Molecular Mechanism Involved in Maleic Hydrazide-Mediated Sucker Control

Payyavula R.; Shen Y.; Kudithipudi C.; Govindarajulu R.; Xu D.

Altria
Altria Client Services

Objectives

- Understand mechanism of Maleic Hydrazide-Mediated (MH) sucker control
- Evaluate gene expression in axillary bud during MH treatment
- Identify candidate genes for sucker inhibition in tobacco
- Integrate selected gene into our sucker control pipeline

Outline

- Tobacco topping
- Experiment design
- Differential gene expression
  - **Cell cycle genes**
  - **MYBs (Myeloblastosis family)**
  - **Ethylene related genes**
- Conclusion and future work

Altria
Altria Client Services

Tobacco Topping and Sucker

Removal of Apical Meristem

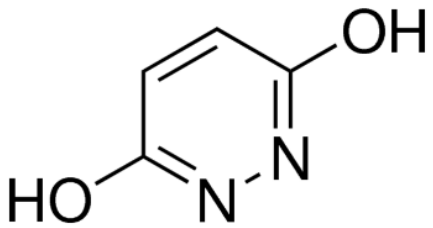
Suckers divert nutrition and impact leaf quality

Altria
Altria Client Services

Tobacco Sucker Control

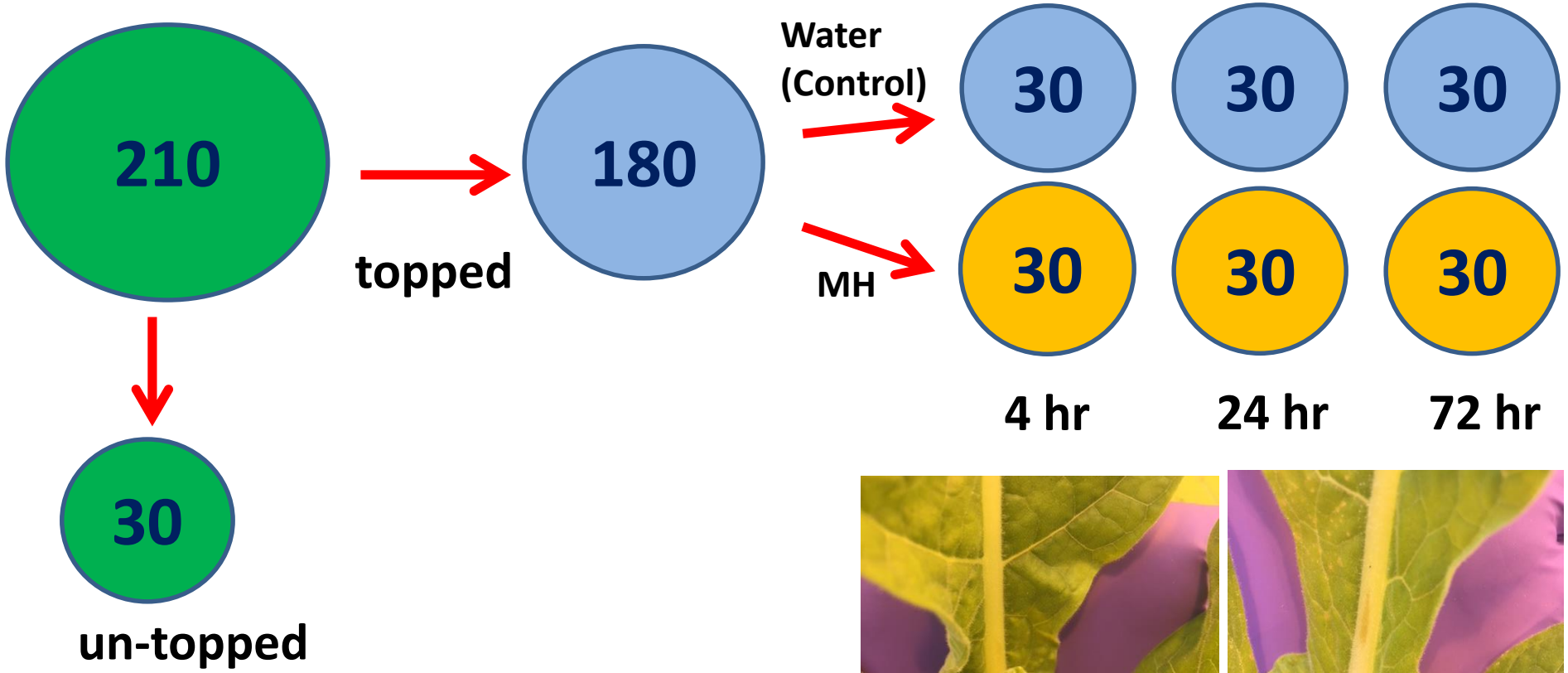
Control Methods

- Manual removal (labor intensive)
- Chemical application
  - Contact : Fatty Alcohols, burn tender suckers
  - Contact-local systemic : Flumetralin
  - Systemic: Maleic Hydrazide (MH)



Altria
Altria Client Services

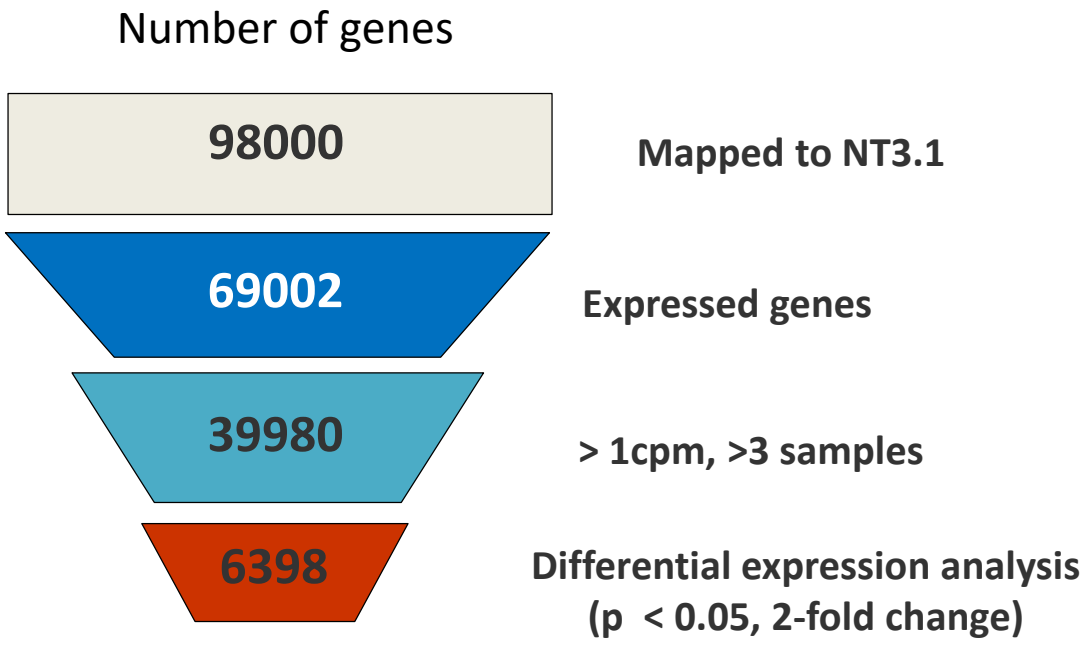
Experiment Design

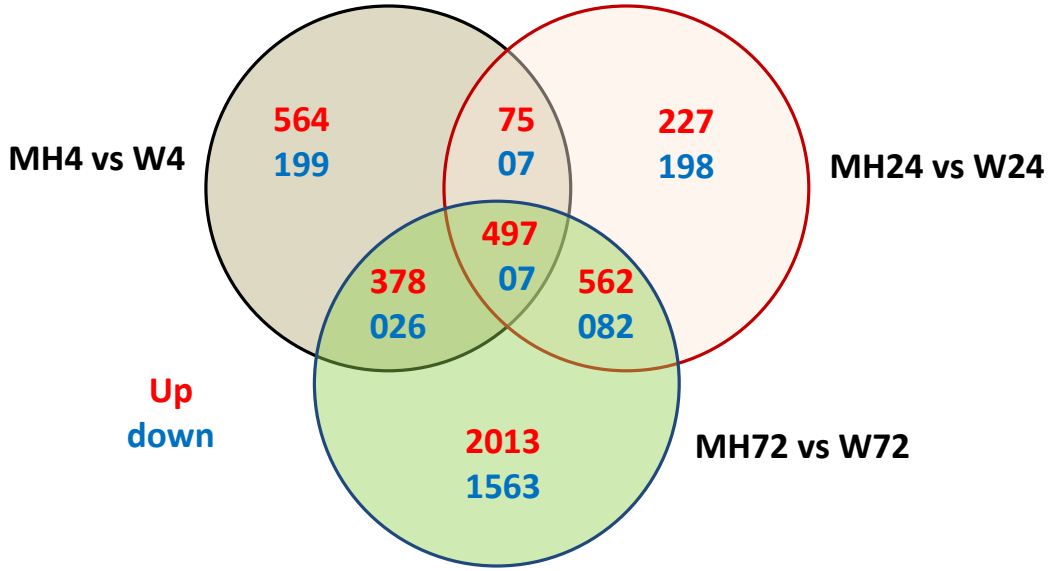


Water MH

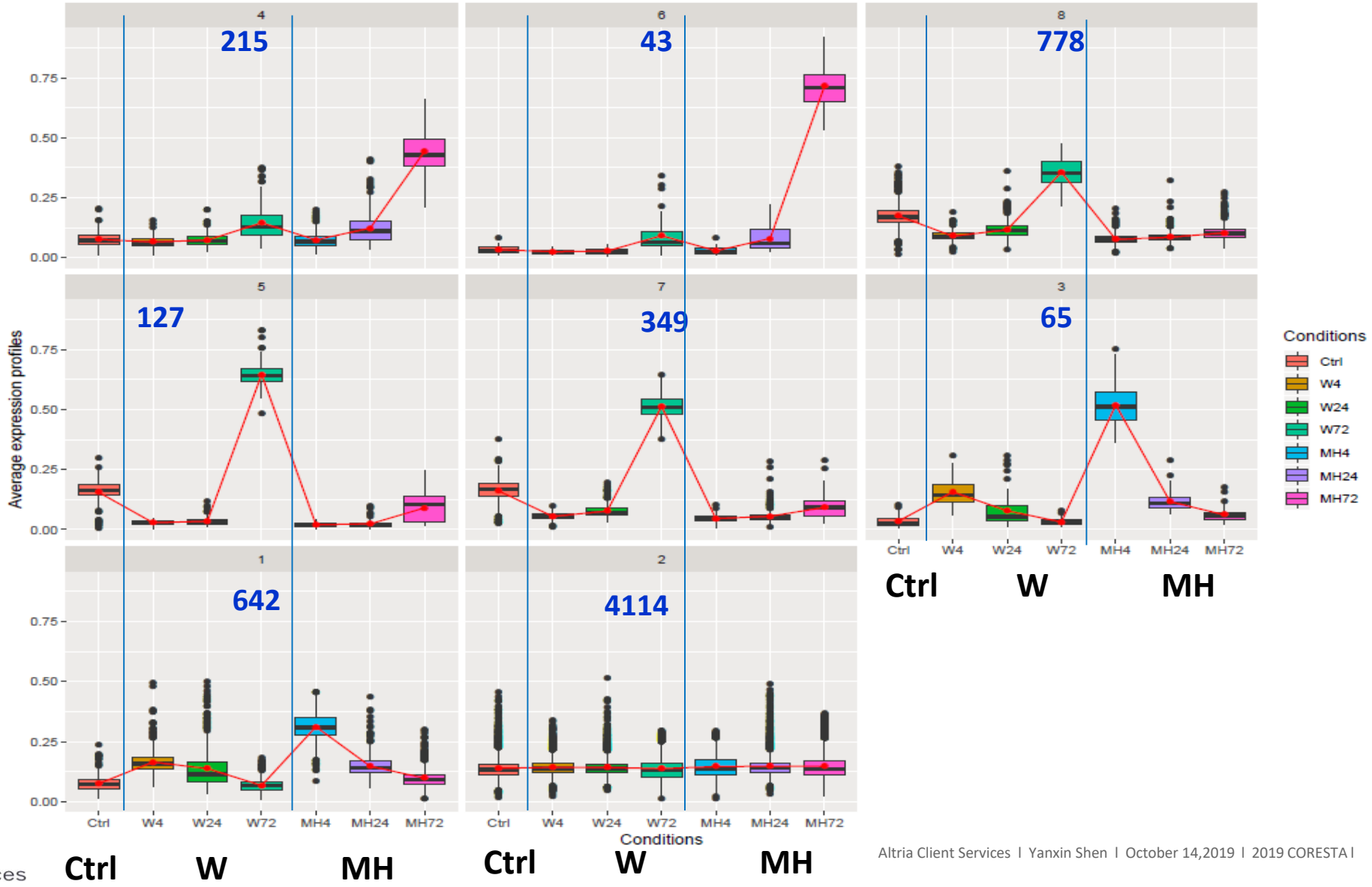
Altria
Altria Client Services

RNA Sequencing Analysis

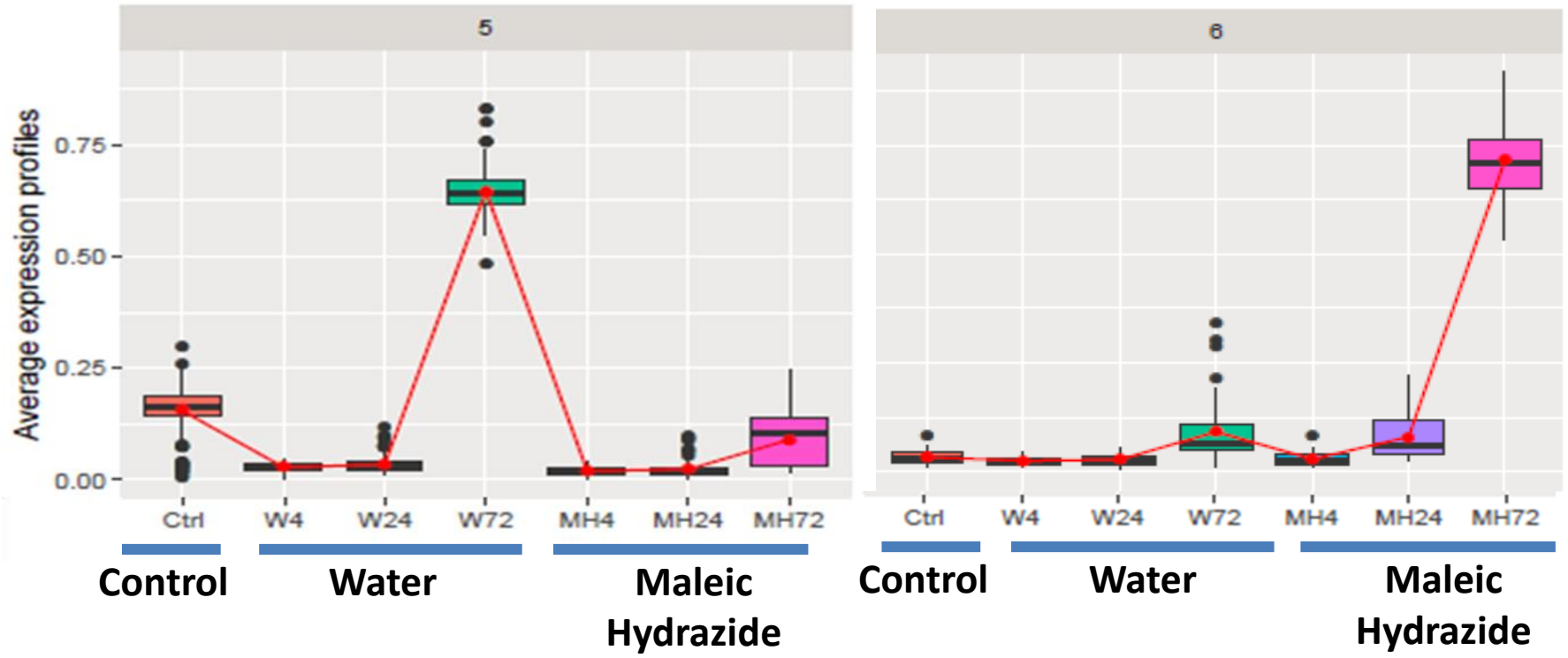




Categories of Differentially Expressed Genes



Examples of Differentially Expressed Genes



Genes Induced by MH-treatment

Gene	# of Genes
Glutathione S-transferase	9
Ribonucleoside-diphosphate reductase	2
P-loop containing nucleoside triphosphate hydrolases	2
Transcription repressor ofp17	1
WRKY transcription factor	1
Cyclin-dependent protein kinase inhibitor SMR4	1

Genes Suppressed by MH-treatment

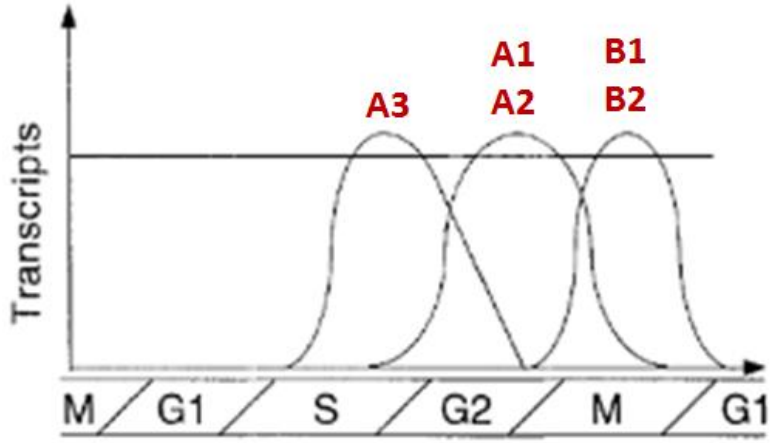
Cell cycle	Cyclin
	Cyclin Dependent Kinase
	MYB transcription factors
Spindle	
DNA synthesis	
Cell division	
Others	Cellulose synthase
	Unknown
	Kinesin-like protein

Cyclin and Cyclin Dependent Kinases

class	Gene	W72/Ctrl	MH72/Ctrl
A1	g50932	1.811	0.072
A1	g63973	1.517	0.074
A2	g3714	1.827	0.321
A2	g37426	2.911	0.560
A2	g85369	4.147	0.826
A2	g92675	1.786	0.464
A3	g65151	2.965	1.584
A3	g60711	3.748	2.066
B1	g89928	1.823	0.079
B1	g74082	1.552	0.098
B1	g23522	1.843	0.047
B1	g95819	2.088	0.075
B1	g22492	1.705	0.087
B1	g94178	2.041	0.060
B1	g22493	1.840	0.043
B2	g11884	1.852	0.119
B2	g13941	2.519	0.068
B2	g49442	1.752	0.071
B2	g58123	1.975	0.147
B2	g60549	1.485	0.093
B2	g65820	1.718	0.072
B2	g66934	1.216	0.121
B2	g69724	3.047	0.149
B2	g80143	0.976	0.032
B2	g82564	2.155	0.073
B3	g40199	1.945	0.694
B3	g71747	2.185	0.727
D	g23478	0.863	1.030
D	g95745	0.406	0.959
D	g78000	1.246	0.653
D	g44547	1.247	0.592
U2	g8070	1.717	0.845

93% reduction

85 – 95% reduction



CDK

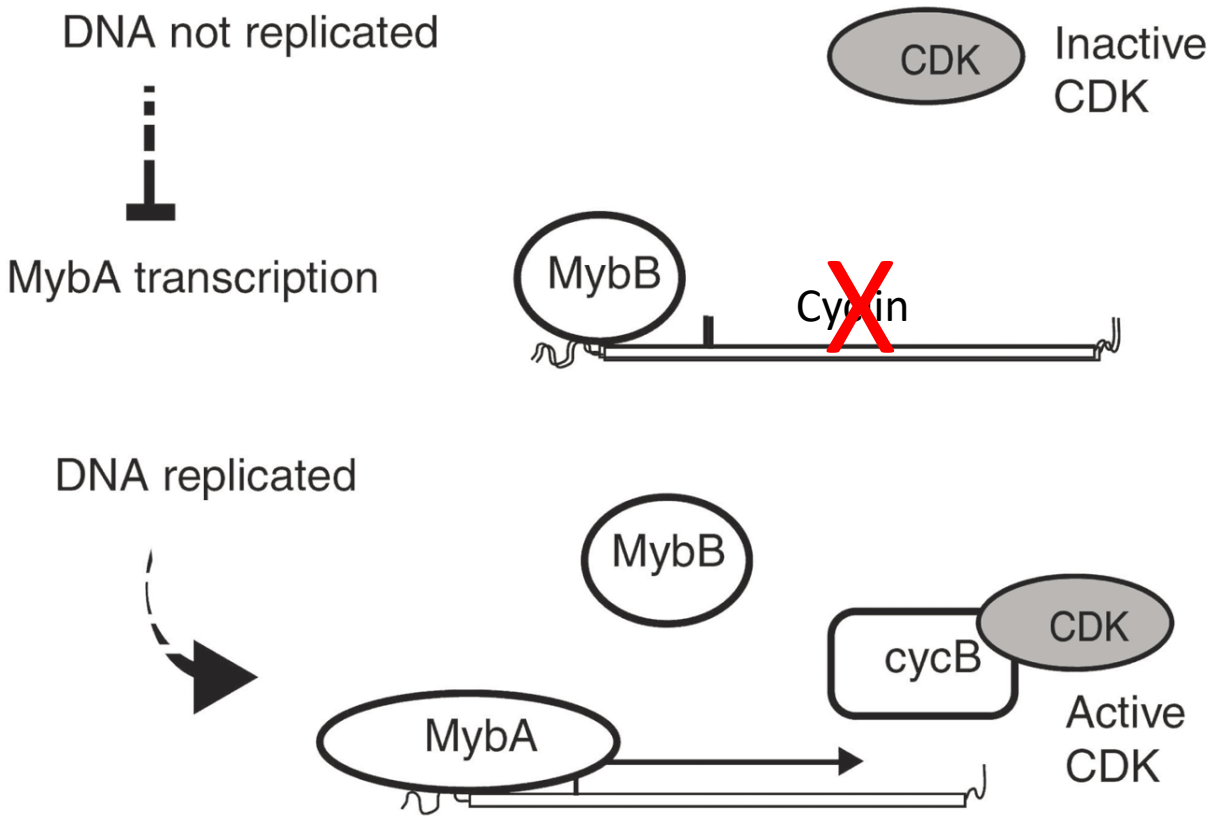
class	Gene	W72/Ctrl	MH72/Ctrl
B1	g95438	2.664	0.407
B1	g23308	2.034	0.439
B2	g49387	1.964	0.138
B2	g65882	1.831	0.096

CDK inhibitor

g45641	0.758	114.916

Altria
Altria Client Services

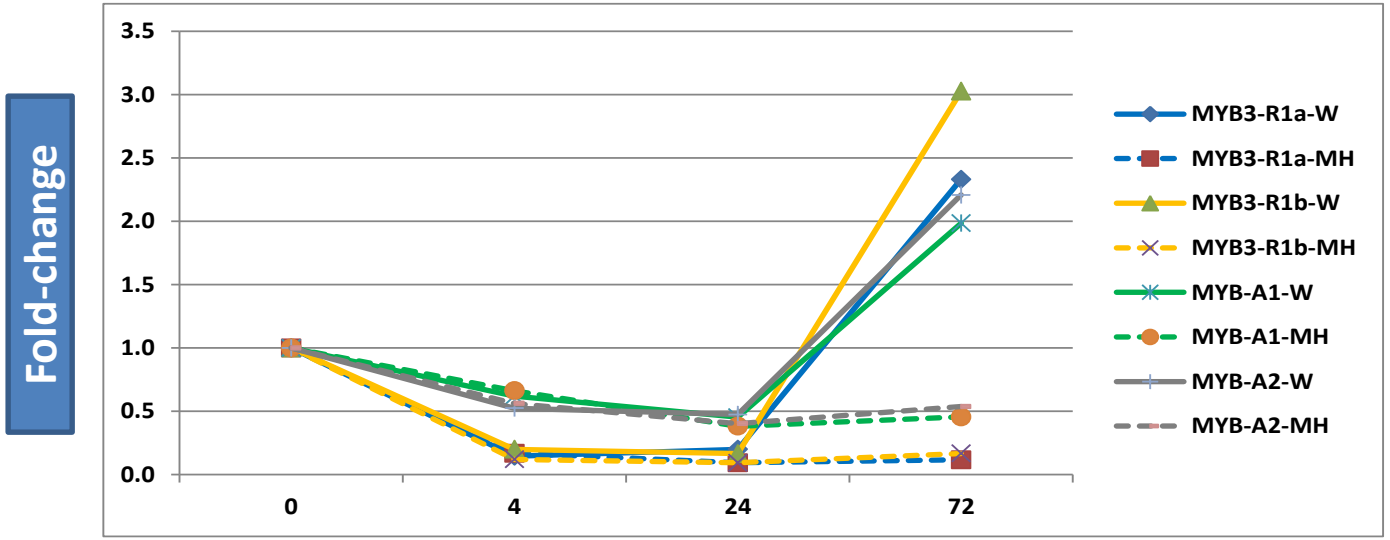
MYB Transcription Factors



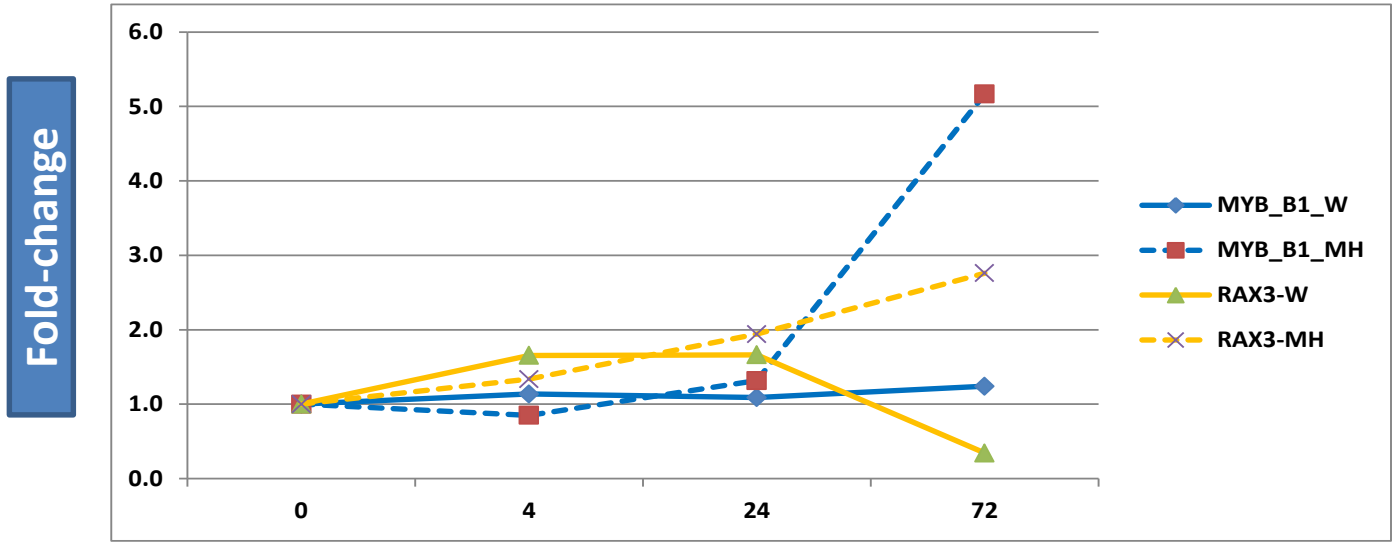
- Unreplicated DNA inhibits the production of activating MybA protein by an unknown mechanism, and inhibitory MybB occupies the promoter, preventing the transcription of M-phase–related genes, including cyclin.
- When DNA replication is complete, MybA is produced and displaces MybB from the promoter.
- Cyclin B is transcribed and activates cyclin-dependent kinase A (CDKA), allowing progression into mitosis

MYB Transcription Factors

+ve regulators



_ ve regulator



Altria
Altria Client Services

Ethylene Related Genes

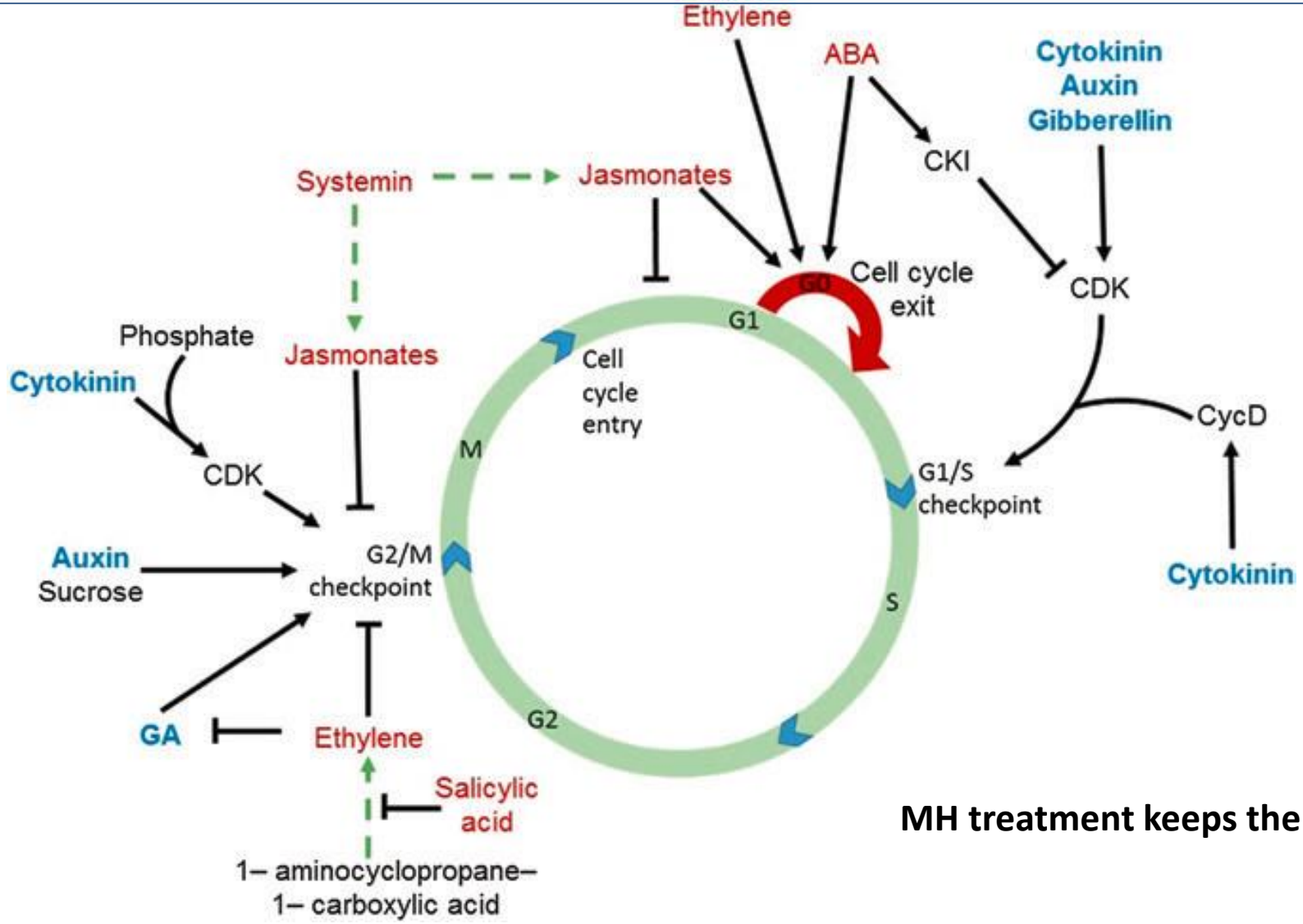
ACS — 1-aminocyclopropane-1-carboxylate synthase

ACO — 1-aminocyclopropane-1-carboxylate oxidase

		Water			MH		
	0	4	24	72	4	24	72
ACO							
g15354	1	1.87	0.97	0.16	2.95	2.02	0.52
g44783	1	19.83	2.61	2.90	64.22	26.35	29.01
g58208	1	1.71	1.54	0.69	1.43	2.39	2.02
g68638	1	1.21	1.29	0.18	1.23	1.48	0.51
g7868	1	29.80	4.67	2.30	100.17	36.06	30.80
g7434	1	0.90	0.57	0.28	2.06	1.58	2.43
g34158	1	7.53	5.73	0.75	18.94	13.74	3.89
g7435	1	1.25	0.88	0.42	3.00	2.28	3.30
g83990	1	1.58	1.33	0.87	2.79	4.45	2.39
ACS							
g2929	1	1.72	2.62	0.46	2.32	1.77	1.59
g93421	1	0.81	1.25	0.33	0.68	0.75	0.76
g20152	1	5.86	6.12	0.77	10.77	12.49	2.43
g58718	1	24.74	20.19	0.68	43.46	38.21	5.66
g64971	1	0.08	0.25	0.05	0.09	0.25	0.22
g76853	1	1.08	0.49	1.20	1.60	0.58	2.92
g93423	1	1.67	2.84	0.82	3.19	2.29	1.68

Ethylene biosynthesis is upregulated by MH

Plant Cell Cycle regulation



MH treatment keeps the cell in G1 to G0 Stage

Altria
Altria Client Services

Conclusion and Future Work

- Ethylene biosynthesis upregulated by MH
- MYBs altered expression
- Cyclins, CDKs: significantly down-regulated
- MH inhibit cell division by affecting G2 and M phase
- Functional analysis of several cell cycle genes will be analyzed to confirm sucker control effects

Thank You

Questions?

For copies of this presentation visit the Altria's Science Website at
www.altria.com/alcs-science

Altria has a new Altria Science Twitter account
Follow us @AltriaScience