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5 Development and Evolution of Molecular Markers and Genetic Maps in *Capsicum* Species

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Abstract

Capsicum species including six main cultivated species, *C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, *C. pubescens,* and *C. assamicum*, commonly known as chili peppers, are economically important crops in the world. Peppers are used as fresh vegetables, spices, pigments, and medical supplies. Over the four decades, a number of molecular marker techniques have been developed to analyze variations on DNA sequences of the genome in biological organisms. For plant breeding, molecular markers can substantially improve selection efficiency and reduce breeding time compared to conventional breeding. Genetic linkage mapping is an important basic tool for localizing gene(s) that are associated with important horticultural traits, marker-assisted selection, comparative mapping, physical mapping, and map-based cloning of the gene of interest. Recently, genetic linkage mapping has become easier owing to the advent of next-generation sequencing technology and its various applicative technologies. Here in this chapter, we reviewed the development and evolution of molecular markers and genetic maps of *Capsicum* spp. in which pepper researchers are interested.

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5.1 Introduction

Molecular markers, which can be classified into biochemical and DNA markers, indicate a visible phenotype or fragment of DNA that is associated with a certain location within the genome (Kumar 1999). Of them, DNA marker is the most important marker because it is the most widely used. The well-known techniques for DNA markers include restriction fragment length polymorphisms (RFLPs; Botstein et al. 1980), random amplified polymorphic DNAs (RAPDs; Williams et al. 1990), cleaved amplified polymorphic sequences (CAPSs; Akopyanz et al. 1992), sequence-characterized amplified regions (SCARs; Paran and Michelmore 1993), amplified fragment length polymorphisms (AFLPs; Vos et al. 1995), simple sequence repeats (SSRs; Hearne et al. 1992), and single-nucleotide polymorphisms (SNPs; Wang et al. 1998). DNA markers can be applied in construction of genetic linkage maps, comparative mapping analysis, understanding germplasm relationships, tagging economically important genes, marker-assisted selection (MAS; Mohan et al. 1997; Ribaut and Hoisington 1998), and map-based cloning of genes (Kumar 1999). Several articles are well reviewed on molecular markers (Mohan et al. 1997; Kumar 1999; Kesawat and Das 2009; Jiang 2013).

Genetic mapping is an important method for positioning genes of interest in genome as well as identifying quantitative trait loci (QTLs)

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responsible for natural phenotypic variation (Xu et al. 2017). Recently, rapid genome-wide SNP detection techniques using next-generation sequencing (NGS; Shendure and Ji 2008), including genotyping-by-sequencing (GBS; Huang et al. 2009), restriction site-associated DNA sequencing (RAD-seq; Baird et al. 2008), and specific-locus amplified fragment sequencing (SLAF-seq; Sun et al. 2013), shortened the time required for genetic map construction.

In this chapter, I will review the development and evolution of various molecular marker techniques, their use for *Capsicum* researches, development of the interspecific and intraspecific genetic maps of *Capsicum* spp., and their application to QTL detection and marker-assisted backcrossing (MABC).

5.2 Development and Evolution of Markers in *Capsicum* Species

5.2.1 Biochemical Marker

Biochemical markers, also known as protein markers or isozyme markers, can be examined by protein electrophoresis to identify the alleles producing isozymes (Kumar 1999). The most commonly used biochemical markers are isozymes which are variant forms of the same enzyme (Vodenicharova 1989). Isozyme markers reveal differences in the amino acid sequence and function as codominant markers. However, their use is limited due to their limited number and various posttranslational modifications.

Three isozyme markers, *Gpi-2*, *Idh-1*, and *Pgm-2*, were mapped on an interspecific genetic map of *Capsicum annuum* 'NuMex RNaky' × *Capsicum chinense* 'PI159234,' and two isozyme markers, *Idh-1* and *Pgm*, were used to compare the genetic maps between pepper and tomato (Livingstone et al. 1999). The isozyme marker *Mnr-1* corresponding to the first region of the menadione reductase (1.6.99.2) was mapped on an intraspecific genetic map of *C. annuum* 'Perennial' × 'Yolo Wonder' (Lefebvre et al. 2002).

5.2.2 DNA Marker

DNA markers, which are based on the difference of DNA sequences, can be classified into two categories, hybridization-based and polymerase chain reaction (PCR)-based markers, depending on the method to detect polymorphisms (Kumar 1999). Hybridization-based markers include restriction fragment length polymorphism (RFLP; Botstein et al. 1980) and variable number of tandem repeats (VNTRs; Nakamura et al. 1987) that generally use probes and Southern blot analysis. PCR-based markers can be more classified into random and specific, depending on the type of primer used. Random PCR-based markers include random amplified polymorphic DNA (RAPD; Williams et al. 1990), arbitrarily primed PCR (AP-PCR; Welsh and McClelland 1990), DNA amplification fingerprinting (DAF; Caetano-Anollés et al. 1991), inter-simple sequence repeats (ISSRs; Gupta et al. 1994; Zietkiewicz et al. 1994), and amplified fragment length polymorphism (AFLP; Vos et al. 1995). Specific PCR-based markers include simple sequence repeat (SSR; Hearne et al. 1992), cleaved amplified polymorphic sequence (CAPS; Akopyanz et al. 1992; Konieczny and Ausubel 1993; Lyamichev et al. 1993), sequence-characterized amplified region (SCAR; Paran and Michelmore 1993), and single-nucleotide polymorphism (SNP; Wang et al. 1998). The characteristics of these markers are given in Table 5.1.

5.2.2.1 Restriction Fragment Length Polymorphism (RFLP)

RFLP analysis follows next steps: DNA extraction, restriction enzyme digestion, agarose gel electrophoresis, Southern blotting, hybridization with radioactive probes such as random genomic clones and cDNA clones, and autoradiography (Botstein et al. 1980). RFLP reveals the differences of fragment length hybridized with probes, which are resulted from the presence or the absence of a cleavage site and insertion or deletion of DNA sequences within a fragment (Botstein et al. 1980). The major strength of RFLP markers is their high reproducibility, codominant

Table 5.1 Overview of the characteristics of some important DNA markers

Classification	Marker	Genomic abundance[a]	Locus specificity[b]	Codominance[b]	Reproducibility[a]	Required DNA quantity[a]	Labor intensity[a]	Cost per analysis[c]	First reported references
Hybridization-based marker									
	RFLP	H	Y	Y	H	H	H	H	Botstein et al. (1980)
	VNTR	M	N/Y	N/Y	H	H	H	H	Nakamura et al. (1987)
PCR-based marker									
Random primers	RAPD	H	N	N	L	L	L	L	Williams et al. (1990)
	ISSR	M-H	N	N	M-H	L	L	L	Gupta et al. (1994), Zietkiewicz et al. (1994)
	AFLP	H	N	N/Y	M-H	M	M	M	Vos et al. (1995)
Specific primers	SSR	H	Y	Y	H	L	L/M	L	Hearne et al. (1992)
	CAPS	L	Y	Y	H	L	L-M	L	Akopyanz et al. (1992)
	SCAR	L	Y	N/Y	H	L	L	L	Paran and Michelmore (1993)
	SNP	VH	Y	Y	H	L	L	L/VL	Wang et al. (1998)
	InDel	H	Y	Y	H	L	L	L/VL	Bhattramakki et al. (2002), Weber et al. (2002)

[a] *VH* very high, *H* high, *M* medium, *L* low
[b] *Y* yes, *N* no
[c] *H* high, *M* moderate, *L* low, *VL* very low

inheritance, and good transferability which can allow synteny studies (Kesawat and Das 2009).

A total of 85 RFLP markers were developed using tomato cDNA probes and mapped by using 46 F_2 individuals derived from the F_1 of *C. annuum* 'CA50' and *C. chinense* 'CA4' to compare the genetic maps between pepper and tomato (Tanksley et al. 1988). A total of 192 molecular markers including RFLPs and isozymes were used for constructing an interspecific pepper genetic map (Prince et al. 1993). A total of 85 markers including RFLP and RAPD covered approximately 820 cM of the integrated pepper linkage map (Lefebvre et al. 1995). Using pepper-derived probes, total 150 RFLP markers were developed and positioned on an interspecific F_2 linkage map of *C. annuum* 'TF68' *C. chinense* 'Habanero' (Kang et al. 2001). To analyze yield-related quantitative trait loci (QTLs) in pepper, 92 RFLP markers were used, resulting in detection of a total of 58 QTLs (Rao et al. 2003). To detect QTLs associated with *Phytophthora capsici* resistance, a RFLP-based linkage map was constructed using 100 F_2 individuals from a cross between *C. annuum* 'CM334' and *C. annuum* 'Chilsungcho' and bacterial artificial chromosome (BAC)-derived markers were developed from RFLP linked to the resistant trait (Kim et al. 2008c).

5.2.2.2 Minisatellites: Variable Number of Tandem Repeat (VNTR)

VNTR analysis is almost the same with RFLP analysis except using probes with minisatellite sequences (Kumar 1999). The polymorphism of VNTR is due to the differences in the number of repeats (Nakamura et al. 1987). No VNTR markers were used for the *Capsicum* studies.

5.2.2.3 Random Amplified Polymorphic DNA (RAPD)

Multiple arbitrary amplicon profiling (MAAP) techniques include random amplified polymorphic DNA (RAPD; Williams et al. 1990), arbitrary primed PCR (AP-PCR; Welsh and McClelland 1990), and DNA amplification fingerprinting (DAF; Caetano-Anollés et al. 1991), which are random PCR markers (Kumar 1999). RAPD markers use generally 10 bp synthetic primers of random sequence, while AP-PCR uses longer arbitrary primers than RAPDs and DAF uses shorter 5–8 bp primers to generate a larger number of fragments (Kesawat and Das 2009).

An integrated linkage map of pepper, including RFLP and RAPD markers, was constructed by alignment of three intraspecific linkage maps (*C. annuum*, Lefebvre et al. 1995). Screening with 400 RAPD primers along with bulked segregant analysis (BSA; Michelmore et al. 1991) allowed the identification of three QTLs for capsaicinoid content in *Capsicum* (Blum et al. 2003). A total of 122 RAPD markers were used for constructing an intraspecific linkage map of *C. annuum* (Sugita et al. 2005).

5.2.2.4 Inter-simple Sequence Repeat (ISSR)

ISSR, which uses microsatellites as primers, involves amplification of DNA segments present at a close distance in between two identical microsatellite repeat regions oriented in opposite directions (Gupta et al. 1994; Zietkiewicz et al. 1994). The primers can be either unanchored or more usually anchored at 3′ or 5′ end with 1–4 degenerate bases extended into the flanking sequence (Reddy et al. 2002). ISSRs have higher reproducibility due to the use of longer primers (16–25 bp) as compared to RAPD primers (10 bp, Reddy et al. 2002).

A total of 17 ISSR markers were used for differentiating the four disputed chili pepper samples (Kumar et al. 2001). Five ISSR primers amplified 204 reproducible bands of which 139 were polymorphic and they were used for assessing the genetic relation to 13 *C. annuum* cultivars (Patel et al. 2011). Eight ISSR primers were used for analyzing genetic variability in six *Capsicum* species (Thul et al. 2012). A total of 219 ISSR clear and reproducible fragments generated with 13 ISSR primers were used to evaluate the effects of in vitro culture on genetic variation in Habanero pepper (*C. chinense* Jacq.; Bello-Bello et al. 2014). Using eight ISSR anchored primers, a total of 38 bands were obtained for assessment of inter- and intraspecific differentiation in two Serrano and two Jalapeno

cultivars of *C. annuum* and one cultivar of *Capsicum pubescens* (Ibarra-Torres et al. 2015). Total 85 ISSR markers were used to construct a genetic map of *Capsicum baccatum* (Moulin et al. 2015).

5.2.2.5 Amplified Fragment Length Polymorphism (AFLP)

The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digested genomic DNA (Vos et al. 1995). AFLP analysis follows next steps: DNA extraction, double digestion with two different restriction enzymes (generally *Eco*RI and *Mse*I), adaptor ligation, pre-selective amplification, selective amplification, polyacrylamide gel electrophoresis (PAGE), and silver staining (Vos et al. 1995; Blears et al. 1998). The banding profiles result from variations in the restriction sites or in the intervening regions (Kesawat and Das 2009).

A total of 430 AFLP markers were used to construct a linkage map of pepper in an interspecific F_1 population derived from a cross between *C. annuum* 'TF68' and *C. chinense* 'Habanero' (Kang et al. 2001). A genetic linkage map of the sweet pepper was constructed by 382 AFLP markers in an intraspecific doubled haploid (DH) population (Sugita et al. 2005). A total of 175 AFLP markers were used for identifying QTLs associated with anthracnose resistance in an intraspecific F_2 population of a cross between *C. baccatum* var. *pendulum* and *C. baccatum* 'Golden-aji' (Kim et al. 2010). Using an introgression BC_1F_2 population made by interspecific crosses between *C. annuum* 'SP26' and *C. baccatum* 'PBC81,' a total of 197 AFLP markers were developed to identify QTLs for resistance to anthracnose caused by *Colletotrichum scovillei* and *C. dematium* (Lee et al. 2010b).

5.2.2.6 Simple Sequence Repeat (SSR)

Microsatellites are known as simple sequence repeat (SSR; Hearne et al. 1992), short tandem repeat (STR), and simple sequence length polymorphism (SSLP) and are the smallest class of simple repetitive DNA sequences (1–6 bp) (Kesawat and Das 2009). SSR markers result from variations on the number of tandem repeats of microsatellites (Hearne et al. 1992). Specific primers (generally 20–25 bp) in the flanking regions of microsatellite can be designed to amplify the microsatellite by PCR (Kesawat and Das 2009). SSR markers can be developed from genomic or genic microsatellite sequences. Expressed sequence tag-SSR (EST-SSR) markers can be easily developed by data mining for SSRs in EST databases (Kantety et al. 2002). Genomic SSRs are more polymorphic compared to genic SSRs (EST-SSRs) and are superior to fingerprinting or varietal identification studies, while genic SSRs are useful for assessment of functional diversity (Varshney et al. 2005).

Forty-six SSR markers were first placed on an interspecific linkage map of *C. annuum* 'TF68' and *C. chinense* 'Habanero' (Lee et al. 2004). In the same population, a total of 150 EST-SSRs were developed through in silico analysis of 10,232 non-redundant EST sequences (Yi et al. 2006). By sequencing 1873 clones derived from the genomic DNA libraries of *C. annuum* 'Manganji,' 106 new SSR markers were developed and mapped on an intraspecific linkage map of *C. annuum* 'Manganji' × 'Tongari' (Minamiyama et al. 2006). In an intraspecific genetic map of *C. annuum* 'YCM334' and 'Tean,' 101 EST-SSR markers were located after screening of total 1667 EST-SSR markers (Truong et al. 2010). Total 151 SSR markers were used to construct an intraspecific linkage map of *C. annuum* 'California Wonder' and 'LS2341' (Mimura et al. 2012). To construct a high-density linkage map of *C. annuum*, 1736 genomic SSR markers and 1344 EST-SSR markers were developed from 6528 clones and 13,003 sequences, respectively (Sugita et al. 2013). To map QTLs affecting the initiation of flower primordia in pepper, 95 SSR markers were validated and integrated into a genetic map of *C. annuum* 'BA3' and *Capsicum frutescens* 'YNXML' (Tan et al. 2015). A total of 113,500 in silico unique SSR loci were identified in nuclear genome of pepper using a homemade bioinformatics workflow, and as a preliminary study, 65 SSR markers were validated among a wide collection of 21 *Capsicum* genotypes

(Cheng et al. 2016b). To map QTLs for fruit length, 400 SSR markers were surveyed, but only 28 markers were mapped in an F_2 population derived from a cross of *C. annuum* 'FL201' and *Capsicum galapagoense* 'TC07245' (Arjun et al. 2018).

5.2.2.7 Cleaved Amplified Polymorphic Sequences (CAPSs)

CAPS analysis, referred to as PCR-restriction fragment length polymorphism (PCR-RFLP), follows next steps: PCR using specific 20–25 bp primers, digestion of the PCR products with a restriction enzyme, and agarose gel electrophoresis of the digested products (Akopyanz et al. 1992; Konieczny and Ausubel 1993; Lyamichev et al. 1993). The DNA fragment length polymorphisms of CAPS result from variation in the occurrence of restriction sites (Kesawat and Das 2009). Advantages of CAPS include the requirement of only low quantities of template DNA (50–100 ng per reaction) for PCR, the codominance of alleles, the high reproducibility, and easier procedure compared to RFLP due to no requiring Southern blot hybridization and radioactive detection steps (Kesawat and Das 2009). A derived cleaved amplified polymorphic sequence (dCAPS) marker is a modified method of CAPS technique where mismatches in a PCR primer are used to create a polymorphism based on the target mutation (Neff et al. 1998).

A CAPS marker was converted from the AFLP marker E41/M49-645 linked to the *Pvr4* resistance gene in *C. annuum* (Caranta et al. 1999). A CAPS marker $SCAC_{568}$ was developed from the $OPAC10_{593}$ RAPD marker linked to *Tsw* gene to assist selection of TSWV resistance in pepper (Moury et al. 2000). Three CAPS markers, Pvr1-S, pvr1-R1, and pvr1-R2, were developed to discriminate between $Pvr1^+$, *pvr1*, $pvr1^1$, and $pvr1^2$ alleles in *Capsicum* spp. (Yeam et al. 2005). The *Rf* locus was mapped by using eight AFLP markers, and of them, the AFRF8 marker was successfully converted to a CAPS marker AFLP8CAPS which was closest to *Rf* with a genetic distance of 1.8 cM (Kim et al. 2006). A CAPS marker PR-CAPS for partial restoration (*pr*) locus was developed from the AFLP marker E-AGC/M-GCA_{112} estimated at about 1.8 cM in genetic distance (Lee et al. 2008). Two CAPS markers, PmsM1-CAPS and PmsM2-CAPS, linked to the ms_1 gene on pepper chromosome 5 were developed (Lee et al. 2010a, 2011a). Two CAPS markers, GMSK-CAPS and GMS3-CAPS, were identified to cosegregate with the ms_k and ms_3 genes, respectively (Lee et al. 2010c, d). A major QTL *CaR12.2* for the resistance was found in an introgression BC_1F_2 population made by interspecific crosses between *C. annuum* 'SP26' (susceptible) and *C. baccatum* 'PBC81' (resistant), and the CaR12.2M1-CAPS marker closely linked to the major QTL *CaR12.2* was developed (Lee et al. 2010b, 2011b). The M3-CAPS marker tightly linked to the major QTL *Phyto.5.2* for resistance to *Phytophthora* root rot was developed using two segregating F_2 populations from a cross of 'Subicho' × 'CM334' and self-pollination of a commercial cultivar 'Dokyacheongcheong' (Lee et al. 2012b). A set of allele-specific markers of *L* locus, including L2-CAPS and L0nu-CAPS markers, was developed using five pepper differential hosts including *C. annuum* 'ECW' (L^0/L^0), *C. annuum* 'Tisana' (L^1/L^1), *C. annuum* 'CM334' (L^2/L^2), *C. chinense* 'PI159236' (L^3/L^3), and *Capsicum chacoense* 'PI260429' (L^4/L^4) (Lee et al. 2012a). A codominant CAPS marker, CL000081-0555, located 1.13 cM away from the *Me1* gene, was developed using an F_2 population of a cross between *C. annuum* 'AZN-1' (susceptible line) and 'PM217' (resistant inbred line derived from 'PI201234'; Uncu et al. 2015). Recently, a CAPS marker 16,830-CAPS, tightly linked to the *Me1* gene, was developed through a fine mapping approach and the *CA09g16830* gene was identified as a candidate gene for *Me1* (Wang et al. 2018).

5.2.2.8 Sequence-Characterized Amplified Region (SCAR)

SCARs are PCR-based markers that are identified by PCR amplification of genomic DNA with a pair of specific primers (Paran and Michelmore 1993). SCARs can be classified into two types, dominant and codominant, depending on inheritance pattern: Dominant SCARs result from the

presence or the absence of amplification of the band; codominant SCARs result from the length polymorphisms caused by insertion or deletion (Paran and Michelmore 1993). SCARs have higher reproducibility compared to RAPD due to the use of longer primers (22–24 bp) designed for specific amplification of a particular locus (Kesawat and Das 2009).

A SCAR marker $SCUBC19_{1432}$ linked to the *Pvr4* locus was developed using segregating progenies obtained by crossing a homozygous resistant ('Serrano Criollo de Morelos-334') with a homozygous susceptible ('Yolo Wonder') (Arnedo-Andrés et al. 2002). Three SCAR markers, $PMFR11_{269}$, $PMFR11_{283}$, and $PMFR21_{200}$, positioned at a distance of 4.0 cM from the L^3 locus, were converted from two RAPD markers, $E18_{272}$ and $E18_{286}$, which were developed by applying the bulked segregant analysis (BSA) method to two DH populations, K9-DH and K9/AC-DH, derived from F_1 hybrid 'K9' that harbors the L^3 gene derived from 'PI159236' (Sugita et al. 2004). Two markers, *atp6*-SCAR and *coxII* SCAR, have been developed to identify the CMS cytoplasm (Kim and Kim 2005). The D4 SCAR marker for the detection of *Phyto.5.2* and a major QTL for resistance to *P. capsici* were developed (Quirin et al. 2005). A SCAR marker L4SC340, which mapped 1.8 cM from the L^4 locus, was developed from an AFLP marker L4-c, which was identified by applying BSA-AFLP method to a near-isogenic BC_4F_2 population generated by using *C. chacoense* 'PI260429' (carrying the L^4 allele) as a resistant parent (Kim et al. 2008a). The presence of a third haplotype ($Rfls^{7701}$) of the sequence linked to the *Rf* gene was reported, and two codominant SCAR markers CaRf-M1 and CaRf-M2 were developed for discriminating between $Rfls^A$, $Rfls^B$, and $Rfls^{7701}$ (Min et al. 2008b). A codominant SCAR marker AFRF4 linked to the *Rf* locus with a genetic distance of 0.1 cM was developed (Min et al. 2009). A newly developed *Rf*-linked marker BAC13T7-SCAR was developed from the sequence of a tomato BAC clone containing three genes which are homologous to petunia *Rf* gene encoding a pentatricopeptide repeat (PPR) protein (Jo et al. 2010). A codominant SCAR marker PR-Bs3 was developed by designing primers to amplify the InDel region of *Bs3* and *bs3* promoters (Römer et al. 2010). A major QTL *CcR9* for the resistance of 'PBC81' to *Colletotrichum truncatum* was identified, and the CcR9M1-SCAR marker closely linked to the QTL *CcR9* was developed (Lee et al. 2010b, 2011b). A marker SCAR_P2 linked to the ms_8 locus on the lower arm of the pepper chromosome 4 was identified (Bartoszewski et al. 2012). A set of allele-specific markers of *L* locus, including L1-SCAR, L3-SCAR, L4-SCAR, and L0c-SCAR markers, was developed (Lee et al. 2012a). A codominant SCAR marker SA133_4 linked to the QTL P5 for *Phytophthora* resistance was developed (Truong et al. 2013). The SCAR_PM54 marker was identified to be fully consistent with artificial nematode (*Meloidogyne incognita* race 2) testing, correctly predicting resistant ('PM687', 'PM217,' and 'Carolina Cayenne') and susceptible ('Yolo Wonder B,' 'California Wonder 300,' and 'CM331') genotypes (Pinar et al. 2016). Two markers SCAR-InDel and SSR-HpmsE032 associated with resistance to *C. scovillei* were validated in two *C. annuum* anthracnose-resistant introgression lines, P_{R1} derived from 'PBC932' and P_{R2} derived from 'PBC80,' showing the selection efficiency of 77% when both markers were used together (Suwor et al. 2017). Recently, a novel powdery mildew resistance locus, *PMR1*, was identified on pepper chromosome 4 using two populations consisting of 102 'VK515' $F_{2:3}$ families and 80 'PM Singang' F_2 plants, and one SCAR marker (ZL1_1826) was developed to cosegregate with the *PMR1* locus (Jo et al. 2017).

5.2.2.9 Single-Nucleotide Polymorphism (SNP) and Insertion/Deletion (InDel)

A single-nucleotide polymorphism (SNP) is a single-nucleotide difference between two DNA sequences or individuals (Wang et al. 1998), and an insertion/deletion (InDel) refers to an insertion or deletion of bases in the genome of an organism (Bhattramakki et al. 2002; Weber et al. 2002).

SNPs and InDels are highly abundant and distributed throughout the genome in plants (Kesawat and Das 2009). They are very useful tool for genetic mapping, marker-assisted breeding, and map-based cloning (Rafalski 2002; Kesawat and Das 2009). Over the past two decades, a number of different SNP genotyping methods have been developed. Various SNP genotyping assays can be classified by a combination of one of the sample preparation techniques (allele-specific hybridization, primer extension, oligonucleotide ligation, and nuclease cleavage) and one of the analysis techniques (gel separation, array, mass spectrometry, and plate reader; Gut 2001). The well-known SNP genotyping methods include TaqMan assay (Livak 1999), allele-specific PCR (AS-PCR) with universal energy-transfer-labeled primers (Myakishev et al. 2001), and high-resolution melting (HRM) analysis (Wittwer et al. 2003; Liew et al. 2004).

TaqMan assay, also known as 5′ nuclease assay, can be used to discriminate alleles that differ from a single-nucleotide substitution, using a fluorogenic probe consisting of an oligonucleotide labeled with both a fluorescent reporter dye (generally FAM or TET) and a quencher dye (Livak 1999). Amplification of the probe-specific product causes cleavage of the probe, generating an increase in specific reporter fluorescence (Livak 1999). However, the biggest problem of this method is production cost of the specific probe required for each TaqMan assay.

AS-PCR with universal energy-transfer-labeled primers was developed for high-throughput SNP genotyping (Myakishev et al. 2001). The technique involves PCR amplification with two different tailed allele-specific primers that contain priming sites for universal energy-transfer-labeled primers. This method can solve the problem of TaqMan assay by using the same universal primers for all analyses (Myakishev et al. 2001). SNP-type assay (Wang et al. 2009) and kompetitive allele-specific PCR (KASP; Semagn et al. 2014) for SNP genotyping adopt this method and can be applied to high-throughput SNP genotyping analysis.

HRM analysis, a method that allows detecting polymorphism in double-stranded DNA by comparing profiles of melting curves, can be used for genotyping SNP, SSR, and InDel markers (Liew et al. 2004; Simko 2016). HRM markers are faster, simpler, and less expensive than other marker systems requiring gel separation or labeled probes because it is directly analyzed within a closed tube with the addition of fluorescent dyes such as LCGreen® Plus, SYTO® 9, EvaGreen®, LCGreen™ I, or SYBR® Green I before PCR amplification (Wittwer 2009).

A total of 40 SNP markers using AS-PCR analysis were developed for cultivar identification in *Capsicum* (Jung et al. 2010). To develop a SNP-based genetic map in an F_2 population derived from a cross of *C. annuum* 'NB1' × *C. chinense* 'Jolokia,' 116 SNP markers using HRM analysis were developed from SNPs identified from next-generation resequencing of parents (Lee et al. 2013). To construct an EST-based linkage map in the F_2 population (*C. annuum* 'NuMex RNaky' × *C. chinense* 'PI159234'), 48 EST-based SNPs markers were developed (Park et al. 2014). To develop an InDel-based linkage map of hot pepper (*C. annuum*), 251 InDel markers were developed (Li et al. 2015). To construct a SNP-based genetic linkage map of *C. baccatum*, a total of 395 HRM markers were developed based on SNPs identified by comparing genome sequences generated through next-generation resequencing of the parents, *C. baccatum* 'Golden-aji' and 'PI594137' (Lee et al. 2016).

5.2.2.10 High-Throughput SNP Genotyping Systems

Next-generation sequencing (NGS) technologies, including 454, Solexa, SOLiD, Polonator, and HeliScope, have had a great influence on biological studies by enabling faster and less expensive analysis of genomes and transcriptomes (Shendure and Ji 2008). NGS technologies have made it easy to detect genetic variations including SNPs and InDels and to develop DNA-based molecular markers in plant genetics and breeding (Varshney et al. 2009). Indeed,

SNP markers are increasingly becoming the go-to marker system because SNPs can be identified so easily through NGS technologies (Ganal et al. 2009; Kumar et al. 2012).

High-throughput SNP genotyping systems, including Illumina Infinium iSelect HD array (International HapMap Consortium 2005), Affymetrix Axiom array (International HapMap Consortium 2005), Douglas Array Tape (www.douglasscientific.com), Fluidigm dynamic arrays (Wang et al. 2009), restriction enzyme-based genotyping-by-sequencing (GBS; Huang et al. 2009), and amplicon sequencing (Bybee et al. 2011), are very useful for plant breeding (Thomson 2014).

Fluidigm dynamic arrays, a flexible, PCR-based SNP genotyping platform, include three formats for nanofluidic integrated fluid circuits (IFCs): 96 samples × 96 SNPs, 48 samples × 48 SNPs, and 192 samples × 24 SNPs (Wang et al. 2009). A 48.48 dynamic array yields 2304 data points with 48 samples and 48 markers, and 96.96 and 192.24 dynamic arrays yield 9216 and 4608 data points, respectively (Wang et al. 2009; Thomson 2014). The dynamic arrays can be used with three types of assays: TaqMan, KASP, or SNP-type assays (Wang et al. 2009; Thomson 2014).

Genotyping-by-sequencing (GBS), a genome-wide genotyping method that enables a rapid and inexpensive analysis of the whole genome using a multiplexed NGS technology, can be applied to various areas of plant genetics and breeding, including SNP discovery, high-density genetic mapping, QTL analysis, genome-wide association studies (GWASs), genomic selection (GS), and low-cost genomics-assisted breeding (GAB) (Deschamps et al. 2012; Poland and Rife 2012). Representative GBS methods include various following protocols: restriction association DNA sequencing (RAD-seq; Baird et al. 2008), genotyping-by-sequencing (GBS; Huang et al. 2009), multiplex shotgun genotyping (MSG; Andolfatto et al. 2011), double-digested RAD-seq (Peterson et al. 2012), double-digested GBS (Poland et al. 2012), sequence-based genotyping (SBG; Truong et al. 2012), restriction enzyme sequence comparative analysis (Monson-Miller et al. 2012), ion torrent GBS (Mascher et al. 2013), restriction fragment sequencing (REST-seq; Stolle and Moritz 2013), and specific-locus amplified fragment sequencing (SLAF-seq; Sun et al. 2013) (Kim et al. 2016).

These techniques can be used in *Capsicum* spp. due to reports of whole-genome sequences of *C. annuum* 'CM334' (Kim et al. 2014), 'Zunla-1' (Qin et al. 2014), *C. annuum* var. *glabriusculum* 'Chiltepin' (Qin et al. 2014), *C. chinense* 'PI159236' (Kim et al. 2014, 2017b), and *C. baccatum* 'PBC81' (Kim et al. 2017b).

For marker-assisted backcrossing (MABC) in *Capsicum*, 412 SNPs evenly distributed on each chromosome were used to develop locus-specific markers for the Fluidigm® EP1™ genotyping system as a high-throughput SNP genotyping method (Kang et al. 2014). GBS analysis was used to detect QTLs conferring resistance to the *cucumber mosaic virus* P1 (CMV_{P1}) strain in pepper (Eun et al. 2016). An ultra-high-density bin map containing 2578 bins was constructed to identify QTLs for horticultural traits in *C. annuum* through next-generation resequencing analysis (Han et al. 2016). With an Illumina Infinium iSelect SNP array (pepper *Cap*SNP15K array), a high-density interspecific genetic map containing 5569 SNPs was constructed to analyze genetic diversity of 339 pepper elite/landrace lines (Cheng et al. 2016a). The PepperSNP16K array, which simultaneously genotyped 16,405 SNPs, was developed using the pepper haplotype map (HapMap) completed through resequencing of inbred lines (Hulse-Kemp et al. 2016). A total of 20 SNP-type assays for Fluidigm dynamic array, which were associated with several disease resistances and high capsaicinoid content, were developed for marker-assisted selection (MAS) of chili pepper (Kim et al. 2017a). Whole-genome resequencing and GBS were used for high-resolution mapping of QTLs controlling capsaicinoid content in *Capsicum* spp. (Han et al. 2018). A high-density genetic map containing 12,727 SNP markers was

constructed to identify QTLs for *cucumber mosaic virus* resistance in pepper using SLAF-seq (Li et al. 2018).

5.3 Genetic Maps in *Capsicum* Species

5.3.1 Interspecific Genetic Linkage Maps

Interspecific genetic linkage maps of *Capsicum* spp. were constructed using crosses including *C. annuum* × *C. chinense*, *C. annuum* × *C. frutescens*, and *C. annuum* × *C. baccatum* (Table 5.2). These maps are overviewed in Table 5.2.

5.3.1.1 *Capsicum annuum* × *Capsicum chinense*

The first pepper genetic linkage map was constructed by using 84 RFLP markers based on a common set of cDNA clones and selected single-copy genomic clones and by using 46 individuals derived from a cross between *C. annuum* 'Doux des Landes (CA50)' and *C. chinense* 'CA4' (Tanksley et al. 1988). A molecular genetic map of pepper covering 720 cM was constructed in an interspecific F_2 population with a total of 192 RFLP and isozyme markers (Prince et al. 1993). A genetic map of pepper consisting of 13 linkage groups that cover a total of 1245.7 cM was created from an interspecific F_2 population (*C. annuum* 'NuMex RNaky' × *C. chinense* 'PI159234'; Livingstone et al. 1999). The SNU pepper genetic map, consisting of 16 linkage groups and covering 1320 cM, was constructed in an interspecific F_2 population (*C. annuum* 'TF68' × *C. chinense* 'Habanero') with 150 RFLP and 430 AFLP markers (Kang et al. 2001). The SNU2 pepper map with 333 markers (46 SSR and 287 RFLP) in 15 linkage groups covering 1761.5 cM was generated in the same population with the SNU map (Lee et al. 2004). The SNU3 pepper map, forming 14 linkage groups and spanning 2201.5 cM, was constructed by adding 139 SSR markers based on expressed sequence tags (ESTs) (Yi et al. 2006). A SNP-based genetic map of pepper was developed in an F_2 population derived from a cross of *C. annuum* 'NB1' × *C. chinense* 'Jolokia' by using 116 SNP (HRM) markers generated from next-generation resequencing of parents (Lee et al. 2013). An EST-based linkage map of pepper (the AC2 map) was constructed in the AC99 F_2 population (*C. annuum* 'NuMex RNaky' × *C. chinense* 'PI159234') by using a total of 512 markers, comprising 214 intron-based polymorphic markers (IBPs), 143 conserved ortholog sets (COSIIs), 48 EST-SNPs (eSNPs), and 107 previously reported markers (Park et al. 2014). QTL mapping for capsaicinoid content was conducted in an interspecific population of 85 RILs derived from *C. annuum* 'TF68' × *C. chinense* 'Habanero' through a genotyping-by-sequencing (GBS) analysis (Han et al. 2018).

5.3.1.2 *Capsicum annuum* × *Capsicum frutescens*

A pepper genetic map was constructed for identifying yield-related QTLs using 248 BC_2 plants derived from a cross between *C. annuum* 'Maor' and *C. frutescens* 'BG2816' (Rao et al. 2003). In the same population, QTLs for capsaicinoid content were analyzed (Blum et al. 2003). An interspecific genetic map (*C. annuum* 'BA3' × *C. frutescens* 'YNXML') containing 129 InDel and 95 SSR markers was constructed for mapping the QTLs affecting the initiation of flower primordia (Tan et al. 2015). A linkage map with 5546 markers separated into 1361 bins on 12 linkage groups representing 1392.3 cM was produced using an interspecific population created between *C. frutescens* 'Tabasco' and *C. annuum* 'P4' and using the PepperSNP16K Infinium array (Hulse-Kemp et al. 2016). A high-density interspecific SNP genetic map of pepper was constructed in 297 F_2 individuals of *C. annuum* × *C. frutescens* using an Illumina Infinium iSelect SNP array (pepper *Cap*SNP15K array) (Cheng et al. 2016a).

Table 5.2 Overview of the interspecific genetic linkage maps of *Capsicum* spp.

Interspecific cross	Parents	Population size and type[a]	Number and type of markers[b]	Number of linkage groups	Total map length (cM)	References
C. annuum × *C. chinense*	'CA50' × 'CA4'	46 F_2	84 RFLPs	19	229	Tanksley et al. (1988)
	'CA50' × 'CA4'	46 F_2	192 RFLPs and isozymes	19	720	Prince et al. (1993)
	'NuMex RNaky' × 'PI159234'	75 F_2	350 AFLPs, 303 RFLPs, 17 RAPDs, 2 isozymes	13	1246	Livingstone et al. (1999)
	'TF68' × 'Habanero'	107 F_2	150 RFLPs, 430 AFLPs	16	1320	Kang et al. (2001)
	'TF68' × 'Habanero'	107 F_2	46 SSRs, 287 RFLPs	15	1762	Lee et al. (2004)
	'TF68' × 'Habanero'	107 F_2	139 EST-SSRs	14	2202	Yi et al. (2006)
	'NB1' × 'Jolokia'	96 F_2	116 HRMs	12	1168	Lee et al. (2013)
	'NuMex RNaky' × 'PI159234'	75 F_2	214 IBPs, 143 COSIIs, 48 eSNPs, 107 other markers	12	2336	Park et al. (2014)
	'TF68' × 'Habanero'	85 RILs	1089 bins (GBS)	12	1127	Han et al. (2018)
C. annuum × *C. frutescens*	'Maor' × 'BG2816'	248 BC_2	92 RFLPs	12	1100	Rao et al. (2003)
	'BA3' × 'YNXML'	154 and 147 F_2	129 InDels, 95 SSRs	13	1250	Tan et al. (2015)
	'P4' × 'Tabasco'	90 F_2	1361 bins (array)	12	1392	Hulse-Kemp et al. (2016)
	'BA3' × 'YNXML'	297 F_2	3826 bins (array)	12	1629	Cheng et al. (2016a)
C. annuum × *C. baccatum*	'SP26' × 'PBC81'	87 BC_1F_2	197 AFLPs, 21 SSRs	13	325	Lee et al. (2010b)

[a]*RILs* recombinant inbred lines
[b]*RFLPs* restriction fragment length polymorphisms, *AFLPs* amplified fragment length polymorphisms, *RAPDs* random amplified polymorphic DNAs, *SSRs* simple sequence repeats, *EST*-SSRs expressed sequence tag-SSRs, *HRMs* high-resolution melting markers, *IBPs* intron-based polymorphic markers, *COSIIs* conserved ortholog sets II, *eSNPs* EST-SNPs, *GBS* genotyping-by-sequencing, *InDels* insertion/deletion markers, *array* Illumina Infinium iSelect SNP array

5.3.1.3 *Capsicum annuum* × *Capsicum baccatum*

An introgression BC_1F_2 population was generated by interspecific crosses between *C. annuum* 'SP26' (susceptible) and *C. baccatum* 'PBC81' (resistant) for QTL mapping analyses of anthracnose resistance, and the introgression map consisting of 13 linkage groups with a total of 218 markers (197 AFLPs and 21 SSRs), covering 325 cM, was constructed (Lee et al. 2010b).

5.3.2 Intraspecific Genetic Linkage Maps

Intraspecific genetic linkage maps of *Capsicum* spp. have been reported in two species, *C. annuum* and *C. baccatum* (Table 5.3). The overview of these maps is given in Table 5.3.

5.3.2.1 *Capsicum annuum*

The first functional detailed map of pepper, containing 100 known-function gene markers and 9 loci of agronomic interest (*L*, *pvr2*, *Pvr4*, *C*, *up*, *Tsw*, *Me3*, *Bs3*, and *y*), was generated using three intraspecific populations including two DH populations of 'H3' × 'Vania' and 'Perennial' × 'Yolo Wonder' and one F_2 population of 'Yolo Wonder' × 'CM334' (Lefebvre et al. 2002). A genetic linkage map of the sweet pepper using an intraspecific DH population, consisting of 382 AFLP, 122 RAPD, 3 RFLP, 7 SCAR, and 4 CAPS markers, was constructed by AFLP using the high-efficiency genome scanning (HEGS) system and RAPD (Sugita et al. 2005). An SSR-based linkage map of *C. annuum*, including 106 new SSR markers distributed across 13 linkage groups and covering 1042 cM, was constructed in an intraspecific DH population derived from 'Manganji' × 'Tongari' (Minamiyama et al. 2006). A RFLP-based pepper linkage map, consisting of 202 RFLPs, 6 WRKYs, and 1 SSR and covering 1482.3 cM, was constructed to detect QTL associated with *P. capsici* resistance using 100 F_2 individuals from a cross between 'CM334' (resistant) and 'Chilsungcho' (susceptible) (Kim et al. 2008c). In the same population, 60 WRKY-based and 71 reverse random amplified microsatellite polymorphism (rRAMP)-based markers were developed and added (Kim et al. 2008b; Min et al. 2008a). A saturated intraspecific genetic map of pepper, containing 281 AFLPs, 101 EST-SSRs, 37 consensus SSRs, and 1 CAPS, was generated for studying QTLs associated with *Phytophthora* root rot resistance using a population of 126 F_8 RILs derived from a cross between 'YCM334' (resistant) and 'Tean' (susceptible) (Truong et al. 2010). The first SSR-based intraspecific genetic map of *C. annuum*, containing 151 SSRs, 90 AFLPs, 10 CAPSs, and 2 STSs and spanning 1336 cM, was constructed using a DH population derived from a cross between 'California Wonder' and 'LS2341' (Mimura et al. 2012). An SSR-based high-density linkage map of *C. annuum*, consisting of 597 SSR markers and covering 2028 cM, was developed by using DH lines derived from an intraspecific cross of 'K9-11' × 'MZC-180' (Sugita et al. 2013). The first InDel-based linkage map of hot pepper (BB-InDel map), containing 251 InDel markers and covering 1178 cM, was made using an F_2 population derived from the intraspecific cross 'BA3' × 'B702' through whole-genome resequencing of two parents (Li et al. 2015). An ultra-high-density bin map of *C. annuum*, containing 2578 bins and spanning 1372 cM, was developed for QTL mapping of horticultural traits using 120 RILs derived from a cross between 'Perennial' and 'Dempsey' (Han et al. 2016). A high-density genetic map of *C. annuum*, containing 12,727 markers on 12 chromosomes and spanning 1785 cM, was constructed using 195 F_2 individuals derived from a cross between 'BJ0747' (resistant) and 'XJ0630' (susceptible) to identify QTLs for CMV resistance using SLAF-seq (Li et al. 2018).

5.3.2.2 *Capsicum baccatum*

An intraspecific genetic map of *C. baccatum*, containing 52 SSRs, 175 AFLPs, and 100 SRAPs, and covering 1896 cM, was developed using 126 F_2 plants derived from a cross between 'Cbp' (resistant) and 'Golden-aji' (susceptible) to identify QTLs associated with anthracnose resistance (Kim et al. 2010). A reference map of *C. baccatum* based on 42 SSRs, 85 ISSRs, and 56 RAPDs, consisting of 16 linkage groups and covering 2547 cM, was constructed using 203 F_2 individuals originated from a cross of 'UENF1616' and 'UENF1732' (Moulin et al. 2015). A SNP-based genetic linkage map of *C. baccatum*, containing 395 HRM markers and covering 1056.2 cM, was generated using an F_2 population from a cross between 'Golden-aji' and 'PI594137' and was compared to *C. annuum* reference physical map (Lee et al. 2016).

Table 5.3 Overview of the intraspecific genetic linkage maps of *Capsicum* spp.

Intraspecific cross	Parents	Population size and type[a]	Number and type of markers[b]	Number of linkage groups	Total map length (cM)	References
C. annuum × *C. annuum*	'H3' × 'Vania'	101 DH	434 AFLPs, 56 RAPDs, 50 RFLPs, 3 morphological markers (*C*, *L*, *pvr2*)	12	1513	Lefebvre et al. (2002)
	'Perennial' × 'Yolo Wonder'	114 DH	325 AFLPs, 164 RAPDs, 133 RFLPs, 1 isozyme, 4 SCARs, 3 morphological markers (*C*, *L*, *up*)	26	1.668	Lefebvre et al. (2002)
	'Yolo Wonder' × 'CM334'	151 F_2	109 AFLPs, 67 RFLPs, 28 RAPDs, 2 SCARs, 2 morphological markers (*C*, *Pvr4*)	18	685	Lefebvre et al. (2002)
	'K9-11' × 'AC2258'	176 DH	382 AFLPs, 112 RAPDs, 3 RFLPs, 7 SCARs, 4 CAPSs	16	1043	Sugita et al. (2005)
	'Manganji' × 'Tongari'	117 DH	123 SSRs, 228 AFLPs, 60 RAPDs, 1 CAPS	13	1042	Minamiyama et al. (2006)
	'CM334' × 'Chilsungcho'	100 F_2	202 RFLPs, 6 WRKYs, 1 SSR	14	1482	Kim et al. (2008c)
	'CM334' × 'Chilsungcho'	100 F_2	163 rRAMPs, 134 AFLPs, 29 SSRs, 9 RFLPs, 2 RAPDs	16	1854	Min et al. (2008a)
	'CM334' × 'Chilsungcho'	100 F_2	41 WRKYs, 199 AFLPs, 26 SSRs, 8 RFLPs, 97 rRAMPs	20	2051	Kim et al. (2008b)
	'YCM334' × 'Tean'	126 RILs	281 AFLPs, 101 EST-SSRs, 37 SSRs, 1 CAPS	14	2178	Truong et al. (2010)
	'California Wonder' × 'LS2341'	94 DH	151 SSRs, 90 AFLPs, 10 CAPSs, 2 STS	12	1336	Mimura et al. (2012)
	'K9-11' × 'MZC-180'	184 DH	597 SSRs	12	2028	Sugita et al. (2013)
	'BA3' × 'B702'	178 F_2	251 InDels	12	1178	Li et al. (2015)
	'Perennial' × 'Dempsey'	120 RILs	2578 bins (WGS)	12	1372	Han et al. (2016)
	'A1' × '2602'	96 SSD F_3	906 SNPs (GBS)	12	1273	Eun et al. (2016)
	'BJ0747' × 'XJ0630'	195 F_2	12,727 SNPs (SLAF-seq)	12	1785	Li et al. (2018)
	'Perennial' × 'Dempsey'	56 RILs	2578 bins (WGS)	12	1372	Han et al. (2018)
C. baccatum × *C. baccatum*	'Cbp' × 'Golden-aji'	126 F_2	52 SSRs, 175 AFLPs, 100 SRAPs	13	1896	Kim et al. (2010)
	'UENF1616' × 'UENF1732'	203 F_2	42 SSRs, 85 ISSRs, 56 RAPDs	16	2547	Moulin et al. (2015)
	'Golden-aji' × 'PI594137'	93 F_2	395 HRMs	12	1056	Lee et al. (2016)

[a]*DH* doubled haploids, *RILs* recombinant inbred lines, *SSD* single seed descent

[b]*AFLPs* amplified fragment length polymorphisms, *RAPDs* random amplified polymorphic DNAs, *RFLPs* restriction fragment length polymorphisms, *SCARs* sequence-characterized amplified regions, *CAPSs* cleaved amplified polymorphic sequences, *SSRs* simple sequence repeats, *WRKYs* WRKY gene-based markers, *rRAMPs* reverse random amplified microsatellite polymorphisms, *EST*-SSRs expressed sequence tag-SSRs, *InDels* insertion/deletion markers, *WGS* whole-genome resequencing, *SNPs* single-nucleotide polymorphisms, *GBS* genotyping-by-sequencing, *SLAF-seq* specific-locus amplified fragment sequencing, *SRAPs* sequence-related amplified polymorphisms, *ISSRs* inter-simple sequence repeats, *HRMs* high-resolution melting markers

5.3.3 Integrated Genetic Linkage Maps

The first integrated linkage map of *C. annuum*, including mainly RFLP and RAPD markers, was constructed by alignment of three intraspecific linkage maps generated by segregating DH progenies (Lefebvre et al. 1995). An integrated genetic linkage map of pepper, consisting of 1528 AFLP, 440 RFLP, 288 RAPD, several known gene sequences, isozymes, and morphological markers and covering 1832 cM, was generated by using pooled data from six individual maps (Paran et al. 2004). An integrated pepper map, containing 169 SSR, 354 RFLP, 23 STS from BAC end sequences, 6 STS from RFLP, 152 AFLP, 51 WRKY, and 99 rRAMP markers on 12 chromosomes, was constructed using four genetic maps of two interspecific (*C. annuum* 'TF68' × *C. chinense* 'Habanero') and two intraspecific (*C. annuum* 'CM334' × 'Chilsungcho') populations (Lee et al. 2009).

5.3.4 Comparative Mapping Between Solanaceous Crops

The first RFLP-based pepper linkage map was compared to the RFLP-based tomato map, suggesting that gene repertoire is conserved but gene order is not (Tanksley et al. 1988). Comparison of the pepper, tomato, and potato genetic maps revealed a total of 30 breaks as part of 5 translocations, 10 paracentric inversions, 2 pericentric inversions, and 4 disassociations or associations of genomic regions (Livingstone et al. 1999). Disease resistance genes (*R* genes) and *R* gene homologues were compared between three solanaceous crops including tomato, potato, and pepper (Grube et al. 2000). Pepper genome was compared to tomato genome using a total of 299 orthologous markers including 263 conserved ortholog set II (COSII) markers, suggesting that the two genomes have become differentiated by a minimum number of 19 inversions and 6 translocations, as well as numerous putative single gene transpositions but share 35 conserved syntenic segments within which gene/marker order is well preserved (Wu et al. 2009). In addition, the genome of cultivated *C. annuum* and wild *C. annuum* (as well as *C. chinense*, *C. frutescens*) was found to differ by a reciprocal translocation between chromosomes 1 and 8 (Wu et al. 2009). Comparative mapping studies were performed in tomato, potato, eggplant, pepper, and diploid *Nicotiana* species (*Nicotiana tomentosiformis* and *Nicotiana acuminata*) using COSII markers, providing the first broad overview of chromosomal evolution in the family Solanaceae (Wu and Tanksley 2010). The eggplant/pepper syntenic map confirmed 10 translocations and 8 inversions already detected, and a set of 151 pepper QTL were located as well as 212 eggplant QTL, including 76 major QTLs (phenotypic variance explained, PVE $\geq$ 10%) affecting key agronomic traits (Rinaldi et al. 2016). Recently, two high-quality de novo genomes (*C. baccatum* 'PBC81' and *C. chinense* 'PI159236') and an improved reference genome (*C. annuum* 'CM334') were reported, showing dynamic genome rearrangements involving translocations among chromosomes 3, 5, and 9 between *C. baccatum* and the two other peppers and suggesting the process of speciation and evolution of the *Capsicum* species (Kim et al. 2017b).

5.3.5 Marker-Assisted Backcrossing

Marker-assisted backcrossing (MABC) is a new breeding approach that can substantially reduce breeding time and cost by using highly polymorphic markers with known positions in each chromosome (Frisch et al. 1999; Herzog and Frisch 2011). A total of 412 SNP markers were developed from EST sequences generated by large-scale transcriptome sequencing of eight accessions (*C. annuum* 'Jeju,' 'LAM32,' 'Tean,' 'CM334,' 'Yuwolcho,' 'PI201234,' and 'YCM334' and *C. chinense* 'SNU-001') using the Illumina Genome Analyzer IIx platform to facilitate MABC in hot pepper (Kang et al. 2014). Moreover, by analyzing the SNP makers via a high-throughput SNP genotyping system

(Fluidigm® EP1™ system), a genetic linkage map of *C. frutescens* 'BG2814-6' × *C. annuum* 'NuMex RNaky' was constructed and a genetic diversity of 27 *Capsicum* accessions was tested (Kang et al. 2014).

5.4 Future Prospects

Various molecular marker techniques and many genetic linkage maps can be used to develop the trait-linked markers or gene-based markers as well as to identify a gene or QTLs for important horticultural traits including male sterility (CMS and GMS), various disease resistances (anthracnose, powdery mildew, phytophthora, bacterial wilt, bacterial spot, CMV, TSWV, PMMoV, PepMoV, and nematode), and fruit traits (color, shape, size, capsaicinoid content, carotenoid content, and sugar content). To date, a few function-known pepper genes including *Bs2*, *pvr1* (*pvr2*), *pun1*, *Bs3*, *pAMT*, *L*, *Pvr4*, *Tsw*, and ms_1 were only identified through map-based cloning or candidate gene approach. Recent genome-wide genotyping technologies such as GBS, Rad-seq, and SLAF-seq will accelerate development of whole-genome genetic maps and trait-linked DNA markers and identification of genes controlling important horticultural traits.

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