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SHORT COMMUNICATION

How do we know that plants listen: Advancements and limitations of transcriptomic profiling for the identification of sound-specific biomarkers in tomato

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ABSTRACT

Sound vibration has been recently identified as an important physical trigger to elicit plant responses. Naturally occurring sound waves modulate diverse aspects of plant physiology, such as root growth, stress responses, and seed germination. However, it has been debated whether plants perceive artificially generated sound vibration and exhibit similar phenotypic changes to those exhibited after perception of natural sound waves. Recently, analysis of RNA-Seq and microRNA-Seq using tomato fruits treated with optimized sound waves to attenuate fruit ripening revealed sound-specific microRNAs, which could be used as sound-specific biomarkers in tomato. These data provide solid molecular evidence of sound perception in plants. Despite these results, there are obvious limitations of biomarkers' specificity and selectivity that need be addressed to facilitate the application of sound treatment in agriculture. Here, the pros and cons of sequencing technologies used to identify sound-associated molecules suggest recommendations for the effective identification of biomarkers responsive to sound treatment in plants.

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Tomato; sound vibration; ripening; ethylene; miRNA; transcriptome; biomarker

Introduction

Plants are sensitive to external stimuli such as chemical triggers and sound waves. Naturally occurring or artificially created sound waves elicit various responses in plants such as plant growth, defense response, abiotic stress tolerance, photosynthesis, and delayed ripening.[1] However, it has been debated whether artificially generated specific sound vibration triggers similar phenotypic changes in plants as naturally occurring sound waves. Moreover, there are no mechanistic genomics- or transcriptomics-based insights into the effect of sound treatment on plants. Recently, analysis of RNA-Seq, one of the recent high-throughput sequencing technologies, employed an investigation of transcriptomic alterations in tomato (*Solanum lycopersicum*) associated with sound vibration[2]. Sound wave (1 kHz) treatment delayed ripening in tomato, and this coincided with the down-regulation of ethylene and cytokinin biosynthesis and signaling genes and up-regulation of genes involved in flavonoid, phenylpropanoid, and glucan biosynthesis. Additionally, two sound-specific microRNAs (miRNAs) were identified. Their transcriptomic activities validated by estimating the expression profiles of their pre-miRNAs and target mRNAs,[2] with the aim of utilizing these miRNAs for measuring the effects of sound treatment on plants in agricultural fields.

Applications of high-throughput sequencing have gradually broadened, allowing for rapid advances in many fields associated with the biological sciences. Notably, gene expression studies using RNA-Seq have begun to replace the use of traditional microarray analysis, providing researchers with the ability to visualize RNA expression in terms of nucleotide sequence. RNA-Seq is an experimental protocol that sequences mRNA molecules within a biological sample and determines the relative abundance of each mRNA molecule.[3] With the growing popularity of high-throughput sequencing technologies such as RNA-Seq, additional innovative applications in plant science are inevitable. However, despite the advances in sequencing technologies, there are critical limitations that need to be resolved to enable the practical application of sound treatment in agricultural fields; for example, poor integrity of the reference genome sequence, insufficient informative data on gene-to-gene interactions across various plant species, and insufficient reports on epigenetic alterations associated with sound treatment. The investigation of sound-induced delay in tomato fruit ripening conferred the pros and cons of sequencing technologies for identifying sound associated molecules, and provide recommendations for the effective identification of sound related biomarkers in plants.[2]

Identification of optimal sound treatment environments

Previously, sound treatment has been recognized as a means to improve crop health and quality. In Asian countries such as China and Korea, researchers and farmers employ sound, referred to as "Green Music", for crop fitness.[4] However, the output of such approaches is inconsistent and varies with the scientist's skills and experimental conditions. Inconsistent sound effects may also be caused by the lack of optimization

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of sound quality. To identify the underlying problem, plant biomarkers responsive to specific sound frequency (hertz; Hz) and volume (decibel; dB) are required. Therefore, sophisticated and efficient screening methodology beyond the classical screening procedure is needed. Next generation sequencing (NGS) technologies, such as RNA-Seq, enable the identification of plant biomarker(s) responsive to specific sound frequency (Hz). These sound-specific biomarkers in plants could help fine-tune the sound quality for more efficient plant responses.

RNA-Seq analysis of transcripts associated with sound treatment

Using 1 kHz sound wave treatment, the ripening delay in tomato successfully confirmed. Based on these phenotypic changes, a genome-wide survey was performed to identify biomarker(s) responsive to sound wave treatment.[2] Among the NGS technologies, RNA-Seq was considered as the first approach to explore transcriptional changes and obtain mechanistic insights into the changes associated with sound vibration. The significant alterations in the expression of genes involved in ethylene, cytokinin, flavonoid, phenylpropanoid, and glucan biosynthesis, with the sound-specific upregulation of two miRNAs, *pre-miR6026* and *pre-miR6024* were detected.[2] Although sound associated transcripts and their biological functions had identified, it could not identify robust biomarkers for estimating the effectiveness of sound treatment. Sound is an external stimulus, which is a limitation. Therefore, additional approaches, such as epigenomics, may be needed to identify effective biomarkers of sound associated molecular changes.

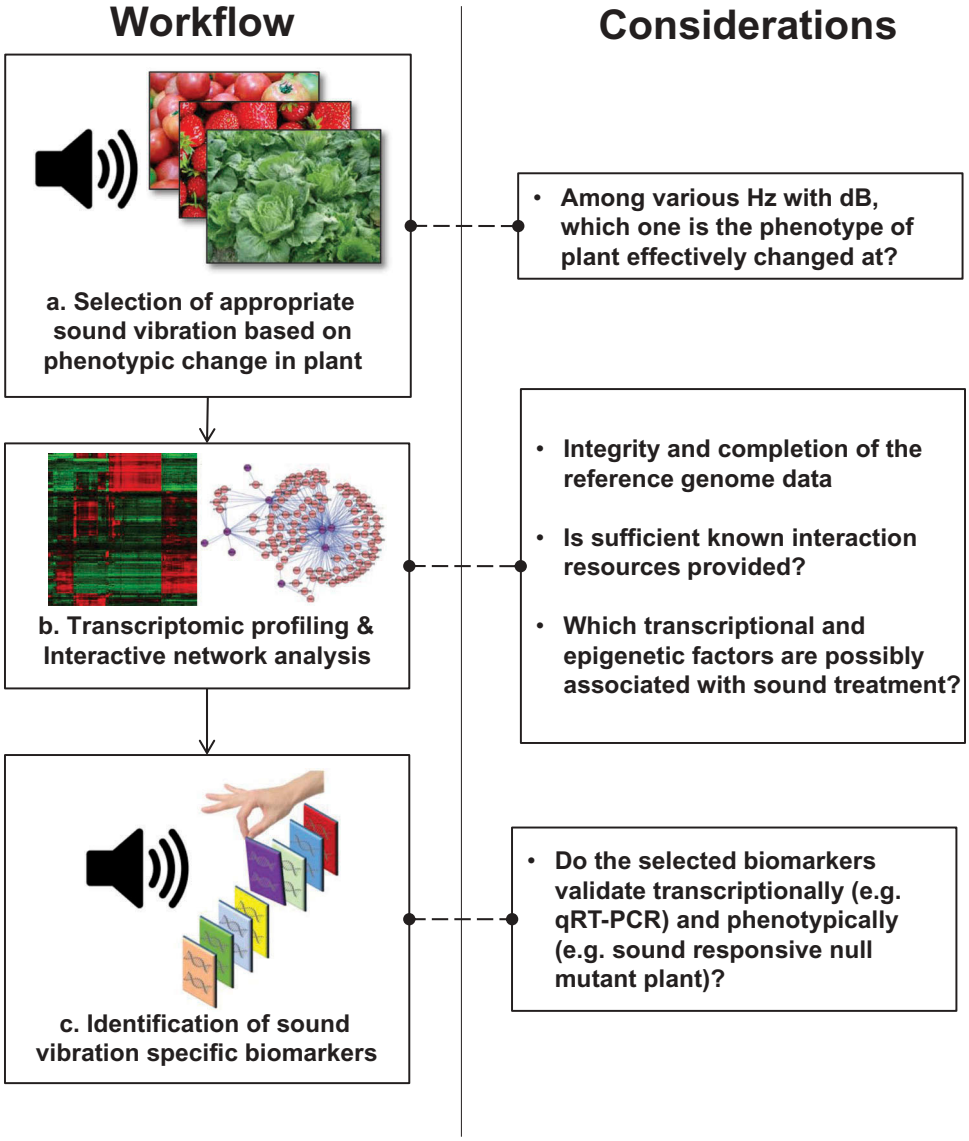

Figure 1. Schematic representation of the workflow and considerations for the selection of sound-specific biomarkers. (a) Selection of appropriate sound vibration based on phenotypic changes in plants for further experimentation. At this stage, the most effective hertz (Hz) and decibel (dB) of sound are selected corresponding to phenotypic changes in plants. (b) Transcriptomic profiling and Interactive network analysis. At this stage, transcriptome and interactive network analyses are conducted to identify sound-specific biomarkers in plants.

Incomplete reference genome sequence of plant species

The complete genome sequence of tomato is available in the genome database of the National Center for Biotechnology Information (NCBI), and the sequence data have been updated recently under the assembly version SL3.0. The tomato genome sequence is annotated as comprising a total of 44,058 transcripts. When explored the genes associated with sound vibration in tomato, approximately 3,500 transcripts were identified as significantly differentially expressed in response to sound treatment. Among these, only ~400 transcripts were curated; the RNA accession numbers of these transcripts started with "NM_". The remaining ~3,100 transcripts were annotated as predicted, and their RNA accession numbers started with "XM_". When searching the annotations of all 44,058 transcripts in tomato, only 2,586 transcripts were curated, and the vast majority of the remaining transcripts were predicted or not curated. Insufficient data resources limit the potential of genome-wide screening and narrow down the selection of practical biomarkers. In addition, insufficient data on known interactions in the tomato genome should be considered. According to the BioGrid resource,[5] which provides interactive data across various species, only 141 interactions between mRNA molecules have been reported in tomato. Small interaction data sets, such as that available for the tomato genome, may limit the assessment of key signaling pathways or the identification of gene-to-gene networks associated with sound treatment. Therefore, as an alternative approach, exploring and selecting molecules affected by sound in a model plant, such as *Arabidopsis thaliana*, and an extended investigation of homologous molecules in various plant species are needed.

Identification of epigenetic factors associated with sound treatment

Since sound is an external stimulus, it was hypothized that plants may contain key epigenetic markers responsive to sound treatment similar to plant respond to chemical stimuli such as microbial metabolites and proteins. Many histone modification markers have been reported in plants, some of which have a considerable impact on gene expression. Among these, H3K36ac, H3K4me3, and H3K36me are active histone markers, whereas H3K27me3 is an inactive histone marker; these histone modifications may be good candidates for epigenetic markers associated with sound wave treatment.[6,7] Although gene expression alterations due to sound vibration have already confirmed, identifying key epigenetic factors, especially histone proteins, that regulate gene expression is greatly needed.

Conclusion

In summary, recent data suggest a brief outline for selecting effective biomarkers responsive to sound (Figure 1). Transcriptome analysis is a methodology that can be used to understand responses of plant to sound. In this article, the advancements and limitations of transcriptomic profiling was described for the identification of biomarkers of plant responses to sound. Taking into account the pros and cons of sequencing technologies, future study will provide a number of recommendations for effective sound biomarker identification in plants.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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