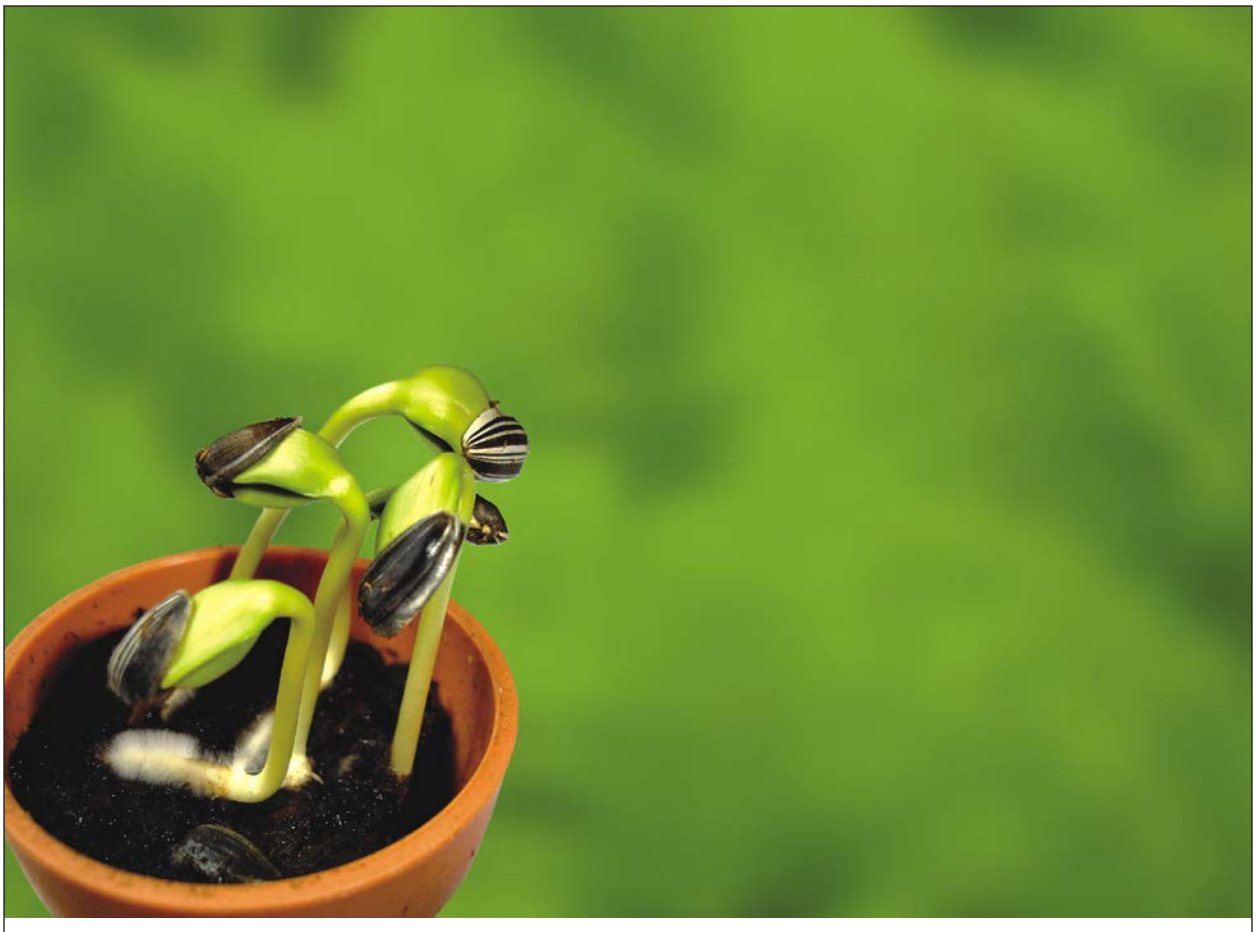

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A Review on Molecular Characterization of Pepper for Capsaicin and Oleoresin

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ABSTRACT

Pepper (*Capsicum* spp.) is one of the most economical and agriculturally important vegetable crops all over world. Pungency and oleoresin (colour) are the most important attributes of peppers used widely in food products, as spice and in diverse pharmacological applications. Development of high oleoresin content and less pungent cultivars has been a major objective in paprika production therefore trait specific marker development based on molecular mapping has proved as a potential tool for MAS (Marker Assisted Selection). For peppers several genetic maps have been published in the past 15 years, these maps are being used for the determination of syntenic relationships, gene tagging, marker-assisted selection and for gene cloning. This review highlights the different genetic linkage maps constructed for pepper and molecular mapping strategies as well as QTL detected for pungency and oleoresin.

Key words: Carotinoids, chilli, gene tagging, linkage maps, pungency, QTL mapping

INTRODUCTION

Pepper (*Capsicum annuum* L.) belongs to the Solanaceae family and it originated from South and Central America where it was domesticated around 7000 BC. The genus *Capsicum* includes 30 species, five of which are cultivated: *Capsicum annuum* L., C. frutescens, *C. chinense*, *C. pubescens* and *C. baccatum* (Bosland and Votava, 2000; Wang and Bosland, 2006) has been cultivated basically for use as spice and vegetable from thousands of year (Andrews, 1995). In the fifteenth century with Columbus expedition *Capsicum* came to Europe, subsequently spread rapidly around the world. *Capsicum annuum* is most widely cultivated in India, however, *C. frutescens*, *C. chinense* and *C. baccatum* are also grown in specific regions, especially in North-East region and state of Kerela. Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu are major chilli growing states in India which together contributes about 75% of the total cultivated area. *Capsicum annuum* is cultivated either for pungent fruited genotype called chilli (synonyms: hot pepper, American pepper, chile, azi, cayenne, paprika etc.) or non-pungent fruited genotype called sweet pepper (synonyms: Capsicum, paprika, bell pepper, Shimla mirch). Mixed application of organic, mineral or bio-N fertilizer had a better effect on growth and productivity of sweet pepper (Ghoname and Shafeek, 2005). Chilli is an economically important crop cultivated for its fruit valued for colour, flavour, spice, vegetable, nutrition that is provided its several products (Kumar *et al*., 2006). In recent year's chilli varieties being utilized for a wide range of food

processing, medicine development, pest and animal control and even in law enforcement which make this crop of immense importance. The majority of cultivars grown in Asian, central and Latin American countries are pungent, while in European countries cultivation of less pungent and non pungent peppers are more common. A novel protocol has been described for callus induction and large scale capsaicin production using various growth harmone (Umamaheswari and Lalitha, 2007). Colour (oleoresin) is one of important attributes of chilli, valued for its use as oleoresin in food as well as cosmetic industries. Red colour in chilli is mainly due to capsanthin and capsorubin pigment collectively known as oleoresin (Bosland and Votava, 2000). The oleoresin is slightly viscous, homogenous red liquid with good flow properties at room temperature. In food processing industries, especially in meat industry, concentrated oleoresin is added to the processed meat to impart attractive colour (Kumar *et al*., 2006). In beverage industries, oleoresins are also used to improve colour and flavors of its products. Oleoresin is considered to be among the best substitute of synthetic colour used in food and cosmetic industries (Kumar *et al*., 2006). On the basis of stability of quality traits in stored chilli powder capsaicin is a more stable trait than oleoresin (Pandey *et al*., 2010).

Construction of Pepper genetic maps using molecular markers: Genetic maps using molecular markers were constructed for most crops in recent years (Paterson *et al*., 2000). These maps are being utilized for the marker development, marker-assisted selection, gene tagging, gene cloning and for syntenic relationship studies. Universal acceptability of trait specific markers became a prime requirement for MAS (Marker Assisted Selection) (Kumar *et al*., 2009). Several genetic maps have been published in the past 20 years, details of genetic maps constructed are given in Table 1. Mapping populations used to construct the most maps were F_2, BC_1 and Doubled Haploids (DH) populations from interspecific crosses of *C. annuum*×*C. chinense* or from intra-specific crosses of *C. annuum* mostly based on RFLP, RAPD, AFLP and SSR markers. The first genetic map with a wide genome coverage that contained 85 markers was constructed using interspecific cross by Tanksley *et al*. (1988). Shortly thereafter using F_2 population of cross with the same *C. chinense* parent and a NuMex R Naky (*C. annuum*) parent allowed the construction of a more detailed map containing 192 markers (Prince *et al*., 1993). The most comprehensive pepper-tomato comparative map using AFLP, RFLP and RAPD markers was constructed by Livingstone *et al*. (1999) who mapped over 1007 loci in 13 linkage groups using the same cross as in Prince *et al*. (1993). Another genetic map based on RFLP markers that are mostly pepper-originated was developed by Kang *et al*. (2001). This map was subsequently expanded to include 46 pepper SSR markers (Lee *et al*., 2004). A BC_2 population from a cross of *C. annuum*×*C. frutescens* was used to construct an RFLP-based map of 92 markers (Rao *et al*., 2003). Recently an F_2 population of *C. annuum* (NuMex RNaky)×*C. frutescens* (BG *28*14-6) was constructed using 728 AFLP, RFLP and SSR markers (Ben-Chaim *et al*., 2006). In addition to these inter-specific maps, several intra-specific *C. annuum* maps have been reported. An map containing 85 markers derived from three partial maps constructed with DH lines was reported by Lefebvre *et al*. (1995). A *C. annuum* map from the cross of Perennial and Maor which consists of 177 AFLP and RFLP markers was constructed by Ben-Chaim *et al*. (2001). An update of the *C. annuum* map constructed from the integration of three individual maps containing RFLP, AFLP and known function genes was reported by Lefebvre *et al*. (2002).

Table 1: Genetic maps in pepper

Cross	Population type	Population size	Marker type	No. of marker	No. of linkage groups	Length (cM)	Reference
Doux des landes (*C. annuum*) ×PI 159234 (*C. chinense*)	BC_1	46	RFLP	85	14	ND	Tanksley *et al.* (1988)
NuMex R Naky (*C. annuum*) ×PI 159234 (*C. chinense*)	F_2	46	RFLP	192	19	720	Prince *et al.* (1993)
NuMex R Naky (*C. annuum*) ×PI 159234 (*C. chinense*	F_2	75	AFLP, RFLP, RAPD	1007	13	1246	Livingstone *et al.* (1999)
TF68 (*C. annuum*)× Habanero (*C. chinense*)	F_2	107	RFLP, AFLP	580	16	1320	Kang *et al.* (2001)
TF68 (*C. annuum*)× Habanero (C. hinense)	F_2	107	RFLP, SSR	333	15	1762	Lee *et al.* (2004)
Maor (*C. annuum*)× Perennial (*C. annuum*)	F_2	180	RFLP, RAPD,	177	12	1740	Ben-Chaim *et al.* (2001)
Maor (*C. annuum*)× BG 2816 (C. frutescens)	BC_2	248	RFLP	92	12	1100	Rao *et al.* (2003)
H3 (*C. annuum*)× Vania (*C. annuum*)	DH	101	AFLP, RFLP,RAPD	543	20	1513	Lefebvre *et al.* (2002)
Perennial (*C. annuum*)× Yo lo Wonder ((*C. annuum*)	DH	114	AFLP RAPD,RFLP	630	26	1668	Lefebvre *et al.* (2002)
Yo lo Wonder (*C. annuum*)× Criollo de Morelos 334 (*C. annuum*)	F_2	151	RFLP, RAPD, AFLP	208	18	685	Lefebvre *et al.* (2002)
Map integration	4 [C.a× C.a] +[C.a ×C.c] +1 [C.a×C.c] ×C.a	101+ 114 DH+151 F2+180 F2+75 F2+83BC_1	AFLP, RFLP, RAPD	2262	81	1832	Paran *et al.* (2004)
NuMex RNaky (*C. annuum*) ×BG 2814-6 (C. frutescens)	F_2	234	AFLP, RFLP,	728	16	1358	Ben-Chaim *et al.* (2006)
NuMex RNaky (*C. annuum*) ×PI 159234 (*C. chinense*)	F_2	100	RFLP, SSR	426	15	1304	Ben-Chaim *et al.* (2006)

ND: Not determined

Construction of integrated genetic maps: For the first time integrated genetic map of pepper was constructed by merging segregation data from six mapping populations (Paran *et al.*, 2004; www.plbr.cornell.edu/psi). These populations included maps built from the interspecific F_2 cross reported by Livingstone *et al.* (1999), an interspecific BC_1 cross of *C. annum*×*C. chinense* and the four intraspecific *C. annuum* maps (Ben-Chaim *et al.*, 2001; Lefebvre *et al.*, 2002). The integrated map included a total of 2262 loci covering 1,832 cM distributed in 13 linkage groups. Map integration improved the average marker density throughout the genome to 1 marker per 0.8 cM; however, because of uneven marker distribution, 15 gaps of at least 10 cM between adjacent markers still remain in the map (Paran *et al.*, 2004).

SSR (Simple Sequence Repeats) markers-based maps: Two interspecific SSR-based maps derived from F_2 populations *C. frutescens*×*C. annuum* (FA03) and *C. annuum*×*C. chinense* (AC99) were created via collaboration between several universities, research institutes and private

companies holding large sets of proprietary SSR markers reported at http://www.sgn.cornell.edu/. The FA03 map contains a total of approximately 728 markers covering 1,358 cM grouped to 12 linkage groups and associated with the 12 chromosomes of pepper. The AC99 map currently contains a total of about 450 markers covering 1,304 cM. Among the markers used to construct these maps, 150 SSR loci are common to both maps and can be used to describe co linearity. Selected tomato RFLP markers with known locations on the pepper genome were also used to anchor these maps to previous linkage maps of both pepper and tomato/potato (Paran *et al*., 2004). The grouping of the two populations was similar, no major translocation was observed. In both maps, the markers were unevenly distributed throughout the genome and marker classes tended to cluster.

Capsaicinoids (pungency): The cultivation and consumption of chilli peppers is mainly due to their ability to produce capsaicinoids responsible for the pungent flavour, accumulate in vesicles or blisters on the epidermis of the chilli placenta (Suzuki *et al*., 1980; Zamski *et al*., 1987). The pungent-oily substances from the fruits of hot pepper was first discovered and isolated by Bucholzin 1816 and the most active ingredient (named capsaicin) was isolated by Thresh in 1846 (Govindarajan, 1986). Capsaicin is considered to be a hydrophobic, colorless, odorless and crystalline alkaloids interact specifically with the mammalian pain receptor, VR1 have been widely used as tropical analgesics (Caterina *et al*., 1997). Capsaicin produced by a condensation reaction in the presence of catalyst capsaicin synthase between vanillylamine derived from either valine or leucine (Bennett and Kirby, 1968; Leete and Louden, 1968). The pungency is expressed in Scoville Heat Units (SHU) and organoleptic test was the first method to measure the pungency but now a day the most common and reliable method to estimate capsaicin is through High-Performance Liquid Chromatography (HPLC). The HPLC analysis has become the standard method for routine analysis of samples because it is rapid and a large number of samples can be handled.

Capsaicinoid Biosynthetic pathways: 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 major ones, capsaicin and dihydrocapsaicin, normally occur in the highest concentration. The other capsaicinoids occur in smaller concentration and known as "minor" capsaicinoids. Three genes *Pal, Ca4h and comt* were selected and their c DNA clones were used to measure transcript level and found that expression of these genes positively correlated with pungency (Curry *et al*., 1999). The biosynthetic pathway of capsaicinoids was studied in terms of organic synthesis and biochemistry using a radiotracer technique. It has been proposed that they are synthesized by the condensation of vanillylamine with C9 to C11 isotype branched-chain fatty acids; the former is derived from phenylpropanoid pathway, the latter from valine and leucine (Bennett and Kirby, 1968; Iwai *et al*., 1979; Leete and Louden, 1968; Suzuki *et al*., 1981). Two pathways (Fig. 1) are involved in the biosynthesis of capsaicinoids (i) fatty acid metabolism and (ii) phenylpropanoid pathway (Ochoa-Alejo and Gomez-Peralta, 1993).

The phenolic structure comes from the phenylpropanoid pathway, in which phenylalanine is the precursor. The formation of ferulic acid from phenylalanine is well understood in other higher plants. Four enzymes, Phenylalanine Ammonia-Lyase (PAL), cinnamic acid-4-hydroxylase (C4H), r-coumaric acid-3-hydroxylase (C3H) and Caffeic Acid-O-Methytranferase (CAOMT) are involved in the process. Capsaicinoids are formed from vanillylamine and isocapryl-CoA via Capsaicinoid Synthetases (CS) (Fujiwake *et al*., 1982; Sukrasno and Yeoman, 1993; Curry *et al*., 1999). During

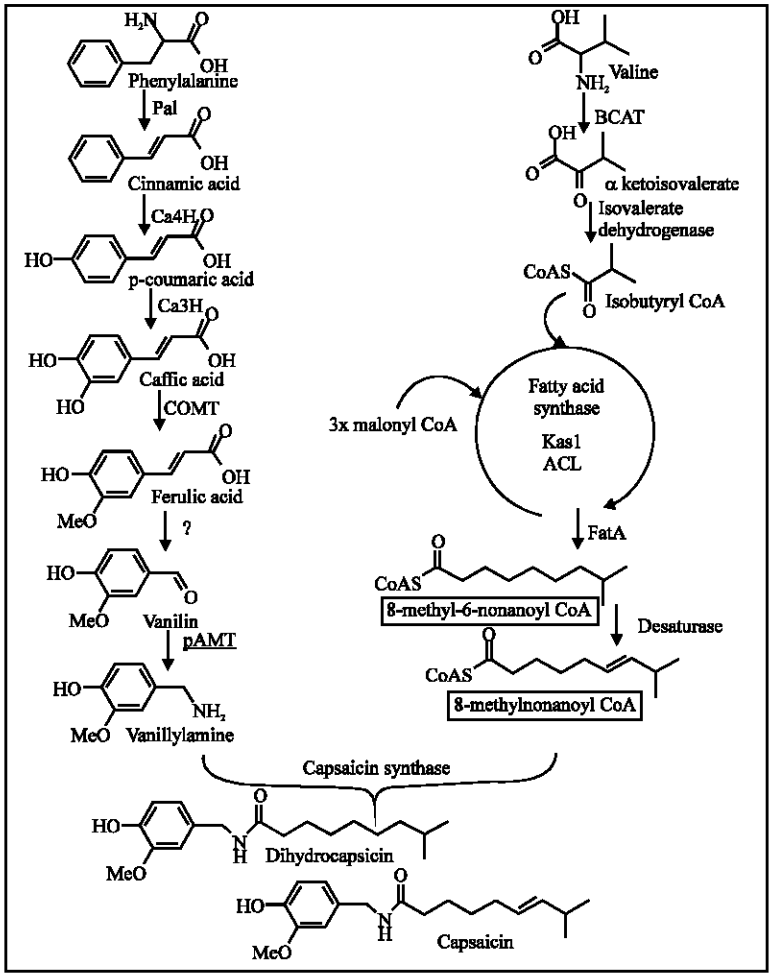

Fig. 1: Proposed pathway for capsaicinoid biosynthesis, phenylpropanoid metabolism (left) with fatty acid synthesis (right) (Adopted from Blum *et al.* (2003))

fruit ripening, capsaicin concentration reaches a maximum and capsaicin later degrades to other secondary products. Most peroxidase activity occurs in the placenta and the outer layer of pericarp epidermal cells. As determined by gel permeation chromatography, the major oxidative products were 5-5'-dicapsaicin and 4'-O-5-dicapsaicinether (Bernal *et al.*, 1995). Peroxidase activity increased at the time when the concentration of capsaicinoids started to decrease (Contreras-Padilla and Yahia, 1998). It was assumed that peroxidases catalyze capsaicinoid oxidation and play a central role in their metabolism. Water deficit affects phenylpropanoid metabolism and the pungency of fruits (Quagliotti, 1971; Estrada *et al.*, 1999). PAL, C4H and CS are involved in capsaicinoid biosynthesis and peroxidase isoenzyme B6 directly affects capsaicin degradation (Bernal *et al.*, 1994). Higher concentrations of PAL are followed by an increase in the pungency of fruits about 10 days later. At the arrest of fruit growth, increased PAL activity in the fruit accelerates the degradation of phenylalanine and the concentration of cinnamic acid and capsaicinoids increased (Ochoa-Alejo and Gomez-Peralta, 1993). Large amounts of cinnamic acid are synthesized seven days after flowering in the presence of PAL, demonstrating that PAL is a key enzyme in the

phenylpropanoid pathway (Ochoa-Alejo and Gomez-Peralta, 1993). Cinnamic acid-4-hydroxylase (C4H) hydroxylates cinnamic acid to r-coumaric acid. Capsaicinoid Synthetase (CS), the last enzyme involved in the biosynthesis of capsaicin, combines vanillylamine and isocapryl-CoA to make capsaicin (Fujiwake *et al*., 1982). Capsaicin concentration begins to decline 50 days after flowering. Cumulative evidence supports that capsaicinoids are oxidized in the fruits by peroxidases. Peroxidases are efficient in catalyzing *in vitro* oxidation of capsaicin and dihydrocapsaicin. These enzymes are mainly located in placental and the outermost epidermal cell layers of the fruits. The products of capsaicin oxidation by peroxidases have been characterized *in vitro* and some of them have been found to appear *in vivo* in the fruits (Di *et al*., 2000).

QTL detected and molecular mapping for pungency: Early genetic studies determined that a single dominant gene, C, was responsible in peppers to produce pungent fruit (Deshpande, 1935; Greenleaf, 1986). A second non-pungency locus was reported named C2, in a wild, non-pungent pepper (Loaiza-Figueroa and Tanksley, 1988) however, this has not been volidated in subsequent studies of the same accession. The line described in this study (BG 3547) does develop pungency, although this occurs later than usual during fruit maturation. In fruits, pungency owing to the presence of capsaicinoids has long been known that a single dominant gene, C, controls the presence or absence of pungency. Blum and coworkers also reported three RFLP markers linked to 'C' one of them co segregated with 'C' and other two RFLP markers also located within 1 cM. Moreover, a CAPs (Cleaved Amplified Polymorphic Sequence) marker linked to 'C' were developed using the sequence of *Capsicum* fibrillin gene, located 0.4 cM from 'C' (Blum *et al*., 2002). Moreover, as we know 'C' locus is responsible for pungency in a qualitative manner, there is a great variation in capsaicinoid content among different pungent (chili) varieties (Zewdie-Tarekegn, 1999). Thus pungency is genotype dependent. However, in the pungent types, the degree of pungency is quantitatively inherited and highly affected by the environments (Zewdie and Bosland, 2000). Using bulk segregant analysis three RAPD markers were found to be linked with capsaicinoids content and converted in to SCAR markers (Blum *et al*., 2003) (Table 3). Using same strategies like bulk segregant analysis on F_2 population two RAPD markers OPD 20-800 and OPY-09-800, were found linked with 'C' locus and more closely linked marker OPY-09-800 was converted into a CAPs marker found 3.6 cM distance from 'C' (Minamiyama *et al*., 2005). The segregation of three capsaicinoids capsaicin, dihydrocapsaicin and norhidrocapsaicin were analyzed by constructing a dense molecular map using SSR (Simple Sequence Repeats). Six QTLs controlling capsaicinoids content were detected on three chromosomes, One gene BCAT has a role in capsaicinoid biosynthetic pathway and one random fruit EST, 3A2, co localized with QTL detected on chromosomes 3 and 4 (Ben-Chaim *et al*., 2006). A tightly linked microsatellite PAP SSR (Plastid lipid associated protein Simple Sequence Repeat) marker was identified from the microsatellite region of fibrillin gene and Found 0.6 cM distance from 'C', PAP SSR screened with three species of *capsicum* and many alleles were found at this locus (Sugita *et al*., 2005). The molecular linkage maps of C locus have been prepared and pungency related gene has been found to be located on chromosome 2 (Lee *et al*., 2004). The genes of capsacinoids biosynthetic pathway have been isolated and characterized (Curry *et al*., 1999). Isolated genes encoding a putative aminotransferase (pAmt) and a 3-keto-acyl-ACP synthase *Kas*. Kim *et al*. (2001) identified three genes coding for enzymes, viz., SB2-66, a putative capsaicinoid synthase (CS), SB2-149, an aminotransferase and SB2-58, a keto-acyl-ACP synthase. SB-2-66 (CS) is linked with C locus and the non-pungent locus has a deletion. Based on sequence of CS, sequence characterized amplified

region (SCAR) markers have been developed and their usefulness in early detection of pungent genotype has been demonstrated (Lee *at al*., 2005).

Oleoresin (carotenoids): The green, orange and red fruit colour originates from the carotenoid pigments. More than 30 different pigments have been identified in the fruits (Bosland and Votava, 2000). These pigments include the green chlorophyll (a, b), the yellow orange and the red pigments which are exclusively produced in pepper fruits. The capsanthin and capsorubin constitute more than 60% of the total carotenoids present in the fruits. The contents of capsanthin and capsorubin increase proportionally with advanced stages of ripening with capsanthin being the more stable (Bosland, 1996). The most highly valued characteristic of pepper genotype for oleoresin production is a high content in carotenoids. This is because; ultimately the commercial value of paprika (non-pungent oleoresin) depends on its coloring capacity which depends directly on relative pigment richness. Other characters of interest are very low content of capsaicinoids, low moisture content and a relatively thin pericarp. Thin pericarp shorten the drying fruits before processing, thereby reducing the cost. Higher temperature and longer time lengths cause a decreasing in capsantin and vitamin C content, Lipoxygenase enzyme could be used as an indicator to decrease losses in quality of some red peppers (Orak and Demirchi, 2005). The basic carotene structure can undergo several structural modifications, namely, cyclization, hydroxylation and epoxidation, yielding the huge variety of carotenoids (more than 600) in nature. During ripening of fruits, there is a spectacular synthesis of carotenoids. All the carotenoids present in the fruits are C40 isoprenoids containing nine conjugated double bonds in the central polyenic chain, although with different end groups (3-hydroxy-5, 6-epoxide) which change the chromophore properties of each pigment, allowing them to be classified in two isochromic families: red (R) and yellow (Y) as described in Fig. 2. The red fraction contains the pigments exclusive to the *Capsicum* genus (capsanthin, capsanthin-5, 6-epoxide and capsorubin) and the yellow fraction comprises of the remaining pigments, viz., zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene and cucurbitaxanthin.

Genetics and gene tagging for carotenoids: A few informations has been reported for the colour control in *Capsicum* (Bosland, 1993). The early study demonstrated that mature red colour of the fruits is dominant over yellow and is controlled by a single gene (Y) and later it was suggested that three independent pairs of genes (cl, c2 and y) control the mature fruit colour (Table 2). The presence of dominant alleles at these three loci result in red mature fruits while the presence of recessive alleles at three loci results in white mature fruits (Popovsky and Paran, 2000). The predominant pigments of the fruits i.e., capsanthin and capsorubin are synthesized by the enzyme Capsanthin-Capsorubin Synthase (CCS). Intronless cDNA clone of CCS has been isolated and the expression studies indicated that CCS is induced during chloroplast differentiation at the time of fruit ripening and it is not express in leaves or green immature fruits (Bouvier *et al*., 1994; Houlne *et al*., 1994; Hugueney *et al*., 1996). The absence of capsanthin and capsorubin in yellow fruits correlates with the lack of expression of CCS enzyme in yellow fruits (Bouvier *et al*., 1994; Houlne *et al*., 1994). For the identification of red and yellow-fruited genotypes a co-dominant DNA markers at seedling stage has been reported (Popovsky and Paran, 2000).

Importance of capsaicin and oleoresin: The pharmaceutical industry uses capsaicin as a counter-irritant balm for external application (Carmichael, 1991). Capsaicinoid extracts is widely used to prepare certain drugs which are applied externally to stop pains of arthritis (rheumatoid

Table 2: Gene tagging with molecular markers for pungency and oleoresin in pepper

Population	Trait	Gene (symbol)	Marker type	Chromosome	Distance (cM)	Reference
C. chinense PI 152225 ×Kelvin and 4751	Fruit colour	Y	CCS (Specific)	ND	ND	Popovsky and Paran (2000)
C Maor (*C. annuum*) ×BG 2816 (C. frutescens)	Pungency	C	RFLP	2	0	Blum *et al.* (2002)
A 5226 (*C. annuum*)× PI 159234 (*C. chinense*)	Anthocyanin	A	RFLP	10	0	Borovsky *et al.* (2004)
(TM2 and TF_2)	Pungency	C	CAPS	ND	ND	Minamiyama *et al.* (2005)

ND: Not determined

Table 3: QTL detected for pungency in pepper

Population	Trait	No. of QTLs	Major effect QTLs	References
Maor (*C. annuum*)×BG 2816 (*C. frutescens*)	Capsaicinoid content	1	Cap	Blum *et al.* (2003)
NuMex RNaky (*C. annuum*)×BG 2814-6 (*C. frutescens*)	Capsaicinoid content	3	Total 7.1	Ben-Chaim *et al.* (2006)

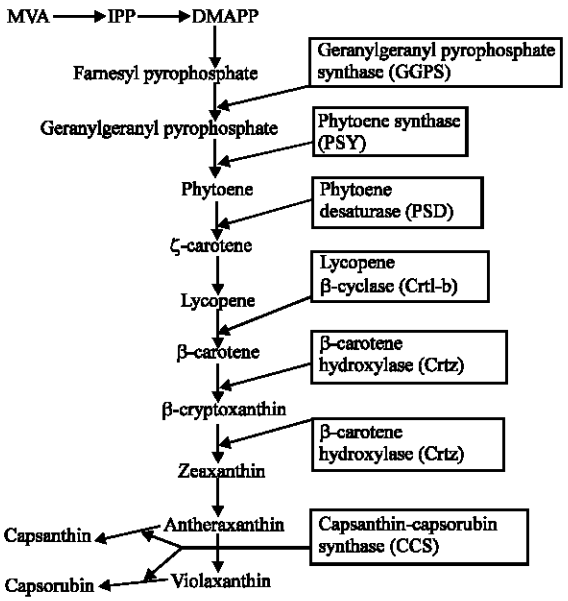

Fig. 2: Carotenoids biosynthetic pathway. Adopted from Lee *et al.* (2004)

arthritis, osteoarthritis), artily diseases (peripheral neuropathies) and to relive cramps. Now day's capsaicin spray is being used for self-defense purposes and also to control the unruly people (Cordell and Araujo, 1993; Bosland, 1996; Kumar *et al.*, 2006). Application of creams containing capsaicin reduces post-operative pain for mastectomy patients and its prolonged use helps in reducing the itching of dialysis patients, pains from shingles (Herpes zoster) and cluster headaches (Bosland, 1996). Capsanthin and capsorubin (major carotenoids exclusively present in pepper fruits) can improve the cytotoxic action of anticancer chemotherapy and considered to be potential of carotenoids as possible resistance modifiers in cancer chemotherapy (Maoka *et al.*, 2001). Lutein, zeaxanthin, capsanthin, crocetin and phytoene have showed more potent anticarcinogenic activity

than beta-carotene and useful for cancer prevention and may be applicable as the concept of 'bio-chemoprevention' which involves transformation-assisted method for cancer chemoprevention (Nishino *et al.*, 2002). The water extract of 'paprika' has been considered as a new anticancer agent and fat soluble component of this drug has been regarded as an anti-promoter of cancer (Mori *et al.*, 2002). Capsaicin has recently been tried as an intravesical drug for overactive bladder (bladder cancer) and it has also been shown to induce apoptotic cell death in many cancerous cells (Lee *et al.*, 2004).

Future scope of work: More effort is needed to determine the QTL position effecting the quality traits pungency and oleoresin. Although considerable progress has been achieved in mapping programs targeting pungency in pepper, however, very few oleoresin mapping strategies has been reported. Future work on construction of linkage maps and QTL mapping in pepper will rely on integration of sequence information from other *Solanaceae* as well as other plant species. Recently some new genetic maps were constructed based on SSR markers can also utilize in the mapping of pepper because of its co-dominant and multi allelic nature. Identification of genes that control pepper development and production will take advantage of more efficient positional cloning and candidate gene mapping approaches. Functional genomics experiments such as determination of expression profiles by microarrays, metabolic profiling and screening mutant populations are the emerging areas in pepper research. These complementary approaches will significantly helpful for pepper improvement.

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