Chapter-2

Review of Literature

2. Review of Literature

In this chapter, attempts have been made to describe the taxonomic status of pepper (*Capsicum* spp.), carotenoids and capsaicinoids present in the pepper fruits, their biosynthetic pathways. Special attempts have been made to provide some innovative uses including spice uses of pepper in a tabular form.

2.1 The genus *Capsicum*

The name genus *Capsicum* perhaps comes from the Latin word 'capsa', meaning chest or box because of the shape of fruits, which enclose seeds very neatly, as in a box (Berke and Shieh, 2000). The *Capsicum* has been known since the beginning of civilization in the Western Hemisphere and has been a part of the human diet since 7500 BC. Hence chilli is among the oldest cultivated crops of the Americas.

The *Capsicum* ($2n = 24$) encompasses a diverse group of plants producing pungent or non-pungent fruits. At present, it is widely accepted that the genus consists of approximately 25 wild and 5 domesticated species. The genus *Capsicum* was domesticated atleast five times by prehistoric peoples in different parts of South and Center America, resulting in five domesticated species, viz., *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* R. & P. (IBPGR, 1983). Among these, *C. annuum* is most widely spread and has world wide commercial distributions. On the basis of gene flow through natural and conventional hybridization, *Capsicum* species are grouped in three species complexes (Kumar *et al.*, 2006a). Except that of *C. pubescens*, wild forms of the remaining four cultivated species are known. The *C. annuum* was domesticated in highland of Mexico and includes most of the Mexican chile (syn. chilli), most of the chilli of Asia and Africa, sweet peppers of temperate countries. However, due to the non-adaptability of *C. annuum* in lowland tropics of Latin America, its cultivation was replaced by *C. frutescens* and *C. chinense* (Pickersgill, 1997). The cultivation of *C. baccatum* and *C. pubescens* are mostly restricted to Latin American countries like Peru, Bolivia, Columbia and Brajil. Although *C. annuum* is most widely cultivated in India, *C. frutescens*, *C. chinense* and *C. baccatum* are also grown in specific regions, especially in North-East region and state of Kerela.

The five cultivated species are represented by genotypes with pungent (hot pepper) or non-pungent (sweet pepper) fruits and have huge variability for fruit size/shape and often genotypes with similar fruit morphology exist across the species. Hence assigning a given genotype to a cultivated species based on fruit size, shape and pungency is difficult. Nonetheless, certain flower and fruit descriptors may be used to assign a genotype to a cultivated species without much doubt (Table 2.1). Recently, RAPD markers specific to *C. chinense* and *C. frutescens* have also been reported (Baral and Bosland, 2004).

2.2 Botany

Tap root system of chilli consists of a main root with lateral roots with uniform distribution on the main axis and the occurrence of adventitious roots is very rare in pepper. Stems are branched, erect or semi-prostrate, fleshy often woody at the base, round or slightly angular growth normally indeterminate. Flowers are small, terminal but due to form of branching, appear to be axillary, small calyx, rotate companulate corolla and 5-6 stamens, which are inserted near the base of corolla. Unlike other members of nightshade (Solanaceae) family, *viz.* tomato, eggplant and potato, chilli leaves lack phenols. Therefore it is believed that nature has provided pepper capsaicinoids (pungency) pathway to protect plants from the enemies, especially mammals. This could be viewed as an analogue of phenol pathway present in other members of nightshade family (Kumar *et al.*, 2006a). The evolutionary significance of pungency in the fruits of chilli has actually been explained and demonstrated as directed deterrence (capsaicin) hypothesis, wherein capsaicin in fruits function selectively to discourage seed predators like mammals without deterring beneficial seed dispersers like birds (Tewksbury and Nabhan, 2001).

Table 2.1. Distinguishable morphology of five cultivated species of *Capsicum*

Species		Distinguishable morphological feature/s
C. annuum	:	White corolla and white filaments
C. frutescens	:	Yellow/greenish corolla and purple filaments
C. chinense	:	Annular constriction on pedicel attachment and yellow/greenish corolla
C. baccatum	:	Yellow or greenish yellow spots on corolla
C. pubescens	:	Hairy stems/leaves and black/brown seeds

2.3 Innovative utility

Chilli fruits are most popular and widely used condiment all over the world. Fruits are consumed in fresh, dried or processed forms as vegetable or spice. Fruits are extensively pickled in salt and vinegar. Sweet (non-pungent) peppers are widely used at green-immature or mature stage as a vegetable. Chilli fruits are being used as a food flavoring, a coloring agent, a pharmaceutical ingredient and many other innovative ways and the uses of a large number of cultivars within the five cultivated species have grown exponentially (Bosland, 1996). Thus fruits of the genus *Capsicum* have as many versatile uses as its diversity (Bosland, 1996; Dewitt *et al.*, 1998; Bosland, 1999; Table 2.2).

Table 2.2. Versatile and innovative uses of pepper

I. Fresh uses: Immature-green and mature-red fruits

- Green or red ripe fruits of hot pepper with variable degree of pungency are invariably added in most of the South Asian curries.
- Immature or mature fruits of sweet peppers are exclusively prepared as vegetable.
- Immature sweet pepper fruits are added in many Chinese cuisines.
- Immature mild pungent fruits are deep fried with gram flour and consumed in India.
- Fresh green non-pungent or mild pungent fruits are consumed as salad.
- In Philippines, leaves are added to soup and stew and consumed because unlike leaves of tomato and eggplant, leaves of *Capsicum* species do not contain phenols. The upper shoots of the plants are sold in bunches, just like other leafy vegetables (Bosland, 1999).

II. Fresh processing: Sauce, paste, pickles, beer!

- Green or red ripe fruits with variable degree of pungency are used to prepare sauce.
- Red ripe and mild pungent fruits are stuffed with certain spices in North Indian states and prepared as pickles. Similarly, green fruits are also pickled in edible oils. Red ripe fruits are also stored in vinegar/citric acid for several years.
- In US, mild pungent fruits are used prepared as salsa and consumed with snacks.
- Red ripe fruits are used in the preparation of tomato ketchup for improving its colour.
- The Black Mountain Brewing Co. in Arizona developed a pepper beer with an idea to produce a spicy beer for a local Mexican restaurant and idea worked (Bosland, 1993).

III. Dried spice: Mature whole fruits and powder

- Dry intact fruits or grounded powder are invariably added in the preparation of almost all South Asian chicken, egg and vegetable curies.

IV. Colouring and flavoring agents: Oleoresins (carotenoids) extracts or powder

- Paprika oleoresin (colour extracts from non-pungent fruits) is natural colouring agent, therefore, it is considered to be among the best substitute of synthetic colour used in food and cosmetic industries.

- Cosmetic industry uses non-pungent oleoresin to prepare its products.
- In food processing industries, especially in meat industry, concentrated oleoresin is added to the processed meat to impart attractive colour.
- In beverage industries, oleoresins are used to improve colour and flavour of its products.
- In certain region of the world (e.g. Japan and South Korea) oleoresins are mixed with chicken feed in order to impart attractive red colour to chicken skin and red colour to yolk.
- Oleoresins are mixed with the feed of flamingoes in zoo and koi in aquariums for improving the feather colour (Bosland, 1996).

V. Ethno-botanical/traditional medicine: Fruit extracts and powder (pungent fruits)

- Traditionally, fruits are consumed to stimulate digestion (stimulates the flow of saliva and gastric juice), raise body temperature and cures common cold.
- Mayas mix fruits with corn flour to produce 'chillatolli', a treatment for common cold. Mayas also use them to treat asthma, coughs, and sore throats. The Aztecs used fruit pungency to relieve toothaches (Bosland, 1999). In many African countries, fruits are consumed with the belief that it improves the complexion and increases passion (Bosland and Votava, 2000).
- Fruits are added to rose-gargles to cure pharyengitis. Fruits are also consumed as it also has carminative effects. The West Indian native, soak fruits in water, add sugar and sour orange juice and drink it during fever (http.//www.dominion.com).
- In Columbia, the Tukano native pore a mixture of crushed fruits and water into their noses to relive a hangover and effectiveness of a night of dancing and drinking alcoholic beverages (Bosland, 1999).
- In Columbia and India, victims of snakebite are given pungent fruits to taste in order to sense the functioning of nervous system caused due to snake venom. In similar fashion, freshly crushed fruits or powder are used to reduce swelling and draw out poison of bee strings, spider bites and scorpion strings (Dewitt *et al.*, 1998).

VI. Modern medicine/pharmaceuticals: Capsaicinoids and carotenoids

- The pharmaceutical industry uses capsaicin as a counter-irritant balm for external application (Carmichael, 1991).
- Capsacinoids (mainly capsaicin) are active ingredient in 'Heet' and 'Sloan's

Liniment', massage liniments used for sore muscles (Bosland, 1996).

- Capsaicinoids are used in the preparation of powder, tincture, plaster ointments and medicated wools.
- Pharmaceutical industry uses capsaicinoid extracts to prepare certain drugs (sprays), which are applied externally to stop pains of arthritis (rheumatoid arthritis, osteoarthritis), artily diseases (peripheral neuropathies) and to relive cramps (Cordell and Araujo, 1993; Bosland, 1996).
- 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).
- Carotenoids found in fruits (β-carotene, acyl derivatives of capsanthin, acyl derivatives of capsorubin) have been shown to be inhibits LDL oxidation *in vitro* with probable lowering the "atherogenic" LDL subfraction production (Medvedeva *et al.*, 2003).
- 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; Molnar *et al.*, 2004).
- 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 cancerchemoprevention (Nishino *et al.*, 2002).
- The water extract of 'paradicsompaprika' (mainly containing capsanthin) 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).

VII. Insecticide/repellent: Capsaicinoids

- Capsaicin extracts are used as an effective repellent against mice damaging the underground cables and protect germinating seeds from squirrels (Bosland, 1996).

VIII. Spiritual: Whole fruits

- In India, fruits are stringed on a thread along with a lime fruit and hung on the entrance of houses/shops with the belief that it will keep evil away (Kumar and

Rai, 2005).

- Red dry fruits are used in a desire to remove the bad consequences of evil eyes on younger babies in North Indian states.
- Traditionally, in New Mexico of US, mature fruits are stringed (called 'ristras') and hung on the entrance of house as a symbol of hospitality (Bosland, 1992).

IX. Ornamental: Whole plants or fruits

- Certain genotypes of pepper are grown for their attractive plant shape, dense and colourful foliage and fruits. Several colours of fruit (at various maturing stage) can be found on a single plant making plant a very attractive ornamental (Bosland and Votava, 2000).

X. Defense/punishment: Capsaicin extracts/ fruit powder

- In India, traditionally villagers keep fruit powder in house as a self-defense weapon against dacoits.
- Sprays containing capsaicin are emerging as a safe weapon for armless people, especially for women in metros of many countries including India.
- Now a day, capsaicin spray has replaced the maces and tear gas in the police departments of many countries to control unruly mobs and criminals.
- In Mexico, India and other Latin American countries, pepper powder is rubbed on children's thumps to prevent sucking (Dewitt *et al.*, 1998). Similary in India, fruit paste is applied on mother's nipple to get rid of prolonged breast feeding.
- Maya threw chilli powder into the eyes of young girls who stared at boy or men and they squirt fruit juice on the private parts of unchaste women (Dewitt *et al.*, 1998).

2.4 Pungency (capsaicinoids)

The pungent-oily substances from the fruits of hot pepper was first discovered and isolated by Bucholz in 1816 and the most active ingredient (named capsaicin) was isolated by Thresh in 1846 (Govindarajan, 1987). The burning sensation (pungency) one gets from eating pepper fruits is caused by alkaloids called capsaicinoids, which are uniquely produced in *Capsicum*. Capsaicinoids are acid amides of C_9-C_{11} branched chain fatty acids and vanillylamine. 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 an instrument called 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. The capsaicin contents (ppm) are multiplied by 15 to convert it to SHU.

2.4.1 Biosynthetic pathways

More than 15 different capsaicinoids are known to be found in pepper fruits, which are synthesized and accumulated in the epidermal cells of placenta of the fruits. Among these, capsaicin and dihydrocapsaicin accounts for more than 80% of the total capsiacinoids that determine the pungency (Bosland and Votava, 2000). These two most common capsaicinoids differ in the degree of unsaturation of a 9-carbon fatty acid chain and other naturally occurring capsaicinoids differ in chain length as well as degree of unsaturation (Curry *et al.*, 1999).

Two pathways 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 Yewman, 1993; Curry *et al.*, 1999). During 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 is 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.,* 1994a). 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 Gómez-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 Gómez-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 i.e. at the site of capsaicinoids. 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).

2.4.2 Genetics and markers

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 *et al.,* 2002). Thus pungency is genotype dependent (Table 2.3). However, in the pungent types, the degree of pungency is quantitatively inherited and highly affected by the environments (Zewdie and Bosland, 2000). 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.,* 2005). 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).

2.5 Colour (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 lutine, xeaxanthin, violaxanthin, anthraxanthin, β-cryptoxanthin and β-carotene; and the red pigments capsanthin, capsorubin and cryptocapsin, 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.

Table 2.3. Name of some popular genotypes with their pungency level (Kumar *et al.,* 2006a)

Name	Pod type	Species	Scoville Units
Naga Jolokia	Cuban	*C. frutescens*	855,000
Orange Habanero	Habanero	*C. chinense*	210,000
Red Habanero	Habanero	*C. chinense*	150,000
Tabasco	Tabasco	*C. frutescens*	120,000
Tepin	Tepin	*C annuum*	75,000
Chiltepin	Tepin	*C. annuum*	70,000
Thai Hot	Asain	*C. annuum*	60,000
Jalapeno M	Jalapeno	*C. annuum*	25,000
Long-Slim Cayenne	Cayenne	*C. annuum*	23,000
Mitla	Jalapeno	*C. annuum*	22,000
Santa Fe Grande	Hungarian	*C. annuum*	21,000

Name	Pod type	Species	Scoville Units
Aji Escabeche	Aji	*C. baccatum*	17,000
Long-Thick Cayenne	Cayenne	*C. annuum*	8,500
Cayenne	Cayenne	*C. annuum*	8,000
Pasilla	Pasilla	*C. annuum*	5,500
NuMex Primavera	Jalapeno	*C. annuum*	5,000
Sandia	New Mexican	*C. annuum*	5,000
NuMex Joe E. Parker	New Mexican	*C. annuum*	4,500
Serrano	Serrano	*C. annuum*	4,000
Mulato	Ancho	*C. annuum*	1,000
Bell	Bell	*C. annuum*	0

2.5.1 Chemistry

The basic carotene structure can undergo several structural modifications, namely, cyclization, hydroxylation and epoxidation, yielding the great 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). 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.

2.5.2 Genetics and markers

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 the mature fruit colour is controlled by three independent pairs of genes (*cl, c2* and *y*). 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). Co-dominant DNA markers for the identification of red and yellow-fruited genotypes at seedling stage are available (Popovsky and Paran, 2000).

2.6 Flavours

Although pepper is commonly known for its pungency, it is often used in meals because of flavour. The pyrazine 2-methoxy 3-isobutyl-pyrazine, the green bell pepper smell, is one of the most potent volatiles known so far. The human can detect this smell at 2 parts per trillion (Bosland and Votava, 2000). In *C. annuum* and *C. frutescens*, 102 volatiles have been found (Keller *et al.,* 1981). The aroma compounds vary greatly between the cultivated species and also between genotypes within the same species. For example, tabasco (*C. frutescens*) contains no pyrazine compounds, while its presence is the characteristic feature of bell pepper (*C. annuum*). The delicate flavours of the fruits can be differentiated after a few years of experience. Ancho is sweetish, mulatto is chocolaty, mirasol is fruity and chilpotle is smokey. Grinding the fruits produces one flavour, toasting produces another and soaking the fruits in water produces yet another flavour (Bosland 1996).

2.7 Spice production and quality

Chilli spices are the powders that are derived from pungent, mild pungent or non-pungent red fruits. Therefore, the main fruit quality parameters colour and pungency. Apart from these, colour retention during storage, fruit wall thickness, fruit size, shape and weight are also important quality parameters. Yet another important quality concern is the development of afflatoxin in both raw and processed pepper spice. The afflatoxin level should be checked at less than 5µg/kg. Fruit stalk (peduncle) should be removed to get a good quality of powder. Colour is influenced by stage of fruits ripeness at harvest, processing and storage of the final spice powder. Similarly, besides genotype dependent, pungency is highly influenced by the environment. For spice purpose, fruits need to be maintained on the plant until they become dark red and slightly shrivelled to obtain the maximum possible colour for the spice product. But it is not possible to leave crops in the field until all fruit become shrivelled. Therefore, a more realistic aim is to harvest fruits when 80% or more fruits reach a dark red or dark red and slightly shrivelled stage. Only those fruits should be processed into spice powder, which are physiologically matured and dried. This results in the best achievable overall colour. The energy efficient heat pump dryers are well suited for drying of fruits because they operate at low temperatures. Fruit drying at low temperature is preferred as the spice otherwise becomes brown instead of a bright red. Therefore, drying temperature should be below 60°C (optimum drying regimes should be 40°C at 20% relative humidity) for heat pump dryers. To accelerate drying, fruit should be cut into small and regular pieces. Final moisture content of about 8% is considered to be ideal, as moisture content above 11% allows mould growth and below 4% causes excessive colour loss. Seeds of different cultivars have varying effects on the rate of colour loss, which is most likely due to the presence of varying antioxidant contents in the seeds. For instance, vitamin E, a fat-soluble antioxidant, has an effect on reducing colour loss. Selecting cultivars with high seed antioxidant levels is therefore necessary to produce a colour stable spice powder. During storage, carotenoid pigments (red colour) are readily oxidised and the spice powder becomes less intensely coloured. The selection of appropriate cultivars, standardization and adoption of drying and storage methods are the management strategies to reduce the instability of the carotenoids (Kumar *et al.,* 2006a).

2.8 Male sterility in *Capsicum*

2.8.1 Nuclear male sterility

In Punjab state, farmers have adopted chilli hybrid seed production technology using a nuclear male sterile line (gms), namely MS-12. The MS-12 posses *ms-10* gene (originally called *mc-509*, induced by E.R. Pochard in France during early 1970). During 1980, this *ms* gene was introduced in India by J.S. Hundal (retired chilli breeder) at Punjab Agricultural University, Ludhiana and this gene was introgressed in a multiple resistant line Punjab Lal (named MS-12). The farmers were imparted training on MS-12 based hybrid seed production and currently many farmers are producing seeds of CH-1 and CH-3 hybrids using MS-12 as common female parent. Therefore, F_1 seeds are available to the other farmers at reasonable price as compared to the F_1 seeds marketed by the seed companies. Since the *ms* gene of MS-12 was induced through mutagenesis and it is paying dividend to the framers, this could be cited as one of the classical examples of indirect use of mutation breeding in crop improvement. In an independent study, *ms-10* gene of MS-12 line has been found to be linked with taller plant height, erect growth of plant and dark purple anther (Dash *et al.*, 2001) and based on these information technology refinement has been advocated and adopted by the farmers (Kumar and Rai, 2005). The *ms-3* gene is being commercially exploited in Hungry (Kumar *et al.,* 2000a).

2.8.2 Cytoplasmic male sterility (cms)

The use of cytoplasmic-nuclear male sterility (cms) facilitates cost effective hybrid seed production. The first cms *Capsicum* plant was identified in an Indian *C. annuum* population (PI-164835) in USA. Subsequently, two more male sterile cytoplasms were isolated, but all the three independently isolated male sterile cytoplasms were found to be genetically identical. Yet another male sterile cytoplasm was located in India during 1990's, which has been transferred in several desirable chilli genotypes and utilized for hybrid development at IIHR, Bangalore. Hitherto, the Peterson's sterile cytoplasm has been commercially exploited in China, Korea and India, although at limited scale due to the temperature sensitivity of male sterility expression (Shifriss, 1997; Zhang *et al.,* 2000; Kumar *et al.,* 2000a; Liu and Gniffke, 2004). In India, at IIVR and IIHR, cms lines have been successfully utilized to develop commercial hybrids (Rai, 2005; Kumar *et al.,* 2006b). The occurrence of restorer (*Rf* allele is common in small fruited (usually hot pepper) and *rf* in large fruited (usually sweet pepper) lines (Shifriss, 1997). The restorer (*Rf*) gene associated Random Amplified Polymorphic DNA i.e. RAPD (Zhang *et al.*, 2000), Cleaved Amplified

Polymorphic Sequence (CAPS) and Amplified Fragment Length Polymorphism i.e AFLP (Kim *et al.,* 2006) markers have been developed. The validation of RAPD markers has been examined (Kumar *et al.,* 2006b) and QTLs associated with fertility restoration have been identified (Wang *et al.,* 2004).

2.8.3 Male sterile cytoplasms in *Capsicum*

After the first cms *Capsicum* plant was identified by Peterson (1958), two more male sterile cytoplasms were isolated, but all the three independently isolated male sterile cytoplasms were found to be genetically identical (Shifriss, 1997). Yet another two male sterile cytoplasms are known in *Capsicum*. The first was located in India during 1990's, which has been transferred in several desirable chilli genotypes and is being utilized for hybrid development (Reddy *et al.,* 2002). The second male sterile cytoplasm was obtained in an interspecific cross between *C. chacoense* (non-cultivated species) and *C. annuum* in United States (Bosland, 2004, personal communication), however, non-availability of restorer for this cytoplasm has been an obstacle to use it in hybrid development. The genetic relationship between the Peterson's sterile cytoplasm and sterile cytoplasm independently isolated in India has not been examined.

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