Single berry development- a new phenotyping and transcriptomics paradigm

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Abstract

Present knowledge on berry development mostly arises from sampling and averaging hundreds of berries representing their diversity in the plot. According to recent studies, such chimeric samples formed from non-synchronized berries are unsuitable for detecting physiological changes faster than two weeks. It is thus necessary to revisit in-depth the physiological and transcriptional bases of berry development.

Over a three-month period, berry expansion characterized through image analysis was adjusted to a noticeably invariant (from-berry-to-berry) double sigmoid model. From this analysis, the second growth period lasts only three weeks on single fruits instead of five on non-synchronized samples. Hundreds of berries were then individually analyzed for tartaric and malic acids, glucose, fructose, and K^+ concentrations to calculate their respective accumulation rates with unprecedented precision. These individual fluxes allowed us to distinguish eleven developmental stages, during which specific pathways were switched ON or OFF. In all investigated genotypes, the new fluxes of malate and sugar quantitatively argue for the activation of a sucrose/H^+ exchange, providing a considerable sink strength during ripening. Finally, an RNAseq study was conducted on single berries from each physiological stage. Triplicates were well resolved on PCA plots of gene expression, while single berries inside the stages remained almost indistinguishable.

Switch genes abruptly set ON or OFF were easily identified and expressed explicitly during:
(i) the successive synthesis of tannins, tartaric, and then malic acids during the green phase;

(ii) the sudden activation of the apoplasmic pathway of phloem unloading of water and sugar in the pericarp, and the breakdown of malic acid during the second growth phase, in line with the immediate expression of specific membrane transporters and primary pump genes, indicating strong compartmentation control on berry ripening;

(iii) these genes were switched off with phloem unloading as abruptly as they were induced.

Moreover, genes inside multigenic families, such as cell wall-related proteins or aquaporins, were differentially expressed between the two growth phases.

Single berry monitoring evidenced sharp developmental phases and enlighted the mechanism underlying the malate/sugar ratio evolution during ripening.

New, high-throughput single berry phenotyping methods are now required to compare unambiguous developmental stages in genetic studies.

Keywords: grapevine, fruit development, image analysis, RNA-Sequencing, gene regulation