



Effects of mineral fertilization on fruit quality in drip-irrigated Marselan grape (*Vitis vinifera*)

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ABSTRACT

Top quality of grape (*Vitis vinifera* L.) berries is essential for producing delicious and customer-favorite wine. Thus, the optimal application of mineral fertilizers is important and necessary. Here, a standard drip-irrigation protocol with five different mineral fertilizer (F) treatments- C0 kg/ha (control), F1 245 kg/ha, F2 490 kg/ha, F3 735 kg/ha and F4 980 kg/ha were laid out in a split-plot design to evaluate their effects on grapevine factors which included shoot growth, berry weight and size, and berry sugar, acid, phenolics and volatiles. The experiment was conducted at the Wuwei Academic Forest Science Center during 2017. The results showed that all fertilizer applications significantly promoted shoot growth. Fruit weight and size increased under F2 and F3 treatments. In addition, soluble solids and reducing sugars grew up under F3 treatment. However, sugar-acid ratios did not differ significantly among all F treatments but phenolic and tannin levels were increased under F3 treatment, as were anthocyanin levels under F1, F2 and F3 treatments. A total of 83 different aroma components were identified in all the F treatments. These were clustered into seven groups. The total amount of aroma components tended to increase, then to decrease with increasing rates of fertilization (from C to F4). The results indicate that mineral fertilizer application has complex effects on the berry quality factors that were evaluated.

Key words: Fertilizers, Grape berry, Quality, Volatile components, Wine grapes

The quality of a grape (*Vitis vinifera* L.) berry depends on its chemical composition and its physicochemical properties. The key quality determinants are the levels of soluble sugars, titratable acidity, pH and the levels of the phenolics, anthocyanins and volatiles (Pereira *et al.* 2006, Zerihun 2015). However, the influence of the standard vineyard managements on wine grape and quality are less often reported. Examples of these standard managements include the applications of mineral fertilizers and of irrigation water which are the most important inputs that directly affect the plant growth and development, yield and quality of produce (Ramniwas *et al.* 2013).

In arid and semi-arid areas of China, water scarcity and water quality are becoming increasingly problematical. However, in these dry areas, grape berry growth and development can also be manipulated and optimized by the appropriate balancing between the applications of water and those of the mineral fertilizers (Gong *et al.* 2016). Thus, drip irrigation in conjunction with mineral fertilization can be used to deliver essential nutrients to the vines and also to maximize both crop yield and crop quality (Ramniwas *et al.* 2013). Therefore, by using a fixed criterion for irrigation

while adjusting the rate of fertilization, the balance between these two can be optimized to maximize water use efficiency and thus yield, while also maximizing fruit quality (Zhai and Li 2005, Fu *et al.* 2014). In recent years, a great deal of viticultural research in China has focused on the relationships between irrigation and mineral fertilization and the growth, yield and quality of the grapes. However, most of this work has been concentrated on table grapes (Hani 2005, Gong *et al.* 2016). Thus, reports on their effects on wine grapes under Chinese conditions are relatively few. In this study the French winegrape cultivar Marselan was used as the test material and the effects of different applications of N-P-K mineral fertilizers on the growth, development and quality of its fruit using a field plot trial design were examined.

MATERIALS AND METHODS

Growing conditions: The experiment was conducted at the Wuwei Academic Forest Science Center, Gansu province (2017) which is located at latitude 38°02'N and longitude 102°42'E, at an altitude of 1632 m amsl. The 10-year old Marselan vines were planted with in-row spacing of 1 m and between-row spacing of 2.5 m. Vines are trained to a 'fan' system.

Trial design: For the trial, we selected vigorous vines of similar growth form and size. The trial design was a randomized complete block with five fertilizer treatments.

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For each treatment, three rows of vines constituted one block with three replications - each row (6 vines) was one replicate. The five fertilizer (F) treatments were: C 0 kg/ha (control), F1 245 kg/ha, F2 490 kg/ha, F3 735 kg/ha and F4 980 kg/ha. The fertilizer contained N:P₂O₅:K₂O in the mass ratio 1:0.75:1.31. The fertilizers were applied dissolved in the water (fertigation) at five growth stages – (i) bud break, (ii) during shoot extension, (iii) at flowering, (iv) pre-veraison and (v) post-veraison. The fertilizer amounts at each application varied as follows: the total N fertilizer amount (see above) was applied progressively during the season at the above five growth stages (i, ii,...v) as 40%, 20%, 10%, 30% and 0%, respectively; similarly for P₂O₅ as 0%, 45%, 20%, 15% and 20% and for K₂O as 0%, 20%, 10%, 30% and 40%. During the growing season, weeds, pests and diseases were controlled using standard industry methods.

Grape berry samples: The maturation phase of grape berries was estimated by fruit soluble solids. The time for the collection of samples was 8:00-10:00 AM on October 8, 2017. The samples were collected uniformly and randomly from both sides of each row, placed in plastic boxes and immediately taken to the laboratory. Individual berries were removed from the tip, middle and base of each cluster and from opposing sides to measure mean single-berry weight, diameter, total soluble solids, titratable acidity, reducing sugar content and skin color. Grape skins were removed from the berries, frozen in liquid nitrogen, ground to a powder and stored at -80°C for measurement of phenolic compounds.

Shoot growth: Twelve vigorous vines were chosen per treatment to measure shoot length and diameter. Shoot length was measured using a tapeline and shoot diameter (at the base) was measured using a Vernier caliper. Both were measured weekly from the beginning of shoot-growth to pinching. Three weeks of measurements were made and assigned as the corresponding three growth phases.

Single berry weight and diameter: Samples of 100 berries at the harvest phase per treatment were randomly selected from different bunches as described above to measure the average single berry weight, average diameter and the index of fruit shape (length/width).

Color parameter of berry skin: Two areas were measured on the skins of each of 100 berries collected from 10 clusters using a colorimeter (Konica Minolta CR-400). Three color indicators were used (Tahmasbpour *et al.* 2014).

TSS, TA and reducing sugars: Samples of 100 berries per treatment were juiced with a squeezing roller and total soluble solids (TSS) was measured by using a TD-45 digital refractometer. Titratable acidity (TA) was measured by titration with sodium hydroxide (Cao *et al.* 2007), samples of 100 berries were separated into 10 technical replicates. Reducing sugars were measured by the Folin regent colorimetry method (Li *et al.* 2000). The sugar-acid ratio was calculated as the ratio of the reducing sugar content divided by the titratable acidity.

Phenolic compounds: The total phenolic content

(TPC) was estimated using the Folin-Ciocalteu method (Singleton and Rossi 1965). The results are expressed as gallic acid equivalents (mg/g). The tannin content (TC) was estimated using the Folin-Danis method (Lu *et al.* 2017). The results are expressed as tannic acid (mg per g). The total anthocyanin content (TAC) was estimated using the pH differential method (Wrolstad 1976). TAC is expressed as malvidin-3-glucoside equivalents (mg per g).

Headspace solidphase micro-extraction (HS-SPME): Samples of 300 berries per treatment were taken from the tip, middle and base of each cluster and were homogenized in a blender. Samples (10 g) of homogeneous juice were placed in a 15 ml centrifuge tube with 1 g crosslinking polyvinylpyrrolidone (PVPP) and 0.5 g delta-gluconolactone, extracted at 4°C and then centrifuged at 8000 rpm to get supernatants for future measurements. Samples (10 ml) of the supernatants were mixed with 1g NaCl, 50 ml octan-2-ol and the total sample placed into small vial. This was tightly capped with a PTFE-silicon septum and heated at 60°C for 30 min on a heating platform while being agitated using a magnetic stirrer at 500 rpm. The SPME (50/30-µm DVB/Carboxen/PDMS), preconditioned according to the manufacturer's instructions, was then inserted into the headspace, where extraction was allowed to occur for 30 min with continued heating and agitation by a magnetic stirrer.

GC-MS analysis: The TRACE 1310-ISQ was used to do the GC-MS analysis. The carrier gas was helium at a flow rate of 1.0 ml/min. Samples were injected by placing the SPME fiber at the GC inlet for 25 min with the split less mode. The oven starting temperature was 40°C which was held for 5 min, then raised to 220°C at a rate of 2°C/min and held at 220°C for 15 min. The mass spectrometer was used in the electron impact mode (MS/EI) at 70 eV recording in the range m/z 35 to 350 U. The mass spectrophotometer was operated in the selective ion mode under auto tune conditions and the injected volumes were 1.0 µL.

The qualitative analysis of the aroma substances was based on the chromatographic retention time and mass spectrum information standards, by comparison with a standard spectrum library and related references. A semi-quantitative analysis was carried out using an internal standard method (2-octanol was selected as internal standard). The content of aroma substances was calculated using the formula:

$$X_i = A_i / A_s \times C_s,$$

where, X_i is the mass concentration of the component (µg/L), C_s is the mass concentration of the internal standard 2-octanol (µg/L), A_s is the peak area of the internal standard and A_i is the peak area of the component to be tested.

Statistical analyses: The data were analyzed using One-Way ANOVA test. Statistically significant differences are indicated with lowercase letters ($P < 0.05$).

RESULTS AND DISCUSSION

Shoot length and shoot diameter growth response to

fertilizer treatment: As shown in Supplementary Fig 1, 2, 3, 4 treatments significantly promoted shoot growth during the three phases. The highest growth was under F3 where total shoot length growth over the three phases was 67.02 cm. Under F3, shoot length increased by 25.14 cm in the second phase (S Fig 1A). However, the shoot diameter growth increased significantly during the first phase. The highest rate of shoot diameter growth was under F3 (1.78 mm) (S Fig 1B). These results indicate fertilizer treatments significantly affect shoot growth in the first two weeks. It is worth noting that F3 is about optimal for shoot growth.

Fruit size and fruit weight response to fertilizer treatment: Fruit weight and fruit size increased with increased fertilization (Table 1). Single fruit weight increased gradually with fruit size (fruit length and fruit width). The treatments F2 and F3 significantly increased fruit weight (~1.062 g and 1.069 g, respectively). However, with further fertilizer applications (F4) single fruit weight decreased by about 10% compared to under F2. However, for fruit size growth significant differences were recorded under F2, F3 and F4. These results indicate the F2 and F3 are effective in increasing fruit weight and fruit size.

Fruit quality response to fertilizer treatment: All four quality measures behaved differently with the increasing level of fertilization. Total soluble solids increased slightly with increasing fertilization but the increase was significant only under F3 (~22.17%) (Table 1). The same trend was observed for reducing sugars where both F3 and F4 increased significantly (to 20.93% and 20.61%, respectively). The effects of fertilizer on titratable acidity show it rose to a maximum under F1 treatment (1.01%). There were no significant differences among the other treatments for sugar-acid ratio, where this remained approximately constant.

Phenolic and anthocyanin response to fertilizer treatment: The phenolics and tannins increased significantly under F3 reaching to 3.42 and 2.69 mg/g, respectively (Table 1). The anthocyanin content also increased

significantly and markedly under F1, F2 and F3. Meanwhile, the fruit color parameters (L^* , a^* and b^*) were not affected significantly by increases in mineral fertilization (Table 1). Furthermore, our results are consistent with those of others that either the higher or lower amount of fertilizers application has the negative effect of fruit quality, where the application foliar microbial fertilizers at 2000 ppm can maximize berry weight and raise soluble solid content, but not applications of either 1000 ppm or 3000 ppm (Kok and Bal 2017). Anthocyanin and phenolic compounds are also affected by fertilizer treatment or by canopy management (Kok 2011, 2013). Research on anthocyanin accumulation in grapes shows that reducing N fertilization can increase anthocyanins and this modifies wine quality in terms both of sensory perception and also health benefits. High levels of K can also reduce anthocyanins (Delgado *et al.* 2006, Jezek *et al.* 2018). But our result show that with the right levels of N, K and P anthocyanin accumulation is increased.

Volatile components response to fertilizer treatment: The aroma compounds in the berry determine the smell and taste of the resulting wine. There were 83 different aroma components detected (Table 2). These can be clustered into seven groups, including 23 higher alcohols, 10 aldehydes, four esters, three fatty acids, three ketones, nine benzene derivatives and 31 hydrocarbons which comprised 13 alkanes, 17 olefins and one alkyne. Also, fertilizer level can induce specific and unique aroma compounds. A total of 43 aroma compounds were found under F4 but only 37 under F3. Moreover, the total amount of the aroma compounds also varied. Under all the treatments we recorded from 900.0 to 3,140.0 $\mu\text{g/L}$. The lowest amount was detected under F and the highest under F2. Under all treatments, the aldehydes, higher alcohols, hydrocarbons and benzene derivatives were the main aroma compounds present, accounting for more than 98.5% of the total aroma components. The minor aroma compounds were the esters, acids and ketones together accounted for just 1.5% of the total. Overall, we show

Table 1 Qualities in grape berries under different fertilization applications

Treatment	C	F1	F2	F3	F4
Single berry weight/g	0.936 $\pm$ 0.010c	0.954 $\pm$ 0.025bc	1.062 $\pm$ 0.025a	1.069 $\pm$ 0.008a	1.031 $\pm$ 0.010ab
Width/mm	10.98 $\pm$ 0.24	11.62 $\pm$ 0.06	11.62 $\pm$ 0.13	11.36 $\pm$ 0.30	11.57 $\pm$ 0.19
Length/mm	11.43 $\pm$ 0.18b	12.03 $\pm$ 0.05ab	12.32 $\pm$ 0.03a	12.47 $\pm$ 0.22a	12.38 $\pm$ 0.20a
Index of fruit shape	1.0413 $\pm$ 0.0064	1.0352 $\pm$ 0.0012	1.0601 $\pm$ 0.0101	1.0986 $\pm$ 0.0291	1.0699 $\pm$ 0.0068
L^*	35.11 $\pm$ 0.42	33.75 $\pm$ 0.37	33.35 $\pm$ 0.91	34.39 $\pm$ 0.89	32.75 $\pm$ 1.60
a^*	-0.64 $\pm$ 0.02	-0.61 $\pm$ 0.02	-0.53 $\pm$ 0.08	-0.69 $\pm$ 0.05	-0.62 $\pm$ 0.06
b^*	-4.66 $\pm$ 0.06	-4.76 $\pm$ 0.22	-4.15 $\pm$ 0.40	-4.82 $\pm$ 0.53	-4.60 $\pm$ 0.34
Reducing sugar/%	17.15 $\pm$ 0.05b	20.04 $\pm$ 0.63ab	19.04 $\pm$ 0.30ab	20.93 $\pm$ 0.82a	20.61 $\pm$ 1.18a
SSC/%	19.83 $\pm$ 0.29b	21.77 $\pm$ 0.24ab	21.20 $\pm$ 0.38ab	22.17 $\pm$ 0.26a	21.70 $\pm$ 0.84ab
TA/%	0.75 $\pm$ 0.02cd	1.01 $\pm$ 0.02a	0.71 $\pm$ 0.02d	0.87 $\pm$ 0.01b	0.81 $\pm$ 0.03bc
Sugar-acid ratio	22.80 $\pm$ 0.51ab	20.80 $\pm$ 0.90b	28.07 $\pm$ 1.16a	24.98 $\pm$ 1.12ab	26.47 $\pm$ 2.35ab
Tanin content/(mg/g)	2.22 $\pm$ 0.03b	2.33 $\pm$ 0.07b	2.38 $\pm$ 0.04b	2.69 $\pm$ 0.08a	2.42 $\pm$ 0.06b
Total phenolic content(mg/g)	2.52 $\pm$ 0.03d	2.69 $\pm$ 0.04cd	2.84 $\pm$ 0.11bc	3.42 $\pm$ 0.04a	3.06 $\pm$ 0.03b
Total anthocyanin content (mg/g)	1.59 $\pm$ 0.02c	1.94 $\pm$ 0.02a	2.03 $\pm$ 0.01a	1.84 $\pm$ 0.05ab	1.69 $\pm$ 0.08bc

Table 2 Contents of aroma components in grape berries under different fertilization applications

Treatment		C	F1	F2	F3	F4
Alcohols	Total amount	168.97±1.22E	388.89±9.03B	730.34±22.30A	349.88±0.54C	254.93±3.21D
	Proportion	13.06	24.85	23.28	23.85	28.32
	Types	9	10	12	9	15
Aldehydes	Total amount	1005.45±11.23B	831.13±5.75D	2036.79±10.99A	853.37±15.22C	455.63±4.51E
	Proportion	77.69	53.11	64.92	58.16	50.62
	Types	8	8	6	6	5
Esters	Total amount	6.51±0.61B	-	-	9.77±0.53A	-
	Proportion	0.50			0.67	
	Types	2	-	-	2	-
Fatty acids	Total amount	-	-	12.35±0.53	11.81±1.40	12.92±0.12
	Proportion	-	-	0.39	0.81	1.44
	Types	-	-	2	2	3
Ketones	Total amount	1.07±0.07b	0.50±0.02c	7.51±0.34a	0.30±0.01cd	0.14±0.01d
	Proportion	0.08	0.03	0.24	0.02	0.02
	Types	2	1	2	1	1
Benzene derivatives	Total amount	21.60±1.28e	62.17±3.79c	93.88±4.10a	76.16±2.26b	42.16±1.27d
	Proportion	1.67	3.97	2.99	5.19	4.68
	Types	5	8	7	5	7
Hydrocarbons	Total amount	90.51±2.57e	282.22±7.54b	256.43±5.09a	165.91±3.12d	134.26±3.60c
	Proportion	6.99	18.03	8.17	11.31	14.92
	Types	14	13	9	12	11
Sum up		1294.12±5.70d	1564.91±9.23b	3137.30±10.29a	1467.21±14.82c	900.04±12.33e
Types		40	40	38	37	42

Unit: Total amount /(mg/g), Proportion/%

that the total number of aroma compounds increases, then decreases, with increasing mineral fertilization.

The total mass aroma compounds 3137.30 µg/L in F2. This was significantly higher than that in the other treatments. Among these were the aldehydes, higher alcohols, ketones and benzene derivatives which are beneficial to taste. These beneficial esters and fatty acids were conspicuously accumulated in F3. Our results illustrate that the application of fertilizers has a complex range of effects on both the 'quality' contents and also the aroma composition of grape berries.

In this study, we found 83 different aroma compounds, which included aldehydes, alcohols, hydrocarbons and benzene derivatives with significant differences being detected between fertilizer treatments. This compares with the study of Zhao *et al.* (2010) where 52 different aroma compounds were detected in Marselan. The different results are likely related to different growing conditions, different soils and managements.

Our results show that trans-2-hexenal was the major aroma compound in all five treatments, being between 30.5 and 52.0% of the total. This result fits with that of Crouzet (1986) and of Gomez *et al.* (1995) that this compound

is commonly the dominant volatile across a number of grape cultivars. The terpenes and isoprene compounds are also important aroma compounds in grapes and these to contribute importantly to the aroma of the fruit (Guth 1997). Here, we report nine different terpenes and isoprene which include: geraniol, myrtenol, verbenol, β-ionone, vanillin, lauryl, orange-acetone. Among these, geraniol and β-ionone were relatively abundant in all five treatments. Furthermore, aldehydes, higher alcohols and hydrocarbons are also major volatiles. The different fertilizer treatments affected the formation and accumulation of most aroma substances, not only in terms of which ones were produced but also in terms of the relative amounts.

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