EFFECTS OF NITROGEN, POTASSIUM FERTILIZERS AND CLUSTERS PER VINE ON YIELD AND ANTHOCYANIN CONTENT IN CABERNET SAUVIGNON GRAPE

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Abstract

The experiment was carried out in a humid subtropical climate located in the south of China, in Xichang, Sichuan province, (27ºN, 102ºE, and 1650 m above mean sea level) during the 2005 and 2006 seasons. Eight year old, irrigated Cabernet Sauvignon vines were planted at Xichang Chia Tai Wine & Spirits Co., Ltd. Vine plants were spaced 1.25 m apart with 2 vines and rows were 2.0 m apart and oriented approximately north/south. Vines were trained to a vertical shoot positioned training system (VSP), and were bilaterally cordon-trained, spur-pruned, and shoots were vertically positioned upright. Vines were irrigated by drip irrigation system. The 3 different levels of N-K were 0-0, 100-20, and 200-60 g/plant and the 3 different levels of clusters per vine were 10, 20, and 30 clusters per vine. Treatments were applied to 1 vine plot, each replicated 6 times in a split plot, in which the main plots were clusters per vine. Vine shoot lengths were maintained at 15 nodes by shoot trimming. It was found that the yield of grapes in year 2005 was higher than 2006. Increasing the rate of N-K in both years did not increase yields, but increased clusters increased yields in both years. Color and phenolic compounds in the berries were higher in year 2006 than 2005. At the 0-0 and 100-20 levels of N-K the phenolic compounds in the grape were lower than other treatments. It was also found that the Cabernet Sauvignon berry contained more malvidin than other anthocyanins.

Keywords: Anthocyanin, phenolic compounds, malvidin

Introduction

The most significant constituent of wine is water (75-90%). This amount of variation (15%) can be explained by the amount of phenolics, organic acids, mineral salts, and pectins which form the wine extract, and which differ from wine to wine. The second largest constituent of wine is ethyl alcohol, which, according to the type of wine, varies from 8% to 13% (v/v) or more. The sugar content of dry wines is generally less than 2 g/l; while in a botrytized sweet wine it can reach almost 200 g/l (Dominé *et al.*, 2004).

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Grape color is an integral and important part of red grape and red wine quality (Somers and Evans, 1974; Gishen *et al*., 2002). It is generally accepted that an increase in grape color coincides with an improvement in the phenol structure, an increase in aroma intensity and an increase in wine quality. The color of red or black grapes is caused by the presence of anthocyanins in the grape skins (Marais, 2005). The synthesis of anthocyanins is stimulated by light, both UV and visible, as well as by nutrient stress especially nitrogen and phosphorus deficiencies, and low temperature (Hopkins, 1995). Revilla *et al*. (1997) showed that the content of phenolics (catechin and procyanidins) in grapes is clearly affected by 4 agroecological factors: the cultivar, the year of production (i.e., the climate condition from year to year), the site of production (the effect of geographic origin of grapes, soil chemistry, and fertilization), and the degree of maturation. Reported levels of anthocyanins in red grapes range from 300 to 7500 mg/kg, but the levels vary highly according to the cultivar, maturity, production year and environmental conditions (Mazza, 1995). The above characteristics are controlled by cultivation managements; namely weed control, pest control, canopy management, crop loading, irrigation, and fertilizer management. These practices are considered as routine works. Among these the most immediate effect, which can give rise to the yield and wine quality are crop load and N-P-K in each specific area. In Xichang China the maturity of grape is in the rainy season in which sunlight is usually low. Thus to obtain high quality, grape cluster management should be conducted. Also soil in this area is high in P and K. Therefore we were interested in finding the rates of N and K on berry yield and quality because the P requirement in grape is low. The aim of this research was to investigate the rates of N and K and the clusters per vine on yield and the anthocyanin content in the grape.

Materials and Methods

Experimental Design

Fertilizer and clusters per vine treatments were applied to 1 vine plot each treatment was replicated 6 times in a split plot, main plot was cluster number and they were arranged in randomized complete block design. For a standard canopy area for each treatment the shoots were thinned to 20 shoots per vine prior to bloom. Vine shoot lengths were maintained at 15 nodes by shoot trimming. Three fertilizer treatments and 3 clusters per vine treatments were applied and repeated in each year of the experiment.

Treatments

Fertilizer treatments:

F1 = Control (no fertilizer)

F2 = N-K (100-20 g/vine) split 3 times soil application of actual N-K, 1st (30-5 g/vine at bud break), 2nd (40-10 g/vine at bloom), and 3rd (30-5 g/vine 30 days after bloom).

F3 = N-K (200-60 g/vine) split 3 times soil application of actual N-K, 1st (60-20 g/vine at bud break), 2nd (80-20 g/vine at bloom), and 3rd (60-20 g/vine 30 days after bloom).

Urea and potassium sulfate, the sources of N and K, were applied in the rows under the treatment vines and incorporated into the soil.

Clusters per vine treatments:

C1 = 10 clusters per vine

C2 = 20 clusters per vine

C3 = 30 clusters per vine

Clusters per vine treatments were applied at veraison.

Population, Samplings and Location of Research

Vineyard: 8 year old, irrigated Cabernet Sauvignon vines were planted in Xichang, Sichuan province, China (27°N, 102°E, and 1650 m above mean sea level) during the

2005 and 2006 seasons at Xichang Chia Tai Wine & Spirits Co., Ltd. Vine plants were spaced at 1.25 m apart with 2 vines and rows were 2.0 m apart and oriented approximately north/south. Vines were trained to a vertical shoot positioned training system (VSP), and were bilaterally cordon-trained, spur-pruned, and shoots were vertically positioned upright. Vines were irrigated by drip irrigation.

Instrumentation and Data Collection

Tissue analysis: Plant tissue samples were collected in each year for each replication at bloom. Samples consisted of 20 petioles per treatment plot. Leaves opposite flower clusters were sampled at bloom. The petioles were separated from blades and placed in paper bags and were promptly dried at 70ºC for 48 h. The N and K contents in the petioles were determined. The N and K contents in the grape berries were determined after harvest.

Component of yield and berry composition: 50 berries for each replication were randomly sampled, and the percentage of soluble solids concentrations was determined with a temperature compensating, hand-held refractometer. A portable pH meter was used to determine pH. Titratable acidity (TA) was determined by titration with 0.1M of NaOH to a pH 8.2 end point and expressed as g/l of tartaric acid. Phenolic compounds were measured by spectrophotometer and high performance liquid chromatography (HPLC). At harvest, the fruit from each vine was harvested and weighed. Other yield components including cluster weights and berry weights were recorded. Cane pruning weights were collected after each growing season. The crop load (fruit weight per vine/pruning weight per vine) was determined for each treatment.

Determination of red pigments (color) and the total phenolic content of the grape berries was by the method of Iland, *et al.* (2000). Detection of grape berry anthocyanin was done by HPLC. The extracts from 3.4.4 were centrifuged at 14000 rpm for 10 min and filtrated with a 13 mm, 0.20 μm syringe filter (GAT Asia Limited). Ten microliters were used for HPLC analysis. The gradient cycle consisted of an initial 5 min isocratic segment (solution A, 100%). Then the linear gradient was changed progressively by increasing solution B (100% CH_3CN) to 10% at 10 min, 15% at 20 min, 20% at 25 min and then increasing solution A to 100% at 30 min. Solution A consisted of 4/4/92 CH_3OH/CH_3CN/87 mmol/L H_3PO_4 in H_2O (v/v/v). Anthocyanins were determined by reverse-phase HPLC using a Luna 5U c18 100A column (4.6 μm, 150 mm) particle diameter 5 μm. A wavelength of 520 μm was used.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows release 13.0, from SPSS Inc., 2004, was used. As a parametric methodology, variance analysis was used.

Results and Discussion

Petiole Analysis and Yield

Results from 2 years of experiments as shown in Table 2 indicated that rainfall distribution had an influence on the yield components of the grape. Yield, berries per clusters and berry weight were higher in 2005 than 2006. This was probably due to insufficient water during maturity (July, August and September) in 2006 (Figure 1). Increasing rates of fertilizer (N-K) did not increase the yields of both years because the soil might contain enough N and was very high in K (Table 1). This phenomena was confirmed by petiole analysis (Table 3). Increasing the number of clusters increased the yields of both years. The number of berries per cluster and berry size was not changed when the fertilizer rates and clusters per vine were increased. The response of the grape vine to K occurs only when soil lacks K over a number of years

(Smolarz and Mercik, 1997). Wolf, *et al*. (1983) reported that the excessive application of K could be toxic to vines and cause yield reduction. A similar result was also found by other investigators (Ahalwat and Yamadagi, 1988) who confirmed that the toxicity was caused by using potassium chloride, KCl.

Results from petiole analysis found that there was no significant increase of N in the petiole when there was an increase in the level of N and cluster numbers in both 2005 and 2006. An increase of K at 60 g and clusters number at 30 clusters per vine could increase K in the petiole only in year 2006 (Table 3). Overall we could say that there was no significant difference in N and K in the petiole because the soil analysis in Table 1 showed a very high K content in the soil. This was in agreement with other investigators (Delgado *et al*., 2006) who found that only the highest application rate of N and K would increase the level of these elements in plant tissue, but the lower rates had no effect. It was also reported that an excessive application of K and cluster thinning tended to increase the petiole and fruit K and fruit pH in most cultivars (Morris *et al*, 1987). The concentration in the petiole resulted in reducing acidity which can result in reducing wine quality (Ruhl, 1989) and berry quality.

Total Soluble Solid and TA

The total soluble solid (TSS), as measured in % Brix, in the berries was not affected by N-K and cluster levels. Increasing N-K levels increased pH and decreased TA; on the other hand, increasing the number of clusters increased TA (Table 4). This finding was agreed with Delgado *et al*. (2006) who reported that both

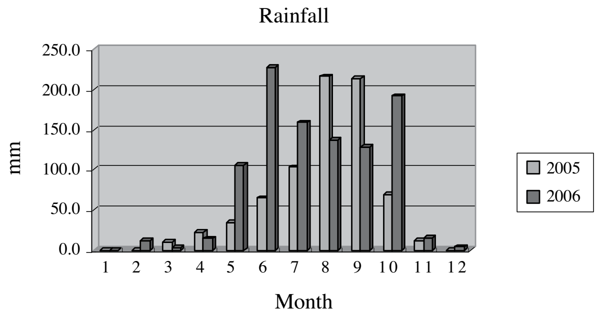

Figure1. Monthly rainfall at experimental station in 2005 and 2006

Table 1. Soil analysis data of the experimental site

Soil analysis	2005	2006
Texture	Clay loam	Clay loam
pH	5.25	5.79
OM (%)	0.70	0.75
N (ppm)	9.90	9.80
P (ppm)	9.80	19.40
K(ppm)	470.00	156.00
Ca (ppm)	300.00	229.00

rates of K (60 and 129 g/vine) caused a reduction in TA. This mechanism seems to be that the excessive K+ migrates to the fruit and enhances the formation of potassium bitatrate which precipitats and causes a lowering of TA. Ruhl (1989) assessed the effect of N application on other juice composition parameters of Riesling, Chardonnay and Cabernet Sauvignon and he found that the effect of N on Chardonnay was significant. The results suggested that the increases in juice pH would result in a poorer quality of end product. Therefore the application of N and K fertilizer in wine grapes should be

Table 2. Influence of fertilizers (N-K) and number of clusters on yield, berry per cluster, and berry weight

Treatments	Yield (kg/vine)		Berry per cluster		Berry weight (g/berry)	
	2005	2006	2005	2006	2005	2006
Fertilizer (N-K)						
F1	2.33a	1.08b	154.37a	61.21b	1.22b	0.93b
F2	2.23a	0.93b	158.58a	54.55ab	1.81b	0.87a
F3	2.18a	0.83a	152.92a	49.84a	1.13a	0.90ab
No. of cluster						
C1	1.24	0.59a	157.49a	64.93b	1.19a	0.90a
C2	2.30b	0.97b	154.41a	54.45a	1.17a	0.89a
C3	3.20c	1.28c	153.67a	46.22a	1.16a	0.92a
Mean	2.25	0.95	155.19	55.20	1.17	0.90

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT

Table 3. Influence of fertilizers (N-K) and number of clusters on petiole N and K at blooming period

Treatments	Petiole N (%)		Petiole K (%)	
	2005	2006	2005	2006
Fertilizer (N-K)				
F1	1.24a	1.04a	4.17b	4.17a
F2	1.21a	1.14b	3.52a	4.15a
F3	1.24a	1.11ab	3.84ab	4.42b
No. of cluster				
C1	1.23a	1.12a	3.79a	4.11a
C2	1.19a	1.07a	3.82a	4.26ab
C3	1.27a	1.10a	3.92a	4.38b
Mean	1.23	1.10	3.84	4.25

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

carefully considered and not over applied. The optimum range of TSS of Cabernet Sauvignon variety was 22.5-28.5°Brix and TA was less than 5.5 g/l (Golan and Shalit, 2010; McDonnell *et al.* 2008). Regarding the number of clusters per wine quality reducing the cluster numbers by thinning at veraison strongly improves the accumulation of sugars during ripening; berry skin anthocyanins and flavanoids were also more concentrated Arfelli *et al.* (1996); Guidoni *et al.* (2002); Gao and Cahoon (1998).

Grape Berry Color and Phenolic Compounds

Data in Table 5 showed that there were no significant differences in grape color when the levels of N-K and the clusters were increased, except that the levels of N-K in 2005 when there was no fertilizer and at the highest rate gave a higher color. A similar

Table 4. Influence of fertilizers (N-K) and number of cluster on TSS (Brix), TA (g/l), and pH of grape berry

Treatments	TSS (Brix)	TA (g/l)	pH
Fertilizer (N-K)			
F1	18.25a	10.79b	3.32a
F2	18.08a	10.50ab	3.36b
F3	18.39a	10.34a	3.40c
No. of cluster			
C1	18.33a	10.07a	3.37a
C2	18.35a	10.85b	3.34a
C3	18.04a	10.70b	3.36a
Mean	18.24	10.54	3.36

In a column and each treatment means followed by a common letter are not significantly different at the5% level by DMRT.

Table 5. Influence of fertilizers (N-K) and number of clusters on color and phenolic compounds in grape berry

Treatments	mg color/g berry		Total phenolic/g berry	
	2005	2006	2005	2006
Fertilizer (N-K)				
F1	0.886b	0.894a	828b	1.292a
F2	0.726a	0.913a	690a	1.221a
F3	0.889b	0.905a	765ab	1.387b
No. of cluster				
C1	0.889a	0.924a	798a	1.322a
C2	0.802a	0.925a	759a	1.300a
C3	0.810a	0.863a	726a	1.279a
Mean	0.834	0.904	761	1.300

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

trend was also obtained on total phenolic content per g berry in both years in which the high levels of N-K gave a higher level. This effect might be due to N because in this area the soil already contained a very high amount of K (Table 1). Delgado *et al*. (2004) reported that an increase in N reduced polyphenol synthesis in the berry skins. However, when K was high an application of N at the maximum level had higher polyphenols. This finding supported this reseach, which found that in 2006 the treatment of the highest N-K levels gave the highest phenolic polyphenol levels, when the N-K ratio was 3.6:4.3. Gao and Cahoon (1998) reported that the fruit's red pigmentation was increased quadratically by cluster thinning. The total anthocyanin in the berry skin was increased linearly by cluster thinning.

Grape Anthocyanin

The major compounds in the anthocyanin group are petunidin (Pt), cyanidin (Cy), and malvidin (Mv). In year 2005, the highest N-K level had a tendency to decrease Pt and Cy but to increase Mv. On the contrary, an increase to the highest cluster numbers had a tendency to increase Pt, Cy, and Mv. However in the lowest number of clusters, Pt and Cy were also high. In year 2006, there were no significant differences in Pt and Cy among fertilizer rates, but at no fertilizer and at the highest rates the grapes were high in Mv. The highest number of clusters gave the highest Pt and Cy but there were no significant differences in Mv content in all levels. The formation of malvidin-glucoside appears to be more prevalent to unfavorable environmental conditions than other anthocynins. In this study we found that petunidin and cyanidin were of the same pattern and they were the opposite of malvidin. Among the 3, malvidin was higher in the grape berries than the other 2 combined. This compound finding was supported by other investigators who found that malvidin was the major anthocyanin in the Cabernet Sauvignon grape (Burns *et al*., 2002).

Conclusions

The rainfall distribution at the experimental site in 2005 and 2006 was different, especially during July-September, when the rainfall in year 2005 was higher than 2006, which resulted in the grape yield in 2005 being higher. Increasing rates of fertilizer N-K did not increase the yield of either year, because

Table 6. Influence of fertilizers (N-K) and number of clusters on grape berry and anthocyanin compounds (mg/ml)

Treatments	Petunidin (Pt)		Cyanidin (Cy)		Malvidin (Mv)	
	2005	2006	2005	2006	2005	2006
Fertilizer (N-K)						
F1	0.379c	0.202a	0.036b	0.078a	0.518a	0.514ab
F2	0.267a	0.194a	0.026a	0.072a	0.562a	0.445a
F3	0.318b	0.217a	0.032ab	0.089a	0.568a	0.575b
No. of cluster						
C1	0.321ab	0.165a	0.035b	0.074a	0.539a	0.495a
C2	0.282a	0.146a	0.028a	0.065a	0.488a	0.464a
C3	0.360b	0.302b	0.031ab	0.100b	0.622b	0.577a
Mean	0.321	0.204	0.031	0.080	0.549	0.511

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by the soil contained enough N and K. But increased cluster numbers increased the yield of both years. There were no differences in N and K in the petiole. The TSS in the berries was not affected by N-K and cluster levels. Increasing rates of N-K increased pH and decreased TA but decreasing cluster levels decreased pH. The application of a high rate of N-K gave a higher total phenolic content per gram berry in both years. The total of phenolic content and the color were higher in year 2006, which indicated that water stress influenced the synthesis of this compound. With regard to the effect of fertilizer N-K and cluster numbers on compounds in the anthocyanin group we found that no fertilizer treatment had a tendency to increase anthocyanin in the grape berry. It was also found that malvidin was the major anthocyanin compound in Cabernet Sauvignon. It could be concluded that, with high fertility, a soil application of N-P-K may not be necessary for the wine grape. Water influenced grape yield but water stress promoted the anthocyanin production. Therefore to produce good quality wine, soil and water management should be considered as a priority means in wine grape production.

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