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BREEDING FOR EXTRACTABLE COLOUR AND PUNGENCY IN *CAPSICUM* - A REVIEW

D. PRASATH* AND V. PONNUSWAMI

Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore – 641 003.

**Indian Institute of Spices Research, P.B. No. 1701, Marikunnu Post, Calicut, Kerala – 673 012 .*

Summary

High yielding varieties developed by plant breeders has resulted in green revolution, caused self sufficiency in food production. As the world market develops more towards healthy eating and natural products, the quality of the raw material from agriculture becomes more critical. The indiscriminate use of synthetic colours for the food colouring has several harmful effects. This necessitated our focus towards natural colours for food colouring, in general and development of high colour chilli varieties for extraction of paprika oleoresin, in particular. *Capsicum* breeding for quality has evolved through pure line selection of local varieties to hybrid development. This review highlights achievements in breeding for high extractable colour and pungency and breeding strategies. The scope of the discussion addresses colour and pungency principles, genetic resources, diversity, species compatibility, variety and hybrid development and biotechnological approaches.

Introduction

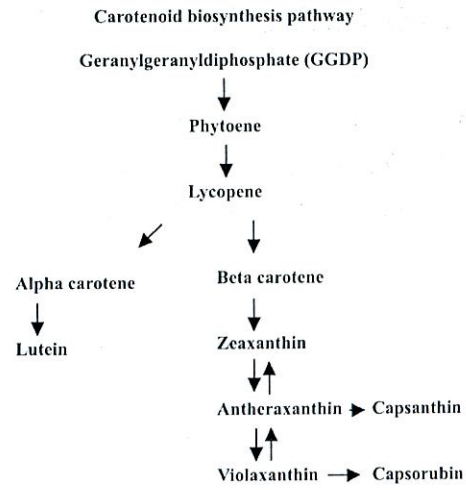
The genus *Capsicum* is indigenous to the Western Hemisphere. Christopher Columbus found *Capsicum* plants in the New World and initially it was confused with black pepper. Capsicums were consumed by the Indians as far back as 7000 B.C. and it is believed that plants were initially taken to Europe and from there they spread to Asia and Africa within 150 years. At present it is widely accepted that the genus *Capsicum*, consists of approximately 25 wild and five cultivated species. Among the five cultivated species, *C. annuum* is the most commonly cultivated for pungent and non-pungent fruits. The groups of spices belonging to the genus *Capsicum* are bell pepper, paprika, chilli and red pepper. All the five cultivated species of *Capsicum* are represented by genotypes with pungent or non-pungent fruits. Hence assigning a given genotype to a specific cultivated species based on fruit size, shape and pungency is difficult. Paprika is the ground product from the mild pungent or non-pungent fruited varieties of *Capsicum*, but in international trait paprika always refers to non-pungent fruits or its powder or oleoresin extracts from such fruits (Bosland and Votava, 2000). Hence besides unique place in the world diet in its ripe dried form (spice) as well as green fruits (vegetable), chilli is also used as an essential condiment in foods for natural red colour. India is rich in many *Capsicum* varieties with different quality attributes and is the leading consumer and exporter of chilli. Besides dry chilli and powder, oleoresin of chilli with low, medium or high pungency is also exported in large quantities. Chilli colourant, which imparts appealing colour, aesthetic flavour and aroma, has many end uses in various food, pharmaceutical and cosmetic preparations. Dried paprika powder and paprika oleoresin are the natural colour sources exempt from certification in United States of America and they can be used directly (Marmion, 1979).

Colour compounds

There are different varieties, forms and uses of *Capsicum* (Smith *et al.*, 1987 and Bosland, 1992). The Hungarian word for plants in the genus *Capsicum* is paprika. Thus Hungarian paprika may be of pungent or non-pungent depending on the cultivar (Somos, 1984). The quality of paprika is based on visual and extractable red colour, pungency level and to a lesser degree, nutrition. International traders use the term "paprika" for non-pungent, red *Capsicum* powder (Bosland, 1993). The colour value is the principal criterion for assessing the quality of paprika. The pigment content ranged from 0.1 to 0.8 per cent. The extractable colour value is measured by a spectrophotometric process, usually expressed in terms of ASTA colour value (American Spice Trade Association). Common extractable colour values present in the industry are 85, 100, 120 and 150 (Anu and Peter, 2000).

It is reported that approximately 20 carotenoids contribute to the colour of *Capsicum* powder.

Carotenoid compounds are yellow to red pigments composed of isoprene units and are normally fat-soluble colours (Bunnell and Bauernfeind, 1962). The keto-carotenoids, capsanthin, capsorubin and cryptocapsin are unique *Capsicum* carotenoids. The major red colour develops from capsanthin and capsorubin, while the yellow orange colour is from beta-carotene and violaxanthin. Capsanthin, the major carotenoid of ripe fruits, contribute up to 60 per cent of the total carotenoids. Capsanthin and capsorubin contents increase proportionately with advanced stages of ripeness, with capsanthin being the more stable of the two (Kanner *et al.*, 1977). The amount of carotenoids in fruit tissues at harvest depends on cultivar, maturity stage and crop growing conditions (Reeves, 1987).



Pungency

Pungency is defined as a "sharp, piercing, stinging, biting or penetrating quality" or "power to excite or stimulate" (Bosland, 1995). Pungency in chilli is due to chemical compounds known as capsaicinoids, which are alkaloid compounds found only in the plant genus, *Capsicum*. The nature of the pungency has further been established as a mixture of seven homologous branched-chain alkyl vanillyamides (Hoffman *et al.*, 1983). Capsaicin, also known as n-vanillyl-8-methyl-6-(e)-noneamide, is the most pungent of this group. It is sparingly soluble in water but highly soluble in fats, oils and alcohol.

The other compounds of the capsaicinoid groups are Dihydrocapsaicin, Nordihydrocapsaicin, Homocapsaicin and Homodihydrocapsaicin. The Scoville organoleptic test is a refined, systematic approach to measure the pungency level. It was the first laboratory test reported and used to measure the pungency in chillies. One 'Scoville Heat Unit' corresponds to about fifteen parts per million (ppm) of the capsaicin. The pure capsaicin has the maximum possible Scoville rating of 15 million units (Scoville, 1912). But nowadays the most common and reliable method to estimate pungency is by high performance liquid chromatography (HPLC).

Mode of action of the pungency

The capsaicinoids are not sensed by the taste buds. Heat sensation from the capsaicinoids results because of irritation of pain receptors. Even at dilutions down to one part per sixteen million sensation of warmth can be detected. Capsaicinoids release a chemical messenger, substance P that signals the brain about pain. Substance P causes the nervous system to telegraph a signal to the brain to flood the nerve endings with endorphins, which are the body's natural pain killers. The release of endorphins at nerve ending gives the body a sense of pleasure. Capsaicinoids are generally not destroyed in the mouth. The body masks their presence (Bosland, 1993).

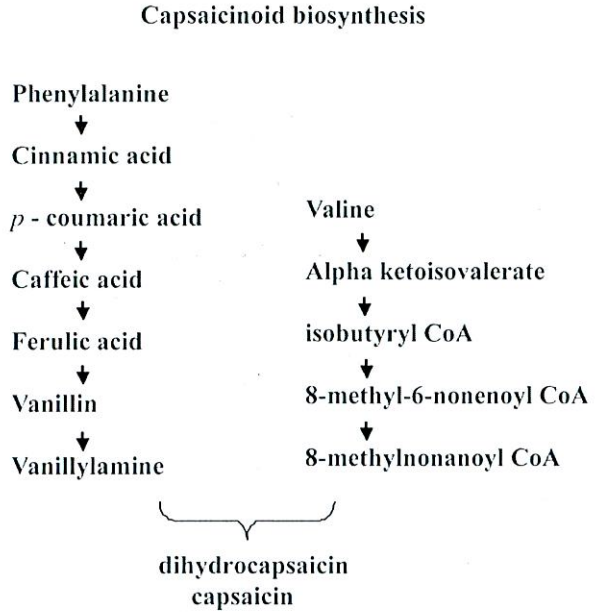
Capsaicin and dihydrocapsaicin together make up 80-90 per cent of the capsaicinoids found in hot peppers. In *Capsicum annuum* species, the total capsaicinoid content ranges from 0.1 to 1.0 per cent and the capsaicin to dihydrocapsaicin ratio is about 1: 1. In *Capsicum frutescens,* the total content of these compounds ranges from 0.4 to1.0 per cent with the ratio around 2: 1. Govindarajan *et al.* (1987) stated that in the case of *Capsicum annuum,* the ratio varied from 0.64 to 1.94.

Biosynthesis of capsaicin

Capsicum species synthesize and accumulate capsaicin specifically in capsaicinoid secreting organs localized in the placenta and the intercellular septum of fruits (Ohta, 1962). Accumulation of capsaicin occurs over a relatively short period during the latter stages of fruit development (Iwai *et al.*, 1979).

The capsaicinoids are produced in glands on the placenta of the fruit. While seeds are not the source of pungency, they occasionally absorb capsaicin because of their proximity to the placenta. No other plant part produces capsaicinoids (Bosland, 1995). The pathway leading to the capsaicinoids has two distinct arms,

one of which contributes the fatty acid moiety and the other the aromatic component (Bennett and Kirby, 1968; Leete and Louden, 1968). The first part of the reaction sequence comprising the aromatic pathway is shared with other pathway of general phenylpropanoid metabolism, which is common to all higher plants. This leads to the formation of a wide range of phenolic compounds, including cinnamates, benzoates, flavanoids, coumarins, tannins, cell wall phenolics and lignin-like substances (Fry, 1984). However, the later part of the reaction sequence from ferulic acid through vanillin and vanillylamine to capsaicin is found only in fruits of *Capsicum frutescens* synthesising capsaicinoids.



The fraction of pungency level in the fruit was studied by many workers such as Iwai *et al.* (1979), Suzuki *et al.* (1980) and Garciglia and Alejo (1990). The whole chilli consists of 40 per cent pericarp containing an inner sheath known as dissepiment, 56 per cent seeds and 4 per cent stalks. The pericarp contains almost all of the pungency, whereas the chilli seeds contain only traces of pungency with a capsaicin content of 0.005 per cent. The pungency of the pericarp is mostly concentrated in the dissepiment. The capsaicin content of chilli oleoresin ranged from 2.5 to 3 per cent. Correspondingly, the capsaicin content of pericarp oleoresin varies from 4.5 to 5.5 per cent. Also, the yield of oleoresin is variable from 6.5 to 10 per cent (Narayanan *et al.*, 1979).

Ranking of world *Capsicum* based on the pungency level (Anon, 2003; Anon, 2005a; Kumar *et al.*, 2006)

Scoville Heat Units	Pod type	Species	Name
0	Bell, Bydagi	*C. annuum*	Bell, Bydagi kaddi
1000	Ancho	*C. annuum*	Mulato
4,000	Serrano	*C. annuum*	Serrano
5,000	New Mexican	*C. annuum*	Sandia
8,000	Cayenne	*C. annuum*	Cayenne
17,000	Aji	*C. baccatum*	Aji Escabeeche
21,000	Hungarian	*C. annuum*	Santa Fe Grande
22,000	Jalapeno	*C. annuum*	Mitla
23,000	Cayenne	*C. annuum*	Long-Slim Cayenne
25,000	Jalapeno	*C. annuum*	Jalapeno M
30,000	Ellachipur	*C. annuum*	Ellachipur Sannam
36,000	Hindpur	*C. annuum*	Hindpur S7
60,000	Asian	*C. annuum*	Thai Hot, Jwala
70,000	Tepin	*C. annuum*	Chiltepin
75600	Kanthari	*C. annuum*	Kanthari White
88,350	Tabasco	*C. frutescens*	Bird eye chilli
120,000	Tabasco	*C. frutescens*	Tabasco
150,000	Habanero	*C. chinense*	Red Habanero
210,000	Habanero	*C. chinense*	Orange Habanero
455,000	-	*C. chinense*	Naga Jolokia
855,000*	-	*C. annuum*	Tezpur

(*Ritesh Mathur *et al.*, 2000)

Iwai *et al.* (1979) examined the fluctuation of pungent principles of hot pepper fruits of *Capsicum annuum* var. *annuum* cv. Karaystzubusta at different growth stages after flowering. Capsaicinoid was detected 20 days after flowering, which reached maximal level at around 40 days after flowering and then decreased gradually. Capsaicin was always the major component, occupying approximately 60 per cent of the capsaicinoids with a capsaicin: dihydrocapsaicin ratio of 1.36: 1.71.

Enzymes involved in the capsaicin metabolism

Phenylalanine ammonia lyase (PAL) is the enzyme that catalyses the first step in the pathway using L-phenylalanie as the substrate and rendering cinnamic acid, which is subsequently converted into r-coumaric, caffeic, and ferulic acids by the action of cinnamic acid – 4 – hydroxylase (CA_4H), r-coumaric acid – 3 – hydroxylase (CA_3H), and caffeic acid –*O*– methyltransferase (CAOMT) respectively. The enzymes that participate in the transformation of ferulic acid into vanillin and vanillylamine are unknown. Finally, the enzymatic condensation of vanillylamine and 8 – methyl – 6 – nonenoic acid is carried out by 'capsaicinoid synthetase (CS)' (Fujiwake *et al.*, 1980).

Fujiwake *et al.* (1982) determined that the biosynthetic site of capsaicinoids is in the placenta of the fruit and produced by the cinnamic acid pathway. Iwai *et al.* (1979) suggested that capsaicinoids production increase with maturity until a maximum and then decreased by rapid turnover and degradation of upto 60 per cent. In general, the fruit tissue showed a low activity of PAL, CA_4H and CS during the period from 0 to 22 days after flowering and a peak of activity for all the enzymes at the time of maximum growth in length of the fruit (Alejo and Peralta., 1993)

Fujiwake *et al.* (1982) observed that the capsaicinoids progressively accumulated during the development of the fruit, reached maximum of 120 mg/g in field grown after 45-50 days after fruit set and then started to decrease gradually. The capsaicinoids started to accumulate just before the increase in length ceased (between day 20 and 35) and active accumulation took place over a 15 day period (day 25 to 40) with an average increase of 333 mg day^{-1} per fruit. After day 40, the level of capsaicinoids per fruit remained virtually constant (Sukrasno and Yeoman, 1993).

Pepper peroxidase isoenzyme B6, is capable of oxidizing capsaicin and the phenolic precursors of capsaicin, with caffeic acid and ferulic acid being the best substrates among them and the basic peroxidase isoenzyme B6 is also located in cell walls (Bernal *et al.*, 1993). Bernel *et al.* (1995) suggested that this isoenzyme may be involved in the insolublisation of phenylpropanoids precursors. These results support the biochemical evidence which points to the existence of an oxidative competitive sink for phenylpropanoid intermediates of capsaicin biosynthesis, which probably competes with capsaicin itself in the cell wall of *Capsicum annuum*.

Genetic diversity

Consideration and assessment of genetic variability existing in a population is the basic requirement for any effective crop improvement programme. Introgression of different desirable traits spread in the diverse genotypes into single genotype is desirable for any crop. The diversity for pungency and colour value of the Indian chillies has been studied by different research workers and are summarized in the following tables.

Genetic resources and conservation

The International Plant Genetic Resources Institute (IPGRI, 1983) has made efforts to organize plant explorations and both working and base collections of *Capsicum* genetic resources. In 1986, the Asian Vegetable Research and Development Center (AVRDC) was invited to maintain a backup of global *Capsicum* collections (Poulos, 1994). In India, the systematic collection, characterization and conservation of *Capsicum* germplasm for quality were undertaken by both by Indian Council of Agricultural Research (Indian Vegetable Research Institute, Varanasi; Indian Institute of Spices Research, Calicut; Indian Agriculture Research Institute, Regional Station, Katrain and Indian Institute of Horticulture Research, Bangalore) and Sate Agricultural Universities (University of Agricultural Research, Dharwad and Tamil Nadu Agricultural University, Coimbatore).

A prospect for the future is to enhance germplasm pool development with other domesticated species of *Capsicum*, particularly from Mexico, Central and South America and parts of Asia and Africa where species in addition to *C. annuum* are cultivated.

Species compatibility

The species complex concept expounded by Pickersgill *et al.* (1988), Eshbaugh (1980) and Mcleod *et al* (1982) sets the framework for relatively successful crosses with in *C. annuum* group (*C. annuum, C. chinense* and *C. frutescens)* the *C. baccatum* group

Colour value of different Indian chillies under different locations (Anon, 2005)

Chilli types	Location	ASTA value	Capsaicin (%)
Guntur Sannam	Guntur, Warangal and Khammam, districts of Andhra Pradesh	32.11	0.226
Tomato Chilli	Warangal, Khammam, East and West Godavari districts of Andhra Pradesh	125.26	0.17
Birds Eye Chilli	Mizoram and some locations of Manipur	41.7	0.589
Byadagi (Kaddi)	Hubli district of Karnataka	156.9	Negligible
Ramnad Mundu	Ramnad district of Tamilnadu	32.95	0.166

Variability in colour value of different genotypes

No. of accessions evaluated	Range of colour value (ASTA units)	Reference
24	22.30 – 118.63	Bajaj *et al.* (1980)
11	90.00 – 136.36	Narayanankutty *et al.* (1992)
40	70.30 – 268.30	Anu *et al.* (2002)
36	56.07 – 187.70	Gadal *et al.* (2003)
59	15.92 – 180.00	Singh *et al.* (2003)
27	32.82 – 208.56	Prasath (2005)

(*C. baccatum* var *pendulum, C. baccatum* var *baccatum, C. praetermissum)* and the *C. pubescens* group (*C. pubescens. C. eximium, C. cardenasii)* and some between taxon groups or to other species such as *C. chacoense* or *C. tovarii*. Until recently chilli quality improvement in India was restricted within the species *C. annuum* only. *C. chinense* and *C. frutescens* are notable for biennial or perennial habit and for highly pungent and deep red fruits. Hybrids between

Variation of capsaicin content in Indian chilli cultivars

No. of accessions studied	Range of capsaicin content (%) among genotypes	Reference
4	0.0075 - 0.0800	Ananthasamy *et al.* (1960)
20	0.3300 - 0.4900	Sooch *et al.* (1977)
14	0.1200 - 0.5300	Sankarikutty *et al.* (1978)
24	0.1500 - 0.9250	Bajaj *et al.* (1980)
7	0.0030 - 0.0039	Maurya *et al.* (1984)
47	0.0130 - 0.1990	Teotia and Raina (1987)
73	0.0580 – 1.8100	Usha Rani (1997)
29	0.4390 - 0.5090	Anandanayaki (1997)
20	0.6600 - 0.7800	Aloni (1999)
40	0.5110 – 0.8750	Sathiyamurthy *et al.* (2001)
59	0.3300 – 0.7000	Singh *et al.* (2003)

C. annuum and *C. chinense* had high values of capsaicin, colour and oleoresin besides yield per plant (Pradeepkumar *et al.* 1993).

Breeding methods

Variety development

Traditionally, *Capsicum* breeding has been restricted to pure line and pedigree selection, and back crossing has been an essential method for introgression of genes, and by interspecific crosses. Two local types of chillies viz., Bydagi Dabbi and Kaddi and Tomato Chilli constitute the major source of raw material for paprika type oleoresin in India (Mathew, 2002). Joshi *et al.*, (1993) identified promising lines Viz., Kt-Pl-19, Kt-Pl-18 and Kt-Pl-8 with high yield and colour value by employing single seed descent method at Katrain, India. A few selections were made from bydage chillies at the Indian Institute of Horticultural Research and the selection was released as 'Arka Abir'.

All the condiment paprika cultivars grown in Hungary belong to *Capsicum annuum* L. covar. *longum* by botanical classification. There are two exceptions (Kalocasi A and M) which are cherry types and classified under *C. annuum* covar. *cerasiforme*. Regarding growth habit, the cultivars classified as continuous (Szegedi), semi determinate (Kalocasi 801) and determinate (Kalocasi D 601) (Somogyi *et al.*, 2003).

Heterosis for pungency and colour value

Although *Capsicum annuum* tolerate inbreeding, various workers have demonstrated considerable extent of heterosis for pungency and colour value. Deshpande (1933) recorded less pungency in hybrids than that of their parents. Absence of heterosis for this pungency was observed by Sharma and Saini (1977). Later, positive heterosis were reported by (Sekar, 1984; Anandanayaki 1997; Tanki, 1999; Hemavathy, 2000; Doshi and Shukla, 2000 and Sathiyamurthy, 2002), averaging between 8.84 and 98.08 per cent. Prasath (2005) reported that the heterosis for extractable colour, which ranged from -47.84 to 32.11 per cent over better parent. The variety, KDC –1, a hybrid derivative of cross between Byadagi and *C. frutescens* was released from UAS, Dharwad, Karnataka. It bears semi-wrinkled red fruits with high colour and low pungency.

Gene action

It has long been known that single dominant gene, *C*, controls the presence or absence of pungency in the fruits (Blum *et al.*, 2002). However, in the pungent types, the degree of pungency is quantitatively inherited and highly influenced by environment (Zewdie and Bosland, 2000). Studies conducted so far have indicated that matured red colour is dominant over yellow colour and is controlled by a single gene (*Y*) and later it was reported that colour compounds (carotenoids) is under the control of four different genes (*y*, c_1, c_2 and c^1) with epistatic interaction (Hurtado-Hernandez and Smith, 1985); Shifriss and Pilovsky , 1992). Popovsky and Paran (2000) reported mature fruit colour is under influence of three independent pair of genes (*c1*, *c2* and *y*).The presence of dominant alleles at these three loci results in red mature fruits, while recessive alleles give white mature fruits.

Biotechnological approaches

The growth of molecular biology and instrumentation paved the way for the latest breeding approach which is easy and quick. This also proved to be very important approach for quality breeding at the present and future conditions. Genes coding for enzymes in capsaicinoid biosynthesis pathway have been isolated (Curry *et al.* 1999; Kim *et al.* 2001 and Aluru *et al.* 2003). A study of placental transcript levels for the suite of structural genes thought to be involved in capsaicin biosynthesis demonstrated a general correlation between transcript level and the degree of pungency, consistent with coordinate regulation (Curry *et al.*, 1999). QTL interval analysis for individual and total capsaicinoid content identified a major QTL, termed *cap*, which explained 34 - 38 per cent of the phenotypic variation for this trait in two growing environments (Blum *et al.* 2003). The molecular linkage map of *C* locus has been prepared and a pungency related gene has been found to be located on chromosome 2. Based on sequences of caspsaicinoid synthase (*CS*), sequence characterized amplified region (SCAR) markers have been developed and their usefulness in early detection of pungent or non-pungent genotypes have been also demonstrated (Lee *et al.*, 2005).

The absence of capsanthin and capsorubin in yellow fruits correlates with the lack of expression of CCS (capsanthin-capsorubin synthase) enzyme in yellow fruits (Bouvier *et al.*, 1994; Houlne *et al.*, 1994). Co-dominant DNA markers for the identification of red and yellow-fruited genotypes at seedling stage have been developed (Popovsky and Paran, 2000). Metabolic engineering of *Capsicum*, with regard to enhanced capsaicin or carotenoid pathway gene has been demonstrated in several systems, including rice (Ye *et al.*, 2000). However, there is a need to get a high pigment and low pungency product from *Capsicum*, which will be of value for pharamaceuticals, consumer purposes and as a food colourant. Attempts to get both a placenta-specific expression of capsaicinoids and a fruit wall specific expression of carotenoids is also needed. Such developments need to be completed for disease resistance, which would result in the overall improvement of the *Capsicum* for pre and post harvest applications for augmenting qualitative and quantitative output of the products from *Capsicum*.

Conclusion

Achievements in *Capsicum* breeding have culminated in the release of specialized market types and the advent of hybrid varieties. Land races and facultative self pollinated varieties also continue to occupy major production areas in developing countries. The recent discovery of new medicinal properties of carotenoids and capsaicinoids of *Capsicum* could be of great potential to become evermore versatile crop in world agriculture. Thus, an international focus is required on the development of *Capsicum* for high colour and low or nil pungency through germplasm, genetics and breeding. As far is India is concerned, majority of the

chilli area is confined to tropics and therefore, our attention should be the development of paprika type chilli genotypes suitable to tropical conditions. Also, systematic attempt has to be made in germplasm collection and evaluation of low pungent, high colour types such as Bydagi and tomato chilli. Furthermore, identification of hybrid combinations with high economic yield coupled with high colourant and oleoresin recovery would help in increasing the colourant and oleoresin yield per unit area substantially.

References

Alejo NO and Peralta JEG (1993). Activity of enzymes involved in capsaicin biosynthesis in callus tissues and fruits of chili peppers (*Capsicum annuum* L.). *Plant Physiol.* 141: 147-152

Aloni B (1999). Effect of fruit weight, height and dry matter production on the morphology of pepper flower and fruit shape. *Ann. Bot.* 83(5): 529-534.

Aluru MR, Mazourek M, Landry LG, Curry J, Jahn M and O'Connell MA (2003). Differential expression of fatty acid synthesis genes, *Acl, Fat* and *Kas*, in *Capsicum* fruit. *J Exp Bot.* 54: 1655-1644.

Anandanayaki D (1997). Genetic studies of yield and quality parameters in chilli (*Capsicum annuum* L.) through diallel analysis. *M.Sc. (Hort.) Thesis*, Tamil Nadu Agric. Univ., Coimbatore.

Ananthasamy TS, Kamat VN and Pandya HG (1960). Capsaicin content in chilli varieties. *Curr. Sci.* 29(7): 271.

Anonymous (2003). Hotness of chillies, chemistry and Scoville units. *http://www.chez-williams.com/chemistry_and_scoville_units.htm.*

Anonymous (2005). Pungency level of Indian chillies. *http://www.indianspices.com*

Anonymous (2005). Colour value of Indian Chillies. *http://www. indianspices.com/html/s0623frm.htm.*

Anonymous (2005). Engineering carotenoid production in *Capsicum* fruits. *http:// www.* uni-hohenheim.de/biotechnologie/www/research/capsicum.php

Anu A and Peter KV (2000). The chemistry of paprika. *Capsicum and Egg Plant Newsl.* 19: 19-22.

Anu, A., Babu, K.N. and Peter, K.V. (2002). Evaluation of paprika genotypes in Kerala. *Indian J. Plant Genet. Resour.* 15(2): 93-99.

Bajaj, K.L., Kaur, G. and Sooch, B.S. (1980). Varietal variation in some important chemical constituents in chilli (*Capsicum annuum* L.) fruits. *Veg. Sci.* 7: 48-54.

Balbaa, S. I., Karawya, M. S. and Girgis, A. N. (1968). The capsaicin content of *Capsicum* fruits at different stages of maturity. *Lloydia.* 31: 272-274.

Ben Chaim, A., Paran, I., Grube, R., Jahn, M., Wijk R.and Peleman J. (2001). QTL mapping of fruit related traits in pepper (*Capsicum annuum*). *Theor. Appl. Genet.* 102: 1016 – 1028.

Bennett, D. J. and Kirby, G. W. (1968). Constitution and biosynthesis of capsaicin. *J. Chem. Soc.* 442-446.

Bernal, M. A., Calderon, A. A., Pedreno, M. A., Munoz, R. R., Barcelo A. and Caceres, F. M. (1993). The subcellular localization of iso-peroxidases in *Capsicum annuum* leaves and their different expression in vegetative and flowering plants. *Ann. Bot.* 72: 415-421.

Bernal, M. A., Calderon, A. A., Ferrer, M. A., Caceres, F. M. and Barcelo. A. R. (1995). Oxidation of capsaicin and capsaicin phenolic precursors by the basic peroxidase isoen-zyme B6 from hot pepper. *J. Agric. Food Chem.*, 43: 352-355.

Blum, E., Kede, L., Mazourek, M., Yoo, E.Y., Jahn, M. and Paran, I. (2002). Molecular mapping of the C locus for the presence of pungency in *Capsicum*. Genome., 45: 702-705.

Blum, E., Mazourek, M., O'Connell, M., Curry, J., Thorup, T., Liu, K., Jahn M. and Paran, I. (2003). Molecular mapping of capsaiciniod biosynthesis genes and quantitative trait loci analysis for capsaicinoid content in *Capsicum*. *Theor. Appl. Genet.* 108: 79 – 86.

Bosland, P.W. (1992). Chillies: a diverse crop. *Hort. Tech.* 2(1): 6-10.

Bosland, (P.W). 1993. Breeding for quality in Capsicum. *Capsicum Newsl.* 12: 25 – 31.

Bosland, P.W. (1995). *Capsicum: history, cultivation and uses*. Spices, herbs and edible fungi. Elsevier Publ., New York. pp. 347-366.

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

Bunnell, R.H and Bauernfeind, J.C. (1962). Chemistry, uses and properties of carotenoids in foods. Food. Tech., 16: 36-43.

Curry, J., Aluru, M., Mendoza, M., Nevarez, J., Melendrez, M. and O'Connell, M.A. (1999). transcripts for possible capsaicinoid biosynthetic genes are differently accumulated in pungent and non-pungent *Capsicum* spp., *Plant. Sci.* 148: 47-57.

Deshpande, R.B. (1933). Studies in Indian chillies 3. The inher-itance of some characters in *Capsicum annuum*

L. *Indian J. Agric. Sci.* 3: 219-300.

Doshi, K.M. and Shukla, P.T. (2000). Expression of heterosis in chilli (*Capsicum annuum* L.). *Capsicum and Egg Plant Newsl.* 19: 66-69.

Esbaugh, W.H. (1980). The taxonomy of the genus *Capsicum* (Solanaceae), *Phytologia*. 47 (3): 153-156.

Fry, S. C. (1984). Incorporation of [^{14}C] cinnamates into hydrolase resistant components of the primary cell wall. *Phytochem*. 23: 54-64.

Fujiwake, H., Suzuki, T., Oka S. and Iwai, K. (1980). Enzymatic formation of capsaicinoids from vanillylamine and iso-type fatty acids by cell – free extracts of *Capsicum annuum* var. *annuum* cv. Karayatsubusa. *Agric. Biol. Chem*. 44: 2907-2912.

Fujiwake, H., Suzuki, T., Iwai, I. (1982). Intercellular distribution of enzymes and intermediates involved in biosynthesis of capsaicin and its analogues in *Capsicum* fruits. *Agric. Biol. Chem*. 46(11): 2685-2689.

Gadal, C.M., Manjunath, A., Nehru, S.D. and Kulkarni, R.S. (2003). Genetic variability and correlation in chilli (*Capsicum annuum* L.) with special reference to quality characters. *Mysore J. Agric. Sci*. 37(4): 325-331.

Garciglia, S. R. and Alejo, N. O. (1990). Increased capsaicin content in PFP-resistant cells of chili pepper (*Capsicum annuum* L.). *Plant Cell Rep*. 8: 617-620.

Govindarajan, V.S., Rajalakshmi, D. and Chand, N. (1987). Capsicum production, technology, chemistry and quality. Part IV. Evaluation of quality. *Critc. Rev. Food Sci. Nutr*. 25 (3): 185-282.

Hemavathy, K. (2000). Studies on physiological basis of heterosis in chillies (*Capsicum annuum* L.). *M.Sc. (Hort.) Thesis*, Tamil Nadu Agric. Univ., Coimbatore.

Hoffman, P.G., Lego, M.C. and Galetto, W.G. (1983). Separation and quantification of red pepper major heat principles by reverse-phase high pressure liquid chromatography. *J. Agric. Food Chem*. 31: 1326-1330.

Houlne, G., Schantz, M.L., Meyer, B., Pozueta-Romero, J. and Schantz, J. (1994). A chromoplast specific protein in *Capsicum annuum*: Characterization and expression of the corresponding genes. *Cur. Genetics*. 26: 524-527.

Hurtado-Hernandez, I.H and Smith, P.G. (1985). Inheritance of matured fruit colour in *Capsicum annuum* L. *J. Hered*. 76: 211-213.

IBPGR, (1983). Genetic resources of Capsicum. *International Board for Plant Genetic Resources, Food and Agriculture Organization of the United Nations. Rome, Italy, 49pp.*

Iwai, K., Suzuki, T. and Fujiwake, H. (1979). Formation and accumulation of pungent principle of hot pepper fruits, capsaicin and its analogues, in Capsicum annuum *var. annuum cv. Karayatsubusa at different growth stages after flowering.* Agric. Biol. Chem. *43: 2493-2498.*

Joshi, S., Thakur. P.C., Verma, T.S. and Verma, H.C. (1993). Selection of spice paprika breeding lines. *Capsicum and Egg Plant Newsl.*, 12: 50 – 52.

Kanner, J., Harel. S., Palevitch, D. and Ben-gera, I. (1977). Colour retention in sweet paprika powder as affected by moisture contents and ripening stage. *J. Food. Tech*. 12: 59-64.

Kim, M., Kim, S., Kim, S. and Kim, B.D. (2001). Isolation of cDNA clones differently accumulated in the placenta of pungent pepper by suppression subtractive hybridization. *Mol. Cells*. 11: 213 – 219.

Kumar, S., Kumar, R. and Singh, J. (2006). *Cayenne/ American pepper*. Hand book of herbs and spices: Vol 3. Woodhead Publishing Ltd, London.pp 299-312.

Lee, C.J., Yoo, E.Y., Shin. J.H., Lee. J., Hwang, H.S. and Kim, B.D. (2005). Non-pungent *Capsicum* contains a deletion in the capsaicinoid synthase gene, which allows early detection of pungency with SCAR markers. *Mol. Cells*. 19: 262-267.

Leete, E. and Louden, M. C. L. (1968). Biosynthesis of capsaicin and dihydrocapsaicin in *Capsicum frutescens. J. Amer. Chem. Soc*. 90: 6837-6841.

Marmion, D.M. (1979). *Handbook of U.S. colorants for foods, drugs and cosmetics*. John Woley and Sons, Inc. New York.

Mathew, A.G. (2002). *Research needs of Indian paprika*. Proc. Interface between Spices Exporters, Farmers and Scientists. Indian Inst. Spices Res., Calicut. pp. 3-6.

Maurya, K.R., Tha, R.C. and Chaudhary, M.L. (1984). Physico-Chemi-cal qualities of some varieties of chillies. *Indian Cocoa. Arecanut Spices J*. 7(4): 120-121.

Mcleod, M.J., Guttman, S. I. and Eshbaugh, W.H. (1982). Early evolution of chilli pepper (*Capsicum*). *Economic Botany*. 36(4): 361-368.

Narayanankutty, C., Narayanankutty, M.C., Rajan, K.C. and Peter, K.V. (1992). Evaluation of selected chilli varieties for yield and quality parameters. *Spice India*. 5(2): 11-13.

Narayanan, C. S., Sumathikutty, M. A., Sankarikutty, B., Rajaraman, K., Bhat, A. V. and Mathew, A. G. (1979). Studies on separation of high pungent oleoresin from Indian chilli. *J. Food Sci. Tech*. 17: 136-138.

Ohta, (1962). Physiological and genetical studies on the pungency of *Capsicum* IV. Secretary organs, receptacles and distribution of capsaicin in the *Capsicum* fruit. *Japanese J. Breed*. 12: 179-183.

Pickersgill, B. (1988). The genus *Capsicum*: a multidisciplinary approach to the taxonomy of cultivated and wild plants. *Biologisches Zentralblatt*.

107: 381-389.

Popovsky, S and Paran, I. (2000). Molecular genetics of the y locus in pepper: its relation to capsanthin-capsorubin synthase and to fruit colour. *Theor. Appl. Genet.* 101: 86-89.

Poulos, J.M. (1994). Pepper breeding (*Capsicum* spp.): achievements, challenges and possibilities. *Pl. Breed. Abstr.* 64 (2): 143–155.

Pradeepkumar, T., Gopalkrishnan, T.R. and Peter, K.V. (1993). Interspecific hybridization in the genus *Capsicum*. *Veg. Sci.* 20(2): 132-141.

Prasath, D. (2005). Studies on development of F_1 hybrids in paprika type chilli (*Capsicum annuum* L.) for high yield, colourant and resistance to anthracnose disease *Ph.D. Thesis*, Tamil Nadu Agric. Univ., Coimbatore, India.

Reeves, M.J. (1987). Re-evaluation of capsicum colour data. *J. Food. Sci.* 52:1047-1049.

Ritesh Mathur., Dangi. R.S. Dass, S.C. and Malhotra, R.C. (2000). The hottest chilli variety in India. *Cur. Sci.* 79(3): 287-288.

Sankarikutty, B., Sumathikutty, M.A. and Narayanan, C.S. (1978). Standardization of extraction of pungency from whole chillies for estimation of capsaicin. *Indian Spices*, 15: 19.

Sathiyamurthy, V.A. (2002). Studies on the development of F_1 hybrids in chilli (*Capsicum annuum* L.) with high capsaicin, oleoresin and yield. *Ph.D. Thesis*, Tamil Nadu Agric. Univ., Coimbatore, India.

Sathiyamurthy, V.A., Veeraragavathatham, D. and Chezhiyan, N. (2001). Studies on the capsaicin content in chilli hybrids. *Capsicum and Egg Plant Newsl.* 21: 44 – 47.

Scoville, W. B. (1912). Note on *Capsicum*. *J. Amer. Pharm. Assoc.* 1: 453.

Sharma, P.P. and Saini, S.S. (1977). Heterosis and combining ability for yield and agronomic characters in pepper. (*Capsicum annuum* L.). *Veg. Sci.* 4(1): 43-48.

Shifriss, C and Pilovsky, M. (1992). Studies of the inheritance of mature fruit colour in *Capsicum annuum* L. *Euphytica*. 60(2): 123-126.

Singh, R., Hundal, J.S. and Neena Chawla. (2003). Evaluation of chilli (*Capsicum annuum* L.) genotypes for quality components. *Indian J. Agric. Sci.* 73(1): 51-53.

Smith, P.G., Villalon, B. and Villa, P.L. (1987). Horticultural classification of pepper grown in the United States. *Hort. Sci.* 22: 11-13.

Sooch, B.S., Thakur, M.L. and Kaur, G. (1977). Evaluation of some chilli (*Capsicum annuum* L.) genotypes for capsaicin and ascorbic acid contents. *Indian Fd. Packer.* 31(6): 9-11.

Somogyi, N., Moor Andrea and Pek Miklos. (2003). *Capsicum- The genus Capsicum*. Medicinal and Aromatic Plants – Industrial profiles; v.33. Taylor and Francis, London.pp 144-162.

Somos, Andras. (1984). *The paprika*. Akademiai Kiado, Budapest, 320pp

Sukrasno, N. and Yeoman, M. M. (1993). Phenylpropanoid metabolism during growth and development of *Capsicum frutescens* fruits. *Phytochem.*, 32(4): 839-844.

Suzuki, K., Fujiwake, H. and Iwai, K. (1980). Intercellular localization of capsaicin and its analogous, capsaicinoid in capsicum fruit. I. Microscopic investigation of structure of the placenta of the *Capsicum annuum* var. *annuum* cv. Karyatsubusa. *Plant cell physiol.* 21: 839-853.

Tainter, D.R and Grenis, A.T. (2003). *Spices and Seasonings: a food technology hand book*. A Jhon Wiley and Sons, Inc., New York.

Tanki. (1999). Heterosis and combining ability studies in hot pepper. *Appl. Biol. Res.* 1(1): 11-14.

Teotia,. M.S. and Raina, B.L. (1987). Post harvest and quality aspects of chillies grown in Punjab, Haryana, Himachal Pradesh and Jammu and Kashmir. A comparative study. *Bever-age Food World.* 14(3): 17.

Usha Rani. (1997). Evaluation of chilli (*Capsicum annuum* L.) germplasm for fruit characters. *J. Res. ANGRAU.* 24(2): 150-153.

Zewdie, Y and Bosland, P.W. (2000). Evaluation of genotype, environment and genotype-by-environment interaction for capsaicinoids in *Capsicum annuum* L. *Euphytica*. 111: 185-190.