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A MOLECULAR PHENOLOGY SCALE OF GRAPE BERRY DEVELOPMENT

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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. Establishing recognizable stages over the whole fruit lifetime represents a fundamental requirement for research and fruit crop cultivation. This is especially relevant in perennial crops like grapevine (*Vitis vinifera L.*) to scale the development of its fruit across genotypes and growing conditions.

MOLECULAR PHENOLOGY MAP CREATION

We applied the here summarized pipeline to a highly comprehensive RNA-sequencing dataset. We identified core transcriptomic traits and three PCs that best described the general progression of berry development. The three PCs defined a three-dimensional scatter of points, with each point corresponding to a specific berry samples. Points were fitted by one-dimensional space using a Bézier curve (black line). Then, thirty marks (red dots) were evenly distributed along this curve to represent stages of what we call the Molecular Phenology Scale (MPhS; Figure 1). **The MPhS allowed the alignment of time-series fruit samples proving to be a complementary method for mapping the progression of grape berry development with greater classification detail** compared to classic time- or phenotype-based approaches (Figure 1).

Details on the MPhS creation have been published in Tornielli *et al.*, 2023

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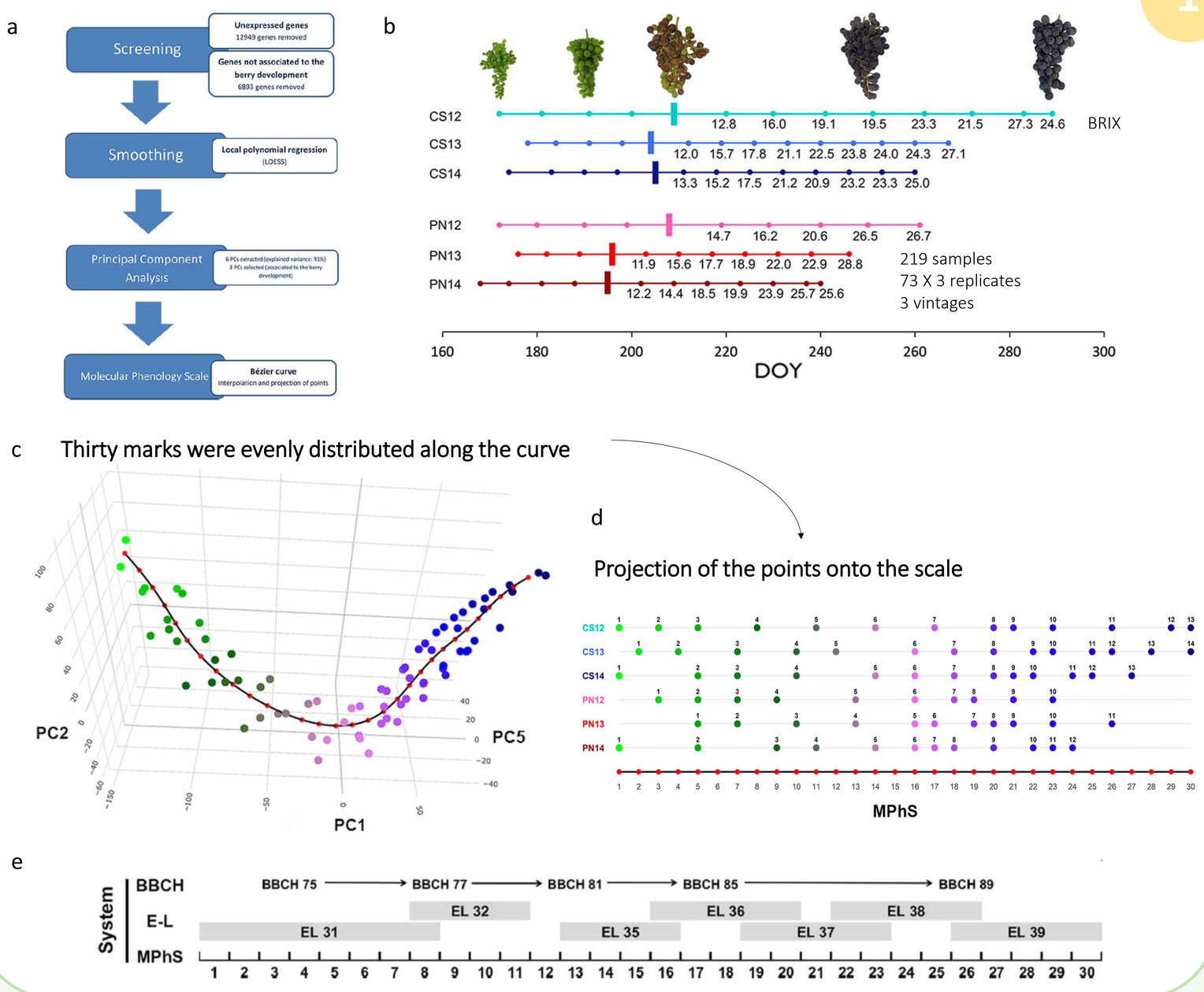
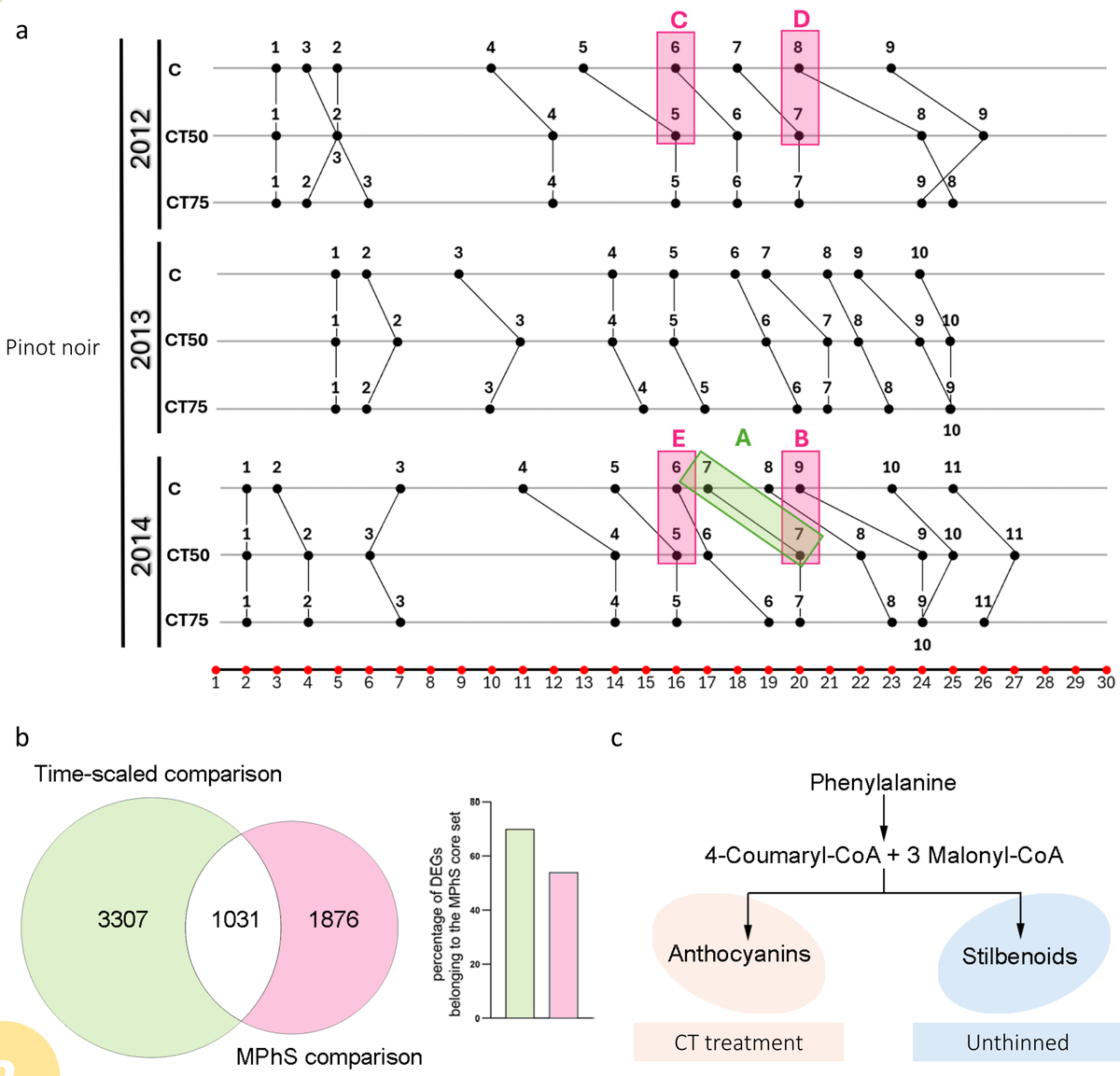


Figure 1: a. Flow chart of the pipeline; b. Cabernet Sauvignon (CS) and Pinot noir (PN) time-series of berry sample collection (Fasoli et al., 2018); c. Three-dimension scatter plot of the three selected PCs interpolated by the Bézier curve (blackline); d. Projection of the smoothed three-year time series of CS and PN to the closest point among the 30 points identified along the Bézier curve. The sample number is reported above each point. Red dots correspond to the 30 steps of the MPhS; e. Alignment between MPhS and two phenotype-based phenological scales (BBCH and the modified E-L) during berry development. .

THE MPhS DEFINES MOLECULAR EVENTS IN GRAPE BERRIES BEYOND DEVELOPMENT PROGRESSION



The MPhS application defines the shifts of fruit development driven by various agronomic and environmental factors (i.e. cluster thinning, defoliation, water limitation, and temperature). In the reported example, the transcriptomic data mapped on the MPhS were retrieved from experiments that compared vines at two thinning levels and unthinned ones in cv. Pinot noir. **The MPhS projection indicates an advanced maturation in response to cluster thinning** (Figure 2a).

By statistical analysis we assess the MPhS potential for emphasizing molecular responses specific to the crop load modulation. In a preliminary analysis focused on samples collected over the 2014 season, we determined and intersected the DEGs between sample 7 of the CT50 sets (CT50_7) with i) its time-based control (C7; comparison A) and ii) its MPhS-based control (C9; comparison B) (Figure 2b). **Comparing grape samples aligned according to the MPhS, it is possible to identify molecular responses genuinely related to the variable factor, beyond any anticipation or delay in the developmental progression** (Figure 2b).

Genes differently modulated in at least one MPhS-aligned comparisons (Figure 2a, B-E comparisons) were investigated and **a differentiation of the phenylpropanoid pathway branches was observed between the two sample sets** (Figure 2c).

Figure 2: a. Projection of transcriptomic datasets of berries collected from Pinot noir cultivar following following no thinning (C), 50% thinning (CT50) and 75% thinning (CT75) at fruit set. Red dots correspond to the 30 stages of the MPhS. Numbered black dots indicate berry samples. Colored rectangles indicate samples taken at the same sampling time (A; green) and samples aligned on the same MPhS stage (B-E; pink) which were investigated for DEGs employing a *t-test*; b. Venn diagram of DEGs identified in comparisons A and B described in Figure 3a. The histogram reports the percentage of specific DEGs belonging to the MPhS core set of genes; c. Differentiation of the phenylpropanoid metabolic branches for cluster thinned and control samples.

Given the global importance of grapevine and the strict relationship between the quality of the grape berries and the environment, the precise definition of fruit growth stages is of crucial interest to develop and apply mitigation strategies, especially in the context of climate change which is anticipating the ripening with a negative impact on quality and on the overall sustainability of the winegrowing sector. It is our efforts to make the R-based MPhS scripts fully available to all users for their research purposes and applications.

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