Vitis 31, 163—168 (1992)

Effect of root infection with *Pseudomonas fluorescens* and *Glomus mosseae* in improving Fe-efficiency of grapevine ungrafted rootstocks[1])

by

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Effetto dell'infezione radicale con *Pseudomonas fluorescens* e *Glomus mosseae* sulla nutrizione ferrica di portinnesti di vite non innestati

Riassunto: Talee legnose di tre genotipi portinnesto con resistenza decrescente alla clorosi da calcare (*V. berlandieri* × *V. rupestris* 140 Ru; *V. berlandieri* × *V. riparia* SO 4; *V. riparia* × *V. rupestris* 101-14) sono state coltivate in vaso su un terreno calcareo non sterilizzato, dopo aver infettato le radici con il battere *Pseudomonas fluorescens* ed il fungo micorrizico *Glomus mosseae*. L'obiettivo della ricerca era quello di controllare l'effetto di questi organismi sul manifestarsi del fenomeno clorotico, valutato mediante la misura del contenuto fogliare di clorofilla e di ferro ferroso. I risultati principali possono essere cosi sintetizzati: (i) i trattamenti con il battere ed il fungo micorrizico hanno aumentato il tenore fogliare di ferro ferroso e di clorofilla nel genotipo più sensibile alla clorosi da calcare (101-14); (ii) la percentuale di infezione micorrizica é risultata correlata positivamente con le concentrazioni fogliari di N, P, Mn e Cu.

Key words: Chlorosis, grapevine, iron, Pseudomonas, VAM fungi.

Introduction

Grapevine belongs to the fruit tree species susceptible to lime-induced chlorosis, usually recognized by yellowed interveinal areas in new leaves. The most important factor responsible for the occurrence of chlorosis is the high bicarbonate concentration in the soil solution (MENGEL *et al.* 1984; KOLESCH *et al.* 1987) related to high pH values (JUSTE *et al.* 1967) causing a low solubility of soil iron minerals. The formation of soluble organic chelates is important for the iron supply of plants growing in these soils. Organic chelates include decomposition products of organic matter, as humic and fulvic acids, organic acids as citrate and malate and siderophores produced by living organisms (REID *et al.* 1986). Siderophores are low molecular weight iron(III) chelating agents produced by different organisms under iron deficiency conditions to supply iron to the cell (NEILANDS 1981). Plant growth-promoting activity of fluorescent pseudomonads, especially of *P. fluorescens*, is well documented (KLOEPPER and SCHROTH 1981). *Pseudomonas* spp. produce hydroxamate siderophores and are able to enhance plant growth (BAKKER *et al.* 1988). This can be explained by their iron chelating capability, making iron less available for pathogenic microorganisms in the rhizosphere (KLOEPPER *et al.* 1980) and by competition for utilization of siderophore iron by microbes and plant roots (JURKEVITCH *et al.* 1986; CROWLEY *et al.* 1991). Vesicular-arbuscular mycorrhizal (VAM) fungi improve plant growth by increasing the uptake of nutrients, especially phosphorus, in nutrient-poor soil (ABBOT and ROBSON 1982). *Vitis* roots are commonly infected by VAM fungi (POSSINGHAM and GROOT OBBINK 1971; SCHUBERT and CRAVERO 1985). Evidences have been obtained on the production of siderophores also by endo-

[1]) Research supported by C.N.R. (Italian Research Council)

mycorrhizae (CRESS *et al.* 1986) enhancing iron uptake in the plants particulary under calcareous conditions. Even if the best way to solve the chlorosis problem in viticulture is the choice of tolerant rootstocks (BAVARESCO *et al.* 1991), it is interesting to test the role of some siderophore producing organisms on chlorosis in ungrafted rootstocks with different degrees of chlorosis resistance.

Material and methods

Soil and plant material. One-year-old grapevine cuttings (about 10 cm long) rooted in sand, were grown in pot in a calcareous soil. Before adding basic nutrients the main soil characteristics were: texture silty-loam; pH 8.2; carbonates 70 %; Olsen P_2O_5 14 ppm, iron (extracted with NH_4OAc 0.5 N + EDTA 20 mM at pH 4.65) 96 ppm. The soil was utilized just one day after the sampling in the field. The genotypes tested were three ungrafted rootstocks, *V. berlandieri* × *V. rupestris* 140 Ru; *V. berlandieri* × *V. riparia* SO 4; *V. riparia* × *V. rupestris* 101-14, with a decreasing resistance to lime-induced chlorosis starting from the first genotype.

The trial considered three treatments: untreated plants (control), root inoculation with *P. fluorescens* EM4 RS, root inoculation with *G. mosseae* inoculum. The soil was not sterilized before the trial and was maintained at field capacity by drip irrigation. Grapevine plants were grown in three liter pots with ten repetitions for each treatment.

Preparation of the bacterial inoculum. The bacterial strain (EM4 RS) of *P. fluorescens* used in this study was isolated from maize, and a spontaneous rifampicin and streptomycin resistant mutant was used (POLINELLI *et al.* 1991). The strain was grown on nutrient broth medium (VIVADER 1967) at 28 °C for 48 h to a density of about 10^9 CFU/ml. The plants to be inoculated were dipped in the bacterial suspension before transplantation.

Preparation of the mycorrhizal inoculum. The stock culture was prepared by mixing one part of soil, one part of sand and one part of peat, the pH corrected to 6.8 with $CaCO_3$ and inoculation with isolated spores of *G. mosseae*. Spores, mycelium and infected root fragments from stock cultures on tomato were used as inocula adding 20 g of stock culture for each pot underneath the root apparatus.

Leaf sampling. 80 d after the potting, the 4th and the 5th leaf (beginning from the shoot tip) were collected, washed in 1 % NaOCl solution and analyzed as follows.

Iron determination. Fe(II) was determined as µg/g (ppm) dry and fresh weight, according to KATYAL and SHARMA (1980).

Chlorophyll determination. Chlorophyll a, b and total chlorophyll was extracted from leaf discs with acetone 80 % (TORRECILLAS *et al.* 1984).

Mineral element analyses. Macronutrients (N, P, K, Ca and Mg) and trace elements (Fe, Mn, Cu, Zn and B) were determined, after wet destruction of the dry matter, by flame photometry (K and Ca), atomic absorption spectrometry (Mg, Fe, Mn, Cu, Zn) or colorimetry (total N, P, B).

Mycorrhizal infection. At the end of the annual growing cycle the roots of five plants per treatment were weighed and the VA mycorrhizal infection (%) was measured by the slide length method (GIOVANNETTI and MOSSE 1980), after staining the roots with 0.05 % trypan blue in lactophenol.

Statistical methods. The statistical plan provided for two-way-ANOVA with interaction; the means were compared by using the LSD test at the level of 5 %. Percent mycorrhizal colonization data were submitted to angular transformation.

Results

The shoot length is affected by the genotype, but not by the treatment (Tab. 1); SO 4 rootstock has the highest shoot length, followed by 140 Ru and 101-14. The leaf Fe(II) content, both on the dry and fresh basis, is affected signficantly by the treatment and genotype. The highest values occur in the most susceptible rootstock (101-14),

Table 1

Range of some physiological parameters depending on the genotype (average values of the 3 treatments) and the treatment (average values of 3 genotypes).

Valori di alcuni parametri fisiologici, in funzione del genotipo e del trattamento.

	Genotype				Treatment			
	140 Ru	SO4	101-14	LSD 0.05	*Pseudomonas*	*G. mosseae*	Control	LSD 0.05
Shoot length (cm)	65	83	46	13	71	58	66	N.S.
Fe^{2+} (µg/g leaf dry wt)	58	60	81	2	71	66	62	2
Fe^{2+} (µg/g leaf fresh wt)	15.8	17.1	17.4	0.4	17.4	16.8	16.0	0.4
Tot. chl. (mg/100 g leaf dry wt)	569	547	638	19	582	599	574	19
Tot. chl. (mg/g leaf fresh wt)	1.55	1.63	1.33	0.05	1.48	1.51	1.52	N.S.
Chl. a/Chl. b	2.13	2.07	1.92	0.04	2.03	2.09	2.01	0.04
Leaf dry matter (%)	27.4	29.5	21.1	0.4	25.4	26.2	26.3	0.4
Root fresh wt (g/plant)	10.8	8.9	3.9	2.3	10.2	7.8	5.6	2.3
VA mycorrhizal infection (%)	42	59	34	2	42	51	41	2

while the lowest one in the most tolerant (140 Ru). Both the bacterial and mycorrhizal inoculations have positive effects on iron uptake by the plant. This is particularly evident in the genotype 101-14 (Tab. 2). On the basis of the dry weight the rootstock 101-14 has the highest chlorophyll content, SO 4 the lowest one (Tab. 1), but considering the fresh weight the rank is the opposite. No visual differences among the treatments occur; nevertheless mycorrhizal treatment increases the chlorophyll content (on the dry weight basis): this is evident for 140 Ru and 101-14 (Tab. 2). The differences among the leaf dry matter (%) of the genotypes are wide (Tab. 1), with the rootstock 101-14 at a very low percentage compared to the other genotypes. The root fresh weight of genotypes 140 Ru and SO 4 is twice as high compared to 101-14, and both treatments significantly increase this value. The root fresh weight is particularly enhanced in the genotype 140 Ru with bacterial inoculum (Tab. 2). SO 4 is more responsive to the VAM inoculum. The VAM infection is significantly higher, over the control, for the genotypes 140 Ru and SO 4, but not for 101-14.

Significant positive correlations have been calculated between the percentage of mycorrhizal infection and N, P, Mn and Cu leaf content, on the fresh weight basis (Tab. 3).

Table 2

Range of some physiological parameters: interaction genotype × treatment; C. = Control (Untreated cuttings); P.f. = *P. fluorescens*; G.m. = *G. mosseae*

Valori di alcuni parametri fisiologici: interazione genotipo × trattamento

		Fe^{++} µg/g dry wt	Fe^{++} µg/g fresh wt	Chl. tot. mg/100 g dry wt	Chl. tot. mg/g fresh wt	Chl a / Chl b	Leaf dry matter %	Root fresh wt g/plant	VAM Infection %
140 Ru	C.	51	14.6	485	1.45	2.12	29.2	5.2	38
140 Ru	P.f.	60	16.1	560	1.50	2.12	26.8	16.7	37
140 Ru	G.m.	63	16.7	661	1.69	2.13	26.1	10.4	51
SO4	C.	63	17.2	665	1.92	1.99	28.1	7.0	52
SO4	P.f.	65	18.1	550	1.57	2.06	28.3	9.6	58
SO4	G.m.	51	16.0	424	1.40	2.17	32.0	10.2	67
101—14	C.	73	16.3	571	1.19	1.91	21.6	4.6	33
101—14	P.f.	87	18.1	634	1.36	1.89	21.1	4.3	33
101—14	G.m.	85	17.7	711	1.44	1.96	20.5	2.7	36
LSD 0.05		2.8	0.7	34	0.08	0.06	1.0	3.9	7

Table 3

Correlation coefficients ('r') between the percentage of mycorrhizal colonization (% VAM) and mineral elements of the leaves, expressed on the basis of the fresh weight

Coefficienti di correlazione tra l'infezione micorrizica (% VAM) ed il contenuto di elementi minerali nelle foglie, espresso sul peso fresco

	N %	P %	K %	Ca %	Mg %	Fe^{2+} ppm	Fe ppm	Mn ppm	Cu ppm	Zn ppm	B ppm	K/Ca	P/Fe	Fe/Mn
% VAM	+0.81 *	+0.92 **	N.S.	N.S.	N.S.	N.S.	N.S.	+0.92 **	+0.89 *	N.S.	N.S.	N.S.	N.S.	N.S.

*: significant for $p \leq 0.05$; **: significant for $p \leq 0.01$; N.S.: not significant

Discussion

The genetic variability of two leaf parameters involved in lime-induced chlorosis, namely ferrous iron and chlorophyll content, is very high. Rootstock 101-14, that commonly develops chlorosis symptoms in calcareous soils, shows no visual symptom when ungrafted, although the chlorophyll content, related to fresh weight, is the lowest. This behaviour, already observed by POUGET and JUSTE (1972) and BAVARESCO (1990), could be explained by the different iron requirement of ungrafted and grafted plants. Despite the lack of visual chlorosis symptoms, rootstock 101-14 differs from the other two (more tolerant to lime-induced chlorosis) having the lowest percentage of leaf dry matter, shoot growth, root fresh weight and VA mycorrhizal infection. This genotype does not increase its natural mycorrhizal colonization when it is treated by *Glomus mosseae*.

The Fe(II) enhancement in the plant infected by *Pseudomonas* emphasizes the role of this bacterium in iron uptake. Evidence has been obtained on iron uptake from *P. putida* pseudobactin by dicots, involving a reductive mechanism (BAR-NESS *et al.*

1991). As reported by JURKEVITCH *et al.* (1986) bacterial infection increases chlorophyll content, but only in 2 genotypes (140 Ru and 101-14), while it does not affect chl a/chl b ratio. Interesting is the positive effect of the *Pseudomonas* root inoculation on the root weight, observed also by BAKKER *et al.* (1988), even if there is a genetic control of the response. The treatment with *Glomus mosseae*, which increases the natural VAM infection level of the genotypes tested, except for 101-14, enhances the ferrous iron content in 140 Ru and 101-14, but not in SO 4, where it decreases. The same behaviour is observed for the chlorophyll content. Rootstock SO 4 benefits from VAM treatment only for the percentage of dry matter.

A positive effect of VAM fungi on iron uptake was observed by CRESS *et al.* (1986) with a test on excised roots, and among the fungal species *G. mosseae* was the best, providing for a large pool of siderophores. This high iron efficiency' of *G. mosseae* could explain the enhancement of ferrous iron and chlorophyll of the genotype 101-14 after the treatment, even if treatment caused no difference in root colonization.

Positive correlations between the percentage of mycorrhizal infection and some mineral elements like N, P, Cu (but not Mn) were observed by others (GEBBING *et al.* 1977; LAMBERT *et al.* 1979; AMES and BETHLENFALVAY 1987) who related the mineral content to dry matter. In this study the fresh matter provided a more effective basis for expressing nutrient concentration in leaf tissue, as suggested by LEIGH (1989) for potassium.

Summary

Woody cuttings from three ungrafted rootstocks, with decreasing resistance to lime-induced chlorosis (*V. berlandieri* × *V. rupestris* 140 Ru; *V. berlandieri* × *V. riparia* SO 4; *V. riparia* × *V. rupestris* 101-14), were potted in unsterile calcareous soil with *Pseudomonas fluorescens* cells and *Glomus mosseae* inoculum to test the effects of these organisms on some physiological parameters involved in chlorosis occurrence. The most significant findings are: (i) *P. fluorescens* and *G. mosseae* treatments increase ferrous iron and chlorophyll leaf content in the rootstock more susceptible to lime-induced chlorosis (101-14); (ii) increased mycorrhizal colonization, over the control, enhances N, P, Mn and Cu concentration in leaf fresh matter.

Acknowledgements

We thank Mr. GIUSEPPE BRUZZI (laboratory staff) for his contribution to this project.

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Received, 2. 1. 1992

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