Irrigation and rootstock effects on the phenolic composition of Vitis vinifera L. cv. Cabernet Sauvignon grapes under semi-arid conditions

Margarita Karamolegkou [a], Stefanos Koundouras [b], Stamatina Kallithraka [a], John T. Tsialtas [b], Eleftheria Zioziou [b], Nikolaos Nikolaou [b] and Yorgos Kotseridis [a]

[a] Department of Food Science & Technology, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece
[b] School of Agriculture, Aristotle University of Thessaloniki, 541 24, Thessaloniki, Greece

Abstract

Phenolic compounds of the skin and seeds are the principal sources of wine color and structural properties. Under semi-arid conditions, irrigation management is the most determining factor of grape and wine quality while rootstocks affect numerous physiological and vegetative parameters of the scion. In the present study, compositional changes of skin and seed phenolic compounds were studied in a factorial experiment, in field-grown grapevines of cv. Cabernet-Sauvignon (*Vitis vinifera* L.) grafted onto 1103P and SO4 rootstocks and subjected to three irrigation levels (FI - 100% of evapotranspiration, DI - 50% of evapotranspiration and NI-not irrigated). The experiment was conducted in a ten-year-old commercial vineyard of central Greece over two years (2005-2006). Limited water supply decreased berry size but did not affect the skin-to-pulp weight ratio. Water limitation did not affect skin anthocyanin concentration at harvest. Irrigation regime (mainly post-veraison) and rootstock genotype affected total flavan-3-ol monomers in seed tissue, mainly as a result of variations in catechin amount. The lower seed phenolic concentration was found in non-irrigated and SO4-grafted vines and was probably related to the modification of cluster exposure due to restricted scion vigor.

Key words: Water status, leaf area index, skin anthocyanins, seed flavan-3-ols.

Introduction

Grape-derived phenolic compounds play a major role in grape composition and wine quality (Glories, 1988) and their levels at ripeness vary greatly with site (Tessic et al., 2002), viticultural practices such as canopy management and leaf removal (Haselgrove et al., 2000), and water regime (Ojeda et al., 2002). Although irrigation seems to be the most important and easily controllable viticultural technique determining wine phenolic content (Fereres and Evans, 2006), limited data exist regarding the effect of vine water status on certain categories of phenolic compounds, most notably, seed phenolics (Kennedy et al., 2002).

Rootstocks affect numerous vegetative and reproductive parameters of the grafted variety, such as canopy growth and yield (Ezzahouani et al., 2005), most probably due to their role on root density and thus, vine water supply (Koundouras et al., 2008). However, limited knowledge exists on the effect of different rootstock genotypes on scion berry attributes in the field (Reynolds and Wardle, 2001), especially under drought conditions.

In the present study, we investigated the effect of irrigation water regimes and rootstock cultivar on berry phenolic components of field-grown *Vitis vinifera* cv. Cabernet-Sauvignon vines with the aim to understand whether differences were related to a direct impact on the biosynthesis of these compounds or to indirect effects related to variations in berry size or canopy microclimate.

Materials and Methods

The experiment was conducted during 2005 and 2006 in a 10-year-old vineyard in Larissa, central Greece (39° 48′ N, 22° 27′ E, 190 m), planted with cv. Cabernet-Sauvignon (*Vitis vinifera* L.) The experimental design was arranged as a 2×3 factorial with two rootstocks of different drought tolerence [1103 Paulsen (*V. rupestris* × *V. berlandieri*) and SO4 (*V. riparia* × *V. berlandieri*)] and three irrigation levels [full irrigation (FI) - 100% of crop evapotranspiration (ETc), deficit irrigation (DI) - 50% ETc and non irrigated (NI)], replicated three times in randomized blocks.

Vine water status was monitored throughout the season by midday measurements of stem water potential (Ψs) using a pressure chamber according to Choné et al. (2001). Vine vigour was assessed by Leaf Area Index (LAI) determinations using the Delta-T

SunScan system (Delta-T Devices, Cambridge, UK). To obtain LAI estimates, four under canopy measurements were recorded per plot, holding the sensor close to the ground and looking along the row.

Grapes were harvested on the 31st August in 2005 and 30th August in 2006, from each plot and yield and fresh weight of berry components was determined. Berries were then pressed and the must was analyzed for soluble solids (^{o}Brix) by refractometry.

A lot of 100 berries from each plot was weighted, manually skinned, and the skins were weighed and freeze-dried. The freeze-dried tissues were then extracted with 100mL of 1% HCl in MeOH. Extraction was carried out under stirring for 48 h and repeated three times in triplicate. Extracts were pooled, and this mixture was used for further procedures analysis either immediately, or after deep-freezing (-70˚ C) for no longer than 4 days. Anthocyanin analysis was carried out according to Arnous et al. (2002). Identification was based on comparing retention times of the peaks detected with those of original compounds, and on UV–vis on-line spectral data. Quantification was performed by establishing calibration curves for each compound determined, using the standards. Results were expressed as mg malvidin per fresh skin weight and per berry.

Berries of the same lot were manually de-seeded, the seeds were counted and weighed, frozen in liquid nitrogen and stored in the freezer (-20 ˚C) until analysed. A lot of 2 g of seeds was ground with a pestle and a mortar subsequently placed in a vial, 8 ml of ethyl acetate was added and vortexed for 3 min. The extract was centrifuged at 6000 rpm for 5 min, at 4 ˚C, and this process was repeated twice more. The clear extracts were then pooled and taken to dryness in a rotary vacuum evaporator (35°C), and the resulting residue was dissolved in 8 mL of MeOH, containing 5% (v/v) perchloric acid. The solution was filtered through Gelman GHP Acrodisc 13 syringe filters (0.45 μm) prior to analyses. Chromatographic analyses were carried out as described previously (34). Quantification was performed by establishing calibration curves for each compound determined, using the standards. Procyanidins are expressed as mg/L (+)-catechin, whereas the rest of the compounds are expressed against their own calibration curves. All analyses were performed in duplicate.

Data were subjected to analysis of variance (ANOVA), using SPSS software (version 16.0, SPSS Inc., IL, USA). Comparison of means were performed using Duncan's multiple range test at $p<0.05$.

Results and Discussion

Vine water status, expressed as midday stem water potential (Ψs), was significantly affected by irrigation regime and was consistently more limiting in NI vines (more negative Ψs), in both years and rootstocks (Table 1). According to van Leeuwen et al., (2008), water deficit was weak in FI, weak to moderate in DI and moderate to severe in NI vines. Rootstock did not alter vine water status but significantly affected vine vigour (Table 1) with 1103P-vines presenting significantly higher LAI (years combined), compared to SO4-grafted vines. Only on 1103P, LAI showed a significant increase with higher water availability, whereas no differences in vigor among irrigation treatments were detected on SO4 (Table 1). The increased vegetative growth of 1103P-grafted vines under favourable water conditions was probably associated to its denser root system, able to provide the scion with higher amounts of water under irrigation.

Table 1. Rootstock and irrigation effects on water status and vegetative growth of Cabernet Sauvignon vines.

	2005		2006	
	Ψ_s (MPa)	LAI (mol/m^2/s)	Ψ_s (MPa)	LAI (mol/m^2/s)
SO4	-0.96	2.83 b	-0.93	3.11 a
1103P	-1.04	3.22 a	-0.97	3.69 b
SO4				
NI	-1.28 c	2.76	-1.13 b	2.94
DI	-0.98 b	2.84	-0.91 a	3.16
FI	-0.63 a	2.88	-0.74 a	3.16
1103P				
NI	-1.39 b	2.99 b	-1.24 b	3.28 b
DI	-0.93 a	3.19 ab	-0.91 a	3.79 ab
FI	-0.80 a	3.47 a	-0.77 a	4.01 a

In each column, statistically significant differences between treatments within factors are indicated by different letters ($p<0.05$).

Yield was similar between years, rootstocks (data not shown) and irrigation treatments (Table 2). Berry growth was significantly affected by water regime on both rootstocks, being lowest in NI and highest in FI vines (Table 2). A significant effect of water deficit on berry growth, especially when applied before veraison, has been

previously reported (Matthews and Anderson, 1989; Shellie, 2006). According to the distribution of fresh mass in berries, skin consisted approximately 20% and seeds 4% of the whole berry weight, on both rootstocks. Although reduction in berry size is often associated with an increase in the skin to pulp ratio, in the conditions of this study skin mass followed variations in berry size, as shown by a positive linear correlation between these parameters on both rootstocks (SO4: $r=0.622$ and 1103P: $r=0.646$, $p<0.01$, $n=18$), leading to a similar skin to berry weight ratio between irrigation treatments (Table 2). However, the proportion of seeds in total berry mass increased with water deficit (seed to berry weight ratio higher in NI) in both rootstocks.

Differences in must sugar content among irrigation treatments were significant only in SO4, with NI berries showing the highest levels (Table 2). The accumulation of soluble solids decreased linearly with berry size in both rootstocks (SO4: $r=-0.579$ and 1103P: $r=-0.479$, $p<0.05$, $n=18$) showing that smaller berry size was associated to a better technological maturity (Roby et al., 2004). Sugar content of the must was also higher in SO4-grafted vines than on 1103P ones (averaging years and irrigation treatments).

Table 2. Rootstock and irrigation effects on reproductive growth parameters of Cabernet-Sauvignon vines. Means are combined over years.

	Yield (kg/vine)	Berry weight (g)	Skin/berry weight ratio	Seed/berry weight ratio	Total soluble solids (g/L)
SO4					
NI	1.46	0.892 b	20.7	7.1 a	294 a
DI	1.90	0.976 ab	23.0	7.4 a	277 ab
FI	2.07	1.094 a	20.4	6.1 b	270 b
1103P					
NI	1.39	0.836 b	19.8	7.9 a	273
DI	2.10	1.043 a	19.7	6.7 b	263
FI	2.36	1.149 a	21.4	6.8 b	259

In each column, statistically significant differences between treatments within factors are indicated by different letters ($p<0.05$).

Concerning the analytical anthocyanin composition of skin extracts, malvidin 3-O-monoglucoside was the major anthocyanin determined (Kallithraka et al., 2005), representing 85% of the total anthocyanin content (together with its coumarate

derivative). Since the skin to berry ratio was not affected by irrigation (Table 2), the anthocyanin content of the berries was for the most part the result of anthocyanin concentration of the skins. Water availability did not affect individual anthocyanin concentration in the skin tissue (expressed on a per berry basis to simulate vinification conditions), except for a higher malvidin coumarate content in grapes of 1103P-grafted vines (Table 3). Total anthocyanin content, calculated as the sum of individual compounds, was not different between rootstocks or among irrigation treatments. This is also supported by the absence of significant correlation between total skin anthocyanins at harvest and mean Ψs values at veraison (SO4: r=-0.451 and 1103P: r=-0.163, non significant, n=18). Our findings did not confirm a generally reported positive effect of reduced irrigation on anthocyanin levels in the grape and wine (Downey et al., 2006).

Table 3. Rootstock and irrigation effects on skin anthocyanins (mg per berry) of Cabernet Sauvignon berries at harvet (Dp, delphinidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin- 3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; MvC, malvidin 3-*O*-coumarateglucoside); NI, non irrigated; DI, deficit irrigated; FI, full irrigated.

	Dp	Pt	Pn	Mv	MvC	Total
SO4	0.048	0.045	0.031	0.539	0.030 b	0.693
1103P	0.045	0.043	0.032	0.554	0.089 a	0.763
SO4						
NI	0.039	0.041	0.024	0.564	0.029	0.697
DI	0.058	0.051	0.037	0.572	0.033	0.751
FI	0.046	0.043	0.033	0.482	0.028	0.632
1103P						
NI	0.042	0.043	0.025	0.588	0.038 b	0.736
DI	0.055	0.050	0.036	0.527	0.123 a	0.791
FI	0.036	0.036	0.035	0.547	0.107 a	0.761

In the same column, statistically significant differences within factors, are indicated by different letters ($p<0.05$).

Changes in the amthocyanin content of berries are often attributed to modification in the grape microcliamte, especially light and temperature conditions. Generally, high light intensities promote the anthocyanin accumulation in skins (Bergqvist et al., 2001). In turn, differences in grape microclimate are often associated with differences

in vine vigor (Cortell et al., 2007), with low vigour canopies tending to have berries with higher anthocyanins due to increased sunlight penetration. In our work, both rootstock and irrigation treatments strongly differed in vegetative vigor parameters (Table 1) but differences in anthocyanin content of skins were not observed. Moreover, skin anthocyanins were not correlated with mean season LAI (r=0.036, not significant, averaged over years and rootstocks, n=36) suggesting that anthocyanin levels were not depended on vigor variations.

Table 4. Rootstock and irrigation effects on seed flavan-3-ol monomers (mg per berry) of Cabernet Sauvignon berries at harvest (C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-*O*-gallate; EGCG, (-)-epigallocatechin- 3-*O*-gallate; EGC, (-)-epigallocatechin); NI, non irrigated; DI, deficit irrigated; FI, full irrigated.

	C	EC	ECG	EGCG	EGC	Total
SO4	0.080 b	0.060 b	0.050 b	0.005 b	0.001 b	0.196 b
1103P	0.104 a	0.087 a	0.067 a	0.009 a	0.002 a	0.269 a
SO4						
NI	0.049 b	0.042	0.037	0.004	0.001	0.133 b
DI	0.087 ab	0.070	0.058	0.006	0.001	0.222 ab
FI	0.104 a	0.068	0.054	0.006	0.002	0.234 a
1103P						
NI	0.074 c	0.069 b	0.057 b	0.008 b	0.002	0.210 b
DI	0.106 b	0.087 ab	0.066 ab	0.010 ab	0.002	0.271 ab
FI	0.134 a	0.104 a	0.079 a	0.011 a	0.002	0.330 a

In the same column, statistically significant differences within factors, are indicated by different letters ($p<0.05$).

Regarding the examination of grape seed extracts, catechin was the most abundant polyphenol accounting for approximately 40% of the total flavan-3-ols content of Cabernet-Sauvignon seeds. Rootstock significantly affected levels of all measured flavan-3-ol in the seeds, with higher values on 1103P compared to SO4 (Table 4). Catechin amount per berry was higher in the irrigated vines on both rootstocks while the other monomers increased with irrigation only on 1103P. The total flavan-3-ol amount (calculated as the sum of individual flavan-3-ol monomers per berry) was also significantly higher in FI vines compared to NI ones, on both rootstocks. Taking into consideration that the seeds to berry weight ratio was higher in the non irrigated vines, the higher flavan-3-ols content in FI was entirely associated to a higher polyphenol

concentration in the seed tissues. Higher water availability during the season (esimated as midday Ψs at veraison) was also strongly associated with higher total flavan-3-ol monomers per berry and this effect was more pronounced in 1103P-grafted vines (linear correlations SO4: $r=0.542$, $p<0.005$ and 1103P: $r=0.571$, $p<0.01$; $n=18$). Kennedy et al. (2000) reported that flavan-3-ol monomers of Cabernet-Sauvignon seeds declined during berry ripening and their rate of loss increased with water deficit.

Moreover, the highly significant positive correlation of total seed flavan-3-ol amount with mean season LAI ($r=0.510$, $p<0.001$, averaged over years and rootstocks, $n=36$) showed that vine vigor significantly affected the levels of seed polyphenols at harvest, possibly by modifying cluster microclimate (Cortell et al., 2007). Our results suggest that rootstock and irrigation effects on seed polyphenols are probably mediated through their respective effect on canopy growth.

Conclusions

This two-year experimentation demonstrated that moderate water deficit generally improved the phenolic of Cabernet-Sauvignon grapes under the semi-arid conditions of central Greece. Limited water availability was associated with a lower contribution of the seeds to the total pool of berry tannins. However, the effect of water availability on skin anthocyanins was not significant. Irrigation-induced changes in seed monomeric flavan-3-ols were most probably related to changes in canopy microclimate affecting the rate of flavan-3-ol decline during berry ripening. Our results also provided evidence regarding rootstock evaluations for dry-land conditions. Rootstock cultivars inducing higher vigour to the scion (i.e. 1103P) can result in a lower phenolic ripeness of the seeds at harvest, thereby negatively affecting red wine quality.

Acknowledgements

The authors would like to express their gratitude to Karipidi Winery in Vounaina, Thessaly, Greece for field management of the experimental vineyard.

References

1. Bergqvist, J., Dokoozlian, N. and Ebisuda, N., 2001. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. Am. J. Enol. Vitic., 52, 1-7.
2. Choné, X., van Leeuwen, C., Dubourdieu, D. and Gaudillère, J.P., 2001. Stem water potential is a sensitive indicator of grapevine water status. Annals of Botany 87, 477-483.
3. Cortell, J. M., Halbleib, M., Gallagher, A. V., Righetti, T. and Kennedy. J. A., 2007. Influence of vine vigor on grape (Vitis vinifera L. cv. Pinot noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit. J. Agric. Food Chem., 55, 6575-6584.
4. Downey, M. O., Dokoozlian, N. K. and Krstic, M. P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wines: a review of recent research. Am. J. Enol. Vitic., 57, 257-268.
5. Ezzahouani, A. and Williams, L. E., 2005. Performance of Dattier de Beyrouth and Alphonse Lavallée grapevines on eight rootstocks under dry-land conditions. J. Int. Sci. Vigne Vin, 39, 91-94.
6. Glories, Y., 1988. Anthocyanins and tannins form wine: organoleptic properties. In Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties; Cody, V., Middleton, E. and Harborne, J. B., Eds.; Alan R. Liss, New York,; pp. 123-134. 3.
7. Fereres, E. and Evans, R. G., 2006. Irrigation of fruit trees and vines: an introduction. Irrig. Sci., 24, 55-57.
8. Haselgrove, L., Botting, D., van Heeswijck, R., Høj, P. B., Dry, P. R., Ford, C. and Iland, P. G., 2000. Canopy microclimate and berry composition: the effect of bunch exposure on the phenolic composition of Vitis vinifera L cv. Shiraz grape berries. Aust. J. Grape Wine Res., 6, 141–149.
9. Kallithraka, S., Mohdaly, A. A., Makris, D. P. and Kefalas, P., 2005. Determination of major anthocyanin pigments in Hellenic native grape varieties (Vitis vinifera sp.): association with antiradical activity. J. Food Compos. Anal., 18, 375–386.
10. Kennedy, J.A., Matthews, M.A. and Waterhouse, A.L., 2000. Changes in grape seed polyphenols during ripening. Phytochemistry, 55, 77-85

11. Kennedy, J.A., Matthews, M.A. and Waterhouse, A.L., 2002. Effect of maturity and vine water status on grape skin and wine flavonoids. Am. J. Enol. Vitic., 53, 268-274.
12. Koundouras, S., Tsialtas, J. T., Zioziou, E. and Nikolaou, N., 2008. Rootstock effects on the adaptive strategies of grapevine (Vitis vinifera L. cv. Cabernet–Sauvignon) under contrasting water status: leaf physiological and structural responses. Agric. Ecosyst. Environ., 128, 86-96.
13. Matthews, M. A. and Anderson, M. M., 1989. Reproductive development in Vitis vinifera L.: responses to seasonal water deficits. Am. J. Enol. Vitic., 40, 52-60.
14. Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A. and Deloire, A., 2002. Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of Vitis vinifera cv Shiraz. Am. J. Enol. Vitic., 53, 261-267.
15. Reynolds, A. G. and Wardle, D. A., 2001. Rootstocks impact vine performance and fruit composition of grapes in British Columbia. HortTechnology, 11, 419-427.
16. Roby, G., Harbertson J. F., Adams D. A. and Matthews, M. A., 2004. Berry size and vine water deficits as factors in grapevine composition: anthocyanins and tannins. Aust. J. Grape Wine Res., 10, 100-107.
17. Shellie, K. C., 2006. Vine and berry response of Merlot (Vitis vinifera L.) to differential water stress. Am. J. Enol. Vitic., 57, 514-518
18. Tesic, D., Woolley, D.J., Hewett, E. W. and Martin, D. J., 2002. Environmental effects on cv Cabernet Sauvignon (Vitis vinifera L.) grown in Hawke's Bay, New Zealand. 2. Development of a site index. Aust. J. Grape Wine Res., 8, 27-35.
19. van Leeuwen, C., Tregoat, O., Choné, X., Gaudillère, J. P. and Pernet, D., 2008. Different environmental conditions, different results - the role of controlled environmental stress on grape quality potential and the way to monitor it. In Proceedings of the Thirteenth Australian Wine Industry Technical Conference; Blair, R., Williams, P., Pretorius, S., Eds.; Winetitles, Adelaide, South Australia, pp. 39-46. (1)