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Exploring the Transcriptomic Landscape: Revolutionizing Plant Disease Management

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Abstract

Plant diseases are one of the most significant constrains to global agriculture production. Detecting plant pathogens are responsible for causing diseases early and keeping a close eye on plant health are critical for limiting disease development and dissemination and applying appropriate management measures successfully. While plant pathogens can be identified morphologically, use of serological and nucleic-acid based techniques can increase the accuracy and specificity including the pathogens that lack visible morphological structures. Since the development of protein and nucleic-acid based diagnostics, pathogen identification has been revolutionized and plant diseases diagnosis has substantially been improved.

Introduction

Transcriptomics, a field within molecular biology, focuses on the examination of messenger RNA molecules. This approach enables high-throughput analysis of gene expression. The origin 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 (Mitra., 2021).

Getting into the transcriptome that 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 (Chakraborty and Jolly, 2017).

Sample preparation and assembly for RNA-seq

The total RNA from biological samples is isolated using kits or manually (TRIzol) protocols m-RNA was extracted from total RNA "Experiments should be employed in triplicate form." The enriched mRNA was fragmented and converted into first-strand cDNA, which was then followed by second-strand generation, A-tailing, adapter ligation, and a limited number of PCR amplifications of the adaptor-ligated libraries. The amplified libraries were analyzed on the Bio-analyzer according to the instructions of the manufacturer. Quantification and qualification of the library were performed using any sensitivity kit by the Nanodrop spectrophotometer. NGS for cDNA of all plants was performed by library on any sequencing platform according to samples. The raw reads were first filtered to exclude the reads containing adaptors or with ambiguous nucleotides ('N'). Below than 20% $Q < 20$ nucleotide bases were also trimmed. The obtained high-quality (HQ) clean reads were used to construct a sequence assembly using Trinity or any other software (Agho *et al.*, 2010).

Current status and future prospects of Transcriptomics for identifying disease resistant genes and plant disease management

A comprehensive transcriptional analysis performed using RNA-seq from plant leaf tissues to gain insight into the mechanisms activated for the defense after infection. Pathogen infection in resistant cultivar resulted in about 5927 differentially expressed genes (DEGs) compared to 1161 DEGs in the susceptible cultivar. The expression levels of genes

related to plant disease resistance such as serine/threonine kinase activity, signal transduction, plant-pathogen interaction, endocytosis, autophagy, mitogen-activated protein kinase (MAPK), and others were significantly enriched in the upregulated DEGs in resistant cultivar, whereas in the susceptible cultivar, only the pathway related to phenylpropanoid biosynthesis was enriched in the upregulated DEGs (Agho *et al.,* 2010).

Unravelling of molecular mechanism of plant pathogen interaction in post genomic area by Transcriptomics

Genes related to plant defense responses were activated and the transcriptome differences between the resistant ZQK9 and susceptible E31 at 0-, 3-, and 5-days post- inoculation (dpi) were identified by RNA-seq which was reflected by a lot of up-regulated DEGs involved in pathogenesis-related (PR) genes, hormones biosynthesis and signal transduction, secondary metabolites biosynthesis and cell wall modification in resistant ZQK9. The dataset provided a basis for identifying candidate resistant genes in melon against *P. capsici* and lay a foundation for further research on the molecular mechanisms. (Wang *et al.,* 2020).

Differential display of eukaryotic messenger RNA by means of Polymerase chain reaction

Effective methods are needed to identify and isolate those genes that are differentially expressed in various cells or under altered conditions. This report describes a method to separate and clone individual messenger RNAs (mRNAs) by means of the polymerase chain reaction. The key element is to use a set of oligonucleotide primers, one being anchored to the polyadenylate tail of a subset of mRNAs, the other being short and arbitrary in sequence so that it anneals at different positions relative to the first primer.

The mRNA subpopulations defined by these primer pairs were amplified after reverse transcription and resolved on a DNA sequencing gel. When multiple primer sets were used, reproducible patterns of amplified complementary DNA fragments were obtained that showed strong dependence on sequence specificity of either primer.

The first time-series transcriptome of *S. betaceum* with a comprehensive expression profile across infection caused by *P. betacei* provided a close interaction between the host transcriptional response and the hemibiotrophic infection strategy of the pathogen, exhibiting a dynamic defense-related gene response throughout the course of infection. The different upregulated genes, related to susceptibility and resistance, that elucidate the continuous response in this compatible interaction: from the recognition of the pathogen and the activation of defense related pathways to the final stages of infection with the expression of genes associated with cell death. T he nature of this interaction as ETS, with a reprogramming of the host transcription caused by the pathogen for essential resources to aid in its colonization (Bautista *et al.,* 2021).

Applications of CRISPR-CAS in agriculture Transcriptomic lineage

The prokaryote-derived CRISPR–Cas genome editing technology has altered plant molecular biology beyond all expectations. Characterized by robustness and high target specificity and programmability, CRISPR–Cas allows precise genetic manipulation of crop species, which provides the opportunity to create germplasms with beneficial traits and to develop novel, more sustainable agricultural systems. Furthermore, the numerous emerging biotechnologies based on CRISPR–Cas platforms have expanded the toolbox of fundamental research and plant synthetic biology. In this Review, we first briefly describe gene editing by CRISPR–Cas, focusing on the newest, precise gene editing technologies such as base editing and prime editing. The most important applications of CRISPR–Cas in increasing plant yield, quality, disease resistance and herbicide resistance, breeding and accelerated domestication.

Conclusion

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.

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