Chapter 1

General Introduction

Over the past few years great attention and concern is given to the conservation of biodiversity in India, which is blessed with splendid plant wealth. World agriculture will be hard hit if the genes lodged in our natural forests and other wild ecosystems are wiped out. Biodiversity is "the species richness occurring as an interacting system in a given habitat" and one of resourceful hotspots of biodiversity is the Western Ghats of South India. The tropical evergreen forests of Western Ghats is the home for the most precious economically important spice crop, the black pepper (*Piper nigrum* L) known as the 'King of Spices'.

Black pepper belongs to the family *Piperaceae* and it is widely consumed across the world. The International Pepper Community (IPC) has projected the world pepper production as 2.71 lakh tones for the year 2007-2008. India holds the supreme position in the world Pepper market because of its celebrated grades like `Malabar Garbled' and `Tellicherry extra bold'. Apart from India, other countries such as Malaysia, Indonesia, Thailand, Vietnam, China, SriLanka, Brazil and Madagascar extensively cultivate this spice crop. The humid climatic conditions and the inevitable consumption in the dietary requirements made this spice crop a synonym of the Asian continent. The finest Indian pepper is grown in the monsoon forests of the Malabar Coast in Kerala and hence Kerala accounts for 95 % of the black pepper produced in India. About 2,37,998 hectares of area in Kerala is under cultivation of black pepper that accounts for the production of 87,605 tons during the year 2005 -2006 (Spices Board of India). According to a study conducted by the Jakarta-based International Pepper Community (IPC), world pepper consumption is growing at a rate of 3.46 percent per annum. This increased demand could be overcome only by an increase in productivity against the various threats ensuing marginal fall in pepper production like the wide spread of disease (*Phytophthora* foot rot) in pepper plantations, unfavorable weather conditions in Kerala region etc.

A. Plant profile

Black pepper is a perennial climber, climbing on support trees with the help of aerial climbing roots. The picture of the plant profile is shown in Fig.1.1a to 1.1d. Commercial propagation is by stem cuttings and the young shoot tips in the runner

shoots are protected by sheathing petiole of the leaf. Flowers are mostly bisexual. Pepper is predominantly a self-pollinated plant. The pollen dispersal is aided by rain or dewdrops and also by the gravitational descending of pollen known as "geitonogamy". The fruit, often called as berry is spherical and has only one seed with a fleshy pericarp and hard endocarp. The dried fruit is the commercial source of this significant spice crop.

B. Origin and Genetic Diversity

Piper sp of Indian subcontinent was first described by van Rheede in his "Hortus Indicus Malabaricus"-the first printed document about the plants of Malabar coasts of Kerala. The genus *Piper* includes more than 1000 species; out of which 110 are of Indian origin (Ravindran and Nirmal Babu, 1994). About 14 species of *Piper* were reported by Gamble, 1925 from South India. The Western Ghats of Indian peninsula is the Primary Centre of Origin of black pepper. Kerala occupies a considerable portion of Western Ghats and is a rich source of gene pool of this spice crop. The genetic variability is high at the centre of origin. The hot and humid climatic condition of the sub mountainous tracts of Western Ghats is ideal for the cultivation of black pepper. It is believed that due to the heavy demand for black pepper, this was taken from the Malabar Coast to the Indonesian Islands and from there it might have been spread to various Pacific Island nations, South East Asian countries and later to tropical Africa and America. Currently pepper is cultivated in about 26 countries.

Ravindran, 1991 suggested three species namely *Piper wightii, Piper galeatum* and *Piper trichostachyon* as the putative parents of *Piper nigrum* based on the morphological and biosystematic studies. This conclusion was based on the nature of the shallow cup like bracts in *Piper nigrum* and this character seemed to be transitional between the connate, shoe-shaped bract in *Piper galeatum* and oblong bracts of *Piper wightii*. Recent surveys by scientists of Indian Institute of Spices Research (IISR) in certain areas of Western Ghats in Idukki district led to the discovery of intermediate populations, apparently comprised of hybrids between *Piper nigrum, Piper sugandhi, Piper trichostachyon* and *Piper galeatum* and their segregating populations. Various degree of sexual dimorphism was met with in these plants.

BLACK PEPPER PLANT PROFILE

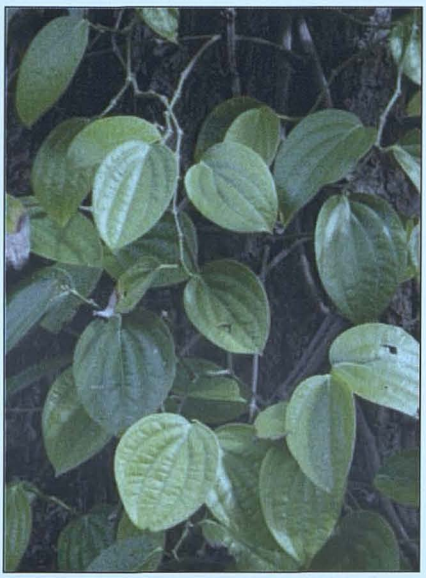

Fig.1.1a. Black pepper foliage

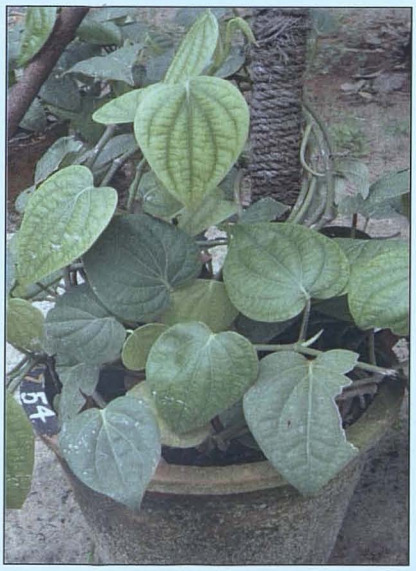

Fig.1.1b. A potted black pepper plant

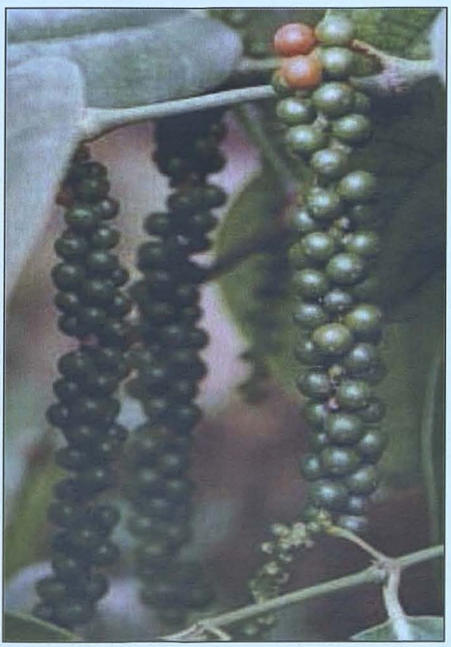

Fig.1.1c. Ripening fruit cluster

Fig.1.1d. Dried berries of black pepper

All these observations emphasized the view that pepper originated in the tropical evergreen forests of Western Ghats. A polyploidy series existed in the *Piper* genus as the reported chromosome number included 2n = 24, 26, 36, 39, 40, 48, 52, 60, 64, 65, 68, 80, 96, 104, 132 and 156. *Piper* sp. from South India and SriLanka showed a basic chromosome number x = 13; whereas species from North India exhibited a basic chromosome number of x = 12. Mathew, 1958 suggested x = 13 as the valid chromosome number of the *Piper* genus. Studies (Mathew 1972) in eleven cultivated and six wild *Piper nigrum* cultivars showed that the chromosome number was 2n = 52 for all except for the wild types having chromosome number 2n = 104. Similar variations were reported for the chromosome number in North Eastern Indian types with 2n = 36; 60 for South Indian types (Dasgupta and Dutta, 1976), 2n = 48 (Sharma and Bhattacharya, 1959) and 2n = 128 (Janaki Ammal, 1945) in *P. nigrum.* Cytological studies led to suggest that *P. nigrum* with 2n = 52 is tetraploid. This chromosome number (52) in *P. nigrum* could be due to its ancient polyploid origin, followed by diploidization during the course of evolution. Another notable feature was the progressive reduction in spike length that had accompanied evolution of the cultivated pepper varieties (Mathew, 1972).

The anticipated picture behind the origin of *P. nigrum* was that chance cross-pollination between different species of *Piper* might have occurred when more than one *Piper* species climbed up the same support trees during the course of evolution. Subsequent gene flow was restricted in these cross pollinated progenies, due to the absence of active pollen transfer mechanism. Later, high successful vegetative propagation led to the maintenance of any variation that have occurred during this chance cross pollination. Gradually survival and spread of these new progenies had occurred during the course of time. It is said that the present day *Piper nigrum* cultivars are the descendants of such segregating populations, which are vegetatively propagated by farmers through cuttings. Breeding and conservation programmes by humans based on qualities like their good fruit set, pungency etc, contributed to this cultivar diversity in black pepper. Cultivar diversity is richest in the state of Kerala followed by the state of Karnataka. Currently, more than 3000 accessions of black pepper could be seen in

the germplasm collection of black pepper maintained at IISR, Calicut (Sarma and Kalloo, 2004). Majority of the black pepper fields are now cultivated with landraces or with popular hybrid varieties. The advanced cultivars have been derived by the clonal selection from land races, though a few are of hybrid origin.

C. Advent of molecular markers

The analysis of genetic diversity between or within different species is a prerequisite for effective utilization and protection of plant genetic resources (Weising *et al.*, 1995). Unlike morphological and biochemical markers that may be affected by environmental factors, a DNA marker portray genome sequence composition, and helps to detect difference in the genetic information carried by different individuals. The hype in molecular marker technology was really a boon to molecular biologists. The concept of using variations at the DNA level as genetic markers started with the Restriction Fragment Length Polymorphism (RFLP) (Grodzicker *et al.*, 1974). This was the first molecular marker generated for genome analysis and mapping. In RFLP technique, the DNA of different individuals is digested with restriction enzymes and the difference in the size of the resulting fragments is visualized through Southern hybridization using labeled probe. The difference in size happen due to evolutionary changes as a result of point mutations, deletions or insertions, inversions or translocations in existing chromosomes leading to change in nucleotide sequence in the DNA of different individuals. But RFLP analysis required large quantities of high quality DNA, and involved several time-consuming and tedious steps and detection systems that used radioisotopes. Subsequently the conventional hybridization based assay of detecting DNA polymorphism was replaced by a more rapid Polymerase Chain Reaction (PCR) based assay.

PCR analysis is fast and easy to perform and required only a few nanograms of DNA. Speed, efficiency, and safety consideration had led investigators to evaluate PCR-based analysis as an alternative to Southern-blot analysis for detection of restriction fragment length polymorphisms. PCR assay gave way to the new DNA markers that were responsible for revolutions in molecular genetics and these included Random Amplified Polymorphic DNA (RAPD) (Williams *et al.*, 1990), Degenerate

Oligonucleotide Primer-PCR (DOP-PCR) (Telenius, 1992), Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.*, 1995), Sequence Characterized Amplified Region (SCAR) (Paran and Michelmore, 1993), Inter Simple Sequence Repeats (ISSR) (Zietkiewicz *et al.*, 1994), microsatellites also known as Sequence Tagged Microsatellite Sites (STMS) or Simple Sequence Repeats (SSRs) (Akkaya *et al.*, 1992), Single Nucleotide Polymorphisms (SNP) (Jordan and Humphries, 1994) etc.

The choice of marker system to be used is critical as each marker has its own requirements, sensitivity and reliability. RAPD analysis is a rapid and simple PCR based assay resulting in the amplification of many discrete DNA products with arbitrary primers; still the generated band profile is irreproducible if the PCR conditions or equipment is changed. Better consistency and reproducibility in the banding profiles are exhibited by AFLP and SSR markers, where AFLP markers has the major advantage of analyzing wide range of genome by a single PCR based assay and SSRs exhibit high discriminatory power of closely related genotypes. On the whole, a comparison with other class of molecular markers suggests that microsatellites are considered as the ideal DNA markers due to their hyper-variable, co-dominant nature, with relative high abundance and random distribution in the genome (Powell *et al* 1996). Hence they are often the marker of choice for genome analysis, genetic mapping and diversity studies in plants (Westman and Kresovicch, 1998).

D. Evaluation of the black pepper germplasm

Despite the economic value and medicinal importance, the research focusing on black pepper has been scarce. A few studies based on morphological characters and other biochemical characteristics showed extensive inter- and intrapopulation genetic variability among the cultivars of black pepper. Significant morphological divergence among 44 major cultivars and seven wild collections of black pepper using 22 characters were already studied (Ravindran, 1991 and Ravindran *et al.*, 1997a and 1997b). They have also carried out a study on comparative chemical affinity among cultivars based on flavonoid profiles. Parthasarathy *et al.*, 2006 described about the biodiversity of *Piper* sp. in South India using Geographical Information System (GIS) based on 15 qualitative characters. From their observation, an absence of random

mating and free gene flow in *Piper* might have led to the isolation of small population that further had undergone divergence through segregation. A multivariate analysis was conducted in fifty cultivars of black pepper in Kerala resulting in the grouping of the cultivars into 12 major clusters (Mathew *et al.*, 2006).

Molecular marker based germplasm evaluation studies in black pepper was really scarce. RAPD based fingerprinting was the most prominent reported analysis which was conducted in 22 cultivars from South India and one accession each of *Piper longum* and *Piper colubrinum* (Pradeepkumar *et al.*, 2003). Male parent specific RAPD markers (George *et al.*, 2005) successfully identified hybrids in 11 black pepper accessions and other hybrid populations (IISR, Calicut). Sasikumar *et al.*, 1999 reported the use of isozyme technology in the identification of two interspecific hybrids of *Piper*. Thus the large genetic variability exhibited among the cultivars of black pepper should be taken into account during crop improvement breeding programmes.

E. Pepper in medicine

Black pepper is an important ingredient of drugs in Ayurveda, Unani and Sidha. It is used either alone or in combination with long pepper (*Piper longum)* and dry ginger (*Zingiber officinale*) - the combination popularly known as "Trikatu"- the three acrids which cures the three disordered humours –*Vata, Pitta* and *Kapha* and helps to maintain normal health. Pepper is antihelminthic and germicidal, employed as antiperiodic in obstinate fevers, useful in diseases of spleen, pain in the liver and muscles, leucoderma, lumbago and paralysis. An infusion of pepper forms a useful stimulant gargle in relaxed sore throat, hoarseness, toothache and inflammation.

F. Impact of the present study

The cultivar diversity is high at center of origin – the Western Ghats, the home for both unexploited and extinct varieties of pepper. Being a predominantly self-pollinated plant propagated through cuttings, the high variability shown by black pepper cultivars was surprising. This could be explained from the evidence in the past history of black pepper for natural and chance cross pollination between different *Piper* species leading to the building up of large populations of hybrids (Ravindran, 1991). The present day wild and cultivated black pepper are the descendants of such progenies and

any 'variations' inherited are maintained by each cultivar through its vegetative mode of propagation. Thus the present day black pepper is a 'precious gem' of unexploited resource of many agronomical important genes that may be applied to improve the major weakness of pepper industry like the low productivity, non-availability of sufficient healthy planting vines, and crop loss by biotic (diseases and pests) and abiotic (drought) stresses. Hence the conservation of genetic diversity within such a species of economic importance is indeed a prerequisite for the success of future breeding programs. Thus there is an immediate need for an efficient system that could systematically bring about the characterization of germplasm of black pepper, exhibiting high genetic variation. Characterization and evaluation of existing germplasm helps to identify and draft the genetic relationships among cultivars thereby avoiding redundancy and to isolate many valuable genes that may have the capability to overcome the major threats of pepper cultivation. Hence, molecular markers, that are unaffected by any external environmental factors were chosen for the present study. Molecular markers give consistent and reliable results that act as a stepping-stone for any crop improvement breeding programmes. The aid of molecular markers could ensure significant advancement in pepper research community.

GATEWAY TO THE CURRENT STUDY

In plants, molecular markers were first developed in 1985-86 by two groups of researchers working independently at Native Plants Incorporated, USA and Cornell University, Ithaca, USA. Subsequently molecular markers have revolutionised and modernised the field of biological sciences with their applicability in detection of variability in DNA sequence and evolved into a favorite tool for molecular biologists because of their greater ease and efficiency. DNA molecular markers have specific addresses on chromosomes and are the "signposts" of chromosomes. In the course of time, a large number of molecular markers were generated and each one of them differed in its information content.

Black pepper is one among the best known and widely consumed spices. Even though high variability existed in black pepper germplasm, the precise relationship between cultivars at the genetic level was unknown. The biodiversity of pepper in Western Ghats could be a good resource of numerous unidentified cultivars which could be lost in course of time, if they are not characterized. With this limited knowledge, the present journey started to unravel the genetic relatedness among black pepper cultivars with the aid of a multi-locus AFLP marker and single locus SSR marker that is discussed in the following chapters……..