Biotechnology 8 (4): 416-424, 2009
ISSN 1682-296X
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Molecular Cloning of Cellulose Synthase Gene, *SpCesA1* from Developing Xylem of *Shorea parvifolia* spp. *parvifolia*

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Abstract: This study reported the isolation and *in silico* characterization of full-length cellulose synthase (*CesA*) cDNA from *Shorea parvifolia* spp. *parvifolia*, an important tropical hardwood tree species. Cellulose synthase (CesA) is a member of processive glycosyltransferases that involved in cellulose biosynthesis of plants. The full-length of *SpCesA1* cDNA with size 3308 and 3120 bp open reading frames encoding a 1040 amino acid was isolated using RT-PCR and RACE-PCR approaches. The predicted *SpCesA1* protein contained N-terminal cysteine rich zinc binding domain, 7 putative transmembrane helices (TMH), 4 U-motifs that contain a signature D, D, D, QxxRW motif, an alternating conserved region (CR-P) and 2 hypervariable regions (HVR). These entire shared domain structures suggest the functional role of *SpCesA1* is involved in cellulose biosynthesis in secondary vascular tissues of *S. parvifolia* spp. *parvifolia*. Sequence comparison also revealed the high similarity (87%) among *SpCesA1* and PtrCesA2 of *Populus tremuloides*. This further implies the involvement of *SpCesA1* in catalyzes the cellulose biosynthesis of secondary cell wall rather than primary cell wall. Thus, identification of new *CesA* genes from tropical tree genomes is essential for enhancing knowledge of cellulose biosynthesis in trees that has many fundamental and commercial implications.

Key words: Cellulose synthase (CesA), cell wall biosynthesis, *Shorea parvifolia* spp. *parvifolia*, RACE PCR, wood formation

INTRODUCTION

Cellulose synthase (CesA) is a key enzyme that responsible for the biosynthesis of cellulose (Campbell *et al*., 1997). More properly designated as "ellulose synthase catalytic subunits", the CesA protein is an integral membrane protein, consisting of approximately 1,000 amino acids. It is imaged by TEM as a rosette consisting of six particles which is termed rosette terminal complexes (RTC) (Brown and Montezinos, 1976). Kimura *et al*. (1999) later confirmed that the RTC are the sites of cellulose synthesis after carrying out immunolocation of putative cellulose synthase catalytic subunits in the rosette subunits. The rosette portion of the terminal complexes (TC) is approximately 25 nm in diameter when viewed in freeze-fractured plasma membranes. Recently, Saxena and Brown (2005) discovered that the rosette portion and its six subunits are localized to the innermost leaftlet of the plasma membrane. They also found that the cytoplasmic portion of the TC contains the globular region of the catalytic subunits and is approximately 40-60 nm in diameter.

Genes encoding CesA proteins in plant were first identified in cotton (*Gossypium hirsutum*) fibers (Pear *et al*., 1996) and later their roles in cellulose synthesis were confirmed in the *Arabidopsis rsw1* mutant by Arioli *et al*. (1998). To date, there are six classes of CesA in higher plants with most of the information coming from *Arabidopsis thaliana*. Multiple *CesA* genes that have been identified in the *Arabidopsis* genome show high similarity to the cotton *CesA* cDNAs (Holland *et al*., 2000; Richmond, 2000). In *Arabidopsis*, it has been found that at least four *CesA* genes, namely *AtCesA1* (*rsw1*), *AtCesA2*, *AtCesA3* and *AtCesA6* are involved in the formation of primary cell walls and mutation or antisense repression of these genes cause a reduction in cellulose synthesis which is associated with the decrease in cell elongation (Arioli *et al*., 1998). Three other *CesA* genes, *AtCesA4*, *AtCesA7* and *AtCesA8* have been found to be responsible for the formation of secondary cell walls (Joshi, 2003). Mutation in these genes has brought about a dramatic reduction in cellulose content and secondary cell wall thickness,

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