American Journal of Bioinformatics

Original Research Paper

Bioinformatics Analysis of Xyloglucan Endotransglycosylase/Hydrolase (XTH) Gene from Developing Xylem of a Tropical Timber Tree *Neolamarckia Cadamba*

[1]Tiong Shing Yiing, [1]Ho Wei Seng, [2]Pang Shek Ling and [1]Ismail Jusoh

[1]Forest Genomics and Informatics Laboratory (fGiL), Department of Molecular Biology,Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak
[2]Applied Forest Science and Industry Development (AFSID), Sarawak Forestry Corporation, 93250 Kuching, Sarawak

Article history
Received 2014-07-31
Revised 2014-10-27
Accepted 2014-10-31

Corresponding Author:
Ho Wei Seng,
Forest Genomics and Informatics Laboratory (fGiL), Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak
Email: wsho@frst.unimas.my

Abstract: This study reported the isolation and *in silico* characterization of a full-length Xyloglucan endotransglycosylase/Hydrolase (XTH) cDNA from *Neolamarckia cadamba*, an important tropical light hardwood plantation tree species. XTH is considered as a key agent to regulate cell wall expansion and is believed to be responsible for the incorporation of newly synthesised xyloglucan into the wall matrix. The full-length of *NcXTH* was firstly predicted using the XTH singletons from the NcdbEST through contig mapping approach. Further validation and confirmation were conducted by amplifying the full-length XTH cDNA using RT-PCR approach. Two full-length XTH cDNAs, namely *NcXTH1* (JX134619) and *NcXTH2* (JX134620) were discovered and the nucleotide sequences were 893 and 1,024 bp in length, respectively. The open reading frames for *NcXTH1* and *NcXTH2* were 858 and 915 bp, respectively. Results predicted that *NcXTH1* and *NcXTH2* proteins carry out XET activity but they are from different XTH family members. This full-length *NcXTH* cDNA can serve as good candidate genes in association genetics study which leads to Gene-Assisted Selection (GAS) in the *N. cadamba* tree breeding programme.

Keywords: *Neolamarckia Cadamba*, Xyloglucan Endotransglycosylase/Hydrolase (XTH), Wood Formation, Contig Mapping, Protein Structure Prediction, Phylogenetics

Introduction

Wood formation or also known as xylogenesis is an ordered and complex developmental process in plants. It involves cell division, cell expansion, secondary wall deposition, lignification and programmed cell death. Most of the enzymes that involved in cell wall biopolymers synthesis are under the Carbohydrate Active enzymes (CAZymes) family. These include Glycosyltransferases (GTs), Glycoside Hydrolases (GHs), Polysaccharide Lyases (PLs) and various Carbohydrate Esterases (CEs) (Geisler-Lee *et al*., 2006). Xyloglucan endotransglycosylase/Hydrolase (XTH) is one of the glycosyltransferase members. XTH is considered as a key agent to regulate cell wall expansion and is believed to be responsible for the incorporation of newly synthesised xyloglucan into the wall matrix. In order for cell wall to expend, cross-linked or connections of microfibrils need to be broken. XTH is able to cut a xyloglucan chain and rejoin the reducing end to another xyloglucan molecule (xyloglucan endotransglycosylase, XET action) or to water molecule (xyloglucan endotranshydrolase, XEH action) (Fry *et al*., 1992; Darley *et al*., 2001; Rose *et al*., 2002).

A few mechanisms have been proposed in XET action. XTHs may join the newly synthesised xyloglucans into a larger xyloglucan polymer chains before integrated with existing xyloglucans in the cell wall layer (Campbell and Braam, 1999a). Transient polysaccharide-enzyme complex is probably formed between XTH and xyloglucan as the intermediate before being transferred and joined to another xyloglucan (Sulová *et al*., 1998). XTHs may also be involved in shifting the size of xyloglucans by XET action that allows cellulose microfibrils to move apart and/or past one another driven by tugor pressure (Fry *et al*., 1992; Talbott and Pickard, 1994). When there is no new xyloglucan released and supplied to the wall, XET

activity could lead to rearrangement of existing xyloglucans or degradation of deposited xyloglucans (Campbell and Braam, 1999a). Studies also suggested that XET activities may play an important role in fruit development. Decrease in *XTH* genes expression during fruit ripening suggest that XET action might contributes to fruit softening (Miedes and Lorences, 2009).

XTHs are family genes. *XTH* is reported as one of the key gene families in GH16 that are involved in cell walls modification (Ye *et al*., 2012). Expression studies of CAZymes in poplar shows that XTHs are one of the most highly expressed cell wall enzyme (Geisler-Lee *et al*., 2006). The evolution of *XTH* family gene through gene duplication and divergence has brought to the multi-members being reported: 33 *XTH* genes in *Arabidopsis* (Yokoyama and Nishitani, 2001); 41 in *populus* (Geisler-Lee *et al*., 2006); 29 in rice (Yokoyama *et al*., 2004; Yokoyama and Nishitani, 2004). Researchers have divided these large family members into a few subfamilies according to the gene structures and expression: Three (Rose *et al*., 2002) or four (Saladié *et al*., 2006) subfamilies in *Arabidopsis*; two main groups in rice (Yokoyama *et al*., 2004); three (Geisler-Lee *et al*., 2006) or four (Ye *et al*., 2012) groups in poplar; three major clusters in tomato and kiwi (Atkinson *et al*., 2009).

XTH proteins are predicted to have several structural features in common: A hydrophobic amino terminus which probably functions as a signal peptide to direct the protein to the cell wall; a highly conserved DEIDFEFLG domain that acts as the catalytic site for both transferase and hydrolase activities; an *N*-linked glycosylation consensus site which its function for XET activity still remain unclear; and pairs (four) of Cysteine (C) residues in the carboxyl-terminal region that might form disulphide bridges (Okazawa *et al*., 1993; Campbell and Braam, 1999b). Although many *XTH* genes and proteins were discovered, their detail functions in vascular tissues and in the formation of secondary walls are less well understood.

To date, there are considerable amounts of full-length XTH cDNA being published in NCBI but no such information available for *Neolamarckia cadamba* trees. *N. cadamba* or locally known as kelampayan belongs to the family of Rubiaceae. It has been selected as one of the fast growing plantation species for planted forest development in Sarawak (Tchin *et al*., 2012; Lai *et al*., 2013; Tiong *et al*., 2014a; 2014b; Ho *et al*., 2014). The state government of Sarawak has introduced the Forest (Planted Forest) Rules (1997) to encourage the development of commercial planted forests and has set a target of 1.0 million hectares for forest plantations to be established by 2020. It is estimated that 42 million of high quality seedlings are required for the annual planting programme. *N. cadamba* is a large, deciduous and fast growing tree that gives early economic returns within 8-10 years. Under normal conditions, it attains a height of 17 m and diameter of 25 cm at breast height (dbh) within 9 years. It is a lightweight hardwood with a density of 290-560 kg/m^3 at 15% moisture content (Joker, 2000). It is one of the best sources of raw material for the plywood industry, besides pulp and paper production. *N. cadamba* can also be used as a shade tree for dipterocarp line planting, whilst its leaves and bark have medical applications. The dried bark can be used to relieve fever and as a tonic, whereas a leaf extract can serve as a mouth wash (WAC, 2004). *N. cadamba* also has high potential to be utilized as one of the renewable resource of raw materials for bioenergy production such as cellulosic biofuels in the near future.

Hence, the objectives of this study were: (i) To obtain the full-length *XTH* cDNA sequences through contig mapping approach by using *XTH* singletons from the Kelampayan tree transcriptome database (NcdbEST) and (ii) to *in silico* characterize the *XTH* genes from *N. cadamba*. The full-length XTH cDNA discovered can serve as good candidate gene for association genetics study in *N. cadamba* to detect the potential genetic variants underlying the common and complex adaptive traits.

Materials and Methods

Hypothetical Full-Length XTH cDNAs Assembly from EST Singletons

Singletons of *XTH* gene were selected from the Kelampayan tree transcriptome database (NcdbEST) (Ho *et al*., 2014). The *XTH* singletons were blast again NCBI database to search for sequence homology and binding position on the respective gene. Subsequently, the singletons were grouped according to the alignment score and position on gene. Singletons which have overlapping fragment were then identified and jointed together to form the full-length sequences via contig mapping approach. Two hypothetical cDNAs (*XTH1* and *XTH2*) were used to design primer pairs for full-length *XTH* cDNAs amplification by using Primer Premier 5 software (PREMIER Biosoft International, USA). The oligonucleotide primers used for *XTH1* were NcXTH1-F (5'-ACAATGGCTTCTCATTTGAACT-3') and NcXTH1-R (5'-TTTGGCTCCTCTCAGATCG-3') and *XTH2* were NcXTH2-F (5'-CTTCTGATTCATCAATGGCTTC-3') and XTH2-R (5'-CATAGAGTTCATGTCCAGTGCA-3').

RNA Isolation and RT-PCR Amplification

Total RNA was isolated from the developing xylem tissues of *N. cadamba* using RNeasy® Midi Kit (QIAGEN GmbH, Germany) with modification. Total RNA was then reverse transcript into cDNA by using Ready-To-Go You-Prime First-Strand Beads (GE Healthcare, UK). RT-PCR amplification was carried out in a total reaction volume of 25 µL containing 1 x Advantage 2 PCR buffer (Clontech, USA), 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 10 pmol of primer pair, 1 x Advantage 2 Polymerase Mix (Clontech,