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AT3 (Acyltransferase) Gene Isolated from *Capsicum frutescens* cv. Cakra Hijau

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ABSTRACT

Chili pepper is widely used and cultivated by Indonesian people. There are three species of chili pepper, i.e. *Capsicum annuum*, *Capsicum frutescens*, and *Capsicum violaceum*. *C. frutescens* has a higher economic value due to its pungency and carotenoid content. *C. frutescens* has several cultivars, one of those is *C. frutescens* cv. Cakra Hijau. This cultivar is resistant against pest and disease and has very high pungency. This special character of chili pepper is capsaicin, its secondary metabolic. Moreover, capsaicin also serves as defense mechanism, antiarthritis, analgesic, and anticancer. This study was aimed to isolate acyltransferase (AT3) gene which encoded capsaicin synthase (CS) enzyme. AT3 gene was isolated through PCR using forward primer 5'-ATG GCT TTT GCA TTA CCA TCA-3' and reverse primer 5'-CCT TCA CAA TTA TTC GCC CA-3'. Data were analyzed using *DNA Baser*, *BLAST*, and *ClustalX*. This study has successfully isolated 404 bp fragments of AT3 gene. These fragments are located at 1918-1434 bp referred to AT3 gene from *C. frutescens* cv. Shuanla. The isolation of upstream and downstream fragments of AT3 gene from *C. frutescens* cv. Cakra Hijau is undergoing.

Keywords: *Capsicum frutescens cv. Cakra Hijau, capsaicin, AT3 gene*

INTRODUCTION

Chili pepper is widely used and cultivated by Indonesian people. There are three species of chili pepper, i.e. *Capsicum annuum*, *Capsicum frutescens*, and *Capsicum violaceum* [1]. In Indonesia, *C. frutescens* are the most widely cultivated [2], and is a high economic valued plant for its pungency and carotenoid content [3]. *C. frutescens* has several cultivars, namely, Sky Line, White Chili, Bara, Cakra Putih, and Cakra Hijau. Cakra Hijau cultivar is resistant to pest and disease, has very high pungency, can be harvested at ±80 days, and potentially produces 12.000 kg/ha chili fruit [4].

Pungency in chili pepper is caused by capsaicin compound. Capsaicin is only found within *Capsicum* genus. Capsaicin has been used in medicine and pharmacy as anti-arthritis and analgesic, [5]. It has also been described as the regulator of fat distribution in the body [6], antibacterial [7], and anticancer [8].

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Several enzymes are involved in Capsaicin biosynthesis. Capsaicin synthase (CS) is the last enzyme which has an important role in capsaicin biosynthesis by condensing vanillylamin with acyl moieties to produce capsaicin. CS also serves as a regulator for the formation of capsaicin [9]. Acyltransferase (AT3) gene is proposed as a gene encode CS enzyme [10]. This statement is supported by Leung[11] who proposesthat AT3 is expressed at placenta and this gene is segregated at C locus. On the other hand, Kim *et al.* [12] reported that AT3 co-localized with Pun1 and a 2.5 kb deletion of AT3 was found to be related to non-pungency in pepper.

The isolation of AT3 gene from *C. annuum* [13] and several *C. frutescens* [14] has been reported. So far, there is no report about AT3 of *C. frutescens* from Indonesia, and this gene data has not been recorded in Gene Bank. This research was aimed to isolate AT3 gene from *C. frutescens* cv. Cakra Hijau.

MATERIALS AND METHODS

C. frutescens cv. Cakra Hijau plants were obtained from Balai Pengkajian Teknologi Pertanian (BPTP) Karangploso, Malang, Indonesia.

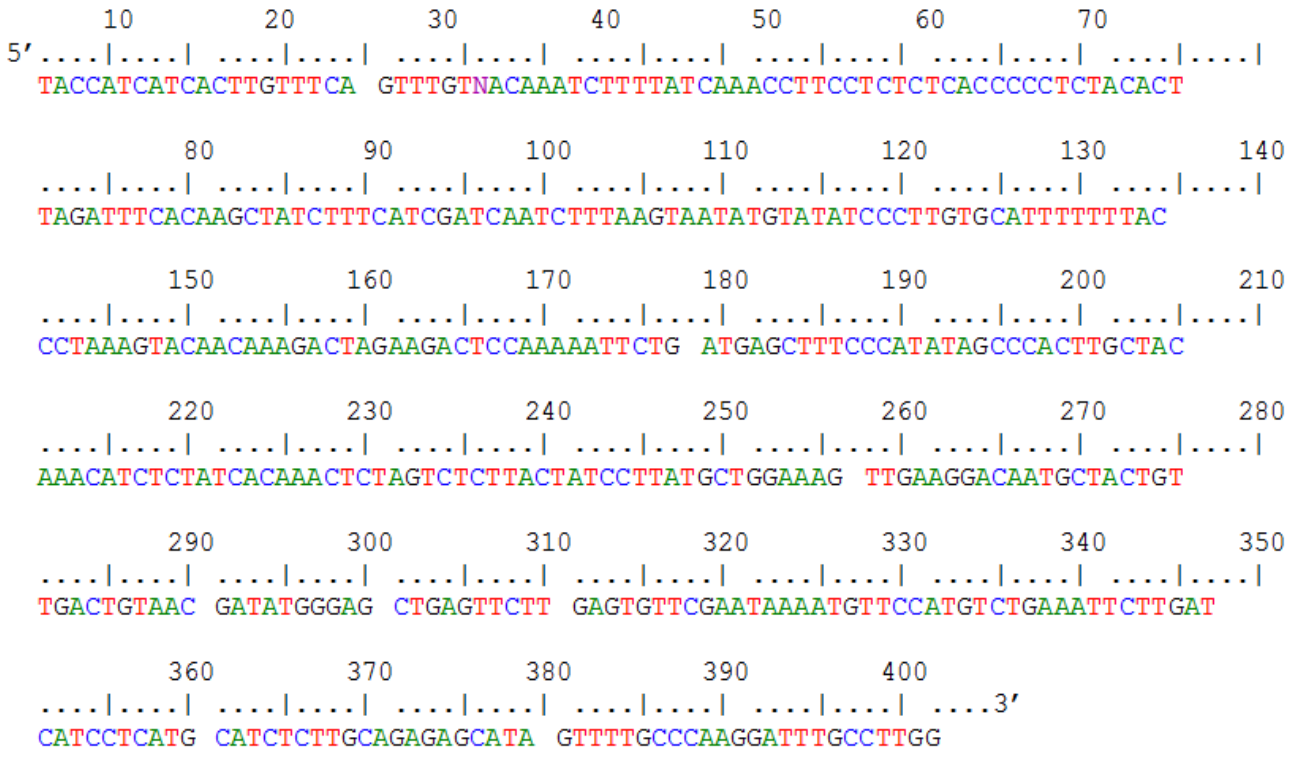

Figure 1. Partial sequence of AT3 gene from *C. frutescens* cv. Cakra Hijau that has been isolated

The DNA total was isolated from leaf by using the plant DNA isolation kit (*Nucleospin ® II, Macherey-Nagel, Germany*). The primers used were 5'-ATG GCT TTT GCA TTA CCA TCA-3' (forward) and 5' –CCT TCA CAA TTA TTC GCC CA-3' (reverse). PCR cycle: 94°C for 5 m (pre denaturation), 94 °C for 1 minute (denaturation), 53 °C for 1 minute (annealing), 72°C for 2 minutes (extension), and 72 °C for 10 minutes (final extension) in 30 cycles.

The PCR products were electrophoresed by using 1 % agarose gel and sequenced by Big Dye transiluminator through ABI 3130 Genetic analyzer machine at Eijkman Institute for Molecular Biology of Jakarta. The sequencing result was analyzed by using *Bioedit*, *Peak trace*, *DNA Baser*, *BLAST*, and *Clustal X* software.

RESULTS AND DISCUSSION

The AT3 gene isolation from *C. frutescens* cv. Cakra Hijau using PCR technique with a pair of primersproduced 404 base pair fragments. The position of amplified target gene located in 1918-1434 bp, refer to AT3 of *C. frutescens* (access code: HM854860.1 and AY819026.1). Suspected AT3 gene sequence result from *C. frutescens* cv. Cakra Hijau are shown in Figure 1. The analyses sequence using BLAST program were compared with AT3 of *C. frutescens* cv. Shuanla (Figure 2A) and *C. frutescens* cv. BG2814.6 (Figure 2B). The result shows *query coverage* of 24 % and 10 %, respectively, with similarity index 99 % of each. According to the result, sequences that have been acquired are AT3 gene.

The DNA sequence from AT3 gene of *C. frutescens* cv. Cakra Hijau was analyzed by using *ClustalX* to make an alignment of amino acid with AT3 of *C. frutescens* cv. Shuanla and *C. frutescens* cv. BG2814.6. The amino acid alignment shows that *AT3* gene of *C. frutescens* cv. Cakra Hijau is located in amino acid the 43rd to 139th of AT3 amino acid sequence of *C. frutescens* cv. Shuanla and *C. frutescens* cv. BG2814.6 (Figure 3).

We lack confidence to assure that the first methyonin in our amino acid sequence is a start codon, regarding that there are 42 amino acid upstream to ours started with methyonin that has not yet been isolated. Furthermore, there is another fragment downstream from amino acid 140th that has not yet been obtained in our study. So far, there is no report for the exact length of AT3 gene from *C. frutescens* cv. Shuanla [15] which shows no stop codon in their reported AT3 sequence.

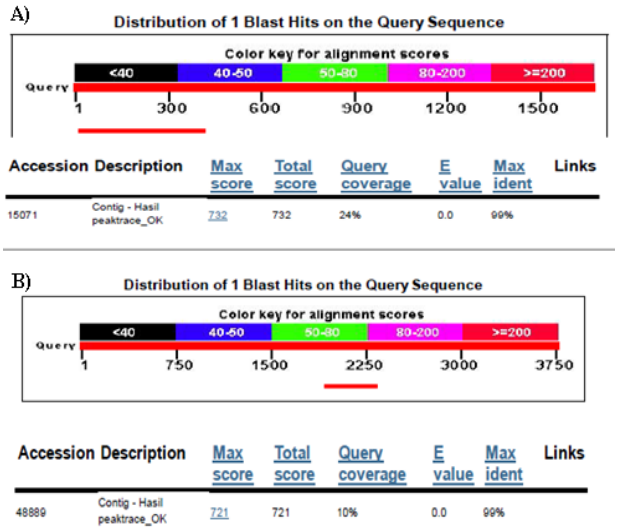

Figure 2. BLAST result of target gene compared with a) AT3 gene of *C. frutescens* cv. Shuanla, b) AT3 gene of *C. frutescens* cv. BG2814.6

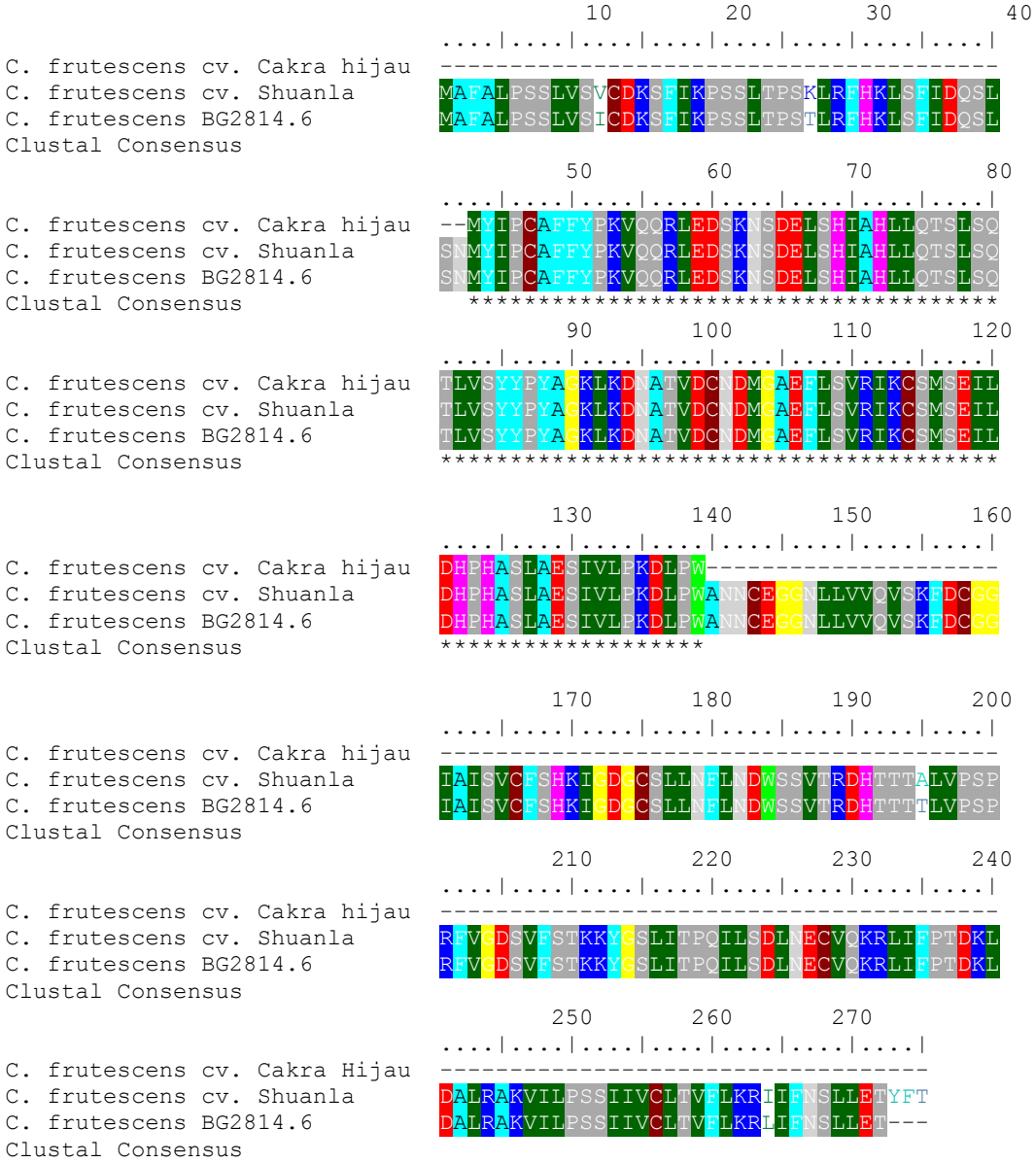

Figure 3. Amino acid alignment between AT3 from *C. frutescens* cv. Cakra hijau with AT3 from *C. frutescens* cv. Shuanla and *C. frutescens* cv. BG2814.6

CONCLUSION

This study successfully isolates 404 bp suspected fragment of AT3 gene from *C. frutescens* cv. Cakra Hijau which encodes 97 amino acids. The sequences obtained are the middle part of AT3 gene. The isolation of up-stream and down-stream part of this gene is necessary to obtain the intact sequence.

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