Pungency is a characteristic trait of pepper (*Capsicum* spp.). Two genes, *Pun1* and *pAMT*, are known as determinative factors of pepper pungency. To date, it has been considered that most bell-type sweet peppers (called piman and paprika, in Japan) possess the identical mutated *Pun1* allele, *pun1*, whereas *pAMT* mutated non-pungent pepper has been found only in non-bell-type pepper. In this study, to reconsider the uniformity of the source of non-pungency in sweet bell pepper and explore new genetic resources, the presence of *pun1* was investigated in 26 sweet bell pepper varieties. Among them, a seemingly common sweet bell pepper ‘Color Piman Yellow’ had the intact *Pun1*, in spite of its non-pungency. Sequencing and linkage analyses revealed that ‘Color Piman Yellow’ possessed a novel mutated *Pun1* allele, *pun10*, that has a nonsense substitution at the 11th exon responsible for non-pungency. This is the first *pAMT* mutant to be found in sweet bell pepper. The finding that there was a *pAMT* mutant in sweet bell pepper suggests the possibility that more *pAMT* mutants exist unconsciously in other sweet bell peppers. The discovery of a new factor of non-pungency contributes to expanding the genetic diversity of sweet pepper varieties.

**Key Words:** *Capsicum*, sweet bell pepper, pungency, *Pun1*, *pAMT*, *pam10*, genetic diversity.

---

**Introduction**

Chili or red pepper (*Capsicum* L. spp., hereafter, pepper) is one of the most widely used Solanaceae spice and vegetable crops in the world. Taxonomically, there are five cultivated species in the genus, including, *C. annuum* L., *C. chinense* Jacq., *C. frutescens* L., *C. baccatum* L. and *C. pubescens* Ruiz and Pavon (Pickersgill 1991, Thul et al. 2012). Among them, *C. annuum* is the most common species and is remarkably differentiated depending on the local preferences and food culture. The fruit shape of *C. annuum* is considerably variable and categorized into several types, for example, cherry (small, spherical and erect type), cone (small and erect type), long (relatively large and long type) and bell (relatively large and blocky type) (Kaname et al. 1989). In Japan, bell-type non-pungent (hereafter, sweet) peppers called “piman” (green peppers named after pimento) and paprika are popular as vegetables.

Pungency is a characteristic trait of pepper. The principal component of the pungency is capsaicinoid, a kind of alkaloid, biosynthesized and accumulated in placental tissue (Kosuge et al. 1961, Kosuge and Inagaki 1962, Ohta 1962, Suzuki et al. 1980, Thresh 1876). The biosynthesis of capsaicinoid includes two pathways, namely, the phenylpropanoid pathway that synthesizes vanillylamine from phenylalanine and the branched-chain fatty acid pathway that synthesizes acyl moieties from valine or leucine (Azagonzález et al. 2011, Koeda et al. 2015). Finally, the products of the two pathways are combined with catalysis by capsaicin synthase, then capsaicin, the major component of capsaicinoid, is generated (Fig. 1). The pungency of pepper is attractive to consumers and has promoted the demands of pepper as a spice, but has also made some peppers unsuitable as an edible vegetable. Therefore, probably the pepper that has lost pungency by mutation has been preferably selected and spread over the world.

Due to scientific interest but also agronomical and commercial importance, the genetic mechanism of pepper pungency has been well-studied. Classical genetic studies revealed that the presence of pungency is determined by the single dominant gene *C* (Lippert et al. 1966, Ramiah and Pillai 1935). The *C* locus was mapped on chromosome 2 by linkage analysis (Blum et al. 2003) and was renamed *Pun1*, which is currently most used (Stewart et al. 2005). Molecular studies have clarified that *Pun1* encodes an acyltransferase that catalyzes the dehydration condensation of vanillylamine with acyl moiety, which is exactly consistent with the

---

This article is an Advance Online Publication of the authors’ corrected proof. Note that minor changes may be made before final version publication.
caused a lack of vanillylamine, the substrate of capsaicinoid, which resulted in the loss of pungency. Linkage analysis revealed that pAMT is located on chromosome 3, suggesting that it is inherited independently from Pun1 (Ben-Chaim et al. 2006). The sweet pepper C. annuum cv. ‘CH-19 Sweet’, originating from Thailand, was the first discovered variety to possess the unfunctional pAMT allele (Yazawa et al. 1989). ‘CH-19 Sweet’ has a 1 bp insertion at the 16th exon of pAMT that results in a frameshift (Lang et al. 2009). Subsequently, a different allele of unfunctional pAMT was found in Japanese landrace C. annuum cv. ‘Himo’ (Tanaka et al. 2010a). Contrary to Pun1, various types of unfunctional mutations were found in pAMT. To date, nine alleles of unfunctional pAMT which generate sweet pepper have been identified (Tanaka et al. 2018). However, they were found only in minor local varieties or landraces but not in modern breeding varieties, like bell-type piman and paprika. This fact implies that the occurrence of mutations of pAMT is not unusual, but its utilization is restricted, probably because the precedent wide distribution of the major pun1 allele in sweet pepper hindered new resources of non-pungency from spreading in modern varieties.

In this study, we investigated the allelic diversity of Pun1 in various sweet pepper varieties belonging to C. annuum and discovered a bell pepper variety ‘Color Piman Yellow’ with novel unfunctional pAMT. This research is the first to report sweet bell pepper containing mutated pAMT. Here, we report the genetic characterization of the variety and discuss the possibility of application of the allele for sweet pepper breeding.

**Materials and Methods**

**Plant materials**

A total of 28 commercial pepper varieties were used (Table 1). All the varieties belonged to C. annuum. Among them, only cultivar ‘Takanotsume’ is pungent and the others are sweet. ‘Himo’ (common name ‘Himotougarashi’) is a sweet cultivar that resulted from a mutated pAMT allele in Japanese landrace C. annuum cv. ‘Himo’ (Tanaka et al. 2010a). Contrary to Pun1, various types of unfunctional mutations were found in pAMT. These varieties were grown in the experimental field of Yamagata University. The genomic DNA was extracted from each of the varieties, using the hexadecyltrimethylammonium bromide method of Murray and Thompson (1980).

**Allele-specific PCR amplification of Pun1**

Table 2 lists the sequences of the primers used in this study. The genotype of Pun1 locus was determined by allele-specific genomic PCR amplification, using two forward primers, namely, U16F specific to the intact allele of Pun1 and pun1-1Fwd2 specific to the mutated allele pun1 designed by Wyatt et al. (2012), with a reverse primer D14R. Each of the forward primers differed in the amplified fragment length. The PCR mixture contained 0.1 µL of Ex Taq DNA polymerase (5 U/µL, Takara Bio. Inc., Japan), 2 µL of 10× Ex Taq Buffer, 1.6 µL of dNTP mixture, 0.5 µL of each

---

**Table 1.** List of the pepper varieties for genetic characterization

| Variety           | Source                  | Pungency | Allele Characteristics |
|-------------------|-------------------------|----------|------------------------|
| Pun1              |                         |          |                        |
| Pun1-1            |                         |          |                        |
| Pun2              |                         |          |                        |
| Pun2-1            |                         |          |                        |
| Pun3              |                         |          |                        |
| Pun3-1            |                         |          |                        |
| Pun4              |                         |          |                        |
| Pun4-1            |                         |          |                        |
| Pun5              |                         |          |                        |
| Pun5-1            |                         |          |                        |
| Pun6              |                         |          |                        |
| Pun6-1            |                         |          |                        |
| Pun7              |                         |          |                        |
| Pun7-1            |                         |          |                        |
| Pun8              |                         |          |                        |
| Pun8-1            |                         |          |                        |
| Pun9              |                         |          |                        |
| Pun9-1            |                         |          |                        |
| Pun10             |                         |          |                        |
| Pun10-1           |                         |          |                        |
| Pun11             |                         |          |                        |
| Pun11-1           |                         |          |                        |
| Pun12             |                         |          |                        |
| Pun12-1           |                         |          |                        |
| Pun13             |                         |          |                        |
| Pun13-1           |                         |          |                        |
| Pun14             |                         |          |                        |
| Pun14-1           |                         |          |                        |
| Pun15             |                         |          |                        |
| Pun15-1           |                         |          |                        |
| Pun16             |                         |          |                        |
| Pun16-1           |                         |          |                        |
| Pun17             |                         |          |                        |
| Pun17-1           |                         |          |                        |
| Pun18             |                         |          |                        |
| Pun18-1           |                         |          |                        |
| Pun19             |                         |          |                        |
| Pun19-1           |                         |          |                        |
| Pun20             |                         |          |                        |
| Pun20-1           |                         |          |                        |
| Pun21             |                         |          |                        |
| Pun21-1           |                         |          |                        |
| Pun22             |                         |          |                        |
| Pun22-1           |                         |          |                        |
| Pun23             |                         |          |                        |
| Pun23-1           |                         |          |                        |
| Pun24             |                         |          |                        |
| Pun24-1           |                         |          |                        |
| Pun25             |                         |          |                        |
| Pun25-1           |                         |          |                        |
| Pun26             |                         |          |                        |
| Pun26-1           |                         |          |                        |
| Pun27             |                         |          |                        |
| Pun27-1           |                         |          |                        |
| Pun28             |                         |          |                        |
| Pun28-1           |                         |          |                        |
**Discovery of pAMT mutant in sweet bell pepper**

The genomic sequence of *Pun1* and *pAMT* were determined for varieties ‘Takanotsume’ and ‘Color Piman Yellow’. About 3 kbp from the promoter region to 3′UTR and 11 kbp from 5′UTR to 3′UTR were amplified for *Pun1* and forward primer (10 µM each), 1 µL of reverse primer (10 µM) and 2 µL of DNA (20 ng/µL). The condition of PCR amplification was 1 cycle at 94°C for 1 min, 30 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 2 min, followed by a final extension at 72°C for 7 min.

### Table 1. Pepper varieties used in this study

| No. | Variety | Fruit type | Pungency | Remarks                      |
|-----|---------|------------|----------|------------------------------|
| 1   | ‘Takanotsume’ | cone       | pungent  | Japanese traditional pungent variety |
| 2   | ‘Color Piman Yellow’ | bell     | sweet    | Unknown origin pungent variety sold in Japan |
| 3   | ‘Banana Piman’  | between long and bell | sweet   | Japanese modern variety |
| 4   | ‘Bimni Puh’ | bell       | sweet    | Russian variety             |
| 5   | ‘California Wonder’ | bell     | sweet    | USA variety, known as typical paprika in Japan |
| 6   | ‘California Wonder Orange’ | bell | sweet | USA variety, known as typical paprika in Japan |
| 7   | ‘Yellow Paprika’ | bell     | sweet    | Unknown origin modern variety sold in Japan |
| 8   | ‘Color Piman Orange’ | bell   | sweet    | Unknown origin modern variety sold in Japan |
| 9   | ‘Color Piman Purple’ | bell    | sweet    | Unknown origin modern variety sold in Japan |
| 10  | ‘Color Piman Red’ | bell    | sweet    | Unknown origin modern variety sold in Japan |
| 11  | ‘Color Piman White’ | bell   | sweet    | Unknown origin modern variety sold in Japan |
| 12  | ‘Gabriel’ | bell       | sweet    | Japanese modern variety     |
| 13  | ‘Hana Kuropi’ | bell     | sweet    | Japanese modern variety     |
| 14  | ‘Kyo-Midor’ | bell      | sweet    | Japanese modern variety, typical piman |
| 15  | ‘Marconi Red’ | long     | sweet    | Italian variety             |
| 16  | ‘Pitari’ | between long and bell | sweet  | Japanese modern variety     |
| 17  | ‘Round of Hungary’ | bell   | sweet    | Hungarian variety           |
| 18  | ‘Sakigake’ | bell      | sweet    | Japanese modern variety, typical piman |
| 19  | ‘Senhorita’ | bell     | sweet    | Japanese modern variety     |
| 20  | ‘Senhorita Gold’ | bell  | sweet    | Japanese modern variety     |
| 21  | ‘Sonia Gold’ | bell    | sweet    | Japanese modern variety     |
| 22  | ‘Victoria’ | bell      | sweet    | Japanese modern variety, typical paprika in Japan |
| 23  | ‘Viva Papricot’ | bell  | sweet    | Japanese modern variety     |
| 24  | ‘Wonder Bell’ | bell    | sweet    | Japanese modern variety, typical paprika in Japan |
| 25  | ‘Wonder Bell Yellow’ | bell | sweet | Japanese modern variety, typical paprika in Japan |
| 26  | ‘Zolotoy Yubirei’ | bell  | sweet    | Russian variety             |
| 27  | ‘Himotougarashi’ | long   | sweet    | Japanese traditional sweet variety |

**Table 2.** Primers used in this study

| Gene | Primer name | Sequence (5’–3’) | Purpose | Reference\(^a\) |
|------|-------------|------------------|---------|-----------------|
| *Pun1* | U16F | CCCTTGTGCATTTTTTTACC | Genotyping | Lang et al. (2006) |
|       | pun1-1fwd2 | GCTCCACCGAAAGACTCAT | Genotyping | Wyatt et al. (2012) |
|       | D14R | CTCTCAATCAAAACACACA | Genotyping, RT-PCR and qRT-PCR | Lang et al. (2006) |
|       | –F1148 | AAATGGTCGTTGTCAAAATC | Amplification of sequence template | this study |
|       | D1R | GTTGACCGTAAACTTCCGTT | Amplification of sequence template | this study |
|       | U1F | ATTTTTGAGGGAAGAACCT | RT-PCR | Lang et al. (2006) |
|       | ex2_F1600 | CCGGGTGATGTTGCACAAGC | qRT-PCR | this study |
| *pAMT* | F1 | TCTTCTCTTTCTTTGACAAT | Amplification of amplicon 1 | Tanaka et al. (2010b) |
|       | 7th-intron-R | AAATGATCATGTTGATGTTTAAA | Amplification of amplicon 2 | Tanaka et al. (2010b) |
|       | F443 | GTTGAAAGATGTGTGGTAT | Amplification of amplicon 2 | Tanaka et al. (2010b) |
|       | R788 | AAATGTGTTGCCAGGAAAGT | Amplification of amplicon 2 | Tanaka et al. (2010b) |
|       | F747 | TCTCAGAGAGCAGAGGTGTA | Amplification of amplicon 3 | Tanaka et al. (2010b) |
|       | R1313 | CCAACATCCTCGACTAGACCA | Amplification of amplicon 3 | Tanaka et al. (2010b) |
|       | 14th-intron-F | AAATGCTTTGCGCCCTAAAAT | Amplification of amplicon 4 | Tanaka et al. (2010b) |
|       | 16F | TGIAAAATGTTGGGATCAAACACAGTC | Amplification of amplicon 4 and qRT-PCR | Lang et al. (2009) |
|       | ex1F5 | AAGGGGAACTTGGTGGCACC | qRT-PCR | this study |
|       | ex17_F10721 | GAAGAACTCAGTCTACAGAGAAGTAA | qRT-PCR | this study |
|       | R10853 | GTTGCAAGAGAACTTCTTTTATAC | qRT-PCR | this study |
|       | ex11-99WF | GGAAATGTGTTGCCAGGAAAGT | Genotyping | this study |
|       | ex11-99MF | GGAAATGTGTTGCCAGGAAAGT | Genotyping | this study |
|       | int11-R2 | TCTGTCACAACACATTAAAACATGCTGA | Genotyping | this study |
| *Ubiquitin* | ubiquitin-F | TTGGTCTCACAATCTTCTCGTGA | RT-PCR and qRT-PCR | Stellari et al. (2010) |
|       | ubiquitin-R | ATACAGCAGTGCTCGTGT | RT-PCR and qRT-PCR | Stellari et al. (2010) |

\(^a\) References are given for the primers developed in previous studies.

---

**Genomic sequencing of *Pun1* and *pAMT***

The genomic sequence of *Pun1* and *pAMT* were determined for varieties ‘Takanotsume’ and ‘Color Piman Yellow’. About 3 kbp from the promoter region to 3′UTR and 11 kbp from 5′UTR to 3′UTR were amplified for *Pun1* and
pAMT, respectively. With respect to Pun1, the entire region was amplified in the PCR mixture containing 0.25 µL of Ex Taq DNA polymerase (5 U/µL, Takara), 5 µL of 10× Ex Taq Buffer, 4 µL of dNTP mixture, 2.5 µL of each forward and reverse primer (10 µM each) and 3 µL of DNA (20 ng/µL). PCR amplification involved 1 cycle at 94°C for 1 min, 30 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 3 min, followed by a final extension at 72°C for 10 min. Obtained promoter sequences were analyzed using the PLACE (https://sogo.dna.affrc.go.jp/cgi-bin/sogo.cgi?lang=en) database of cis-acting regulatory DNA elements (Higo et al. 1999). As for pAMT, the target was amplified dividedly into four amplicons: amplicon 1 (from primer F1 to primer 7th-intron-R), amplicon 2 (from F443 to R788), amplicon 3 (from F747 to R1313) and amplicon 4 (from 14th-intron-F to R1616). For amplicons 1 and 3, the PCR mixture contained 0.25 µL of LA Taq DNA polymerase (5 U/µL, Takara), 5 µL of 10× LA Taq Buffer II, 8 µL of dNTP mixture, 5 µL of MgCl₂ mixture (provided with LA Taq), 2.5 µL of each forward and reverse primer (10 µM each) and 3 µL of DNA (20 ng/µL). The PCR for amplicons 1 and 3 was done according to Tanaka et al. (2010b): 1 cycle at 94°C for 2 min, 35 cycles at 98°C for 10 s, 55°C for 30 s and 68°C for 15 min, followed by a final extension at 68°C for 15 min. For amplicons 2 and 4, the content of the PCR mixture and the PCR condition was the same as that of Pun1 amplification. The PCR products were electrophoresed in 1% agarose gel and purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Germany), according to the manufacturer’s protocol. Nucleotide sequencing was performed by Eurofins Genomics sequencing service, Japan. Supplemental Table 1 lists the primers used for the genomic sequencing.

Phenotyping of fruit pungency and segregation analysis
A total of 93 F₂ plants were obtained by crossing of ‘Color Piman Yellow’ with ‘Takanotsume’. The pungency phenotype was determined by an organoleptic test of mature fruits of each plant. For each plant, multiple fruits were tested, and if at least one was pungent, the plant was classified as pungent. The genotype of Pun1 of each F₂ plant was identified by the direct sequencing of the Pun1 promoter region. The genotype of pAMT of each F₂ plant was identified by the PCR-based DNA marker we designated (Table 2). The PCR mixture contained 0.4 µL of Tks Gflea DNA polymerase (1.5 U/µL, Takara), 10 µL of 2× Gflea PCR Buffer, 1 µL of each forward and reverse primer (10 µm each) and 2 µL of DNA (20 ng/µL). The PCR amplification condition was 1 cycle at 94°C for 1 min, 30 cycles at 94°C for 30 s, 67°C for 15 s and 72°C for 30 s, followed by a final extension at 72°C for 30 s.

Expression analysis of Pun1 and pAMT
Total RNA was extracted from the placental tissues of 20 and 30 DAF (days after flowering) fruits, using an RNaseasy Plant Mini Kit (Qiagen, the Netherlands). The extracted total RNA was quantified using a Qubit 3.0 fluorometer (Life Technologies, USA) and was diluted with sterile distilled water to 125 ng/µL. cDNA synthesis was conducted using a PrimeScript II 1st strand cDNA Synthesis Kit (Takara), according to the manufacturer’s protocol.

Semi-quantitative RT-PCR analyses were conducted using the primer pair U1F/D14R for Pun1, and ex15F/R1616 for pAMT, respectively. The PCR mixture contained 0.1 µL of Ex Taq DNA polymerase (5 U/µL, Takara), 2 µL of 10× Ex Taq Buffer, 1.6 µL of dNTP mixture, 1 µL of each forward and reverse primer (10 µM each) and 2 µL of DNA (20 ng/µL). The PCR condition was 1 cycle at 94°C for 1 min, 30 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 30 s. Ubiquitin-conjugating enzyme E2 was amplified as the positive control, using ubiquitin-F and ubiquitin-R primers. The PCR condition was 1 cycle at 94°C for 1 min, 25 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 30 s.

Quantitative RT-PCR analyses were conducted using ex2_F1600 primer and D14R primer for Pun1, ex17_F10721 and R10853 for pAMT, and ubiquitin-F and ubiquitin-R for ubiquitin as the normalizer, respectively. The PCR mixture contained 12.5 µL of SYBR Premix Ex Taq II (Tli RNaseH Plus, Takara), 1 µL of each forward and reverse primer (10 µM each) and 2 µL of cDNA solution synthesized as described above. PCR amplification was conducted using a TP850 Thermal Cycler Dice Real-Time System Single (Takara), under the following condition: 95°C for 30 s, 40 cycles at 95°C for 5 min, 60°C for 30 s, 1 cycle at 95°C for 15 s, 1 cycle at 60°C for 30 s and 1 cycle at 95°C for 15 s. Three fruits at 20 and 30 DAF were examined per variety.

Genotyping of pAMT in C. annuum non-pungent cultivars
Using the DNA marker of pAMT, 25 sweet pepper varieties belonging to C. annuum were genotyped. The composition of the PCR mixture and the PCR condition were the same as described in the segregation analysis.

Results

Allele-specific PCR amplification of Pun1
The Pun1 genotype of the 27 pepper varieties was determined by allele-specific PCR (Fig. 2). Twenty-five varieties, except ‘Takanotsume’ and ‘Color Piman Yellow’, showed amplification specific to the mutated pun1 allele (1,055 bp fragment). ‘Takanotsume’ and ‘Color Piman Yellow’ exhibited amplification specific to the intact Pun1 (1,602 bp fragment). This result for ‘Takanotsume’ was consistent with the expectation because it is a common pungent variety, possessing the fully functional Pun1. For ‘Color Piman Yellow’, however, this result was unexpected. ‘Color Piman Yellow’ is a seemingly normal sweet bell pepper, likely to possess the mutated pun1 allele. This finding implied that the non-pungency of ‘Color Piman Yellow’ has resulted in an unknown mutated Pun1 allele or a mutation...
Discovery of pAMT mutant in sweet bell pepper

**Genomic sequencing of Pun1**

We determined 2,996 and 2,939 bp genomic sequences of Pun1 from the promoter region to 3′UTR for ‘Color Piman Yellow’ and ‘Takanotsume’, respectively. No nucleotide difference between the two varieties was detected in the coding region and 3′UTR. A 1 bp deletion and a 1 bp nucleotide substitution were found at 875 and 665 bp upstream positions from the start codon in the promoter region, respectively. Searching on PLACE database indicated that these two single base nucleotide changes are not involved in any regulatory motifs. These results strongly suggested that Pun1 is not responsible for the non-pungency of ‘Color Piman Yellow’.

**Linkage analysis of Pun1 and pungency**

Using an F2 population obtained from the crossing between ‘Color Piman Yellow’ and ‘Takanotsume’, the linkage of pungency with Pun1 was investigated. The 1 bp deletion in the promoter region was used to determine the genotype of Pun1. All ten F1 plants were pungent. Seventy and 23 F2 plants were pungent and sweet, respectively (Table 3). The observed segregation ratio of the F2 plants well fitted the expected ratio of 3:1 (p = 0.952), indicating that the non-pungency of ‘Color Piman Yellow’, similarly to other sweet peppers, is controlled by a single recessive gene.

The genotype of Pun1 also segregated in the F2 population with fitting the single gene model. The independence of pungency phenotype from Pun1 genotype was evident in the F2 population (Table 4) (p = 0.985). This result indicates that the non-pungency of ‘Color Piman Yellow’ is not caused by Pun1.

**Genomic sequencing of pAMT**

Since the independence of non-pungency of ‘Color Piman Yellow’ from Pun1 was clarified, the nucleotide variation of pAMT, another candidate gene generating non-pungency of pepper, was investigated. In total, 1,380 bp genomic sequences, including all the 17 exons and their splicing junctions, were determined for ‘Color Piman Yellow’ and ‘Takanotsume’. Seven SNPs were found in the exons, among which, three were synonymous, and the other four were non-synonymous (Table 5). Among them, a non-synonymous substitution at the 11th exon generates a stop codon, resulting in the lack of about 40% C-termini from the translated amino acid sequence (Fig. 3). In addition, the substitution itself was involved in the pyridoxal

---

**Table 3. Phenotype of fruit pungency in F1 and F2**

| Combination and generation | No. of plants | Phenytype | Expected ratio | Chi-square (p-value) |
|---------------------------|---------------|-----------|----------------|---------------------|
| ‘Color Piman Yellow’ (P1) | 5             | Pungent   | 20             | 19                  |
| ‘Takanotsume’ (P2)        | 5             | Sweet    | 10             | 6                   |
| ‘Himotougarashi’ (P3)     | 5             | Pungent   | 23             | 1                   |
| P1 × P2 F1 F1             | 20            | Pungent   | 31             | 19                  |
| P1 × P2 F1 F2             | 10            | Sweet    | 7              | 6                   |
| P1 × P3 F1 F1             | 10            | Pungent   | 31             | 1                   |
| P1 × P3 F1 F2             | 7             | Sweet    | 20             | 2.20E-16            |

**Table 4. Linkage analysis of phenotype with Pun1 and pAMT genotype in F2 population between ‘Color Piman Yellow’ and ‘Takanotsume’**

| Gene       | Phenotype | No. of plants | Genotype* | Chi-square (p-value) |
|------------|-----------|---------------|-----------|---------------------|
| Pun1       | Pungent   | 70            | TT        | 0.985               |
|            | Sweet     | 23            | TY        | 6                   |
| pAMT       | Pungent   | 70            | YY        | 1                   |
|            | Sweet     | 23            | TT        | 2.20E-16            |

*TT, TY and YY denote homozygous for ‘Takanotsume’ allele, heterozygous and homozygous for ‘Color Piman Yellow’ allele, respectively.

**Table 5. Nucleotide substitutions found in exons of pAMT**

| Exon no. | Position (bp)* | Nucleotide substitution | Synonymous/non-synonymous |
|----------|---------------|-------------------------|--------------------------|
| 7        | 447           | C                       | synonymous               |
| 9        | 630           | A                       | non-synonymous, Lys to Asn |
| 11       | 819           | C                       | non-synonymous, Tyr to stop codon (TAG) |
| 12       | 918           | A                       | synonymous               |
| 15       | 1129          | A                       | non-synonymous, Thr to Ala |
| 15       | 1149          | A                       | synonymous               |
| 15       | 1150          | G                       | non-synonymous, Asp to Asn |

*Position in the entire exon sequences, with the first nucleotide of exon 1 shown as 1.
caused by the mutation of pAMT but by a tightly linked gene, it is more probable that these exceptional phenotypes were caused by an unstable expression of pungency in pungent pepper and incomplete deficiency of capsaicinoid analogs in sweet pepper. When we decided the phenotype, we investigated multiple fruits (1–12) from a single plant, and if at least one fruit was pungent, the plant was classified as pungent. However, the level or even presence/absence of pungency differed among fruits in a single pungent plant. Indeed, most of the F2 plants classified as pungent (40 of 70) had several non-pungent fruits in phenotyping. In some cases, even the plant whose almost fruits were sweet was classified as pungent, due to the presence of pungent fruit. Hence, it is stochastically possible that all the fruits examined were sweet even if the plant has the pungent genotype. This mixture phenomenon of pungent and sweet fruits has already been described in the pepper varieties ‘Shishitou’ and ‘Manganji’, both of which have the intact Pun1 and pAMT (Doi et al. 2013, Matsushima 2015). With respect to the exception for the pamt10 homozygous plant classified as pungent, the inconsistency was probably caused by an incomplete deficiency of capsaicinoid analogs. The exceptional plant had only one weakly pungent fruit in nine examined. The previous study of Lang et al. (2009) reported that even

5-phosphate (PLP) binding domain that is critical for pAMT enzymatic activity (Fig. 3B). The sequences of the splicing junctions of 16 introns retained the GT-AG motif. These results suggest that the nonsense mutation at the 11th exon is responsible for the non-pungency of ‘Color Piman Yellow’. This mutation has not been reported previously; thus, we designated this allele pamt10. It is the first found pAMT mutated allele in bell pepper. For the following segregation and diversity analyses, we developed PCR primers that can distinguish the nonsense substitution as a DNA marker specific to pamt10 (Fig. 4). The determined sequences of Pun1 and pAMT were deposited in DDBJ databases with the following accession numbers LC423554–LC423557.

**Linkage analysis of pAMT and pungency**

The genotype of pAMT in the F2 had a 1:2:1 segregation ratio and strongly co-segregated with the phenotype of fruit pungency. The 22 pAMT intact allele homozygous plants and 47 heterozygous plants were pungent while the 20 pamt10 homozygous plants were sweet. Only four F2 plants were exceptions, that is, three heterozygous plants were sweet, and one pamt10 homozygous plant was pungent. Although this incomplete co-segregation suggests the possibility that the non-pungency of ‘Color Piman Yellow’ is not caused by the mutation of pAMT but by a tightly linked gene, it is more probable that these exceptional phenotypes were caused by an unstable expression of pungency in pungent pepper and incomplete deficiency of capsaicinoid analogs in sweet pepper. When we decided the phenotype, we investigated multiple fruits (1–12) from a single plant, and if at least one fruit was pungent, the plant was classified as pungent. However, the level or even presence/absence of pungency differed among fruits in a single pungent plant. Indeed, most of the F2 plants classified as pungent (40 of 70) had several non-pungent fruits in phenotyping. In some cases, even the plant whose almost fruits were sweet was classified as pungent, due to the presence of pungent fruit. Hence, it is stochastically possible that all the fruits examined were sweet even if the plant has the pungent genotype. This mixture phenomenon of pungent and sweet fruits has already been described in the pepper varieties ‘Shishitou’ and ‘Manganji’, both of which have the intact Pun1 and pAMT (Doi et al. 2013, Matsushima 2015). With respect to the exception for the pamt10 homozygous plant classified as pungent, the inconsistency was probably caused by an incomplete deficiency of capsaicinoid analogs. The exceptional plant had only one weakly pungent fruit in nine examined. The previous study of Lang et al. (2009) reported that even

![Fig. 3. Schematic structure of pamt10. (A) Exon–intron structure of pAMT and the position of the nonsense substitution found in pamt10. Boxes and lines indicate exons and introns, respectively. (B) Amino acids sequences of translated proteins of pAMT of ‘Color Piman Yellow’ and ‘Takanotsume’. Consensus amino acids are highlighted in black. The pyridoxal 5-phosphate (PLP) binding domain is underlined.](image-url)
Discovery of pAMT mutant in sweet bell pepper

In this study, we discovered a novel mutated pAMT allele that caused non-pungency in ‘Color Piman Yellow’, a seemingly common sweet bell pepper variety. In spite of the universal use of sweet pepper as a vegetable, the source of non-pungency was restricted, that is, most non-pungent varieties possess the identical allele, pun1. Recent studies found unfunctional pAMT alleles as a secondary factor for pepper non-pungency, but they were only in minor local cultivars or landraces and have not been previously observed in bell pepper (Tanaka et al. 2018), probably because the non-pungency of pepper is remarkably preferable to consumers. As Stewart et al. (2005) mentioned, it has been considered that pun1, originating in a single mutation, has spread rapidly during the modern breeding process of sweet pepper, consequently, generating the sole resource of non-pungency in bell pepper. Our finding in this study can be an epoch-making report that changes the collective knowledge of sweet bell pepper. The result indicates the possibility of the hidden presence of mutated pAMT alleles in common sweet bell-type peppers. Future researchers may find more mutated pAMT alleles in sweet bell pepper. Although it is not clear whether the pamt10 was generated in a bell-type pepper or was introduced from a non-bell-type pepper, the fact that a variety heterozygous for pamt10 was found in the genetic resources examined suggests the possibility

the variety homozygous for unfunctional pAMT allele had a slight pungency because of the presence of capsaicinoid analog.

To completely deny the possibility that a tightly linked gene caused non-pungency of ‘Color Piman Yellow’, we conducted an allelism test using C. annuum cv. ‘Himo’. ‘Himo’ is homozygous for an unfunctional pAMT allele pamt2 and the non-pungency of ‘Himo’ was caused by this allele (Tanaka et al. 2010a). If the non-pungency of ‘Color Piman Yellow’ is not caused by the pAMT mutation, the pungency was complementarily expressed in F1 between ‘Color Piman Yellow’ and ‘Himo’. As a result, all ten F1 plants exhibited sweet (Table 3). Thus, we conclude that the newly discovered mutated pAMT allele pamt10 is the responsible factor for the non-pungency of ‘Color Piman Yellow’.

Expression analysis of Pun1 and pAMT

To clarify the change of gene expression in ‘Color Piman Yellow’, RT-PCR was carried out. Although amplicons of pAMT were not observed in ‘Color Piman Yellow’, a considerably low level of expression was detected in quantitative real-time RT-PCR (Fig. 5A, 5B). Compared to ‘Takanotsume’, the levels of relative expression of pAMT in ‘Color Piman Yellow’ were more than five hundred times less at 20 DAF and twenty three times less at 30 DAF.

As for Pun1, although the expression was confirmed in both ‘Color Piman Yellow’ and ‘Takanotsume’ (Fig. 5A, 5C), the level of relative expression of ‘Color Piman Yellow’ was significantly less than that of ‘Takanotsume’ at 20 DAF (Fig. 5C).

Genotyping of pAMT in C. annuum non-pungent cultivars

To reveal the distribution of pamt10 in sweet pepper genetic resources, genotyping using the pamt10 specific DNA marker was conducted. Among the 25 varieties examined, only ‘Yellow Paprika’, a sweet cultivar possessing pun1, was heterozygous for pamt10 and no other variety had pamt10 (Fig. 6).

Discussion

In this study, we discovered a novel mutated pAMT allele pamt10 that caused non-pungency in ‘Color Piman Yellow’, a seemingly common sweet bell pepper variety. In spite of the universal use of sweet pepper as a vegetable, the source of non-pungency was restricted, that is, most non-pungent varieties possess the identical allele, pun1. Recent studies found unfunctional pAMT alleles as a secondary factor for pepper non-pungency, but they were only in minor local cultivars or landraces and have not been previously observed in bell pepper (Tanaka et al. 2018), probably because the non-pungency of pepper is remarkably preferable to consumers. As Stewart et al. (2005) mentioned, it has been considered that pun1, originating in a single mutation, has spread rapidly during the modern breeding process of sweet pepper, consequently, generating the sole resource of non-pungency in bell pepper. Our finding in this study can be an epoch-making report that changes the collective knowledge of sweet bell pepper. The result indicates the possibility of the hidden presence of mutated pAMT alleles in common sweet bell-type peppers. Future researchers may find more mutated pAMT alleles in sweet bell pepper. Although it is not clear whether the pamt10 was generated in a bell-type pepper or was introduced from a non-bell-type pepper, the fact that a variety heterozygous for pamt10 was found in the genetic resources examined suggests the possibility
that more \textit{pamt}^{10} exists unconsciously in common sweet bell pepper.

The fact that non-pungency of ‘Color Piman Yellow’ was caused by \textit{pamt}^{10} was proved by the presence of a nonsense substitution in the middle of the coding region, co-segregation of the genotype with the pungency phenotype and the allelism test. Furthermore, a drastic decrease in \textit{pAMT} expression was detected by RT-PCR. Compared to ‘Takanotsume’, the level of \textit{pAMT} expression in ‘Color Piman Yellow’ was more than five hundred times less at 20 DAF. Tanaka et al. (2018) also reported a significant reduction in the transcript of the mutated \textit{pAMT} allele in sweet pepper and insisted that this phenomenon was caused by the mechanism called nonsense-mediated mRNA decay (NMD), which rapidly degrades mRNA, to suppress the accumulation of premature protein negatively affecting the cells (Conti and Izaurralde 2005). The dramatic reduction of \textit{pAMT} expression in ‘Color Piman Yellow’ was also possibly caused by the NMD mechanism. In addition, significant reduction in the expressional level of \textit{Pun1} was also detected in ‘Color Piman Yellow’ at 20 DAF. Since the sequence of the coding and promotor regions of \textit{Pun1} of ‘Color Piman Yellow’ does not contain any significant nucleotide changes, the result may indicate the presence of unknown interaction between \textit{pAMT} and \textit{Pun1} expression.

‘Color Piman Yellow’ loses its pungency by \textit{pamt}^{10}; the novel discovered mutated \textit{pAMT} allele with a nonsense mutation. However, in F\textsubscript{2} with ‘Takanotsume’, a \textit{pamt}^{10} homozygous plant produced a pungent fruit even though the frequency was quite low. Similarly, several previous studies noted the accumulation of small amounts of capsaicinoid in \textit{pAMT} mutated sweet pepper (Lang et al. 2009, Park et al. 2015, Tanaka et al. 2010a, 2015). These facts suggest that loss of function of \textit{pAMT} does not generate a complete loss of capsaicinoid, probably because of non-enzymatic synthesis or a complementary pathway by other genes. Nevertheless, the non-pungency of ‘Color Piman Yellow’ is remarkably stable, as it is commercially supplied as a sweet bell variety. Such a difference in stability of pungency should depend on the genetic background. Although the major determinative genes of pungency are \textit{Pun1} and \textit{pAMT}, the level and stability of pungency are affected by numerous unidentified minor genes, such as transcriptional factors (Arce-Rodríguez and Ochoa-Alejo 2017, Ben-Chaim et al. 2006, Blum et al. 2003, Keyhaninejad et al. 2014, Stewart et al. 2005).

As described in previous studies, sweet pepper possessing unfunctional \textit{pAMT} accumulates capsinoid, an analog of capsaicinoid (Koeda et al. 2014, Lang et al. 2009, Park et al. 2015, Tanaka et al. 2010a, 2010b, 2015, 2018). Although capsinoid has about one-thousandth the pungency of that more \textit{pamt}^{10} exists unconsciously in common sweet bell pepper.

The fact that non-pungency of ‘Color Piman Yellow’ was caused by \textit{pamt}^{10} was proved by the presence of a nonsense substitution in the middle of the coding region, co-segregation of the genotype with the pungency phenotype and the allelism test. Furthermore, a drastic decrease in \textit{pAMT} expression was detected by RT-PCR. Compared to ‘Takanotsume’, the level of \textit{pAMT} expression in ‘Color Piman Yellow’ was more than five hundred times less at 20 DAF. Tanaka et al. (2018) also reported a significant reduction in the transcript of the mutated \textit{pAMT} allele in sweet pepper and insisted that this phenomenon was caused by the mechanism called nonsense-mediated mRNA decay (NMD), which rapidly degrades mRNA, to suppress the accumulation of premature protein negatively affecting the cells (Conti and Izaurralde 2005). The dramatic reduction of \textit{pAMT} expression in ‘Color Piman Yellow’ was also possibly caused by the NMD mechanism. In addition, significant reduction in the expressional level of \textit{Pun1} was also detected in ‘Color Piman Yellow’ at 20 DAF. Since the sequence of the coding and promotor regions of \textit{Pun1} of ‘Color Piman Yellow’ does not contain any significant nucleotide changes, the result may indicate the presence of unknown interaction between \textit{pAMT} and \textit{Pun1} expression.

‘Color Piman Yellow’ loses its pungency by \textit{pamt}^{10}; the novel discovered mutated \textit{pAMT} allele with a nonsense mutation. However, in F\textsubscript{2} with ‘Takanotsume’, a \textit{pamt}^{10} homozygous plant produced a pungent fruit even though the frequency was quite low. Similarly, several previous studies noted the accumulation of small amounts of capsaicinoid in \textit{pAMT} mutated sweet pepper (Lang et al. 2009, Park et al. 2015, Tanaka et al. 2010a, 2015). These facts suggest that loss of function of \textit{pAMT} does not generate a complete loss of capsaicinoid, probably because of non-enzymatic synthesis or a complementary pathway by other genes. Nevertheless, the non-pungency of ‘Color Piman Yellow’ is remarkably stable, as it is commercially supplied as a sweet bell variety. Such a difference in stability of pungency should depend on the genetic background. Although the major determinative genes of pungency are \textit{Pun1} and \textit{pAMT}, the level and stability of pungency are affected by numerous unidentified minor genes, such as transcriptional factors (Arce-Rodríguez and Ochoa-Alejo 2017, Ben-Chaim et al. 2006, Blum et al. 2003, Keyhaninejad et al. 2014, Stewart et al. 2005).

As described in previous studies, sweet pepper possessing unfunctional \textit{pAMT} accumulates capsinoid, an analog of capsaicinoid (Koeda et al. 2014, Lang et al. 2009, Park et al. 2015, Tanaka et al. 2010a, 2010b, 2015, 2018). Although capsinoid has about one-thousandth the pungency of
capsaicinoid, it is known that it has the same physiological activity as capsaicinoid, such as metabolism enhancement (Ohnuki et al. 2001). Due to its low pungency and high physiological activity, capsinoid is used commercially as a health supplement. Since ‘Color Piman Yellow’ was revealed to be a pAMT mutant, it is possible that this variety also accumulates capsinoid, indicating the possibility for utilization of ‘Color Piman Yellow’ as healthy food.

In conclusion, this study is the first to reveal the presence of pAMT mutant in common sweet bell pepper. The finding of new genetic factors broadens the genetic variation of non-pungency. The DNA marker specific to pamt10 designated in this study will help the future utilization of this allele for breeding of sweet bell pepper varieties.

Acknowledgments

We thank Dr. Mineo Senda, Hirosaki University, and Dr. Tomoki Hoshino, Yamagata University, for their critical advice. We also thank Dr. Toshinori Abe, Yamagata University, for his kind support throughout this work. We are grateful to Yuki Nishida, Koharu Sawada, Rina Suto, Shoko Chiba and Akitsu Koide for their assistance in phenotyping of pungent pepper fruits. This study was financially supported by the Yamazaki Spice Promotion Foundation, and the Urakami Foundation for Food and Food Culture Promotion.

Literature Cited

Abraham-Juárez, M.d.R., M.d.C. Rocha-Granados, M.G. López, R.F. Rivera-Bustamante and N.Ochoa-Alejo (2008) Virus-induced silencing of Cont, pAmt and Kas genes results in a reduction of capsaicinoid accumulation in chili pepper fruits. Planta 227: 681–695.

Arce-Rodríguez, M.L. and N.Ochoa-Alejo (2017) An R2R3-MYB transcription factor in capsaicinoid biosynthesis. Plant Physiol. 174: 1359–1370.

Aza-González, C., H.G. Náñez-Palenius and N.Ochoa-Alejo (2011) Molecular biology of capsaicinoid biosynthesis in chili pepper (Capsicum spp.). Plant Cell Rep. 30: 695–706.

Ben-Chaim, A., Y. Borovsky, M. Falise, M. Mazourek, B.-C. Kang, I. Paran and M. Jahn (2006) QTL analysis for capsaicinoid content in Capsicum. Theor. Appl. Genet. 113: 1481–1490.

Blum, E., M. Mazourek, M.O’Connell, J. Curry, T. Thorup, K. Liu, M. Jahn and I. Paran (2003) Molecular mapping of capsaicinoid biosynthesis genes and quantitative trait loci analysis for capsaicinoid content in Capsicum. Theor. Appl. Genet. 108: 79–86.

Conti, E. and E. Izurralde (2005) Nonsensed-mediated mRNA decay: molecular insights and mechanistic variations across species. Curr. Opin. Cell Biol. 17: 316–325.

Curry, J., M. Altur, M. Mendoza, J. Nevarez, M. Melendez and M.A. O’Connell (1999) Transcripts for possible capsaicinoid biosynthetic genes are differentially accumulated in pungent and non-pungent Capsicum spp. Plant Sci. 148: 47–57.

Doi, M., S. Matsubara and S. Koeda (2013) Expression of capsaicin synthesis-related genes in Capsicum annuum L. Shishitou fruits. Hort. Res. (Japan) 12 (Suppl. 1): 321.

Higo, K., Y. Ugawa, M. Iwamoto and T. Korenaga (1999) Plant cis-acting regulatory DNA elements (PLACE) database: 1999. Nucleic Acids Res. 27: 297–300.

Kaname, T., N. Yamamoto, S. Suzuki and D. Chon (1989) Capsicum L. In: Hotta, M., K. Ogata, A. Nitta, K. Hoshikawa, M. Yanagi and K. Yamazaki (eds.) Useful Plants of the World, Heibonsha, Tokyo, pp. 213–215.

Keyahinejad, N., J. Curry, J. Romero and M.A. O’Connell (2014) Fruit specific variability in capsaicinoid accumulation and transcription of structural and regulatory genes in Capsicum fruit. Plant Sci. 215–216: 59–68.

Kim, M., S. Kim, S. Kim and B.-D. Kim (2001) Isolation of cDNA clones differentially accumulated in the placenta of pungent pepper by suppression subtractive hybridization. Mol. Cells 11: 213–219.

Kiri, E., T. Goto, Y. Yoshida, K. Yasuba and Y. Tanaka (2017) Non-pungency in a Japanese chili pepper landrace (Capsicum annuum) is caused by a novel loss-of-function Pun1 allele. Hort. J. 86: 61–69.

Koeda, S., K. Sato, K. Tomi, Y. Tanaka, R. Takisawa, M. Hosokawa, M. Doi, T. Nakazaki and A. Kitajima (2014) Analysis of non-pungency, aroma, and origin of a Capsicum chinense cultivar from a Caribbean island. J. Japan. Soc. Hort. Sci. 83: 244–251.

Koeda, S., K. Sato, Y. Tanaka, R. Takisawa and A. Kitajima (2015) A Comt1 loss of function mutation is insufficient for loss of pungency in Capsicum. Am. J. Plant Sci. 6: 1243–1255.

Kosuge, S., Y. Inagaki and H. Okumura (1961) Studies on the pungent principles of red pepper. Part VIII. J. Agric. Chem. Soc. Japan 35: 923–927.

Kosuge, S. and Y. Inagaki (1962) Studies on the pungent principles of red pepper. Part XI. J. Agric. Chem. Soc. Japan 36: 251–254.

Lang, Y., S. Yanagawa, T. Sasanuma and T. Sasakuma (2006) A gene encoding a putative acyltransferase involved in pungency of Capsicum. Breed. Sci. 56: 55–62.

Lang, Y., H. Kisaka, R. Sugiyama, K. Nomura, A. Morita, T. Watanabe, Y. Tanaka, S. Yazawa and T. Miwa (2009) Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in Capsicum annuum cv. CH-19 Sweet. Plant J. 59: 953–961.

Lee, C.-J., E.Y. Yoo, J.H. Shin, J. Lee, H.-S. Hwang and B.-D. Kim (2005) Non-pungent Capsicum contains a deletion in the capsaicin synthase gene, which allows early detection of pungency with SCAR markers. Mol. Cells 19: 262–267.

Lipper, L.F., P.G. Smith and B.O. Bergh (1966) Cytogenetics of the vegetable crops. Garden pepper, Capsicum sp. Bot. Rev. 32: 24–55.

Matsushima, K. (2015) A low-pungency S3212 genotype of Capsicum annuum L. Shishitou fruits. Jap. J. Breed. 12: 179–183.

Matsushima, K. (2015) Fluctuation of pungency in a Japanese chili pepper landrace (Capsicum chinense) molecular insights and mechanistic variations across species. Curr. Opin. Plant Biol. 20: 593–927.

Matsushima, K. (2015) Fluctuation of pungency in a Japanese chili pepper landrace (Capsicum chinense). Jap. J. Breed. 66: 400–411.

Matsuzono, S. and Y. Inagaki (1962) Studies on the pungent principles of red pepper. Part IX. J. Agric. Chem. Soc. Japan 36: 937–944.

Matsumura and C. Masuta (2015) Evidence of capsaicin synthase activity of the Pun1-encoded protein and its role as a determinant of capsaicinoid accumulation in pepper. BMC Plant Biol. 15: 93.

Ohnuki, K., S. Hoshikawa, M. Furuya, Y. Takagawa, T. Matsumura and C. Masuta (2015) Evidence of capsaicin synthase activity of the Pun1-encoded protein and its role as a determinant of capsaicinoid accumulation in pepper. BMC Plant Biol. 15: 93.

Ogawa, K., K. Murota, H. Shimura, M. Furuya, Y. Takagawa, T. Matsumura and C. Masuta (2015) Evidence of capsaicin synthase activity of the Pun1-encoded protein and its role as a determinant of capsaicinoid accumulation in pepper. BMC Plant Biol. 15: 93.

Ohnuki, K., S. Hoshikawa, M. Furuya, Y. Takagawa, T. Matsumura and C. Masuta (2015) Evidence of capsaicin synthase activity of the Pun1-encoded protein and its role as a determinant of capsaicinoid accumulation in pepper. BMC Plant Biol. 15: 93.

Ohta, Y. (1962) Physiological and genetical studies on the pungency of Capsicum, IV. Secretory organs, receptors and distribution of capsaicin in the Capsicum fruit. Jap. J. Breed. 12: 179–183.

Park, Y.-J., T. Nishikawa, M. Minami, K. Nemoto, T. Iwasaki and T. Miwa (2009) Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in Capsicum annuum cv. CH-19 Sweet. Plant J. 59: 953–961.

Park, Y.-J. (2015) Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in Capsicum annuum cv. CH-19 Sweet. Plant J. 59: 953–961.

Park, Y.-J. (2015) Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in Capsicum annuum cv. CH-19 Sweet. Plant J. 59: 953–961.

Park, Y.-J. (2015) Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in Capsicum annuum cv. CH-19 Sweet. Plant J. 59: 953–961.
Pickersgill, B. (1991) Cytogenetics and evolution of Capsicum L. In: Tsuchiya, T. and P.K. Gupta (eds.) Chromosome Engineering in Plants: Genetics, Breeding, Evolution, Part B, Elsevier, Amsterdam, pp. 139–160.

Ramiah, K. and M.R. Pillai (1935) Pungency in chillies (Capsicum annuum). A Mendelian character. Curr. Sci. 4: 236–237.

Stellari, G.M., M. Mazourek and M.M. Jahn (2010) Contrasting modes for loss of pungency between cultivated and wild species of Capsicum. Heredity 104: 460–471.

Stewart, C., Jr., B.-C. Kang, K. Liu, M. Mazourek, S.L. Moore, E.Y. Yoo, B.-D. Kim, I. Paran and M.M. Jahn (2005) The Pun1 gene for pungency in pepper encodes a putative acyltransferase. Plant J. 42: 675–688.

Stewart, C., Jr., M. Mazourek, G.M. Stellari, M. O’Connell and M. Jahn (2007) Genetic control of pungency in C. chinense via the Pun1 locus. J. Exp. Bot. 58: 979–991.

Suzuki, T., H. Fujiwake and K. Iwai (1980) Intracellular localization of capsaicin and its analogues, capsaicinoid, in Capsicum fruit 1. Microscopic investigation of the structure of the placenta of Capsicum annum var. annum cv. Karayatsubusa. Plant Cell Physiol. 21: 839–853.

Tanaka, Y., M. Hosokawa, T. Miwa, T. Watanabe and S. Yazawa (2010a) Newly mutated putative-aminotransferase in nonpungent pepper (Capsicum annum) results in biosynthesis of capsinoids, capsaicinoid analogues. J. Agric. Food Chem. 58: 1761–1767.

Tanaka, Y., M. Hosokawa, T. Miwa, T. Watanabe and S. Yazawa (2010b) Novel loss-of-function putative aminotransferase alleles cause biosynthesis of capsinoids, nonpungent capsaicinoid analogues, in mildly pungent chili peppers (Capsicum chinense). J. Agric. Food Chem. 58: 11762–11767.

Tanaka, Y., T. Sonoyama, Y. Muraga, S. Koeda, T. Goto, Y. Yoshida and K. Yasuba (2015) Multiple loss-of-function putative aminotransferase alleles contribute to low pungency and capsinoid biosynthesis in Capsicum chinense. Mol. Breed. 35: 142.

Tanaka, Y., S. Fukuta, S. Koeda, T. Goto, Y. Yoshida and K. Yasuba (2018) Identification of a novel mutant p4MT allele responsible for low-pungency and capsinoid production in chili pepper: accession ‘No. 4034’ (Capsicum chinense). Hort. J. 87: 222–228.

Thresh, C.F. (1876) Capsaicin, the active principle of capsicum fruits. Pharmaceut. J. 315: 21.

Thul, S.T., M.P. Darokar, A.K. Shasany and S.P.S. Khanuja (2012) Molecular profiling for genetic variability in Capsicum species based on ISSR and RAPD markers. Mol. Biotechnol. 51: 137–147.

Wyatt, L.E., N.T. Eannetta, G.M. Stellari and M. Mazourek (2012) Development and application of a suite of non-pungency markers for the Pun1 gene in pepper (Capsicum spp.). Mol. Breed. 30: 1525–1529.

Yazawa, S., N. Suetom, K. Okamoto and T. Namiki (1989) Content of capsaicinoids and capsaicinoid-like substances in fruit of pepper (Capsicum annum L.) hybrids made with ‘CH-19’ Sweet as a parent. J. Japan. Soc. Hort. Sci. 58: 601–607.