# The effect of different levels of postveraison water deficit on the phenolic composition and concentration of the Kékfrankos berry (*Vitis vinifera* L.)

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## Abstract
The effect of moderate and severe water deficit was examined on berry skin phenolic concentration and composition of the Kékfrankos variety (*Vitis vinifera* L.). Moderate water stress induced higher concentration of anthocyanin derivatives compared to the non-stressed plants with, the exception of Cya-3-g. Concentrations of some anthocyanin derivatives (Mal-3-g and Peo-3-g) were also higher in the severely stressed berry skins than in the control berries. No differences were found between the stressed and the non-stressed plants in the case of Cya-3-g. Similarly, concentration of some flavonol components (ie. protocatechuic acid, gallic acid, vanillic acid, *trans*-resveratrol etc.) increased as a result of water deficit. On the other hand, others such as quercetin-3-glucuronide decreased as the water deficit increased. In general, water deficit had a great effect on grape phenolic concentration; however it can be modified by the changes in berry skin/flash ratio.

## Introduction

Water deficit is one of the most important environmental factors, which influence berry ripening, and thus its chemical composition (Schultz 1996). Anthocyanins and other phenolic compounds play a major role in grape and wine quality. Their concentrations depend on many factors such as variety, vintage, terroir and cultural practices (Deloire et al. 2005). One of the most important factors that affect the amount of phenolic compounds is water deficit. Water deficit is frequent nowadays, especially in countries with a hot climate. Due to climate change this phenomenon can also be observed in the cool climate viticulture regions, because uneven precipitation, more frequent heat waves, and droughts can easily result in water shortage (Schultz 2000). Furthermore, water plays an important role in fruit development. Therefore, there is an increasing need to understand how water deficit affects the ripening process and the quality of the grape. It is well known that water deficit causes reduced berry size and weight due to decreased pericarp volume (McCarthy 1997, Ojeda et al. 2001). Therefore it induces beneficial changes in wine composition due to the modified skin/pulp ratio of the berry (Roby et al. 2004). However, grape varieties may produce different responses to water deficit in physiology (Villangó et al. 2013) as well as in ripening processes. The aim of our study was to reveal the effect of different levels of postveraison water deficit on berry skin and the whole berry phenolic concentration and composition of the Kékfrankos variety (*Vitis vinifera* L.).

## Materials and methods

### Experimental design

The experimental design was adjusted as described earlier by other authors (Villangó et al. 2013, Zsófi et al. 2014, 2015) Briefly: Six-year-old Kékfrankos vines grafted

onto Teleki-Kober 5BB rootstock in 50 l plastic containers were exposed to water deficit in 2011 under greenhouse conditions. The level of the water deficit was adjusted by measuring the plot weight, and stomatal conductance (gs) by a CIRAS-1 infrared gas analyzer (PP Systems, UK). Three levels of water deficit were applied based on stomatal conductance values (Galmés et al. 2007, Pou et al. 2008): Nil stress (gs above 150 mmol H2O $m^{-2}s^{-1}$), moderate ($g_s$ between 50 and 150 mmol H2O $m^{-2}s^{-1}$ and severe stress ($g_s$ under 50 mmol H2O $m^{-2}s^{-1}$). The desired water deficit treatments were achieved after 10 and 15 unirrigated days. Harvest was carried out on 5th August.

**Berry sampling and analytical measurements**

Whole grape bunches were harvested. The berries were removed with pedicels from the clusters and visually examined before analysis. 24 clusters of four plants (six bunches per plant) per treatment were harvested, respectively. 40 berries for analytical measurements were taken from each cluster (1-2 berries/cluster) Skins of the berries were pealed and weighted in order to measure their phenolic composition.
The extraction of phenolics from grape skins was carried out according to Sun et al. (1996). The following solvent was used during the maceration: methanol:water (60:40) with 1% HCl–methanol. 20 ml solvent was used for each sample. The maceration of skins took place for 48 hours in the dark. The total amount of skins of ten berries was used for one replicate and four replicates were done for each treatment. After that the samples were filtrated and stored in a cool and dark place before the analysis.

**Qualitative and quantitative determination of phenolic components in grape skin extracts by HPLC**

**Flavonoids**

Grape skin extracts were analyzed on a modular Shimadzu HPLC system equipped with LC20-AD pump, DGU-14A degasser, SIL10-ADvp autosampler, CTO-10ASvp column oven and SPD-10Avp UV-VIS detector. 10 µl of the samples were injected onto a Kinetex 2.6 µ XB-C18 100A (100 x 4.6 mm) column at a flow rate of 1 ml/min. For separation of different flavonoid compounds eluent A and B were water and acetonitrile, respectively, both of them supplemented with 1 % acetic acid. During the HPLC analysis the following solvent gradient was used: initially 0% B; at 16.40 min 16.3% B; at 16.90 min 18.4% B, which was held until 20.30 min; at 24.90 min 19.4% B; at 27.50 min 20.4% B; at 27.51 min 100% B until 30.40 min; at 30.41 min 0% B until 37.0 min. Flavonoid content of the samples was identified and quantified using standard reference compound of caftaric acid (8.1 min), *t*-caffeic acid (11.7 min), *t*-piceid (18.2 min), quercetin-3-O-galactoside (19.1 min), quercetin-3-O-glucuronid (19.8 min), kaempferol-3-O-glucoside (21.8 min) and *t*-resveratrol (24.8 min) at 320 nm and gallic acid (3.5 min), protocatechuic acid (6.0 min), (+)-catechin (10.7 min), vanillic acid (10.9 min), (-)-epicatechin (15.1 min) at 280 nm. $R^2$ values of the calibration curves were above 0.99 for each compound.

**Anthocyanins**

Grape skin extracts were poured into the sample vials of the automatic sampler (L-7200) of the Hitachi LaChrom HPLC system involving D-7000 controller, L-7612 degasser, L-7100 quaterner pump, L-7455 dioda array detector and Jasco 860-CO column thermostat. 20 µl of the samples were injected onto the Hypersil ODS (250x4.6 mm, 5 µm) column coupled with Uniguard (C18 10x4 mm, 5 µm) guard column and separated at 40 °C with the mobile phase flow rate of 0.8 ml/min. The eluent A and B were water/formic acid/acetonitrile, 87 : 10 : 3 (V/V%) and 40 : 10 : 50 (V/V%), respectively. The gradient program started from 6% B growing to 30% for 15 min, to 50% for 15 min, to 60% for 5 min and linearly decreased to the starting value for 6 min, and was held for 4 min, resulting in 45 min total runtime. The anthocyanins were identified and quantified using standard compound of delphinidin-3-glucoside ( Del-3-g, 11.1 min), cyanidin-3-glucoside ( Cya-3-g, 13.6 min), petunidin-3-glucoside ( Pet-3-g, 15.2 min), peonidin-3-glucoside (Peo-3-g, 17.7 min) and malvidin-3-glucoside ( Mal-3-g, 18.9 min) monitoring the detector signal at the wavelength of 518 nm.

**Statistics**

Statistical analyses were carried out by one way ANOVA and mean separation was made by Tukey's test ($p < 0.05$).

**Result and Discussion**

Water deficit affects sugar concentration, berry texture properties and berry weight parameters as was reported in previous studies (Roby et al. 2004, Ollé et al. 2011, Zsófi et al. 2014, 2015). Also, water stress resulted in changes in skin phenolic concentration (Ojeda et al. 2002) via the alteration of gene expression in the berries covering cell wall metabolism, primary and secondary metabolism, signaling, stress, and hormones (Berdeja et al. 2015). In our study the amount of anthocyanin derivatives changed as a result of water shortage (Table 1.). Moderate stress caused a significant increase in total anthocyanin concentration, but no changes were observed in the case of Cya-3-g in berry skin. Plants under severe stress also produced more anthocyanins compared to the control, but the increase was lower than in the case of mild-to moderate stress. However, the concentration per berry mass of these components of the severe and the moderate water stressed berries became close to equal as a result of decreased berry size and thus higher skin/flash ratio (Roby and Matthews 2004, Zsófi et al. 2014, 2015). Similar results could be observed in the case of (+)-catechin and (-)-epicatechin. Also, water stress resulted in higher concentrations of phenolic acids in the berry skin, among them the caftaric acid was the most sensitive, because at the higher water deficit higher concentration was observed. Derivatives of quercetin and kaempferol seem to be less sensitive to water effect compared to the other examined compounds. Indeed, very little increase was observed as a result of moderate water deficit, and no any differences were found between the control and the severe water deficit treatments. Concentration of *t*-resveratrol showed a significant increase during water shortage and the highest amount of this component was caused by severe water deficit. Its glucoside form *t*-piceid was produced the most during mild stress, while severe stress resulted in lower concentration (Table 2).

In summary, moderate water deficit generally resulted in an increase in several phenolic components in the berry skins, with some exceptions. Also, severe water shortage increased the concentration of these secondarily metabolites. However, no differences were found between the water stressed treatments in concentrations per berry mass due to the increased skin/flash ratio.

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| | mg/kg/FW skin | | | mg/kg/FW berry | | |
|---|---|---|---|---|---|---|
| | **Nil** | **Moderate** | **Severe** | **Nil** | **Moderate** | **Severe** |
| **Del-3-g** | 15.686$^{a}$ | 19.964$^{b}$ | 14.239$^{a}$ | 0.337$^{a}$ | 0.349$^{a}$ | 0.291$^{b}$ |
| **Cya-3-g** | 4.513$^{a}$ | 4.659$^{a}$ | 4.411$^{a}$ | 0.092$^{a}$ | 0.081$^{a}$ | 0.090$^{a}$ |
| **Pet-3-g** | 36.375$^{a}$ | 47.726$^{b}$ | 35.935$^{a}$ | 0.757$^{a}$ | 0.835$^{b}$ | 0.732$^{a}$ |
| **Peo-3-g** | 22.834$^{a}$ | 27.042$^{b}$ | 28.745$^{b}$ | 0.467$^{a}$ | 0.472$^{a}$ | 0.586$^{b}$ |
| **Mal-3-g** | 1440.554$^{a}$ | 2058.196$^{b}$ | 1740.969$^{c}$ | 29.505$^{a}$ | 35.939$^{b}$ | 35.424$^{b}$ |
| **Σ** | **1519.962$^{a}$** | **2157.587$^{b}$** | **1824.299$^{c}$** | **31.158$^{a}$** | **37.676$^{b}$** | **37.123$^{b}$** |

**Table 1** Anthocyanin concentration of the berry skin (mg/kg/FW skin) and the whole berry (mg/kg/ FW berry) in the water stressed treatments. Each value represents the average of 2-4 replicates. Different letters indicate significant differences between the treatments according to Tukey's test ($p<0.05$).

| | mg/kg/FW skin | | | mg/kg/FW berry | | |
|---|---|---|---|---|---|---|
| | **Nil** | **Moderate** | **Severe** | **Nil** | **Moderate** | **Severe** |
| **(+)-catechin** | 50.17$^{a}$ | 63.84$^{b}$ | 67.17$^{b}$ | 1.23$^{a}$ | 1.13$^{a}$ | 1.42$^{b}$ |
| **(-)-epicatechin** | 38.89$^{a}$ | 44.66$^{b}$ | 41.99$^{ab}$ | 0.62$^{a}$ | 0.88$^{b}$ | 0.96$^{b}$ |
| **protocatechuic acid** | 43.46$^{a}$ | 59.25$^{b}$ | 60.30$^{b}$ | 0.76$^{a}$ | 1.04$^{b}$ | 1.19$^{b}$ |
| **gallic acid** | 12.12$^{a}$ | 16.41$^{b}$ | 12.44$^{a}$ | 0.21$^{a}$ | 0.29$^{b}$ | 0.27$^{b}$ |
| **vanillic acid** | 102.98$^{a}$ | 126.15$^{b}$ | 112.38$^{ab}$ | 1.81$^{a}$ | 2.22$^{b}$ | 2.41$^{b}$ |
| **caftaric acid** | 67.88$^{a}$ | 85.77$^{b}$ | 117.90$^{c}$ | 1.11$^{a}$ | 1.52$^{b}$ | 2.53$^{c}$ |
| ***t*-caffeic acid** | 11.14$^{a}$ | 23.78$^{b}$ | 21.60$^{b}$ | 0.41$^{a}$ | 0.43$^{a}$ | 0.47$^{b}$ |
| **quercetin-3-O-galactoside** | 83.39$^{a}$ | 93.72$^{b}$ | 81.25$^{a}$ | 1.45$^{a}$ | 1.65$^{b}$ | 1.74$^{b}$ |
| **quercetin-3-glucuronide** | 231.64$^{a}$ | 215.69$^{a}$ | 191.06$^{b}$ | 4.03$^{a}$ | 3.82$^{a}$ | 4.11$^{a}$ |
| **kaempferol-3-O-glucoside** | 82.77$^{a}$ | 71.43$^{b}$ | 66.05$^{b}$ | 1.20$^{a}$ | 1.31$^{a}$ | 1.42$^{b}$ |
| ***t*-Resveratrol** | 4.77$^{a}$ | 5.17$^{a}$ | 8.39$^{b}$ | 0.11$^{a}$ | 0.14$^{b}$ | 0.19$^{c}$ |
| ***t*-Piceid** | 2.25$^{a}$ | 2.86$^{b}$ | 2.41$^{a}$ | 0.04$^{a}$ | 0.05$^{b}$ | 0.05$^{b}$ |

**Table 2** Concentration of the phenolic compounds of the berry skin (mg/kg/FW skin) and the whole berry (mg/kg/ FW berry) in the water stressed treatment. Each value represents the average of 2-4 replicates. Different letters indicate significant differences between the treatments according to Tukey's test ($p<0.05$).

## Literature Cited

Berdeja, M., Nicolas, P., Kappel, C., Dai, Z.W., Hilbert, G., Peccoux, A., Lafontaine, M., Olla,t N., Gomès, E., Delrot, S. 2015. Water limitation and rootstock genotype interact to alter grape berry metabolism through transcriptome reprogramming. Hortic Res. 2:1-13.

Deloire, A., E. Vaudour, V. Carey, V. Bonnardot, and C. van Leeuwen. 2005. Grapevine responses to terroir: A global approach. J. Int. Sci. Vigne Vin. 39:149-162.

Galmés, J., A. Pou, M. Alsina, M. Tomàs, H. Medrano, J. Flexas. 2007. Aquaporin expression in response to different water stress intensities and recovery in Richter-110 (*Vitis sp*.): relationship with ecophysiological status. Planta 226:671–681.

McCarthy, M.G. 1997. The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). Aust. J. Grape Wine Res. 3:2-8.

Ojeda, H., A. Deloire, A. Carbonneau. 2001. Influence of water deficits on grape berry growth. Vitis 40:141-145.

Ojeda, H., C. Andary, E. Kraeva, A. Carbonneau, and A. Deloire. 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.

Ollé, D., J.L. Guiraud, J.M. Souquet, N. Terrier, A. Ageorges, V. Cheynier and C. Verries. 2011. Effect of pre- and post-veraison water deficit on proanthocyanidin and anthocyanin accumulation during Shiraz berry development. Aust. J. Grape Wine Res. 17:90-100.

Pou, A., J. Flexas, M. Alsina, Mdel, J. Bota, C. Carambula, F. de Herralde, J. Galmés, C. Lovisolo, M. Jiménez, M. Ribas-Carbó, D. Rusjan, F. Secchi, M. Tomàs, Z. Zsófi, H. Medrano. 2008. Adjustments of water use efficiency by stomatal regulation during drought and recovery in the drought-adapted *Vitis* hybrid Richter-110 (*V. berlandieri* × *V. rupestris*). Physiol. Plant. 134:313–323.

Roby, G., and M.A. Matthews. 2004. Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. Aust. J. Grape Wine Res. 10:74-82.

Roby, G., J.F. Harbertson, D.A. Adams, M.A. Matthews. 2004. Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins. Aust. J. Grape Wine Res. 10:100-107.

Schultz, H.R. 1996. Water relations and photosynthesis responses of two grapevine cultivars of different geographical origin during water stress. Acta Hortic. 427:251-266.

Schultz, H.R. 2000. Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects. Aust. J. Grape Wine Res. 6:2-12.

Sun, B.S., M.I. Spranger, and J.M. Ricardo da Silva. 1996. Extraction of grape seed proanthocyanidins using different organic solvents, In: J. Vercauteren, C. Chèze, M.C. Dumon, J.F. Weber (Eds): Polyphenols Communications 96:169-170. Groupe Polyphenols, Bordeaux.

Villangó, S., Z. Zsófi, B. Bálo. 2013. Pressure–volume analysis of two grapevine cultivars (‘Kékfrankos’ and ‘Portugieser’ *Vitis vinifera* L.): water deficit, osmotic conditions and their possible relations with drought tolerance. Vitis 52:205–206.

Zsófi, Z., S. Villangó, Z. Pálfi and X. Pálfi 2015. Combined effect of berry size and postveraison water deficit on grape phenolic maturity and berry texture characteristics (*Vitis vinifera* L. 'Portugieser'). Vitis 54:161–168.

Zsófi, Z., S. Villangó, Z. Pálfi,, E. Tóth, and B. Bálo 2014. Texture characteristics of the grape berry skin and seed (*Vitis vinifera* L. cv. Kékfrankos) under postveraison water deficit. Sci. Hortic. 172:176-182.