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EXPRESSION PROFILING OF GENES INVOLVED IN FRUIT WAX BIOSYNTHESIS IN DIFFERENT GENOTYPES OF BLUEBERRY (*Vacinium corymbosum* L.)

sylwia.keller@inhort.pl

Keller-Przybyłkowicz S., Stanisław Pluta, Łukasz Seliga, Agnieszka Walencik, Krystyna Strączyńska, Renata Czarnecka
The National Institute of Horticultural Research (InHort)
ul. Konstytucji 3 Maja 1/3, 96-100 Skierniewice

1. Objectives

The highbush blueberry (*Vaccinium corymbosum* L.) is common berry fruit species cultivated commercially in the world temperate regions. Fruit quality, including postharvest shelf life of berries is an important factor in the breeding and selection of this species. The fruit wax coat intensity of blueberry cultivars, significantly vary, which is particular importance in this aspect.

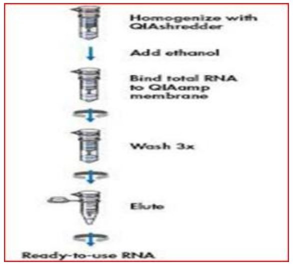
Based on transcriptome analysis of fruit samples of 'Duke' and 'Bluecrop' we have selected four genes, involved in wax and long fatty acids biosynthesis pathways. The differentially expressed and functionally annotated genes (DEG's) were finally analyzed in accordance to their expression profiling.

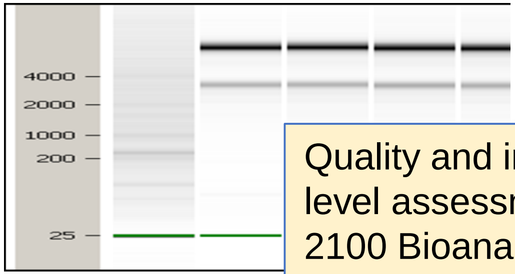
The goal of presented studies

Identification of genomic sequences correlated with fruit waxy coating, and selection of molecular markers useful for the selection of highbush blueberry breeding materials in terms of the studied traits.

2. Material and Methods

Total RNA from ‘Bluecropp’ and ‘Duke’ (Plant Isolation Kit, Qiagen - fruit peal and fruit flesh)



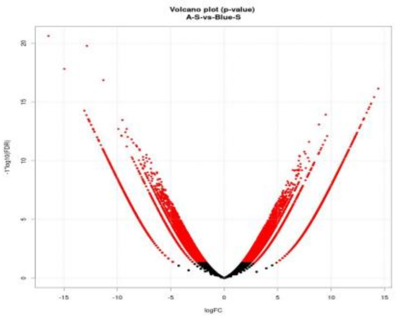


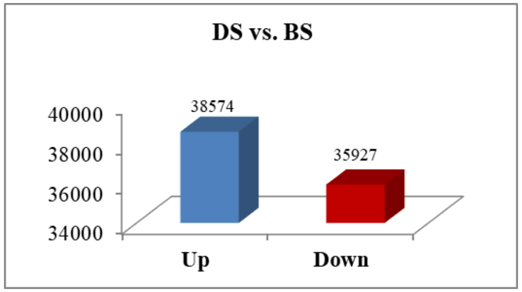
Transcriptome sequence reads in the Illumina system (MiSeq, Genomed).

Quality and integration level assessment (Agilent 2100 Bioanalyzer and Expert 2100 software),

Transcriptome analysis

Comparative bioinformatics analysis of cDNA libraries -’Bluecropp’ (BS) and, Duke’ (DS) (heat map, volcano – p value)
Functional annotation of DEG’s to the KEGG
(Kyoto Encyclopedia for Genes and Genomes) database
Mapping to reference genome
(https://www.vaccinium.org/analysis/49)
- Searching for significant sequences in Uniprot protein databases - BLASTX and BLASTP.
- Final processing of results – edgeR package

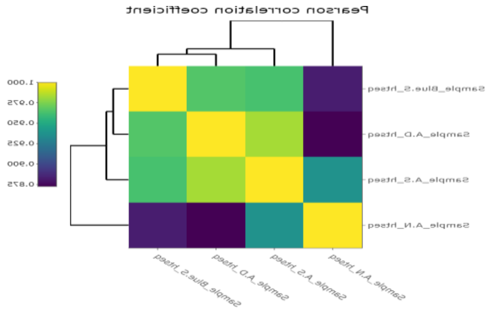




Expression profiling DEG’s

Thermal profile:
95 °C for 5 min. –activation, 15 s
95 °C – sample denaturation,
60 °C annealingfor 20 s – 40 cyl.

Number of transcript calculation: amplicon conc. in each qPCR cycle, Rotor-Gene 6000 Series Software v. 1.7 (Corbett).



The relative expression calculation: $2^{-\Delta\Delta Ct}$ method.
Data visualization: GraphPadPRISM 8.1, calculated in comparison to the ‘Bluecrop’ and normalized to the VcGAPDH reference gene, assigned as the mean standard error ±SEM.

3. Results and Discussions

The comparison of 6 cDNA libraries, obtained from the transcriptomes of fruit peal of ‘Duke’ and ‘Bluecrop’ (producing fruits with low and intensive wax coat, respectively), over 74,500 genes were identified (38,574 - overexpressed and 35,927 -inhibited).

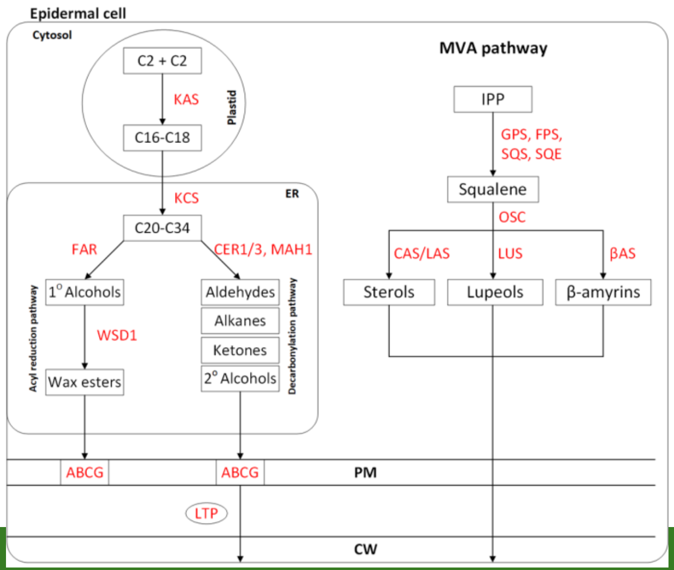
GO enrichment analysis

Three main functional groups.
BP - biological processes (totaly 1,199 genes),
CC- cellular components (totaly 245 genes)
MF - molecular factors (totally 626 genes)

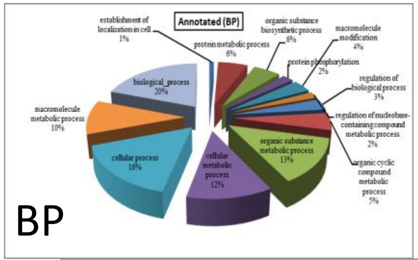
KEGG enrichment analysis

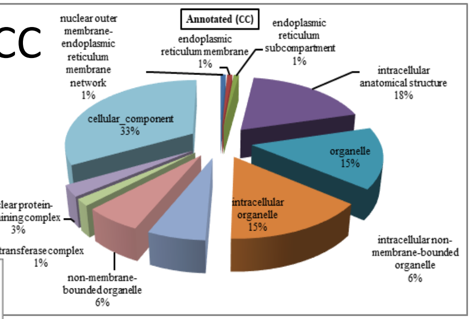
For the gene expression profiling we have selected: **CER3-like** (fatty acid acyl-CoA reductase), **WSD1** (wax degradation protein), **VcAldOx** (aldehyde oxidase) and **VcAlcoDeh**, (alcohol dehydrogenase) - fatty acid decarboxylation process

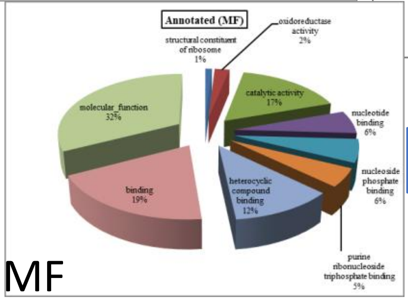
Cutin and wax biosynthesis pathway



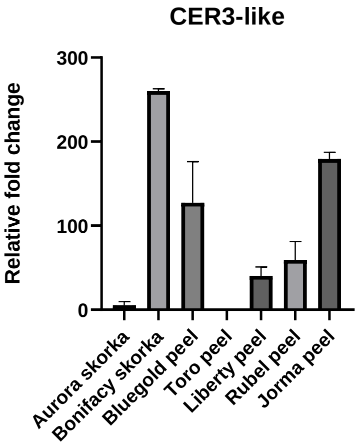
Structure of transcriptome data

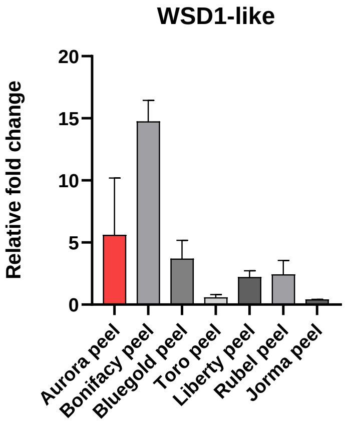






Validation of DEGs



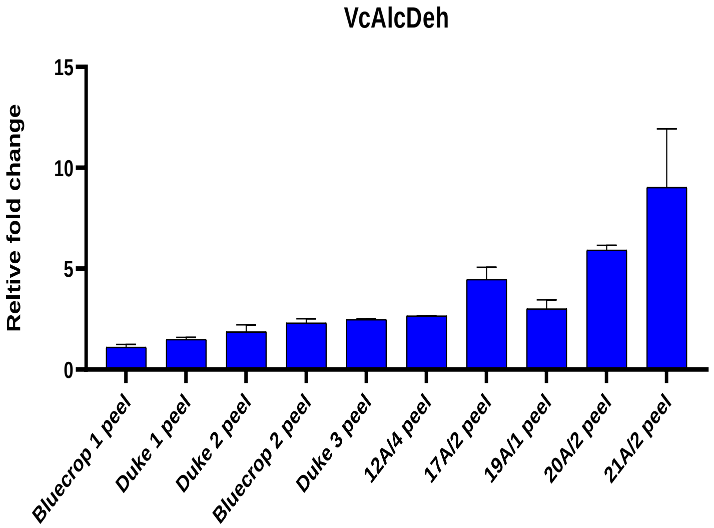


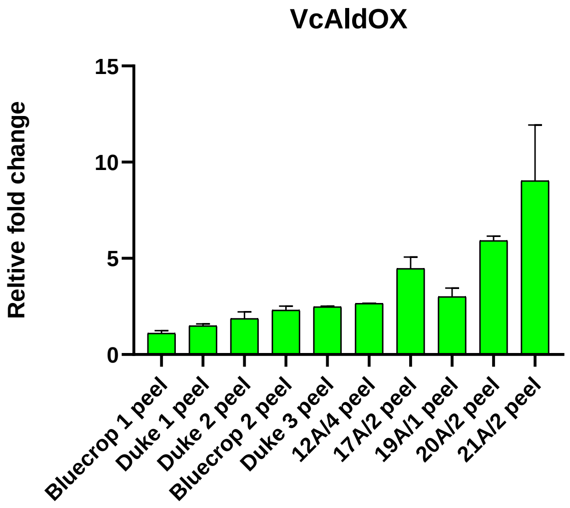
Cv. – waxy fruits ‘Boniface’ and ‘Bluegold’

- fatty acyl-CoA reductase domain (**CER3-like**), 260- and 140-fold increase in the activity
- **WSD1** gene in samples collected from these varieties was 2-15 times higher, respectively.

3. Results and Discussions (cont…)

Expression profiling of selected genes performed for the fruit peel samples of hybrid cultivated in InHort: ‘Bluecrop’, Duke 1, Duke 2, Duke 3, 1A/1, 2A/1, 12A/4, 17A/2, 19A/2, 20A/2, 21A/2.





Observations:

significantly high level of activity of both selected genes in the fruit samples of cultivars producing fruits with lower wax coating: clons developed at InHORT: 17A/4, 19A/1, 20A/2, 21A2.

4. Conclusions and perspectives

1. Activity of the selected genes from wax biosynthesis pathway directly depend on the genotype studied and the level of waxiness intensity of highbush blueberry fruit.
2. We confirmed the role of specific genes activity regulating mechanism of wax coat formation on the fruit surface of the blueberry cultivars from the breeding collection.
3. Selected genes seems to be potential functional molecular markers, and could be applied for marker assisted selection (MAS breeding process) in monitoring waxy fruit trait of highbush blueberry.

Acknowledgements

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