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# Cluster shading modifies amino acids in grape (*Vitis vinifera* L.) berries in a genotype- and tissue-dependent manner

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ABSTRACT

Amino acid composition of the grape berry at harvest is important for wine making. The present study investigates the complex interplay between tissue, cultivar and light conditions that determine berry amino acid content. Twenty amino acids were assessed in the berry skin and pulp of two grape cultivars (Gamay Noir and Gamay Fréaux), grown under either light exposure or cluster shading conditions. In all samples, cluster shading significantly reduced most amino acids, except gamma-aminobutyric acid (GABA) and phenylalanine. However, the magnitude of the decrease was stronger in the skin (67.0% decrease) than in the pulp (30.4%) and stronger in cv. Gamay Noir (69.7%) than in Gamay Fréaux (30.7%). Cluster shading also significantly modified amino acid composition by decreasing the proline content while increasing the GABA content. These results are of oenological interest for shaping the amino acid composition of the must and improving wine quality.



## 1. Introduction

Amino acids account for the majority of nitrogenous compounds in grapes (Kliewer, 1969, 1970) and play an important role in berry quality and nutritional value (Stines et al., 2000). Through direct interaction with the palate, amino acids such as GLU and GABA, bring pleasant sensory feeling to consumers (Yamaguchi & Ninomiya, 2000). Moreover, PHE and branched chain amino acids are precursors of various phenylpropanoids and volatile organic compounds in grape berries, and therefore contribute significantly to grape flavors. The free amino acid content of the berries has significant implications for wine quality. During wine making, amino acids are main nitrogen source for yeasts and are also precursors of aroma compounds (Herbert, Cabrita, Ratola, Laureano, & Alves, 2005; Hernández-Orte, Cacho, & Ferreira, 2002). Because of their importance in determining grape and wine quality, it is of both scientific and economic interest to study amino acid accumulation profile and its responses to viticultural and environmental factors in grape berries.

Light is known to impact the biosynthesis of amino acids in many plants. Leaves of *Arabidopsis thaliana* accumulate more amino acids containing high C/N ratios (such as GLU) in the light, while amino acids with low C/N ratios (such as ASN) accumulated in the dark (Coruzzi, Last, Dudareva, & Amrhein, 2015). Similarly, dark treatment increases ASN in detached leaves of tobacco (*Nicotiana tabacum*, Vickery et al., 1937), wheat (*Triticum aestivum*, Peeters & Laere, 1992), and pea (*Pisum sativum*, Joy, Ireland, & Lea, 1983). These studies focused mostly on the autotrophic leaves, and whether light has a similar effect on amino acid composition in heterotrophic organs such as ripening fruits is still an open question. In grape, the effect of cluster shading on the amino acid profile of berry has not been well documented, although sunlight has been widely acknowledged as one of the most important factors affecting berry composition, especially sugars, organic acids and anthocyanins (Dokoozlian & Kliewer, 1996; Guan et al., 2014, 2016).

The free amino acid profiles in mature berries also vary considerably among berry tissues (skin, pulp and seed), but there are contradictory reports concerning the relative contributions of these tissues. For example, Lamikanra and Kassa (1999) found that seeds contained most of the amino acids in mature 'Noble' berries (50%), followed by the pulp (38%), and the skin (12%). In contrast, in Cabernet Sauvignon and Riesling, most of the berry amino acids are localized in the pulp (66–77%), with only 8.5–11% in the seeds, and 15–23% in the skin (Stines et al., 2000). These tissue-specific differences in amino acid concentration appear to depend on cultivars, which exhibit remarkable diversity in the concentration (443–1174 mg/L) and composition of free amino acids in berries (Garde-Cerdán et al., 2009). A closer analysis of literature data revealed that grape berries with white or pale grey colors used for white wine production, such as Chardonnay, Chenin blanc, and Pinot blanc had higher total amino acid concentration than the red wine cultivars (e.g. Alicante Bouschet, Cabernet Sauvignon, and Pinot noir) (Kliewer,

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1970). Among red cultivars, Merlot and Petit Verdot with relatively higher color index contained low total amino acid concentration than Syrah with relatively lower color index (Garde-Cerdán et al., 2009). Therefore, it seems that the bulk biosynthesis of anthocyanin may compete for C-skeletons for the biosynthesis of amino acids. However, this hypothesis is difficult to investigate due to the complex interactions between genotypes and environments that regulate the biosynthesis of anthocyanins and amino acids.

Cultivars issued from somatic mutations with near identical genetic background but distinct anthocyanin accumulation profiles may provide a valuable system to investigate these questions. In a previous study, we compared cv. Gamay Noir with its somatic mutant cv. Gamay Fréaux and found that both cultivars have similar concentrations of sugars and organic acids in berries, but with distinct anthocyanins concentration in both skin and pulp (Guan et al., 2016). These features make the two cultivars particularly suitable to investigate the potential relationship between amino acids and anthocyanins in grape.

In the present study, seasonal variations of amino acid concentrations were studied in both skin and pulp of Gamay Noir and Gamay Fréaux, grown under either regular sunlight exposure or cluster shading. The present work aimed at gaining insight into the effects of cluster shading on amino acid accumulation in grape berries and comparing the amino acid profiles of berry skin and pulp in both Gamay Noir and Gamay Fréaux.

## 2. Material and methods

### 2.1. Plant materials

The experiments were conducted with red wine grape (*Vitis vinifera* L.) cv. Gamay Noir (white-fleshed) and Gamay Fréaux (teinturier cultivar with colored flesh) from a germplasm collection vineyard in Bordeaux, France. The vineyard management, shading treatment with opaque boxes and weekly sampling from 1 week after treatment (WAT) until maturity were detailed in Guan et al. (2016). Berry skin and pulp were separated, ground in liquid nitrogen and then stored in −80 °C for later analysis.

### 2.2. Extraction and analysis of free amino acids

Aliquots (500 mg) of frozen sample powder were extracted at 80 °C successively with 2 mL of 80% and 50% (v/v) ethanol. The supernatants were combined and evaporated in a Speed-Vac concentrator (Savant Instruments, Inc., Hicksville, NY) before dissolution in 2 mL of ultra-pure water (Millipore, Billerica, MA, USA) for later analysis of amino acids.

Amino acids were determined by using HPLC (Waters, Milford, MA, USA) after derivation with 6-aminoquinolyl-*N*-hydroxysuccinimidyl-carbamate (AccQ-Fluor Reagent Kit, Waters), as described in Martínez-Lüscher et al. (2014). All the amino acids were identified and quantified with external chemical standards purchased from Sigma (St Louis, MO, USA).

### 2.3. Analysis of sugars and organic acids

Glucose and fructose were measured enzymatically as described in Guan et al. (2016). Tartaric and malic acids were determined using the autoanalyser TRAACS 800 (Bran & Luebbe, Plaisir, France) according to the method of Berdeja et al. (2014).

### 2.4. Statistical analysis

To get overviews of correlations among amino acids, principal component analysis (PCA) and heatmaps were produced using R software (R development Core Team, 2010). Dynamic profiles of total and six family amino acids were drawn using sigmaplot 11.0 (Systat Software Inc.).

## 3. Results

### 3.1. Total amino acid concentration and amino acid composition

Twenty amino acids were quantified in berry skin and pulp of cv. Gamay Noir (GN) and Gamay Fréaux (GF). The total amino acid concentration was calculated by summing all these amino acids and the developmental profiles of total amino acid concentration varied with tissue, cultivar, and light condition (Fig. 1a, b). Skins showed higher total amino acid concentrations than pulps for both cultivars and light conditions along berry development. Under light-exposed conditions, the concentration of GF skin and pulp greatly increased between 3 and 4 WAT, slowed down thereafter, and finally remained relatively constant around maturity. On the other hand, the concentration of GN skin and pulp increased slower than GF skin and pulp before 5 WAT, but later on they increased faster than GF from 5WAT to maturity. These differences in accumulation dynamics led to higher total amino acid concentrations in the skin and pulp of GF than those of GN during the early berry development stages (4–5 WAT), but the reverse situation was observed at maturity. At maturity, GN contained much higher total amino acids concentration in both skin (4157 pmoles/mg) and pulp (1599 pmoles/mg) than GF (2645 pmoles/mg and 1133 pmoles/mg in the skin and pulp, respectively) under light-exposed conditions. Cluster shading greatly repressed the accumulation of total amino acids in the skin of both cultivars, and this effect was the most marked in GN skin and the less marked in GF pulp. Total amino acid concentration in shaded skin at maturity was significantly reduced, reaching 19.9% (GN)–46.2% (GF) of that in light-exposed one. This reduction was much less in shaded pulp, and reached 59.3% and 7.5% in GN and GF pulp, respectively.

The amino acids analyzed can be classified into six families based on their biosynthesis pathways and links to central metabolic pathways, including glutamate (GLU, GLN, ARG, GABA and PRO), aspartate (ASN, ASP, ILEU, LYS, MET and THR), pyruvate (VAL, LEU and ALA), serine (GLY, SER and CYS), aromatic amino acids (PHE and TYR) and histidine (HIS) families (Fig. A). Amino acid composition was relatively conserved during berry development and only the composition at maturity is shown (Fig. 1c, d). Amino acid compositions under sunlight exposure varied with tissues but were relatively conserved between grape cultivars: PRO, ARG, GLU and ALA were present in high proportion (11.5%–23.9%) in berry skin, while PRO, ALA and GABA were dominant in berry pulp, ranging from 11.4% to 33.8%. GLN, THR, SER in both skin and pulp, GABA and ASP in the skin, and GLU and ARG in the pulp were present in small proportions, ranging between 2.7% and 8.8%. The relative abundance of the remaining amino acids was less than 1%. Cluster shading significantly modified amino acid composition in berry skin and pulp of both cultivars: the proportion of PRO greatly decreased, whereas the proportion of GABA significantly increased. The proportions of the other amino acids were only slightly affected by shading.

### 3.2. Coordinated developmental changes of amino acid concentrations according to metabolic family

Heatmaps and hierarchical clustering analysis were performed to provide an overview of accumulation profiles of the 20 amino acids in berry skin and pulp of both cultivars under light exposure and cluster shading conditions (Fig. 2). Heatmaps clearly showed that most individual amino acids increased dramatically from 3 to 4 WAT onward, and the accumulation was repressed by cluster shading. In addition, amino acids from the same family shared similar accumulation profile, and they could be clustered into one group with minor exceptions (Fig. 2). Based on these features, only the dynamic changes of six amino acid families are presented in detail (Fig. 3). The exceptions for amino acids whose profile differ from the other members of a given family are presented in Fig. B.