Summary and Conclusions

The inexorable spicy nature of black pepper accounts for its supreme position and hence preferred as the 'King of Spices'. At present, various biotechnological approaches are enhancing towards the dream of producing a pepper genotype with high yield, quality, and resistance to biotic and abiotic factors. The germplasm characterization in black pepper is extremely important as the cultivar diversity is high at center of origin – the Western Ghats, the home for unexploited varieties of pepper.

The present study revealed certain facts which are summarized below:

1a. AFLP analysis revealed the high level of polymorphism (>90%) and the extensive range of similarity value (6.01 to 98.13) among black pepper cultivars.

1b. The selected AFLP primer pair combinations; EAGC/MCAA, EAGG/MCTA and EAGC/MCTG were efficient to discriminate almost all of the genotypes successfully to a certain extent. AFLP markers outgrouped certain cultivars like acc. nos. 5, 6, 15, 3, 4, 2 and 1 that diverged at less than 75% similarity and identified four unique cultivars with less than 60% similarity with the rest of the genotypes like Konnamankara (acc.no.25), Thevanmundi (acc.no.12), Karimunda (acc.no.16) and Local 'e' (acc.no.7).

2a. The alternative approach by 'Genome Walking' proved to be a turnover to the less fruitful conventional microsatellite enrichment technique applied in *Tsp*5091 and genomic AFLP fragment assembly of black pepper. Very few reports have utilized Genome Walking protocol for development of SSR markers. To the best of our knowledge, this is the first report for successful development of SSR markers in black pepper by an efficient Genome Walking approach. Seven new SSR primer pairs were developed for genotyping, out of which four were found to be the most suitable based on the polymorphic banding pattern. A comprehensive analysis with all the four SSR primer pairs discriminated almost all the genotypes, thereby establishing the distinctiveness of each accession. Among the SSR primers, PnAG30 could be considered as the most informative marker with PIC content >0.95.

2b. The species-specific genotyping using Real-Time PCR was in concurrence with the results observed with SSR analysis and thus proved to be successful in discriminating different species of black pepper by generating characteristic DNA

melting peaks. Still, Real-Time analysis did not show a high resolution when compared to the automated fluorescent analysis. The SSR primer, PnGT2 marker was found to be the most efficient which discriminated all the four *Piper* species studied.

2c. A precise picture regarding the genetic correlation of black pepper cultivars was revealed by SSR analysis and the major findings are noted below:

i. Certain local unnamed landraces showed close association with popular cultivars at the genetic level. {The close association was between Local e (acc.no.7) with Karimunda (acc.no.3) and Vattamundi (acc.no.9); Local f (acc.no.8) with Neelamundi (acc.no.10) and Local g (acc.no.22) with Vokkale (acc.no.30)}.

ii. Panniyur -1, the only hybrid variety showed more similarity with Nedumundi (acc.no.6), Local f (acc.no.8), Karinthakara (acc.no.23), Kaniakadan (acc.no.42). Kuching, a popular Malaysian cultivar showed more similarity with Panchami (acc.no.38), Kalluvally(acc.no.41), Karimunda(acc.no.36) and Sreekara(acc.no.37) which emphasized the introduction of black pepper from South India to Malaysia. Sreekara is the selection from Karimunda variety (KS-14). SSR analysis demonstrated this genetic similarity by grouping Karimunda (acc.no.36) and Sreekara (acc.no.37) together in the PCA plot.

2d. SSR analysis was able to identify local cultivar 'b' (acc.no.2), Karimunda (acc.no. 16) and Wild (acc.no.34) as the most divergent genotypes since these outgrouped from all the rest of the cultivars.

3. Eventhough the cultivars analyzed for AFLP fingerprinting were less, an overall comparison of AFLP and SSR analysis revealed that certain cultivars outgrouped from the rest after both the analysis. This indicated the genetic distinctiveness of these cultivars which includes Local a (acc.no.1), Local b (acc.no.2), Local c (acc.no.4), Local d (acc.no.5), Thevanmundi (acc.no.12), Karimunda (acc.no.16) and Konnamankara (acc.no.25).On the whole, AFLP and SSR markers together unambiguously distinguished Karimunda (acc.no.16) as the most divergent genotype.

4a The role of SSR motifs in the function of plant genes has been poorly detected. This is the first report from black pepper regarding transcribed regions bearing

microsatellite motifs and identification of putative miRNA precursors associated with microsatellites. The approach of 'SMART technology' successfully generated full length cDNA library in black pepper and the presence of microsatellites in the transcribed regions of genes was confirmed. Screening more clones available in the generated library could enhance the discovery of agronomically important genes associated with microsatellites in black pepper.

4b. Putative miRNAs precursor was identified from the isolated mRNA sequence of black pepper and this may play an important role in cellular and developmental processes. The close association of dinucleotide repeat like (CT) in the 'pre-miRNAs' proved that microsatellites may have specific roles in various biological processes which are yet to be elucidated.

The major conclusions drawn from the study are:

1. Black pepper, being a perennial spice crop, fingerprinting studies negotiates the traditional methods for identification of cultivars, which will take several years. AFLP and SSR markers together proved its potential by bringing out the genetic relatedness and precise discrimination of the cultivars and revealed the high genetic variability among the popular cultivars.
2. Real-Time PCR analysis could be utilized for an initial rapid genotyping analysis in varieties with no other previous history of polymorphism data reported.
3. The development of gene derived microsatellite markers in black pepper could directly identify variation in the transcribed regions of genomes, thus enhance their value in marker assisted selection, comparative genetic analysis etc.
4. The identified miRNA has to be validated in detail to study its regulatory role in various biological processes. The presence of miRNAs in black pepper suggested an ancient origin of these important regulatory units. These initial results are relevant for several genetically applied studies and thus eliminate the concept that microsatellites are just 'junk DNAs' without any specific function.