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A candidate gene approach identified phytoene synthase as the locus for mature fruit color in red pepper (*Capsicum* spp.)

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Abstrat The color of mature pepper fruit is determined by the composition of carotenoids. The fruit color of red pepper is genetically determined by three loci, *y*, *c1*, and *c2*. We have been developing a genetic map of hot pepper using RFLP and AFLP markers in the F_2 population of an interspecific cross between *Capsicum annuum* cv TF68 and *Capsicum chinense* cv Habanero. The color of the ripe fruit of TF68 is red and Habanero is orange. The red color is dominant over orange in the F_1 and the locus controlling this character has been marked in our SNU Linkage Group 7. To identify the gene or markers tightly linked to the red/orange locus, several candidate genes involved in the carotenoid biosynthesis pathway, namely FPS, GGPS, PSY, PDS, LCY and CCS, were examined. One of the candidate genes, phytoene synthase, cosegregated completely with fruit color in the F_2 population. QTL analysis of the pigment content of F_2 individuals quantified by HPLC also indicated that phytoene synthase is the locus responsible for the development of fruit color. The color, pigment content and genetic behavior of Habanero also suggest that phytoene synthase may be responsible for the *c2* gene discriminating between red and orange cultivars.

Keywords Candidate gene · *Capsicum* · Carotenoid, phytoene synthase · QTL

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Introduction

The various colors of ripe pepper fruits are not only attractive but also provide vitamin A. The carotenoids are synthesized in the chromoplast. In contrast to the tomato, where lycopene accumulates as the major carotenoid, deep red capsanthin is produced as a major pigment in the red pepper.

The inheritance of mature fruit color in the pepper has been a favorite subject of study for a long time but each locus has not yet been fully identified. Hurtado-Hernandez and Smith (1985) found that there were eight phenotypes in the F_2 segregation of the cross of white with red fruits. According to them, there are three independent loci determining fruit color, which are known as *c1*, *c2* and *y*. Recently, the gene for capsanthin-capsorubin synthase (CCS), which plays a role in the conversion of antheraxanthin to capsanthin and violaxanthin to capsorubin, has been considered as a candidate gene for the *y* locus (Lefebvre et al. 1998). However, the *c1* and the *c2* loci still need to be identified. To plant breeders, these loci have high economic values since they determine the mature red, orange, and yellow color of commercial pepper cultivars. Moreover, dry pepper fruits are important sources of natural red pigments for the food and cosmetic industry.

Until now, many genes involved in carotenoid biosynthetic pathway have been characterized and cloned in pepper (Dogbo and Camara 1987; Dogbo et al. 1988; Hugueney et al. 1992; Kuntz et al. 1992; Römer et al. 1993; Bouvier et al. 1994; Hugueney et al. 1995a; Bouvier et al. 1996; Hugueney et al. 1996). However, the direct connection between these genes and their phenotypic expression could not be established due to the absence of appropriate mutants and the availability of transformation techniques in peppers. The function of isolated genes could be postulated through biochemical studies or gene-introgression into other organisms such as tobacco, tomato or bacteria.

By utilizing the molecular genetic-analysis system that has been developed in our laboratory, carotenogenic genes were located in the linkage map and served as can-

didate genes for fruit-color development (Kang et al. 2000). If a certain gene-specific probe shows complete linkage with the fruit color locus, it could serve as an important candidate controlling the level of carotenoid content for fruit color. In addition, quantitative analysis of carotenoids in the ripe fruits of red pepper by HPLC and QTL analysis would provide a relatively accurate description of the relationship between the candidate genes and the carotenoid contents.

Material and methods

Plant materials

Pepper accessions *Capsicum annuum* cv TF68 (red) and *Capsicum chinense* cv Habanero (orange) were employed as parents and a total of 103 F_2 individuals were used as a population for genetic linkage analysis. DNA from these plants was extracted from tissues of whole leaves by a procedure described by Nahm et al. (1997). Individual identity of the plants was maintained by recording the DNA markers, ripe fruit colors, and carotenoid contents of each plant.

Cloning of candidate genes involved in carotenoid biosynthesis

Genes involved in the carotenoid biosynthesis pathway were cloned by employing the PCR-amplification method. The mRNA or genomic DNA sequences of farnesyl pyrophosphate synthase (FPS) (Hugueney et al. 1996), geranylgeranyl pyrophosphate synthase (GGPS) (Kuntz et al. 1992), phytoene synthase (PSY) (Römer et al. 1993), phytoene desaturase (PDS) (Hugueney et al. 1992), lycopene β-cyclase (LCYB) (Hugueney et al. 1995a), and capsanthin-capsorubin synthase (CCS) (Bouvier et al. 1994; Lefebvre et al. 1998) of *C. annuum* have been identified. Oligonucleotide primers to amplify the partial genomic fragments were designed according to the following sequences:

FPS-u (5′-CTTTCATCTGCCTCTGAAAATGAGTG-3′),
FPS-d (5′-CAAACATCTCATTCCCAAAAGGAG-3′),
GGPS-u (5′-CTTTCTCCAAGTGAAA TTGCACCAC-3′),
GGPS-d (5′-TCGATCACCTTCATTTCCATTTGG-3′),
PSY-u (5′-ATGTCTGTTGCCTTGTTATGGGTTG-3′),
PSY-d (5′-CCTGATTTCATGTTCTTG TAGAAGGC-3′),
PDS-u (5′-ATGTTGGAATTGGTCTTTGCGCCTG-3′),
PDS-d (5′-CACAATAGCTCCACTAGGCTAAAC-3′),
LCYB-u (5′-GCACCTTGTTGGGAAAA TATGGATACGC-3′),
LCYB-d (5′-GATCCCAGATAAGTCGAATTCATTC-3′),
CCS-u (5′-CCTTTTCCATCTCCTTTACTTTCCATT-3′),
CCS-d (5′-AAGGCTCTCTATT GCTAGATTGCCCAG-3′).

Using the following protocol, 94°C (5 min), 35 cycles at 94°C (1 min), 55°C (1 min) and 72°C (2 min), the total genomic DNA (200 ng) of young pepper leaves of TF68 and Habanero was amplified in the presence of 50 ng of both upstream and downstream primers. The PCR products were purified and cloned in the pGEM-T EASY vector (Promega, Wis., USA), sequenced using a Thermo Sequenase cycle sequencing kit (Amersham Pharmacia Biotech, N.J., USA) and were also used as probes for RFLP analysis.

Southern-blot analysis

Restriction digests were done using 0.5 to 1 units of five restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I) per microgram of DNA. Over 20 µg of pepper DNA were loaded and separated on 0.8% agarose gels in 0.5× TBE buffer (45 mM Tris-borate, 1 mM EDTA). Two different types of filters using Hybond N+ (Amersham Pharmacia Biotech) were prepared: (1) survey filters to check for polymorphism between the parental DNAs digested with five different restriction enzymes, and (2) progeny filters with genomic DNA from both parents and all 103 F_2 progenies digested with the same restriction enzymes, for generating segregation data.

Probes were labelled with [α-^{32}P]dCTP (Amersham Pharmacia Biotech) usi ng the random-hexamer protocol (Promega). Labelled probes were denatured by base treatment for 10 min with 0.2 M NaOH, and then added to the filters in 40 ml of hybridization buffer. Hybridization was carried out for 1 day with mid-stringency (6× SSC, 0.5% SDS, 5× Denhardt reagent, over 100 µg/ml of salmon sperm DNA) at 65°C. Filters were washed at low stringency (2× SSC), then at high stringency (0.5 or 0.1× SSC), and then placed on Agfa CP-BU film (European Communities) with one intensifying screen at –80°C for 1–5 days depending on the strength of the signal.

Linkage analysis

Linkage analysis was performed using the software package MAPMAKER V3.0 (Lander et al. 1987). Markers and their corresponding distances (cM) were included in the framework map only if the LOD value for the ripple was >3. The Kosambi mapping function was employed to convert recombination frequencies to map distances in cM (Kosambi 1944).

Quantification of carotenoids by HPLC

Fruits were harvested at five different stages; immature green, mature green, breaker, ripe, and over-ripe stages. Fruits of F_2 were harvested only at the ripe stage. Freshly picked fruit was cut into four sections and then put in liquid nitrogen to freeze. The frozen material was freeze-dried and ground to powder in a mill. The lyophilized powder was stored at –20°C until used. HPLC was performed using Nova-Pak C18 (8×100 mm, Waters) as described previously (Eskins and Dutton, 1979; Mínguez-Mosquera et al. 1995; Kim et al. 1997). The chromatograms were evaluated quantitatively by relating the heights of the individual peaks to those of a known quantity of authentic standards (Hoffman LaRoche, Basel, Swiss).

QTL analysis

Interval-mapping analysis for pigment content was performed by the program MAPMAKER/QTL 1.1. An LOD score of 3.0 was considered significant. LOD peaks were used to position QTLs on the linkage map and the phenotypic variance accounting for significant QTLs in the F_2 was estimated. The locus thought to be responsible for carotenoid content was examined by the analysis of variance (ANOVA).

Results

Assignment of the locus governing fruit color on the linkage map

The fruit color of the parents was either red or orange. F_1 plants bore the same red-colored fruits as TF68. Out of 103 F_2 plants, 78 had a red color but 25 had an orange color with a varying degree of intensity. This ratio of 3:1 fit very well for the Mendelian segregation (χ^2=0.029) of a single locus determining the color, with red dominant over orange. This locus for fruit color was assigned to linkage group 7 after calculating the recombination frequencies between the red and orange color-determining locus and other molecular markers (Kang et al. 2000).

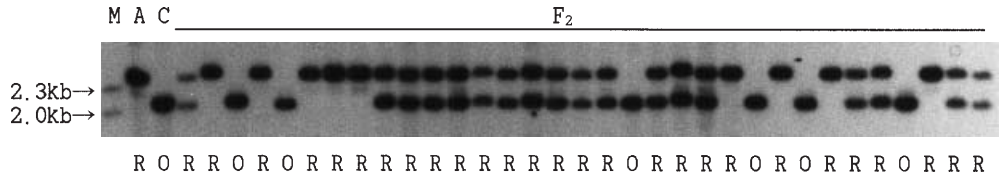

Fig. 1 Hybridization of the PSY probe to a Southern blot containing DNA from parents and F_2 plants digested with *Eco*RI. *Upper and lower bands* represent the PSY alleles from TF68 and Habanero, respectively. The probe was an *Eco*RI-digested, 1.5-kb genomic fragment of PSY at the 5′ end. *M λ/Hin*dIII size marker; *A* TF68; *C* Habanero; *O* orange; *R* red

Relationship between candidate genes and fruit color

The genomic fragments of several genes related to the carotenoid biosynthetic pathway, including FPS, GGPS, PSY, PDS, LCYB and CCS, were successfully amplified by PCR in both TF68 and Habanero (data not shown). There were no differences in the size and intensity of the amplified fragments of candidate genes between TF68 and Habanero. The expected size of the DNA fragments was obtained in the case of GGPS, LCYB and CCS. The size of the PSY genomic DNA fragment was larger by about 1.4 kb than the expected mRNA fragment, which implies that it contained introns. The amplified fragments of the pepper FPS and PDS were the partial genomic regions at the 3′ end.

The amplified fragments of candidate genes were cloned and their identity was confirmed by sequencing. They were used as gene-specific probes for Southern-blot analysis and showed polymorphism between parents, which enabled us to convert them into RFLP markers and to locate them on the linkage map (Kang et al. 2000). The candidate genes, GPS, PDS, LCYB, CCS and PSY, were assigned on linkage groups 7, 2, 10, 4 and 7, respectively. FPS could not be located on the map due to the absence of polymorphism. Likewise, zeaxanthin epoxidase and ζ-carotene desaturase could not be located on the map, because they did not show any polymorphism in RFLP analysis. Lycopene epsilon cyclase was not tested. In addition to these, transketolase 2 (Bouvier et al. 1998a), β-carotene hydroxylase (Bouvier et al. 1998b), and the plastid fusion and/ or translation factor (Hugueney et al. 1995b) were also cloned and positioned on linkage groups 1, 4 and 6, respectively.

Interestingly, one of the candidate genes, PSY, revealed polymorphism between the parents and showed complete linkage with the locus determining mature fruit color (Fig. 1). They were assigned together on the same locus of linkage group 7. F_2 individuals having PSY alleles, either homozygous for TF68 genotype (AA) or heterozygous for the TF68 and Habanero (AC), all demonstrated red fruit color. The segregation ratio for PSY was 34:44:25 (χ^2=3.757^{ns}) for the genotypes AA, AC and CC. It was also confirmed through a progeny test with 53 F_3 individuals that plants bearing the red fruit possessed TF68-derived PSY allele(s), while others with orange fruit only had the Habanero-derived PSY (data not shown).

Quantification of carotenoid in pepper fruit

HPLC was performed to analyze the carotenoid contents in the freeze-dried fruit powder of TF68, Habanero, and 89 out of their 103 F_2 individuals. Three major carotenoids, capsanthin, capsorubin and zeaxathin, were quantified using authentic standards (Hoffman LaRoche) (Fig. 2). Quantification of the amount of β-carotene, cryptocapsin, β-cryptoxanthin and lycopene were impossible because only trace amounts were detected. In the early developmental stage of the fruits (immature green, mature green), the amount of total carotenoid in TF68 fruit was similar to

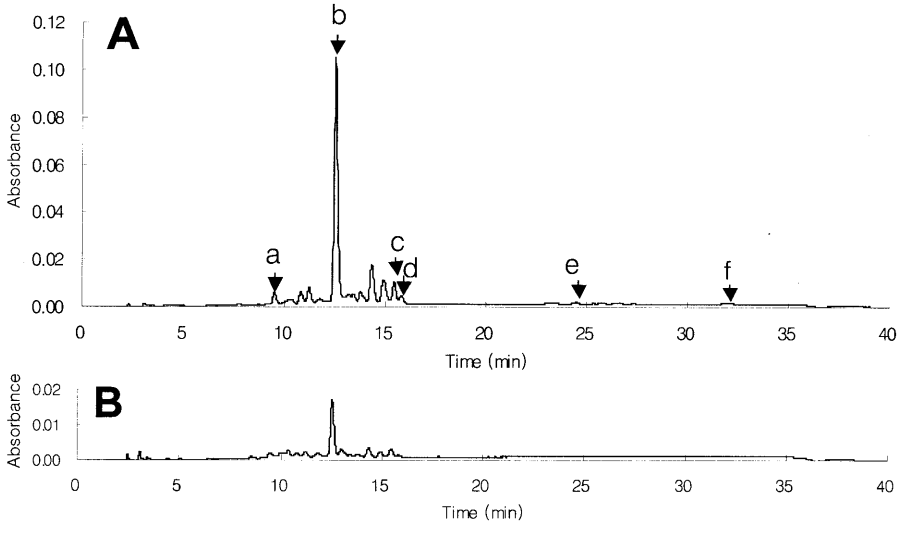

Fig. 2 HPLC elution profiles of pigments extracted from TF68 (**A**) and Habanero (**B**) mature fruit pericarps. Peaks were detected at 470 nm by a Waters diode-array detector. *a* capsorubin; *b* capsanthin; *c* zeaxanthin; *d* cryptocapsin; *e* β-cryptoxanthin; *f* β-carotene

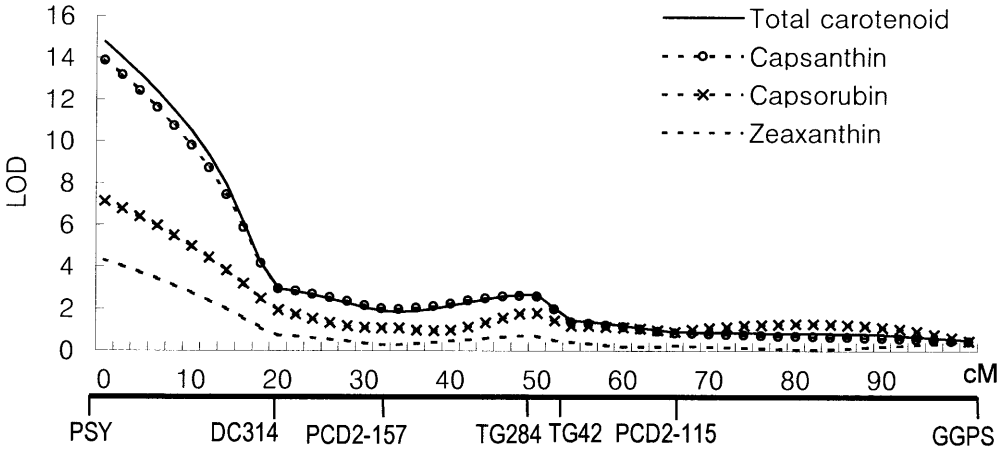

Fig. 3 The results of interval mapping analysis for the capsanthin, capsorubin, zeaxanthin and total carotenoid content. The *thick bar* below the horizontal axis represents the SNU linkage group 7 and on which the RFLP markers are positioned

that of Habanero. The amount of carotenoid in TF68 fruit dramatically increased as it ripened and turned red. Habanero fruit, however, showed only a gradual increase in carotenoid content (data not shown). The total carotenoid content of red-colored fruits was about 6-times higher than that of orange fruits. The HPLC profile of the orange parent did not reveal the lack of any single carotenoid peak. The orange had the same carotenoid composition as the red fruit but in a reduced amount in relation to the varying intensity of orange color. The keto carotenoids such as capsanthin and capsorubin are responsible for the red color in mature pepper fruit. The capsanthin content of red fruit was 1.07 mg/g of dry weight on average, about 6-times higher than the 0.18 mg/g dry weight of orange fruit. The capsanthin content showed wide variation (from 0.10 to 3.25 mg/g of dry weight) in the F_2 population. The frequency distributions of capsanthin, capsorubin, and zeaxanthin content in the F_2 population showed skewness toward the mean value of each pigment-content of the red fruits (data not shown).

QTL analysis

The relationship between the phytoene synthase and carotenoid content was tested with interval mapping using MAPMAKER/QTL 1.1 Interval analysis for the capsanthin, capsorubin, zeaxanthin and total carotenoid content showed that they were detected only at the PSY locus at LOD >3.0 (Fig. 3). There were no other loci showing a significant LOD score for carotenoid content among the entire linkage groups except PSY. This result suggests that PSY may be the locus responsible for the carotenoid content and fruit-color difference between TF68 and Habanero, and that this phenotype is not governed by QTLs.

The mean values of the carotenoid content of the three PSY genotype classes (TF68 homozygous, Habanero homozygous, and heterozygous) in the F_2 population showed that individuals possessing the TF68 PSY allele(s) have about a 6-times higher carotenoid level than those possessing only Habanero PSY alleles (Table 1). There was no significant difference in carotenoid levels between plants homozygous and heterozygous for the TF68 PSY allele. To test whether phytoene eynthase plays the decisive role in fruit-color phenotype, ANOVA was performed. Statistical analysis of the variances of the carotenoid content of three PSY genotype classes in the F_2 population showed that the PSY locus was associated significantly ($P<0.00001$) with the content of individual pigments such as capsanthin, capsorubin and zeaxanthin (Table 1). The phenotypic variances (R^2) of capsanthin and total carotenoid content explained by the PSY locus were 51.3% and 53.4%, respectively. This result strongly suggests that the carotenoid level is determined by the composition of the PSY allele.

Wide variation within individual and total carotenoid content is supposed to result from harvesting at different maturation stages, or genetic diversity throughout the F_2 population, which can affect carotenoid biosynthesis. Actually, fruits of TF68 and Habanero have many characteristics different from each other such as size, fruit shape, chlorophyll content, pungency, and thickness of pericarp. Besides, the fact that plants heterozygous for the red pepper-derived PSY allele have an amount of carotenoid pigment similar to those homozygous for PSY implies that red color is completely dominant over orange color.

Discussion

A linkage map containing carotenoid genes is useful for the characterization of color mutants

The genetic linkage map containing genes related to carotenoid biosynthesis was found to be very useful in characterizing the locus which controls fruit color resulting from an accumulation of the carotenoid pigment. Proper use of the genetic map would increase the probability for identifying genes corresponding to functionally characterized loci and subsequently minimize the cost and labor needed for intense gene isolation, such as map-based cloning. Several genetic linkage maps in the pepper have been developed (Tanksley et al. 1988; Prince et al. 1993; Lefebvre et al. 1995; Livingstone et al. 1999), but the map containi

Table 1 Quantity of three major carotenoids and total carotenoid, and their significance values in mature fruit pericarps from 89 F_2 progenies. Three different PSY genotype classes, homozygous for TF68 (AA) and Habanero (CC) and heterozygous (AC), were applied for evaluating significance by the GLM method

Carotenoid	Mean[a]			R^2	*F*	*P*[b]
	AA	CC	AC			
Capsanthin	1.12±0.52[c]	0.18±0.07	1.03±0.36	0.513	45.23	<0.00001
Capsorubin	0.21±0.13	0.04±0.02	0.21±0.13	0.308	19.18	<0.00001
Zeaxanthin	0.08±0.07	0.01±0.01	0.08±0.06	0.201	10.84	<0.00001
Total carotenoid[c]	2.05±0.91	0.32±0.13	1.90±0.63	0.534	49.28	<0.00001

[a] Mean value and standard deviation of carotenoid with the unit: mg/g of dry weight
[b] Probability that the marker genotype had no effect on the trait
[c] The total carotenoid content was calculated by absorbance at 470 nm (Lichtenthaler 1987)

ng ripening-related genes such as those involved in carotenoid biosynthesis has not been available. Thus, the hot pepper linkage map including several candidate genes investigated in this study will be very useful in identifying other fruit ripening-related loci when coupled with an analysis of gene functions (Kang et al. 2000).

Map positioning also allows the exclusion of certain candidate genes for a known mutation. A major limitation in identifying candidate genes at present is the significantly small number of mutations that have been characterized by genetic analysis. Consequently, though positioning relative to other morphological loci on known chromosomes makes it possible to eliminate numerous candidate genes based on linkage to different chromosomes, the identification of tightly linked candidate genes is viable only for those mutant loci which have been mapped relative to DNA marker loci.

Phytoene synthase plays a major role in red-color development

The result of molecular genetic analysis showing that *psy*, the recessive mutant gene, is the locus responsible for determining the red and orange fruit colors of hot pepper is surprising since phytoene itself is a colorless, fluorescent pigment. Phytoene synthase is the enzyme catalyzing the condensation of two molecules of the C20 compound geranylgeranyl pyrophosphate (GGPP) to give rise to the first C40 carotenoid compound, phytoene. This first step is very important in the further synthesis of carotenoids and may play a rate-limiting step. In this study, the reduced pigmentation in orange fruit may be due to a shortage of phytoene as a substrate for further enzymatic steps. The amount of all the investigated carotenoid pigments in orange fruit is about 1/6 of that of the red fruit. Capsanthin and capsorubin, the major pigments responsible for red color in pepper fruit, are also produced from phytoene via sequential desaturation, cyclization, hydroxylation and epoxydation steps. Thus, the orange-color phenotype may be the result of the reduced activity of phytoene synthase rather than the lack of a red pigment. To confirm this hypothesis, the structural differences between TF68 and Habanero of the PSY genes as well as the promoter regions need to be compared, and the expression pattern and the enzyme activity should also be investi gated. However, it cannot be ruled out that there exist other minor genes or factors which regulate the amount of carotenoids in the *psy* mutant. Heritability estimates should be performed in the future in order to determine the contribution of non-genetic factors to the traits.

Compared with the TF68 PSY genomic and cDNA sequences, Habanero PSY is suspected to perform abnormal splicing, resulting in aberrant transcripts (unpublished data). The reduced amount of carotenoid in Habanero might be due to the impaired catalytic activity of this enzyme. Thus, this enzyme is thought to be involved in the rate-limiting step of carotenoid synthesis. The loss of function or reduced activity of this enzyme has been known to cause defective carotenoid synthesis in various carotenogenic tissues in plants. For example, in tomato the *r* mutant has pale-yellow fruit flesh with more intensely yellow-colored fruit skin. And it is also associated with a paler, lemon-yellow flower corolla. The ripe fruit of the *r* mutant contains virtually no carotenoids (Jenkins and Mackinney 1955). Another mutant, r^y, has a phenotype similar to *r* but has a normal-colored corolla. These mutants were shown to encode defective PSY sequences and the *r* mutation could be complemented by over-expressing the wild-type sequence in transgenic mutant plants (Fray and Grierson 1993). The *y1* gene of maize, responsible for the yellow endosperm/ green leaf phenotype, also encodes phytoene synthase (Buckner et al. 1990, 1996).

The plant phytoene synthase was originally isolated from pepper (Dogbo et al. 1988). But the PSY mutant in pepper has not been identified yet and thus its role in phenotype determination could not be revealed. This is in part due to the difficulty of the transformation and regeneration of red pepper.

Phytoene synthase might be the putative *c2* locus

Hurtado-Hernandez and Smith (1985) reported that there were three gene pairs involved in ripe fruit-color expression, and that there were eight colors in the F_2 of a cross between the normal red ($y^+c1^+c2^+$)- and white (*yc1c2*)-fruited peppers. According to them, the orange color re-

sults from the highly reduced red pigment due to the presence of the *c2* factors which are involved in its recessive genetic expression. Kormos and Kormos (1960) reported that the recessive expressions *c1* and *c2* reduced the pigmentation of y^+ and *y* by the inhibition of β-carotene. When *c2* was present the pigment reduction was approximately 1/10, and with *c2* only traces of the pigments of y^+ and *y* were expressed.

Another candidate gene, CCS, has been known to determine red and yellow color in pepper fruit (Lefebvre et al. 1998) and yellow color in the pericarp is due to the deletion of CCS. But primers designed to successfully amplify this genomic fragment generated the same expected length of CCS both in TF68 and Habanero. Southern-blot analysis revealed that CCS is present in single copy in both parents. Also, CCS was located in linkage group 4 whereas the color-determining locus and phytoene synthase were both located in linkage group 7. From these results it was concluded that the gene determining the mature fruit color of TF68 and Habanero is not the *y* locus gene for *ccs* but the *c2* locus gene for *psy*.

In this study, orange Habanero fruit had about 1/6 the amount of total carotenoid pigment compared to TF68. Also, PSY was not linked to the other candidate gene CCS, which implies that the PSY and CCS segregate independently. This fact is also consistent with the results of the above-mentioned study in which three fruit-color loci are known to be unlinked. Further genetic analysis with cultivars having similar phenotypes should be done to confirm that the PSY is responsible for determining red and orange color.

With the aid of molecular markers, the breeding of fruits of different colors will be accelerated since the PSY- and CCS-specific markers make it possible to select red pepper at the seedling stage without waiting until the mature fruit stage. For example, trying to introgress red color into yellow- or orange-fruited pepper with a strong pungency, such as Habanero, will take less time since these markers are very useful in the selection of lines with desirable characters.

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References

Bouvier F, Hugueney P, d'Harlingue A, Kuntz M, Camara B (1994) Xanthophyll biosynthesis in chromoplasts: isolation and molecular cloning of an enzyme catalyzing the conversion of 5,6-epoxycarotenoid into ketocarotenoid. Plant J 6:45–54

Bouvier F, d'Harlingue A, Hugueney P, Marin E, Marion-Poll A, Camara B (1996) Xanthophyll biosynthesis: cloning, expression, functional reconstitution, and regulation of β-cyclohexenyl carotenoid epoxidase from pepper (*Capsicum annuum*). J Biol Chem 271:28861–28867

Bouvier F, d'Harlingue A, Suire C, Backhaus RA, Camara B (1998a) Dedicated roles of plastid transketolases during the early onset of isoprenoid biogenesis in pepper fruits. Plant Physiol 117:1423–1431

Bouvier F, Keller Y, d'Harlingue A, Camara B (1998b) Xanthophyll biosynthesis: molecular and functional characterization of carotenoid hydroxylases from pepper fruits (*Capsicum annuum* L.). Biochim Biophys Acta 1391:320–328

Buckner B, Kelson TL, Robertson DS (1990) Cloning of the *y1* locus of maize, a gene involved in the biosynthesis of carotenoids. Plant Cell 2:867–876

Buckner B, San Miguel P, Janick-Buckner D, Bennetzen JL (1996) The *y1* gene of maize codes for phytoene synthase. Genetics 143:479–488

Dogbo O, Camara B (1987) Purification of isopentenyl pyrophosphate isomerase and geranylgeranyl pyrophosphate synthase from *Capsicum annuum* chromoplasts by affinity chromatography. Biochem Biophys Acta 920:140–148

Dogbo O, Laferriere A, d'Harlingue A, Camara B (1988) Carotenoid biosynthesis: isolation and properties of the envelope of spinach chloroplasts. In: Reid E (ed) Plant organelles, vol 9. Ellis Horwood Publishers, Chichester, pp 47–59

Eskins K, Dutton HJ (1979) Sample preparation for HPLC of higher pigments. Anal Chem 51:1885–1886

Fray RG, Grierson D (1993) Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. Plant Mol Biol 22:589–602

Hugueney P, Römer S, Kuntz M, Camara B (1992) Characterization and molecular cloning of a flavoprotein catalyzing the synthesis of phytofluene and ς-carotene in *Capsicum* chromoplasts. Eur J Biochem 209:399–407

Hugueney P, Badillo A, Chen HC, Klein A, Hirschberg J, Camara B, Kuntz M (1995a) Metabolism of cyclic carotenoids: a model for the alteration of this biosynthetic pathway in *Capsicum annuum* chromoplasts. Plant J 8:417–424

Hugueney P, Bouvier F, Badillo A, d'Harlingue A, Kuntz M, Camara B (1995b) Identification of a plastid protein involved in vesicle fusion and/ or membrane protein translocation. Proc Natl Acad Sci USA 92:5630–5634

Hugueney P, Bouvier F, Badillo A, Quennemet J, d'Harlingue A, Camara B (1996) Developmental and stress regulation of gene expression for plastid and cytosolic isoprenoid pathways in pepper fruits. Plant Physiol 111:619–626

Hurtado-Hernandez H, Smith PG (1985) Inheritance of mature fruit color in *Capsicum annuum* L. J Hered 76:211–213

Jenkins JA, Mackinney G (1955) Carotenoids of the apricot tomato and its hybrids with yellow and tangerine. Genetics 40:715–720

Kang BC, Nahm SH, Huh JH, Yoo HS, Lee MH, Kim BD (2001) An Interspecific (*Capsicum annuum*×*C. chinense*) F_2 linkage map in pepper using RFLP and AFLP markers. Theor Appl Genet 102:531–539

Kim BD, Kang BC, Nahm SH, Kim BS, Kim NS, Lee MH, Ha KS (1997) Construction of a molecular linkage map and development of a molecular breeding technique. J Plant Biol 40:156–163

Kim S, Kim YH, Lee ZW, Kim BD, Ha KS (1997) Analysis of chemical constituents in fruits of red pepper (*Capsicum annuum* L. cv Bugang). J Korean Soc Hortic Sci 38:384–390

Kormos J, Kormos K (1960) Die Genetischen Typen der Carotinoid-systeme der Paprikafrucht. Acta Bot 6:305–319 (cited by Hurtado-Hernandez and Smith et al.)

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Kuntz M, Römer S, Suire C, Hugueney P, Weil JJ, Schantz R, Camara B (1992) Identification of a cDNA for the plastid-located geranylgeranyl pyrophosphate synthase from *Capsicum annuum*: correlative increase in enzyme activity and transcript level during fruit ripening. Plant J 2:25–34

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lefebvre V, Palloix A, Caranta C, Pochard E (1995) Construction of an intraspecific integrated linkage map of pepper using molecular markers and doubled-haploid progenies. Genome 38:112–121

Lefebvre V, Kuntz M, Camara B, Palloix A (1998) The capsanthin-capsorubin synthase gene: a candidate gene for the *y* locus controlling the red fruit colour in pepper. Plant Mol Biol 36:785–789

Lichtenthaler H (1987) Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. ln: Packer L, Douce R (eds) Methods Enzymol. 148:350–382

Livingstone KD, Lackney VK, Blauth JR, Van Wijk R, Jahn MK (1999) Genome mapping in *Capsicum* and evolution of genome structure in *Solanaceae*. Genetics 152:1183–1202

Mínguez-Mosquera MI, Hornero-Méndez D, Garrido-Fernandez J (1995) Detection of bixin, lycopene, canthaxanthin, and b-apo-8′-carotenal in products derived from red pepper. J Anal Chem 78:491–496

Nahm SH, Yu JW, Kang BC, Kim BD (1997) Selection of parental lines for a hot pepper mapping population using RFLP and AFLP analysis. J Korean Soc Hortic Sci 38:693–697

Prince JP, Pochard E, Tanksley SD (1993) Construction of a molecular linkage map of pepper and a comparison of synteny with tomato. Genome 36:404–417

Römer S, Hugueney P, Bouvier F, Camara B, Kuntz M (1993) Expression of the genes encoding the early carotenoid biosynthetic enzymes in *Capsicum annuum*. Biochem Biophys Res Commun 196:1414–1421

Tanksley SD, Bernatzky R, Lapitan NL, Prince JP (1988) Conservation of gene repertoire but not gene order in pepper and tomato. Proc Natl Acad Sci USA 85:6419–6423