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Bunch Exposure of Syrah Vines Affect Bunch and Berry Quality

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ABSTRACT

Exposure of bunches to sunlight play an important role in deciding bunch and berry quality. Berries exposed completely to sunlight were found with minimum diameter. Present study revealed that increased total soluble solid (TSS) up to 22.4°B with reduced acidity was found in the completely sunlight exposed berries as compared to the partially exposed or complete shade. Complete exposure of bunches registered with lower juice pH. Bunches exposed to complete sunlight recorded higher concentration of hydroxybenzoic acid, gallic acid and ellagic acid than other treatments. Gallic acid in sunlight exposed berries was higher while lowest in completely shaded bunches. Total flavan-3-ols was lower in shaded bunches than under complete sunlight exposed bunches. Higher anthocyanins was recorded in bunches completely exposed to sunlight than those in shade. However, amino acids were less in the sunlight exposed bunches compared to shade bunches. Proline was higher (37.350 mg/L) in shaded bunches in comparison to complete sunlight exposure.

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INTRODUCTION

Grape (*Vitis vinifera* L.) is grown on a variety of soil and water conditions under different climatic conditions. Quality of grapes depends on variety, growing conditions and adopted vineyard management practices. Grape quality achieved during harvest leads to quality of prepared wines. The sensorial properties of red wine, such as color, flavor and mouth feel depends on several components extracted from grapes during fermentation of process of wine aging. Prevailing high temperature during veraison to harvest stage under tropical region of Maharashtra affect grape quality. It fasten sugar accumulation in grape berries and at the same time reduces the acidity very fast. Ultimately berry quality parameters comprising of TSS, acidity and juice pH affect under high temperature conditions.

Sunlight and temperature at ambient levels are essential for the process of photosynthesis. Environmental stress variables like sunlight, temperature caused to solar injury in plants or fruits. Grape berries exposed to sunlight are generally higher in sugar, malate and pH compared to berries ripened under canopy shade (Morrison 1988). Increased fruit exposure to sunlight, can be achieved by removal of excess shoots, leaf removal in the fruiting zone of canopy repeated combing and shoot positioning of the vine. This exposure increases light interception in the day time and temperature may differentiate the composition of the grape berries in shaded vs exposed. Canopy management practices positively affect composition of wine grapes. Reduction in TSS content and anthocyanin accumulation in berries of Nebbiolo grapes was noted by fruit zone shading while sun burn damage was observed in excessive sunlight exposure and not resulted in improvement in content of TSS or anthocyanins (Chorti *et al.* 2010).

Combination treatments of leaf removal either with shoot thinning or cluster thinning were imposed on Cabernet Sauvignon vines under tropical conditions. Jogaiah *et al.* (2013) noted that excess light exposure or excess shade to clusters is not congenial for producing better quality grapes. Berries collected from vines which received either treated to leaf removal treatment or in combination with either cluster thinning or shoot thinning were recorded with maximum anthocyanin and phenol content. Observed trend was not definite for compounds such as rutinhydrate and kaempferol while measured non-flavonoid compound ellagic acid was maximum in bunches where cluster and shoot thinning both were imposed. Various researchers conducted studies on cluster exposure to improve grape and wine quality under temperate areas where prevailing temperature is low and many times insufficient sunshine hours are also experienced. While in tropical conditions where temperature is always higher and crop exposed to more sunshine hours few research activities are conducted. But cluster exposure affects grape and wine quality in different manners. To understand effect of cluster exposure on grapes and wine of Syrah under tropical conditions of Maharashtra state of India, present study was conducted.

MATERIALS AND METHODS

The experiment was conducted at the farm of ICAR-National Research Center for Grapes, Pune (18.32 °N and 73.51°E) (India). Pune has tropical wet and dry climate with an average temperature ranging between 20 to 28°C. Four-year-old vines of Syrah grape grafted on 110R rootstock were selected for this study. The vines were planted in N-S direction at spacing of 2.66 meter between the rows and 1.33 meter between the vines with a total number of 3403 vines per hectare. Vines were trained to mini-Y-trellises. Fruit pruning was done on 12th October 2015. Activity on uniform shoot growth and bunch development was initiated 10 days after fruit set. The clusters were divided into three different categories i.e., (a) Bunches under complete sunlight - Bunches were completely exposed to sunlight and outside of the leaf canopy, (b) Bunches under partial sunlight. Bunches were partially exposed to sunlight, i.e., half inside the canopy and half outside the canopy and (c) Bunches under complete shade these bunches were completely covered with the leaf canopy. The experiment was laid out in Randomized Block Design with three treatments. Each treatment was repeated seven times. Five healthy vines were tagged for recording the observations under each treatment. Vineyards were managed under uniform cultural practices and managed by judicial application of irrigation, fertilizer.

Samples were collected at harvest when the berries in a bunch attained total soluble solids of 22 °Brix. Hundred berries were randomly selected from each replicate, and processed in a blender. TSS, total acidity, pH and volatile acidity in juice was measured by OenoFoss a FTIR based wine analyzer.

The estimation of the phenolic compounds was performed according to procedure suggested by Patil *et al.* (2011). One hundred representative berries were homogenized in a mixer grinder. One gram of homogenized sample was extracted with 5 mL of 0.1% formic acid in methanol. The sample was vortexed for 2 min and centrifuged at 5000 rpm for 5 min. One mL of supernatant was transferred to Eppendorf tube and again centrifuged at 10000 rpm at 4ºC for 10 min. The supernatant was filtered through a 0.2 μm nylon membrane filter, and the filtrate was used for analysis.

Standards of amino acid and phenolic compounds had purity of >90%. Amino acids stock solutions were prepared by dissolving 10 (±0.1 mg) reference standards in 10 mL of HPLC water resulting in a final concentration of approximately 1000 μg/mL. Phenolic compounds standard solutions were prepared by dissolving 10 mg into 10 mL in methanol. A working standard mixture of 10 μg/mL was prepared in water (amino acids) and methanol (phenolic compounds) by mixing and diluting individual standard stock solutions. Caliberation standard

Table 1. Effect of sun exposure on berry quality parameters of Syrah wine grapes.

Treatments details	Avg bunch wt (g)	100 berry wt (g)	Berry diameter (mm)	TSS (°Brix)	TA (g/L)	VA (g/L)	pH
Complete sun exposure	95.70^{c}	88.11^{b}	9.74^{c}	22.4^{a}	6.5^{b}	0.12^{a}	3.45^{b}
Partial sun exposure	102.78^{b}	88.24^{b}	10.29^{b}	21.6^{b}	6.6^{ab}	0.07^{c}	3.69^{a}
Complete shade	128.10^{a}	98.61^{a}	11.25^{a}	19.5^{c}	6.7^{a}	0.08^{b}	3.78^{a}
LSD 5 %	3.904	2.557	0.296	0.345	0.155	0.001	0.093

solutions were prepared in the range of 10-1000 ng/mL by serial dilution of 10 µg/mL solution.

For estimation of amino acids, five gram of homogenized sample was taken in 50 mL polypropylene tube followed by addition of 10 mL of acidified methanol : Water (20 : 80). The sample was vortexed for 2 min, centrifuged for 5 min at 5000 rpm. The supernatant was passed through 0.2 µm nylon membrane filter and 20 µL clean extract was injected to liquid chromatography tandem mass spectrometry (LC-MS/MS).

High-performance liquid chromatography (Waters 2695 separation module) hyphenated to triple quadrupole (Quattro Premier Micro mass) mass spectrometer equipped with electro spray ionization (ESI) probe. An aliquot of 20 µL was injected via auto-sampler. The chromatographic separation was performed Atlantis® T_3 (100×3.0 mm, 5µm column), with a flow rate of 0.350 mL/ min. The mobile phase was composed of A - methanol : Water (10 : 90, v/v) with 0.5% Formic acid and B - methanol : Water (90:10, v/v) with 0.5% Formic acid; gradient 0–0.5 min 10% B, 3.0 min 60%B, 7.0–13.0 min 98% B, 14.0-20.0 min 10% B. The column temperature was maintained at 35ºC. The LC-MS/MS analysis was performed in positive polarity by multiple reaction monitoring (MRM) with a dwell time of 10 ms. The other LC-MS/MS parameters included capillary voltage of 3.0 kV, source temperature 120ºC, desolvation temperature 500ºC, desolvation gas flow 900 L/hr and cone gas 50 L/hr. The data acquisition and analysis were performed by using Mass Lynx 4.1v software (Waters Corporation, Manchester UK).

Analysis of variance was performed for each variable using the SAS statistical package 9.3 (SAS Institute, Cary, NC). Least significant differences among treatments were calculated.

RESULTS AND DISCUSSION

Average bunch weight under full sunlight exposure was less (95.70 g) and was increased with the shading of the bunch to 128.10 g. A linear relation between 100 berry weights was also recorded in the sunlight exposure treatments indicating that the berry and bunch weight depends on exposure of bunch to sunlight (Table 1). Berry diameter also showed same trend and minimum diameter was recorded in bunches under complete exposure. The exposure of bunches to different micro-climate may lead to changes in temperature of grape berries. Previous studies have shown that the effect of temperature (Hale and Buttrose 1974) on berry size and composition vary during the fruit ontogeny. Direct sunlight exposure greatly influenced berry temperature (Scafidi *et al.* 2013). However, Haselgrove *et al.* (2000) reported non-significant differences in the berry weight of Syrah grapes when comparison was made between berries from fully exposed or completely shaded bunches. In the present study reported differences in berry weight are attributed to the direct effect of sunlight.

TSS was observed in the range of 19.5 °B to 22.4 °B among the treatments. With the increase in bunch exposure to sunlight, there was an increase in total soluble solids from 19.5 °B (complete shade) to 21.6 °B (partial sun exposure) and 22.4 °B (complete sunlight exposure). In general, higher TSS is found in complete sunlight exposure followed by partial sunlight exposure. Same trend was recorded in present study also. Total acidity was 6.7 g/L in the berries from complete shade whereas 6.5 g/L for complete sunlight exposure and acidity content in berries was

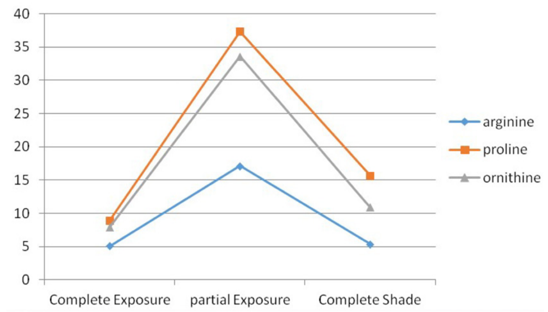

Fig. 1. Content of important amino acids in Syrah wine grapes.

reduced with increased exposure to sunlight. However, no correlation was recorded for the volatile acidity of the grape berries obtained from different sunlight exposures. Low juice pH was estimated in the berries harvested from bunches completely exposed to sunlight than berries harvested from other treatments. The increase in sugar and reduction in titratable acidity in this study agree with Smart *et al.* (1990). However, in other studies on different wine grapes exposed to sunlight with variable levels revealed non-significant differences in TSS content of berries (Downey *et al.* 2004, Cortell and Kennedy 2006 and Spyad *et al.* 2002). Under the tropical condition where the humidity is less might have played a major role in increasing sugar and reducing acidity. Increased bunch exposure to sunlight expressed a decline in acidity is due to malic acid degradation. This effect was enhanced by high temperature experienced by exposed fruit (Kliewer 1971). The present study also confirms the findings of Bergqvist *et al.* (2001) who reported that exposed clusters have lower juice pH than the shading clusters. Decline in acidity and increment in pH was observed in shaded but not in sun-exposed clusters of Merlot (Spayd *et al.* 2002). Our results indicated that shading of berries resulted in higher berry weight, acidity, and pH values than bunches exposed to complete sunlight. These results confirm the findings of previous work on the influence of micro-climate changes in fruit zone of vine on berry physical characteristics (Rustioni *et al.* 2011). However, the grape grown under excessive sunlight exposure might be undesirable for some reason such as berry desiccation and sunburn (Mustafa 2014). While Jogaiah *et al.* (2013) stated that excess light exposure or excess shade to clusters is not congenial for producing better quality grapes under tropical conditions. Mustafa (2014) concluded that partial shade to the bunch may be beneficial due to the sufficient sunlight availability.

The concentration of amino acids recorded under different treatments viz., arginine, proline and ornithine, is presented in Fig. 1. Proline is found with higher concentration while arginine was in minimum concentration. Berries from partially shaded bunches contained maximum quantity of estimated amino acids. Among them proline was found in higher amount (37.350 mg/L) under partial shade while it was minimum (8.970 mg/L) in complete sunlight exposure. Ornithine was another major compound found in higher amount with lowest in sunlight exposed bunches (7.940 mg/L) than highest (33.600 mg/L) in partial shade. Arginine was ranged from 5.080 mg/L in complete exposure to 17.150 mg/L in complete shade. At harvest, berry size and quality mainly depend on water, sugars like glucose, fructose, and sucrose (Conde *et al.* 2007), amino acids especially arginine, proline, glutamic acid (Hernandez *et al.* 1999). Content of amino acids is found to change in cultivars and according to micro-climate conditions in response to sun exposure including other factors such as vine nutrition, vineyard management, soil type, soil moisture content, vine virus status, grape maturity and growing season (Bell and Henschke 2005 and Pereira *et al.* 2006). Teixeira *et al.* (2013) reported that arginine was the most abundant amino acid in grapes from all cultivars and regions, eventually reflecting its role as a precursor of the remaining amino acids. They also reported that the grape berry is considered a sink for primary key metabolites and relies on the use of available carbohydrate resources produced by photosynthesis to support its growth and development.

Phenolic profiling of berries collected from different bunch exposure treatment was analyzed by using LCMS/MS (Fig. 2) and details are presented in Table 2. Bunches exposed to complete sunlight recorded higher amount of hydroxyl-benzoic acid than others. Among the hydroxybenzoic acids, the ellagic acid concentration was higher while; gallic acid was presented with lower concentration compared to berries under complete sunlight exposed bunches.

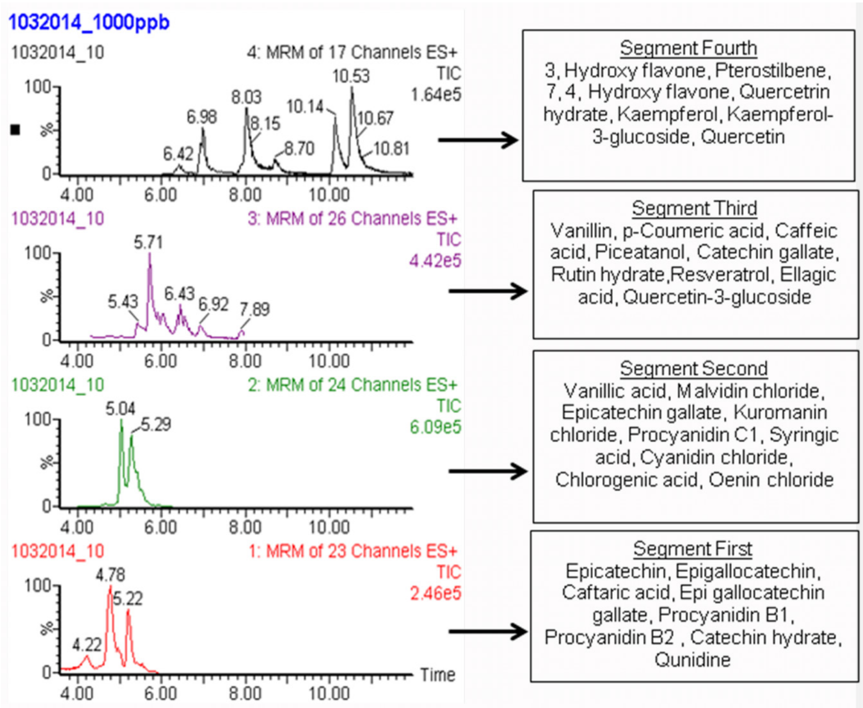

Fig. 2. Total Ion chromatograph (TIC) for phenolic compounds.

Quantity of ellagic acid observed in sunlight exposed berries was 15.49 mg/L followed by 11.92 mg/L in partial shade and 9.67 mg/L in shaded bunches. In group of hydroxycinnamic acids, caftaric acid was found in major quantity as compared to other acids in all the treatments. The shaded bunches exhibited a lower amount of caftaric acids (1.41 mg/L), and it was observed that the concentration was increased from partial bunch exposure (1.87 mg/L) to complete bunch exposure to sunlight (2.67 mg/L).

Among the flavon-3-ols, rutin hydrate was recorded with higher concentrations. The total flavan-3-ols was lower in shaded bunches (23.37 mg/L) than under complete sunlight exposed bunches (34.58 mg/L). The concentration of quercetin and myricetin was lower in all bunch exposure treatment compared to other flavan-3-ols. The trend of stilbenes concentration was different and maximum concentration of for. Picetannol was recorded in berries from complete shaded bunches while minimum concentration was found in complete exposure to sunlight. The hydroxycinnamates are the third most abundant class of phenolics in grape berries after proanthocyanins and anthocyanins. In warmer climatic conditions, high light exposure can increase the concentrations of phenolics because of higher activity of phenylalanine ammonialyase (Roubelakis-Angelaki and Kliewer 1986). Greater synthesis of flavonols in grapes, as a result of increased sunlight exposure caused by defoliation, has already been reported by several workers (Haselgrove *et al.* 2000). In the present study, increased concentration of flavonols in the grape berries as a result of bunch exposure (either partial or complete) is a positive and desired result. These compounds play an important role in co-pigmentation phenomenon and color stabilization in red wines (Boulton 2001). To a certain extent, flavonol profile demonstrated that some of them can be used as chemical markers for authentication and varietal differentiation of grapes and wine (Garde-Cerdian *et al.* 2009). The results observed in the present study were similar to the results of Crippen and Morrison (1986) who reported that sunlight exposed fruits are generally higher in anthocyanin and phenolics compared to non-exposed fruits. The bunch exposure to

Table 2. Effect of sun exposure on phenolic composition and anthocyanin accumulation in Syrah red wine grapes (mg/L).

Treatments	Complete sun exposure	Partial exposure	Complete shade
Non flavonoids (Hydroxybenzoic acid)			
Gallic acid	0.985	0.909	0.523
Ellagic acid	15.49	11.92	9.67
Vanillic acid	2.023	1.931	1.763
Sorbic acid	1.748	1.641	1.234
Non flavonoids (Hydroxycinnamic acid)			
Caftaric acid	2.674	1.87	1.41
P-cumaric acid	0.497	0.507	0.654
Chlorogenic acid	1.59	1.391	1.298
Flavonols (Flavan 3-ols)			
Catechin hydrate	1.216	0.998	0.6398
Epicatechin	1.897	1.723	154
Qucertinn	0.102	0.099	0.074
Rutin hydrate	29.40	24.12	19.76
Myricitin	0.92	0.871	0.627
Kampherol	1.047	0.953	0.732
Stilbenes			
Picetannol	14.18	14.94	17.21
Resveratrol	0.527	0.445	0.218
Anthocyanins			
Cynadin	17.43	13.05	12.21
Delphinidin	33.14	32.27	29.11
Peonidin	23.96	21.70	16.98
Petunidin	53.21	47.10	39.87
Malvidin	134.59	127.15	121.78

sunlight had a significant influence on phenolic content. The presence of higher amount of phenolics in mature grape berries in red wine varieties may help to improve the wine quality. Increase in color intensity, total phenolics through sunlight exposure of bunch has already been explained by Song *et al*. (2015).

The higher amount of anthocyanin was found in bunches exposed to complete sunlight (262.33 mg/L) than those in the complete shade (219.95 mg/L). Among the anthocyanins, malvidin concentration was more than petunidin and delphinidin in bunches exposed to sunlight (Table 2). Bunches exposed to partial shade had moderate anthocyanin content. Synthesis of anthocyanins and its regulation in response to sunlight conditions have studied in several types of grapes (Matus *et al.* 2009). Intense sunlight caused excessive sunburn in exposed berries and reduced the anthocyanin accumulation. The associated high temperature can also inhibit the color development. It is reported that optimum temperature for anthocyanins synthesis in grapes occurs between 15 and 27 ºC and temperature above 30 ºC induces degradation of these compounds (Borsani *et al.* 2010). According to Mori *et al.* (2007), high temperature (maximum 35 °C) reduced the total anthocyanin content to less than half of that in the control berries (maximum 25 °C). In the same study, HPLC analysis showed that the concentration of anthocyanins with the exception of malvidin derivatives (3-glucoside, 3-acetylglucoside, and 3-p-coumaroylglucoside), decreased considerable in the berries grown under high temperature as compared to the control. Guidoni *et al.* (2002) stated that cluster thinning changed the proportion of anthocyanins, increasing cyanidin and peonidin agrees with the result of the present study. Considering the present results, the high temperature in fruit zone created due to complete sunlight exposure of bunches may not be good option under the tropical regions where the temperature exceeds 30°C during the period of veraison to harvest. The present finding gives a clue that canopy manipulation or canopy arrangement was done on a given trellis in such a way so that bunches exposes to either full or partial sunlight. However, under the tropical condition, partial shade may help to achieve better quality wine.

CONCLUSION

In this study, bunches exposed to complete and partial sunlight showed changes in berry diameter, TSS contents of berries and juice pH indicating the influence of sunlight on fruit composition. The phenolics composition in grape berries and subsequent reflection in wine indicated that under tropical condition bunches could be exposed to partial sunlight to achieve a better quality of wine. The shading of bunches under the canopy was also responsible for the delay in veraison and harvest by 2-3 days compared to bunches exposed to complete sunlight. This was also positively correlated with the reduction in anthocyanin and phenolics. Canopy management practices that can improve micro-climate in the fruit zone without affecting the fruit quality in tropical region can be useful. The result indicated that the canopy that provides a high amount of diffused light in the fruiting zone rather than direct sunlight might work well under the tropical condition where the temperature during veraison to ripening ranges between 30 to 40 °C. Trellis system may also help to protect the bunch from direct sunlight. The detailed study about canopy may help to obtain the quality wine under tropical condition. The grape bunches protected under the canopy reduced canopy temperatures and clearly improved the wine quality through improved fruit composition. This clearly indicates detail investigation as a practical means of protecting bunches from high temperature under tropical condition.

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