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Research Note

Effect of nitrogen and potassium fertilizer on induced resveratrol synthesis in two grapevine genotypes

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K e y w o r d s : grapevine, nitrogen, potassium, resveratrol.

S u m m a r y : One-year-old grapevine cuttings of the interspecific hybrid Castor and *V. vinifera* L. cv. Bacchus were grown in pots with 4 combined doses of nitrogen and potassium, in order to test their effect on the resveratrol synthesis of the leaves, after elicitation with mucic acid (0.01 %). The tests were performed on leaf discs, inside bio-assay dishes. The most significant findings were: a) the fertilizer treatments had a higher effect on the resveratrol synthesis of Castor than in Bacchus; b) high potassium supply together with low nitrogen rate promoted the resveratrol synthesis of Castor; c) high potasium rates did not balance the negative effect of high nitrogen supply on resveratrol synthesis.

Introduction: Induced stilbene synthesis is a genetic feature of *Vitis* species, associated with the resistance to some pathogens (Langcake and Mc Carthy 1979; Dercks and Creasy 1989; Hoos and Blaich 1990; Jeandet *et al.* 1992). In spite of the genetic control, environmental variables, including mineral element supply as well as nitrogen and potassium (Bavaresco and Eibach 1987; Bavaresco and Zamboni 1990; Bavaresco 1993), often have an effect on stilbene synthesis. The aim of this research is to test the effect of combined rates of nitrogen and potassium, on induced resveratrol synthesis in a hybrid and a *V. vinifera* cultivar, which are known to have a different response to elicitation.

Materials and methods: One-year-old grapevine cuttings were grown on 12 l pots of a mixture of sand, soil and peat (2:1:1), with basic nutrient (macronutrient and trace-elements) supply. The varieties tested were the interspecific hybrid Castor (Oberlin 595 F1 x Foster's White Seedling) and the *V. vinifera* cv. Bacchus [(Silvaner x Riesling) x Müller-Thurgau], coming from an original supply from Inst. für Rebenzüchtung Geilweilerhof.

At the second growth year, when the average shoot lenght was about 1 m, the 4 N-K treatments were applied as follows (per pot): A) 0.8 g N + 1 g K_2O; B) 3.2 g N + 1 g K_2O; C) 0.8 g N + 4 g K_2O; D) 3.2 g N + 4 g K_2O. Nitrogen was applied as NH_4NO_3 and K_2O as K_2SO_4.

Resveratrol synthesis and N and K leaf concentrations were checked 25 d after the fertilizer supply, on the 5th leaf from the shoot tip, after washing in 1 % NaOCl solution and rinsing in water. Each plant was trained to 2 shoots and clusters were removed. The plants were placed in the open, on a platform with a hail protection net, and they were irrigated by a drip system.

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Resveratrol synthesis was induced by exploiting the elicitor characteristic of mucic acid, following the method of Stein and Hoos (1984). The experimental plan provided for 3 replicates; each of them included the leaves coming from 3 plants chosen at random inside the pool of 9 plants per treatment. Leaf discs were placed on filter cardboard strips soaked with a mucic acid solution (0.01 %), inside bio-asssay dishes, for 48 h in the darkness, in order to allow the resveratrol to leak into the cardboard. After that, the leaf discs were removed, the cardboard dried at 40 °C and extracted with methanol for 24 h in the dark. The methanol was removed by rotary evaporation at 40 °C and resveratrol was collected by 2 x 1 ml of MeOH/H_2O (1:1). Resveratrol was identified by quantitative TLC; the amount of each sample was 2 µl. Resveratrol concentration was expressed as scan units (s.u.) using a Camag TLC-Scanner II under 325 nm UV radiation, connected with a Merck-Hitachi Chromato-Integrator D-2000.

Oven-dry leaves (the remaining blade tissue after the discs) were digested in H_2SO_4/H_2O_2, and analysed for the macronutrient and trace element concentration. N, P and B were measured by colorimetry; K and Ca were measured by flame emission photometry; Mg, Fe, Zn and Cu were measured by AAS (Cottenie 1980).

The statistical plan provided for a two-way ANOVA with interaction (genotype x treatment), and the mean values were compared by LSD test at a 5 % level. Linear regression statistics between resveratrol and mineral elements were calculated.

Results: The treatments affected the resveratrol synthesis in a different way, depending on the genotype (Table). The high N rates depressed resveratrol synthesis of Castor, while the effect of potassium was significant only in treatment D (low N and high K supply) which induced the highest synthesis. Nitrogen depressed resveratrol synthesis of Bacchus only in treatment D, when the mineral element was supplied together with the high rate of potassium.

Nitrogen leaf concentration of Castor was weakly affected by the treatments, while in the case of Bacchus a significant effect occurred (treatment C to treatment D).

Potassium leaf concentration was affected in a significant way by the treatments in both the genotypes; treatments C and D, corresponding to the high K supply, increased the K leaf concentration.

Significant linear regressions were calculated between resveratrol and N ($r = -0.80^*$), P ($r = -0.95^{**}$), Zn ($r = -0.82^*$); and not significant regressions between resveratrol and K (-0.68), Cu (-0.49), Mn (-0.07), Ca (-0.01), Mg (0.04), B (0.53), Fe (0.54).

Discussion: The high resveratrol synthesis of the interspecific hybrid Castor and the low one of *V. vinifera* cv. Bacchus confirms evidences already given (Stein 1984; Bavaresco 1993).

Castor did react to the different fertilizer supplies, changing in a significant way the leaf concentration of resveratrol, while Bacchus did react to a lower extent.

Table

Effect of fertilizer doses on resveratrol synthesis and N and K concentration of the leaves. Doses (N + K_2O, g/pot): A 0.8 + 1.0; B 3.2 + 1.0; C 0.8 + 4.0; D 3.2 + 4.0)

		Resveratrol (s.u.)	N %	K %
Castor	A	18.100	2.67	1.12
	B	13.470	2.86	0.91
	C	20.940	2.46	1.35
	D	13.650	2.67	1.50
Bacchus	A	5.540	2.84	1.48
	B	5.800	2.82	1.45
	C	5.920	2.63	1.83
	D	2.150	3.10	1.86
LSD 0.05		2.770	0.22	0.23

A: 0.8 g N/pot + 1 g K_2O/pot B: 3.2 g N/pot + 1 g K_2O/pot
C: 0.8 g N/pot + 4 g K_2O/pot D: 3.2 g N/pot + 4 g K_2O/pot

Increased nitrogen rates, supplied either with low or with high potassium doses, decreased resveratrol synthesis of Castor; this depressing effect of N on resveratrol synthesis, pointed out also by the negative correlation between the two parameters, was already observed in previous trials (BAVARESCO and EIBACH 1987).

According to MARSCHNER (1986), disease resistance increases in response to K, up to the optimal K level for growth; beyond this level no further increase in resistance can be achieved. This assertion can fit also with resveratrol, if it is considered a phytoalexin, and in a previous trial (BAVARESCO 1993) increased potassium rates, under the same basic nutrient supply, did enhance resveratrol synthesis in the earlier shoot growth stage. In the present work Castor did benefit from potassium supply only when it was added to a low nitrogen rate; the effect of combined doses of N and K was first studied by LAST (1962) in barley infected by powdery mildew and the author found a little decreasing of the infection when potassium was added to nitrogen. The interesting thing to emphasize is that high potassium rates do not balance the negative effects of high nitrogen supplies on resveratrol synthesis, and this finding is better stressed in the case of Bacchus.

The range of resveratrol synthesis in Castor was related to weak shiftings in the N leaf concentration. In the case of Bacchus the resveratrol synthesis corresponding to treatments A, B and C did not change because no substantial variation of leaf N occurred; when the leaf N concentration did increase in a significant way (treatment D), the resveratrol synthesis was more than halved. The hybrid anyway seems to be more reactive than Bacchus to variations of the leaf N concentration.

The lack of a significant effect of treatment B on the N leaf concentration of Castor and Bacchus could be due to intenser shoot growth (dilution effect).

The negative correlation between resveratrol and P could mean that the element depressed disease resistance, even though the variation in the P leaf concentration is not due to different P fertilizer rates; the biological meaning of this correlation, as well as the one with Zn, is anyhow not well established.

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