Substitution of a Dysfunctional *pAMT* Allele Results in Low-Pungency but High Levels of Capsinoid in *Capsicum chinense* 'Habanero'

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ABSTRACT Capsinoids are the class of secondary metabolites identified in non-pungent peppers exhibiting the same bioactive properties as capsaicinoid. Previously, it has been demonstrated that capsinoid production is controlled by the capsaicin synthase (*CS*) gene and the putative-aminotransferase (*pAMT*) gene. In this study, we report that *C. chinense* 'SNU11-001' containing high levels of capsinoid has an early stop codon in *pAMT* resulted from 403 bp and 8 bp insertions deletion in the third and sixth exons. In order to know whether *CS* expression is correlated with the level of capsinoid, *CS* and *pAMT* expressions were determined using SNU11-001 and four *Capsicum* accessions with different pungency level. RT-PCR analysis showed higher transcription levels of *CS* in pungent accessions but no clear differences in *pAMT* expression. To investigate the effect of the substitution of the *pAMT* allele of *C. chinense* 'Habanero' with the dysfunctional *pAMT* allele of SNU11-001, an F₂ population was constructed by a cross between aforementioned parental lines. Molecular markers were developed to distinguish *CS* and *pAMT* genotypes of SNU11-001 and Habanero and F₂ plants were genotyped. All F₂ plants having the *pAMT* genotype of SNU11-001 contained high levels of capsinoid while very low levels of capsaicinoid. There was no significant difference in levels of capsinoid among the F₂ plants regardless of *CS* genotypes. This may be due to strong *CS* expression of both parental lines. In conclusion, our results show that it is possible to develop a new Habanero cultivar with high capsinoid content by introducing a dysfunctional *pAMT* allele.

Keywords Capsaicinoid, Capsinoid, Capsiate, *pAMT*, *CS*, SNU11-001

INTRODUCTION

The pungency is a unique characteristic of genus *Capsicum* caused by capsaicinoids in fruits. Capsaicinoids are alkaloids derived from placenta tissues of pepper and contain many biomedical functions such as antioxidation, cancer prevention, weight reduction, and cardiovascular regulation (Thiele *et al.* 2008; Xiu-Ju *et al.* 2011). Capsaicin, one of the capsaicinoid analogues, is synthesized by capsaicin synthase (*CS*) through condensation of vanillyl amine derived from the phenylpropanoids pathway and 8-methyl-6-nonenic acid from the valine pathway. Vanillin is converted to vanillylamine by putative aminotransferase (*pAMT*) (Xiu-Ju *et al.* 2011).

Capsinoid, capsaicinoid-like substance was first reported by Yazawa in 1989. Capsiate, one of the capsinoid analogues,

has the same structure as capsaicin except for replacement of a peptide bond (NH) by an ester bond (O). The replacement of peptide bond with ester bond causes nonpungency or low-pungency of capsinoid. Low-pungency of capsiate makes it more palatable and less toxic than capsaicin. Capsinoid are unstable and easily degraded in the aqueous phase. Therefore, capsinoid has advantage over capsaicinoid in biomedical uses (Sharma *et al.* 2013).

Several genetic studies have been conducted on biosynthesis of capsinoid. Biosynthesis of capsinoid is caused by mutations in the *pAMT* gene resulting in suppression of the formation vanillylamine from vanillin (Lang *et al.* 2009; Tanaka *et al.* 2010a). Dysfunction of *pAMT* shunts synthesis vanillylamine into vanillyl alcohol. Tanaka *et al.* (2010) identified several loss-of-function in *pAMT* alleles that rendered production of capsinoid in pepper. Capsaicin

Received Jun 17, 2015; Revised Jun 23, 2015; Accepted Jun 25, 2015; Published June 30, 2015

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synthase encoded by the *Pun1* locus is required for biosynthesis of both capsaicinoid and capsinoid (Han *et al.* 2013).

Quantitative control of capsinoid synthesis can be affected by other factors besides *pAMT* and *CS*. Capsaicinoid accumulation is affected by environmental conditions and genetic constitutions. Genetic studies on capsaicinoid content have been conducted using QTL analysis and molecular mapping. Six QTLs in capsaicinoid accumulation were identified explaining 31% of the phenotypic variation (Ben-Chaim *et al.* 2006). The same genetic factors controlling capsaicinoid accumulation may be involved in capsinoid accumulation. Genetic study of capsinoid biosynthesis has been performed since capsinoid was discovered in 1980s. However, quantitative control of capsinoid in pepper has not been elucidated.

The purpose of this research was to investigate the genetic factors affecting capsinoid accumulation and to test the possibility to develop a new Habanero cultivar with high capsinoid content by introducing a dysfunctional *pAMT* allele. To achieve the objectives, analysis of capsaicinoid and capsinoid content and genotype analysis of *pAMT* and *CS* were performed in an F₂ population derived from crossed between *C. chinense* ‘SNU11-001’ and *C. chinense* ‘Habanero’.

MATERIALS AND METHODS

Plant materials

A total of six *Capsicum* cultivars containing different levels of capsaicinoid and capsinoid were used. *C. chinense* ‘SNU11-001’ contains the highest level of capsinoid and the lowest level of capsaicinoid. *C. annuum* ‘Early Calwonder (ECW)’ produces no capsaicinoid and capsinoid. *C. annuum* ‘Yuwol-cho’ and *C. annuum* ‘Takanotsune’, which are Korean and Japanese landraces, respectively, have mild pungency. *C. chinense* ‘Habanero’ and ‘Jolokia’ are very pungent cultivars.

SNU11-001 and Habanero were used to construct a mapping population. Nine F₁ and 215 F₂ plants were grown in Seoul National University farm (Suwon, Korea).

pAMT and *CS* genotype analysis

For genotyping of *pAMT* of SNU11-001, two types of molecular markers were developed. To design SCAR markers, *pAMT* sequence was obtained from *C. annuum* genome database (<http://cab.pepper.snu.ac.kr>). The first primer set, at the third intron F and R, was designed to detect insertion of repeat sequence on the third intron of the *pAMT* gene which is specific to *C. chinense*. The second marker, the third intron *Tcc*-R3 and third intron (R), was designed to detect the transposable element on the third intron in the *pAMT* gene which is specific to SNU11-001.

To distinguish the *CS* genotype between SNU11-001 and Habanero, two CAPS markers were developed. First marker was designed in the first exon using *AluI* site and the other marker was designed in the second exon using *RsaI* site. The latter marker was used for the *CS* genotyping, since it showed clearer band pattern than the former.

To determine the *pAMT* genotype of SNU11-001, polymerase chain reaction was performed in a 25 µl final volume containing 50 ng template DNA concentration, 10 pmol of reverse and forward primers, dNTP, 10x HiPi buffer and 1 unit of *Taq* polymerase.

PCR was performed with following conditions: 94 °C preheating for 5 minutes followed by 35 cycles of 94 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 1 minutes and a final extension of 10 minutes at 72 °C. PCR condition to determine the *CS* genotype was similar to that of the *pAMT* genotype analysis. Except the annealing temperature which was standardized at 57 °C.

DNA extraction

Total genomic DNA was extracted from young leaves by CTAB method as described previously (Han *et al.* 2013). Nanodrop (Nanodrop Technology, Inc., Wilmington, DE, USA) was used to determine the concentration of genomic DNA. DNA samples were dissolved in the final volume of 30 µl in TE buffer (pH7.0).

RNA Isolation and cDNA synthesis

Total RNA was isolated from the placenta 20 days after fruit setting by TRIzol reagent (Invitrogen, Korea) method (Han *et al.* 2013). RNA samples were diluted in RNase-

free water (Hybrid-R, GeneAll Biotechnology, Seoul, Korea). RNA concentration was measured at Nanodrop. To synthesize cDNA, reverse-transcriptional PCR was performed in a 20 µl PCR volume containing M-MLV 5x reaction buffer, dNTP, M-MLV RT 200 units and mixture of mRNA and oligo dT for 1 hour at 42 °C.

HPLC analysis of capsaicinoid and capsinoid

Three fruits from individual plants were harvested from all *Capsicum* accessions and SNU11-001 x Habanero F₁ and F₂ plants. Whole fruits including seeds were chopped and stored at -20 °C. HPLC analysis was performed in the Foundation of Agri. Tech. Commercialization and Transfer (FACT, Suwon, Korea) according to the method described by Han *et al.* (2013).

RESULTS

Capsaicinoid and capsinoid content of the five cultivars

HPLC analysis was executed to measure capsaicinoid and capsinoid concentrations of five cultivars; SNU11-001, ECW, Yuwol-cho, Takanotsume and Habanero (Table 1, Fig. 1). It was assumed that *CS* transcriptional level might be correlated with capsaicinoid content. Capsaicinoid and capsinoid content were measured at four different stages. Habanero contained the highest capsaicinoid contents (9195.3 ± 591.29 µg/gDW) among five cultivars at stage 2. Yuwol-cho and Takanotsume showed similar capsaicinoid levels, 3433.52 ± 588.23 µg/gDW and 3153.73 ± 518.04 µg/gDW, respectively. However, capsaicinoid content of Takanotsume was higher than those of Yuwol-cho at stage

Table 1. Comparison of capsaicinoid and capsinoid content in five cultivars by HPLC analysis.

<i>Capsicum</i> cultivars	Species	Stage ^{y)}	Capsaicinoid (µg/gDW)			Capsinoid (µg/gDW)		
			Capsaicin	Dihydrocapsaicin	Total	Capsiate	Dihydrocapsiate	Total
SNU11-001	<i>C. chinense</i>	1	17.9±5.26	15.5±4.79	33.4±10.04	2910.43±974.2	647.85±138.33	3558.28±1112.54
		2	16.13±4.28	0	16.13±7.15	6106.47±1609.48	749.52±186.96	6855.98±1795.53
		3	25.72±6.6	24.36±11.4	50.09±17.94	6669.92±613.8	771.88±90.47	7441.81±693.84
		4	17.72±5.08	17.31±2.99	35.03±7.94	4352.9±925.64	487.96±110.79	4840.86±1032.86
ECW	<i>C. annuum</i>	1	-	-	-	-	-	-
		2	nd ^{z)}	nd	nd	nd	nd	nd
		3	nd	nd	nd	nd	nd	nd
		4	nd	nd	nd	nd	nd	nd
Yuwol-cho	<i>C. annuum</i>	1	116.52±10.29	125.45±4.09	241.97±6.19	87.59±3.58	22.9±3	110.48±0.59
		2	1572.32±325.99	1861.19±317.44	3433.52±588.23	273.17±145.78	79.21±15.7	352.37±159.43
		3	717.43±84.11	1063.48±152.96	1780.9±235.44	101.74±29.01	18.81±2.89	120.55±31.74
		4	516.92±204.75	841.51±186.46	1358.44±391.21	30.25±8.13	12.53±0.27	42.78±7.86
Takanotsume	<i>C. annuum</i>	1	-	-	-	-	-	-
		2	1632.78±203.98	1520.95±322.53	3153.73±518.04	364.53±21.62	85.46±8.32	449.98±29.49
		3	1362.76±92.97	1563.18±161.88	2925.93±324.81	275.4±22.97	82.58±7.09	357.98±29.96
		4	1780.93±174.57	2172.31±359.59	3953.24±399.23	187.81±36.12	40±6.76	227.81±42.84
Habanero	<i>C. chinense</i>	1	4771.1±677.47	4211.51±474.46	8982.62±1130.04	470.63±83.72	118.47±16.52	589.1±99.95
		2	4655.94±566.67	4539.36±53.88	9195.3±591.29	488.82±102.01	114.84±23.02	603.66±128.81
		3	2113.27±0*	2227.2±0*	4340.47±0*	169.34±0*	45.75±0*	215.1±0*
		4	2456.59±143.4	2651.17±140.63	5107.75±277.74	255.44±59.45	58.28±6.37	313.71±65.54

^{z)}nd=not detected

^{y)}stage, 1: 23 days after fruit set, 2: 30 days after fruit set, 3: 37 days after fruit set, 4: 45 days after fruit set

*indicates that this experiment was not repeated.

Fig. 1. Comparison of (A) capsaicinoid and (B) capsinoid content according to fruit developmental stages in five cultivars.

3 and 4. ECW and SNU11-001 was marked with nondetectable capsaicinoid ($16.13 \pm 7.15 \mu\text{g/gDW}$). On the other hand, the highest capsinoid level ($6855.98 \pm 1795.53 \mu\text{g/gDW}$) was detected in SNU11-001 followed by Habanero, whereas no capsinoid was detected in ECW.

Construction of segregating populations for capsinoid study

To investigate relationship between capsinoid production and Nonitalic activity, four F_1 populations using SNU11-001 and four cultivars showing various levels of pungency (ECW, Yuwolcho, Takanotsume, Habanero) were constructed. In the interspecific crosses, *C. annuum* lines were used as a maternal parent and others as paternal parents to reduce the cross incompatibility. Only one F_2 population was developed derived from the cross between SNU11-001 and Habanero due to interspecific cross incompatibility.

pAMT and *CS* expression patterns

pAMT and *CS* expression patterns were tested in five cultivars. The primers for *pAMT* and *CS* were designed using an allele-specific sequences. The cDNA of *pAMT* and *CS* were amplified as 1455 bp and 1206 bp in size, respectively. cDNAs were prepared from RNA extracted from fruits after 20 and 45 days after fruit set were used (Fig. 2).

pAMT transcripts were amplified at immature stage in all cultivars. Two *pAMT* transcripts with different sizes were detected in SNU11-001. The nonpungent cultivar ECW also expressed the *pAMT* gene. However, at mature stage, no *pAMT* expression was detected in all cultivars except Habanero. The *CS* gene was not expressed in ECW as expected. *CS* expression was detected in other cultivars including SNU11-001 at the immature stage. Habanero showed the highest *CS* expression among the tested cultivars. By contrast, almost no *CS* transcript was detected at the mature stage in all cultivars.

Fig. 2. *pAMT* and *CS* expression patterns in five cultivars by RT-PCR. Immature and mature stages correspond to 20 and 45 days after fruit set respectively. *Actin* was used as control. S SNU11-001, E ECW, Y Yuwol-cho, T Takanotsume, H Habanero.

cDNA sequence analysis of *pAMT* and *CS*

Two partial *pAMT* transcripts were obtained from SNU11-001 (Fig. 3). The longer transcript 1,118 bp in size contained a 403 bp insertion and 8 bp deletion in the third and sixth exons. The longer transcript was similar to that of Aji Dulce strain 2 (Tanaka *et al.* 2010b). The smaller transcript had 45 bp deletion and 8 bp insertions. Both transcripts contained an early stop codon.

To identify sequence differences of *CS* between SNU11-001 and Habanero, full sequences of the coding region in both cultivars were obtained (Fig. 4) and 4 SNPs were identified. Three SNPs resulted in amino-acid changes but one was a synonymous mutation. First two non-synonymous mutations were located in the first exon whereas the other was occurred in the second exon.

Fig. 3. Two types of loss-of-function *pAMT* in SNU11-001. (A) Two types of *pAMT* transcript were detected in SNU11-001. (B) The longer transcript contains a 403 bp insertion between the third and the fourth exons and another 8 bp insertion but smaller transcript has 45 bp deletion and 8 bp insertion.

SNU11-001	MAFALPSSSLVSVCDKSFIFKPSLTPSKLRFHKLFSFIDQSLSNMYIPCAFFYPKVQQRLED	60
Habanero	MAFALPSSSLVSVCDKSFIFKPSLTPSKLRFHKLFSFIDQSLSNMYIPCAFFYPKVQQRLED	60

SNU11-001	SKNSDELSHIAHLLQTSLSQTLVSYYPYAGKLDNATVDCNDMGAFLSVRIKCSMSEIL	120
Habanero	SKNSDELSHIAHLLQTSLSQTLVSYYPYAGKLDNATVDCNDMGAFLSVRIKCSMSEIL	120

SNU11-001	DHPHASLAESIVLPKDLPWANNCEGGNLLVVQVSKFDCGGIAISVCFSHKIGDGCSSLNF	180
Habanero	DHPHASLAESIVLPKDLPWANNCEGGNLLVVQVSKFDCGGIAISVCFSHKIGDGCSSLNF	180

SNU11-001	LNDWSSVTRDHTTTTLVPSRPFVGDVSFSTQKYGSLITPQILSDLNECVQKRLIFPTDKL	240
Habanero	LNDWSSVTRDHTTTTLVPSRPFVGDVSFSTQKYGSLITPQILSDLNECVQKRLIFPTDKL	240

SNU11-001	DALRAKVAEESGVKNPTRAEVVSALLFKCATKASSMLPSKLVHFLNIRTMIKPRLPRNT	300
Habanero	DALRAKVAEESGVKNPTRAEVVSALLFKCATKASSMLPSKLVHFLNIRTMIKPRLPRNA	300

SNU11-001	IGNLSSIFSIEATNMQDMELPTLVRNLRKEVEVAYKKDQVEQNELILEVVESMREGKLPF	360
Habanero	IGNLSSIFSIEATNMQDMELPTLVRNLRKEVEVAYKKDQVEQNELILEVVESMREGKLPF	360

SNU11-001	ENMDGYENVYTCNLCKYPYYTVDFGWGRPERVCLGNGPSKNAFFLKDYKAGQGVEARVM	420
Habanero	ENMDGYENVYTCNLCKYPYYTVDFGWGRPERVCLGNGPSKNAFFLKDYKAGQGVEARVM	420

SNU11-001	LHKQMSEFERNEELLEFLA	440
Habanero	LHKQMSEFERNEELLEFLA	440

Fig. 4. Amino acid sequence alignment of the *CS* gene in SNU11-001 and Habanero. Four mutations were detected. Three of them in the box are non-synonymous mutation and another marked with triangle is synonymous mutation.

pAMT and *CS* marker development and genotype analysis

Two molecular markers were designed. One marker was developed for the *pAMT* gene to detect the *pAMT* mutant and the other marker was based on the *CS* gene to

distinguish *CS* of SNU11-001 and Habanero. *pAMT* marker was designated as SNU-*pAMT*669. The insertion of transposable element (*Tcc*) on the third intron of the *pAMT* gene was specific to SNU11-001. This SCAR marker was developed from the sequence of *Tcc* in the third intron of

Fig. 5. Development of molecular markers to select *pAMT* mutant. (A) The SCAR marker set was designed from the sequence of *Tcc* in the third intron of SNU11-001 to select *pAMT* mutant plant. (B) PCR analysis of *pAMT* marker. Striped box corresponds to exon and black bar indicates marker.

Fig. 6. Development of molecular markers to distinguish the *CS* genotypes of SNU11-001 and Habanero. (A) SNP position and restriction sites distinguishing SNU11-001 and Habanero. The CAPS marker set was developed in the second exon using *Rsa I* site to distinguish *CS* genotypes of SNU11-001 and Habanero. (B) *CS* marker analysis using SNU11-001, Habanero, and F₁ hybrids

SNU11-011 (Fig. 5). Therefore, the primer set differentiated *pAMT* mutant cultivars which contain *Tcc* element. On the other hand, *CS* marker was developed to discriminate between normal *CS* in two cultivars using a SNP (Fig. 6). This marker set was based on the synonymous mutation in second exon which can be detected by *RsaI* site. This CAPS marker was used to genotype *CS* alleles in SNU11-001 x Habanero F_2 population.

pAMT and *CS* genotyping analysis was performed for SNU11-001 x Habanero F_2 plant using SCAR and CAPS markers described above. Out of 215 F_2 individuals, 76, 84, and 49 plants turned out to have *pAMT/pAMT*, *pAMT/pamt*, and *pamt/pamt* genotypes, respectively (Table 2). Overall, the segregation ratio did not fit an expected ratio 1:2:1 ($p < 0.05$) and the number of *pAMT/pamt* heterozygote was less than expected. When F_2 plants were subjected to *CS* genotyping, CS^S/CS^S , CS^H/CS^H and CS^S/CS^H genotypes were 50, 150 and 108, respectively (Table 3).

Capsinoid and capsaicinoid content in plants having the *pamt/pamt* genotype

Capsaicinoid and capsinoid content was measured for 42 *pamt/pamt* plants (Table 4). All tested plants contained

very low levels of capsaicinoid whereas capsinoid content were relatively high ranging from 1485.61 ± 115.58 to 6050.75 ± 698.74 $\mu\text{g/gDW}$ (Table 4). Capsinoid content in plant No. 76 was marked approximately 4 times higher as compared to No. 170. Capsinoid content of No. 76 was comparable to that of SNU11-001 (Table 4).

Using the 42 *pamt/pamt* plants, correlation between the *CS* genotype with capsinoid content was investigated. Capsinoid content in plants having CS^S/CS^S , CS^H/CS^H and CS^S/CS^H were 3033.95d with, 2664.02 ± 198.43 , and 2933.66 ± 309.53 $\mu\text{g/gDW}$, respectively (Table 4). These results demonstrate that there is no significant difference in capsinoid content between *CS* genotypes.

DISCUSSION

This research was conducted to investigate the genetic factors affecting capsinoid accumulation and to test the possibility to develop pepper cultivars with high capsinoid content by introducing a dysfunctional *pAMT* allele. We showed that substitution of the *pAMT* allele of ‘Habanero’ with the dysfunctional *pAMT* allele of ‘SNU11-001’ results

Table 2. Genotype analysis of *pAMT* and *CS*.

F_2	Pop. size	Expected ratio	<i>pAMT</i> genotype			χ^2 (p value)	<i>CS</i> genotype			Undetermined	χ^2 (p value)
			<i>pamt/pamt</i>	<i>pAMT/pamt</i>	<i>pAMT/pAMT</i>		CS^S/CS^S	CS^S/CS^H	CS^H/CS^H		
(SNU11-001 x Habanero)	215	1:2:1	49	84	76	14.7674 (0.00062130)				6	
		1:2:1					50	108	50	7	0.308 (0.8574)

CS^S indicates *CS* of SNU11-001 type and CS^H corresponds to *CS* of Habanero type.

Table 3. Inheritance pattern of *pAMT* and *CS* in SNU11-001 x Habanero F_2 population.

<i>pAMT</i> genotype	Number of individuals	<i>CS</i> genotype	Number of individuals
<i>pamt/pamt</i>	49	CS^S/CS^S	14
		CS^S/CS^H	25
		CS^H/CS^H	10
<i>pAMT/pAMT</i> <i>pAMT/pamt</i>	160	CS^S/CS^S	36
		CS^S/CS^H	83
		CS^H/CS^H	40

Table 4. Capsaicinoid and capsinoid content in *pamt/pamt* F₂ plants with different *CS* genotypes.

<i>pamt</i> mutant individual	<i>CS</i> type	Capsaicinoid (μg/gDW)			Capsinoid (μg/gDW)		
		Capsaicin	Dihydrocapsaicin	Capsaicinoid	Capsiate	Dihydrocapsiate	Capsinoid
6	<i>CS^S/CS^H</i>	50.15noid7	56.64noid8	106.79oid.85	2461.66id50.09	628.466id	3090.06id53.29
15	<i>CS^H/CS^H</i>	31.4806id1	38.6306id8	70.1106id15	1639.35id80.97	536.255id.65	2175.61id27.96
18	<i>CS^S/CS^H</i>	39.5561id6	46.9761id46	86.5261id07	2602.74id10.73	380.784id.37	2983.52id28.45
23	<i>CS^S/CS^H</i>	30.9352id	28.3752id2	59.3752id2	2132.12±284.9	608.632±284	2740.75±284.9t
26	<i>CS^S/CS^S</i>	15.3475±28	13.8875±28	29.2275±28	1968.62±284.9t	357.522±284.	2326.15±284.9t
38	<i>CS^S/CS^H</i>	36.1315±28	50.9915±284	87.1215±284	2400.28±284.9t	417.548±284.	2817.82±284.9t
39	<i>CS^S/CS^H</i>	17.3482±284	20.6482±28	56.97±30.67	1660.430.67.9	222.2430.67	1993.720.67.9t
42	<i>CS^S/CS^H</i>	23.1.720.	30.45720.6	48.93720.67	2584.320.67.9t	530.2820.67.9	3114.620.67.9
48	<i>CS^S/CS^S</i>	23.0562	14.0862	37.1362	2679.750.	363.8450	3043.590.
64	<i>CS^S/CS^S</i>	6.25.590.	6.46.590.	12.71590.6	2233.2±177.1	335.33±177.1	2568.52177.19t
66	<i>CS^S/CS^S</i>	41.4652177	38.0652177.	79.5252177.	1821.85177.19t	476.295177.1	2298.14177.19t
69	<i>CS^S/CS^S</i>	37.8.1	45.5714	83.3714	2023.7117	368.5611	2392.2717
76	<i>CS^S/CS^H</i>	48.0427177	46.1627177	94.262717	4965.44177.19t	1085.31±96.63	6050.75±96.63t
83	<i>CS^S/CS^S</i>	38.7575±9	37.7275±96	76.4775±96	4126.75±96.63	609.025±96.6	4735.72±96.63t
91	<i>CS^H/CS^H</i>	16.8.72±	4.87.72±9	21.6772±96	3955.06±96.63t	679.286±96.6	4634.34±96.63t
93	<i>CS^S/CS^H</i>	2.73.34±9	4.31.34±9	7.04.34±	2025.29±96.63t	316.079±96.6	2341.36±96.63t
96	<i>CS^H/CS^H</i>	18.4936±9	14.1936±96	32.6736±96	1444.06±96.63	396.946±96.	1841946±96
99	<i>CS^S/CS^H</i>	19.3946±9	21.7646±96	41.0646±96.	3352.42±96.63t	620.182±96.6	3972.62±96.63
102	<i>CS^S/CS^H</i>	27.9.62±9	32.3262±96	60.2262±96	1358.05±96.612	185.675±96.6	1543.71±96.612
105	<i>CS^S/CS^H</i>	24.9271±9	20.5971±9	45.5171±96.	2807.51±96.612	351.311±96.6	3158.81±96.612
112	<i>CS^S/CS^H</i>	31.9381±9	42.1681±96	74.0981±9	2935.99±96.612	364.329±96.6	3300.31±96.612
113	<i>CS^H/CS^H</i>	37.1431±96	38.2731±96	75.4131±96	3280.82±316.12	455.382±316.	3736.22±316.1
116	<i>CS^S/CS^H</i>	25.6522±31	15.3822±31	41.0222±31	1504.93±316.12	396.943±316	1901.87±316.12
124	<i>CS^S/CS^S</i>	36.9487±31	28.2287±31	65.1687±31	4521.08±316.12e	416.358±316.	4937.43±316.12
137	<i>CS^S/CS^H</i>	13.0743±31	15.7843±31	28.8443±31	2075.93±316.1	437.743±316.	2513.64±316.1
138	<i>CS^S/CS^H</i>	50.8464±31	54.8264±31	105.664±316	2436.68±316.12	511.158±316.	2947.84±316.12
143	<i>CS^S/CS^H</i>	42.0384±31	54.4884±316	96.5884±31	1313.25±316.12	477.325±316.	1790.57±316.12
144	<i>CS^S/CS^H</i>	41.5357±19	54.6757±19	96.2757±1	2147.34±196.12	416.634±19	2563.94±196.12
158	<i>CS^H/CS^H</i>	70.3594±19	41.3594±1	111.654±196.	4570.33±196.12	625.233±196.	5195.55±196.12
162	<i>CS^S/CS^H</i>	35.6255±19	48.5755±19	84.1855±19	2381.47±196.12	313.657±196	2695.13±196.12
164	<i>CS^S/CS^H</i>	18.0213	12.2213	30.2413	1295.06±1	518.116±	1813.17±1
169	<i>CS^H/CS^H</i>	37.6217±19	53.0917±19	90.7117±19	2488.37±196.12	310.027±196.	2798.47±196.1
170	<i>CS^S/CS^H</i>	104.647±196	28.8747±19	133.477±196.	1234.92±196.12	250.792±196	1485.61±196.12
172	<i>CS^S/CS^H</i>	14.75±7.74	38.59±7.746	53.33±7.74	1490.46.746.12	291.666.746.	1782.12.746.12
176	<i>CS^H/CS^H</i>	33.7912.74	33.5812.74	67.3712.746	1967.44.746.12	347.384.746.	2314.81.746.12
187	<i>CS^H/CS^H</i>	11.7481.74	12.1981.74	23.9481.746	1607.09.746.1	244.929.746.	1852.02.746.1
189	<i>CS^S/CS^H</i>	111.742.746.	46.174	157.832.746.	2026832.746	445.222.746.	2471.22.746.12
190	<i>CS^S/CS^S</i>	39.8222.74	71.5222.7	111.322.746.	1841.77.746.12	339.517.746.	2181.28.746.12
195	<i>CS^S/CS^S</i>	22.1728.74	26.6128.746	48.7828.746	2160.44.746.12	382.494.746.	2542.93±242.4
204	<i>CS^H/CS^H</i>	63.7493±242	54.3793±242	118.113±242.	2408.69±242.42	348.919±242	2757.59±242.42
205	<i>CS^S/CS^H</i>	27.3759±24	56.5359±242	83.9159±242	1890.82±242.4	419.542±242.4	2310.35±242.42
213	<i>CS^S/CS^S</i>	31.3635±24	45.6335±24	77.6335	1998.59±242.42	311.94±33.04	2310.5333.0442

Capsaicinoid and capsinoid concentration at 30 days after fruit set was measured. Four plants (22, 51, 134 and 184) were not determined. * indicates that this experiment was not repeated. S: SNU11-001 H: Habanero.

in high levels of capsinoid in F₂ plants.

In this study we identified and used a pepper accession *C. chinense* 'SNU11-001'. cDNA sequence structure of SNU11-001 is similar to Aji Dulce strain 2 in that SNU11-001 has *Tcc* element in third intron and 8 bp insertion (Tanaka *et al.* 2010b). However, SNU11-001 is distinguished from Aji Dulce strain 2, since SNU11-001 has additional 45 bp deletion and remarkably higher capsinoid content as compared to Aji Dulce strain 2. Furthermore, capsinoid content was higher because it was measured in whole fruits of SNU11-001 while it was extracted from placenta and seeds in Aji Dulce strain 2 (Tanaka *et al.* 2010b). It is expected that SNU11-001 would be useful for breeding cultivars with high capsinoid content.

A similar study was reported by a Japanese research group (Tanaka *et al.* 2014). A cultivar named 'Maru Salad' was developed by crossing non-pungent pepper 'Murasaki' and 'CH-19 Sweet' which also has a dysfunctional *pAMT* allele. This cultivar contains approximately 700 µg/gDW capsinoid, which is much lower than those of 'CH-19 Sweet' (5825 ± 286 µg/gDW) and of F₂ plants derived from SNU11-001. In this study, Habanero was selected to generate a population because of its high levels of capsaicinoid. It was assumed that the strong CS activity of Habanero causing high content of capsaicinoid could also contribute to capsinoid content.

However, we cannot rule out other factors that are also involved in the control of capsinoid content because plants with *CS^S/CS^S*, *CS^H/CS^H* and *CS^S/CS^H* contained almost similar concentration of capsinoid (3033.95 ± 383.82 µg/gDW, 2622.69 ± 207.26 µg/gDW and 2933.66 ± 309.53 µg/gDW, respectively). Other genes in capsaicin or capsinoid pathway could be *Pal*, *Ca4h*, *Comt*, and *Kas* (Curry *et al.* 1999; Aluru *et al.* 2003). If the factors causing high capsaicinoid content in Habanero had have the same effect on capsinoid content, QTL responsible for capsaicinoid might also control accumulation of capsinoid (Blum *et al.* 2003; Ben-chaim *et al.* 2006). For further study, F₂ populations using SNU11-001 and other cultivars with various pungency levels need to be developed to validate the relationship between CS expression levels and capsinoid content.

ACKNOWLEDGMENTS

This work was supported by a grant (code: 710001-07) from the Vegetable Breeding Research Center through R&D Convergence Center Support Program, Ministry for Food, Agriculture, Forestry and Fisheries and by the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center, No. PJ01120501), Rural Development Administration, Republic of Korea.

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