A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Identification of Candidate Genes Responsible for Low-pungency in the EMS-induced Mutant of Pepper (*Capsicum annuum* L.)

EMS 돌연변이 계통을 이용한 고추의 캡사이시노이드 생합성 경로 조절 유전자 발견

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UNDER THE DIRECTION OF DR. BYOUNG-CHEORL KANG
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
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ABSRACT

Capsaicinoids are the unique compounds found in *Capsicum,* and 90% of capsaicinoids comprise capsaicin and dihydrocapsaicin. Capsaicinoids biosynthesis can be controlled by two types of genes including structural and regulatory genes. However, only a few genes in the capsaicinoid biosynthetic pathway have been discovered. In this study, the low-pungent mutant line '1559-1-2h' was selected as a genetic resource to identify a novel genetic factor controlling capsaicinoid biosynthesis. The average capsaicinoid contents of the control and a parental line,

including ‘Yuwolcho’ (17,339 ± 11,527 ug/g DW) and ‘MicroPep Red’ (11,722±5,749 ug/g DW), were significantly higher than of ‘1559-1-2h’ (167±243 ug/g DW). Inheritance studies revealed that low-pungency in ‘1559-1-2h’ is controlled by a major gene. This major gene was named ‘*Pun6*’ in this study. Then a genetic study was conducted to discover the gene governing *Pun6* that regulates the low-pungency of the *C. annuum* mutant line ‘1559-1-2h’. To identify the *Pun6* genetic region, Bulked Segregant RNA-Seq (BSR-Seq) was performed using an F_2 population derived from a cross between the low-pungent mutant with the ‘MicroPep Red’. The BSR-Seq analysis revealed that the *Pun6* locus responsible for low pungency is located on chromosome 6. The physical position of *Pun6* was predicted to be from 204.8 to 219.2 Mbp, and 11 candidate genes contain SNPs in the coding sequence were identified. Considering together with the results of BSR-Seq and fine mapping, the position of *Pun6* was predicted to be around 208.0 Mbp. The information obtained in this study will be useful to identify a novel gene regulating capsicinoid biosynthesis in *Capsicum*.

Keywords: Pepper, Pungency, Capsaicinoid, Ethyl methanesulfonate (EMS), Mutant, Bulked Segregant RNA-Seq (BSR-Seq)

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSR-Seq	Bulked Segregant RNA-Seq
EMS	Ethyl methanesulfonate
bp	Base pairs
DPA	Days after anthesis
CDS	Coding sequence
pAMT	Putative aminotransferase
KR	Ketoacyl-ACP reductase
TF	Transcription factor
DEG	Differentially expressed gene
SNP	Single-nucleotide polymorphism
HPLC	High performance liquid chromatography
MR	MicroPep Red
gDNA	Genomic DNA
CTAB	Cetyltrimethylammonium bromide
PCR	Polymerase Chain Reaction
QTL	Quantitative trait locus
qRT	Quantitative reverse transcription
BSA	Bulked segregant analysis
HRM	High resolution melting
MG	Mature green

INTRODUCTION

Pepper (*Capsicum* spp.) is a very important economical crop that is grown all over the world. The genus *Capsicum* belonged to the Solanaceae family, and contains 5 domesticated species including *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens* and 22 wild species (Bosland, 1994; Eshbaugh, 1980; Walsh and oot, 2001). Among the species, *C. annuum* is the most widely cultivated and shows various spiciness and fruit shapes. *Capsicum* is the only plant genus that can synthesize capsaicinoids, a spicy alkaloid mixture (Aza-González et al., 2010). Capsaicinoids provide physiological and pharmacological health benefits including analgesia, anti-cancer, anti-inflammatory, anti-oxidative, and anti-obesity (Negulesco et al., 1987; Govindarajan and Sathyanarayana, 1991; Luo et al., 2010; Liu and Nair, 2010; Aza-González et al., 2010). Capsaicinoids start to be produced in the epidermis of the pepper placenta from 20 days after anthesis (DPA) and increased to 40 DPA, at which pepper fruit starts to turn red, however, this can be varied depending on the cultivars (Suzuki and Iwai, 1984; Zamski et al., 1987; Rowland et al., 1983; Iwai et al., 1979; Estrada et al., 2000; Zhang et al., 2016).

Capsaicinoids were first isolated by Thresh in 1876 and the exact formula and chemical structures were discovered (Thresh, 1876). Capsaicinoids are alkaloid compounds and consist of vanillylamine and acyl CoA derivatives (Aza- González et al., 2011). The two major capsaicinoids are capsaicin and dihydrocapsaicin

(Govindarajan et al., 1987; Collins et al., 1995; Zewdie et al., 2001). In the late 1960s, the biosynthesis pathway for capsaicinoids was postulated (Bennet and Kirby, 1968; Leete and Louden 1968). The phenylpropanoid and the branched-chain fatty acid pathways are required for capsaicinoid biosynthesis. The phenylpropanoid pathway produces vanillylamine from phenylalanine, and the branched-chain fatty acid pathway provides branched fatty acids from valine (Bennett and Kirby, 1968; Leete and Louden, 1968).

The capsaicinoid biosynthesis pathway was first investigated based on the phenylpropanoids biosynthesis pathway (Fujiwake et al., 1982; Sukrasno et al., 1993; Curry et al., 1999), followed by fatty-acid biosynthesis (Curry et al., 1999; Aluru et al., 2003; Stewart et al., 2005; Mazourek et al., 2009). To understand the mechanisms regulating the level of pungency, studies on capsaicinoid biosynthesis-related enzymes and genes were conducted, and potential capsaicinoid biosynthesis enzymes and genes were proposed (Curry et al., 1999; Alura et al. al., 2003; Blum et al., 2003; Kim et al., 2001). Many candidate genes for the capsaicinoid biosynthetic pathway have been proposed based on the chemical structure of precursors. For the phenylpropanoid pathway, enzymes for each precursor are well characterized including phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumaronyl-CoA ligase (4CL), coumarate 3-hydroxylase (C3H), caffeoyl-CoA O -methyltransferase (CCoAOMT), hydroxycinnamoyl-CoA hydratase/lyase (HCHL), and putative aminotransferase (pAMT) (Fujiwake et al., 1982; Sukrasno and

Ywoman, 1993; Stewart et al., 2005; Mazourek et al., 2009; Aza-González et al., 2010). Meanwhile, in the branched-chain fatty acid pathway, acyl carrier protein (Acl), acyl-ACP thioesterase (Fat), ketoacyl-ACP synthase (Kas), and branched-chain amino acid aminotransferase (BCAT) have been proposed as major enzymes (Alura et al., 2003); Mazourek et al., 2009).

Among proposed capsaicinoid biosynthetic genes, only a few structural genes, including *Pun1*, *pAMT*, and *CaKR1*, have been extensively studied. *Pun1*, the first discovered gene for controlling pungency, plays a key role at the final stage in capsaicinoid biosynthesis. *Pun1* is localized on chromosome 2 and is also known to be the *C* gene (Deshpande, 1935; Blum et al., 2002; Stewart et al., 2005). The *Pun1* gene encodes acyltransferase 3 (AT3), which condenses vanillylamine and 8-methyl-6-nonenoyl-CoA, and contains two exons in the coding sequence (CDS) region (Stewart et al., 2005; Stewart et al., 2007; Mazourek et al., 2009). Four non-functional alleles of *Pun1* have been reported. In the low-pungent cultivar *C. annuum* 'ECW', $pun1^1$ is characterized by having a 2.5 kb deletion spanning the promoter and first exon (Stewart et al., 2005). $pun1^2$ is the second non-functional allele discovered in *C. chinense* 'NMCA30036' (Stewart et al., 2007). In the $pun1^2$ allele, there is a 4 bp deletion in the first exon, which prevents the formation of functional proteins by creating a frameshift and premature stop codon (Stewart et al., 2007). A third non-functional allele, $pun1^3$, was found in *C. frutescens* 'PI594141' (Stellari et al., 2010). $pun1^3$ has a large deletion at the end of the second exon (Stellari et al.,

2010). Finally, *pun1*^{4} was reported to have a 1 bp insertion in the second exon, which results in changing 20 amino acids and deletion of 50 amino acids due to the frame shift, in *C. annuum* cultivar 'Nara Murasaki' (Kirii et al., 2016).

The last step of the phenylpropanoid pathway for capsaicinoid biosynthesis is predicted to be carried out by the enzyme known as putative amino transferase (*pAMT*) (Curry et al., 1999; Blum et al., 2003). There are three copies of this gene, which has 17 exons and 16 introns, in the pepper genome (Lang et al., 2009). If *pAMT* does not function, most vanillin is converted to vanillyl alcohol instead of vanillylamine to form capsicnoid (Sutoh et al., 2006; Kobata et al., 1998). Twelve alleles were found in the three *Capsicum* species (*C. annuum*, *C. chinense*, and *C. frutescens*), and most alleles were found in *C. chinense*. *pamt*^{1}, *pamt*^{3}, *pamt*^{5}, *pamt*^{6}, *pamt*^{8}, and *pamt*^{9} alleles have a small InDel in the exons, whereas *pamt*^{2} and *pamt*^{10} contain a single SNP, resulting in amino acid substitution and a premature stop codon (Lang et al., 2009; Tanaka et al., 2010a; Tanaka et al., 2010b; Koeda et al., 2014; Park et al., 2015; Tanaka et al., 2018; Tsurumaki and Sasanuma, 2019). The non-functional alleles of *C. chinense* including *pamt*^{4}, *pamt*^{5}, *pamt*^{7}, *pamt*^{L1}, and *pamt*^{L2}, contain hAT family transposon insertions of 2.3-2.8 kb into the exon or intron regions, which result in frameshift mutation and abnormal splicing (Tanaka et al., 2010b; Tanaka et al., 2015; Tanaka et al., 2019). Due to the structural similarity of the transposon, they were named Tcc1 (2.3 kb) and Tcc2 (2.8 kb) (Jang et al., 2015; Tanaka et al., 2010b; Tanaka et al., 2015; Tanaka et al., 2019).

Recently, a putative ketoacyl-ACP reductase (*CaKR1*) in *C. chinense* was reported as a novel structural gene in the capsaicinoid biosynthetic pathway (Koeda et al., 2019). In *C. chinense* 'No.3341', a non-functional allele is resulted from an insertion of a 4.5 kb hAT-Tam3/Ac transposable element at the first exon, which prevents the production of the final product of the branched-chain fatty acid pathway, 8-methyl-6-nonenoic acid (Koeda et al., 2019). The insertion of transposon produces truncated transcripts leading to the absence of the catalytic domain (Koeda et al., 2019).

The regulatory gene of capsaicinoids has also been studied. It was discovered that *Pun3* is a transcription factor that controls the transcription of genes involved in capsaicinoid production (Han et al., 2019; Zhu et al., 2019). *Pun3* consists of three exons and two introns and encodes the Solanaceae-specific MYB transcription factor (TF), MYB31 (Han et al., 2019; Zhu et al., 2019). In *C. annuum* 'YCM334', the SNP in the second exon of *Pun3* causes a premature stop codon, which leads to non-functional protein (Zhu et al., 2019; Han et al., 2019). Zhu et al., (2019) suggested that WRKY TF binding site (TTGAC) in the promoter region of *Pun3* could explain the extreme pungency of *C. chinense*.

Because there are limited genetic variations in natural genetic resources, various induced mutations have been used to obtain new resources for genetic study and breeding in many crops. One of the chemical mutagens, ethylene methanesulfonate (EMS) regularly applied for mutagenesis. EMS induces GC to AT

and AT to GC conversion, leading to amino acid substitutions or premature translational aborts (Sega, 1984). EMS can also induce insertions and deletions of base pairs (InDel) (Malling and de Serres, 1968). There have been many genetic researches with EMS-induced populations of *C. annuum*. Jabeen and Mirza (2002) treated EMS on a *C. annuum* accession and observed phenotypic variability including leaf area, number of leaves, number of branches, height of plants, days to flowering, days to fruiting, number of fruits per plant and chlorophyll content (Jabeen and Mirza, 2002). Bosland (2002) generated a flaccidity phenotype in bell pepper with a EMS mutant population (Bosland, 2002). Paran et al. (2007) produced an EMS mutant population using *C. annuum* for understanding the shoot architecture in *Capsicum* (Paran et al., 2007). Another mutant population containing 5,023 M2 mutants was generated using the Korean landrace pungent pepper *C. annuum* 'Yuwolcho' (Jeong et al., 2011; Hwang et al., 2014).

Bulked segregant analysis (BSA) is a method that can be used to rapidly identify genetic markers associated with the desired phenotype using two bulked DNA pools. 'Fast-forward genetics', which combines BSA with next-generation sequencing (NGS) technologies, can quickly identify target genes (Schneeberger and Weigel, 2011, Klein et al., 2018). The most widely used NGS technique is RNA-Seq. The read count of each transcript of the NGS data in RNA-Seq represents the relative expression level of the genes. RNA-Seq can also mine polymorphisms of DNA sequences, such as single nucleotide polymorphisms (SNPs), which can also be

converted into genetic markers. Bulked Segregant RNA-Seq (BSR-Seq) is a strategy used for genetic mapping by combining RNA-Seq technology and BSA technology (S. Liu et al., 2012). BSR-Seq can be used to discover new polymorphisms and delimit the candidate locus, understanding the information on the overall pattern of gene expression (S. Liu et al., 2012; Byun et al., 2022).

Previously, a mutant '1559-1-2h' showing stable low pungency was selected from 122 M2 lines by Gibb's reagent screening and then analyzed by high-performance liquid chromatography (HPLC) (Jeong et al., 2012; Hwang et al., 2014). In this study, the genetic study of the low-pungent EMS-induced mutant '1559-1-2h,' named '*Pun6*' was conducted. The candidate locus responsible for low-pungency was identified on chromosome 6 with BSR-Seq and SNP markers, and candidate region for '*Pun6*' were fine-mapped.

MATERIALS AND METHODS

Plant materials

The pungent *C. annuum* accessions, 'Yuwolcho' and 'MicroPep Red' ('MR') were used as control and parental lines. '1559-1-2h' (*C. annuum*) which is a fixed low-pungent mutant line derived from an EMS-induced 'Yuwolcho' mutant population was used for identifying a novel genetic factor controlling pungency in this study. A total of 136 F_2 plants derived from a cross between 'MR' (pungent) and '1559-1-2h' (low-pungent) were grown from 2020 to 2021 in the greenhouse of Seoul National University (Suwon, Korea). The fruits of plant materials are shown in the Figure 1.

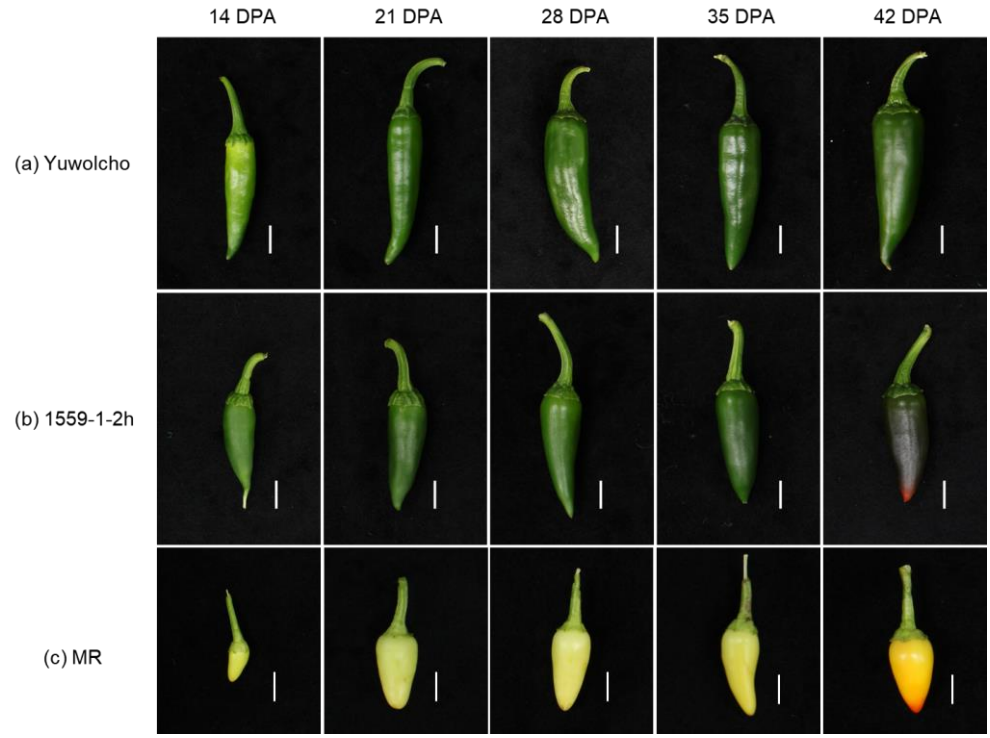

Figure 1. Pepper fruits used in this study. Fruits of (a) ‘Yuwolcho’, (b) ‘1559-1-2h’, and (c) ‘MR’ were harvested at developmental stages of 14, 21, 28, 35 and 42 DPA. Pungent fruits are (a) ‘Yuwolcho’ and (c) ‘MR’, and low-pungent mutant is (b) ‘1559-1-2h’. The scale bar in the picture is 1 cm.

Nucleic acids extraction

Genomic DNA (gDNA) was extracted from young leaves of the plants using a modified cetyltrimethylammonium bromide (CTAB; Sigma-Aldrich, Saint Louis, Missouri, USA) procedure (Lee et al., 2017). The extracted gDNA was dissolved 1X TE buffer. The extracted gDNAs were quantified and the quality of DNA was checked by Epoch microplate spectrophotometer with Take3 micro-volume plate (BioTek, Winooski, Vermont, USA). DNA was diluted to a concentration of 20 $ng \cdot \mu L^{-1}$ with triple distilled water (TDW) for further studies. Total RNA samples prepared from the placenta tissues of pepper fruits were used for the bulked RNA-Seq (BSR-Seq) analysis and qRT-PCR for gene expression analyses. The tissues were immediately frozen in liquid nitrogen and grounda to the fine powder. Total RNA was extracted using MG RNAzol kit (MGmed, Seoul, Korea). Approximately 1 μg of RNA was used for cDNA synthesis by reverse transcription (RT) PCR using AccuPoser RT PreMix (Bioneer, Daejeon, Korea)

Poly chain reaction (PCR) amplification and sequence analysis of *Pun1*, *pAMT*, *Pun3*, and *CaKR1*

To analyze sequence variations of pungency controlling genes (*Pun1*, *pAMT*, *Pun3*, and *CaKR1*) from 'Yuwolcho' and '1559-1-2h', coding sequences were obtained by PCR and sequenced. Target genes were amplified with a 25 μL of mixture containing 100 ng of gDNA, 2 μL of 10mM dNTPs, 5 μL of 5X GXL buffer, 0.5 μL of *Taq* DNA polymerase (PrimeStar GXL; Takara, Shiga, Japan), and 10 pmol of each primer. The amplified products were resolved in 1% agarose gel (Lonza, Lockland, USA) and gel eluted and purified using with LaboPass Gel and PCR Clean-up kit (CMA0112, Cosmogenetech, Seoul, Korea) according to the manufacturer's instructions. The amplicons were sequenced by Sanger sequencing at Macrogen (Macrogen, Seoul, Korea) and analyzed using SeqMan (Ver 5.00, DNASTAR Inc., Madison, WI, USA).

Capsaicinoids extraction

For HPLC analysis, at least three fruits from individual plants of 'Yuwolcho', 'MR', '1559-1-2h', F_1 and F_2 population were harvested. The placental tissues from dissected pepper fruits were freeze-dried for three days with a freeze dryer (Bondiro, IlShinBioBase, Dongducheon, Korea). Freeze-dried samples were ground, and the fine powders were extracted with 7.5 ml of ethyl acetate and acetone (both Honeywell Burdick & Jackson, Muskegon, Michigan, USA) mixture (6:4) by shaking at 37°C for 24 h with a precise shaking incubator (WiseCube WIS-20, DAIHAN Scientific, Wonju, Korea). The supernatant of 3 ml from the mixtures were taken and evaporated in a vacuum concentrator (Automatic Environmental SpeedVac System AES1010, Operon, Gimpo, Korea). The obtained pellets were dissolved in 1 ml of 99.9% methanol (Honeywell Burdick & Jackson, Muskegon, Michigan, USA) and filtered with a 0.2 μm pore syringe filter (Acrodisc LC 13 mm Syringe Filter, PALL, Port Washington, New York, USA).

High-performance liquid chromatography (HPLC) analysis

With the capsaicinoid extracts, HPLC analysis was used to determine the amount of capsaicinoids in the placenta of pepper fruits according to developmental stages. The analysis was conducted at the National Instrumentation Center for Environmental Management (NICEM; Seoul, Korea) following the method of Han et al. (2013). Total capsaicinoid content was calculated by the sum of capsaicin and dihydrocapsaicin contents. Pure capsaicin and dihydrocapsaicin were used as reference standards (Sigma-Aldrich, Saint Louis, Missouri, USA).

BSR-seq

For the BSR-Seq analysis, equal amounts of RNA from 18 pungent (P) and low-pungent (LP) fruits from the F_2 plants were sampled. These RNA samples were then pooled to create three separate pools, each of which had six individual RNA samples. RNA-Seq library was constructed using the TruSeq Standard mRNA LT Sample Prep Kit from Illumina (San Diego, CA, USA), and the RNA sequencing was performed in Macrogen (Seoul, Korea). Raw reads of sequences were aligned to pepper reference genome Dempsey v1.0 (Lee et al., 2022), using STAR version 2.7.5a (Dobin et al., 2013). The alignments of each pool were analyzed following the quantitative trait locus (QTL) sequencing analysis pipeline (Takagi et al., 2013). Samtools was used to extract SNPs from the alignment files of two pools. SNPs were filtered and plotted by internal Perl and R scripts of the QTL-Seq pipeline. The number of reads aligned on the reference genomes was calculated as the Δ(SNP – index) value for each pool. Consequently, the difference of Δ(SNP – index) value between P and LP pools was used to define Δ(SNP – index) value.

Quantitative reverse transcription (qRT) PCR

The real-time analysis was performed using the Rotor-Gene 6000 real-time PCR thermocycler (Corbett Research, Sydney, Australia) with the following PCR amplification conditions: 95°C for 5 min; 45 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s. The qRT-PCR was performed in a 20 μL reaction volume containing 2 μL of 5x diluted cDNA, 2 μL of 10mM dNTPs, 2 μL of 10x reaction buffer, 0.5 μL of SYTO 9 (Thermofisher Scientific Korea, Seoul, Republic of Korea), 0.5 μL of 10 pmol primers, and 0.4 μL of R Taq DNA polymerase (Takara Bio). The relative expression levels of genes were normalized against the threshold cycle value of the internal reference gene, *Actin* (Schmittgen & Livak, 2008). Primers used for qRT-PCR were listed in Table 1.

High resolution melting (HRM) analyses

HRM markers were analyzed by amplifying the short genomic regions that are above 100 base pairs (bp) and below 300 bp, including each target SNP, for the fine physical mapping of the low-pungency locus. Using a Roter-Gene 6000 real-time PCR (Qiagen, Hilden, Germany) and Rotor-Gene Q series software version 2.1.0, the amplification and HRM analysis were conducted. The HRM was performed in a 20 μL reaction volume containing consisting of 100 ng of gDNA, 10X HiPi reaction buffer, 1 nM dNTPs, 0.3 μl of homemade *Taq* DNA polymerase, 0.6 μl of SYTO 9 (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and 250 pM of primers including forward and reverse direction primers. The amplification conditions consist of 95°C for 5 mins; 55 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 40 s; 60°C for a min. Then, for the HRM analysis, the temperature rose 0.1°C in every step including 90 s of pre-melt conditioning and 2 s of waiting for the step. HRM normalized graphs and melting curves were examined for genotyping. Primers used for HRM analysis were listed in Table 1.

Table 1. Primers used in this study.

Purpose	Primer	Primer sequence (5' to 3')
Mapping of *Pun6*	HRM.QTL.5-2 (197,109,506 Mb)	F: ATCATTAAGACGGTCCGCGA
		R: GCAGCAGGAGCAGTACAGT
	HRM.QTL.6.6-1 (206,574,812 Mb)	F: CCTCCATTTTTGCTCGGTGA
		R: GAGAACATTGCTGTTTCTGTTTGA
	HRM.QTL.6.5-1 (207,373,847 Mb)	F: ACTCCACTTCTTCTTCTCACACA
		R: GTGTAGAGTCTGAAAGGGGCA
	INDEL.QTL.6.1-2 (208,000,352 Mb)	F: ACAGTAAAGTTGGTTAAACCCCCA
		R: GTCAGTGGTCATAGTTACCGCA
	HRM.QTL.6.2-2 (208,129,726 Mb)	F: AAGCATGGGAACAACTGGAGA
		R: CTCCTAAATCGCGTGGAACA
	Chr6_213_HRM_1 (213,663,640 Mb)	F: GGCTTGCCCTACCATCACT
		R: AAGGAAATACACTATCATGAAACCC
	Chr6_215_short_HRM_1 (215,690,540 Mb)	F: ACCAGGTCATCAATGTAGTAGCT
		R: GGCAACAAGATATCAAACGACAATT
Gene expression analysis	ACT_qRT	F: ATTCTCACCTTGAAGTATCCCA
		R: ATAGCAACATACATGGCAGG
	pAMT_qRT	F: GTCTCTCTGGGCTTCCTCCA
		R: GTTCGGCAATGAAAGCAGCT
	BCAT_qRT	F: CGCCCTTAAACAAACAGCCC
		R: TGGTGCTGTTCCTTCCTTGA
	BCKDH_E1α_qRT	F: GCATTTACAGGGGATGGTGGA
		R: CACAACGCCATCACTTCGAA
	KAS_qRT	F: TGAGTTTGGTAGATGCGGGAG
		R: ACCAATCATCGACTTTGTCCCA
	ACL_qRT	F: AGTATTACTTGTGCGGCTAAACC
		R: TCTTCTTCCACGGAGATGCC
	FatA_qRT	F: AGGACTTGTGCCACGAAGAG
		R: TCTTGCTGGCATTCACGTCT
	Pun1_qRT	F: CACTTCGAGCTAAGGTGGCA
		R: TTCCAATGGCATTTCGTGGT
	Pun3_qRT	F: CTTCCCAAGTATGCTGGACTAG
		R: TGGTAAGTGAGCAGCAATCG

RESULTS

Evaluation of pungency level in parental lines.

To evaluate the capsaicinoid contents of wild type ‘Yuwolcho’, ‘MR’, and the EMS mutant line ‘1559-1-2h’ (Figure 1), HPLC analysis was performed using the pepper fruit placenta in mature green (MG) stage which was estimated to be between 28 and 35 days post anthesis (DPA). The total amount of capsaicinoid contents was quantified as the sum of capsaicin and dihydrocapsaicin. The HPLC analysis revealed that the average capsaicinoid contents were significantly higher in ‘Yuwolcho’ (17,339±11,527 ug/g DW) and ‘MR’ (11,722±5,749 ug/g DW) than in ‘1559-1-2h’ (167±243 ug/g DW), as shown in the Figure 2 and Table 2. The ‘1559-1-2h’ mutant showed low pungency, suggesting that it could be used as suitable material to discover the genetic factor for controlling low pungency. ‘MR’ was selected as the parental line of the mapping population and contained high capsaicinoid contents similar to wild type cultivar ‘Yuwolcho’ (Table 2).

In order to confirm the change in pungency levels of wild type ‘Yuwolcho’ and mutant line ‘1559-1-2h’ at the five developmental stages (14, 21, 28, 35, and 42 DPA), HPLC analysis was performed using the pepper fruit placenta for each developmental stage. Capsaicinoid content was calculated by adding capsaicin and dihydrocapsaicin. As shown in the Figure 3 and Table 3, capsaicinoid contents of ‘Yuwolcho’ were 6, 1,677, 3,932, 12,055, and 5,360 ug/g DW at 14, 21, 28, 35, and

42 DPA, respectively. The capsaicinoid contents of the mutant line ‘1559-1-2h’ were 8, 9, 95, 37, and 5 at 14, 21, 28, 35, and 42 DPA, respectively.

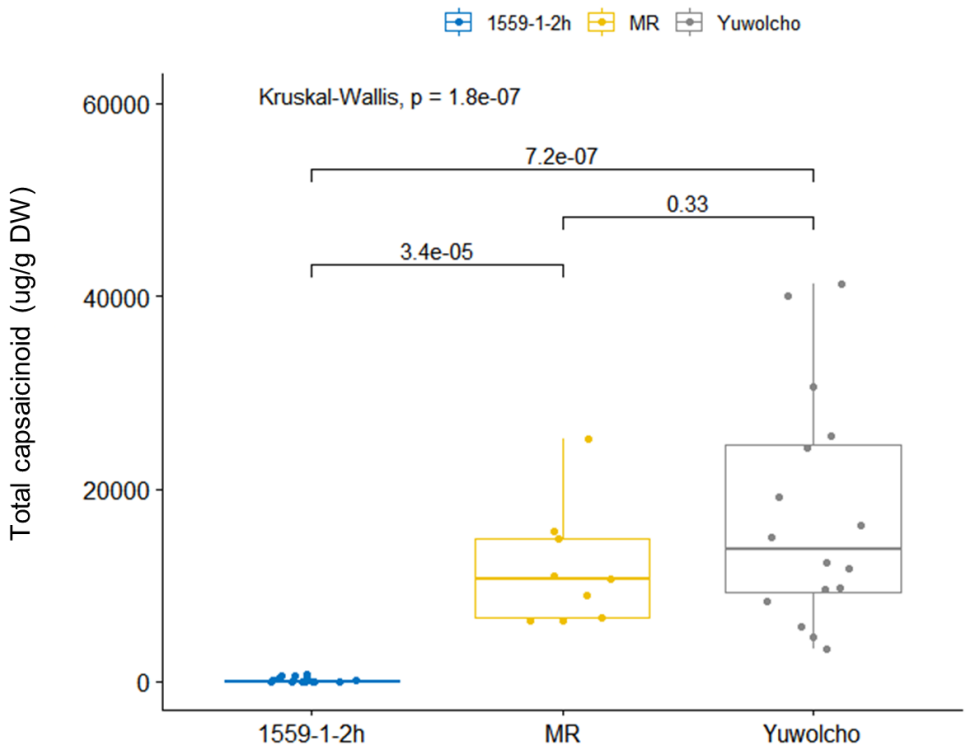

Figure 2. Capsaicinoid contents of parental and control lines. Total capsaicinoid contents of the parental and control lines (‘1559-1-2h, ‘MR’, and ‘Yuwolcho’) were presented by combining capsaicin and dihydrocapsaicin contents of the pepper fruit placenta at mature green stage. At least three plants of each accession were investigated, and three fruits of each plant were harvested and pooled. Distributional differences of the parental lines were verified by the Kruskal-Wallis test.

Table 2. Total capsaicinoid contents of 'Yuwolcho', 'MR', '1559-1-2h', and '1559-1-2h' X 'MR' F_1.

Line	Sample number	Stage	Capsaicin	DHCapsaicin	Total capsaicinoid
			ug/g DW	ug/g DW	ug/g DW
'Yuwolcho'	1	MG	1,661	1,802	3,462
	2	MG	2,166	2,456	4,622
	3	MG	2,792	2,870	5,662
	4	MG	4,755	3,617	8,372
	5	MG	5,488	4,017	9,506
	6	MG	5,281	4,472	9,753
	7	MG	6,786	4,997	11,783
	8	MG	6,610	5,759	12,370
	9	MG	8,104	6,947	15,051
	10	MG	7,917	8,310	16,228
	11	MG	11,561	7,592	19,153
	12	MG	15,058	9,192	24,250
	13	MG	13,921	11,545	25,466
	14	MG	19,649	10,918	30,566
	15	MG	25,616	14,363	39,979
	16	MG	25,277	15,923	41,201
'MR'	1	MG	3,923	2,460	6,382
	2	MG	3,504	2,898	6,402
	3	MG	4,141	2,452	6,593
	4	MG	5,237	3,712	8,950
	5	MG	5,578	5,056	10,634
	6	MG	5,934	5,044	10,978
	7	MG	7,847	7,000	14,847
	8	MG	8,953	6,605	15,558
	9	MG	14,423	10,732	25,155

‘1559-1-2h’	1	MG	0	0	0
	2	MG	0	0	0
	3	MG	0	0	0
	4	MG	0	0	0
	5	MG	1	0	1
	6	MG	2	0	2
	7	MG	13	5	18
	8	MG	40	0	40
	9	MG	27	16	43
	10	MG	33	18	51
	11	MG	69	34	102
	12	MG	76	33	109
	13	MG	66	50	116
	14	MG	96	42	138
	15	MG	244	103	347
	16	MG	460	207	667
	17	MG	457	219	676
	18	MG	497	199	696
‘1559-1-2h’ X ‘MR’ F_1	1	BK	6,572	5,636	12,208
	2	BK	8,060	5,983	14,042

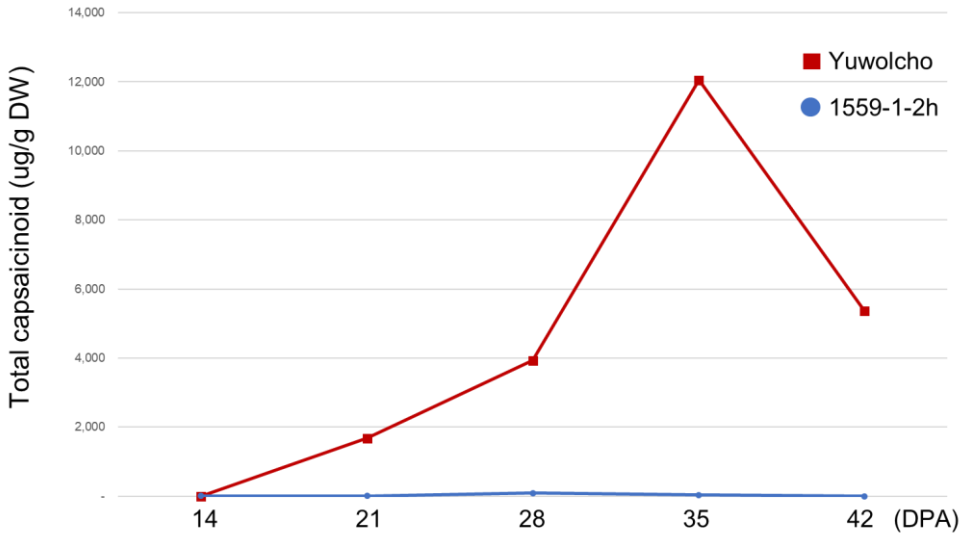

Figure 3. Levels of capsaicinoid in 'Yuwolcho' and '1559-1-2h' at five developmental stages. Fruit placenta extracts from 'Yuwolcho' and '1559-1-2h' were analyzed for their capsaicinoid content at five developmental stages. The capsaicinoid content was quantified by the sum of capsaicin and dihydrocapsaicin levels. Three pepper fruits of each stage were harvested and pooled.

Table 3. Levels of capsaicin and dihydrocapsaicin (DHCapsaicin) in 'Yuwolcho' and '1559-1-2h' at five developmental stages (14, 21, 28, 35, and 42 DPA).

Sample name	Capsaicin	DHCapsaicin	Total capsaicinoid
	ug/g DW	ug/g DW	ug/g DW
Yuwolcho_14_DPA	5	0	5
Yuwolcho_21_DPA	914	763	1,677
Yuwolcho_28_DPA	2,154	1,779	3,932
Yuwolcho_35_DPA	6,687	5,367	12,055
Yuwolcho_42_DPA	3,404	1,956	5,360
1559-1-2h_14_DPA	8	0	8
1559-1-2h_21_DPA	9	0	9
1559-1-2h_28_DPA	60	35	95
1559-1-2h_35_DPA	24	13	37
1599-1-2h_42_DPA	0	5	5

Segregation of pungency in the F_2 population

The low-pungent mutant '1559-1-2h' was crossed with the pungent variety 'MR', and the F_1 plants produced pungent fruits as expected, indicating that the pungency is dominant over low-pungency in '1559-1-2h' (Table 4). The HPLC analysis was used to determine the capsaicinoid contents of 136 plants from the F_2 population. The pungency phenotype distribution of the F_2 population showed a right-skewed density curve with a very sharp decrease in plant number around 3,000 ug/g DW (Figure 4). Therefore, the low-pungent plant in the population was classified as plants with capsaicinoid content under 3,000 ug/g DW. Among 136 F_2 plants, 90 plants with the pungency phenotype and 46 plants with the low-pungency phenotype were observed. The Chi-square test showed that the ratio of pungency to low-pungency phenotypes was 2:1 (P>0.05) (Table 4), indicating that the low-pungency trait is controlled by a major gene. This major gene was named '*Pun6*' in this study.

Table 4. Segregation of pungency phenotypes in an F_2 population.

Line	Number of plants			Pungent: low-pungent
	Pungent	Low-pungent	Total	
'Yuwolcho'	16	0	16	1:0
'MR'	9	0	9	1:0
'1559-1-2h'	0	18	18	0:1
'1559-1-2h' X 'MR' F_1	2	0	2	1:0
'1559-1-2h' X 'MR' F_2	90	46	136	2:1 (P>0.05)

* Total capsaicinoid contents of the low-pungent plant are under 3,000 ug/g DW.

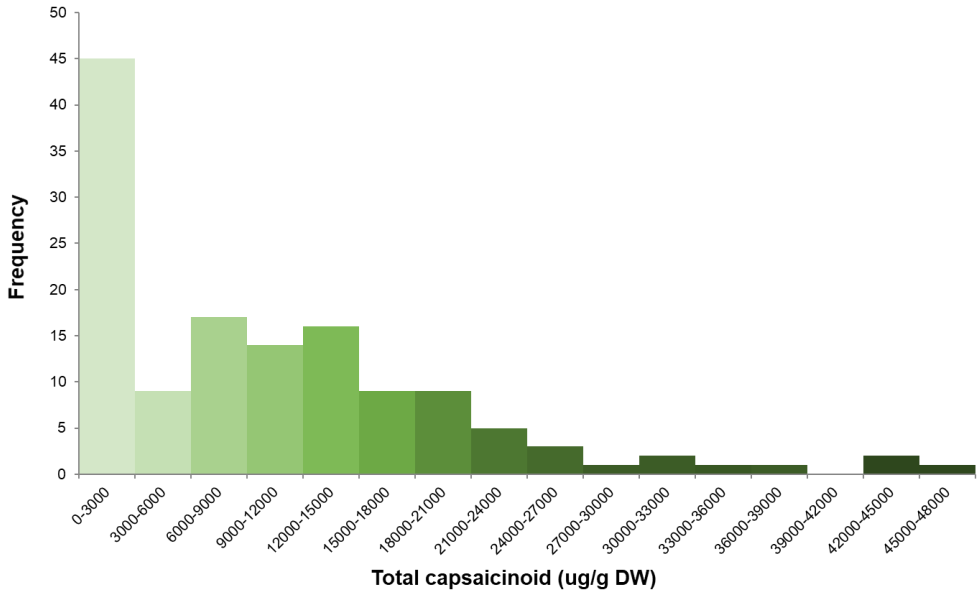

Figure 4. Distribution of pungency phenotypes in an F_2 population. Histogram for '1559-1-2h' X 'MR' F_2 population. The histogram was drawn using the total capsaicinoid of the 136 individuals in F_2 population. The pungency phenotype distribution of the F_2 population showed a right-skewed density curve and inflection point was shown around 3,000 ug/g DW.

Complementarity tests with *pun1*, *pun3*, *pamt*, and *cakr1* alleles

As *Pun1*, *Pun3*, *pAMT*, and *CaKR1* are known to regulate pungency trait in pepper, the sequence variations of these genes in '1559-1-2h' were investigated. Sequence analysis revealed no specific mutations in all exons of *Pun1*, *Pun3*, *pAMT*, and *CaKR1* between pungent the wild type 'Yuwolcho' and low-pungent '1559-1-2h' (Appendix 1-4).

Allelism tests were performed to test whether '1559-1-2h' has non-functional alleles of *Pun1*, *Pun3*, and *CaKR1*. Capsaicinoid content was 4,027±1,242 ug/g DW in the F_1 plants derived from a cross between '1559-1-2h' and 'ECW' with the non-functional *pun1* allele (Table 5). The pungency phenotype was also observed in the F_1 plants derived from a cross between '1559-1-2h' and 'YCM334' with non-functional *pun3* allele, and the capsaicinoid content of F_1 plants was 4,687±996 ug/g DW (Table 5). The capsaicinoid content of F_1 plants from a cross between '1559-1-2h' and 'No.3341' with the non-functional *cakr1* allele was 36,996±3,469 ug/g DW (Table 5). As a result of allelism tests, the *pun6* gene in '1559-1-2h' was discovered to be non-allelic to non-pungency alleles at the *Pun1*, *Pun3*, and *CaKR1* genes.

Table 5. Allelism tests between non-pungent accessions harboring *pun1*, *pun3*, and *cakr1*. Total capsaicinoid contents of mature stage placenta of F_1 plants (ug/g dry weight).

X (crossing)	***C. annuum* 'ECW' (*pun1/pun1*)**	***C. annuum* 'YCM334' (*pun3*/*pun3*)**	***C. chinense* 'No.3341' (*cakr1*/*cakr1*)**
***C. annuum* '1559-1-2h' (*pun6*/*pun6*)**	4,027±1,242	4,687±996	36,996±3,469

Expression analysis of genes for capsaicinoid biosynthetic pathway

To compare the expression levels of capsaicinoid biosynthetic genes between ‘Yuwolcho’ and ‘1559-1-2h’, real-time PCR was performed with RNA samples extracted from placenta tissues at five different fruit stages (14, 21, 28, 35, and 42 DPA). The expression levels of all the genes peaked at 21 DPA in ‘Yuwolcho’ and then began to decline at 28, 35, and 42 DPA (Figure 5). This gene expression change patterns showed a decalcomanic form for the graph of the capsaicinoid contents of ‘Yuwolcho’ at five developmental stages (Figure 3). The capsaicinoid biosynthetic genes were expressed the highest at 21 DPA, leading to the synthesis of capsaicinoids, whereas capsaicinoid content peaked at 35 DPA. All the genes were confirmed to be expressed very low in ‘1559-1-2h’. Expression levels of each gene including *pAMT*, *BCAT*, *BCKDH E1α*, *KAS*, *ACL*, *FatA*, *Pun1*, and *Pun3* in ‘Yuwolcho’ were found to be 89, 277, 96, 68, 6, 71, 43, and 21-fold higher to the low-pungent mutant line ‘1559-1-2h’ (Figure 5). These findings suggested that the *Pun6* gene may function as transcription factor, regulating the expression of genes involved in capsaicinoid biosynthesis.

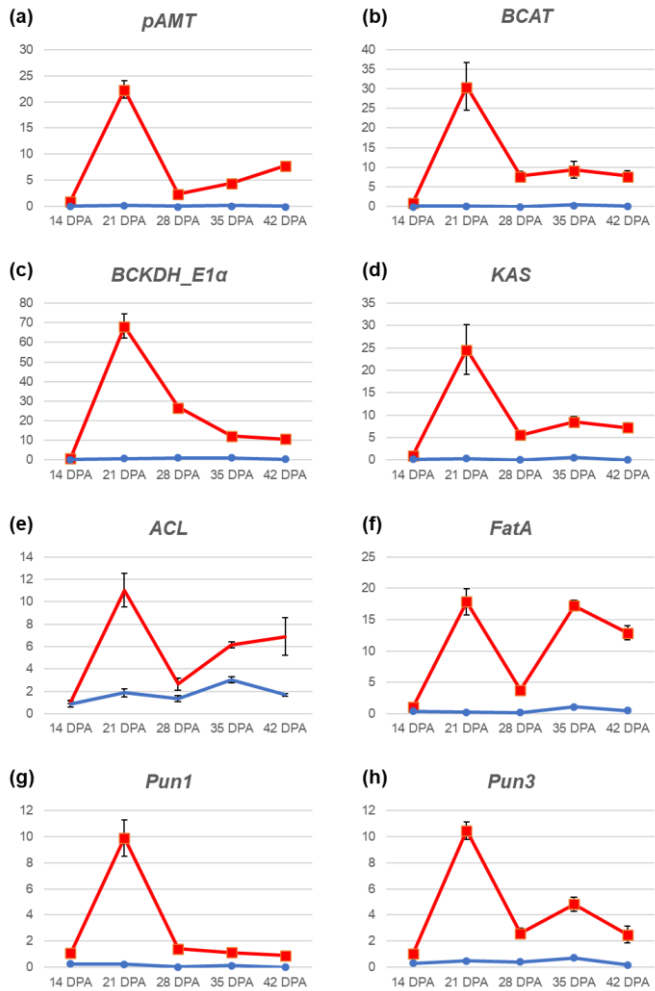

Figure 5. Real-time PCR expression of genes involved in capsaicinoids biosynthesis at five developmental stages. (a) *pAMT* belongs to the phenylpropanoid biosynthesis pathway in the capsaicinoids biosynthesis. (b) *BCAT* belongs to the amino acid precursor preparation. (c) *BCKDH_E1α* belongs to the branched-chain α-ketoacid dehydrogenase. (d) *KAS*, (e) *ACL*, and (f) *FatA* belong to the fatty acid biosynthesis pathway. (g) *Pun1* participates in the downstream mechanism merging the fatty acid and phenylpropanoid pathways. (h) *Pun3* involves in the regulation of capsaicinoids biosynthesis genes. Mean values were obtained from triplicates. The ratio of target gene average C_p to reference C_p was plotted.

BSR-Seq for identification of the *Pun6* locus

BSR-Seq analysis was used to identify the *Pun6* locus. In an F_2 population derived from crossing '1559-1-2h' and 'MR', 18 plants with the highest capsaicinoid content and 18 plants with the lowest capsaicinoid content were selected for high- and low-pungent bulks, respectively, and three RNA pools of six individuals were prepared for each bulk. The Illumina sequencing platform was used to sequence each RNA pool. As shown in Table 6, RNA-seq generated 201,803,754 raw reads in the pungent pool and 196,068,672 raw reads in the low-pungent pool. Total read bases per RNA pool were 20.4 Gbp in pungent bulk and 19.8 Gbp in low-pungent bulk with a coverage of 454X and 441X for each bulk. The raw sequences were aligned to the reference genome, Dempsey v1.0, which covered approximately 97% of the total coding region length. A total of 393,948 raw SNPs were called, and the SNPs with non-polymorphism and low-quality values were filtered out, yielding 753 filtered SNPs. A total of 753 significant SNPs were identified by calculating Δ(SNP – index) values between pungent and low-pungent pools. These significant SNPs were analyzed, and the peak with the highest value was found on chromosome 6 (Figure 6). The physical position of candidate genes was predicted to be from 204.8 to 219.2 Mbp.

Table 6. Summary of BSR-Seq analysis for pungent and low-pungent bulks.

Information	Pungent	Low-pungent
No. of individuals in pools	18	18
No. of raw reads	201,803,754	196,068,672
Total size (Gbp)	20.4	19.8
No. of filtered reads	200,101,336	193,769,836
Coverage	~454X	~441X
Mapped reads percentage (%)	97.05	97.19

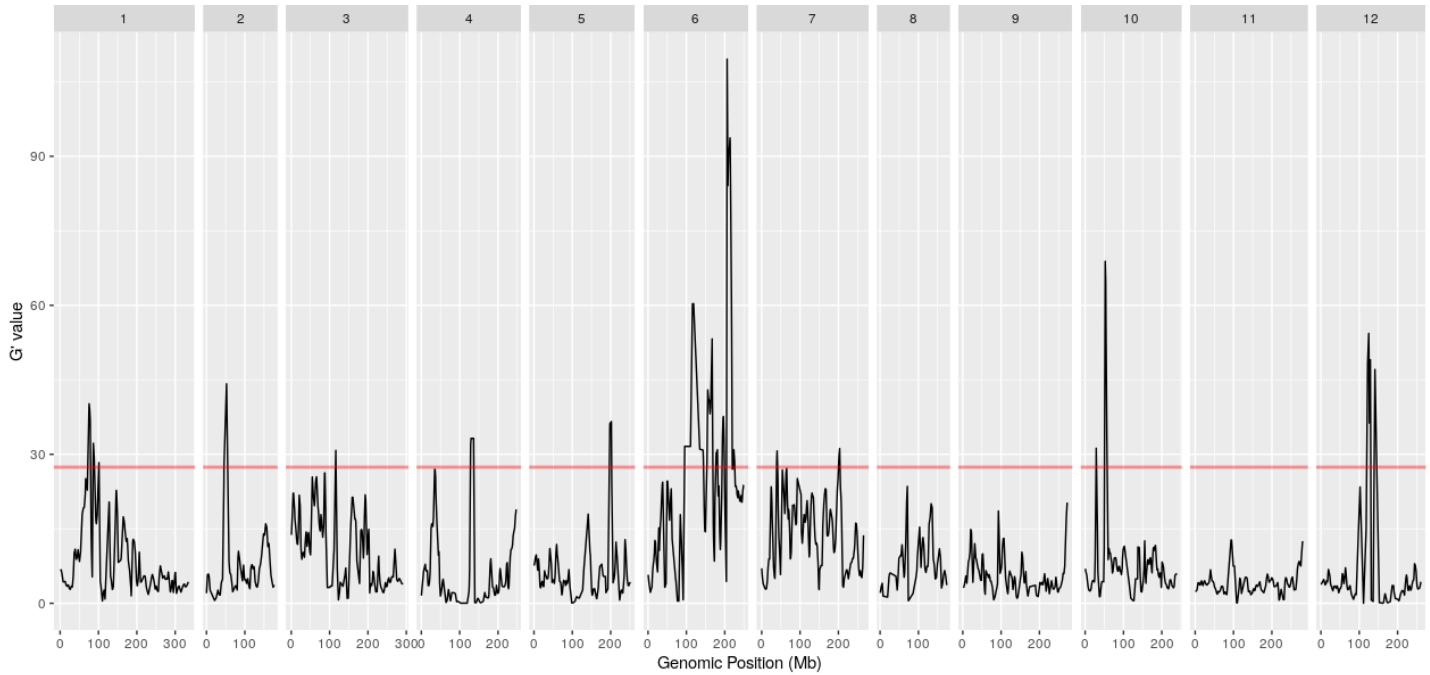

Figure 6. Estimation of the candidate region for *Pun6*. A total of 753 significant SNPs were identified by calculating Δ(SNP – index) between the BSR-Seq data of pungent and low-pungent pool. Chromosome 6 is expected to be the location of candidate genes.

Fine mapping of the *Pun6* locus

In the BSR-Seq analysis, the physical position of the *Pun6* locus was predicted to be 204.8 -219.2 Mbp. To delimit the *Pun6* locus, the F_2 population of 136 individuals was genotyped using 6 HRM markers and a INDEL marker of SNPs near the candidate region (Table 7 and Appendix 6-7). When genotyping results and pungency phenotypes were compared, markers that reduced the number of recombination were discovered around 208 Mbp (Table 7 and Figure 7-8). Based on the recombination pattern, the *Pun6* locus could be predicted to be around 208.0 Mbp in chromosome 6 of the Dempsey v1.0 reference (Figure 7).

Considering together with the candidate region 204.8 - 219.2 Mbp from BSR-Seq and fine mapping, the candidate gene was predicted to be around 208.0 Mbp. Within the delimited region, 11 candidate genes with SNPs in CDS were listed (Table 8).

Table 7. Segregation of genotypes and phenotypes in an F_2 population.

Marker	SNP location (bp)	# Recombinant
HRM.QTL.5-2	197,109,506	26
HRM.QTL.6.6-1	206,574,812	20
HRM.QTL.6.5-1	207,373,847	24
INDEL.QTL.6.1-2	208,000,352	17
HRM.QTL.6.2-2	208,129,726	16
Chr6_213_HRM_1	213,663,640	27
Chr6_215_short_HRM_1	215,690,540	26

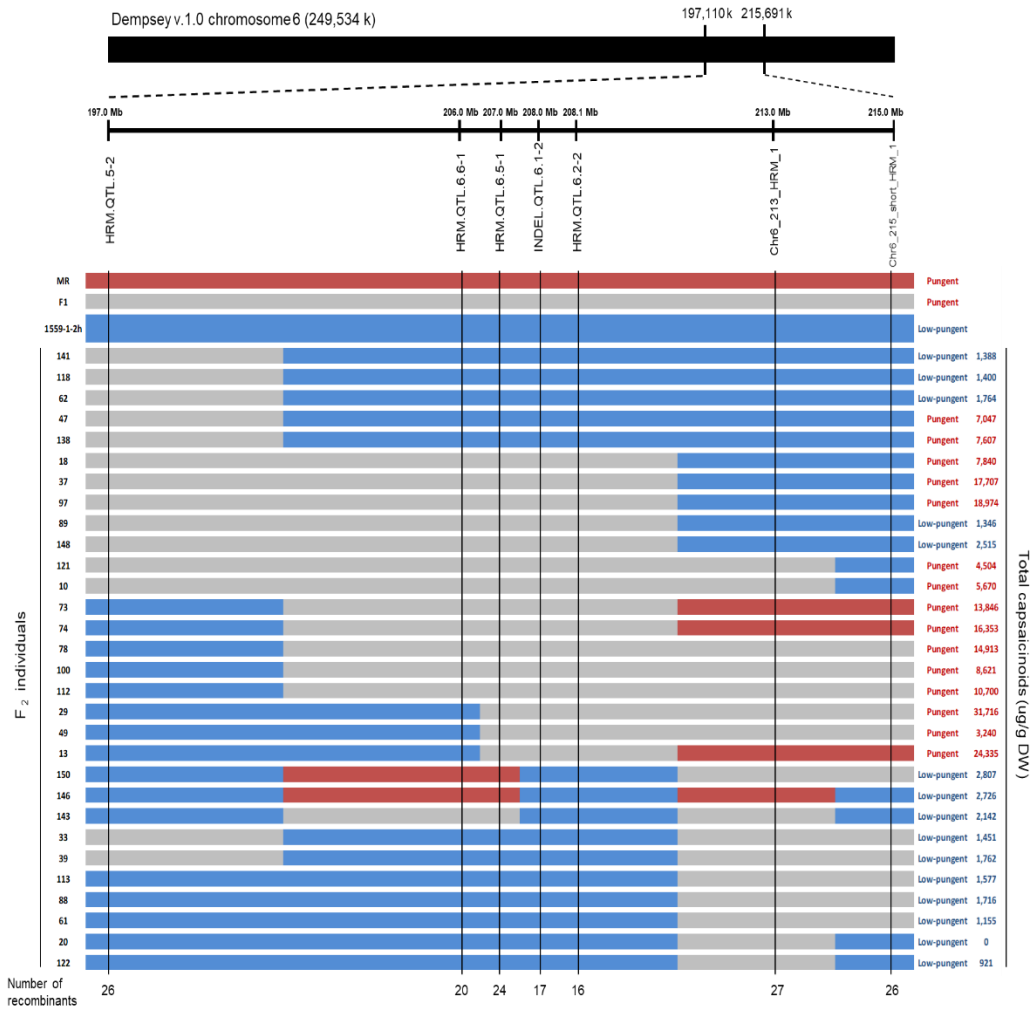

Figure 7. Fine mapping and recombination patterns of F_2 recombinants in chromosome 6. The *Pun6* region was mapped on chromosome 6, ranging from 197,110 kb to 215,691 kb. The individuals of F_2 population were genotyped by using 6 HRM marker sets and a INDEL marker set. Red, blue, and gray bars indicate the genotypes of 'MR' (homozygous), '1559-1-2h' (homozygous), and 'F_1'(heterozygous).

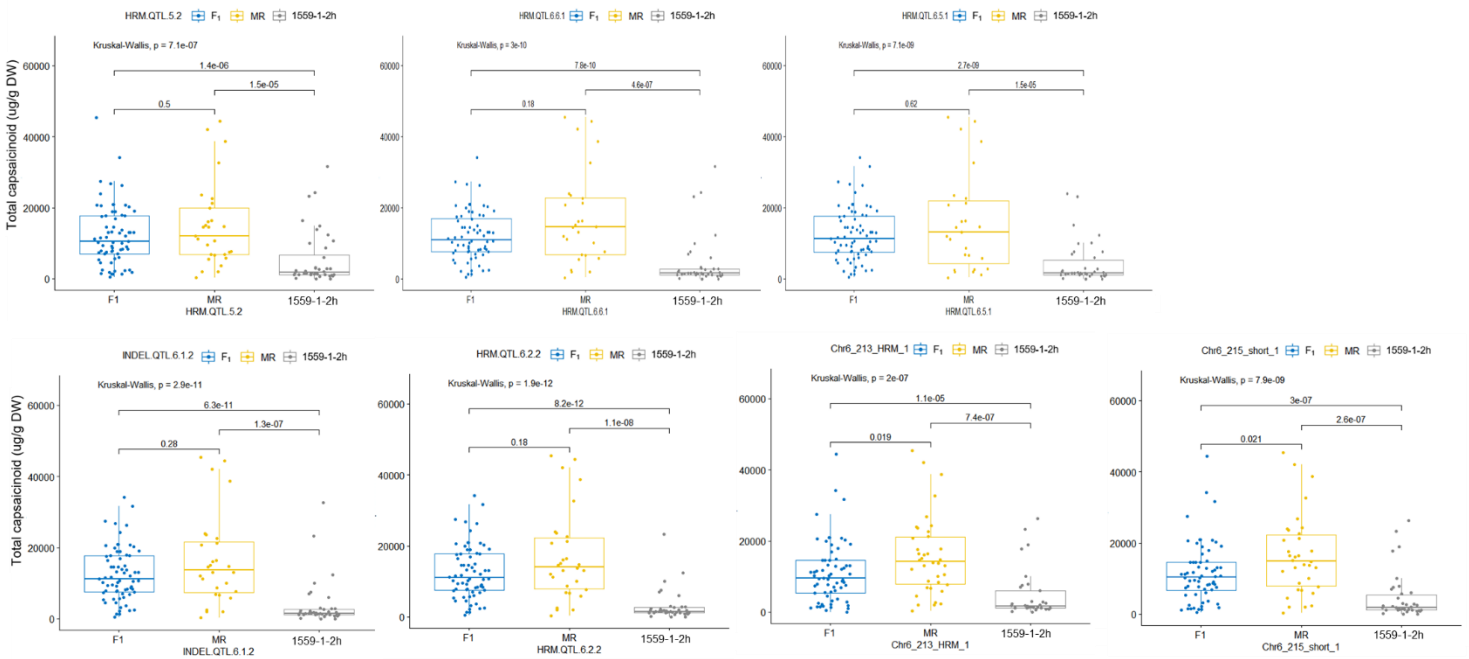

Figure 8. Box plots for genotypes and phenotypes of the F_2 population using 7 SNP marker sets. Total capsaicinoid contents were presented according to the genotypes of the homozygous ('MR' and '1559-1-2h') and heterozygous ('F_1') for the F_2 populations. Distributional differences of the parental lines were verified by the Kruskal-Wallis test.

Table 8. Selected variations for protein effect on candidate genes from the bulked segregant RNA-seq region (204.8 – 219.2 Mbp) of '1559-1-2h' and MR.

Position (Mb)	deltaSNP	Gprime	SNP effect	Target gene
208.95	-0.660550459	102.2004459	missense_variant	DEMF06G16430
208.95	-0.66	102.200649	missense_variant	DEMF06G16430
209.07	-0.266666667	102.2372862	missense_variant	DEMF06G16470
209.07	-0.297468354	102.2372933	missense_variant	DEMF06G16470
209.07	-0.530864198	102.2373154	missense_variant	DEMF06G16470
209.63	0.590062112	102.4190246	missense_variant	DEMF06G16590
209.63	0.523481569	102.4190479	missense_variant	DEMF06G16590
209.63	0.554048583	102.4190564	stop_gained	DEMF06G16590
209.63	0.466880771	102.4191328	missense_variant	DEMF06G16590
209.63	0.544283037	102.419174	missense_variant	DEMF06G16590
209.63	0.53135932	102.4191772	missense_variant	DEMF06G16590
210.98	-0.582417582	101.7259937	missense_variant	DEMF06G16990
213.63	0.580909944	100.5490143	missense_variant	DEMF06G17630
213.63	0.580909944	100.5490141	missense_variant	DEMF06G17630
213.66	-0.722826087	100.5438014	missense_variant	DEMF06G17660
213.67	-0.522613065	100.5432183	missense_variant	DEMF06G17670
213.67	-0.373134328	100.5432085	missense_variant	DEMF06G17670
213.67	-0.378378378	100.543207	missense_variant	DEMF06G17670
215.69	0.778263881	85.13546487	stop_gained	DEMF06G17970
216.43	-0.550925926	77.06101485	missense_variant	DEMF06G18170
218.13	0.390663391	55.94876293	missense_variant	DEMF06G18670
218.13	0.404803051	55.94836747	missense_variant	DEMF06G18670
218.84	0.369827703	44.75771981	missense_variant	DEMF06G18940
218.84	0.467105263	44.7379148	missense_variant	DEMF06G18940

DISCUSSION

In this study, the low-pungent line '1559-1-2h' derived from EMS mutant population of 'Yuwolcho' was used to identify the gene for controlling pungency in pepper. BSR-Seq was performed using an F_2 population constructed by crossing low-pungent mutant '1559-1-2h' with pungent variety 'MR'. The BSR-Seq analysis revealed that the locus responsible for low-pungency was located on chromosome 6, and this locus was named *Pun6*. By combining the results of BSR-Seq and fine mapping, the *Pun6* locus region was predicted to be 204.8 – 219.2 Mbp, and 11 candidate genes for *Pun6* were identified.

The mutant line, '1559-1-2h', was derived from *C. annuum* 'Yuwolcho', a pungent landrace in Korea. To fix the low-pungency phenotype, the mutant line was self-pollinated for five generations. The capsaicinoid content of the '1559-1-2h' was significantly lower than that of the 'Yuwolcho' and 'MR' mutants. Therefore, the low-pungent mutant '1559-1-2h' was a suitable material for identifying a new candidate gene for controlling pungency.

Sequence analysis and allelism tests were used to confirm that the low pungency in '1559-1-2h' was not due to mutations in known pungency controlling genes such as *Pun1*, *Pun3*, *pAMT*, and *CaKR1*. No mutations causing non-pungency were found in the CDS sequences of *Pun1*, *Pun3*, *pAMT*, and *CaKR1* in '1559-1-2h'. The allelism tests were performed by crossing '1559-1-2h' with 'ECW' having non-

functional *Pun1*), 'YCM334' having non-functional *Pun*1, and 'No.3341' having non-functional *CaKR1*. F_1 plants derived from the crosses showed pungency phenotype, indicating that *Pun6* is not allelic to *Pun1*, *Pun3*, *pAMT*, and *CaKR1*. For an allelism test, '1559-1-2h' was also crossed with 'SNU11-001' (non-functional *pAMT*), but the F_1 seeds did not germinate due to the interspecies barrier between *C. annuum* and *C. chinense*. In summary, the sequence analysis and allelism test results of the known pungency controlling genes indicated that '1559-1-2h' has functional *Pun1*, *Pun3*, *pAMT*, and *CaKR1*, and other gene is responsible for its low-pungency of '1559-1-2h'.

To understand the expression patterns of the genes involved in capsaicinoid biosynthesis in 'Yuwolcho' and '1559-1-2h', real-time PCR and RNA-Seq analysis were performed. The results showed that the overall gene expression levels of the genes in the capsaicinoid biosynthesis pathway such as *pAMT*, *BCAT*, *BCKDH E1α*, *KAS*, *ACL*, *FatA*, *Pun1*, and *Pun3*, in '1559-1-2h' were significantly lower than those in 'Yuwolcho'. Although sequence analysis revealed that '1559-1-2h' had functional *Pun1*, *Pun3*, *pAMT*, and *CaKR1* genes, their overall expression levels were significantly reduced in '1559-1-2h'. These findings indicated that *Pun6* may encode a transcription factor that regulates the expression of *Pun1*, *Pun3*, *pAMT*, *CaKR1,* and other capsaicinoid biosynthesis genes.

The inheritance of the pungency phenotype was evaluated by HPLC analysis for F_1 and F_2 populations derived from a cross between the low-pungent

‘1559-1-2h’ and the pungent cultivar ‘MR’. All F_1 hybrid plants exhibited a pungent phenotype, and the segregation ratio of pungent and low-pungent in the F_2 population satisfied the classical Mendelian model 2:1 (P>0.05). This indicates that the trait is controlled by multiple genes, and a major gene named *Pun6*. In BSR-Seq analysis, a major gene *Pun6* was predicted to be in chromosome 6 in which the highest peak was showed, but other minor genes could be found in chromosome 10 and chromosome 12.

As a result of genetic mapping using BSR-Seq with the pungent pool and low-pungent pool derived from the F_2 population, the *Pun6* locus was mapped to 204.8 -219.2 Mbp in chromosome 6 of reference genome Dempsey v1.0 (Lee et al., 2022). The detailed location of the *Pun6* on chromosome 6 was delimited using the SNP markers, and its location was estimated to be around 208.0 Mbp. Within the delimited region, the 11 candidate genes could be found. Among them, 3 genes had stop gained variants and 9 genes had missense variants. These genes could be a candidate gene for *Pun6*.

The markers of 0 cM of genetic distance have not yet been found. More markers should be designed with SNPs around 208.0 Mbp. If markers in which all genotypes and phenotypes are linked without a recombinant are discovered, stronger candidate genes will be identified. However, since pungency of this locus appears to be also controlled by a minor locus on chromosome 10 or 12, the phenotype data of

the F_2 group is difficult to be 100% reliable. Therefore, it is necessary to confirm the pungency of the fruit of F_3 of the recombinant plants of the F_2 group.

To validate candidate genes responsible for the low-pungent of '1559-1-2h', the sequences of the candidate genes in the 'Yuwolcho' and '1559-1-2h' should be analyzed. In addition, it is necessary to examine the function of the candidate genes by knocking it down using virus-induced gene silencing (VIGS) or knocking it out using genome editing.

In conclusion, this study discovered a novel locus *Pun6* that regulates the pungency of the *C. annuum*. Trancriptome analysis suggested that *Pun6* was predicted to encode a transcription factor that regulates the expression of capsaicinoid biosynthetic genes. As the results of BSR-Seq, the physical position was predicted to be from 204.8 to 219.2 Mbp, and 11 genes that have SNPs of protein effect were predicted in the region. Considering together with the results of BSR-Seq and fine mapping with SNP markers, the *Pun6* locus was predicted to be around 208.0 Mbp.

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ABSTRACT IN KOREAN

고추 과실의 매운맛을 유발하는 캡사이시노이드 (Capsaicinoid)는 고추속 (*Capsicum*)에서 발견되는 독특한 화합물이다. 캡사이노이드의 90%는 캡사이신과 디하이드로캡사이신으로 구성된다. 캡사이시노이드 생합성은 다양한 구조 유전자 및 조절 유전자에 의해 조절되며, 현재까지 *Pun1*, *pAMT*, *Pun3*, *CaKR1* 유전자에 대한 기능이 밝혀졌다. 본 연구에서는 캡사이시노이드 생합성을 조절하는 신규 유전인자를 규명하기 위한 유전자원으로, 유월초의 돌연변이 계통인 '1559-1-2h'를 선정하였다. 저신미를 갖는 돌연변이 계통 '1559-1-2h'와 고신미를 갖는 'MicroPep Red' ('MR')과의 교배를 통해 얻은 F2 집단의 분리비가 2:1 이라는 것을 확인했으며, 이를 통해 '1559-1-2h'의 저신미는 하나의 주요 유전자와 여러 개의 미동 유전자에 의해 조절된다는 것을 확인하였고, 이 주요 유전자좌를 *Pun6* 라고 명명하였다. *Pun6* 유전자좌를 확인하기 위해 '1559-1-2h'와 'MR'과 교배를 통해 얻은 F2 집단을 사용하여 Bulked Segregant RNA-Seq (BSR-Seq)을 수행했다. BSR-Seq 분석 결과를 바탕으로 단일염기다형성 (SNP) 마커들을 활용하여 후보 지역을 208.0Mb 근처로 예측하였다.

주요어: 고추, 신미, 캡사이시노이드, Ethyl methanesulfonate (EMS), 돌연변이, Bulked Segregant RNA-Seq (BSR-Seq)

학번: 2021-25689

>**pun1**_acylsugar acyltransferase 3-likecapsicum annuum_Zunla-1_ch2 Gene ID: 107859694

143394174-143448270

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reference       ATGGCTTTTGCATTACCATCATCACTTGTTTCAGTTTGTGACAAATCTTTTATCAAACCT      60
Yuwolcho        ATGGCTTTTGCATTACCATCATCACTTGTTTCAGTTTGTGACAAATCTTTTATCAAACCT      60
1559-1-2h       ATGGCTTTTGCATTACCATCATCACTTGTTTCAGTTTGTGACAAATCTTTTATCAAACCT      60
                ************************************************************

reference       TCCTCTCTCACCCCCTCTACACTTAGATTTCACAAGCTATCTTTCATCGATCAATCTTTA      120
Yuwolcho        TCCTCTCTCACCCCCTCTACACTTAGATTTCACAAGCTATCTTTCATCGATCAATCTTTA      120
1559-1-2h       TCCTCTCTCACCCCCTCTACACTTAGATTTCACAAGCTATCTTTCATCGATCAATCTTTA      120
                ************************************************************

reference       AGTAATATGTATATCCCTTGTGCATTTTTTTACCCTAAAGTACAACAAAGACTAGAAGAC      180
Yuwolcho        AGTAATATGTATATCCCTTGTGCATTTTTTTACCCTAAAGTACAACAAAGACTAGAAGAC      180
1559-1-2h       AGTAATATGTATATCCCTTGTGCATTTTTTTACCCTAAAGTACAACAAAGACTAGAAGAC      180
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reference       TCCAAAAATTCTGATGAGCTTTCCCATATAGCCCACTTGCTACAAACATCTCTATCACAA      240
Yuwolcho        TCCAAAAATTCTGATGAGCTTTCCCATATAGCCCACTTGCTACAAACATCTCTATCACAA      240
1559-1-2h       TCCAAAAATTCTGATGAGCTTTCCCATATAGCCCACTTGCTACAAACATCTCTATCACAA      240
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reference       ACTCTAGTCTCTTACTATCCTTATGCTGGAAAGTTGAAGGACAATGCTACTGTTGACTGT      300
Yuwolcho        ACTCTAGTCTCTTACTATCCTTATGCTGGAAAGTTGAAGGACAATGCTACTGTTGACTGT      300
1559-1-2h       ACTCTAGTCTCTTACTATCCTTATGCTGGAAAGTTGAAGGACAATGCTACTGTTGACTGT      300
                ************************************************************

reference       AACGATATGGGAGCTGAGTTCTTGAGTGTTCGAATAAAATGTTCCATGTCTGAAATTCTT      360
Yuwolcho        AACGATATGGGAGCTGAGTTCTTGAGTGTTCGAATAAAATGTTCCATGTCTGAAATTCTT      360
1559-1-2h       AACGATATGGGAGCTGAGTTCTTGAGTGTTCGAATAAAATGTTCCATGTCTGAAATTCTT      360
                ************************************************************

reference       GATCATCCTCATGCATCTCTTGCAGAGAGCATAGTTTTGCCCAAGGATTTGCCTTGGGCG      420
Yuwolcho        GATCATCCTCATGCATCTCTTGCAGAGAGCATAGTTTTGCCCAAGGATTTGCCTTGGGCG      420
1559-1-2h       GATCATCCTCATGCATCTCTTGCAGAGAGCATAGTTTTGCCCAAGGATTTGCCTTGGGCG      420
                ************************************************************

reference       AATAATTGTGAAGGTGGTAATTTGCTTGTAGTTCAAGTAAGTAAGTTTGATTGTGGGGGA      480
Yuwolcho        AATAATTGTGAAGGTGGTAATTTGCTTGTAGTTCAAGTAAGTAAGTTTGATTGTGGGGGA      480
1559-1-2h       AATAATTGTGAAGGTGGTAATTTGCTTGTAGTTCAAGTAAGTAAGTTTGATTGTGGGGGA      480
                ************************************************************

reference       ATAGCCATCAGTGTATGCTTTTCGCACAAGATTGGTGATGGTTGCTCTCTGCTTAATTTC      540
Yuwolcho        ATAGCCATCAGTGTATGCTTTTCGCACAAGATTGGTGATGGTTGCTCTCTGCTTAATTTC      540
1559-1-2h       ATAGCCATCAGTGTATGCTTTTCGCACAAGATTGGTGATGGTTGCTCTCTGCTTAATTTC      540
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reference       CTTAATGATTGGTCTAGCGTTACTCGTGATCCTACGACAACAACTTTAGTTCCATCTCCT      600
Yuwolcho        CTTAATGATTGGTCTAGCGTTACTCGTGATCCTACGACAACAACTTTAGTTCCATCTCCT      600
1559-1-2h       CTTAATGATTGGTCTAGCGTTACTCGTGATCCTACGACAACAACTTTAGTTCCATCTCCT      600
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reference       AGATTTGTAGGAGATTCAGTCTTCTCTACACAAAAATATGGTTCTCTCATTACGCCACAA      660
Yuwolcho        AGATTTGTAGGAGATTCAGTCTTCTCTACACAAAAATATGGTTCTCTCATTACGCCACAA      660
1559-1-2h       AGATTTGTAGGAGATTCAGTCTTCTCTACACAAAAATATGGTTCTCTCATTACGCCACAA      660
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reference       ATTTTGTCCGATCTCAACCAGTGCGTACAGAAAAGACTCATTTTTCCTACAGATAAGTTA      720
Yuwolcho        ATTTTGTCCGATCTCAACCAGTGCGTACAGAAAAGACTCATTTTTCCTACAGATAAGTTA      720
1559-1-2h       ATTTTGTCCGATCTCAACCAGTGCGTACAGAAAAGACTCATTTTTCCTACAGATAAGTTA      720
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reference       GATGCACTTCGAGCTAAGGTGGCAGAAGAATCAGGAGTAAAAAATCCAACAAGGGCTGAA      780
Yuwolcho        GATGCACTTCGAGCTAAGGTGGCAGAAGAATCAGGAGTAAAAAATCCAACAAGGGCTGAA      780
1559-1-2h       GATGCACTTCGAGCTAAGGTGGCAGAAGAATCAGGAGTAAAAAATCCAACAAGGGCTGAA      780
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reference       GTTGTTAGCGCTCTTCTTTTCAAATGTGCAACAAAGGCATCATCATCAATGCTACCATCA      840
Yuwolcho        GTTGTTAGCGCTCTTCTTTTCAAATGTGCAACAAAGGCATCATCATCAATGCTACCATCA      840
1559-1-2h       GTTGTTAGCGCTCTTCTTTTCAAATGTGCAACAAAGGCATCATCATCAATGCTACCATCA      840
                ************************************************************

reference       AAGTTGGTTCACTTCTTAAACATACGTACTATGATCAAACCTCGTCTACCACGAAATGCC      900
Yuwolcho        AAGTTGGTTCACTTCTTAAACATACGTACTATGATCAAACCTCGTCTACCACGAAATGCC      900
1559-1-2h       AAGTTGGTTCACTTCTTAAACATACGTACTATGATCAAACCTCGTCTACCACGAAATGCC      900
                ************************************************************

reference       ATTGGAAATCTCTCGTCTATTTTCTCCATAGAAGCAACTAACATGCAGGACATGGAGTTG      960
Yuwolcho        ATTGGAAATCTCTCGTCTATTTTCTCCATAGAAGCAACTAACATGCAGGACATGGAGTTG      960
1559-1-2h       ATTGGAAATCTCTCGTCTATTTTCTCCATAGAAGCAACTAACATGCAGGACATGGAGTTG      960
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reference       CCAACGTTGGTTCGTAATTTAAGGAAGGAAGTTGAGGTGGCATACAAGAAAGACCAAGTC     1020
Yuwolcho        CCAACGTTGGTTCGTAATTTAAGGAAGGAAGTTGAGGTGGCATACAAGAAAGACCAAGTC     1020
1559-1-2h       CCAACGTTGGTTCGTAATTTAAGGAAGGAAGTTGAGGTGGCATACAAGAAAGACCAAGTC     1020
                ************************************************************

reference       GAACAAAATGAACTGATCCTAGAAGTAGTAGAATCAATGAGAGAAGGGAAACTGCCATTT     1080
Yuwolcho        GAACAAAATGAACTGATCCTAGAAGTAGTAGAATCAATGAGAGAAGGGAAACTGCCATTT     1080
1559-1-2h       GAACAAAATGAACTGATCCTAGAAGTAGTAGAATCAATGAGAGAAGGGAAACTGCCATTT     1080
                ************************************************************

reference       GAAAATATGGATGGCTATAAGAATGTGTATACTTGCAGCAATCTTTGCAAATATCCATAC     1140
Yuwolcho        GAAAATATGGATGGCTATAAGAATGTGTATACTTGCAGCAATCTTTGCAAATATCCATAC     1140
1559-1-2h       GAAAATATGGATGGCTATAAGAATGTGTATACTTGCAGCAATCTTTGCAAATATCCATAC     1140
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reference       TACACTGTAGATTTTGGATGGGGAAGACCTGAAAGGGTGTGTCTAGGAAATGGTCCCTCC      1200
Yuwolcho        TACACTGTAGATTTTGGATGGGGAAGACCTGAAAGGGTGTGTCTAGGAAATGGTCCCTCC      1200
1559-1-2h       TACACTGTAGATTTTGGATGGGGAAGACCTGAAAGGGTGTGTCTAGGAAATGGTCCCTCC      1200
                ************************************************************

reference       AAGAATGCCTTCTTCTTGAAAGATTACAAAGCTGGGCAAGGCGTGGAGGCGCGGGTGATG      1260
Yuwolcho        AAGAATGCCTTCTTCTTGAAAGATTACAAAGCTGGGCAAGGCGTGGAGGCGCGGGTGATG      1260
1559-1-2h       AAGAATGCCTTCTTCTTGAAAGATTACAAAGCTGGGCAAGGCGTGGAGGCGCGGGTGATG      1260
                ************************************************************

reference       TTGCACAAGCAACAAATGTCTGAATTTGAACGCAATGAGGAACTCGTTGAGTTCATTGCC      1320
Yuwolcho        TTGCACAAGCAACAAATGTCTGAATTTGAACGCAATGAGGAACTCGTTGAGTTCATTGCC      1320
1559-1-2h       TTGCACAAGCAACAAATGTCTGAATTTGAACGCAATGAGGAACTCGTTGAGTTCATTGCC      1320
                ************************************************************

reference       TAA   1323
Yuwolcho        TAA   1323
1559-1-2h       TAA   1323
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Appendix 1. *Pun1* CDS sequences of 'Yuwolcho' and '1559-1-2h'. No mutation was observed in low-pungent mutant '1559-1-2h' compared with wild-type 'Yuwolcho' CDS sequences of *Pun1*.

>Chr07:200083109-200100827_**CC.CCv1.2.scaffold939.1_Pun3_(+/+)**

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reference       ATGGTGAGAACACCTTGCTACGACGAAAATGGAATGAAGAAGGGGACATGGACTCCTGAA      60
Yuwolcho        ATGGTGAGAACACCTTGCTACGACGAAAATGGAATGAAGAAGGGGACATGGACTCCTGAA      60
1559-1-2h       ATGGTGAGAACACCTTGCTACGACGAAAATGGAATGAAGAAGGGGACATGGACTCCTGAA      60
                ************************************************************

reference       GAAGATAGGAAGTTAACAGCATATATTGCAAAATATGGCTCATGGAACTGGCGCCAACTT      120
Yuwolcho        GAAGATAGGAAGTTAACAGCATATATTGCAAAATATGGCTCATGGAACTGGCGCCAACTT      120
1559-1-2h       GAAGATAGGAAGTTAACAGCATATATTGCAAAATATGGCTCATGGAACTGGCGCCAACTT      120
                ************************************************************

reference       CCCAAGTATGCTGGACTAGCAAGGTGTGGAAAGAGCTGCAGACTTCGATGGATGAATCAC      180
Yuwolcho        CCCAAGTATGCTGGACTAGCAAGGTGTGGAAAGAGCTGCAGACTTCGATGGATGAATCAC      180
1559-1-2h       CCCAAGTATGCTGGACTAGCAAGGTGTGGAAAGAGCTGCAGACTTCGATGGATGAATCAC      180
                ************************************************************

reference       TTACGGCCAAATGTTAAAAGAGGGAATTATACCAAAGAAGAAGATGAAATCATCTTGAAC      240
Yuwolcho        TTACGGCCAAATGTTAAAAGAGGGAATTATACCAAAGAAGAAGATGAAATCATCTTGAAC      240
1559-1-2h       TTACGGCCAAATGTTAAAAGAGGGAATTATACCAAAGAAGAAGATGAAATCATCTTGAAC      240
                ************************************************************

reference       CTCCATGCTCAACTTGGAAATAGGTGGTCGGCGATTGCTGCTCACTTGCCAGGAAGATCA      300
Yuwolcho        CTCCATGCTCAACTTGGAAATAGGTGGTCGGCGATTGCTGCTCACTTGCCAGGAAGATCA      300
1559-1-2h       CTCCATGCTCAACTTGGAAATAGGTGGTCGGCGATTGCTGCTCACTTGCCAGGAAGATCA      300
                ************************************************************

reference       GACAATGAGATAAAGAATCATTGGCACACAAAACTTAAGAAGCGCGGTACTAATTATGCG      360
Yuwolcho        GACAATGAGATAAAGAATCATTGGCACACAAAACTTAAGAAGCGCGGTACTAATTATGCG      360
1559-1-2h       GACAATGAGATAAAGAATCATTGGCACACAAAACTTAAGAAGCGCGGTACTAATTATGCG      360
                ************************************************************

reference       ACAAACTCAAGTGATGAATCAAGCAAGAAATGTAAGAATAATACTAAGAAGAGGTATACT      420
Yuwolcho        ACAAACTCAAGTGATGAATCAAGCAAGAAATGTAAGAATAATACTAAGAAGAGGTATACT      420
1559-1-2h       ACAAACTCAAGTGATGAATCAAGCAAGAAATGTAAGAATAATACTAAGAAGAGGTATACT      420
                ************************************************************

reference       GAAAGTAATACCAATAAAAATACAAGTCATAATAATATGCAGGAAAATATAGTACTGGAA      480
Yuwolcho        GAAAGTAATACCAATAAAAATACAAGTCATAATAATATGCAGGAAAATATAGTACTGGAA      480
1559-1-2h       GAAAGTAATACCAATAAAAATACAAGTCATAATAATATGCAGGAAAATATAGTACTGGAA      480
                ************************************************************

reference       AGTTTAGAATGGTCACCAAAGGAATCATCAAGTGAAGAACTCTCCTCTTACAGTACCACT      540
Yuwolcho        AGTTTAGAATGGTCACCAAAGGAATCATCAAGTGAAGAACTCTCCTCTTACAGTACCACT      540
1559-1-2h       AGTTTAGAATGGTCACCAAAGGAATCATCAAGTGAAGAACTCTCCTCTTACAGTACCACT      540
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reference       AATTATCAACAGCAACATAAAGTGTTTCAAGAGGAAATAACTAGTGGAAGCTTTTGGACA      600
Yuwolcho        AATTATCAACAGCAACATAAAGTGTTTCAAGAGGAAATAACTAGTGGAAGCTTTTGGACA      600
1559-1-2h       AATTATCAACAGCAACATAAAGTGTTTCAAGAGGAAATAACTAGTGGAAGCTTTTGGACA      600
                ************************************************************

reference       GAACCATTTGTAGTAGAAAGTTTCAATACTACTAGAACTGATTTTCTAGCTCCTTCAATT      660
Yuwolcho        GAACCATTTGTAGTAGAAAGTTTCAATACTACTAGAACTGATTTTCTAGCTCCTTCAATT      660
1559-1-2h       GAACCATTTGTAGTAGAAAGTTTCAATACTACTAGAACTGATTTTCTAGCTCCTTCAATT      660
                ************************************************************

reference       GATTACTGTGGACTTGTGTGTCCACCTTCACCATATATAGGTCATGAATTTCTTTCCTCC      720
Yuwolcho        GATTACTGTGGACTTGTGTGTCCACCTTCACCATATATAGGTCATGAATTTCTTTCCTCC      720
1559-1-2h       GATTACTGTGGACTTGTGTGTCCACCTTCACCATATATAGGTCATGAATTTCTTTCCTCC      720
                ************************************************************

reference       TTTGACTTTGATCATTATAATTATTGGTAA      750
Yuwolcho        TTTGACTTTGATCATTATAATTATTGGTAA      750
1559-1-2h       TTTGACTTTGATCATTATAATTATTGGTAA      750
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Appendix 2. *Pun3* CDS sequences of 'Yuwolcho' and '1559-1-2h'. No mutation was observed in low-pungent mutant '1559-1-2h' compared with wild-type 'Yuwolcho' CDS sequences of *Pun3*.

>Pepper.v.1.55.chr03:26788049-26810708_(pAMT)_(+/-)_RC

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reference       ATGGCCAATATTACTAATGAATTTATGGGACATGATATGTTGGCACCCTTTACTGCGGGA     60
Yuwolcho        ATGGCCAATATTACTAATGAATTTATGGGACATGATATGTTGGCACCCTTTACTGCGGGA     60
1559-1-2h       ATGGCCAATATTACTAATGAATTTATGGGACATGATATGTTGGCACCCTTTACTGCGGGA     60
                ************************************************************

reference       TGGCAGAGTGATATGGAACCTTTAGTTATAGAAAAGTCGGAGGGCTCTTATGTCTATGAC    120
Yuwolcho        TGGCAGAGTGATATGGAACCTTTAGTTATAGAAAAGTCGGAGGGCTCTTATGTCTATGAC    120
1559-1-2h       TGGCAGAGTGATATGGAACCTTTAGTTATAGAAAAGTCGGAGGGCTCTTATGTCTATGAC    120
                ************************************************************

reference       ATAAATGGGAAGAAGTATCTTGACACTTTATCTGGTTTATGGTGCACAACATTAGGGGGA    180
Yuwolcho        ATAAATGGGAAGAAGTATCTTGACACTTTATCTGGTTTATGGTGCACAACATTAGGGGGA    180
1559-1-2h       ATAAATGGGAAGAAGTATCTTGACACTTTATCTGGTTTATGGTGCACAACATTAGGGGGA    180
                ************************************************************

reference       AGTGAGACTCGACTTGTTGAAGCTGCAAATAAACAACTCAATACATTGCCATTTTATCAT    240
Yuwolcho        AGTGAGACTCGACTTGTTGAAGCTGCAAATAAACAACTCAATACATTGCCATTTTATCAT    240
1559-1-2h       AGTGAGACTCGACTTGTTGAAGCTGCAAATAAACAACTCAATACATTGCCATTTTATCAT    240
                ************************************************************

reference       TCATTTTGGAATCGAACCACAAAACCTTCTTTGGATCTTGCAAAGGAGCTCCTAAATATG    300
Yuwolcho        TCATTTTGGAATCGAACCACAAAACCTTCTTTGGATCTTGCAAAGGAGCTCCTAAATATG    300
1559-1-2h       TCATTTTGGAATCGAACCACAAAACCTTCTTTGGATCTTGCAAAGGAGCTCCTAAATATG    300
                ************************************************************

reference       TTTACTGCAAATAAAATGGCCAAAGTTTTTTTCACTAATAGCGGATCAGAAGCCAATGAC    360
Yuwolcho        TTTACTGCAAATAAAATGGCCAAAGTTTTTTTCACTAATAGCGGATCAGAAGCCAATGAC    360
1559-1-2h       TTTACTGCAAATAAAATGGCCAAAGTTTTTTTCACTAATAGCGGATCAGAAGCCAATGAC    360
                ************************************************************

reference       ACTCAGGTGAAGCTGGTGTGGTATTACAATAATGCCCTTGGGAGGCCACAGAAAAAGAAA    420
Yuwolcho        ACTCAGGTGAAGCTGGTGTGGTATTACAATAATGCCCTTGGGAGGCCACAGAAAAAGAAA    420
1559-1-2h       ACTCAGGTGAAGCTGGTGTGGTATTACAATAATGCCCTTGGGAGGCCACAGAAAAAGAAA    420
                ************************************************************

reference       ATTATTGCTCGAGCAAAAGCATATCATGGTTCCACTTACATTTCTGCTGGTCTCTCTGGG    480
Yuwolcho        ATTATTGCTCGAGCAAAAGCATATCACGGTTCCACTTACATTTCTGCTGGTCTCTCTGGG    480
1559-1-2h       ATTATTGCTCGAGCAAAAGCATATCACGGTTCCACTTACATTTCTGCTGGTCTCTCTGGG    480
                ************************** ***********************************

reference       CTTCCTCCAATGCATCAAAAATTTGATTTGCCACCTCCATTTGTTCTGCACACTGAGTGC    540
Yuwolcho        CTTCCTCCAATGCATCAAAAATTTGATTTGCCACCTCCATTTGTTCTGCACACTGAGTGC    540
1559-1-2h       CTTCCTCCAATGCATCAAAAATTTGATTTGCCACCTCCATTTGTTCTGCACACTGAGTGC    540
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reference       CCTCATTATTGGGCCTATCACTTGCCAGGTGAAACCGAAGAGGAATTCTCTACTAGGTTG      600
Yuwolcho        CCTCATTATTGGGCCTATCACTTGCCAGGTGAAACCGAAGAGGAATTCTCTACTAGGTTG      600
1559-1-2h       CCTCATTATTGGGCCTATCACTTGCCAGGTGAAACCGAAGAGGAATTCTCTACTAGGTTG      600
                ************************************************************

reference       GCAAATAATTTGGAAAGTCTTATACTCAACGAGGGGCCTGAAACAGTAGCTGCTTTCATT      660
Yuwolcho        GCAAATAATTTGGAAAGTCTTATACTCAAAGAGGGGCCTGAAACAGTAGCTGCTTTCATT      660
1559-1-2h       GCAAATAATTTGGAAAGTCTTATACTCAAAGAGGGGCCTGAAACAGTAGCTGCTTTCATT      660
                ***************************** ******************************

reference       GCCGAACCAGTCCTAGGAGCAGCAGGTGTAATACTTCCTCCCGCAACATATTTTGATAAG      720
Yuwolcho        GCCGAACCAGTCCTAGGAGCAGCAGGTGTAATACTTCCTCCCGCAACATATTTTGATAAG      720
1559-1-2h       GCCGAACCAGTCCTAGGAGCAGCAGGTGTAATACTTCCTCCCGCAACATATTTTGATAAG      720
                ************************************************************

reference       GCACTTTCTTCTGGATATATGCCAATTGCCGCTGTCCTTGTAAGCCAGAAAATTTCTAGT      780
Yuwolcho        GCACTTTCTTCTGGATATATGCCAATTGCCGCTGTCCTTGTAAGCCAGAAAATTTCTAGT      780
1559-1-2h       GCACTTTCTTCTGGATATATGCCAATTGCCGCTGTCCTTGTAAGCCAGAAAATTTCTAGT      780
                ************************************************************

reference       GTCATCCTTTCTGAAAGCAATAAAATTGGTGCCTTTTGCCATGGATTTACTTATTCCGGA      840
Yuwolcho        GTCATACTTTCTGAAAGCAATAAAATTGGTGCCTTTTGCCATGGATTTACTTATTCCGGA      840
1559-1-2h       GTCATACTTTCTGAAAGCAATAAAATTGGTGCCTTTTGCCATGGATTTACTTATTCCGGA      840
                ***** ********************************************************

reference       CACCCTGTTGCGTGCGCAGTTGCATTGGAAGCATTGAAGATCTATAAGGAAAGAAATATT      900
Yuwolcho        CACCCTGTTGCGTGCGCAGTTGCATTGGAAGCATTGAAGATCTATAAGGAAAGAAATATT      900
1559-1-2h       CACCCTGTTGCGTGCGCAGTTGCATTGGAAGCATTGAAGATCTATAAGGAAAGAAATATT      900
                ************************************************************

reference       ACTGAGGTGGTGAACAAAATATCACAAAAGTTTCAAGAAGGTTTGAAAGCATTCGCCGAC      960
Yuwolcho        ACTGAGGTGGTGAACAAAATATCACAAAAGTTTCAAGAAGGTTTGAAAGCATTCGCCGAC      960
1559-1-2h       ACTGAGGTGGTGAACAAAATATCACAAAAGTTTCAAGAAGGTTTGAAAGCATTCGCCGAC      960
                ************************************************************

reference       AGTCCCATAATTGGGGAGATAAGGGGAACTGGTTTGGCACTTTCTACAGAGTTTGTGAAC      1020
Yuwolcho        AGTCCCATAATTGGGGAGATAAGGGGAACTGGTTTGACACTTTCTACAGAGTTTGTACGAC      1020
1559-1-2h       AGTCCCATAATTGGGGAGATAAGGGGAACTGGTTTGACACTTTCTACAGAGTTTGTACGAC      1020
                *********************************** ******************  **

reference       AATAAATCTCCTAATGATCCCTTTCCATATGAATGGGCTGTCGGTACATATTTTGGAGCA      1080
Yuwolcho        AATAAATCTCCTAATGATCCCTTTCCATATGAATGGGCTGTCGGTACATATTTTGGAGCA      1080
1559-1-2h       AATAAATCTCCTAATGATCCCTTTCCATATGAATGGGCTGTCGGTACATATTTTGGAGCA      1080
                ************************************************************

reference       CAATGTGCTAAGTACGGGATGTTGGTAAGTTCCACTGGTGATCATGTAAATATGGCTCCA      1140
Yuwolcho        CAATGTGCTAAGTACGGGATGTTGGTAAGTTCCACTGGTGATCATGTAAATATGGCTCCA      1140
1559-1-2h       CAATGTGCTAAGTACGGGATGTTGGTAAGTTCCACTGGTGATCATGTAAATATGGCTCCA      1140
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reference       CCATTTACCTTGAGTCTTGAAGAACTTGATGAGTTGATACGCATATATGGGAAAGCATTG      1200
Yuwolcho        CCATTTACCTTGAGTCTTGAAGAACTTGATGAGTTGATACGCATATATGGGAAAGCATTG      1200
1559-1-2h       CCATTTACCTTGAGTCTTGAAGAACTTGATGAGTTGATACGCATATATGGGAAAGCATTG      1200
                ************************************************************

reference       AAGGATACTGAAAAGAGAGTTGAAGAACTCAAGTCTCAGAAGAAGTAA        1248
Yuwolcho        AAGGATACTGAAAAGAGAGTTGAAGAACTCAAGTCTCAGAAGAAGTAA        1248
1559-1-2h       AAGGATACTGAAAAGAGAGTTGAAGAACTCAAGTCTCAGAAGAAGTAA        1248
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Appendix 3. *pAMT* CDS sequences of 'Yuwolcho' and '1559-1-2h'. No mutation was observed in low-pungent mutant '1559-1-2h' compared with wild-type 'Yuwolcho' CDS sequences of *pAMT*.

>Pepper.v.1.55.chr10:227625481-227631490_**CaKR1_(+/-)**

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reference       ATGGCTCTCCATGCTGAACCAGTACTACCCCAGCTGGAGCCATGGCGGCAACTCGCCCGA      60
Yuwolcho        ATGGCTCTCCATGCTGAACCAGTACTACCCCAGCTGGAGCCATGGCGGCAACTCGCCCGA      60
1559-1-2h       ATGGCTCTCCATGCTGAACCAGTACTACCCCAGCTGGAGCCATGGCGGCAACTCGCCCGA      60
                ************************************************************

reference       AAAGTAGTAATGGTGACCGGAGCATCGTCCGGAATCGGCCGTGAATTCTGCCTTGATCTA     120
Yuwolcho        AAAGTAGTAATGGTGACCGGAGCATCGTCCGGAATCGGCCGTGAATTCTGCCTTGATCTA     120
1559-1-2h       AAAGTAGTAATGGTGACCGGAGCATCGTCCGGAATCGGCCGTGAATTCTGCCTTGATCTA     120
                ************************************************************

reference       TCCAAAACTGGTTGTAGAATCATCGCCGCTGCTCGTCGGATCGATCGATTGAAATCCTTA     180
Yuwolcho        TCCAAAACTGGTTGTAGAATCATCGCCGCTGCTCGTCGGATCGATCGATTGAAATCCTTA     180
1559-1-2h       TCCAAAACTGGTTGTAGAATCATCGCCGCTGCTCGTCGGATCGATCGATTGAAATCCTTA     180
                ************************************************************

reference       TGTGATGAGATCAACGGAATTGCCTCGAATCCGTCGAAGGAATTACGAGCAGTTGCGATT     240
Yuwolcho        TGTGATGAGATCAACGGAATTGCCTCGAATCCGTCGAAGGAATTACGAGCAGTTGCGATT     240
1559-1-2h       TGTGATGAGATCAACGGAATTGCCTCGAATCCGTCGAAGGAATTACGAGCAGTTGCGATT     240
                ************************************************************

reference       GAGCTAGATGTTAGTGCTAATGGAGGTGTCATTGAAGCTGCTGTTAAGAAAGCTTGGGAT     300
Yuwolcho        GAGCTAGATGTTAGTGCTAATGGAGGTGTCATTGAAGCTGCTGTTAAGAAAGCTTGGGAT     300
1559-1-2h       GAGCTAGATGTTAGTGCTAATGGAGGTGTCATTGAAGCTGCTGTTAAGAAAGCTTGGGAT     300
                ************************************************************

reference       GTGTTTGGTAGGATTGATGCTTTGGTTAATAACGCCGGTATTCGAGGCAGTGTGCACTCT     360
Yuwolcho        GTGTTTGGTAGGATTGATGCTTTGGTTAATAACGCCGGTATTCGAGGCAGTGTGCACTCT     360
1559-1-2h       GTGTTTGGTAGGATTGATGCTTTGGTTAATAACGCCGGTATTCGAGGCAGTGTGCACTCT     360
                ************************************************************

reference       CCACTGGATTTGACAGAGGAGGAATGGGAAAAGATCCACAAAACGAACCTAAGAGGAGCA     420
Yuwolcho        CCACTGGATTTGACAGAGGAGGAATGGGAAAAGATCCACAAAACGAACCTAAGAGGAGCA     420
1559-1-2h       CCACTGGATTTGACAGAGGAGGAATGGGAAAAGATCCACAAAACGAACCTAAGAGGAGCA     420
                ************************************************************

reference       TGGTTGGTGACCAAATATGTTAGTATGCATATGCGTGCTGCTAATCAAGGAGGATCAGTT     480
Yuwolcho        TGGTTGGTGACCAAATATGTTAGTATGCATATGCGTGCTGCTAATCAAGGAGGATCAGTT     480
1559-1-2h       TGGTTGGTGACCAAATATGTTAGTATGCATATGCGTGCTGCTAATCAAGGAGGATCAGTT     480
                ************************************************************

reference       ATTAATATCTCTTCTATCGCTGGTCTTAATCGCGGGCAATTACCAGGTGGTCTTGCTTAT     540
Yuwolcho        ATTAATATCTCTTCTATCGCTGGTCTTAATCGCGGGCAATTACCAGGTGGTCTTGCTTAT     540
1559-1-2h       ATTAATATCTCTTCTATCGCTGGTCTTAATCGCGGGCAATTACCAGGTGGTCTTGCTTAT     540
                ************************************************************

reference       GCATCTTCAAAAGAAGCTCTCAACAGCATTACAAAGGTGATGGCCATTGAATTGGGACAA     600
Yuwolcho        GCATCTTCAAAAGAAGCTCTCAACAGCATTACAAAGGTGATGGCCATTGAATTGGGACAA     600
1559-1-2h       GCATCTTCAAAAGAAGCTCTCAACAGCATTACAAAGGTGATGGCCATTGAATTGGGACAA     600
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reference       TACAAGATCAGAGTGAACTCAATATCACCGGGACTTTTCAAATCTGAGATAACAGAAGGT    660
Yuwolcho        TACAAGATCAGAGTGAACTCAATATCACCGGGACTTTTCAAATCTGAGATAACAGAAGGT    660
1559-1-2h       TACAAGATCAGAGTGAACTCAATATCACCGGGACTTTTCAAATCTGAGATAACAGAAGGT    660
                ************************************************************

reference       CTTGTACAGAAGGACTGGCTTCAAAATATTGAATTGAGAACCGTTCCCTTGAGAACTCAT    720
Yuwolcho        CTTGTACAGAAGGACTGGCTTCAAAATATTGAATTGAGAACCGTTCCCTTGAGAACTCAT    720
1559-1-2h       CTTGTACAGAAGGACTGGCTTCAAAATATTGAATTGAGAACCGTTCCCTTGAGAACTCAT    720
                ************************************************************

reference       GGAACATCAAATCCCGCTTTGACTTCAGTAGTACGTTACTTAATCCACGATTCATCAGAA    780
Yuwolcho        GGAACATCAAATCCCGCTTTGACTTCAGTAGTACGTTACTTAATCCACGATTCATCAGAA    780
1559-1-2h       GGAACATCAAATCCCGCTTTGACTTCAGTAGTACGTTACTTAATCCACGATTCATCAGAA    780
                ************************************************************

reference       TATGTTTCAGGCAACATGTTCATCGTTGATGCTGGTGCTACTTTACCAGGTGTCCCAATT    840
Yuwolcho        TATGTTTCAGGCAACATGTTCATCGTTGATGCTGGTGCTACTTTACCAGGTGTCCCAATT    840
1559-1-2h       TATGTTTCAGGCAACATGTTCATCGTTGATGCTGGTGCTACTTTACCAGGTGTCCCAATT    840
                ************************************************************

reference       TTCTCATCCCTCTAG 855
Yuwolcho        TTCTCATCCCTCTAG 855
1559-1-2h       TTCTCATCCCTCTAG 855
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Appendix 4. *CaKR1* CDS sequences of 'Yuwolcho' and '1559-1-2h'. No mutation was observed in low-pungent mutant '1559-1-2h' compared with wild-type 'Yuwolcho' CDS sequences of *CaKR1*.

Appendix 5. Selected SNPs of candidate region (204.8 to 219.2 Mbp) from the bulked segregant RNA-seq of '1559-1-2h' and MR.

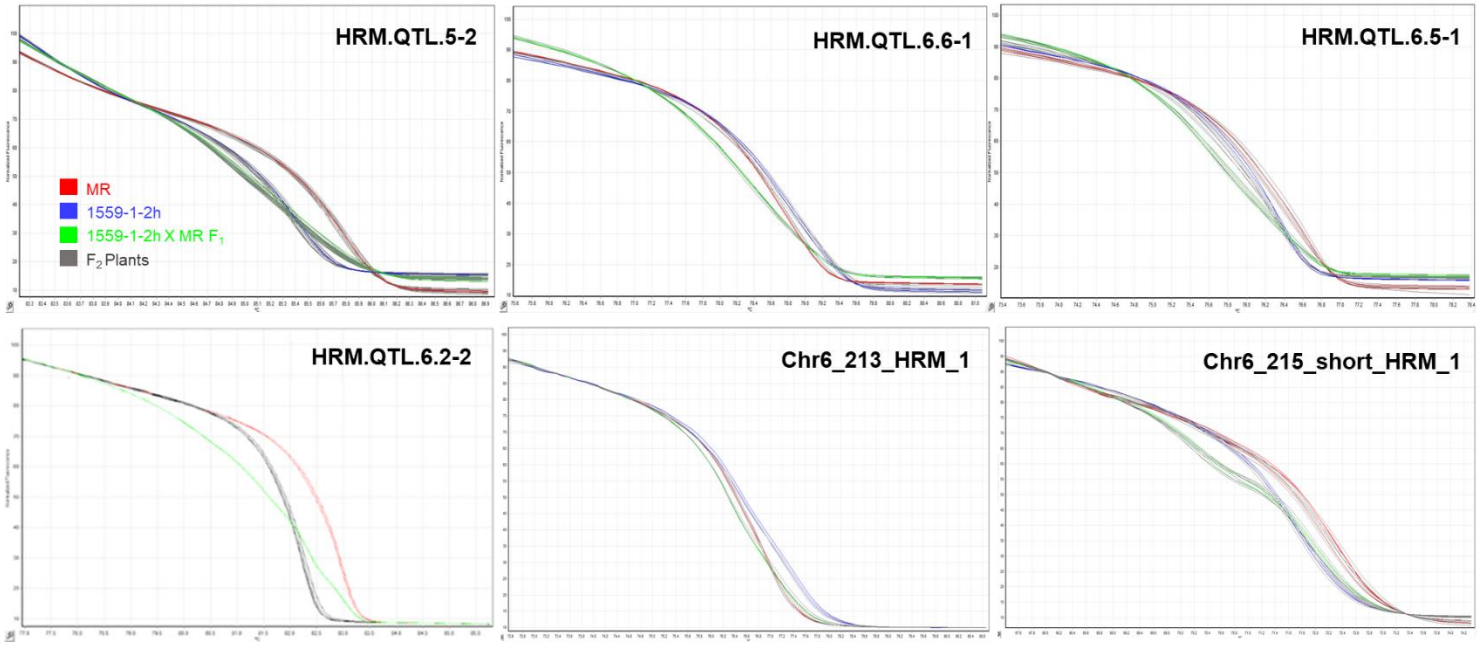
CHR	POS	REF	ALT	deltaSNP	tricubeDeltaSNP	Gprime	pvalue
6	206420085	T	C	-0.74603	0.008784195	140.1906	0
6	206470111	T	C	-0.46559	0.011929424	139.0682	0
6	206470113	A	G	-0.46559	0.01192955	139.0682	0
6	206476137	C	T	-0.31672	0.01230829	138.9331	0
6	206578672	G	T	-0.3871	0.018754859	136.6327	0
6	206627237	A	C	-0.53623	0.021808232	135.5431	0
6	206627883	C	T	-0.5092	0.021848848	135.5286	0
6	206925850	C	A	0.525424	0.040582595	128.8437	0
6	206925851	A	T	0.525424	0.040582658	128.8437	0
6	207204892	A	C	-0.75455	0.058126493	122.5833	0
6	207207740	C	A	-0.5415	0.058305552	122.5194	0
6	207625596	T	A	-0.52857	0.084576948	113.1448	0
6	208000353	C	T	0.804362	0.108138628	104.737	0
6	208129727	C	T	0.741379	0.116362471	101.934	1.11E-16
6	208129733	G	A	0.741379	0.116362971	101.934	1.11E-16
6	208129744	C	T	0.716981	0.116363889	101.934	1.11E-16
6	208658761	G	A	-0.57143	0.160495182	102.1054	1.11E-16
6	208782072	T	A	0.568966	0.170781948	102.1454	1.11E-16
6	208782082	T	G	0.558824	0.170782782	102.1454	1.11E-16
6	208782292	A	G	0.546512	0.170800301	102.1455	1.11E-16
6	208782454	G	T	0.5625	0.170813815	102.1455	1.11E-16
6	208782566	C	G	0.54595	0.170823158	102.1456	1.11E-16
6	208782688	C	T	0.558111	0.170833335	102.1456	1.11E-16
6	208783155	G	A	0.637732	0.170872293	102.1457	1.11E-16
6	208783241	C	G	0.715103	0.170879467	102.1458	1.11E-16
6	208783254	G	C	0.696256	0.170880552	102.1458	1.11E-16
6	208783344	T	C	0.692982	0.17088806	102.1458	1.11E-16
6	208783418	C	T	0.686047	0.170894233	102.1458	1.11E-16

6	208783446	C	G	0.690722	0.170896569	102.1458	1.11E-16
6	208785758	C	A	0.629903	0.171089439	102.1466	1.11E-16
6	208785842	C	T	0.798165	0.171096446	102.1466	1.11E-16
6	208951989	A	G	-0.66055	0.184956647	102.2004	1.11E-16
6	208952616	G	A	-0.66	0.185008953	102.2006	1.11E-16
6	209065693	C	A	-0.26667	0.194441985	102.2373	1.11E-16
6	209065715	G	A	-0.29747	0.19444382	102.2373	1.11E-16
6	209065783	C	A	-0.53086	0.194449492	102.2373	1.11E-16
6	209066730	T	*,G	-0.80876	0.194528492	102.2376	1.11E-16
6	209067368	C	T	-0.70833	0.194581715	102.2378	1.11E-16
6	209068122	C	T	-0.54762	0.194644615	102.2381	1.11E-16
6	209103035	A	C	-0.60759	0.197557103	102.2494	1.11E-16
6	209104297	C	T	-0.40506	0.197662381	102.2498	1.11E-16
6	209116595	C	G	-0.42308	0.198688296	102.2538	1.11E-16
6	209626578	T	A	0.597968	0.241231748	102.419	1.11E-16
6	209626610	T	C	0.590062	0.241234418	102.419	1.11E-16
6	209626682	C	T	0.523482	0.241240424	102.419	1.11E-16
6	209626708	A	C	0.554049	0.241242593	102.4191	1.11E-16
6	209626944	G	C	0.466881	0.241262281	102.4191	1.11E-16
6	209627071	C	T	0.544283	0.241272875	102.4192	1.11E-16
6	209627081	A	G	0.531359	0.241273709	102.4192	1.11E-16
6	209766213	A	T	-0.02646	0.252880284	102.4643	1.11E-16
6	209766225	A	T	0.003497	0.252881285	102.4643	1.11E-16
6	209766227	T	G	0.016508	0.252881451	102.4643	1.11E-16
6	209798297	A	G	-0.78723	0.255556773	102.4747	0
6	210123086	C	A	0.660494	0.277762737	102.5112	0
6	210984004	A	C	-0.58242	0.273831138	101.726	1.11E-16
6	210984255	A	G	-0.61058	0.273829992	101.7258	1.11E-16
6	211014516	G	T	-0.56842	0.273691797	101.6982	1.11E-16
6	211151869	C	G	0.646154	0.27306454	101.5729	1.11E-16
6	211162882	C	T	0.441325	0.273014246	101.5628	1.11E-16

6	211164473	T	C	0.657371	0.273006981	101.5614	1.11E-16
6	211164501	A	G	0.671698	0.273006853	101.5614	1.11E-16
6	211164518	T	C	0.661789	0.273006775	101.5614	1.11E-16
6	211164531	A	G	0.674176	0.273006716	101.5613	1.11E-16
6	211164547	C	G	0.692742	0.273006643	101.5613	1.11E-16
6	211164680	G	T	0.584615	0.273006035	101.5612	1.11E-16
6	211170968	G	A	-0.6	0.27297732	101.5555	1.11E-16
6	211277876	C	T	-0.51111	0.272489097	101.458	1.11E-16
6	211277923	G	A	0.538017	0.272488883	101.4579	1.11E-16
6	211359924	T	C	0.619853	0.272114404	101.3831	1.11E-16
6	211359972	A	G	0.596876	0.272114185	101.3831	1.11E-16
6	211360046	C	T	0.573601	0.272113847	101.383	1.11E-16
6	211360106	G	T	0.428691	0.272113573	101.383	1.11E-16
6	211360274	G	T	0.496758	0.272112806	101.3828	1.11E-16
6	211360353	G	T	0.554931	0.272112445	101.3827	1.11E-16
6	211360408	T	G	0.567072	0.272112194	101.3827	1.11E-16
6	211360468	G	A	0.62963	0.27211192	101.3826	1.11E-16
6	211360683	A	T	0.634217	0.272110938	101.3824	1.11E-16
6	211360848	C	G	0.470111	0.272110185	101.3823	1.11E-16
6	211363505	T	C	0.70875	0.272098051	101.3799	1.11E-16
6	211453212	C	A	0.722414	0.271688381	101.298	1.11E-16
6	211453213	C	T	0.722414	0.271688377	101.298	1.11E-16
6	211453703	A	G	0.798178	0.271686139	101.2976	1.11E-16
6	211453711	C	T	0.783791	0.271686102	101.2976	1.11E-16
6	211453742	G	T	0.646516	0.271685961	101.2976	1.11E-16
6	211453828	G	C	-0.59504	0.271685568	101.2975	1.11E-16
6	211453849	A	C	0.579622	0.271685472	101.2975	1.11E-16
6	211560688	G	A	0.787879	0.271197565	101.2	1.11E-16
6	212391565	T	A	0.68006	0.233187114	100.7335	1.11E-16
6	212391571	T	C	0.676292	0.233186549	100.7335	1.11E-16
6	212424498	A	G	0.480762	0.230085813	100.7286	1.11E-16

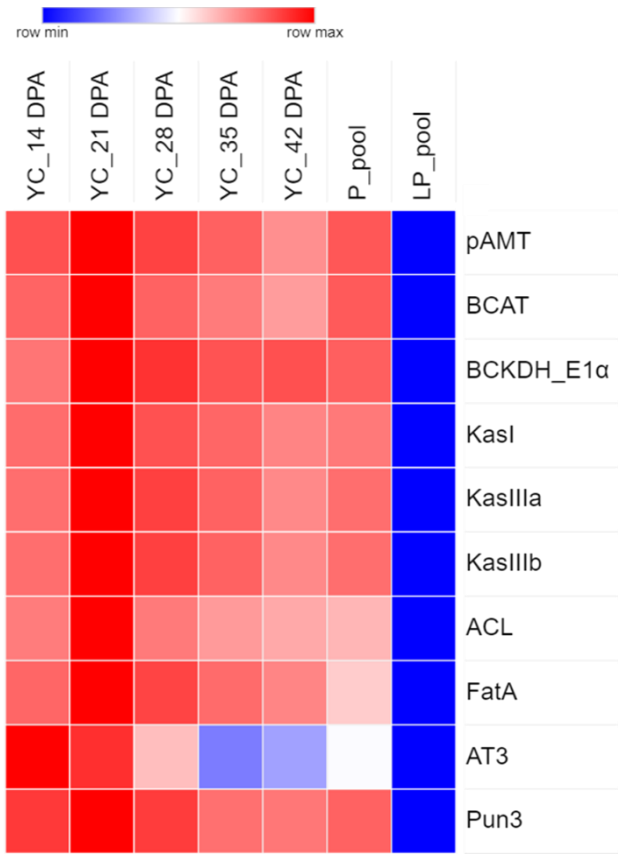
6	213612327	A	G	-0.65263	0.118227934	100.5515	1.11E-16
6	213614292	A	G	0.467888	0.11804289	100.5512	1.11E-16
6	213628885	A	G	0.58091	0.116668667	100.549	1.11E-16
6	213628886	T	A	0.58091	0.116668573	100.549	1.11E-16
6	213663840	C	T	-0.72283	0.113376954	100.5438	1.11E-16
6	213667750	G	A	-0.52261	0.11300875	100.5432	1.11E-16
6	213667776	C	T	-0.41627	0.113006301	100.5432	1.11E-16
6	213667809	A	G	-0.3913	0.113003193	100.5432	1.11E-16
6	213667816	A	C	-0.37313	0.113002534	100.5432	1.11E-16
6	213667826	G	A	-0.37838	0.113001593	100.5432	1.11E-16
6	213676840	T	A	-0.64478	0.112152744	100.5419	1.11E-16
6	215690540	G	*,A	0.778264	-0.094692747	85.13546	3.22E-15
6	215695197	C	A	-0.0278	-0.095177408	85.09431	3.22E-15
6	216299951	C	G	0.022903	-0.101388426	78.59111	1.72E-14
6	216370300	T	C	0.612099	-0.098878075	77.76858	2.13E-14
6	216372407	T	A	-0.76667	-0.098802889	77.74394	2.14E-14
6	216389108	A	G	-0.67805	-0.098206926	77.54867	2.26E-14
6	216389145	A	G	-0.61573	-0.098205606	77.54824	2.26E-14
6	216389868	G	T	-0.51573	-0.098179806	77.53978	2.26E-14
6	216389892	A	C	-0.50466	-0.09817895	77.5395	2.26E-14
6	216389997	G	C	-0.59706	-0.098175203	77.53828	2.26E-14
6	216390027	A	C	-0.66296	-0.098174132	77.53793	2.26E-14
6	216390034	G	T	-0.65082	-0.098173883	77.53784	2.26E-14
6	216430720	T	G	-0.5	-0.096722033	77.06214	2.58E-14
6	216430816	A	T	-0.55093	-0.096718608	77.06101	2.58E-14
6	216441462	G	T	0.540779	-0.096338713	76.93654	2.66E-14
6	216672816	A	G	0.585714	-0.088083021	74.23152	5.52E-14
6	216799246	T	A	0.504665	-0.083571461	72.75328	8.29E-14
6	216859960	A	G	-0.64122	-0.081404928	72.0434	1.01E-13
6	217217932	C	T	0.650273	-0.068630968	67.85794	3.31E-13
6	217261957	A	G	-0.48958	-0.067059969	67.3432	3.84E-13

6	217557941	C	T	0.505108	-0.056498004	63.88251	1.07E-12
6	217559737	G	C	0.358388	-0.056433915	63.86151	1.08E-12
6	218132079	C	A	0.390663	-0.049343042	55.94876	1.28E-11
6	218132104	T	A	0.404803	-0.049343277	55.94837	1.28E-11
6	218406728	T	C	0.286839	-0.051919601	51.60418	5.49E-11
6	218501464	G	A	-0.54667	-0.052808346	50.10558	9.22E-11
6	218501656	T	G	-0.47863	-0.052810147	50.10254	9.23E-11
6	218782714	A	G	-0.54696	-0.05544683	45.65657	4.55E-10
6	218839249	G	A	-0.45995	-0.055977201	44.76226	6.34E-10
6	218839536	C	T	0.369828	-0.055979893	44.75772	6.35E-10
6	218840788	T	G	0.467105	-0.055991639	44.73791	6.40E-10
6	218840861	T	C	0.501377	-0.055992324	44.73676	6.40E-10
6	218841016	G	A	0.478824	-0.055993778	44.73431	6.41E-10
6	218841026	A	G	0.472693	-0.055993872	44.73415	6.41E-10
6	218841039	A	T	0.480173	-0.055993994	44.73394	6.41E-10
6	219223265	T	C	-0.72	-0.059579762	38.68763	6.72E-09

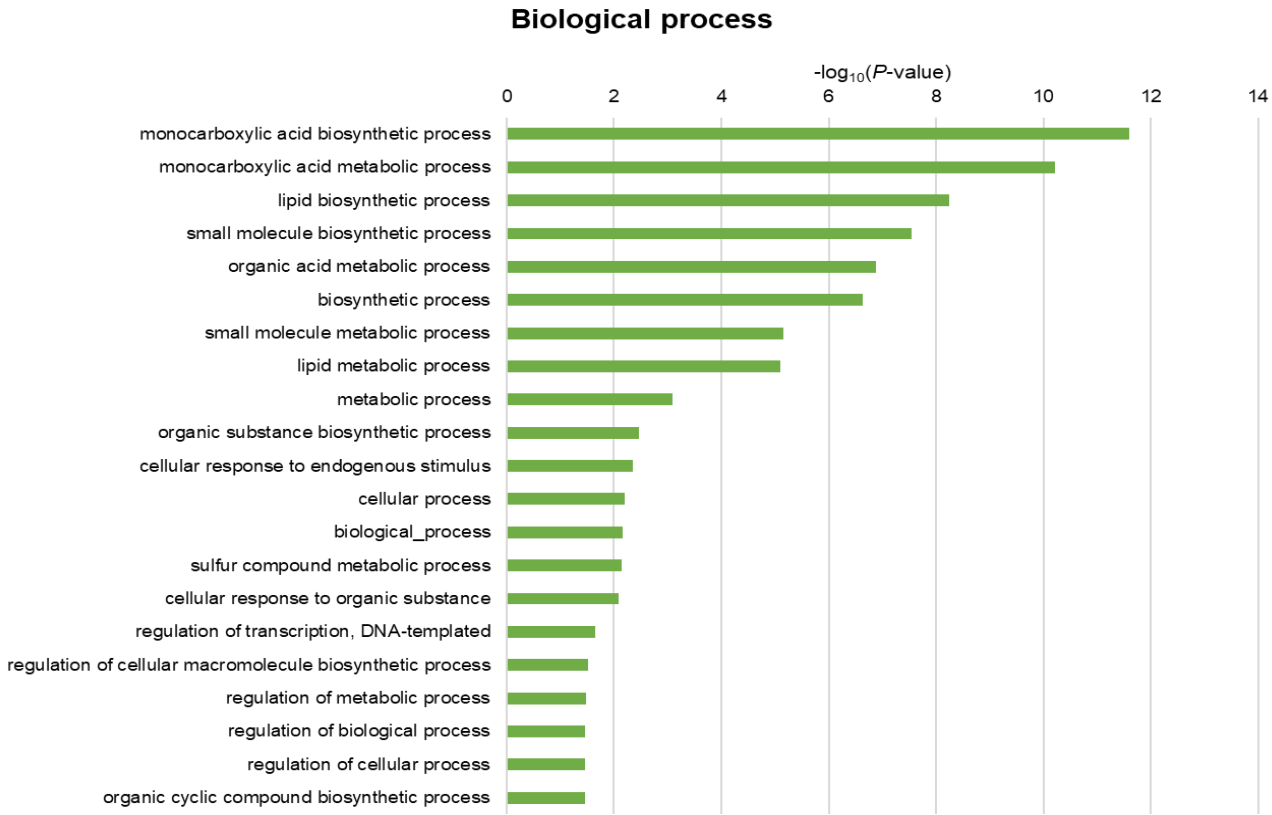


Appendix 6. HRM marker analyses. A total of 6 HRM markers were used for genotyping 136 F_2 plants. Red, blue, green, and gray lines indicate the 'MR', '1559-1-2h', 'F_1', and segregating F_2 population, respectively. The bottom axis represents temperature and the left axis for normalized fluorescence.

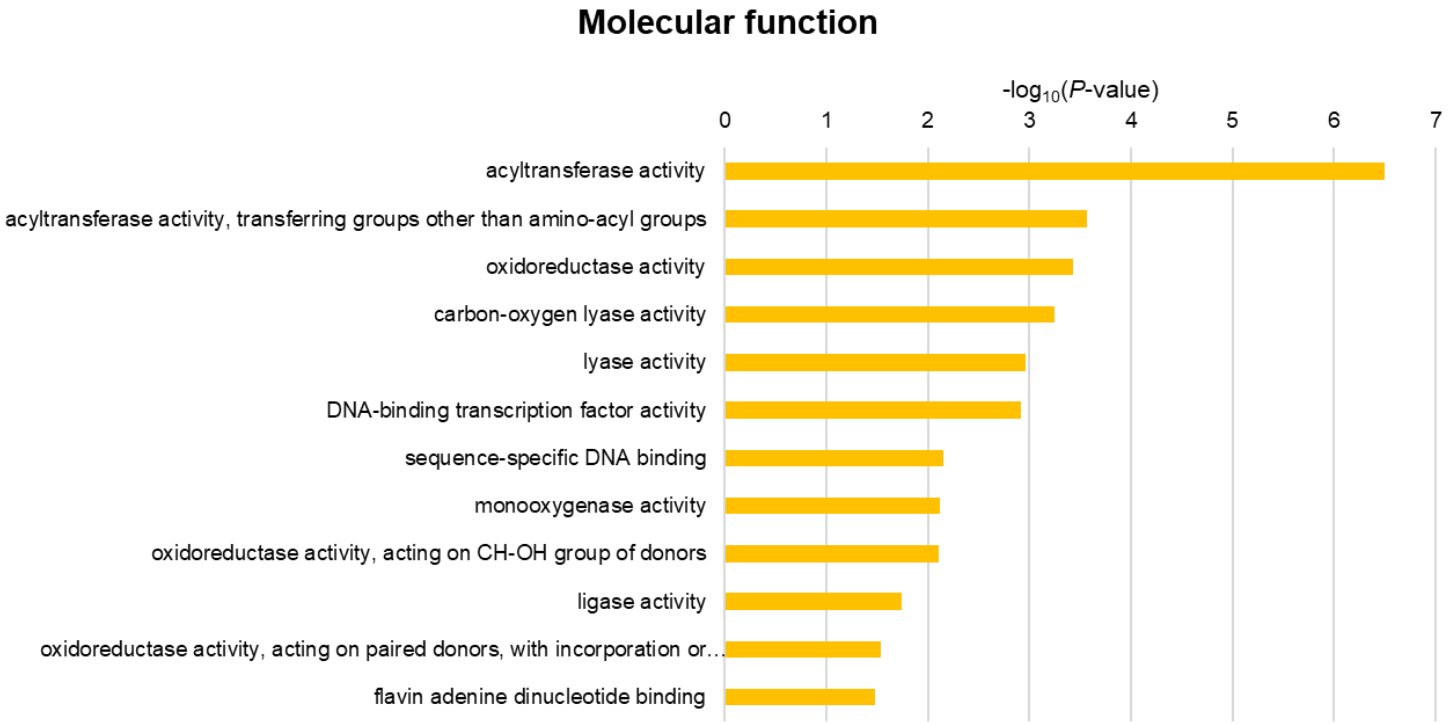
Appendix 7. INDEL marker analyses. An INDEL marker was used for genotyping the F_2 population



Appendix 8. Expression of genes involved in the capsaicinoid biosynthesis pathway in 'Yuwolcho' ('Yuwolcho'), pungent (P) and low-pungent (LP) pools according to the developmental stages by RNA-Seq. The heat map showed the expression patterns of capsaicinoid biosynthesis genes. The heat map is based on Log_2(average FPKM+1) values.



Appendix 9. Transcriptome analysis using DEGs. GO term enrichment analysis was performed in the biological process category.



Appendix 10. Transcriptome analysis using DEGs. GO term enrichment analysis was performed in the biological process category.

Appendix 11. Annotated genes in the *Pun6* region on chromosome 6 and relative gene expression levels in DEGs analysis.

Gene	Physical location (Mbp)	Annotation	Relative gene expression Log_2(average FPKM+1)		Log_2 fold change
			Low-pungent	Pungent	
DEMF06G01010	1.6	Putative late blight resistance protein homolog R1A-3	7.84	6.71	-1.14
DEMF06G04580	25.0	Serine/threonine-protein kinase PCRK1 (EC 2.7.11.1) (Protein PTI-COMPROMISED RECEPTOR-LIKE CYTOPLASMIC KINASE 1)	5.37	6.68	1.34
DEMF06G06020	45.4	Ran-binding protein 1 homolog b	8.17	9.37	1.21
DEMF06G06030	45.4	Ran-binding protein 1 homolog c	7.55	8.70	1.16
DEMF06G09210	115.9	alpha-(1,6)-fucosyltransferase [Trema orientale]	12.43	11.89	-0.54
DEMF06G10230	143.7	Anaphase-promoting complex subunit 1 (Cyclosome subunit 1) (Protein EMBRYO DEFECTIVE 2771)	8.00	7.12	-0.88
DEMF06G10480	148.3	Peroxisomal membrane protein PMP22 (22 kDa peroxisomal membrane protein)	6.82	8.72	1.91
DEMF06G11260	161.7	NAC domain-containing protein 79 (ANAC79) (ANAC80) (AtNAC79) (AtNAC80)	5.51	7.02	1.53
DEMF06G11320	163.5	Aquaporin PIP2-2 (Plasma membrane intrinsic protein 2-2) (AtPIP2;2) (Plasma membrane intrinsic protein 2b) (PIP2b) (TMP2b)	4.14	6.71	2.62
DEMF06G11720	171.8	3-ketoacyl-CoA synthase 11 (KCS-11) (EC 2.3.1.199) (Very long-chain fatty acid condensing enzyme 11) (VLCFA condensing enzyme 11)	7.12	12.12	5.01
DEMF06G12370	178.6	Staphylococcal-like nuclease CAN2 (EC 3.1.31.-) (Calcium-dependent nuclease 2) (AtCAN2) (Ca(2+)-dependent nuclease 2)	12.53	13.32	0.80

DEMF06G13250	188.2	2-oxoisovalerate dehydrogenase subunit alpha 1, mitochondrial (EC 1.2.4.4) (Branched-chain alpha-keto acid dehydrogenase E1 component alpha chain) (BCKDE1A) (BCKDH E1-alpha)	10.35	13.85	3.50
DEMF06G15570	204.8	Phosphomevalonate kinase, peroxisomal (EC 2.7.4.2) (5-phosphomevalonate kinase) (AtPMK)	8.05	9.04	0.99
DEMF06G16010	206.9	Probable nucleoredoxin 1 (AtNrx1) (EC 1.8.1.8)	6.88	9.30	2.43
DEMF06G16560	209.4	Probable indole-3-pyruvate monooxygenase YUCCA4 (EC 1.14.13.168) (Flavin-containing monooxygenase YUCCA4)	3.66	8.76	5.22
DEMF06G17360	212.5	Acyltransferase Pun1 (EC 2.3.2.35) (Acyltransferase 3) (Capsaicin synthase) (Protein PUNGENT 1)	5.11	9.19	4.11
DEMF06G18800	218.5	Glycosyltransferase BC10 (EC 2.4.-.-) (Protein BRITTLE CULM 10) (Protein FRAGILE CULM 116)	5.92	7.77	1.87
DEMF06G19570	222.7	extensin-like [Capsicum annuum]	7.88	9.88	2.00
DEMF06G19580	222.8	putative late blight resistance protein -like protein R1B-23-like [Capsicum annuum]	6.44	8.63	2.21
DEMF06G19590	222.8	UDP-glucosyltransferase UGT13248 (HvUGT13248) (EC 2.4.1.-) (Deoxynivalenol-UDP-glucosyltransferase)	1.52	4.81	3.81
DEMF06G21660	228.7	Biotin carboxyl carrier protein of acetyl-CoA carboxylase 2, chloroplastic (AtBCCP2) (BCCP-2)	10.70	11.65	0.96
DEMF06G21690	228.8	UDP-glucose 6-dehydrogenase 1 (UDP-Glc dehydrogenase 1) (UDP-GlcDH 1) (UDPGDH 1) (EC 1.1.1.22) (Gm-UGD1)	5.23	8.22	3.02
DEMF06G22890	231.2	GrpE protein homolog 1, mitochondrial	7.47	8.60	1.13
DEMF06G23780	235.2	Homeobox-leucine zipper protein ROC8 (GLABRA 2-like homeobox protein 8) (HD-ZIP protein ROC8) (Homeodomain	5.76	9.03	3.29

		transcription factor ROC8) (Protein RICE OUTERMOST CELL-SPECIFIC 8)			
DEMF06G23830	235.3	CASP-like protein 4D1 (RcCASPL4D1)	9.40	5.35	-4.08
DEMF06G24050	235.7	Bidirectional sugar transporter SWEET9 (AtSWEET9) (Protein SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTERS 9)	12.10	10.58	-1.51
DEMF06G24270	236.1	Late embryogenesis abundant protein At5g17165	14.36	15.19	0.83
DEMF06G25060	237.4	Hyoscyamine 6-dioxygenase (EC 1.14.11.11) (Hyoscyamine 6-beta-hydroxylase)	10.26	8.90	-1.36
DEMF06G25660	238.9	7-dehydrocholesterol reductase (7-DHC reductase) (EC 1.3.1.21) (Protein DWARF 5) (Sterol Delta(7)-reductase)	10.24	11.61	1.37
DEMF06G26390	240.2	Histone H2B.2	10.37	11.29	0.93
DEMF06G26520	240.4	Zinc finger protein ZAT5	6.72	8.89	2.18
DEMF06G28030	243.5	Unknown protein from spot 103 of 2D-PAGE of thylakoid (Fragment)	8.54	7.73	-0.81
DEMF06G29100	245.3	B3 domain-containing transcription repressor VAL1 (Protein HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE 2) (Protein VP1/ABI3-LIKE 1)	11.16	11.58	0.42
DEMF06G29690	246.2	40S ribosomal protein S8	8.86	8.10	-0.76
DEMF06G29890	246.4	Oleoyl-acyl carrier protein thioesterase, chloroplastic (EC 3.1.2.14) (18:0-acyl-carrier protein thioesterase) (18:0-ACP thioesterase) (Acyl-[acyl-carrier-protein] hydrolase) (Fragment)	10.31	13.31	3.01
DEMF06G30030	246.7	Caffeic acid 3-O-methyltransferase (CAOMT) (COMT) (EC 2.1.1.68) (S-adenosysl-L-methionine:caffeic acid 3-O-methyltransferase) (Fragment)	0.00	5.17	7.57
DEMF06G30920	248.2	Fatty-acid-binding protein 1 (AtFAP1) (Chalcone-flavanone isomerase family protein 1)	8.62	10.89	2.27