Combined effect of berry size and postveraison water deficit on grape phenolic maturity and berry texture characteristics (*Vitis vinifera* L. cv. Portugieser)

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Abstract

The effect of berry size and moderate water deficit on skin phenolic maturity and berry texture behaviour was studied on Portugieser variety (*Vitis vinifera* L.) under green house conditions. In all berry weight categories (I: < 1,1 g; II: 1,11 - 1,4 g; III: 1,41 - 1,7 g; IV: 1,71-2 g; V: > 2,01 g) water deficit resulted in reduced sugar concentration due to decreased photosynthetic activity. Interestingly, lower phenolic concentration for unit skin mass was measured in the drought stressed treatment compared to the control, irrespective of berry size. However, the concentration of the phenolic components for one berry was lower in the well watered treatment. This phenomenon was due to the increased skin/flesh ratio of the water stressed vines. Berry skin hardness was probably in connection with its phenolic concentration for unit skin weight. Changes in several berry texture parameters were accompanied by changes in berry size. Berry hardness and skin elasticity increased with berry size in both treatments. On the other hand, skin break force, skin break energy, skin thickness showed increase/decrease only in the case of the stressed vines. This result suggests that texture properties of the water-stressed berries depend on berry size to a greater extent compared to the berries of the non-stressed vines. This phenomenon may be explained by the faster ripening of the smaller and of the water stressed berries.

Introduction

Grape quality parameters depends on many environmental factors. One of the main aspects of the complex biochemical process that is responsible for grape ripening is water deficit (OJEDA *et al.* 2002, ROBY *et al.* 2004, ZSÓFI *et al.* 2014). This factor has a direct effect on grape phenolic composition and concentration. Generally, mild to moderate water deficit has a beneficial effect on the phenolic concentration of the berry skin as a result of the increased intensity of some metabolic pathway (CASTELLARIN *et al.* 2007a, CASTELLARIN *et al.* 2007b). In addition, the polymerization degree of proanthocyanins increases in the skin due to water withholding and thus it has a beneficial effect on the sensorial quality of the wine (OJEDA *et al.* 2002). However, in some cases severe water deficit produced less phenolic components in the berry skins compared to moderate water stress treatments (ZSÓFI *et al.* 2014). Furthermore, berry size, skin/flesh/seed proportion and thus wine quality are also influenced by water supply (ROBY AND MATTHEWS 2004,ZSÓFI *et al.* 2009).

Beside the quantitative approach of grape skin and seed phenolic maturity, the extractability of the phenolic components (ie. anthocyanin) from the grape during wine making is also an important aspect of wine quality. It seems that the extractability of skin anthocyanin is strongly influenced by grape berry texture properties. It was found that there was a close relationship between skin thickness/elasticity/hardness and anthocyanin extractability (RÍO SEGADE *et al.* 2008, RÍO SEGADE *et al.* 2011a, ROLLE *et al.* 2011b, ROLLE *et al.* 2012). However, there can be differences among varieties in skin mechanical parameters, which show a correlation to anthocyanin extractability (ROLLE *et al.* 2012). Also, berry skin and seed mechanical behaviour show high variability under different environmental conditions (RÍO SEGADE *et al.* 2011a) as well as during the ripening (ZSÓFI *et al.* 2014) and there is also variablility among several grape varieties (LETAIEF *et al.* 2008b, RÍO SEGADE *et al.* 2011b). Indeed, (RÍO SEGADE *et al.* 2011a) showed that different terroirs have a significant effect on berry texture properties and the phenolic maturity index. Also, PORRO *et al.* (2010) showed that water stress and different nutrition levels resulted in increased berry skin thickness. Similar results were obtained by ZSÓFI *et al.* (2014). They found that different water stress treatments increase berry skin thickness, hardness and seed hardness to a different extent in the case of the Kékfrankos variety. However, in the case of some texture parameters the differences between the treatments decreased as the ripening process went forward (ZSÓFI *et al.* 2014), but varieties may have different responses under different conditions, as was suggested by (LETAIEF *et al.* 2008a).GIORDANO *et al.* (2013) also reported that irrigation treatments had no influence on berry skin thickness and skin hardness of Muscat blanc variety. However, optimal irrigation level has a beneficial effect of free volatile components under alpine environment.

Besides environmental factors, berry size is also an important factor in creating grape quality. Indeed, the sugar concentration of smaller berries is generally higher compared to the bigger ones (ROBY *et al.* 2004, BARBAGALLO *et al.* 2011, ZSÓFI *et al.* 2011). Also, very similar results were obtained in the case of the phenolic concentration (tannins and anthocyanins) of berry skin mass (ROBY AND MATTHEWS 2004, ZSÓFI *et al.* 2014). Furthermore, ROBY AND MATTHEWS (2004) showed that relative skin and seed mass (% of the whole berry fresh mass) was consequently higher in smaller berries and in water stressed berries.

As a consequence, berry size and water deficit may have a combined effect on berry mechanical properties. Therefore a description of the berry skin texture behaviour under different water conditions may also provide valuable data from a practical point of view. The aim of this present paper is to study the effect of mild-to-moderate water deficit and berry size on Portugieser (*Vitis vinifera* L.) berry analytical parameters and skin mechanical properties.

Material and Methods

Experimental design and plant material

Six-year-old Portugieser (*Vitis vinifera* L.) red grapevines grafted on Teleki-Kober 5BB rootstock were submitted to water deficit under greenhouse conditions, as described in (VILLANGÓ *et al.* 2013) and in (ZSÓFI *et al.* 2014). Briefly: The experiment was carried out in Eger, Hungary in a greenhouse of the Research Institute for Viticulture and Enology. The greenhouse was opened at the front during the experiment; furthermore the air temperature of the greenhouse was half-controlled by an automatic system, which regulated the opening of the upper windows. Plants were planted into 50L white plastic containers in a mixture of perlite (20%), loamy soil (30%) and peat (50%) (v/v). Three shoots and two clusters per shoot were left in each pot; lateral shoots of the plants were removed during plant development from each treatment. Two regimes of water supply were examined, defined by the leaf daily stomatal conductance (g_s) according to several authors (FLEXAS AND MEDRANO 2002, MEDRANO *et al.* 2002, CIFRE *et al.* 2005) and as applied in other works previously (GALMÉS *et al.* 2007, POU *et al.* 2008): nil stress (g_s above 150 mmol H_2O $m^{-2}s^{-1}$, as 100% field capacity) and moderate (g_s between 50-150 mmol H_2O $m^{-2}s^{-1}$, as 50% field capacity). The level of water stress was maintained by watering the plants with the amount of daily water loss.

Irrigation was carried out twice a day, early in the morning and in the afternoon. Eight plants were kept as control, with irrigation twice a day (in the evening and in the morning). Irrigation was stopped from veraison for 8 plants for the moderate stress treatment, and the daily water loss was measured by a scale (Kern, DS 100K1, Balingen, Germany). Changes in leaf stomatal conductance of the treatments were monitored daily (except cloudy days) in the morning, 11:30 (local time) by a CIRAS-1 infrared gas-analyser (PP System, UK) during the experiment. As a result of water withholding stomatal conductance decreased. Moderate water deficit (g_s values were ranging between 50-150 mmol $m^{-2}s^{-1}$) was achieved by the 9th day after the irrigation stopped. After the desired water deficit was achieved the weights of the pots were recorded. All pots of water deficit treatments were weighted twice a day during the rest of the experiment and the water loss was calculated. The level of water stress was maintained by watering the plants with the amount of daily water loss each day until the end of the experiment. Also, stomatal conductance was monitored in this period, in order to check the plant response of the treatments (Fig. 1).

The harvest was conducted 24 days later when the desired water deficit was achieved (27 July). For each treatment the harvest was made at the same time (Fig. 1).

Measurements

Physiological measurements

In situ gas-exchange parameters were measured with a CIRAS-1 infrared gas-analyser (PP System, UK) in 6-8 replicates per sampling at 11.30 am (local time). Measurements were taken on different plants, on mature, undamaged leaves that had grown fully-exposed to the sun. During the gas-exchange measurements there were no significant differences between the samplings with regard to light intensity (PAR), relative humidity (RH) and air temperature (T) (please see the description of the greenhouse conditions). All measurements were taken within 1 hour in order to obtain comparable data (ZSÓFI *et al.* 2014).

Berry sampling and analytical measurements

Grape bunches were harvested from the plants of the treatments, berries were removed with pedicels from the clusters and visually tested before analysis. 48 clusters of eight plants (nine bunches per plant) per treatment were harvested, respectively. All berries for measurements were taken from each cluster and five berry categories were defined: I: < 1,1 g; II: 1,11 - 1,4 g; III: 1,41 - 1,7 g; IV: 1,71 - 2 g; V: > 2,01 g. In each category the diameter of 25 berries (100 berries/treatment) was measured and their volume was calculated. High correlation was found between berry volume and berry weight in both treatments ($r^2 = 0.974$ and 0.961 respectively) as was reported by ROBY AND MATTHEWS (2004) in the case of the Cabernet sauvignon grapevine and by ZSÓFI *et al.* (2011) in the case of the Kékfrankos variety (data not shown).

Altogether 100 berries per treatment were taken for texture analyses, 25 from each berry category. Skin and seed weight of 40 berries was also measured by an analytical scale (Kern EG 300-3M, Albstadt, Germany) from all berry size categories. Skins of the berries were pealed 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 of 20 mL this solvent was used for each sample. The maceration of skins took place for 48 hours in a dark room. 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. Phenolic components were measured by a spectrophotometer (UVmini-1240 CE UV-VIS, Shimadzu, Japan). The bisulfite bleaching method was used to determine the anthocyanin content of grape skin extracts (RIBÉREAU-GAYON AND STONESTREET 1965). Total phenolics of the grape skin extracts were analysed by the Folin-Ciocalteau method (SINGLETON AND ROSSI 1965). Results are expressed in gallic acid equivalents (GAE mg/l). Catechin was measured with the vanillin assay according to (AMERINE AND OUGH 1980).

From each category berries were divided into three parts, and were crunched and pressed. Juice sugar concentration was measured with Rebelein's method (SCHMITT 2005).

Measurements of berry mechanical properties

TA.XTplus Texture Analyser (Stable Micro System, Surrey, UK) with HDP/90 platform and 30 kg load cell was used to follow grape mechanical properties. 25 berries were used for all type of mechanical measurements from each berry category. The Exponent 5.1 software was used for data evaluation. All operative conditions were applied according to (LETAIEF *et al.* 2008b); see Table 1. Briefly: P/35 probe was used to determine berry hardness (BH, N). Berries with their pedicel were gently removed from the bunch; they were laid on the plate of the analyser. After this, they were compressed to 25% of their diameter. P/2N needle probe was applied to conduct a puncture test. Also, berries with their pedicel were removed from the bunch, laid on the plate of the analyser and then punctured on the lateral face (LETAIEF *et al.* 2008a). Skin break force (F_{sk}, N), skin break energy (W_{sk}, mJ) and Young modulus of berry skin (E_{sk}, N/mm) were calculated from the puncture test by macros. Berry skin thickness was measured using of P/2 probe with 2 mm diameter. For this measurement approximately 0.25 cm^2 skin was removed from the lateral face of the berry. The skin was carefully and gently cleaned from pulp, placed on the platform, and the test was conducted as described by other authors previously (LETAIEF *et al.* 2008a, LETAIEF *et al.* 2008b, RÍO SEGADE *et al.* 2008).

Statistical analyses

Statistical analyses were conducted by the Sigma Stat (Systat Software Inc., San Jose, CA, USA) 8.0 software. Values were compared by one-way ANOVA test and Tukey's multiple range test was used for mean separation.

Results

Leaf gas-exchange

Stomatal conductance (g_s) of the stressed plants ranged between 114-136,1 mmol $m^{-2}s^{-1}$. These values were significantly lower compared to the non-stressed plants (242-315 mmol $m^{-2}s^{-1}$ (Fig. 1). Therefore, stomatal responses induced decreased CO_2 incorporation and transpiration rate per unit leaf area in the water stressed treatment. Net assimilation rate of the non-stressed treatment was ranging between 10,9-13,3 μmol m^{-2} s^{-1}, values of the stressed plants were between 5,9-8,9 μmol m^{-2} s^{-1}. Transpiration rate of the non-stressed treatment was between 4,6-6,9 mol m^{-2} s^{-1}, values of the moderately stressed plants were between 2,6-3,7 mol m^{-2} s^{-1} (data not shown).

Skin weight, berry sugar and phenolic concentration

Sugar concentration of the non-stressed berries were higher (ranged between 224-198 g) compared to the stressed treatment (ranged between 203-188 g) in all berry size categories. In the case of both treatments smaller berries presented higher sugar concentration than the bigger ones (non-stressed: I: 224 g/L II: 209 g/L III: 205g/L IV: 201 g/L V: 198 g/L; stressed: : I: 203 g/L II: 195 g/L III: 191 g/L IV: 190 g/L V: 188 g/L).

Skin weights of the water stressed berries were significantly higher compared to the control treatments in each category. Skin weights of the water stressed berries were between

0,15-0,24 g, control berries presented skin weights between 0,11-0,19 g. Therefore skin/flesh ratio higher in the stressed treatment compared to the well watered treatment (Fig. 2.).

Anthocyanin, catechin and total polyphenol concentrations for one kg of the berry skin were significantly higher in the non-stressed treatment compared to stressed berries in several categories (Fig. 3. A, B, C). In contrast, in most cases the anthocyanin and catechin concentration of the water stressed treatment calculated for one berry was higher compared to the control (Fig. 3. D, E). No differences were found between the treatments in total phenolic concentration (Fig. 3. F).

Berry mechanical properties

In each berry size category skin thickness (Sp_{sk}) was significantly higher in the case of the water-stressed treatment compared to the non-stressed vines (Fig. 4). In contrast, skin break force (F_{sk}) and skin elasticity (E_{sk}) of the water-stressed berries showed lower values than the non-stressed berries. In berry categories I-IV no significant differences were found between the treatments in the case of skin break energy (W_{sk}). W_{sk} of the berries in category V was significantly higher in the non-stressed treatments (Fig. 5). Berry hardness (BH) of the stressed vines was significantly lower in each berry size category. Interestingly, the smaller the berry size, the softer the berry in both treatments (Fig. 6). Also, a slight increase was observed in W_{sk} in both treatments as the berry weight increased. Interestingly, a decreasing trend was measured as the berry weight increased in F_{sk}, E_{sk} and Sp_{sk}; however this was observed only in the case of the stressed berries.

Discussion

Grape and wine quality is influenced by several factors. Water deficit is one of the main components that may influence berry composition and the amount of quality parameters such as sugar, acids, anthocyanins etc. Indeed, several authors found that mild to moderate water deficit has a beneficial effect on the concentration of the quality parameters of the grape berries as well as the wines (OJEDA *et al.* 2002, ROBY *et al.* 2004, CASTELLARIN *et al.* 2007a, CASTELLARIN *et al.* 2007b, ZSÓFI *et al.* 2009, ZSÓFI *et al.* 2014). Water deficit has a direct effect on berry growth and thus on berry size and the proportion of the berry parts such as seeds, skin and flesh. Water deficit reduces berry size and, in parallel, results in thicker berry skin and thus lower skin/flesh ratio, as was reported by (ROBY AND MATTHEWS 2004) in the case of the Cabernet sauvignon variety. We found very similar results in the case of the Portugieser variety: in each berry size category the water stressed treatments presented higher skin weigh and skin thickness compared to the control vines. This phenomenon resulted in higher skin/flesh ratio.

Also, water deficit resulted in decreased sugar concentration as a result of decreased photosynthetic activity. Similar results were found by (MATTHEWS AND ANDERSON 1988) and ZSÓFI *et al.* (2014) where the water-stressed treatments had lower Brix^{o}/sugar concentration compared to the non-stressed treatment. However, other studies have reported that mild to moderate water stress often results in an increased sugar concentration in the berries compared to the non-stressed vines. This phenomenon was explained as a result of reduced berry size, the change in assimilate partitioning (KELLER 2010) and the modified sink-source ratio of the grapevine (ZSÓFI *et al.* 2011). In both treatments lower sugar concentration was accompanied by bigger berry size, as was also reported earlier by other authors (ROBY *et al.* 2004, ZSÓFI *et al.* 2011), and explained by the different dilution of sugars.

Interestingly, skin phenolic concentration (anthocyanin, catechin, total polyphenol – calculated for one kg berry skin) of the water stressed berries was significantly lower in each berry size categories. This result is in contrast with other findings (OJEDA *et al.* 2002, BUCCHETTI *et al.* 2011, LIANG *et al.* 2014, ZSÓFI *et al.* 2014), where phenolic concentration for unit grape skin weight was higher as a result of water deficit. However, taking the calculation for one berry, the concentration of anthocyanins and catechin of the stressed berries was higher for each berry category, with the exception of category II. It was reported that a possible reason for the increased anthocyanin concentration of the berry is the higher skin/flesh ratio as a result of water deficit (ROBY *et al.* 2004). Indeed, our results showed that the skin/flesh ratio of the drought-stressed berries was higher by approximately 30-50% compared to the control berries. The phenolic concentration of the berry skin extraction (20 ml) of the drought stressed treatment was also higher in each berry weight category compared to the non-stressed treatment (data not shown). This finding matches other results such as (NADAL 2010). Taking the effect of berry size on skin phenolic concentration, it seems that smaller berries (with higher sugar concentration) have a higher phenolic concentration calculated for one kg berry skin. This result is in accordance with the findings of (ROLLE *et al.* 2011a). They showed that berries with higher sugar concentration presented higher anthocyanin and catechin concentration. BARBAGALLO *et al.* (2011) also showed in Syrah grapevine, that the largest berries have lower quality characteristics, with yellow-green seed colour. On the other hand in the smallest berries brown seed colour indicate faster ripening rate.

Texture characteristics of the water-stressed berries showed significant differences almost in each berry category. The lower hardness (BH) of the stressed berries indicates a softer pulp texture as a result of changes in cell wall structure (GOULAO AND OLIVEIRA 2008) and thus faster ripening. It has already been suggested by other authors that berry size must be an influence on grape berry texture behaviour (LE MOIGNE *et al.* 2008, MAURY *et al.* 2009). This phenomenon is probably also in connection with berry size in both treatments. Smaller berries presented lower hardness, indicating faster ripening. These findings are in accordance with the berry quality parameters within the treatments.

Berry skin thickness (Sp_{sk}) of the well watered plants was lower in each berry category. Increase of skin thickness as a result of water deficit has also been described in other studies (ROBY AND MATTHEWS 2004). In these studies, the higher skin mass of the water stressed berries was explained by the increased cell wall volume. Indeed, the increase of apoplast volume (i.e. cell wall) has already been well documented in other reports in other plant organs (i.e. grapevine leaves), as a result of water deficit (PATAKAS AND NOITSAKIS 1999).

Interestingly, berry skin break force (F_{sk}) was significantly lower in the stressed treatment. This is in contrast with other findings, where this parameter was higher in the water stressed treatments in the case of the Kékfrankos variety (ZSÓFI *et al.* 2014). A possible explanation for this result could be the concentration of the phenolic compounds in the skin. Phenolic compounds are bound to cell wall polysaccharides and proteins by peroxidase, and thus stiffen the cell walls and limit cell expansion (KELLER 2010). Indeed, in this study, the lower F_{sk} value is accompanied by lower phenolic concentration for unit skin weight, which may result in softer berry skin. Similar results were obtained by (ANDREWS *et al.* 2002, ROLLE *et al.* 2011b). They found that mechanical properties of the Nebbiolo grape variety did not relate to accumulation of red pigments in the skins. However, parameters of the puncture test seem a good estimator for the accumulation and the extractability of flavonoids, proanthocyanidins and flavanols.

Changes in skin break energy (W_{sk}) showed a very similar pattern to F_{sk} related to the treatments. Low E_{sk} values of the stressed grape berries indicated more elastic skin properties as was shown by (ZSÓFI *et al.* 2014) in the case of the Kékfrankos variety.

Changes in several berry texture parameters were accompanied by changes in berry size. Berry hardness and skin elasticity increased with berry size in both treatments. On the other hand, skin break force, skin break energy, skin thickness showed increase/decrease only in the case of the stressed vines. This result suggests that texture properties of the water-stressed berries depend on berry size to a greater extent compared to the berries of the non-stressed vines. This phenomenon may be explained the faster ripening of the smaller and of the water stressed berries. This result is also supported by (ROBY AND MATTHEWS 2004) . They found that the decreasing trend of the relative berry skin mass of the water stressed plants within six berry size categories was very similar in two different vintages (1999, 1998). In contrast, different trends were observed in the case of the irrigated and control treatments in each year respectively. In addition, they found that skin/pulp/seed proportions can be different according to berry size and different water supply. Furthermore, this finding partly matches the results of (ROLLE *et al.* 2011a, ROLLE *et al.* 2011b). They found tendencies in several texture parameters with berries having different flotation behaviour and density in the case of Mencía and Nebbiolo red grape cultivars (*Vitis vinifera* L.). However, it was very vineyard-dependent, which suggests that this phenomenon largely depended on the local environmental conditions (i.e. water deficit, vineyard exposure, soil etc.).

In summary, berry size and water deficit have a profound effect on berry texture behaviour and quality parameters. Water deficit increased the concentration of the phenolic compounds per berry; however, this value was lower for unit skin weight. It seems that the effect of water deficit on berry texture behaviour largely depends on the variety. Also, the differences among berry size categories and trends in texture parameters mainly manifested themselves in the water stressed treatments, with the exception of berry hardness.

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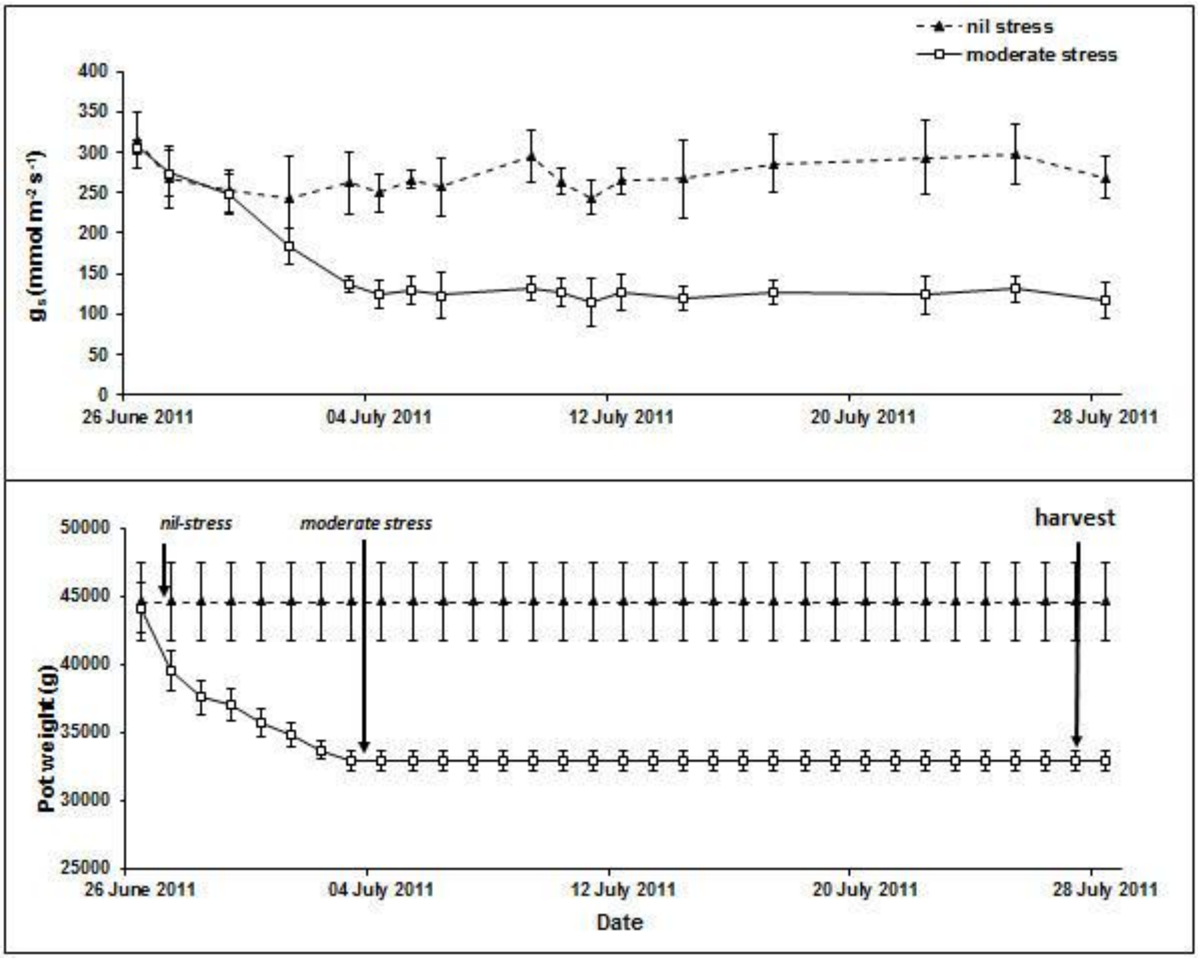

Fig. 1. Changes in stomatal conductance (g_s) (A) and pot weights (g) (B) during the experiment. Each g_s symbols represent the average ± standard error of 6-8 replicates. Also, pot weight symbols represent the average ± standard error of 8 replicates. The starting dates of the water supply treatments and the dates of harvest are indicated by arrows. There were significant differences among the treatment after achieved the desire water deficit according to Tukey's test (P<0,05).

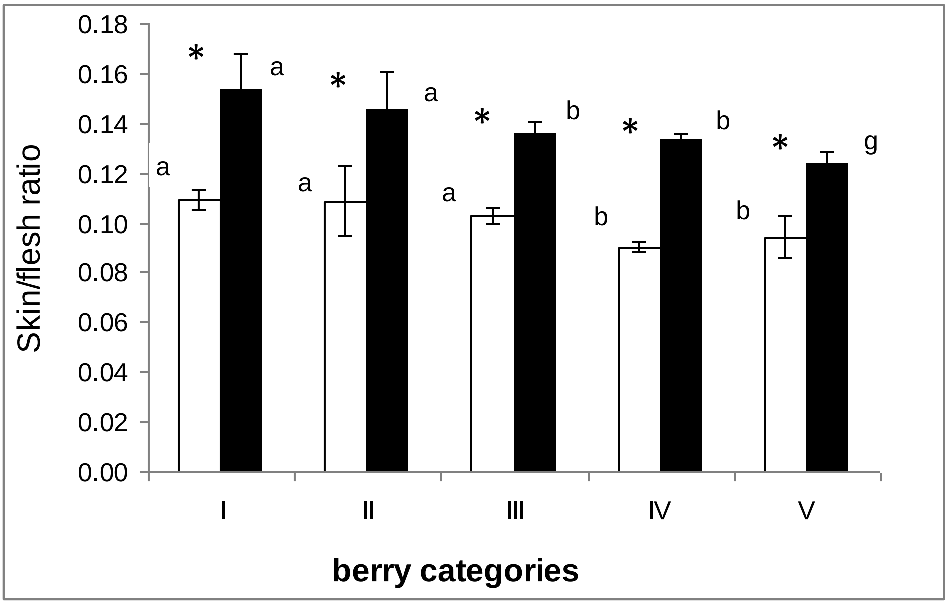

Fig. 2. Changes in berry skin/flesh ratio of the treatments in different berry weight categories. Each column represents the average ± standard error of 40 replicates. Columns marked * are significantly different from each other. Different letters indecate significant differences between the berry weight categories (greek letters – moderate water stress; roman letters – nil stress) according to Tukey's test (P<0,05).

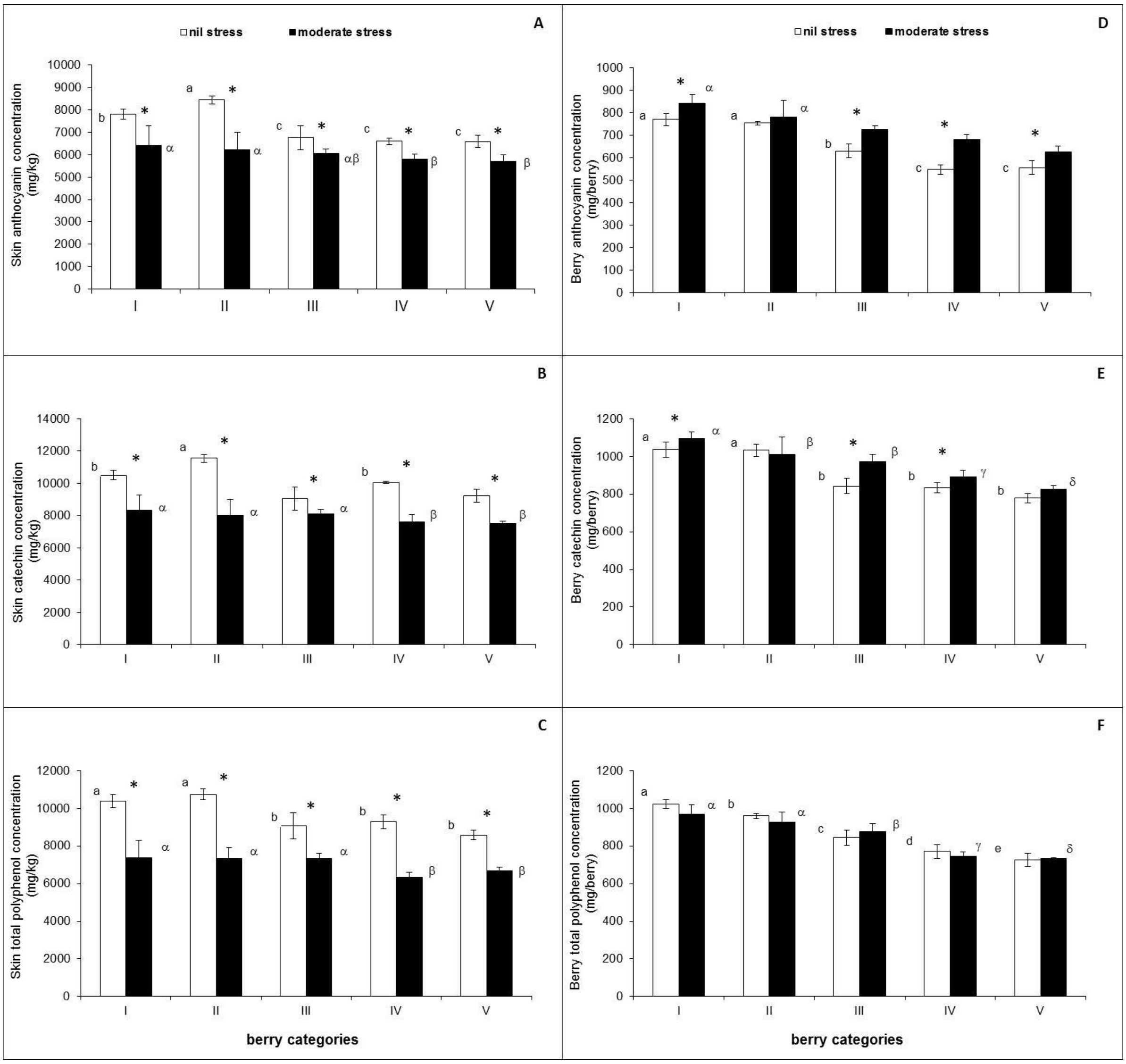

Fig. 3. Anthocyanin (A, D) catechin (B, E) and total polyphenol (C, F) concentrations of the skin and berry in different berry weight categories. Each column represents the average ± standard error of three replicates. Columns marked * are significantly different from each other. Different letters indecate significant differences between the berry weight categories (greek letters – moderate water stress; roman letters – nil stress) according to Tukey's test (P<0,05).

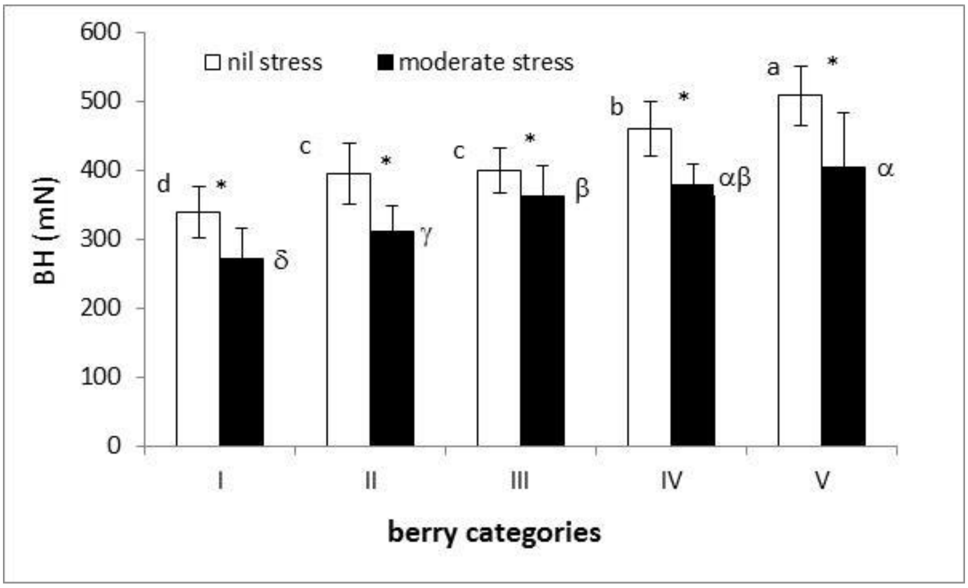

Fig. 4. Changes in berry hardness (BH) of the treatments in berry weight categories. Each column represents the average ± standard error of 25 replicates. Columns marked * are significantly different from each other. Different letters indecate significant differences between the berry weight categories (greek letters – moderate water stress; roman letters – nil stress) according to Tukey's test (P<0,05).

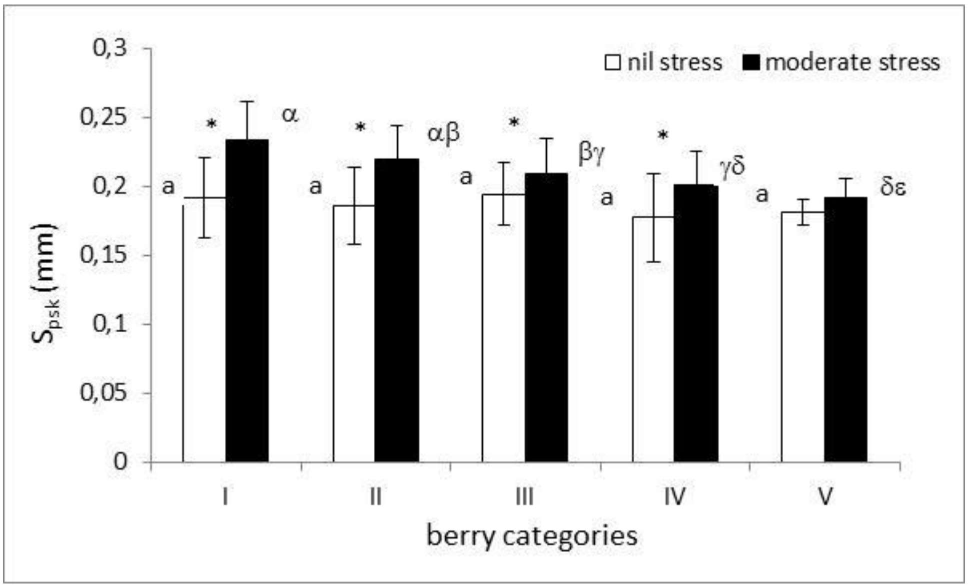

Fig. 5. Changes in berry skin thickness of the treatments in berry weight categories. Each column represents the average ± standard error of 25 replicates. Columns marked * are significantly different from each other. Different letters indecate significant differences between the berry weight categories (greek letters – moderate water stress; roman letters – nil stress) according to Tukey's test (P<0,05).

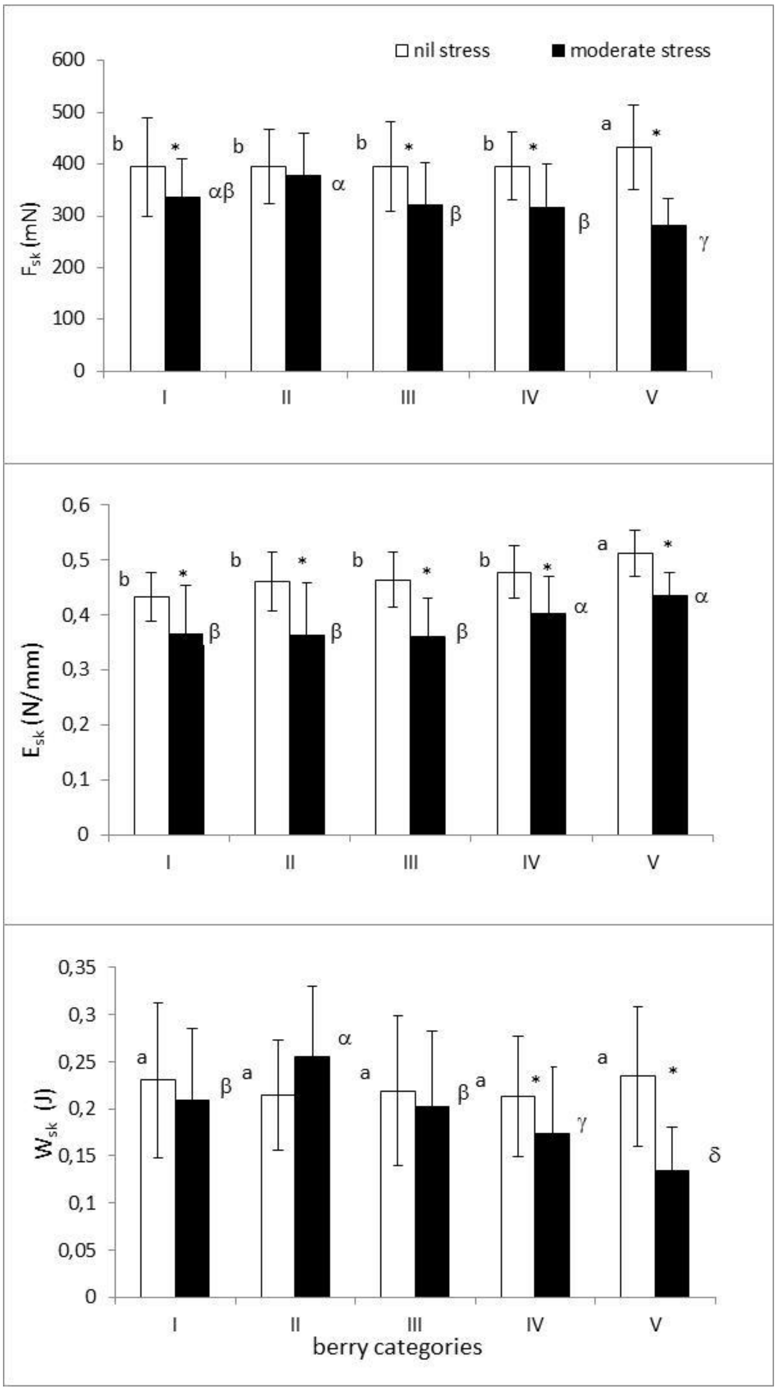

Fig. 6. Results of puncture test conducted on the berries according to berry weights. F_{sk}=skin break force, E_{sk}=skin Young's modulus, W_{sk}=skin break energy. Each column represents the average ± standard error of 25 replicates. Columns marked * are significantly different from each other. Different letters indecate significant differences between the berry weight categories (greek letters – moderate water stress; roman letters – nil stress) according to Tukey's test (P<0,05).

Table 1. Operative conditions of the berry texture analyses (after Letaief *et al.* 2008a).

	Probe	**Test speed**	**Compression**	**Mechanical property**
Berry skin thickness	P/2 2mm diameter	0,2 mm s^{-1}	-	**S_{psk}:** berry skin thickness (mm)
Berry skin hardness	P/2N needle	1 mm s^{-1}	3 mm	**F_{sk}:** berry skin break force (N) **W_{sk}:** berry skin break energy (mJ) **E_{sk}:** Young's modulus of the skin (N/mm)
Berry hardness	P/35 35 mm diameter	1 mm s^{-1}	25% of the berry diameter	**BH:** measure of force necessary to attain a given deformation (N)

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