Transcriptomics: An Emerging Tool (Approach) For Plant Disease Management

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Abstract

Transcriptomics, a field within molecular biology, focuses on the examination of messenger RNA molecules. This approach enables high-throughput analysis of gene expression. The origins of transcriptomics date back to the early 1990s, and its significance has grown substantially since then. Transcriptomics encompasses a range of methods that collectively capture all the alterations in transcription within both plant and pathogen transcriptomes during their interaction in plant-pathogen relationships.

Introduction

Transcriptomics, a subfield of molecular biology, is concerned with the investigation of mRNA molecules generated in an individual or a population of specific cell types. The term "transcriptome" was initially coined and introduced by Charles Auffray in 1996. The transcriptome encompasses the entirety of RNA molecules, encompassing mRNA, rRNA, tRNA, and various non-coding RNAs, synthesized within an individual cell or a group of cells. Before the advent of transcriptomics, studies primarily focused on individual transcripts, and this practice predates the availability of transcriptomics approaches by several decades. In those earlier studies, transcript quantification involved aligning fragments with known genes. Nevertheless, these methods have largely been surpassed by high-throughput sequencing techniques that analyze complete transcripts, thereby providing additional insights into transcript structure. Currently, six key techniques are present viz., differential displayed-reverse transcriptase PCR (DDRT-PCR), cDNA-amplified fragment length polymorphism (cDNA-AFLP), Subtractive Suppression Hybridization (SSH), DNA microarrays, serial analysis of gene expression (SAGE), and RNA sequencing (RNA-Seq) (Chakraborty and Jolly, 2017).

Techniques of Transcriptomics:

Differential Displayed Reverse Transcriptase-PCR (DDRT-PCR)

DDRT-PCR is a method employed for the detection of cDNAs that exhibit differential expression between two or more samples. It relies on a crucial approach that involves the use of a pair of oligonucleotide primers. One of these primers is anchored to the poly-A tail of mRNAs, while the other is of a shorter length and possesses an arbitrary sequence, allowing it to attach at various positions relative to the first primer. This technique proves particularly valuable in the context of stress conditions, as it can effectively pinpoint a significant number of genes that exhibit differential expression (Liang and Pardee, 1992).

cDNA Amplified Fragment Length Polymorphism (cDNA-AFLP)

AFLP, a PCR-based approach, is utilized to visualize variations in genomic DNA within a genome. cDNA-AFLP is an extension of this method, designed to assess gene expression levels by amplifying reverse transcribed cDNAs, rather than identifying simple DNA polymorphisms (Vos et al., 1995). The underlying principle involves two rounds of restriction enzyme digestion, each targeting distinct recognition sites. Adaptors are subsequently linked to the sticky ends resulting from the restriction digestion, with the adaptor sequences serving as binding sites for primers in the subsequent PCR amplification. This technique employs a combination of short oligonucleotide primers, with selective nucleotides added to the 3' ends of the primers, extending into the restriction fragments. Consequently, only those restriction fragments with nucleotide sequences matching the selective nucleotide sequences of the primers will undergo amplification. These subsets of amplified fragments are then subjected to denaturing polyacrylamide gel electrophoresis (PAGE) to visualize the fragments derived from transcripts.

Suppression Subtractive Hybridization (SSH)

The SSH technique involves the comparison of two mRNA populations and the generation of gene clones that

exhibit either overexpression or exclusive expression in one population but not in the other. SSH combines the precision of subtractive hybridization with the sensitivity of PCR. It also facilitates the creation of subtracted cDNA libraries and is grounded in a process that involves hybridization and the suppression of PCR, incorporating normalization and subtraction in a single operation (Diatchenko et al., 1996). In brief, this method categorizes cDNAs containing differentially expressed transcripts as "tester," while the normal cDNAs are referred to as "driver." These tester and driver cDNAs are first hybridized, and the sequences formed through this hybridization are subsequently eliminated. As a result, the remaining unhybridized cDNAs represent genes expressed in the tester population but absent from the driver mRNA pool. These target genes can be further enriched through subsequent PCR.

DNA Microarray

DNA Microarray is a hybridization technique in which cDNAs derived from unknown samples are subjected to hybridization with known complementary DNA probes fixed on a slide or array. This process allows for the simultaneous measurement of the expression levels of numerous genes in a single experiment. These arrays consist of short nucleotide oligomers, referred to as probes, which are meticulously arranged on a solid substrate. The abundance of transcripts is ascertained by the hybridization of fluorescently labeled transcripts to these probes. The fluorescence intensity observed at each probe location on the array serves as an indicator of the transcript abundance for that specific probe sequence. In the realm of transcriptomics, microarrays typically fall into one of two broad categories: low-density spotted arrays and high-density short probe arrays (Boonham et al., 2007).

Serial Analyses of Gene Expression (SAGE)

SAGE, originally an advancement of the EST technique, was designed to enhance the tag generation throughput and enable some level of quantification for transcript abundance (Velculescu et al., 1997). In its fundamental process, mRNA is first isolated and then converted into double-stranded cDNA, which is subsequently cleaved using a specific restriction enzyme (known as the anchoring enzyme). The resulting cleaved cDNAs are divided into two equal portions, each of which is ligated to distinct linker fragments containing primer binding sites. These linker fragments feature recognition sequences for a type IIS restriction endonuclease, responsible for cleaving the cDNAs. The two sets of cDNAs, now equipped with linkers, are then ligated together to create ditags, featuring linkers at both ends. Subsequent PCR amplification is carried out on these ditags. They are later concatenated, cloned, and subjected to sequencing. The quantification of expression profiles is achieved by tallying the tags, identifying those originating from the same RNA molecule, and distinguishing between tags associated with well-studied, known genes and those representing novel transcripts (Cerutti et al., 2003).

Expressed sequence tag (EST)

EST is a distinctive, unaltered, concise, single sequence that originates from either the 5' or 3' end of randomly selected cDNA libraries in specific cells, tissues, or organs of interest. It represents a segment of DNA sequence that encodes solely the coding region of a gene, and typically ranges from 200 to 700 base pairs in length. These arbitrarily selected cDNA clones are subsequently subjected to sequencing, offering a means to explore the collection of transcribed sequences within that tissue, particularly under varying experimental conditions. The initial step involves converting RNA into cDNA through the action of a reverse transcriptase enzyme before sequencing the resulting cDNA (Marra et al., 1998). Until the emergence of high-throughput techniques like sequencing by synthesis, the Sanger method of sequencing was the primary approach. When coupled with diverse bioinformatic analyses, this method proves instrumental in the discovery of novel genes.

RNA-Sequencing (RNA-Seq)

In the mid-2000s, RNA-Seq was first introduced concurrently with the emergence of next-generation sequencing technology. RNA-Seq represents the amalgamation of high-throughput sequencing techniques with computational approaches to capture and quantify the transcripts found within an RNA sample (Ozsolak

and Milos, 2011). It is a sequencing method that leverages next-generation sequencing (NGS) to unveil both the presence and abundance of RNA molecules in a biological sample at a specific moment, providing insights into the dynamic and ever-changing cellular transcriptome. Precisely, RNA-Seq enables the examination of various aspects, including alternative splicing of gene transcripts, post-transcriptional modifications, gene fusion, mutations/Single Nucleotide Polymorphisms (SNPs), and alterations in gene expression across time or in response to different conditions or treatments. Beyond just mRNA transcripts, RNA-Seq extends its scope to encompass various RNA populations, encompassing total RNA, small RNA categories like miRNA, tRNA, and ribosomal profiling.

Conclusion

Transcriptomics stands as a well-established framework for unraveling the intricacies of plant-pathogen interactions. Through transcriptome profiling, we gain valuable insights into the crucial biochemical and signaling pathways that undergo manipulation when these interactions occur within the host plant. This abundance of data equips us with the tools needed to develop more efficient strategies for managing these interactions effectively. Furthermore, there's a pressing demand for enhanced analytical systems and more efficient methods for comparing large-scale data. This evolution in our understanding of genome-wide expression during plant-pathogen interactions is instrumental in shaping the effective management of plant diseases.

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