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Dibyabharati Sahu
Department of Plant Breeding and Genetics, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

Swapan K Tripathy
Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

Biswanath Sahoo
Scientist Horticulture, KVK, Ranital, Bhadrak, Odisha, India

Swarajya Laxmi Nayak
Division of Food Science and Postharvest Technology, IARI, New Delhi, India

Dibya Ranjan Dalei
Department of Plant Breeding and Genetics, SOA University, Bhubaneswar, Odisha, India

Corresponding Author:
Swapan K Tripathy
Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

Role of molecular markers in vegetables improvement

Dibyabharati Sahu, Swapan K Tripathy, Biswanath Sahoo, Swarajya Laxmi Nayak and Dibya Ranjan Dalei

Abstract
Vegetables serve as the major source of nutrients in the daily diet in developing countries. But, this group of plants are most vulnerable to various pest and diseases. Most of the vegetables are annuals and restricted to location specific environments. Their growth and yield of economic products are drastically reduced under a variety of abiotic stresses. A number of conventional breeding methods are available for genetic improvement of vegetable crops. But, selection of desirable plants in the breeding programme often becomes misleading due to inadequate biotic and abiotic threshold conditions and other environmental factors. Recent advances in development of molecular markers have made it possible for reliable selection and to speed up the breeding cycle in vegetable crops. Therefore, in the present pursuit, the authors presented a detailed review of the role of molecular markers to assist breeding programme of important vegetable crops.

Keywords: Molecular markers, tagging of genes/QTLs, marker aided selection, breeding programme, vegetable crops

Introduction
Molecular markers are the landmarks whose position in the genome is known. Molecular markers directly reveal the polymorphism at the level of DNA. It has been characterized into three types a) Hybridization based marker (First generation marker) viz., restriction fragment length polymorphism (RFLP) (Botstein *et al*., 1980) [4]; PCR based markers(second generation markers) *viz*., Simple sequence repeat (SSR), Random amplified polymorphic DNA (RAPD) (Williams *et al*., 1990) [40], sequence characterized amplified regions (SCARs) (Michelmore *et al*., 1991) [24]; and quasi types *viz*., amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995) [39]. Earlier, RFLP marker system was massively used in DNA fingerprinting but with the discovery of PCR, SSR marker replaced the use of RFLP marker system owing to its automation and simplicity. However, RFLP markers are still now a marker of choice for identification of uncharacterized genes and identification of multiple insertion of transgene in the genome of transgenic plants. Now-a-days, a number of genome-wide sequencing techniques made it possible for high-throughput genotyping and unravelling the abundant genetic variation down to the single nucleotide variation(SNPs: single nucleotide polymorphisms) in the entire genome. Therefore, SSR markers have now been replaced by SNP marker system (third generation markers) which seems to be the most useful to construct saturated linkage maps, precision DNA fingerprinting, phylogenetic and evolutionary studies, gene tagging and marker assisted selection (MAS).

Genetic Linkage Maps
It is a graphical representation of an arrangement of genes or markers in different array of loci on the basis of recombination frequency that takes place between homologous chromosomes during crossing over. Recombination frequency increases with the increase in marker distance from each other. The populations used to construct a genetic linkage map are segregating populations like F_2 population, recombinant inbred lines, backcross inbred lines, near isogenic lines, double haploid population .A molecular genetic linkage map is essential for providing information about physical location of a candidate gene or a cluster of minor genes (QTL) on the chromosomes. Also it helps in map based or positional cloning of oligogene when there is close linkage between molecular marker and gene of interest. Comparison of linkage groups of different crops of solanaceae family provides the information about rearrangement of genes during the time of evolution of the two species (brinjal and tomato) from a common ancestor (Doganlar *et al*., 2002) [10].

High density linkage maps are available in tomato and potato (Tanksley *et al*., 1992) [38]. Genetic linkage maps are also available in cucumber by using SLAF (Specific Length Amplified Fragment) and SNP markers (Zhu *et al*., 2016) [43].

Assessment of Genetic Diversity

Heterotic performance of hybrids depends on degree of genetic diversity between the parents. More the parents are diverse more is the chance of exploitation of heterosis. So assessment of genetic diversity plays a crucial role in hybrid development and characterization of germplasms collected from different region. Dominant markers like RAPD is used for the analysis of brinjal (Demir *et al*., 2010) [8], pepper and capsicum breeding lines (Ilbi *et al*., 2003) [17] which revealed very narrow genetic base with more than 50% of the DNA bands being common among all the lines. Kaur *et al*. (2014) evaluated the collection of faba bean (*Vicia faba* L.) genotypes for intra- and inter-population diversity using a set of 768 genome-wide distributed single nucleotide polymorphism (SNP) markers, of which 657 obtained successful amplification and detected polymorphisms

Arian Dijkhuizen *et al*. (1996) [2] determined the potential use of restriction fragment length polymorphisms (RFLPs) for estimating genetic relationships in two sets of cucumber (*Cucumis sativus* L.) germplasm. The RFLP-derived genetic relationships among this germplasm were in agreement with predictions based on fruit type and pedigree information. Young-Juan *et al*. (2014) [42] who developed a rapid and reliable PCR-restriction fragment length polymorphism (RFLP) marker to identify the *Amaranthus cruentus* species by comparing sequences of the starch branching enzyme (SBE) locus among the three cultivated grain amaranths. The result indicated that MseI recognize the sequence 5'-T/TAA-3' in intron 11 from A. cruentus SBE. A 278 bp portion restriction digestion of the SBE gene revealed 174-bp and 104-bp fragment in *A. cruentus*, while *A. caudatus* and *A. hypochondriacus* remained undigested (278-bp).

During recent decades, Simple sequence repeats (SSR) also known as microsatellites, have become the most popular source of genetic markers owing to their high reproducibility, multi-allelic nature, co-dominant inheritance, abundance, and wide genome coverage. SSR markers have been successfully adopted to analyze genetic diversity in a variety of different plant species. SSR and sequence related amplified polymorphism (SRAP) markers were used by Ruiz and Martinez (2005) [32] to study the genetic variability of some traditional tomato cultivars of Spain. RAPD and SSR markers are also proved to be effective in differentiating the genotypes of *Solanum aethiopicum* and *Solanum melongena* (Ansari and Singh (2013, 2014) [1]. Shim and Jorgensen (2000) [35] carried out AFLP analysis in diversity studies between wild and cultivated carrots. Muminoric *et al.* (2005) [26] used 12 AFLP and 10 inter-simple sequence repeat (ISSR) primers to estimate genetic diversity in 68 varieties of cultivated radish. They revealed substantial genetic variability in cultivated radish germplasm and even within cultivated material. The black radish and French breakfast radish types formed a separate cluster. In another study, AFLP marker analysis detected a greater genetic variability among American than among Spanish accessions of *Cucurbita maxima* (Ferriot *et al*., 2004) [12].

Tagging of target genes

Gene tagging is a pre-requisite for successful MAS and map based gene cloning. Tagging of valuable resistant genes *viz*., TMV resistance (Tm-2 locus in tomato), nematode resistance, Miresistance, Fusarium oxysporum resistance, and powdery mildew resistance etc. has been made in important vegetable crops. Huang *et al.* (2000) [16] tagged powdery mildew resistance gene 'ol-1'on chromosome 6 of tomato using RAPD and SCAR markers. Lee *et al*. (2015) [21] identified 674,521 SNPs between the two cabbage lines, with an average of one SNP per 662.5 bp. A total of 43,018 SNPs were identified from 173 common bean accessions using DNA sequencing. In recent times, Diversity Arrays Technology (DArT)- a microarray hybridization based technique has been used for genetic diversity, population structure, association mapping and construction of linkage map and genetic studies in various vegetable crops. With the recent development of Next generation sequencing, a still new approach such as DArTseq™ is rapidly gaining popularity as a preferred method of genotyping by sequencing and this facilitate whole genome scanning to identify InDel SNP markers (Cruz *et al*., 2013 and Raman *et al.*, 2014) [7, 30] in vegetable crops.

DNA fingerprinting for varietal and hybrid identification

This has direct bearing on varietal differentiation and reliable identification inany crop plants as well as whole living organism. Though RFLP was the early bird in DNA profiling, but now-a-days large number of molecular marker have been used for DNA fingerprinting of cultivars and breeding lines in a number of vegetable crops *viz*., tomato (Kaemmer *et al*., 1995) [18], beans (Hamann *et al*., 1995) [14], pepper (Prince *et al*., 1995) [29], and potato (McGregor *et al*., 2000) [23]. Molecular markers mostly the co-dominant (SSR) and dominant (RAPD and ISSR) markers like RAPD and ISSR are being widely used for test of hybridity. Hybrids share both the parental alleles, while either of the genetically pure parents reveal single band(s). Besides, the molecular marker systems are useful for maintaining genetic purity of plant varieties (Mongkolporn *et al.* 2004) [25] of different vegetable crops.

Detection of QTLs

Development of linkage map gives a clear picture about physical localization of a genes in the chromosomes. It provides evidence that not only the oligogenes, but minor genes (polygenes) have role in inheritance of characters. Mapping of polygenes started with the finding of Sax (1923) [33] who reported the linkage between seed coat colour (qualitative trait) and seed size (quantitative trait) in common bean (*Phaseolus vulgaris).* Therefore, mapping of polygenes was initiated based on the principle of association between a quantitative trait phenotype and genetic marker. However, such strategy failed to map exact location of polygenes due to their huge number and scattered position in the genome, and cumulative effect towards the expression of character. Later, different clusters of minor genes were identified in the genomic region which were associated with expression of quantitative traits. Each such genomic region comprising cluster of minor genes in different chromosomes is termed as quantitative trait locus (QTL). Characterization and mapping of QTL can be done by observing the segregation pattern of individual genes that are present within that QTL. Extensive QTL mapping has been conducted in many vegetable crops for identification of QTL. Development of linked molecular markers to particular gene or QTL is a pre-requisite for MAS. However, the use of biparental mapping populations (F_2, Backcross, DH, RIL) provides low mapping resolution due to

the occurrence of only a few recombination events. However, multi-paent advanced generation intercross (MAGIC) mapping population can be a better alternative for construction of highly saturated map. Besides, association mapping is a powerful technique that uses historical recombination events for QTL detection in natural populations or germplasm collections. This mapping approach has several advantages relative to linkage analysis, including 1) higher mapping resolution, 2) less time-consuming, and 3) aminability of greater number of alleles for mining. In tomato, QTLs have been identified for late blight disease by using different mapping populations. Besides, yield QTLs (Brekketet *et al*., 2019) [5] and QTLs for glandular trichomes have been identified in chromosome 1 by using SNP markers (Bennewitz *et al*., 2018) [3]. In cucumber, QTLs for fruit peduncle length (Song *et al.* 2016) [37] and cucumber mosaic virus (Shi *et al*., 2018) [34] have been detected on chromosome 6 by using SSR markers. Further, the identification of QTLs provides an opportunity for developing new linked molecular markers for ease in marker assisted selection. Some major QTLs associated with specific traits in vegetable crops are presented in Table 1.

Table 1: QTLs identified in different vegetable crops.

Crops	Traits	QTL/gene	Ch. No.	Marker	Population used	Source	Reference
Tomato	Late blight and yield	QTL	11	*SNP*	F_2	*Koralik*	Brekketet *et al.*, 2019 [5].
	Glandular trichomes	QTL	1	SNP	BC	*S. habrocha-ites*	Bennewitz *et al.*, 2018 [3].
	Fruit mineral content	QTL	-	SSR	RIL	*S. pimpine-llifolium*	Capel *et al.*, 2017 [6].
	Early flowering	QTL	1	SNP	F_2	BoneMM cultivar	Ruangrak *et al.*, 2018 [31].
	Late blight	QTL	2,3,10	SNP	F_2	PI163245	Ohlson *et al.*, 2018 [28].
Cucumber	Fruit peduncle length	Qfpl6.1	6	SSR	F_2	Inbred line 1101	Song *et al.*, 2016 [37].
	Cucumber mosaic Virus	CMV6.1	6	SSR	RIL	Inbred line 02245	Shi *et al.*, 2018 [34].
	Alternaria leaf spot	Psl5.1, psl5.2	5	SSR	RIL	GY 14	Slomnicka *et al.*, 2018 [36].
	Powdery mildew	Pm 1.1, pm 1.2	1	SSR	F2.3	WI 2757	He *et al.* 2013 [15].
	Low temperature germination ability	qLTG1.2	1	-	RIL	LGT tolerate variety	Yagcioglu *et al.*, 2019 [41].
		qLTG2.1	2	-	RIL	LGT tolerate variety	Yagcioglu *et al.*, 2019 [41].

Marker Assisted Selection (MAS)

It is an indirect selection method that facilitates selection of the target traitusing tightly linked molecular marker(s). This has several advantages over phenotypic selection. These include a) early detection of the target trait at even seedling stage (prior to its expression) to select plants for hybridization in the same season, b)effective for high as well as low heritable character, c) high selection efficiency compared to phenotypic selection. d) Speeding up breeding cycle. Some molecular marker like SSR is able to distinguish between homozygote and heterozygote. So, while transferring the recessive genes, the selfing needed after every generation of back cross can be bypassed.

Molecular marker aided selection is generally carried out in three ways *viz*., marker-assisted backcrossing (MABC), Marker assisted recurrent selection (MARS) and Genomic selection (GS). MABC has been used to introgress large effect QTLs and oligogenes using the backcross scheme. Besides, it is well suited for recurrent parent genome recovery and the elimination of donor parent genome flanking the target gene for minimizing linkage drag in a faster way as compared to conventional backcross breeding. While, MARS paves the way for population improvement programme by increasing the frequency of QTLs that have significant effect towards expression of character. In this context, GS enriches the population with QTLs irrespective of having significant or non-significant effect towards expression of characters. Ultimately it increases the frequency of minor QTL also in the population.

Marker assisted selection (MAS) has manifold applications in vegetable breeding. Tomato leaf curl virus is one of the devastating disease which is highly prevalent during autumn-winter season in plains of Northern as well as Eastern India. Six resistant genes (Ty1, Ty2, Ty3, Ty4, Ty5, Ty6) have been identified for the trait. Foreground Selection for Ty-3 gene was done through closely linked molecular markers, P6-25 and SCAR-1. The Ty3 gene has been transferred into the genetic background of Pusa Ruby, PusaRohini and Pusa-120 which are considered as the most adapted and preferred varieties by consumers. In water melon, 3 microsatellite markers MCPI_11, BVWS02441, CYSTSIN linked to powery mildew resistant gene were identified by Natalia *et al.* (2015) [21]. on chromosome 2. These linked molecular markers can be used for indirect selection or introgression of powdery mildew resistant gene into new population. Some molecular markers linked to different disease resistant genes in different vegetable crops are mentioned below.

Table 2: Molecular markers linked to disease resistance in different vegetable crops

Crop	Trait	Gene/QTL	Ch. No	Markers	Genetic distance (cM)	Reference
Tomato	Yellow leaf curl virus	Ty-3	-	ACY (Indel)	-	Nevame *et al.*, 2018 [27].
	Bacterial wilt	Bwr-6, Bwr-12	6,12	SNP	-	Kim *et al.*, 2018 [20].
	Fusarium wilt	Frl	9	TG101(RFLP)	-	Devron *et al.*, 2018
Cucumber	Powdery mildew	Pm-s	5	pmsSR27 pmSSR17	0.1 0.7	Lieu *et al.*, 2017
	CMV	cmv6.1	6	SSR11	-	Shi *et al.*, 2018 [34].
	ALS	Psl5.1	5	IS_16325300	1.6	
Watermelon	Powdery mildew	Pm	2	MCPI_11, CYSTSIN	2.6	Gama *et al.*, 2015 [13].

Conclusion

Conventional plant breeding supplemented with molecular markers has already proved to be a dynamic tool for vegetable crop improvement. The development of new genomic tools and next generation sequencing technology will help in developing saturated linkage map which ensures more precise

location of QTL. Besides, development of tightly linked molecular markers can facilitate the marker assisted selection with high selection efficiency. Such molecular tools are highly valuable to harness diverse genomic resources for development of superior vegetable cultivars. Moreover, current and future genomic advances will lead to the next green revolution.

Conflict of Interests

The authors declare that there is no conflict of interests.

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