Conference Paper

Synthesis of cDNA Pun1 gene from *Capsicum frutescens* L. cv. Cakra Hijau

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Abstract

Capsicum frutescens L. cv. Cakra Hijau is a local cultivar that has been widely cultivated in Indonesia due to the pungency. Pungent on *Capsicum* is generated by capsaicin compound encoded by *Pun1* gene. The sequences of *Pun1* gene containing with two exons that located on the upstream and downstream, which are separated by introns in the middle. This study aimed to synthesis of cDNA of *Pun1* gene from isolated total mRNA using two primers: F1/R1 (**F1** 5′-ATG GCT TTT GCA TTA CCA TCA-3′; and **R1** 5′-CTT AGC TCG AAG TGC ATC TA-3′) to synthesis the exon-1 sequences and F2/R2 (**F2** 5′-GAA GGT GGC AGA AGA ATC AG-3′; and **R2** 5′-TTA GGC AAT GAA CTC AAG GA-3′) to synthesis the exon-2 sequences. The cDNAs resulted from RT-PCR were visualized on 1.5% agarose gel electrophoresis. From this study we obtained a 1323 bp fragment cDNA.

Keywords: *Capsicum frutescens* L. cv.Cakra Hijau; *Pun1* gene; synthesis of cDNA.

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Received: 11 February 2017
Accepted: 08 March 2017
Published: 26 March 2017

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the ICBS Conference Committee.

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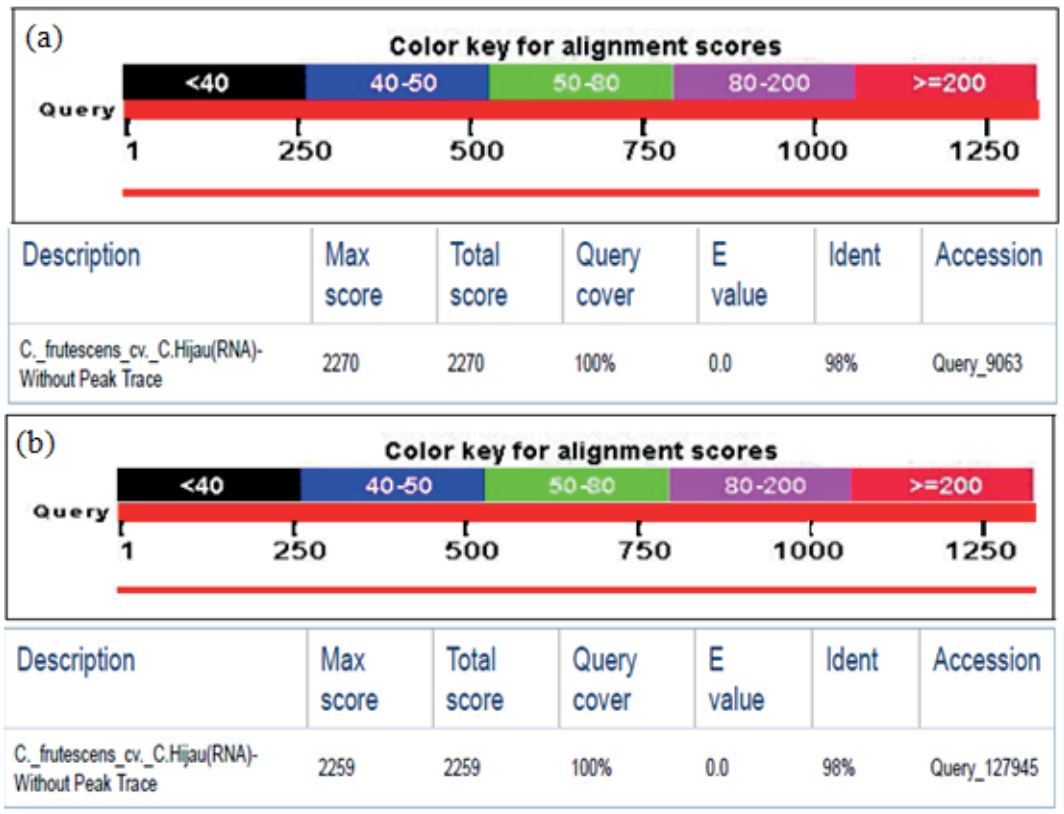
1. Introduction

Pepper is a member of Solanaceae's family, *Capsicum*'s genus [1]. It has many cultivars in Indonesia, one of the local cultivar is *Capsicum frutescens* L. cv. Cakra Hijau. *Capsicum frutescens* L. cv. Cakra Hijau is able to adapt well in lowland and high, resistant against disease and has high level of pungency [2].

The pungency of *Capsicum frutescens* L. cv. Cakra Hijau comes from a secondary metabolite named capsaicin (trans-8-methyl-N-vanillyl1-6-nonenamide). The accumulation and biosynthesis of capsaicin are localized in the epidermal cells of the placental, specifically in the interlocular septum which defines the fruit locules and is derived from the tissue connecting the placenta to the pericarp [3, 4]. Capsaicin biosynthesis involves two secondary metabolic pathways, there are phenylpropanoid and fatty acid pathways [5] begin at approximately 20 d post-anthesis (dpa), gradually increasing throughout fruit development, maximum in 40 d to 50 d post-anthesis (dpa) [3, 4]. The enzymes involved in biosynthesis pathway of capsaicin were phenilalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (Ca4H), coumaric acid 3-hydroxylase (Ca3H), caffeic acid o-methyl transferase (COMT), and amino transferase



Description	Max score	Total score	Query cover	E value	Ident	Accession
C._frutescens_cv._C.Hijau(RNA)-Without Peak Trace	2270	2270	100%	0.0	98%	Query_9063

Description	Max score	Total score	Query cover	E value	Ident	Accession
C._frutescens_cv._C.Hijau(RNA)-Without Peak Trace	2259	2259	100%	0.0	98%	Query_127945

Figure 1: BLAST analysis result comparison between *Pun1* gene cDNA sequences of *C. frutescens* cv. Cakra Hijau with *Pun1* gene of: (a) *C. frutescens* cv. BG2814-6; (b) *C. frutescens* cv. Shuanla.

(AMT), but the main enzyme is capsaicin synthase (CS) encoded by the *Pun1* gene [3, 6].

Pun1 gene had been reported for three species i.e *Capsicum annum*, *Capsicum chinense,* and *Capsicum frutescens* [3]. *Pun1* gene of *Capsicum frutescens* had been reported from *Capsicum frutescens* L. cv. BG2814-6 and *Capsicum frutescens* L. cv. Shuanla. Total DNA sequences of *Pun1* gene from *Capsicum frutescens* L. cv. Cakra Hijau have been isolated in several previous studies [7–10]. There are two exons that located on the upstream and downstream, which are separated by introns in the middle. Total sequences of *Pun1* gene without introns may be obtained from messenger RNA (mRNA) that can be used as templates in the synthesis of cDNA by reverse transcriptase enzyme [11]. In vitro cDNA synthesis can be done by using a Reverse Transcription-Polymerase Chain Reaction (RT-PCR) [12]. This study aimed to synthesis of cDNA *Pun1* gene from *Capsicum frutescens* L. cv. Cakra Hijau.

2. Material and Method

mRNA isolation from placenta and epidermal interlocular septums of *Capsicum frutescens* L. cv. Cakra Hijau that had reached 20–40 d post-anthesis (dpa). It was done by following the Tri Reagent RNA isolation protocol. The obtained mRNA quantitatively measured using a spectrophotometer analysis engine Nano Drop 2000

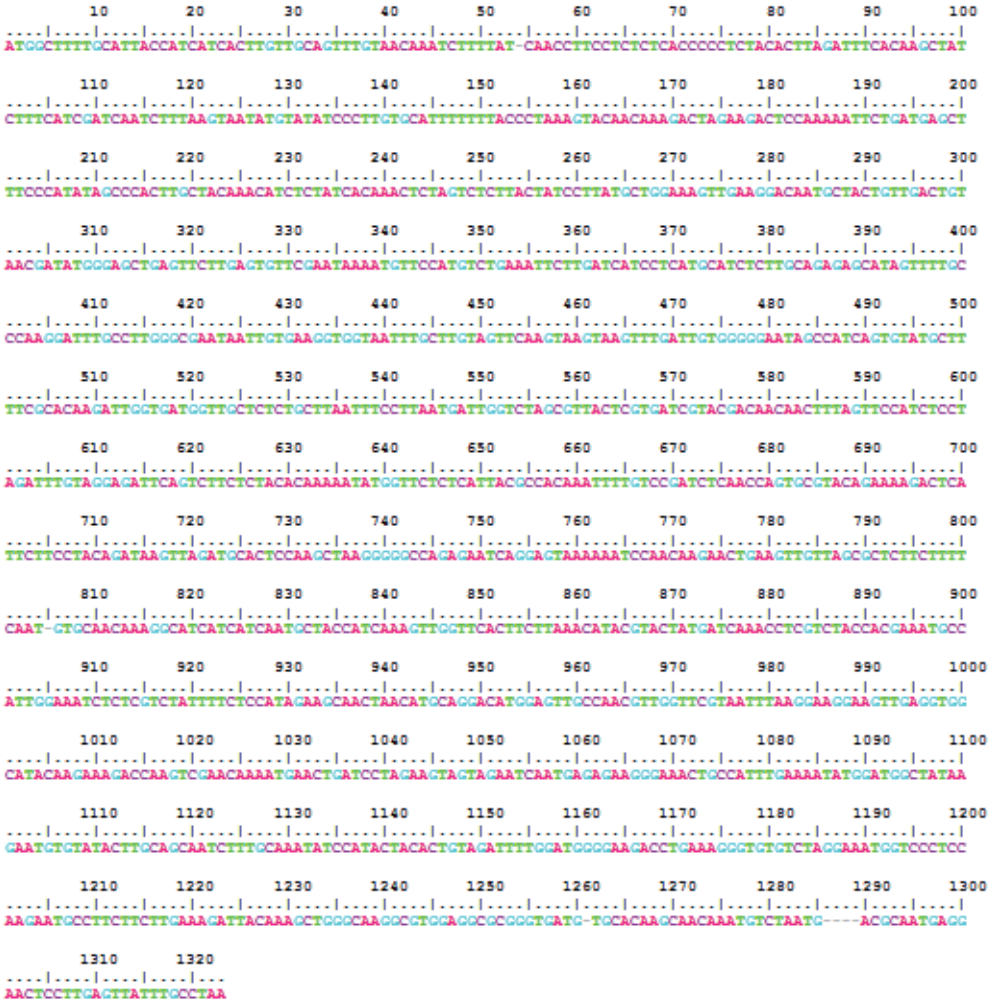


Figure 2: *Pun1* gene cDNA sequences of *C. frutescens* cv. Cakra Hijau.

on A260/A280 nm wavelengths. mRNA amplification was performed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using primers designed based on DNA sequences of *Pun1* genes from *Capsicum frutescens* L. cv. Cakra Hijau that had reported previously. The primers used F1/R1 (**F1** 5′-ATG GCT TTT GCA TTA CCA TCA-3′; and **R1** 5′-CTT AGC TCG AAG TGC ATC TA-3′) to synthesis the exon-1 sequences and F2/R2 (**F2** 5′-GAA GGT GGC AGA AGA ATC AG-3′; and **R2** 5′-TTA GGC AAT GAA CTC AAG GA-3′) to synthesis the exon-2 sequences.

mRNA was converted into cDNA using reverse transcriptase enzyme in the Qiagen OneStep RT-PCR. RT-PCR was performed in 40 cycles, as follow: reverse transcription 50°C for 30 min, initial PCR activation step 95°C for 15 min, denaturation 94°C for 1 min, annealing optimization 54°C for 1 min, extension 72°C for 1 min, and final extension 72°C for 10 min. The cDNAs resulted from RT-PCR were analyzed by 1.5% agarose gel electrophoresis and sequenced by the Big Dye Transluminator through ABI 3130 Genetic analyzer machine at First Base, Malaysia. The sequencing results was analyzed using *DNA Baser, BLAST, SIXFRAME* and *Clustal X* software.

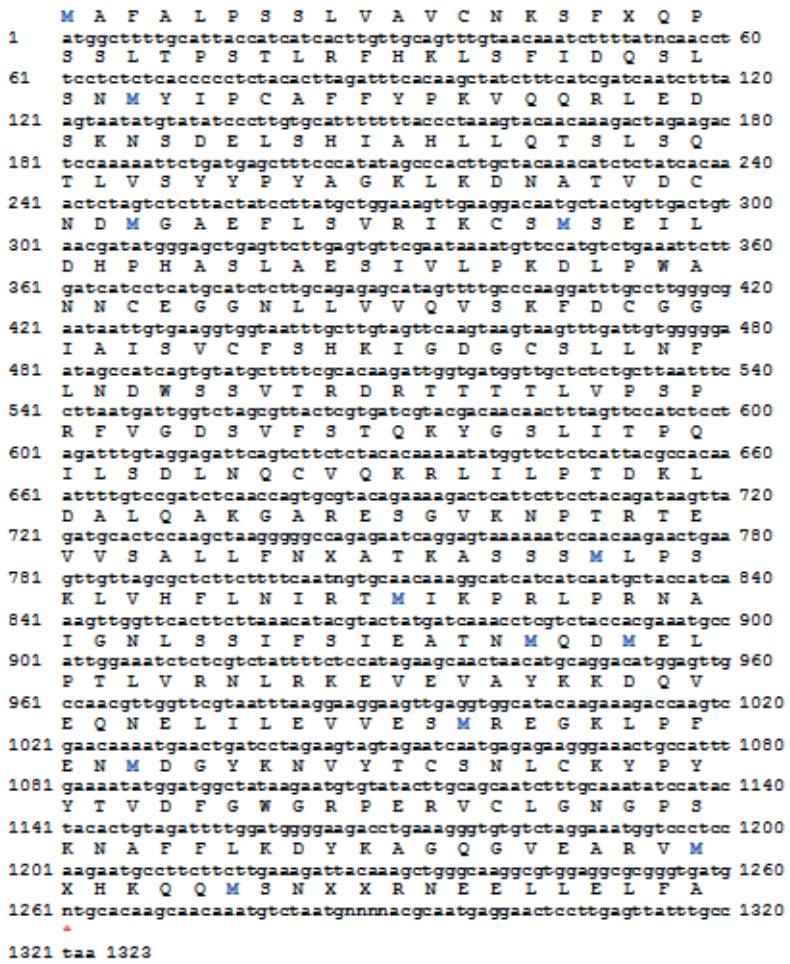


Figure 3: ORF from SIXFRAME analysis results of cDNA *Pun1* gene of *C. frutescens* cv. Cakra Hijau. M: start codon or methionine; *: stop codon.

3. Results and Discussions

Analysis of cDNA *Pun1* genes in *C. frutescens* cv. Cakra Hijau using BLAST analysis showed that query coverage cDNA sequences of the *Pun1* genes *C. frutescens* cv. Cakra Hijau was 100% of *C. frutescens* cv. BG2814-6 and *C. frutescens* cv. Shuanla; sharing 98% similarity with *Pun1* of *C. frutescens* cv. BG2814-6 and *C. frutescens* cv. Shuanla (Figure 1).

Analysis using DNA Baser generate consensus sequences throughout 1323 bp (Figure 2).

Determination of the Open Reading Frame (ORF) was performed using SIXFRAME. Open reading frame analysis revealed that ORF #1 is the most possible reading frame of the target gene marked by the reading of the start codon at the beginning and the stop codon at the end of the cDNA gene target (Figure 3).

Alignment of *Pun1* gene cDNA sequences and *Pun1* gene sequences done using ClustalX analysis program. It shows the similarities and differences (Figure 4).

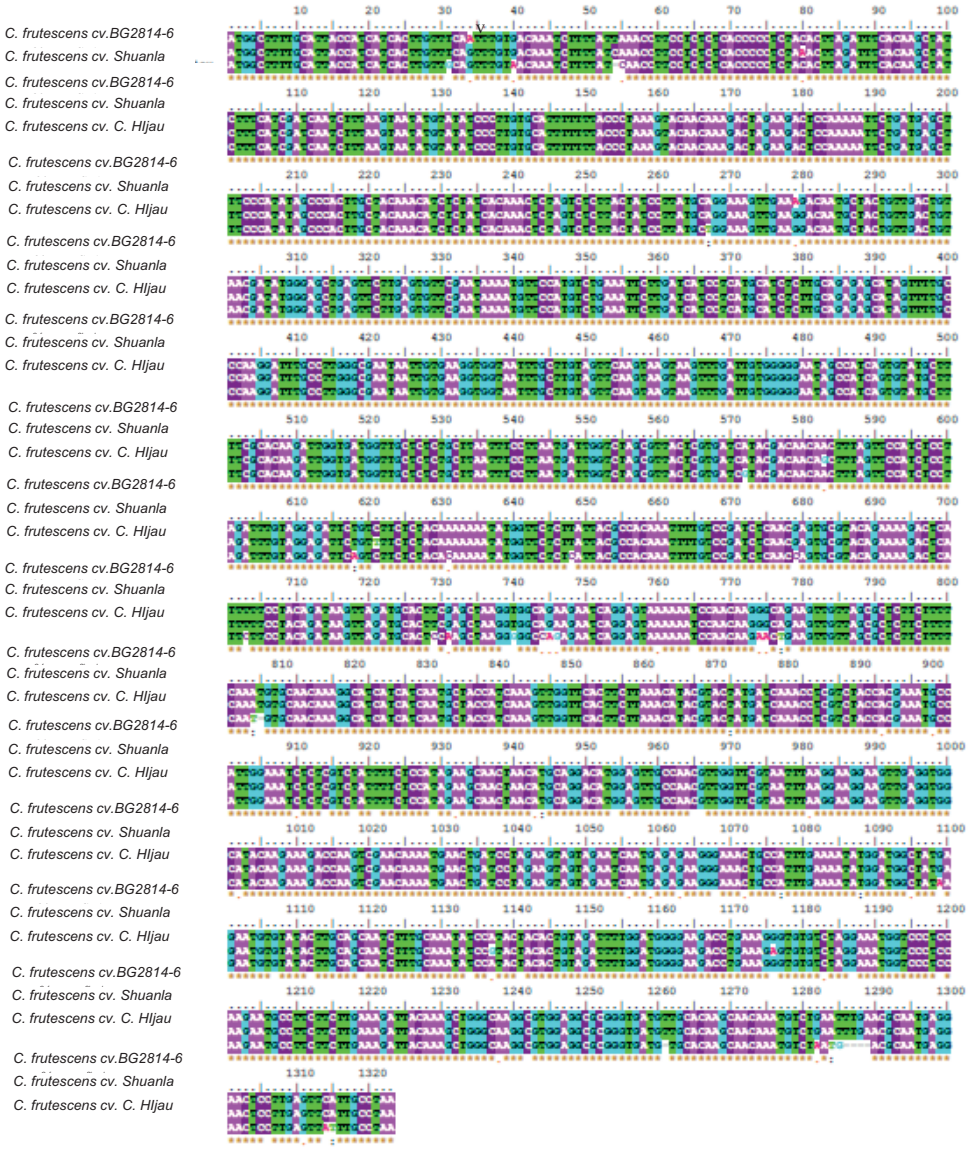


Figure 4: Alignment of *Pun1* gene cDNA sequences of *C. frutescens* cv. Cakra Hijau with sequences of *C. frutescens* cv. BG2814-6 and *C. frutescens* cv. Shuanla. (*): the same nucleotide base (conserve); (.)/(:): the different nucleotide base; (-): the missing nucleotide base (deletion).

Alignment of amino acid *Pun1* gene cDNA sequences from *C. frutescens* cv. Cakra Hijau and comparison *Pun1* gene sequences produces 441 amino acid sequence preceded by a start codon and ends with a stop codon (Figure 5). There are some differences and unreadable amino acids in the alignment result. It formed because of differences in the nucleotide bases [13] and the deletion of the cDNA sequences.

Isolation of genes encoding a protein can be obtained by isolating mRNA of those transcripted [11]. RT-PCR techniques were developed to analyze the very small amount RNA molecules of the transcript in the cell. Therefore PCR cannot be done using RNA as a template. The first process was reverse transcription of the mRNA molecules to obtain cDNA molecules. cDNA molecules then used as a template in the PCR process.

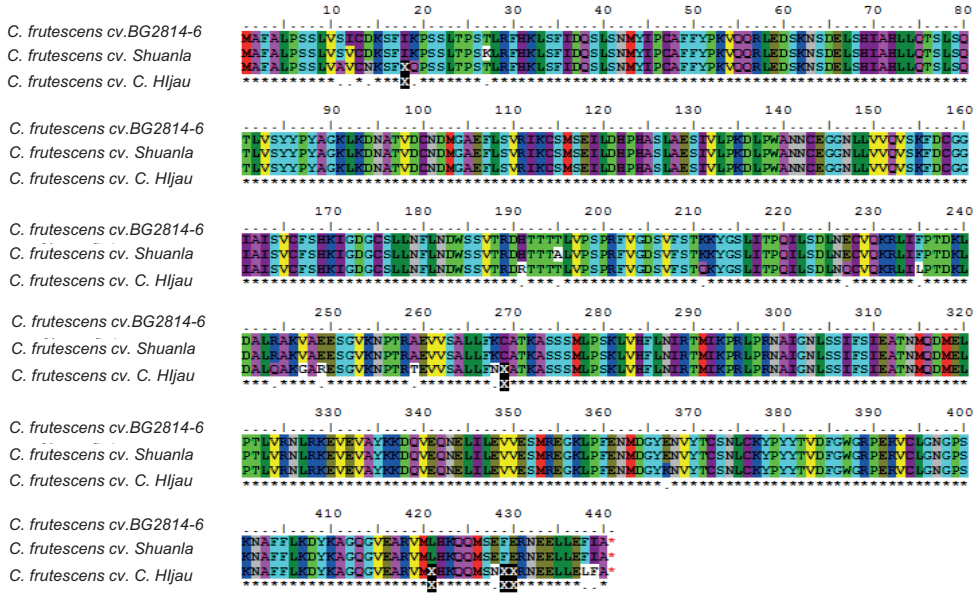


Figure 5: Alignment of *Pun1* gene cDNA amino acid sequences of *C. frutescens* cv. Cakra Hijau with *C. frutescens* cv. BG2814-6 and *C. frutescens* cv. Shuanla. *: the same amino acid (conserve); (.): the different amino acid; (X): amino acids that are unreadable; (*): stop codon.

RT-PCR technique is particularly useful for detecting gene expression, RNA amplification prior to cloning and analysis, as well as for the diagnosis of infectious agents or genetic diseases [14].

4. Conclusions

RT-PCR in cDNA synthesis of *Capsicum frutescens* L. cv. Cakra Hijau produced 1323 bp fragment cDNA. Further research is necessary to obtain the total cDNA sequences without deletion with the pairs of primers as a first step of cloning.

Acknowledgements

The authors thank to Dwi Listyorini and Eko Sri Sulasmi for their positive guidelines to solve our problem in this research. All seniors at Biotechnology Laboratory, State University of Malang, Moh. Habibi, Andi Madihah, and Septi Kurniama Sari for discuss in this research. Moreover, this study was supported by PKM Dikti 2013 to R.R.J group and PKM Dikti 2014 to E.N.K group (Elhah Nailul Khasna and Ria Reinnata Juliandari).

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