POLYPHENOL ACCUMULATION IN VITIS SPECIE LEAVES DURING THE VEGETATIVE SEASON

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POLYPHENOL ACCUMULATION IN *VITIS* SPECIE LEAVES DURING THE VEGETATIVE SEASON

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INTRODUCTION

Grapevine has approximately 60 species; *Vitis vinifera* is the most known and widely cultivated specie with high productivity and specific traits in berry characteristics that have favoured the spread of viticulture in the world. American and Asian *Vitis* species generally present low productivity and low berry quality, but they often possess good tolerance to environmental abiotic (for instance drought, to cite one) or biotic stressors (Li *et al*., 2014). Reasons of resistance or low susceptibility to biotic stressors have to be searched also in vegetative organs (leaves, stems, shoots) beyond that in berries. Moreover, vegetative organs are used in traditional medicine as a good source of bioactive polyphenolic compounds and they are known to display several health beneficial effect (Ali *et al*., 2010). Yet, knowledge of individual polyphenolic compounds and their accumulation trend during the season in different species is scarce.
Therefore, we have undertaken a study regarding the quantification and identification of main polyphenols in the healthy leaves of a number *Vitis* species during the vegetative season. In this work we report results of one Asian specie - *Vitis amurensis* (**Fig. 1**) and one American specie – *Vitis berlandieri* (**Fig. 2**). *Vitis amurensis* is highly cold and disease-resistant, displaying low susceptibility to downy mildew and powdery mildew (Liu *et al*., 2013); *Vitis berlandieri* is resistant to *Phylloxera*, tolerant to chlorosis (Ollat *et al.* 2016) and blood of many commercially used rootstocks.

EXPERIMENTAL OUTLINE

Healthy leaves were sampled in the collection vineyard of DISAFA, University of Turin located at Grugliasco (Piedmont, Italy) at five different time points: 1 - 28th of May (DOY 148), 2 – 22nd of June (DOY 173), 3 – 14th of July (DOY 195), 4 – 3rd of August (DOY 212), 5 - 28th of August (DOY 240) in 2015. Leaves were immediately transported to the laboratory, where blades and veins were separated and stored until further analysis at -20 °C. After the extraction of polyphenols with a suitable solvent, specifically optimized for leaf tissue, total polyphenols were measured spectrophotometrically. Quantification of individual polyphenolic compounds was performed by HPLC-DAD according to a previously published method (Ferrandino *et al*. 2010). For individual polyphenolic compound identification, the pool of extracts of all sampling dates for each specie blades and veins was prepared and analyzed by HPLC-ESI-MS.

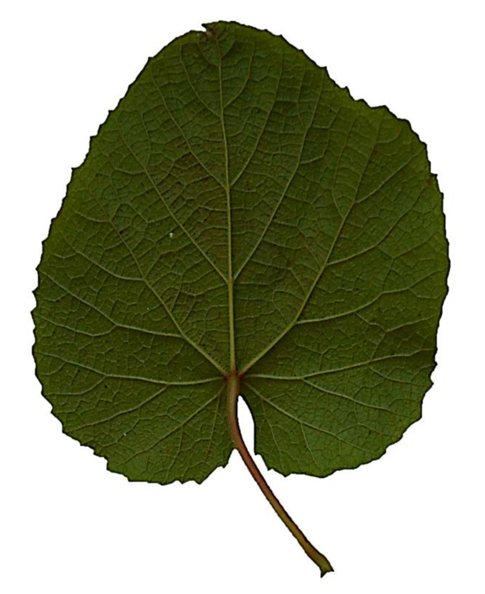

Figure 1. *Vitis amurensis*

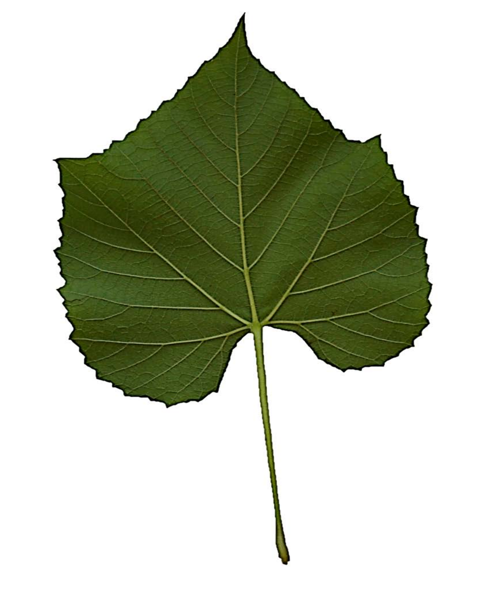

Figure 2. *Vitis berlandieri*

RESULTS AND DISCUSSION

In leaf blades the concentration of total polyphenols ranged from 31.2 to 56.3 g/kg of fresh weight and it was significantly higher in *V. amurensis* during the whole season. Concentration of total polyphenols in leaf veins was similar between the two species and twice lower than in blades (**Fig. 3A**). Total flavonols displayed even greater differences between the two species as to concentrations, trends (**Fig. 3B**) and profiles (**Fig. 4).** In *V. amurensis* concentration of total flavonols increased until DOY 215 (3rd of August), whereas in *V. berlandieri* total flavonols decreased from the beginning of the season onwards (Fig. 3B). In *V. amurensis,* comparing to *V. berlandieri,* total flavonol concentration was significantly higher in blades during all the season and in veins at the first and at the last samplings. Moreover, at DOY 215 the concentration in *V. berlandieri* blades and *V. amurensis* veins was practically equal. Remarkably high concentration of flavan-3-ols was found in *V. amurensis* leaf blades, ranging from 530.6 to 1383.8 mg/kg of fresh weight; in *V. amurensis* veins flavan-3-ol concentration was important, sometimes significantly higher respect to *V. berlandieri* veins and blades (**Fig. 3C**).

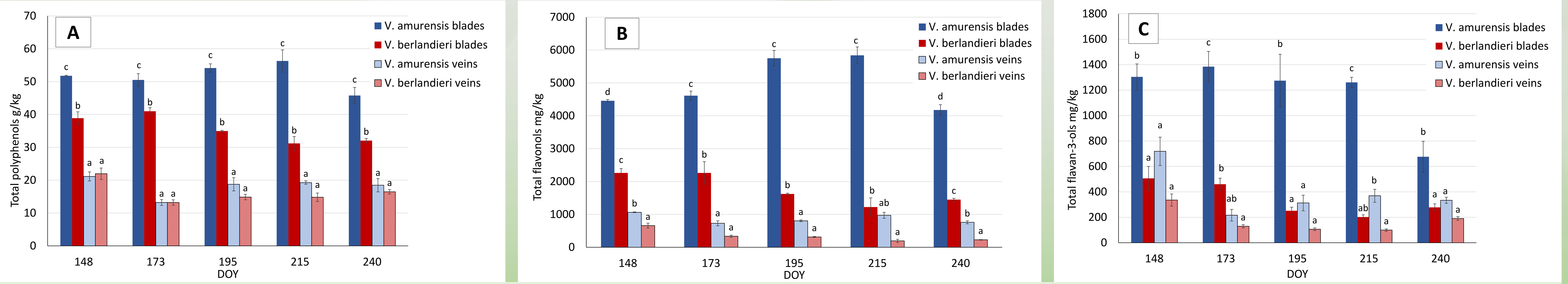

Figure 3. Accumulation of polyphenolic compounds in *Vitis* specie leaf blades and veins during the vegetative season. Total polyphenols (A) expressed as grams of (+)-catechin equivalents per kg of leaf blade/vein fresh weight; ; total flavonols (B) are means of the sum of detected flavonols expressed as milligrams per kg of leaf blade/vein fresh weight; total flavan-3-ols (C) are means of the sum of detected flavan-3-ols expressed as milligrams per kg of leaf blade/vein fresh weight. All data are presented as means ± standard errors (n=3); means are separated by ANOVA and significant differences between specie blades and veins were evaluated by Tukey-B post-hoc test at P ≤ 0.05.

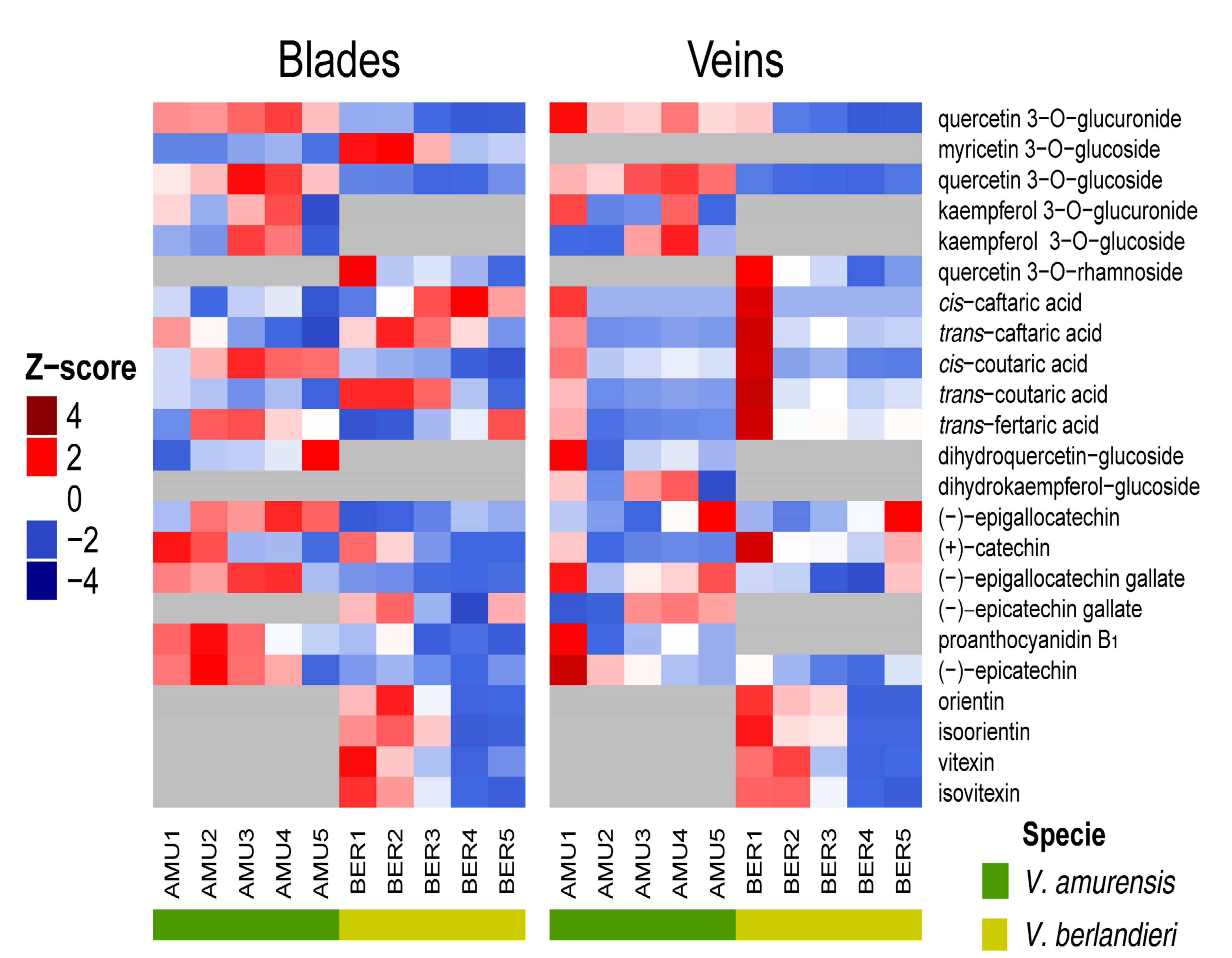

Figure 4. Evolution of polyphenols in the leaves of *Vitis amurensis* and *Vitis berlandieri.* Heatmap of blades and veins represented as Z-scores of each individual compound calculated by subtracting to each average value (variety and date) the general average of the entire population divided by the standard deviation. AMU = *V. amurensis*; BER = *V. berlandieri*. Number after variety acronym refer to 1 – DOY 148 (28th of May); 2 – DOY 173 (22nd of June); 3 – DOY 195 (14th of July), 4 – DOY 215 (3rd of August); 5 – DOY 240 (28th of August).

GENOTYPIC AND SEASONAL PATTERN OF POLYPHENOLIC COMPOUNDS IN LEAVES

Two heatmaps were constructed for the visualization of phenolic compound changes during the vegetative season in blades and veins (**Fig. 4**).

- *V. amurensis* had higher concentration of the two prevalent **flavonols** in leaves – quercetin 3-O-glucoside and quercetin 3-O-glucuronide. Instead, quercetin 3-O-rhamnoside was exclusively detected in *V. berlandieri* that also displayed a higher concentration of myricetin 3-O-glucoside.
- Caftaric acid and *trans*-coutaric acid were prevalent in *V. berlandieri* and *cis*-coutaric and *trans*-fertaric acids were prevalent in *V. amurensis*. Hydroxycinnamates showed a peak of maximum concentration at the first sampling date in veins of both species.
- The accumulation of (+)-catechin, (-)-epicatechin and proanthocyanidin B_1 decreased during the season. On the contrary concentration of (-)-epigallocatechin and (-)-epicatechin gallate increased.
- In *V. berlandieri* leaves we found **flavones,** rarely detected in grapevine and to our knowledge, identified in leaves here for the first time. Vitexin and orientin were prevalent over their isoforms and their concentration progressively reduced during the season **(Fig. 4)**.
- **Flavanonols** were found exclusively in *V. amurensis*: dihydroquercetin-glucoside in blades and veins and dihydrokaempferol-glucoside in veins.

In *V. amurensis* high concentration of total polyphenols, flavonols and flavan-3-ols were detected; veins displayed a high concentration of flavanonols (**Fig. 5**). These peculiarities could contribute to explain the known specie low susceptibility to pathogens and its ability to cope with abiotic stressors.

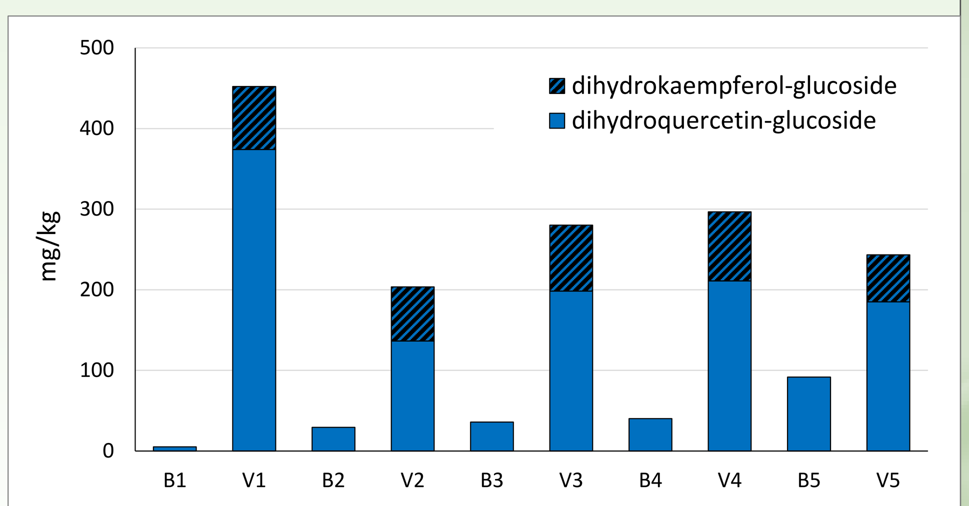

Figure 5. Total flavanonol concentration in *V. amurensis* leaf blades and veins. Results expressed as means (N=3) of the sum of individual flavanonols in mg/kg of leaf blade/vein fresh weight. B –blades, V – veins (numbers stand for DOY, see Fig. 4).

CITED LITERATURE

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