Isolation And Characterization Of Hypervariable Region (HVR II) From Cellulose Synthase Gene In Neolamarckia Macrophylla

Chia-Nin Chang, Wei-Seng Ho, Shek-Ling Pang

Abstract: The present study is aimed to isolate and characterize the hypervariable (HVRII) region in cellulose synthase gene from the developing xylem tissues of a tropical timber tree species, *Neolamarckia macrophylla*. *N. macrophylla* is locally known as red kelampayan and it has been selected as one of the important reforestation tree species in Malaysia. RT-PCR was carried out by using the degenerate primers and one of the three amplified DNA bands was successfully sequenced and characterized. The sequence was named as *NmCesA1HVRII* and it was clustered in a distinct clade that is associated with secondary cell wall development. This study has generated a useful genomic resource for a better understanding about the HVRII region of CesA gene in *N. macrophylla* and its function which is important in future applications, genetic improvement of *N. macrophylla*. This also facilitates the future selection of trees with optimal cellulose content required for certain specific industries as well as synthesizing of artificial cellulose, hence increasing the economic development and growth in the country.

Index Terms: *Neolamarckia macrophylla*, reverse transcription-polymerase chain reaction (RT-PCR), cellulose synthase (*CesA*), hypervariable region II (HVRII)

1 INTRODUCTION

Cellulose is a linear and unbranched polymer which made up of glucose monosaccharide units [1]. It can be found in the cell wall of plants. Cellulose is being synthesized at the plasma membrane by “rosette” complexes through freeze-fracture studies [2], [3]. Rosette complexes are identified as cellulose-synthesizing complexes where there is localization of cellulose synthase. Each cellulose-synthesizing complex contains at least three isoforms of cellulose synthase [4]. Previous studies on different plant species have proved that there are ten or more homologs present in cellulose synthase (*CesA*) gene family [4]. The cellulose synthase enzymes are believed can be further categorized into two types which function in cellulose synthesis in primary and secondary cell wall of plants [5]. Betancur et al. [6] stated that cellulose synthase enzymes from the primary cell wall phylogenetic clades can also support the secondary cell wall thickening besides cellulose synthase enzymes from the secondary cell wall phylogenetic clades.

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In addition, there are at least six classes of CesA proteins exist in plants. In general, CesA protein consists of a zinc finger, two hypervariable regions (HVR I and II), several transmembrane domains and conserved residues [1], [7], [8], [19]. The hypervariable region II (HVRII) is made up of around 500bp to 600bp and the amino acid sequences between highly conserved motifs of ALYG and VISCG are associated with this HVRII region. This region might be involved in the regulation of quantity and quality of cellulose synthesized in plants [8]. It also plays a role in interaction with other unique cell-type-specific proteins involved in the cellulose biosynthesis [7] that may regulate and affect the wood quality and properties [4], [9], [10]. *Neolamarckia macrophylla* has other synonyms names which are *Anthocephalus macrophyllus, Bancalus macrophyllus* and *Nauclea macrophylla* [11]. It can be found in Indonesia, Malaysia, Vietnam, Filipina, Sri Lanka, Myanmar, Thailand, China and Papua New Guinea. It is a fast growing plantation tree species. The timber is durable and possesses red colour stem. It has fast growing rate where its height and diameter increases by 3 m and 7 cm, respectively per year. It is ready for logging within 4 to 6 years. It has high market price and value due to its good characteristics. In addition, it is resistant to pests and diseases. It is also valuable in the production of furniture and plywood as well as timber trade. It can be produced in large quantity and high productivity with good quality. Hence, this species is a good selection of tropical timber tree for research activities [11]. Despite the high economic value of *N. macrophylla* as one of the timber resources, little is known about the HVRII region in *CesA* gene of this important tree species. Therefore, the present study was aimed to isolate and characterize the HVRII region of *CesA* gene in *N. macrophylla*. It is hoped that this study could pave the way for a better understanding about the HVRII region of *CesA* gene in *N. macrophylla* and its function which is important in future applications.

2 MATERIALS AND METHODS

2.1 Total RNA Extraction and RT-PCR

Total RNA of *N. macrophylla* was extracted from the developing xylem tissues by using the RNeasy Midi Kit (Qiagen, USA). First-strand cDNA was synthesized according