APPLICATIONS OF A NOVEL MOLECULAR PHENOLOGY SCALE TO ALIGN THE STAGES OF GRAPE BERRY DEVELOPMENT

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Abstract:

Context and purpose of the study

Phenology scales widely adopted by viticulturists (i.e., BBCH or modified E-L systems) are classification tools that describe seasonal and precisely recognized stages of fruit growth and development based on specific descriptors such as visual/physical traits or easy-to-measure compositional parameters. Although some stages can be unequivocally described (e.g., fruit set, veraison), defining comparable developmental stages, from berry formation to full ripening, for grapes of the same cultivar when grown in different conditions or for grapes of different cultivars can be challenging. In this work, molecular-based information was accessed to build a Molecular Phenology Scale (MPhS), suitable to map the ontogenic development of the fruit with high precision.

Material and methods

We exploited the transcriptomic data generated from grape berries of different cultivars sampled weekly from vines grown in the same location over consecutive vintages and focused on conserved annual dynamics rather than on the biological significance of the expression program. The statistical pipeline that was applied consisted of an unsupervised learning procedure yielding an innovative combination of semiparametric, smoothing, and dimensionality reduction tools. By interpolating the transcriptomic samples dispersed in a three-dimension Principal Component Analysis (PCA) we built a 30-stage MPhS that was then used to align samples from several grape berry transcriptomic datasets featuring comparisons between different treatments or growing conditions.

Results

The transcriptomic distance between fruit samples was precisely quantified by means of the MPhS that also enabled to highlight the complex dynamics of the transcriptional program over berry development computing the variation rate of the MPhS stages by time. The performance of the scale was assessed projecting both RNA-seq and microarray transcriptomic samples onto the MPhS. The results allowed to align samples on the MPhS and to highlight differences related to variables like the grape variety, the cultivation site, the vintage, or applied treatments e.g., cluster thinning, defoliation, water limitation and temperature regimes.
The MPhS allowed aligning time-series fruit samples and proved to be an advanced method for defining the stage of grape berry development with higher detail compared to classic time- or phenotype-based approaches.

Keywords: Molecular phenology, berry development, phenological scales, transcriptomics. growth stage.

1. Introduction

Fruit growth and development consist of a continuous succession of physical, biochemical, and physiological changes driven by a genetic program that dynamically responds to environmental cues. Developmental expression patterns are extremely dynamic and, especially under fluctuating environmental conditions (Alderman et al., 2017; Menzel et al., 2006; Nissanka et al., 2015), rapid changes may happen within short time windows, challenging the setup of meaningful comparisons to study the fruit response to any factor.

Establishing recognizable stages over the whole fruit lifetime represents a fundamental requirement for fruit crop research and cultivation. This is especially relevant in perennial crops like the grapevine to scale the development of its fruit across genotypes and growing conditions.
The development of the grape berry is tracked by adopting phenological scales describing seasonal and precisely recognized stages of fruit growth and development based on specific descriptors such as visual/physical traits or easy-to-measure compositional parameters (Gillaspy et a., 1993; Labadie et al., 2019). The most adopted phenology scales, namely the modified E-L and the extended BBCH systems (Coombe, 1995; Lorenz et al., 1995), are widely used in models describing known or hypothetical cause-effect relationships between growth stages progression and environmental driving factors (Hess et al., 1997; Parker et al., 2011, 2020). However, the precise definition of developmental stages can be challenging as the fruit traits used for stage description are highly influenced by genotype, climate, water availability, agronomical practices, and crop load (Parker et al., 2015; Pastore et al., 2017; Sadras et al., 2012).
The advent of next-generation sequencing represents an opportunity to exploit the expression kinetics of large sets of genes to stage fruit development and enrich the available classification systems incorporating information at a molecular level (Chuine et al., 2017; Gildor et al., 2018).
In this work, we used the most informative portion of several grapevine fruit transcriptomic datasets (Fasoli et al., 2018; Massonnet et al., 2017) to build a molecular phenology scale. The MPhS was used to reinterpret previously published transcriptomic datasets and to evidence its potential to trace and compare fruit developmental stages from different genotypes, growing conditions, vintages and applied treatments.

2. Material and methods

We used the data described in Fasoli et al. (2018). We removed genes exhibiting uninteresting profiles (i.e., no expression in some experimental conditions or expression not associated to berry development) from the 29,971 in the grapevine transcriptome, based on the criteria described in Tornielli et al. (2023). This screening step exploited the information of an additional dataset (Massonnet et al., 2017), composed of 10 cultivars, observed for 4 timepoints during fruit development in a single vintage. The resulting dataset comprised 10,129 genes.
This step was followed by the application of a local polynomial regression that allowed smoothing the gene expression patterns over the time points and averaging the replicates. We then performed a PCA with the data matrix obtained by column-standardization of the smoothed gene expression and extracted six PCs accounting for a 91% explained variance. We selected Principal Components (PCs) 1 and 2 that best described the general progression of berry development, and PC5 that improved the discrimination of early-stage samples, whereas the effect of genotype and vintage (PC3, 4 and 6) was excluded. The PC1, 2 and 5 defined a three-dimensional scatter of points, with each point corresponding to an experimental condition (one time point for one cultivar in one year) that were then fitted by one-dimensional space using a Bézier curve (Rabut, 2002). Thirty marks were evenly distributed along the curve to represent steps of the MPhS. To map the same transcriptomic samples used to build the MPhS, the points of the 3D scatter were projected onto the MPhS and assigned to the closest among the set of 30 evenly spaced marks previously identified along the curve.

The MPhS was used to align samples from several transcriptomic datasets featuring comparisons between berry development of different varieties, treatments, or growing conditions. The relative samples were mapped onto the MPhS using the core set of 10,129 genes selected for the scale definition. The procedure to project observations coming from different case studies onto the MPhS is fully described in Tornielli et al. (2023).

3. Results and discussion

3.1. Molecular phenology map creation

To create a molecular scale of grapevine berry development we relied on the RNA-sequencing dataset consisting of 219 samples published by Fasoli et al. (2018). Samples were collected from fruit set to full maturity from Cabernet Sauvignon (CS) and Pinot noir (PN) vines every 7–10 days across three years. The technological

and molecular data, and the related methodologies are reported in the original paper. the transcriptomic data was analyzed using statistical and data mining tools to identify the core set of genes that define the berry development progression. The comparison with the transcriptomic dataset of Massonnet et al. (2017) was also part of the initial screening to ensure the congruency of gene expression in white and other red skinned grapevine varieties. Fitting the information of a three-dimension PCA by one-dimensional space using a Bézier curve, we defined thirty evenly distributed marks along the curve to represent steps of the MPhS (Fig. 1). Time information was exploited by only considering timepoint succession disregarding their distance so the MPhS units are not time but ideal steps of the berry development, which can take longer or shorter, providing the flexibility of accounting for a multiplicity of factors.

Plotting the MPhS by the day of the year (DOY) confirmed differences among years and evidenced a transcriptional progression delayed in 2012 in both varieties, whereas the alignment on the phenological flowering phase (days after flowering, DAF) resulted in nearly overlapping curves (Fig. 2). This representation unraveled common kinetics, reflecting a non-linear relationship between time and MPhS stages with some MPhS stages that were more rapidly passed through than other by developing berries. For both varieties the highest rate of variation of the MPhS stage by time was recorded well prior the assessed veraison stage, confirming that the ripening transcriptional program is established one-to-two weeks before berry phenotypic changes can be visually appreciated (Fig. 2).

3.2. Mapping other transcriptomic samples onto the MPhS

The performance of the MPhS was tested for previously published berry transcriptomes (performed by RNAseq or microarray platforms) describing berry development for different varieties and at varying growing conditions. The relative samples were mapped onto the MPhS using the core set of 10,129 genes selected for the scale definition. Mapping the samples from the work of Dal Santo and co-authors (2018) that explored the genotype by environment interaction (GxE), revealed that berries of both Sangiovese and Cabernet Sauvignon collected at the pre-ripening stage were rather misaligned, mapping between stages 17 and 21 of the MPhS. Moreover, this analysis highlighted that the cultivar Sangiovese reached maturity at different MPhS stages by cultivation site and year, whereas Cabernet Sauvignon samples appeared much more aligned (Fig. 3A), affirming the marked transcriptomic plasticity of Sangiovese.
We then projected onto the scale transcriptomic datasets from studies comparing the effect of different environmental conditions (e.g., water availability, air temperature) or agronomic treatments (e.g., cluster thinning, defoliation) on the transcriptome of berries collected at various developmental stages. In Fig. 3B, the projection of the transcriptomic samples of four berry developmental stages of cv Sangiovese vines subjected to Pre Flowering Defoliation (PFD) treatments (Zenoni et al., 2017) versus control are shown. Such projection clearly revealed that samples from defoliated vines collected at stages 2 (hard and green berries at veraison) and 3 (soft, yet still not colored berries at veraison) were at an advanced developmental stage compared to the corresponding samples collected from untreated vines. However, at ripening (sugar level 18 °Brix) such advance evened out, and samples from defoliated vines resulted aligned with control.

4. Conclusions

In this study, we selected about ten thousand genes from several transcriptomic datasets for their consistent expression throughout berry development regardless genotype and season and built a molecular phenology scale to map the ontogenetic development of the fruit with high precision and to align berry development of different grapes. The projection of samples from various transcriptomic studies highlighted shifts of fruit development driven by factors like the genotype the cultivation site, the vintage, or applied treatments such as cluster thinning, defoliation, water limitation and temperature regimes, allowing the precise assessment of the ‘transcriptomic distance’ between samples.

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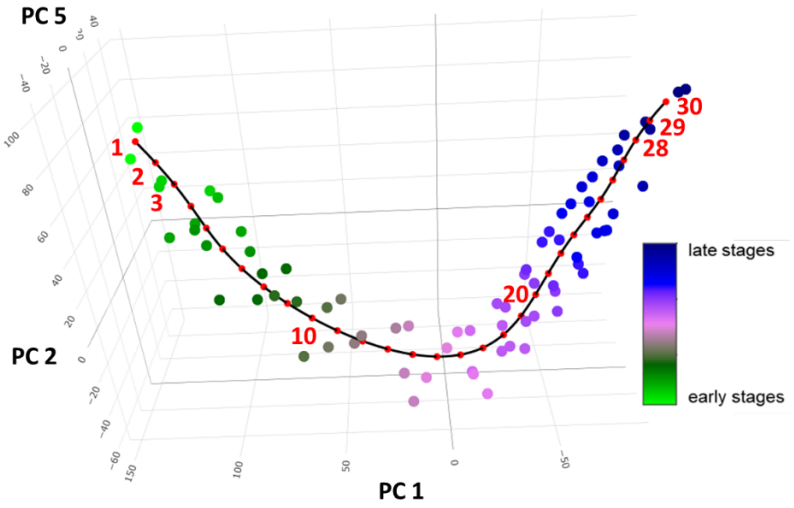

Figure 1: Molecular phenology scale (MPhS) creation using Cabernet Sauvignon (CS) and Pinot noir (PN) time series of berry transcriptomic sample collection (Fasoli et al., 2018). Three-dimension scatterplot of the three selected PCs interpolated by the Bézier curve (black line). Red dots along the Bézier curve define a set of 30 evenly spaced molecular stages (indicated in red). Scattered points correspond to the smoothed samples and changing color highlight berry progression from immature to ripe stages. An interactive plot of the curve is accessible at the link https://bodai.unibs.it/grapevine-gea/mphs/.

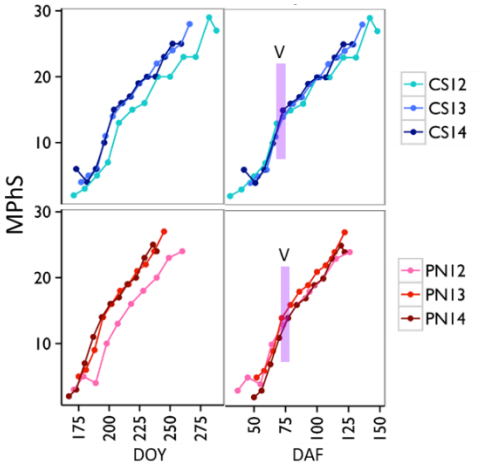

Figure 2: Relationship between Molecular Phenology Scale and time during fruit development. Trends of MPhS stages by day of the year (DOY) (left) and day after flowering (DAF) (right) in CS and PN transcriptomic samples projected on the scale, over the three years. 'V' indicates the veraison time.

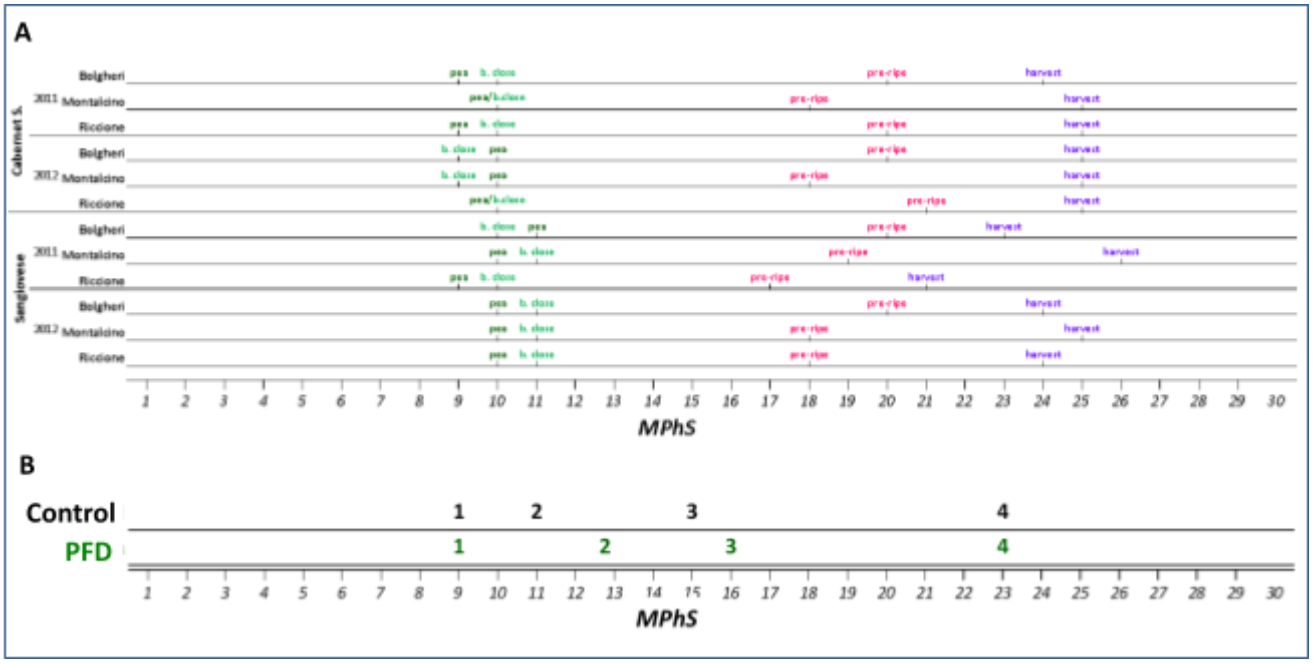

Figure 3: Examples of scaling berry transcriptomic samples onto the MPhS. **(A)** Projection of transcriptomic berry samples of cv Sangiovese and cv Cabernet Sauvignon compared across three growing sites and over two years (Dal Santo et al., 2017). Berries were collected at four developmental stages defined by the BBCH phenological scale: Pea Size (BBCH 75), Bunch Closure (BBCH 79), Pre Ripening (BBCH 83) and Harvest (BBCH 89). **(B)** Projection of transcriptomic berry samples of cv Sangiovese berries sampled from control and Pre Flowering Defoliation (PFD) treatments (Zenoni et al., 2017) at four development stages: post-fruit set (Stage 1); hard and green berries at veraison (Stage 2); soft, yet still not colored berries at veraison (Stage 3); berries at ~18°Brix (Stage 4).