Influence of the rootstock and potassium fertilizer on phytoalexin synthesis in Pinot blanc grown in a calcareous soil

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S u m m a r y : Plants of cv. Pinot blanc, grafted on *Vitis berlandieri x V. riparia* Kober 5 BB, *V. berlandieri x V. riparia* SO 4, *V. berlandieri x V. rupestris* 1103 P, were grown in pots at two levels of potassium supply (0 g K_2O/pot and 2 g K_2O/pot) in order to test the phytoalexin synthesis in the leaves.

The experimental plan included also the macronutrient, trace element and chlorophyll contents of the leaves.

The most important findings are:

a) Resveratrol synthesis decreases from Kober 5 BB to SO 4 and 1103 P, while leaf chlorophyll content increases.

b) Resveratrol synthesis is higher in the plants without potassium supply.

K e y w o r d s : rootstock, potassium, nutrition, lime, soil, phytoalexin, resveratrol, chlorophyll, mineral, Botrytis, resistance.

Introduction

Phytoalexins are low-molecular, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms (PAXTON 1981). Vitaceae, including *Vitis vinifera*, synthesize stilbene phytoalexins (resveratrol and the viniferins), which on the one hand are able to inhibit *Botrytis cinerea* growth (LANGCAKE and McCARTHY 1979; BLAICH *et al.* 1982; STEIN and BLAICH 1985), on the other hand they are used as precursors for structural defence mechanisms (HOOS and BLAICH 1988).

Environmental variables, including fertilizer supply, often have a predictable effect on phytoalexin synthesis, altering plant resistance. Mineral elements are, in fact, directly involved in all mechanisms of defence as integral components of cells, substrates, enzymes and electron carriers, or as activators, inhibitors and regulators of metabolism (MENGEL and KIRKBY 1978).

High levels of nitrogen compared to low levels reduce the quantities of phytoalexin accumulated in grapevine hybrids and *V. vinifera* varieties (BAVARESCO and EIBACH 1987).

In viticulture, the mineral nutrition of the plant is greatly affected by the rootstock which has selective uptake for mineral elements, as K, Ca, Mg, Fe.

The aim of the work is to clarify the effect of three rootstocks with different K uptake and of potassium supply on the resveratrol synthesis in Pinot blanc grown on calcareous soil, which is most favourable for the production of sparkling wines.

Materials and methods

Plants of Pinot blanc grafted on three different rootstocks (*V. berlandieri x V. riparia* Kober 5 BB, *V. berlandieri x V. riparia* SO 4, *V. berlandieri x V. rupestris* 1103 P) were grown in pots (9 l volume) containing a calcareous soil. Before adding basic nutrients, the soil main characteristics were: pH = 8.4, active lime = 17 %, total N = 0.5 %, P_2O_5 = 24 ppm, exch. K_2O = 67 ppm, Fe = 96 ppm, CEC = mEq/100 g.

Potassium treatments were 0 g K_2O/pot and 2 g K_2O/pot, added as K_2SO_4 in water solution to the soil surface (1 g K_2O at the stage of 5 expanded leaves and 1 g K_2O at flowering).

At the beginning of veraison, the 5th leaf (beginning from the shoot tip) was sampled and, after washing with 1 % NaOCl solution, the leaves were investigated as follows:

Table 1: Effects of rootstock and potassium supply on resveratrol synthesis and on chlorophylls and potassium contents of the leaves

	ROOTSTOCK				SUPPLY		
	Kober 5BB	SO4	1103 P	LSD 0.05	$+K_2O$	$-K_2O$	LSD 0.05
Resveratrol (s.u.)	3390	2020	1840	1050	1980	2850	860
Tot. Chl (mg/g fresh wt)	0.82	0.85	0.96	0.06	0.83	0.93	0.05
Tot. Chl (mg/100 g dry wt)	241	265	313	19	259	287	15
Chl a/Chl b	1.96	2.03	1.92	0.08	2.02	1.93	0.06
K %	0.53	0.98	0.96	--	1.05	0.60	--

Resveratrol synthesis was obtained by exploiting the elicitor activity of mucic acid (method of STEIN and HOOS 1984). Leaf discs (17 mm Ø) were placed on filter cardboard imbued by mucic acid solution (0.01 %). After phytoalexin extraction and distillation, resveratrol was identified by using thin-layer chromatography; the amount of each sample was 2 μl. The resveratrol values were expressed as scan units (s. u.) using a Shimadzu-Chromato-Scanner CS 920, under 325 nm UV radiation.
Chlorophylls (a, b, total) were expressed as mg/g fresh wt and mg/100 g dry wt. They were extracted from leaf discs with 80 % acetone for 72 h in darkness, at +4 °C (TORRECILLAS *et al.* 1984). Corresponding equations have been used for determination of the chlorophylls (STRAIN and SVEC 1966).
Mineral elements content, macronutrients (N, P, K, Ca, Mg) and some trace elements (Fe, Mn, B) were analyzed after wet destruction of the dry matter by using the methods of COTTENIE (1980).

The statistical plan included two-way-ANOVA with interactions (the means were compared by using the LSD test at the level of 5 %) and linear regressions.

Results

Resveratrol synthesis of Pinot blanc leaves is affected by both rootstock and potassium supply (Table 1). The highest value is found in Kober 5 BB (3390 s. u.) and the lowest one in 1103 P (1840 s. u.). The plants without potassium fertilization show higher resveratrol content (2850 s. u.) than those supplied with potassium (1980 s. u.). Kober 5 BB and SO 4 show similar responses to potassium supply (resveratrol reduction), whereas 1103 P does not change stilbene synthesis in a significant way (Table 2).

Rootstock and potassium supply influence the leaf chlorophylls content significantly (Table 1). Total chlorophyll increases, changing between Kober 5 BB (0.82 mg/g fresh wt and

Table 2: Resveratrol synthesis and contents of chlorophylls and leaf mineral elements depending on rootstock and potassium supply

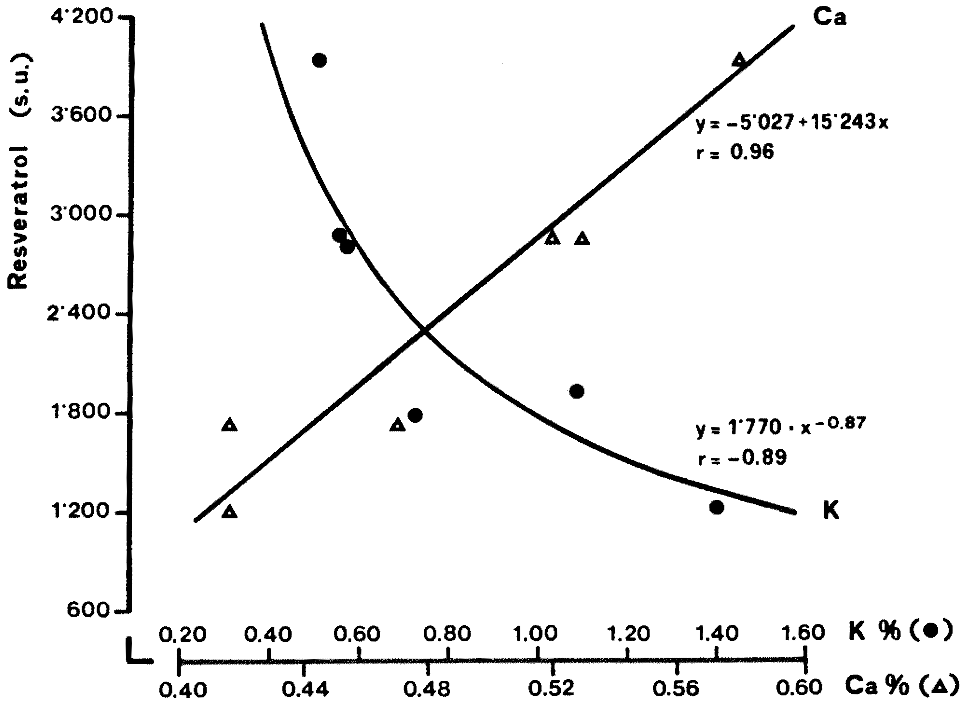
		Resveratrol s.u.	Tot. Chl mg/g f.wt	Tot. Chl mg/100g dry wt	Chl a ----- Chl b	N%	P%	K%	Ca%	Mg%	Fe ppm	Mn ppm	B ppm
Pinot b./K 5BB	+K	2 850	0.72	215	1.99	2.09	0.27	0.56	0.52	0.36	75	55	7.3
	-K	3 930	0.92	266	1.93	2.60	0.28	0.50	0.57	0.64	58	47	6.4
Pinot b./SO4	+K	1 200	0.72	233	2.03	2.32	0.26	1.40	0.42	0.37	64	43	10.3
	-K	2 850	0.98	298	2.03	2.76	0.26	0.57	0.53	0.62	77	53	8.1
Pinot b./1103 P	+K	1 910	1.04	328	2.04	2.40	0.20	1.20	0.47	0.42	61	41	11.0
	-K	1 770	0.89	299	1.81	2.87	0.28	0.72	0.42	0.52	136	34	8.2
LSD 0.05		1 490	0.09	26	0.10	--	--	--	--	--	--	--	--

241 mg/100 g dry wt), SO 4 (0.85 mg/g fresh wt and 265 mg/100 g dry wt) and 1103 P (0.96 mg/g fresh wt and 313 mg/100 g dry wt).

By potassium supply the chlorophyll content decreases, changing from 0.93 mg/g fresh wt and 287 mg/100 g dry wt to 0.83 mg/g fresh wt and 259 mg/100 g dry wt, respectively.

The negative effect of potassium supply on the chlorophyll content is observed with each rootstock, except for 1103 P which has a positive influence (Table 2).

The leaf potassium content is strongly related to the rootstock genotype; the lowest value occurs with Kober 5 BB (0.53 %), the highest one with SO 4 (0.98 %) (Table 1). The plants supplied



Correlations between leaf potassium and calcium content, respectively, and resveratrol.

show a higher content (1.05 %) than those without potassium fertilizer (0.60 %): this difference is very plain in SO 4, whereas it is very weak in Kober 5 BB (Table 2).

Resveratrol synthesis is negatively related to leaf potassium content; on the other hand, the phytoalexin synthesis increases by increasing the leaf calcium content (Fig.).

Discussion

The results obtained emphasize the role of the rootstocks on mineral element uptake which modifies some physiological parameters involved in disease resistance (resveratrol) and chlorosis occurrence (chlorophylls). Kober 5 BB does not seem suitable for calcareous soils because of its susceptibility to chlorosis; besides this, the leaf potassium content is lowest, as well as nitrogen and iron with Kober 5 BB.

The high resveratrol content in case of Kober 5 BB is probably related to the leaf nitrogen content which is lowest among the genotypes. This situation can have given rise to a shift of the balance between the primary and secondary metabolic pathways onto the shikimate pathway, providing for a large pool of phenolics (Graham 1983).

The behaviour of 1103 P is opposite: it seems suitable for calcareous soils because of its chlorosis tolerance and for its high nutritional level.

With 1103 P, the resveratrol level is lowest, probably due to the high nitrogen leaf content.

As a rule, resistance increases in response to potassium supply, up to an optimal level; in deficient plants, the synthesis of high-molecular compounds (proteins, starch and cellulose) is impaired, and low-molecular organic compounds (sugars, amino acids, amides) favourable to parasitic attack, accumulate (Marschner 1986).

According to this assertion, some authors observed in grapevine a positive effect of potassium supply in decreasing development of or damage by downy mildew, powdery mildew and grey mould (SCHAFFNIT *et al.* 1930, GÄRTEL 1959, KIRALY 1976; quoted by PERRENOUD 1977).

Nevertheless, evidence has not yet been obtained that potassium supply affects phytoalexin synthesis in grapes.

The negative effect of potassium supply, observed in the present work, could be due to two reasons:

1. The potassium leaf content is in a deficiency range (CHAMPAGNOL 1984): potassium supply was not enough to allow an optimal element level in the leaves.
2. Potassium is an antagonist to calcium which is positively related to resveratrol synthesis. Calcium seems more effective than potassium in promoting phytoalexin synthesis.

Conclusions

The most significant findings are that:

a) resveratrol synthesis decreases from Kober 5 BB to SO 4 and 1103 P, while chlorophyll leaf content increases;

b) potassium supply has a negative effect on resveratrol synthesis.

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