

Review Article

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Molecular Marker Application in *Capsicum* spp: A Supplement to Conventional Plant Breeding

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ABSTRACT

Chilli, is spice cum vegetable crop having various nutritious, medicinal and industrial application values in it. It consists of many species of which only five are cultivated. Among these, *Capsicum annuum* is widely cultivated across the world followed by *Capsicum frutescens*. Many of the biotic and abiotic stress also effect the crop production. Molecular markers usage in this crop has hasten the crop breeding programme to select diverse parental line, screening for biotic stress, transfer of recessive alleles, identifying and mapping, introgression of these beneficial genes and helps in marker assisted selection (MAS). Now most advanced next generation sequencing and genotyping technologies have also been generating more genomic resources which have to be used efficiently for crop improvement in future.

Keywords

Capsicum, Marker, Diversity, Mapping, MAS.

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Introduction

Chilli or *Capsicum*, is spice cum vegetable crop, belongs to solanaceae family (Greenleaf, 1986). It is native to Central and South America (Pickersgill, 1997) and in 17th century it was introduced to India by Portuguese traders. Chilli is diploid in nature with chromosome number $2n=24$, genome size is 2700 Mb and total available genes are 30,701 (Solgenomics). It consists of several species, of which, only five species viz., *Capsicum annuum*, *Capsicum frutescens*, *Capsicum pubescence*, *Capsicum chinense* and *Capsicum baccatum* are cultivated in different parts of the world (Perry *et al.*, 2007). Three complexes have been identified in the *Capsicum* genus as given in Figure

1 (Taranto *et al.*, 2016). Chilli is often cross pollinated crop and frequency of cross-pollination in the field can range from just 2% to as high as 90% (Pickersgill, 1997).

Chilli contains steam volatile oils, carotenoids, fatty oils, vitamins viz., A, C, E along with mineral elements like molybdenum, manganese, folate, potassium and thiamine etc. (Bosland and Votava, 2003). It has industrial application for paprika oleoresin, capsaicinoids and carotenoids along with non-food uses for defense, spiritual, ethnobotanical (Kumar *et al.*, 2006). A large number of carotenoids provide high nutritional value and the color to chilli. The

pungency is due alkaloid capsaicinoids, these are synthesized in the placental tissue localized inside of the fruits (Wahyuni *et al.*, 2013). According to an estimate for 2016-17, in India, chillies were cultivated on 845,000 ha with a total production of 2.12 million t of dry fruits (NHB, 2017).

Chilli production in India is constrained by biotic stresses like fungal diseases *viz.*, damping off (*Pythium aphanidermatum*), anthracnose or fruit rot (*Colletotrichum capsici*), fusarium wilt (*Fusarium solani*), powdery mildew (*Leveillula taurica*); bacterial disease *viz.*, bacterial wilt; viral diseases *viz.*, chilli veinal mottle virus, leaf curl, murda complex, tospovirus; nematode like Root-knot nematode (*Meloidogyne*) and insect pests *viz.*, gram pod borer (*Helicoverpa armigera*), tobacco caterpillar (*Spodoptera litura*), thrips (*Scirtothrips dorsalis*), aphids (*Aphis gossypii* and *Myzus persicae*), red spider mite (*Tetranychus*), broad/yellow mite (*Polyphagotarsonemus latus*).

Genetic markers and its types

A trait that is polymorphic, easily and reliably identified, and readily followed in segregating generations and indicates the genotype of the individuals that exhibit the trait is known as genetic marker. There are three types as mentioned below

Visible/morphological markers

Flower pigmentation, fruit shape *etc* these traits represent actual phenotype which is easily scorable by naked eye, simple, rapid, inexpensive and these assays do not require sophisticated equipments.

Some of the limitations are, available in lesser number, scored on whole plants, require specific environment for expression and are developmental stage specific.

Protein markers- isozymes

Detected as electrophoretic variants of proteins. They also generated by small changes in the coding sequences of the concerned genes that alter the amino acid sequences of the concerned proteins using small amount of tissue either taken from seedling stage or from seeds and this analysis is easy. Major drawbacks are, it varies with tissue and developmental stage along with environment.

DNA markers- A molecular marker is a DNA sequence that is readily detected

As constant landmarks in the genome and transmitted by the Mendelian laws of inheritance from one generation to the next and detected as differential mobility of fragments in a gel or by hybridization with specific probe. Maximum numbers of markers are available throughout the genome exhibit more polymorphism located in DNA at the level of nucleotide bases which is source of biological function these arise from DNA mutations such as point mutation, insertions or deletions or errors in replication of DNA (Paterson, 1996). These are independent of environment and developmental stage of crop.

Markers available are Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Simple Sequence Repeats (SSRs), Inter-Simple Sequence Repeats (ISSRs), Amplified Fragment Length Polymorphisms (AFLPs) and Single Nucleotide Polymorphisms (SNPs). Some novel markers include Randomly amplified microsatellite polymorphisms (RAMPO), Expressed Sequence Tag Polymorphisms (ESTPs), Conserved Orthologue Set (COS) markers, Amplified Consensus Genetic Markers (ACGMs), Gene Specific Tags (GSTs), Resistance Gene Analogues (RGAs) and

Exon Retrotransposon Amplification Polymorphism (ERAP) (Gupta and Rustgi, 2004). Targeting Fingerprinting Markers (TFMs) are Conserved DNA-Derived Polymorphism (CDDP), Cytochrome P450-Based Analogues (PBA), Intron-Targeting Polymorphism (IT), Start Codon Targeted (SCoT) Polymorphisms, Sequence-Related Amplified Polymorphism (SRAP) and Targeted Region Amplified Polymorphism (TRAP), Conserved Region Amplification Polymorphism (CORAP), Some Mobile element-based molecular markers include: Inter-Retrotransposon Amplified Polymorphism (IRAP) and Retrotransposon Microsatellite Amplified polymorphism (REMAP) Retrotransposon Based Insertion Polymorphism (RBIP), Retrotransposon Based Sequence Specific Amplification Polymorphism (SSAP) as mentioned literature (Semagn *et al.*, 2006; Kumar *et al.*, 2009; Ismail *et al.*, 2016). The effective marker has following criteria (Jiang, 2013)

High level of polymorphism (Clear distinct allelic features).

Even distribution across the whole genome.

Co-dominance inheritance (so that heterozygotes can be distinguished from homozygotes).

Single copy and no pleiotropic effect.

Low cost to use.

No detrimental effect on phenotype (Selectivity neutral).

Markers can be easily exchangeable between laboratories.

In *Capsicum sp.*, most of the research workers isolated genomic DNA from young leaf tissue following the method of Doyle and Doyle (1990) with minor modifications.

Molecular markers in *Capsicum sp.* are mainly being used for the following purposes

Germplasm characterization, conservation and utilization

For efficient, effective conservation and utilization of genetic resources, identification and characterization of germplasm is an important step. Molecular markers assist in ex-situ germplasm preservation; to sampling, management and development of core collection there by making decisions on multiplication and maintenance of plant accessions to make them exploitable by plant breeders. Similarly for in-situ it aid in recognition of the most representative populations within the 'gene pool' of a landrace (Sergio and Barcaccia, 2005). It is a tool for precise germplasm identification and builds crop plant collection based on presence of valuable genes and traits and also powerful tool for removing duplicates and to establish core collection (Barcaccia, 2009).

rDNA (18S and 5S) specific probe and heterochromatic banding pattern used for characterize cultivars (Romero-da Cruz *et al.*, 2017). SSR markers were designed for conserved coding regions of *C. annuum* could be able to amplify region in *C. pubescens*. SSR and ISSR markers used to characterize the cultivar of *C. annuum* and *C. pubescens* sp (Ibarra-Torres *et al.*, 2015).

Genetic diversity analysis, heterotic pools prediction, DNA fingerprinting and protection of varieties.

The choice of using marker technique in diversity analysis is due their capacity to detect genetic diversity at higher level of resolution (the DNA), speed and information obtained from small quantity of plant material. It has shown that molecular markers are important tools in characterizing

estimating genetic variability within and between species and populations (Nayak *et al.*, 2005).

Thul *et al.*, 2012 conducted diversity analysis by using floral characteristics and the molecular markers (RAPD and ISSR) and revealed that *C. annuum* accessions formed a single cluster in the molecular analysis as maintaining their flower characteristic. *C. chinense* accessions shared flower features with the accessions of *C. frutescens* and were found to be closer at genotypic level. *C. luteum* was found to be rather closer to *C. baccatum* complex, both phenotypically and genetically. The accession of *C. eximium* presenting purple flowers falls apart from the groupings, this found to be helpful towards delineation of the species specificity.

Genetic diversity also investigated by RAPD primer (Makari *et al.*, 2009). AFLP marker based diversity study conducted by Krishnamurthy *et al.*, 2015 and this inquiry shown that high number of polymorphic bands suggests that AFLPs are efficiently discriminator and powerful marker for classification, finger printing and diversity analysis.

Toledo-Aguila *et al.*, (2016) conducted characterization study with microsatellite marker and opined that all of this genetic diversity found in the native and wild chili populations of Mexico must be protected and conserved for future studies. Principal component and clustering done indicates collection from different geographic region of Mexico. So this diversity can be exploited by selection. Morphological and molecular markers (RAPD and ISSR) employed in selecting parents for production segregating population. The molecular markers are valid tags for the investigation of genetic diversity in *C. annuum* germplasm (Rana *et al.*, 2014). SSR markers used in diverse parent selection

for further breeding process including hybridization through which limits narrowing of genetic base and also guides in stalking of desirable genes. (Hossain *et al.*, 2014).

ISSR markers were also used in genetic variation experiment reported that UBC841, LOL12, and LOL10 could be very useful due to their polymorphism (Pena-Ortega *et al.*, 2016). Diversity Arrays Technology or (DArT) along with next generation sequences to discover higher number of markers (Mongkolporn and Kethom, 2016). SNP markers identified by DArT sequencing were used to study origin of species in various places and Clustering (Silvar *et al.*, 2016). Genotyping by sequencing (GBS) analysis generated SNP markers used in diversity analysis of *C. annuum* (Taranto *et al.*, 2016).

These molecular markers help in heterotic group or pool construction. A heterotic group is a set of genotypes displaying similar hybrid performance when crossed with individuals from another, genetically distinct germplasm group. It helps in broadening genetic base and limits the uniformity of genotypes. Krishnamurthy *et al.*, 2013 revealed that intermediately divergent parents produced remarkable heterotic cross by using AFLP and morphological markers. Isozyme markers (Peroxidase (PO) and Polyphenol oxidase (PPO) isozymes) can also be used for variety registration (Kumar *et al.*, 2014). DNA profiling by RAPD (Sanatombi *et al.*, 2010; Prasad *et al.*, 2013), AFLP and ISSR (Gaikwad *et al.*, 2013) may be a useful tool for cultivar identification as well as for variety protection.

Taxa identification, phylogenetic relationship and identification of adulterants

Phylogeny is the history of descent of a group of taxa such as species from their common

ancestors including in a study on the basis of their relationships so groupings indicates the degree of genetic similarities and dissimilarities among them (Patwardhan *et al.*, 2014).

By the phylogenetic research results using RAPD markers on *C. chinense* from different geographic regions it has shown that within *C. chinense* three clusters are possible (Moses and Umaharan, 2012). Based on molecular marker study using RAPD, it is revealed that Naga king chilli (Bhoot Jolokia in Assam) is possibly an interspecific hybrid of *C. chinense* and *C. frutescens* (Bosland and Baral, 2007). An allopolyploid cultivar 'Dalle Khursani' ($2n = 4x = 48$) a *C. annuum* complex its genomic DNA analysis with 30 RAPD based molecular markers only two primers are reproducible revealed that *C. annuum* and one with *C. frutescens* and two with *C. chinense* indicating close affinity with *C. annuum* (Jha *et al.*, 2017).

Attempting towards sequence analysis of the nuclear ribosomal DNA (nrDNA) Internal Transcribed Spacers (ITS) region (Figure 2), the phylogenetic relationship of Naga King Chili showed a clear grouping from *C. chinense* and *C. frutescens* (Kehie *et al.*, 2016).

Dhanya and Sasikumar (2010) mentioned that PCR based markers has been used for adulterant detection in chilli powder of by using species specific primers (mainly RAPD and SCAR markers).

The mapping of genes/QTL for qualitative and quantitative traits and Marker assisted selection (MAS)

A genetic map is a schematic representation of genetic markers in the specific order, in which they are located in a chromosome along with the distances between them. In

most of the cases, Haldane (1919) and Kosambi (1944) mapping functions helps for converting recombination frequency into genetic distance.

For constructing the genetic maps we require mapping population which means population that is suitable for linkage mapping of genetic markers. etc. various types of mapping populations are F₂, F₂-derived F₃ (F₂:₃), backcross (BC), backcross inbred lines (BILs), doubled haploids (DHs), recombinant inbred lines (RILs), near-isogenic lines (NILs), chromosomal segment substitution lines (CSSLs), immortalized F₂, advanced intercross lines, recurrent selection backcross (RSB) populations, and interconnected populations as given in the Figure 3 (Singh and Singh, 2015). Most commonly used populations are F₂ (Prince, *et al.*, 1993; Livingstone *et al.*, 1999; Djian-Caporalino *et al.*, 2001; Kang *et al.*, 2001; Ben-Chaim *et al.*, 2001; Arnedo-Andres, *et al.*, 2002; Blum *et al.*, 2003; Lee *et al.*, 2009; Maharijaya *et al.*, 2015; Venkatesh *et al.*, 2015), DH (Djian-Caporalino *et al.*, 2001; Sugita *et al.*, 2005; Minamiyama *et al.*, 2006) and RIL (Ogundiwin *et al.*, 2005; Barchi *et al.*, 2007; Naegele *et al.*, 2014; Yarnes *et al.*, 2013).

Conventional plant breeding is supplemented with molecular breeding approaches including both, the transgenic crops and the marker-assisted selection (MAS). The use of molecular markers that are tightly-linked to target loci as a substitute for or to assist phenotypic screening or selection is known as MAS. Markers helps in indirect selection of desired alleles in earlier stages of plant life and early in breeding line development, polygenic and showing low heritability which requires additional breeding cycles (selfing) and/or expressed late in development. Progeny testing can be bypassed if co-dominant marker systems are used to identify recessive alleles present in the desired

genotype (Frey *et al.*, 2004). Markers are really beneficial for introgression of genes from breeding lines or wild relatives, MABC, marker-assisted recurrent selection (MARS) and pyramiding of genes.

Molecular marker application for biotic stress

To develop cultivar with host-plant resistance, considered as an economically viable and eco-friendly approach to manage biotic stress. Conventional breeding has met with limited success due polygenic control of resistance traits, wide range of pathogen strains distributed in different environments, Complexity of host-pathogen interaction and wide variability pathogenicity. So MAS is an effective and reliable approach. Specific geographic region isolates of pathogen-specific QTL controlling resistance is critical to breed for a cultivar with durable resistance (Truong *et al.*, 2012).

For mapping QTL's conferring to anthracnose resistance mapping population derived from *C.annuum* variety 'Bangchang' X *C.chinense* PBC 932, the QTL map with 214 SNPs and covered 824cM. Another mapping population obtained from *C. baccatum* 'PBC80' x 'CA1316, the map having 403 SNPs and 1270cM coverage (Struss *et al.*, 2016). Suwor *et al.*, 2017 conducted research on anthracnose disease resistance lines selection by marker assisted selection in introgressed lines PR1 (derived from PBC 932) and PR2 (derived from PBC 80) crossed to susceptible parent (PS). Validation of SCAR-Indel and SSR-HpmsE032 markers on F2 of the three way population revealed that their individual ability to predict correctly the resistant genotype was 65per cent; together it was 77 per cent.

QTL Pc.5.1 confers major QTL effect for resistance to root rot in germplam (Lefebvre

et al., 2013). For Phytophthora root rot, two types of resistance one is oligogenic which follow gene for gene hypothesis by doing experiment on RIL population (Sy *et al.*, 2008) and another one polygenic as studied by using intraspecific (*C. annum*) DH population (Lefebvre and Palloix, 1996). A Perennial accession has 4 main QTLs controlling resistance to *P. capsici*. To make rapid progress in introgression, a DH285 line (has 3QTL) derived from the cross Perennial X Yolo Wonder YW, having all the chromosomal regions to be transferred was used as donor parent and YW, a bell pepper line used as recipient. Three cycle of Backcrossing has carried along with screening of markers linked to resistance allele's presence and lastly for recipient genetic background and they identified additive and epistatic effect of QTLs (Thabuis *et al.*, 2004). Xu *et al.*, (2014) revealed that SRAP-Me6/Em15 marker linked with *Phytophthora* blight resistance.

The *Me7* gene as a resistance gene localized on long arm of the chromosome P9 (other *Me* genes like *Me1*, *Me3*, *Me4*, *Me7*, *Mech1*, *Mech2* are also located on chromosome 9). To fine map the *Me7* gene using F2 individuals with SNP markers developed by reference genome information were used, yet no closer marker has identified. Nearly 22 NB-LRR candidates were identified in the flanking region by using Ren-Seq analysis (Changkwian *et al.*, 2016). The tightly linked markers 375A and 226B can be used in marker assisted selection (MAS) to develop RKN resistant lines (Toth *et al.*, 2016).

Two closely linked markers to the powdery mildew resistance gene PMR1 were developed and this gene was located on chromosome 4. These markers can be used for fine mapping of gene (Jo *et al.*, 2016). Bs3 gene mediates the perception of avrBs3-expressing strain of Bacterial spot pathogen,

PCR based co-dominant DNA marker PR-Bs3 which help in diagnosis of pepper line (Romer *et al.*, 2010). This becomes a tool in MAS to resistance breeding of bacterial spot.

Gene pyramiding strategy and molecular markers along with biological assays can be effectively used to transfer multiple virus resistance genes to the sweet Charleston line Y-CAR (Ozkaynak *et al.*, 2014).

Resistant CMV genes are recessive in nature and these resistance genes from *C. annum* French Perennial and *C. frutescens* (BG2814-

6) to commercial varieties (bell, jalapeno and Anaheim) transferred by backcross method and tried to map CMV resistance QTL by PCR based marker after RFLP and RAPD marker conversion. Morphological traits were also employed along with RAPD molecular markers to identify CMV tolerant BC3 individuals Plants were 99.9 % more similar to their recurrent parent, by this breeder effort reduced to few backcrossing cycles and it also avoided progeny test in each backcross (Herison *et al.*, 2012), to introgress recessive gene governed CMV tolerance (Herison *et al.*, 2004).

Table.1 QTLs associated with important trait of interest in pepper

Trait	Mapping population	QTLs detected	Effect of the QTLs	Reference
Number of flowers per node	46 F2 progeny	28.8+39.9%		Prince <i>et al.</i> , (1993)
Fruit-related traits	180 F3 family progeny <i>C. annum</i> Maor × <i>C. annum</i> Perennial	6–67% the QTL	according to	Ben Chaim <i>et al.</i> , (2001b)
Yield and fruit-related traits	248 interspecific BC2 progeny [(<i>C. annum</i> cv. Maor × <i>C. frutescens</i> BG2816) × BG2816] × BG2816]	1–25% the QTL	according to	Rao <i>et al.</i> , (2003)
Fruit related traits Fruit length (FL); Fruit diameter (FD); Fruit shape (FS)	94 Doubled haploid progeny California Wonder (<i>C. annum</i>) X LS2341 (JP187992) (<i>C. annum</i>)	FL- 51-52%; FD-37-38%; FS-61-68%	of the total phenotypic variation	Mimura <i>et al.</i> , 2012
Capsaicinoid content	242 Interspecific F2 progeny [(<i>C. annum</i> cv. Maor × <i>C. frutescens</i> BG2816)	34–38%	according to the experimental year	Blum <i>et al.</i> , (2003)
Resistance to <i>Phytophthora capsici</i>	94 Doubled haploid progeny Perennial × Yolo Wonder	21–90%	according to the resistance components	Lefebvre and Palloix (1996)
Resistance to potyviruses	94 Doubled haploid progeny Perennial × Yolo Wonder	66–76%	according to the potyvirus strain	Caranta <i>et al.</i> , (1997a)
Restriction of cucumber mosaic virus installation in host-cells	94 Doubled haploid progeny Perennial × Yolo Wonder	Together explaining 57%	of the phenotypic variation	Caranta <i>et al.</i> , (1997b)
Resistance to cucumber mosaic virus	180 F3 family progeny <i>C. annum</i> Maor × <i>C. annum</i> Perennial	7–33%	according to the QTL	Ben Chaim <i>et al.</i> , (2001a)
Restriction of cucumber mosaic virus long distance movement	101 Doubled haploid progeny H3 × Vania	4.0–63.6%	according to the QTL	Caranta <i>et al.</i> , (2002)
Resistance to <i>Leveillula taurica</i>	101 Doubled haploid progeny H3 × Vania	Together explaining more than 50%	of the phenotypic variation	Lefebvre <i>et al.</i> , (2003)
Resistance to <i>Ralstonia solanacearum</i> bacterial wilt (BW)	94 Doubled haploid progeny California Wonder X LS2341.	33%	of the resistance derived from 'LS2341'	Mimura <i>et al.</i> , (2009)
Resistance to thrips	196 F2 plants from <i>C. annum</i> AC 1979 X <i>C. chinense</i> 4661	50 %	of the genetic variation	Maharijaya <i>et al.</i> , (2015)

Lefebvre, 2005

Fig.1 Main Capsicum complexes (the name of the complex is highlighted) (Taranto *et al.*, 2016)

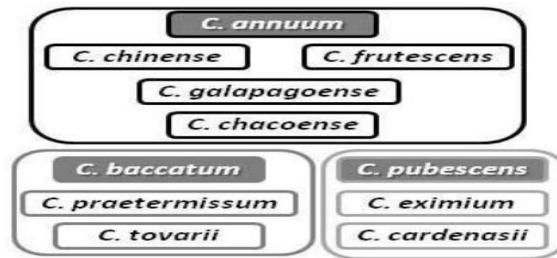


Fig.2 Organization of the eukaryotic nuclear ribosomal DNA tandem repeats [External transcribed sequence (ETS); Non-transcribed spacer (NTS); Internal transcribed spacer (ITS)]

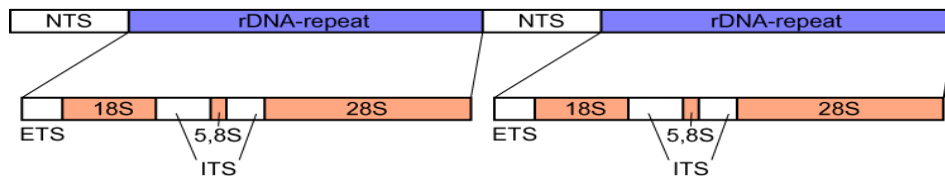
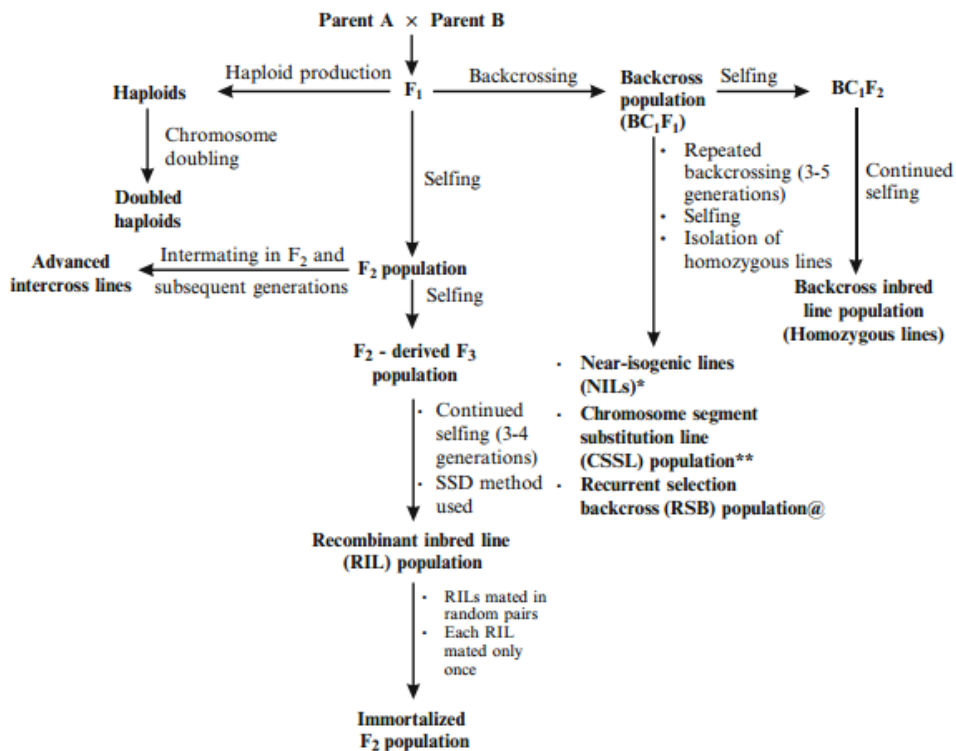


Fig.3 Simplified graphical chart of the various biparental mapping populations.*Introgression of a gene by repeated backcrossing combined with selection for the gene. ** Repeated backcrossing without selection; each line has a distinct chromosome segment from the donor parent. (Singh and Singh, 2015)



Basically Chilli veinal mosaic virus resistance is recessive in nature (Green and Kim 1994), but Indian landrace of *C. annuum* NW4 has dominant source of resistance gene. AFLP markers linked to this gene was converted into high-resolution melting markers for single nucleotide polymorphism (SNP) detection (chromosome 6) further CAPS marker obtained (Lee *et al.*, 2013). Incorporation of the *Tsw* resistance gene from *C. chinense* was evaluated using the linked codominant SCAR T2 marker to *Tsw*. (Salamon *et al.*, 2016). Pvr 4 loci confer dominant resistance to three pathotypes of PVY and PepMoV; markers linked to the resistance genes which were developed by next generation sequencing (NGS) technology along with BSA coupled with comparative genomics of pepper and tomato. Single nucleotide variants discovered were converted to CAPS markers. This helped in fine mapping of Pvr4 interval in pepper to 630 kb (Devran, 2015).

Pvr7 and Pvr4 is tightly linked dominant resistance genes, A CAPS marker linked to Pvr4 helped to localize this gene (Grube *et al.*, 2000). RAPD marker converted to SCAR (SCUBC191423) linked to *Pvr4* gene helps in MAS (Arnedo-Andres *et al.*, 2002). Chilli veinal mottle virus (ChiVMV) resistance in 'IHR 2451' was monogenic recessive and K5-HD6 a RGAP marker which co-segregated with ChiVMV resistance which helps in the marker assisted selection (Naresh *et al.*, 2016). QTL associated with some important trait is given in Table 1.

According various researchers most of the genes for viral pathogens are present in the *C. chinense* L. PI159236. Majority of viral disease research is taken up in tomato and potato, by comparative maps explains conservation of marker content and also facilitates transfer of information between Solanaceae family (Livingstone *et al.*, 1999).

As virus screening methods were laborious and expensive. These obstacles can be overcome by usage of MAS.

Abiotic Stress

Cross between popular high yielding, heat sensitive malaysian chilli variety Kulai and heat tolerant AVPP0702 done to introgress heat shock protein (*Hsp*) genes by following backcross method along with 252 SSR marker assisted selection to overcome linkage drag and time consumption problem of traditional breeding method. Recipient allele recovered to maximum of 83.40% in one of the BC1F1 plant (BC1P7) and was positive to *Hsp*-linked markers (*Hsp70-u2* and AGI42). Genome recovery of the recurrent parent after three successive generation of backcrossing was upto 95.37% (Usman *et al.*, 2017).

Quality

From *C. baccatum* species for agronomically important traits 61 QTLs identified on 9 linkage groups (Moulin *et al.*, 2015). Segregating doubled haploid progenies with RFLP and RAPD markers intraspecific integrated linkage map were constructed to locate L (hypersensitive resistance to TMV), C (fruit pungency), up (erect habit of the fruits) genes on chromosomes (Lefebvre *et al.*, 1995). *C. chinense* is high pungent one (Tanaka *et al.*, 2016). RFLP marker converted to CAPS markers. These markers also help in recessive c gene selection (Blum *et al.*, 2002).

Markers help in handling large population of pepper for developing new low-pungent capsinoid containing cultivars development (Tanaka *et al.*, 2014). Babak *et al.*, 2016 developed STS marker particularly fruit quality gene i.e., analogue of the *rin* (*ripening inhibitor*) mutation specific to capsicum after comparative analysis of marker allele fragment sequence with tomato.

Male sterility

Chilli male sterility has been reported by Peterson (1958). Inability of flowering plant to produce functional pollen grain is referred as male sterility. In majority of the crops male sterility is exploited for hybrid seed production (heterosis breeding) by preventing self-pollination. In chilli CMS, GMS and CGMS types are applicable. Markers mainly used to identify tight association with trait of interest, Possible to convert diverse lines into restorers and Maintenance of fertility restorer trait. S-cytoplasm (Kim and Kim, 2005) and the restorer-of-fertility *Rf* gene (Gulyas *et al.*, 2006) are associated with molecular markers. For selection restorer and maintainer plants more widely distributed *Rf* gene which are tightly linked to markers would be required to exercise marker assisted selection (MAS) (Kumar *et al.*, 2004). It permits classification of lines as either B or R without need for test crosses. For *Restorer-of-fertility (Rf)* based expression CaPPR6 (pentatricopeptide repeat protein) was identified as candidate gene by using BSA-AFLP and comparative mapping fine mapping and ColMod1-CAPS, which is located near *CaPPR6* could be remarkable in MAS (Jo *et al.*, 2016).

GMS is two lines breeding transferred among parental lines, in this most of the breeding lines are restorers, and no negative cytoplasmic effect (Mandaliya and Thaker, 2016). So GMS type of hybrid seed production was followed due to ease in introgression of genetically controlled recessive male sterility genes by backcross method where selfing is done after every backcross. In this case homozygous (*MsMs*) and heterozygous (*Msms*) genotype identification was difficult, this was resolved by developing marker which is associated with control of male sterility in paprika by following Bulk segregant analysis in F₂ population by making bulk of male sterile and

male fertile and amplified fragment length polymorphism (AFLP), a dominant marker. Markers Egat/Mcgg (AFLP) was converted into PmsM1-CAPS (cleavage amplified polymorphic sequence) marker is located about 2 to 3 cM from the *ms* locus (Lee *et al.*, 2010). Lee *et al.*, (2010) reported that E-AGC/M-GTG AFLP marker converted into co-dominant sequence characterized amplified region (SCAR) marker based on sequence information for *ms*₁ locus selection located about 3 cM distant from SCAR, which helps in GMS system. These codominant markers help in heterozygote selection without progeny test.

Detection of polymorphism, environmental interaction with genotype, time required for detection of recessive alleles are some of the constraints in conventional plant breeding strategies. Rapid, early detection of traits, screening of difficult traits and efficient molecular marker approaches hasten the crop improvement programme and also helps to breed for durable biotic stress tolerant eco-friendly cultivars with respect to major pepper diseases. MAS acts as complementary tool for traditional plant breeding. Linkage and QTL mapping for various traits are providing an additional genomic resource to the plant breeders to speed up new cultivar development in future.

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