Vine and Berry Responses to Severe Water Stress in Different Stages in *cv*. Syrah (*Vitis vinifera* L.)

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This experiment was carried out during the 2009 growing season in order to evaluate the ecophysiology and quality characteristics of *cv*. Syrah (*Vitis vinifera* L.) grafted onto SO4 in the ECOTRON vineyard in Montpellier SupAgro/INRA in France. The aim of this study was to analyse the effect of sudden and severe water stress (SWS) on the ecophysiological changes, volume losses and shrivellings in grape berries. Also possibilities of existence of recovery, and its possible relationship with SWS and final grapes composition at different phenological stages in cv. Syrah were studied. Three water regime levels; Control (only very mild water stress) and 2 severe water stress levels, SWS1 and SWS2 were established depending on the Ψ_{pd}. Stressed periods were started at the BV, MM and EM phenological stages. A randomized block design was used. The experimental plots consisted of 54 vines totally. All data analyses were performed with MSTAT-C Statistical Software (Michigan State University) and LSD tests were done for significant differences of measured traits between groups. Minimum Ψ_{pd} values in BV were -0.28MPa, -1.79MPa, -2.16MPa¸ in MM were -0.14MPa; -1.58MPa, -2.72MPa, in EM were -0.16MPa; -2.37MPa, -2.76MPa for Control, SWS1 and SWS2 respectively. Notice that such low predawn total leaf water potential are really exceptional, and at that level the regulation of the osmotic potential is critical. In the berry volume a limited recovery determined after about 13.00-26.00% volume loss for SWS1 and SWS2 respectively along stressed periods. At harvest; berry volume (cm^3), 100-berry weight (g), total soluble solids (°Brix), pH, total acidity (g tartaric acid L^{-1}), Tartaric acid (g L^{-1}), K (g L^{-1}), TPI and Anthocyanins (mg L^{-1}) were analysed. Berry volume loss rate (%) and mg sugar per 1g berry were calculated. SWS had a negative effect on the sugar per 1g berry, 100 berry weight, berry volume, TSS and positive effect on the anthocyanin concentrations and TPI at three phenological stages. This can open new ways for irrigation monitoring in combination with too high berry sugar content due to the climate change.

Keywords: Syrah, leaf water potential (Ψ_{leaf}), water stress, volume loss, recovery.

Introduction

Climate irregularity and consequently water stress are some of the most important worldwide problems in recent years. Water stress may greatly influence grape and vine metabolism. Furthermore, stress levels at different stages may lead to important physiological alterations that will affect vine yield and grape composition.

The water status of the vines has a significant influence on berry growth, at the herbaceous growth stage and during the period from veraison to maturity (Ojeda et al., 2001; Carbonneau and Bahar, 2009). Early water deficits modify the structural properties of the cell components and consequently cell wall extensibility, thereby limiting the subsequent enlargement of pericarp cell. After veraison berry size reduction due to water deficits is thought to be a consequence of a limitation of cell enlargement (Ojeda et al., 2001). Since development of the pressure chamber (Scholander et al., 1965), measurement of leaf water potential (Ψ_L) has been used as a tool to assess the water status of plants (Jones, 1990). Also in vineyard leaf water potential is considered the most practicable method for the control vine water status**.** Carbonneau (1998) and Deloire et al. (2004) use both pre-dawn (Ψ_{pd}) and mid-day (Ψ_{md}) leaf water potential as a criterion to evaluate vine water status at different developmental stages. Moreover Deloire et al. (2005) and Rogiers et al. (2015) proposed different levels of Ψ_{pd} for various vine styles. Depending on the intensity of the water stress and the period at which it occurs, it may or not be favourable for the harvest and the wine it is used to produce (Deloire et al., 2004).

Severe water stress is known for affecting negatively berry ripening (Coipel et al., 2006). Intensity of water stress is also correlated to production levels. Also berry solutes like organic acids, sugars, anthocyanins and soluble phenolic compounds are sensitive to vine water status (De la Hera Orts et al., 2005; Carbonneau and Bahar, 2009).

Many researches were conducted as to determine the effect of sudden and severe water stress on vine and berry metabolism. Some water fluxes were simulated in Ecotron (SupAgro/Montpellier) by Carbonneau and Bahar (2009). The manipulation of water limitation towards some extreme values and short periods around veraison, allowed the control of berry size and the differentiation in primary metabolites such as sugars and secondary metabolites such as polyphenols. Bahar et al. (2011a) revealed the importance of sudden and extreme water stress (EWS) on vine physiology and berry composition of three cultivars, namely Chardonnay, Merlot and Cabernet-Sauvignon. Their results showed that, both Ψ_{pd} and Ψ_{md} values were similar when Ψ_{pd} was reduced to -2.1MPa in all three varieties. The results also indicated that all the leaves were dropped and the clusters were fully exposed to sun after EWS treatment, these lead to smaller berries, thus increase of anthocyanin concentration, FCI and PTI values at harvest time. Also there was an increase in pH and total acidity values. On the contrary a decrease in 100 berry weight, berry volume, TSS, sugar concentration, sugar content per berry, K and tartaric acid was dermined. Bahar et al. (2011b) analyzed the effects of Severe Water Stress (depend on Ψ_{leaf}) to determine the ratio of berry shrivelling, possibilities of recovering, changes of berry composition during the stress period in the lag phase and its relationship with yield and grape composition. Because of SWS more than 50-60% of the leaves dried from the base to upper part of shoots in vines. Despite SWS the vines did not die and there was recovering after irrigation. Full recovery of berry sugar loading and concentration was not possible just after the period of stress.

The response of polyphenol in terms of hue attesting the real change in colour at veraison (days following the period of stress) was quite independent of the evolution of sugars which lead to berries rich enough in polyphenol with less sugars. Thus berry characteristics at harvest which correspond to a sugar maturity for both SWS1 and SWS2 water limitation between bunch closure and veraison stages leads to a significant increase of total polyphenol and anthocyanin.

The manipulation of water limitation towards some extreme values and short periods between bunch closure and veraison, allows to control berry size, primary metabolites and secondary metabolites. Because of SWS after 20% volume loss (shrivelling) there was a possibility of recovering of the shrivelled berries. The growth of berries continued but at harvest time almost 50% of the clusters shrivelled after the stress period. According to Korkutal et al. (2011) pollen viability and pollen germination ratios were not affected by early water stress conditions, but berry set ratio, phenologic stages and berry development were negatively affected. It was determined that in cv. Merlot, Ψ_{pd} values below -0.4MPa, should be avoided between 19th to 29th stages of Eichhorn and Lorenz (1977). Bahar et al. (2012) indicated that under severe water stress yield levels significantly and sustainably affected sugar concentration, titratable acidity and cluster weights. During the period of decrease of berry volume, berry sugar loading was stabilized by any stress during its application meanwhile it was increasing for the control. Because of water loss there was an increase in berry density and decrease in berry volume. It looks like there was an increase in sugar concentration but actually there was no increase in sugar content per berry.

The manipulation of water limitation towards some extreme values and short periods between bunch closure and veraison, allows to control berry size and to differentiate primary metabolites. According to yield level and SWS level and SWS period there was a possibility of recovering after about 15-20% volume loss in the shrivelled berries. After the stress period the growth of berries continued but at harvest time almost 58-81% of the clusters were partly or completely damaged according to stress levels.

As it was seen there are many possibilities for the development of new strategies in combating drought in vineyards. The aim of this study was to analyse the effect of sudden and severe water stress (SWS) on the ecophysiological changes, volume losses and shrivellings in grape berries. Also possibilities of existence of recovery, and its possible relationship with SWS and final grapes composition at different phenological stages in cv. Syrah were studied.

Materials and Methods

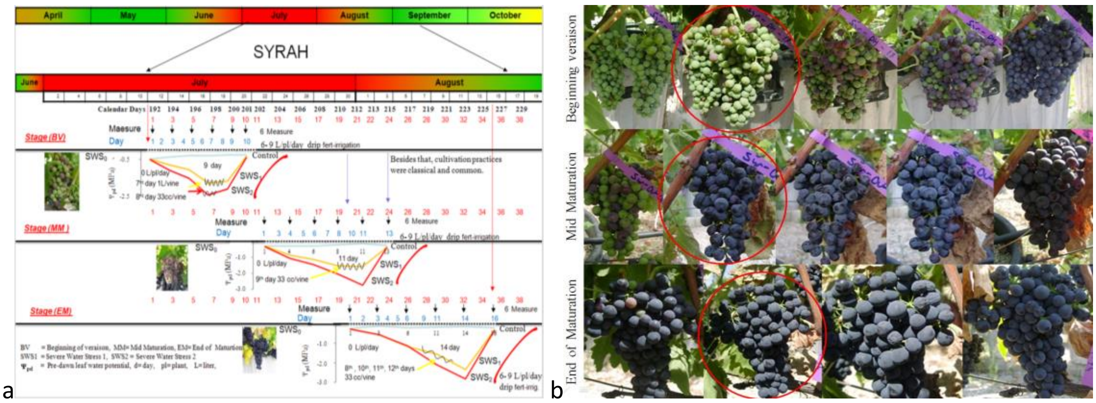
Plant material and location

The experiment was carried out during the 2009 growing season on Syrah (*Vitis vinifera* L.) grafted onto SO4 in the ECOTRON of the campus of Montpellier SupAgro/INRA in France inserted in the general experimental vineyard. The eight-year-old grapevines were grown in pots under natural conditions which had 70L volume. The pots were isolated from rainfall and had a controlled drainage and potting media with a mixture of coarse sand

and perlite. Row spacing was 3.5-0.8m and rows were N–S oriented and bilateral cordon trained vines were pruned on a Lyre architecture. Six arms and each had 2-3 nodes which remained, for a shoot load of 10-12 shoots per vine.

The calculated volumes of nutrient solution (6-9 L day^{-1}) were applied regularly every 6 hours for a day through two drip emitter for each plant except during water stressed period of growing season in severe water stress 1 (SWS1) and severe water stress 2 (SWS2) while its permanent for Control [well irrigated (WI)] group. Stress periods of vines were started in 192^{nd}(BV), 203^{rd}(MM) and 211^{th}(EM) days of growth under well watered conditions in the ECOTRON at the Beginning Veraison (BV), Mid Maturation (MM) and End of Maturation (EM) phenological stages (stages 35, 36, 37) (Eichhorn and Lorenz, 1977). Ψ_{pd} of control vines was maintained consistently between -0,30MPa and -0,05MPa during the experimental periods in all three developmental stages.

Two stress levels Severe Water Stress1 (SWS1) and Severe Water Stress2 (SWS2) were established in relation to the reference of maximal transpiration, and monitored in function of the vine response measured as the pre-dawn leaf water potential (Ψ_{pd}) (Carbonneau, 2001). The Ψ_{pd} values for the SWS1 in BV stage were n∈[-1,83, -0,34] MPa, for MM n∈[-1,58, -0,27] MPa, as for EM the values were n∈[-2,35, -0,26] MPa. For the SWS2 the Ψ_{pd} values in BV stage were n∈[-2,46, -0,41] MPa, for MM n∈[-2,72, -0,31] MPa, as for EM the values were n∈[-2,81, -0,27] MPa. The changes in Ψ_{pd} values for all treatments were indicated in detail according to certain days in (Figure 1). For two stress level, vines were irrigated with negligible quantity water during the stressed period. The experimental period for BV was 10 days, as for MM it was 13 days and for EM was 16 days. After the stressed period, potted vines were irrigated one time on the saturation point. Besides that, cultivation practices were classical and common (Figure 1).



a b

Figure 1. Experimental design of research (a) and general appearance of shrivelled grape berries (b).

Physiological measurement

The Ψ_{leaf} of each vine was determined with a Scholander pressure chamber (Scholander et al., 1965). Pre-dawn (Ψ_{pd}) and mid-day (Ψ_{md}) leaf water potentials were measured 6 times from beginning to the end of stress period for each growing stage (total 18-times measurement). Measurements were carried out about 35 days on freshly cut, healthy and fully expanded (mature) leaves from each vines for each of the two stress levels and control. After the first measurements, water and mineral supply cut off during days until pre-dawn leaf water potential (Ψ_{pd}) to be approximately equivalent with mid-day leaf water potential (Ψ_{md}). Those modalities and their distribution over time were illustrated in Figure 1.

Grape and juice analysis

During stressed period in early morning (06:00 to 07:00 AM), 10 berries per vines were sampled from different parts of various clusters and transported to the laboratory. Berry volumes (cm^3) were measured immediately after sampling by Dyostem apparatus (Sferis technology). After that, classical measurements were made on berries. Berries were weighed with an electronic balance and processed to determine 100-berry weight (g) and then juice extractions were analyzed for total soluble solids [(TSS) (Brix°)], pH and total acidity (g L^{-1}). Total soluble solids [(TSS) (Brix°)] were measured using an Abbé-type refractometer. Juice pH was measured using a pH-meter. Total acidities were measured by pH-meter with a base to an end point of pH 7.0 (20°C), and results were expressed as a g-tartaric acid L^{-1}. Potassium (K) analysis were conducted by flame photometer and expressed as a g L^{-1}. Tartaric acid (g L^{-1}) were analysed according to Cemeroglu (2007). TPI was quantified according to Ribéreau-Gayon (1970). Anthocyanins (mg L^{-1}) were analysed according to Mode d'Opératoire MO-LAB-23 UE Pech Rouge INRA-France (Anonymous, 2007).

Berry volume loss rate was calculated according to the following formula:
Berry volume loss rate (%)= [100 x (Berry volume 2, 3...n – Berry volume 1)]/Berry volume 1

Miligram sugar per 1g berry was calculated according to the following formulas:
Sugar (mg $berry^{-1}$) = [(1/3) x sugar cons. (g L^{-1})] x [(1/100) x 100 berries weight (g)] Carbonneau and Bahar (2009) and mg sugar per 1g berry= (Sugar (mg $berry^{-1}$) x 1)/Berry weight (g)

Experimental design and statistical analysis

A randomized block design was used. There were three blocks of 3 rows. The experimental plots consisted of 54 vines totally (18 vines for SWS1, 18 vines for SWS2, and 18 vines for Control) (Table 1). All data analyses were performed with MSTAT-C Statistical Software (Michigan State University) and LSD tests were done for significant differences of measured traits between groups.

Results and Discussion

During beginning of veraison stage (BV) in Control vines Ψ_{pd} values changed between -0,27MPa and -0,48MPa. Similarly, in other stages [mid-maturation (MM) and end of maturation (EM)] Control vines were non stressed or moderate stressed. Throughout MM and EM stages in Control vines Ψ_{pd} values were between -0,14MPa : -0,30MPa and -0,12MPa : -0,25MPa respectively (Figure 2 a; 3 a and 4 a). In relation to these values average Ψ_{md} in Control (wel irrigated) vines were remained almost -1,25MPa and changed between -0,99MPa and-1,47MPa; -0,84MPa and -1,51MPa; -1,19MPa and -1,53MPa in BV, MM and EM stages respectively (Figures 2 b; 3 b and 4 b).

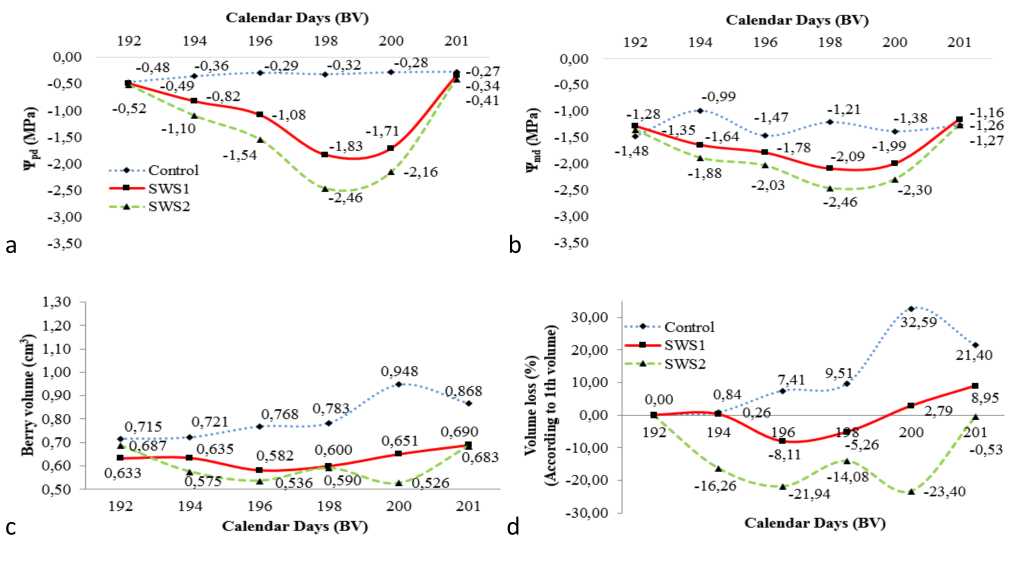


Figure 2. Changes in Ψ_{md}, berry volumes and berry volume losses, depending on Ψ_{pd} during BV.

In addition to Ψ_{pd} levels Ψ_{md} values were also affected by daily conditions (sunlight intensity, sunlight exposure duration, temperature, relative humidity, wind speed and direction, etc.) and in control, they did not fall below the critical limit of -1.6 MPa (Ψ_{md}) and remained compatible with Ψ_{pd} (Figure 2 a, b; 3 a, b and 4 a, b). First water stresses were seen after 194th, 204th and 213th calendar days in BV, MM and EM stages respectively after irrigation cut of. Severe water stresses were started in SWS1 and SWS2 at 5th (BV) and 6th (MM and EM) days after irrigation stopping and were finished at 200th, 211th and 224th calendar days. When Ψ_{pd} values decreased below to -1.6 MPa Ψ_{md} values were generally below to -2.0MPa in SWS1 and SWS2. Also when Ψ_{pd} decreased below to -2.1 MPa both Ψ_{pd} and Ψ_{md} values were similar in all three phenological stages (BV: Ψ_{pd}= -2.46MPa and Ψ_{md}= -2.46MPa); MM: Ψ_{pd}= -2.72MPa and Ψ_{md}= -3.40MPa and EM: Ψ_{pd}= -2.81MPa and Ψ_{md}= -2.95MPa) in SWS2. 1-2 days after re-irrigation water stresses finished and Ψ_{leaf} values returned to normal at all periods and stress levels. There was a recovery after re-irrigation in stressed vines (Figures 2 a,b,3 a,b and 4 a,b). Similar findings obtained by Carbonneau and Bahar (2009), Bahar et al. (2011a), Bahar et al. (2011b), Korkutal et al. (2011), Bahar et al. (2012).

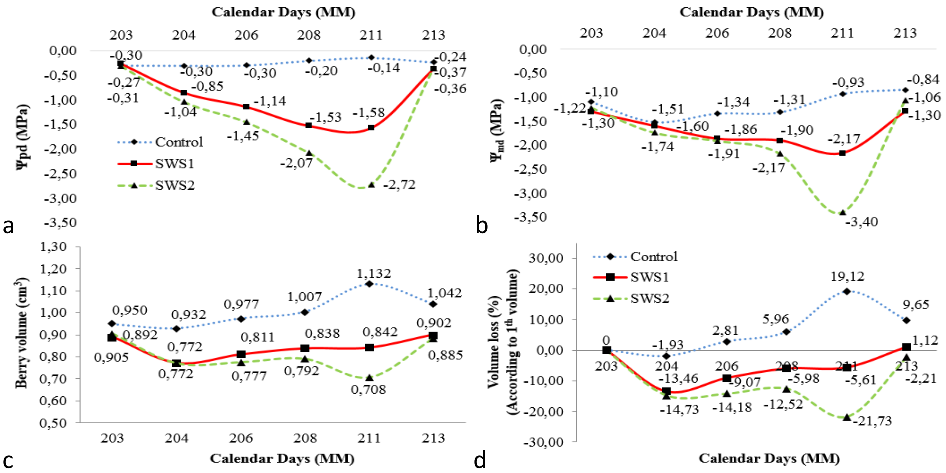


Figure 3. Changes in Ψ_{md}, berry volumes and berry volume losses, depending on Ψ_{pd} during MM.

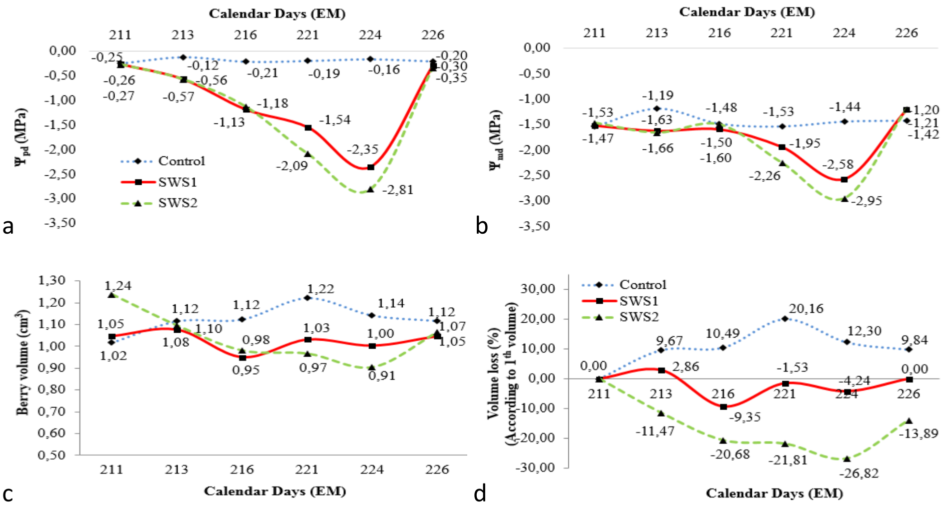


Figure 4. Changes in Ψ_{md}, berry volumes and berry volume losses, depending on Ψ_{pd} during EM.

There were considerable volume losses in berries according to stress levels while berry volumes continued to increase normally in non-stressed vines (Figures 2 c, 3 c and 4 c). These volume losses range from 8.11% (BV) to 9.35% (EM) in SWS1.

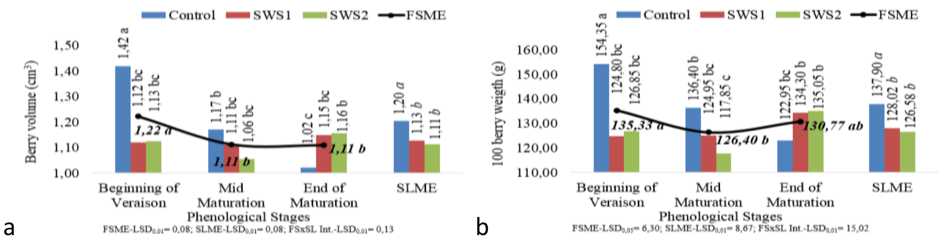


Figure 5. Changes of berry volumes (a) and 100 berry weights (b) in BV, MM and EM stages according to stress levels.

In SWS2, the volume losses were greater than SWS1 and changed from 21.73% (MM) to 26.82% (EM). Shrivelling finished after re-irrigation but there were a limited recovery in all grape berries according to stress levels (Figures 2 d, 3 d and 4 d). Therefore, the final berry volumes and berry weights were also negatively affected by both stress levels (SWS1: 1.13 b and 128.02 b; SWS2: 1.11 b and 126.58 b) during BV (1.22 a; 135.33 a) MM (1.11 b; 126.40 b) and EM (1.11 b; 130.77 ab) stages. (Figure 5 a, b).

Photosynthetic radiation use efficiency strongly depended on both, pre-dawn leaf water potential and light-satured stomatal conductance (Escalona, 2003). TSS ratios decreased with increases in stress levels during BV (21.67 a), MM (19.67 b) and EM (19.87 b) periods. These declines in concentration were reflected to the mg sugar/1 g berry. Especially in SWS2, the decline in sugar concentration (19.77 b) and sugar quantity per 1 g berry (63.12 b) has become very apparent in MM (18.10 e; 56.80 e) and EM (19.10 de; 60.57 de) stages respectively (Figure 6 a, b).

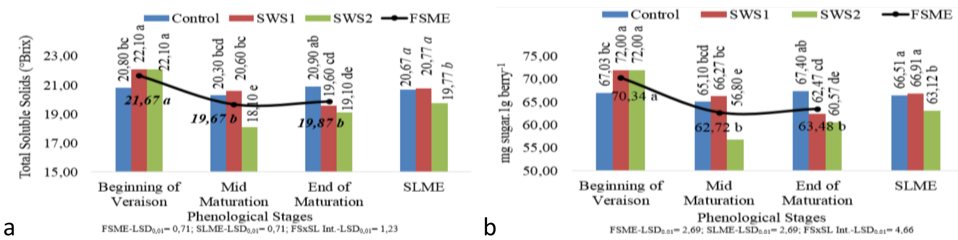


Figure 6. Changes of total soluble solids (TSS) (a) and mg sugar/1 g berry (b) in BV, MM and EM stages according to stress levels.

The final pH and K content of grape juice increased in parallel with the increase in stress levels during BV, MM and EM stages. Therefore, the highest pH was observed in SWS2 (4.11 a), followed by SWS1 (4.09 a) and similar results obtained by Bahar et al. (2011a). The highest potassium content was determined during the BV period in SWS1 (3.88 a). Higher water stresses in the early period (in BV) increased the pH (4.09 a) and K levels (3.51 a) in grape juices. Grape berry potassium accumulation is important because elevated levels of berry potassium can have a negative effect on wine quality by increasing berry and wine pH (Gawel et al., 2000).

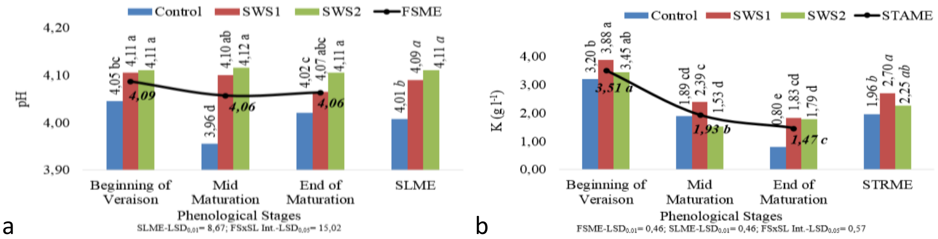


Figure 7. Changes of pH (a) and K (Potassium) (b) in BV, MM and EM stages according to stress levels.

Total acidity was higher in SWS1 (4.36 b) and SWS2 (4.54 a) than control vines (4.11 c). It also varied depending on the periods of stress applied. Especially the effect of stress applied in the later stages of maturation is more apparent (BV: 4.20 b; MM: 4.50 a; EM: 4.31 b). Bahar et al. (2011b) obtained similar results. As noted by Carbonneau and Bahar (2009) depending on the stress level tartaric acid also increased and SWS2 (2.51 a) was the highest.

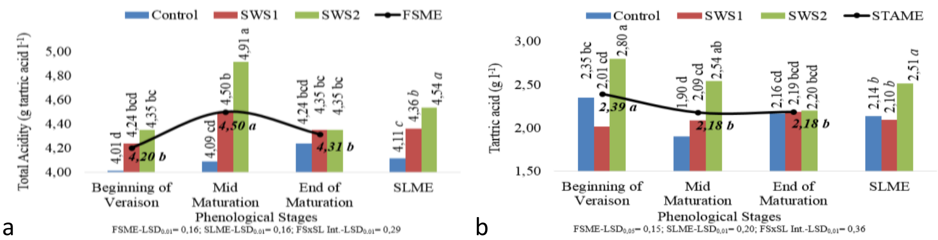


Figure 8. Changes of total acidity (a) and tartaric acid (b) concentrations in BV, MM and EM stages according to stress levels.

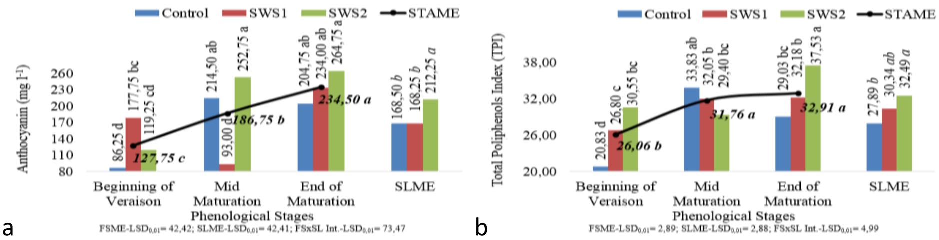


Figure 9. Changes of anthocyanin concentrations (a) and TPI (b) in BV, MM and EM stages according to stress levels.

Conclusions

As a result of evaluating the data obtained from this study: It was determined that sudden and severe water stresses applied at different stages caused significant shrivelling (about 27.00%) in the grape berries. After the sudden and severe water stresses, there was a recovery in the vines and limited one in grapes berries. There was also a decrease in berry volume, 100 berry weight, TSS and mg sugar per 1 g berry depending on periods and stress levels. Furthermore there was an increase in pH, K, total acidity, tartaric acid, anthocyanin and TPI quantities depending on periods and stress levels. In conclusion the stress levels applied at different stages can be strategically used to decrease or increase some problematic criterias depending on terroir in wine varieties such as re-equilibrating the ratio polyphenols/sugars of the grape berry.

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