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Progress in Functional and Gene-Targeted Markers for Plant Genomics and Breeding

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Abstract: -

Public genomic databases have started a shift in the kinds of PCR-based methods frequently employed in plant science and have given new directions for the development of molecular markers. Other techniques have been developed in addition to widely used arbitrarily amplified DNA markers. Techniques for targeted fingerprint markers are based on the proven techniques for randomly amplified DNA, but use cutting-edge methodological improvements such adding gene or promoter regions to the primers. Due to the concomitant occurrence of dominant and co-dominant bands, these markers offer good repeatability and improved resolution. These semi-random markers have potential collision and non-homology issues, similar to those identified with randomly produced fingerprints, despite their promising properties. Fingerprints can also be created using transposable elements, which are abundant in plant genomes. By using particular targeted sites, these markers boost genome coverage and generate bands that appear to be homologous. The main disadvantage of the majority of these methods is that primer construction requires genetic knowledge about retrotransposons, which prevents universal applications. Length polymorphism found in arrays of multi-copy gene families, such as cytochrome P450 and β-tubulins, is used by another class of recently developed technologies to enable cross-species amplification and transferability. A particular class of markers uses shared traits of plant resistance genes to display genetic variation or create bands associated with a certain phenotype. While resistance genes may be subject to particular evolutionary pressure, conserved DNA-based techniques have limited genome coverage and may fail to detect genetic variation. Using various gene-targeting techniques combined with the usage of RNA information, markers can also be created from functional and/or transcribed sections of the genome. These methods have the ability to provide phenotypically connected functional indicators, particularly when fingerprints are produced from the transcribed or expressed section of the genome. Larger datasets will undoubtedly be produced by these newly established methods, but their drawbacks should also be recognized and thoroughly examined.

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Introduction

Many intriguing novel alternative molecular marker techniques have been created in plant genetics in recent years, primarily as a result of rapidly expanding genomic research including a shift from random DNA markers to gene-targeted functional markers. The generation of functional markers, which are found in or near candidate genes of interest, has become very simple due to the widespread availability of various public genome databases. These markers are useful for identifying people or lines carrying certain genes, creating linkage maps, and studying genetic variability and diversity, for instance. They can be used to identify qualities that are challenging to detect using phenotypic assays, as well as to select and couple parental genotypes or reduce a linkage drag in back-crossing. In addition to its various uses in forensics, disease testing, and population assessment, molecular markers are also used in phylogenetics and systematics, conservation biology, molecular ecology, and developmental biology. A historical example may demonstrate the significance of a mark's unique characteristics. Sheep breeders in Brnowere debated the relationship between wool qualities (color, fitness, density, etc.) and how to successfully combine beneficial traits in offspring between 1816 and 1820. A Hungarian aristocrat from Keszthely (Georgikon), Imre (Emmerich) Festetics (1764-1847), participated actively in these conversations and conducted several crossover experiments. In a series of publications regarding inbreeding published between 1819 and 1822, he developed certain rules of heredity based on his findings and was the first to allude to such principles as "Genetic laws of Nature" (“Die genetische Gesæ der Natur”), a generation before Gregor Mendel. He coined the term "genetic" eight decades before Bateson and Johannsen. Unfortunately, the markers of choice were characteristics like wool density and length that are subject to polygene inheritance, and results like to Mendel's would have required exacting methodologies and reliable statistical approaches, like what is now known as quantitative trait loci (QTL) mapping. Festetics, however, simplified his findings into four "genetic laws," emphasizing that racial characteristics in sheep are innate and can be "concentrated" by inbreeding. Additionally, he connected inheritance (Vererbung) with health and vigor independent of outside influences and said that while animals with comparable features may produce different offspring, grandparents' traits may recur in subsequent generations. There is no proof that Mendel ever read or referenced Festetics' work, which was housed in the Brno library, despite the fact that the development of genetics was clearly

delayed. Fortunately, Mendel later decided to study the monogenic features (markers) in peas (Pisum L.), which enabled him to formulate the rules of heredity. An ideal marker should be polymorphic, autonomous, and trustworthy, offering adequate resolution in a rapid, easy, and reasonably priced manner. Numerous additional factors can potentially be significant, depending on the type of investigation. Understanding the relationship between a marker and a desired trait (phenotype) is crucial for plant breeding, but it has little bearing on genetic diversity or phylogenetic research. However, because tissue for phylogenetic studies is sometimes very limited, molecular approaches that require relatively modest amounts of DNA or organismal material considerably enhance these studies but in plant breeding projects, where a lot of fresh plant material is nearly always available, this is mostly irrelevant.

Arbitrarily amplified DNA markers (AADs)

It's important to talk about some of the early PCR-based techniques before discussing more current advancements. Banding patterns are the result of amplification from several priming sites since this collection of techniques uses genetic markers that are found at many locations throughout the genome. They can be helpful in resolving a variety of issues that might be challenging to handle with single-locus techniques, such as those related to introgression and hybridization studies, by sampling several loci at once. The lack of a priori sequence information from the examined organism is the main benefit of technologies based on randomly amplified markers. The majority of dominant markers are produced by sampling many loci throughout the entire genome. These techniques provide a comparatively high number of markers per sample, are somewhat inexpensive, and are technically straightforward. Numerous well-known multi-locus strategies include inter-sample sequence repeats (ISSR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and a few other methods that involve certain adaptations of these. They have numerous uses and are still in use. They are collectively known as arbitrarily amplified DNA markers (AADs; omitting single-locus methods like microsatellites or SSRs). AADs have been used in thousands of plant science investigations over the past 20 years for a variety of reasons. To get a general idea of the number of studies that have used AAD markers, we conducted an informal Google Scholar search and compared the results to those for the other marker types discussed here. Although the percentages shown in our pie chart should be read cautiously, it appears that AADs are still widely used methods.

Gene-targeted and functional markers (GTMs and FMs)

Anonymous dominant markers (AADs) and functional or gene-targeted markers differ primarily in how they are produced. Any DNA segment exhibiting polymorphism can be used to create a molecular marker, which is then tagged with a primer of varying length. However, a straightforward recombination can frequently invalidate the usefulness of such neutral markers, restricting the application of arbitrarily amplified DNA markers. To put it another way, non-targeted amplicons can be found in either the transcribed or non-transcribed regions of the genome; their purpose is unknown. In a number of plant species, including potatoes (*Solanum tuberosum* L., soybeans (*Glycine max* (L.) Merr., ryegrass (*Lolium perenne* L.), and maize (*Zea mays* L.), structural and functional genomic research projects have produced additional information that enables the systematic development of targeted markers derived from polymorphic sites within genes that affect phenotypic trait variation. Differentiating between gene-targeted markers (GTM) and functional markers (FM) is crucial since not all GTM are engaged in phenotypic trait variation and may therefore not become functional. Untranslated portions of expressed sequence tags can likewise be tagged by gene-targeted markers. Functional markers are formed from polymorphic sequences and are more likely to be involved in phenotypic trait variation, according to the concept put forward by Andersen and Lýberstedt. The marker systems covered below are all (gene)-targeted markers with the potential to become functional based on this conceptual framework. Numerous novel marker systems of this kind have been created recently.

Benefits and drawbacks of conserved DNA-based indicators

Selection criteria for the use of molecular markers include repeatability, cost-effectiveness, speed and convenience of information processing, and the amount and kind of genetic data that will be acquired. These requirements are certainly met by AAD markers, however as was previously said, there are a number of issues with their use. According to certain research, depending on the method used, amplification of AADs from the genome may be biased. Certain AADs, like AFLPs, are known to frequently cluster in the centromeric regions of plants. Additionally, it has been shown that some clusters are limited to specific chromosomal regions because of the enrichment of AFLP markers in those regions, which in a number of species suggests recombination suppression. The acquired fragments exhibit variability in a variety of plant species, according to the results of research based on conserved DNA and gene

family related markers. This suggests that these markers could be helpful tools for assessing genetic diversity within or within populations.

Markers based on resistance genes (RGMs)

Resistance-gene markers constitute a distinct category of gene-targeted markers, since they leverage specific characteristics of genes associated with plant defense systems. Prior to examining the specifics of these indicators, it is essential to succinctly outline certain prevalent characteristics of plant disease resistance. Plants have developed both active and passive defensive systems to safeguard themselves from diseases. Active pathways include adaptive and innate immune responses. Adaptive immunity is predicated on the RNA interference mechanism and mostly operates against viral pathogens. Innate immunity is broad and allows the plant to protect itself against a wide array of diseases through pathogen and pattern recognition receptors (PPRs) and resistance proteins (R proteins). PPRs identify microbe or pathogen-associated molecular patterns that are conserved among pathogens of a specific class. R proteins, in turn, identify distinct avirulence (Avr) factors that are not conserved across pathogens. R protein-mediated signaling results in the generation of reactive oxygen species and triggers a specific form of programmed cell death known as the hypersensitive response, which eliminates the afflicted cells. Recent study suggests that cell death does not inhibit pathogen dissemination; rather, its propagation is hindered in the adjacent viable tissue by an unidentified mechanism. R-protein mediated innate immunity is referred to as gene-to-gene resistance, wherein each R gene corresponds to a unique pathogenic Avr gene.

Therefore, it is anticipated that several R genes within each plant genome can provide resistance to a wide array of diseases. Furthermore, R genes are subject to diversifying selection to adapt to the fast evolution of pathogens. Despite varying responses to distinct pathogens, different R genes possess some conserved sections (domains). According to these domains, R proteins can be categorized into four subclasses. The majority of R proteins possess a central nucleotide binding site (NBS) that functions as a molecular switch to regulate the protein's activation status, along with a C-terminal leucine-rich repeat domain (LRR) essential for Avr factor recognition. Consequently, the classification of R proteins is predicated on variations in the N-terminal domain. NBS-LRR type R proteins featuring N-terminals exhibit homology with Drosophila Toll and human Interleukin receptors, and they are together categorized as TIR-NB-LRR proteins. Non-TIR NBS-LRR proteins are

designated as CC-NBS-LRR proteins due to the presence of a coiled coil (CC) domain in their N terminus. Furthermore, there exist two categories of R proteins that possess an extracellular LRR at their N terminus. One of these types, known as receptor-like kinases (RLKs), possesses a cytoplasmic protein kinase domain. Receptor-like proteins (RLPs), in contrast, do not possess this cytoplasmic protein kinase domain. R genes from many plant species include common domains, enabling their utilization for screening plant genomes for R genes and suspected R genes (e.g., resistance gene analogs, RGAs), as well as for the development of genetic markers.

Conclusion

The number of research employing these sophisticated techniques is growing, even though the application of some recently discovered marker techniques in plant science is not yet as widespread as that of well-established methods like AADs. This could be explained by the possibility for these marketing platforms to offer additional information sources. Even if they have been proved to be more effective than these conventional methods, certain recently created techniques can be considered underutilized resources for researchers, and none have gained the same level of popularity as RAPD or AFLP. Significant attempts have been made to create new and more effective markers for important agricultural plants (such as potatoes, rice, and maize), but relatively little study has been done on creating markers for underutilized crops. In other scientific domains, such as molecular ecology and phylogenetics, where the organisms of interest lack economic significance and there is no prior sequence or genome information available for primary design, some marker approaches are still unavailable. A key drawback of certain recently established techniques is the necessity for preliminary genetic data, which occasionally necessitates additional and time-consuming laboratory work. The development of gene-targeted markers will be less expensive as DNA sequencing prices decrease with the advent of high throughput techniques. The growing number of research based on newly created marker systems indicates that these methods may be helpful for a variety of applications. Furthermore, compared to AADs, which are mostly based on unknown and occasionally vast genomic rearrangements, these approaches appear to be more specific. In some areas of plant biology where they haven't been employed yet, it may be anticipated that the majority of the techniques covered here could offer more organized data sets that could be used either alone or in combination with sequence level characters.

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