INTRODUCTION
1. INTRODUCTION

The genus *Eucalyptus* commonly referred to as ‘eucalypts’ are long-lived, evergreen species belonging to the family Myrtaceae (Brooker, 2000). Most of the species are native to Australia and have been introduced to India, France, Chile, Brazil, South Africa and Portugal in the first quarter of 1800s (Doughty, 2000). There are more than 900 species and subspecies of eucalypts, mostly native to Australia, and very few species are distributed in adjacent areas of New Guinea and Indonesia (Boland *et al*., 2006; Australia's State of the Forests Report, 2013). It is one of the widely planted hardwood species in the world because of its superior growth, adaptability and wood properties and occupies 20.07 Mha globally. India ranks second in terms of total land area under eucalypts plantation [3.943 Mha] after Brazil [4.259 Mha] (Booth, 2012).

The genetic improvement programs in eucalypts involve selection of elite plants for clonal propagation, establishment of seed orchard, hybridization orchard and generation of full-sib hybrids with desirable traits. The most commonly targeted trait for improvement in this genus is the wood property traits, due to its industrial implications. In general, *Eucalyptus* wood density is widely regarded as the controlling factor influencing paper and pulp quality (Malan and Arbuthnot, 1995). The rapid rise in eucalypts plantation throughout the world during the past 60 years is mostly attributed to its superior fibre and pulping properties along with the increased global demand for short-fibre pulp (Turnbull, 1999; Bhuiyan, 2008). Eucalypts are still in early stages of domestication implying that understanding the genetic and genomic architecture of this genus is pivotal for breeding programs (Poke *et al*., 2005; Myburg *et al*., 2007; Grattapaglia and Kirst, 2008). The small genome size with less repetitive DNA, high diversity and ability to produce large progeny sets has facilitated extensive genetic and genomics research in different species of eucalypts in the past two decades to advance the process of domestication for targeted improvement of the species.

In the last few decades, major thrust was given to understanding the genes governing the complex processes of wood formation by large-scale genomic approaches in both annuals and perennials (Mellerowicz *et al*., 2001). The research on this complex process was initiated by understanding the gene functions in simple model systems like *Zinnia*
elegans (Fukuda, 1992, 1997; Milioni et al., 2001) and Arabidopsis, which possess highly developed vascular systems for carrying out xylogenetic processes (Turner and Somerville, 1997; Taylor et al., 1999; Zhong et al., 2008). Based on these model systems in annuals, studies using functional genomics approach has been undertaken in woody perennials with Populus as model system (Mellerowicz et al., 2001) followed by Pinus (Brown et al., 2003; Gonzalez-Martinez et al., 2007) and E. grandis (Hertzberg et al., 2001; Lorenz and Dean, 2002; Paux et al., 2004) to understand secondary growth and xylem differentiation. However, the genetic control of wood formation in trees is likely to be in variance with their annual counterparts emphasizing the need to explore the functions of genes involved in wood formation. Since wood formation is complex quantitative trait, it is predicted that they are controlled by many genes with an additive effect on the phenotype (Neale and Savolainen, 2004). Recently, it was predicted that there is a tight genetic control for several wood property traits and therefore targeted breeding programs using genomic platforms can provide phenotypes with superior quality wood traits suitable for commercial use (Wu et al., 2013).

Wood is primarily composed of lignin, cellulose and non-cellulosic polysaccharides (including neutral and pectic polysaccharides), which associate to form a complex structure that strengthens the massive form distinctive to woody perennials. Wood properties influence pulp and paper quality. The pulp yield is directly related to the cellulose content, while variation in hemicellulose content is associated with modifications in pulp cohesiveness, and pulping efficiency is correlated with lignin content (Evtuguin and Neto, 2007).

Cellulose forms the structural framework of plant cell wall and is the world's most abundant biopolymer, with over 180 billion tons of organic matter produced in nature every year (Delmer, 1999; Singh, 2011). Cellulose deposition acts as a foundation step for proper cell wall formation, growth and development that forms the most essential biochemical process during cell growth and differentiation (Zhong et al., 2008). The cellulose content in secondary wall is higher than the lignin and plays a vital role as the useful constituent for several industrial applications (Van Acker et al., 2013). However, the quality and quantity of cellulose deposited in the primary and secondary cell walls of plants vary in accordance with their biological function. The molecular basis of such
cellulose heterogeneity in the primary and secondary cell wall is poorly understood in tree species (Kalluri and Joshi, 2004; Grushetskaya, 2010).

Cellulose is synthesized in the plasma membrane by a large membrane-bound multi-enzyme complex known as the Cellulose synthase complex (CSC) (Delmer and Amor, 1995; Diotallevi and Mulder, 2007). Cellulose synthases (CesA), the enzyme involved in the cellulose biosynthesis, are the most enigmatic and elusive components of cell wall synthesis machinery. Plant CesAs belong to a multigene family with a co-ordinated and complex expression pattern. Specialized plant cells (e.g., xylem and phloem fibers) sequentially deposit primary and secondary cell walls that differ significantly in the quantity and quality of cellulose along with their biological function.

It is reported that the grouping of specific CesAs may be necessary and active in different cell types at different time points. Nevertheless, this combination of CesA may also be necessary in the same cell type at various stages, facilitating different functions (Mendu et al., 2011). Recent reports in several plant species reveal that two groups of CesA gene families exist which are associated with either primary or secondary cell wall deposition. Various catalytic subunits of cellulose synthase genes have been reported from green plants which are deposited in NCBI and include 5774 sequences from 260 plant species (http://www.ncbi.nlm.nih.gov/nuccore) of which around 2000 sequences are reported from trees. Recently, the complete genome sequence of E. grandis (‘BRASUZ1’) was released with genome size of 640Mb that has been organized into 11 pseudo molecule (Myburg et al., 2014). The location of CesA genes were mapped in the complete genome of E.grandis and deposited in Phytozome v10, where CesA1 (Eucgr.D00476.1) was positioned in 4th chromosome scaffold, CesA2 (Eucgr.A01324.1) in 1st chromosome, CesA3 (Eucgr.C00246.1) in 3rd chromosome, CesA4 (Eucgr.G033380.1) in 7th chromosome, CesA5 (Eucgr.H00939.1) at 8th chromosome and CesA6 (Eucgr.B01532.1) at 2nd chromosome.

Eucalyptus tereticornis Sm., commonly known as forest red gum has an extensive natural distribution from southern Papua New Guinea (PNG) to southern Victoria of Australia (5° 20’S – 38° 08’S). In Australia, it generally occurs in a long narrow strip along the eastern coastal regions and in high rainfall areas of the northern Queensland (Potts and Pederick, 2000). This species ranks among the most extensively planted
Eucalyptus in the tropics and subtropics (Zobel et al., 1987; Evans, 1992). It has been introduced in several countries to provide wood for fuel, poles, construction and pulp. E. tereticornis is one of the most widely planted hardwood tree in the Indian subcontinent to provide raw material for pulp wood industries. The popularity of the species, as that of other widely planted eucalypts is attributed to its rapid growth and production of desirable wood, when grown in a wide range of environmental conditions (Gan et al., 2003). Nevertheless, molecular studies in this species are limited to the use of molecular markers towards genetic diversity analysis within the species, seed orchards, species identification and genetic/QTL mapping (Poke, 2004; Tiwari et al., 2013).

The molecular aspects of cellulose biosynthesis are presently unknown in E. tereticornis, hence the present study was undertaken to identify different gene families of cellulose synthases, their expression in different tissue types and isolation and characterization of developing xylem specific CesA genes. Additionally, the specific expression of CesA genes during hormone signalling was also studied. This study would help to improve the understanding on genes involved in synthesis and deposition of cellulose during wood formation in E. tereticornis. The Objectives of the present study included:

**OBJECTIVES**

- In silico sequence analysis of cellulose synthase super family members to design specific primers.
- Expression profiling of cellulose synthase gene families in *E. tereticornis* tissues.
- Isolation of full length developing xylem specific cellulose synthase genes from *E. tereticornis*.
- Effect of phyto-hormones on the expression of developing xylem specific cellulose synthase gene transcripts.