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TERPENOID PROFILES AND BIOSYNTHETIC GENE EXPRESSION PATTERN IN ASTI DOCG WHITE MUSCAT GRAPES AT RIPENING AS AFFECTED BY DIFFERENT CANOPY MANAGEMENT PROTOCOLS

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Abstract

Aim: The main goal of this study was to find an efficient canopy management to limit the high temperature-related aroma losses of White Muscat grapes, and consequently to preserve the quality standards of Asti DOCG wines.

Methods and Results: Four different strategies have been tested in two vineyards of the Asti DOCG production area: pre-flowering leaf removal (m1), post-berry set leaf removal (m2), leaf removal at veraison (m3), and clusters thinning (m4). Control vines (m0) did not receive any thinning or defoliation. Grapes were collected at four time points: seven days before the commercial harvest, at the commercial harvest scheduled for "Asti spumante" wine, at the commercial harvest scheduled for "Moscato" wine and overripening. Free and glycosylated terpenoids content (GC-MS) as well as the expression of key genes involved in terpenoids biosynthesis and metabolism (RT-qPCR) were analysed separately in skin and pulp. The results revealed a peak of volatile accumulation, which occurred early and late throughout the sampling times. The treatments m3 and m4 were, in general, those more effective in enhancing the aroma profiles in both tissues analysed. Correspondingly, in these grapes, specific genes, such as *VvDXS3* and *VvGT14* resulted up-regulated. Other genes, such as *VvHDR,* showed different expression pattern resulting, in general, more expressed in pulp than skin, regardless the applied treatment.

Conclusions: Based on these preliminary trials carried out in a specific production area of White Muscat, it seems that m3 and m4 treatments had a significant effect on the volatile's accumulation in both grape skin and pulp. m1 treatment resulted to be the less effective in inducing changes in the aroma profile and the terpenoid biosynthetic pathway.

Significance and Impact of the Study: Moscato d'Asti DOCG is one of the most characteristic enological products of Piemonte (North-West Italy) wine grapes-growing area. It comes exclusively from White Muscat grapes which are exalted by the climatic and geographical conditions of the production area. Indeed, the interactions between vine and environment, limestone terrain and micro-climate typical of hilly zones leads to a characteristic fruity and sweety aroma. The characteristic aroma of Muscat wine is attributed to the presence of specific terpenoids, mainly linalool, nerol, geraniol, trans-piran linalool oxide and citronellol. The grapevine terpenoids pathway is strongly regulated by endogenous and environmental factors and among them, temperature and light exposure plays a crucial role. As recently observed, the content of these compounds is strongly decreasing due to the increasing temperatures. Higher temperature during the growing season is forcing growers to find ways to reliably control grape composition preserving the typical aroma of Asti DOCG wines. The present study could offer important information to address grower's choice in term of canopy management that are better suited to the changing climate.

Keywords: Canopy management, Moscato d'Asti DOCG, terpenoid content and biosynthesis, climate change

Introduction

Moscato d'Asti DOCG is one of the most characteristic products of Piemonte (North-West Italy) wine-growing area. It comes from White Muscat grapes which are exalted by the climatic and geographical characteristics of the production area. Indeed, the interactions between vine, environment, limestone terrain and micro-climate typical of hilly zones leads to the characteristic fruity and sweet aroma. The volatile aroma is of primary interest to assess the quality of a wine, in particular concerning wines from aromatic variety like Muscat-type. The unique aromatic bouquet of Muscat grapes is related to the presence of different terpenes, mainly linalool, nerol, geraniol, α-terpineol and citronellol in their form of alcohols, alkenes, aldehydes, and oxides (Ribereau-Gayon *et al.,* 1975). Two different biochemical processes are responsible for the biosynthesis of the terpenoids. The methylerythritol phosphate (MEP) pathway, which occurs in the plastids, and the mevalonate (MVE) pathway, which occurs in cytosol (Luan *et al.,* 2002). Both pathways result in two structural C5 units, isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) (Schwab *et al.,* 2015). The two isomers are condensed by different enzymes with prenyl transferase activity: geranyl diphosphate synthases (GPS), trans-geranylgeranyl diphosphate synthases (GGPS) and (E,E)-farnesyl diphosphate synthases (FPS). The enzymes responsible for the transition of GPP, FPP and GGPP to the different classes of terpenoids are the terpene synthases (TPSs) (Ribéreau-Gayon *et al.,* 2006). This complex biosynthetic pathway is still under studied, and its regulation is still unclear. Terpenes content is strongly affected by biotic and abiotic factors such as genotype, development stage, vineyard management and environmental factors (mainly light and temperature) (Kalua *et al.,* 2009; Hjelmeland *et al.,* 2015; Liu *et al.,* 2015; Wang *et al.,* 2018; Modesti *et al.,* 2020). As a confirmation of this, in the recent years, due to the climate change and increasing temperature, terpenes content in grapes is strongly decreasing (Ribéreau-Gayon *et al.,* 2006) and this has a marked impact on the final quality of Muscat wines and consumer acceptance. Hence, it is crucial to find and develop effective strategies to mitigate these negative effects, especially when dealing with DOCG products with a pre-fixed quality standard, even in term of aroma characteristics. Specific vineyard managements have been already tested to modulate terpenes biosynthesis and accumulation. Defoliation is a commonly used technique aimed to regulate microclimate around and inside the clusters. Indeed, leaf removal besides facilitating air circulation throughout the cluster, increases light exposure and berries temperature (Xiaofeng *et al.,* 2020; Feng *et al.,* 2015). Many studies have pointed out that with different intensity and timing, different effects can be reached. For instance, defoliation at veraison stage positively affect Blaufrankisch grapes composition in term of anthocyanins content (Pavic *et al.,* 2019), while in Tempranillo and Semillon grapes, a pre-bloom stage defoliation increases free and bound aromatic compounds (Alessandrini *et al.,* 2018; Moreno et al., 2017). Another common practices that can be used to modulate volatile content is cluster thinning. Cluster thinning is a viticultural practice aimed to reduce the crop load. It is generally performed to improve fruit quality or to conform with commercially expected crop loads (Uzes and Skinkis, 2016). Cluster thinning reduces the yield and it may indirectly increase grapes quality (Gamero *et al.,* 2014; Keller *et al.,* 2005; Santesteban *et al.,* 2011; Uzes and Skinkis, 2016). As for defoliation, the timing and the intensity of cluster thinning strongly affect the results. Cluster thinning performed during the growth lag phase of the berries seems to be the most appropriate time to maximize the possible positive effects (Jackson *et al.,* 1993). On the other hand, it seems that removing clusters earlier in the growing season, may negatively affect leaf transpiration rates and, as a consequence, leaf photosynthesis rates. This may lead to important physiological deficit (Naor *et al.,* 1997).

Little is known about the effects of different timings of leaf and cluster removal on the content of bound and free terpenes in Muscat grapes, and very few studies have taken into consideration molecular aspects and the modulation of gene expression. We hence studied the effects of three basal leaf removal timing (pre-flowering, post-berry set, at veraison) and clusters thinning on terpenes pathway genes expression and accumulation of free and bound terpenes in Muscat grapes. The present study will hopefully help growers to find and select an efficient canopy management to limit the high temperature-related aroma losses.

Materials and Methods

The trials have been carried out in 2019 growing season. Two vineyards of the Asti DOCG production area were selected for the study: Marenco vineyard located in Strevi (Alba, Italy) and Vignaioi Santo Stefano s.s. (Ceretto's group) located in Calosso (Asti, Italy) (hereafter Ceretto vineyard). In both vineyards the agronomic practices follow the disciplinary of production for the Appellation of Controlled and Guaranteed Origin (DOCG) of Consorzio per la tutela dell'Asti. A fully randomised block design was used for the trial, with three blocks in total, and each biological replicate has been obtained randomly collecting bunches from 6 plants. In each block all the treatments were applied on vines, randomly selected. Four different vineyard management protocols have been

tested: pre-flowering (BBCH 55-57) leaf removal (m1), post-berry set (BBCH 71-73) leaf removal (m2), leaf removal at veraison (BBCH 81-85) (m3), and clusters thinning (BBCH 81-85) (m4). Defoliation have been carried out removing 5 proximal leaves, from the shoot base to the second bunch. The lateral shoots were not defoliated. Cluster thinning has been carried out reducing the production rate of 30%. Control vines (m0) did not receive any thinning or defoliation. Grapes were collected at four time points: seven days before the commercial harvest, at the commercial harvest scheduled for "Asti spumante" wine, at the commercial harvest scheduled for "Moscato" wine and at overripening (hereafter T1, T2, T3 and T4 respectively). Sugars has been measured using a digital refractometer and expressed in g/L. Titratable acidity has been measured titrating 7.5 mL of filtered must with 0.1 N sodium hydroxide (NaOH), expressed in g/L of tartaric acid equivalent. At each sampling, skin and pulps were separate and the seeds discarded, then were immediately frozen in liquid nitrogen and stored at -80 °C for the following analyses. Free and glycosylated terpenoids content were quantified using Gas chromatography–mass spectrometry (GC-MS). A GC Agilent 7890 B and Agilent 7010, with split/splitless injector (PAL RSI 85) set at 230° C have been used for the analyses. The GC oven heating program were 40 °C x 1 min, 60 °C/min up to 60 °C and 4 °C/min up to 230 °C. Source temperature was set at 230 °C. Volatiles were separated on a JeW DB WAX polyethylene glycol column (30 m, 0.25 mm ID, 0.25 µm film). Helium was used as carrier gas with a flow rate of 1 mL min^{-1}. Terpenes quantification was carried out on three biological replicates (one per block). For molecular analysis frozen tissues were grounded to powder under liquid nitrogen. RNA was extracted from 100 mg of grounded tissue following the protocol of Spectrum™ Plant Total RNA Kit (Sigma-Aldrich,Italy), including DNA digestion with the On-Column DNase I Digestion Set (Sigma-Aldrich,Italy). Reverse transcription of the RNA templates to cDNA was carried out using ReadyScript™ cDNA Synthesis Mix (Sigma-Aldrich,Italy). Relative expression of key genes involved in terpenoids biosynthesis and metabolism (*VvDXS3, VvHDR, VvGT7, VvGT14, VvTer* and *VvLinNer1*) were analysed using a real-time quantitative PCR (RT-qPCR). *VvActin* was used as housekeeping gene. RT-qPCR was carried out on three biological replicates (one per block) and on two technical replicates. Each set of data was tested to detect outliers. One-way ANOVA was performed on the data following a post hoc Tukey's honestly significant difference (HSD) test (with $p = \leq 0.05$) for multiple comparation using GraphPad Prism version 7 (GraphPad Software, La Jolla California USA). Gene expression level and terpenoids levels were then assembled in one data set, separately for each vineyards and time. The original concentrations of terpenes (µl / 1000 berries), sugars (mg/l) and gene expression level were first auto scaled by mean-centring and division by the standard deviation of each variable, then has been rescaled based on the maximum and minimum content of each specific features and are presented in heatmaps where the color scale range from green (low level) to red (high level). The multivariate statistical analyses have been carried out employing Metaboanalyst online tool (Chong *et al.,* 2019).

Results and Discussion

Sugar content and acidity in berries from different treatments during the sampling time are reported in Table 1. In Ceretto vineyard control grapes (m0) always shows the lowest sugar level compared with the other treatments, the only exception is shown at T3 with m1 and m2 showing the lowest sugar content. Sample acidity throughout time course appeared similar between treatments. In Marenco vineyard sugars level showed a high variability between samples. Grapes heterogeneity in this vineyard could partly explain some contradictory results discussed in the following paragraphs and related to terpenoids content in Marenco sample pulp.

Table 1: Sugars content (g/l) and total acidity (g/l) in berries (pulp + skin) collected from Marenco (upper panel) and Ceretto (lower panel) vineyards. The columns represent the different sampling times, from left to right: T1, T2, T3 and T4. The lines represent the the different treatments.

MARENCO	T1		T2		T3		T4	
	Sugars (g/l)	Acidity (g/l)	Sugars (g/l)	Acidity (g/l)	Sugars (g/l)	Acidity (g/l)	Sugars (g/l)	Acidity (g/l)
m0	180	7,42	196	6,36	202	6,15	217	5,89
m1	157	8,08	202	6,45	198	5,72	214	5,68
m2	168	7,69	199	6,40	207	5,78	220	5,67
m3	157	8,85	201	6,65	198	6,32	201	5,66
m4	166	7,53	206	6,27	209	5,58	228	5,31
CERETTO	**T1**		**T2**		**T3**		**T4**	
	Sugars (g/l)	Acidity (g/l)	Sugars (g/l)	Acidity (g/l)	Sugars (g/l)	Acidity (g/l)	Sugars (g/l)	Acidity (g/l)
m0	171	8,01	196	6,12	215	5,56	207	5,64
m1	175	7,30	196	6,51	206	5,63	225	5,16
m2	174	7,85	209	6,25	206	5,50	217	5,46
m3	192	6,92	204	6,24	216	5,25	213	5,68
m4	175	7,87	207	5,98	220	5,42	227	5,29

Figure 1: Glycoconjugate and free volatile terpenes, identified in berries (pulp + skin) collected from Marenco (left panel) and Ceretto (right panel) vineyards. The columns represent the different sampling times, from left to right: T1, T2, T3 and T4. The lines represent the identified terpenes and each cell of the heatmap represents the average of the concentrations measured at the specific sampling time in all the performed treatments (m0-m4). The original concentrations of terpenes (µl / 1000 berries) has been rescaled based on the maximum and minimum content of each specific features and are here presented by a color scale ranging from green (low level) to red (high level) using Metaboanalyst online software.

Regardless the treatments, in two sampling times the berries resulted richer in terms of terpenoids: seven days before the commercial harvest for "Asti spumante" wine (T1) and overripening (T4) (Figure 1). However, the T4 has been considered less interesting from a practical aspect mainly due to two reasons. i) climatic conditions: high temperatures induce anticipated and unbalanced ripening, pushing the Italian wine sector to anticipate the harvest time; ii) oenological purpose: grapes would result with a too high sugar content. These preliminary observations suggest that, regardless the treatment, a slightly early harvest (2-4 days, within the limits of sugar accumulation curve) could lead to an increase in the terpene fraction.

Terpenoids Accumulation in Skin and Pulp

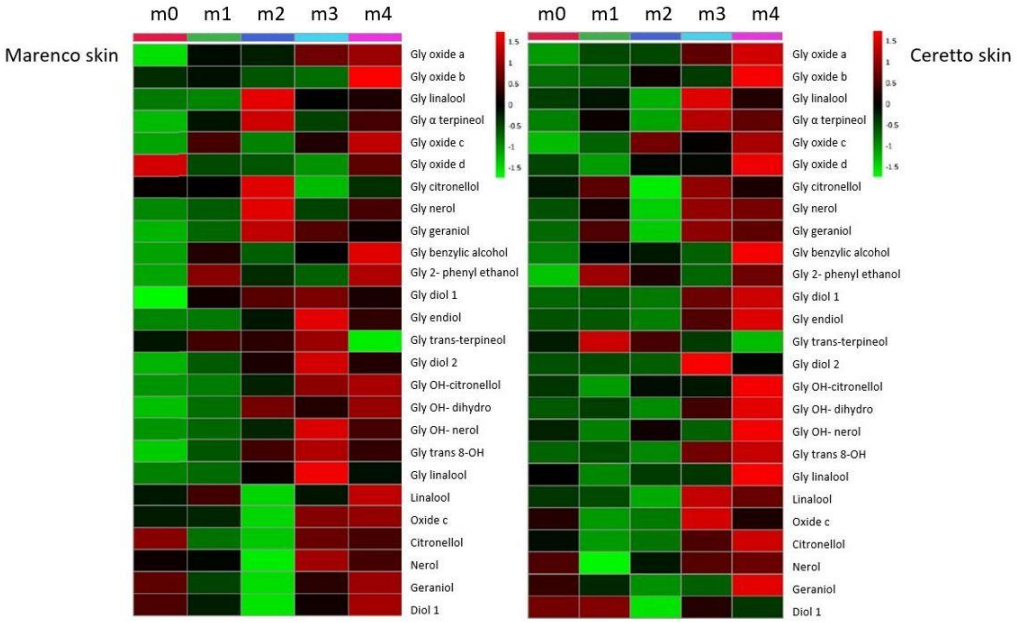

Figure 2: Glycoconjugate and free volatile terpenes, identified in berries skin collected from Marenco (left panel) and Ceretto (right panel) vineyards. The columns represent the different treatments, from left to right: m0 in red, m1 in green, m2 in blue, m3 in blue and m4 in pink. The lines represent the identified terpenes and each cell of the heatmap represents the average of the concentrations measured in the specific treatment in all the performed sampling times (T1-T4). The original concentrations of terpenes (µl / 1000 berries) has been rescaled based on the maximum and minimum content of each specific features and are here presented by a color scale ranging from green (low level) to red (high level) using Metaboanalyst online software.

Heatmap analysis of terpenes accumulation trend in berries skin (Figure 2) and pulp (Figure 3) have been performed. Heatmaps include samples collected from the two vineyards (Marenco in the right panels and Ceretto in the left panels) and from different treatments irrespectively of the sampling times. In grapes skin, m3 (leaf removal at veraison) and m4 (clusters thinning) treatments resulted richer in terpenes content in both vineyards, while grapes collected from vines defoliated in pre-flowering (m1) and control vines (m0) showed a general decrease in terpenes accumulation in both vineyards (Figure 2).

As for the skins data, in the pulp of Ceretto vineyard (right panel, Figure 3) terpenes show an accumulation in the m3 and m4 treatments (leaf removal at veraison and clusters thinning, respectively), and a lower level in all the other treatments. Here, the positive effect of late defoliation and cluster thinning is particularly marked. On the other hand, in Marenco vineyard (left panel, Figure 3), the accumulation trend is not as evident as in the other tissue/vineyard. Indeed, even though m4 still shows some compounds which result higher compared with the other treatment, control vines (m0) and post berry set basal leaf removal (m2) showed a more general higher level of terpenes. Additionally, in contrast with what observed in the other vineyards/tissues, m3 resulted to be the treatment with the lowest level of terpenoids. As mentioned earlier, the high grapes heterogeneity could be the reason for this inconsistent result. Indeed, seems that terpenoids content in strongly affected by the sugar content even though the information is contradictory. Some studies have shown that monoterpene contents decreased when sugar content increases (Xiaofeng *et al.,* 2020, Yuan and Qian, 2016). On the other hand,

Reynolds and Wardle (1989) reported that sugar content and bound monoterpenes were dependent processes and their accumulation occurred simultaneously.

Regarding defoliation treatment, many studies reported that leaf removal increase terpenes content in grapes and wine (Reynolds *et al.,* 1996; Zoecklein *et al.,* 1998). It must be mentioned that defoliation alters vine growth, especially if it is applied at early growth period, such as pre-bloom, and the physiological development and carbohydrate assimilation will be affected (Poni *et al.,* 2006). In this case, it is possible that an inhibition of terpenes biosynthesis occurs. On the other hand, the photosynthetic capacity of basal leaves at veraison is lower, so it reduces the influence on source-sink balance and increases the impact of light and temperature (Pastore *et*

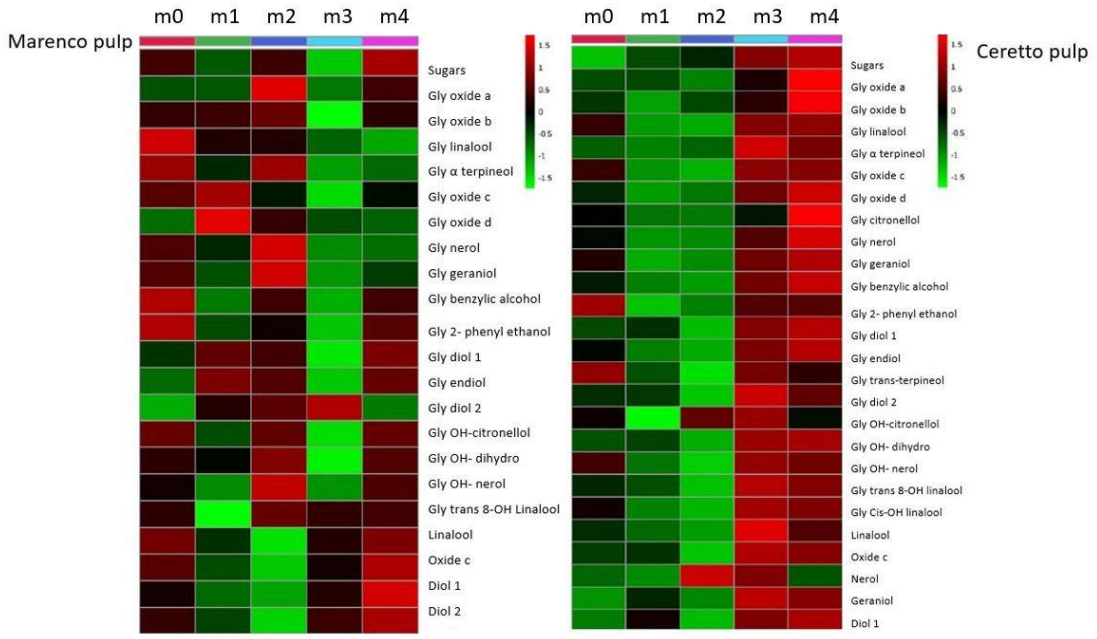

Figure 3: Glycoconjugate and free volatile terpenes, identified in berries pulp collected from Marenco (left panel) and Ceretto (right panel) vineyards. The sugar content is also reported. The columns represent the different treatments, from left to right: m0 in red, m1 in green, m2 in blue, m3 in blue and m4 in pink. The lines represent the identified terpenes and the sugar content and each cell of the heatmap represents the average of the concentrations measured in the specific treatment in all the performed sampling times (T1-T4). The original concentrations of terpenes (µl / 1000 berries) and sugars (mg/l) has been rescaled based on the maximum and minimum content of each specific features and are here presented by a color scale ranging from green (low level) to red (high level) using Metaboanalyst online software.

al., 2013). This may in part explain our results of increasing terpenoids content in late defoliation treatments comparing with early-stage defoliation. For cluster thinning, few papers deal with the effect of the treatment on volatile aroma composition. Most of the studied demonstrated that cluster thinning often reduce yield, increase sugars, color, and sometimes increase volatile organic compounds in fruit and in wines (Mayr *et al.*, 2014; Davies *et al.*, 2015; Condurso *et al.*, 2016; Yu *et al.*, 2020; Reynolds *et al.*, 2007; Guidoni *et al.*, 2002). In particular, it has been demonstrated that thinned vines increased concentration of terpenes in Syrah grapes (Condurso *et al.,* 2016). On the contrary, others studied did not report any difference in terpenes concentration in grapes from thinned and not-thinned vines (Yu *et al.,* 2020), highlighting that the response of terpenes to cluster thinning needs to be further investigated.

However, many studies conclude that aroma compounds accumulation is strongly influenced by the sun-light exposition of grapes and berries temperature (Yu *et al.,* 2020; Rutan *et al.,* 2018; Reynolds *et al.,* 2007). Leaf removal and cluster thinning promoted exposure of the cluster to light and increase temperature around the berries. One of the main problems in studying terpenoids biosynthesis/accumulation after defoliation or clusters thinning is that there are just few studies about the impact of light and/or temperature on this process (Hubert *et al.,* 2018). It is still unclear if the general reported effects of these treatment are due to the sun-light exposure, temperature or - more probably - to the interaction between temperature and light intensity (Feng *et al.,* 2017; Kotseridis *et al.,* 2012). Some studies found that a slight increase of daily sun-light exposition increased terpenoid concentrations in grapes (Zoecklein *et al.,* 2008), while others did not find any difference in terpenoids

accumulation, or sometimes a decreasing, after increased sunlight exposition (Bureau *et al.,* 2000; Scafidi *et al.,* 2013).

Terpenoids Accumulation and Gene Expression Trend in T1 and T4

To better understand the treatments effects on terpenes biosynthesis, we studied the expression level of key genes of the terpenoids metabolic pathway by qRT-PCR. We selected specific upstream (*VvDXS3, VvHDR, VvGT7 and VvGT14*) and downstream (*VvTer and VvLinNer1*) genes. All these genes encode for essential enzymes for terpenoid biosynthesis (Vranová *et al.,* 2013). As already mentioned, two sampling times resulted richer in terpenoids content: seven days before the commercial harvest for "Asti spumante" wine (T1) and overripening (T4) (Figure 1). However, since the T4 is considered less interesting for climatic and oenological reasons, here we present just the T1 results (Figure 4). Terpenes for which significant differences have been detected are here analysed together with the gene expression level of samples at T1 by mean of hierarchical clustering analysis and shown as heatmaps. Data are presented as fold change values by normalization on the level of the corresponding control samples (m0) and transformation in logarithmic scale.

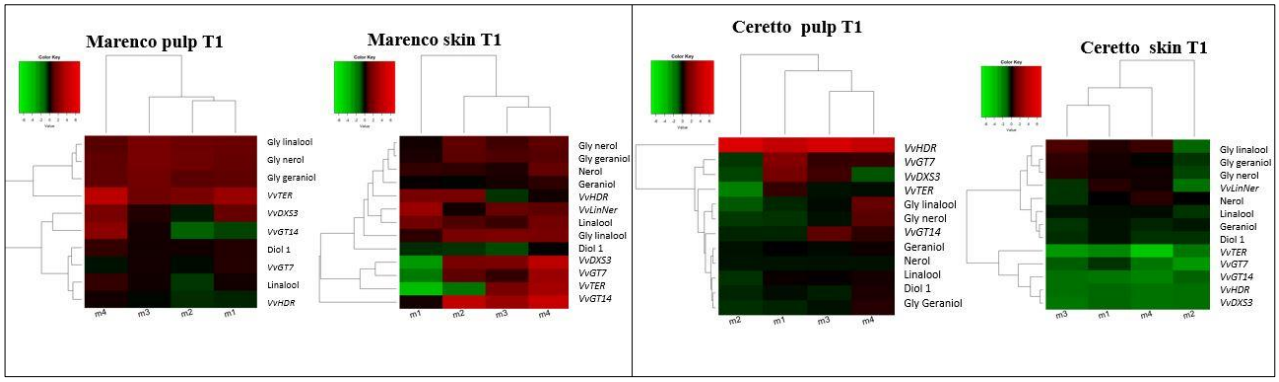

Figure 4: Glycoconjugate and free volatile terpenes and gene expression level at T1 identified in berries pulp and skin collected from Marenco (left panel) and Ceretto (right panel) vineyards. The columns represent the different treatments. The lines represent the identified terpenes and the relative expression level. The original concentrations of terpenes (µl / 1000 berries) and relative gene expression data are presented as fold change values by normalization to the level in the corresponding control (m0) and transformation in logarithmic scale as following: log2 (FC) = log2 [replicate/mean (m0)] and are here presented by a color scale ranging from green (low level) to red (high level) using Metaboanalyst online software.

In accordance with the general terpenes accumulation trend already discussed, at T1 most of the analysed genes seems to be upregulated in both tissues and vineyards. The most remarkable results were observed in Marenco vineyards (Figure 4, left panel). In both pulp and skin m3 and m4 treatments showed the highest expression level of *VvTer, VvGT14, VvDXS3* and *VvGT7*. In skin, m3 and m4 drove to the highest expression level of *VvLinNer* as well. These results are in accordance with the highest level reached in m3 and m4 of some important terpenes: linalool, nerol and geraniol, free and glycoconjugate. The cluster analysis shows that in pulp the different glycosylated terpenes and the different genes are grouped in two clusters showing similar trend. In Marenco skin, most of the terpenes, free and glycosylated, clustered together with *VvHDR* and *VvLinNer* while the rest of the genes (i.e. *VvDXS3, VvGT7, VvTER and VvGT14)* are grouped separately and shows similar expression trend. On the other hand, in Ceretto vineyard the trend is less clear. In pulp tissue there is a slight over expression of *VvHDR, VvGT7* and *VvGT14* (clustered together) in both m3 and m4 which reflect a slight accumulation of linalool, nerol, geraniol and diol-1. In the skin, all the analysed genes are strongly down-regulated, regardless the treatment. However, a slight accumulation of linalool, geraniol and nerol is still reached in m3 and m4, thus confirming that these two treatments may be suitable for preserving the aroma of Moscato grapes, increasing – or in any case maintaining – high level of terpenes. In general, in all the sampling time (data not shown) we did not detect a clear and direct relationships between gene expression level and terpenes accumulation. The relationships between genes expression and compounds accumulation is not often straight forward. Other studies showed that in many cases the expression level of most of the TPS genes is not consistent with the accumulation of their associated terpenes in grapes (Falara *et al.,* 2011; Matarese *et al.,* 2014).

Conclusion

The data reported in relation to gene expression analysis and terpenes content show that defoliation treatments at veraison (m3) and clusters thinning (m4) are those that induce a more pronounced effect on the accumulation of terpenes, both in skin and pulp. These results are in some cases associated with an up-regulation of key genes involved in terpenoid biosynthesis. On the other hand, post berry set defoliation (m1) results the less interesting treatment driving to a lower accumulation of volatile compounds in the berries and, in some cases, to a reduced activity of the terpenes metabolic pathway. Additionally, our data suggest that the highest level of terpenes accumulation reach two peaks: seven days before the commercial harvest for "Asti spumante" wine (T1) and in overripening (T4). Given the climate conditions, which in the Italian wine scenario is anticipating -year after year- the harvest time, this could suggest that a slightly early harvest (which can be also considered in combination with m3 or m4 treatment application) could lead to an increase in the terpenoids fraction. Considering the importance of terpenes for grapes and wine aroma, further research should be carried out on the effects of temperature, light, and their interaction with key genes of terpenes biosynthesis and accumulation.

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References

Alessandrini, M., Battista, F., Panighel, A., Flamini, R., Tomasi, D., 2018. Effect of pre-bloom leaf removal on grape aroma composition and wine sensory profile of semillon cultivar. Journal of the Science of Food and Agriculture, 98: 1674-1684.

Bordiga, M., Rinaldi, M., Locatelli, M., Piana, G., Travaglia, F., Coïsson, JD., Arlorio, M., 2013. Characterization of Muscat wines aroma evolution using comprehensive gas chromatography followed by a post-analytic approach to 2D contour plots comparison, Food Chemistry, 140(1–2): 57-67.

Condurso,, C., Cincotta, F., Tripodi, G., ..., 2016. Effects of cluster thinning on wine quality of Syrah cultivar (*Vitis vinifera* L.). European Food Research and Technology, 242: 1719–1726.

Davies, C, Nicholson, EL, Bottcher, C, Burbidge, CA, Bastian, SE, Harvey, KE., 2015. Shiraz wines made from grape berries (Vitis vinifera) delayed in ripening by plant growth regulator treatment have elevated rotundone concentrations and "pepper" flavor and aroma. Journal of Agricultural and Food Chemistry, 63: 2137–2214.

Feng, H., Yuan, F., Skinkis, PA., Qian, MC., 2015. Influence of cluster zone leaf removal on Pinot Noir grape chemical and volatile composition. Food Chemistry, 173: 414-423

Gamero,, E., Moreno, D., Talaverano, I., Prieto, MH., Guerra, MT., Valdés, ME., 2014. Effects of irrigation and cluster thinning on Tempranillo grape and wine composition. South African Journal of Enology and Viticulture, 35: 196–204.

Guidoni, S, Allara, P, Schubert, A., 2002. Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. American Journal of Enology and Viticulture, 53: 224–226.

Hjelmeland, AK., Ebeler, SE., 2015. Glycosidically bound volatile aroma compounds in grapes and wine: a review. American Journal of Enology and Viticulture, 66: 1–11.

Jackson, DI., Lombard, PB., Kabinett, LQ., 1993. Environmental and management practices affecting grape composition and wine quality: a review. American Journal of Enology and Viticulture, 44: 409–430.

Kalua, CM., Boss, PK., 2009. Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). Journal of Agricultural and Food Chemistry, 57: 3818–3830.

Keller, M., Mills, LJ., Wample, RL., Spayd, SE., 2005. Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. American Journal of Enology and Viticulture, 56: 91–103.

Liu, B., Xu, X-Q., Lan, Y-B., Zhu, B-Q., Wang, J., 2015. The free and enzyme-released volatile compounds of distinctive *Vitis amurensis* var. Zuoshanyi grapes in China. European Food Research and Technology, 240: 985–997.

Luan, F., Wüst, M., 2002. Differential incorporation of 1-deoxy-Dxylulose into (3S)-linalool and geraniol in grape berry exocarp and mesocarp. Phytochemistry**,** 60: 451–459.

Mayr, CM, Geue, JP, Holt, HE, Pearson, WP, Jeffery, DW, Francis, IL., 2014. Characterization of the key aroma compounds in Shiraz wine by quantitation, aroma reconstitution, and omission studies. Journal of Agricultural and Food Chemistry, 62: 4528–4536.

Modesti, M., Shmulevitz, R., Brizzolara, S., Tonutti, P., 2020. Short-term low temperature treatments of harvested wine grapes (cv. Vermentino) affect the volatile organic compound profile of the berries. Advances in Horitcultural Science, 34(1S): 2733.

Moreno, D., Valdés, E., Uriarte, D., Gamero, E., Talaverano, I., Vilanova, M., 2017. Early leaf removal applied in warm climatic conditions: Impact on Tempranillo wine volatiles. Food Research International, 98: 50-58.

Naor, A., Gal, Y., Bravdo, B., 1997. Crop load affects assimilation rate, stomatal conductance, stem water potential and water relations of field-grown Sauvignon blanc grapevines. Journal of Experimental Botany, 48: 1675–1680.

Pavic, V., Kujundzic, T., Kopic, M., Jukic, V., Braun, U., Schwander, F., Drenjancevic, M., 2019. Effects of defoliation on phenolic concentrations, antioxidant and antibacterial activity of grape skin extracts of the varieties blaufrankisch and merlot (*Vitis vinifera* L.). Molecules, 24.

Reynolds, AG., Schlosser, J, Sorokowsky, D., Roberts, R, Willwerth, J., de Savigny, C., 2007. Magnitude of viticultural and enological effects. II. Relative impacts of cluster thinning and yeast strain on composition and sensory attributes of Chardonnay Musqué. American Journal of Enology and Viticulture, 58: 25–41.

Ribereau-Gayon, P., Boidron, JN., Terrier, A., 1975. Aroma of Muscat grape varieties. Journal of Agricultural and Food Chemistry, 23: 1042-1047.

Rutan, TE., Herbst-Johnstone, M., Kilmartin, PA., 2018. Effect of cluster thinning *Vitis vinifera* cv. Pinot noir on wine volatile and phenolic composition. Journal of Agricultural and Food Chemistry, 66: 10053–10066.

Santesteban, LG., Miranda, C., Royo, JB., 2011. Thinning intensity and water regime affect the impact cluster thinning has on grape quality. Vitis – Journal of Grapevine Research, 50: 159–165.

Schwab, W., Wüst, M., 2015. Understanding the constitutive and induced biosynthesis of mono- and sesquiterpenes in grapes (*Vitis vinifera*): A key to unlocking the biochemical secrets of unique grape aroma profiles. Journal of Agricultural and Food Chemistry, 63(49): 10591–10603.

Uzes, D., Skinkis, PA., 2016. Factors influencing yield management of Pinot noir vineyards in Oregon. Journal of Extension, 54(3): 2012–2014.

Wang, Y., He, Y-N., Chen, W-K., He, F., Chen, W., Cai, X-D., Duan, C-Q., Wang, J., 2018. Effects of cluster thinning on vine photosynthesis, berry ripeness and flavonoid composition of Cabernet Sauvignon. Food Chemistry, 248: 101–110.

Xiaofeng, Y., Xin, M., Yali, T., Ying, W., Bowen, W., Xuliang, J., Zhenwen, Z., Yanlun, J., 2020. Effect of cluster zone leaf removal on monoterpene profiles of Sauvignon Blanc grapes and wines. Food Research International, 131: 10902.

Yuan, F., Qian, MC., 2016. Development of C13-norisoprenoids, carotenoids and other volatile compounds in *Vitis vinifera* L. cv. Pinot noir grapes. Food Chemistry, 192: 633-641.

Wang, Y., H., Y-N., He, L., He, F., Chen, W., Duan, C-Q., Wang, J., 2019. Changes in global aroma profiles of Cabernet Sauvignon in response to cluster thinning. Food Research International, 122: 56-65.