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Effect of varying levels of potassium on berry quality of Cabernet Sauvignon grapes under tropical conditions

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ABSTRACT

Potassium, an essential nutrient for grapevine has profound effect on berry growth and development and subsequently on wine quality. A sharp increase in berry potassium levels is observed due to potassium redistribution from leaves to berries after veraison. But excessive levels of potassium in berries at harvest may reduce the quality of must and have a negative impact on wine quality, particularly in red wines. Potassium has been implicated in reduction in tartaric acid in juice with overall increase in the pH of wine. To standardize potassium requirement of Cabernet Sauvignon on 110R rootstock in relation to quality parameters of berries, present investigation was carried out during 2014-15 at ICAR-NRC for Grapes, Pune. The experiment was laid out as per RBD having five graded doses of potassium as sulphate of potash viz. 0, 50, 100, 500, 600 kg K_2O/ha per year. Each treatment was replicated 4 times. Except control, more fructose content in berries were observed in comparison to glucose in all potassium treated vines. Potassium applications resulted in decreased tartaric and malic acid content in berries. More caftaric acid (53.3 ppm), chlorogenic acid (86.7 ppm), epicatechin (34.7 ppm) and quercetin hydrate (251 ppm) content was recorded in vines treated with 100 kg of potassium per hectare. Increasing trend in petiole content of K, Ca, Na and Cu was found. Juice acidity and pH increased with increasing potassium application.

Key words: Potassium, berry quality, sugars, phenols, acids, juice pH.

INTRODUCTION

Nutrition is a key component of vineyard management. It has the potential to influence various factors in vine production that includes, fruit set, fruit quality and the processed products such as raisins, juice and wine (Adsule *et al.*, 1). Tropical climate poses a challenge to production of quality wine in India. In grape growing regions of India, the soils are mainly alkaline and calcareous in nature, which affects the potassium supply to the vines. Judicious use of fertilizers is important to attain better yield and quality of grapes. Among macronutrients, potassium plays a major role in grapevine growth and development. Bhargava and Sumner (3) have identified physiological stages at which potassium is needed at optimum dosage for back pruning such as fruit bud differentiation stages, bud fixing stage and cane maturity stage. After forward pruning, adequate potassium is required for translocation of sugar into berries. It has an important role in maintaining sugar-acid ratio.

Potassium (K^+) is the main cation (positively-charged ion) in must and wine (Blouin and Cruège, 5). Early in the season, when the growth rate is high, much of the K^+ accumulates in the leaves. After véraison, a sharp increase in berry K^+ is observed as a result of K^+ redistribution from leaves to berries (Blouin and Cruège, 5 and Ollat and Gaudillère, 15). The pH increases over the harvest season in wine grapes. The increase in pH is most probably due to the increase of potassium after véraison (Saayman, 17). During véraison, availability of the respiratory substrate, sucrose (via photosynthesis), becomes limited due to degradation of chlorophyll. The berry is therefore forced to shift its metabolism from sugar to malic acid respiration. Grape phenolics contribute to color, flavor, texture and astringency of wine and to its antioxidant properties. The degree of ionization of anthocyanins, which is the percentage of total anthocyanins present in the coloured forms, decreases as pH increases (Somers, 19). Anthocyanins are located in berry skin (Somers and Pocock, 21) where K concentration is generally higher than in the pulp (Iland and Coombe, 10 and Walker *et al.*, 13). Therefore, berry K levels are an important consideration for red wine than for white wine.

Potassium is released during the homogenisation process from the skin cells during fermentation process. Extra potassium combines with tartaric acid to form potassium bitartrate (Ribéreau-Gayon *et al.*, 16). Grape is the only fruit in which tartaric

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acid is found in the form of potassium bitartarate which affects pH and stability of grape juice products. Higher K levels in grape berries may have a negative impact on wine quality, mainly because it decreases free tartaric acid resulting in an increase in the pH of grape juice, must and wine (Boulton, 6 and Gawel *et al*., 8). Although tartaric acid is often found at higher concentrations than malic acid and is the stronger acid of the two, its concentration is relatively constant. During the fermentation of red wine, the skin is left for maceration and during the extraction of anthocyanins more K may also be extracted. As grape growing and wine making in India is mainly confined to semi-arid tropics, where problems of salinity and low water availability (Sharma *et al*., 18), could pose challenge for production of quality wine grapes from red grapes having balanced acidity and sugars. Considering importance of potassium in production of quality wine grapes for making acceptable wines, present study was conducted to understand the effect of different potassium levels on berry and wine quality of Cabernet Sauvignon.

MATERIALS AND METHODS

This study was carried out during cropping season of 2014-15 on Cabernet Sauvignon vines grafted on 110R rootstock at ICAR-National Research Centre for Grapes, Pune Maharashtra. Vines were planted in 2007 at spacing of 8x4 ft and pruned twice in a year once in April and another in October as foundation pruning and fruit pruning, respectively.

The experiment included 5 graded potassium applications arranged in a randomized block design with four replicates and each replication having 12 vines. The graded doses of potassium viz. 0, 50, 100, 500, 600 kg K_2O/ha per year were added as sulphate of potash with irrigation water having EC-1.62 dSm^{-1}; pH-8.87; Ca^{2+} -42.4 ppm; Mg^{2+} -67.56ppm; Na^+ -206.31 ppm; Cl^- -223.65 ppm; HCO_3^{2-} - 488 ppm, SO_4^{2-} - 125.14 ppm and 37.0 ppm NO_3^--N. The fertilizers were applied in split doses a) 10% applied at foundation pruning, b) 30% of the total dose was applied in 6 equal parts at 45, 60, 75, 90, 105 and 120 days after foundation pruning and rest 60% of the dose was split into 6 equal parts at 45, 60, 75, 90, 105 and 120 days after fruit pruning. Farm yard manure @ 25 t/ha was applied uniformly across the treatments. Soil samples were collected from the root zone of the vines, representing 40 cm soil surface diameter below the emitter and at one feet depth. Soil pH (1: 2.5 soil: water) was 7.90, EC (1:2 soil: water) = 0.85 dS m^{-1}, organic carbon=1.35%, calcium carbonate=9.5%, mineralizable N= 135 ppm, and available P (Olsen's P) = 152 ppm. Ammonium acetate extractable K^+, Ca^{++}, Mg^{++}, and Na^+ content in soil were 650 ppm, 6215 ppm, 3150 ppm, and 750 ppm, respectively, whereas water soluble chloride content was 205 ppm.

To study content of minerals viz.; N, P, K, Ca, Mg, Na, Cu, Fe, Mn, Zn, in petiole, physiologically mature leaves free from damage or defects were collected at fruit bud differentiation, full bloom and veraison stage. The collected leaf samples were immediately transported to laboratory. The separated petioles were washed carefully to remove surface contamination with liquid soap solution followed by tap water and then distilled water. For nutrient analysis, the samples were placed in paper bags and dried in a Hot Air Oven at 70°C for 72 hours. Dried petiole samples were crushed using sample mill grinder (Labtec). The digested sample was taken for further analysis of macro and micro nutrients. Nitrogen was estimated by using Kjeldahl method by automatic distillation and titration unit (Gerhardt). All other nutrients like Na, K, Ca, Mg, P, Cu, Fe, Mn and Zn were simultaneously analyzed by Inductively Coupled Plasma Mass Spectrometer (Thermo Scientific).

Berries were harvested when the TSS Acidity ratio of the berry juice reached 20:1 as recommended. Samples were collected as per standard protocol. Fully ripe fruits, free from defects or decay were collected. In addition, number of bunches, bunch weight, size of berries, 100 berries weight and total yield (kg/plot) were also measured. Skin was separated from the berries and skin individually as well as whole berry samples were crushed and then further taken for nutrient content, organic acid content, phenolic content and sugar content using ICP-MS, High performance liquid chromatography with UV and ELSD detector, respectively. For estimation of Nitrogen content, skin and whole berry samples were freeze dried using freeze dryer (Virtis) and then taken for digestion using sulphuric acid and perchloric acid and then by Kjeldahl method.

Berry sample as per treatment and replication were used for preliminary fruit composition analysis like juice pH, total acidity, total soluble solids, volatile acidity using Oenofoss.

HPLC analysis of organic acids, sugars and phenolic compounds: The fruit samples were stored at -20°C. These stored samples were utilized for analysis of different parameters by using HPLC. After removing samples from freezer, samples were thawed overnight under refrigerated conditions. Later the fruits were macerated in cheese cloth and the resultant must was centrifuged and the supernatant was used for HPLC analysis.

Analysis of organic acids (Tartaric acid and malic acid) was done with Agilent technologies 1260 series HPLC system with Diode array detector

(DAD) at wave length of 214 nm and band width of 4.0. The column used was Agilent Zorbax eclipse plus C 18 (4.6 ×100 mm 5um). The separation was done with mobile phase of A- 95 % Acidified water with orthophosphoric acid (pH 2.0) and B- 5 % absolute methanol with flow rate of 0.8ml/min. Column temperature was 25° C. The injection volume was 10µl and total run time was 7 minutes.

HPLC analysis of glucose and fructose was done with Agilent technologies 1260 series system with Evaporative Light Scattering Detector (ELSD) in isocratic mode. The amino column of 250 µ 4 or 4.6 mm, 5µ particle size was used. The injection volume was 10 µ L and total run time was 6 min.

LC-MS/MS (Agilent Technologies with series hyphenated to API 4000 Qtrap (ABS Sciex) mass spectrometer equipped with electrospray ionization (ESI+) probe 1200) was used for quantification of individual phenolic content in samples.

Analysis of variance procedures was performed for petiole nutrient content, yield and fruit quality properties according to Little and Hills (14). Mean separation was performed with least significant difference (LSD) at p ≤0.05.

RESULTS AND DISCUSSION

There are evidences to show conclusively that N, P and K involved in bud initiation and differentiation of grape bunches in the previous year determine the potential yield component for the current year crop (Bhargava, 4). The nutrient status in petioles at phenological stages namely fruit bud differentiation, flowering and veraison, has own role as content of nutrients in vine conclusively determined the productivity of grapevines. Effect of potassium application on petiole nutrient status is presented in Table 1. Significant differences were noted in petiole nutrient content at fruit bud differentiation stage. At full bloom, P, K, Ca, Mn and Zn showed significant differences while at veraison, N, P, K, Ca, Na, Cu, Fe and Zn had significant differences in petioles. NPK content improves bud fruitfulness, which determines the productivity, has been shown to increase with adequate N, high P and optimum K (Bhargava and Sumner, 3). N+P or P+K induced early flower bud initiation in grapevines. Application of potassium in K deficient vineyards markedly increased the fruitfulness of latent buds of Thompson

Table 1. Effect of K application on content of nutrients in petiole at different phenological stages.

Stage	Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Fruit bud differentiation	T1	0.94	0.69	1.37	1.08	1.04	0.02	2.5	41	89	6.5
	T2	0.97	0.79	1.04	0.95	1.25	0.02	2.2	36	105	7.4
	T3	1.00	0.68	1.21	0.96	1.13	0.02	2.2	43	97	6.4
	T4	0.90	0.74	1.14	0.80	1.18	0.03	2.3	40	128	8.2
	T5	0.85	0.76	1.28	0.75	1.26	0.02	1.6	43	129	7.6
	LSD	0.02	0.01	0.03	0.011	0.013	0.0009	0.36	1.65	2.01	0.30
	Significance	***	***	***	***	***	***	**	***	***	***
Flowering	T1	0.92	0.56	1.14	1.12	0.96	0.05	9.6	40	126	43.9
	T2	0.93	0.65	0.89	1.36	1.01	0.06	10.7	33	120	35.2
	T3	0.95	0.62	0.96	1.39	0.96	0.03	12.4	36	112	28.7
	T4	0.95	0.61	1.10	1.35	1.01	0.05	13.2	44	125	31.4
	T5	0.95	0.62	1.26	1.41	1.02	0.08	11.1	37	131	28.3
	LSD	0.04	0.06	0.10	0.15	0.08	0.03	2.96	8.77	8.53	10.07
	Significance	NS	*	***	**	NS	NS	NS	NS	**	*
Veraison	T1	0.71	0.40	1.14	1.59	0.84	0.02	19.0	211	504	35.3
	T2	0.69	0.37	1.28	1.51	0.81	0.02	15.5	162	596	41.8
	T3	0.74	0.38	1.30	1.90	0.79	0.02	17.4	134	667	43.1
	T4	0.76	0.47	1.46	2.04	0.81	0.02	15.7	220	572	51.0
	T5	0.78	0.45	1.58	2.11	0.82	0.03	14.5	154	561	42.6
	LSD	0.04	0.05	0.18	0.32	0.05	0.004	1.05	10.85	155.11	0.94
	Significance	**	**	**	**	NS	*	***	***	NS	***

Seedless grapes and its mutant (Bhargava, 4). Overall decreasing trend was noted in petiole nutrient content from fruit bud differentiation to verasion. According to nutrient uptake dynamics in general, the uptake of the main nutrient elements are the largest from budbreak to veraison, and during the ripening process continues to decrease. The exception to this is magnesium, because the level of it is almost constant in the full season (Szőke, 22). But in present data, Mg also decreased. Application of K increased micronutrients namely Cu, Fe, Mn and Zn content in petiole from bud differentiation to veraison stage. Leaf petiole analyses indicated that K applied at high levels caused a significant increase in leaf petiole contents of N, P, K, Ca, Cu, Cl and Na, but reduced Fe compared with the control. Both the increments and the reductions, which occurred in the chemical composition of the petiole contents, varied according to K doses (Al-Moshileh and Al-Rayes, 2). Potassium promotes fruitfulness through its enzyme activating property. Application of potassium was found to increase the bunch number per vine (Gopalswamy, 9). Increasing potassium levels increased petiole K content at veraison which were later reflected in K content of juice (Fig.2). According to Saayman (17), the increase in pH is most probably due to the increase of potassium after véraison, which was observed in our study.

Organic acids and sugars in berries were significantly affected by doses of K. By increasing the application of potassium, content of studied organic acids in berries were decreased. Maximum content of these acids were recorded in control (Table 2). Potassium is the major cation in grape juice. By application of potassium, pH content in juice was increased and maximum potassium content was recorded in T5 (Fig. 1). High juice K decreases free acids and increases overall pH which is clearly indicated in Fig 2. Grape juice with a high pH often results in unstable musts and wines that are more susceptible to oxidative and biological spoilage, and often produces a wine with high pH and low acidity with a flat taste (Somers, 20). Data in Table 2, showed, increased content of sugars (Fructose and Glucose) by adding more K and maximum sugars were recorded in T5 where 600 kg K was applied. But ratio of glucose fructose was reversed. In control this ratio was 1.03 while application of 50 kg K changed this ratio as 0.77, however more application of potassium resulted in increasing trend of glucose fructose ratio. The sink strength of the berries for solutes during ripening could possibly be controlled at the phloem unloading step (Coombe, 7). Potassium may be involved in the translocation of solutes into the berry through its roles in phloem loading and unloading (Lang 13) as sugars are the major soluble solids in grape berries, particularly after veraison. Martin et al. (11) reported higher potassium supply increases total soluble solids content and decreased the total acidity of berries. The rapid increase in the levels of glucose and fructose in the berry vacuole is likely to be driving both cell and fruit expansion that occurs post-veraison (ripening) and potassium may play an important role in the accumulation of sugars as potassium is essential for the biosynthesis and transport of sugars from the leaves to the grapes. The Cabernet Sauvignon variety falls in high fructose forming group. As in Indian conditions harvesting coincides with warmer season so the majority of biochemical reactions take place more rapidly and glucose respires much faster than fructose. So the glucose fructose was higher in control. But application of potassium may enhance the production of fructose.

In present study it was observed that, application of potassium increased content of phenolics namely

Table 2. Different Phenolics and organic acid in berries affected by different doses of potassium.

Treatments	Phenols						Organic Acids			
	Gallic Acid (ppm)	Caftaric Acid (ppm)	Vanillic Acid (ppm)	Quercertin Hydrate (ppm)	Resveretrol (ppm)	Kampherol (ppm)	Tartaric Acid (mg/g)	Malic Acid (mg/g)	Lactic Acid (mg/g)	Citric Acid (mg/g)
T1	62.5	37.0	9.2	1269	0.47	2.37	4.16	3.92	0.47	0.14
T2	57.3	44.0	8.0	1373	0.45	2.62	4.02	3.86	0.32	0.21
T3	50.0	54.1	9.6	1473	0.33	2.43	3.89	3.60	0.51	0.14
T4	45.0	48.8	9.1	1788	0.47	2.66	3.28	2.81	0.22	0.30
T5	45.9	35.4	9.1	1970	0.51	2.61	3.11	2.72	0.23	0.24
SEM±	1.81	3.0	0.59	96	0.06	0.12	0.05	0.05	0.02	0.01
CD (p=0.05)	3.93	6.6	1.29	210	0.13	0.27	0.11	0.12	0.05	0.02
Significance	S	S	NS	S	NS	NS	S	S	S	S

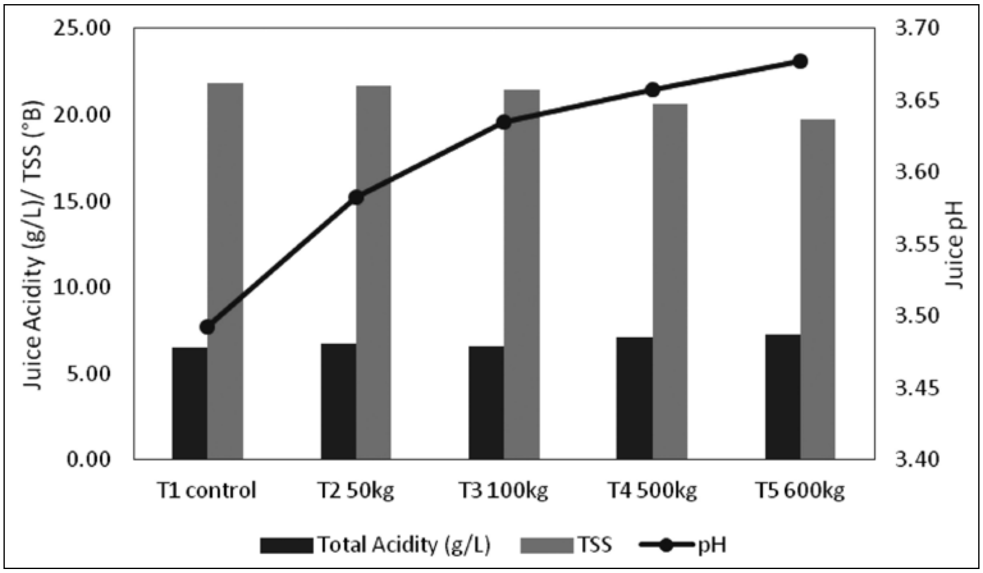

Fig. 1. Effect of potassium levels on juice pH, acidity and TSS.

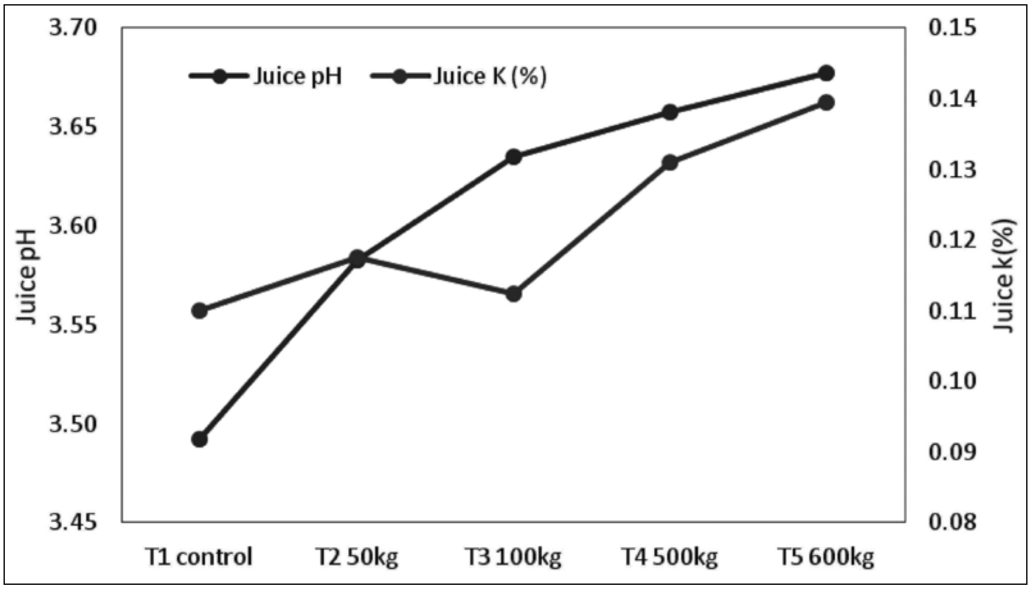

Fig. 2. Effect of potassium levels on juice K content and juice pH.

caftaric acid and chlorogenic Acid. Potassium applied @ 100kg/ha resulted in maximum caftaric acid and chlorogenic acid content in berries while Potassium applied @ 200kg/ha was noted with maximum resveratrol content. However, differences were non-significant in resveratrol content. Adequate potassium nutrition helps to increase both the colouring and polyphenolic content of berries (Sommers, 20). Application of potassium @ 100 kg K_2O/ ha in combination with farm yard manure will be sufficient for improving berry quality of Cabernet Sauvignon vines.

From the study, it can be concluded that petiole potassium levels increased with increasing potassium application during flowering and veraison stage. The potassium applied @ 100 kg/ha led to higher caftaric acid (53.3 ppm), chlorogenic acid (86.7 ppm), epicatechin (34.7 ppm) and quercetin hydrate (251 ppm) content. TSS content of juice decreased with increasing potassium application. The glucose content initially higher than fructose under control treatment but later on declined with the application of potassium. The juice pH and potassium levels increased with increasing potassium application.

Application of potassium @ 100 kg K_2O/ ha in combination with farm yard manure will be sufficient for improving berry quality of Cabernet Sauvignon vines.

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