1. INTRODUCTION

Forest trees are highly complex plant systems grow extensively as wild populations and harbor enormous genetic diversity. Biological diversity in tree species is extremely essential to evolve and adapt to changing environment, to support the ecosystem functions and for intentional genetic improvement. World’s forest cover is about 4 billion hectares which cover 31 percent of total land area and serve as premier source of timber and fuel wood. Thirty percent of the world’s forests (~ 1.2 billion hectare) are primarily used for production of wood and non-wood forest products (FAO, 2010). The ever increasing demand for wood and the huge decline in the native forests and associated loss of biodiversity diverts the attention towards plantation forestry. Plantation forestry aims to increase the productivity from limited land area, which involves the cultivation of fast growing short rotation tree crops like *Eucalyptus, Acacia, Populus,* etc.

Among the industrial plantation tree species, *Eucalyptus* wood has high global demand for short-fiber pulp. Compared to other tree species, it displays high fiber count along with uniformity of fibers, which is exploited for production of coated and uncoated free-sheet paper, bleach board, sanitary products, etc. It also finds limited uses for top liner on cardboard boxes, as a corrugating medium, and as filler in long-fiber conifer products such as newsprint and containerboard. Eucalypts are excellent sources of carbon sequestration, with carbon sequestering capacity of 14 tons/ha/yr in fast growing tropical plantations. Eucalypts have an average annual fixation rate of 10 tons of carbon per hectare. Each eucalypt tree can fix 20 kg of CO$_2$ per year. This attribute is particularly important when eucalypts are used for reforestation of degraded lands or areas abandoned by intensive agriculture. These factors drive the commercialization of eucalypt plantations in many countries. Plantation forestry of *Eucalyptus* occupy an area of more than 20.0 million ha world-wide. Majority of the plantations exists in Brazil and India with 6.3 Mha and 3.9 Mha, respectively (http://git-forestry.com/Global_Eucalyptus_Map.htm).
Eucalyptus has been targeted as a major genus of the Myrtaceae family due to its immense applications being the source of timber or fiber for multiple industrial purposes and its wider adaptability to various climatic conditions. They are native to Australia and its adjacent islands and are grown as exotic plantations, outside its native range. With around 700 species, they are planted throughout Asia, South America, southern Europe, New Zealand and Australia like 90 countries world-wide (FAO, 2005). The worldwide importance of Eucalyptus species coupled with its relatively small genome size (~ 700 Mbp) has driven the whole-genome sequencing efforts. Joint Genome Initiative’s (JGI's) comparative plant genome database, Phytozome (http://www.phytozome.net/) has made publically available the annotated E. grandis genome assembly, E. globulus re-sequencing data, and all supporting EST sequences during the year 2011. The Kazusa DNA Institute in Japan has produced a draft assembly of the genome of E. camaldulensis. The importance of this genus has been motivating most of the genomic research in the family and further considered keystone species for ecological studies in their natural ranges. The species dominate multiple places and the species-richness makes the taxonomy of the family difficult. Moreover the vast synteny occurring within the species assist in the significant advances in other related species which may be possible from the improvement in Eucalyptus species.

Among the genus, E. tereticornis and E. camaldulensis are two most important species commonly planted in India, due to their suitability to the temperature and climatic conditions of the country. Hence most of the paper industries in India depend upon Eucalyptus plantations for their pulp resources. Indian Newsprint and Paper industry mainly depend on the species like Eucalyptus and therefore it is highly essential that the planting stock should be of high genetic quality which influences the yield from plantations. Tree improvement programs are being carried out in many research organizations and research departments of paper industries for identification of quality planting stock. Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore has implemented genetic improvement program for two eucalypts species namely, E. tereticornis and E. camaldulensis in the year 1995 under the World Bank funded Forestry Research Education and Extension (FREE) Project with the co-ordination from CSIRO, Australia. Provenance trials and progeny trials for these species were established since 1995. Superior performing plus trees were identified from these trials to give impetus to clonal forestry.
program with pure species instead of the use of landraces. Recently a network project has been sanctioned to IFGTB by the Department of Biotechnology, Government of India, for the improvement of these species by mapping Quantitative Trait Loci (QTL) for pulping and rooting traits. The work is in progress with the collaborators from the state forest departments and paper mills of Karnataka and Tamil Nadu.

Breeding and propagation technologies play a significant role in tree improvement programs. Breeding of eucalypts for industrial plantation forestry developed rapidly in many countries after 1960s. During early stages of eucalypt domestication, large genetic gains have been obtained through species and provenance selection followed by individual selection and establishment of seed orchards. A major step forward in eucalypt plantation technology occurred after establishment of the plantation of the first commercial stands of selected clones in the 1970s. Since then, vegetative propagation has enhanced the genetic gains of breeding programs by capturing both additive and non-additive effects into elite clones. Breeding experience a major constrain of capturing years and years to achieve a sustainable genetic gain. Vegetative propagation gains significance by making it possible to multiply desirable genotypes in a short period of time. Eucalypt hybrids deployed as clones currently make up a significant proportion of the existing commercial plantations and are recognized as some of the most advanced genetic materials in forestry.

Genetic improvement in *Eucalyptus* breeding primarily involves the focus on the traits related to productivity and wood quality, the main sources for the pulp and paper industry. In addition, stress tolerance, synthesis of essential oil, are also investigated for particular pharmaceutical purposes. In the course of increasing productivity, growth traits such as seedling height or vigor, and the morphological traits are also considered into study. However, limited studies target on vegetative propagation competence of elite clones, the trait which shows variation within and across the species. Clonal propagation along with hybrid breeding has become a powerful combination of tools for the improvement of primary traits. Productivity of clonal eucalypt plantations was reported to reach up to 100 m³/ hectare/year when the best clones are used on the best sites. IFGTB has released four best performing clones of *E. camaldulensis* during the year 2010, with best productivity and rooting capabilities.
Adventitious root formation on vegetative cuttings is a key parameter influencing the viability of a clonal forestry program for any species. It is the most complex phenomenon, influenced by physiological status of the donor plant, environmental factors including temperature, humidity treatments with rooting hormones and genetic architecture of the plant system. Although physiological and environmental factors play major role in the success of vegetative propagation, the trait exhibits moderate to high heritability. In eucalypts, easy to root and rooting recalcitrant species were identified and high within species variability was observed. Variable rooting potential (0 to 100 %) among the selections are considered as a hindrance to the clonal propagation. Hence, many breeding programs target intra- and inter-specific hybridization for the transfer of vegetative propagation traits. In species like Eucalyptus inter specific hybrids provide an opportunity to capture significant genetic gain which are propagated by means of clonal methods. Adventitious rooting is a quantitatively inherited trait i.e. controlled by many genes/loci (polygenic).

In addition to the significant gains achieved through the intensive analysis of the rooting traits, understanding the genetic architecture of these traits is essential for further improvement. Improvement of adventitious rooting could be enhanced through the dissection of the QTLs controlling vegetative propagation traits employing various molecular markers. Molecular markers, the breeder’s tool, are continuously exploited in genetic diversity estimation, propagation systems, seed orchard management and marker-trait associations to accelerate the genetic improvement of a particular species. Marker assisted breeding have high economic value in forest tree species as each tree occupies a considerable amount of land area for several years. Different type of DNA markers like RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeats), SSR (Simple Sequence Repeats), SNP (Single Nucleotide Polymorphism) and DArT (Diversity Array Technology) are available for various genetic studies. Among them RFLP, SSR and SNP markers are of greatest interest to many molecular breeders. The low throughput and employing of labeled probes make RFLP marker less preferable. SSRs are preferred markers for genetic studies, because of their co-dominant mode of inheritance, and are highly informative, highly polymorphic, locus specific and are easily transferable across the species within the genus. Moreover, they are the potential
markers for genome and QTL mapping studies. Also, these markers are amenable to automated genotyping strategies.

Mapping of QTLs through linkage mapping is one of the tools in identifying the genetic basis of quantitative traits. Identification of specific genes or alleles that are associated with the trait, improves the potential to shorten the breeding cycle of forest trees. In outcrossing tree species QTL mapping strategy essentially depend on inter-specific hybrid generation, pseudo-testcross strategy based linkage map construction and localization of QTLs on the consensus map. However construction of genetic linkage maps and QTL identification are difficult tasks because of their highly out-crossing nature, inbreeding depression and high heterozygosity and long generation time. Further, the stability and resolution of QTLs are affected by several factors like the type of mapping population and the number of individuals analyzed, heritability of the trait, statistical methods used, genome size, linkage disequilibrium and type of marker system. In tree species construction of organized pedigrees from controlled breeding crosses is not possible to take up additional fine scale mapping. Consequently, the utilization of QTL markers to marker assisted selection becomes challenging. Hence, linkage disequilibrium based association mapping approach, which does not require specific pedigreed populations as in conventional QTL approach, gains importance.

Association mapping (AM) has been suggested as an alternative approach for dissecting the genetic architecture. In the recent years, LD based association mapping has been successfully applied in crop species for the identification of QTLs and genes controlling the phenotypic traits. AM refers to significant association of a molecular marker with a phenotypic trait. AM relies on Linkage Disequilibrium (LD) which refers to non-random association between two markers or two genes/QTLs or between a gene/ QTL and a marker locus. The technique involves the examination of populations of unrelated individuals (although they may be related through distant ancestry), wherein the phenotypic variation among individuals is correlated with DNA polymorphism.

Linkage disequilibrium reflects the size of chromosomal segments remaining intact in a population. During meiosis, crossing over occurs between the homologous chromosomes from the two parents leading to recombination in the chromosomes and hence the offspring consists of
derivatives of DNA from either parent. In such process if the two loci are closer on the chromosome the chances of crossover are less and those loci will therefore be more likely inherited together due to physical linkage. This kind of segregation results in linkage disequilibrium of the particular loci, which is useful in identification of trait variations. Information about population structure and linkage disequilibrium is essential for employing association mapping for complex trait identification. Most of the LD studies have focused on model plants and crops like maize and wheat, with very few analyses in forest trees. Both DNA markers and candidate genes were the target for linkage disequilibrium estimation in plant species to facilitate genomic and gene based association studies.

The pattern and extent of LD determines the number of DNA markers required for successful identification of DNA markers linked to a particular phenotypic variation. LD can vary within the genome and among the populations. Unlike crop plants, the pattern of LD was not explored in the forest tree species. Also, limited studies have been carried for dissection of QTLs in forestry species using AM approach. Hence, the present study aims at estimating the LD in the eucalypts species using SSR loci in natural and synthetic populations followed by associating the markers with the adventitious rooting traits.

The present study includes the following objectives:

1. Analysis of population structure in natural populations of *E. camaldulensis* and *E. tereticornis* and their linkage disequilibrium estimation using SSR loci.

2. Association analysis and tagging of DNA markers for the adventitious rooting traits in *E. camaldulensis* and *E. tereticornis* populations.