

APPLICATION OF MARKERSASSISTED SELECTION IN VEGETABLE BREEDING AND IMPROVEMENT

Abstract

The advancements in technology have high-throughput sequencing platforms that allows the sequencing and assembling of numerous plant genomes, providing valuable information about the genetic makeup of different crops. The molecular maps have been instrumental in identifying the locations of genes and QTLs (quantitative trait loci) that are responsible for important variations (phenotypic) in plants. By identifying precise segments of the genome linked to favorable characteristics, breeders can selectively cultivate plants possessing these traits, facilitating the advancement of superior plant varieties. Molecular markers have been pivotal within breeding initiatives; these markers consist of distinct DNA sequences correlated with specific traits, enabling breeders to swiftly and precisely opt for plants harboring the preferred characteristics. This expedites the process and conserves resources when juxtaposed with traditional breeding methodologies reliant on visual scrutiny and phenotypic evaluations. Through the employment of these tools and methodologies, breeders are empowered to create novel vegetable cultivars endowed with augmented attributes, ultimately bolstering the global food reservoir and enhancing human well-being.

Keywords: Gene pyramiding, marker assisted selection, QTLs, vegetables

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I. INTRODUCTION

In the realm of vegetables, India stands as the foremost producer of ginger and okra, while securing the second position in potato, onion, cauliflower, brinjal, cabbage, and other productions, as reported by the FAO in 2021. In the fiscal year 2022-23, the country achieved exports of fresh fruits and vegetables valued at Rs. 13,185.30 crores / 1,635.95 USD million. This export comprised Rs. 6,219.46 crores / 770.70 USD million in fresh fruits and Rs. 6,965.83 crores / 865.24 USD million in vegetables, as stated by APEDA. Despite the perpetual potential for enhancement in vegetable production, challenges such as yield reduction and quality deterioration due to multiple factors persist. In light of the sluggish, labor-intensive, and expensive nature of conventional breeding methods, recent advancements in genetics and genomics have introduced novel tools, techniques, and approaches that can be harnessed to augment plant breeding programs, as highlighted by Tiwari et al.(2021).

Numerous crop species, including various vegetables, have witnessed the development and publication of molecular markers, genetic linkage maps, marker assays, and complete genome sequences (Singh, 2007). The adoption of different strategies within MAS (Collard and Mackill, 2008), marker-assisted backcrossing (MABC) (Collard and Mackill, 2008), marker-assisted recurrent selection (MARS) (Charmet et al., 1999), and genomic selection (GS) (Heffner et al., 2009) hinges on factors like available resources and the intricacy of the species and its breeding process. These methodologies, at varying stages of development, are tailored to each specific vegetable crop. Within this context, this chapter elaborates on Marker Assisted Selection as well as its application for select vegetables.

II. APPLICATION OF MARKER ASSISTED SELECTION (MAS)

By utilizing closely linked molecular markers, the MAS method enables the indirect selection of the desired trait. This has several advantages over phenotypic selection. So, prior to its expression, even in the seedling stage, detection of the target trait is possible which could be used for hybridization in the same season. This method helps in the effective selection of high as well as low heritable characters. Time period taken for breeding can also be reduced. SSR (Simple Sequence Repeats) is one such molecular marker that distinguishes between homozygote and heterozygote. With the help of this, recessive genes can be transferred; that bypasses the selfing needed in every generation of back cross. (Sahuet al., 2019).

III. TRANSFER OF ABIOTIC AND BIOTIC STRESS RESISTANCE

To enhance traits like grain yield and resistance to both biotic and abiotic stresses, it is recommended to incorporate a greater number of Quantitative Trait Loci (QTLs) into crops. Additionally, a range of analyses encompassing expression-QTL (eQTL), protein-QTL (pQTL), and metabolite-QTL (mQTL) are employed for multiple trait assessment. The Marker Assisted Selection (MAS) technique has shown successful implementation through the utilization of markers linked to resistant genes or QTLs against biotic stressors such as gall midge (*Contarinia quinquenotata* L.), bacterial blight (*Xanthomonas campestris* L.), late blight (*Phytophthora infestans* L.), mosaic viruses, powdery mildew (*Podosphaera xanthii*

L.), in addition to various abiotic stresses (Table 1) including heat, salinity, drought, cold, as well as quality enhancement (Dormatey et al., 2020).

Table 1: QTLs Responsible for Abiotic and Biotic Stress Resistance in Vegetables

Crop	Trait resistance	QTL	MAS Assay	Citation
Chilli	CMV	cmr2	KASP	Choi et al., 2018
	CMV	Cmr1	HRM	Kim et al., 2017b
	Anthracnose	AnRGO 5	SCAR	Zhao et al., 2020
Tomato	Late blight	Ph-2	CAPS	Zhang et al., 2014a
	Bacterial speck	Pto	CAPS/SCAR	Zhang and Dilip, 2021
Eggplant	Fusarium oxysporum f. sp. melongenae	Fm1	SSR	Miyatake et al., 2016
Lettuce	Bacterial leaf spot (QTLs)	qXCR	-	Sandoya et al., 2019
	Leafminer (Liriomyza trifolii) (QTLs)	qSTP	-	Kandel et al., 2021

IV. VARIETAL PURITY AND IDENTIFICATION

Marker Assisted Selection helps to differentiate one variety from the other. Even unique alleles can be identified with the help of markers that leads to differentiate cultivars. Fifty EST-SSR primers were used on seven onion varieties that showed two varieties viz., Bhima Red and Bhima Raj were found to be closely related to each other (Anandhan et al., 2014).

Highest discrimination power of AFLP is revealed by carrying out DNA fingerprinting techniques in tetraploid potato genotype followed by multi-locus SSR, RAPD, ASSR, and single locus SSR. ISSR markers were found to be highly polymorphic in pepper (Gaikwad et al., 2001). AFLP generates very high number of markers per assay. So, Comparing ISSR and RAPD, the marker index of AFLP is higher (Ansari, 2015).

In Capsicum species, genetic variability and diversity were explored, through which 383 accessions were resequenced and pan genome was constructed in pepper, which represented *C. chinense*, 13 *C. frutescens*, 11, 355 *C. annuum* and 4 *C. baccatum* species (Ou et al., 2018).

V. GENE PYRAMIDING

Through MAS pyramiding of genes responsible for different characters is possible. Marker-assisted backcrossing helps in improving a targeted trait by transferring one to more genes/ QTLs from donor to other genotype which is superior. Comparing conventional backcrossing, MABC depends on desirable genes or QTLs which is linked with a marker instead of phenotypic performance. Furthermore, MARS streamlines the process of intercrossing and genotype selection within a single cropping season. This enhancement

simplifies the recurrent selection efficiency, refining the procedure for selection and facilitating the incorporation of numerous genes of interest (Dormatey et al., 2020).

VI. QUALITY IMPROVEMENT

MAS has been successfully implemented in various vegetable crops to improve their quality. MAS allows breeders to select plants with desired traits more efficiently and accurately. For example, in tomatoes, MAS has been used to improve traits such as shelf life, fruit, colour, shape and size. In cucumbers, MAS has been employed to enhance traits like disease resistance, fruit quality, and yield. Similarly, in other vegetables like peppers, eggplants, and lettuce, MAS has been effective in improving traits such as flavor, nutritional value, and disease resistance. These advancements in crop improvement techniques contribute to the production of high-quality vegetables for consumers (Table 2).

Table 2: QTLs Responsible for Quality Improvement in Vegetables

Crop	Trait	QTL	MAS Assay	Reference
Chilli	Pungency	pAMT	SCAR	Jeong et al., 2015
	Pungency	Pun3	CAPS	Han et al., 2019
	Orange fruit colour CaOr (ZEP)	CaOr (ZEP)	HRM, CAPS	Lee et al., 2021
Eggplant	Peel colour	-	SCAR, AFLP	Liao et al., 2009
Lettuce	Anthocyanin content	qACI	-	Wei et al., 2021

Sex determination MAS is a greater technique when it comes to determine the sex of the crop. Also through identification of male sterility gene or a sex determining locus linked with marker, hybrid development is quickly possible (Table 3).

Table 3: QTLs Responsible for sex Determination in Vegetables

Crop	Trait	QTL	MAS Assay	Citation
Eggplant	Male sterility	Rf	SCAR	Khan and Isshiki, 2016
Chilli	Fertility restorer	Rf	CAPS	Jo et al., 2016
	Nuclear male sterility	ms ₃ , ms _w	CAPS, dCAPS	Naresh et al., 2018
	Nuclear male sterility	ms1	HRM	Jeong et al., 2018
	Nuclear male sterility	msc-1	Indel marker	Cheng et al., 2018
Spinach	Sex determining locus	X/Y	-	Qian et al., 2017; She et al., 2021
	Monoecious gene	X ^m	-	Yamamoto et al., 2014
Chicory	Nuclear male-sterility	NMS1	AFLP, SCAR, STS	Gonthier et al., 2013

VII. MAPPED GENES AND QTLs

Markers offer a means to create genetic maps for different crops, serving as valuable resources for effective breeding. Recently, an ultra-high density genetic map was established using an RIL population (*S. lycopersicum* × *S. pimpinellifolium*), where 141,083 SNP markers were organized into 2,869 genomic bins (Gonda et al., 2019). This genetic mapping effort yielded significant advantages for the fresh market tomato industry, particularly in terms of fruit shape and quality (Celik et al., 2017; Capel et al., 2017; Gonda et al., 2019; Gao et al., 2019; Safaei et al., 2020).

Although generating mapping populations and conducting their phenotypic assessments is a time-consuming process, it has led to extensive gene mapping in lettuce across various populations such as F₂ and early BC generations, BILs, RILs, NILs, and diversity panels. Notably, RIL populations resulting from intraspecific crosses of distinct cultivated lettuce varieties are commonly employed to pinpoint QTLs, for instance, Iceberg x Batavia (Hayes et al., 2014, Simko et al., 2015).

VIII. CONCLUSION

MAS proves to be a valuable asset within crop improvement initiatives. It allows breeders to select plants with desired traits more efficiently by using molecular markers linked to those traits. In the case of gene pyramiding, where multiple resistance genes are desired, MAS can greatly aid in the selection process. By using highly polymorphic markers, MAS helps in accurately identifying and differentiating varieties and cultivars, as well as assessing their purity. This is particularly important in maintaining the genetic integrity of breeding lines. Moreover, MAS can be especially useful in improving disease resistance in crops, even when the resistance is controlled by recessive genes. By selecting plants with the desired markers associated with resistance, breeders can effectively pyramid multiple resistance genes and develop cultivars with enhanced disease resistance. Overall, MAS enables breeders to make more informed and precise selections, leading to more efficient and effective crop improvement programs. By expediting the creation of enhanced cultivars possessing desired attributes such as disease resistance, MAS plays a pivotal role in bolstering food security and promoting sustainability in agriculture.

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