RESEARCH ARTICLE

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IDENTIFICATION OF THE SECONDARY METABOLITE CAPSIATE IN CAPSICUM GERMPLASM ACCESSIONS

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SUMMARY

Besides capsaicin (CAP), capsiate (CAT) is now recognized as an important secondary metabolite of pepper (*Capsicum* spp.) for use as a health food, dietary supplement, and pharmaceutical. However, genetic resources for high levels of CAT are scarce worldwide. Sources of variation are needed to develop new commercial pepper varieties with high levels of CAT. This study was primarily conducted to evaluate high CAT yields with or without CAP. Moreover, two DNA markers, which were associated with CAT and CAP, were used to identify the presence of these two secondary metabolites. Results indicated that the accession KKU-P31146 contained the highest levels of CAT (505.3 µg/g DW) without Sum CAPs among the 19 tested accessions. Although the KKU-P62268 pepper accession showed the highest Sum CATs (3794.1 µg/g DW), it contained lower levels of CAT than KKU-P31146. The dCAPS (*p-AMT)* molecular marker amplified a DNA fragment of 269 bp, whereas a 1670 bp DNA fragment was not amplified by the SCAR (*Pun1)* molecular marker. In addition, the genotypic data from the two known molecular markers were associated with CAT and Sum CAPs in all tested accessions. In particular, this association was clearly observed in KKU-P31146. The novel discovery here is that KKU-P31146 should be considered as a new source for breeding high-CAT accessions due to its high CAT and no CAP. An obvious association between the CAT-related genotypic and phenotypic traits of KKU-P31146 indicated that the two known markers are useful for the selection of pepper accessions with the target trait.

Keywords: Chili, capsinoids, capsaicinoids, nonpungent pepper, breeding, antiobesity

Key findings: The key finding here is that the KKU-P31146 pepper accession contained the highest CAT along with no CAP among the 19 tested accessions. Thus, KKU-P31146 should be considered as a new source for breeding high-CAT accessions. Genotypic data that were directly associated with high CAT without CAP were clearly observed in KKU-P31146, wherein the dCAPS (*p-AMT*) marker amplified a DNA fragment of 269 bp and no DNA product was amplified by the SCAR (*Pun1*) marker.

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INTRODUCTION

Capsinoids (CATs) are newly identified secondary metabolites in chili pepper (*Capsicum* spp.) with a molecular structure similar to that of capsaicinoids (CAPs); however, they do not cause the burning sensation associated with pungency from CAPs and are more palatable (Tanaka *et al*., 2009). The most predominant CATs in pepper are capsiate (63%) and dihydrocapsiate (DI-CAT) (27%) with ~10% of other components (Snitker *et al.,* 2009). The pungency of chili pepper is caused by a class of secondary metabolites called CAPs, which consist of capsaicin (CAP) and derivative components (Bosland and Votava, 2000; Basu and Krishna, 2003). The two major CAPs are CAP and dihydrocapsaicin (DI-CAP), and these components account for ~90% of CAPs (Barbero *et al*., 2010; Jeeatid *et al.,* 2018a). CATs and CAPs are biosynthesized from the same precursors, phenylalanine and valine (Sutoh *et al*., 2006). CATs are synthesized by mutations in the putative aminotransferase (*p-AMT*) gene, which presumably catalyzes the formation of vanillyamine from vanillin (Tanaka *et al*., 2010). The loss of function of the *p-AMT* gene switches the CAP pathway to the CAT pathway (Lang *et al*., 2009). CAPs are widely used in the pharmaceutical industry and have potential bioactivities, including anti-inflammation, anticancer, topical analgesic effect for pain treatment, and fat accumulation suppression (Knotkova *et al*., 2008; Yang *et al*., 2010). CATs have been found to have properties similar to CAPs, such as suppressing fat accumulation (Kawada *et al*., 1986). CAT is the major component of CATs in enhancing fatty acid oxidation by CAT administration (Faraut *et al*., 2009). Subsequently, CAT decreases total energy intake during negative energy balance, fat, carbohydrate, cholesterol, or fiber intake (Inoue *et al*., 2007). Moreover, CAT exerts no effect on the desire to eat sweet food or food rich in carbohydrates (Ludy *et al*., 2012). The application of CAPs in food supplement industries has been limited due to their pungency (Tanaka *et al*., 2010). In contrast to CAPs, the utilization of nonpungent cultivars with high CAT content for food and pharmaceutical industries is not limited by their pungency (Park *et al*., 2015). The only high-CAT cultivar developed to date is the *Capsicum annuum* L. cultivar 'CH-19 Sweet' (Yazawa *et al*., 1989), which has been reported as the cultivar with the highest CAT content of 5825 ± 286

µg/g DW (Tanaka *et al*., 2014). However, access to 'CH-19 Sweet' is limited. Information on the variation in the CAT contents of *Capsicum* germplasm is limited, and some of the genetic resources of high CATs have been unavailable. The cultivars 509-45-1 and Shima have been released to the public as high-CAT cultivars; however, their CAT content is several-fold lower than that of 'CH-19 Sweet.' The development of new high-CAT varieties is important for CAT production. Information on the genetic resources of CAT variation in pepper is needed, especially with nonpungent pepper accessions. Thus, finding a new source of nonpungent accessions with high CAT would be a great benefit for plant breeders.

In general, the variations in CATs and CAPs in *Capsicum* germplasm have classically been evaluated by using high-performance liquid chromatography (HPLC) (Canto-Flick *et al.,* 2008). Currently, some potential markers, such as dCAPS and SCAR markers, have been reported as an effective tool for CAT and CAP selection. The dCAPS marker can be used effectively to screen *C. annuum* L. accessions with different CAT levels (Lang *et al*., 2009). The dCAPS (*p-AMT*) marker amplifies a DNA fragment with 317 bp, which is homozygous dominant in nonCAT genotypes, wherein the 269-bp DNA fragment is homozygous recessive in CAT genotypes and 317- and 269-bp fragments are heterozygous for CAT and CAP genotypes (Tanaka *et al*., 2010; Tanaka *et al*., 2014). However, the dCAPS (*p-AMT*) marker has not been validated for high-CAT genotypes in *Capsicum* species other than *C. annuum* L. Meanwhile, the SCAR (*Pun1*) marker can validate the three *Capsicum* species (*C. annuum* L., *Capsicum chinense* Jacq., and *Capsicum frutescens* L.) by producing a 1670-bp DNA fragment for CAP genotypes (homozygous dominant and heterozygous) while not producing any fragments for non-CAP genotypes (homozygous recessive) (Stewart *et al*., 2005; Lee *et al*., 2005; Tanaka *et al*., 2014).

Therefore, this study was conducted to evaluate 19 pepper accessions from different genetic resources for agronomic traits and CAT and CAP levels. The dCAPS and SCAR markers were used to validate *Capsicum* accessions with different levels of CATs and CAPs, respectively. The information obtained in this study is important for the identification of genetic resources for high CAT contents and the screening of superior genotypes by using marker-assisted selection.

MATERIALS AND METHODS

Plant materials and experimental design

The experiment was conducted in a plastic net house at the experimental farm of Khon Kaen University, Khon Kaen Province, Thailand (16° 28′N, 102° 48′E, 200 m above sea level) during May–September 2014. Nineteen *Capsicum* accessions from different genetic backgrounds were used in this experiment. Six accessions were introduced from the United States Department of Agriculture of the United States of America (USA), two accessions were kindly donated by Kyoto University (KU) of Japan, and 11 accessions were from Khon Kaen University (KKU) of Thailand. These accessions have different growth habits, fruit shapes,

Table 1. Descriptors of the 19 chili pepper accessions used in this experiment.

No.	Accessions	Species	Fruit characteristic	Source
1	KKU-P31146	*C. annuum* L.	small and triangular	USA
2	KKU-P61036	*C. annuum* L.	medium and blocky	Japan
3	KKU-P11197	*C. annuum* L.	small and elongate	Thailand
4	KKU-P31118	*C. annuum* L.	small and elongate	USA
5	KKU-P11003	*C. annuum* L.	small and elongate	Thailand
6	KKU-P61273	*C. annuum* L.	small and triangular	USA
7	KKU-P11007	*C. annuum* L.	small and elongate	Thailand
8	KKU-P11016	*C. annuum* L.	small and elongate	Thailand
9	KKU-P11039	*C. annuum* L.	large and blocky	Thailand
10	KKU-P11076	*C. chinense* Jacq.	small and elongate	Thailand
11	KKU-P63032	*C. chinense* Jacq.	medium and campanulate	USA
12	KKU-P61281	*C. chinense* Jacq.	medium and campanulate	USA
13	KKU-P33093	*C. chinense* Jacq.	small and almost round	USA
14	KKU-P12013	*C. chinense* Jacq.	medium and campanulate	Thailand
15	KKU-P13006	*C. chinense* Jacq.	medium and campanulate	Thailand
16	KKU-P13049	*C. chinense* Jacq.	small and elongate	Thailand
17	KKU-P18021	*C. chinense* Jacq.	small and triangular	Thailand
18	KKU-P62268	*C. frutescens* L.	small and elongate	Japan
19	KKU-P11173	*C. frutescens* L.	small and elongate	Thailand

and CAT and CAP levels. The material used belonged to three *Capsicum* species, including *C. annuum* L.*, C. chinense* Jacq., and *C. frutescens* L. (Table 1). A randomized complete block design with three replications and five plants per experimental unit was used. There were 95 plants in a replication and 285 plants in total. The plants were grown in 12 L containers and irrigated daily through a microdrip irrigation system at field capacity. Fertilizer was applied to the crop as described by Patricia (1999).

Measurement of CAT and CAP levels in fruits

Ten green mature fruits (30 days after anthesis; DAA) and 10 ripe mature fruits (40 DAA) per plant were harvested, and their CATs and CAPs levels were analyzed. The fruits were dried in a freeze dryer (Scanvac Coolsafe55-9 Model, LaboGene) at −53 °C for 65 h. Dried fruits were ground in a blender and stored at −20 °C until analysis. CATs were extracted and quantified with HPLC (10AT-VP Shimadzu, Japan) in accordance with the modified method described by Singh (2009). CATs were expressed as µg per g of dry weight (µg/g DW). The Sum CAT concentration was calculated as the sum of CAT and DI-CAT. CAT yield was calculated by multiplying the CAT concentration and dry fruit yield per plant. Meanwhile, CAPs were extracted and measured with HPLC (10AT-VP Shimadzu, Japan) in accordance with the modified "short run" methodology (Collins *et al*., 1995). CAPs were expressed as µg per g of dry weight (µg/g DW). The Sum CAP concentration was calculated as the Sum of CAP and DI-CAP. The Sum CAP concentration was converted into Scoville heat units (SHU) as described by Collins (1995).

Statistical analysis

Analysis of variance (ANOVA) was performed for all parameters in accordance with a randomized complete block design. Treatment means were compared through Duncan's multiple range tests at 5% probability level ($P < 0.05$) (Gomez and Gomez, 1984). Correlations among traits were determined by performing Pearson's correlation analysis (Statistix10 software program, Tallahassee, FL, USA).

Marker validation

The associations of the two markers, dCAPS (*p-AMT*) and SCAR (*Pun1*), associated with CAT and CAP content in three *Capsicum* species were validated (Table 2). For genotyping, DNA was extracted from young actively growing leaves (30 days old) of each pepper plant, and polymerase chain reaction (PCR) was performed by using Phire®Plant Direct PCR Kit (Thermo Fisher Scientific, UK). For the dCAPS (*p-AMT*) marker, the PCR reaction mixture contained 10 µL of 2× Phire plant direct PCR master mix, 1 µL of forward primer (0.7 µM), 1 µL of reverse primer (20 µM), and 1 µL of DNA template adjusted to a final volume of 20 µL with sterile distilled water. The thermocycler reaction included 1 cycle at 98 ° C for2 min followed by 35 cycles at 98 °C for 10 s, 62 °C for 30 s, and 72 °C for 1 min with a final extension of 7 min at 72 °C. For DNA digestion, 5 µL of PCR amplicon was mixed with 1 µL of buffer (provided with the enzyme) and 1 µL of *Dra*I restriction enzyme (TOYOBO, Japan). This mixture was then adjusted to 10 µL with double-distilled water, vortexed, and incubated at 37 °C for 60 min. The digested products were separated through electrophoresis on a 1% agarose gel, stained with red safe, and visualized by using a UV transilluminator. For the SCAR (*Pun1)* marker, the same PCR mixture thermocycler reaction as the dCAPS marker was used, with the exception of the annealing temperature of 60 °C. The PCR products were separated via electrophoresis as described above.

RESULTS

Determination of capsinoids and capsinoid yield

Among three species, *C. annuum* L. had the highest dry fruit yield (125.3 g/plant) followed by *C. frutescens* L. and *C. chinense* Jacq. (121.2 and 113.1 g/plant, respectively). Within the *C. annuum* L. group, KKU-P11197 gave the highest dry fruit yield (191.6 g/plant), followed by KKU-P11003 and KKU-P31146 (162.0 and 156.7 g/plant, respectively). Within *C*

Table 2. Markers associated with Sum CAP and Sum CAT content used for the validation study.

Markers	Primer sequence (5'–3')	Product size	Reference
SCAR (*Pun 1*)	F: ATGGCTTTTGCATTACCATCA	1670 bp	Lee *et al*. (2005)
	R: TCAAACACCACAAAAGACTTGG	-	
dCAPS (*p-AMT*)	F: GGCACTTTCTACAGAGTTTGT	317 bp	Lang *et al*. (2009)
	R: TAAAATATTATAACAAATGTAAA GTGATATTACCTCATCAAGTTCTTT	269 bp	

frutescens L., KKU-P62268 and KKU-P11173 had high fruit yield (131.1 and 111.4 g/plant), whereas KKU-P13006 had the highest dry fruit yield (180.3 g/plant) followed by KKU-P33093 (149.0 g/plant) and KKU-P11076 (141.9 g/plant) in the *C. chinense* Jacq. group.

The accessions of *C. annuum* L. had the highest CAT yield (4.9 mg/plant) followed by those of *C. frutescens* L. and *C. chinense* Jacq. (0.9 and 0.5 mg/plant, respectively). Within *C. annuum* L., KKU-P31146 had the highest CAT yield (15.8 mg/plant) followed by KKU-P11007 and KKU-P11197 (7.9 and 3.9 mg/plant, respectively). However, comparing the accessions of *C. annuum* L. and *C. frutescens* L. revealed that the accessions of *C. frutescens* L., including KKU-P62268 (1.6 mg/plant) and KKU-P11173 (0.2 mg/plant), had lower CAT yield. Similarly, within *C. chinense* Jacq., KKU-P33093 had the highest CAT yield (1.5 mg/plant) followed by KKU-P13049 (0.8 mg/plant) and KKU-P11076 (0.7 mg/plant) (Table 3).

Among the three species of *Capsicum*, *C. frutescens* L. had the highest Sum CATs (1902.6 µg/g DW) followed by *C. annuum* L. (337.3 µg/g DW) and *C. chinense* Jacq. (55.8 µg/g DW) (Table 3). Within *C. frutescens* L., KKU-P62268 had the highest Sum CATs (3794.1 µg/g DW). KKU-P31146 had the highest Sum CATs (912.5 µg/g DW) in *C. annuum* L. Within *C. chinense* Jacq., KKU-P33093 had the highest Sum CATs (99.5 µg/g DW). The *C. annuum* L. accession KKU-P31146 had high CAT and DI-CAT (505.3 and 407.2 µg/g DW, respectively). Among accessions of *C. chinense* Jacq., KKU-P33093 had high CAT and DI-CAT (50.2 and 49.1 µg/g DW, respectively). Between two accessions of *C. frutescens* L., KKU-P62268 had higher CAT and DI-CAT (60.8 and 3733.3 µg/g DW, respectively) than KKU-P11173 (11.0 µg/g DW and nd, respectively).

Determination of capsaicinoids

Among the three species, *C. chinense* Jacq. had the highest CAP, DI-CAP, and Sum CAPs of 19402.3, 5941.8, and 23058.3 µg/g DW, respectively. The accessions within *C. frutescens* L. had the next-highest CAPs parameters of 5293.8, 1017.7, and 6311.5 µg/g DW, whereas within *C. annuum* L., the means for CAPs, Sum CAP, and DI-CAP were 2483.4, 697.3, and 3180.7 µg/g DW, respectively (Table 4). Within *C. chinense* Jacq., KKU-P12013 had the highest CAP, DI-CAP, and Sum CAPs (50126.4, 20473.4, and 70599.8 µg/g DW, respectively), which were significantly higher than those of the other accessions within the species (nd to 49618.8 µg/g DW, nd to 14377.6 µg/g DW, and nd to 63996.4 µg/g DW, respectively). Within *C. annuum* L., KKU-P31118 had the highest CAP, DI-CAP, and Sum CAPs (5739.4, 1662.1, and 7401.5 µg/g DW, respectively), which were significantly higher than those of the other accessions within the species (nd to 3401.7 µg/g DW, nd to 1175.7 µg/g DW, and nd to 4225.6 µg/g DW, respectively). Within *C. frutescens* L., KKU-P62268 had higher CAP, DI-CAP, and Sum CAPs (6927.9, 1004.8, and 7932.7 µg/g DW, respectively) than KKU-P11173 (3659.7, 1030.6, and 4690.2 µg/g DW, respectively). In addition, CAP, DI-CAP, and Sum CAPs were not detected in KKU-P13049 (*C. chinense* Jacq.) and KKU-P31146 (*C. annuum* L.).

Table 3. Concentrations of capsiate **(**CAT**)**, dihydrocapsiate **(**DI**-**CAT**),** and sum of capsiate and dihydrocapsiate **(**Sum CATs**)** in 19 chili pepper accessions**.**

Accessions	µg/g of dry fruit weight			Dry fruit yield (g/plant)	CAT yield (mg/plant)
	CAT	DI-CAT	Sum CATs		
Capsicum annuum L.					
KKU-P31146	505.3^{a}	407.2^{b}	912.5^{b}	156.7^{c}	15.8^{a}
KKU-P61036	76.7^{d}	99.1^{d}	175.8^{e}	97.3^{h}	1.5^{d}
KKU-P11197	101.2^{c}	36.6^{f}	137.9^{f}	191.6^{a}	3.9^{c}
KKU-P31118	nd	nd	nd	128.7^{f}	nd
KKU-P11003	109.1^{c}	169.6^{c}	278.7^{d}	162.0^{c}	3.5^{c}
KKU-P61273	38.8^{fg}	nd	38.8^{ij}	29.7^{k}	0.2^{ef}
KKU-P11007	294.2^{b}	409.1^{b}	703.3^{c}	135.5^{ef}	7.9^{b}
KKU-P11016	77.3^{d}	37.1^{f}	114.4^{g}	95.3^{h}	1.5^{d}
KKU-P11039	Nd	nd	nd	130.6^{f}	nd
Means	171.8	193.1	337.3	125.3	4.9
Capsicum chinense Jacq.					
KKU-P11076	23.3^{gh}	nd	23.3^{jk}	141.9^{de}	0.7^{ef}
KKU-P63032	15.3^{h}	42.9^{f}	58.1^{hi}	110.6^{g}	0.4^{ef}
KKU-P61281	19.4^{gh}	39.1^{f}	58.6^{hi}	75.2^{i}	0.3^{ef}
KKU-P33093	50.2^{ef}	49.1^{f}	99.5^{g}	149.0^{d}	1.5^{d}
KKU-P12013	11.7^{h}	39.6^{f}	51.4^{hi}	72.5^{i}	0.2^{ef}
KKU-P13006	Nd	73.5^{e}	73.5^{h}	180.3^{b}	nd
KKU-P13049	28.2^{gh}	38.1^{f}	66.3^{h}	132.7^{f}	0.8^{e}
KKU-P18021	16.0^{h}	nd	16.0^{k}	42.6^{j}	0.1^{f}
Means	23.5	47.1	55.8	113.1	0.5
Capsicum frutescens L.					
KKU-P62268	60.8^{de}	3,733.3^{a}	3,794.1^{a}	131.1^{f}	1.6^{d}
KKU-P11173	11.0^{h}	nd	11.0 ^{k}	111.4^{g}	0.2^{ef}
Means	35.9	3,733.3	1,902.6	121.2	0.9
Grand Mean	89.9	398.0	389.0	114.7	2.5
F-test	**	**	**	**	**
CV **(%)**	11.9	3.0	3.4	4.1	15.7

****** Significant at $P < 0.01$**.** Different superscript lower case letters indicate least significant differences within each column by Duncan**'**s**-**test ($P < 0.05$)**.**

Correlation

The correlation coefficients among CAT, DI-CAT, Sum CATs, CAP, DI-CAP, Sum CAPs, and fruit yield are presented in Table 5. Highly significant and positive correlations were observed between CAT yield and CAT (0.99); Sum CATs and DI-CAT (0.99); Sum CAPs and DI-CAP (0.99); Sum CAPs and CAP (0.97); DI-CAP and CAP (0.95); and CAT yield and fruit number (0.58). Meanwhile, significant and positive correlations were found between dry fruit yield and fruit number (0.50) and CAT and fruit number (0.53). Nevertheless, the correlation between fruit number and fruit width was highly significant and negative (0.62).

Table 4. Concentrations of capsaicin **(**CAP**)**, dihydrocapsaicin **(**DI**-**CAP**),** and sum of capsaicin and dihydrocapsaicin **(**Sum CAPs**)** in 19 chili pepper accessions**.**

Accessions	µg/g of dry fruit weight			Sum CAPs (SHU)
	CAP	DI-CAP	Sum CAPs	
Capsicum annuum L.				
KKU-P31146	nd	nd	nd	nd
KKU-P61036	104.2^{k}	46.6^{j}	150.8^{k}	2262.6^{k}
KKU-P11197	2358.4^{gh}	786.0^{f-i}	3144.4^{gh}	47174.3^{gh}
KKU-P31118	5739.4^{d}	1662.1^{e}	7401.5^{d}	111044.4^{d}
KKU-P11003	3401.7^{ef}	540.1^{g-j}	3941.8^{e-g}	59138.8^{e-g}
KKU-P61273	1450.4^{i}	486.4 $^{h-j}$	1936.8^{ij}	29058.3^{ij}
KKU-P11007	3050.0^{ef}	1175.7^{f}	4225.6^{ef}	63396.7^{ef}
KKU-P11016	2941.1^{fg}	362.2^{ij}	3303.3^{f-h}	49558.8^{f-h}
KKU-P11039	822.1^{j}	519.5^{g-j}	1341.6 ^{j}	20127.9^{j}
Means	2483.4	697.3	3180.7	47720.2
Capsicum chinense Jacq.				
KKU-P11076	1996.1^{hi}	442.5^{ij}	2438.5^{hi}	36584.9^{hi}
KKU-P63032	7767.0^{b}	3031.4^{c}	10798.4^{c}	162007.7^{c}
KKU-P61281	3422.9^{ef}	307.7^{ij}	3730.6^{e-g}	55969.8^{e-g}
KKU-P33093	5203.1^{d}	2174.6^{d}	7377.6^{d}	110685.8^{d}
KKU-P12013	50126.4^{a}	20473.4^{a}	70599.8^{a}	1059200.2^{a}
KKU-P13006	49618.8^{a}	14377.6^{b}	63996.4^{b}	960130.8^{b}
KKU-P13049	nd	nd	nd	nd
KKU-P18021	17681.5^{i}	785.4^{f-i}	2466.9^{hi}	37010.7^{hi}
Means	19402.3	5941.8	23058.3	345941.4
Capsicum frutescens L.				
KKU-P62268	6927.9^{c}	1004.8^{f-h}	7932.7^{d}	119013.5^{d}
KKU-P11173	3659.7^{e}	1030.6^{fg}	4690.2^{e}	70367.1^{e}
Means	5293.8	1017.7	6311.5	94690.3
Grand Mean	8839.5	2894.5	11734.0	176043.0
F-test	**	**	**	**
CV **(%)**	4.2	9.9	4.6	4.6

****** Significant at $P < 0.01$**.** Different superscript lower case letters indicate least significant differences within each column by Duncan**'s-**test ($P < 0.05$)**.**

Marker validation

The DNA of 19 accessions with different Sum CAT levels were validated with the dCAPS (*p-AMT*) marker. As expected, dCAPS (*p-AMT*) analysis identified three patterns of PCR product fragments upon digestion by using *Dra*I. A DNA fragment of 317 bp was amplified in KKU-P31118 and KKU-P11039 (*C. annuum* L.), whereas a DNA fragment of 269 bp was amplified in KKU-P31146 and KKU-P13049 (*C. annuum* L.). Both DNA fragments were also observed in the other 15 accessions of *C. annuum* L., *C. chinense* Jacq., and *C. frutescens* L. (Figure 1). Furthermore, the DNA samples of 19 accessions with different Sum CAP levels were analyzed with the SCAR (*Pun1*) marker. The amplification of the SCAR marker resulted in a common DNA fragment of 1670 bp for most accessions within three *Capsicum* species except in KKU-P31146 (*C. annuum* L.) and KKU-P13049 (*C. chinense* Jacq.) (Figure 2).

Table 5. Correlation coefficients among capsinoids, capsaicinoid contents, and dry fruit yield**.**

Characters	Fruit width	Fruit length	Fruit number	Dry fruit yield	CAT	DI-CAT	Sum CATs	CAT yield	CAP	DI-CAP
Fruit width	-									
Fruit length	0.47	-								
Fruit number	–0.62**	–0.44	-							
Dry fruit yield	–0.10	0.17	0.50*	-						
CAT	–0.33	–0.18	0.53*	0.29	-					
DI-CAT	–0.23	–0.22	0.36	0.11	0.11	-				
Sum CATs	–0.27	–0.24	0.43	0.15	0.25	0.99**	-			
CAT yield	–0.32	–0.18	0.58**	0.37	0.99**	0.1	0.24	-		
CAP	0.39	0.18	–0.30	–0.04	–0.27	–0.05	–0.09	–0.25	-	
DI-CAP	0.40	0.23	–0.27	–0.01	–0.22	–0.08	–0.11	–0.21	0.95**	-
Sum CAPs	0.41	0.23	–0.26	0.04	–0.24	–0.04	–0.08	–0.22	0.97**	0.99**

* and ** Significant at $P < 0.05$ and $P < 0.01$, respectively**.**

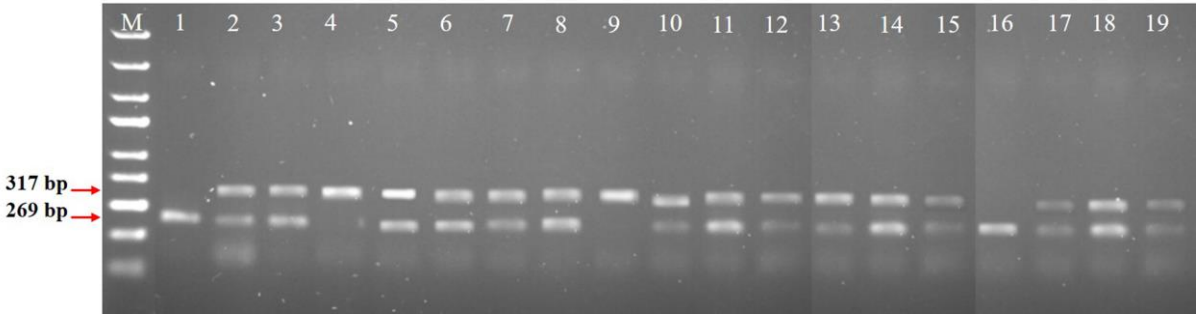

Figure 1. DNA fingerprints of dCAPS marker **(***p-AMT***)** analysis for capsinoid substances**.** M**:** DNA ladder marker, wells 1–9 **(***C. annuum* L.**)**, 10–17 **(***C. chinense* Jacq.**),** and 18–19 **(***C. frutescense* L.**).** Numbers 1–19 indicate the accessions described in Table 1**.**

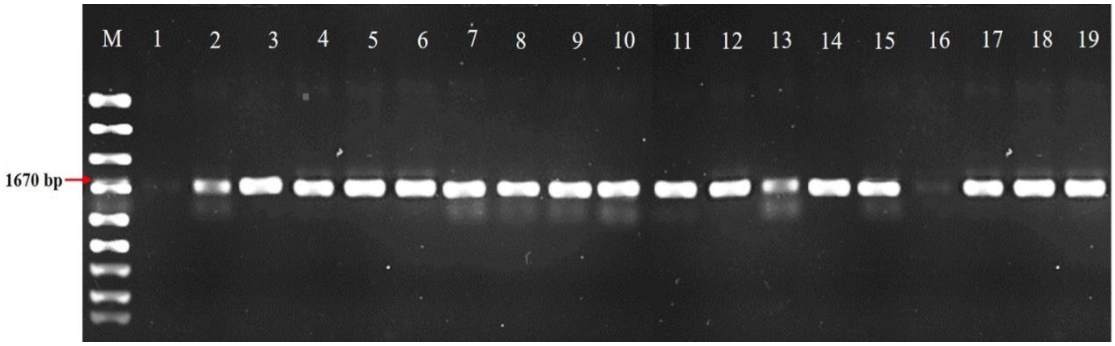

Figure 2. DNA fingerprints of SCAR marker (Pun1) analysis for capsaicinoid substances. M: DNA ladder marker, wells 1–9 (*C. annuum* L.), 10–17 (*C. chinense* Jacq.), and 18–19 (*C. frutescense* L.). Numbers 1–19 indicate the accessions described in Table 1.

DISCUSSION

In comparing CAT and DI-CAT, CAT content is the key substance to use as a good criterion for selecting target genetic resources for improving new high-CAT cultivars in breeding programs. This is due to its mechanism as the most effective component for suppressing fat accumulation (Ludy *et al*., 2012; Ohnuki *et al*., 2001; Kawabata *et al*., 2006). Interestingly, among the 19 evaluated accessions, KKU-P31146 was the accession with the highest CAT content. Although KKU-P62268 had the highest Sum CATs, it had considerably lower CAT than KKU-P31146. Although DI-CAT, which was found to be highest in KKU-P62268, has been reported to have anticancer, anti-inflammation, and antioxidant activities, it has not been reported to suppress fat accumulation (Pyun *et al*., 2008; Rosa *et al*., 2002). In particular, KKU-P31146 had high CAT yield because its CAT content was multifold higher than that of other accessions. Although some accessions, such as KKU-P11007, KKU-P11003, and KKU-P11197, had high fruit yield, their CAT contents were quite low. Consequently, their final CAT yields were not as high as those of KKU-P31146. Considering CAT yield performance, KKU-P31146 had the highest CAT yield due to the combination of CAT content and dry fruit yield weight (Jeeatid *et al*., 2018b). However, the capability of this accession (KKU-P31146) to be a good parent and its inheritance for the CAT trait should be clarified in further studies.

Notably, Sum CAPs were not detected in two accessions (KKU-P31146 and KKU-P13049) among the 19 accessions used in this experiment. However, the CAT content of KKU-P31146 was multifold higher than that of KKU-P13049. The results might be attributed to the fact that the high CAT accumulation of by the CAT biosynthesis pathway in these two accessions completely converts vanillin into vanillyl alcohol (Sutoh *et al*., 2006). In contrast to the pathways in the accessions of KKU-P11039 and KKU-P31118, these pathways completely convert vanillin into vanillylamine, resulting in low to high Sum CAPs (Abraham-Juárez *et al*., 2008). Meanwhile, in other 15 accessions, these pathways convert

vanillin into vanillyl alcohol and vanillylamine, resulting in low-to-medium Sum CATs and Sum CAPs (Lang *et al*., 2009). As expected, some accessions of *C. chinense* Jacq. had higher Sum CAPs than the other two species (*C. annuum* L. and *C. frutescens* L.) (Bosland and Baral, 2007). The accessions with the highest Sum CAPs, KKU-P12013 and KKU-P13006, of *C. chinense* Jacq. had high Sum CAPs (approximately one million Scoville heat units; SHU), which was similar to the world's hottest pepper Bhut Jolokia (Dewitt and Bosland, 2009). Thus, these two accessions could be used in the CAP extraction industry to produce various pharmaceutical products (Jeeatid *et al*., 2018b).

The dCAPS (*p-AMT*) marker can be used to distinguish all three *Capsicum* species in our populations effectively even though it has been reported to identify only *C. annuum* L. (Lang *et al*., 2009; Tanaka *et al*., 2014). The 269-bp DNA fragment is considered to be the homozygous recessive gene for the *p-amt* allele (aa) (Sutoh *et al*., 2006), which controls high CATs in 'CH-19 Sweet' (Lang *et al*., 2009). Meanwhile, the 317 bp DNA fragment is considered as the homozygous dominant gene for the *p-AMT* allele (AA) that controls CAPs in the CH-19 cultivar (Tanaka *et al*., 2014). This association was obviously shown by the accession with high CAT and no CAPs (KKU-P31146). The results revealed that the dCAPS (*p-AMT*) marker might be considered as a neutral marker for the selection of different *Capsicum* species with Sum CATs and the absence of Sum CAPs. In addition, the SCAR (*Pun1*) marker could not amplify a common DNA fragment of 1670 bp in KKU-P31146. These results indicated that the SCAR (*Pun1*) marker did not produce any fragment in nonCAPs genotypes (homozygous recessive), and these genotypes did not produce Sum CAPs (Lee *et al*., 2005). As expected, SCAR (*Pun1*) is a marker known to be useful for selecting *Capsicum* accessions with Sum CAPs given that *Pun1* is the only locus known to date to have a qualitative effect on Sum CAP accumulation (Blum *et al*., 2003; Stewart *et al*.,2007; Stellari *et al*., 2010; Truong *et al*., 2009). The *Pun1* locus has a qualitative effect on CAP biosynthesis in cultivated accessions belonging to *C. annuum* L., *C. chinense* Jacq., and *C. frutescens* L. (Stewart *et al*., 2005). Nevertheless, the genotypic information obtained by using the dCAPS (*p-AMT*) marker without the SCAR (*Pun1*) marker is efficient and useful for validating the Sum CATs and Sum CAPs of the pepper accessions evaluated here.

CONCLUSION

The key findings of this experiment are that KKU-P31146 with high CAT content should be considered as a new source for improving new high-CAT accessions with good agronomic traits in breeding programs due to its high CAT content. In addition, the known dCAPS (*p-AMT*) and SCAR (*Pun1*) markers are effective as neutral markers for validating pepper accessions with different CATs and CAPs, respectively. The CAT inheritance of their offspring should be determined thereafter.

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