

## 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.

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

## 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 potential produces of 12 t · ha<sup>-1</sup>, 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].

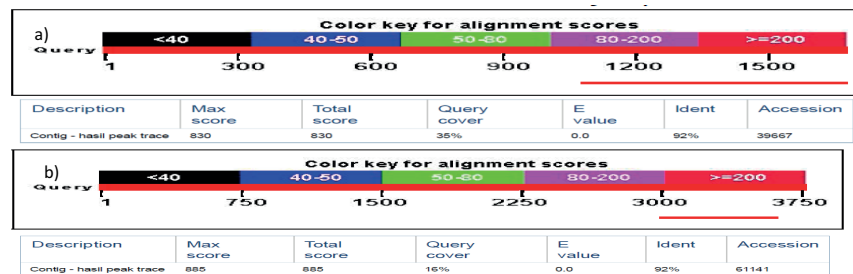
Capsaicin compound produced by condensation of vanyllamine derived from Phenylalanine with 8 methyl-6 nonenoic acid derived from valine or leucine, which the activated capsaicin synthase (CS) [6, 7]. Capsaicin synthase functioned as the last enzyme of capsaicin biosynthesis that encoded by a putative *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
TTGAAGGTTGGCAGAGAATCAGGAGTAGAAAA CCCAACAGGGCAGAGTTGTTAGCGCTCTTCTTTTCAATGTGCAACAAAGGCATCATCATCAATGCT
110     120     130     140     150     160     170     180     190     200
ACCATCAAAGTTGGTTCAC TTCTTAAACATACGTACAAATGATCAAACCTCGTCTACCCCGAAATACCATTGGAATATCTTGTCCATGTTCTCCACAGCA
210     220     230     240     250     260     270     280     290     300
GCAACTAACGAGCAGGACATTGAGTTGCCAAGTTTGGTTCGTAATTTGAGGAAGGAAGTTGAGGTGGCGTACAAGAAAGACCAAGTCGAACAAAATGAAC
310     320     330     340     350     360     370     380     390     400
TGATCCTAGAAAGTAGTAGAATCCATAAGAAAAGGTAATAATGCCCTTTGAAAATAAGGATGGATATCGAATGTTTATATTTGCAGTAATCTTTGCAATA
410     420     430     440     450     460     470     480     490     500
CCCATACGACACTGTAGATTTTGGATGGGAAGACCTGAAAGTGTGTATAGCAAAATGGTCCCTTCAGAAATGCCTTCTTGAAGATTACAAAGCT
510     520     530     540     550     560     570     580     590     600
GGCGAGGTGTGGAGGCGCGGTGATGTTGCACAAGCAACAAATGCTGAAATTTGAACGCBAATGAGGAACCTTTGAAATTTATTCCTAAATTAATCCAA
610     620     630     640     650     660     670     680     690     700
GTTTTGGAGTAACTGGATGTTGATTGAAGAGGATTTTACCAATTAAGGTAGGAATAATGTAATGTGTTGTTTCATAAAACAACCTTCACCGGAAGTT
710
TACGGTTCAACAAA
    
```

**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

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10      20      30      40      50      60      70      80      90      100
TGGC TTTTGC TT CC TC TC CTTG TTTT C G TTTT TC C CTTCCCTCTCTC CCCCCTCT C CTT G TTTT C GCT T
110     120     130     140     150     160     170     180     190     200
CTTTC TCG TC C TCTTT TGT T TGT T TCCCTTGTGC TTTT TTT CCCTAAGT C C C AAG CT G G CTCC A A A T TCTG TG GCT
210     220     230     240     250     260     270     280     290     300
TTCCC T T GCCC CTTGCT C C C TCTCT TC C C C TCT GTCTCTT CT TCCCT TGC TGG GTTG GGG C A TGCT CTGTTG CTGT
310     320     330     340     350     360     370     380     390     400
CG T TGGG GCTG GTTCTTG GTGTTTCAATAAATAATTTCCATGCTCTGAATTTCTTGATCATCCTCATGCATCTTGCAGAGAGCATAGTTTTCC
410     420     430     440     450     460     470     480     490     500
CC AAGGATTTGCC TGGGCAATAATTTGAAGGTGGTAATTTGCTTGTAGTTCAAGTAAGTAAGTTTGAATTGGGGGAA TAGCCATCAGTGTATGCTT
510     520     530     540     550     560     570     580     590     600
TTCCG C AAGATTTGGTGATGGTTGCTCTCTGCTTAATTTCTTAATGATTTGGTCTAGCGTTACTCGTGA TCC TACGACAA CACTT TAGTTCATCCTT
610     620     630     640     650     660     670     680     690     700
AGATTTGTAGGAGATT CAGTCTTCTCTA C A C A A A A T A T G G T T C T C A T T A C G C C A C A A A T T T T G T C C G A T C T C A A C C A G T G C G T A C A G A A A G A C T C A
710     720     730     740     750     760     770     780     790     800
TTTTTCTTACAGATAAGTTAGATGCACTTCGAGCTAAGGTAATACTACCATCGTCCATTAATTGTTTGTCTTACGTAATTTTGA AAA G A A T A A T T T A A
810     820     830     840     850     860     870     880     890     900
TAGTCTTCTTGA G A C A T A T T T C A C T T A A C A A G C C T A G G C T A T T T A G T C T A T T T G T A G A A G C T A C T C T T A A A C G C C C T A C T T A G T T A A T A G C A C T C C A C T T
910     920     930     940     950     960     970     980     990     1000
ATTGGTGTCAAAA C T A C T C T T G G A C A T G C A T T T A C T T A A T A A C A C T C C A C T T A A T T A T C G A A C A G T A A A G T G G A A A A T A T A A A A A A T T G C A G T A A T A A
1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
A T A C T T G T A G T T T T C C G A A A T G A A A G T A C T G A A T A A T T A T T T A A A A T A A A T T A G T T G G T T G A C A T T A A T T T G G G A T T G A A G T T G C C A G A A G A A T C
1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AGGAGTAGAAA C C C A A C A A G G G C A G A G X T T G T T A G C G C T C T T T T C A A T X G T G C A A C A A G G C A T C A T C A A T G C T A C C A T C A A A G T T G G T T C A C
1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
TTCTTAAA C A T A C G T A C A A T G A T C A A A C C T C G T C T A C C C C G A A A T A C C A T T G G A A A T A T C T T G T C C A T G T T C T C C A C A G C A G C A A C T A A C G A C A G G A C A
1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
TTGAGTTGCCAAGTTTGGTTTGGTAATTTGAGGAAGGAGTTGAGGTGGCGTCAAGAAAGACC A A G T C C A A C A A A A T G A A C T G A C C C T A G A A G T A G T A G A
1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
ATCCA T A A G A A A G G T A A A A T G C C T T T T G A A A A T A A G G A T G G A T A T C A G A A T G T T T A T A T T T G C A G T A A C T T T G C A A A T A C C C A T A C G A C A C T G T A G A T
1510    1520    1530    1540    1550    1560    1570    1580    1590    1600
TTTGGATGGGG A A G C C T G A A A G T C T G T G T A T A G C A A A T G G T C C C T T C A G A A A T G C C T T C T T G A A A G A T T A C A A A G C T G G G C G A G G T G T G G A G G C C
1610    1620    1630    1640    1650    1660    1670
GGGTGATGTTGCAC A A G C A A C A A A T G C T G A A T T T G A A C C G A A T G A G G A A C T C T T T G A A T T T A T T G C C T A A

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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%

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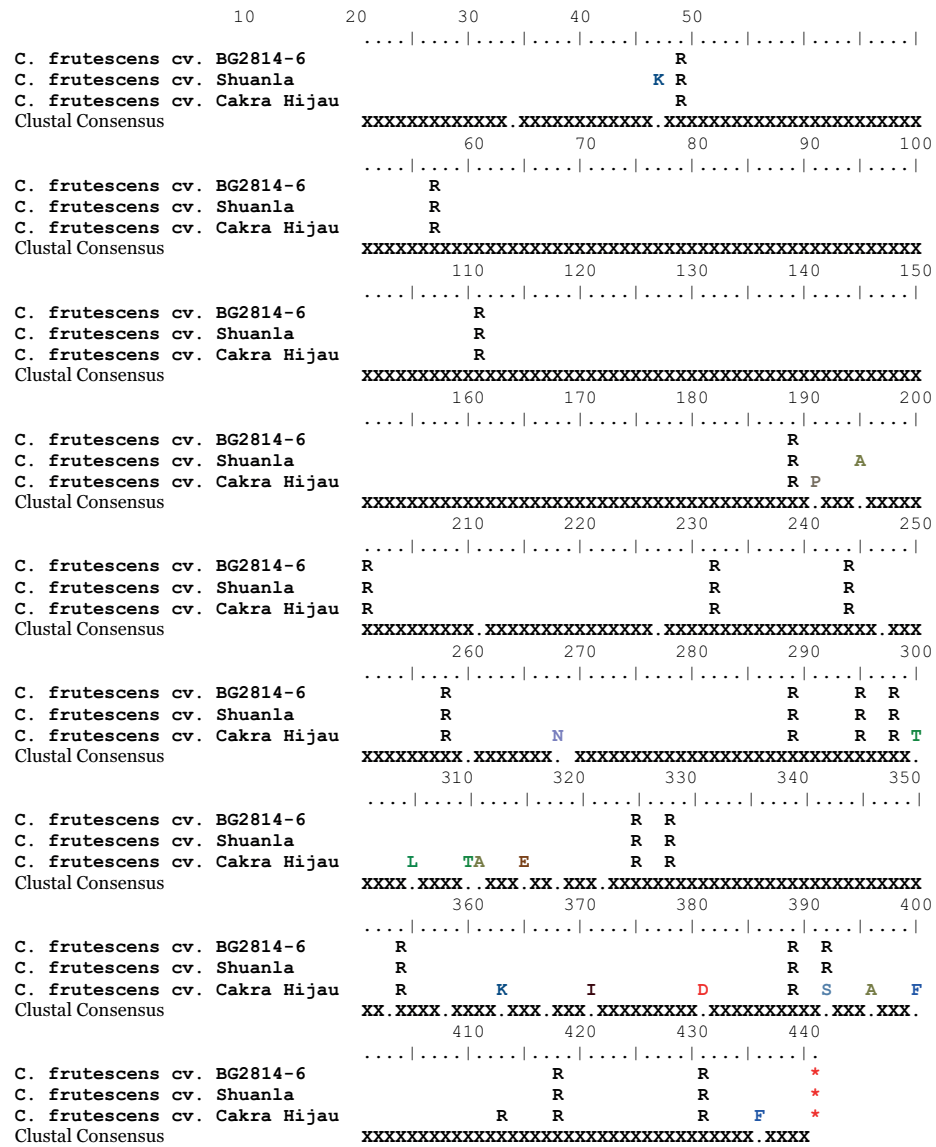
      M A F A L P S S L V S V C N K S F I K P
1   atggcttttgcattaccatcatcacttgtttcagtttgtaacaaatcttttatcaaacct 60
   S S L T P S T L R F H K L S F I D Q S L
61  tcctctctcaccctctacacttagatttcacaagctatctttcatcgatcaatcttta 120
   S N M Y I P C A F F Y P K V Q R L E D
121 agtaatatgtatatcccttggcattttttaccctaaagtacaacaaagactagaagac 180
   S K N S D E L S H I A H L L Q T S L S Q
181 tccaaaaattctgatgagctttcccatatagcccacttgctacaacatctctatcacia 240
   T L V S Y Y P Y A G K L K D N A T V D C
241 actctagtctcttactatcctttatgctggaagttgaaggacaatgctactgttgactgt 300
   N D M G A E F L S V R I K C S M S E I L
301 aacgatatgggagctgagttcttgagtgttcgaataaaatgttccatgtctgaaattctt 360
   D H P H A S L A E S I V L P K D L P W A
361 gatcaccctcatgcatctcttgacagagcatagtttggccaagatttgccttggcg 420
   N N C E G G N L L V V Q V S K F D C G G
421 aataattgtgaaggtggtaatttgcttgtagttcaagtaagtaagttgatgtggggga 480
   I A I S V C F S H K I G D G C S L L N F
481 atagccatcagtgatgcttttccgacaagattggtgatggttgcctactgcttaatttc 540
   L N D W S S V T R D P T T T T L V P S P
541 cttaatgattgggtctagcgttactcgtgatcctacgacaacaactttagttccatctcct 600
   R F V G D S V F S T Q K Y G S L I T P Q
601 agattgtaggagattcagttcttctacacaaaaatatggttctctactacgcacaaa 660
   I L S D L N Q C V Q K R L I F P T D K L
661 attttgcgatctcaaccagtgctacagaaaagactcatttttctacagataagtta 720
   D A L R A K L A E E S G V E N P T R A X
721 gatgcacttcgagctaagttggcagaagaatcaggagtagaaaaccaacaagggcagna 780
   V V S A L L F N X A T K A S S S M L P S
781 gttgttagcgtcttcttttcaatngtgcaacaaagggcatcatcaatgctaccatca 840
   K L V H F L N I R T M I K P R L P R N T
841 aagttgggtcacttcttaacatacgtacaatgatcaaacctcgtctaccccgaaatacc 900
   I G N I L S M F S T A A T N E Q D I E L
901 attgaaatatcttgcctatgttctccacagcagcaactaacgagcaggacattgagttg 960
   P S L V R N L R K E V E V A Y K K D Q V
961 ccaagtttgggttcgtaatttgaggaaggaagttgaggtggcgtacaagaaagaccagtc 1020
   E Q N E L I L E V V E S I R K G K M P F
1021 gaacaaaatgaactgatcctagaagtagtagaatccataagaaaaggtaaaatgcctttt 1080
   E N K D G Y Q N V Y I C S N L C K Y P Y
1081 gaaaataaggatggatatcagaatgtttatatttgcagtaatctttgcaaataccatatac 1140
      D T V D F G W G R P E S V C I A N G P F
1141 gacactgtagattttggatggggaagacctgaaagtgtgtgtatagcaaatgggtcccttc 1200
   K N A F F L K D Y K A G R G V E A R V M
1201 aagaatgccttcttcttgaagattacaagctggcgaggtgtggagggcgggtgatg 1260
   L H K Q Q M S E F E R N E E L F E F I A
1261 ttgcacaagcaacaaatgtctgaatttgaacgcaatgaggaactccttgaatttattgccc 1320
      *
1321 taa 1323

```

**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 1323 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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