

## Conference Paper

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

Shelly Zairina, Elhah Nailul Khasna, Ria Reinnata Juliandari, Eko Sri Sulasmi, and Dwi Listyorini

Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Malang, Jl. Semarang 5, Malang 65145, Indonesia

## 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.

Corresponding Author:

Shelly Zairina

shelly.zairina@yahoo.com

Received: 11 February 2017

Accepted: 08 March 2017

Published: 26 March 2017

Publishing services provided  
by Knowledge E

© Shelly Zairina et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

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

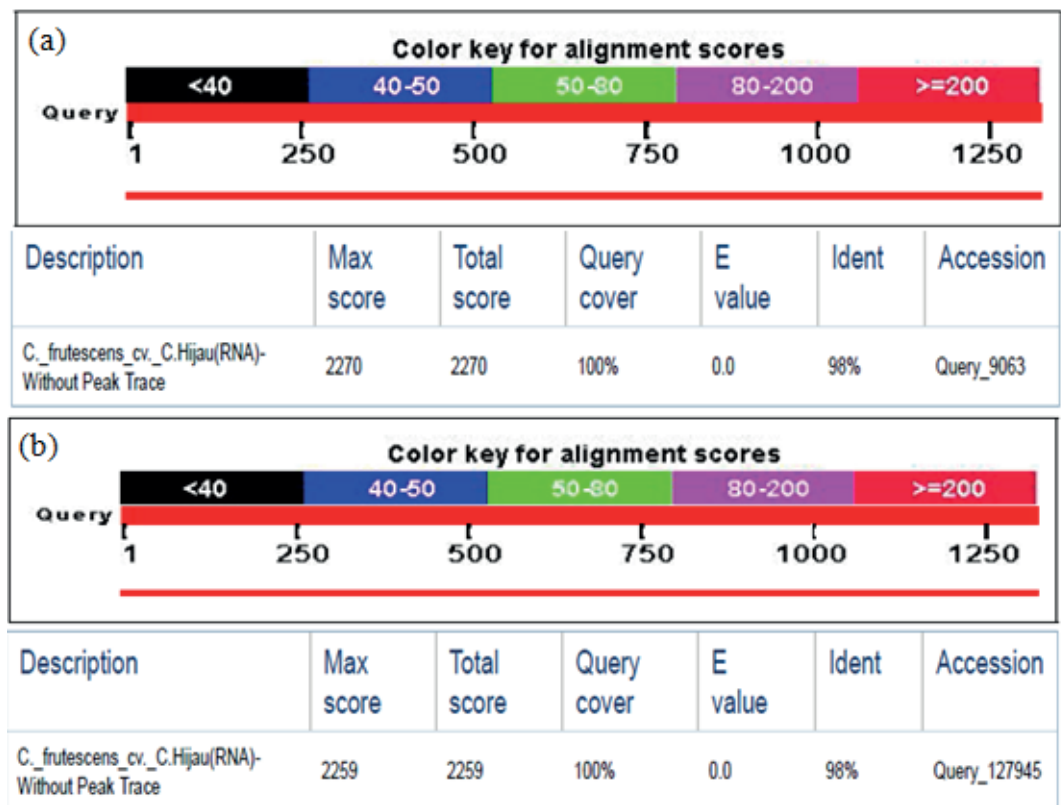
**Keywords:** *Capsicum frutescens* L. cv. Cakra Hijau; *Pun1* gene; synthesis of cDNA.

## 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-vanillyl-6-nonenamide). The accumulation and biosynthesis of capsaicin are localized in the epidermal cells of the placental, specifically in the interocular 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 phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (Ca4H), coumaric acid 3-hydroxylase (Ca3H), caffeic acid o-methyl transferase (COMT), and amino transferase

## OPEN ACCESS



**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 interocular 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

```

10 20 30 40 50 60 70 80 90 100
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ATGCGCTTTTCATTACCATCATCACTGTTGCAAGTTTGTAAACAAATCTTTTAT-CAACCTTCCTCTCCACCCCTCTACACTTACATTTCCACAAOCTAT

110 120 130 140 150 160 170 180 190 200
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CTTTCATCGATCAATCTTTAAAGTAATAATGTATATCCCTTTCGOCATTTTTTACCCCTAAAAGTACAAACAAAGACTAGAAAGCTCCAAAAATTCCTGATGAGCT

210 220 230 240 250 260 270 280 290 300
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCCCATATAGCCCACTTCTACAAACATCTCTATCACAACACTCTACTCTTACTATCCCTTATCCCTGGAAAGTTCAAGGCAAACTGCTACTGTTGACTGCT

310 320 330 340 350 360 370 380 390 400
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAGCATATGGGAGCTGAGCTTCTGAGTGTTCGAATAAAAATGTTCCATGTCTGAAATTTCTTGCATCATCCCTCATGOCATCTCTTGCAGAGAGCATAGCTTTTCC

410 420 430 440 450 460 470 480 490 500
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CCAAAGGATTTGCCCTGGCCGAATAATTGTCAAGCTGCTAAATTTGCTGTAGTCTCAACTAAAGTAAAGTTTGATTTGGGGGAAATGACCATCAGCTGATGCTT

510 520 530 540 550 560 570 580 590 600
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCCACAAAGATTTGGTGTATGGTTCCCTCTCTGCTTAAATTTCCCTTAATGATGCTCTAGCGTTACTCTGCTGATCGTACGACAAACAACTTTAGTCCATCTCCCT

610 620 630 640 650 660 670 680 690 700
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AGATTTGTAGGAGATTCAGCTTCTCTACACAAAAATATGGTTCTCTCATTACGCCACAAATTTTGTCCCATCTCAACCACTGCTCCACAAAAAGACTCA

710 720 730 740 750 760 770 780 790 800
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
TTCTTCTACAGATAAGTTAGATCCACTCCAAAGCTAAAGGGGCCACAGAAATCAAGGACTAAAAAATCCAAACAAAGAACTGAAAGTTTGTAGCCCTCTCTTTT

810 820 830 840 850 860 870 880 890 900
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CAAT-GTCCAAACAAAGCCATCATCATCAATGCTAACATCAAAAGTTGCTTCCACTTCTTAAACATACGCTACTATGATCAAAACCTGCTTACCACGAAATGCC

910 920 930 940 950 960 970 980 990 1000
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
ATTCCAAATCTCTCCCTATTTTCTCCATAGAAOCAAATCAACATOCAGGACATGGAGTTCCCAACGTTGCTGCTAAATTTAAGCAAGCAAGTTCAAGCTGC

1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
CATACAAAGAACCCAAAGTCCAAACAAATCAACTGATCCTAGAACTAGTAAATCAATGAGAGAAAGGAACTGCCATTTGAAAAATATGCATGCTATATAA

1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
GAAATGCTATACTTCCAGCAATCTTTCCAAATATCCATACTACACTCTGATTTTGCATGGGGCAAGCACTGAAAAGGCTGCTCTAGAAAATGCTCCCTCC

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
AAAGATGCCCTTCTCTTCAAAGATTACAAAGCTGGGCCAAGGCGTGGAGGCCGCGGCTGATG-TCCACAAAGCAACAAATGCTTAATG---ACCCATGAGG

1310 1320
.....|.....|
AACTCCCTTCAAGTTATTTCCCTAA

```

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.

```

M A F A L P S S L V A V C N K S F X Q P
1 atggcttttgcattaccatcacttctgttgcagtttgaacaaatcttttatncaacct 60
S S L T P S T L R F H K L S F I D Q S L
61 tectctctcaccctctacacttagatttcacaagctatctttcatcgatcaaatcttta 120
S N M Y I P C A F F Y P K V Q Q R L E D
121 agtaaatgtatatacccttgcattttttaccctaaagtacaacaagactagaagac 180
S K N S D E L S H I A H L L Q T S L S Q
181 tccaaaaattctgatgagctttcccatatagcccacttgcacaacaactctctatccaaa 240
T L V S Y Y P Y A G K L K D N A T V D C
241 actctagtctcttactatcttctgtggaagttgaaggacaatgctactgttgaactgt 300
N D M G A E F L S V R I K C S M S E I L
301 aacgatatgggagctgagcttctgtgagttctgaataaaatgttccatgtctgaaattctt 360
D H P H A S L A E S I V L P K D L P W A
361 gatcctcctcatgcctctcttgcagagagcatagttttgcccaggatttgccttggggcg 420
N N C E G G N L L V V Q V S K F D C G G
421 aataaattgtgaaggtggaatttctgttagttcaagtaagtaagtttgattgtggggga 480
I A I S V C F S H R I G D G C S L L N F
481 atagccatcagtgatgcttttccgacaagattggtgaggttgcctctctgcttaatttc 540
L N D W S S V T R D R T T T T L V P S P
541 cttaaatgattggtctagcgttactctgtgacgacaacaactttagttccatctctct 600
R F V G D S V F S T Q K Y G S L I T P Q
601 agattttaggagattcagttctctctacacaaaaataggttctctcattacgccaaa 660
I L S D L N Q C V Q K R L I L P T D K L
661 attttgcgatctcaccagtgctgacagaaaagactcattctctcctacagataaagta 720
D A L Q A K G A R E S G V K N P T R T E
721 gatgcactccaagctaaaggggcccagagaatcaggagtaaaaaatccaacaagaactgaa 780
V V S A L L F N X A T K A S S S M L P S
781 gttgttagcgtctctcttttcaatngtgcacaaaaggcatcatcaatgctaccatca 840
K L V H F L N I R T M I K P R L P R N A
841 aagttggttccattcttaaacatagctactatgatcaaacctcgtctcacaagaaatgoc 900
I G N L S S I F S I E A T N M Q D M E L
901 attggaaatctctctctctctctctctctctctctctctctctctctctctctctctct 960
P T L V R N L R K E V E V A Y K K D Q V
961 ccaacgttgggtctgtaatttaaggaaggaagttgaggtggcacaagaagaagccaagtc 1020
E Q N E L I L E V V E S M R E G K L P F
1021 gaacaaaatgaactgatcctagaagtagtagaatcaatgagagaagggaaactgocattt 1080
E N M D G Y K N V Y T C S N L C K Y P Y
1081 gaaaatattggatggctataagaatgtgtatacttgcagcaatctttgcaaatatccatc 1140
Y T V D F G W G R P E R V C L G N G P S
1141 tacactgtagattttggatgggggaagcctgaaaggggtgtgtctaggaatggctccctcc 1200
K N A F F L K D Y K A G Q G V E A R V M
1201 aagaatgcctctctcttgaagattacaaagctgggcaagggcgtggggcggggtgatg 1260
X H K Q Q M S N X X R N E E L L E L F A
1261 ntgcacaagcaacaaatgtctaatgnnnaacgcaatgaggaactcctttaggttatttggc 1320
.
1321 taa 1323

```

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

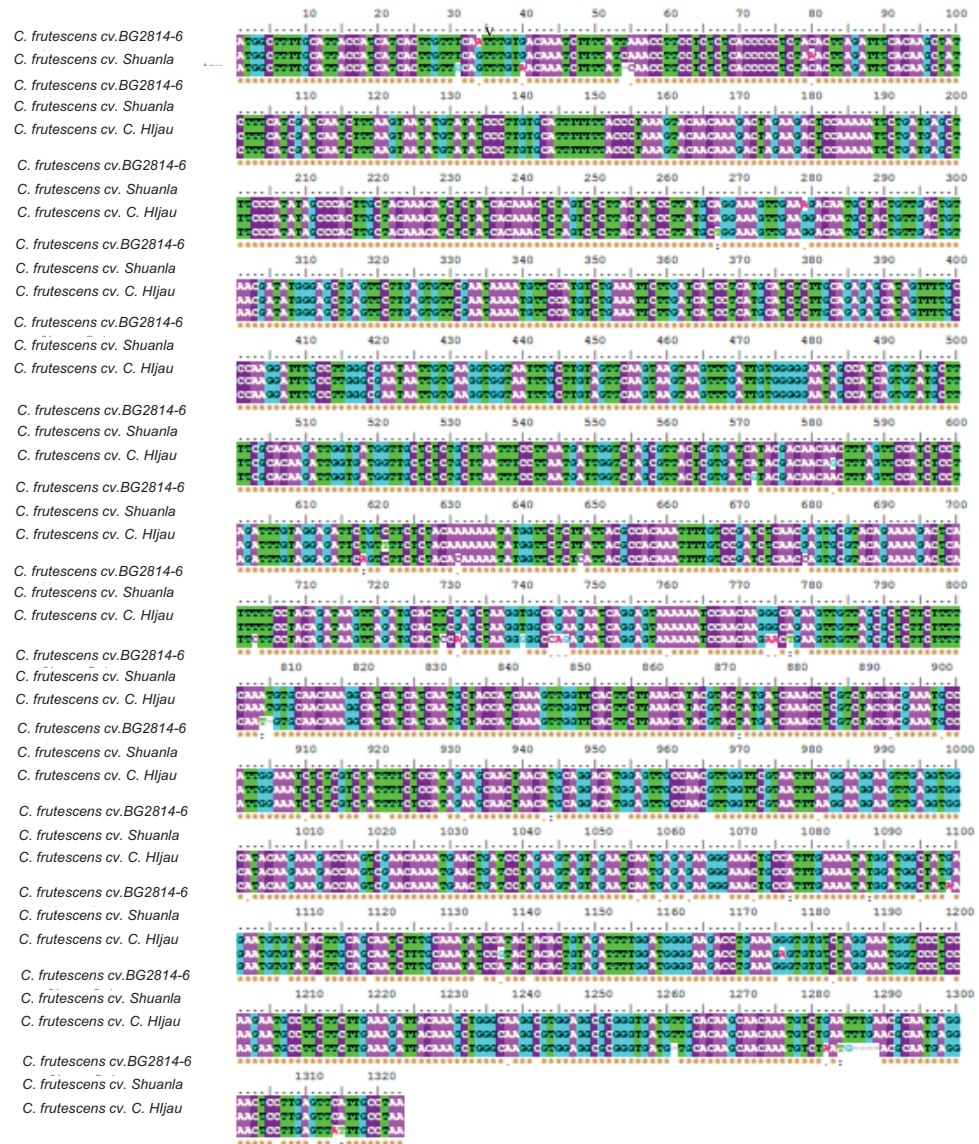
### 3. Results and Discussions

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 transcribed [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.



- [3] C. Stewart Jr., B.-C. Kang, K. Liu et al., "The Pun1 gene for pungency in pepper encodes a putative acyltransferase," *Plant Journal*, vol. 42, no. 5, pp. 675–688, 2005.
- [4] C. Stewart Jr., M. Mazourek, G. M. Stellari, M. O'Connell, and M. Jahn, "Genetic control of pungency in *C. chinense* via the Pun1 locus," *Journal of Experimental Botany*, vol. 58, no. 5, pp. 979–991, 2007.
- [5] M. De Lourdes Reyes-Escogido, E. G. Gonzalez-Mondragon, and E. Vazquez-Tzompantzi, "Chemical and pharmacological aspects of capsaicin," *Molecules*, vol. 16, no. 2, pp. 1253–1270, 2011.
- [6] BCN. Prasad, K. Vinod, HB. Gururaj, R. Parimal, R. Giridhar, and GA. Ravishankar, "Characterization of capsaicin synthase and identification of its gene (*csy1*) for pungency factor capsaicin in epper (*Capsicum* sp.)," *PNAS*, vol. 103, no. 36, pp. 13315–13320, 2006.
- [7] M. Habibi, A. Madhihah Manggabarani, E. Sri Sulasmi, and D. Listyorini, "AT3 (Acyltransferase) Gene Isolated From *Capsicum frutescens* cv. Cakra Hijau," *Journal of Tropical Life Science*, vol. 3, no. 2, pp. 83–86, 2013.
- [8] AM. Manggabarani, RR. Juliandari, EN. Khasna, S. Zairina, ES. Sulasmi, and D. Listyorini, "Isolation of upstream and downstream fragment of Acyltransferase (AT3) gene from *Capsicum frutescens* L. cv. Cakra Hijau," in *The 3rd International Conference on Biological Science, ., KnE Life Sciences*, P. Rarastoeti, AST. Woro, RN. Tri, NM. Siti, and R. Ganies A, Eds., pp. 21–26, 2013.
- [9] S. K. Sari, Isolasi fragmen tengah ketiga gen Pun1 dari *Capsicum frutescens* L. kultivar Cakra Hijau The third middle fragmen of gene Pun1 isolation from *Capsicum frutescens* L. Cakra Hijau cultivar. Undergraduated thesis FMIPA Universitas Negeri Malang; 2014. in Bahasa Indonesia.
- [10] Juliandari. R. R., Isolasi ujung 3' Gen Pun1 *Capsicum frutescens* L. kultivar Cakra Hijau The 3' edge of gene Pun1 isolation from *Capsicum frutescens* L. Cakra Hijau cultivar. Undergraduated thesis. FMIPA Universitas Negeri Malang; 2015. in Bahasa Indonesia.
- [11] Fatchiyah, E. L. Arumingtyas, S. Widyarti, and S. Rahayu, Prinsip dasar analisis biologi molekular Base principal of molecular biology analysis. Jakarta: Erlangga; 2011. in Bahasa Indonesia.
- [12] EL. Fatchiyah Arumingtyas, S. Widyarti, and S. Rahayu, Prinsip dasar analisis biologi molekular Base principal of molecular biology analysis. Jakarta: Erlangga; 2011. in Bahasa Indonesia.
- [13] N. M. Luscombe, R. A. Laskowski, and J. M. Thornton, "Amino acid-base interactions: A three-dimensional analysis of protein-DNA interactions at an atomic level," *Nucleic Acids Research*, vol. 29, no. 13, pp. 2860–2874, 2001.
- [14] T. Yuwono, Teori dan aplikasi polymerase chain reaction Polymerase chain reaction theory and application. Yogyakarta: Andi; 2006. in Bahasa Indonesia.