

Allele Mining: On The Road to Vegetable Crop Improvement

Bichhinna Maitri Rout¹, Rajani², Kumar Chiranjeeb³

¹Ph.D Scholar, Division of Vegetable Science, ICAR-IARI, New Delh

²Assistant Professor, Dept. Of Plant Breeding and Genetics,

GIET University, Gunupur, Odisha

³Assistant Professor, Dept. of Soil Science, GIET University, Gunupur, Odisha

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Abstract

Allele mining is a promising approach to dissect naturally occurring allelic variations or to suitable alleles of a candidate gene controlling key agronomic traits which have potential in vegetable crop improvement. It basically helps in tracing the evolution of alleles, identification of new haplotypes and development of allele-specific molecular markers for use in marker-assisted selection (MAS). Its methodology paved a way for breakthrough discovery of fresh alleles hidden in hereditary diversity, which is vital for crop improvement.

Keywords: **Marker assisted selection, Allele, Haplotype, Markers**

Introduction:

Huge genetic variation exists in crop gene pools which can be identified and novel genes having agronomic importance can be used for developing improved cultivars by making use of superior alleles of the gene. Allele mining is a practical and promising approach which makes use of naturally occurring allelic variation of genes with desirable traits for crop improvement. Potent abiotic stress resistant alleles as well as new haplotypes can be discovered using the technique of allele mining which paves way for developing allele specific markers for improved marker assisted selection. The main objective of allele mining lies in identification and isolation of unknown and superior alleles from within genetic resource collections, present at a known locus that are candidates for conferring important traits. A large number of allele mining studies have been performed in recent years for dissection of useful alleles in imparting disease resistance.

Intensive breeding efforts have concentrated the favourable alleles already selected during early domestication and thus contributed to further narrowing of the gene pool. The available germplasm resources need to be screened to fish out potent alleles to improve qualitative agronomic traits of crops. Handling the entire germplasm is a whooping task, whether for conventional plant breeding or for allele mining and hence must involve sampling strategies to narrow it down to a manageable size while maintaining the variability. Development of core and mini collections out of the entire collection is an effective strategy to simplify the conservation of germplasm resources and proper utilization of the existing variation in gene banks.

Allele Mining And True Allele Mining

Allele is an alternative form of a gene found at a given locus on a chromosome. Mining is nothing but searching for new alleles in the wild germplasm. So, allele mining means, searching for useful alleles of genes from a wide range of cultivars, related species and even across species. In other words, it is a research field aimed at identifying allelic variation of relevant traits within genetic resource collections.

Mutation is considered as an evolutionary driving force which underlies existing allelic diversity in any crop species. For creation of new alleles or causing variations in the existing allele and allelic combinations, mutations in the genic regions of the genome either as single nucleotide polymorphism (SNP) or as insertion and deletion (InDel) are important. The mutations in coding regions and/or regulatory regions may have tremendous effect on the phenotype by altering the encoded protein structure and function. True allele mining includes analysis of non-coding and regulatory regions of the candidate genes in addition to analyzing sequence variations in the coding regions of genes.

Importance of Allele Mining:-

It helps in tracing the evolution of alleles.

Also helps in identification of new haplotypes and development of allele-specific markers for use in marker-assisted selection (MAS).

This capability will be important for giving breeders direct access to key alleles conferring:

- ✓ resistance to biotic stresses
- ✓ tolerance to abiotic stresses



- ✓ greater nutrient use efficiency
- ✓ enhanced yield
- ✓ improved quality

It can also provide insight into molecular basis of novel trait variations and identify the nucleotide sequence changes associated with superior alleles.

Approaches For Allele Mining:-

There are three approaches for allele mining such as modified tilling procedure called eco-tilling-based allele mining, sequencing-based allele mining and association mapping-based allele mining.

A) Modified Tilling procedures called Eco-Tilling

TILLING (Targeting Induced Local Lesions IN Genomes) is a technique that can identify single base-pair allelic variation in target gene (more specifically induced point mutations) while Eco-Tilling technique detects natural mutation. It allows the rapid detection of variation in many individuals and is cost effective because only one individual for each haplotype need to be sequenced.

Tilling consists of several major steps: Development of a mutagenized population, DNA preparation and pooling, and mutation discovery. At first, random mutations are induced in genomes by using chemical mutagens. Seeds are mutagenized by treatment with ethylmethanesulfonate (EMS) etc. The resulting M1 plants are self-fertilized, and M2 individuals are used to prepare DNA samples for mutational screening. DNA is extracted from test samples. The DNA samples are pooled and arrayed into 96 wells containing microtiter plates. Screening for mutations begins with PCR amplification of a target fragment using gene-specific infrared dye-labeled primers. The forward primer is 5'-end labeled with a fluorescent dye that is detected at 700 nm (IRDye 700) and the reverse primer is labeled with the IRDye 800 nm. These PCR products are denatured and re-annealed to allow the formation of mismatches or heteroduplexes, which represent naturally occurring single nucleotide polymorphisms (SNPs) and induced SNPs. Samples, are then incubated with a single-strand specific nuclease to digest mismatched base pairs. For mismatch-specific cleavage, several enzymes, including S1 nuclease⁵, T4 endonuclease VII⁶ and Cel-17 have been used. Cleaved

bands representing mutations or polymorphisms are visualized using denaturing polyacrylamide gel.

B) Sequencing-based allele mining

Sequencing based allele mining involves amplification of alleles in diverse genotypes through PCR followed by identification of nucleotide variation by DNA sequencing techniques. In other words, multiplication of specific segment of deoxy-ribose nucleotides as well as, we can identify various allele among the cultivars through sequence based allele mining.

C) Next generation sequencing for allele mining

Sequencing is a technique which enables us to understand arrangement of this nucleotide in DNA molecules. In the last few years, “massively parallel” methods have also emerged and lead to the development of “next generation” sequencing platforms with increased throughput and accuracy. These methods used for resequencing, alignment of the sequence data and their comparison with reference genome. The first of this type was commercialized by 454 Life Sciences and this technique relied on pyrosequencing while eliminating the need for cloning. With this 454 sequencing platform, it is possible to produce 100 Mb of sequence with 99.5% accuracy and increase read length averaging over 250 bases. Another massively parallel sequencer Illumina/Solexa genome analyzer has been developed and this is capable of sequencing one billion bases (1 Gb) of 30-40 base sequence reads in a single run in a short timer period of 3-4 days.

D) Association mapping-based allele mining

This strategy is used to establish regions of the genome associated with critical phenotypes by association or linkage-disequilibrium mapping. The approach relies on the assumption that alleles responsible for a phenotype, along with the markers which flank the locus, are inherited as a block. Using DNA markers has been suggested as a means to identify useful alleles in the vast reservoirs of genetic diversity.

E) Bioinformatic tools required for allele mining

Allele mining requires various sophisticated bioinformatic tools *viz.*, PLACE, plantCARE, TRANSFAC, JASPAR, MEME, Plantprom DB, DCPD, SCPD, BioEdit, ClustalW etc. These

tools useful for sequence alignment in order to compare new genome sequence to reference genome i.e, sequenced genome data.

Applications Of Allele Mining

- Promoter mining
- Allele identification
- Allele variation characterization
- Allele synteny
- Haplotype identification
- Analysing the haplotype diversity
- Evaluation study/ Evolutionary relationship
- Similarity analysis
- Allele specific markers development

Status Of Allele Mining In Vegetable Crops

Crop	Allele/locus	Trait/name of the protein
Tomato	<i>Pto</i>	Disease resistance
Pea	<i>TII</i>	Trypsin inhibitors characterization
<i>Phaseolus</i> sp.	Lectin locus	Storage and defense proteins
Potato	<i>Rpi-blb1</i>	Late blight resistance

Conclusion

The contribution of genetic diversity in crop improvement is limited until today because of the inadequacy of novel alleles having agronomical importance. Allele mining in this scenario strongly proclaims precise detection of naturally occurring mutations at a particular gene sequence. The candidate genes associated with key agronomic traits are further utilised in crop architecture modification. In addition, it confirms molecular basis of novel trait variations using efficient data mining tools and high-throughput sequencing platforms to catalogue superior alleles and their behaviour from diverse genetic backgrounds.

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