

Dual Transcriptomics in Plant-Insect Interactions: Decoding the Molecular Battle Between Crops and Insect Pests

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ABSTRACT

Insect pests pose a threat to crop production because they cause damage to plant tissues, decrease yield, and quickly adapt to host defenses. Plants respond by signaling with hormones, producing secondary metabolites, strengthening cell walls, and other defense mechanisms, and insects respond with salivary effectors, detoxification enzymes, digestive proteins, and regulatory molecules. Dual transcriptomics, also known as dual RNA sequencing, is the study of the gene expression of the crop and the insect at the same feeding interaction. This two-sided approach allows researchers to gain insight into the simultaneous occurrence of plant defense and pest adaptation in real time. Using bioinformatics tools, the mixed RNA data can be split into plant and insect reads, which can be used to identify defense genes, pest vulnerability genes and important molecular pathways. This information can aid in resistant crop breeding, RNA interference control, and more targeted pest management.

Keywords: Dual RNA sequencing; Plant–insect molecular interaction; Transcriptomic profiling; Insect detoxification genes; Precision pest management; Bioinformatics

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Introduction

Crop plants and insect pests are one of the most dynamic biological conflicts in agriculture. Plants are not just food sources, but can sense insect feeding and respond with physical, chemical and molecular defenses. In turn, insect pests have developed several counterstrategies, such as salivary effectors, detoxification enzymes, digestive proteins and regulatory molecules that enable them to cope with plant defenses. This ongoing battle is sometimes referred to as a molecular arms race in which the crop tries to defend itself and the insect tries to overcome the defense [9].

This relationship has been examined for many years by entomologists and plant pathologists only from one point of view. Some studies were directed at the expression of plant genes after insect attack, and others were directed at the genes of the insects that are involved in feeding, detoxification or adaptation. Such single-organism transcriptomic studies have proven useful for the understanding of general plant defense responses to arthropod herbivorous [5]. But they cannot fully reflect on the two-way molecular communication during herbivory.

To get a more integrated view, dual transcriptomics – or dual RNA sequencing – examines the gene expression of both the plant and insect simultaneously. This facilitates the study of the coordinated changes in crop defense pathways and insect adaptive responses during crop infestation. In a recent dual RNA-seq study of wheat and the English grain aphid, *Sitobion avenae*, the simultaneous transcriptomic analysis of both the crop plant and the insect pest showed how dynamic molecular warfare can be uncovered between the two [1]. Hence, dual transcriptomics is becoming a valuable resource to understanding plant–insect interactions and to aid future sustainable pest management strategies.

What is Dual Transcriptomics?

Transcriptomics involves analysis of all the RNA transcripts generated from genes in each cell, tissue or organism under a given set of conditions. It reveals which genes are turned on, turned down, or turned up in some biological process. Transcriptomics is useful in studies of plant–insect interactions, where it can be used to investigate the response of the crop plant to insect attack and the adjustment of the insect pest's gene expression when it feeds on the plant.

Dual transcriptomics or dual RNA sequencing is the simultaneous investigation of the expression of genes in two interacting organisms. It enables scientists to study both the plant defense mechanism and the insect adaptation mechanism in plant–insect interaction in a single feeding event. This is unlike conventional transcriptomic studies, in which either the plant or the insect alone is typically studied.

Researchers can obtain RNA from the infested plant tissue, the feeding insects or both, depending on the type of experiment being conducted (dual transcriptomics). The goal is to record the changes in gene-expression of both partners during the interaction. Plant-derived reads show the response of the plant to defend itself; insect-derived reads show the genes needed for feeding, detoxification, salivary effectors and adaptation. Bioinformatics software like HISAT2 and STAR are used to align RNA reads with the

correct reference genome and DESeq2 is used to identify changes in gene-expression [2].

In the case of wheat, they studied wheat and English grain aphid (*S. avenae*) transcriptomes 6, 24, 48, and 72 hours after aphid infestation. This time-series approach led to the identification of changes in the wheat defense pathways and aphid genes associated with the production of secretory proteins during feeding [1].

Thus, dual transcriptomics can offer a more comprehensive view of plant–insect interactions and can be used to assist in the development of resistant crops, the discovery of functional genes, and to enable targeted pest management.

Dual Transcriptomics in Plant-Insect Interactions

Decoding the molecular battle between crops and insect pests

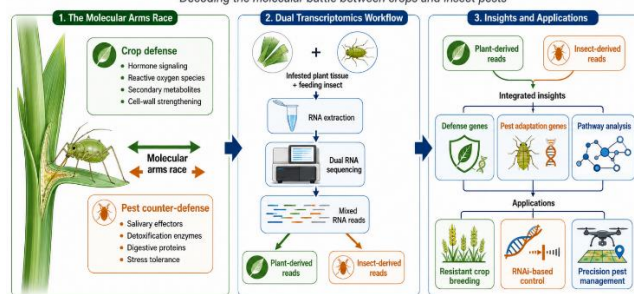


Fig. 1: Dual transcriptomics reveal the molecular arms race between crops and insect pests.

Plant Defense: The Crop Side of the Battle

Crop plants are not passive victims when attacked by insects. They cannot avoid herbivores, but they can detect the damage caused by herbivores and trigger a complicated defense mechanism. Initial cues can be from damaged cells, insect saliva, oral secretions or herbivore associated molecules. These early warning signals result in internal changes, such as calcium movement, reactive oxygen species, protein kinase activity, and defense related hormone pathways [5, 9].

The type of feeding by the insect is crucial to the plant response. Chewing insects also typically cause obvious damage to the tissues and are known to trigger production of compounds containing jasmonic acid such as proteinase inhibitors, toxic secondary metabolites, cell wall strengthening, and compounds that interfere with insect digestion. Phloem-feeding insects like aphids and whiteflies, on the other hand, feed on sap and cause less obvious damage by inserting their stylets into the plant tissues. They activate a different combination of the feeding-induced responses of jasmonic acid, salicylic acid, oxidative stress and vascular defense [4, 10]. These defense responses are now more easily observed on the level of gene-expression by transcriptomic studies. The temporal transcriptomic profile in sugarcane aphid-infested sorghum revealed that defense is not a static process, as the stress-response, metabolic and defense-related genes varied over time after infestation. This is because sorghum can modify its

molecular response with ongoing aphid pressure [3]. Transcriptomic analysis of the resistant variety Qingliu revealed that the attack of leaf folder resulted in more changes in leaf defense, while the attack of brown planthopper resulted in more changes in sheath tissues in rice. The study correlated resistance with jasmonic acid and salicylic acid signaling phenylpropanoid metabolism, cell wall modification and cuticular wax formation [4].

A transcriptomic meta-analysis also revealed that plant responses to arthropod herbivores include early recognition, signaling genes and transcription factors. These signals correlated to jasmonate signaling, glucosinolate biosynthesis, terpenoid production and other secondary metabolic pathways in response to common herbivores like cabbage butterfly larvae and spider mites [5].

Plant transcriptomics can be used to decipher the crop side of the equation and identify defense genes that can be used in breeding resistant crop varieties and minimize reliance on insecticides.

Insect Response: The Pest Side of the Battle

Insect pests are not just feeding machines; they also react to plant defense. They can alter the expression of genes associated with digestion, detoxification, stress tolerance, nutrient transport and saliva production during feeding. These responses enable insects to persist, survive defense chemicals and adjust to resistant crop tissues.

Saliva is one of the most important weapons in this counter-response. Piercing-sucking insects like aphids and whiteflies inject saliva during probing and feeding on plant tissues. A subset of the salivary molecules is known to be detected by the plant and trigger defense responses, while others are known as effectors as they suppress plant defense or alter host physiology [9,10]. A dual transcriptomic and proteomic analysis of the pea aphid salivary secretome led to the prediction of several effector molecules, indicating that the salivary glands are a major source of pest counter-defense molecules [6].

Saliva is not the only indication of insect adaptation. For example, the whitefly is an successful phloem feeder, with molecular mechanisms that allow it to cope with the nutritional and defensive problems of plant sap [10]. Recent studies also indicate that insect salivary microRNAs can be transferred to plant tissues and act as inhibitors of plant immunity by cross-kingdom RNA interference, thus the pest can interfere with the regulation of plant genes when attacking the plant [7].

This pest side is revealed by multi-omics studies. Combination of transcriptomic and proteomic analyses has been used to correlate candidate effector genes and proteins with virulence and avirulence phenotypes in aphids, to explain how some insect populations are able to overcome certain host resistance responses [8]. Likewise, dual RNA-seq of the wheat-English grain aphid system identified aphid responses and wheat responses at various infestation times and identified aphid genes associated with secretory proteins [1].

Knowing the insect side of the equation is crucial because these pest genes could be future targets for RNA interference, resistance monitoring and/or species-specific pest management.

Bioinformatics Workflow of Dual Transcriptomics

Dual transcriptomics produces a mixed RNA data set of both the crop and the insect. Millions of short reads are produced after the extraction and sequencing of RNA. At this point the reads are not specifically identified as plant or insect and bioinformatic analysis is required to structure the data.

The initial stage is quality control, in which low quality reads and technical errors are eliminated. The clean reads are then aligned to the reference genome/transcriptome of both organisms. Reads can be assigned to the proper source with tools like HISAT2 and STAR. Reads derived from plants are used to investigate the defense response of the crop and reads derived from insects are used to investigate the response of the pest [2].

Following read separation, the counts of genes of each organism are created. DESeq2 is a used tool for identifying genes that are expressed at different levels after infestation (differentially expressed genes). In the plant, these genes could be associated with hormone signaling, secondary metabolites or cell wall defense. They can be associated with salivary proteins, digestion, detoxification or nutrient transport in the insect.

Lastly, pathway analysis and gene ontology tools are used to translate long lists of genes into biological significance. This workflow was useful in the wheat-aphid dual RNA-seq study to link wheat defense pathways with the responses of aphid secretory proteins throughout the infestation time [1].

Applications, Challenges and Future Prospects

Dual transcriptomics can be used to make pest management more precise, through identification of the useful genes on both sides of the interaction. In crops, defense genes, like lipoxygenase genes related to jasmonic acid related resistance, are found in rice [4]. Detoxification genes like the cytochrome P450 genes have been found to be responsible for insect resistance to plant defense chemicals [10]. These genes can be used for resistant breeding, RNA interference and species-specific pest control [7,8]. Although valuable, dual transcriptomics are technically challenging. It demands quality RNA, proper sampling, robust plant and insect reference genomes and skilled bioinformatics support. When a mix of sequencing data is received, separating the data can be challenging, particularly if low amounts of insect RNA are detected. Also, confirmation of the results with gene-expression methods using qPCR or proteomics or metabolomics is required.

It is envisioned that dual transcriptomics can be integrated with other 'omics' analyses, such as metabolomics, proteomics, microbiome analysis and artificial intelligence, to gain deeper insight into crop-pest interactions soon. This integration might account for the failure of some resistant varieties when insect effectors, detoxification capacities or feeding habits change. These can contribute to the development of more sustainable agriculture by changing the way that pest management is carried out, from broad spectrum chemical control to more precise biological control in the field.

Conclusion

The era of dual transcriptomics has opened a new window in the future to access the hidden molecular warfare in the conflict between crops and insect pests. How plants activate defense pathways; the other one - how insects responded via detoxification genes, digestive enzymes, and other adaptive mechanisms. This is a two-sided picture of things that is important because herbivory is not just the plant responding to its being attacked, it is also a dynamic process of delivery and "translation" of signals, defenses, and counter-defenses.

This method may be useful to research crop protection, and identify genes associated with resistance in plants and success or vulnerability in insects. This information can be used to aid in resistant crop breeding, RNA interference-based control and more accurate monitoring of pests. While sampling, reference genomes, and validation are all still essential components of a dual transcriptomics experiment, the future applications of its use are promising. It is an effort to link molecular biology, bioinformatics and applied entomology and to help usher in smart, sustainable and science-based approaches in agriculture to control pests.

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