Multiple Non-pungent \textit{Capsicum chinense} Accessions with a Loss of Function CaKR1 Allele Originating from South America

Sota Koeda\textsuperscript{1*}, Ryutaro Nakano\textsuperscript{1}, Takaya Sawaki\textsuperscript{2}, Kosuke Sato\textsuperscript{2}, Yoshiyuki Tanaka\textsuperscript{3} and Shinya Kanzaki\textsuperscript{1}

\textsuperscript{1}Faculty of Agriculture, Kindai University, Nara 631-8505, Japan
\textsuperscript{2}Graduate School of Agriculture, Kyoto University, Kizugawa 619-0218, Japan
\textsuperscript{3}Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

In \textit{Capsicum}, loss of function mutation of acyltransferase (\textit{Pun1}), putative aminotransferase (\textit{pAMT}), putative ketoacyl-ACP reductase (\textit{CaKR1}), and R2R3-MYB transcription factor (\textit{CaMYB31}) have been reported to be the genetic causes of non-pungency. In the present study, 245 \textit{C. chinense} accessions were initially screened for non-pungency attributes. Six candidates with identification numbers, No. 3327, No. 3356, No. 3529, No. 4026, No. 4028, and No. 4034 were selected by tasting test, and the non-pungency attribute was confirmed by high-performance liquid chromatographic analysis. Expression and sequence analysis inferred that the non-pungency of No. 3529 was due to the non-expression of \textit{Pun1}. Analysis of \textit{pAMT} confirmed that No. 3356 (\textit{pamt5}) and No. 4034 (\textit{pamt9}) had loss of function mutations. Because the non-pungency of No. 3327, No. 4026, and No. 4028 did not seem to be caused by mutation of either \textit{Pun1} or \textit{pAMT}, the \textit{CaKR1} mutation was further examined using a polymerase chain reaction-based, co-dominant marker. Genotyping clarified that No. 3327, No. 4026, and No. 4028 had the same mutated \textit{CaKR1} allele as non-pungent No. 3341. Moreover, a crossing test with a pungent Habanero and No. 3341 clearly revealed that the non-pungency in No. 3327, No. 4026, and No. 4028 was a result of a loss of function mutation of \textit{CaKR1}. Our previous and present studies have shown that non-pungent cultivars of \textit{C. chinense} possessing \textit{pamt} are widely distributed in Central America, South America and the West Indies (Caribbean), while non-pungent cultivars possessing \textit{Cakr1} originate from Bolivia and Peru. Some artificial selection may have occurred that was based on a preference for non-pungent peppers in the local region of origin.

Key Words: acyltransferase (\textit{Pun1}), capsaicinoid, pepper, putative aminotransferase (\textit{pAMT}), putative ketoacyl-ACP reductase (\textit{CaKR1}).
In contrast, most of the reported non-pungent cultivars lack ketoacyl-ACP reductase (Koeda et al., 2014). Almost all the non-pungent pepper fruit is one of its most important traits, numerous studies have been conducted to better understand this phenomenon. To date, a loss of function mutation of acyltransferase (Pun1), putative aminotransferase (pAMT), putative ketoacyl-ACP reductase (CaKR1), and R2R3-MYB transcription factor (CaMYB31) have been reported to be the genetic causes of loss of pungency (Stewart et al., 2005; Lang et al., 2009; Arce-Rodríguez and Ochoa-Alejo, 2017; Han et al., 2019; Koeda et al., 2019). Almost all the non-pungent C. annuum cultivars possess pun1, and only a few cultivars have pamt or mutated CaMYB31. In contrast, most of the reported non-pungent cultivars of C. chinense possess pamt, and only single accessions are reported to have Cakr1 and pun12 (Stewart et al., 2007; Tanaka et al., 2010, 2015, 2018; Koeda et al., 2014, 2019). In the present study, non-pungent peppers were screened from 245 accessions of C. chinense to reveal the distribution of non-pungency in peppers with different genetic mechanisms and places of origin.

Materials and Methods

Plant materials, crossing combinations, and growth conditions

For initial screening of non-pungent peppers, 245 accessions of C. chinense from Antigua and Barbuda, Barbados, Bolivia, Brazil, Colombia, Fiji, Guyana, Jamaica, Peru, Trinidad and Tobago, and Venezuela were used. Initial screening was conducted at the experimental farm of Kyoto University in 2013 and 2014. A pungent pepper, Habanero, and a non-pungent accession, No. 3341 carrying the recessive allele of putative ketoacyl-ACP reductase (CaKR1; Cakr1/Cakr1) were used for crossing. F1 and F2 populations that were obtained by crossing Habanero with No. 3327, No. 4026, or No. 4028 to determine the inheritance pattern of fruit pungency. F1 populations were prepared by crossing No. 3341 with No. 3327, No. 4026, or No. 4028. All plants were grown in an unheated greenhouse at Kindai University from March to October in 2018 and 2019.

Phenotyping of fruit pungency

The capsaicinoid contents of the fruits were confirmed using high-performance liquid chromatographic analysis (HPLC). After the fruit had been freeze-dried, the capsaicinoids were extracted and quantified according to the method described by Koeda et al. (2014). The capsaicinoid content was then calculated as the sum of capsaicin and dihydrocapsaicin.

RT-polymerase chain reaction (PCR) analysis and cDNA sequence analysis of Pun1 and pAMT

The full-length cDNA sequences of Pun1 and pAMT were determined for No. 3327, No. 3356, No. 3529, No. 4026, and No. 4028. Pepper fruits were harvested at 25 days post-anthesis (dpa) and the placenta and interlocular septum were separated for RNA extraction. Total RNA was extracted and reverse-transcribed according to the method described by Koeda et al. (2014). For RT-PCR, CaActin (AY572427) was used as a positive internal control. The full-length cDNA sequence of Pun1 was amplified using Pun1-F and Pun1-R primer sets (Koeda et al., 2014), and the full-length cDNA sequence of pAMT was amplified using F1 and R1481 primer sets (Tanaka et al., 2010). PCR and electrophoresis were performed according to Koeda et al. (2015). The full-length sequences of Pun1 and pAMT amplified by RT-PCR were cloned into the pTAC-1 cloning vector (BioDynamics Laboratory, Tokyo, Japan). Nucleotide sequencing was performed in an ABI PRISM 3100 genetic analyzer with an ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA).

Genotyping of the CaKR1 allele

DNA was extracted from the pepper leaves using the Nucleon PhytoPure kit (GE Healthcare, IL, USA). Primers 1, 2, and 3 for the PCR-based, co-dominant marker were used to determine the allelic state at CaKR1 for parental cultivars and crossing progenies according to Koeda et al. (2019).

Real-time qRT-PCR

Pepper fruits were harvested at 10, 25, and 45 dpa and the placenta and interlocular septum were separated for RNA extraction. DNase treatment of RNA, cDNA synthesis, and real-time quantitative reverse-transcription PCR (real-time qRT-PCR) were conducted according to Koeda et al. (2019). At least three biological replicates were analyzed, with three technical replicates for each. The relative abundance of transcripts was normalized to the C. annuum CaActin (AY572427) reference control gene, and relative quantities were calculated using the 2−ΔΔCt method.
**Results and Discussion**

Six candidates, No. 3327, No. 3356, No. 3529, No. 4026, No. 4028, and No. 4034, were screened as non-pungent from the 245 accessions (C. chinense) by tasting test. The non-pungency of No. 4034 was shown to be caused by the loss of a functional mutation of pAMT (Tanaka et al., 2018). No. 3327, No. 3356, No. 4026, and No. 4028 were shown to have the typical round fruit of C. chinense; fruit height and width were approximately 3.5–4.0 cm, and fruit weight was approximately 15 g (Fig. 1). In contrast, the fruit morphology of No. 3529 was distinct, and it had a slender fruit shape. Capsaicinoid accumulations of five candidate accessions were evaluated using HPLC (Table 1). In Habanero, capsaicinoids were detected from mature fruits of 45 dpa. In contrast, capsaicinoids were not detected in No. 3327, No. 3356, No. 3529, No. 4026, or No. 4028.

We investigated the expression levels of Pun1 and pAMT to elucidate the genetic basis of the non-pungent phenotype of No. 3327, No. 3356, No. 3529, No. 4026, and No. 4028. First, the expression of Pun1 was analyzed by RT-PCR. Pun1 fragments of 1.3 kbp were amplified from Habanero, No. 3327, No. 3356, No. 4026, and No. 4028, but not from No. 3529 (Fig. 2). The non-expression of Pun1 seemed to be the underlying mechanism of non-pungency for No. 3529. In C. annuum cultivars, deletion mutations in promotor regions caused non-expression on Pun1, resulting in non-pungency (Stewart et al., 2005). No. 3529 was originally collected by Norio Yamamoto and categorized as C. chinense/C. frutescens, but we observed the typical white flowers of C. annuum in No. 3529, and No. 3529 had a distinct, slender fruit morphology as compared with the other five accessions, which had the typical round shape of C. chinense (Fig. 1). These results strongly suggest that the No. 3529 C. annuum accession possesses the pun1 allele. When cDNA sequences of Pun1 were compared for Habanero, No. 3327, No. 3356, No. 4026, and No. 4028, the deduced amino acid sequences of Pun1 completely matched. These results indicate that the non-pungency of No. 3327, No. 3356, No. 4026, and No. 4028 was not caused by Pun1 mutations.

The expression level of pAMT was also analyzed by RT-PCR. Expression of pAMT was detected for Habanero and the other five candidates. When cDNA sequences of pAMT were compared with pungent Habanero and PI159236, mutations were detected in No. 3356 and No. 3529 (Fig. 3). cDNA sequence analysis revealed that the pAMT cDNA of No. 3356 contained a 403-bp insertion, resulting in a frame-shift mutation that was identical to the pam5 of Aji Dulse Strain 2 (C. chinense) (Tanaka et al., 2010). This insertion led to truncated proteins of 59 amino acids lacking the pyridoxal 5-phosphate (PLP) binding domain (Fig. 3), which is essential for aminotransferase activity and mutations in this domain resulted in loss of pungency (Lang et al., 2009). No. 3529 had two SNPs, and one of them was a guanine (G) to adenine (A) mutation in the PLP domain. However, because this SNP was also observed in pungent PI159236, it could not be the cause of non-pungency in No. 3529. These results indicated that the non-pungency of No. 3327, No. 4026, and No. 4028 was not caused by pAMT mutations.

The loss of mutational functionality in CaKR1 was

---

**Table 1.** Capsaicinoid content in the fruit of Habanero, No. 3327, No. 3356, No. 3529, No. 4026, and No. 4028.

| Cultivar and accessions | Capsaicinoid content (μg·g⁻¹ DW) |
|-------------------------|----------------------------------|
| Habanero                | 15353 ± 2485                     |
| No. 3327                | N.D.                             |
| No. 3356                | N.D.                             |
| No. 3529                | N.D.                             |
| No. 4026                | N.D.                             |
| No. 4028                | N.D.                             |

Average and standard deviation were calculated for the results of three plants. N.D. indicates not detected.

---

Fig. 1. Non-pungent accessions screened from germplasms. Fruit morphology of No. 3327, No. 3356, No. 3529, No. 4026, and No. 4028. Bars indicate 2 cm.

Fig. 2. RT-PCR analysis for full-length Pun1 in No. 3327, No. 3356, No. 3529, No. 4026, No. 4028, and Habanero.
shown to cause non-pungency in a single C. chinense accession, No. 3341, in our previous study (Koeda et al., 2019). No. 3341 had an insertion of a 4.5-kb transposable element (TE) sequence in the first intron, resulting in the production of a truncated transcript missing the region coding for the catalytic domain. A co-dominant marker system for CaKR1 based on the TE insertion was utilized for No. 3327, No. 4026, and No. 4028 (Fig. 4A). A 599-bp amplicon was detected for Habanero, while amplicons of 1447-bp were detected for No. 3341, No. 3327, No. 4026, and No. 4028, indicating that these newly studied non-pungent peppers have the same mutated CaKR1 allele as No. 3341. Moreover, when gene-specific primers amplified the 3′ end of CaKR1 and it was used for real-time qRT-PCR, expression of CaKR1 was detected only in Habanero and not in No. 3341, No. 3327, No. 4026, or No. 4028 (Fig. 4B). These results strongly suggest that the mutation in CaKR1 is responsible for the non-pungency in No. 3327, No. 4026, and No. 4028.

We further conducted crossing tests with Habanero, No. 3341, No. 3327, No. 4026, and No. 4028 (Table 2). F1 progenies obtained by crossing No. 3327, No. 4026, or No. 4028 with Habanero were pungent, and F2 progenies obtained by crossing with No. 3341 were non-pungent. Moreover, F2 progenies obtained by crossing with Habanero were pungent when individuals possessed wildtype CaKR1 in homozygous or heterozygous states, but non-pungent when they possessed a mutated allele (CaKr1) in homozygous state, which represents complete co-segregation between the phenotype and genotype. These results clearly show that non-pungency in No. 3327, No. 4026, and No. 4028 is the result of a CaKR1 loss of function mutation.

In the present study, 245 C. chinense accessions were used for preliminary screening for non-pungent accesses, and this is a relatively large number. We have reported many non-pungent peppers (C. chinense) for...
Fig. 5. Places of origin for non-pungent C. chinense cultivars carrying the loss of function allele of CaKR1, pAMT, and Pun1. Black circles and white circles indicate the original collection place for the Cakr1 mutant and pamt mutant. Gray circles indicate country of origin for the pamt mutant. Double circles indicate country of origin for the pun12 mutant.

Table 2. Phenotypic segregation of the non-pungent phenotype of No. 3327, No. 4026, and No. 4028.

| Parental cultivar and cross combination | Genotype of CaKR1 locus$^z$ | Population size (n) | Number of plants$^y$ |
|----------------------------------------|-----------------------------|---------------------|----------------------|
|                                        |                             |                     | Pungent  | Non-pungent |
| P1 Habanero                            | WT/WT                       | 10                  | 10       | 0          |
| P2 No. 3341                            | mu/mu                       | 10                  | 10       | 0          |
| P1 No. 3327                            | mu/mu                       | 10                  | 0        | 10         |
| P4 No. 4026                            | mu/mu                       | 10                  | 0        | 10         |
| P5 No. 4028                            | mu/mu                       | 10                  | 0        | 10         |
| F1 (P1 × P2)                           | WT/mu                       | 7                   | 7        | 0          |
| F1 (P1 × P3)                           | WT/mu                       | 7                   | 7        | 0          |
| F1 (P1 × P4)                           | WT/mu                       | 7                   | 7        | 0          |
| F1 (P2 × P3)                           | mu/mu                       | 15                  | 0        | 15         |
| F1 (P2 × P4)                           | mu/mu                       | 15                  | 0        | 15         |
| F2 (P1 × P3)                           | WT/WT                       | 8                   | 8        | 0          |
| F2 (P1 × P4)                           | WT/WT                       | 9                   | 9        | 0          |
| F2 (P1 × P5)                           | WT/WT                       | 15                  | 0        | 15         |
| F2 (P1 × P3)                           | WT/mu                       | 9                   | 9        | 0          |
| F2 (P1 × P4)                           | WT/mu                       | 10                  | 10       | 0          |
| F2 (P1 × P5)                           | WT/mu                       | 17                  | 0        | 17         |
| F2 (P1 × P3)                           | WT/WT                       | 11                  | 11       | 0          |
| F2 (P1 × P4)                           | WT/WT                       | 7                   | 7        | 0          |
| F2 (P1 × P5)                           | WT/WT                       | 10                  | 0        | 10         |

$^z$ Genotyping was conducted by PCR using primers 1, 2, and 3 reported in Koeda et al. (2019). WT and mu indicate wildtype and mutated allele of CaKR1.

$^y$ Pungency of each individual was evaluated using HPLC.

which the country of origin is evident (Fig. 5). No. 3341 (Cakr1) and No. 3327 (Cakr1) originated from the suburbs of Cobija in Bolivia. No. 4026 (Cakr1), No. 4028 (Cakr1), and No. 4034 (pamtn) originated from the suburbs of Alto Rio Purus, located near the border of Peru and Brazil, which is an upstream region of the Rio Purus River. No. 2 (pamtn) originated from Manaus in Brazil, No. 80 (pamtn) from Trinidad island in Trinidad and Tobago, No. 3356 (pamtn) from Guayaramerin in Bolivia, Belize sweet (pamtn) from Belize, LP2 (pamtn) from Puerto Rico, and the Aji dulce strain 2 (pamtn) from Venezuela, NMCA30036 (pun12) from Colombia (Koeda et al., 2014; Bosland and Coon, 2015). These results show that non-pungent cultivars possessing pamtn are widely distributed in Central America, South America and the West Indies (Caribbean). In contrast, non-pungent cultivars with Cakr1 originated only from Bolivia and Peru, which is a relatively limited region. Moreover, NMCA30036 is the only non-pungent cultivar possessing pun12, and we could not find additional accessions with pun12, even though we screened a large number of germplasms. Phylogenetic research inferred that the origin of Capsicum is Bolivia (McLeod et al., 1982; Moscone et al., 2007), a continuous belt of land from south-eastern Brazil to the Andes (Bianchetti, 1996; Pozzobon et al., 2006), or regions of Peru, Ecuador and Colombia (Carrizo Garcia et al., 2016). In Central America, South America and the West Indies,
the majority of peppers are pungent cultivars. On the other hand, although they represent a minority compared to the pungent cultivars, several non-pungent cultivars are also important in local cuisine (Koeda, 2012; Koeda et al., 2014). Some artificial selection may have occurred with these non-pungent peppers motivated by preference in the local region of origin. Although there are many possibilities, we hypothesize that differences in mutated genes (\( \text{Pun1}, \text{pAMT}, \text{or CaKR1} \)) may be affecting other fruit characteristic traits. \( C. \ chinense \) cultivars such as Habanero and Scotch Bonnet are highly pungent and have highly aromatic flavors, which \( C. \ annuum \) cultivars lacks (Moreno et al., 2012; Koeda et al., 2014). There may be some overlap or indirect interaction between the capsaicinoid biosynthetic pathway and volatile aroma-producing compound biosynthetic pathways. Our team is currently analyzing the relationship between pungency/non-pungency and the aroma of \( C. \ chinense \) fruit.

**Acknowledgements**

Research materials were kindly provided from Norio Yamamoto (National museum of ethnology, Japan) and Sota Yamamoto (Kagoshima University, Japan). We would like to thank Toshiho Sakakibara and Masaru Matsuda (Experimental farm of Kyoto University) for supporting the initial screening of non-pungent accessions.

**Literature Cited**

Andrews, J. 1984. Peppers: the domesticated Capsicums. University of Texas Press, Austin.

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

Bennett, D. J. and G. W. Kirby. 1968. Constitution and biosynthesis of capsaicin. J. Chem. Soc. C 4: 442–446.

Bianchetti, L. B. 1996. Aspectos morfológicos, ecológicos e biogeográficos de dez táxons de \( C. \ annuum \) ocorrentes no Brasil. PhD thesis, Universidade de Brasília, Brazil.

Bosland, P. W. and D. Coon. 2015. ‘NuMex Trick-or-Treat’, a no-habit Habanero pepper. HortScience 50: 1739–1740.

Bosland, P. W. and E. J. Votava. 2000. Peppers: vegetable and spice cultivars. CAB Publishing, New York.

Carrizo García, C., M. H. Barfuss, E. M. Sehr, G. E. Barboza, R. Samuel, E. A. Moscone and F. Ehrendorfer. 2016. Phylogenetic relationships, diversification and expansion of chili peppers (\( C. \ annuum \), Solanaceae). Ann. Bot. 118: 35–51.

Eshbaugh, W. H. 1993. Peppers: history and exploitation of a serendipitous new crop discovery. p. 132–139. In: J. Janick and J. E. Simon (eds.). New crops. Wiley, New York.

Han, K., S. Jung, J.-H. Lee, D.-G. Lee, J.-K. Kwon and B.-C. Kang. 2019. A MYB transcription factor is a candidate to control pungency in \( C. \ annuum \). Theor. Appl. Genet. 132: 1235–1246.

Koeda, S. 2012. Cultivation and consumption of capsaicins in tropical regions—\( C. \ chinense \) and \( C. \ frutescens \)—. Agric. Hortic. (Nogyo-oyobi-Engei) 87: 29–33 (In Japanese).

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

Koeda, S., K. Sato, H. Saito, A. J. Nagano, M. Yasugi, H. Kudoh and Y. Tanaka. 2019. Mutation in the putative ketoacyl-ACP reductase \( \text{CaKR1} \) induces loss of pungency in \( C. \ chinense \). Theor. Appl. Genet. 132: 65–80.

Koeda, S., K. Sato, R. Takisawa and A. Kitajima. 2015. Inheritance of non-pungency in ‘No. 3341’ (\( C. \ chinense \)). Hort. J. 84: 323–326.

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

McLeod, M. J., S. I. Gutman and W. H. Eshbaugh. 1982. Early evolution of chili peppers (\( C. \ chinense \)). Econ. Bot. 36: 361–368.

Moreno, E., A. Fita, M. C. González-Mas and A. Rodríguez-Burruezo. 2012. HS-SPME study of the volatile fraction of \( C. \ chinense \) accessions and hybrids in different parts of the fruit. Sci. Hortic. 135: 87–97.

Moscone, E. A., M. A. Scadalferro, M. Gabriele, N. M. Cecchini, Y. Sánchez García, R. Jarret, J. R. Daviña, D. A. Ducasse, G. E. Barboza and F. Ehrendorfer. 2007. The evolution of chili peppers (\( C. \ annuum \), Solanaceae): a cytogenetic perspective. Acta Hortic. 745: 137–169.

Perry, L., R. Dickau, S. Zarrillo, I. Holst, D. M. Pearsall, D. R. Piperno, M. J. Berman, R. G. Cooke, K. Rademaker, A. J. Ranere, J. S. Raymond, D. H. Sandweiss, F. Scaramelli, K. Tarble and J. A. Zeidler. 2007. Starch fossils and the domestication and dispersal of chili peppers (\( C. \ chinense \)) in the Americas. Science 315: 986–988.

Pickersgill, B. 1997. Genetic resources and breeding of \( C. \ chinense \). Euphytica 96: 129–133.

Pozzobon, M. T., M. T. Schifino-Wittmann and L. B. Bianchetti. 2006. Chromosome numbers in wild and semidomesticated Brazilian \( C. \ chinense \) (Solanaceae) species: do \( r=12 \) and \( r=13 \) represent two evolutionary lines? Bot. J. Linn. Soc. 151: 259–269.

Stewart, C. Jr., B. C. Kang, K. Liu, M. Mazourek, S. L. Moore, Y. Y. Eun, B. D. Kim, I. Paran and M. M. Jahn. 2005. The \( \text{Pun1} \) 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 \( \text{Pun1} \) locus. J. Exp. Bot. 58: 979–991.

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

Tanaka, Y., M. Hosokawa, T. Miwa, T. Watanabe and S. Yazawa. 2010. Novel loss-of-function putative aminotransferase alleles cause biosynthesis of capsinoids, nonpungent capsaicinoid analogues, in mildly pungent chili peppers (\( C. \ chinense \)). 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 \( C. \ chinense \). Mol. Breed. 35: 142.