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# Genetic and molecular regulation of colour and pungency in Hot pepper (*Capsicum* spp): A review

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ABSTRACT

Chilli (*Capsicum* spp) is an important horticultural crop both from its economic importance point of view and its nutritional value. It is an excellent source of natural colours and nutraceutical compounds. Chilli is a popular food ingredient in many parts of the world because of its two special attributes pungency and colour. It is pungent because of capsaicinoid alkaloids which get accumulated in the placenta of maturing fruits. The most abundant components of these compounds are capsaicin and dihydrocapsaicin. Capsaicinoids give a peppery flavour to meals and have various other interesting properties and applications such as antioxidant, antimutagenic and antitumoral activities. In addition, it possesses a number of pigments which impart it different colours. Various molecular explanations have been proposed for specific colour and pungency in peppers. An understanding of the regulation of the carotenoid as well as capsaicinoid pathways is necessary to manipulate these two traits in chilli. This article deals with the current state of knowledge of the molecular biology of these two traits in the genus *Capsicum*.

**Key words:** Capsaicin, Capsaicinoids, Capsicum, Carotenoids, Oleoresin

Chilli (*Capsicum* spp) occupies an important place in human culture since prehistoric times. It is an indispensable adjunct and a popular condiment in the world of food. Besides adding flavor, it also enhances the nutraceutical value of the diet. Chilli has been identified in herbal medicines as one of the purest and most effective natural stimulating botanical which is packed with potassium, magnesium, ascorbic acid and iron. It has long been used for pain relief especially for alleviating the pain of arthritis, headaches, burns and neuralgia as they are known to inhibit pain messengers. It is also claimed that they have the power to boost immune system and are also helpful in getting rid of parasites of gut (Khyadagi 2009, Anon 2009). It has a very beneficial effect on the circulatory system. Studies have shown that it counteracts the cholesterol build up and reduces platelet aggregation, thus reducing the risk of heart attacks and strokes. It also lowers high blood pressure and increases peripheral circulation (Anon 2009). Fresh chilli contains ratin (Vitamin P) which besides having antioxidant property, strengthens blood capillaries and regulate permeability. Chilli when consumed, creates a stimulating burning sensation in the mouth and general glow all over the body. The stimulation is regarded as a favourable state, helping to increase absorption of nutraceuticals and drugs (Khyadagi 2009). Fresh chillies are an excellent source of vitamin A, tocopherol and ascorbic acid as well as neutral and acidic phenolic compounds which are important antioxidants (Howard *et al.* 2000). Chillies help in digestion of starchy foods as they stimulate taste buds and increase flow of saliva (amylase). When eaten fresh with salads they serve as a good vitamin supplement in addition to appetizing property. Chillies are used throughout the world as a spice and also in the making of beverages and medicines. Chilli is an indispensable item in the Indian kitchen and is consumed daily as a condiment in one form or the other. It is the cheapest spice available in India and is eaten across all groups (Anon 2009, Khyadagi 2009). Different nomenclatures are used to describe the chillies in different parts of the world. They are variously referred to as chillies, chile, hot peppers, bell peppers, red peppers, pod peppers, cayenne peppers, paprika, pimento and generic term *Capsicum*. There are more than 400 different varieties of chillies found all over the world. Different varieties are grown for vegetables, spices, condiments, sauces and pickles. The varieties differ in colour from red to yellow. Chillies with a bright red colour command higher prices than those which are dull red or orange or yellow in colour and deep red fruits tend to retain their colour longer than those which are of lighter shade during storage. The world's hottest chilli Naga Jolokia is cultivated in hilly terrain of Assam in a small town Tezpur in India (Hosamani 1993, Khyadagi 2009, Anon 2009).

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*Nutraceutical traits*

Hot red chilli peppers, which belong to the genus *Capsicum,* are among the most heavily and frequently consumed spices throughout the world. Colour and pungency are the two most important nutraceutical traits in chilli. Their principal pungent ingredient is capsaicin which has analgesic and anti-inflammatory activities and is used in topical creams and gels (e.g. Axsain and Zostrix) to mitigate neurogenic pain. Capsaicin has been found to have chemopreventive and chemotherapeutic effects as it has been found to preferentially repress the growth of some transformed human and mouse cells (Surh 2002). Research conducted at Cancer Research Institute, Adyar, Madras, indicated that green chillies retard cancer due to the natural presence of an enzyme *Asperginase* which is effective only when applied or used in pure or isolated form (Khyadagi 2009).

In food and beverage industries, chilli is being used in the form of oleoresin which permits better distribution of colour and flavour in food. The natural pigments in chillies are increasingly used as organic food colour. Colour oleoresins are used in pharmaceutical, cosmetic and face making products, textile, meat, food and confectionary industries. Natural colour is gaining more importance for its eco-friendly and medicinal uses (Khyadagi 2009). The colour imparted by the oleoresin ranges from red to orange, depending upon the concentration used. Commercial oleoresins are available in strengths ranging from 40000 to 100000 ASTA (American Spice Trade Association) colour units (Reddy and Sasikala 2013**)**. Because of the presence of coloured pigments chillies are also good sources of provitamin A carotenoids, viz. β-carotene, α- carotene, β-cryptoxanthin and oxygenated carotenoids or xanthophylls which can vary in composition and concentration due to differences in genetics and degree of ripening (Markus *et al.* 1999).

*Capsaicinoids*

The burning sensation generated from eating hot peppers is caused by alkaloids called capsaicinoids. These compounds are uniquely produced in the fruit of members of the genus *Capsicum*. All capsaicinoids share a common aromatic moiety, vanillylamine, and differ in the length and degree of unsaturation of a fatty acid side chain (Bennett and Kirby 1968, Leete and Louden 1968, Curry *et al.* 1999). More than 22 different capsaicinoids are known to be found in pepper fruits which are synthesized and accumulated in the epidermal cells of placenta of the fruits (Bosland and Walker 2010). The two most common capsaicinoids, capsaicin and dihydrocapsaicin differ in the degree of unsaturation of a 9-carbon fatty acid side chain; other naturally occurring capsaicinoids such as nordihydrocapsaicin, homodihydrocapsaicin, homocapsaicin, norcapsaicin and nornorcapsaicin, differ in chain length as well as degree of unsaturation. Capsaicinoids accumulate in the placenta of maturing *Capsicum*. These compounds are used widely in food products, as spice, and in diverse pharmacological applications. Pepper genotypes exhibit a wide range of capsaicinoid accumulation as a consequence of both environmental and genetic variability (Harvell and Bosland 1997, Zewdie and Bosland 2000a, 2000b, Anon 2009). Capsaicin is a potent inhibitor of substance P, a neuropeptide associated with inflammatory processes. The hotter the chilli pepper, the more capsaicin it contains. The highest concentrations of capsaicin are found in the ovary and in the lower flesh (tip) and the lowest content of capsaicin can be found in the seeds (Supalkova *et al.* 2007). The seeds are not the source of pungency but they occasionally absorb capsaicin because they are in close proximity to the placenta. No other plant part produces capsaicinoids (Arora *et al.* 2011). The hottest varieties include Naga Jolokia, habañero and Scotch bonnet peppers. Jalapeños are next in their heat and capsaicin content, followed by the milder varieties, including Spanish pimentos, and Anaheim and Hungarian cherry peppers. Capsaicin is being studied as an effective treatment for sensory nerve fiber disorders, including pain associated with arthritis, psoriasis, and diabetic (Anon 2009). Ecologically, a function of capsaicinoids in pepper is to deter eating of chilli fruits by mammals by binding a thermoreceptor, TRPV1, found on nonreceptive nerve fibers, thereby creating a sensation of burning pain (Caterina *et al.* 1997, Tewksbury and Nabhan 2001). Additionally, capsaicinoids have been found to exhibit antimicrobial properties (Billing and Sherman 1998, Tewksbury *et al.* 2008).

*Biosynthesis of capsaicinoid*

Capsaicinoids have been studied since the beginning of 1800s. The structure of capsaicin, the predominant form of the molecule, was solved in 1923 (Nelson and Dawson 1923, Fig 1). A general biosynthetic pathway for capsaicinoid synthesis was first outlined in 1968 using radiotracer studies to investigate capsaicinoid precursors. It has been reported that capsaicinoids are synthesized by the condensation of vanillylamine and a branched chain fatty acid and that the vanillyamine moiety was synthesized from phenylalanine and the branched-chain fatty acid was derived from valine (Bennett and Kirby 1968, Leete and Louden 1968). This biosynthetic phenylpropanoid pathway involved the sequential synthesis of phenylalanine, cinnamic, p-coumaric, caffeic and ferulic acids, and then the formation of vanillin and vanillylamine (Bennett and Kirby 1968). Several studies thereafter established the participation of phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), coumarate 3-hydroxylase (C3H) and caffeic acid O-methyltransferase (COMT) in phenylpropanoid-mediated capsaicinoid biosynthesis (Fujiwake *et al.* 1982a, b, Sukrasno and Yeoman 1993). It was discovered in 1981 by Suzuki *et al.* that acyl moieties were derived from either valine or leucine. Participation of some other enzymes, such as 4-coumaroyl-CoA ligase (4CL), hydroxycinnamoyl transferase (HCT), caffeoyl-CoA O-methyltransferase (CCoAOMT) was established by Stewart *et al.* (2005) and Mazourek *et al.* (2009).

Table 1 Varieties of chilli pepper with varying pungency level

| Species | Cultivar | Pungency (SHU) | References |
|---|---|---|---|
| *Capsicum annuum* L. | CalWonder, NuMex Conquistador, Bell, Bydagi Kaddi | 0 | Curry *et al*. 1999, Prasanth and Ponnuswami 2008 |
| | Meiteimorok, Haomorok | 0-100 | Sanatombi and Sharma 2008 |
| | Mulato, NuMex 6-4, NuMex Joe E. Parker | 1000-1200 | Curry *et al*. 1999 |
| | Serrano | 4000 | Prasanth and Ponnuswami 2008 |
| | Sandia | 5000 | Prasanth and Ponnuswami 2008 |
| | Cayenne | 8000 | Prasanth and Ponnuswami 2008 |
| | Santa Fe Grande, Mitla, Long Slim Cayenne, Jalapeno M | 21000-25000 | Prasanth and Ponnuswami 2008 |
| | Ellachipur Sannam | 30000 | Prasanth and Ponnuswami 2008 |
| | Hindpur S7 | 36000 | Prasanth and Ponnuswami 2008 |
| | Thai Hot, Jwala | 60000 | Prasanth and Ponnuswami 2008 |
| | Chiltepin | 70000 | Prasanth and Ponnuswami 2008 |
| | Kanthari White | 75600 | Prasanth and Ponnuswami 2008 |
| | Tezpur | 855000 | Prasanth and Ponnuswami 2008 |
| *Capsicum chinense* Jacq | PI 1721 | 0 | Curry *et al*. 1999 |
| | Umorok, Chiengpi | 100-500 | Sanatombi and Sharma 2008 |
| | Red Habanero | 150000 | Prasanth and Ponnuswami 2008 |
| | Orange Habanero | 210000 | Prasanth and Ponnuswami 2008 |
| | Naga Jolokia | 455000 | Prasanth and Ponnuswami 2008 |
| *C. baccatum* | Aji Escabeeche | 17000 | Prasanth and Ponnuswami 2008 |
| *Capsicum frutescens* L. | Uchithi, Mashingkha | 100-150 | Sanatombi and Sharma 2008 |
| | Bird eye chilli | 88350 | Prasanth and Ponnuswami 2008 |
| | Tabasco | 120000 | Prasanth and Ponnuswami 2008 |

Curry *et al.* (1999) utilized the differential accumulation of capsaicinoid biosynthetic genes in pungent and nonpungent peppers to understand the capsaicinoid biosynthetic pathway. Considering the fact that it was already known that the phenylpropanoid pathway was involved in supplying precursors for capsaicinoid biosynthesis, and that the PAL, C4H and COMT encoding genes were already cloned in other plants (Estabrook and Senguptagopalan 1991, Gowri *et al.* 1991, Fahrendorf and Dixon 1993), they decided to isolate some of the phenylpropanoid-pathway genes from chilli peppers. Transcript levels of enzymes on the capsaicinoid pathway were monitored in *Capsicum annuum* and *Capsicum chinense* fruit as a function of development, tissue type and genotype. Clones for *Pal*, *Ca*4*h*, and *Comt* were isolated from a cDNA library of habanero (*C. chinense*) placenta. These cDNA clones were used to measure transcript levels in different fruit tissues throughout development in six cultivars differing in pungency. Transcript levels for all three genes were positively correlated with degree of pungency in placental tissue; habanero, the most pungent chilli fruit, had the highest transcript levels, CalWonder, a non-pungent fruit, had the lowest levels. Two transcripts were characterized: one showing high homology to a 3-keto-acyl-ACP synthase (*Kas* gene), which might be involved in the biosynthesis of the branched-chain fatty acid, and the other with high homology to a putative aminotransferase (*pAmt* gene) which might be involved in the conversion of vanillin to vanillylamine. Northern blot expression analyses were carried out for the two newly found sequences and, much like *Pal, C4h* and *Comt*, the transcripts showed maximal accumulation during the first developmental stages in the chilli pepper fruits with the highest pungency. By using tissue-specific expression analysis in fruits, it was also found that both the 3-ketoacyl- ACP synthase (*Kas*) and the putative aminotransferase *(pAmt*) sequences were only expressed at significant levels in placental tissues, where the capsaicinoids were synthesized. Subsequently, Aluru *et al.* (2003) carried out a differential screen of a Habanero (*C. chinense*) placenta cDNA library and recovered three cDNA sequences with high similarity to branched-chain fatty acid biosynthesis enzyme genes: an acyl carrier protein (*Acl)*, a thioesterase (*Fat*) and a b-keto-acyl-ACP synthase (*Kas*). They established that the transcript accumulation of those three sequences was positively correlated with pungency levels in several Capsicum varieties. Since genes for capsaicinoid biosynthesis are expressed at a high level in placenta tissues from highly pungent chilli pepper fruits, Kim *et al.* (2001) isolated cDNA clones differentially accumulated in the placenta of pungent pepper *C. chinense* cv. Habanero by suppression subtractive hybridization. They

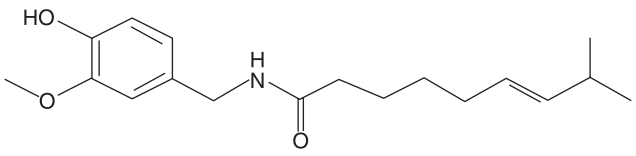


Fig 1 Structure of Capsaicin

observed that 39 cDNA sequences were highly expressed in placenta tissues from 30 DPA (days post anthesis) of highly pungent *C. annuum* cv Habanero fruits, but not in either 10 DPA Habanero and non pungent *C. annuum* cv Haehwa III placenta tissues. The cloned sequences were analyzed by northern blot analysis and two clones, viz. SB2-149 and SB1-158 showed a high similarity to the *pAmt* and *Kas* genes which had already been reported to be involved in the capsaicinoid biosynthetic pathway (Curry *et al.* 1999). These authors also suggested that another clone, SB2-66 might be the gene coding for capsaicinoid synthase (CS), responsible for the condensation of vanillylamine to a branched-chain fatty acid moiety in the capsaicinoid biosynthetic pathway as this clone showed homology to a group of coenzyme A-dependent acyl transferases. In addition, they reported that a clone SB2-115 showed high homology to long chain fatty-acid alcohol oxidases from *Arabidopsis* and *Candida tropicalis* and this clone could participate in fatty acid biosynthesis, and the products might be used for capsaicinoid production.

Stewart *et al.* (2005) worked in detail on the SB2-66 clone and observed that this clone co-segregated with the pungency trait and it was mapped to a locus in close proximity to *Pun 1* (locus C), which modifies the pungency level (Blum *et al.* 2002). They also isolated SB2-66 genomic DNA using genome walking and compared with certain sequences from pungent and nonpungent chilli peppers and observed that nonpungent fruits have a 2.5-kb deletion encompassing part of the putative promoter and the first exon. That allele was named *pun1*, and since the SB2-66 clone had acyltransferase domains it was labeled *At3*. The expression pattern for *At3* was determined by northern blot assays in pungent and non pungent peppers and it was observed that *At3* expression was specifically located in the placental tissues from pungent peppers and that its maximal accumulation was observed at 20 DPA (days of post anthesis). They also observed that with the exception of BCAT and *Acl,* other candidate genes namely, *Pal, C4H, Comt, pAmt, Kas* and FatA were either undetectable or their levels were significantly reduced in non-pungent peppers. These results suggested that *At3* might participate in the regulation of other capsaicinoid-related genes. In order to demonstrate that *At3* was related to capsaicinoid production, Stewart *et al.* (2005) utilized virus-induced gene silencing (VIGS) approach with Tobacco rattle virus (TRV) and the vector was used to silence the At3 gene, it was observed that capsaicinoid production was reduced by 50% compared with a control plant. Lee *et al.* (2005) confirmed the observations of Stewart *et al* (2005) that the gene corresponding to the SB2-66 clone might be the capsaicinoid synthase. They analyzed the $F_2$ population from a cross between a non-pungent *C. annuum* and a mildly pungent *C. annuum*. According to their results, the capsaicinoid synthase; (a) co-segregated with the pungency trait, (b) expressed only in the fruit placenta, and (c) co-segregated with locus C, (these authors proposed that the SB2-66 clone was gene C), which is thought to be responsible for Chilli pepper fruit pungency and (d) non-pungent peppers had a 2,529-bp deletion in the 50-region of the putative capsaicinoid synthase gene.

Later, Stewart *et al.* (2007) observed a 4-bp deletion in the first exon of *At3* gene in a non-pungent *C. chinense* NMCA 30036 chilli pepper and named this allele as pun12. In order to confirm their role in capsaicinoid production, Abraham-Juarez *et al.* (2008) attempted Pepper huasteco yellow veins virus induced silencing of *Comt, pAmt* and *Kas* genes and observed that this silencing resulted in a reduction of capsaicinoid accumulation in chilli pepper fruits which proved the participation of *Comt, pAmt* and *Kas* in capsaicinoid-biosynthesis supporting the previously proposed capsaicinoid biosynthetic pathway.

The participation of the *pAmt* gene in the capsaicinoid pathway was also ascertained by Sutoh *et al.* (2006) and Lang *et al.* (2009). The pAMT activity was measured in cell-free extracts from *C. annuum* cv. CH-19 sweet placenta, which showed that the conversion of vanillin into vanillylamine and capsaicinoid production were reduced to 60 and 9%, respectively, compared with pungent varieties. They compared the sequences of *pAmt* from non-pungent *C. annuum* cv. CH-19 sweet and pungent pepper Habanero (*C. chinense*), and observed that a T nucleotide insertion in the *pAmt* sequence of CH-19 sweet pepper had produced a stop codon, which affects the production of active pAMT. It was concluded that pAMT actively participates in capsaicinoid biosynthesis by regulating the phenylpropanoid precursors channeled into this pathway.

In order to know the exact process of branched chain fatty acids synthesis, various authors (Blum *et al.* 2003, Stewart *et al.* 2005, Thiele *et al.* 2008 and Mazourek *et al.* 2009) performed series of experiments. A desaturase has been reported to convert 8-ethylnonanoic acid into 8-methyl-6-nonenoic acid by Blum *et al.* (2003) and Stewart *et al.* (2005). However, the desaturation reaction has been suggested by Thiele *et al.* (2008) to take place before the thioesterase FAT removes the branched-chain fatty acids, and no modification occurs once the fatty acid is attached to the vanillylamine moiety. This observation was based on the detection of 8-methyltrans-6-nonenoic acid, the branched-chain fatty acid used for capsaicin synthesis, in the thioester pool (acyl-ACP and acyl-CoA) isolated from two chilli pepper placenta tissues (*C. chinense* var. Habanero orange and *C. annuum* var. Jalapeno). Furthermore, the fatty acid moieties attached to ACP and CoA corresponded to those found in capsaicinoid molecules. On the other hand, Mazourek *et al.* (2009) recently proposed that in addition to isobutyryl-CoA, some other intermediaries like acetyl-CoA, isovaleryl-CoA, anteisovaleryl-CoA and propinyl-CoA could

be used as substrates for capsaicinoid biosynthesis. Complete Capsaicinoid biosynthetic pathway adapted from Blum *et al.* (2003 ), Mazourek *et al.* (2009) and Aza- Gonzalez *et al.* (2011) is represented in Fig 2.

Liu *et al.* (2013) applied RNA-seq for the mixture of placenta and pericarp of pungent pepper (*Capsicum frutescens* L.). Their results predicted three new structural genes Dihydroxyacid dehydratase, Thr deaminase and Prephenate aminotransferase (DHAD, TD, PAT), which filled gaps of the capsaicinoid biosynthetic pathway predicted by Mazourek *et al.* (2009). TD and DHAD mainly participate in valine, leucine and isoleucine biosynthesis, as well as pantothenate and CoA (Co-enzyme A) biosynthesis. PAT belongs to the family of transferases, specifically the transaminases, which transfer nitrogenous groups.

*Molecular mapping of genes responsible for pungency*

The information regarding the genetic control of quantitative variation of capsaicinoid is limited. The earlier genetic studies suggest that a single dominant gene "C" was responsible for pungency in pepper fruits (Deshpande 1935, Greenleaf 1986). A C2 locus responsible for non-pungency was also reported in wild, nonpungent genotypes of pepper by Loaiza-Figueroa and Tanksley (1988) which, however, could not be validated in subsequent studies on the same accession. Three RFLP markers were found linked to 'C' (Blum *et al.* 2002), one of which coseggregatd with "C" and the other two were located within 1 cM distance of 'C'. These authors also developed a CAPs marker (Cleaved Amplified Polymorphic Sequence) linked to "C" using the sequence of capsicum Fibrillin gene which was located at 0.4 cM from 'C'. It was observed that pungency is a genotype dependent trait and the 'C' locus is responsible for pungency in a qualitative manner (Zewdie-Tarekegn 1999), however the degree of pungency in pungent genotypes is quantitatively inherited and influenced by environments (Zewdie and Bosland 2000b). Later, Blum *et al.* (2003) identified a major QTL for capsaicinoid content, termed *cap*,

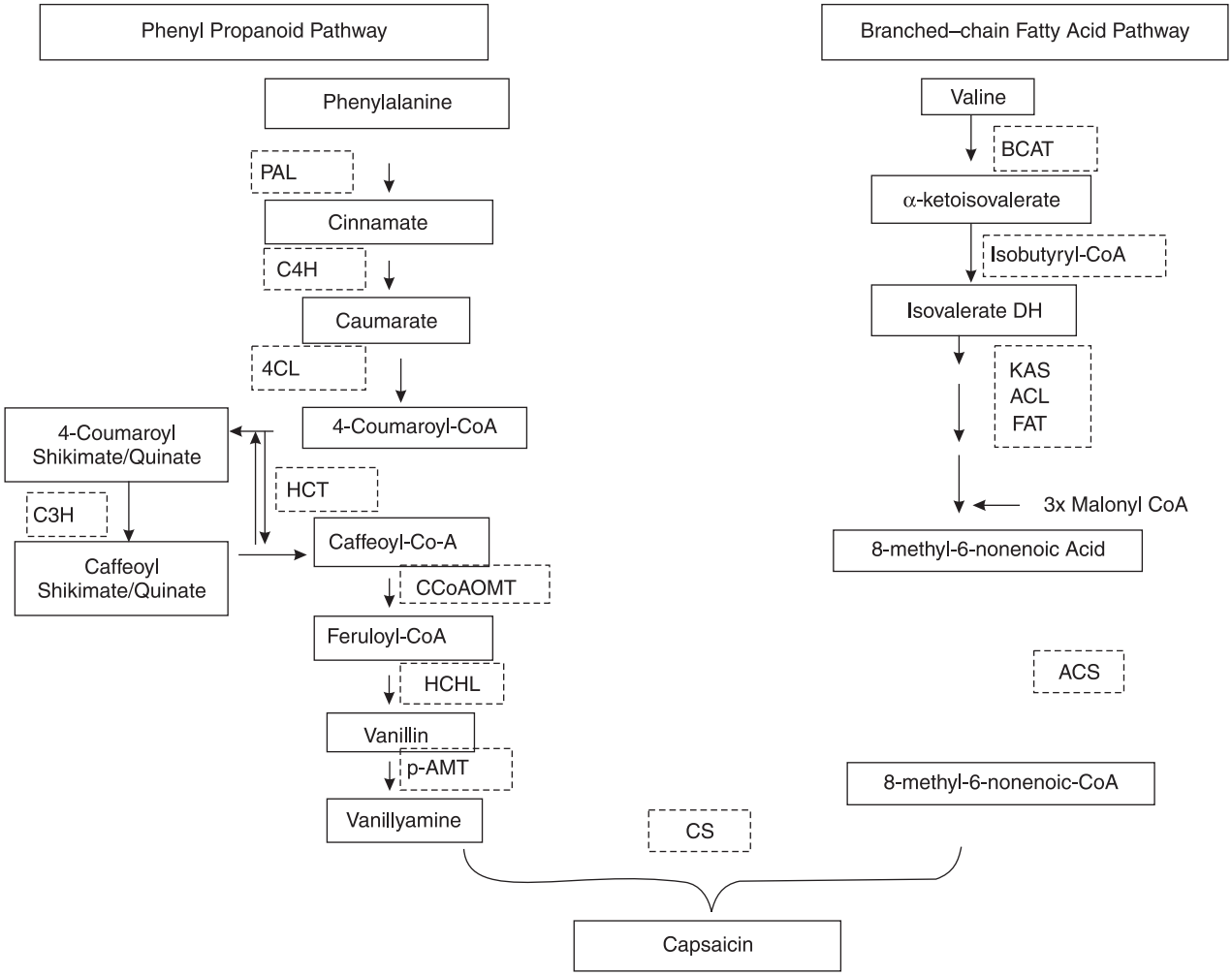


Fig 2 Capsaicinoid biosynthetic pathway (adapted and modified from Curry *et al*. 1999; Blum *et al*. 2003 ; Mazourek *et al*. 2009; Aza- Gonzalez *et al*. 2011). PAL:Phenylalanine ammonia lyase; C4H : cinnamate 4-hydroxylase; 4CL: 4-coumaroyl-CoA ligase; HCT : hydroxycinnamoyl transferase; C3H: coumaroyl shikimate/quinate 3-hydroxylase, CCoAOMT : caffeoyl-CoA 3-O-methyltransferase; HCHL: hydroxycinnamoyl-CoA hydratase/lyase; pAMT: putative aminotransferase; BCAT: branched-chain amino acid transferase; KAS: ketoacyl-ACP synthase; ACL: acyl carrier protein; FAT : acyl-ACP thioesterase; ACS: acyl-CoA synthetase; CS: capsaicin or capsaicinoid synthase.

on chromosome 7. They analysed quantitative variation in the accumulation of capsaicin and dihydrocapsaicin in the fruit of chilli peppers in a cross between the non-pungent *Capsicum annuum* parent cv. Maor and a pungent *Capsicum frutescens* parent, accession BG 2816. They employed the bulked segregant analysis method and screened bulked DNA from $F_2$ individuals at the extremes of the distribution of capsaicinoid content with RAPD primers and identified three loci that were polymorphic between the bulks. These RAPD markers were converted to SCARs and subsequently mapped with additional RFLP markers to chromosome 7 of pepper. QTL interval analysis for individual and total capsaicinoid content identified a major QTL, termed cap, which explained 34–38% of the phenotypic variation for this trait in two growing environments. In study by Blum *et al.* (2003), position of no known structural or regulatory genes correlated with *cap* for which they suggested that *cap* may be yet an unknown structural gene or the regulator of the pathway. Using the same bulk segregant approach, Minamiyam *et al.* (2005) identified two RAPD markers linked to 'C' locus one of which was converted into CAPs marker and was found at a distance of 3.6 cM from 'C'.

In order to understand the genetic control of capsaicinoid biosynthesis in *Capsicum* by defining the genomic regions that control the presence and accumulation of three major capsaicinoid analogues (capsaicin, dihydrocapsaicin and nordihydrocapsaicin), Ben-Chaim *et al.* (2006) analyzed the segregation of these three capsaicinoids in an inter-specific cross between a mildly pungent *Capsicum annuum* NuMex RNaky and the wild, highly pungent *C. frutescens* accession BG 2814-6 using simple sequence repeat (SSR) markers and confirmed the existence of a QTL in the *cap* region. They detected six QTL controlling capsaicinoid content on three chromosomes. One gene from the capsaicinoid biosynthetic pathway, BCAT, and one random fruit EST, 3A2, co-localized with QTL detected in this study on chromosomes 3 and 4. The major contribution to the phenotypic variation of capsaicinoid content (24–42% of the total variation) was attributed to a digenic interaction between a main-effect QTL, cap7.1, and a marker located on chromosome 2 that did not have a main effect on the trait. It was also proposed that a second QTL, *cap7.2* is likely to correspond to the QTL, *cap*, which has a pronounced influence on capsaicinoid content.

As has been discussed earlier, the identity of *pun1,* a putative acyltransferase named *At3*, was reported by Stewart *et al.* (2005). The *pun1* locus, is responsible for non-pungency throughout *C. annuum* based on inferred breeding pedigrees and sequencing results (Webber 1911, Stewart *et al.* 2005). As mentioned earlier in the section on biosynthesis of capsaicinoids non-pungent *C. annuum* genotypes had a 2.5 kb deletion spanning the putative promoter and first exon of *At3*. The *pun1* allele defined by this large deletion is the only known mutation to date that has a qualitative effect on the presence/absence of capsaicinoids (Webber 1911, Blum *et al.* 2002). It has also been suggested that it may be possible to regulate capsaicinoid production by regulating the presence of blisters that contain capsaicin (Somos 1984, Stewart *et al.* 2007). Votava and Bosland (2002) termed these blister structures 'vesicles' and stated that the absence of these capsaicinoid-accumulating vesicles is inherited as a single recessive gene at a new locus designated 'loss-of-vesicles' (*lov)*. This locus, *lov*, was proposed as a second locus, in addition to *Pun1*, that has a qualitative effect on pungency. Stewart *et al.* (2007) further, attempted to test the genetic relationship between blisters, capsaicinoid biosynthesis, *lov*, and *Pun1*. They had identified the recessive allele of the *Pun1* locus and showed that in the allelic state at *Pun1*, transcript accumulation of capsaicinoid biosynthetic genes and capsaicinoid accumulation are highly correlated. They also concluded that mutations at a single locus are responsible for non-pungency within *C. annuum* and *C. chinense* and that this locus is also responsible for the presence/absence of the blistered structures that contain capsaicinoids. Further, Sanchez-Sanchez *et al.* (2010) on the bases of their study on manzano chilli materials suggested that fruit pungency is regulated mainly by dominant genes, more than by additive effect genes, and that extranuclear genes also have a significant influence, a result that had not been previously reported. In line with these reports, very recently, Yarnes *et al.* (2013) identified 12 QTL associated with capsaicinoid levels. The identification of these QTLs and new alleles of *Pun1* will be useful for marker-assisted selection for pungency in breeding programmes. In addition, manipulation of such genes in future will be useful in the metabolic engineering of natural products for the benefit of humanity.

*Regulation of colour*

Capsicum species produce fruits that synthesize and accumulate carotenoid pigments, which are responsible for the yellow, orange and red colours of fruits. Carotenoids are the antenna pigments in plants which are associated with the light-harvesting and reaction centres on the thylakoid membranes in chloroplasts (Della Penna and Pogson 2006). Carotenoids provide several nutraceutical benefits and specific carotenoids (β-carotene and β-cryptoxanthin) are essential dietary components as vitamin A precursors (von Lintig 2010). Consumption of coloured peppers is becoming increasingly popular these days even in developed countries (USDA 2011) especially because these fruits are rich sources of pro-Vitamin A carotenoids and anti-oxidant xanthophylls (Deli *et al.* 2001, Wall *et al.* 2001, Wahyuni *et al.* 2011). In order to manipulate carotenoid levels in chilli through genetic engineering, a full understanding of the regulation of the carotenoid pathway is necessary (Schaub *et al.* 2005, Diretto *et al.* 2010, Bai *et al.* 2011). The Carotenoid biosynthesis pathway in plants is depicted in Fig 3 (adapted from Gómez-García and Ochoa-Alejo 2013).

The type and quantity of carotenoid accumulation in chilli pepper fruits is under a fine genetic control (Gómez-García and Ochoa-Alejo 2013). Although ancestral peppers were red but humans selected for fruit with additional colours (Paran and van der Knaap 2007). Several authors

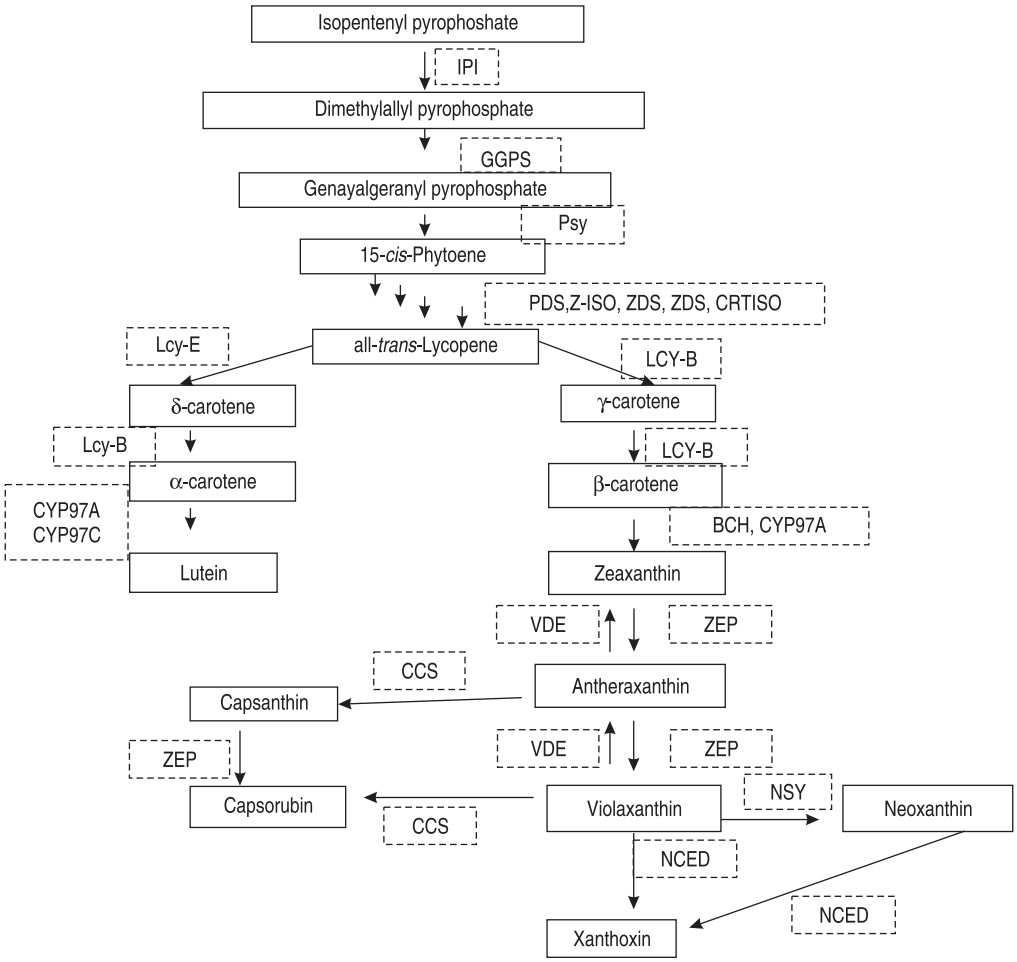


Fig 3 Carotenoid biosynthetic pathway in plants (adapted from Gómez-García and Ochoa-Alejo 2013). IPI: isopentenyl pyrophosphate isomerase; GGPS: geranylgeranyl pyrophosphate synthase; PSY: phytoene synthase; PDS: phytoene desaturase; Z-ISO: ζ-carotene isomerase; ZDS: ζ-carotene desaturase; CRTISO: carotene or carotenoid isomerase; LCY-B: lycopene-β-cyclase; LCY-E: lycopene-ε-cyclase; BCH: β-carotene hydroxylase; CYP97A: β-carotene hydroxylase (cytochrome 450 type); CYP97C: ε-carotene hydroxylase (cytochrome 450 type); ZEP: zeaxanthin epoxidase; CCS: capsanthin-capsorubin synthase; VDE : violaxanthin de-epoxidase; NSY: neoxanthin synthase; NCED: 9-cis-epoxycarotenoid dioxygenase.

have characterized the carotenoid pathway in chilli fruit, with the intent to investigate ways to increase β-carotene accumulation in the fruit (Huh *et al.* 2001, Lang *et al.* 2004, Ha *et al.* 2007, Guzman *et al.* 2010, Rodriguez-Uribe *et al.* 2012). Three loci (three independent pairs of genes: *c1*, *c2* and *y*) have been predicted to control the red, yellow, or orange fruit colour in pepper (Hurtado-Hernandez and Smith 1985). As a result of *Capsicum* mapping data, the biochemical identities of two of the three loci proposed to control pepper fruit colour are known; Y is Ccs and C2 is Psy (Popovsky and Paran 2000, Thorup *et al.* 2000). Red colour has been proposed to be dominant over white and yellow, as in the F1 cross of a red pepper with a white and yellow pepper only red F1 progeny was produced (Romer *et al.* 1993, Hugueney *et al.* 1995, Bouvier *et al.* 1996, Ha *et al.* 2007, Guzman *et al.* 2010). It was also hypothesized that the yellow chilli pepper fruit colour phenotype might be the result of a *CCS* gene deletion (Lefebvre *et al.* 1998). A co-segregation of the *y* locus and *CCS* in populations generated from crosses between chilli pepper plants (red × white and red × yellow) indicated the correspondence of the two genes (Popovasky and Paran 2000). Different authors (Popovasky and Paran 2000, Huh *et al.* 2001, Lang *et al.* 2004) have reported that *CCS* determined the chilli pepper fruit color by altering the carotenoid pattern. A new *CCS* chilli pepper (*C. annuum*) variant in the yellow fruit line CK7 with a premature stop codon derived from a C to G change (1095 bp downstream of the start codon), and also a downstream frame-shift caused by a 1 bp nucleotide deletion (1265 bp downstream of the start codon) was described by Li *et al* ( 2013). This variant exhibited positive expression of the mutant CCS protein.

Huh *et al.* (2001) reported that out of various candidate genes of carotenoid biosynthetic pathway, such as *GGPS*, *PSY*, *PDS*, *LCY-B* and *CCS*, only the *PSY* gene completely co-segregated with the colour in the $F_2$ population, suggesting that this gene corresponded to the *c2* locus responsible for the accumulation of red color. These authors further suggested that the association of *PSY* locus with the content of individual pigments such as capsanthin,

capsorubin and zeaxanthin and carotenoid levels in fruits was determined by the composition of the *PSY* alleles. In addition, *PSY* and *CCS* were also reported to segregate independently of each other.

In earlier studies (Lang *et al.* 2004), the orange colour of chilli pepper fruits was considered to be the consequence of a *CCS* gene deletion, but studies in Habanero Chilli pepper fruits (*C. chinense*) have demonstrated that the orange colour in the fruit is the result of a point mutation at the splice acceptor site of the fifth intron of the *PSY* gene which causes both a frame-shift and a premature translational termination. This mutant exhibited a recessive *c2* homozygous allele (Kim *et al.* 2010). However, recently Borovsky *et al.* (2013) reported that *CHY2* gene which encodes a β-carotene hydroxylase controls the orange colour in chilli pepper as a mutation in this gene resulted in accumulation of orange carotenoids (β-carotene) in the fruits instead of the red ones of the red-fruited progenitor "Maor". In general, carotenoid biosynthesis and accumulation has also been found to be influenced by factors like reactive oxygen species (ROS) and growth of plants in light-dark conditions (Bouvier *et al.* 1998, Simkin *et al.* 2003).

Not much information is available on transcriptional control of Carotenoid biosynthesis. Expression studies on selected carotenoid structural genes show that pigment-related transcripts are detected as the fruit begins to ripen (Romer *et al.* 1993, Ha *et al.* 2007). Ha *et al.* (2007) used cDNAs for phytoene synthase (Psy), phytoene desaturase (Pds), β-carotene hydroxylase (CrtZ-2), and capsanthin-capsorubin synthase (Ccs) amplified from Korean red pepper as probes in northern blot studies. Their results demonstrate high levels of transcripts in those peppers with high amounts of carotenoids. Recently, a quantitative comparative expression analysis of the *PSY*, *LCY-B*, *BCH* (*CRTZ-2*) and *CCS* genes in orange-fruited chilli pepper cultivars (*C. annuum*) showed a unique carotenoid profiles as well as distinct patterns of transcription of carotenogenic enzymes for each chilli pepper type (Rodriguez-Uribe *et al.* 2012). In one cultivar with the *ccs-3* mutant allele (Fogo), *CCS* gene transcripts were detected, but no CCS enzyme was produced, whereas in two other cultivars (Orange Grande and Oriole) with four wild-type genes, no *CCS* transcripts were recorded, and no capsanthin or capsorubin was produced. In the case of "Canary", this cultivar expressed the four wild-type genes, but no CCS enzyme was produced, and consequently no red carotenoids accumulated. Their results suggested that non-structural genes (potentially transcription factors) might regulate colour development in *Capsicum*.

*Future prospects*

Colour and pungency in *Capsicum* fruits is a broad field of study as chilli pepper fruits contain a wide variety of carotenoid pigments and a number of capsaicinoids with a large amount of structural diversity. However, an in depth research at enzymatic and genetic levels is needed to elucidate different carotenoid biosynthesis pathways operating in chilli pepper fruits, some of which are responsible for production of minor carotenoids. Similarly, biosynthesis of capsaicinoids has been a matter of research for decades and number of genes involved in capsaicinoid biosynthesis are still being updated. Two of the loci controlling fruit colour in *Capsicum* have been annotated with carotenoid biosynthetic activities, Y is Ccs and C2 is Psy (Lefebvre *et al.* 1998, Popovsky and Paran 2000, Thorup *et al.* 2000, Huh *et al.* 2001). The nature of the gene(s) at C1 is still unknown. Studies have revealed that the control of colour development is more complex than a deletion in a structural gene for a biosynthetic step (Rodriguez-Uribe *et al.* 2012). Further, transcriptional and translational control of the genes on the capsaicinid and carotenoid pathway also appears to play a significant role in the expression of fruit pungency and colour. Unfortunately, not much is known about the regulation of capsaicinoid and carotenoid biosynthesis in *Capsicum* at the gene and enzyme level as only a few studies of transcription factors or other genes that impose global regulatory functions on capsaicinoid and carotenoid metabolism in plants have been described. Therefore, it is imperative to focus more research on the transcriptional and translational control of these two important biosynthetic pathways in *Capsicum*. Further, from a breeding point of view, efforts need to be directed to identify QTL linked to colour and pungency in chilli. Although several reports are available on the mapping of QTLs for pungency in chilli, not much information is available for mapping programmes targeting oleoresins in chilli. Therefore, this is certainly a potential area of research in future as the identification of new QTLs will be useful for marker-assisted selection for pungency and colour in future breeding programmes.

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