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Nutrient uptake by grape in a Brazilian soil affected by rock biofertilizer

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Abstract

PK rock biofertilizers made from rocks and elemental sulphur inoculated with *Acidithiobacillus* improve yield of many short cycle plants similarly to soluble fertilizers. This study aims to evaluate the potential of PK rock biofertilizers for grape cultivation in the Brazilian San Francisco Valley. Three sources of P and K were compared: (a) soluble fertilizers, (b) biofertilizers plus elemental sulphur inoculated with *Acidithiobacillus*, and (c) ground phosphate and potash rocks, all at three application rates. A control treatment without P and K fertilization was added. Earthworm compound was applied as N source in all treatments. Grape (*Vitis vinifera* cv. Italia Pirovano) was cultivated in a dystrophic Planossol (medium texture) at the San Francisco River in the Brazilian Semiarid. P, K, Ca, Mg, S-SO_4^{2-} and Fe concentrations were analyzed in grape leaves and fruits. The results showed adequate leaf contents of S-SO_4^{2-}, K, and Fe with PK biofertilizer application plus earthworm compound, which indicates this may be alternative to soluble fertilizer for grape in soils with low available P and K.

Keywords: *Acidithiobacillus*, *Vitis vinifera*, mineral solubility, nutrients absorption, sulphur oxidative bacteria.

1. Introduction

Grape (*Vitis vinicola* L.) is a major crop in several European countries, the United States, Australia, Chile, Argentina and Brazil. In Brazil, it is traditionally found in the uplands of the sub-tropical Rio Grande do Sul state. More recently, though, it is extending to the tropical semiarid San Francisco River valley, Northeastern Brazil, where two grape harvests per year are possible, when adequately irrigated. Viticulture is always intensively managed, with high agrochemical inputs, including fertilizers (Leão, 2003). Since fertilization represents up to 30% of the production costs in grape agricultural systems (Christensen and Kearney, 2000), it should be continually researched to increase its efficiency, while reducing the cost.

A possibility for this cost reduction is using slow-release rock and mineral fertilizers, such as phosphate rocks, biotite and other K-rich volcanic rocks as well as "green rock fertilizers" like ground basalt. These rocks and minerals need to be processed by physical, chemical or biological means to enhance nutrient availability (van Straaten, 2007). *Acidithiobacillus* is a soil bacterium which is not very abundant in agricultural soils, but is highly efficient in elemental sulphur oxidation to sulfuric acid which can increase nutrient availability from both phosphate and potassium bearing rocks (Stamford *et al*., 2009).

Rock biofertilizers from P and K rocks plus sulphur inoculated with *Acidithiobacillus* have been applied with excellent results in different crops and soils of the rain forest zone and in the semiarid region of the Brazilian Northeast, as reported by Stamford *et al.,* (2006, 2007, 2009), but no literature was found on biofertilizer usage on grapes, or on long-term field cultures.

The study aims to evaluate the effects of PK rock biofertilizers under field conditions on the nutritional status (leaves and fruits) of grapes cultivated in the San Francisco Valley of the Brazilian semiarid region.

2. Material and Methods

2.1. Biofertilizer production

The Biofertilizers were produced with "Gafsa" natural phosphate, containing 13.6% of total P; and with potassium bearing rock (biotite) from Santa Luzia, Paraiba, Brazil, containing 9% of total K. The data for P and K powdered rocks, P and K biofertilizer mixed with organic matter (OM) are presented in Table 1.

Table 1. Available P and K, and pH of the mixture biofertilizers plus organic matter (earthworm compound) and PK bearing rock minerals (natural phosphate and biotite).

Chemical element	P Biofertilizer + OM	Phosphate	K Biofertilizer + OM	Biotite
	---------- (%) ----------			
Total N	1.21	0.00	1.20	0.00
Total P_2O_5	27.48	28.98	1.99	1.21
Available P*	5.00	6.00	0.09	0.05
Total K_2O	0.96	0.00	7.81	9.06
Available K	0.04	0.00	0.09	0.98
Total CaO	41.76	43.89	ND(1)	ND
Total MgO	0.40	0.32	7.33	8.58
Total Fe_2O_3	0.23	0.18	15.48	16.29
Total MnO	ND	ND	1.50	0.94
pH - water $_{(1:2.5)}$	5.1	6.4	5.0	6.1

(1) ND = not determined;
*Available P by the Embrapa (1997) extracted by Mehlich 1.

Biofertilizers were prepared separately for P and K, by mixing each rock with fine powdered elemental sulphur (200 mesh) on a 10:1 mass to mass ratio, and inoculated with *Acidithiobacillus* sulphur oxidative bacteria. The sulphur oxidative bacteria were cultivated in specific medium (El Tarabily *et al*., 2006), in 125 mL Erlenmeyers, at 180 rpm and 24-25 ºC, for 5 days. *Acidithiobacillus* was applied at 10^7 viable cells mL^{-1} through inoculation of a 1:10 (v:v) mixture with distilled water, which was pulverized to 20 cm deep layers of the rock: sulphur mixture. The mix was kept near field capacity for 60 days, after which pH in water, available P and K were determined following Embrapa (1997) with the following results: BP- pH 3.3; available P 50 (g kg^{-1}) and BK- pH 3.0; available K 10 (g kg^{-1}).

The P and K biofertilizer were mixed 1:4 (m:m) with earthworm compound (OM), to neutralize their excessive acidity, with final pH 6.1 and 6.0, respectively (Table 1).

2.2. Soil and experimental conditions

The field experiment was conducted at a production farm (08º59’49” S, 40º16’19” W and altitude 300 m) of the Botticelli Company, located at the San Francisco Valley, Pernambuco state, semiarid region of the Brazilian Northeastern. The climate is classified as “BSwh” according to the Köppen classification.

The soil used was a Dystrophic Planossol medium texture (Embrapa, 1999) representative of the region, with low available P and K, predominantly used for grape and mango. Soil chemical and physical properties were analyzed in soil samples collected before the experiment. The soil was analyzed using Embrapa (1997) methodology with the following results: pH (H_2O 1.0:2.5) = 5.8; available P = 15 mg dm^{-3}; soluble S-SO_4^{-2} = 26 mg dm^{-3}; exchangeable cations (cmol$_c$ dm^{-3}): Ca^{2+} = 1.88; Mg^{2+} = 0.37; K^+ = 0.18; micronutrients (mg dm^{-3}): B = 10.8; Zn = 4.8; Cu = 6.6; Mn = 5.2; total N = 0.7 g kg^{-1} and organic matter = 19.5 g kg^{-1}; global density (g cm^{-3}) 1.67; bulk density (g cm^{-3}) 2.63; texture (g kg^{-1}) sand = 650; silt =169 and clay = 181.

The table grape cultivar “Italia Pirovano” was used due to its high commercial and agricultural value in the region (Leão, 2003). Grape seedlings at 90 days were transplanted to furrows (40 x 40 x 40 cm) in 210 m^2 plots (35.0 m long and 6.0 m wide), with two rows, spaced at 3.5 x 3.0 m for 20 plants per plot and a total of 1404 plants in the experiment (1.228 ha).

All plants received, at seedling transplantation, the same basic fertilization following the IPA (2008) recommendations and 2 L plant^{-1} of cow manure as producers practice. The fertilizers were applied 20 cm deep and 20 cm distant from plant base.

Soil water was maintained near field capacity using computer controlled micro irrigation. All grape cultural practices, including pesticide application, were accompanied weekly after pruning, by Embrapa (Brazilian Agricultural Research Company), as required by Fruit Integrated Program (FIP) fresh fruit export guidelines. Grapes were pruned to two shoots, each with eight to ten gens, two months before each harvest and shoots were wrapped on the fruiting wires.

2.3. Experimental design and data collection

The experiment was a factorial arrangement with fertilizer sources (soluble fertilizer; biofertilizer; powdered rock) and rates (50, 100 and 150% of Pernambuco state grape fertilization recommendation, IPA (2008), and was conducted in a randomized block design, with four replicates. Fertilization was after each pruning (12 and 18 months after seedling transplantation - AST), using the same procedure described for basic fertilization. Fertilizers sources and rates (P+K) were: (a) soluble P fertilizer (single superphosphate = 300, 450 and 600 kg ha^{-1}) + soluble K fertilizer (potassium sulphate = 70, 140 and 210 kg ha^{-1}); (b) biofertilizer from phosphate rock (BP= 300, 450 and 600 kg ha^{-1}) + biofertilizer from potash rock (BK= 70, 140 and 210 kg ha^{-1}); (c) powdered rock phosphate (PR = 1,000; 1,500 and 2,000 kg ha^{-1}) + biotite bearing rock (BR = 1,000; 1,500 and 2,000 kg ha^{-1}).

A control treatment with earthworm compound (1 L plant^{-1}) and without P and K fertilization (P_0K_0) was used for comparative purpose. The earthworm compound was commercially available in the regional market with: pH (H_2O) 7.9; total N 5.0 (g kg^{-1}); available P 2.0 (mg kg^{-1}) and available K 5.0 (mg kg^{-1}).

Harvest was 26 month after transplant and berry composition was measured at harvest 32-berry samples per plot. Fruits were macerated and the juice was analyzed for pH, soluble SO_4^{-2} and total P, K, Ca, Mg and Fe content according to Malavolta *et al*., (1987). Leaves were sampled at the same time, dried, ground and analyzed for P, K, Ca, Mg, S-SO_4^{2-} by the same methodology.

2.4. Statistical analysis

Data was analyzed by ANOVA, with significant effects evaluated by Tukey test (p=0.01) using SAS. The regression equation and R^2 values between biofertilizer treatments (sources and rates) and concentration of nutrients on grape juice were calculated.

3. Results and Discussion

Plants which did not receive any PK fertilizer did not produce at 26 months, due to the low natural fertility, and high plant requirements. There was a significant source x rate interaction for berry nutrient content and pH (Table 2), with higher P and K content for soluble fertilizer, while PK rocks did not show any increment with increasing fertilizer rates. At the same time, PK biofertilizer presented the higher S-SO_4, Mg and Fe contents, likely due to the sulfuric acid made during biofertilizer production. This effect would be direct for S-SO_4, and through the acid effects on biotite structure for Fe and Mg. The higher Fe contents for biofertilizer than for soluble fertilizer confirm earlier observations by Christensen and Kearney (2000) and Faria *3.,* (2004), which found reduced Fe contents after high fertilization rates with soluble fertilizers.

Table 2. Chemical analyses in grape berries as affected by sources and rates of PK fertilizers (rock biofertilizers, soluble fertilizers and ground rocks).

PK Fertilization	Chemical analyses in grape berries						
	pH (H_2O)	P	S-SO_4^{-2}	K	Ca	Mg	Fe
	mg L^{-1}						
Biofertilizer $_{50}$	3.2Bb	25Cb	27Ba	902Bb	64Cb	94Ba	9Ca
Biofertilizer $_{100}$	3.3ABb	33Bb	36Aa	1003Ab	74Bb	102Aa	13Ba
Biofertilizer $_{150}$	3.4Ab	40Ab	40Aa	1040Ab	87Ab	113Aa	14Aa
Fertilizer $_{50}$	4.0Ca	38Ca	15Bb	1092Ca	46Bc	29Bc	3Ac
Fertilizer $_{100}$	4.3Ba	47Ba	19ABb	1631Ba	60Ac	36Bc	3Ac
Fertilizer $_{150}$	4.6Aa	53Aa	23Ab	1996Aa	68Ac	54Ac	3Ac
Rocks $_{500}$	2.9Bc	15Ac	7Bc	656Bc	96Ca	42Bb	6Cb
Rocks $_{1000}$	3.0ABc	17Ac	13Ab	733Ac	119Ba	46Bb	7Bb
Rocks $_{1500}$	3.1Ac	20Ac	14Ac	749Ac	132Aa	56Ab	9Ab
CV (%)	2.99	10.09	9.23	3.34	7.13	5.78	5.43

Values followed by different letters are significantly different (α=0.05), by Tukey test. Small letters compare means between PK sources and capital letters different application rates. The control treatment produced no fruits.

Both biofertilizer at 150% of the recommended rate and soluble fertilizer at 100 or 150% of the recommended rate presented berry P content higher than the 40 mg.dm-3 threshold for adequate wine production (Rizzon, 2000; Rizzon and Miele 2002a, 2004; Curvelo-Garcia, 2005). On the other hand, soluble fertilizer at both 100 and 150% of the recommended rate had high K berry contents, exceeding the maximum of 1000 mg.L-1, as described by Corrêa *et al*., (2006), Curvelo-Garcia (2005), Rizzon and Miele (2002b, 2004). Fe berry content is also important in enology, with a minimum threshold of 10 mg.dm-3, In enology there is also a 10.0 mg dm-3 minimum concentration of Fe in berries (Mira 2004, Curvelo-Garcia, 2005), which was only exceeded by 100 and 150% of the recommended rate applied with biofertilizer (Table 2).

Berry pH was about 3.3 for biofertilizer and 4.0 for soluble fertilizer, in which case there may by wine stability problems due to increased microbiological and physical-chemical alterations (Rizzon and Miele, 2002a; Nunes, 2003; Mira 2004; Curvelo-Garcia, 2005). Rizzon and Miele (2002b) described that pH increases may be caused by increased concentrations of K in soil.

As for the berries, there was no effect of application rate for ground rock on leaf K content, almost none in P and Mg, and only an increase over no fertilization for Fe (Figure 1). While leaf P and K contents increased with application rate for both soluble and biofertilizers, the increase was much stronger for soluble fertilizer, with the reverse pattern for Mg leaf content, similarly to berry nutrient concentrations. These results are similar to those found for sugarcane and melon (Stamford *et al*., 2006, 2008). Faria *et al*., (2004) found leaf K content in the San Francisco Valley in the 2.0 to 3.5 g kg^{-1} range, much higher than the values observed in this study, of 0.1 to 0.3 g kg^{-1}. and much lower than the 12 to 20 g kg^{-1} threshold for malic acid accumulation which can lower the wine quality (Jacobs, 2002).

The regression equations for nutrients in leaves are shown in Table 3. The effect of fertilizers applied in recommended rate showed that fertilizers and biofertilizers were significant and with higher values than rocks. For Mg the regression showed that this nutrient is closely related with Biofertilizers probably due to the content of Mg in biotite rock. The higher correlation may be because P, K, Mg and Fe are nutrients released by acidity promoted from the metabolic reaction carried out by *Acidithiobacillus* bacteria.

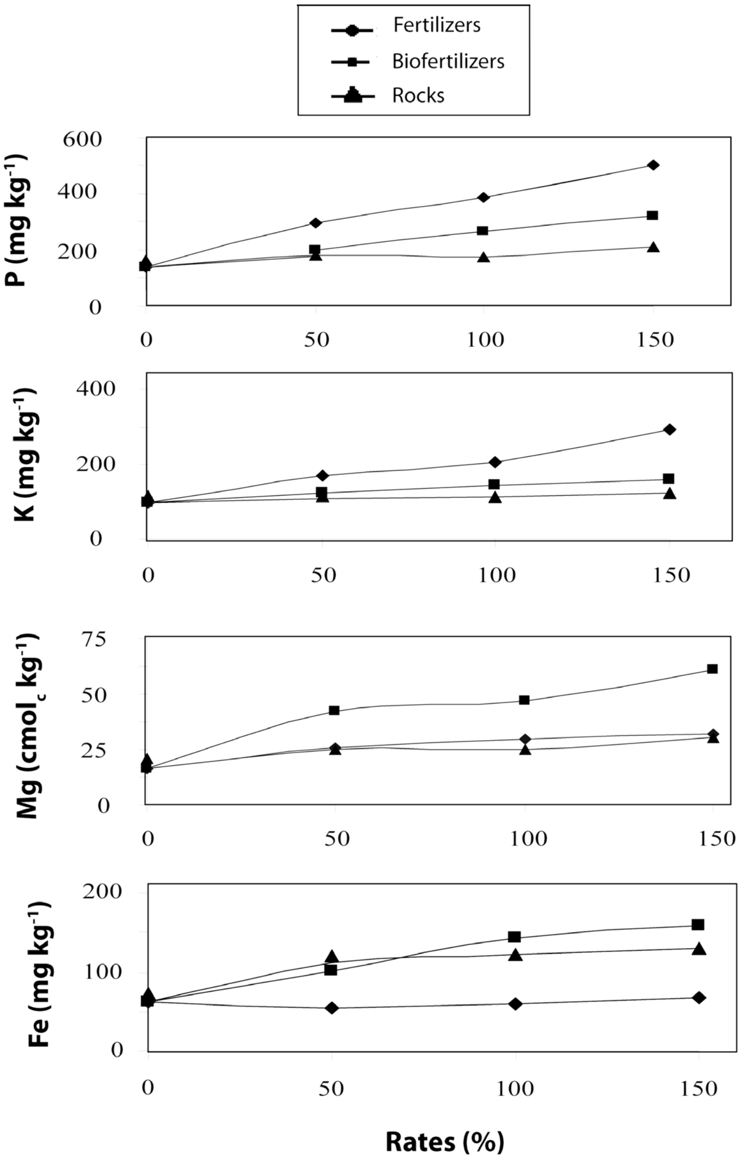

Figure 1. P, K, Mg and Fe in grape leaves, harvested at 26 months after seedling transplantation, as affected by sources (fertilizers, biofertilizers and rocks) and rates (P_2O_5 and K_2O) of fertilizers treatments.

Table 3. Regression equation and coefficient of determination, for nutrients (P, S-SO_4^{-2}, K, Ca, Mg e Fe), in leaves harvested at 26 months after seedling transplantation, as affected by the fertilizers treatments (fertilizers, biofertilizers and rocks) applied in recommended rate.

Analysis in leaves	Fertilizer treatment	Regression Equation	R^2
Total P	Fertilizers	Y= 0.1531 + 0.00237x	0.90**
	Biofertilizers	Y= 0.1388 + 0.00124x	0.96**
	Rocks	Y= 0.1437 + 0.00044x	0.63*
Total K	Fertilizers	Y= 1.3712 + 0.00929x	0.95**
	Biofertilizers	Y= 0.9850 + 0.00582x	0.97**
	Rocks	Y= 0.9781 + 0.00165x	0.83**
Total Mg	Fertilizers	Y= 0.2827 + 0.00085x	0.57*
	Biofertilizers	Y= 0.3216 + 0.00499x	0.92**
	Rocks	Y= 0.3173 + 0.00256x	0.47*
Total Fe	Fertilizers	Y= 58.078 – 0.01896x	0.61*
	Biofertilizers	Y= 61.282 + 1.02949x	0.80**
	Rocks	Y= 64.517 + 1.06272x	0.85**

(1) In equation 'x' correspond to recommended rate (P_2O_5 and K_2O) for irrigated grape and 'Y' variable analyzed. ** Significant (*p*=001) and * Significant (*p*=005).

4. Conclusions

PK biofertilizers show potential for wine grape production, due to grape berry nutrient content usually better for wine production than observed with soluble fertilizer. This fact will be explained especially in reference to P, K, Fe and SO_4^{-2} nutrients released from the phosphate and potash rocks by the acidity promoted by the oxidative bacteria *Acidithiobacillus* to produce the PK biofertilizers.

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