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Marker Assisted Breeding in Vegetables

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Abstract
Molecular markers have great potential to improve the efficiency and precision of conventional plant breeding through marker-assisted selection (MAS). It is also important to identify outstanding parents, improve the selection of elite alleles at loci controlling important traits, and pyramid favorable alleles at multiple loci associated with a single trait or multiple traits. Currently, the efforts of marker-assisted breeding in vegetable breeding for horticultural crop improvement will benefit the targeted traits through new approaches like marker-assisted back crossing, genomic selection, etc. This will enhance efficiency, reduce costs, and save time in the ongoing breeding programme.

Keywords: Introduction, Types of markers, Types of molecular markers, How molecular markers are useful, Application of MAS in vegetable breeding

Introduction
Marker-assisted breeding (MAB), also known as molecular breeding, refers to a modern breeding process where selection of targeted traits is based on specific molecular markers. Marker-assisted breeding uses DNA markers associated with desirable traits to select a plant or animal for inclusion in a breeding program early in its development. This approach dramatically reduces the time required to identify varieties or breeds which express the desired trait in a breeding program.

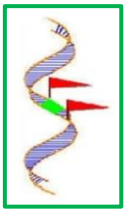
Types of Markers


Morphological Markers: Morphological markers are usually visual indicators of phenotypically differing characters, such as color, shape, and size of the flower, seeds, or leaves; type of development of plants, inflorescences, or root system; pigmentation; or habit.

Biochemical Markers: Biochemical markers are the proteins produced by gene expression. Isozymes, allozymes, proteins are successful biochemical markers. Isozymes are the different molecular form of the same enzyme which are easily identified by their activities. Biochemical markers have been successfully applied in the detection of genetic diversity, population structure, gene flow and population subdivision. They are co-dominant, easy to use and cost effective. However, they are less in number; they detect less polymorphism and they are affected by various extraction methodologies, plant tissues and different plant growth stages .

Cytological Markers: It includes all chromosomal activities means differences of chromosome, i:e number, size, shape, banding patterns etc. Markers that are related with variations present in the numbers, banding patterns, size, shape, order and position of chromosomes are known as cytological markers. These variations reveal differences in the

distributions of euchromatin and heterochromatin. For example, G bands are produced by Giemsa stain, Q bands are produced by quinacrine hydrochloride and R bands are the reversed G bands. These chromosome landmarks can be used in the differentiation of normal and mutated chromosomes. Such markers can also be used in the identification of linkage groups and in physical mapping .



Molecular Markers (Variation in DNA fragments): Molecular markers are specific fragments of DNA that can be identified within the whole genome. Molecular markers are found at specific locations of the genome. They are used to 'flag' the position of a particular gene or the inheritance of a particular character. Molecular markers are phenotypically neutral. The green section indication the presence of a desirable gene in an organism genetic code that is associated with two genetic markers.

Types of Molecular Markers

RFLP (Restriction Fragment Length Polymorphism): RFLP was the first molecular marker technique and the only marker system based on hybridization. Individuals of same species exhibit polymorphism as a result of insertion/deletions (known as InDels), point mutations, translocations, duplications and inversions. Isolation of pure DNA is the first step in the RFLP methodology. This DNA is mixed with restriction enzymes which are isolated from bacteria and these enzymes are used to cut DNA at particular loci (known as recognition sites). This results in a huge number of fragments with different length. Agarose or polyacrylamide gel electrophoresis (PAGE) is applied for the separation of these fragments by producing a series of bands. Each band represents a fragment having different lengths. Base-pair deletions, mutations, inversions, translocations and transpositions are the main causes for the variation resulting in the RFLP pattern. These variations lead to the gain or loss of recognition sites, resulting in fragments of various length and polymorphism. The restriction enzymes will not cut the fragment if a single base-pair variation occurs in the recognition site. However, if this point mutation occurs in one chromosome but not the other, it is called heterozygous for the marker, as both bands are present .

SSR (Simple Sequence Repeats): Microsatellites are also called as SSRs; short tandem repeats and simple sequence length polymorphisms. SSRs are tandem repeat motifs of 1–6 nucleotides that are present abundantly in the genome of various taxa. Microsatellites can be mononucleotide (A), dinucleotide (GT), trinucleotide (ATT), tetranucleotide (ATCG), pentanucleotide (TAATC) and hexanucleotide (TGTGCA). Microsatellites are distributed in the genome; however, they are also present in the chloroplast and mitochondria. Studies have also confirmed the presence of SSRs in protein-coding genes and expressed sequence tags (ESTs). SSRs represent the lesser repetition per locus with higher polymorphism level. This high polymorphism level is due to the occurrence of various numbers of repeats in microsatellite regions and can be detected with ease by PCR. Occurrence of SSRs may be due to slippage of single-strand DNA, recombination of double-strand DNA, transfer of mobile elements (retrotransposons) and mismatches. Common motifs present in SSRs are Mono: A, T; Di: AT, GA; Tri: AGG; Tetra: AAAC. Mainly the sequences which are flanking the SSRs are conserved and are used in the development of primers. Development of a genomic library and sequencing a segment of the studied genome will result in the development of these primers. The development of SSR markers involves the development of an SSR library and then detection of specific microsatellites. After this, the detection of favourable regions for primer designing is done and then PCR is performed. Interpretation and evaluation of banding patterns are performed and assessment of PCR products is performed for investigation of polymorphism. SSR markers are considered a marker of choice, as they are co-dominant, with high reproducibility and greater genome abundance, and they can be used efficiently in plant mapping studies.

AFLP (Amplified fragment length polymorphism): Amplified fragment length polymorphism (AFLP) is a PCR-based fingerprinting technique that was first described by Vos et al. . Since then several modified protocols have been reported, but all typically include five main steps: (a) restriction of genomic DNA and ligation of adaptors (most often performed together) to restricted fragments; (b) preselective PCR amplification of a subset of the restricted fragments; (c) selective PCR amplification, reducing further fragment number; (d) electrophoretic separation of amplified DNA fragments; (e) scoring and interpretation of the data. We detail below one of the protocols that uses a RedTaq polymerase.

The power of AFLP analysis derives from its ability to quickly generate large numbers of marker fragments for any organism, without prior knowledge of genomic sequence. In addition, AFLP requires only small amounts of starting template and, in comparison with other fingerprinting techniques such as RAPD (random amplified polymorphic DNA) and ISSR (inter-simple sequence repeats) it exhibits much higher reproducibility. Despite the fact that AFLP is a relatively labour-intensive method, it can be easily multiplexed and frequently is used to amplify in the same batch hundreds of genomic fragments from hundreds of individuals.

SCoT (Start Codon Targeted): Start codon targeted (SCoT) polymorphism marker, a targeted fingerprinting marker technique, has gained considerable importance in plant genetics, genomics, and molecular breeding due to its many desirable features. SCoT marker targets the region flanking the start codon, a highly conserved region in plant genes. Therefore, it can distinguish genetic variations in a specific gene that link to a specific trait. It is a simple, novel, cost-effective, highly polymorphic, and reproducible molecular marker for which there is no need for prior sequence information. In the recent past, SCoT markers have been employed in many commercially important and underutilized plant species for a variety of applications, including genetic diversity analysis, interspecific/generic genetic relationships, cultivar/hybrid/species identification, sex determination, construction of linkage map, association mapping/analysis, differential gene expression, and genetic fidelity analysis of tissue culture-raised plants. The main aim of this review is to provide up-to-date information on SCoT markers and their application in many commercially important and underutilized plant species, mainly progress made in the last 8-10 years.

EST (Expressed sequence tag): (ESTs) are randomly selected clones sequenced from cDNA libraries. Each cDNA library is constructed from total RNA or poly (A) RNA derived from a specific tissue or cell, and thus the library represents genes expressed in the original cellular population. A typical EST consists of 300–1000 base pairs (bp) of DNA and is often deposited in a database as a “single pass read” that is sufficiently long to establish the identity of the expressed gene. EST analysis has proved to be a rapid and efficient means of characterizing the massive sets of gene sequences that are expressed in a life-stage-specific manner in a wide variety of tissues and organisms; the approach was first applied to the screening of a human brain cDNA library (Adams et al., 1991). The ESTs derived from human brain were also able to provide reference for EST analysis of other organisms. In the helminth field, ESTs have extensive application in the discovery of new genes and identification of novel vaccine candidates and drug targets (Blaxter et al., 2002; Hu et al., 2003; Lee et al., 2003; Mitreva et al., 2004). Over 400,000 ESTs were generated from more than 30 different species of nematode during the parasitic nematode EST project by 2003 (Parkinson et al., 2003). However, EST pools do not represent the whole but only partial profiles of the gene expression of an organism. For instance, EST analysis has potential pitfalls when it comes to redundancy of the data because of overexpressed genes. In addition, the EST sequencing of large numbers of housekeeping genes that are not developmentally regulated tends to neglect rare transcripts (Knox, 2004). In most cases, EST information is

employed in initial parasite protein screening together with subsequent transcriptomic and proteomic analyses (Brindley et al., 2009).

How can molecular markers are useful?

- Molecular markers allow working with genotype information directly
- Analyse the effect of the genotype on the phenotype
- Characterization of germplasm
- Assessment of genetic diversity
- Variety identification
- Marker assisted selection

Application of mas in vegetable breeding

- Genetic diversity studies
- Resistance breeding
- Varietal identification/ Finger printing
- Hybrid seed purity testing
- Novel allele determination at earlier stages
- Sex determination
- Gene tagging
- Map-based cloning

Conclusion

Marker assisted selection is a methodology which is likely to become more valuable as selection can be performed in early segregating populations and at early stages of plant development for pyramiding the resistance genes, with the ultimate goal of producing varieties with durable or multiple disease resistance in crop. As a new technology, MAB is not a replacement for but a valued supplement to conventional breeding. Integration of MAB into conventional breeding programs represents an optimistic strategy for future crop improvement. This technique would be most useful for tracing the inheritance of QTLs and major genes where the procedures for screening the plants (as in disease resistance genes) are labor intensive or require plants to be screened for several traits at the same time and in an environment not conducive for one or more of these traits to be expressed.

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