Conference Paper

Pun1 Gene Isolation from *Capsicum frutescens* L. cv Cakra Hijau

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Abstract

Capsicum frutescens L. is one of chili peppers with high pungency. *Capsicum frutescens* has several cultivars, one of those is *C. frutescens* cv. Cakra Hijau. This cultivars is known resistance to pests and diseases as well. Pungency is due to the accumulation of capsaicinoids. *Pun1* is an important gene responsible for pungency. The full-leght genomic sequence of *Pun1* is 1897 bp, containing two exons of 738 bp and 590 bpand one intron of 348 bp in between. This study was aimed to isolate *Pun1* gene that free from intron. mRNA was isolated with TriReagent furthermore RT-PCR method used Qiagent One-Step RT-PCR and two pairs of primer : F1/R1 (F15'-ATG-GCT-TTT-GCA-TTA-CCA-TCA-3'/R15'-CTT-AGC-TCG-AAG-TGC-ATC-TA-3') and F2/R2 (F25'-GAA-GGT-GGC-AGA-AGA-ATC-AG-3'/R25'-TTA-GGC-AAT-GAA-CTC-AAG-GA-3'). The result of this study are isolated 738 bp exon-1 and 590 bp exon 2 of *Pun1* gene.

Keywords: Capsaicin; *Capsicum frutescens* L. cv. Cakra Hijau; exon; *Pun1* gene.

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1. Introduction

Capsicum frutescens L. is widely cultivated in tropical and subtropical regions of the world including Indonesia [1, 2]. Cakra Hijau is one of local cultivar from *C. frutescens* that have economic value, it is resistant to pest and disease as well and has high level of pungency [3]. The pungency level is due to the accumulation of capsaicinoids [4]. Capsaicinoid are synthesized and accumulated in the placental tissue [5]. Capsaicinoid was first detected at 20th days after anthesis then gradually increased and reached maximal level around 40 d after anthesis, on the 50 d, the capsaicinoid content decreased significantly [6].Capsaicin synthase (CS) is the last enzym in the biosynthesis of capsaicin and have important role in condensation of vanillylamine from phenylpropanoid with 8-methyl-6-nonenoic acid from valine pathway [7]. The *CS* expression starts at 10 dpa, reaches a maximum at 20 dpa, then suddenly decreases and almost stops at 40 dpa [8]. *Pun1* gene encodes CS enzyme, this gene probably functions as an acyltransferase to complete the capsaicinoid synthesis. Non-pungent were observed to have a 2,5 kb deletion spanning the putative promotoer and exon-1

of *Pun1* gene and a 4 bp deletation in part of exon-1 which distrub translation and so deletion of part of exon-2 including the stop codon [9–11].

DNA isolation of *Pun1* gene from *C. frutescens* cv. Cakra Hijau had been reported [12, 13]. This study aimed to isolate coding region from RNA of *Pun1* gene *C. frutescens* cv. Cakra Hijau to complete the exising data.

2. Materials and Methods

2.1. Plant material

Seeds of *Capsicum frutescens* cv. Cakra Hijau were obtained from Balai Pengkajian Teknologi Pertanian (BPTP) [Assesment Institute for Agricultural Technology—AIAT)], Karang Ploso, Malang, Indonesia.

2.2. RNA extraction

Total RNA was extracted from placental tissue approximately 100 mg for each reaction using TriReagent (Invitrogen) according to the manufactures instruction. Quantity of RNA was measured using NanoDrop DR2000.

2.3. Reverse Transcriptase-PCR

Complementary DNA (cDNA) was synthesized using Qiagen OneStep RT-PCR (Qiagen) as per the manufacturer's instruction. In all, 500 ng of total RNA was used for each reaction. PCR primer were design based on *C. frutescens* cv. Shuanla sequence from Gene Bank (accession number HM854860). The primer used were F1/R1 (F15'-ATG-GCT-TTT-GCA-TTA-CCA-TCA-3'/R15'-CTT-AGC-TCG-AAG-TGC-ATC-TA-3') and F2/R2 (F25'-GAA-GGT-GGC-AGA-AGA-ATC-AG-3'/R25'-TTA-GGC-AAT-GAA-CTC-AAG-GA-3'). RT-PCR cycles a is follows: reverse transcription 50°C for 30 min, initial PCR activation step 95°C for 15 min, denaturation 94°C for 1 min, annealing 54°C for 1 min, extension 70°C for 1 min, and final extension 70°C for 10 min. RT-PCR product were checked using 1.5% gel electrophoresis and sequenced by ABIPRISM 3730x1 Genetic analyzer machine at First Base Laboratories, Malaysia.

2.4. Analysis

The sequence was analyzed using DNA Baser, BLASTN, Clustal X and SIXFRAME (as it is an online programe so please add the website address).

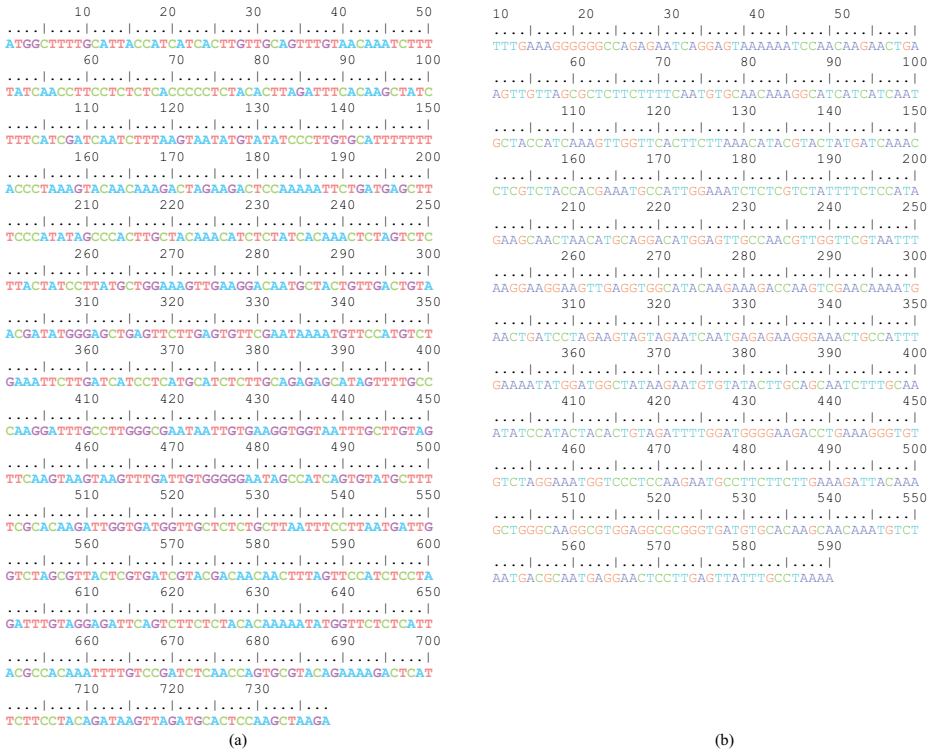


Figure 1: Sekuen *Pun1* gen from *C. frutescens* cv. Cakra Hijau isolated using primers: (a) F1/R1; (b) F2/R2.

3. Result and Discussion

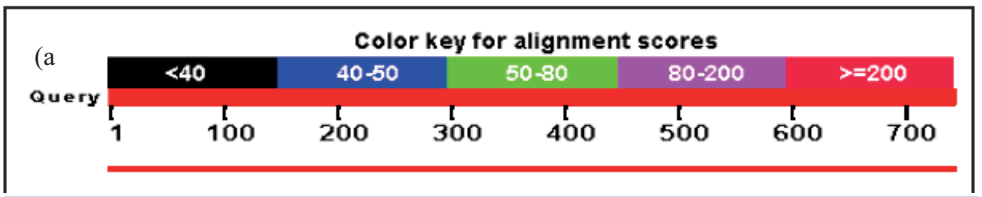
In this study, combination primer forward and reverse F1/R1 primer obtained 738 bp exon-1 meanwhile combination primer forward and reverse F2/R2 primer obtained 590 bp exon-2 (Figure 1).

BLASTN analysis result that query coverage of F1/R1 Sequence is 100% from total sequence exon-1 *Pun1* gene from *C. frutescens* cv. Shuanla and *C. frutescens* cv. BG2814-6, and have 98% similarity sequence with exon-1 *Pun1* gene from *C. frutescens* cv. Shuanla and *C. frutescens* cv. BG2814-6 (Figure 2).

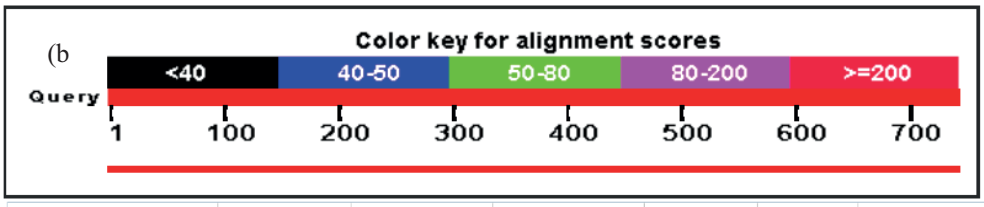
BLASTN analysis result that query coverage of F2/R2 Sequence is 99% from total sequence exon-2*Pun1* gene from *C. frutescens* cv. Shuanla and *C. frutescens* cv. BG2814-6 and have 98% and 97% similarity sequence with exon-1 *Pun1* gene from *C. frutescens* cv. Shuanla and *C. frutescens* cv. BG2814-6, respectively (Figure 3).

F1/R1 sequence was analyzed with SIXFRAME (www.seqtool.edu) showing first open reading frame (ORF) is the most possible reading frame of the target gene because it contained start codon in the beginning of fragment (Figure 4). Exon-1 from *Pun1* gene containing a start codon [9].

Open reading frame analysis from F2/R2 sequence show that second ORF is the most possible reading frame of the target gene because it just have one stop codon

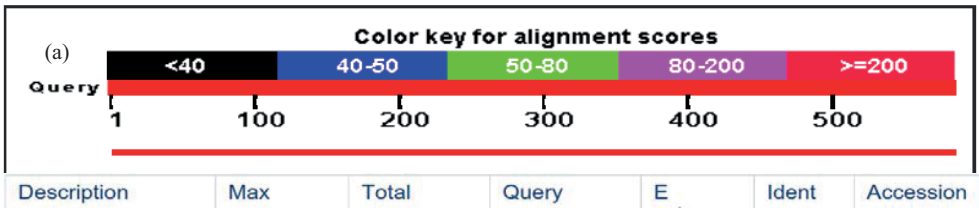


Description	Max score	Total score	Query cover	E value	Ident	Accession
C.frutescens_cv._C.Hijau	1279	1279	100%	0.0	98%	Query_105379

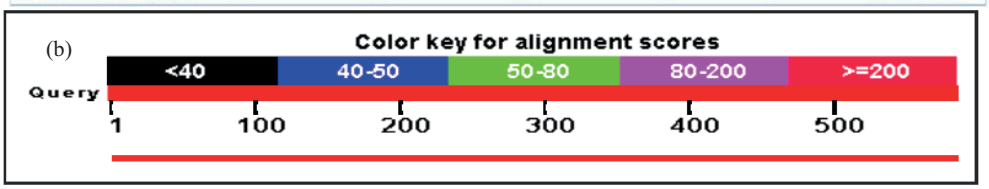


Description	Max score	Total score	Query cover	E value	Ident	Accession
C.frutescens_cv._C.Hijau	1279	1279	100%	0.0	98%	Query_120227

Figure 2: BLASTN analysis result of F1/R1 sequence *Pun1* gene from *C. frutescens* cv. Cakra Hijau compared with *Pun1* gene from: (a) *C. frutescens* cv. BG2814-6 (b) *C. frutescens* cv. Shuanla.



Description	Max score	Total score	Query cover	E value	Ident	Accession
C.frutescens_cv._C.Hijau	994	994	99%	0.0	98%	Query_66303



Description	Max score	Total score	Query cover	E value	Ident	Accession
C.frutescens_cv._C.Hijau	983	983	99%	0.0	97%	Query_176493

Figure 3: BLASTN analysis result of F2/R2 sequence *Pun1* gene from *C. frutescens* cv. Cakra Hijau compared with *Pun1* gene from: (a) *C. frutescens* cv.; BG2814-6; (b) *C. frutescens* cv. Shuanla.

in the end of sequence and not found another stop codon in the middle of sequence (Figure 5). Exon-2 *Pun1* gene containing a stop codon [11].

Sequences from exon-1 and exon-2 *Pun1* gene from *C. frutescens* cv. Cakra Hijau was analyzed using ClustalX to align amino acid sequence. The result of this analysis revealed that exon-1 *Pun1* gene of *C. frutescens* cv. Cakra Hijau located in the 1st up to 246th amino acid sequence of *C. frutescens* cv. BG2814-6 and *C. frutescens* cv. Shuanla (Figure 6).

```
     M  A  F  A  L  P  S  S  L  V  A  V  C  N  K  S  F  I  X  P
1    atggcttttgcattaccatcatcacttgttgcagtttgtaacaaatcttttatcaancct  60
     S  S  L  T  P  S  T  L  R  F  H  K  L  S  F  I  D  Q  S  L
61   tcctctctcacccccctctacacttagatttcacaagctatctttcatcgatcaatcttta  120
     S  N  M  Y  I  P  C  A  F  F  Y  P  K  V  Q  Q  R  L  E  D
121  agtaatatgtatatcccttgtgcatttttttaccctaaagtacaacaaagactagaagac  180
     S  K  N  S  D  E  L  S  H  I  A  H  L  L  Q  T  S  L  S  Q
181  tccaaaaattctgatgagctttcccatatagcccacttgctacaaacatctctatcacaa  240
     T  L  V  S  Y  Y  P  Y  A  G  K  L  K  D  N  A  T  V  D  C
241  actctagtctcttactatccttatgctggaaagttgaaggacaatgctactgttgactgt  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  gatcatcctcatgcatctcttgcagagagcatagttttgcccaaggatttgccttgggcg  420
     N  N  C  E  G  G  N  L  L  V  V  Q  V  S  K  F  D  C  G  G
421  aataattgtgaaggtggtaatttgcttgtagttcaagtaagtaagtttgattgtgggggа  480
     I  A  I  S  V  C  F  S  H  K  I  G  D  G  C  S  L  L  N  F
481  atagccatcagtgtatgcttttcgcacaagattggtgatggttgctctctgcttaatttc  540
     L  N  D  W  S  S  V  T  R  D  R  T  T  T  T  L  V  P  S  P
541  cttaatgattggtctagcgttactcgtgatcgtacgacaacaactttagttccatctcct  600
     R  F  V  G  D  S  V  F  S  T  Q  K  Y  G  S  L  I  T  P  Q
601  agatttgtaggagattcagtcttctctacacaaaaatatggttctctcattacgccacaa  660
     I  L  S  D  L  N  Q  C  V  Q  K  R  L  I  L  P  T  D  K  L
661  attttgtccgatctcaaccagtgcgtacagaaaagactcattcttcctacagataagtta  720
     D  A  L  Q  A  K
721  gatgcactccaagctaaga  739
```

Figure 4: The first ORF F1/R1 sequence *Pun1* gene of *C. frutescens* cv. Cakra Hijau. M: Methionin or Start codon.

```
     F  E  R  G  A  R  E  S  G  V  K  N  P  T  R  T  E  V  V  S
2    tttgaaaggggggccagagaatcaggagtaaaaaatccaacaagaactgaagttgttagc  61
     A  L  L  F  X  C  A  T  K  A  S  S  S  M  L  P  S  K  L  V
62   gctcttcttttcaantgtgcaacaaaggcatcatcatcaatgctaccatcaaagttggtt  121
     H  F  L  N  I  R  T  M  I  K  P  R  L  P  R  N  A  I  G  N
122  cacttcttaaacatacgtactatgatcaaacctcgtctaccacgaaatgccattggaaat  181
     L  S  S  I  F  S  I  E  A  T  N  M  Q  D  M  E  L  P  T  L
182  ctctcgtctatttttctccatagaagcaactaacatgcaggacatggagttgccaacgttg  241
     V  R  N  L  R  K  E  V  E  V  A  Y  K  K  D  Q  V  E  Q  N
242  gttcgtaatttaaggaaggaagttgaggtggcatacaagaaagaccaagtcgaacaaaat  301
     E  L  I  L  E  V  V  E  S  M  R  E  G  K  L  P  F  E  N  M
302  gaactgatcctagaagtagtagaatcaatgagagaagggaaactgccatttgaaaatatg  361
     D  G  Y  K  N  V  Y  T  C  S  N  L  C  K  Y  P  Y  Y  T  V
362  gatggctataagaatgtgtatacttgcagcaatctttgcaaatatccatactacactgta  421
     D  F  G  W  G  R  P  E  R  V  C  L  G  N  G  P  S  K  N  A
422  gattttggatggggaagacctgaaagggtgtgtctaggaaatggtccctccaagaatgcc  481
     F  F  L  K  D  Y  K  A  G  Q  G  V  E  A  R  V  M  X  H  K
482  ttcttcttgaaagattacaaagctgggcaaggcgtggaggcgcgggtgatgntgcacaag  541
     Q  Q  M  S  N  X  X  R  N  E  E  L  L  E  L  F  A  *
542  caacaaatgtctaatgnnnnacgcaatgaggaactccttgagttatttgcctaaaa  597
```

Figure 5: The second ORF F1/R1 sequence *Pun1* gene of *C. frutescens* cv. Cakra Hijau.*: Stop codon.

Analyzed of exon-2 *Pun1* gene from *C. frutescens* cv. Cakra Hijau show that sequance located in the 240th up to 440th amino acid sequence of *C. frutescens* cv. BG2814-6 and *C. frutescens* cv. Shuanla (Figure 7).

Alignment analysis showed that there was over lapping amino acid in *C. frutescens* cv. Cakra Hijau from 240th to 246th amino acid. Further study is needed to confirmthis result.

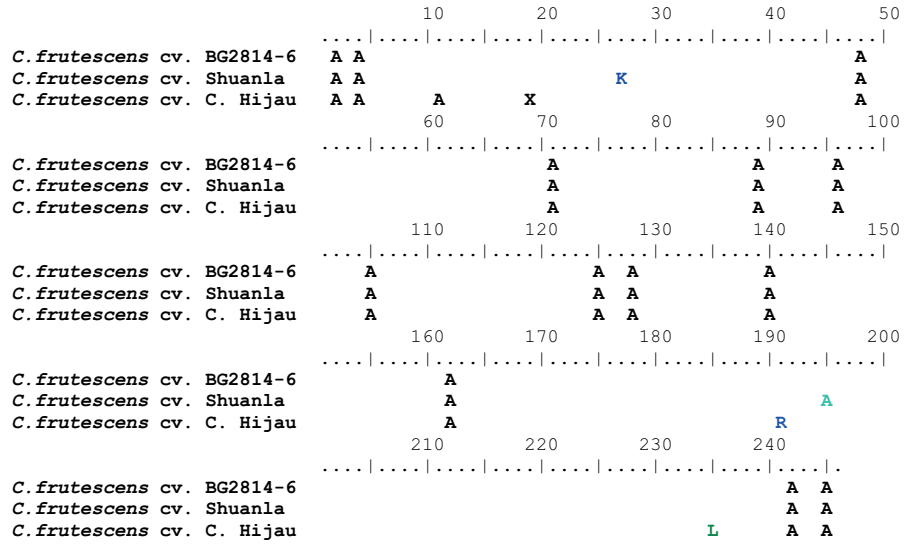


Figure 6: Aligenment of amino acid sequence from exon-1 *Pun1* gene *C. frutescens* cv. Cakra Hijau with *C. frutescens* cv. BG2814-6 and *C. frutescens* cv. Shuanla.

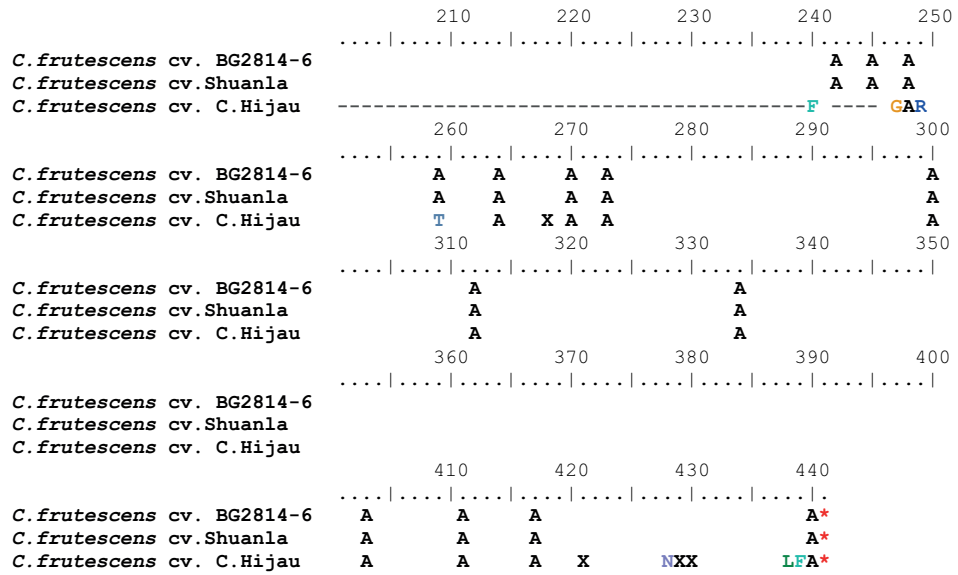


Figure 7: Alignment of amino acid from exon-2 *Pun1* gene *C. frutescens* cv. Cakra Hijau with *C. frutescens* cv. BG2814-6 and *C. frutescens* cv. Shuanla. .*: Stop codon.

4. Conclusions

This study has successfully isolated 738 bp length of exon-1 and 590 bp length of exon-2 of *Pun1* gene from *C. frutescens* cv. Cakra Hijau. Further study is needed because there was an over lapped amino acid by 6 bp length.

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