**Application of Markers Assisted Selection in Vegetable Breeding and Improvement**

**Bharathi S1, Gadha Sreekumar2\*, Manjusha M R3, Thiruppathi M4, Lalu Prasad5 and Harini M6**

1,2Ph.D. Scholar, Departmentof Vegetable Science, HC & RI, TNAU, Periyakulam

3,4Ph.D. Scholar, Departmentof Fruit Science, HC & RI, TNAU, Periyakulam

5Ph.D. Scholar, Departmentof Vegetable Science, Acharya Narendra Deva University of Agriculture & Technology, Ayodhya, Uttar Pradesh

6M.Sc. Scholar, Departmentof Vegetable Science, HC & RI, TNAU, Periyakulam

*Corresponding author mail id:* [*hortiresearchg@gmail.com*](mailto:hortiresearchg@gmail.com)

**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

**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.

**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. (Sahu *et al.*, 2019).

**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 |

**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).

**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).

**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 | ms3, msw | 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 |

**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).

**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.

**Reference**

Tiwari, J. K., Yerasu, S. R., Rai, N., Singh, D. P., Singh, A. K., Karkute, S. G. & Behera, T. K. (2022). Progress in marker-assisted selection to genomics-assisted breeding in tomato. *Critical Reviews in Plant Sciences*, *41*(5), 321-350.

Charmet, G., Robert, N., Perretant, M. R., Gay, G., Sourdille, P., Groos, C. & Bernard, M. (1999). Marker-assisted recurrent selection for cumulating additive and interactive QTLs in recombinant inbred lines. *Theoretical and Applied Genetics*, *99*, 1143-1148.

Collard, B. C., & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1491), 557-572.

Sahu, D., Tripathy, S. K., Sahoo, B., Nayak, S. L., & Dalei, D. R. (2019). Role of molecular markers in vegetables improvement. *Journal of Pharmacognosy and Phytochemistry*, *8*(6), 456-460.

Ansari, A. M. (2015). Molecular markers in vegetable improvement. *Horticultural Biotechnology Research*, *1*, 5-10.

Anandhan, S., Mote, S. R., & Gopal, J. (2014). Evaluation of onion varietal identity using SSR markers. *Seed Science and Technology*, *42*(2), 279-285.

Dormatey, R., Sun, C., Ali, K., Coulter, J. A., Bi, Z., & Bai, J. (2020). Gene pyramiding for sustainable crop improvement against biotic and abiotic stresses. *Agronomy*, *10*(9), 1255.

Ou, L., Li, D., Lv, J., Chen, W., Zhang, Z., Li, X. & Zou, X. (2018). Pan-genome of cultivated pepper (*Capsicum*) and its use in gene presence–absence variation analyses. *New Phytologist*, *220*(2), 360-363.

Celik, I., Gurbuz, N., Uncu, A. T., Frary, A., & Doganlar, S. (2017). Genome-wide SNP discovery and QTL mapping for fruit quality traits in inbred backcross lines (IBLs) of *Solanum pimpinellifolium* using genotyping by sequencing. *BMC genomics*, *18*(1), 1-10.

Gonda, I., Ashrafi, H., Lyon, D. A., Strickler, S. R., Hulse‐Kemp, A. M., Ma, Q. & Foolad, M. R. (2019). Sequencing‐based bin map construction of a tomato mapping population, facilitating high‐resolution quantitative trait loci detection. *The plant genome*, *12*(1), 180010.

Gao, Z., Zhang, H., Cao, C., Han, J., Li, H., & Ren, Z. (2020). QTL mapping for cucumber fruit size and shape with populations from long and round fruited inbred lines. *Horticultural Plant Journal*, *6*(3), 132-144.

Hayes, R. J., Galeano, C. H., Luo, Y., Antonise, R., & Simko, I. (2014). Inheritance of decay of fresh-cut lettuce in a recombinant inbred line population from ‘Salinas 88’×‘La Brillante’. *Journal of the American Society for Horticultural Science*, *139*(4), 388-398.

Simko, I. (2015). Analysis of bibliometric indicators to determine citation bias. *Palgrave Communications*, *1*(1), 1-9.

Choi, S., Lee, J. H., Kang, W. H., Kim, J., Huy, H. N., Park, S. W. & Kang, B. C. (2018). Identification of cucumber mosaic resistance 2 (cmr2) that confers resistance to a new cucumber mosaic virus isolate P1 (CMV-P1) in pepper (*Capsicum* spp.). *Frontiers in Plant Science*, *9*, 1106.

Kim, H., Yoon, J. B., & Lee, J. (2017). Development of Fluidigm SNP type genotyping assays for marker-assisted breeding of chili pepper (*Capsicum annuum* L.). *Horticultural Science and Technology*, *35*(4), 465-479.

Zhao, Y., Liu, Y., Zhang, Z., Cao, Y., Yu, H., Ma, W. & Wang, L. (2020). Fine mapping of the major anthracnose resistance QTL AnRGO5 in *Capsicum chinense* ‘PBC932’. *BMC plant biology*, *20*(1), 1-8.

Zhang, C., Liu, L., Wang, X., Vossen, J., Li, G., Li, T. & Du, Y. (2014). The Ph-3 gene from *Solanum pimpinellifolium* encodes CC-NBS-LRR protein conferring resistance to *Phytophthora infestans*. *Theoretical and applied genetics*, *127*, 1353-1364.

Zhang, J., & Panthee, D. R. (2021). Development of codominant SCAR markers to detect the Pto, Tm22, I3 and Sw5 genes in tomato (*Solanum lycopersicum*). *Plant Breeding*, *140*(2), 342-348.

Miyatake, K., Saito, T., Negoro, S., Yamaguchi, H., Nunome, T., Ohyama, A., & Fukuoka, H. (2016). Detailed mapping of a resistance locus against Fusarium wilt in cultivated eggplant (*Solanum melongena*). *Theoretical and applied genetics*, *129*, 357-367.

Kandel, R., Lu, H., & Sandoya, G. V. (2021). Identification and mapping of quantitative trait loci for resistance to *Liriomyza trifolii* in romaine lettuce cultivar ‘Valmaine’. *Scientific Reports*, *11*(1), 998.

Sandoya, G. V., Maisonneuve, B., Truco, M. J., Bull, C. T., Simko, I., Trent, M. & Michelmore, R. W. (2019). Genetic analysis of resistance to bacterial leaf spot in the heirloom lettuce cultivar Reine des Glaces. *Molecular Breeding*, *39*, 1-11.

Jeong, H. S., Jang, S., Han, K., Kwon, J. K., & Kang, B. C. (2015). Marker-assisted backcross breeding for development of pepper varieties (*Capsicum annuum*) containing capsinoids. *Molecular breeding*, *35*, 1-10.

Han, K., Jang, S., Lee, J. H., Lee, D. G., Kwon, J. K., & Kang, B. C. (2019). A MYB transcription factor is a candidate to control pungency in *Capsicum annuum*. *Theoretical and Applied Genetics*, *132*, 1235-1246.

Lee, S. Y., Jang, S. J., Jeong, H. B., Lee, S. Y., Venkatesh, J., Lee, J. H. & Kang, B. C. (2021). A mutation in Zeaxanthin epoxidase contributes to orange coloration and alters carotenoid contents in pepper fruit (*Capsicum annuum*). *The Plant Journal*, *106*(6), 1692-1707.

Yi, L., SUN, B. J., SUN, G. W., LIU, H. C., LI, Z. L., LI, Z. X. & CHEN, R. Y. (2009). AFLP and SCAR markers associated with peel color in eggplant (*Solanum melongena*). *Agricultural Sciences in China*, *8*(12), 1466-1474.

Wei, T., Van Treuren, R., Liu, X., Zhang, Z., Chen, J., Liu, Y. & Liu, H. (2021). Whole-genome sequencing of 445 *Lactuca* accessions reveals the domestication history of cultivated lettuce. *Nature Genetics*, *53*(5), 752-760.

Khan, M. M. R., & Isshiki, S. (2016). Cytoplasmic male sterility in eggplant. *Horticult J,* 85 (1): 1–7.

Naresh, P., Lin, S. W., Lin, C. Y., Wang, Y. W., Schafleitner, R., Kilian, A., & Kumar, S. (2018). Molecular markers associated to two non-allelic genic male sterility genes in peppers (*Capsicum annuum* L.). *Frontiers in Plant Science*, *9*, 1343.

Qian, W., Fan, G., Liu, D., Zhang, H., Wang, X., Wu, J., & Xu, Z. (2017). Construction of a high-density genetic map and the X/Y sex-determining gene mapping in spinach based on large-scale markers developed by specific-locus amplified fragment sequencing (SLAF-seq). *BMC genomics*, *18*(1), 1-10.

Cheng, Q., Wang, P., Liu, J., Wu, L., Zhang, Z., Li, T. & Shen, H. (2018). Identification of candidate genes underlying genic male-sterile msc-1 locus via genome sequencing in *Capsicum annuum* L. *Theoretical and Applied Genetics*, *131*, 1861-1872.

Jeong, K., Choi, D., & Lee, J. (2018). Fine mapping of the genic male-sterile ms 1 gene in *Capsicum annuum* L. *Theoretical and applied genetics*, *131*, 183-191.

Yamamoto, K., Oda, Y., Haseda, A., Fujito, S., Mikami, T., & Onodera, Y. (2014). Molecular evidence that the genes for dioecism and monoecism in *Spinacia oleracea* L. are located at different loci in a chromosomal region. *Heredity*, *112*(3), 317-324.

Gonthier, L., Blassiau, C., Mörchen, M., Cadalen, T., Poiret, M., Hendriks, T., & Quillet, M. C. (2013). High-density genetic maps for loci involved in nuclear male sterility (NMS1) and sporophytic self-incompatibility (S-locus) in chicory (*Cichorium intybus* L., Asteraceae). *Theoretical and applied genetics*, *126*, 2103-2121.