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Allele Mining: Applications and Challenges in Crop Improvement

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Plant breeding has been successful in developing high-yielding varieties by accumulating beneficial alleles from plant genetic resources worldwide. However, a significant portion of these alleles has not been utilized and there is potential to exploit untapped genetic variation in wild relatives and land races of crop plants to develop superior cultivars. Genomic databases have enabled the discovery and annotation of new genes, facilitating the development of allele-specific markers and access to key alleles conferring resistance to biotic and abiotic stresses, greater nutrient use efficiency, enhanced yield and improved quality. Allele mining, or the dissection of naturally occurring variation at candidate genes/loci, is a useful tool for identifying allelic variants from germplasm collections, providing new germplasm for delivering novel alleles to targeted trait improvement and categorizing germplasm entries for conservation. Efficient utilization of plant genetic resources (PGR) is crucial for developing crops with superior traits such as disease resistance and drought tolerance. In this context, allele mining is a valuable tool for identifying and utilizing superior alleles. However, despite its potential benefits, allele mining faces several challenges that need to be addressed to ensure its efficient use in crop improvement and secure the future food supply.

Concept

Allele mining is an effective method to uncover naturally occurring allelic variation in candidate genes that control key agronomic traits with the potential to improve crops. It involves the identification of new and superior alleles for various traits such as disease resistance, drought tolerance and quality, which can be integrated into breeding programs to develop new high-yielding crop varieties that address emerging challenges. Despite the collection of a vast number of germplasm lines, the genetic variation available has not been fully explored and exploited. Hence, there is ample opportunity to discover numerous superior alleles using allele mining, thereby unlocking the potential genetic diversity. True allele mining involves the consideration of variations in both expressed and non-expressed regions of the gene. It includes the 5'UTR, promoter, introns, exons, 3'UTR, splice sites etc (figure 1).

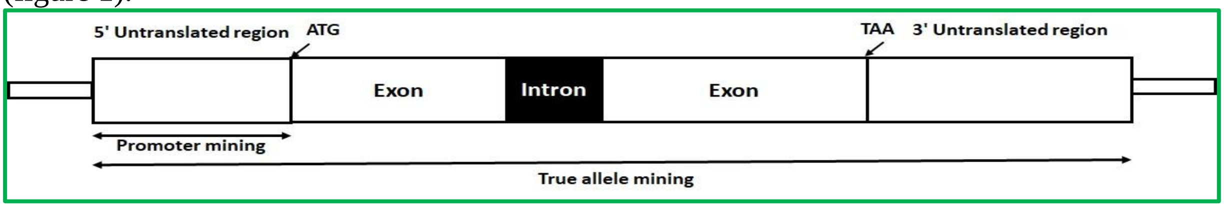

Figure 1: Allele mining for a gene

Sufficient evidence exists to indicate that intronic mutations significantly contribute to creating allelic diversity, which can potentially modify the phenotype. Recently, sequence variation in a gene's regulatory regions has gained more attention, as it directly affects gene expression. There are two main methods for identifying sequence polymorphisms in a gene in naturally occurring populations. The first is a modified TILLING (Targeting Induced Local Lesions in Genomes) procedure, known as EcoTilling. The second method is sequencing-based allele mining.

Applications

Allele mining is a crucial process for identifying superior alleles by examining the gene of interest from a diverse range of genetic resources. It enables the study of the evolution rate of alleles, allelic similarity/dissimilarity at a candidate gene, allelic synteny with other family members and the identification of nucleotide sequence changes associated with superior alleles. Moreover, allele mining may also lead to the development of molecular markers that can discriminate among related species and facilitate the introgression of novel alleles through Marker-Assisted Selection (MAS) or deployment via genetic engineering (GE).

1. Discovering and accessing allelic variation that impacts plant phenotype is crucial for harnessing genetic resources in crop improvement. Allele mining appears to be a promising method, albeit largely untested, for unlocking the diversity present in collections of genetic resources housed in world gene banks.
2. Allele mining can identify nucleotide variations in a candidate gene that are linked to phenotypic variation for a trait. This allows for the evaluation of new haplotypes and resulting phenotypic changes, which can be used to study haplotype diversity. Understanding the frequency and type of common haplotype changes in populations can aid in association mapping studies.
3. Identification of sequence variations in key genes can facilitate the development of allele-specific molecular markers, which can aid in the precise introgression of "superior and/or novel" alleles into suitable genetic backgrounds using Marker-Assisted Selection (MAS). A comparison of nucleotide sequences of the Waxy gene in rice across 18 different accessions revealed the presence of five distinct alleles, each characterized by a unique mutation, which were associated with observed phenotypic alterations.
4. Sequence information obtained from allele mining studies can be used to assess syntenic relationships among loci/genes across species/genera. This technique has been employed to isolate superior homologue alleles for aluminium tolerance in rye using syntenic allele sequence information from wheat. It has also been used to isolate agronomically superior alleles in *Phaseolus vulgaris* and other grasses.

Challenges

Efficient and effective utilization of allele mining to tap the potential genetic diversity in breeding field crops faces several challenges.

1. The primary challenge in efficiently utilizing allele mining to unlock existing genetic variation is selecting the appropriate germplasm to screen. Screening the entire collection is impractical, so a mini core collection should be created that represents maximum diversity. Computational tools like "Powercore" can be used to efficiently select genotypes and avoid redundancy.
2. Accurate and efficient phenotyping is crucial in allele mining as it plays a key role in identifying potential superior alleles. Inaccurate phenotyping can lead to erroneous results and hinder the success of the allele mining process.
3. Relevant and efficient bioinformatics tools should be used to handle the huge amounts of sequencing and other data created in the course of the allele mining process.

4. During genic diversity studies, identifying the putative promoter region, regulatory regions, and exonic regions can be challenging. It is important to note that variations in both expressed and non-expressed regions can have a significant impact on the phenotype. Thus, care must be taken to accurately characterize the gene.
5. High cost is a major obstacle in sequence-based allele mining. Nevertheless, the use of efficient sequencing techniques such as Next Generation Sequencing can significantly reduce the sequencing costs and make the process more cost-effective.

Conclusion

In conclusion, plant breeding has been successful in developing high-yielding cultivars, but there is still untapped potential in utilizing genetic variation from wild relatives and land races of crops for trait improvement. Genomic databases have facilitated the identification of new genes and allele-specific markers for accessing key alleles conferring resistance to biotic and abiotic stresses, enhanced yield, and improved quality. Allele mining is a valuable tool for identifying naturally occurring variation and providing new germplasm for targeted trait improvement and conservation, but it faces challenges such as accessing and characterizing germplasm collections, identifying allelic variants, and assessing their effects on complex traits. Addressing these challenges is crucial for the efficient utilization of plant genetic resources and securing the future food supply.