**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 quantitative trait loci (QTLs) that are responsible for important phenotypic variations in plants. By pinpointing the specific regions of the genome associated with desirable traits, breeders can selectively breed plants with those traits, leading to the development of improved cultivars. Molecular markers have played a crucial role in breeding programs and they have specific DNA sequences associated with a particular trait, allow breeders to quickly and accurately select plants with the desired traits. This saves time and resources compared to traditional breeding methods, which rely on visual inspection and phenotypic assessments. By utilizing these tools and techniques, breeders can now develop new cultivars of vegetables with enhanced characteristics, ultimately contributing to the global food supply and improving human health.

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

**Introduction**

India is the largest producer of ginger and okra among vegetables and ranks second in the production of potatoes, onions, cauliflowers, brinjal, cabbages, etc. (FAO, 2021). During 2022-23, India exported fresh fruits and vegetables worth Rs. 13185.30 crores / 1635.95 USD millions which comprised Fresh Fruits worth Rs. 6,219.46 crores/ 770.70 USD Millions and vegetables worth Rs. 6,965.83 crores / 865.24 USD Millions (APEDA). Always there is a scope for improvement of vegetables due to decline in yield or quality caused by various factors. Due to slow, labour-intensive, and costly processes of traditional breeding approaches, recent progress in genetics and genomics through the development and deployment of novel tools, techniques, and approaches can used to enhance plant breeding programs (Tiwari *et al*., 2021). Molecular markers, genetic linkage maps, marker assays, and whole-genome sequence have been developed and published for many crop species, including several vegetables (Singh, 2007). Depending on the availability of resources and the complexity of the species and its breeding, Marker-assisted selection (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) can be used that are in various stages of development for each vegetable crop. The present chapter describes about the Marker Assisted Selection for some of the vegetables and its application.

**Application of marker assisted selection**

With the help of tightly linked molecular markers, Marker Assisted Selection (MAS) method facilitates indirect selection of the target 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 biotic and abiotic stress resistance**

For traits, such as grain yield and resistance to biotic and abiotic stress, the pyramiding of more number of QTLs into crops is recommended. And, expression- QTL (eQTL), protein- QTL (pQTL), and metabolite- QTL (mQTL) types of analysis are applied on the multiple traits. MAS has been successfully utilized with the help of markers linked to resistant genes or QTLs against biotic stresses, such as gall midge (*Contarinia quinquenotata* L.), bacterial blight (*Xanthomonas campestris* L.) late blight (*Phytophthora infestans* L.), mosaic viruses, powdery mildew (*Podosphaera xanthii* L.), and many abiotic stresses (Table 1), such as heat, salinity, drought, and cold as well as quality improvement (Dormatey *et al*., 2020).

**Table 1: QTLs responsible for biotic and abiotic stress resistance in vegetables**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Crop** | **Trait** | **QTL** | **MAS Assay** | **Citation** |
| **Chilli** | CMV resistance | cmr2 | KASP | Choi *et al*., 2018 |
| CMV resistance | Cmr1 | HRM | Kim *et al*., 2017b |
| Anthracnose (caused by  *Colletotrichum acutatum*) | AnRGO 5 | SCAR | Zhao *et al*., 2020 |
| **Tomato** | Late blight  (*Phytophthora infestans*) | Ph-2 | CAPS | Zhang *et al.,* 2014a |
| Bacterial speck  (*Pseudomonas syringae*) | 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 355 *C. annuum*, 4 *C. baccatum*, 11 *C. chinense*, and 13 *C. frutescens* 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. Also, Marker-assisted recurrent selection (MARS) enables genotype selection and intercrossing in a single cropping season which eases the efficacy of recurrent selection and improves the selection process promotes the integration of multiple number of genes of inteest (Dormatey *et al*., 2020).

**Quality improvement**

Marker-assisted selection (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 fruit size, shape, color, and shelf life. 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** | **Citation** |
| **Chilli** | Fruit pungency | pAMT | SCAR | Jeong *et al*., 2015 |
| Fruit pungency | Pun3 | CAPS | Han *et al*., 2019 |
| Orange fruit color CaOr (ZEP) | CaOr (ZEP) | CAPS, HRM | Lee *et al*., 2021 |
| **Eggplant** | Peel color | - | AFLP, SCAR | 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 provides way to develop genetic maps of different crops which can be utilized as an effective breeding material., An ultra-high density genetic map was constructed recently based on a RIL population (*S. lycopersicum* × *S. pimpinellifolium)*, in which 141,083 SNP markers were grouped into 2,869 genomic bins (Gonda *et al*., 2019). Through genetic mapping research, significant benefit was obtained by the fresh market tomato industry has related to shape and quality of the fruits (Capel *et al*., 2017; Celik *et al*., 2017; Gao *et al*., 2019; Gonda *et al*., 2019; Safaei *et al*., 2020)

It is a lengthy process to generate mapping populations and to do its phenotypic characterization. In lettuce from various populations like F 2 and early BC generations, BILs, RILs, NILs, and diversity panels, mapping has been done on many genes. Mostly the RIL populations from the intraspecific crosses of two types of the cultivated lettuce are used to identify QTLs; for example: Iceberg x Batavia (Hayes *et al*., 2014a; Simko *et al*., 2015a)

**Conclusion**

Marker-assisted selection (MAS) is indeed a valuable tool in crop improvement programs. 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. It helps accelerate the development of improved varieties with desired traits, including disease resistance, ultimately contributing to food security and agricultural sustainability.

**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.