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EFFICACY AND SPECIFICITY OF PRIMERS DESIGNED FROM CONSERVED AND OPEN READING FRAME SITES OF CODING DNA SEQUENCES AND EXPRESSED SEQUENCE TAGS IN TEAK (*TECTONA GRANDIS*)

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ABSTRACT: Eight pairs of primers were designed from three CDS nucleotide sequences (*MYB1*, *MYB2* and *CAD1*) and one EST sequence (*CAD1)* of teak (*Tectona grandis* L.f.) through primer-BLAST tool by referring to their conserved and an ORF site (start and stop region). Seven pairs of the primers efficiently amplified teak genomic DNA. However, only two primer pairs showed specific single band amplification of the targeted genomic site. The remaining primers showed two or more bands of close to the expected size. The amplified single band (CADA, CADB and MYB2A) and the gel eluted double band (CAD1A) were sequenced and aligned with NCBI database for identification of target nucleotide sequences. The primers designed from EST of *CAD1* (2 pairs) were 100% efficient and specific for their targets. Thus, we conclude that the primer pairs obtained from EST sequence is more efficient and specific for amplification of the target site in the genomic DNA than those from CDS sequences.

Keywords: *BLAST, E-value, PCR amplification, query cover, sequencing, sequence homology*

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Polymerase chain reaction is an *in vitro* enzymatic technique used to synthesize multiple copies of specific segment of DNA from a pool of complex DNA (Mullis, 1990). An amplification of a particular gene requires specific primer pairs. The designing such primers is a tedious work. Xu et al. (2002) designed gene specific probe using open reading frame (ORF) site for microarray analysis. Cheua-ngam and Volkaert (2006) obtained gene specific primers from conserved region of the reference DNA sequence of teak. The coding DNA sequence (CDS) derived from mRNA consists of 5' un-translated region (5'UTR) followed by a ORF with a start codon (ATG), exonic region, stop codon (TAA, TAG, TGA) and 3' un-translated region (3'UTR). The CDS sequence translates into a protein, except 3' and 5' UTR regions. Similarly, the expressed sequence tag (EST) is a small sub sequence of CDS derived from cDNA library (Picoult-Newberg et al., 1999) and represents a portion of expressed gene. It is obtained through single shot of sequencing of cDNA library. Therefore, both CDS and EST are coding DNA sequence and are suitable for the specific amplification of the target gene.

There are some publically available tool for designing primers such as Primer-BLAST (Ye et al., 2012), Primer3 (Rozen and Skaletsky, 2000), primer3plus (Untergasser, 2007) etc. Primer-BLAST is a more advanced tool because it not only designs primer from the flanking region of interest but also checks their potential target in the genome (Ye et al., 2012). Conserved DNA fragments exhibit very little variation throughout the evolution. On the contrary, the start and stop position of a reference sequence represent a frame of DNA that translates into protein. Consequently, we selected four NCBI deposited cDNA, i.e. three CDSs and one EST of teak (Galeano et al., 2014; Galeano et al., 2015; Gomez 2015) to design gene specific primers from their conserved site and an ORF site followed by checking their efficacy and robustness for specific amplification of the targeted gene sequence in the teak genome.

MATERIALS AND METHODS

Designing of primers

Four reference genes were selected to design gene specific primers in teak (*Tectona grandis* L.f.). The primers were designed from CDS sequences of *MYB1* transcription factor (KR092428), *MYB2* transcription factor (KR092429) (Galeano et al., 2015) and *CAD1* (KX058438.1) (Gomez 2015) and EST sequence of *CAD1* (JZ515980) (Galeano et al., 2014) through Primer-BLAST (https://www.ncbi.nlm.nih.gov) software of NCBI. Primer-BLAST software has an advantage that we can specify the start and stop position of DNA template to design primers of our own selected site. Therefore, the selected template DNA was firstly