A systems biology approach to investigate the mechanisms that promote ripening and regulate post-harvest fruit withering in the cherry-like tomato landrace “pomodorino del piennolo del Vesuvio”

Rosa Caiazzo, PhD

The 3rd Plant Genomics Congress 11-12 May 2015 London, UK
Cultivation area

Protected Designation Origin

VESUVIO
Peculiarities of “Piennolo”

- cultivation techniques
  - no irrigation and apical buds constantly removed
- jointless pedicels
- long shelf life
- thick skin which limits dehydration
MICROSCOPIC ANALYSIS

Cuticle development

- Ailsa craig
- Fogliariccia
- Lucariello

Weight (mg) vs. Thickness (μm)

- $r = 0.35$
- $r = 0.64$
- $r = 0.62$
To identify differential expressed genes and determine metabolite content during fruit development and post-harvest storage

To generate a model of biochemical and molecular mechanisms involved in the post-harvest withering
STRATEGIES

- Next-generation sequencing
  - “digital gene expression profiling”
- Metabolite content evaluation (HPLC, GC/MS)
- Optical microscopy analysis of cuticle
Genotype: *Lucariello*
Tissues: Exocarp and Mesocarp
Biological replicates: 3

MG
mature green

BR
breaker

RR
red ripe

60DPH
60 days post-harvest

150DPH
150 days post-harvest
WORKFLOW

- RNA extraction
- Library construction & sequencing
- Quality control on raw reads & adapter trimming
- Alignment on reference
- Summarization
- Normalization
- DEG call
WORKFLOW

RNA extraction
Library construction & sequencing
Quality control on raw reads & adapter trimming
Alignment on reference
Summarization
Normalization
DEG call
WORKFLOW

RNA extraction
Library construction & sequencing
Quality control on raw reads & adapter trimming
Alignment on reference
Summarization
Normalization
Directional single-end sequencing on Illumina HiSeq1500
DEG call
WORKFLOW

- RNA extraction
- Library construction & sequencing
- Quality control on raw reads & adapter trimming
- Alignment on reference
- Summarization
- Normalization
- DEG call

FastQC, FASTX-Toolkit and Trimmomatic
RNA extraction
Library construction & sequencing
Quality control on raw reads & adapter trimming
Alignment on reference
Summarization
Normalization
DEG call

Reference: SL2.50
TopHat2
Statistics on read mapping

# reads with multiple matches
# unmapped reads
# reads uniquely mapped on exocarp tissue
# reads uniquely mapped on mesocarp tissue
RNA extraction
Library construction & sequencing
Quality control on raw reads & adapter trimming
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Normalization
DEG call

HTSeq-count. Reads were aggregated over genes as biological units
**Aligning reads to the tomato reference genome**

Results of the mapping of RNA-Seq reads along the reference genome (SL2.50)

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<th>Samples</th>
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<th>AlternativeTSS</th>
<th>AlternativePolyA</th>
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**Genomic coordinates of annotated genes (iTAG 2.40)**

- **Exocarp**
  - Alternative TSS: 306071 (0.92%)
  - Alternative PolyA: 959247 (2.88%)
  - Internal exon-extension: 9563
  - Intron-retention: 13833

- **Mesocarp**
  - Neighboring exon: 7119
  - Genes: 2184913 (6.56%)
  - Gene desert: 200541 (0.60%)
ALIGNING READS TO THE TOMATO REFERENCE GENOME

- Exons-only
- Alternative TSS
- Internal exon extension
- Multiple annotations
- Neighboring exon
- Gene desert
- Intron only

90% 7% 3%
WORKFLOW

- RNA extraction
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- DEG call

EdgeR Trimmed mean of M-value
NORMALIZATION

Exocarp
Mesocarp

Raw Data

Normalized Data

Exocarp
Mesocarp

MG  BR  RR  60DPH  150DPH
CORRELATION AMONG BIOLOGICAL REPLICATES

0.8 < r < 1
WORKFLOW

1. RNA extraction
2. Library construction & sequencing
3. Quality control on raw reads & adapter trimming
4. Alignment on reference
5. Summarization
6. Normalization
7. DEG call

EdgeR

$pdj < 0.05 \& \log_{10}FC \leq -2 \& e \geq 2$
WORKFLOW

RNA extraction
Library construction & sequencing
Quality control on raw reads & adapter trimming
Alignment on reference
Summarization
Normalization
DEG call

DESeq
pdj < 0.05 & LogFC ≤ -2 or ≥ 2
DEG called with EdgeR & DESeq

EXOCARP

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E = EdgeR  D = DESeq
DEG MESOCARP involved in the withering?
GO enrichment analysis (mesocarp): RR vs 60DPH

- Response to desiccation
- Response to water deprivation
- Hexose metabolic process
- Monosaccharide metabolic process
- Response to acid chemical
- Response to water
- Response to inorganic substance
- Response to oxygen-containing compound
- Response to chemical
- Response to abiotic stimulus
- Response to stress
- Response to stimulus

- Terpenoid metabolic process
- Terpenoid biosynthetic process
- Coenzyme biosynthetic process
- Isoprenoid biosynthetic process
- Isoprenoid metabolic process
- Cofactor biosynthetic process
- Cell redox homeostasis
- Cellular homeostasis
- Homeostatic process
- Regulation of biological quality
- Organic acid metabolic process
- Carboxylic acid metabolic process
- Oxoacid metabolic process
- Single-organism process
GO enrichment analysis (exocarp): RR vs 60DPH

- Glutamine family amino acid metabolic process
- Response to desiccation
- Response to water deprivation
- Lipid catabolic process
- Response to abiotic stimulus
- Response to acid chemical
- Response to chemical
- Response to inorganic substance
- Response to oxygen-containing compound
- Response to water
- Single-organism catabolic process
- Cation transport
- Ion transport
- Metal ion transport
- Carbohydrate metabolic process
- Coenzyme biosynthetic process
- Cofactor biosynthetic process
- Single-organism organelle organization
- Organelle organization
- Organic acid metabolic process
- Carboxylic acid metabolic process
- Oxoacid metabolic process
- Single-organism cellular process
- Single-organism metabolic process
- Single-organism process
Metabolism overview (exocarp): RR vs 60DPH
Functional classification of DEG (exocarp): RR vs 60DPP

- Lipid Metabolism
- Secondary metabolism
- Development
- Cell wall
- Hormone metabolism
- Transport
- Stress
Functional classification of DEG(mesocarp):RR vs 60DPH

-Lipid metabolism
-Secondary metabolism
-Development
-Cell wall
-Hormone metabolism
-Transport
-Stress
Genes involved in electron transport chain

transport_exocarp

transport_mesocarp
Genes involved in electron transport chain

Metal ion transporter are associated with the cold storage of different fruit (Mellidou et al., 2014)

- during post-harvest, cells need to synthesize protective compounds to maintain their cellular compartmentalization and to detoxify accumulated metabolic intermediates
Genes involved in the cuticle biosynthesis
Wax metabolites

- 143 metabolites identified
- Separation between early and late stages
- Wax metabolites cluster in 3 groups: common pathways
WAXES

N° of wax metabolites

- SPECIFIC
- NON SPECIFIC
WAX SYNTHESIS

Current Opinion in Plant Biology
Involved in the fatty acid elongation process for the production of aliphatic wax components and, consequently, confers **resistance to cuticular water loss** (Hooker *et al.*, 2002).
The β-ketoacyl-CoA synthase (KCS family)

Gene ID: Solyc05g013220
Significant reduction in fruit cuticle thickness proportional to the level of GDSL1 silencing (Girard et al., 2012).
GDSL esterase/acylhydrolase family
The analysis of the cuticle confirmed our hypothesis, that cuticle plays a key role in the post-harvest withering of "piennolo" tomato fruits.

- Up regulation in post-harvest of genes involved in:
  - resistance to cuticular water loss
  - increase of cuticle thickness
Mobilization of metal ions

- Metal ion homeostasis is up to regulate post-harvest disorders (such as internal browning, linked to cell wall modifications, similar to those observed during fruit softening, or the response to biotic stress)
The alignment along the reference genome proved that tomato genome annotation needs further revisions and RNA-Seq expression profile studies can be influenced by these inaccuracies.
CONFIRM RNA-SEQ DATA

DEVELOPMENT OF TRANSCRIPT/METABOLITE CORRELATION MATRICES
We acknowledge

for plant cultivation

for sequencing service

This work was carried out in the frame of the "GenoPom-pro - Integrating post-genomic platforms to enhance the tomato production chain" project (PON02_00395_3082360) and is supported by the PON R&C 2007-2013 grant funded by the Italian Ministry of Education, University and Research in cooperation with the European Funds for the Regional Development (FESR).
THANK YOU!
Pomodorino del Piennolo del Vesuvio

La coltivazione del “Pomodoro del Piennolo” in auge le pendici del Vesuvio hanno radici antiche e le loro produzioni artistiche si ritrovano nella scena dei presepi della tradizione napoletana. Le bacche raccolte a grappoli, i cosiddetti “piennoli”, sono conservate fino alla primavera successiva per poi essere usate come condimento per la pasta o altre pietanze.

Rosa Catazzo, Nuzio D’Agostino, Patrizia Spigno, Riccardo Riccardi, Mario Parisi.
**CAROTENOIDS**

Bars with the same letter are not significantly different according to the Tukey HSD test ($p \leq 0.05$).

- **Lycopene** and **β-carotene** are higher in the cuticle and reach the maximum at the **RR stage**
- **Lycopene** increases in the **mesocarp** at **150 days post harvest**
Ascorbic acid increases during the berry development and decreases in post-harvest.

Bars with the same letter are not significantly different according to the Tukey HSD test (p≤0.05).