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ALLELE MINING: MODERN TOOLS OF BIOTECHNOLOGY

Popular Article

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ABSTRACT

Allele mining is the process of finding the superior allele from the natural population. In other words, introgression of novel or superior allele from wild relatives into cultivated one. 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 synteny with other members of the family can also be studied. Allele mining may also pave way for molecular discrimination among related species, development of allele-specific molecular markers, facilitating introgression of novel alleles through MAS or deployment through genetic engineering (GE).

Introduction

Mining is extraction of any non-renewable or highly valuable resources such as Petroleum, natural gas, minerals or even water *etc*. Likewise, Allele mining is the process of finding the superior allele from the natural population. In other words, introgression of novel or superior allele from wild relatives into cultivated one. 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. Allele mining is conducton 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. It helps in tracing evolution of allele, identification of new haplotypes and development of allele specific marker. It is independent of genome size, ploidy level and reproductive system of plants.

Approaches for allele mining

Two major approaches are available for the identification of sequence polymorphisms for a given gene in the naturally occurring populations. They are (i) modified TILLING (Targeting Induced Local Lesions in Genomes) procedure called EcoTilling and (ii) sequencing based allele mining.

TILLING -based allele mining

It is nothing but a (Targeting Induced Local Lesions In Genomes), to determine variation in individual through artificially changed mutation. 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. M1seeds 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 primer 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 (Till *et al*. 2003).

Sequencing-based allele mining

This technique involves amplification of alleles in diverse genotypes through PCR followed by identification of nucleotide variation by DNA sequencing. Sequencing-based allele mining would help to analyze individuals for haplotype structure and Unlike EcoTilling, sequencing-based allele mining does not require much sophisticated equipment or involve tedious steps, but involves huge costs of sequencing.

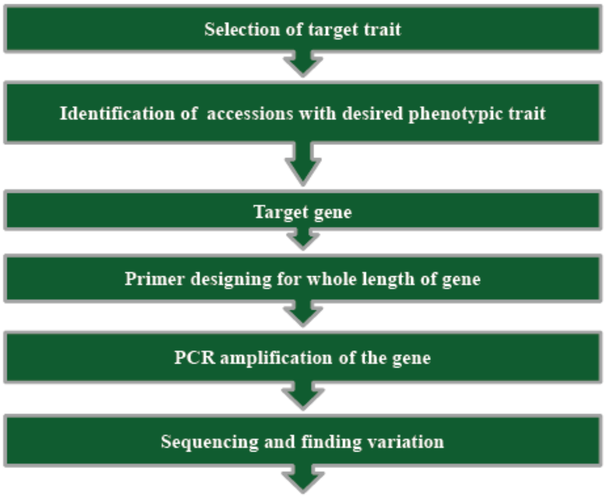
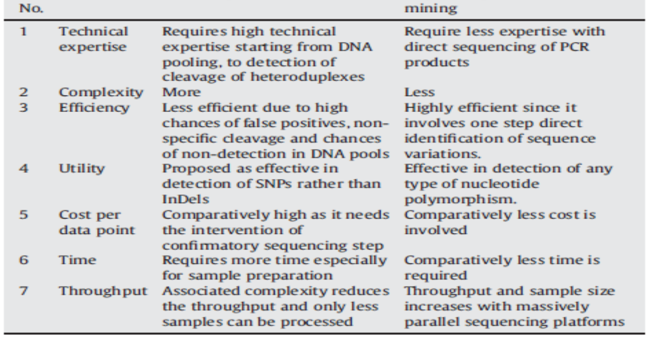


Fig 1. Steps involved in allele mining



S. No.	Parameters	EcoTilling	Sequencing-based allele mining
1	Technical expertise	Requires high technical expertise starting from DNA pooling, to detection of cleavage of heteroduplexes	Require less expertise with direct sequencing of PCR products
2	Complexity	More	Less
3	Efficiency	Less efficient due to high chances of false positives, non-specific cleavage and chances of non-detection in DNA pools	Highly efficient since it involves one step direct identification of sequence variations.
4	Utility	Proposed as effective in detection of SNPs rather than InDels	Effective in detection of any type of nucleotide polymorphism.
5	Cost per data point	Comparatively high as it needs the intervention of confirmatory sequencing step	Comparatively less cost is involved
6	Time	Requires more time especially for sample preparation	Comparatively less time is required
7	Throughput	Associated complexity reduces the throughput and only less samples can be processed	Throughput and sample size increases with massively parallel sequencing platforms

Fig 2. Comparison of techniques available for identifying variation in naturally occurring alleles

Application 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 synteny with other members of the family can also be studied. diversity to infer genetic association studies in plants.

Allele mining may also pave way for molecular discrimination among related species, development of allele-specific molecular markers, facilitating introgression of novel alleles through MAS or deployment through genetic engineering (GE).

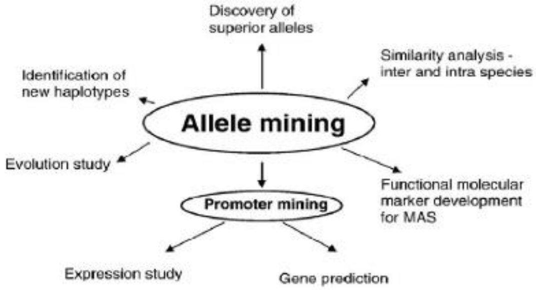


Fig 3. Applications of allele mining

Conclusion

Allele mining pave way for molecular discrimination among related species, development of allele-specific molecular markers, facilitating introgression of novel alleles through MAS or deployment through genetic engineering (GE).

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