Identifying best parameters to characterize genotypes capability of retaining adequate malic acid at harvest and in final wines

Authors: Tommaso FRIONI[1]*, Riccardo COLLIVASONE[1], Silvia PAGANI[1], Ginevra CANAVERA[1], Matteo GATTI[1], Mario GABRIELLI[2], Stefano PONI[1]

[1]Dept. Of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Piacenza, PC, Italy.

[2]Dept. Of Sustainable Food Processing, Università Cattolica del Sacro Cuore, Piacenza, PC, Italy.

*Corresponding author: tommaso.frioni@unicatt.it

Keywords: Acidity, Malic acid, Phenotyping, Germoplasm, Wine quality

Introduction

Under current climate change pressures, obtaining grapes with adequate acidity at harvest is one of the main challenges for growers, especially if the goal is producing sparkling wines. This issue arises from two main occurrences: i) higher temperatures enhance degradation of malic acid; ii) grape maturity may occur under suboptimal climatic conditions due to an advanced phenology (Palliotti et al. 2014). For this reason, the introduction of new varieties or the reconsideration of minor and insofar neglected cultivars or clones are gaining enormous interest and popularity (Palliotti et al. 2014, Poni et al. 2020, Antolìn et al. 2020). However, criteria for the identification of performing genotypes in terms of 'acidity at harvest' are complex. Late veraison and ripening can be inappropriate traits, since they do not necessarily mean that grapes maintain adequate acidity in relation to a satisfying sugars concentration (Palliotti et al. 2014, Frioni et al. 2020). Considering the evolution and the destination of organic acids in grapes, pre-veraison malic acid pool could represent a promising trait. In the same framework, an eventual genetic control over malic acid degradation rates could lead to different conclusions. The same can be said for the ratio between minimum acidity and starting malate abundancy, or for sugars/acidity ratio. However, any of the above-mentioned parameters seems to underestimate some of the factors involved, or in any case to provide an incomplete overview of grapes ripening.

Research Objectives

In this work we compared fruit, musts and wines composition of a local, widely grown white grape variety in the Colli Piacentini area (cv. Ortrugo, ORT) with those of a minor autochthonous variety, namely, Barbesino (BRB), over four consecutive seasons. Our goal was to clarify the relationships between the evolution of titratable acidity during ripening and a wide range of parameters concerning organic acids catabolism, in two contrasting genotypes. Our hypothesis was that that minimum grape malic acid concentration is a genetic trait deriving from a correct interpretation of malic acid degradation rates. General aim of this study was the identification of the proper parameters to describe the attitude of a genotype to retain an adequate acidity at harvest.

Material and methods

Description of experimental sites

The experiment was carried out over four consecutive seasons (2017-2021) in a vineyard germplasm collection at Mossi 1558 Estate (Albareto, Ziano Piacentino, Italy, 44° 97' 93'' N 09 40' 99'' E,

270 m asl). Details of the site and of vineyard management can be found in Frioni et al. (2020). The plot consists of several local and international varieties (about 40 vines per cultivar), including Ortrugo, the most widely cultivated white cultivar in the area, and Barbesino, a minor local variety currently cultivated in less than one hectare surface in the area. Ortrugo (ORT) and Barbesino (BRB) represented the two treatments of this study. The rows were divided into three uniform sections to maintain three biological replicates along the study.

Monitoring fruit composition

In this work, grapes sugars (TSS), titratable acidity (TA), malic acid concentration (MA) and MA degradation rates (MAdr) were weekly analysed from pre-veraison to the end of the season. Sampled berries were brought to the laboratory, weighed, and crushed to obtain a juice. Musts were analyzed immediately for total soluble solids (TSS) using a temperature-compensated desk refractometer, whereas pH and titratable acidity (TA) were measured by titration with 0.1 N NaOH to a pH 8.2 end point and expressed as g/L of tartaric acid equivalents. Grapes organic acids were analyzed by HPLC as reported in Frioni et al. (2020). The TSS/TA and tartaric/malic acid ratios (HT/HM) were then calculated. Malic acid degradation rate was calculated as the difference in malate concentrations between two consecutive sampling dates, divided by the number of elapsed days.

Havest parameters

Nine tagged ORT and BRB vines were harvested when ORT scored TSS of about 20 °Brix. At harvest, test vines were individually picked, the mass of clusters was weighted, and total cluster number per vine counted. Concurrently, three representative clusters per vine—usually inserted on basal, median, and apical cane portions—were taken to the laboratory for further subsampling. Fruits were individually weighted, and the main rachis length measured to calculate the cluster compactness index expressed as cluster mass-to-rachis length ratio. From each of the three clusters, a 50 berry sub-sample was taken by carefully cutting each berry at the pedicel with small sharp scissors and then crushing, and the obtained must was then used for technological maturity determinations.

Winemaking and wines composition

In 2020, about 30 kg of grapes per replicate for each treatment (ORT, BRB, three replicates per two treatments) were hand-harvested, and each grape sample was destemmed and gently pressed with a hydraulic press to obtain approximately 20 L of juice for each batch. The juices were moved separately to 30-litre stainless steel vats, 50 mg/L potassium metabisulphite (Sigma-Aldrich - St. Louis, MO, USA) was added, and the juices were inoculated with *Saccharomyces cerevisiae* at 30 g/hL (L'Enotecnica, Nizza Monferrato, Italy). The fermentations were performed at 17 ± 1°C and monitored daily by measuring wine density until the end of the process (constant density for three consecutive days). At the end of the alcoholic fermentations, the wines were racked, added to potassium metabisulphite at 40 mg/L, bottled in 330 mL glass crown-capped bottles, and stored at 8°C for two months prior to analyses. The oenological parameters were determined in each wine sample; ethanol content, pH, titratable acidity (TA), volatile acidity (VA), free, combined, and total SO_2 were measured using OIV methods. Organic acids were analyzed as reported by Izquierdo-Llopart et al. (2020)

Statistical analysis

Data collected over 4 years were subjected to a two-way ANOVA (treatment, year), and the evolution of parameters assessed over multiple samplings during the season was analyzed using the function repeated measures ANOVA in IBM SPSS Statistics 24.0 (SPSS Inc., Chicago, IL, USA). Furthermore, comparison of wine composition was subjected to a one-way ANOVA, and statistically

significant differences between samples were tested using a post-hoc comparison test (SNK test at $P < 0.05$).

Results

ORT had a significantly lower cluster number per vine. However, ORT had also a notably higher cluster size (300g vs 152g in BRB), resulting in a vine yield comparable to BRB. Interestingly, BRB had looser clusters than ORT (-45%). Overall, given the average yield of cv. Ortrugo, steering toward less productive varieties is not an option for the local industry, although BRB seems to guarantee comparable yield, with lower susceptibility to rots.

ORT and BRB had a similar sugar (TSS) accumulation dynamic, whereas BRB exhibited a delayed loss of titratable acidity (TA). BRB had a much higher pre-veraison malic acid pool (+4.3 g/L), a delayed onset of malic acid degradation, and a higher malate concentration at harvest. However, BRB, showed significantly higher malic acid degradation rates (MAdr) from veraison to harvest (Fig. 1). Malic acid breakdown during grape ripening is mainly due to oxidation catalyzed by malate dehydrogenase and malic enzyme in the first metabolic stages of berry respiration process (Ford et al. 2012). Temperature is considered the main driver of malate oxidation, and high temperatures post-veraison foster malic acid loss (Ford et al. 2012). Another factor affecting malate degradation rates via respiration is the availability of substrate, namely, malic acid abundance (Famiani et al. 2014). In our work, MAdr seemed to be strictly related to the abundance of the substrate and to be a quite contradictive parameter to evaluate capability of a variety to maintain adequate TA at harvest.

The high acidity at harvest in BRB seemed related to a different minimum malic acid concentration. Despite air temperatures were compatible with the malic acid degradation, no further loss of malic acid occurred below the threshold of 1.2 g/L. Conversely, in ORT, a very low malic acid concentration (~ 0.35 g/L) was achieved earlier in each of the seasons, demonstrating that there is a strict varietal control over grapes minimum malic acid concentration.

Finally, plotting malic acid concentration (MA) against MAdr revealed that the higher BRB MAdr were essentially a smokescreen, since BRB had consistently lower MAdr than ORT for any value of MA < 10 g/L (Fig. 2). As a result, at harvest BRB wines had consistently higher TA than ORT. These results were reflected also in final wines, with BRB wines showing consistently higher TA and malic acid concentration.

Conclusions

Our data demonstrate that that MAdr can be a misleading parameter when selecting cultivars for high grapes acidity, since a relevant abundancy of pre-veraison MA could lead to high MAdr, independently by final TA or MA at harvest. Conversely, MAdr should be evaluated as a function of instantaneous MA concentration, to be considered a reliable parameter for identifying genotypes guaranteeing higher acidity. In detail, our data suggest that low MAdr at MA concentration < 10 g/L could represent a new benchmark when breeding or phenotyping for acidity. Our work also demonstrates that currently neglected cultivars could help preserve must acidity, as compared to traditional varieties having early ripening, maintaining the links with terroir and local traditions at the same time.

Acknowledgements

The work was partially funded by Emilia-Romagna Rural development 2014-2020 for Operational Groups (PSR Emilia-Romagna 2014-2020 Mis. 16.01.01) projects EIP-AGRI ValorInVitis (n. 5004320, call 2016) and EIP-AGRI SalViBio (n. 5150112, call 2019).

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Figures

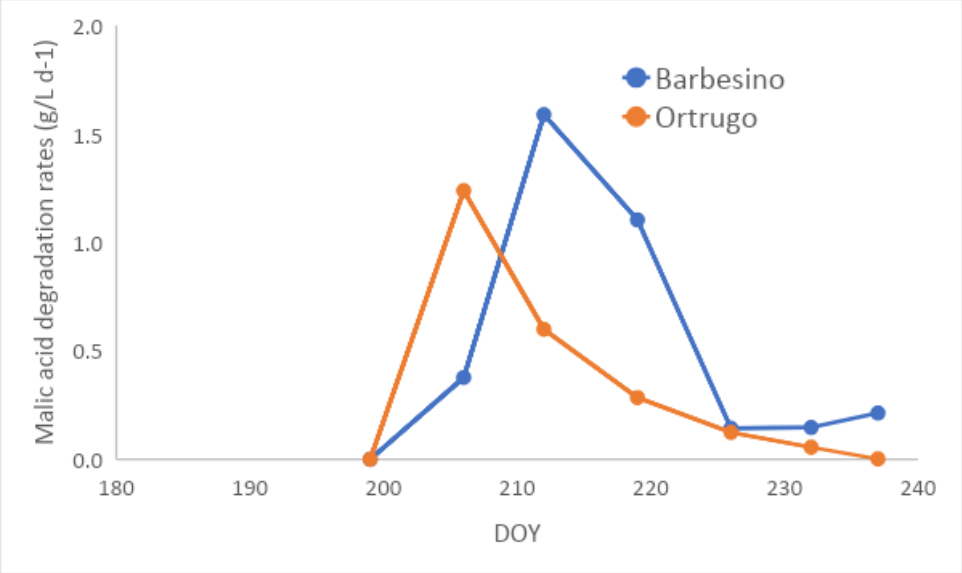

Figure 1. Seasonal evolution of grapes malic acid degradation rates (g/L) in cv. Ortrugo and cv. Barbesino in 2017.

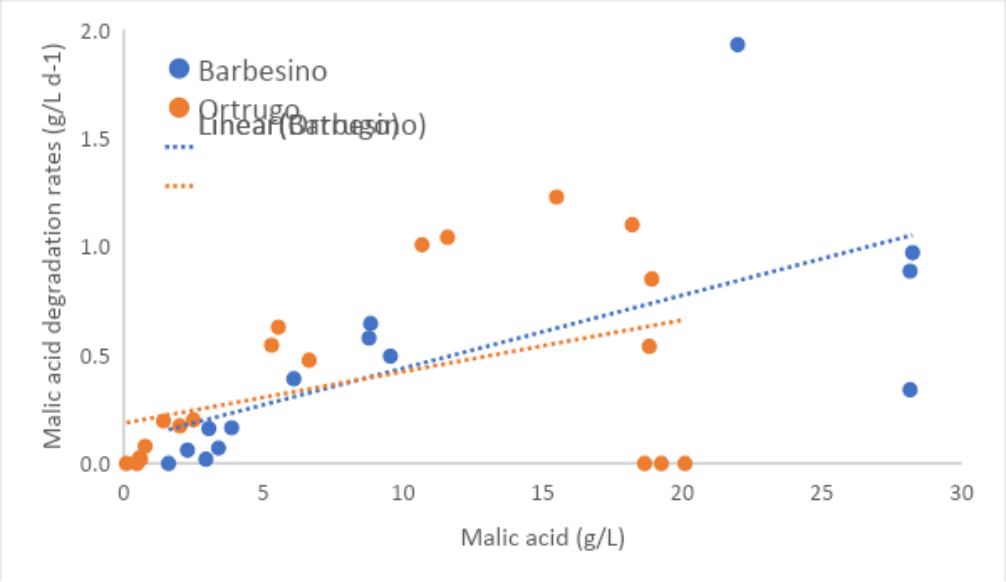

Figure 2. Relationship between grapes malic acid concentration (g/L) and grapes malic acid degradation rates (g/L day^{-1}) in cv. Ortrugo and cv. Barbesino in 2018.