tennakoon. K. U. and Cameron. D. D. 2006. The anatomy of *Santalum album* (sandalwood) haustoria. *Canadian Journal of Botany*. **84**: 1608-1616.

Tennakoon, K. U. and Pate, J. S. 1996. Heterotrophic gain of carbon from hosts by the xylem-tapping root hemi-parasite *Olax phyllanthi*. *Oecologia*. **105**: 369-376.

Tennakoon, K. U. and Pate, J. S. 1997. Xylem fluxes of fixed N through nodules of the legume *Acacia littorea* and the haustoria of an associated N dependent root hemiparasite *Olax phyllanthi*. *Journal of Experimental Botany*. **48**: 1061-1069.

Tennakoon, K. U. and Pate, J. S. 1998. Biological and physiological aspects of the woody root hemi-parasite *Santalum acuminatum* (Quandong) and its common hosts. In *Sandal and its Products*. Edited by A. M. Radomiljac, H. S. Ananthapadmanabha, R. M. Welbourn, and K. Satyanarayana Rao. ACIAR Proceedings. No.84. ACIAR, Canberra, Australia. pp. 58-59.

Tennakoon, K. U., Pate, J. S. and Stewart, G. R. 1997. Haustorium related uptake and metabolism of host xylem solutes by the root hemiparasitic shrub *Santalum acuminatum* (R. Br.) A. DC. (Santalaceae). *Annals of Botany*. **80**: 257-264.

Thomas, S. and Balasundaran, M. 1999. Detection of sandal spike phytoplasma by polymerase chain reaction. *Current science*. **76**: 1574-1576.

Thomas, S. and Balasundaran, M. 2001. Purification of sandal spike phytoplasma for the production of polyclonal antibody. *Current Science*. **80**: 1489-1494.

Thompson, B. E. 1986. Seedling morphological evaluation-what you can tell by looking. In *Evaluating seedling quality: principles, procedures, and predictive abilities of major tests*. Edited by M. L. Duryea. Oregon State University, Portland, Oregon, USA. pp. 59-71.

Trueman, S., Warburton, C., James, E., Fripp, Y. and Wallace, H. 2001. Clonality in remnant populations of *Santalum lanceolatum*. *Sandalwood Research Newsletter*. **14**: 1-4.

Tsumura, Y., Ohba, K. and Strauss, S, H. 1996. Diversity and inheritance of intersimple sequence repeat polymorphism in Douglas Fir (*Pseudotsuga menziesii*) and Sugi (*Cryptomeria japonica*). *Theoretical and Applied Genetics*. **92**: 40-45.

Tuttle, C. L., South, D. B., Golden, M. S. and Meldahl, R. S. 1987. Relationship between initial seedling height and survival and growth of loblolly pine seedlings planted during a droughty year. *Southern Journal of Applied Forestry*, **11**: 139-143.

Uma Shaanker, R., Ganeshaiah, K. N. and Rao, N. M. 2000. Conservation of sandal genetic resources in India: problems and prospects. In International Conference of Science and Technology for Managing Plant Diversity in the 21st century, Kuala Lumpur, Malaysia. pp. 73.

Uniyal, D. F., Thapaliyal, R. C. and Rawat, M. S. 1985. Vegetative propagation of sandal by root cuttings. *Indian Forester*. **111**: 145-148.

Vakalounakis, D. J. and Fragkiadakis, G. A. 1999. Genetic diversity of *Fusarium oxysporum* isolates from cucumber: Differentiation by pathogenicity, vegetative compatibility and RAPD fingerprinting. *Phytopathology*. **89**: 161-168.

Veerendra, H. C. S. and Bagchi, S. K. 1986. Analysis of inter tree variability in anatomical characters of *S. album.* L. *Indian Forester*. **112**: 80-84.

Venkatesan, K. R. 1980. A fresh look at the management of sandal. Sandal Research Centre. Bangalore. In Proceedings of Second Forestry Conference, F.R.I. & Colleges, Dehra Dun.

Venkatesan, K. R. 1995. Sandal and Social Forestry. In *Recent Advances in Research and Management of Santalum album* L. *in India*. Edited by R. A. Srimathi, H. D. Kulkarni, and K. R. Venkatesan. Associated Publishing Company, New Delhi. pp. 199-206.

Venkatesan, K. R. and Srimathi, R. A. 1981. The sandal population - a genetic survey. Paper presented at All-India Sandal Seminar held at Salem, Tamil Nadu. pp. 1-9.

Vidaver, W., Binder, W., Brooke, R. C., Lister, G. R. and Toivonen, P. M. A. 1989. Assessment of photosynthetic activity of nursery grown *Picea glauca* seedlings using an integrated fluorometer to monitor variable chlorophyll fluorescence. *Canadian Journal of Forestry Research*. **19**: 1478-1482.

Viswanath, S., Dhanya, B. and Rathore, T. S. 2007. Financial viability of sandal (*Santalum album* L.) cultivation practices. In Proceedings of the National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.), IWST, Bangalore, 12-13 December, 2007. Edited by S. Gairola, T. S. Rathore, G. Joshi, A. N. Arun Kumar, and P. Aggarwal. Institute of Wood Science and Technology, Bangalore. pp. 158-164.

Voigt, K., Schleier, S. and Bruckner, B. 1995. Genetic variability in *Gibberella fujikuroi* and some related species of the genus *Fusarium* based on random amplification of polymorphic DNA (RAPD). *Current Genetics*. **27**: 528-535.

Vos, P., Hogers, R., Bleeker, M.,Van der Lee T., Hornes, M., Fritgers, A., Pof, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*. **23**: 4407-4414.

Wagner, A., Michalek, W. and Jamiolkowska. 2006. Chlorophyll fluorescence measurements as indicators of fusariosis severity in tomato plants. *Agronomy Research*. **4**: 461-464.

Waldhoff, D., Furch, B. and Junk, W. J. 2002. Fluorescence parameters, chlorophyll concentration, and anatomical features as indicators for flood adaptation of an abundant tree species in central Amazonia: *Symmeria paniculata*. *Environmental and Experimental Botany*. **48**: 225-235.

Wang, G. G., Qian, H. and Klinka, K. 1994. Growth of *Thuja plicata* seedlings along a light gradient. *Canadian Journal of Botany*. **72**: 1749-1757.

Welsch, J. and Mc Clelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*. **18**: 7213-7218.

Whitkus, R., Cruz, M., Mota-Bravo, L. and Gomez-Pompa, A. 1998. Genetic diversity and relationships of cacao (*Theobroma cacao*. L.) in Southern Mexico. *Theoretical and Applied Genetics*. **96**: 621-627.

Wilder-Ayers, J. A. and Tolliver, J. R. 1987. Relationships of morphological root and shoot characteristics to the performance of outplanted bare root and containerized seedlings of loblolly pine. In Proceedings, 4th Biennial Southern Silvicultural Research Conference. Gen. Tech. Rep. Se-42 Asheville, NC: USDA Forest Service, Southeastern Forest Experiment Station. pp. 206-211.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingy, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. **18**: 6231-6235.

Wolfe, A. D. and Liston, A. 1998. Contributions of PCR-based methods to plant systematics and evolutionary Biology. In *Molecular Systematics of Plants.II. DNA Sequencing*. Edited by D. E. Soltis, P. S. Soltis. and J. J. Doyle. New York. Chapman and Hall. pp. 43-86.

Wolfe, K. and Morgan-Richards, M. 1998. PCR markers distinguish *Plantago* major subspecies. *Theoretical and Applied Genetics*. **96**: 282-286.

Woo, S. L., Noviello, C. and Lonito, M. 1998. Sources of molecular variability and applications in characterization of the plant pathogen *Fusarium oxysporum*. In *Molecular Variability of Fungal Pathogens*. Edited by P. Bridge, Y. Couteaudier and J. Clarkson. CAB International, UK. pp. 187-208.

Yeh, F. C. 2000. Population Genetics. In *Forest Conservation Genetics-Principles and Practice*. Edited by A.Young, D. Boshier and T. Boyle. CSIRO Publishing, Australia. pp. 21-37.

Yeh, F. C., Yang, R. And Boyle, T. 1999. POPGENE version 1.31: Microsoft Windows based Freeware for Population Genetic Analysis. A Quick User's Guide. A joint project developed by Centre for International Forestry Research and University of Alberta, Canada. pp. 25.

Ying Xiong, Q., De Yuan, H., Cheng, X. F. and Kenneth, M. C. 2004. Genetic variation in the endangered and endemic species *Changium smyrnioides* (Apiaceae). *Biochemical Systematics and Ecology*. **32**: 583-596.

Young, A., Boshier, D. and Boyle, T. 2000. *Forest Conservation Genetics: Principles and Practice*. Edited by A. Young, D. Boshier and T. Boyle. CSIRO Publishing, Collingwood VIC, Australia. pp. 1-3.

Zhang, S., Keping, M. and Lingzhi, C. 2003. Response of photosynthetic plasticity of *Paeonia suffruticosa* to changed light environments. *Environmental and Experimental Botany*. **49**: 121-133.

Zietkiewicz, E., Rafalski, A. and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction. *Genomics*. **20**:176-183.

*original not seen