Report: Identification of Topping- and Suckercide-Responsive Genes in Tobacco by Next-Generation Gene Sequencing

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Introduction

Shoot branching, or sucker formation, is the formation of axillary buds and subsequent growth in the axils of leaves. It is an undesirable characteristic for commercial crop production. In tobacco, sucker formation affects leaf quality, alkaloid levels and biomass, and when controlled by chemical suckercides, such as *Flupro* or *Offshoot*, it results in increased production costs and chemical pollution. Therefore, effective, non-chemical sucker control that does not compromise whole plant architecture and uniformity is desirable. However, development of such a system presents a significant challenge due to our currently limited understanding of the molecular mechanism for sucker formation. In addition, our understanding of how suckercides affect gene expression is extremely limited. Elucidation of structural and regulatory gene network function during induction and inhibition of sucker formation, in response to topping and suckercide application, respectively, is of biological and agricultural importance.

Objective:

To collect tobacco tissues after topping and treatment of suckercide and use RNA sequencing to measure the differential gene expression in the samples.

Summary of Progress

RNA-seq is a rapid and powerful platform to capture dynamic transcriptome of a species in response to different biotic and abiotic factors. We analyzed the differential gene expression using RNA-sequencing in tobacco (*Nicotiana tabacum*) that are topped, or treated after topping by two different suckercides, the contact-localized-systemic, *Flupro*® (FP), and contact, *Off-Shoot-T*®. Among the differentially expressed genes (DEGs), 179 were identified as common to all three conditions. DEGs, largely related to wounding, phytohormone metabolism and secondary metabolite biosynthesis, exhibited significant upregulation following topping, and downregulation after suckercide treatments. DEGs related to photosynthetic processes were repressed following topping and suckercide treatments. Moreover, topping and FP-treatment affect the expression of auxin and cytokinin signaling pathway genes that are possibly involved in axillary shoot formation. Our results provide insights into the global change of plant gene expression in response to topping and suckercide treatments. The regulatory elements of topping-inducible genes are potentially useful for the development of a chemical-free sucker control system. One peer-reviewed paper describing this work has been published in December 2015 (Singh et al. 2015. RNA-sequencing reveals global transcriptomic changes in Nicotiana tabacum responding to topping and treatment of axillary-shoot control chemicals. Scientific Reports. 5:18148)

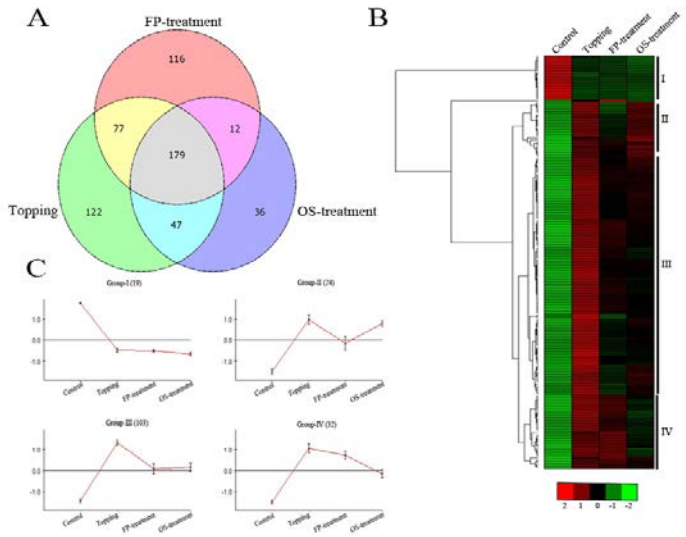

Figure 1. RNA-Seq analyses of differentially expressed genes (DEGs) in leaves of topped, FP- and OS-treated tobacco. (A) Venn diagram showing the overlap of DEGs between topped, FP-, and OS-treated samples. (B) Hierarchical cluster analysis and heatmap showing expression of common DEGs in topped, FP-, and OS-treated samples. (C) Expression profile of common DEGs associated with four different clusters.

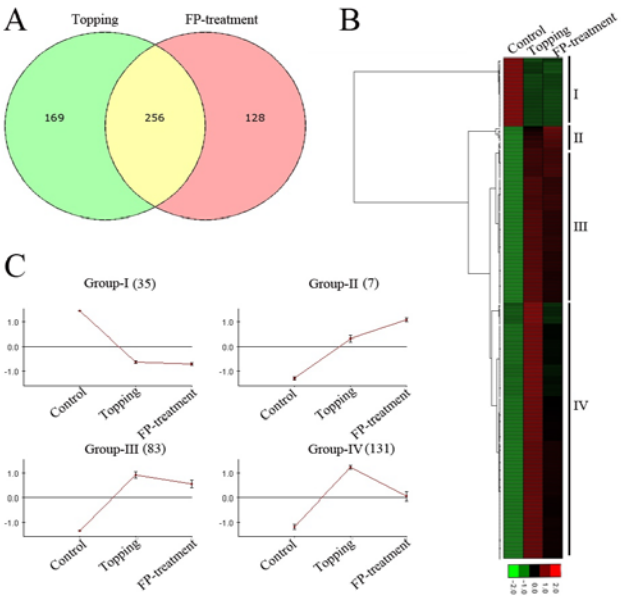

Figure 2. RNA-Seq analyses of DEGs in leaves of topped and FP-treated tobacco. (A) Venn diagram showing the overlap of DEGs between topping and FP-treatment. (B) Hierarchical cluster analysis and heatmap showing expression of common DEGs in topped and FP-treated leaves. (C) Expression profile of common DEGs associated with four different clusters.

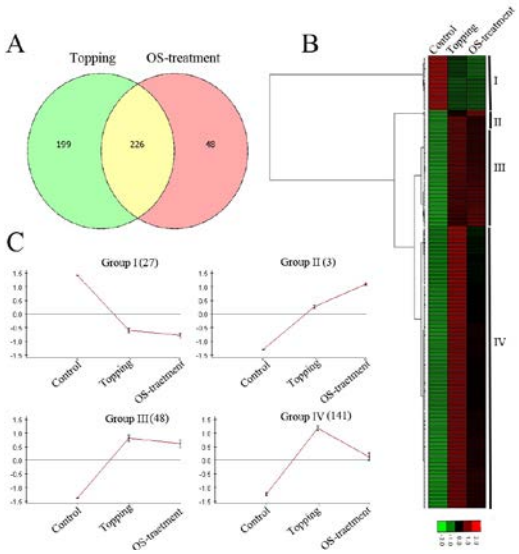

Figure 3. RNA-Seq analyses of DEGs in leaves of topped and OS-treated tobacco. (A) Venn diagram showing the overlap of DEGs between topping and OS-treatment. (B) Hierarchical cluster analysis and heatmap showing expression of common DEGs in topped and OS-treated leaves. (C) Expression profile of common DEGs associated with four different clusters.

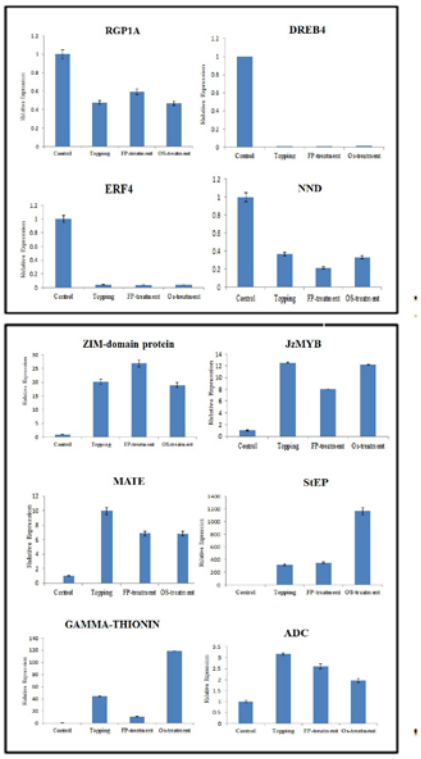

Figure 4. Validation of RNA-seq results using quantitative real-time polymerase chain reaction (qRT-PCR) analysis. The tobacco œ-tubulin was used as an internal control for normalization. Data represent mean ± SD of three independent replicates.

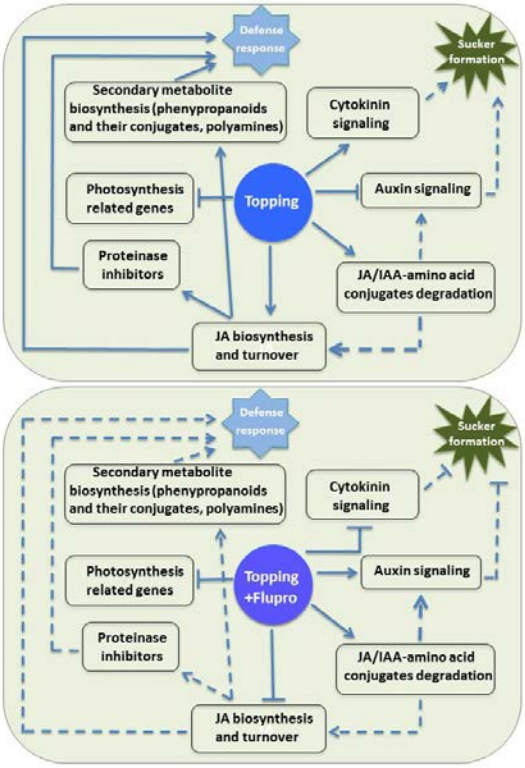

Figure 5. A hypothetical depiction of plant responses to topping and FP-treatment based on the differential gene expression patterns. The upper panel shows the major effects of topping on key biological processes, and the lower panel shows the effects of FP-treatment.