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DOES *Beta vulgaris* L. HAVE *LCYB* GENE?

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ABSTRACT

Beta carotene is a pigment that generally shared by all plants. This pigment synthesis is catalized by lycopene beta cyclase enzime. This enzyme is encoded by the *LCYB* gene. This research aimed to obtain the sequence of *LCYB* gene isolated from beet (*Beta vulgaris* L.) leaves. Several experiments have been done to isolate the *LCYB* gene. In the first experiment, the gene primers are designed using Pick Primer software based on conserved region of *Taraxacum officinale* (accession no.AB247456.1) and *Lilium lancifolium LCYB* (accession no. GU471230.1).The forward primer is (5'-TGTCGTGGTGGATCTTGTGG-3') and the reverse is (5'-ACACCTGTTGAGCGACAGAC-3').At the first experiment, the gene target's sequence was not obtained as the phylogenetic relationship between *Beta vulgaris*, *Taraxacum officinale*, and *Lilium lancifolium were* too far.In the second experiment, the primers are designed using *LCYB* gene in *Arabidopsis thaliana* (accsession no.NM_111858.2), with considering *Arabidopsis thaliana* as a plant model. The primers are (5'-TGGGACAGCAGGAATGGTTC-3') as the forward primer and (5'-GAGAAGAGCGACAACCCGAA-3') the reverse. By using BLAST analysis in the second experiment, it suggests that the fragment amplified by forward primer has similarity with *trnL* gene.This study seems to amplify the *trnL* gene instead of *LCYB* gene. This facts leads to bigger question whether or not the *Beta vulgaris* L has the *LCYB* gene.

Key words : Beta carotene, Lycopene Beta Cyclase, *Beta vulgaris* L, *LCYB* gene

INTRODUCTION

Carotenoids is a precursor in vitamin A biosynthesis (Giuliano *et al.*, 2008). This pigment is a C40 isoprene derived from the C20 precursor geranygeranylpirophosphat (GGPP) (Yamamizo *et al.*, 2009). Deficiency of vitamin A resulted on night blindness (Ye *et al.*, 2000), which inturn stimulates the research on plant breeding in order to produce various plants with high carotenoid content (provitamin A) (Giuliano *et al.*, 2008). Beet is one of plants which contains high carotenoids (Berman *et al.*, 2004).Genetic engineering affects the expression of genes encoding carotenoid pigments that have an impact on levels of carotene (Bai *et al.*, 2011; Giuliano *et al.*, 2008). Beta-carotene,one of carotenoids pigments (Brown, 2010) is converted by *Lycopene Beta Cyclase* enzyme. The enzyme is encoded by the *Lycopene Beta Cyclase* (*LCYB*) gene (Cunningham *et al.*, 1998). A report about *LCYB* gene sequence in *Beta vulgaris* L. has not yet been available. This research aimed to isolate *LCYB* from beet, yet resulted on a question does *Beta vulgaris* L. has *LCYB* gene?

MATERIALS AND METHODS

In this study, DNA were isolated using Nucleospin ®II DNA Isolation Kit, (Macherey-Nagel, Germany). In the first experiment the primer *LCYB* gene were designed using PickPrimer software based on conserved region of *Tarax acumofficinale* (accession no.AB247456.1) for forward primer (5'-TGTCGTGGTGGATCTTGTGG-3 ') and *Lilium lancifolium* (accession no.GU471230.1) for reverse primer (5'-ACACCTGTTGAGCGACAGAC-3 '). Since first

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study resulted an unspecific sequence we therefore continued the study with second experiment using primers for *LCYB* gene which was designed based on conserved region of *Arabidopsis thaliana LCYB* (accsession no.NM_111858.2).
The forward primer (5'-TGGGACAGCAGGAATGGTTC-3') and reverse primer (5'-GAGAAGAGCGACAACCCGAA-3'). PCR results were examined using electrophoresis gel agarose in 1.5% and 1Kbp DNA Marker. The data was analysed using BLAST, Clustal X and BioEdit software.

RESULTS AND DISCUSSION

Total DNA *B.vulgaris* L.obtained was of 245.6 ng/µL. The target gene was amplified by PCR through several stages of annealing temperature optimizations. The first experiment produced a thin band of 200 bp fragment which showed a nonspecific sequences for both forward and reverse primer (Figure 1). Analysis using DNA Base did not showed a consensus sequence from forward and reverse fragment (Figure 2). Using BLAST analysis the forward fragment has query coverage of 6% with maximum identity 100% to *Taraxacum officinale LCYB* while the reverse fragment has a query coverage of 12% and maximum identity 100% to *Lilium lancifolium LCYB*. We suggested that the failure to obtain the target gene was generated primer by miss-design since later we find that *B. vulgaris* L. too far from *Taraxacum officinale* and *Lilium lancifolium* phylogenetically (Graur *et al*., 2000).

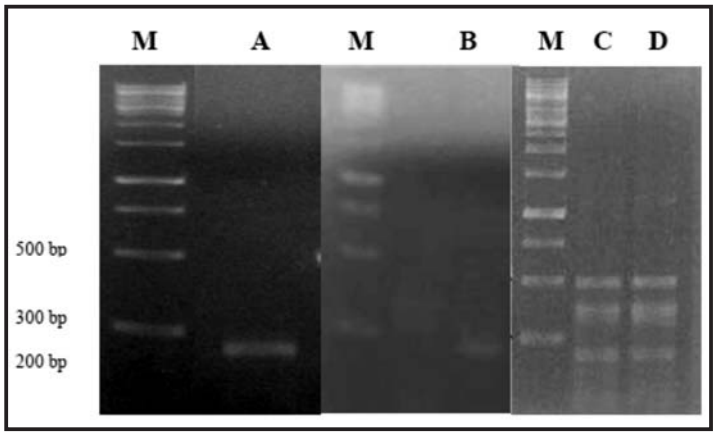

Figure1. Resultsof PCR at annealing temperature A. 61ºC (200 bp); B. 57ºC (200 bp); C. 52 ºC (200 bp, 400 bp, and 500 bp); D.55ºC (200 bp, 400 bp, and 500 bp);M=Marker 1 kbp DNA

Based on that result we performed the second experiment which resulted a 200bp fragment, 400bp and 500bp(GeneRuler 1kbp onagarose gel1.5% (Figure 1).Analysis using DNA Baser did not obtained a consensus between forward and reverse (Figure 3). BLAST analysis showed DNA fragments obtained are not specifi. The forward sequence (AF) showed similarity to *trnL* with amaximum identity 98% and query coverage34% of *B. vulgaris* L. This failure may be caused by primers design failure as a result of the lack conserved region of *LCYB* gene. Another possibility was based on the fact that carotenoid biosynthesis in different plants is catalyzed by not only a single type of enzyme, yet a family proteins and different genes.

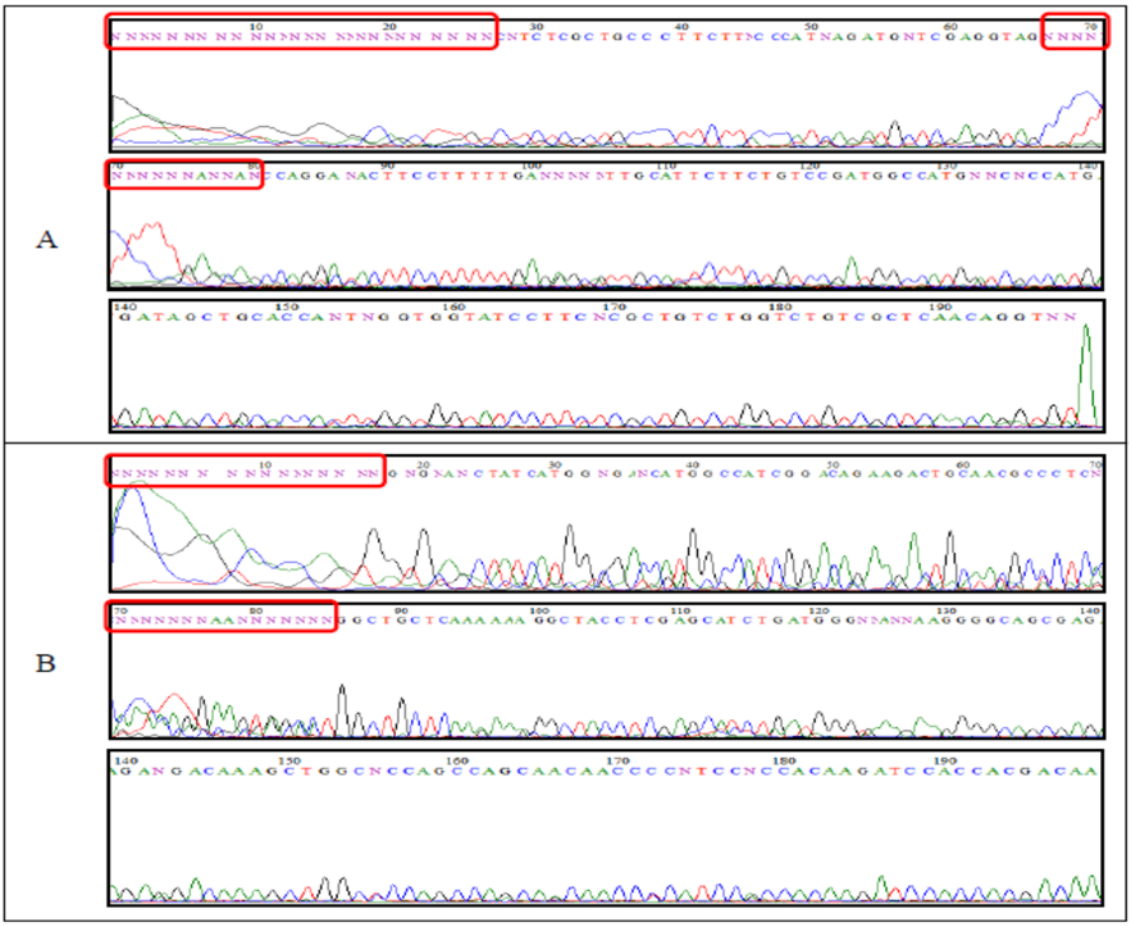

Figure2. The results of the first experiment sequencing; A.ForwardPrimer (197 bases); B. Reverse Primer(199 bases).

The enzyme catalyze the biosynthesis of carotenoid in *Arabidopsis thaliana*, maize, and rice encoded by a single gene *LCYB,*in tomatoes and other plants encoded by family gene: the two enzymes which similar to *LCYB* are *LYCB1/CRTL-B1* (expressed inchloroplasts) and *LCYB2/CRTL-B2/CYCB* (expressed inchromoplas) (Ruiz-Sola *et al.*, 2012), which in citrus grape fruits *LCYB* is encoded by two genes,*CitLCYB1* and *CitLCYB2* which have a similarity of 93%. On the other than, *Capsanthin Capsorubin Synthase* (*CCS*) in chilli and *Neoxanthin Synthase* (*NSY*) on tomato and potato has a mechanism similar to*LCYB* (Alquézar *et al.*, 2009). Remain a question does β-carotene syntesis in beet catalyzed by one of those enzymes sgared by other plant or there is a different enzymes doing so or regulated through different pathway?

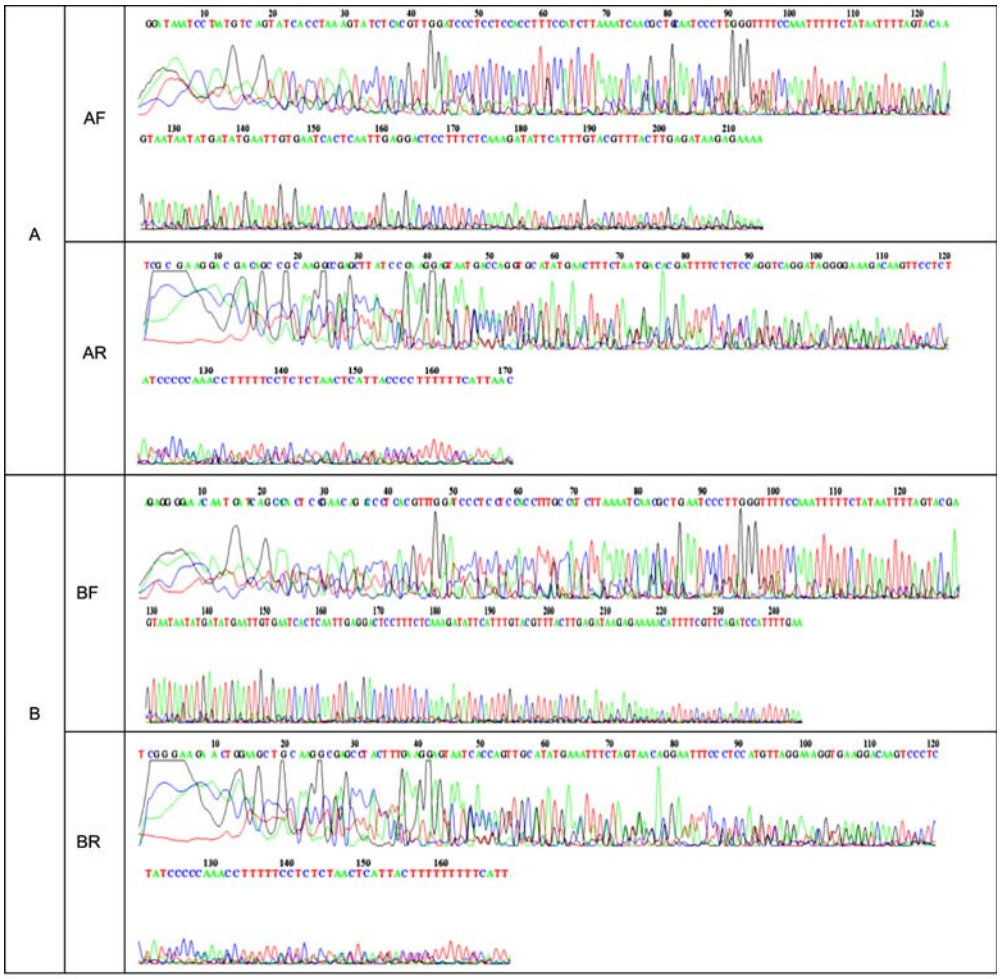

Figure3. The results ofthe secondexperimentsequencing; A.Fragment 500 bp (AF: *forward*, AR: *reverse*) ; B. Fragment 400 bp (BF: *forward*, BR: *reverse*).

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