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Review on strategies for capsicum breeding under poly house cultivation

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Abstract

Capsicum is commonly known as sweet pepper or bell pepper, is the most important commercial and high value vegetable crop rich in Vitamin C mainly grown in Asia, Africa and South America and domesticated throughout the world. Greenhouse cultivation of capsicum is spread all over the world covering about 5-10 %. A breeding program involves several activities such as germplasm bank maintenance, evaluation of genetic diversity, selection of superior genotypes, progenitor's selection, hybridization, and evaluation of segregating populations. The major objective is for very high productivity of quality produce during off seasons with minimum usage of pesticides. Capsicum has a tendency towards autogamy and even though it is hermaphroditic in nature, self-fertilization cannot be avoided to 100 percent. However, the major constraint of using these varieties for commercial cultivation is the high seed cost, because there is a lack of efficient and economic hybrid seed production technology. Male sterility can be used to minimize the hybrid seed cost, which is high because of research involved in selecting parents, testing progeny, assessing performance, maintaining different lines, etc., Exploitation of heterosis in bell pepper has attracted attention of vegetable breeders to meet the requirements of the increasing population. Bell pepper varieties have evolved from local land races to advanced hybrid development. Besides conventional breeding methods, molecular genetics, tissue culture, and genetic engineering are becoming increasingly important. Production of haploids/doubled haploids through anther culture. F1 plants, results in true breeding plants in less than one year. Markers help us to determine the location (map) of genes that control important traits. Greenhouse vegetable production, however, is a highly intensive enterprise requiring substantial labour and capital inputs. Because of this, potential growers should carefully consider with a holistic vision all the factors necessary for a successful enterprise.

Keywords: Capsicum, genotypes, hybrids, seed, male sterility, vegetable and protected cultivation

Introduction

Capsicum (*Capsicum annuum* var. *annuum* Grossum group), commonly known as sweet pepper or bell pepper, is the most important commercial and high value vegetable crop rich in Vitamin C. The name is derived from Capsicum = Greek word 'kapto', meaning "to bite" or "to swallow." Almost 400 types of chillies are grown throughout the world. Among the 5 cultivated species of the genus *Capsicum*, *C. annuum* is the most widely cultivated in India for its pungent (chilli syn. hot pepper) and non-pungent (sweet pepper syn. capsicum, bell pepper) fruits. The cultivation of *C. frutescens*, *C. chinense*, and *C. baccatum* is limited and usually restricted to homestead gardening in different regions. It comprises numerous chemicals including steam volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, proteins, fibres and mineral elements (Bosland & Votava, 2000)^[5].

A breeding program involves several activities such as germplasm bank maintenance, evaluation of genetic diversity, selection of superior genotypes, progenitor's selection, hybridization, and evaluation of segregating populations. These activities are necessary, in general, to develop new cultivars. A considerable number of researchers around the world are dedicated to Capsicum breeding programs. Their great challenge is to select high-yield cultivars resistant to pests and diseases, protect them against biotic and abiotic stresses, and improve their fruit quality, and ornamental potential, according to the purpose for use in industry or for fresh consumption. Market type, fruit or plant, has a number of traits that makes it commercially acceptable. Continuous breeding aimed at production and quality depends on the incorporation of new allelic forms into the new cultivars.

To achieve their goals, breeders adopt the available breeding methods. In this chapter we further detail aspects of genetic variability, hybridization, genetic of quantitative traits, breeding methods, and postproduction of ornamental peppers showing the main results found by different groups of chili pepper breeders.

Capsicum annuum, bell, sweet pepper—with cultivated varieties including bell, sweet and paprika peppers—is a perennial herbaceous plants in the Solanaceae (nightshade family), which originated in Central and South America and the Caribbean and was domesticated over 5,000 years ago. Peppers from *C. annuum* have been developed into numerous varieties that are now cultivated around the world for sweet and hot varieties of green and red bell peppers and chilli peppers, that are one of the world's most widely used spices, with dried forms including paprika, chili powder, and cayenne.

The National Bureau of Plant Genetic Resources (NBPGR), New Delhi facilitates collection, regeneration, characterization, conservation and distribution of capsicum germplasm to researchers in India. However, at NBPGR indigenous collections constitute only 18% of the total Capsicum collections, while the majority of the accessions are exotics (Kalloo *et al.*, 2005). Currently, China is the major producer and exporter of bell pepper. China produces about 53% of the entire world's pepper with a production of 14 million tons/year. After China, the most important peppers producing country is Mexico (6.5% of worldwide pepper production), followed by Indonesia, Turkey and Spain (4% of worldwide pepper production each).

Greenhouse vegetable cultivation is spread all over the world covering about 5-10 % of vegetable cultivated area in most of the countries in Europe, Asia and USA. The major objective is for very high productivity of quality produce during off seasons with minimum usage of pesticides. Increases are expected to continue for at least the next few years, according to trade sources, which report that many greenhouse growers expect to increase their growing area anywhere from 20 to 50 percent because of increasing demand.

If India has to emerge as an economic power in the world, our agricultural productivity should equal those countries, which are currently rated as economic power of the world. We need a new and effective technology which can improve continuously the productivity, profitability, sustainability of our major farming systems. One such technology is the green house technology. Although it is centuries old, In India, research on greenhouse vegetable production has been going on for the past few years which has brought in remarkable change in agricultural production techniques which has made possible to realize yield levels in tomato and capsicum which are comparable to any international levels of greenhouse productivity. Presently in the greenhouse main vegetables cultivated are bell pepper, tomato, cucumber, eggplant, climbing beans, melons and some exotic vegetables.

In general, yields are much higher than for outdoor production: 375 MT/ha/year compared to 100 MT/ha/year in tomatoes. Maximum greenhouse yields are estimated at 500 MT/ha/year. Yields are increasing worldwide because of better cultivars and more intensive use of technology. From the trials at IIHR, Hesaraghatta, Bangalore also the tomato and bell pepper yields have touched 300 tonnes and 200 tonnes per hectare per year.

Vegetable cultivation in cost effective low and medium-cost greenhouse is a technical reality in India and slowly it is being adopted by the resourceful farmers in and around big towns

and cities as a peri-urban horticultural system. Such production system provides: i) Extended the growing season of vegetables and made their availability year round ii) Possibilities of growing new vegetables such as cherry tomatoes, coloured capsicums etc. iii) The quality seedling production, which is slowly catching up is one more profitable venture and necessity in the present agricultural scenario as more and more F1 hybrids are adopted by the farmers. iv) Hybrid seed production of selected high value vegetables such as capsicums and cucurbits under protected environment is also a major step to increase vegetable production and farm income in India. Greenhouse vegetable production, however, is a highly intensive enterprise requiring substantial labour and capital inputs. Because of this, potential growers should carefully consider with a holistic vision all the factors necessary for a successful enterprise.

The central idea behind greenhouse vegetable is to provide a) safe food of high quality. b) To produce vegetables throughout the year c) Use of less water and pesticides than commonly observed in open field grown vegetables. d) Year-round production is key to maintaining the greenhouse's profitability. However, this does not necessarily mean that growers should be producing the same crop year-round. (Summer tomatoes bring more money than do winter ones) e) Another option would be to raise a crop other than fresh vegetables, such as vegetable seedlings or use for production of F1 hybrid seeds.

Fig 1: Diversity of Capsicum

Breeding Objectives in polyhouse capsicums:

1. High yield
2. Indeterminate growth habit
3. Earliness
4. Desirable fruit colour (green, red and yellow), shape and size
5. iv) Preferred fruit quality (acceptable flavour, high sugar/ acid ratio, high pigment content and vitamin C)
6. Resistance/ tolerance to biotic stress (major diseases — viruses, bacterial & fungal and pests thrips & mites)
7. Resistance/ tolerance to abiotic stress (heat, water, salinity)

In breeding capsicums, the choice of breeding method depends on the breeding objective and the plant material being used as parents (Greenleaf, 1986). The strategy of the breeder is to assemble into a cultivar the superior genetic potential for yield, protection against production hazards, and

improved quality. Cultivars have been developed using selection within introductions and hybridization followed by selection. Selection methods have included mass, single plant, backcross, and pedigree. Pure lines showing resistance against biotic (diseases, insects, nematodes, etc.) and abiotic (environment and soil conditions) stresses are selected during routine breeding process. The breeding technique with limited success is genetic transformation. The rates of out crossing (7% to 91%) recorded by several investigators argue that *Capsicum* should be considered to be facultative cross-pollinating species in field research (Odland & Porter, 1941; Franceschetti, 1971; Tanksley, 1984) [38, 53]. The out-crossing is associated with natural insect pollinators. The amount of cross-pollination has an effect not only on the precautions needed for seed production, but also on the breeding methodologies used by the plant breeder (Bosland, 1993) [3]. Natural pollinators such as insects must be excluded to insure self-pollination. The recent development of molecular marker-assisted selection techniques may provide new tools in the breeding of capsicums. This area of research is rapidly adding to our knowledge of capsicums and will undoubtedly be important in the future. Since under protected cultivation only hybrids are of importance therefore, the methodology of hybrid development and abiotic and biotic stresses are of significance for capsicum improvement.

Selection of cultivars

Cultivars suitable for open field condition are usually not suitable for polyhouse cultivation as relatively long duration and high-yielding hybrids are ideal. The selection of cultivars and hybrids depend on plant type and their growth habit. *Capsicum* cultivars and hybrids should be indeterminate type. The plants are grown upright as a single stem rather than bush.

Peppers require comparatively a long growing period to reach transplant size and normally transplants of 35 to 40 days old are used. Paired row method of planting is adopted with 60X30 cm spacing and between paired rows a spacing of 90 cm is maintained accommodating about 44000 plants per ha. Compared to other vegetables it grows well with higher humidity conditions in the range of 60 — 70 %. Plants should be pruned to the two strongest stems, and these should be supported by tying to a wire suspended 6-8 feet above the plants. All lateral stems are removed for the first five to seven nodes above the ground. Above this, plants are allowed to branch from the two main stems and again two sub branches to retain four branches throughout. One to two fruit will set per node. Plants are often vibrated or trellis wires tapped to ensure proper pollination and fruit set.

Heterosis

The goal of increasing productivity in the quickest possible time can be achieved by utilizing heterosis breeding. Hybrid vigor in plant hybrids was first studied by Kolreuter in 1763. After reporting the phenomenon of heterosis by Shull (1914) [49] many-fold improvements have been made in various aspects of exploiting heterosis in vegetable crops. An F1 hybrid variety is the result of a cross between two homozygous (but genetically distinct) pure lines. The prerequisite is that all the F1 plants should resemble each other phenotypically. *Capsicum* has a tendency towards autogamy and even though it is hermaphroditic in nature, self-fertilization cannot be avoided to 100 percent. Heterosis in sweet pepper has been reported for early and total yield by Diku (1976) [12], and Novak and Chemalo (1975) [36].

Expression in the F1 depends upon the selected parents' combining ability. It also depends upon the genetic divergence; generally the wider the divergence, the higher the heterosis. The economic feasibility of hybrid cultivars depends upon the cost of hybrid seeds in relation to the value of increased yield. Heterosis breeding is feasible in bell pepper due to its low seed rate per hectare and only 400-600 grams of seed are sufficient to raise the seedlings for planting one hectare. *Capsicum* tolerates inbreeding, although there is some degree of heterosis for reproductive traits including seed yield component (Mishra *et al.*, 1991) [30]. Heterosis averaging between 2 and 55 percent has been reported for various traits including early maturity, plant height and certain yield components (Gopalkrishnan *et al.*, 1987; Khalil *et al.*, 1987; Lee and Shin, 1989; Lippert, 1975; Joshi *et al.*, 1995; Meshram and Mukewar, 1986) [17, 23, 25, 27, 22, 29].

Combining ability studies for yield components have indicated that additive effects in general were significant for yield components such as fruit length, fruit circumference, fruit weight, primary branches per plant and fruit weight per plant; few components showed significant dominance effects (Pandey *et al.*, 1981; Joshi and Singh, 1983) [39, 22]. In bell pepper, hybrid varieties have been successfully developed. However, the major constraint of using these varieties for commercial cultivation is the high seed cost, because there is a lack of efficient and economic hybrid seed production technology. Male sterility can be used to minimize the hybrid seed cost, which is high because of research involved in selecting parents, testing progeny, assessing performance, maintaining different lines, etc., before recommending an F1 hybrid for commercial cultivation.

Techniques for controlled pollination, such as male sterile lines to economize hybrid seed production. The most successful and viable technique currently practised is hand emasculation and hand pollination.

The main requirement for hybrid development in bell pepper is homozygous parental lines that ideally would give a large number of seed per fruit from single pollination. The main task of improvement is to assemble into cultivar superior genetic elements necessary for increasing yield, quality and protection against abiotic and biotic stresses. To achieve this, one should be familiar with pepper genetics and its reproductive behavior. Bell pepper flowers are complete, that is they have calyx, corolla, anthers, and stigma. It can cross-pollinate because of its protogynous condition (Bosland and Votava, 2000) [5] and exerted position of stigma in some cases.

Hybrid seed production

In bell pepper, hand emasculation and hand pollination are mostly used for production of F1 hybrid seed on a commercial scale. First formed healthy mature buds (a day before flower opening) are selected for emasculation and anthers are removed by forceps. The remaining buds in the cluster should be removed because abortion rate of this kind of buds are high. Pollen is collected from the male parent and transferred to the stigma with the help of forceps, brush, thumb nail or any other suitable tool. The stigma is fully mature in the bud stage and anthers mature either in the bud or after two to three hours of flower opening. For pollen collection, flowers that have not opened are picked in the afternoon and pollen can be collected by vibrating the flowers or by removing the petals preferably in the morning hours. Pollen can also be stored for 3 to 4 days under cool dry conditions depending upon temperature and humidity. Pollination is done in the emasculated flowers after 15 to 20 hours because pollination

on the same day of emasculation results in poor fruit and seed set. Pollination can also be attempted just after emasculation if matured buds are emasculated but the percentage of fruit setting may be less. Pollinated flowers are marked with a clip or a string. The ratio of female and male parent plants is normally kept 4:1. In bell pepper, maximum fruit setting is observed in the temperature range of 20-27°C, with the night temperature being the critical temperature. In bell pepper, single cross hybrids in which two inbred lines are involved are the only type of commercial hybrids (Kalloo, 1986). The hybrid seed yield is low but it becomes economical as the price of the seed is relatively high. Bell pepper fulfils the basic requirement for F1 hybrid production, i.e., homozygous parental lines that produce a large number of seeds from single hand pollination. The lines with the greatest seed quality potential should always be used as female parent if there is no difference in the reciprocal hybrids.

Hand emasculation and hand pollination

Hand emasculation is the most reliable method in this crop suggested by Sharma (1995) [39]. Emasculation can be carried out on flower buds one day before flower opening, preferably in the evening. Emasculated buds may be protected by non-absorbent cotton plugs, perforated butter paper bags, or cheesecloth, but it is not necessary. The buds are tagged with the description of male parent, date of pollination, etc., or use different color threads or twist ties in the pedicels such as red, yellow or blue for hybrids and white for selfs. For pollination, pollen is collected from full grown buds at the time of anthesis and placed on the stigma by brush or needle. The stigma is generally receptive before anthesis. After pollination, no protection is necessary. The set fruit should be protected carefully, using optimum plant protection measures to avoid its rotting or insect damage. Allow the set fruit to mature on the plant till they turn red/golden depending upon the variety. The seeds are extracted by cutting the fresh mature fruit. Dry the seeds to the moisture content <7 percent before packing. A plant in the open field condition with 8 to 9 cross-pollinated would give good yield of hybrid seed. For this, crossing should be attempted on 10 to 12 flowers per plant. However, under controlled conditions hybrid seed yield can be enhanced by pollinating more flowers and leaving 15-18 cross-pollinated fruits per plant is the extended period of growth.

Male sterility

F1 hybrids of bell pepper are popular in the USA and Europe and are gaining popularity in India after the initiation of research and seed production work in vegetables by a large number of private sector seed companies. The first hybrid variety in this crop in India was Bharat developed by Indo-American Hybrid Seeds, Bangalore during 1973 followed by marketing of large number of hybrid cultivars by several other seed companies. The greater success of hybrid cultivars in bell pepper could be attributed to;

1. Sufficiently large flowers, easy emasculation and pollen in abundance
2. Large number of crossed seeds/ fruit
3. Heterosis for yield
4. Easy deployment of dominant genes conferring resistance to diseases
5. Highly remunerative price of hybrid seed
6. High yield potential of hybrids under better management and inputs

In bell pepper, genetic male sterile (gms) lines are being used to a limited extent for hybrid seed production. GMS lines are maintained by hand pollination using heterozygous fertile plants as males. Poulos (1994) [42] reported that genic male sterility was the first controlled pollination strategy adopted by seed industry in the production of F1 hybrid seed after a long history of hand pollination and emasculation. Although much hybrid seed is still produced by hand emasculation and pollination, sterile lines are being used to reduce the labour cost and to improve hybrid purity. A number of gms genes have been reported and spontaneous mutants occur frequently (Shifriss, 1973; Csillery, 1989; Yu, 1990) [46, 10, 56]. The discovery of molecular and physical seedling markers that are linked to gms alleles is impending. Cytoplasmic male sterility (cms) has been tested by Daskalov and Mihailov (1988) [10] and Yu (1990) [56] for commercial hybrid seed production in bell pepper. They have reported that there are some problems in the stability of sterility and its restoration. Daskalov and Mihailov (1988) [10] proposed a scheme using a cms seed parent containing a lethal gene and a female sterile pollen parent to improve the efficiency of hybrid seed production without hand pollination. Chemical gametocides may complement the use of male sterility of bell pepper hybridization but limited success has been reported (Salgare, 1989) [44]. Novel sterility systems are likely to be adopted in an effort to curtail the labor cost of producing hand pollinated hybrids and to assure hybrid purity.

A. Genetic male sterility (MS)

Shifriss and Frankel (1969) [47] found spontaneous genetic male sterile plants in the cultivar All Big. Male sterility was determined by a single recessive gene. Due to this sterility, parthenocarpic fruits developed in unfertilized flowers throughout the season. Shifriss and Rylsky (1972) [48] discovered a second gene encoding genetic male sterility in the cultivar 'California Wonder'. It too, was found to be inherited as a single recessive gene. The authors suggested that the sterility discovered in 1969 may be described as MS1 and second as MS2. In India ms-10Ims509 is being utilized where as in Hungary ms-3 are being utilized for hybrid seed production commercially.

The use of genetic male sterility is limited in hybrid seed production due to the inefficiency in roguing out male fertile plants, since 50 percent of the plants are sterile and 50 percent are fertile. Therefore, 50 percent fertile plants must be rogued out from the field Either use a molecular marker to identify male sterility in the early growth stages so that fertile plants can be rogued at the seedling stage. The mechanics of using genetic male sterility for F1 hybrid production require (i) normal fully fertile inbred line as male and (ii) female inbred line which is maintained by crossing together. Known heterozygous (Msms) and male sterile (msms) plants. Seed must always be harvested from msms plants and should give equal proportions of plants of the same genetic constitution as the parents. Msms plants should not be allowed to intercross with one another since some homozygous male fertile (MsMs) will be produced and will upset the system (Watts, 1980) [55]. In practice, it would be advisable to cross groups of up to five msms plants with a single Msms plant, to ensure that other gene differences do not build up.

B. Cytoplasmic male sterility (CMS)

Male sterile plants are occasionally observed as spontaneous mutants in commercial fields (Bosland, 1996) [4]. Cytoplasmic male sterility (CMS) was first discovered by Peterson (1958)

in *C. annuum* P.I. 164835 from India. But this line was unstable in fluctuating environments and produced pollen at low temperatures.

The cytoplasmic male sterility is expressed under the presence of sterile mt-genome located in S-cytoplasm and recessive allele of restorer *r* located in the nuclear genome. The CMS occurs due to incompatibility between nuclear and mt-genome, because many mitochondrial enzymes are encoded jointly by the nuclear and mitochondrial genes. Such incompatibility leads to dysfunction of the mitochondria, especially in the pollen producing organs such as tapetum, stamens or anther walls. This causes reduced efficiency of mitochondria to give energy to tapetum cells, leading to failure of tapetum and pollen development.

CMS is another system of sterility by which hybrids are produced. In this system a maintainer male can regularly produce 100 percent male sterile plants in the female. These plants are used in F1 production with a male fertile line, which produces fully fertile F1 progenies. CMS is three line hybrid breeding involving A line (male sterile; S-rr), B line (maintainer; N-n-) and C line (restorer; S or N-RR). Some commercial seed companies use the CMS system to produce F1 hybrids in hot pepper but not in sweet peppers.

C. Hand pollination without emasculation

Use of male sterility for economizing hybrid seed production was first reported by Shifriss & Rylski (1973) [46]. Breuits & Pochard (1975) [6] developed the hybrid Lamuyo by using male sterile gene *ms-509*. Use of androcides has also been suggested by Kohli *et al.* (1981) [24] for sterility induction. Spraying GA3 @ 1000 mg/l at 10 days interval from the onset of flowering for three times has caused complete male sterility which lasted throughout the cropping season. Chauhan (1980) [8] used malic hydrazide and dalapon to develop *ms* plants in *Capsicum*. These gametocides caused complete male sterility.

Maintenance of inbred/pure lines

In bell peppers, pure lines are maintained and used as inbreds as in other self-pollinated crops. The uniformity of hybrids solely depends upon the uniformity of parents, therefore 2 to 3 years of selfing to remove all heterogeneity from the parents should be followed. Only homozygous pure lines should be used for crossing. For inbreds seed production, self-pollination should be done in isolation. The multiplication of elite pure lines starts with the positive selection of mother plants. The progenies of the plants are kept separately as A lines. The seeds of individual A lines are usually sufficient to plant B lines in a block experiment where original stock of the variety is planted as a purity check. Further generations of selfing, designated as C and D lines in increasing amounts are needed to provide sufficient quantity of the bulk seed required for hybrid seed production in isolation. Treating the seeds with trisodium phosphate can minimize contamination with infectious diseases. Selection of true type plants is the first step. The number of plants to be selected depends upon the requirement of breeder seed stock. Selection should be done rigorously and plants appearing off-types as inferior in any regard or differing in any character should be rogued discarded. Critical examination of selected plants for stem, leaf, and fruit characteristics (bearing habit, color, shape, size, etc.) is absolutely necessary for the production of foundation seed of inbreds. The selected individual plants can be covered by a thin three-ring muslin cloth bag (preferable 40 to 45 cm diameter and 60-80 cm length) to prevent crosspollination. At

full maturity stage, true to type selfed fruit can be harvested from each of the individual plants. Seeds of the above individual plant progenies should be grown in isolation where the same crop was not grown in the preceding year. An isolation distance of 400 m is minimum. Individual plant progeny rows should be observed very carefully. A minimum of 3 to 5 field inspections is required for bell pepper. In early stages of plant growth, inspection may be based on vegetative morphological characters of leaf, stem, plant type, etc. Second and third inspections may be based on fruiting habit, fruitsize, shape, color, etc. The fourth and fifth inspections should be made at full maturity stage looking for true to type fruits. If there is any off-type plant, it should be removed. Only selfed fruits are taken for extracting seeds. Fruits of individual plants may be harvested separately after attaining physiological maturity (Munshi & Sharma, 1997) [33]. Fruits of individual plants are critically examined and if there is no heterogeneity, they can be bulked for extracting seeds. Seeds of all inbreds are maintained separately. After proper cleaning and drying, the seed is stored at 15°C and 40% relative humidity for use as parental lines. Bosland & Votava (2000) [5] suggested use of tissue culture to regenerate plantlets from pollen microspore tissue. Plantlets produced from pollen microspores are haploid. By treating with colchicines, the chromosome number is doubled. These double haploid plants are homozygous at every locus and save many generations of self-pollination.

Biotechnology and molecular breeding

Micropropagation

Micropropagation is the clonal propagation by using very small plant tissue *in vitro*. The major benefits of this method are:

1. Production of high quality, disease free, super elite planting material for commercial seed production
2. Rapid spread of new varieties.
3. Rejuvenation of old varieties/clones for improving their yield and quality.
4. Interstate/ international exchange of germplasm avoiding the risk of pathogens and insects (Gosal & Gill 2004).
5. Multiplication of F1 hybrids, if permitted is potentially cheaper. Regeneration of plant tissue explants from pepper has been achieved (Agarawal *et al.*, 1989; Fari, 1986; Harini & Sita. 1993; Morrison *et al.*, 1986a; Sripichitt *et al.*, 1987) [15, 21, 31].

Anther /Pollen Culture

The most important use of haploids often advocated, is their use in the production of instant homozygous lines, which may be directly used as cultivars or may be used in breeding programmes. Production of haploids/doubled haploids through anther culture from F1 plants, results in true breeding plants in less than one year, which are otherwise obtained after 7-8 generations through conventional methods (Gosal *et al.*, 1996) [18]. Success with anther culture for tissue regeneration and differentiation is genotype-specific and dependent on environmental conditions (Ochoa Alejo and Ireta-Moreno, 1990) [37]. Despite the varying degrees of out-crossing in bell pepper, populations are managed most commonly as for self-pollinated species, with the final goal of developing homozygous lines.

Using doubled haploids as a tool for reducing generation time and fixing homozygosity has been a long time interest of many workers. Anther culture seems to be the most applicable for rapidly fixing new genotypes for the development of elite breeding lines (Li & Jiang, 1990; Morrison *et al.*, 1986a) [31].

Depending on the genotype, haploid regeneration from anther culture in pepper is generally between 1 to 20 percent, although success up to 50 percent has been reported (Dumas de Vault *et al.*, 1981; Morrison *et al.*, 1986b; Munyon *et al.*, 1989) [13, 32, 34].

Embryo and Protoplast culture

Embryo culture is the practical approach to obtain interspecific and intergeneric hybrids among otherwise hard to cross parents haploids from wide crosses (Bains *et al.*, 1995). It has been successfully used to transfer desirable genes from wild relatives into cultivated varieties of several field and vegetable crops. Somatic cell hybridization involving fusion of protoplasts from different species is considered an important approach to combine characteristics even from otherwise sexually incompatible species and to obtain cybrids (cytoplasmic hybrids) and organelle recombination not possible through conventional methods (Hinnisdaels *et al.*, 1988). Resistance to some diseases like Potato leaf roll virus, PVX and PVY have been incorporated into *Solanum tuberosum* from *Solanum brevidens* and *Solanum phureja* through protoplast fusion (Chahal & Gosal 2002) [8].

Molecular markers for Bell Pepper improvement

A Genetic markers represent genetic differences between individuals of same or different species. Generally, they do not represent the target genes themselves but act as 'signs' or 'flags'. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene 'tags'. Like genes all the genetic markers occupy specific genomic positions within chromosomes. There are three major types of genetic markers: (1) morphological (also 'classical' or 'visible') markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA. Markers help us to determine the location (map) of genes that control important traits

- Morphological markers are visually characterized phenotypic characters such as flower colour, seed shape, growth habits or pigmentation.
- Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining. The major limitations of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant. Crop breeders have used both these markers, albeit to a little extent.
- DNA markers (also referred to as molecular markers) are a piece of a specific DNA segment that is close to a gene of interest and can be used to track the gene during marker assisted selection (MAS). These are most widely used type of marker predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA. These markers are selectively neutral because they are usually located in noncoding regions of DNA. Unlike morphological and biochemical markers, DNA markers are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant.

DNA markers reveal genetic differences that can be visualised by using a technique called gel electrophoresis and staining with chemicals (ethidium bromide or silver) or detection with radioactive or colourimetric probes. Markers that reveal differences between any two individuals are called polymorphic markers, whereas markers that do not discriminate between genotypes are called monomorphic markers. Polymorphic markers may also be described as codominant or dominant. This description is based on whether markers can discriminate between homozygotes and heterozygotes. Codominant markers indicate differences in size whereas dominant markers indicate difference based on their presence or absence.

Strictly speaking, the different forms of a DNA marker (e.g. different sized bands on gels) are called marker 'alleles'. Codominant markers may have many different alleles whereas a dominant marker only has two alleles.

In India research on molecular markers has been initiated recently. So far most of these works are been confined to use of RAPD markers for the tagging of desirable traits of major vegetable crops and genetic purity testing of hybrid seeds.

Markers for tagging of ms and Rf genes

Male sterile line MS-12 (ms-10 ms-10) of chilli is being utilized by farmers to produce seeds of CH-1 and Ch-3 hybrids. In order to tag ms-10 gene, and F2 population (MS - 12 x KA-2) has been developed and individual plant has been characterized. Presently, RAPD primers are being screened using DNA samples of pooled F₂, and the parents in order to identify informative primers (Kumar *et al.*, 2006). Likewise F2 population derived from the cross between stable CGMS (CCA-4261) and strong restorer line (Pant C-1) has been developed to identify marker close to Rf gene.

Markers for hybrid purity and heterosis testing

Evaluation of hybrids for heterosis and combining ability in field is expensive, molecular markers have been used to correlate genetic diversity and predicting heterosis. It has been reported that measures of similarity based on RFLP and pedigree knowledge could be used to predict superior hybrid combinations. However, both low and high correlations between heterosis and DNA based genetic distance have been observed. For years the only method to check the hybrid seed purity has been, the grow out test. Now the RAPD and RFTP are being used for testing hybrid purity. If the male parent is homozygous for a chosen band, all hybrids would present that band and selected.

Two RAPD markers (OPW19800 and OPP13 1400) have been found to be useful for genetic purity of CGMS (CCA-4261) based commercial hybrid (CCH_2 or kashi Surkh) because both are reproducible and were present only in the male parent (Kumar *et al.*, 2006). At present, efforts are on for confirming the reproducibility of these markers in individual plants of the hybrid and parents and in case found non-reproducible, this RAPD marker will be converted into SCAR marker.

Markers for identification of male sterile cytoplasm

A cytoplasmic male sterile system is desirable for use in hybrid seed production as it eliminates the need for hand emasculation. CMS is a maternally inheritable trait characterized by the inability to produce viable pollen but without affecting the female fertility, and it is often associated with mitochondrial DNA rearrangements, mutations and editing. The co-dominant markers are useful in identifying the

homozygous restorer genotypes after the back crossing for production of restorer lines one year earlier than the conventional methods. Recently, SCAR (sequenced characterized amplified region) marker associated with male sterile cytoplasm of chilli has been reported from Korea (Kim and Kim 2005). Three reported SCAR markers (Cox II control, atp6 SCAR and Cox II SCAR) were screened for 8 male sterile (CGMS) lines and 8 fertile (maintainer) lines. Results showed that the SCAR markers have enormous potentiality for characterization of the CGMS gene and paved the universal usefulness of these markers.

Bio-diversity evaluation

Traditionally, bio-diversity evaluation and conservation is based on anatomy, morphology, embryology, physiology etc, based on the data of low genetic resolution. Recent advances in molecular technology have provided powerful genetic tools for rapid and authentic identification. Identity of crop variety has assumed great importance for predicting plant breeder's right/ farmers right, which can be maintained through DNA fingerprinting- the technique developed by Alec Jeffery in 1985 in human but now of great use in plant sciences.

Marker assisted selection

Plant breeders have relied heavily on generating new gene combinations and selecting these new gene combinations empirically. The response in the improvement had been tremendous so far and its efficiency can further be improved by marker-assisted selection. The essential requirements for marker-assisted selection in a plant breeding programme are:

- Marker(s) should co-segregate or be closely linked (less than 1cM) with the desired trait.
- An efficient means of screening large populations for molecular marker(s) should be available. PCR based technique to some extent fulfil this.
- The screening technique should have high reproducibility across laboratories, be economical to use and should be user friendly.

Marker assisted selection can be practiced more efficiently for characters whose phenotypic selection is difficult. For example, transferring a fertility restorer gene from one line to another line through backcrossing needs test crossing before subsequent backcrossing. If such genes are tagged with molecular markers, desirable plants with fertility restorer gene (in heterozygous condition), can be identified and backcrossed.

Similarly, screening for abiotic stresses is very difficult. If desirable genes conferring tolerance to abiotic stresses are tagged, these can be selected easily in segregating generations. Also genetic markers can be assayed in non-target areas such as growth chamber, green houses or off-season nurseries, thus permitting more rapid progress. The efficiencies of scale and time accorded by DNA markers are valuable in breeding most species but are of special value in breeding species that have large stature or long generation time (such species as orchard or forest trees) where fewer individuals might save several hectares and fewer generations may save several decades (Paterson *et al.*, 1991) [40]. Marker assisted selection (MAS) is being used efficiently in the following areas.

i. Gene pyramiding.

MAS has been incorporated in several breeding programmes to pyramid several disease and insect resistance genes in a

single line for increasing durability of resistance. In conventional breeding procedures, it is generally not possible to combine several genes in one line due to 'vertifolia effect' i.e. a dominant gene showing resistance to several races masks the effect of other genes, which are showing resistance to one or more races. Such genes whose effect is masked cannot be selected precisely using conventional techniques. Similarly, combining a dominant and a recessive gene may not be possible. However, it is possible under both the situations using MAS.

ii. Marker assisted alien introgression

Alien introgression has been one of the major activities of most plant breeding programmes. Stephens (1961) [51] eloquently summarized the hazards of interspecific breeding as:

"When the chromosomes of different species are sufficiently alike to recombine more or less freely, their recombinant products are likely to be greatly inferior to the parental combinations. At present, there is no way of controlling this total disruption of the parental genotypes which result from segregation".

Genetic markers provide a means of controlling the degree of disruption of parental genotype. Selection in the first backcross generation using molecular markers, one can restore most of the recurrent parent genotype while retaining the desirable genes from the wild parent. This can be done by selecting against the markers from the wild parent outside the region carrying target genes (Young and Tanksley, 1988) [58]. Using this technique Young and Tanksley (1989) [59] selected for Tm2a gene with minimum backcrosses thus saving time and eliminating linkage drag.

iii. Simultaneous identification and pyramiding of QTLs from primitive cultivars and alien species.

Most of the characters of economic importance are governed by quantitative genes. Conventional breeding has relied exclusively on empirical selection of these quantitative trait loci (QTLs) in cultivated species. Until recently, there was no way out for dissecting and directed manipulation of such loci. Molecular markers, however, have made it possible to dissect individual QTLs, both in cultivated and alien germplasm and further directed selection of these QTLs. First decade of the new millennium should see the large-scale application of this technique in crop improvement programme.

Genetic transformation

During the past 20 years, the combined use of recombinant DNA technology, gene transfer methods and tissue culture techniques has led to the efficient transformation and production of transgenics in a wide variety of crop plants (Chahal & Gosal 2002) [8]. In fact, transgenesis has emerged as an additional tool to carry out single gene breeding or transgenic breeding of crops. Unlike conventional breeding, only the cloned gene(s) of agronomic importance is being introduced without co transfer of other undesirable genes from the donor. The recipient genotype is least disturbed, which eliminates the need for repeated backcrosses. Above all, the transformation method provides access to a greater gene pool as the gene(s) may come from viruses, bacteria, fungi, insects, animals, human beings, unrelated plants and even from chemical synthesis in the laboratory.

(A) Engineering for insect resistance:

There have been two approaches to develop insect resistant

transgenic plants by transferring insect-control-protein genes.

I) Introduction of bacterial gene

Bacillus thuringiensis synthesizes an insecticidal crystal protein, which resides in the inclusion bodies produced by the *Bacillus* during sporulation. This crystal protein when ingested by insect larvae is solubilized in the alkaline conditions of the midgut of insect and processed by midgut proteases to produce a protease resistant polypeptide which is toxic to the insect. *Lepidopteran specific* Bt gene from *Bacillus thuringiensis* subsp. *kurstaki* has been widely and successfully used in tobacco, tomato, potato, cotton, rice and maize for developing resistance against several lepidopteran insect pests (deMaagd *et al.*, 1999 and Gosal *et al.*, 2003) [19]. The use of redesigned synthetic Bt gene has also been used in some of these crops and in several instances the synthetic versions have exhibited upto 500-fold increase in the expression (Brandy 2001). Some of the transgenics have been released in the field for commercial cultivation. Bt brinjal in India is under process of release.

II) Introduction of plant gene(s)

Several insecticidal proteins of plant origin such as lectins, amylase inhibitors and protease inhibitors can retard insect growth and development when ingested at high doses (Jongsma *et al.*, 1997, Tamayo *et al.*, 2000) [52]. Some genes like CpTi, PIN-1, PIN 11, A-1, and GNA have been cloned from different plants and being used in the transformation programmes aiming for the insect resistance in plants.

(B) Engineering for disease resistance

I) Virus resistance

The genetic engineering of virus resistant plants has exploited new genes derived from viruses themselves in a concept referred to as 'pathogen-derived resistance (PDR)'.

Coat protein - mediated resistance (CP-MR)

Introduction of viral coat-protein gene into the plant, make the plant resistant to virus from which the gene for the CP was derived. It was first demonstrated for TMV in tobacco (Powel Abel *et al.*, 1986) [43]. Subsequently, virus resistant transgenics have been developed in tomato, melon, rice, papaya, potato and sugarbeet. Several CP-MR varieties of potato, cucumber and tomato are under field evaluation.

ii) Fungal resistance

Genetic engineering for fungal resistance has been limited. But several new advances in this area now present an optimistic outlook.

Antifungal protein-mediated resistance

Introduction of chitinase gene in tobacco and rice has been shown to enhance the fungal resistance in plants (Nishizawa *et al.*, 1993; Lee *et al.*, 1995) [35, 25]. Chitinase enzyme degrade the major constituents of the fungal cell wall (chitin and 1,3 glucan). Coexpression of chitinase and glucanase genes in tobacco and tomato plants confers higher level of resistance than either gene alone. Use of genes for ribosome inactivating proteins (RIP) along with chitinase has also shown synergistic effects. A radish gene encoding antifungal protein 2 (Rs-AFP2) was expressed in transgenic tobacco and resistance to *Alternaria longipes* was observed (Broekaert *et al.*, 1995). Other pathogenesis related proteins/peptides include osmotin, thionins and lectins etc (Florack & Stiekema 1994).

Antifungal-compound mediated resistance:

The low molecular weight compounds such as phytoalexins possess antimicrobial properties and have been postulated to play an important role in plant resistance to fungal and bacterial pathogens (Leckband and Lorz 1998). Expression of a stilbene synthase gene from grapevine in tobacco resulted in the production of new phytoalexin (resveratrol) and enhanced resistance to infection by *Botrytis cinerea*: Active oxygen species (AOS) including hydrogen peroxide also play an important role in plant defense responses to pathogen infection (Wu *et al.*, 1995) [57]. Transgenic potato plants expressing an H2O2 generating fungal gene for glucose oxidase were found to have elevated levels of H2O2 and enhanced levels of resistance both to fungal and bacterial pathogens particularly to *Verticillium* wilt.

iii) Bacterial resistance

Genetic engineering for bacterial resistance has relatively met with little success. The expression of a bacteriophage T4 lysozyme in transgenic potato tubers led to increased resistance to *Erwinia carotovora*. Besides, the expression of barley -thionin gene significantly enhanced the resistance of transgenic tobacco to bacteria *Pseudomonas syringae*. Advances in the cloning of several new bacterial resistance genes such as the Arabidopsis RPS2 gene, tomato Cf 9 and tomato Pto gene may provide better understanding in the area of plant bacterial interactions.

Conclusion

Protected cultivation of vegetables offers distinct advantages of quality, productivity and favourable market price to the growers. Vegetable growers can substantially increase their income by protected cultivation of vegetables in off-season as the -vegetables produced during their normal season generally do not fetch good returns due to large availability of these vegetable in the markets. In India, breeding bell pepper cultivars specially designed for protected cultivation is a new area and little/no work has been done to develop varieties/hybrids. Presently, whatever hybrids are available are supplied by private seed companies and they are fleecing the green house growers by charging exorbitantly. Therefore, there is emergent need to develop/ breed indigenous location specific hybrids/ varieties tailored for specific purpose having multiple and multigenic resistance. Bell pepper varieties have evolved from local land races to advanced hybrid development. Use of biotechnology will likely aid in the future development of improved bell pepper varieties. Besides conventional breeding methods, molecular genetics, tissue culture, and genetic engineering are becoming increasingly important. Hybrid seed production in capsicum has produced a substantial increase in its production. Exploitation of heterosis in bell pepper has attracted attention of vegetable breeders to meet the requirements of the increasing population. There are alternative systems to produce hybrid seed, but the production of bell pepper hybrid seed commercially still relies on making crosses between two parents by conventional hand pollination, a very labor intensive and expensive process. The future outlook for bell pepper cultivation under protected structures is good but demands indigenous approach for developing economically viable commercial hybrids with resistance to biotic and abiotic stresses rather than leaving the farmers at the doorsteps of private companies.

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