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Influence of canopy management practices on fruit composition of wine grape cultivars grown in semi-arid tropical region of India

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Effect of canopy management practices on berry composition of red and white grape cultivars grown in Pune region of India was examined. Cabernet Sauvignon and Sauvignon Blanc vines were selected for the study. Both the cultivars exhibited significant variation in fruit composition parameters in response to various canopy management practices. Combination treatment of leaf removal (LR) either with shoot thinning (ST) or cluster thinning (CT) exhibited high total soluble solids (TSS), lowest acidity (malic acid), lower potassium content and higher anthocyanin content. The vines which received ST+CT+LR treatment and control vines recorded least anthocyanin concentration and phenolic compounds indicating excess light exposure or excess shade to clusters is not congenial for producing better quality fruits. Leaf removal treatment in combination with either shoot thinning or cluster thinning was found to be superior under semi-arid tropical conditions to obtain good quality fruits. Reasons for such variations in fruit composition parameters under different management practices are discussed.

Key words: Anthocyanins, canopy management, organic acids, phenolic compounds, wine grapes.

INTRODUCTION

Wine grape cultivation is gaining strong impetus in tropical climatic conditions of the world. Tropical viticulture has only been practiced commercially, since approximately 50 years. Countries such as Brazil, India, Thailand and Venezuela play a leading role in the tropical grape production. However, it can be noted that there is a trend towards the expansion of tropical viticulture in the world, since there are vineyards being implemented in different countries in America (Bolivia, Colombia, Peru, Guatemala), in Africa (Madagascar, Namibia, Tanzania) and Asia (Vietnam, China). The production technology in the tropical regions differs significantly from the one employed in the traditional temperate regions. It is necessary to break the bud dormancy in order to foster bud burst, and special management techniques have to be employed to overcome problems of low fertility and to control vigor.

It is generally opined that wine grapes require a temperate climate that includes predominantly winter rainfall, frost-free late spring, and warm to hot summers to ensure ripening, i thus the global wine industry has been analysed predominantly in terms of Old World and New World wines from regions characterised by those criteria (McLennan, 1996). However, this largely ignores the nascent frontier of new climate wines, including the new altitude wines of tropical zones. Between 1996 and 2006 the area under commercial grape cultivation in tropical zones in Africa, Asia and Central and

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South-America, north and south of the equator, between the Tropic of Cancer (23.27°N) and the Tropic of Capricorn (23.27°S) increased by 155% from 55,000 ha to over 140,000 ha. The increase was most rapid in Asian countries like India, Thailand, Myanmar and Vietnam where new vineyards for table grape and wine production are established every year (http://estructuraehistoria.unizar.es/gihea/documents/GwynCampbell.pdf)

Canopy management practices in wine grape cultivation have been developed with an aim of optimizing sunlight interception, photosynthetic capacity and fruit microclimate to improve fruit yield and wine quality, especially in vigorous and robust growing varieties with dense canopies. For wine making, significant benefits have been obtained from comprehensive approaches, to control shoot vigour through the use of different methods of trellis system, training systems, pruning methods, deficit irrigation, rootstocks and canopy management practices (Smart, 1985; Smart et al., 1990). Canopy management practices like leaf removal improved the bunch and berry characteristics with respect to reduced incidence of botrytis incidence and increased anthocyanin and reduced malic acid content in Graciano and Carignan grapes (Tardaguila et al., 2010). Many workers have shown positive effects of canopy management practices on composition of wine grapes in recent years. Fruit zone shading reduced total soluble solids and anthocyanin accumulation in Nebbiolo grapes. Excessive sunlight exposure caused sun burn damage and did not increased TSS or anthocyanin concentration (Chorti et al., 2010). Similarly cluster thinning increased TSS by 25 brix and showed positive impact on wine anthocyanin, berry skin tannins and seed tannins in Corot Noir grapes (Sun et al., 2012). Gatti et al. (2012) in their studied on effect of cluster thinning and pre-flowering leaf removal on fruit composition of Sangiovese grapes observed high brix level corresponded to the highest TA in defoliated vines and conversely the lowest TA and high pH in early cluster thinning and lag phase cluster thinned vines.

Initially, wineries in tropical climate used to follow production technologies similar to traditional old world wine producing countries. But, these production technologies did not work well and hence new and specialized techniques and equipments are being used in tropical wine grape production. The quality of grapes has been improved tremendously, after the establishment of two pruning and single cropping cultivation practices. Though sunlight is not a limitation in semi-arid tropics of India, excess sunlight can harm the production and thereby reduce the wine quality. The other major drawback in tropical climate is the more vigorous nature of vines which needs to be curtailed to improve fruit composition, especially in wine grapes. No systematic research on canopy management practices to improve fruit composition of wine grapes has been attempted in major wine grape growing regions of India. Hence, this preliminary investigation was undertaken, to study the influence of important canopy management practices on fruit composition of Sauvignon blanc and Cabernet Sauvignon grapes.

MATERIALS AND METHODS

This experiment was conducted at the experimental vineyard of National Research Centre for Grapes, Pune during two growing seasons of 2010-2011 and 2011-2012. Pune is located in Midwest Maharashtra state (India) at an altitude of 559 m above the mean sea level. It lies in 18.32° N latitude and 7.51° E longitude. The vines were grown on calcareous black cotton soil (clay content was 44.5%) exhibiting swelling and shrinkage properties. The average bulk density of the root zone up to a depth of 30 cm was 1.25 g/cm^3. The average electrical conductivity (EC) of the irrigation water during the experimentation was 1.98 dS/m with an average pH value of 7.78. The rainfall during 2010-2011 and 2011-2012 was 484 and 540 mm respectively.

Four year old Cabernet Sauvignon and Sauvignon Blanc grapes grafted on 110R rootstock were selected for this study. The vines were planted at a spacing of 2.5 m between rows and 1.2 m between vines within a row. The row orientation was in the direction of North – South. The vines were trained to double cordon small T system. The pruning biomass of the vines was in the range of 1 to 1.25 kg. The concept of balanced pruning is not in practice in tropical viticulture of India, where double pruning and single cropping is being practiced. Hence, approximately 40 to 45 shoots are encouraged per vine in a spacing mentioned above.

Canopy management practices such as shoot thinning (ST), leaf removal (LR) and cluster thinning (CT) were imposed either singly or in different combinations. Shoot thinning was done at 45 days after pruning, wherein approximately 32 shoots (eight shoots on either side of double cordon) were retained per vine by removing weak and non-bearing shoots. Cluster thinning was done after fruit set stage (3 to 4 mm stage) to maintain 40 basal one or two clusters per vine and leaf removal was performed during version stage by removing two leaves above and two below the cluster to expose bunches. The 7 treatments were ST, CT, LR, ST+LR, CT+LR, ST+CT, ST+CT+LR along with control as eighth treatment. Each treatment was replicated thrice with three vines per replication. Except shoot thinned vines, the vines with other canopy management treatments had approximately 40-45 shoots per vine oriented towards east and west side of the cordon.

Fruit composition parameters

After harvesting, about 250 berry samples were collected from each treatment replication wise. Half of the samples were utilized immediately for analysis of basic fruit composition parameters such as total soluble solids (TSS), titratable acidity (TA) and juice pH. The fruit samples were also analyzed for total proteins, total phenols, and potassium content. The remaining half of the berry samples was stored in -20°C for analysis of organic acids and phenolic compounds using high performance liquid chromatography (HPLC) and LC-MS/MS respectively. The frozen samples were analyzed for organic acids and phenolic compounds within 20 days after harvest.

Estimation of total phenols, proteins and potassium content

The total phenol content of the fruit extract was determined using the Folin- Ciocalteu method (Singleton and Rossi, 1965), using gallic acid as the standard. The total protein content was estimated

as per the procedures of Lowry's method (Lowry et al., 1951). Both these estimations were done using UV spectrometer. Juice potassium content was estimated using flame photometer method. In Cabernet Sauvignon grapes, anthocyanin and phenolic concentration was determined as explained below.

Spectrometric analysis anthocyanins

Frozen berries were removed from cold storage and thawed overnight under refrigerated conditions (4°C) and approximately 100 berries were weighed and homogenized in a grinder. One gram of homogenate was taken in 10-ml plastic centrifuge tubes and 10 ml of 50% (v/v) aqueous ethanol was added and the mixture was agitated for 1 h at 400 rpm. Then the mixture was thereafter centrifuged at 1800 rpm for 10 min. The supernatant (extract) was used for estimation of anthocyanins and total phenols. For analysis of anthocyanins and total phenols, about 200 µl of extract was transferred to acrylic cuvettes and 3.8 ml of 1.0 M HCl was added and covered with paraffin film and mixed by inverting. The mixture was incubated for 3 h at room temperature and the color was measured at 520 nm for anthocyanins and 280 nm for total phenols.

Estimation of organic acids

A new method was developed for estimation of organic acids in grapes and wines using ultra HPLC 1260 Series (Communicated for publication). The method was developed based on the common organic acids present in grape must such as tartaric acid, malic acid, citric acid and lactic acid.

Standard solutions of organic acids

All acids and reagents used were of analytical grade. The standard organic acids were purchased from Thomas Baker. All organic acids used for standards were dissolved in double distilled water. For method development both D and L tartaric acid were used. The concentrations of organic acids varied from 1 to 100 mg/L. The prepared standard solutions of organic acids were stored at 4°C.

Solvents

The mobile phase consisted of acidified water of pH 2 adjusted with Othophosphoric acid and 100% methanol (Volume ratio of 95.0:5.0). Prior to use, the solvent was filtered through vacuum filter and then sonicated for 5 to 10 min in an ultrasonic bath to remove air bubbles.

Equipment

The HPLC used was 1260 Agilent Series with EZ chrome software for data acquisition and analysis.

Chromatographic conditions for determination of organic acids

A Zorbax Eclipse plus C18 column (4.6 × 100 mm × 5 µ), with an injection volume of 10 µl, pressure 45-46 Bar, temperature 25°C, wavelength 210 nm, flow rate 0.80 ml /min. For precision and accuracy validation, grape extract were spiked with organic acids to such an amount that the final concentration of the added acid varied from 20, 40, 60 mg/L. From stock solution of 10,000 mg/L of different organic acids, aliquot of 2, 4 and 6 µl was added to 10 µl of samples to get final concentration of 20, 40 and 60 mg/L respectively. Three replicates of the spiked samples were prepared and injected and assay was calculated to measure the repeatability of retention times, peak area of standard and samples.

Similarly, an aliquot of grape extract was diluted with mobile phase and 10 µl of the obtained solution was injected to the system. Before injection, all standards and sample solutions were filtered through 0.45 µm nylon membrane filter units. The chromatogram of standard organic acids with their intensity and retention time is shown in Figure 1. The concentration of L- tartaric acid was very negligible in fruit samples and hence, only D- tartaric acid was estimated in all samples.

Estimation of phenolic compounds

The estimation of phenolic compounds was performed as per the procedures of Patil et al. (2011). The stock solutions of individual standards were prepared by weighing 10 ± 0.01 mg of each analyte and dissolving them in 10 ml methanol and were stored in glass vials under refrigerated conditions. The concentration of each compound was calculated using the weight of the standard and weight of solvent and also the purity of the compound. A 25 ppm intermediate working standards was prepared by diluting the above stock solutions in 10 ml. The chromatogram of standard phenolic compounds showing their intensity and retention time is shown in Figure 2.

Sample extraction for LC-MS/MS analysis

One hundred representative berries were homogenized in a mixer grinder. One gram of homogenized sample was extracted in 5 ml of 0.1% formic acid in methanol. The sample was vortexed for 2 min and centrifuged at 5000 rpm for 5 min. Supernatant (1 ml) was injected to LC-MS/MS by passing through 0.2 µm nylon membrane filter paper.

LC-MS/MS analysis

The LC-MS/MS analysis was done with an Agilent Technologies 1200 series hyphenated to API 4000 Qtrap (ABS Sciex) mass spectrometer equipped with electrospray ionization (ESI +) probe. The separation of the phenolic compounds was done on a Precenton SPHER-60 C_{18} 60 A° (150 × 2 mm × 5 µm) with mobile phase A-1% formic acid in water, B-1% formic acid in water: acetonitrile (1:1) and C- acetonitrile. Oven temperature was 35°C and injection volume 10 µl. Mass parameters were curtain gas 20 psi, ion spray voltage 5500 V and source temperature 550°C. Flow rate: 0.400 ml/min.

Statistical analysis

The experiment was conducted as randomized block design with three replications and the data was analysed using SAS Version 9.3. Tukey's test was used for comparing treatment means.

RESULTS

Sauvignon blanc

Significant difference was recorded for berry weight and basic fruit composition parameters among different canopy management practices. Maximum 100 berry

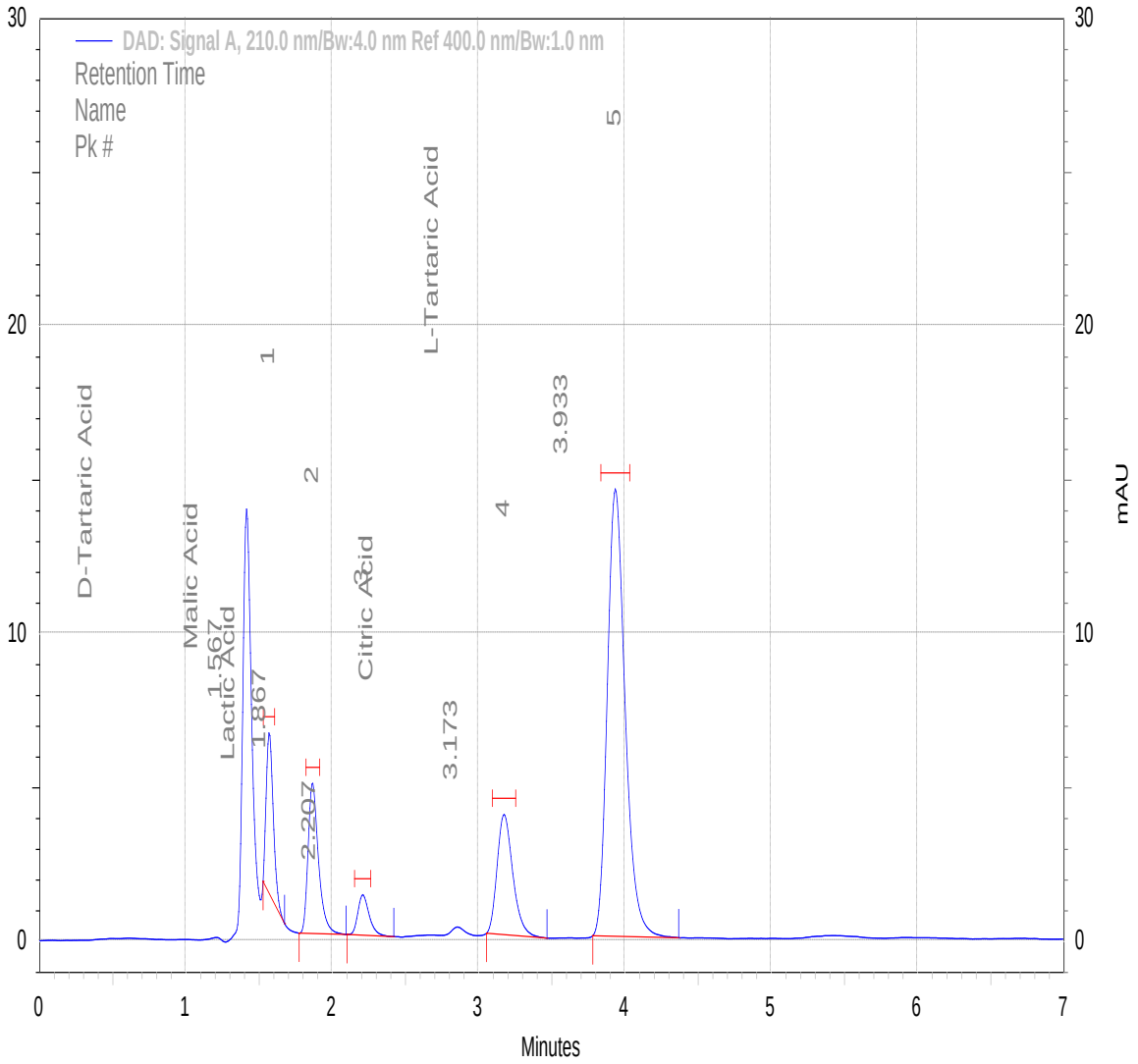

Figure 1. Chromatogram showing intensity of organic acid standards using HPLC.

weight (115.12 g) was recorded on cluster thinned vines. Highest TSS was recorded on CT, LR and ST combination vines (22°B). Least TSS was on control vines (19.83°B) and shoot thinned vines (19.10°B). Highest titratable acidity (TA) was recorded on ST or control vines (1.18%), while least was on LR vines or LR+ST vines (0.90%). Maximum total phenol concentration was recorded on CT+LR vines (6.01 mg/g) while it was least on ST or control vines (3.29 mg/g). Maximum potassium concentration in juice was recorded on control vines (0.17%) while it was least on vines which received LR treatment in combination with ST and / or CT treatments (0.10%) (Table 1). Among organic acids, highest Tartaric acid (1.32 g/L) was recorded on vines which received combination of all the three treatments while it was least on cluster thinned vines. Malic acid was highest (1.51 g/L) on control vines, while it was least on vines which received leaf removal (1.03 g/L) or the combination of cluster thinning and leaf removal and / or shoot thinning (Table 1).

Among phenolic compounds analyzed, maximum concentration of phenols was in flavonoid category. Catechin concentration was highest which varied significantly among treatments with highest being recorded on either CT+ST or CT + LR vines. Least was on control vines. Significant difference was recorded in major flavonol compound quercetin with highest being recorded on CT+ST and CT+LR vines with least on control vines and ST+LR treated vines. Among hydroxybenzoic acids, gallic acid was in higher concentration with highest being recorded on CT+LR and CT+ST vines. Maximum ellagic acid was recoded on ST+LR and ST+CT+LR vines with least on either ST or LR vines. Among non-flavonoid compounds, concentration of chlorogenic acid was highest in Sauvignon Blanc followed that of cafteric acid and gallic

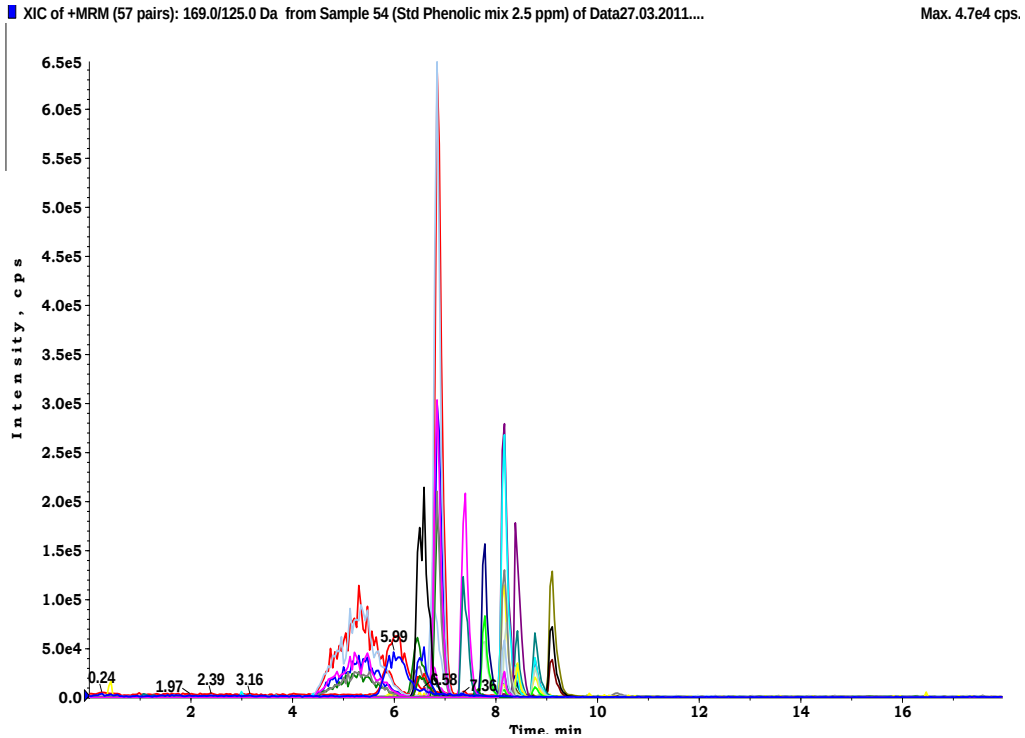

Figure 2. Chromatogram showing intensity of phenolic compound standards using Lc-MS/MS.

acid. But no definite trend could be seen among canopy management practices in concentration of non-flavonoid phenolics. Highest concentration of resveratrol was recorded on vines which received CT+ST treatment (Table 2).

Cabernet Sauvignon

The berry weight and basic fruit composition parameters of Cabernet Sauvignon grapes in response to canopy management practices is shown in Table 3. Maximum berry weight was recorded on vines which received cluster thinning treatment (86.68 g) while it was least on CT+ST vines (59.29 g). Highest TSS was recorded on vines which received combination of all the three practices viz., CT+ST+LR (23.03°B) while it was least on control vines (20.13°B). Highest pH was recorded on vines which received LR treatment (3.85) while it was least on CT +LR, CT+LR+ST and control vines. The TA was highest on ST+LR vines (1.13%) followed by control (1.07%) vines, while it was least on CT+LR vines (0.87%). Vines which received either LR treatment or in combination with either CT or ST recorded maximum anthocyanin and phenol content. Significant difference in juice potassium content was recorded with highest potassium content on either shoot thinned vines (0.199%) or on control vines (0.221). Least potassium was recorded on LR vines (0.145%) followed by those vines which received combined treatment of ST+CT+LR (0.160%). Least anthocyanin concentration was recorded on vines which received ST (1.66 mg/g) treatment followed by those on control vines (1.76 mg/g). Significant differences in organic acid concentration were recorded among canopy management treatments (Table 3). Highest tartaric acid was recorded on vines which received LR treatments (1.65 g/L) followed by the vines which received all the three treatments (1.49 g/L). Highest malic acid content was recorded on control vines (1.84 g/L) while it was least on ST+CT+LR (1.31 g/L) vines.

As expected, a highest concentration for most of the phenolic compounds was recorded in Cabernet Sauvignon grapes as compared to Sauvignon Blanc grapes (Tables 2 and 4). Significant differences were recorded for most of the flavonoid phenolic compounds of which, catechin concentration was highest followed by that of quercetin and epicatechin contents. In both the cultivars, least catechin concentration was recorded on control vines and vines which received CT+ST+LR treatment compared to those vines which received either single treatment or combination of any two treatments. Highest quercetin was recorded in vines which received either ST or CT treatments followed by the vines which received combination of either ST+LR and / or CT+LR treatment. No definite trend was observed for other flavonoid compounds such as rutine hydrate and kaempferol. The major non-flavonoid compound measured was ellagic acid with highest on vines which received CT+ST treatment (3.84 mg/L) followed by those

Table 1. Influence of canopy management practices on basic fruit composition parameters and organic acids in Sauvignon Blanc grapes (values are average of two years).

Treatment	100 Berry wt. (g)	TSS (^oB)	pH	Acidity (%)	Phenol (AU/g)	Protein (mg/g)	Potassium (%)	Tartaric acid (g/L)	Malic acid (g/L)	Citric acid (g/L)
ST	100.33	19.10	3.19	1.18	3.82	181.95	0.126	1.10	1.19	0.042
CT	115.12	20.33	3.17	1.00	4.46	228.7	0.119	0.97	1.23	0.043
LR	99.21	20.16	3.06	0.90	4.86	211.44	0.109	1.00	1.03	0.025
CT+ST	108.03	20.60	3.17	0.98	4.98	253.68	0.121	1.32	1.43	0.045
CT+LR	99.64	21.33	3.19	1.05	6.01	257.26	0.115	0.99	1.15	0.039
ST+LR	90.94	21.13	3.21	0.93	4.56	229.98	0.111	1.37	1.21	0.043
ST+CT+LR	102.32	21.26	3.16	0.93	4.51	241.65	0.117	1.32	1.27	0.045
Control	98.26	19.83	3.27	1.14	3.29	202.73	0.173	1.20	1.51	0.039
SEM ±	3.747	0.379	0.0136	0.0467	0.332	24.621	0.0107	0.042	0.047	0.0015
Significance*	0.0148	0.012	<0.001	0.0051	0.0016	0.406	0.0149	<0.0001	<0.0001	<0.0001

*:Values below 0.05 are significant at p≤0.05 and above are not significant.

Table 2. Influence of canopy management practices on phenolic compounds (mg/L) in Sauvignon Blanc grapes (Values are average of two years).

Treatments	Flavonoid phenolics					Non flavonoid phenolics						
	Flavan -3-ols		Flavonols and Flavonl algycons			Hydroxy benzoic acids			Hydroxy cinnamates			Stilbene
	Catechin	epicatechin	Quercetin	Rutine hydrate	Kaempferol	Gallic acid	Vanillic acid	Ellagic acid	Cafteric acid	Coumaric acid	Chlorogenic acid	Resveratrol
ST	2.22	0.875	0.79	0.615	0.020	0.562	0.293	0.351	0.566	0.021	0.760	0.28
CT	1.98	0.865	2.25	0.600	0.018	0.545	0.231	0.348	0.549	0.024	0.763	0.25
LR	1.81	0.839	2.12	0.644	0.011	0.544	0.112	0.177	0.563	0.068	0.745	0.18
CT+ST	2.29	0.893	2.43	0.644	0.014	0.564	0.319	0.583	0.559	0.087	0.774	0.36
ST+LR	1.86	0.803	1.67	0.676	0.016	0.545	0.230	2.08	0.563	0.086	0.724	0.18
CT+LR	2.21	0.860	2.34	0.644	0.008	0.573	0.194	0.35	0.545	0.106	0.760	0.10
CT+ST+LR	1.70	0.771	2.26	0.654	0.038	0.555	0.214	1.69	0.581	0.032	0.724	0.15
Control	1.52	0.776	0.98	0.624	0.005	0.538	0.250	0.524	0.549	0.060	0.680	0.23
SEM ±	0.165	0.0295	0.199	0.0164	0.0095	0.0055	0.052	0.129	0.0059	0.370	0.0241	0.0304
Significance*	0.0447	0.0641	<0.0001	0.099	0.4215	0.0032	0.2481	<0.001	0.0118	0.645	0.2018	0.0005

*: Values below 0.05 are significant at p≤0.05 and above are not significant.

on control (2.27 mg/L) vines and on CT+ST+LR vines (2.83 mg/L). Concentration of gallic acid,vanillic acid, cafteric acid and coumaric acid were in same proportion with no definite trend among different canopy management practices (Table 4).

Table 3. Influence of canopy management practices on basic fruit composition parameters and organic acids in Cabernet Sauvignon grapes (Values are average of two years).

Treatment	100 Berry wt. (g)	TSS (°B)	pH	Acidity (%)	Anthocyanin (mg/g)	Phenol (AU/g)	Protein (mg/g)	Potassium (%)	Tartaric acid (g/L)	Malic acid (g/L)	Citric acid (g/L)
ST	20.20	3.65	0.97	1.66	5.98	219.76	0.199	83.96	1.23	1.80	0.035
CT	21.33	3.52	0.94	2.39	6.17	233.28	0.191	86.68	1.40	1.55	0.038
LR	21.60	3.45	1.06	2.70	6.92	593.55	0.145	85.29	1.65	1.54	0.034
CT+ST	21.63	3.51	0.89	2.31	6.28	251.92	0.197	59.29	1.40	1.83	0.034
CT+LR	20.96	3.43	0.87	3.28	6.81	260.15	0.163	76.53	1.34	1.55	0.024
ST+LR	20.45	3.45	1.13	3.08	5.98	219.76	0.162	76.08	1.15	1.66	0.033
ST+CT+LR	23.03	3.43	0.95	2.71	6.13	226.34	0.160	78.3	1.49	1.31	0.32
Control	20.13	3.85	1.07	1.76	3.67	299.51	0.221	78.12	1.17	1.84	0.034
SEM ±	0.364	0.0863	0.0433	0.143	0.608	0.0133	0.012	2.890	0.070	0.0847	0.0011
Significance*	0.0007	0.0382	0.006	<0.001	0.0463	0.0415	0.0142	0.065	0.0019	0.0003	<0.0001

*: Values below 0.05 are significant at p≤0.05 and above are not significant.

Table 4. Influence of canopy management practices on phenolic compounds (mg/L) in Cabernet Sauvignon grapes (values are average of two years).

Treatments	Flavonoid phenolics					Non flavonoid phenolics						
	Flavan -3-ols		Flavonols and Flavonol algycons			Hydroxy benzoic acids			Hydroxy cinnamates			Stilbene
	Catechin	epicatechin	Quercetin	Rutine hydrate	Kaempferol	Gallic acid	Vanillic acid	Ellagic acid	Cafteric acid	Coumaric acid	Chlorogenic acid	Resveratrol
ST	6.32	1.109	1.18	1.046	0.0003	0.685	0.510	5.45	0.548	0.616	0.715	ND
CT	6.16	1.100	1.23	0.354	0.0023	0.696	0.509	3.23	0.549	0.619	0.739	ND
LR	3.99	0.687	1.73	0.270	0.0250	0.565	0.490	3.35	0.537	0.597	ND	ND
CT+ST	3.80	0.800	2.57	ND	0.0613	0.611	0.485	3.84	0.573	0.598	ND	ND
ST+LR	4.10	0.811	2.57	ND	0.0006	0.654	0.506	3.43	0.548	0.619	ND	0.0006
CT+LR	4.47	0.825	2.90	ND	0.0140	0.670	0.503	3.22	0.560	0.594	0.716	0.001
CT+ST+LR	3.07	0.604	3.39	ND	0.0176	0.614	0.506	2.83	0.559	0.602	0.00	0.0006
Control	2.06	0.679	1.85	ND	0.0001	0.610	0.504	2.27	0.572	0.643	0.00	0.004
SEM ±	0.149	0.0325	0.0930	0.114	0.0095	0.0049	0.0074	0.497	0.0026	0.0056	0.0057	0.0008
Significance*	<0.001	<0.000	<0.000	<0.0001	0.0045	<0.001	0.224	0.0206	<0.000	0.0002	<0.0001	0.0004

*: Values below 0.05 are significant at p≤0.05 and above are not significant. ND: Not detected.

DISCUSSION

Canopy management practices along with balanced pruning, training and trellising are primarily focused on altering canopy components and cluster microclimate during fruit development mostly in favour of improved light distribution in the canopy (Kliewer and Smart, 1989). Altering the physical appearance of canopy, by judicious

canopy management practices also has physiological implications that virtually always comprise a change in source: sink relationships in the grapevine through improvement in photosynthetic activity and translocation of photosynthates from leaves to sinks such as berries (Johnson et al., 1982; Hunter and Visser, 1988; Candolfi – Vasconcelons and Koblet, 1990; Hunter et al., 1995; Koblet et al., 1996). These canopy management practices include a range of techniques which can be applied in a vineyard to alter the position or amount of leaves, shoots and fruits in space to harness maximum benefits of microclimate. The main objective of this pilot study was to examine the impact of such canopy management practices on changes in fruit composition of Cabernet Sauvignon and Sauvignon Blanc grapes especially in sub-tropical climate of India.

Berry weight was highest in vines which received single treatment of cluster thinning in both the varieties, while it was least on control vines or vines which received shoot thinning treatments. The increased berry weight in cluster thinned vines may be due to diversion of photosynthates in to remaining clusters on the vine. Bunches developed on control vines showed least berry weight. Shoot thinning must have reduced the total photosynthetic capacity of the vines which resulted in reduced accumulation of photsynthates in the developing clusters. The present observation on reduced berry weight on control vines is in accordance to findings of Ristic et al. (2007), where shading (control vines) reduced the size of the berry by 20%. The increase of cluster weight in cluster thinned vines is related to increase in the availability of nutrients to retained clusters on the vines as compared with the un-thinned vines which have more number of clusters. The control vines produced fruits of least weight; the reason for this may be due to competition between the high number of leaves in the shoots and more number of clusters.

Both TA and juice pH were highest in control vines suggesting increased malic and potassium levels. No canopy management practices on those vines might have resulted in more shade inside the canopy especially in bunch zone. This may also suggest delayed fruit ripening on such vines. Similar findings of increased TA in shaded conditions and reduced TA in leaf removed vines were observed by Wolf et al. (1986) and Kliewer and Lider (1970). In contrast, reduced TSS and increased acidity and juice potassium on the same vines explain the importance of exposed canopy to harness sufficient sunlight into the canopy. The decrease in pH in control vines may be due to increased shading of bunches which resulted in bunches to remain cooler, leading to lower pH (Bergqvist et al., 2001) and due to decreased malic acid metabolism (Lakso and Kliewer, 1978). Shaded berries accumulated more potassium, malic acid, and sometimes anthocyanin content (Kliewer and Smart, 1989).

Importance of canopy/cluster exposure to optimum sunlight is evident with respect to most of the biochemical composition which differed significantly between treatments. Although it is worth to notice that leaf removal decreased fruit weight in Gewurztraminer, Seyval, Cabernet Sauvignon, Sangiovese and Trebbiano grapes, many investigators found that sunlight exposed fruits are generally rich in total soluble solids and reduced titratable acidity, compared to non-exposed or canopy shaded (Kliewer and Lakso, 1968; Ferree et al., 2004; Kliewer and Dokoozlian, 2005; Santesteban and Royo, 2006; Main and Morris, 2004). But, in contrast some workers found that defoliation had no effect on soluble solids and titratable acidity (Vasconcelos and Castagnoli, 2000; Howell et al., 1994; Poni et al., 2006). In our study, though we could not observe significant differences in soluble solid concentration among treatments, the TSS was considerably highest on vines which received LR treatment either alone or in combination. The increased TSS on such vines might be either due to remobilization of stored carbohydrates, an increase in photosynthetic activity of remaining leaves and improvement in the light microclimate of remaining leaves and an increase in sink strength as explained by Kliewer and Antcliff (1970), and increased fruit temperature and changes in pattern of assimilate movement (Bledsoe et al., 1988) which needs to be confirmed by measuring light intensity, leaf area index and berry temperature under tropical climate.

Influence of leaf removal performed at different stages of berry development was studied by different workers. Leaf removal is usually performed in the fruit zone during vegetative season between fruit set and ripening (Poni et al., 2006). If it is done at veraison stage, it affects synthesis of primary and secondary metabolites and this effect is directly related to leaf layer number, photosynthetic rate and canopy surface area. Several experiments have shown increased sugars, flavor, flavonoids and decreased acidity when leaf removal was done at veraison stage (Percival et al., 1994; Poni et al., 2006; Zoecklein et al., 1992). In contrast, leaf removal at veraison on plants with low canopy density does not affect grape sugar, acidity and color (Reynolds et al., 1986). The more vigorous nature of vines induced in tropical climate may be benefitted by partial defoliation to improve grape composition and wine quality as suggested by Hunter et al. (1991) that partial defoliation in vigorous varieties is an endeavour to alter grape composition. Some of the investigations have revealed that partial defoliation increases total soluble solids and reduce titratable acidity, malic acid, pH and K level in the fruits (Kliewer and Bledsoe, 1987; Kliewer et al., 1988). In contrast, some of the workers have failed to demonstrate these alterations in fruit compositions (Koblet, 1984; Williams et al., 1987) or in some cases they could see reduced sugar accumulation (Sidahamed and Kliewer, 1980).

The goal of shoot thinning is to reduce canopy density, although the ideal shoot number per meter of cordon is dependent on the cultivar, spacing and site. When shoot

thinning is optimized, the vine is more efficient in radiation interception (Smart, 1985). Appropriate shoot thinning can improve fruit composition in *Vitis* v*inifera* cultivars (Smart, 1988). In the present study, vines which received treatment shoot thinning alone or control vines had lesser tartaric acid content while leaf removal along with cluster thinning resulted in higher tartaric acid and lower malic acid concentration. The shoot thinning alone may increase the vegetative growth of remaining shoots, leading to diminished leaf and cluster exposure as explained by Reynolds et al. (1996) resulting in lesser tartaric acid content and increased malic acid and potassium content resulting in increased juice pH.

Juice potassium content displayed significant difference among treatments in both the varieties wherein, vines which received leaf removal in combination with ST and CT recorded least potassium content than on control vines and ST vines. Between the two varieties studied, Cabernet Sauvignon grapes recorded more potassium concentration compared to Sauvignon Blanc grapes. The increased potassium concentration in control vines of both the varieties is in accordance with the findings of Smart et al. (1985); Bledsoe et al. (1988) and Rojas-Lara and Morrison (1989). In addition, Boulton (1980) identified potassium as a major factor in determining the pH of wines and grapes. He could establish positive correlation between potassium concentration and juice pH. In the present study, this relationship could be observed in Sauvignon Blanc grapes where control vines recorded highest pH and higher potassium content, while similar relationship could not be established in Cabernet Sauvignon grapes. This might be due to the concentration of malic acid which also determines juice pH. Highest concentration of malic acid was recorded in control vines and shoot thinned vines in Cabernet Sauvignon grapes which also recorded highest juice pH. The increased juice pH may be due to degradation of malic acid by respiratory enzymes as it is weaker than tartaric acid. According to Philip and Kuykendall (1973), combination of higher tartaric and lower malic acid is considered as superior grape quality. Thus in present study the acidity balance was therefore apparently changed favourably by leaf removal. As the ratio of malic acid and tartaric acid determines total titratable acidity, there was significant variation in titratable acidity with different canopy management treatments. The current finding of reduced malic acid in leaf removal treatment in combination with either cluster thinning and/or shoot thinning is in accordance with the findings of Kliewer (1967); Wolf et al. (1986); Kliewer and Bledsoe (1987) and Kliewer et al. (1988).

Reduced anthocyanin concentration was recorded on both control vines and on vines which received ST and ST+CT+LR treatments. Vines in control treatment might have developed higher number of leaves with maximum shade inside the canopy. It is likely that both light and berry temperature (either in excess or reduced quantity) may be the factor in accumulation of anthocyanins. This is in accordance to reduced anthocyanins in control (shaded) vines and fully exposed clusters in Shiraz (Haselgrove et al., 2000), where fully exposed clusters recorded relatively higher berry temperature due to more exposure to sunlight which might have reduced anthocyanin accumulation or increased degradation. The increased anthocyanin accumulation in clusters on vines which received LR, ST+LR and CT+LR treatments suggest that, if light conditions within the canopy are such that bunches / cluster zone receives sufficient sunlight of moderate intensity, then light is not necessarily limiting factor for anthocyanin accumulation (Keller and Hrazdina, 1998). However, these effects may also be temperature dependent as explained by Mabrouk and Sinoquet (1998).

Influence of canopy management practices on berry composition is more pronounced with respect to anthocyanin concentration in Cabernet Sauvignon grapes. In control vines, it is likely that light is a limiting factor for anthocyanin accumulation. Similarly on vines which received combination of all the practices, the sunlight falling on clusters may be high thus increasing berry temperature to inhibit anthocyanin synthesis and/or to increase anthocyanin degradation. This hypothesis is supported by earlier literatures that synthesis of anthocyanin is directly regulated by both light exposure and temperature condition to which grape is subjected (Pirie and Mullins, 1980; Crippen and Morrison, 1986; Smart et al., 1988).

As far as phenolic compounds are concerned, significant difference could be seen in Cabernet Sauvignon than on Sauvignon Blanc. Concentration of major phenolic groups flavan – 3 – ols (catechin and epicatechin) and flavonols (quercetin and myricetin) were least in both control vines and those which received CT+ST+LR treatment, while it was highest in vines which received combination treatment of LR+ST or LR+CT or CT+ST. In Cabernet Sauvignon grapes, highest quercetin was recorded in vines which received either ST or CT treatments followed by the vines which received combination of either ST+LR and / or CT+LR treatment. Among non-flavonoid compounds, ellagic acid was highest with maximum concentration recorded on either ST or CT vines followed by ST+CT vines. Control vines and vines which received single treatments (ST, LR or CT) recorded lesser concentration of quercetin in both the varieties compared to those vines which received combination treatments. This is in accordance to the findings of Ristic et al. (2007), wherein shaded clusters of Syrah could accumulate only trace quality of flavonols such as quercetin compared to exposed clusters. This clearly explains the benefit of canopy management practices to expose clusters to light as quercetin accumulation may be a light dependent process. The increased anthocyanin concentration in those treatments may be also attributed to quercetin concentration as

quercetin is important for co-pigmentation.

Light exposure through canopy modification has been shown to significantly influence flavonol accumulation in grapes and wine (Goldberg et al., 1980; McDonald et al., 1998; Haselgrove et al., 2000; Spayd et al., 2002). These observations report that fruit exposed to light mainly via changes in canopy structure have greater levels of flavonols, particularly quercetin glucosides, than shaded fruit. An increase in flavonols from sun exposed fruit may have implications with the stability of the wines particularly if flavonols act as co-pigments for anthocyanins.

Vineyards in semi-arid tropical climate with heavy black soils exhibit excessive vegetative growth. This result in disturbances in source: sink relationship leading to denser canopies and an inferior canopy microclimate for the continuous maximum photosynthetic activity of leaves. These factors may detrimentally affect grape and wine composition in particular resulting in reduced soluble sugars, tartaric acid and anthocyanin and higher concentration of malic acid, potassium and must pH. Though this preliminary study showed improved fruit composition with respect to TSS, acidity, juice pH, anthocyanins (in Cabernet Sauvignon) and few phenolic compounds in both the varieties under study, still detailed study with respect to intensity and time of leaf removal, bunch load, bunch exposure in different canopy sides (viz, east or west) etc. needs to be taken up to derive final conclusions. Measuring other parameters such as light intensity in different canopies, berry temperature, leaf area index, photosynthetic rate etc, will help to understand relationship between canopy management practices and canopy microclimate thus helps to follow, appropriate management practices to improve wine grape composition in semiarid tropical climate.

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