CHAPTER 2

REVIEW OF LITERATURE
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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 occurrence 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 AluI 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; Srekanth, 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 Arctic (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. Furthermore, 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 competition 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* possesses 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 CO₂ 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 F₀ (minimal fluorescence), Fᵥ (variable fluorescence) and Fₘ (maximum fluorescence) parameters of chlorophyll a fluorescence for testing.

2.4.3. Fᵥ/Fₘ

The ratio of variable fluorescence to maximal fluorescence (Fᵥ/Fₘ) of dark-adapted leaves is used commonly to assess the relative state of PSII. Fᵥ/Fₘ 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, \( F_v/F_m \) 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 discriminate 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 differentiation $G_{ST}$ (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 (90-94°C), primer annealing (30-36°C) and primer extension (72°C). The template DNA is denatured by subjecting to temperature between 90-94°C. In annealing step, temperature is rapidly
reduced to 30-36°C 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 (Arnaud *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 comparative 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 (Snyder 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 been 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 *Colletotrichum 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).