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Dissecting foliar physiology and chemical properties with integrated high-throughput phenotyping and molecular markers in grape improvement

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Summary

An advanced phenotyping protocol using a hyperspectral spectrometer to better understand foliar chemical composition and physiological processes in relation to grapevine yield, quality, biotic and abiotic resistance was initiated with in-house and public spectral resources. The initial result for foliar pigments calibration was promising for classification and measurements to support breeding. We demonstrated the potential of adapting public spectral resources in supporting modern phenotyping in programs with limited resources and when combined with our current effort in deploying marker-assisted selection, the dual innovations provide new information to fast-tracking grapevine research and trait improvement.

Key words

Grapes, foliar, chemical composition, breeding, high-throughput phenotyping, hyperspectral, QTL

Introduction

Plant breeders measure a range of attributes from various plant tissues, organs or systems in making selection decisions. The leaves contain important biochemical components that determine numerous physiological processes. Leaf pigments such as chlorophyll are essential in the conversion of the atmospheric light into energy and indirectly affect production and yield (Filimon *et al.*, 2016; Oláh *et al.*, 2010). Leaf structural and chemical compositions impact resistance to diseases and pests, and provide information on nutrition and abiotic stress status (Manning and v. Tiedemann, 1995; Wang *et al.*, 2001). In grapes, considerable efforts have been invested in understanding processes and factors that influence the accumulation and distribution of organic compounds and their

relative impact on vine yield and fruit quality (Bubola *et al.*, 2020; Gil-Muñoz *et al.*, 2009; Salazar-Parra *et al.*, 2018). The differences in the composition, rate and efficiency of some important physiological processes as well as responses to biotic and abiotic factors are likely regulated by variations in genotypes and various genotype interactions (Das and Agarwala, 2011; Moreno *et al.*, 2017). Also, various trait interactions at different phenological stages contribute to varying products in terms of trait composition and concentrations. However, the efficient, large-scale and cost-effective phenotyping measures to support these investigations are still underdeveloped, especially in grapes.

The optical properties of plants including the leaves permit the modern adoption of spectrophotometers in acquiring information to promote greater efficiency and throughput in crop improvement. Hyperspectral spectrophotometers provide a fine resolution of hundreds to thousands of spectral bands in the visible light spectra as well as the near-infrared and short wavelength infrared, enabling the simultaneous characterization of a material's composition (Adão *et al.*, 2017; Li *et al.*, 2017). It reduces the cost of characterizing individual chemical composition and minimizes the laborious processes associated with the alternative chemical laboratory extraction methods while saving time. It has been adopted in improving phenotyping accuracy and has an added advantage of expanding the scope of measurable parameters needed in making improvement decisions. Often, when combined with DNA marker information, it provides an opportunity to meet the demands for more resilient, sustainable and excellent quality products (Ikeogu *et al.*, 2019; Juliana *et al.*, 2019; Marsh *et al.*, 2021).

We present the initial effort in integrating a hyperspectral spectrophotometer to improve phenotyping throughput and expand trait measurements on grape leaves. Calibration results are presented for pigments here. In the absence of the full resources for deploying spectral technology, there are



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various publicly available resources that could be utilized in promoting spectral-based phenotyping where they do not adequately exist (Serbin *et al.*, 2014; Singh *et al.*, 2015; Z. Wang *et al.*, 2020). We derived total phenolics for a mapping family with spectral data using a coefficient from the Ecological Spectral Model Library (EcoSML) and Ecological Spectral Information System (EcoSIS) supported by the National Aeronautics and Space Administration (NASA). The newly derived total phenolics were used for quantitative trait loci (QTL) analysis with the newly developed *rhAmpSeq* markers for grape improvement (Karn *et al.*, 2021; Zou *et al.*, 2020). The integration of hyperspectral-enabled high-throughput phenotyping and marker-assisted selection provide a great opportunity to accelerate the understanding and improvement of novel and complex processes/traits in grapes. Information on leaf attributes and their interactions to other traits and processes at different phenological stages could be beneficial in early performance evaluation and increased selection accuracy.

Material and Methods

Spectral data were collected using an SVC-HR1024i device from the Spectra Vista Corporation (SVC) that measures between the 350 nm – 2500 nm range and covering the visible, near-infrared, and shortwave infrared range. Between 1-2 scans were measured per leaf and between 3-6 newly expanded leaves were measured per vine in 2020 and 2021. The scanned leaf samples were collected and sent to the laboratory at the Department of Entomology, Purdue University, for pigment quantification as well as other metabolites – sugars, phenolics, flavonoids, etc. Laboratory chemical extraction and quantification for pigments was carried out using HPLC. Data were collected from a diverse panel of grapevines including two mapping families – ‘Horizon’ × Illinois 547-1 and NY84.0101.03 × *Vitis amurensis* ‘PI 588634’) and a subset of the cold-hardy *Vitis* repository of the Plant Genetic Resources Unit (PGRU), USDA-ARS, Geneva, New York, USA.

For pigment calibrations, the total calibration set (n~110) was divided into training and validation sets at a ratio of 70%:30%. A calibration model was developed with a partial least square regression on the training set and the performance of the model was validated on both the training and the validation sets. Accuracy was reported as the correlation between the predicted values and the laboratory extracted values in both the training and the validation sets.

Also, we used total phenolics coefficients from the EcoSIS (<https://ecosis.org/>) to derive values for the spectral data collected on the Horizon × Illinois 547-1 mapping family for 269 and 241 vines in 2020 and 2021, respectively. QTL analysis

was performed with the newly derived foliar total phenolics in 2020 and 2021. The calibration and QTL analyses were performed using *caret* and *rQTL* packages in RStudio on R environment (Arends *et al.*, 2010; Broman *et al.*, 2003; R Core Team, 2022; RStudio Team, 2021)

Results

Appreciable variation exists across the samples used for developing foliar pigment calibrations – neoxanthin, violaxanthin, lutein, zeaxanthin, chlorophyll a, chlorophyll b and beta-carotene (Table 1). The correlation between actual and predicted values in the training (n~79) and the validation sets (n~32) for the foliar leaf pigments ranged from 0.46 to 0.99 in the training and 0.28 to 0.63 in the validation sets (Fig. 1). The initial correlations obtained are valuable for selection in breeding for these traits. There is room for improving the accuracy by adding more calibration samples and the adoption of modern machine learning tools.

A QTL near 1 Mb on chromosome 11 was detected for foliar total phenolics in both 2020 and 2021 (with peak markers chr11_601198 and chr11_1025585, respectively). The total variation in foliar total phenolics explained by the QTL was 9% in 2020 and 27% in 2021 (Table 2).

Conclusion

The integration of high-throughput phenotyping and marker-assisted selection using cutting-edge technologies is im-

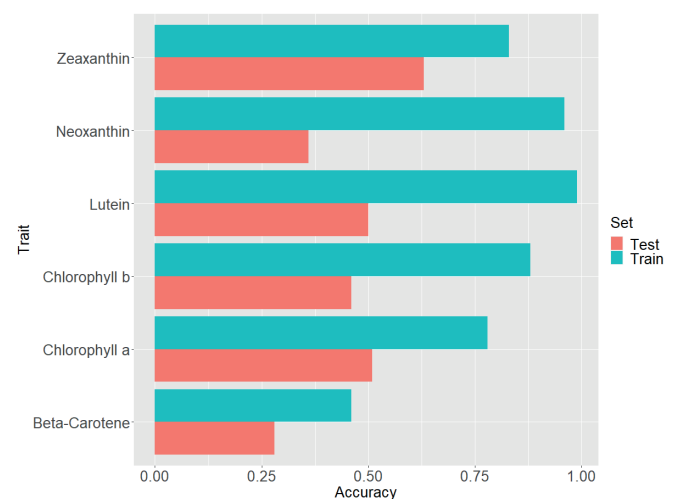


Fig. 1: Prediction accuracy of grape pigments in the training and validation/test sets.

Table 1: The minimum, maximum and mean values of pigments in milligrams per gram (mg/g) dry weight used for calibration

	Beta-carotene (mg·g ⁻¹)	Chlorophyll a (mg·g ⁻¹)	Chlorophyll b (mg·g ⁻¹)	Lutein (mg·g ⁻¹)	Neoxanthin (mg·g ⁻¹)	Violaxanthin (mg·g ⁻¹)	Zeaxanthin (mg·g ⁻¹)
min	0.000029	0.002472	0.091928	0.147608	0.050851	0.022923	0.036294
max	0.002646	2.691116	0.416821	0.598884	0.141906	0.222469	0.406629
mean	0.000219	0.942902	0.172628	0.309721	0.087422	0.053194	0.161033

Table 2: The QTL for total phenolics in 2020 and 2021

Year	n	Marker	Chr.	Pos (bp)	Lod score	Threshold (5%)	Percentage Variation
2020	269	11_601198	11	601198	5.32	4.28	8.70
2021	241	11_1025585	11	1025585	16.2	4.23	26.68

portant in the modern grapevine genetic improvement. The expanded information from foliar composition could enhance selection accuracy and deepen the breeders' understanding of factors and processes that influence grapevine yield, quality and resistance to adverse biotic and abiotic effects. The use of publicly available resources could drive meaningful research and provide information to programs with limited resources. Validation of derived values is being investigated as more laboratory quantifications become available.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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