Plant Archives Vol. 24, No. 1, 2024 pp. 353-361 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.1.050

APPLICATION OF MOLECULAR MARKERS IN VEGETABLE IMPROVEMENT : A REVIEW

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(Date of Receiving-17-10-2023; Date of Acceptance-24-01-2024)

ABSTRACT

Vegetables are the group of plants, which constitute major part of human diet. They can be consumed raw or cooked. Vegetable crops are highly susceptible to abiotic and biotic stresses which largely reduce the yield and crop productivity. Several traditional breeding approaches are available for the crop improvement but these are time consuming, labour intensive, cost ineffective and less accurate processes. The recent technological innovations have made this possible to study and manipulate the genetic variability of crop plant. It is evident that development of molecular markers and their utilization in plant breeding programs facilitates reliable selection and hasten the crop improvement programme. Assessment of genetic variability, molecular mapping, QTL mapping, marker assisted selection, DNA fingerprinting, introgression of resistance genes, gene pyramiding, genomic selection, marker assisted backcrossing, marker assisted recurrent selection and gene tagging are the novel techniques for crop improvement.

Key words: Molecular markers, Introgression, Gene pyramiding, Genomic selection, Marker assisted selection.

Introduction

Plant undergoes various unfavourable environmental conditions throughout their life cycle, and these unfavourable environmental conditions include, Biotic and Abiotic stress. Biotic stresses are caused by insect, pest, bacteria, fungi, viruses, nematodes, weeds and others. Biotic stresses reduce the annual crop productivity about 20-40% (Bommarco *et al.*, 2013). And abiotic stress consist of drought, salinity, cold, heat, flood and heavy metal stress. About 70% of annual yield is reduced due to abiotic stresses (Tigchelaar *et al*., 2018; Zorb *et al*., 2019). Such stresses negatively affect the physiological and biochemical parameters of crop plants (Vaughan *et al*., 2018; Ziska *et al*., 2010). These are the major constraints that limit the crop productivity. So the plant breeders have to develop such varieties, which are resistant or tolerant to the biotic and abiotic stresses. Conventional plant breeding or classical or traditional breeding deals with the development of new varieties by the use of older tool and methods and rely on natural processes (Botstein *et al*., 1980). But the major drawbacks with the traditional breeding approaches are: they are generally slow takes about 10-14 years for development of improved variety, labour intensive and costly process. Although, recent progress in genetics and genomics accompanied by the development of novel tools and techniques to enhance the plant breeding programmes. Breeding for improved varieties can make use all the recent technologies to shorten the selection cycles, use of markers is one such technology (Williams *et al*., 1990). Markers are basically a tag, sign or something which is helpful in the identification of the trait (Vos *et al*., 1995). Marker-assisted selection (MAS), marker-assisted backcrossing (MABC) (Collard and Mackill, 2008) and marker-assisted recurrent selection (MARS) (Charmet *et al*., 1999) can be used in precision breeding. Markers are classified into four type's *viz*., morphological, biochemical, cytological and molecular markers (Kumar *et al*., 2003). Morphological markers are (also known as naked eye polymorphisms) visually

characterized phenotypic traits like plant height, disease response, shape or color of flower, fruits or seeds, surface of plant part, growth habits or pigmentation and many other visual characters and those gene loci that have direct effect on the morphology of plant (Hearne *et al*., 1992). These markers enable the assessment and evaluation of genetic variability among the population and diversity based on single phenotypic difference (Reddy *et al*., 2007). Biochemical markers (also known as protein polymorphism) or isozymes are molecular form of enzyme that is based on the protein staining but having different electrophoretic mobilities. Basically, these biochemical markers are encoded by different genes and have same functions (Kumar *et al*., 2012). Such markers are related to variations in protein and amino acid banding patterns. Isozymes and Protein based markers are successful Biochemical markers. They can also be used to estimate the gene frequency, genotypic frequency and successfully help in the detection of genetic diversity, gene flow and gene structure (Fukuoka *et al*., 1994). To investigate the genetic difference between cultivated lettuce and wild lettuce genotypes biochemical markers where used (Cole *et al.*, 1991; Collard and Mackill, 2008: Dziechciarkova *et al*., 2004). Cytological markers are the variations associated with the chromosome's morphology such as variations in chromosomal number, size, shape, order, position, sequence specificity, meiotic behavior of chromosome (Pashley *et al*., 2006). A cytological marker detects the differences in the euchromatin and heterochromatin, mutated chromosomes and normal and used in the identification of mapping and linkage groups (Feng *et al*., 2018). Morphological markers, biochemical markers and cytological markers are classical markers (Ashraf *et al*., 2012). A marker is a DNA sequence or gene with known location on a chromosome which serves as flag post or signpost or landmark which is directly or indirectly linked to the trait gene of interest and is generally co-inherited with the trait (Lyamichev *et al*., 1993). Molecular markers or genetic markers are the nucleotide sequences which are estimated by level of polymorphism present between the nucleotide sequences of different individuals. The level of polymorphism is based on insertion, deletion, duplication, translocation and point mutations whereas they did not affect the activity of genes (Ghareyazie *et al*., 1995).

Properties desirable for ideal DNA markers (Jiang, 2013; Joshi *et al.*, 2011)

1. High level of polymorphism (Clear distinct allelic features)
2. Co-dominant inheritance (can distinguish between heterozygote and homozygote)
3. Frequent occurrence in genome
4. Easy and fast assay
5. High reproducibility
6. Insensitive to environment
7. Markers can be easily exchangeable between laboratories
8. Non-epistatic

Types of molecular markers

On the basis of various polymorphism-searching methods, DNA markers have been used in different systems (Collard *et al.* 2005) (Fig. 1).

Markers available as mentioned literature (Semagn *et al*., 2006; Kumar *et al*., 2009; Ismail *et al*., 2016) (Table 1).

Applications of Molecular markers

Assessment of Genetic Diversity

Assessment of genetic diversity plays a crucial role in *ex-situ* germplasm conservation, germplasm characterization, management, utilization and in hybrid development. Molecular markers are the efficient tool which shows the adaptation, performance and agronomic qualities of the germplasm (Demeke *et al*., 1997). Molecular markers have made the evaluation of genetic diversity and classification of genetic material easier (Ridout *et al*., 1999). Ruiz and Martinez (2005) used SSR and SRAP markers for the study of genetic variability in some traditional tomato cultivars of Spain. RAPD (Random amplified polymorphic DNA) and SSR (Simple sequence repeats) markers were effectively used in differentiating among the genotypes of *Solanum aethiopicum* and *Solanum melongena* by Ansari and Singh (2014). Several researchers in studies revealed that SSR markers are efficient markers in assessment of

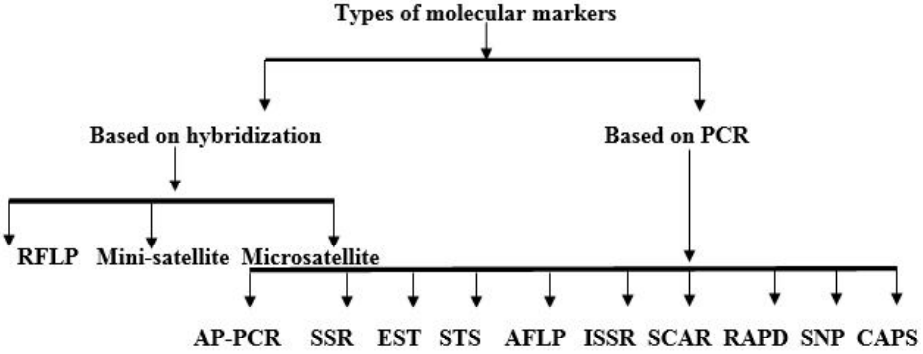

Fig. 1 : Types of molecular markers.

Table 1 :

Markers	Full Form	References
	Hybridization based molecular marker	
RFLP	Restriction fragment length polymorphism	Botstein *et al*. (1980)
	PCR based molecular marker	
RAPD	Random amplified polymorphic DNA	Williams *et al*. (1990)
AFLP	Amplified fragment length polymorphism	Vos *et al*. (1995)
STS	Sequence tagged site	Fukuoka *et al*. (1994)
EST	Expressed sequence tags	Pashley *et al*. (2006)
SCAR	Sequence characterized amplified region	Feng *et al*. (2018)
CAPS	Cleaved amplified polymorphism sequence.	Lyamichev *et al*. (1993)
ALP	Amplicon length polymorphism	Ghareyazei *et al*. (1995)
SNP	Single nucleotide polymorphisms	Kumar *et al*. (2012)
ISSR	Inter simple sequence repeat	Reddy *et al*. (2002)
SSR	Simple sequence repeats	
ScoT	Start codon targeted	Zhang *et al*. (2015)
REMAP	Retrotransposon Microsatellite Amplified Polymorphism	
SRAP	Sequence Related Amplified Polymorphism	Robarts and Wolfe *et al*. (2014)
IT	Intron-Targeting Polymorphism	
TRAP	Targeted Region Amplified Polymorphism	
SSLP	Microsatellite simple Sequence length Polymorphism	Jarmen and Wells (1989)
SSCP	Single stranded conformation Polymorphism	Orita *et al*. (1989)

Genetic diversity, genetic relationships and population structure improvement, DNA fingerprinting and molecular variance, genetic variation, evolutionary relatedness in Capsicum (Lee *et al*., 2021; La Cruz *et al*., 2020; Singh *et al*., 2020). Sharmin *et al*. (2018) also used SSR markers for the assessment of Genetic variability and genetic diversity in Brinjal. EST-SSR have been successfully used for the evaluation of genetic diversity and genetic relationships within and among potatoes from different geographical regions (Salimi *et al*., 2016). Gonias *et al*. (2019) carried out SSR and SCAR analysisto determine genetic diversity and resistance against fungal diseases in tomato.

DNA fingerprinting for varietal and hybrid identification

DNA fingerprinting is one of the important techniques for the varietal identification and detection of any genotype of crop (Provan *et al*., 2001). It is the only practical technique for ensuring the presence of multiple beneficial genes/QTL in a single variety or genotype. Any type of marker can be used for DNA fingerprinting but RFLPs markers were preferred earlier (Dirlewanger *et al*., 1998). Nowadays large number of markers are used *viz*., RAPD and AFLP markers are used for the identification of varieties of Pepper (Prince *et al*., 1995; Paran *et al*., 1998), Beans (Stockton *et al*., 1994), Carrot (Gwanama *et al*., 2000), Sweet potato (Danin *et al*., 2001), Tomato (Bredemeijer *et al*., 1998; Noli *et al*., 1999) and Potato (McGregor *et al*., 2000; Ashkenazi *et al*., 2001).

Gene tagging

Gene tagging is detection/identification of DNA sequences in genome that can perform as a tag for desired genes. Tagging of valuable resistant genes is a pre-requisite for Marker Assisted Selection (MAS) and map basedcloning (Provan *et al*., 2001). There are so many Molecular markers linked to major resistant genes in different vegetable crops. In tomato, Ty2 gene linked with RFLP, which is resistant against Yellow leaf curl virus (Hanson *et al*., 2000), Tm2 gene against Tomato mosaic virus (Sobir *et al*., 2000), Cmr gene against Cucumber mosaic virus (Stamova and Chetalat, 2000). In pea, Mo is a resistant gene against common mosaic virus linked with molecular marker RFLP, similarly Erlinked with RAPD, which is resistant to *Erysiphe polygone* (Dirlewanger *et al*., 1994). In cucumber, Fo gene is linked with SSP which resistant against *Fusarium oxysporum* f. sp. *melonis* (Wechter *et al*., 1998). Huang *et al*. (2000) using RAPD and SCAR markers tagged powdery mildew resistance gene ol-1 on chromosome 6 of tomato. Wechter *et al*. (1995) using RAPD marker tagged Fo and m2 genes which are resistant to *Fusarium oxysporum* f. sp. *melonis*. According to Baghour *et al*. (2019), double

transgenic tomato plants overexpressing both *LeNHX2* and *SlSOS2* show an increased fruit yield and a better performance under NaCl stress than WT and single transgenic plants over expressing only one of these genes. Hansona *et al*. (2016) developed fresh tomato lines resistant to begomo viruses (tomato yellow leaf curl), *Phytophthora infestans* (late blight), *Ralstonia solanacearum* (bacterial wilt), *Stemphyllium* spp. (gray leaf spot), *Fusarium oxysporum* f. sp. *lycopersici* race 2 and *Tobacco mosaic* virus through integrated application of pedigree method and molecular markers to confirm the presence of targeted gene. Zhang *et al*. (2022), investigated the potential regulatory factors *viz*., Solyc01g008390 and Solyc01g008410 genes of cold tolerance related to molecular marker TGS377 located on chromosome 1 of the tomato.

Development of saturated genetic maps

Unlike morphological and biochemical markers, molecular markers are not affected by the environmental factors and developmental stage of the plant. Molecular/ DNA markers can be used in number of ways in breeding studies. In Lettuce, 41 RFLP markers were used for the construction of linkage map (Landry *et al*., 1987). Yayeh (2005) identified first genetic linkages in male fertile garlic accessions using SNPs, SSRs and RAPDs. Zhang *et al*. (2004) constructed linkage map for watermelon by using RAPD and SCAR markers. Montero-Pau *et al*. (2017) reported SNP-based saturated genetic map and QTL analysis of fruit-related traits *i.e*. fruit weight and fruit length in Zucchini using Genotyping-by-sequencing. A molecular genetic map of Cassava (*Manihot esculenta* Crantz) has been constructed using 132 RFLPs, 30 RAPDs, 3 microsatellites and 3 isozyme markers segregating from the heterozygous female parent of an intra specific cross (Fregene *et al*., 1997). Grzebelus *et al*. (2014) analyzed set of 900 Diversity Arrays Technology (DArT) markers for comparing 65 wild and 94 cultivated carrot accessions, to develop saturated linkage genetic map and to detect the genetic diversity of Carrot.

Detection of QTLs

Gene mapping gives a clear picture about the physical location of genes in the chromosomes. The prime objective of plant breeders engaged in resistance breeding is to identify and detect linkage between makers and QTLs (Moreno *et al*., 1998). QTL mapping is a unique technique for identification and detection of loci associated with quantitative components of resistance to infections in crop plants (Provan *et al*., 2001). Different types of QTLs have been identified in vegetable crops using molecular markers, some of them are presented in Tables 2 and 3.

Marker Assisted Selection (MAS) for trait of interest

Marker Assisted Selection is a selection method which uses molecular markers to facilitatethe phenotypic selection in crop improvement (Li *et al*., 2001). Marker assisted selection has several advantages over phenotypic selection. Plant breeders use this selection method for the identification and detection of suitable dominant and recessive alleles across the generation the population (Kalendar *et al*., 1999). Basically, in MAS, QTLs related to important agronomical traits and valuable resistant genes and their highly related DNA markers that are tightly linked to the trait of interest are used (Jaccoud *et al*., 2001). Geographic region as well as Pathogen specific QTLs controlling resistance have been identified by Truong *et al*. (2012) using intra-specific recombinant inbred line population of pepper. MAS breeding involve

Table 2 : QTLs/ genes identified in different vegetable crops for abiotic stress resistance.

Crop	Trait	Gene/QTL	Ch. No	Markers	Reference
Tomato	Salt tolerance	QTL	6	SSR	Liu *et al*. (2021)
	Cold stress germination	-	1, 4 and 8	RFLP	
Cucumber	Salt tolerance	-	3	SSR	Kere *et al*. (2017)
	Low temperature	-	5 and 6	SSR	Dong *et al*. (2019)
	Low temperature	qLTG1.2	1	SNP	Yagcioglu *et al*. (2019)
Pea	Salt index	LG3	3 and 7	SNPs	Leonforte *et al*. (2013)
	Winter frost damage	LG 3	3,5,6 & 7	SNPs & SSR	Klein *et al*. (2014)
Common bean	Drought	Pv01, Pv08 Pv03, Pv09, Pv04. Pv07	-	SNP	Mukeshimana *et al*. (2014)
Cowpea	Salinity	LG1	-	SSR	Chankaew *et al*. (2012)

Table 3 : QTLs/ genes identified in different vegetable crops for biotic stress resistance.

Crop	Trait	Gene/QTL	Ch. No	Markers	Reference
Tomato	YLC virus	Ty-3	-	ACY (Indel)	Nevame *et al*. (2018)
	Bacterial wilt	Bwr-6, Bwr-12	6,12	SNP -	Kim *et al*. (2018)
	Fusarium wilt	Frl	9	TG101(RFLP)	Devron *et al*. (2018)
	Meloidogyne javanica	Mi3	-	RAPD	Yaghoobi *et al*. (1995)
	Late blight	QTL	9 &12	SNP	Panthee *et al*. (2017)
Cucumber	Powdery mildew	Pm-s	5	pmsSR27 pmSSR17s	Liu *et al*. (2017)
	CMV	cmv6.1 -	6	SSR11	Shi *et al*. (2018)
	ALS	Psl5.1, psl5.2	5	IS_16325300 1, SSR	Slomnicka *et al*. (2018)
Pea	*Fusarium oxysporum* f sp. *Melonis*	Fo		SSP	Wechter *et al*. (1998)
	Pea common mosaic virus	Mo	-	RFLP	Dirlewanger *et al*. (1994)
	Erysiphe polygone	Er	-	RAPD	

Table 4 : Marker assisted selection in different vegetable crops.

S. no.	Crop	Marker/gene	Lines used	Trait improved	Reference (s)
1.	Cabbage	In Del markers A1 and M10	D21, D29, D70, D120 and D162	Head splitting and Fusarium wilt resistance	Li *et al*. (2020)
2.	Tomato	TG101 (RFLP) and Fr1 gene	Pusa Ruby	Fusarium wilt resistance	Devran *et al*. (2018)
		SNP and Bwr-6 and Bwr12	Pusa Rohini, Pusa 120	Bacterial wilt resistance	Kim *et al*. (2018)
		ACY (InDel) and Ty-3 gene	Pusa Rohini, Pusa 120	Yellow leaf curl virus resistance	Nevame *et al*. (2018)
3.	Cucumber	SSR11 pmsSR27 pmSSR17	Cmv6.1 Pm-s	CMV resistance Powdery mildew resistance	Shi *et al*. (2018) Liu *et al*. (2017)
4.	Watermelon	MCPI11, CYSTSIN and Pm gene	Arka Manik	Powdery mildew resistance	Gama *et al*. (2015)
5.	Pea	Pea SCAR and er-2 gene	JI2480	Powdery mildew resistance	Katoch *et al*. (2010)
6.	Onion	*Orf725*	A and B lines of onion in Brazilian germplasm	Cytoplasmic male sterility	Ferreira and Santos (2018)

four important schemes *viz*., Gene pyramiding, marker-assisted recurrent selection (MARS), marker assisted backcrossing (MABC) and Genomic Selection (GS) (Roy *et al*., 2015). MAS is an effective approach, where conventional plant breeding is supplemented with molecular markers which facilitates selection efficiency and reliability.

Conclusion

There are several traditional and modern strategies that can be utilized for the crop improvement. Out of which, molecular breeding or use of molecular/DNA markers isone of the best strategy for minimizingyield losses due to various abiotic and biotic stresses. Application of molecular markers in the field of plant breeding is a boon to plant breeders. Molecular mapping, QTL mapping, MAS, DNA fingerprinting, introgression of resistance genes, Gene pyramiding and Gene tagging etc has given a new direction to conventional breeding methods. Molecular tools are highly valuable to utilize diverse genomic resources for development of superior vegetable cultivars. The past years have witnessed tremendous development of molecular markers from first generation to third generation markers, but still available markers are not enough. Currently, there is unavailability of molecular markers for the several important traits controlled by many genes or polygenes. But upcoming years are likely to see continued innovations and

advancement in the molecular marker technology.

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