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Molecular markers in improvement of *Capsicum* spp. – a review

Deepu Mathew[1]

Field Research Laboratory
Defence Research and Development Organization
C/o 56 APO, India.
E-mail: deepumathew@movemail.com

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Abstract

Use of various molecular markers in improvement of *Capsicum* spp. including genetic diversity analysis, fingerprinting, mapping the genes of interest with special reference to pungency and stress resistance, seed purity analysis, phylogenetic studies, genomic library construction and linkage analysis has been well demonstrated. This paper reviews the advancement and information available on the use of RAPD, RFLP, AFLP, ISSR, SPAR, CAPS and SCAR markers in crop improvement programmes in *Capsicum* spp.

Keywords: *Capsicum* spp., crop improvement, molecular markers.

Introduction

The genus *Capsicum* includes a group of economic plants that are grown as spices, vegetables and colouring agents throughout the world and rated as the world's most demanded spice crop. Improvement in any crop plant refers to positive heritable changes brought about in its genome to enhance economic output. In *Capsicum*, fruit yield is polygenic (Mathew 2004), decided by plant morphology and modified by environment including biotic and abiotic stresses, thus opening wide scope for crop improvement especially in ideotype architecture and stress resistance. Molecular markers have greatly enhanced the scope of detailed genetic analysis and improvement of crop plants. These markers act as excellent tools to study genetic diversity, linkage analysis, gene tagging and genome mapping. These are also useful to protect plant varieties by fingerprinting and to eliminate duplication in germplasm.

The discovery of Polymerase Chain Reaction (PCR) (Mullis *et al*. 1986; Mullis & Faloona 1987) led to the development of a class of dominant genetic marker system named Random Amplified Polymorphic DNA (RAPD) which involves PCR amplification of total genomic DNA using single random primer (usually 10 base pair long) and separation of amplified fragments by agarose gel electrophoresis. In this reaction, a single species of primer binds to the genomic DNA at two different sites on opposite strands of the DNA template. If these sites are within amplifiable distance, the fragment will be amplified. The presence of each amplification product identifies complete or partial nucleotide sequence homology between the genomic DNA and the oligonucleotide primer at each end of the

[1]Present address: Krishi Vigyan Kendra, Kelappaji College of Agricultural Engineering and Technology (Kerala Agricultural University), Tavanur-679 573, Kerala, India.

amplified product. On an average, each primer will direct the amplification of several discrete loci in the genome, making the assay an efficient way to screen for nucleotide sequence polymorphism between individuals (Welsh & Mc Clelland 1990; Williams *et al*. 1990).

Restriction Fragment Length Polymorphism (RFLP) involves digestion of genomic DNA with cleaving restriction endonucleases (Grodzicker *et al*. 1974), fractionating the fragments electrophoretically and then preferentially visualizing fragments containing particular homologous sequences by hybridizing them to specific DNA probe (southern hybridization). The co-dominant nature of this marker makes it an efficient tool in fingerprinting, construction of linkage maps and differentiating heterozygotic individuals due to high level of allelic diversity. However, involvement of radioactive probes and labour intensive and expensive nature restricts the use of RFLP.

Amplified Fragment Length Polymorphism (AFLP) markers involve restriction of the DNA and ligation of oligonucleotide adapters, selective amplification of sets of restriction and gel analysis of the amplified fragments. AFLP is a very efficient dominant marker to reveal restriction fragment polymorphisms, generate fingerprints of any DNA regardless of the origin or complexity and construct genetic marker maps (Vos *et al*. 1995). However, the technology is intensive and involves the use of radioactive probes.

Microsatellite markers involve the amplification of tandem repeats in the genomic DNA. Highly conserved nature of these repeated sequences (1–6 bp) make them unique for each individual (Litt & Luty 1989). Microsatellite markers are co-dominant in nature and PCR based, giving high-level polymorphism even between near isogenic lines. These markers are less elaborate, demanding comparatively lesser time, do not require radioactive probes and are capable of performing all the functions of RFLP, including phylogenetic studies and gene mapping.

The advancement and information available on the use of molecular markers in crop improvement programmes in *Capsicum* are reviewed in this paper.

RAPD markers

Genetic diversity assessment and fingerprinting

Genetic diversity assessment and fingerprinting were the initial applications of RAPD. Even though the fingerprints from RAPD are not acceptable due to poor repeatability, especially after the invention of inter simple sequence repeats (ISSR), this marker is highly useful in genetic diversity studies and for initial screening of germplasm for characters of interest. The fingerprinting of *Capsicum* accessions using RAPD was first demonstrated by Heras *et al.* (1996), though the possibility of using RAPD in *Capsicum* was first postulated by Cao (1994). He used 11 *C. annuum* L. lines from Spain and more than 50% of the bands in the profile were common, which made them conclude that the genetic base of *Capsicum* spp. in Spain is narrow. In the same year Wang *et al.* (1996), used RAPD markers for fingerprinting both wild and domesticated *Capsicum* spp. from China. Based on the profile, they classified 14 accessions into *C. chinense* Jacq. (2), *C. annuum* (4), *C. annuum* var. *annuum* (5) and *C. baccatum* L. (3). Later, a standard protocol to isolate high quality genomic DNA from *Capsicum* leaves was standardized by Prince *et al*. (1997) and was slightly modified by Wang *et al*. (1998).

Global mandate on *Capsicum* germplasm is vested with Asian Vegetable Research and Development Centre (AVRDC), Taiwan. Variation among the *Capsicum* germplasm accessions available at AVRDC was determined using RAPD markers using the profiles generated by 110 random primers on 134 representative accessions belonging to 6 species (Rodriguez *et al.* 1999). Multidimensional scaling analysis of the genetic distances among accessions resulted in clustering corresponding to their previous species assignments, except for six accessions. Diagnostic RAPDs that discriminate the different species were also identified. Three lines, which

were misclassified based on the morphological descriptions, were corrected and 10 pairs of duplicated accessions were identified.

A more illustrious example on the use of RAPD markers in genetic diversity estimation in *Capsicum* is from Mexico, which is the primary centre of origin of this genus (Vavilov 1951) and especially, *C. annuum* (Harlan 1971), where Votava (2000) has done extensive work on the characterization of *Capsicum* accessions from that region. He was successful in assigning the accessions into different species based on RAPD markers. The diversity within *C. pubescens* Ruiz & Pavon was estimated by Votava & Bosland (2001). They were able to distinguish between *C. eximum* Hunz., *C. cardenasii* Heiser & Smith and *C. annuum* species. *C. cardenasii* and *C. eximum* were distantly related to *C. pubescens* and closely related to each other; while *C. annuum* showed maximum variation from *C. pubescens*. Similarly, the diversity within *Capsicum* germplasm collection from Nepal was estimated by Baral & Bosland (2002). Both morphological and RAPD markers were used for the characterization and the observed variation was compared with that of Mexican accessions. Further, they observed that all Nepalese accessions formed one cluster while Mexican accessions formed eight clusters underlining a comparatively narrow genetic base of this genus in Nepal. Further, they argued that 'founder effect' probably due to inter-continental migrations is the bottleneck for evolution of *Capsicum* in Nepal and unique RAPD markers identified makes Nepalese accessions a new source of variation and important from the view of plant breeding. A *Capsicum* germplasm collection from Korea was fingerprinted using RAPD markers and the variability, especially with regard to bell peppers was found to be narrow (Kang *et al.* 1997a). Other than landraces and germplasm lines, *Capsicum* hybrids could also be characterized using RAPD markers (Votava *et al.* 1996).

Maintaining maximum variability with limited number of accessions is the objective of gene banks as well as characterization processes (Paran 2003). Identification and conservation of a representative sample, which would depict the characteristics of a group of accessions, will help to save space in gene banks. For this sake, an algorithm to identify maximally diverse core collections in *Capsicum* was designed using 134 AVRDC lines, each representing a minimum of 18.5% of population as revealed through RAPD markers. Per cent representation by each accession was core size dependent (Marita *et al.* 2000).

RAPD is a dominant marker, which often fails to distinguish between the lines of close genetic make up or near isogenic lines. This condition arises when the polymorphic bands generated using random decamers is limited or nil. Yang & Park (1997) proved an alternative for this by increasing the polymorphism through a combination of RFLP and RAPD (RF-RAPD) in *Capsicum* to distinguish even closely related lines.

Spontaneous doubled haploid (DH) plants developed from F_1 hybrids of sweet pepper in anther culture were used to produce DH-R_2 generation. PCR based markers such as RAPD, ISSR and SSR were employed in the molecular studies of these *Capsicum* lines. DH-R_2 plants were discriminated at least by one primer and 35.2% of scorable bands were polymorphic facilitating the characterization of the lines (Gyulai *et al*. 1999). The authors argue that all the polymorphic bands generated in the experiments could originate from locus specific sequence rearrangements in the genome as a result of the meiotic recombination that occurred during the male gametogenesis, which resulted in new RAPD/SSR primer-binding sites in the genome.

Though sweet peppers belong to *C. annuum* (var. *grossum*), classification based on morphological descriptors misleads it to be highly divergent from other *C. annuum* accessions. Similarly, *C. annuum* and *C. baccatum*, though genetically highly distant, appears to be genetically very close with only corolla spot as a distinctive difference. A so-

lution for these problems was obtained through RAPD markers which revealed the exact genetic distances between five different species as well as that between bell peppers and hot peppers belonging to the same species (Mathew 2004). In a similar study, 34 *Capsicum* accessions were analyzed using RAPD and AFLP. RAPD separated large-fruited less pungent group from small-fruited highly pungent group, but inter-cluster distances were low. Divergence within blocky type is comparatively lesser and accordingly, RAPD markers couldn't differentiate four blocky types but AFLP was successful. On an average, AFLP markers amplified six times more products than RAPD and were four times more efficient in variability detection in *Capsicum* (Paran *et al.* 1998). RAPD markers are as good as ISSR markers in genetic distance evaluation and this was shown by Wang & Fan (1998), who used RAPD and ISSR markers to characterize 90 *Capsicum* accessions. Both the markers had high degree of polymorphism and were able to detect the genetic diversity and intra specific polymorphism among *Capsicum* germplasm from 16 countries.

Mapping characters of interest

Disease resistance

RAPD markers were used for genetic mapping of T_{sw} locus for resistance to topso virus in *Capsicum* (Jahn *et al.* 2000). This tagged locus was mapped to distal portion of chromosome 10. Further, its relationship with *Sw-5* gene coding for resistance to the same pathogen in tomato was studied and it was found that these loci in tomato and pepper do not share a recent common evolutionary ancestor. For carrying out this study, a mapping population (B_4 backcross population) was developed by crossing *C. chinense* (resistant) and *C. annuum* (susceptible). Susceptible and resistant parents and B_4 populations were screened using 400 primers of which 5 had shown polymorphism for resistance to topso-virus. One marker amplified with Q-6 primer co-segregated with resistance.

RAPD markers corresponding to hypersensitive reaction to tomato spotted wilt virus in *Capsicum,* governed by single dominant gene T_{SW} was determined (Moury *et al.* 2000). The mapping population comprised of resistant parent (*C. chinense*), susceptible parent (*C. frutescens* L.), resistant bulk, susceptible bulk and resistant F_2 (P_1 X P_2) individuals. DNA from the mapping population was screened using 250 decamer primers and 4 primers (OP-AC10_{593}, OP-AH13_{800}, OP-AF 16_{250} and OP-BO1_{750}) were found to differentiate the marker specific for T_{SW} locus. RAPD was also useful in locating markers tightly linked to *Bs-2* locus which confers resistance against the bacterial pathogen *Xanthomonas campestris* (Pammel) Dowson pv. *vesicatoria* in *Capsicum* spp. (Tai *et al*. 1999).

Genic cytoplasmic interactions

A major (*Rf*) and few minor genes restore the fertility in cytoplasmic male sterile lines of *Capsicum*. Two RAPD markers linked to a major fertility restorer gene were detected by Zhang *et al*. (2000). They screened 520 decamer primers to find the marker OP 131_{400}, which is tightly linked with the gene of interest having a genetic distance of 0.37 centi Morgan (cM) and the marker OW 19_{800} was on the opposite side with a genetic distance of 8.12 cM. The authors also reported that these markers are absent in sweet pepper and could be useful in transferring *Rf* into pepper lines. This marker helps in reducing backcross population for test crosses and also in genetic analysis of minor genes. A similar work by Kumar *et al*. (2002) details the RAPD protocol for tagging of fertility restorer and male sterility genes in chilli. RAPD was also useful in genetic analysis of stunted growth by nuclear cytoplasmic interactions in interspecific hybrids of *Capsicum* (Inai *et al.* 1993).

Hybrid seed purity determination

The ability of RAPD marker to distinguish between both the parents and F_1 enables it to be used for hybrid seed purity analysis. Ballester & Vicente (1998), for the first time, reported RAPD as a powerful tool for hybrid seed purity determination. Of the 100

random decamers screened, at least one was useful for testing the purity of all the 5 hybrids studied. Based on the observations, they concluded that despite 99 dominant inheritance, RAPD is efficient for testing hybrid seeds. Choe *et al*. (1998) had given further evidence to this when they used RAPD and phosphoglucomutase (PGM) isozyme analyses simultaneously in cultivar identification and seed purity determination. Wang *et al.* (2002) estimated the seed purity of hot pepper cultivar Yuejiano No. 1 F_1 using RAPD markers and claimed that the result of the RAPD test in random samples using two primers (given as P_1 and P_2 but identity of the primers are not disclosed) was same as that of grow-out test. Zhou *et al*. (1999) also determined the purity of *C. frutescens* hybrids using RAPD technique. Out of 39 arbitrary primers screened, three were useful to differentiate male and female parents and F_1 lines.

RFLP markers

Genetic diversity assessment

RFLP is the first generation marker and its high level precision is well defined. In *Capsicum* also, this marker has proved to be very efficient for various applications. Genetic distances among 25 accessions from various regions of Mexico were estimated using RFLP markers (Prince *et al.* 1990, 1992). A combination of isozyme-RFLP data was successfully used for characterization and distinguishing different species. Accessions from south Mexico belonged to *C. frutescens* and formed a single cluster. *C. annuum* from north-east and north-west Mexico formed separate clusters. *C. annuum* line from Yuccatan province showed substantial genetic variability from other lines of the same species. Thus, spatial isolation of variability was evident from the study. *C. chinense* showed maximum genetic variability from all other species under study. Prince (1995) also finger-printed *Capsicum* cultivars using RFLP markers and surveyed the polymorphism within this genus. Inter-specific genetic variation was evident from 4 distinct clusters delineated among 21 accessions of cultivated as well as wild peppers (*C. annuum*, *C. baccatum*, *C. chinense*, *C. chacoense* and *C. frutescens*). Three tight clusters comprised of accessions belonging to *C. annuum*, *C. frutescens* and *C. baccatum* were formed along with a fourth cluster comprising one accession each of *C. chinense* and *C. chacoense*. Dendrogram constructed from RFLP and RAPD analyses of the intra-specific data were similar but not the same. The authors concluded that southern blotting as well as RAPD–PCR should be useful for DNA fingerprinting and discrimination of closely related *C. annuum* genotypes.

Total genomic DNA from 13 accessions of *C. annuum* var. *annuum* and 1 accession of *C. baccatum* var. *pendulum* were cut separately with 10 restriction enzymes and polymorphism was analyzed using 41 nuclear probes distributed across the different linkage groups. Genetic distances between accessions were calculated from 141 resultant nuclear DNA restriction fragments (Nei & Li 1979). The genetic variation was comparatively larger between *C. annuum* and *C. baccatum* than that among *C. annuum* cultivars. Distances between small-fruited cultivars were larger than the distance between the bell peppers, which clustered together, once again showing the narrow genetic base in bell peppers (Lefebvre *et al*. 1993). These results were further supported by Mathew (2004).

Hybrid seed purity analysis

The procedure for conversion of an RFLP assay into PCR for determination of purity in hybrid *Capsicum* cultivar was given by Livneh *et al.* (1992). Initially, RFLP to distinguish between F_1s and the parents were established (Liveneh *et al.* 1990). For this conversion, the sequences of the ends of the probes used in the previous RFLP assay were determined. From these sequences, suitable primers were devised for inverse PCR of heterogeneous DNA fragments derived from the male parent. The inverse PCR product was cloned and partially sequenced. These se-

quences, in turn, made it possible to determine primers on both sides of locus of mutation and to develop conventional PCR assay.

Genomic library construction and linkage analysis

This is the most precise and exclusive application of RFLP. A *Capsicum* genomic library for RFLP analysis was done by Kim *et al*. (1992). From the 560 clones in genomic library evaluated using colony hybridization, 3 groups of clones were recognized namely, single copy sequence, low copy number sequence and multiple copy number sequence. Cloning and expression of a squalene synthase cDNA from hot pepper was done by Lee *et al*. (2002). *Capsicum* genes CASAR 8.2A, B and C induced by biotic and abiotic stresses could be used as markers for pathogen infection, abiotic elicitors and abiotic stresses. These genes were successfully isolated from *Capsicum* cDNA library constructed with mRNAs from *Capsicum* plants infected by *X. campestris* pv. *vesicatoria* (Lee & Hwang 2003).

The co-dominant nature and high level accuracy makes RFLP suitable for linkage analysis. A linkage map of *Capsicum* using RFLP was initially constructed by Kang *et al.* (2000). Genomic DNA was restriction digested and the fragments were cloned in *Escherichia coli.* cDNA library was constructed and used for RFLP assay followed by bulk segregant analysis (BSA). From this linkage distances were estimated. Subsequently, a high-density integrated linkage map for *Capsicum* was developed by Paran *et al*. (2004).

Mapping genes of interest

In *Capsicum* spp., capsanthins decide fruit colour, the synthesis of which is controlled by capsanthin-capsorubin synthase gene (CCS). Presence of CCS gene imparts red colour and deletes yellow colour. Mapping populations were developed through red x yellow crosses and RFLP and PCR polymorphism from CCS gene were analyzed. Pungency co-segregated completely with fruit colour and it was also reported that the deletion of CCS gene by induced mutation would lead to yellow fruit colour and deletion of specific band in an RFLP profile (Lefebvre *et al.* 1998). In a similar study, RFLP proved useful to map chlorophyll-retained mutations in *Capsicum* (Efrati *et al*. 2005).

Differentiating precisely the lines with and without pungency is an important step in paprika varietal development. Development of a tool to distinguish the non-pungent lines at the earliest stage of growth helps to reduce the time requirement for this breeding work. Single dominant gene locus (*C*) on chromosome 2 of *Capsicum* decides the pungency. Blum *et al.* (2002) mapped *C* locus precisely using the mapping populations (both parents and F_2 segregating populations) developed by *C. frutescens* (pungent) x *C. annuum* var. *grossum* (non-pungent). On linkage analysis using MAPMAKER, it was observed that RFLP marker TG 205 co-segregates with the *C* locus (Lander *et al*. 1987). This marker codes for fibrillin gene and is located at 0.4 cM from the *C* locus. Tightly linked marker was sequenced and CAPS primers were designed based on the sequences, which upon analysis has clearly shown which are pungent, non-pungent and heterozygous. Later, mapping of capsaicinoid biosynthesis genes and QTL analysis for capsaicinoid content was completed (Blum *et al*. 2003). QTL mapping for fruit shape and size in chromosome 2 and 4 in *Capsicum* was done by Zygier *et al*. (2005) and QTL for fertility restoration in cytoplasmic male sterile *Capsicum* was mapped by Wang *et al*. (2004).

AFLP markers

Genetic distance estimation

High level resolving power makes AFLP suitable for characterization of even near isogenic lines (NILs). Kang *et al.* (1997b) employed AFLP in differentiating NILs and F_2 from interspecific hybrids. Two sets of NILs, one for pod separation and other for resistance to *Phytophthora capsici* Leon. were used to screen putative AFLP markers. After 32 primer combinations were tried, 10 and 5 polymorphic loci were observed in NILs for pod separation and *Phytophthora* resistance, respectively.

From the studies it was observed that AFLP because of its dominant nature, segregated independently in Mendelian manner. Another important observation from this study was that the template DNA concentration significantly influences the reproducibility of even precise markers.

Selection of parental lines for the development of mapping population should be based on their genetic distances, to represent the maximum variability. Using AFLP, Nam *et al.* (1997) estimated the genetic distances among 12 accessions of *C. annuum* and 1 each of *C. baccatum*, *C. pubescens* and *C. chinense* accessions. This study concluded that *C. pubescens* and *C. baccatum* were more distant from *C. annuum*, and hence, non-crossable. Subsequently, accessions from *C. annuum* and *C. chinense* were selected as mapping parents in linkage map construction rather than more distant lines since they are fairly distant and crossing is possible, though with difficulty (Pickersgill 1966, 1997).

The variation within *C. annuum* species was estimated using AFLP markers by Paran *et al.* (1998). A dendrogram based on RAPD markers separated the large-fruited sweet cultivars from small-fruited pungent peppers and the former group showed less divergence than the latter. The percentage of polymorphic markers was fewer for AFLP than RAPD. However, AFLP primers amplified on an average of six times more products than RAPD. While four blocky type cultivars were indistinguishable using RAPD, two AFLP primer pairs were sufficient to distinguish four cultivars from each other. Studies by Kochieva & Ryzhova (2003) also point to the higher efficiency of AFLP markers in revealing the genetic distances among germplasm accessions. Using 9 primer pairs, they were able to generate 16.5% polymorphism among cultivars. AFLP was employed in fingerprinting Guatemala's *Capsicum* genetic resources also (Guzman *et al*. 2005).

AFLP markers were employed in assessing variability among Indian *Capsicum* accessions (Gaikwad *et al.* 2001). Maximum similarity was observed between K2 and PMK1. Loose clusters were distinguished on the basis of geographic locations, with the accessions from South India being close to each other. The polymorphic bands obtained in this study were seven times more than that reported by Paran *et al.* (1998). These results indicate the presence of a high level of genetic diversity in the Indian accessions of *C. annuum* and support the argument of India being the secondary centre of diversity of chilli pepper.

Mapping genes of interest

Genetic mapping of the region containing *Bs*-2 locus coding for resistance to *X. campestris* pv. *vesicatoria* in *Capsicum* was done using AFLP markers (Tai *et al.* 1999). Markers linked to this gene were identified using RAPD and AFLP in near isogenic lines. Linkage map of the region was developed using BSA and tightly linked markers were detected. It was observed that AFLP marker A2 co-segregate with *Bs*-2. A2 marker was sequenced and SCAR primers were developed. It was reported that SCAR primers S2 and S19 are highly useful in marker-assisted selection of resistant lines.

The *Pvr* 4 resistance gene in *Capsicum* confers complete resistance to three pathotypes of potato virus Y and pepper mottle virus. In order to use this gene in marker-assisted selection, tightly linked AFLP markers were obtained using BSA. The closest co-dominant AFLP marker was converted to a co-dominant CAPS marker using data from the alignment of two allele sequences (Caranta *et al.* 1999).

Microsatellite markers

Microsatellites are tandem repeats of DNA sequences of only a few base pairs (1–6 bp) in length, the most abundant being the dinucleotide repeats. The term microsatellite was introduced by Litt & Luty (1989) to characterize the simple sequence stretches amplified by PCR. These tandem repeats in the junk/non-coding DNA is highly conserved and hence most important in phylogenetic studies in all the species including *Capsicum*

(Walsh & Hoot 2001). These are also known as Short Tandem Repeats (STR), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Sequence Tagged Microsatellite Sites (STMS) and are different from minisatellites that are often referred as VNTRs (Variable Number Tandem Repeats). Other 12 terminologies in literature pertaining to microsatellites are detailed by Gupta *et al.* (1996) and Gupta & Varshney (2000). A precise methodology for isolation of satellite DNA from *Capsicum* was given by Black & Prince (1996).

For the first time, Nagy *et al.* (1998) developed and characterized microsatellite markers in *Capsicum*. Later in the same year, a comparison on efficiency of microsatellite and RAPD markers on germplasm identification was done by Wang & Fan (1998). They used 90 accessions collected from Europe, Asia and Australia (16 countries). Both markers had high-level polymorphism. ISSR primer SCRI 1418 was most useful to fingerprint all the accessions. The results suggested that both ISSR and RAPD markers in addition to being simple and time efficient, provides rapid identification of polymorphism within *Capsicum.*

Gyulai *et al.* (1999) used PCR based markers such as RAPD, ISSR and SSR for the analysis of F_1 hybrid derived dihaploid (DH-R_2) *Capsicum* lines and among the various ISSR primer sequences analyzed, $(ACTG)_4$ was most polymorphic. A similar study by Mongkolporn & Dokmaihom (2004) also concludes that ISSR and RAPD are efficient tools for genetic purity tests in hybrid *Capsicum*.

Development of pepper SSR markers from sequence databases with the objectives to search the gene bank databases for the presence of SSR containing sequences from *Capsicum*, to assess the frequency of different motifs and to examine the polymorphism of selected markers in a panel of genotypes was done by Sanwen *et al.* (2000). SSR primer sequences, which are more suitable for amplifying *Capsicum* DNA were given. The Agricultural Biotechnology Centre, Godollo, Hungary, has isolated approximately 400 microsatellite markers from *Capsicum*. Of these, 10–15 markers proved to be successful for practical hybrid seed production. SSR helped to distinguish homozyogotic and heterozygotic lines and to differentiate between Hungarian accessions though the polymorphism among cultivated varieties was extremely low (Juhasz *et al*. 2002). Further, adaptation and optimizing of routinely applicable protocols of automatised fragment analysis techniques for genotype identification, hybrid testing and variety protection are also being done (Nagy *et al*. 2004). Lee *et al*. (2004) and Minamiyama *et al*. (2005a) also reported the isolation and characterization of large number (1837) of new SSR markers in *Capsicum*. Known *et al*. (2005) have successfully demonstrated the use of SSR markers to complement the tests of uniformity and stability in *Capsicum* over generations.

Retrotransposon based SSAP, AFLP and SSR markers were found efficient in comparative analysis of genetic diversities within tomato and *Capsicum* germplasms (Tam *et al.* 2005). A combination of microsatellite and AFLP technique to obtain maximum level of polymorphism among the *Capsicum* lines has also been reported (Acqadro *et al.* 2002). Single Primer Amplification Reaction (SPAR) marker (Gupta *et al.* 1994) is a kind of microsatellite marker. Ballester & Vincente (1998) used SPAR marker for the analysis of F_1 hybrid seed purity in *Capsicum*. The primer $(GATA)_4$ was useful in identifying the hybrid purity by distinguishing male and female parents and F_1 hybrids.

CAPS markers

Cleaved Amplified Polymorphic Sequence (CAPS) marker for the *Pvr* 4 locus in *Capsicum,* which offers resistance to potato virus Y and pepper mottle virus was developed by Caranta *et al.* (1999). In this study, DNA from susceptible and resistant parents, the bulks, F_2 and segregating individuals were scored for the AFLP marker E41-M49-645 and two markers co-segregating with the resistance were detected, eluted, sequenced and con-

firmed by BLAST search (Basic Local Alignment Search Tool – *www.ncbi.nlm.nih.gov/blast*). Subsequently, CAPS primers were designed using the Gene Fisher package. In a similar work, Arnedo-Andres *et al*. (2002) has developed Sequence Characterized Amplified Region (SCAR) and RAPD markers linked to the same gene locus. Further, Grube *et al.* (2000) used CAPS markers linked to *Pvr* 4 to localize *Pvr* 4, *Pvr* 7 and *Tsw* resistance loci to a linkage group 10 on an interspecific map of pepper. A comparative mapping of first identified dominant potyvirus resistant gene cluster in *Capsicum* was done in this study.

Moury *et al.* (2000) have developed CAPS marker to assist the selection of tomato spotted wilt virus resistant lines in *Capsicum*. RAPD markers linked to *Tsw* dominant loci were identified and linkage was analyzed using BSA. SCAR markers for detection of Phyto 5.2 QTL offering resistance to *P. capsici* in *Capsicum* were developed by Quivin *et al*. (2005). Similarly, SCAR markers for capsaicinoid synthase gene responsible for pungency were designed by Lee *et al*. (2005) and Minamiyama *et al*. (2005b). Design of CAPS marker to distinguish hot and sweet peppers with the help of RFLP (Blum *et al*. 2002) has been already discussed. Since the non-pungent capsicums involve a deletion in this gene, early detection of pungency is possible. SCAR markers were also useful for purity testing of F_1 hybrid seeds in *Capsicum* (Jang *et al*. 2004).

Conclusion

Right from the first attempt (Cao 1994), molecular markers have contributed substantially for the improvement of *Capsicum*. RAPD analysis has proved to be very efficient in screening large number of germplasm for genes of interest and genetic diversity and seed purity analyses. RFLP was effective for fingerprinting, genomic library construction, linkage analysis, screening transformants and SCAR/CAPS marker designing. AFLP and microsatellites proved to be efficient in fingerprinting and gene mapping. Combination of markers such as M-AFLP (Acqadro *et al.* 2002) and RF-RAPD (Yang & Park 1997) were found to enhance their efficiency. Only the most prominent characters especially those governed by dominant genes have so far received maximum attention. Diversion of efforts in understanding the genetic mechanism of resistance for all the major stresses and qualitative parameters through suitable markers remains to be most prioritized pre-requisite. The present day work is character specific and total genomic DNA oriented which has led to the formulation of total linkage map of the genus and the culmination should be sequencing of the genome and applications of the sequence to the goal of developing genotypes to suit specific requisites.

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