

Allele Mining

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Introduction

Today, we have 8 billion human population on this planet. These large numbers of populations require large number of resources for survival. In 21st century, climate change has altered the dimensions of food production all over the world. Global warming, deforestation, pollution is one of the major reasons of this situation. These conditions have impacted various dimensions of humankind. The total food production of the planet is declined due to these factors. The crops are not fulfilling their yield targets. The crop plant is being deteriorated by various biotic and abiotic factors.

Scientists and breeders have been developing various crop plant varieties to encounter these problems. Rice is main staple food that is consumed over the largest quantity all over the world but its total worldwide production has decreased a lot. IRRI (International Rice Research Institute) has developed many disease resistant, flood resistant, early, dwarf, high yielding, and drought resistant, insect & pest resistant varieties. The landraces, wild varieties and cultivars are unable to sustain this climatical imbalance. Every other scientist linked with plant sciences is trying to solve this problem by various scientific methodologies. As of now, many approaches are like transgenic plants, biotic & abiotic resistant crops, hybrids, composites, synthetics are developed. The race for invention of better crop plants leads to various agronomical, genetical, physiological aspects of crop plant. Ultimately, an ideal or novel crop plant require bunch of ideal/desirable characters. This plant characters are governed by different genes, which define the phenotype of plant. Alleles are the alternative forms of a gene that specifies its nature of occurrence in particular living organism. In nature there are many forms of genes present for a character within its genome and it is known as allelic variation. Superior alleles make crop plant to carry ideal genetic makeup which can better sustain numerous biotic and abiotic stresses. Major of the depletion in the crop

production is due to different diseases and pests attacking on the crop plant. Various diseases are linked with infestation on molecular level. It is being discovered by scientists that many varieties of same crop plant have shown different responses to diseases. Many wild varieties of crop plant are found with disease resistant genes for the same disease, when came in contact with the diseases. Disease resistance genes and Plant disease resistance genes (R genes) are a major part of the plant response to pathogen attacks. Disease resistance is often mediated via recognition between pathogen a virulence genes and plant resistance genes. The common defence responses in Chapter III 11 plants also consists callose and lignin deposition, salicylic acid synthesis, and hypersensitive response resulting in cell death (Hammond-Kosack and Jones, 1997), thus, restricting the pathogen development. Evolution of disease resistance genes is mediated via mechanisms such as recombination between alleles/genes, point mutations, gene conversion events and illegitimate recombination (Michelmore and Meyers, 1998; Kuang *et al*, 2004, Wicker *et al*, 2007). In case of powdery mildew, most of the single resistance genes confer complete resistance but are only effective against specific races of the pathogen. This race-specific resistance is based on the detection event between the product of the plant disease resistance gene and the respective a virulence gene of the pathogen (Flor,1971)

Allele Mining

- Allele mining is a research field leads to identification of allelic variation of linked traits within genetic resources collections.
- Allele mining is approach that is used to find naturally occurring allelic variation that are suitable alleles of candidate gene controlling key agronomic trait, which has key role in crop improvement.
- Identification and access to allelic variation that affects the plant phenotype is of key importance for the use of genetic resources, such as in plant variety development.
- Considering the large numbers of accessions that are stored collectively by gene banks, genetic resources collections are believed to held a wealth of unrecognized allelic variants.
- The challenge is how to reveal this variation.

Types of Allele Mining

- ❖ **Modified Tilling or Eco-Tilling**

❖ PCR based sequencing allele method

Tilling and Eco-Tilling

Many unique molecular biology tools have been considered to detect mutations in genes to find the function of these genes (reverse genetics). TILLING is a valuable and non-transgenic reverse genetic technique to study gene function that allows screening for mutations in genes with known nucleotide sequences in a plant mutant population (McCallum et al., 2000).

Tilling helps in the direct identification of induced point mutations in a gene by heteroduplex evaluation (Till *et al.*, 2003). TILLING is suitable for most plants and enables the identification of single-base-pair (bp) allelic variation in a target gene in a high-throughput manner. TILLING has several benefits over other techniques used to detect single-bp polymorphisms. Gel-based assays, such as single-strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE), do not pinpoint the location of the polymorphism or type of polymorphism contained in the DNA fragment (DeFrancesco and Perkel, 2001). Array hybridization techniques are only effective in identifying approximately 50% of simple nucleotide polymorphisms (SNPs) (Borevitz *et al.*, 2003).

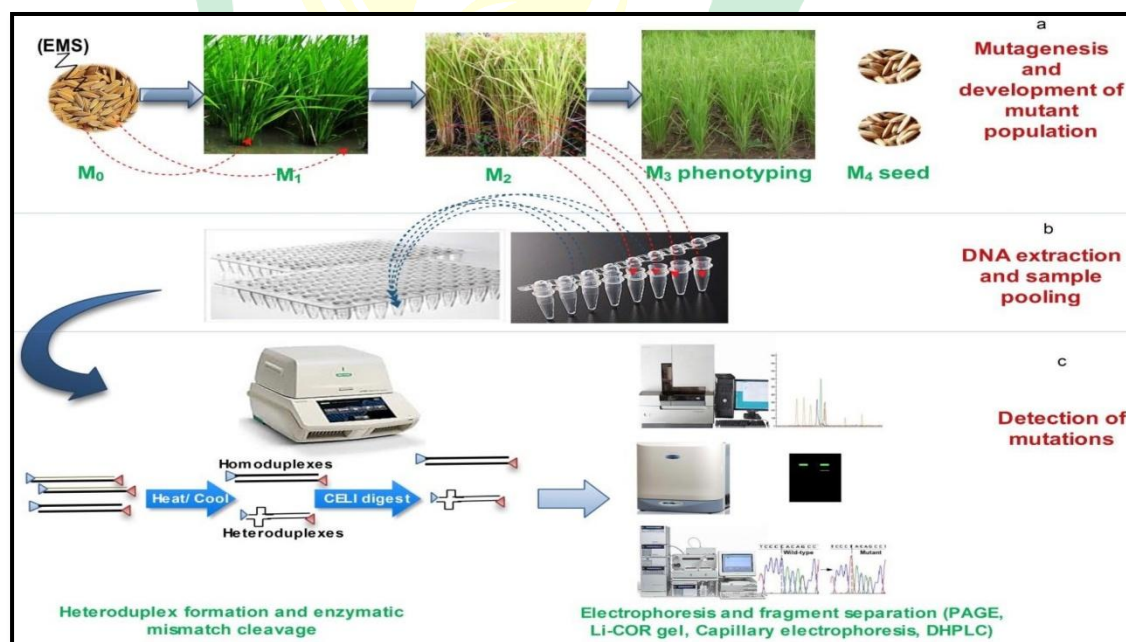


Figure 1: A summary of the TILLING Procedure Importance of allele mining

Sequencing-Based Allele Mining

Sequencing-based allele mining involves PCR-based amplification of alleles of a gene in varied genotypes and then DNA sequencing to recognize nucleotide variance in the alleles (Figure 4). Various alleles among the cultivars through this approach can be identified. The method would help to analyze individuals for haplotype structure and diversity to infer genetic association studies in plants. Applying this approach to recognize the effect of mutations on gene structure, the sequences are analyzed for the location of point mutations or SNPs and insertions or deletions (InDels) to build haplotypes. Sequencing-based allele mining is a very effective method to expand the rice blast R gene source and manage damaging blast disease. Additionally, this method can be applied to the management of additional crop diseases. Sequencing-based allele mining does not require much sophisticated machinery or involve tedious steps (Ramkumar et al., 2010), but the sequencing cost of the targeted genes for this technique is actually high, which will be the major expense for this type of allele mining program. However, with the advanced sequencing methods and tools like massively parallel sequencer using supported oligonucleotide ligation and detection system (SOLiD) the sequencing value is being reduced (Hutchison, 2007). TILLING and Eco-TILLING have been introduced to be economical approaches for haplotyping and SNP discovery. These types of tools require more elegance and entail several steps, for example the creation of DNA pools, particular conditions for nuclease cleavage, and polyacrylamide gel detection using the LI-COR genotype.

Importance of allele mining

- It helps in detecting the evolution of alleles.
- Recognition of new haplotypes and development of allele-specific markers for use in marker-assisted selection.
- This capacity will be important for giving plant breeders direct to key alleles conferring.
- Resistance to biotic stresses.
- Tolerance of abiotic stresses.
- Greater nutrient use efficiency.
- Enhanced yield.

- Improved quality, including human nutrition.

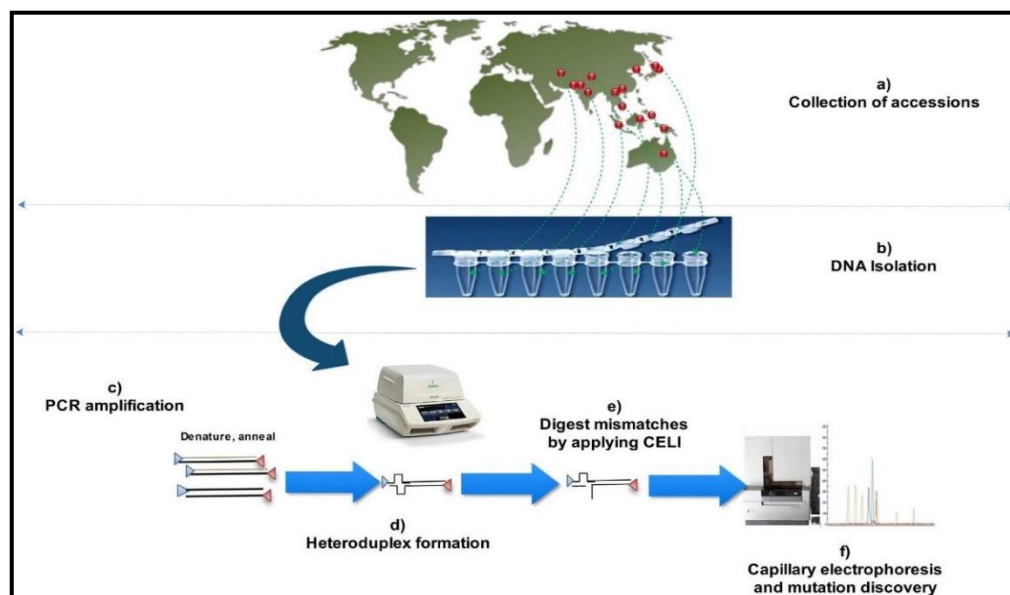


Figure 2. Outline of the Basic Steps for Typical Eco-TILLING to determine natural polymorphisms

Steps in Allele Mining:

- Selection of target trait (Trait Priority)
- Identification of accessions linked with desired phenotypic trait
- Selection of genes underlying the chosen target trait (Gene Targets)
- Primer designing for whole length of gene
- PCR amplification from the identified accessions
- Sequencing- based mining
- PCR
- Sequencing and identification of variation
- Comparison of sequence data with phenotypic data and identification of superior alleles

TILLING

vs

Eco-TILLING

- Mutant population is formed.
- Sequence information required.

- Natural population or association mapping population is utilized.
- Sequence information is not required.

Applications of allele mining:

- **Detection of superior alleles.** Characters vary in their degree of excellence and effectiveness in positive or negative direction due to the allelic variation. allele mining helps us to assess a superior or most performing allele for a trait, i.e., disease resistance, height, flood resistance.
- **Similarity analysis in inter and intra species.** To find out similarities between species and within a species allele mining can help us out. It incorporates us to arrange or classify the collection of genomes.
- **Functional molecular marker development for MAS.** Allele mining helps us to develop markers for selection and hybridization purposes, as we know the sequences of DNA.
- **Identification of new haplotypes.** The allele mining is a tool which evolves new variation which were unknown to world before it gives us discovery to new haplotypes and allelomorphs.
- **Evolutionary study.** Allele mining unfolds all the alleles present for a trait in nature, so it becomes very easy to study the evolution of specific organism or plant.
- **Promoter mining is done for expression study and gene prediction.** Allele mining helps us to predict the gene behaviour and expression on the basis of currently available genome collection data and the number of known alleles.

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