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ISOLATION OF *FLAVONOID 3'5'HYDROXILASE* GENE FROM SNAKEWEED (*Stachytarpheta indica* auct. non. (L.) Vahl)

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ABSTRACT

Stachytarpheta is one of Indonesian medicinal plants which have a wide species diversity. The bioactive component in snakeweed herb including naringenin, flavonol, and flavon are mainly derivate from phenyl propanoid (flavonoid). The gene encodes F3'5'Hydroxilation (*F3'5H*) involved in the biosynthesis of flavonoid product, by means of catalysis hydroxylation on the C atom #3 and #5 of benzene ring. Recently, has not yet meet publication about *F3'5'H* from snakeweed herb. The aim of this study was to isolate *F3'5H* gene from *Stachytarpheta indica*, using primers which were designed from conserve region of *Petunia hybrida F3'5'H* gene; *HF1* and *HF2* alleles. Forward primer is 5'-TGATGCTGCTAAAGCATTCT-3' and reverse primer is 5'GTGCACGCAGGTGACATATG-3'. The amplified fragments were un-specific non-consensus sequences, suggested that two homolog gene locus were isolated. Sequence analysis showed that both share two different domains; conserve-upstream and homolog-downstream domains. It is suggested that *Stachytarpheta indica* may possesses different alleles for *F3'5'H*, i.e. *HF1* and *HF2*, as of *Petunia hybrida.*

Key words: *F3'5'H* gene, *Stachytarpheta indica*, snakeweed

INTRODUCTION

Stachytarpheta indica or snakeweed (UK) and also locally known as Pecut kuda is herb which commonly used by Indonesian as medicinal plant, since it has some of pharmaceutical chemical compound including flavonoid (Roger, 2012). Based on its molecular structure flavonoid are grouped into flavonols, flavones, flavonon, isoflavone, cathecin, anthocyanidins, and chalcone (Ferreyra *et al*., 2000). Many enzymes involved in the flavonoid biosynthetic pathway have different functions, one of which is Flavonoid 3'5'Hydroxilation that catalyzes hydroxylation on C#3 and C#5. This enzyme is involved in anthocyanin biosynthesis (cyanidin, pelargonidin, delphinidin) (Dovichi *et al*., 2011). The purple snakeweed is well known in Indonesia, beside other types such as white, blue, and pink snakeweeds (Whinkel-Shierly, 2010).

There is a report that describes the characteristic of F3'5'H in other plant and its contribution in fruit development (Bogs *et al*., 2005). This compound also involved in the production of flavonoid derivate compound (Olsen *et al*., 2010). The F3'5'H enzyme is encoded by *F3'5H* genes that has been successfully isolated from the Asterid group such as Solanales (Solanum and Petunia), Gentianales (Gentiana), and Lamiales (Mol *et al.,* 1999). The related research about molecular analysis of the Verbenaceae *F3'5'H* gene has not yet been reported. Isolation of *F3'5'H* genes on snakeweed herb needs to be focused for the first step to support further research about development of active compound in snakeweed and could be used for the human benefit especially for pharmaceutical biotechnology improvement.

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MATERIALS AND METHODS

The genomic DNA was extracted from fresh leaf of purple snakeweed using Plant DNA II Isolation Kit (Macherey-Nagel) with modifications. Primers were designed based on conserve regions of *F3'5'H Petunia hybrida* (*GeneBank accession No.*: A29011 (*HF1*), A29013 (*HF2*)). PCR amplification used forward primer 5'TGATGCTGCTAAAGCATTCT'3 and reverse primer is 5'GTGCACGCAGGTGACATATG'3. Amplification cycle was as follows 94°C for 5 minutes of initial denaturation, 33 cycles of 94°C for 1 minutes of denaturation, 55°C for 1 minutes of annealing, 72°C for 1 minutes extension and the PCR result wes subjected to 1,5% agarose gel electrophoresis. Sequencing data was converted into fasta file using BIOEDIT software, ClustalX software was used to align amplifies fragments sequences, and BLAST program was used to align the sequence with reference genes sequences from gene bank (NCBI) and ensure the query product.

RESULT AND DISCUSSION

DNA amplification using PCR technique produced DNA fragment of 182 bp length from forward primer and 183 bp from reverse primer. The sequences are not specific shown by undetected bases (N notation) mainly found at downstream domain which is seen as double sequence from the middle fragment up to the 3'-end (Figure 1). Peak Trace reading was not significantly help for most of those bases. This result presumably is caused by more than one DNA template in the reaction as a result of primer designing failure, since primer design have an important role to make sure that the amplified DNA is a specific or single (Thompson, 2006) and primer that has been designed un-specifically caused more than one binding side (McGill, 2012). Examining the chromatogram in Fig. 1 it is suggested that the double fragments of amplified by forward primer is started from base #75, while one amplified by reverse primer is started from base #70 (Figure 1).

The electrophoresis visualization of amplified DNA shows only one band (Figure 2). Taking a consideration on the report that there are two representative locus for *F3'5'H* gene; *HF1* and *HF2* locus, in *Petunia hybrida* (Holton *et al.*, 1993) and that the primer designing was referred on the conserved domain of *F3'5'H* of *Petunia hybrida*, this fact leads to the possibility that the primers initiated the amplification of different template on conserved area of sequences which encode *F3'5'H* of *Stachytarpeta indica*.

It can also be suggested that the amplified fragments are copies of different alleles of *F3'5'H*, allegedly *HF1* and *HF2* locus, with the similar length. Furthermore, regarding to the reading of bases sequence throughout the chromatogram it may supposed to be that the amplified fragments of this purple *Stachytarpeta indica* sharing conserved-upstream and homolog-downstream domains as depicted in Figure 3.

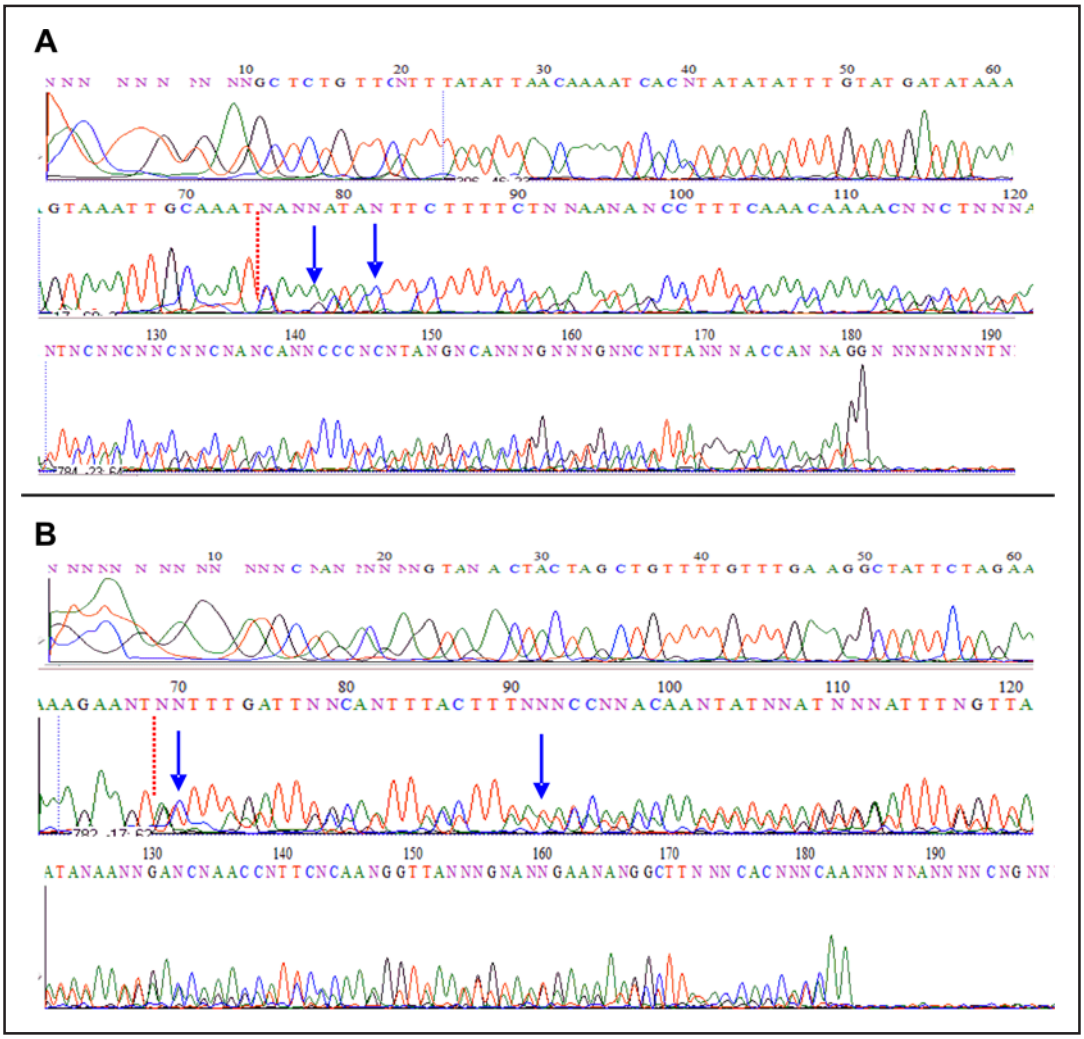

Figure 1. Chromatogram of amplified *F3'5'H* fragments showing unspecific sequence started from red dot lines. A: forward fragment, B: reverse fragment.
N for un-spesific bases; Blue arrows indicate double Peak

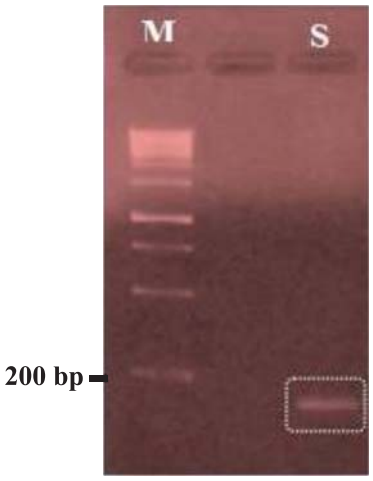

Figure 2. Electrophoresis visualization of amplified fragments. S: sample lane; M: 1kbp DNA Marker lane; Dotted-box band: target fragment.

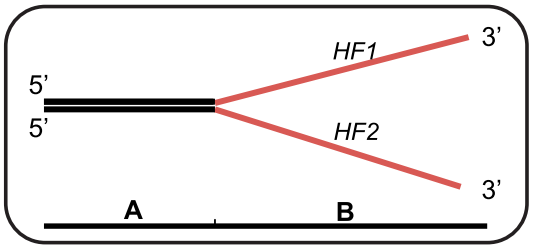

Figure 3. The illustration of two isolated fragments of *F3'5'H* gene from purple *Stachytarpheta indica*. A. Conserve domain, B. Homolog domain

CONCLUSION

The isolation of *F3'5'H* gene from purple *Stachytarpheta indica* using primers which were designed referred to *F3'5'H* gene of *Petunia hybrida* resulted on unspecific non-consensus fragments which sharing a conserved-upstream domain. From careful examination it is suggested that *Stachytarpheta indica* may possesses different alleles for *F3'5'H*, i.e. *HF1* and *HF2*, as of *Petunia hybrida*. Further studies are needed to unveil this doubt.

REFERENCES

Bogs, J., A. Ebadi, D. McDavid, and Robinson. 2006. Identification of The Flavonoid Hydroxylases From Grapevine and Their Regulation During Fruit Development. *Plant Physiology* 140:279-291.

Dovichi, S., and F. Lajolo. 2011. Flavonoid and Their Relationship to Diseases of The Central Nervous System. *Journal Brazilian Sociaty Food Nutrition Sao Paulo* 36: 123-135.

Ferreyra, M., S. Rius, and P. Casati. 2000. Flavonoid: Biosynthesis, Biological Function, and Biological Application. *Plant Science* 3:1-15.

McGill. 2012. *Trubleshooting Sequencing Product*. McGill University and Genome Quebec innovation Centre Sequencing Service.

Mol, J., E. Grotewold, and R. Koes. 1999. How Genes Paint Flowers and Seeds. *Trends Plant Science* 3: 212-217.

Olsen, K., A. Hehn, H. Judge, R. Slimestad, R. Larbat, F. Bourgaud, and C. Lillo. 2010. Identification and Characterization of CYP75A31, A New Flavonoid 3'5'Hydroxilase Isolated From *Solanum Lycopersicum*. *Plant Biology* 10: 21-26.

Thompson, B.D. 2006. *Finding Homologous Genes With Primers Designed Using Evolutionary Models*. Dissertation. North Caroline State University.

Winkel-Shirley, B. 2010. Flavonoid Biosythesis A Colourfull Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. *Plant Phsiology* 126:485-493.

Thomas, A., Brugliera, Lester, Tanaka, L. Menthing, and C. Stevenson. 1993. Cloning and Expression of Cytochrome P450 Genes Controlling Flower Colour. *Nature* 366: 276-279.