Conference Paper

Isolation 3′-end Fragment of Pun1 gene from *Capsicum frutescens* L. cultivar Cakra Hijau

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Abstract

Pun1 gene is the one of candidate gene that responsible to determine pungency in Capsicum. In previous researches, 1 310 bp fragment of 1 671 bp *Pun1* gene from *Capsicum frutescens* L. cv. Cakra Hijau had been isolated. The purpose of this research was to isolate of 3′-*end* fragment and get full length of *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau. DNA isolation was done using modified procedure. The method used to isolate the gene was PCR with a pair of primer, *forward primer* 5′-GAA-GGT-GGC-AGA-AGA-ATC-AG-3′ and *reverse primer* 5′-TTG- TTG ACC-GTA-AAC-TTC-CG-3′. The result successfully to get 715 bp length DNA fragment. The assembly of this fragment into previous research produced a full length of 1 671 bp *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau consist of 738 bp first exon fragment, 348 bp intron fragment, and 585 bp second exon fragment.

Keywords: *C. frustescens* L. cv. Cakra Hijau; gene isolation; *Pun1* gene.

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1. Introduction

Capsicum frutescens L. cv. Cakra Hijau is one of local cultivar in Indonesia that has very high pungency level, with green coloured raw fruit and red coloured ripe fruit, the potencial produces of 12 t · ha^{-1}, harvesting time approximately 80 d after planted and resistant from pests and diseases [1]. The pungency from *C. frutescens* L. cv. Cakra Hijau caused by capsaicin compound. Capsaicin has been used as antioxidant [2], component of dietary medicine [3], anticancer [4], and antibacterial [5].

Capsaicin compound produced by condensation of vannilylamine derived from Phenylalanine with 8 methyl-6 nonenoic acid derived from valine or leucine, which the activated capsaicin synthase (CS) [6, 7]. Capsaicin synthase functioned as the last enzyme of capsaicin biosynthesis that encoded by a putative *acyltransferase 3* gene (AT3), the one of candidate is *Pun1* gene [7]. Furthermore, the *Pun1* gene often presumed to be a master regulator of the pathway [8].

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        10        20        30        40        50        60        70        80        90        100
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TTGAAGGTTGGCAGAAGAATCAGGAGTAGAAAACCCAACAAGGGCAGAGTTGTTAGCGCTCTTCTTTTCAATGTGCAACAAAGGCATCATCATCAATGCT
        110       120       130       140       150       160       170       180       190       200
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ACCATCAAAGTTGGTTCACTTCTTAAACATACGTACAATGATCAAACCTCGTCTACCCCGAAATACCATTGGAAATATCTTGTCCATGTTCTCCACAGCA
        210       220       230       240       250       260       270       280       290       300
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GCAACTAACGAGCAGGACATTGAGTTGCCAAGTTTGGTTCGTAATTTGAGGAAGGAAGTTGAGGTGGCGTACAAGAAAGACCAAGTCGAACAAAATGAAC
        310       320       330       340       350       360       370       380       390       400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TGATCCTAGAAGTAGTAGAATCCATAAGAAAAGGTAAAATGCCTTTTGAAAATAAGGATGGATATCAGAATGTTTATATTTGCAGTAATCTTTGCAAATA
        410       420       430       440       450       460       470       480       490       500
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCCATACGACACTGTAGATTTTGGATGGGGAAGACCTGAAAGTGTGTGTATAGCAAATGGTCCCTTCAAGAATGCCTTCTTCTTGAAAGATTACAAAGCT
        510       520       530       540       550       560       570       580       590       600
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGGCGAGGTGTGGAGGCGCGGGTGATGTTGCACAAGCAACAAATGTCTGAATTTGAACGCAATGAGGAACTCTTTGAATTTATTGCCTAATTAATTCCAA
        610       620       630       640       650       660       670       680       690       700
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GTTTTGGAGTAACTGGATGTTTGATTGAAGAGGGATTTTACCAAATAAAGGTAGGAATAATTGTAATGTGTGGTTCATCAAAACAACTTCAACGGAAGTT
        710
....|....|....|
TACGGTTCAACAAAA
```

Figure 1: The result of isolation 3'-*end* fragment from *Capsicum frutescens* L. cv. Cakra Hijau in consensus sequence.

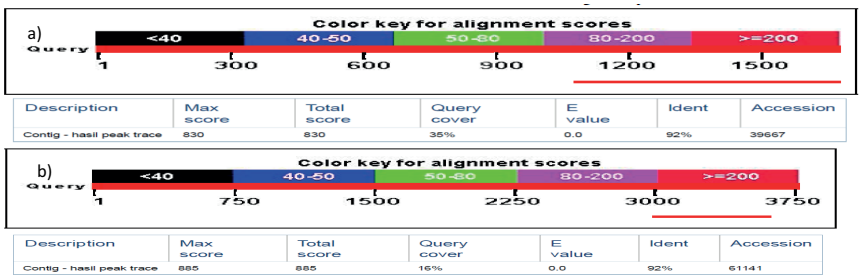

Figure 2: Position of *Pun1* gene from *C. frutescens* L.cv. Cakra Hijau with *Pun1* gene from other cultivar: (a) *C. frutescens* L. cv. Shuanla, (b) *C. frutescens* L. cv. BG 2 814-6.

Pun1 gene from *C. frutescens* L. that has published in gene bank is *C. frutescens* L. cv. BG 2 814-6 with length of 3 757 bp and *C. frutescens* L. cv. Shuanla with length of 1 671 bp [9]. Based on previous researches, 1 310 bp fragment from 1 671 bp *Pun1* gene *C. frutescens* L. cv. Cakra Hijau had been isolated. The purpose of this research was to isolate of 3'-end fragment and get full length of *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau.

2. Material and Method

The sample on this research used young leaves of *C. frutescens* L. cv. Cakra Hijau from BPTP Jatim, Karang Ploso, Malang, Indonesia. DNA isolation was done using DNA Plant Isolation Kit (Geneaid) protocol with modified procedure. *Pun1* gene was amplified using PCR technique with a pair of primer that designed based on *Pun1* gene sequences of *C. frutescens* L. cv. Shuanla [9], forward primer 5'-GAA-GGT-GGC-AGA-AGA-ATC-AG-3' and reverse primer 5'-TTG- TTG ACC-GTA-AAC-TTC-CG-3'. PCR cycle was done with 30 cycle, as follow: denaturation 94°C for 1 min, annealing 54°C for 1 min, and extension

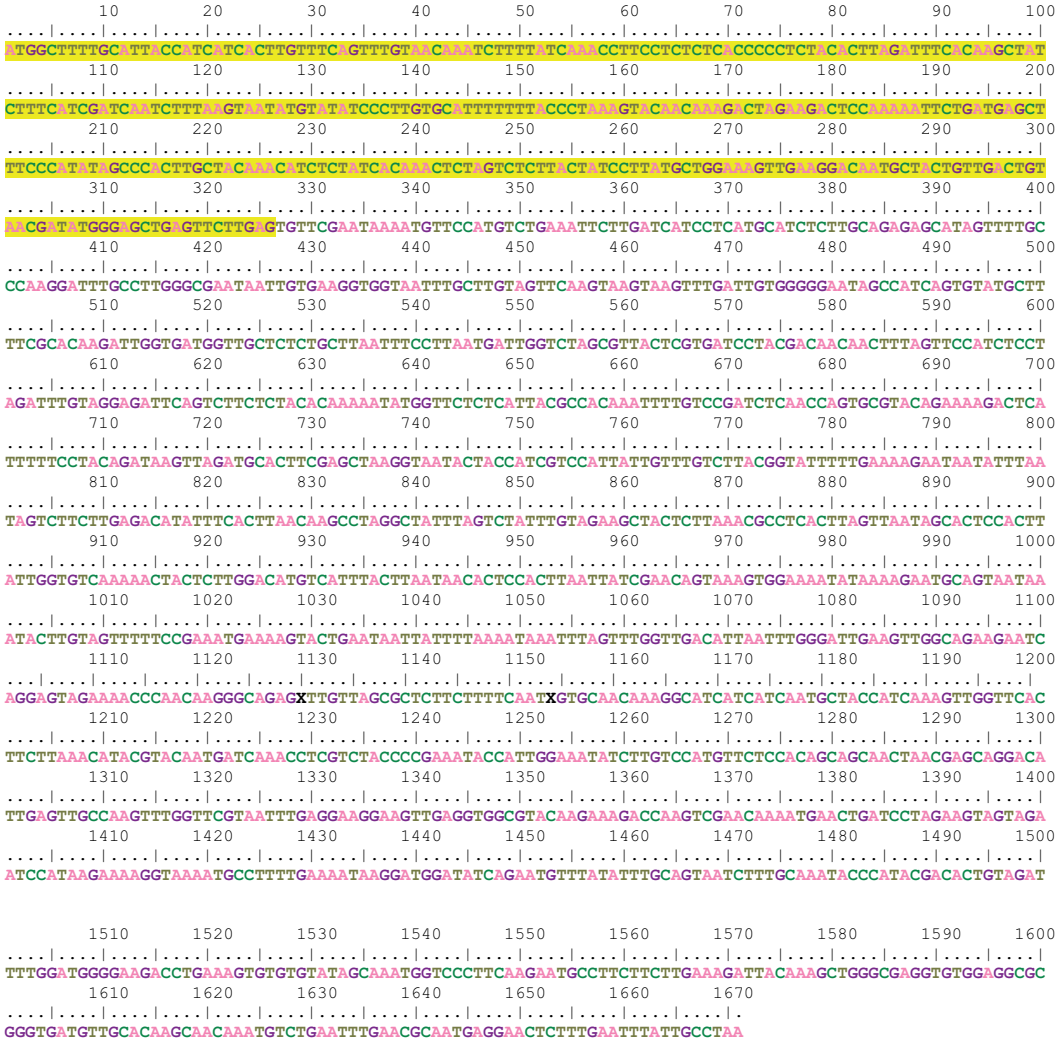

Figure 3: The full length of *Pun1* Gene from *C. frutescens* L. cv. Cakra Hijau.

72°C for 1 min. PCR products were checked by electrophoresis process with completely agarose gel 1,5% for 30 min. DNA sequencing was done by DNA machine type ABI Prism 3 730 × 1 in First Base Laboratory, Malaysia. DNA sequence was analyzed using Bioedit, DNA Baser, BLAST, ClustalX, and Sixframe.

3. Results and Discussions

DNA amplification using forward primer (F5) and reverse primer (R6) successfully to obtained consensus 3'-end fragment sequence with 715 bp length (see Figure 1).

The consensus sequence of 3'-end fragment between *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau, *C. frutescens* L. cv. Shuanla and *C. frutescens* L. cv.BG 2814-6 has analyzed using BLAST program showed high similarity of 92% (see Figure 2). *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau and *C. frutescens* L. cv. Shuanla showed 35%

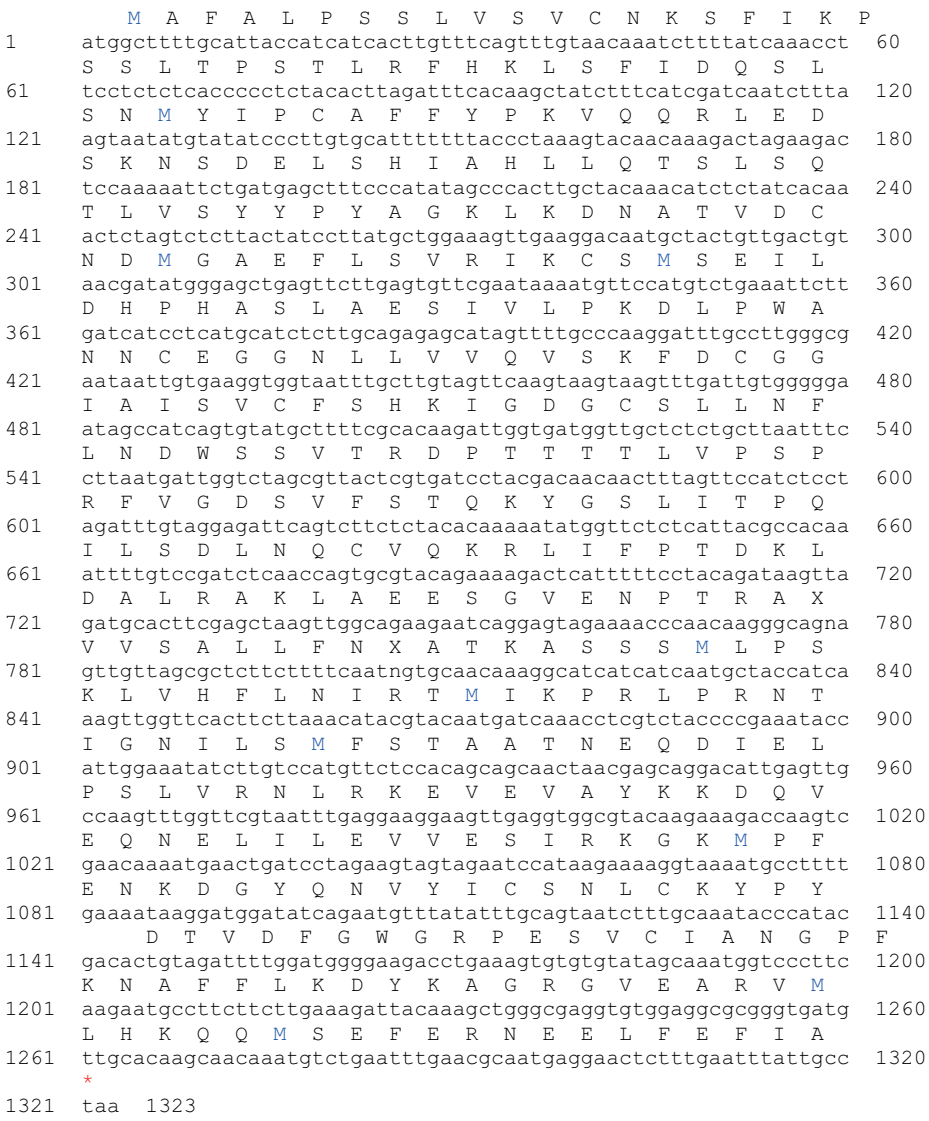

Figure 4: The #1 ORF of complete fragment of merged *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau. Red asterisk depict Stop codon; M: Methionin or Start codon.

of query coverage value, meanwhile *C. frutescens* L. cv. Cakra Hijau and *C. frutescens* L. cv. BG 2814-6 showed 16% of query coverage value (see Figure 2).

Previous researches has succeed to isolated DNA on upstream fragment with 326 bp length (see Figure 3, marked with yellow colour) [11], first middle fragment with 317 bp length (see Figure 3, marked with green colour) [10], second middle fragment with 260 bp length (see Figure 3, marked with yellow colour) [11], last middle fragment with 407 bp length (Figure 3, marked with blue colour) [12], and 3'-end fragment with 715 bp length (see Figure 3, marked with grey colour). The result of 3'-end fragment of *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau used to complete the full length of *Pun1* gene (see Figure 3).

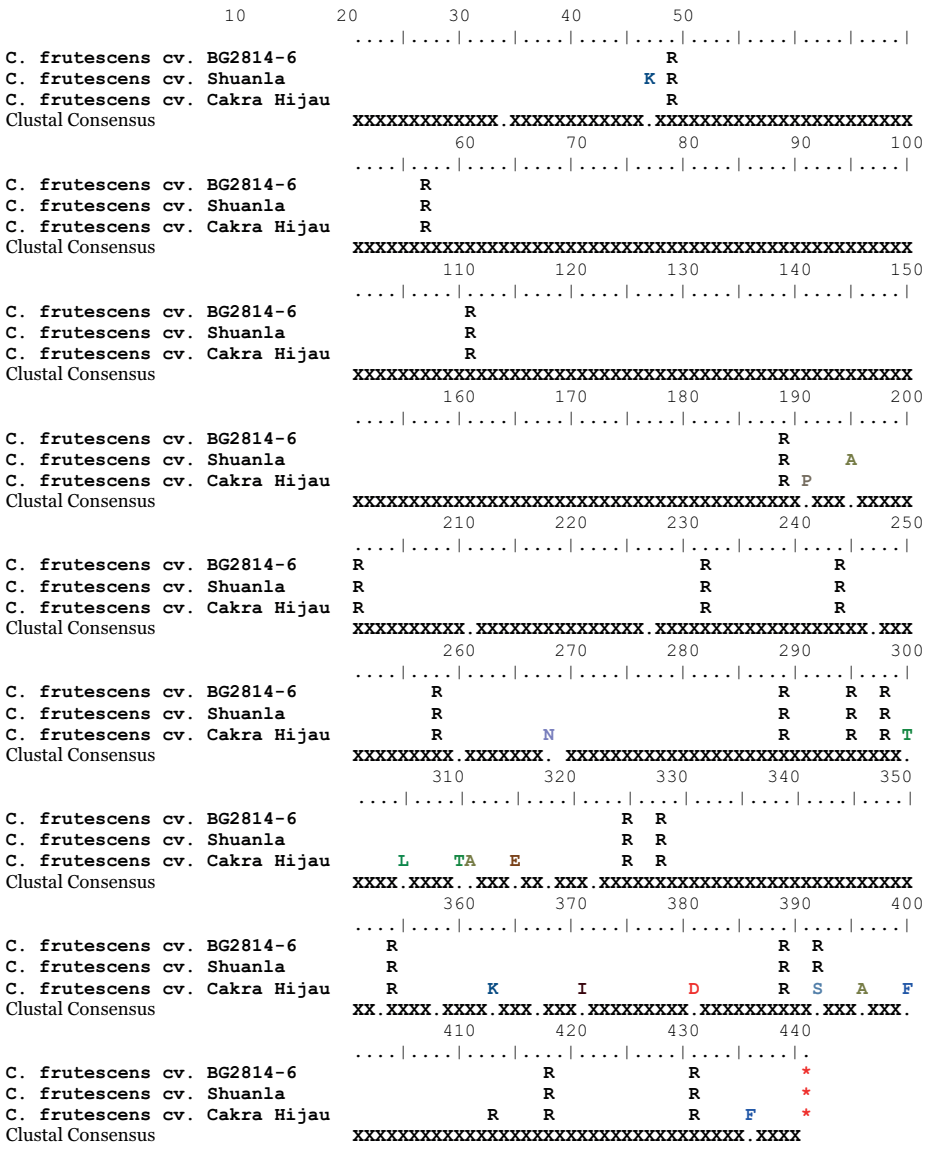

Figure 5: The alignment of amino acid *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau, Shuanla, and BG 2814-6. Marked (X) conserve of amino acid; (.) different of amino acid; (-) amino acid doesn't know.

The full length of *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau consist of two sequence exon, separated by an intron of 348 bp length. The first exon of 738 bp length and second exon of 585 bp length. The sequence exon merging produced of 1 323 bp length of *Pun1* gene. It was analysed using ORF #1 is the most possible reading of *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau for it does not contain stop codon (see Figure 4).

The exon was translated to obtained amino acid structure using Bioedit program. The amino acid results from *C. frutescens* L. cv. Cakra Hijau was compared to amino acids from *C. frutescens* L. cv. Shuanla and *C. frutescens* L. cv. BG 2814-6. The alignment of the three cultivars, *C. frutescens* L. cv. Shuanla, *C. frutescens* L. cv. BG 2814-6, and *C.*

frutescens L. cv. Cakra Hijau showed that there are 26 different amino acids of 441 total of amino acids (see Figure 5).

4. Conclusions

This study successfully obtained 3'-end fragment of 715 bp length which completed the full length of *Pun1* gene from *C. frutescens* L. cv. Cakra Hijau. Currently, we are attempting to modify cDNA to complete the *Pun1* gene insertion from *C. frutescens* L. cv. Cakra Hijau in other Solanaceae family.

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