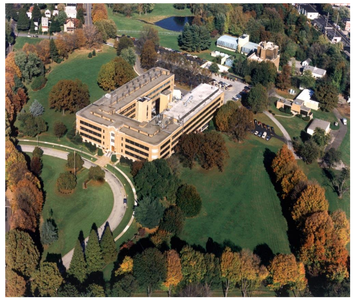


Transcriptomic Analysis on the Regulation of Tomato Ripening by the Ethylene Inhibitor 1-methylcyclopropene

Yanhong Liu[1]*, Jamshed Bobokalonov[2], Tasnuva Shahrin[2] & LinShu Liu[2]

[1]Molecular Characterization of Foodborne Pathogens Research Unit, Eastern Regional Research Center, Agricultural Research Service, USDA, 600 E. Mermaid Lane, Wyndmoor, PA 19038

[2]Dairy and Functional Foods Research Unit, Eastern Regional Research Center, ARS, USDA, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

*Correspondence: Yanhong Liu, Tel: 1-215-233-6587. e-mail: Yanhong.Liu@ars.usda.gov

ABSTRACT

Tomato is a climacteric fruit whose ripening is regulated by the plant hormone ethylene. 1-methylcyclopropene (1-MCP) is a competitive ethylene inhibitor that can delay the fruit ripening process. To understand the molecular mechanism of how 1-MCP inhibits tomato fruit ripening, transcriptomics (RNA-Seq) was used to identify genes that were differentially expressed in 1-MCP-treated (Day 1) tomato fruits. Of the 35340 genes in the tomato genome, about 50% were expressed with 1-MCP treatment. There were 5683 genes identified as significantly differentially expressed. Quantitative reverse transcription PCR (qRT-PCR) assays were used to validate the RNA-Seq data. Our results showed that 1-MCP treatment resulted in the down-regulation of fruit ripening-related genes, including genes involved in ethylene synthesis, signal transduction and carotenoid biosynthesis. Our results provide insight at the whole genome level regarding gene regulation by 1-MCP during fruit ripening. Understanding the molecular basis of 1-MCP inhibition on tomato ripening may help farmers and food processors to better use 1-MCP in agriculture and food industry.

INTRODUCTION

- Fruit ripening is a complex developmental process that coincides with seed maturation, changes in color, texture, taste and flavor. It is regulated by temperature, gas content in the atmosphere, humidity, and plant hormones such as ethylene.
- Application of exogenous ethylene to unripe tomatoes stimulated fruit ripening whereas inhibitors blocking ethylene synthesis and delay tomato ripening.
- 1-MCP is an inhibitor of ethylene though blocking of ethylene receptors. It is non-toxic, it has been used to prolong the post-harvest shelf life of climacteric fruits.
- The molecular mechanism of 1-MCP inhibition of fruit ripening remains unknown.
- Tomato (Solanum lycopersicum) is a good model system because the tomato has a relatively small genome and it has been fully sequenced.
- RNA-Seq has been applied to differential gene expression analysis and used to study the transcriptome changes under 1-MCP treatment to understand the effect of 1-MCP on tomato fruit ripening at the genomic level.
- Our research provides insight on ethylene regulation in tomato fruit ripening.

MATERIALS AND METHODS

Mature green tomatoes were purchased from East Coast Fresh Cuts (Savage, MD, USA), received just prior to performing the experiment and used as is. Tomatoes were treated with EthylBlokTM (Agrofresh, Spring House, PA, USA) as described previously, using airtight glass jars to create a “closed system” to generate 1-MCP at a fixed concentration. Each jar was equipped with a Petri dish in the bottom containing a stir bar and 1 g of the reagent, a tube with one side reaching the dish and another side connected with a 30 ml syringe was fixed with the lid through an airtight rubber horse. All jars were placed on stirrer plates at 22±1ºC. After loading tomatoes, 5 or 6 (200-300 g per a tomato) in each, the jars were closed, 10 ml of water were injected to each Petri Dish, the stirrer was turned on simultaneously and continued for 24 h. A control experiment was conducted simultaneously without EthylBlok^{TM}. After thus treatment, all tomatoes were removed from the jars, and placed in open space at 22±1ºC. Pictures were taken at day 0, 1, 2, 3, 7, 10, 13, 14 and 20.

Total RNA was extracted from tomato pericarps using the RNeasy Plant Mini Kit (Qiagen, Valacia, CA, USA). RNA integrity was assessed using the Agilent Bioanalyzer 2100 system with RIN numbers between 8.8 and 9.3. Library construction and genome sequencing were performed by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). The libraries were sequenced using an Illumina HiSeq 2000 platform, and 150 bp paired-end reads were obtained.

RT-PCR was used to validate the gene expression data obtained from RNA-Seq experiments.

CONCLUSIONS

- The whole genome level regarding gene regulation by 1-MCP during tomato ripening was provided.
- The expression level of the gene encoding for phytoene synthase 1 (psy1) (Solyc03g031860) was significantly inhibited (only 8% compared to untreated) in 1-MCP treated tomato fruits and down-regulation of this gene abolishes normal carotenoid accumulation.
- The expression levels of two ethylene receptors, ethylene receptor homolog (ETR4) (Solyc06g053710) and Never ripe (Solyc09g075440) were significantly down regulated (0.07 and 0.15, respectively) in 1-MCP-treated tomato fruits, which means 1-MCP binds to specific ethylene receptors, possibly ETR4 and Never ripe, therefore reducing their production.
- The ethylene signal transduction pathway ultimately leads to the activation of transcriptional regulators belonging to the Ethylene Response Factor (ERF) family of transcription factors. The 9 ERFs, 5 of them (Solyc07g008250, Solyc12g009560, Solyc01g065980, Solyc02g077370 and Solyc09g066360) were significantly down-regulated (0.02 to 0.15) whereas two of them (Solyc04g051360 and Solyc04g014530) were significantly up-regulated (350 and 72-fold, respectively) in 1-MCP-treated tomato fruits. Our data suggest that 1-MCP delayed ripening by inhibiting ERFs, therefore inhibiting ripening-related gene expression.

Figure 1. Color changes for 1-MCP treated and non-treated tomatoes. Pictures were taken at Day 1, 2, 3, 6, 7, 10, 13, 14, and 20.

Table 1. Throughput and quality of RNA-Seq data

Sample name	Raw reads	Clean reads	Q20 (%)	Total mapped	Multiple mapped	Uniquely mapped
U0-1	73720680	71720882	97.5	65851125 (91.82%)	1028752 (1.43%)	64822373 (90.38%)
U0-2	81324926	78662492	97.55	72002946 (91.53%)	1097549 (1.4%)	70905397 (90.14%)
U1-1	118771830	114590170	98.3	64748336 (56.5%)	1024390 (0.89%)	63723946 (55.61%)
U1-3	122902580	119541298	98.16	65657537 (54.92%)	1092604 (0.91%)	64564933 (54.01%)
T1-1r	123401776	119975950	97.95	60089836 (50.08%)	692821 (0.58%)	59397015 (49.51%)
T1-2r	113749964	110636956	98.28	56339418 (50.92%)	617191 (0.56%)	55722227 (50.36%)

Table 2. DEGs associated with carotenoid metabolism

Gene ID	Annotation	TreD1vsConD1	
		Fold change	P value
Solyc03g031860	Phytoene synthase 1	0.08	7.27E-57
Solyc04g040190	Lycopene beta-cyclase	1.81	0.01
Solyc03g007960	Beta-carotene hydroxylase-2	13.03	3.70E-41

Table 3. DEGs associated with cell wall degradation

Gene ID	Annotation	TreD1vsConD1	
		Fold change	P value
Solyc07g017600	Pectinesterase	38.468	9.03E-24
Solyc09g010210	Endo-1,4-beta-glucanase precursor	60.597	8.32E-05
Solyc02g091680	Probable beta-D-xylosidase 6-like	0.620	0.01
Solyc01g104950	Beta-xylosidase	0.245	0.01
Solyc10g047030	Beta-D-xylosidase 1 precursor	0.109	0.04
Solyc09g005850	Probable pectate lyase 4-like	2.170	1.86E-05
Solyc03g031840	Expansin precursor	1.635	0.02
Solyc06g051800	Expansin 1	2.453	0.02

Table 4. DEGs associated with DNA methylation

Gene ID	Annotation	TreD1vsConD1	
		Fold change	P value
Solyc12g100330.1	Cytosine-specific methyltransferase	1.82	0.0002
Solyc01g006100.2	Cytosine-specific methyltransferase	17.17	3.43E-08
Solyc08g005400.2	Cytosine-specific methyltransferase	2.18	3.74E-05
Solyc02g062740.2	DNA(Cytosine-5-)-methyltransferase 3	0.67	0.008
Solyc05g053260.2	DNA methyltransferase	0.68	0.03
Solyc09g009080.2	Repressor of silencing 1	2.91	4.04E-09
Solyc11g007580.1	HhH-GPD family protein	1.54	0.006

RESULTS

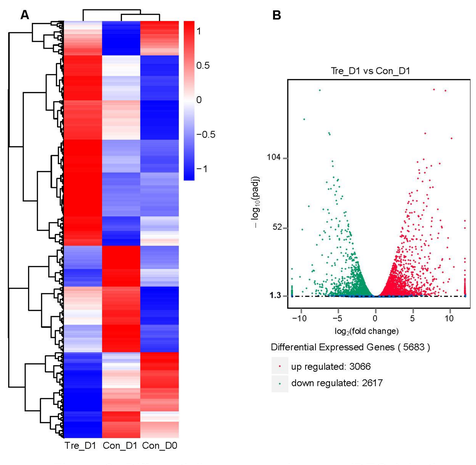


Figure 2. Differentially expressed genes (DEGs) in the MCP-treated samples (Day1)

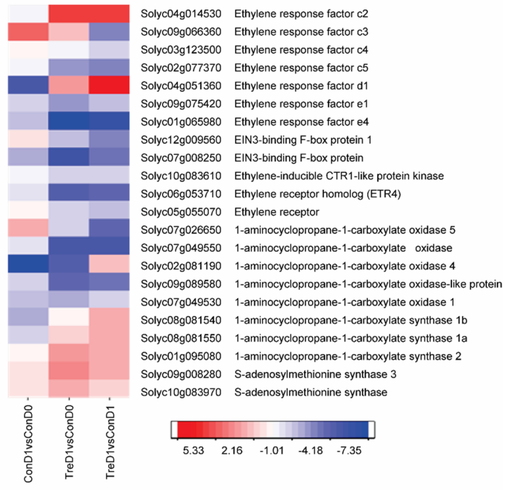
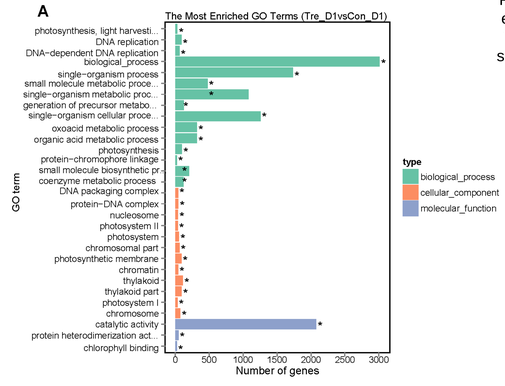


Figure 4. Heat map of the differentially expressed genes (DEGs) in ethylene synthesis and signal transduction after 1-MCP treatment. The RPKM values were normalized and converted to Z-scores to scale the gene expression levels. Red and blue colors indicate high and low expression, respectively



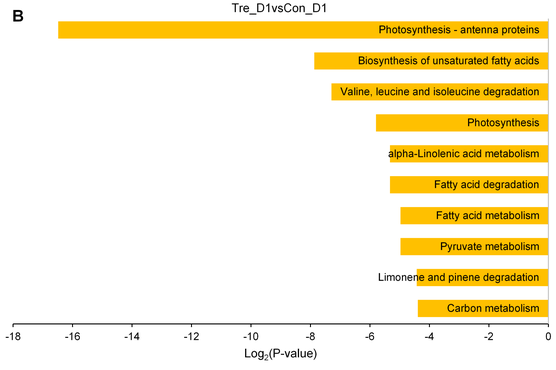


Figure 3. GO and KEGG enrichment analysis of DEGs in 1-MCP-treated tomatoes. (A) The top thirty most enriched GO terms in TreD1vsConD1. The x-axis is the number of differentially expressed genes and the y-axis is GO terms enriched. Asterisks (*) indicate significantly enriched GO terms. Different colors are used to identify biological process. (B) The top ten most enriched KEGG pathways in TreD1vsConD1 samples

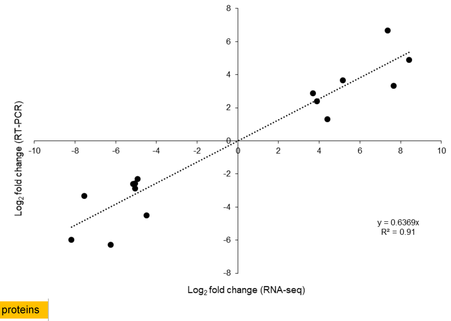


Figure 5. Correlation of gene expression data from RNA-Seq and qRT-PCR