Annual Report

Project: Next generation sequencing aided study of influence of the systemic suckercide maleic hydrazide on global changes of tobacco gene expression

Investigators: Sitakanta Pattanaik and Ling Yuan, KTRDC

Abstract

Topping of tobacco triggers several developmental and metabolic pathways that control axillary shoot (sucker) formation. One commonly used agronomic practice to control sucker formation is to apply chemical suckercides that inhibit axillary shoot formation. Maleic hydrazide (MH) is a systemic suckercide which is widely used by tobacco farmers to control sucker formation. A detailed understanding of gene expression responding to topping and suckercide application is critical to tightly control sucker formation. In this study, we used an analytical platform, RNA-sequencing (RNA-seq) to determine the influence on gene expression by MH on chemically and manually topped tobacco plants. Axillary and apical buds collected from tobacco plants treated with or without MH were used for RNA isolation and library preparation. RNA-seq data obtained from those libraries were processed and genes differentially expressed in control and MH-treated samples were identified. Collectively, our comparative transcriptome analysis provides important information on the influence of MH on global changes in gene expression in tobacco.

Introduction

Proliferation of axillary buds (suckers) is an undesirable characteristic for commercial crop production. In tobacco, sucker formation affects leaf quality, alkaloid levels and biomass. Chemical suckercides, such as flurprimidol or maleic hydrazide (MH), are widely used to control sucker formation. MH can also potentially be used for chemical topping to replace the labor-intensive manual topping. However, 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.

In this study, we used RNA-seq technology to reveal the differential gene expression induced by MH. RNA-seq is a powerful and well-established technology that continues to advance, facilitated by the rapid improvement of sequencing technology and analysis software. This study took advantage of this versatile analytical platform. Axillary and apical buds collected from tobacco plants treated with or without MH were used for RNA isolation and library preparation. RNA-seq data obtained from those libraries were processed and genes differentially expressed in control and MH-treated samples were identified.

Results

RNA isolation and library construction

Tobacco plants grown in field were used for MH treatment and sample collection. Apical and axillary buds were collected from plants: a) control (untopped/untreated), b) untopped and treated with MH (chemical topping), c) topped, and d) topped and treated with MH (manual topping) (Figure 1). Apical and axillary buds were collected from 3-5 individuals, for each treatment, and immediately frozen in liquid nitrogen. Total RNA was extracted using the RNeasy plant mini kit (Qiagen, USA) following manufacturer's instructions. RNA quantity was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Quality of RNA samples were determined using Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). RNA samples with RNA integrity number (RIN) above 8 were used for library preparation.

RNA-sequencing

High throughput sequencing was performed at the Duke Center for Genomic and Computational Biology (GCB, Duke University). RNA-seq libraries were prepared following Illumina sequencing protocols and sequenced, using the Illumina high-throughput DNA sequencing platform.

Sequence analysis

RNA sequencing libraries from apical and axillary buds of tobacco, that were collected from un-topped, topped, or treated with MH (Figure 1) generated a total of 783 million (M) reads. After removal of low quality reads, 668 M high-quality reads were obtained. Each biological sample (un-topped, topped, or MH-treated) was represented by an average of more than 100 M reads (Table 1). On an average more than 75% of the total reads from each condition were successfully mapped to the reference sequence (Table 1). We have identified more than 11,000 genes which are differentially expressed in chemically topped, or manually topped and MH-treated apical or axillary buds. Compared with the control, 455 (93 up-regulated, 362 down-regulated) and 2,789 (2,383 up-regulated, 406 down-regulated) genes were differentially expressed in chemically topped apical (CT-ap) and axillary buds (CT-ax), respectively. In addition, we identified 692 (538 upregulated and 154 downregulated) and 11,902 (7,891 upregulated and 4,011 downregulated) differentially expressed genes (DEGs) in the axillary buds of mechanically topped (MT-Ax), and MH-treated (MTS-Ax) tobacco, respectively (Figure 2). Gene ontology (GO) analysis revealed that genes involved in meristem development, phytohormome signaling and transport were affected by MH treatment. Among the different phytohormones, expression of genes related to auxin and cytokinin metabolism and signaling were affected following MH treatment. In addition, expression of some of the regulatory genes related to meristem development were also affected following MH treatment. Collectively, our comparative transcriptome analysis provides important information on the influence of MH on global changes in gene expression in tobacco.

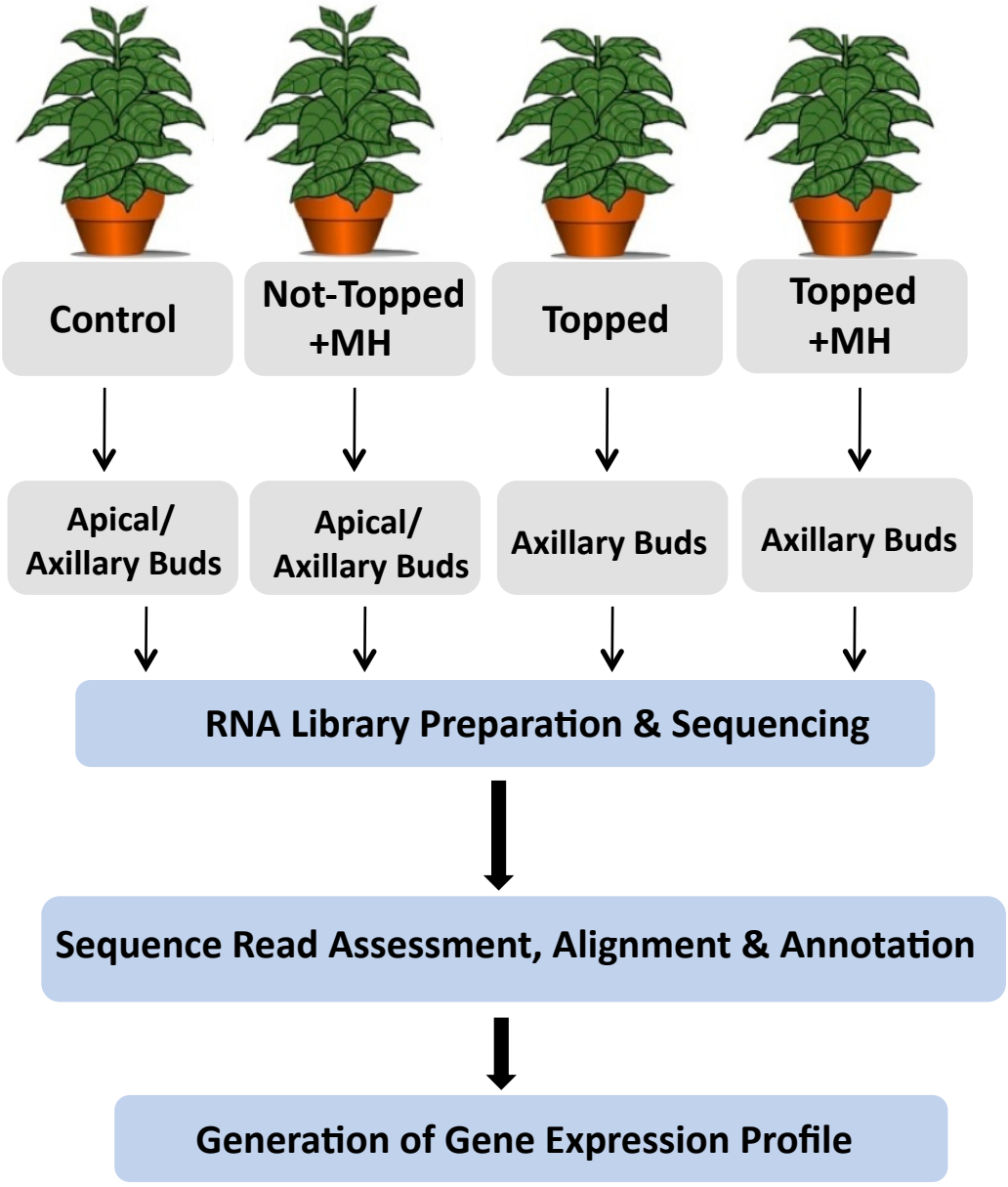

Figure 1. Schematic diagram of the experimental design adopted for this project. Apical and axillary bus were collected from control (untopped), untopped and treated with maleic hydrazide (MH), topped and topped and treated with MH.

Table 1 Summary of sequencing and read mapping

Treatment	Symbol	Experiment	Total raw reads (million)	Total clean reads (million)	Total raw reads per biological sample (million)	Total clean reads mapped to reference transcriptome (%)
Control-axillary buds	CNT-ax	1	41.93	36.34	109.16	76.47
		2	44.47	38.81		75.9
		3	39.63	34.01		73.96
Control-apical buds	CNT-ap	1	51.17	31.11	118.23	81.41
		2	47.94	41.82		78.9
		3	51.93	45.3		79.06
Chemically topped-axillary buds	CT-ax	1	45.55	39.79	110.72	76.06
		2	37.67	31.92		76.26
		3	44.51	39.02		75.8
Chemically topped-apical buds	CT-ap	1	35.74	31.11	111.56	80.38
		2	49.1	43.14		79.83
		3	42.58	37.31		79.58
Manually topped-axillary buds	MT-ax	1	41.03	35.54	102.9	75.63
		2	37.28	32.69		76.09
		3	39.57	34.67		74.28
Manually topped-MH-treated-axillary buds	MTS-ax	1	35.24	30.67	115.71	77.01
		2	57.58	50.37		77.19
		3	39.59	34.67		76.72

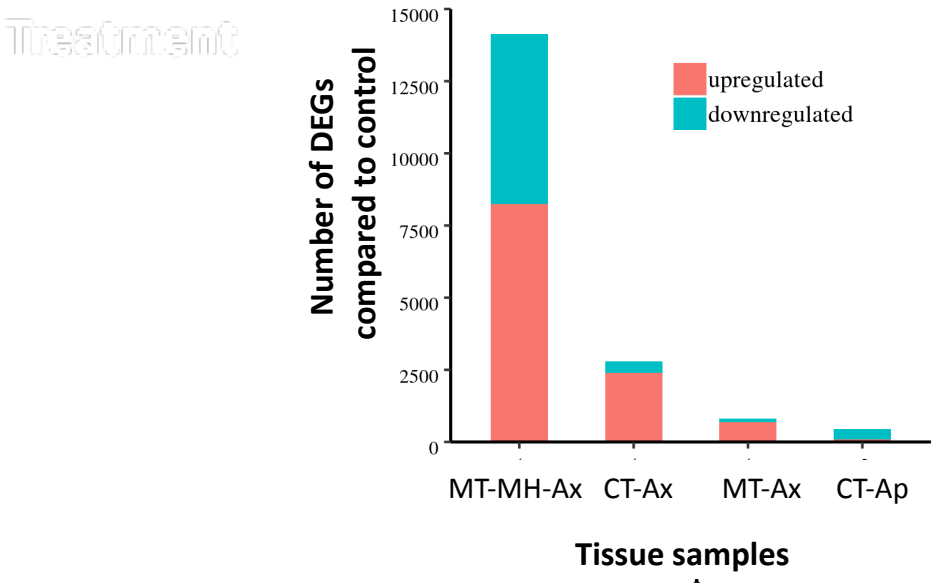

Figure 2. Differential gene expression analysis in manually and chemically topped apical and axillary buds. Ap, apical buds; Ax, axillary buds; CT, chemically topped;MT, manually topped.