



ALLELE MINING IN CROP IMPROVEMENT

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Introduction

Progress in plant breeding in terms of development of superior and high yielding varieties of agricultural crops is possible by accumulation of beneficial alleles from vast plant genetic resources existing worldwide. But still, a significant portion of these superior alleles cannot be used, because those alleles are left behind during evolution and domestication. This genetic variation existing in wild relatives and land races of crop plants and it can be used for development of agronomically superior cultivars. Introgressions of novel alleles occur from wild crop plants into cultivated varieties. It clearly demonstrated that certain alleles and their combinations potentially make dramatic changes in character expression when it moved to a suitable genetic background. Hence, the vast germplasm resources need to for novel alleles to further enhance the genetic potential of crop varieties for various agronomic traits.

Evolution of new alleles

It is alternative forms of gene. In other words, genes occur at similar loci of homologous chromosomes. Mutation is an evolutionary driving force which causes existing allelic diversity in any crop species. For creation of new alleles or causing variations in the existing allele and allelic combinations, generally mutations will occur in the genic regions of the genome either as Single Nucleotide Polymorphism (SNP) or as Insertion and Deletion (InDel) are important. Even though most of the mutations are deleterious, in genera 10.1% of the mutations are vital leading to changes will occur in gene function which may be highly necessary for the survival of the plant (Singh, 2005).

What is allele mining?

Mining is extraction of any non-renewable or highly valuable resources such as Petroleum, natural gas, minerals, or even water etc. It is a finding of superior allele from the natural population. In other words introgression of novel or superior allele from wild relatives into cultivated one.

Allele mining in crop improvement

It is a strategy of finding valuable and unknown alleles at a known locus, is called as allele mining. These valuable and unknown novel alleles can be used for further crop improvement such as resistance to biotic and abiotic stresses, increase greater nutrient use efficiency, enhance yield in crops and improve quality such as cooking, protein, starch including human nutrition (Latha *et al.*, 2004). Allele mining is conduct on specific genes that are involved in the particular mechanism of stress tolerance express by identify accessions. In Cultivated rice and its relatives it will give an opportunity to test the evolutionary range over which PCR-based allele mining can be successful

Approaches for allele mining: There are two major approaches. They are-

TILLING -based allele mining: It is nothing but a (Targeting Induced Local Lesions In Genomes), to determine variation in individual through artificially changed mutation (Comai *et al.*, 2004). It is a powerful reverse genetics tool for functional genomics where knockout methodologies cannot be applied. Tilling allows the identification of allelic variation of target gene in a high-throughput manner. The use of the Tilling technique to survey natural variation in genes is called Ecotilling. Tilling make use of chemical mutagens to introduce random mutation. Seeds are mutagenized with EMS, which causes G/C-to-A/T point mutations (Nagy, *et al.*, 2003). M1 seeds are selfed to produce M2 seeds. M2 progeny from single seed descent are used for screening. For screening, DNAs are pooled eightfold to maximize the efficiency of mutation detection. PCR is performed using 5'-end -labeled gene specific primers to target the desired locus, and heteroduplexes are formed by heating and cooling the PCR products. CEL I nuclease is used to cleave at base mismatches, and products representing induced mutations are visualized with denaturing polyacrylamide gel electrophoresis. The detailed procedure has been given by Till, *et al.* (2003).

Sequencing based allele mining: It is amplification of alleles in diverse genotypes through PCR and identify nucleotide variation by DNA sequencing. 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. Unlike Eco Tilling, sequencing based allele mining does not require much sophisticated equipment or involve tedious steps, but involves huge costs of sequencing. A comparison between these two procedures is given below. Eco Tilling also depend on the enzymatic cleavage of heteroduplexed DNA (formed due to single nucleotide mismatch in sequence between reference means genome sequence available and test genotype) with a single strand specific enzymes use nuclease (i.e. Cel-1, S1nuclease) under specific conditions followed by detection through Li Cor genotypers at point mutations, there will be a cleavage by the nuclease to produce two cleaved products whose sizes will be equal to the size of full length product. The presence, type and location of point mutation or SNP will be confirmed by sequencing the amplicon from the test genotype that carry the mutation. TILLING and EcoTilling has been proposed cost effective approaches for haplotyping and SNP discovery, these techniques require more sophistication and involve several steps starting from making DNA pools of reference and test genotypes, specific conditions for efficient cleavage by nuclease, detection in polyacrylamide gels using Li- Cor genotyper and confirm through sequencing (Raghavan *et al.*, 2007).

Applications of allele mining

Allele mining can be effectively used for discovery of superior alleles, through 'mining' the gene of interest from diverse genetic resources. It can also provide insight into molecular basis of novel trait variations and identify the nucleotide sequence changes associated with superior alleles. In addition, the rate of evolution of alleles; allelic similarity/dissimilarity at a candidate gene and allelic relation with other members of the family can also be study. Allele mining may also give way for molecular difference among related species, development of allele-specific molecular markers, for essay introgression of novel alleles through MAS or through genetic engineering (GE).

Identification of new haplotypes: Allele mining can be potentially employed in the identification of nucleotide variation at a genomic region (candidate gene) associated with

phenotypic variation for a trait. Through this, one can evaluate the frequency, type and the extent of occurrence of new haplotypes and the resulting phenotypic changes.

Development of allele-specific markers for MAS: Identification of sequence variation will pave the way to develop allele-specific marker assay for precise introgression of the identified ‘superior and/or novel’ alleles to suitable genetic background.

Allelic synteny and evolutionary relationship: Using the sequence information obtained from allele mining studies, syntenic relationships can be assessing among the identified loci/genes across the species/genera.

Conclusion

The use of genetic diversity is limited due to the resources which are at hand for characterization of all these lines. Therefore, we need to (i) develop strategies to assemble focused sets of material for specific traits based on criteria for selection of the lines but also (ii) to identify genes underlying agronomically important traits and (iii) establish the molecular tools for rapid characterization of new alleles. Allele mining is a promising approach to dissect naturally occurring allelic variation at candidate genes controlling key agronomic traits which has potential applications in crop improvement programs. Allele mining can be effectively used for discovery of superior alleles, through ‘mining’ the gene of interest from germplasm. It can also provide insight into molecular basis of novel trait variations and identify the nucleotide sequence changes associated with superior alleles. In addition, the rate of evolution of alleles, allelic similarity and dissimilarity at a candidate gene and allelic relationship with other members of the family. Allele mining may also pave way for introgression of novel alleles through Marker Assisted Selection.

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