ENVIRONMENTAL INFLUENCE ON GRAPE PHENOLIC AND AROMATIC COMPOUNDS IN A NEBBIOLO SELECTION (*VITIS VINIFERA* L.)

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Abstract
Nebbiolo (*Vitis vinifera* L.) is one of the most important wine red cultivar of North-west Italy. A better understanding of the complex relations among grape aromatic and phenolic maturity and environmental factors may strongly contribute to the improvement of the quality of Nebbiolo wines. In order to investigate this matter, a trial was set comparing the agronomic behavior and grape composition, with particular regards to aromatic and phenolic compounds, of a Nebbiolo clonal selection (CVT 63) when grown in two locations environmentally different of Piedmont Region (North-west Italy): La Morra and Lessona, areas of production of the renowned "Barolo" and "Lessona" VQPRD wines respectively. These locations differ in terms of climate, soil texture, row exposition and sloping. Agronomic performances of clone CVT 63, assessed over three growing seasons, differed very much depending on the environment, while grape composition (soluble solids, total flavonoids and total anthocyanins) resulted comparable between the two locations. The environmental imprint, however, was clearly present on some grape qualitative aspects such as the anthocyanin profile and the dotation in aromatic substances. Despite the similar amount of grape total anthocyanins assessed in the two sites, the grapes produced in Lessona, showed a profile characterized by an higher % of peonidin-3-glucoside (di-substituted anthocyanin) and a lower % of malvidin, petunidin and delfinidin-3-glucoside (tri-substituted anthocyanins). The modifications in the profile due to different environments may have strong implications on the evolution of wine color, being peonidin-3-glucoside less stable to degradation during fermentation and wine ageing. In terms of aromatic substances, the grapes produced in La Morra resulted richer in benzenoids, terpenes and norisoprenoids, and this bound aroma dotation may give an important contribution to the intensity and complexity of wine bouquet. Our results confirmed the environmental impact on grape qualitative composition and the consequent potential imprinting of the "terroir" on the characteristics of Nebbiolo wines.

Keywords: grape, environment, Nebbiolo, phenols, aromas, clone

1 INTRODUCTION

Nebbiolo (*Vitis vinifera* L.) is one of the most renowned wine red cultivar of North-west Italy from which several high quality VQPRD wines are produced, among them "Barolo" and "Lessona". The main area of Nebbiolo production is Piedmont, where the cultivar is grown in several districts which differ very much in terms of climatic and soil conditions. This is particularly true between Langhe, the area of production of "Barolo", and the North Piedmont, where the wine "Lessona" is obtained. In Langhe, for example, the soil is loamy-clayed with an average pH of 8.4 while in Northern Piedmont is loamy-sandy with an average pH of 4.8.
Both the wines are released on the market after two or three years of ageing to allow the rich phenolic endowment to evolve and soften determining the final color and body of the wine. The contribution of aromatic compounds to the complexity of wine bouquet is also regarded with growing interest for non-aromatic cultivar such as Nebbiolo. Recent studies demonstrated as several aromatic molecules (alcohols, terpenes, norisoprenoids, etc.) which are under bound form in the grapes, during fermentation and ageing modify their structure by hydrolysis lowering their olfactory perception threshold and so contributing to the complexity of wine bouquet. The final enological result therefore depends on the compensative/synergistic influence of local environment on the enological potentiality of the cultivar. A better understanding of the complex relations among grape phenolic and aromatic maturity and growing factors may strongly contribute to the improvement of the quality of Nebbiolo wines. In order to investigate this matter, a trial was set comparing the agronomic behavior and grape composition, with particular regards to aromatic and phenolic compounds, of the selection Nebbiolo CVT 63 when grown in two environmentally different locations of Piedmont.

2 MATERIALS AND METHODS

2.1 Experimental vineyards

The clone CVT 63 of cv. Nebbiolo, widely propagated by nurseries, was chosen for the trial. Vines of this selection grafted onto Kober 5BB rootstock were planted in two experimental vineyards located in La Morra (Langhe area) and Lessona (North-Piedmont area) respectively. The vines were vertically trained and Guyot pruned. The two sites differ in terms of soil texture (sandy to clayed), soil pH (acid to basic), slope (flat to hilly) and vine density (4300 to 5000 vine/hectare) as reported in table 1. Observations were carried out over three years (2008-2010) and started when the vineyards were 15 years-old and fully productive. Climatic data are reported in figure 1.

2.2 Data collection

The main agronomic parameters (yield, number of clusters, cluster weight, pruning wood weight) and juice composition were assessed individually over three years on 20 replicates of single contiguous vines selected in the middle of the vineyards. Particular attention was given to phenolic and aromatic composition of grapes. Total flavonoids and total anthocyanins were analysed by spectrophotometry (Di Stefano and Cravero, 1991) and the anthocyanin profile by HPLC-DAD (Pomar *et al*., 2005). The aromatic compounds were analysed by gas chromatography-mass spectrometry (GC-MS) (Mazza *et al*., 2006).

3 RESULTS AND DISCUSSION

In the vineyard of Lessona, the cultural management devoted to quality optimization (severe pruning, bunch thinning, etc.) and the environment characterized by sandy soil, levelled down the yielding performances of the selection (table 2), but induced a very good grape maturity. In this contest, the clone CVT 63 showed a good attitude to phenolic synthesis as typical of Nebbiolo with grapes rich in total flavonoids and total anthocyanins. In the vineyard of La Morra, the clone yielded more than in Lessona (higher shoot fertility and bigger bunches) without appreciable reduction of soluble solids and a comparable grape phenolic concentration. In terms of grape composition the only appreciable difference was the more energetic pH due to a lower salification. The good environmental stability of qualitative characters of clone CVT 63 in addition to the better orography (well exposed hillside) and the more structured soil of the vineyard in La Morra may have compensate the possible negative side-effects due to the higher grape production.

The total amount of grape anthocyanins is an important starting factor for the quality of a red wine, however also the anthocyanin profile (i.e. the reciprocal percentages of the different anthocyanins) may have an essential role in determining the intensity and the stability of wine color. The anthocyanin profile resulted quite different between the grapes produced in La Morra or in Lessona, despite their similar total amount (table 2 and figure 2).

The grapes in Lessona were characterized by an anthocyanin profile with a higher % of di-substituted peonidin-3-glucoside (less stable, rapidly undergo enzymatic degradation at the end of the fermentative process) and a lower % of more stable tri-substituted anthocyanins (delfinidin-3-glucoside, petunidin-3-glucoside and malvidine-3-glucoside), while the opposite trend was registered in the grapes produced in La Morra. These aspects are of particular relevance in winemaking because tri-substituted anthocyanins in grapes are potentially more determinant for the intensity and the stability of the color in the future wine. In addition the grape of La Morra possessed a lower pH, which is another feature potentially suitable to support color brightness and violet nuance intensity in the wine.

As discussed previously, the aromatic compounds are regarded as an important contribution to the bouquet of wines obtained by non-aromatic cultivars (i.e. cultivars characterized by grapes with a very low or none olfactory perception of aromatic molecules). Terpenes (particularly geraniol responsible of the rose scent) and the norisoprenoids (precursors of β-damascenone and β-ionone which release scents of exotic fruits and violet flower respectively) concur to the complexity of wine bouquet in aged Nebbiolo wines. For the same clone CVT 63, the grapes in La Morra resulted richer in bound aromatic substances compared to the ones in Lessona (figure 3). These results are particularly interesting because obtained at the same degree of grape maturity (soluble solids were similar in the two sites) and despite the much higher yield produced in La Morra. The richer dotation in grape aromatic molecules is then to be ascribed to the environmental features of La Morra such as the better sun exposure (norisoprenoid concentration, for example, is favored by light) and the richer mineral dotation of the calcareous and loamy-clayed soil.

4 CONCLUSION

This study indicated a strong influence of the different environments on the agronomic performances of a same clone of Nebbiolo (CVT 63) when grown in two different locations of Piedmont Region (North-west Italy) as well as a certain degree of environmental stability of the clone qualitative expression. The different environments did not influence sugar accumulation nor the quantitative amount of phenolic substances in grape skin (total flavonoids and total anthocyanins), however, it had a great impact on the qualitative profile of anthocyanins, with potentially important implications on wine color, as well as on the amount of grape bound aromatic substances which concur to the intensity and complexity of wine bouquet.
The results of the trial confirmed how the characteristics of a specific "terroir" give a local imprinting to the composition of grapes which will determine the typicality of the VQPR wines.

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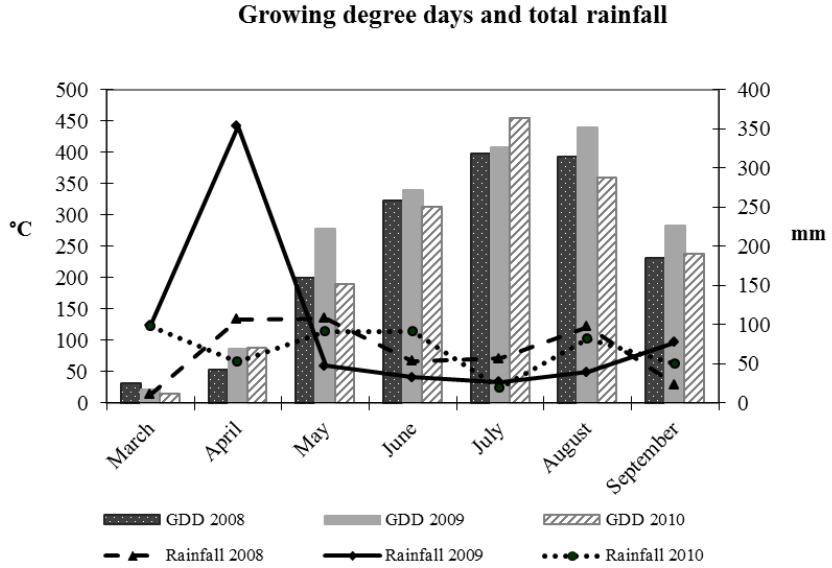

Figure 1: Degree Days (GDD) and total monthly rainfall in the areas of the trial.

Table 1: Environmental features of the experimental vineyards.

VINEYARDS	LA MORRA (CN)	LESSONA (BI)
Orography	hilly	flat
Training system	vertical	vertical
Pruning	Guyot	Guyot
Vine/hectare	4300	5000
Sand (%)	13,3	46,6
Loam (%)	61,1	34,4
Clay (%)	25,6	19
pH	8,43	4,78

Table 2: Field performances and grape composition of Nebbiolo clone CVT 63 grown in two different sites (average data 2008-10; *, **, * = statistical significance at p ≤ 0,05, 0,01, 0,001).**

DATA	*Lessona*	*La Morra*	F
Shoot fertility (n° inflor./shoot)	0,50	1,00	**
Yield (kg/vine)	1,08	2,06	***
Yield (t/hectare)	6	9	
Bunch wt (g)	207	351	***
Berry wt (g)	1,55	1,80	**
Pruning wood wt (g/vine)	988	790	**
Soluble solids (g/L)	24,8	24,3	ns
Titratable acidity (g/L)	7,5	6,4	*
pH	3,24	2,99	**
Tartaric acid (g/L)	4,5	4,7	ns
Malic acid (g/L)	2,2	1,1	*
Potassium (mg/L)	1004	616	**
Total flavonoids (mg/kg berries)	3307	3330	ns
Total anthocyanins (mg/kg berries)	747	648	ns

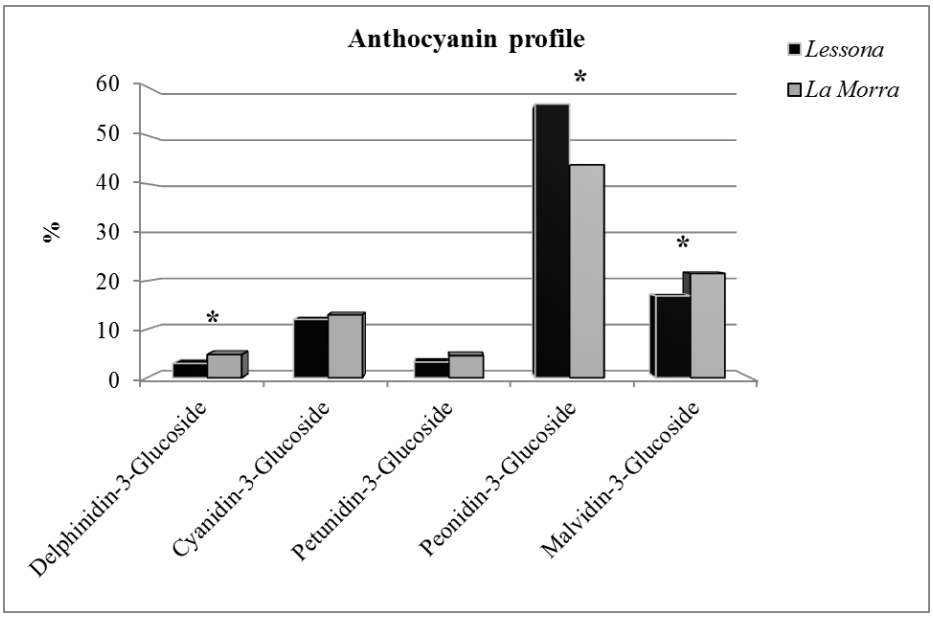

Figure 2: Grape anthocyanin profiles of Nebbiolo clone CVT 63 grown in two different sites (average data 2008-10; ∗= statistical significance at $p \leq 0,05$).

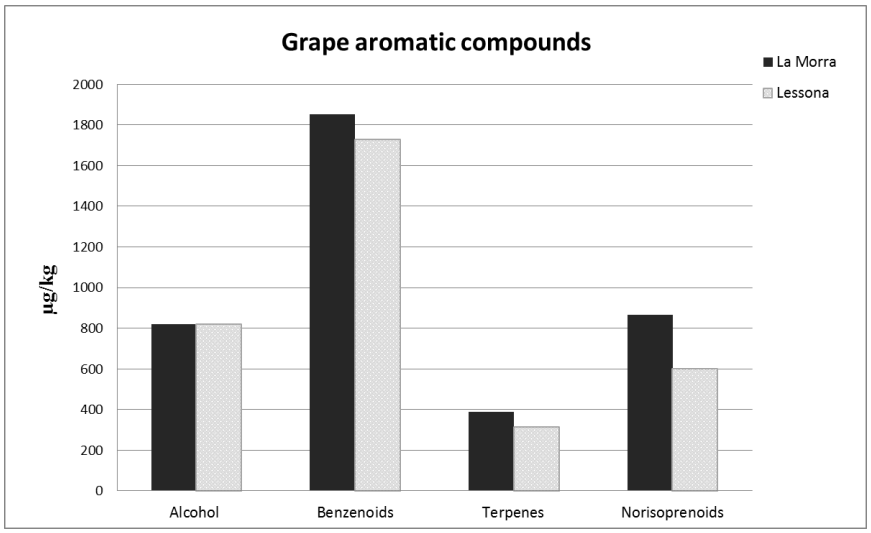

Figure 3: Grape bound aromatic compounds of Nebbiolo clone CVT 63 grown in two different sites (average data 2008-10).