



## Relationship between post veraison temperature and the dynamics of anthocyanin development and berry quality in red wine grape (*Vitis vinifera*) cultivars Shiraz and Cabernet Sauvignon

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### ABSTRACT

The present investigation was carried out to understand the temperature induced dynamics of berry anthocyanin development and berry quality in wine grape (*Vitis vinifera* L.) cultivars Shiraz and Cabernet Sauvignon. Temperature variations during fruit maturation period were achieved by altering the pruning dates. Vines were pruned at weekly intervals starting from third or fourth week of October to the end of November. The changes in berry quality parameters such as total soluble solids (TSS), titratable acidity, total anthocyanins, total phenols and total flavonoids were recorded at weekly intervals starting from veraison to harvest. All the quality parameters were significantly higher in early pruned vines of both the cultivars indicating that the best time for pruning to get good quality wine grapes is during the third or fourth week of October. The major quality parameter affected by the temperature during the berry maturation stage was total anthocyanin content. Maximum and minimum temperature (weekly average) during different week from veraison to harvest were recorded. Early pruned vines exposed to a mean day/night temperatures of 30.7°C/14.9°C and 29.6°C/14.1°C in cv. Shiraz and late pruned vines exposed to a mean day/night temperatures of 32.0°C/16.2°C and 32.1°C/16.0°C in cv. Cabernet Sauvignon during 2010 and 2011 respectively. The pattern of anthocyanin development during the berry maturation period (veraison to harvest) indicated that the maximum anthocyanin accumulation was in the second and third week of March; the concentration decreased thereafter irrespective of dates of pruning in both the cultivars during the two years of the study. Correlation coefficients between the skin anthocyanin content and mean maximum and minimum temperatures showed a negative relationship in both the cultivars indicating that the increase in both maximum and minimum temperatures caused a reduction in anthocyanin content at harvest in late pruned vines.

**Key words:** Anthocyanin, Cabernet Sauvignon, Grapes, Pruning, Shiraz, Temperature,

Anthocyanins are the major pigments responsible for the skin colour in red wine grapes (*Vitis vinifera* L.). Further, they also contribute to the sensory quality and antioxidant capacity of the wine. Health benefitting effects of red wines has been attributed to the anthocyanin content and their antioxidant capacity. One of the important attributes responsible for the consumer acceptability of wine is the anthocyanin induced colour intensity (Burin *et al.* 2011). Therefore the market value of red wine grapes is largely determined by the intensity of skin colour. The intensity of the skin colour which is due to the anthocyanin content is influenced by TSS, maturity level and the night temperature

during maturity (Koshita *et al.* 2007, Koshita *et al.* 2011). However, in hot tropical regions anthocyanin accumulation is inhibited may be due to higher temperatures during the fruit maturation period (Spayd *et al.* 2002, Downey *et al.* 2006).

High day and night temperatures (35°C/25°C day/night) significantly reduce the anthocyanin concentrations in the berry skins of cv. Cabernet Sauvignon when compared to the lower temperatures (25°C/20°C day/night) (Yamamoto *et al.* 2010). However, greater control of anthocyanin accumulation seems to be the night temperature (Mori *et al.* 2005) especially during the initial stages of berry development (Yamane and Shibayama 2006). Lower anthocyanin accumulation at higher temperatures is due to the alterations in the expression of anthocyanin biosynthetic genes and the production of ABA (Yamane *et al.* 2006). In addition to reducing the biosynthesis of anthocyanins, higher temperatures also degrade the already formed anthocyanins in the berries (Mori *et al.* 2007). This indicates the importance of harvesting grapes at right stage for maintaining good quality.

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In peninsular India, the major grape growing region, vines do not undergo dormancy. Therefore, horticulturists have adapted the crop to tropical conditions by practicing double pruning and single cropping system. Two pruning systems practiced are, foundation pruning and forward pruning. Forward pruning is taken for fruiting and pruning time is decided according to the climate of the area. Wine grapes prefer warm bright days, dry cold night temperature and mild winters under tropical regions. For complete maturation of the red wine grapes the growing season length should be sufficiently long. Presently in order to provide sufficiently long fruit development period to produce quality fruits for wine making the forward pruning is taken up during October to November.

The climate change scenarios projected for India indicate greater raise in minimum temperature in addition to the increase in maximum temperature. The quality of grapes in terms of aroma, flavor, acid-sugar balance and fruit colour are likely to be adversely affected by increased temperature. However, the adverse effects of high temperature could be minimized by coinciding the time of veraison during cooler days by altering the pruning dates. Therefore, the present study was carried out in cultivars Shiraz and Cabernet Sauvignon to understand the relationship of anthocyanin development and other quality parameters with temperature by changing the pruning dates.

#### MATERIALS AND METHODS

Red wine grapes were harvested from nine years old vines from the Orchard of Grover's Vineyard located between 13°17'31" N and 77°32'35" E with an elevation of 880 m above mean sea level which is near Bengaluru, Karnataka, India. The vines in the orchard are grafted on Dog ridge rootstock. The forward pruning of the shoots was done leaving five buds from the base and 2% Dormex was applied on all the buds to induce bud break. After the bud break distal 2 or 3 shoots expanded and flowers blossomed after 30 days and bloom period lasted for 10 days. Thereafter, the shoots were halted retaining 16 leaves per shoot to support the developing berries. An average berry yield of 3.5 kg in cv. Shiraz and 3.0 kg in cv. Cabernet Sauvignon per vine was obtained.

In order to understand the requirement of optimum temperature conditions for better quality wine grapes, vines were pruned at different times during the years of 2009-2010 and 2010-2011. Pruning at different times resulted in veraison dates coinciding with different temperature regimes during berry development. Vines were pruned at weekly intervals from 18 October to 18 November in cv. Shiraz and from 22 October to 18 November in cv. Cabernet Sauvignon. The dynamics of changes in berry quality were analyzed in samples taken at weekly intervals from veraison to harvest. The quality parameters such TSS, total acidity of the juice and total anthocyanins, total phenols and total flavonoids of the skin were assessed. To assess various quality parameters a sample of 100 randomly selected berries from 15 bunches was pooled and skin, pulp and seeds were separated. A

known quantity of skin was transferred immediately to a conical flask containing acidic methanol (99:1, Methanol:HCl) solution and incubated for 72 hr at room temperature for further analysis of total anthocyanins, total phenols and total flavonoids in triplicates.

The TSS was analyzed by digital refractometer and results were expressed as °Brix at 25°C.

Acidity was determined by titration method (AOAC, 942.15). A known volume (5 mL) of juice was diluted to 50 mL with distilled water and 10 mL of the diluted juice was titrated against 0.01 N NaOH using phenolphthalein as indicator. Acidity was expressed as percent of tartaric acid equivalents.

Total anthocyanins were estimated using the method of Fuleki (1969). Two grams of berry skin was homogenized with acidic methanol and extracted three times. Extracts were pooled and the volume was made up to 100 mL with acidic methanol. Total anthocyanin content was estimated by measuring the absorbance at 540 nm using spectrophotometer. Total amount of anthocyanin present in the sample was calculated using cyanidin hydrochloride as standard and expressed as milligrams of cyanidin hydrochloride equivalents per 100 g fresh weight.

Total phenols were estimated using Folin-Ciocalteu method (Singleton and Rossi 1965). Two grams of berry skin was homogenized with acidic methanol. The extract was mixed with Folin-Ciocalteu reagent and the color was developed using sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) reagent. Intensity of color developed was read by measuring the absorbance at 700 nm. Results were expressed as mg of gallic acid equivalents per 100 g fresh weight.

Total flavonoids were estimated using the methods of Chun *et al.* (2003). Two grams of berry skin was homogenized with acidic methanol. The extract was mixed with 5% sodium nitrite ( $\text{NaNO}_2$ ) followed by 10% aluminum chloride ( $\text{AlCl}_3$ ). After 1 min, 4 N sodium hydroxide (NaOH) was added and the brick red colour developed was read at 510 nm after diluting with double distilled water. Flavonoid content was expressed as mg of catechin equivalents per 100 g fresh weight.

Analysis of variance was carried out to understand the statistical significance of the differences among the quality parameters. Data were subjected to the analysis of variance using ANOVA and means were compared with critical difference at  $P \leq 0.05$ . The Pearson correlation coefficients ( $r$ ) were calculated among all variables using Microsoft Excel software.

#### RESULTS AND DISCUSSION

Vines of Shiraz and Cabernet Sauvignon were pruned at weekly intervals to alter the dates of veraison with an aim to expose the berry colour development stage to different temperatures. Minimum and maximum temperatures (weekly average) during different weeks from veraison to harvest were recorded. In the first experiment the berries were harvested at weekly intervals after veraison up to full maturity to understand the dynamics of anthocyanin

development under different temperatures. Quality parameters such as TSS, % acidity, total phenols and total flavonoids were also analyzed. In the second experiment berries were harvested at full maturity stage and the relationship of various quality parameters (TSS, % acidity, total anthocyanins, total phenols and total flavonoids) with the temperature during the colour development stage was assessed.

In the first experiment, rate of anthocyanin development in berries from veraison to harvest was studied in vines pruned at different dates, to understand the effect of minimum and maximum temperatures on the dynamics of anthocyanin development and various quality parameters. In Shiraz, the results indicated that irrespective of pruning dates, anthocyanin content reached the maximum concentration

in the 2<sup>nd</sup> and 3<sup>rd</sup> week of March during the first and the second year of study, respectively (Fig 1). In the early pruned vines (22 and 29 October) total anthocyanin content started decreasing after second week of March whereas, in the late pruned vines (5 and 12 November) the reduction was marginal for the year 2010. Similar trend was observed during the year 2011, but the extent of reduction was less and the maximum content was observed during the third week of March. Similar results were also observed for Cabernet Sauvignon. The temperatures (weekly average) during this period indicated that the accumulation of anthocyanin takes place only up to a certain temperature beyond that the content decreases may be due to degradation of already formed anthocyanins by higher temperatures (Mori *et al.* 2007). Flavor components as well as total anthocyanins, FRAP

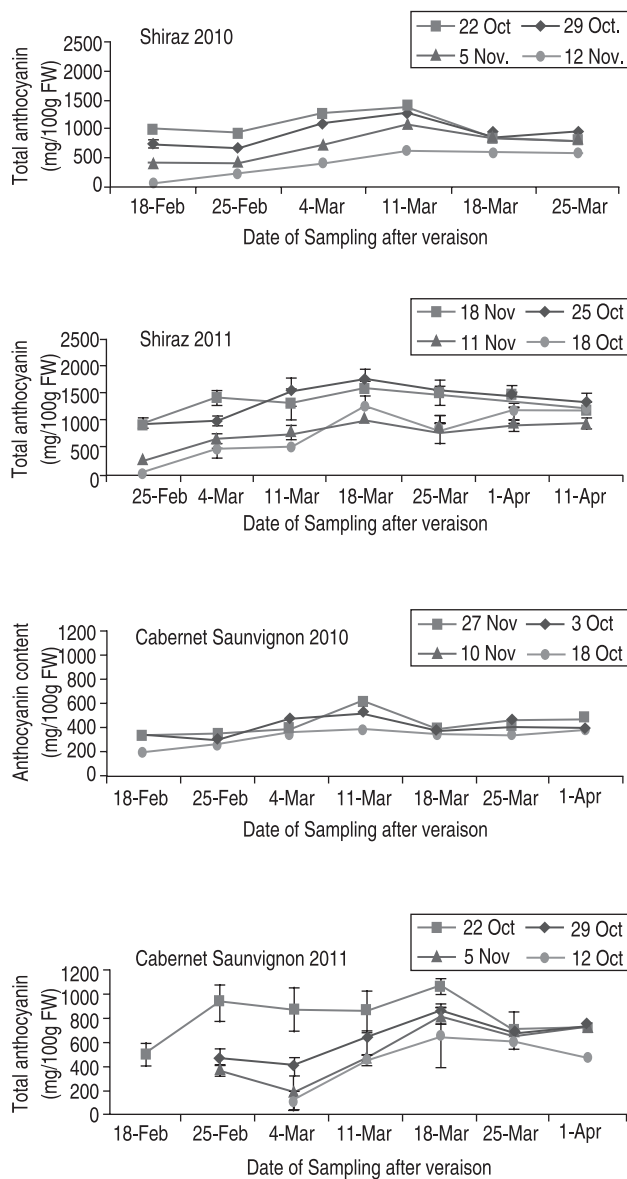


Fig 1 The trend of total anthocyanins accumulation in cv Shiraz and Cabernet Sauvignon during weekly intervals sampling and acquired maximum during 28 days after veraison of every year in each variety.

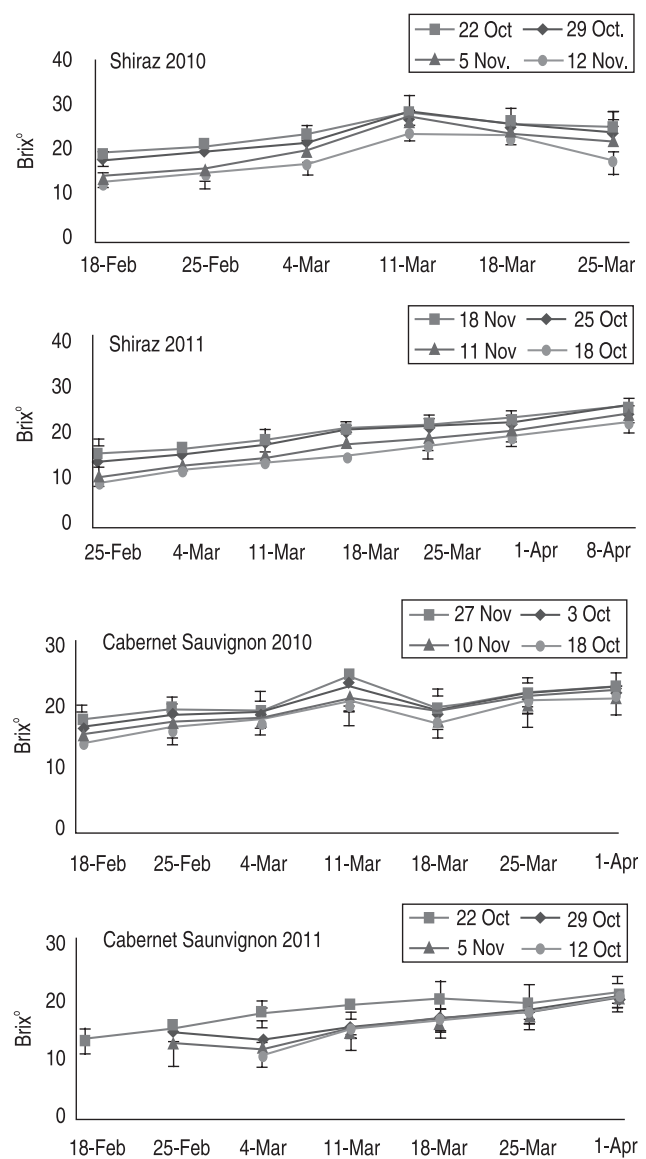


Fig 2 The trend of total soluble solids (TSS) accumulation in c Shiraz and Cabernet Sauvignon during weekly intervals sampling and acquired maximum during 28 days after veraison of every year in each variety.

and total phenols were found to decrease significantly after the full maturity stage in Shiraz grapes (Shivashankara *et al.* 2013). Therefore, red wine grapes need to be harvested at that threshold week irrespective of the pruning dates to achieve the maximum quality.

The various fruit quality parameters were analyzed to understand the effect of minimum and maximum temperatures on the dynamics of metabolites development. Data on total phenols and total flavonoids development indicated that irrespective of pruning dates, it reached the maximum concentration in the 2<sup>nd</sup> week of March during first year but it maintained in second year in both the cultivars. Ivanova *et al.* (2012) reported that the concentration of total phenolics and total flavonoids in grape berry skins were increased during ripening. Results on TSS indicated that irrespective of pruning dates, it reached maximum in the 2<sup>nd</sup> week of March later it decreased during first year but in the second year TSS increased till the harvest in both the cultivars (Fig 2). Even the berries exposed to high temperatures also exhibited higher levels of TSS (Tomasi *et al.* 2003). Data on acidity revealed that irrespective of pruning dates, it decreased from veraison to full maturity harvest stage in both the cultivars during 2010 and 2011. Our results are in line with some of the earlier workers (Shiraishi *et al.* 2010) reported that titratable acidity decreases during berry development.

Night temperatures were reported to have a greater influence on the total anthocyanin content (Koshita *et al.* 2007) in grapes. However, our studies indicated that in addition to the night temperatures, maximum temperatures also influence the development of anthocyanins in red wine grapes. Stronger relationship with maximum temperature might be due to the degradation of anthocyanins by higher temperatures (Mori *et al.* 2007). Our results indicated that the total anthocyanins of berry skin in cv. Shiraz is significantly and negatively influenced by both maximum and minimum temperatures. Higher the maximum or minimum temperature during berry colour development lower is the total anthocyanin content. Regression analysis of the parameters indicated the extent of reduction in anthocyanin content for 1°C increase in maximum ( $R^2=0.5168$ ) and minimum temperatures ( $R^2=0.4983$ ) (Fig 3A-C). However, in cv. Cabernet Sauvignon the regression analysis revealed a weaker relationship between the temperature and anthocyanin (Fig 3D-F).

In order to produce good quality wine, berries must have the right proportion of total anthocyanins, total phenols, sugars and acids. Concentration of total anthocyanins in berries is responsible for the wine colour intensity. In the second experiment, our results indicated that the anthocyanin content of the berries can be increased by advancing the pruning dates under Bangalore conditions mainly due to the prevalence of low temperature during the berry maturation period. Even if there is a delay in the pruning dates the grapes need to be harvested before the temperature rise begins to avoid the degradation of anthocyanins. Berries from early pruned vines were exposed to low temperature

during the berry colour development stage leading to higher production of anthocyanins as compared to late pruned vines (Table 1). Berry sugar content was also found to influence the anthocyanin concentration in Cabernet Sauvignon grapes (Hunter *et al.* 1991). In hot climatic conditions, berries record higher sugar and lower acidity levels than cooler climates (Pandell 1999). Even the berries exposed to high temperatures also exhibited higher levels of TSS and lower titratable acidity (Bergqvist *et al.* 2001). However, a few studies did not find the temperature to influence TSS (Haselgrove *et al.* 2000, Tomasi *et al.* 2003). In present study, late pruned vines which were exposed to higher temperatures when compared to the early pruned vines recorded lower TSS (°Brix), total phenols and total flavonoids but higher titratable acidity when compared to early pruned vines. Acidity is more sensitive to temperature during the stage I of berry growth compared to the later stage of berry growth in 'aki queen' grape berries (Yamane *et al.* 2006).

Total phenols contribute to the astringency of the wine. Grape phenolic composition is greatly affected by climatic conditions and vine management practices (Jackson and Lombard 1993). The quantity of phenolic compounds in grapes depends on the variety of grapevine and is also influenced by viticultural and environmental factors (Ojeda *et al.* 2002). Ferrer-Gallego *et al.* (2012) reported that the changes in the climatic conditions affect the phenolic composition in berries. High temperatures have been reported to cause lower total phenol content (Bergqvist *et al.* 2001) compared to lower temperatures. The inhibitory effect of high temperatures on total flavonols was also reported by Spayd *et al.* (2002). In this study also, total phenols, total flavonoids and total anthocyanins contents were higher in early pruned vines which experienced lower temperatures when compared to late pruned vines.

Results indicated that all the quality parameters of cv Shiraz were significantly higher in early pruned wines (18 October) when compared to the late pruned wines (18 November). Among the quality parameters, total anthocyanins showed best correlation with temperature compared to other quality parameters. Total anthocyanins content was 1323.4 and 2339.4 mg/100g fresh weight of berry skin in early pruned vines during the years 2010 and 2011 respectively, whereas it was 610.8 and 981.3 mg/100g fresh weight respectively in late pruned vines. Total phenols, total flavonoids and TSS were also found to be higher but titratable acidity was lower in early pruned vines. The mean day/night temperatures during the veraison to harvest period in early pruned vines were 30.7°C/14.9°C and 29.6°C/14.1°C in 2010 and 2011 respectively and in the late pruned vines the temperatures during the same period were 32.0°C/16.2°C and 32.1°C/16.0°C in 2010 and 2011, respectively (Table 1).

In the cv. Cabernet Sauvignon also a similar relationship was observed with the dates of pruning as that of cv. Shiraz. Total anthocyanins content was higher in early pruned vines during both the years. However, total phenols and total flavonoids did not show such a clear trend with

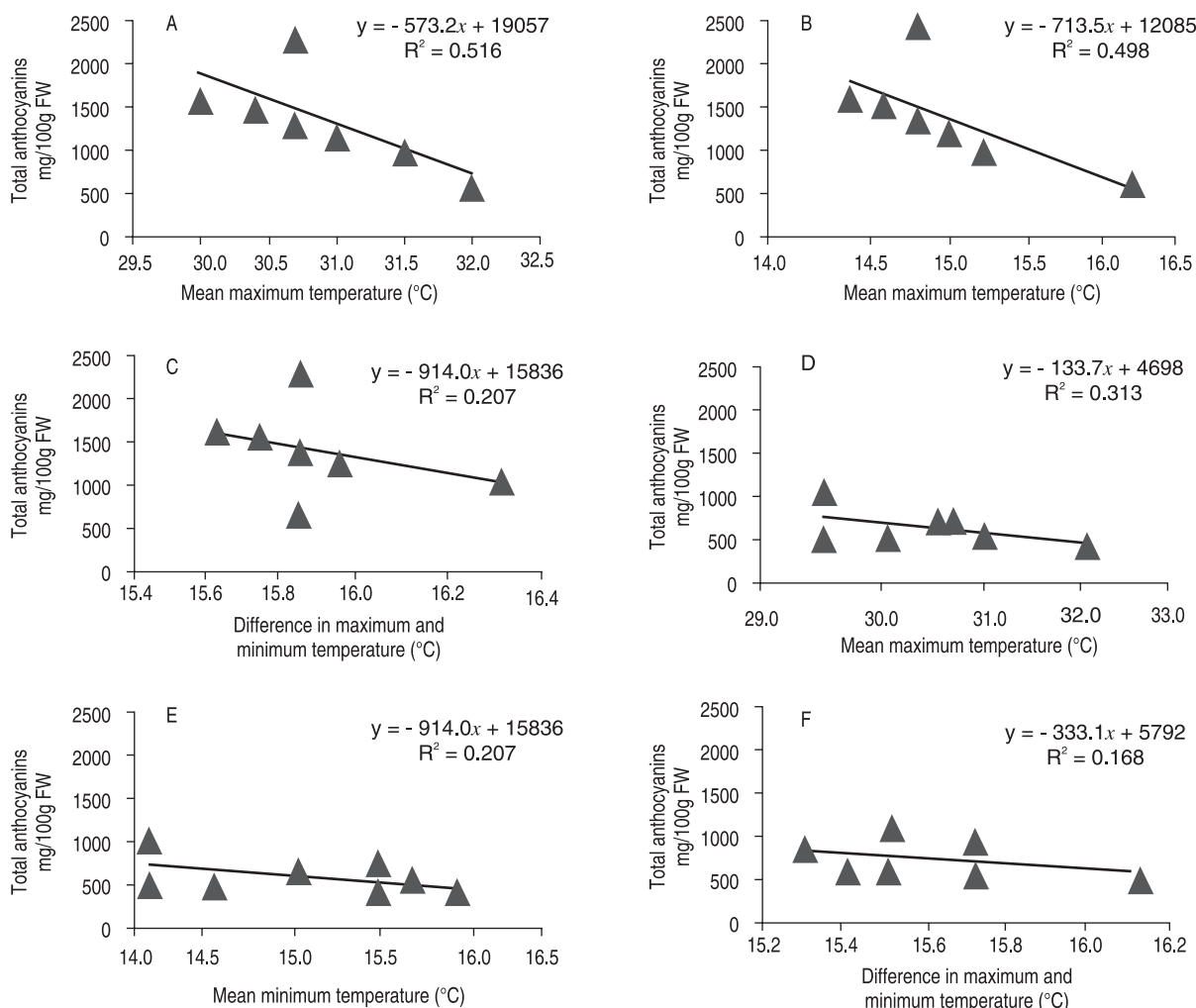


Fig 3 Regression lines between skin total anthocyanin content and mean maximum (A, D), mean minimum (B, E) and difference between maximum and minimum temperatures (C, F) in cv Shiraz and Cabernet Sauvignon respectively.

maximum and minimum temperatures. The mean day/night temperatures of 30.7°C/14.9°C and 29.6°C/14.1°C during 2010 and 2011 respectively was observed during the period of veraison to harvest in early pruned vines and in the late pruned wines a mean day/night temperatures 31.5°C/15.3°C and 31.1°C/15.7°C was observed during the same period in 2010 and 2011 respectively (Table 1). One of the reasons for the lack of strong relationship in cv. Cabernet Sauvignon may be due to lesser temperature variability between early and late pruned vines. There was only 1°C difference in maximum and minimum temperatures during the berry maturation period of early and late pruned vines.

Correlation coefficients between the weather parameters that prevailed during colour development period and quality parameters indicated that total anthocyanins, total phenols and total flavonoids recorded negative relationship with maximum and minimum temperatures and also with the differential temperature between the maximum and minimum temperatures (Table 2). Anthocyanin content exhibited a stronger relationship with maximum temperature ( $r=-0.912$ ), minimum temperature ( $r=-0.855$ ) and the differential temperature ( $r=-0.692$ ) than total phenols and

total flavonoids. Total phenols showed a strong relationship only with mean minimum temperature ( $r=-0.759$ ). Other quality parameters did not have a good relationship with temperatures. Whereas in cv. Cabernet Sauvignon correlation coefficients between anthocyanins and maximum temperature as well as minimum temperatures were  $r=-0.663$  and  $r=-0.476$ , respectively. Similar correlation coefficients were observed for total phenols.

*Conclusions*

Results clearly indicated that the total anthocyanin content in cv Shiraz and Cabernet Sauvignon decreased with increase in both mean maximum and minimum temperatures. It is also observed that the anthocyanins accumulation takes place only up to certain maximum and minimum temperatures irrespective of the date of veraison therefore reducing the number of days available for pigment accumulation in late pruned treatments. Pruning study shows that early pruning during the last week of October gives better quality grapes in cv. Shiraz and Cabernet Sauvignon. In the late pruned vines also harvest dates cannot be delayed beyond the third week of March otherwise the degradation

Table 1 Berry quality parameters in cv. Shiraz and Cabernet Sauvignon during 2010 and 2011 under different dates of pruning

	Pruning date	Max °C	Min °C	Difference	TSS (°Brix)	Acidity (%)	Total anthocyanins (mg/100g fw)	Total phenols (mg/100g fw)	Total flavonoids (mg/100g fw)
Shiraz 2010	22 Oct.	30.7	14.9	15.8	25.6	0.23	1323.4	2771.1	786.4
	29 Oct.	31.0	15.1	15.9	24.1	0.27	1192.2	2417.5	643.8
	5 Nov.	31.5	15.3	16.3	21.8	0.39	908.6	2245.2	660.7
	12 Nov.	32.0	16.2	15.8	20.5	0.53	610.8	2384.9	584.7
	Mean	31.3	15.4	15.9	23.0	0.36	1008.7	2454.7	668.9
CD(P≤0.05)				0.33	0.14	125.0	179.7	64.8	
Cabernet Sauvignon 2010	27 Oct.	30.7	14.9	15.8	19.1	0.35	482.7	1603.4	399.1
	3 Nov.	30.0	14.5	15.6	18.4	0.32	508.6	1541.5	364.5
	10 Nov.	30.4	14.7	15.7	18.6	0.29	497.8	1731.8	367.0
	17 Nov.	31.5	15.3	16.3	17.9	0.49	363.9	1561.8	399.9
	Mean	30.7	14.8	15.8	18.5	0.36	463.2	1609.6	382.6
CD(P≤0.05)				1.06	0.10	92.3	48.5	40.4	
Shiraz 2011	18 Oct.	29.6	14.1	15.5	24.7	0.41	2339.4	3531.1	742.7
	25 Oct.	30.2	14.5	15.7	22.5	0.40	1549.6	2384.9	501.2
	11 Nov.	31.1	15.5	15.5	21.2	0.43	1512.3	2249.7	468.3
	18 Nov.	32.1	16.0	16.1	19.4	0.48	981.3	1614.7	374.0
	Mean	30.8	15.0	15.7	21.9	0.43	1595.6	2445.1	521.5
CD(P≤0.05)				0.46	0.04	371.2	583.0	169.0	
Cabernet Sauvignon 2011	22 Oct.	29.6	14.1	15.5	16.2	0.39	997.5	2454.7	494.4
	29 Oct.	30.7	15.0	15.7	17.6	0.4	734.2	1650.7	384.8
	5 Nov.	30.8	15.5	15.3	17.4	0.48	697.5	1641.7	393.1
	12 Nov.	31.1	15.7	15.4	15.7	0.53	509.5	1808.4	477.6
	Mean	30.5	15.1	15.5	16.7	0.45	734.7	1888.9	437.5
CD(P≤0.05)				0.18	0.08	80.2	217.5	58.8	

CD - Critical Difference

Table 2 Correlation matrix between temperature and berry quality parameters in cv. Shiraz and Cabernet Sauvignon during 2010 and 2011 under different dates of pruning

	Max (°C)	Min (°C)	Difference	TSS	Acidity	Total anthocyanins	Total phenols	Total flavonoids
<i>Shiraz</i>								
Max °C	1							
Min °C	0.971	1						
Difference	0.629	0.429	1					
TSS	-0.761	-0.784	-0.331	1				
Acidity	0.424	0.513	-0.023	-0.829	1			
Total anthocyanins	-0.912	-0.855	-0.692	0.572	-0.230	1		
Total phenols	-0.817	-0.759	-0.585	0.784	-0.313	0.736	1	
Total flavonoids	-0.499	-0.538	-0.094	0.853	-0.618	0.298	0.790	1
<i>Cabernet Sauvignon</i>								
Max °C	1							
Min °C	0.882	1						
Difference	0.450	-0.019	1					
TSS	0.031	-0.213	0.471	1				
Acidity	0.654	0.768	-0.055	-0.667	1			
Total anthocyanins	-0.663	-0.476	-0.547	-0.502	-0.039	1		
Total phenols	-0.609	-0.523	-0.347	-0.629	0.006	0.796	1	
Total flavonoids	-0.150	-0.018	-0.314	-0.853	0.494	0.504	0.798	1

of anthocyanins starts.

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