

In vitro method to screen grapevine genotypes for tolerance to lime-induced chlorosis

by

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Summary: Herbaceous nodes of four grapevine genotypes with different tolerance to lime-induced chlorosis (*V. berlandieri* x *V. rupestris* 140 Ru; *V. berlandieri* x *V. riparia* SO 4; *V. riparia* MICH. Gloire de Montpellier; *V. vinifera* L. cv. Chardonnay) were cultured *in vitro*. The effects of three levels of FeNaEDTA (5, 15, 30 mg/l) and of four mixtures of iron and bicarbonate in the MS medium were compared and chlorosis rating, ferrous iron content of the leaves and fresh weight of the plantlets were assayed. The chlorosis rating of the tested genotypes ranked according to their known degree of tolerance/susceptibility to lime-induced chlorosis.

Key words: chlorosis, *Vitis*, *in vitro* culture.

Introduction

Soil bicarbonate concentration seems to be the most important factor affecting the occurrence of lime-induced chlorosis in grapevine (JUSTE *et al.* 1967; MENGEL *et al.* 1984; KOLESCH *et al.* 1987). Tolerant rootstocks (Fercal, 41 B, 140 Ru, 333 EM, etc.) are now available for the vine-growers of the many calcareous areas world-wide (POUGET 1980), but the ideal rootstock has not yet been obtained although a lot of selection methods have been tested; for literature see JESSEN *et al.* (1988) and RODRIGUEZ DE CIANZIO (1991). The aim of this trial was to assess an *in vitro* method to screen genotypes, at a plantlet level, modifying the composition of the medium.

Materials and methods

The trial lasted two years with different experimental plans. During the first year, the behaviour of some genotypes cultured on media with an increasing Fe level, was studied, while during the second year the effect of combined treatments of Fe and bicarbonate was assayed.

Experiment 1: Effect of different Fe levels in the medium: Herbaceous nodes of the rootstocks *V. berlandieri* x *V. rupestris* 140 Ru, *V. berlandieri* x *V. riparia* SO 4, *V. riparia* x *V. rupestris* 101-14 and 3309 C, *V. riparia* MICH. Gloire de Montpellier (inducing a decreasing degree of chlorosis from the first one), and the *V. vinifera* cv. Chardonnay, were sampled in the field on June. Nodes from the middle part of the shoot were sterilized and cultured inside test tubes (1 node/tube) containing a medium with full strength MS salts, and the following organic components (mg/l): glycine 2; myo-inositol 200; thiamine 0.1; pyridoxine 0.5; nicotinic acid 0.5; BAP 1; sucrose 30 g/l; agar 6 g/l. The test tubes were placed inside a growth chamber (16 h light, 3500 lux, 20 ± 2 °C). After bud burst, when the shoot was about 2 cm long, the four subcultures began in order to amplify the number of plantlets. Cultures were transferred to fresh medium at 12-15 d intervals. During this time the rootstocks 101-14, 3309 C and *V. riparia* G.M. did not develop well and these genotypes were lost. After the 4th subculture, when the shoot length was about 2-3 cm, the best plantlets of each genotype were

transferred inside 4 flasks containing 8 individuals; at this stage the different treatments were applied as follows:

A) 5 mg/l FeNaEDTA; B) 15 mg/l FeNaEDTA; C) 30 mg/l FeNaEDTA. C was the normal FeNaEDTA content of the MS medium. The other compounds of the medium remained the same as before. The plantlets were cultured 60 d in these conditions, and transferred every 15 d in fresh medium. The plantlets did not develop roots, and the uptake of nutrients occurred throughout the callus. After these 60 d the following parameters were assayed: (1) average fresh weight of the plantlets; (2) percentage of dry matter of the leaves; (3) ferrous iron content of the leaves, by the method of KATYAL and SHARMA (1980), with 1-10-o-phenantroline at pH = 3; the values were expressed on the basis of both fresh and dry weight; (4) chlorosis rating, by the scale of POUGET and OTTENWAEELTER (1978): the chlorosis symptoms increase from score 0 to 5.

Experiment 2: Effect of combined rates of Fe and bicarbonate in the medium: Herbaceous nodes of *V. berlandieri* x *V. rupestris* 140 Ru, *V. berlandieri* x *V. riparia* SO 4, *V. riparia* MICH. Gloire de Montpellier, and *V. vinifera* L. cv. Chardonnay were cultured *in vitro* as described above. The treatments were as follows:

A) 5 mg/l FeNaEDTA + 300 mg/l NaHCO₃ (pH 7.4); B) 5 mg/l FeNaEDTA + 600 mg/l NaHCO₃ (pH 7.9); C) 30 mg/l FeNaEDTA + 300 mg/l NaHCO₃ (pH 7.5); D) 30 mg/l FeNaEDTA + 600 mg/l NaHCO₃ (pH 7.8).

The trial ended at the first subculture in the new media, because an unexpected contamination occurred, and therefore only the chlorosis rating was assayed.

Results

Experiment 1 (Table): The highest shoot weight of 140 Ru corresponded to the highest Fe level in the medium, while the highest shoot weight of SO 4 and Chardonnay occurred with treatment B (15 mg/l); the differences, anyway, were not statistically significant.

The percentage of dry matter of the leaves enhanced by increasing the Fe level in the medium in the case of 140 Ru and SO 4, but not for Chardonnay.

The ferrous iron content in the leaves was affected in a positive way by the increasing levels of Fe in the medium. Under the same amount of FeNaEDTA, considering treatment A, the Fe uptake of 140 Ru was the highest (8.9 µg/g FW and 51 µg/g DW), followed by Chardonnay and SO 4, while with the maximum Fe level in the medium, cv. Chardonnay takes up more Fe than the other genotypes.

Table

Effect of the iron rates on some physiological parameters of the three genotypes.

	Shoot weight g	Leaf dry matter %	Fe (II) µg/g FW	Fe (II) µg/g DW	Chlorosis rating *
A	0.69	14.3	8.6	51	1.0
140 Ru B	0.53	17.7	10.1	57	0.6
C	0.75	18.3	12.8	70	0.3
A	0.60	18.2	4.5	24	1.5
SO4 B	0.62	20.4	12.7	62	0.6
C	0.34	25.9	16.4	64	0.3
A	0.38	19.9	6.6	33	1.7
Chardon. B	0.64	18.8	9.9	52	0.8
C	0.45	21.4	16.2	77	0.3
LSD 0.05	N.S.	---	1.05	4.3	0.77

A: treatment with 5 mg FeNaEDTA/L in the medium
B: treatment with 15 mg FeNaEDTA/L in the medium

C: treatment with 30 mg FeNaEDTA/L in the medium
*: 0: none; 5: severe

The chlorosis ratings seemed to be in good correlation with the leaf Fe contents; 140 Ru was the genotype relatively more green among the other ones cultured in the medium with the lowest Fe level.

Experiment 2 (Figure): The Fe rate affected the chlorosis score of 140 Ru and SO 4 more than the bicarbonate. These two genotypes reacted in the same way to the different media, with chlorosis rating 3 for the plantlets growing on the media with 5 mg/l FeNaEDTA, and chlorosis rating 1 for those cultured on the media with 30 mg/l FeNaEDTA. *V. riparia* G.M. behaved like a genotype more susceptible than the other ones, particularly in the media with 30 mg/l FeNaEDTA, in which the chlorosis rating was 3; also with treatment B, *V. riparia* G.M. was more chlorotic (chlorosis score 4) than the other genotypes. Chardonnay behaved like 140 Ru and SO 4, except for the treatment D which induced a light chlorosis (score 2).

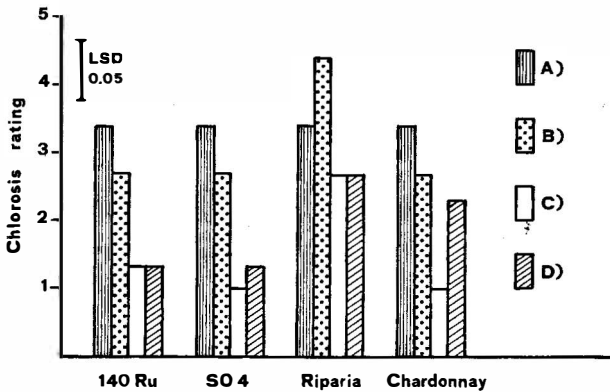


Figure: Effect of combined doses of iron and bicarbonate on the chlorosis rating of the tested genotypes. A) 5 mg/l FeNaEDTA + 300 mg/l NaHCO₃, B) 5 mg/l FeNaEDTA + 600 mg/l NaHCO₃; C) 30 mg/l FeNaEDTA + 300 mg/l NaHCO₃, D) 30 mg/l FeNaEDTA + 600 mg/l NaHCO₃.

Discussion

The different concentrations of iron in the media of experiment 1 were effective in screening the tested genotypes. Unfortunately those most susceptible to chlorosis were lost during the culture, anyway, the left ones behaved according to their known degree of tolerance. 140 Ru rootstock was able to take up the highest amount of iron under stress conditions, namely when it was cultured in the medium with 5 mg/l FeNaEDTA, like reported by BAVARESCO *et al.* (1991) in a trial with excised roots. This resistant rootstock did not show any chlorotic symptom, while SO 4, which is less tolerant than 140 Ru, was a little chlorotic when it was cultured in the medium with the lowest iron level. By increasing the iron level of the medium, the iron uptake was higher in the case of SO 4. The highest iron rate, anyway, flattened the response of the genotypes in terms of chlorosis symptoms, since all plants were green. The behaviour of Chardonnay was similar to the one of SO 4.

In experiment 2 the response of 140 Ru and SO 4 rootstocks was more affected by the iron rate than by the bicarbonate, while in the case of *V. riparia* the effect of the bicarbonate was more evident. Chardonnay reacted to both iron and bicarbonate rates. The response of the genotypes (particularly *V. riparia* G.M.) to the bicarbonate levels in the media, confirms the findings of many authors for the bicarbonate to be the most important factor for chlorosis occurrence in grapevine. It is interesting to point out that the susceptible genotype got chlorotic *in vitro* conditions, while in a previous pot trial with calcareous soil the ungrafted susceptible rootstock did not show any chlorosis (BAVARESCO 1990); even CHADMI and BRANCHARD (1986) did not observe chlorosis with 3309 C cultured *in vitro* on a medium with 10 meq/l bicarbonate.

NETZER *et al.* (1991), on the other hand, observed a good correlation between the response of grapevine anthers of different genotypes, cultured in media with increasing KHCO_3 levels, and the known degree of chlorosis resistance of the whole plant.

The results obtained emphasize the suitability of *in vitro* culture to test the tolerance/susceptibility to chlorosis of grapevine genotypes.

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