2016

한국육종학회-차세대BG21사업단-GSP사업단 공동심포지엄

Gene, Genome & New Technology for Plant Breeding

일시: 2016년 6월 29(수)~7월 1(금)

장소: 청주 라마다플라자 호텔

강민영

주　　최 : 사단법인 한국육종학회

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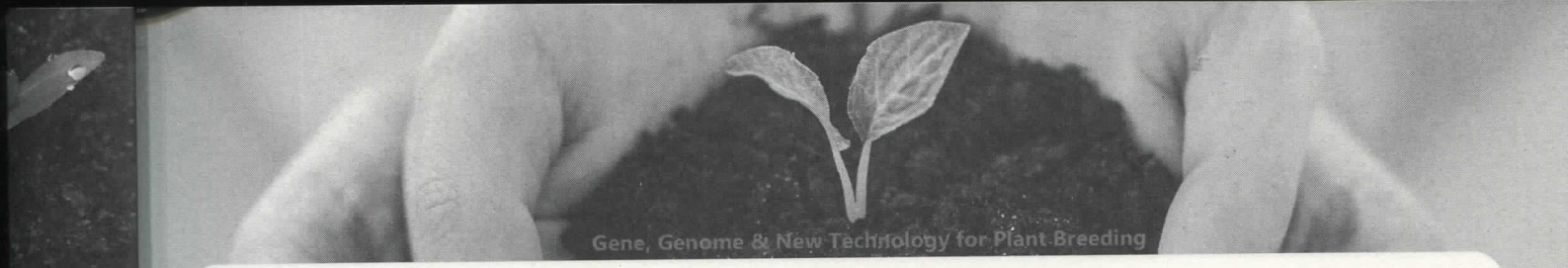
Symposium Program

2016 Annual Symposium of Korean Society of Breeding Science
Date and Place : June 29 - July 1, 2016 & Ramada Hotel at Cheongju

1st day [June 29. Wednesday]	
19:00~	General Meeting of Organizing Committee

2nd day [June 30. Thursday]	
09:00~09:50	Registration
09:50~10:00	Opening Ceremony ▸ Dr. Kang-Sup Lee (Secretary General, RDA, Korea)
	Opening Address – Dr. Young-soo Chung (Organizer, Dong-A University, Korea)
	Welcome Address – Dr. Yong-Gu Cho (President of KSBS, Chungbuk National University, Korea)
〈〈 Plenary Session 〉〉	
10:00~10:40	▸Chair : Prof. Hee-Jong Koh (Seoul National University) ▸Doubled Haploid (DH) Technology in Maize Breeding: Application and Techno Production of DH Lines – Dr. Chaikam, CIMMYT, Mexico
10:40~11:20	▸Finding Mineral Element Transporters for Better and Safe Production of Rice – Dr. Jian Feng Ma, Okayama University, Kurashiki, Japan
11:20~12:00	▸Function of Fibrillin Protein in Photosynthetic Metabolism – Dr. Hyun Wook Kim, SeJong University, Korea
12:00~13:20	Lunch & Poster
13:20~14:00	▸Chair: Prof. Deun-Gun Oh (Korea National College of Agricultural and Fisheries) ▸Revisiting Domestication to Revitalize Crop Improvement: The Florigen Revolution – Dr. Zach Lippman, Cold Spring Harbor Laboratory, USA
14:00~14:40	▸Manipulating Fruit and Vegetable Quality Traits – Dr. David Brummell, Plant & Food Research, New Zealand
14:40~15:20	▸Designing Crops for Global Food Security: Digitizing Plant Phenotypes – Dr. Maurice Moloney, University of Saskatchewan, Canada



2nd day [June 30. Thursday]

〈〈 Oral Presentation 〉〉	
15:40~17:40	OA. Breeding for yield increase and resistant variety
	OB. Breeding for quality improvement and Genetic variation
	OC. Molecular breeding and biotechnology
17:40~18:00	General Meeting
18:00~18:20	Poster Presentation
18:20~	Banquet

3rd day [July 1. Friday]

〈〈 Concurrent Session 〉〉	
	Introduction of genomics integrated Interfaces for breeders in major crops (The Agricultural Genome Center)
	▸ Chair : Dr. Yei-Soo Yu (PHYZEN)
09:00~09:25	▸Development of Bioinformatics pipeline and web database for Solanaceae genome application – Dr. SungHwan Jo (SEEDERS)
09:25~09:50	▸Constructing a Genomics-Assisted Bioinformatic Platform for Legumes – Prof. Hong-Kyu Choi(Dong-A University)
09:50~10:15	▸Genomics Integrated Breeding Platform for Soybean: Korean Soya Base – Dr. Namshin Kim (Korea Research institute of Bioscience and Biotechnology)
10:15~10:40	▸Research Updates of Rice Breeding Activities using Genome Information, and Development of Resources-Sharing Systems: Seed Materials, Genomic Information, Software and Research Experience – Prof. Yong-Jin Park (Kongju National University)
	– The National Center for GM Crops & Plant Molecular Breeding Center –
	▸ Chair : Dr. Hyun Suk Cho (National Academy of Agricultural Science)
09:00~10:40	▸Versatile Application of CRSPR/Cas9 System in Plant Research – Prof. Sangsu Bae (Hanyang University)
	▸Transgenic Plants Producing Green-vaccine for CSFV(classical swine fever virus) Lead on Plant Biotechnology-based Product on Market – Dr. Eun-Ju Sohn (BioApp Inc.,)

3rd day [July 1, Friday]

〈〈 Concurrent Session 〉〉	
	– Golden Seed Project for Vegetable & Horticulture –
	▸Chair : Prof. Yong-Pyo Lim (GSP Vegetable Seed Center)
09:00~10:40	▸Seed Industry Development by Strengthening Plant Breeder's Right and Seed Market C – Dr. Yi Seung In (Korea Seed & Variety Service)
	▸Current condition and strategy for expansion of seed testing service in KSVS – Dr. Eun Hee Soh (Korea Seed & Variety Service)
	▸ Chair : Prof. Ill-Sup Nou (GSP Horticulture Seed Center)
	▸Breeding for Pyramiding Target-genes and Selection of F1 Hybrids by Marker A Selection in Tomato – Dr. Myung Kwon Kim (Tomato Life Science & Research)
	▸Gene Identification, Expression Analysis and Breeding for Enhanced Glucos Biosynthesis in Brassica – Dr. Arif Hasan Khan Robin (Sunchon National University)
10:40~11:00	Coffee Break
〈〈 4부 Plenary Session 〉〉	
	– Golden Seed Project for Cereal –
	▸Chair : Dr. Young-Chan Cho (National Institute of Crop Science)
09:00~10:40	▸Specialty Corn Breeding at Sweet Seeds in Thailand to The Tropical World – Dr. Taweesak Pulam (Sweet Seeds Co., Ltd., Thailand)
	▸The Status of Rice Production and Breeding in China – Dr. Han Longzhi (Institute of Crop Sciences of Chinese Academy of Agricultural Scien
	▸Application of Biotechnology in Developing New Rice Varieties For High Temp Tolerance in the Philippines – Dr. Norvie Manigbas (PhilRice, Philippines)
	▸Introduction of rice in rice production and the change of major diseases in rice du period of climate change in Vietnam – Dr. Lai Tien Dung (Plant Protection Institute Research, Vietnam)
10:40~11:00	Coffee Break
〈〈 Plenary Session 〉〉	
	▸ Chair: Prof. Yong-Won Seo (Korea University)
11:00~11:40	▸RNA-guided genome editing in stem cells, animals, and plant – Dr. Jin Soo Kim, Seoul National University, Korea
11:40~12:20	▸CRISPR Genome Editing in Outcrossing Woody Perennials – Dr. CJ Tsai, UGA, USA
12:20~13:00	Awards Ceremony & Closing Remark

PC-58

Candidate Gene Analysis for the Genes Controlling the Yellow Color in *Capsicum annuum* Cultivar Micropep

Ayoung Jung[1], Juhun Lee[1], Jin-Kyung Kwon[1], Suna Kim, Byoung-Cheorl Kang[1*]

[1]서울대학교 농업생명과학대학

Carotenoids are major pigments coloring yellow, orange and red in plants. In *Capsicum*, phytoene synthase (*PS*
capsanthin-capsorubin synthase (*CCS*), β -Carotene hydroxylase (*Crtz-2*) and lycopene β -cyclase (*Lcyb*) were identified
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examined these four carotenoid biosynthesis genes and measured the carotenoid contents of *C. annuum* Micropep
(MR), Micropep Yellow (MY) and their F_2 population. Micropep lines have dwarf phenotypes. Mutation of the
candidate genes, *PSY* and *CCS*, were identified in the MY line. These mutations of *PSY* and *CCS* genes cosegregated
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Ayoung Jung[1], Juhun Lee[1], Jin-Kyung Kwon[1], Suna Kim[2] and Byoung-Cheorl Kang[1*]

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Abstract

Carotenoids are major pigments coloring yellow, orange and red in plants. In *Capsicum*, phytoene synthase (*PSY*), capsanthin-capsorubin synthase (*CCS*), β-Carotene hydroxylase (*Crtz-2*) and lycopene β-cyclase (*Lcyb*) were identified to be involved in the carotenoid synthesis pathway. Orange and yellow colors in pepper can be resulted from mutations in these four genes but the relationship between the colors and the four candidate genes has not been fully elucidated. We examined these four carotenoid biosynthesis genes and measured the carotenoid contents of *C. annuum* MicroPep Red (MR), MicroPep Yellow (MY) and their F_2 population. MicroPep lines have dwarf phenotypes. Mutation of the two candidate genes, *PSY* and *CCS*, were identified in the MY line. These mutations of *PSY* and *CCS* genes cosegregated with the fruit colors in the F_2 population with a phenotypic ratio of 9:6:1(red:orange:yellow). In the F_2 population, red peppers (*PSY/CCS*) accumulate higher levels of total carotenoid than those of yellow peppers (*psy/ccs*). In the orange peppers, we were able to classify the carotenoid content profile according to genotypes of *PSY* and *CCS*. Total carotenoid content in type 1 (*PSY/ccs*) was much higher than that in type 2 (*psy/CCS*) but capsanthin was accumulated more in type 2 than type 1. These results show that *PSY* and *CCS* genes affect the fruit colors of pepper in different ways in the carotenoid pathway. Moreover markers developed in this study can be used to distinguish the fruit colors of pepper.

Introduction

Carotenoids are piments which determine the fruit color synthesized in plastid. They have role in photosynthesis, attract pollinator or seed despensor and protect against photo-oxidation. The inheritance of mature fruit color in pepper is controlled by three loci: *Y, C1* and *C2* (Hernandez and Smith 1985). Candidate gene approach identified the CAPSANTHIN–CAPSORUBIN SYNTHASE (*CCS*) as a candidate for Y (Lefebvre et al. 1998; Popovsky and Paran 2000; Lang et al. 2004). The PHYTOENE SYNTHASE (*PSY*) has been found as a candidate for C2 (Thorup et al. 2000; Huh et al. 2001).

Mutations in *CCS* can result in a yellow fruit color (Lefebvre et al. 1998; Ha et al. 2007) and mutations in *PSY* have been associated with the orange fruit color of Habanero pepper (Hernandez and Smith 1985; Huh et al. 2001). Some orange peppers showing yellow and orange colors are associated with a mutation in *CCS* (Popovsky and Paran 2000; Langetal. 2004).

To gain a better understanding of the genetic control of fruit color variation in pepper, we measured carotenoids contents of MicroPep F_2 population and analysed the *CCS* and *PSY* genes.

Materials & Methods

Plant materials

Pepper accessions *Capsicum annuum* cv MicroPep (red) and *Capsicum annuum* cv MicroPep (yellow) were employed as parents and a total of 298 F_2 individuals were used as a population for genetic analysis.

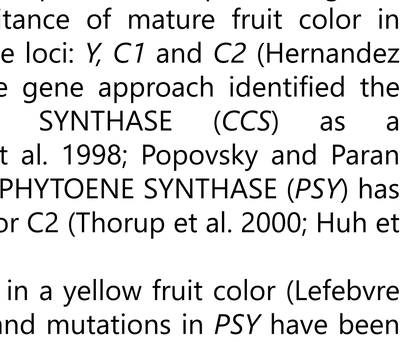

Figure 1. Mature fruits of pepper used in this study. (A) MicroPep red, yellow; (B) MicroPep red × yellow F_1; (C) MicroPep red × yellow F_2 . Bar 3cm.

Carotenoid analysis

HPLC analysis of carotenoids in mature fruits was performed in Prof. Kim's Laboratory. UV-visible detector was used to measure of carotenoid contents.

PCR analysis

Genomic DNA was extracted from leaves. Two sets of oligonucleotides were used to amplify the *CCS* and *PSY* genes with DNA.

Results & Discussion

Carotenoid profile

The major carotenoid in the red fruits was capsanthin (Table 1). In the yellow fruits, only trace amount of carotenoids were accumulated. In the orange fruits, we were able to profile the carotenoid contents according to the genotypes of *PSY* and *CCS*. In type 1 (*PSY/ccs*) and type 2 (*psy/CCS*), the major carotenoids forming compounds were zeaxanthin and capsanthin, respectively. Total carotenoid content in type 1 was much higher than that in type 2, but capsanthin, the final carotenoid product, was more accumulated in type 2 than type 1. Distinguishing the individual fruits between type 1 and type 2 by naked eyes was difficult, but overall view of populations demonstrated that the type 2 population tended to be more reddish color than type 1.

Results & Discussion

Table 1. Carotenoid accumulation in *Capsicum* fruit expressed as μg g-1 fresh wt pericarp, in mature fruit. F_2 red, orange, yellow: fruits with red, orange, yellow color in MicroPep red × MicroPep yellow F_2. F_2 Orange fruits have two genotype.

		MicroPep red	MicroPep yellow	F_2 red	F_2 orange (type 1)	F_2 orange (type 2)	F_2 yellow
Genotype	*PSY*	+	-	+	+	-	-
	CCS	+	-	+	-	+	-
neoxanthin		37.75±0.22	2.02±0.05	51.94±3.42	42.41±4.8	6.43±0.18	13.15±0.59
capsorubin		231.23±29.83	0.08±0.04	151.91±7.51	ND	12.06±1.04	0.75±0.09
violaxanthin		ND	1.81±0.01	63.7±1.42	19.76±3.63	3.74±0.28	4.17±0.05
capsanthin		893.94±102.06	4.01±0.01	466.59±18.01	4.58±0.05	29.76±3.03	4.5±0.02
antheraxanthin		ND	1.76±0.01	ND	13.11±0.89	2.25±0.07	2.2±0.12
zeaxanthin		303.58±25.2	2.38±0	81.11±3.15	76.85±5.52	6.41±0.77	3.88±0.11
lutein		ND	3.13±0.07	10.17±1.08	32.84±2.13	4.37±0.11	4.46±0.12
α-cryptoxanthin		14.55±0.87	1.3±0.51	44.9±2.97	72.62±2.83	ND	2.31±0.25
β-cryptoxanthin		42.86±1.54	1.02±0.04	45.14±6.4	9.95±0.63	0.76±0.03	1.36±0.11
α-carotene		40.82±2.18	0.92±0.08	65.51±6.59	36.27±1.58	0.14±0.01	0.68±0.1
β-carotene		59.4±6.4	2.71±0.04	124.51±13.73	12.68±0.52	2.12±0.02	2.64±0.09
total carotenoids		1624.12±119.47	18.51±0.28	1053.8±37.27	310.25±6.34	57.28±4.26	33.39±0.76

* ND: Not detected.

PCR polymorphism

PCR amplification was performed using the total DNA of two parents and F_2 population with primers for the *PSY, Crtz-2, LCYB* and *CCS* genes. Fragments of the *PSY* and *CCS* genes were obtained from the pepper plants with red fruits, but not from those with yellow fruits. No polymorphism of PCR products was detected between the two parents for *Crtz-2* and *LCYB*. The presence or absence of the amplified fragment almost completely cosegregated with the red, orange or yellow fruit color, respectively, in the F_2 population (Figure 2A, Table 2). In *CCS*, insertion over 7kb was detected from MicroPep yellow (Figure 3). In case of *PSY*, We found mutation from -17kb to *PSY* gene region (Table 3). From cDNA, we could not amplify fragment of the *PSY* gene from the plants with yellow fruits (Figure 2B). We identified premature stop codon in *CCS* from the plants with yellow fruits (Figure 4).

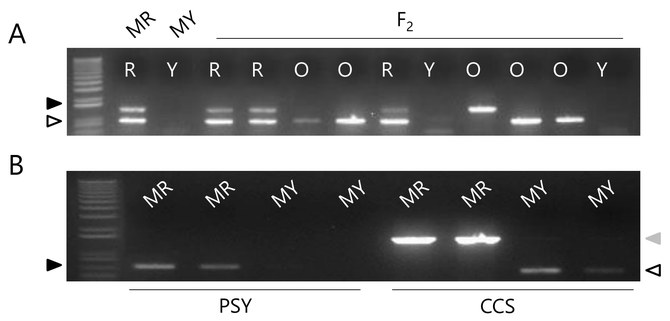

Figure 2. PCR polymorphism of the *CCS* and *PSY* genes in the F_2 segregating population. (A) PCR amplification from gDNA. ▶1,399 bp in *CCS*, ▷ 943 bp in *PSY* (B) PCR amplification from cDNA. ▶589 bp in *PSY*, ▶ 1,475 bp, ▷ 481 bp in *CCS* MR: MicroPep red, MY: MicroPep yellow, R, O, Y: F_2 plants with red, orange and yellow fruits, respectively.

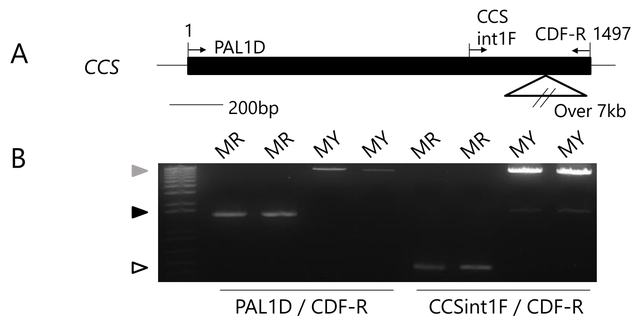

Figure 3. The *CCS* gene structure and mutation analysis in *CCS*. (A) The *CCS* genomic regions consist of one exon (box) in MicroPep red. (B) PCR polymorphism of the *CCS* gene. There is an insertion over 7kb in MicroPep yellow. ▶ 9 kb, ▶ 1,475 bp, ▷ 401 bp

MR 1 /……/ 106 / 107 / 108 /……/ 498 / *
ATG/……/AAG/GTA/TGT/……/CTT/TGA

MY 1 /……/ 106 / 107 / 108 / 109 / 110 / 111 / 112 / 113 / 114 / 115 / *
ATG/……/AAG/ATT/GTA/CGT/ACC/CGT/ATC/GGC/GGT/GGA/TAA

Figure 4. Coding sequences of *CCS*. Mutations in sequences are in red. The numbers depict the amino acid positions and the asterisk indicates the stop codon. Premature stop codon was detected in MicroPep yellow.

Table 2. Analysis of genotype and phenotype in the F_2 segregating population.

A

Genotype		Fruit color: Red	Orange	Yellow	Total
PSY/CCS	+/+	158	5	2	165
	+/-		48	4	52
	-/+		65		65
	-/-			14	14
Total		158	118	20	296

B

	Expected ratio	Actual ratio	χ^2	*P*
Phenotype	9:6:1	158:118:20	1.00	0.61
Genotype	9:3:3:1	170:54:70:16	3.30	0.33

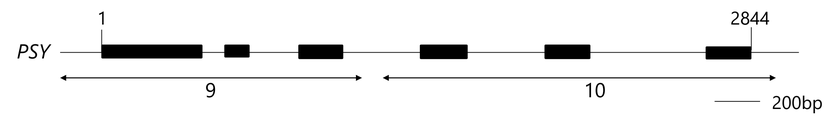

Figure 5. The *PSY* gene structure. The *PSY* genomic regions consist of six exons (box) and five introns (solid line) in MicroPep red. Number indicate primer set (Table 3) used for PCR polymorphism test of *PSY*.

Table 3. Summary of PCR polymorphism test of the *PSY* genes.

Primer set number	Region	Forward primer location (bp)	Reverse primer location (bp)	Product size	Red	Yellow
1	Upstream	-22004	-21008	996	+	+
2		-18552	-17693	859	+	+
3		-17712	-16765	947	+	-
4		-14206	-13678	528	+	-
5		-10008	-9079	929	+	-
6		-6496	-5921	575	+	-
7		-5146	-3841	1305	+	-
8		-3943	-665	3278	+	-
9	Gene	-228	1171	1399	+	-
10		1194	2980	1786	+	-
11	Downstream	2973	4927	1954	+	+
12		4823	5936	1113	+	+

References

Cunningham, F. X. (2002). Regulation of carotenoid synthesis and accumulation in plants. *Symposium A Quarterly Journal In Modern Foreign Literatures*, *74*(8), 1409–1417.

Guzman, I., Hamby, S., Romero, J., Bosland, P. W., & O'Connell, M. a. (2010). Variability of carotenoid biosynthesis in orange colored Capsicum spp. *Plant Science*, *179*(1-2), 49–59.

Ha, S. H., Kim, J. B., Park, J. S., Lee, S. W., & Cho, K. J. (2007). A comparison of the carotenoid accumulation in Capsicum varieties that show different ripening colours: Deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *Journal of Experimental Botany*, *58*(12), 3135–3144.

Acknowledgement

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