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Iron absorption by excised grapevine roots

by

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Absorption du fer par les racines coupées de la vigne

Résumé. — Des racines coupées de *Vitis vinifera* cv. Verduzzo et des porte-greffes V. *berlandieri* × V. *riparia* 420 A et V. *berlandieri* × V. *rupestris* 1103 P. ont été étudiées quant au fer absorbé de solutions de FeEDTA. La vitesse d'absorption diminue en fonction du temps. A 0—2 °C l'absorption est inhibée seulement de 50—60 %. Le BPDS, chélant spécifique du Fe^{2+}, inhibe presque complètement l'absorption, ce qui démontre que la réduction $Fe^{3+} \rightarrow Fe^{2+}$ est nécessaire à 0—2 °C ainsi qu'à 30 °C. Lorsque le pH passe de 5 à 8 la vitesse d'absorption se redouble pour le cv. Verduzzo, elle augmente d'à peu près cinq fois pour le 1103 P. et d'environ dix fois pour le 420 A. Les constantes cinétiques apparentes de l'absorption sont aussi très différentes pour les trois vignes: V_{max} et K_m sont toutes les deux de l'ordre de 1103 P. > Verduzzo > 420 A.

On discute la présence d'une part remarquable de l'absorption qui n'est pas échangeable et qui apparemment ne dépend pas du métabolisme, ainsi que la possibilité de caractériser les cultivars et les porte-greffes suivant leurs besoins en substances nutritives minérales au moyen de mesurages de l'absorption des ions.

Introduction

Vegetative and productive alterations shown by grapevines can be caused by nutritional disorders often due to rootstock characteristics. Difficulties to carry out exact and timely diagnoses arise from a number of possible causes such as unsuitable choice of rootstock, of fertilizers or cultivation technique, as well as from the diversified aptitude of different rootstock-scion combinations to adapt to the pedoclimatic environment.

A great deal of work has been done to characterize rootstock behavior in relation to both scions and soils. However, from the biochemical point of view very little is known about mechanisms performing and regulating nutrient absorption and translocation through the plant.

Indeed, the cytoplasmic membrane of root cells constitutes the boundary, separating plants from the environment and the site where the plant-soil relationships arise. At this level the nutritional requirements of plants together with their capacity of genetically determined selective uptake meet the supply of available soil nutrients.

Up to now, biochemical research on ion absorption has mainly examined macronutrient uptake by roots of annual plants, while less attention has been devoted to trace elements (Epstein 1976, Pitman 1976).

Furthermore, as far as woody plants are concerned, the lack of physiologically suitable and genetically homogeneous plant material limited the application and development of biochemical analysis of nutrient uptake.

For grapevine these difficulties have been overcome (Maggioni 1979 a) and it has been possible to tackle the study of the uptake apparatuses to characterize mecha-

nisms of nutrient absorption with special attention to micro-nutritional deficiencies representing a conspicuous part of vegetative and productive alterations.

It has been shown that iron uptake by soybean roots requires an obligatory reduction to the divalent form either by a redox system bound to plasma membrane or through the action of reducing substances released by roots (CHANEY *et al.* 1972, BROWN and AMBLER 1974). Iron precipitation at root periphery without corresponding uptake and translocation is the major component of iron absorption by 15 to 18 d old barley roots (CLARKSON and SANDERSON 1978). On the other hand, 3 to 6 d old oat roots show that uptake capacity decreases with age and is affected by inhibitors of electron flow (MAGGIONI 1979 c). The present work considers the effect of temperature, pH and iron concentration on iron uptake and shows that excised roots of different grapevine species markedly vary in their ability to absorb iron in that they are differently affected by the parameters taken into account.

Materials and methods

Roots were obtained by the already described method (MAGGIONI 1979 a). Briefly, single-node woody cuttings of grapevine *Vitis vinifera* cv. Verduzzo friulano and of the rootstocks *V. berlandieri* × *V. riparia* 420 A and *V. berlandieri* × *V. rupestris* 1103 PAULSEN (clone I.S.V. 1), 8 to 10 cm long were rinsed in running water for 24 h and the bases dipped into a 5000 ppm solution of indole-3-butyric acid for 5 s.

Cuttings were inserted into a close mesh net taut on the rim of a 5 l beaker containing 3 l of distilled water vigorously aerated by blowing compressed air through a porous set so that air between water and net was constantly humidity saturated. The containers were placed in a climatic room at 25 ± 2 °C and 95 % relative humidity in the dark to avoid interferences by photosynthesis. After an average 16 d period for Verduzzo, about 30 d for 1103 P. and 44 d for 420 A the first roots appeared.

Uptake trials were performed by using roots 7—10 cm long from which the apical 1 cm sections were removed and the next 4 cm sections were used in groups of four for each replicate.

The uptake solutions contained radioactive FeEDTA (usually 0.2—0.4 μCi/ml) prepared by adding EDTA (ethylenediamine-tetraacetic acid) to $^{59}FeCl_3$ in HCl (Radiochemical Center, Amersham), then neutralized with solid Tris (tris-(hydroxy methyl)aminomethane); they also contained 1 mM $CaSO_4$ and were buffered with 1 mM Tris-MES at desired pH (MES: 2(N-morpholino)ethane sulfonic acid). During the experiments they were stirred and oxigenated by bubbling air. Temperature was controlled by operating in a Dubnoff water bath. After the preset time in the labelled solution roots were rapidly rinsed and iron uptake was evaluated after an exchange, intended to remove passively absorbed iron, carried out for 30 min with ice-cold non-radioactive 20 mM FeNaEDTA solution containing 1 mM $CaSO_4$ buffered with 1 mM Tris-MES.

Root sections were weighted, placed into the vials and radioactivity was measured with a Packard 2425 spectrometer after oxidation of the organic matter with conc. H_2O_2 at 80 °C and addition of 10 ml of scintillation fluid, prepared as previously reported (MAGGIONI 1979 a).

Concentrations were chosen in the order of magnitude of iron available in soils (OLSON 1965) and close to levels causing iron chlorosis in annual plants (CHRIST 1974).

Oxigen consumption by roots was measured with an O_2 electrode (YSI 53).

Values shown in the figures are the means of two experiments; each experiment included at least three replicates.

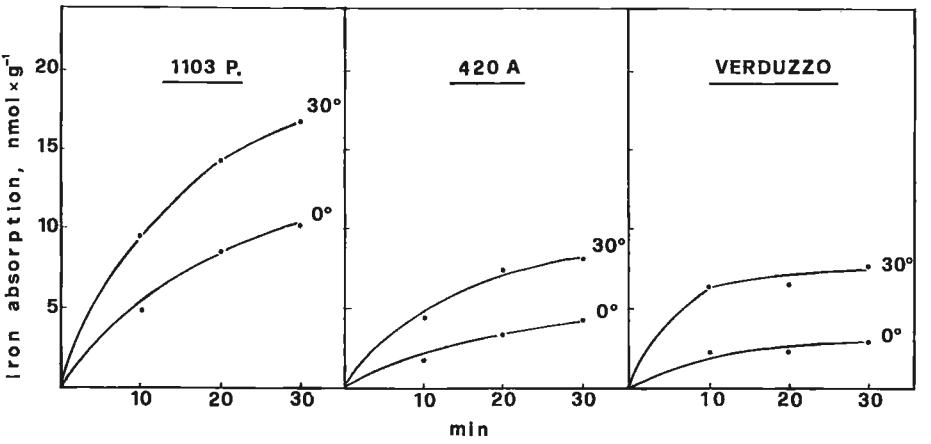

Fig. 1: Time-course of iron absorption at 30 and 0 °C from 10 μM FeEDTA solutions at pH 5.5 by roots excised from cuttings of 1103 P., 420 A and Verduzzo.

Absorption du fer en fonction du temps à 30 et 0 °C des solutions de FeEDTA 10 μM à pH 5,5 par les racines coupées de boutures de 1103 P., 420 A et Verduzzo.

Results

The time-course of iron absorption from 10 μM FeEDTA solutions by grapevine roots at pH 5,5 is shown in Fig. 1. The uptake rate decreased with time. For the rootstocks it was rather constant for about 20 min, then it decreased. For the Verduzzo roots, the higher uptake rate noticed during the first 10 min was followed by a much lower one in the subsequent time. Despite the different initial rate, the quantities of iron taken up by Verduzzo and 420 A within 30 min were almost the same, whereas the quantity absorbed within 30 min by 1103 P. was about twice as much.

As temperature decreased from 30 °C to 0—2 °C all the three grapevines reduced the quantity of iron absorbed. After 30 min uptake this inhibition varied from 40 % for 1103 P. to 50 % for 420 A and to 60 % for Verduzzo.

In order to compare these inhibitions to those of better known plant roots, the same experiment was carried out with oat roots. It resulted that iron uptake by oat roots at low temperature was inhibited by 90—95 %. Furthermore, oxygen consumption was measured at both temperatures to evaluate the metabolic activity of root tissues. Respiration was inhibited by more than 90 % for both root types as a consequence of lowering temperature.

The problem of the valence form of iron taken up (Fe^{3+} or Fe^{2+}) was studied by evaluating the effect of the chelating agent BPDS (batho-phenanthroline sulfonate) known to bind Fe^{2+} strongly while it displays much lower affinity for Fe^{3+}. Therefore, it inhibits uptake of iron when this is absorbed as Fe^{2+} (Chaney *et al.* 1972). When BPDS was used at concentrations up to 1 mM, iron uptake by roots of the three types of grapevine was reduced to 1—4 nmol $\times$ g^{-1} $\times$ h^{-1}, inhibition at 30 °C being as high as 92 % (Table 1). Similar results were found by operating at 0—2 °C. Therefore, iron must go through a reduction process from Fe^{3+} to Fe^{2+} to be absorbed by grapevine roots, no matter how temperature changes.

The effect of pH was measured by varying the pH of the uptake solutions from 5 to 8. Fig. 2 shows that absorption increased as the pH increased but not in the same way for the three grapevines. 1103 P. increased 4—5 fold its already high absorption rate reaching about 200 nmol × g^{-1} × h^{-1}. 420 A gave the lowest values at pH 5 and 6 but at higher pH its rate increased rapidly to over 100 nmol × g^{-1} × h^{-1}. Ver-

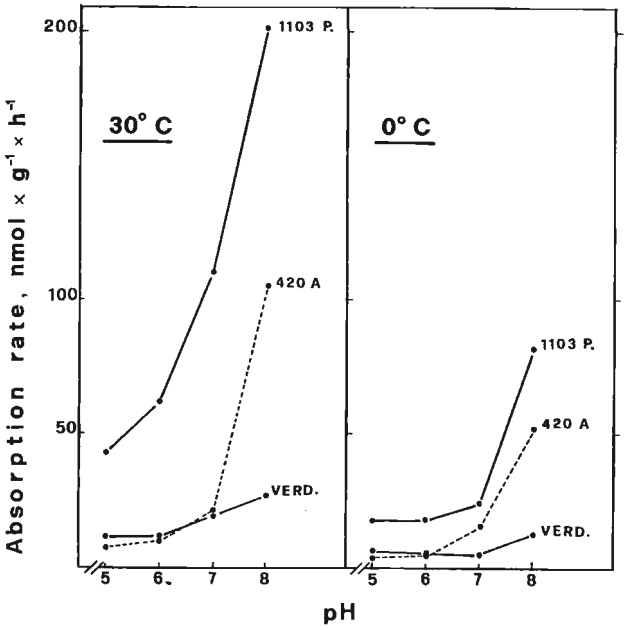

Fig. 2: The influence of pH on the rates of iron absorption from 10 μM FeEDTA solutions at 30 and 0 °C by roots excised from cuttings of the three grapevines studied.
Effet du pH sur la vitesse d'absorption de fer des solutions 10 μM de FeEDTA à 30 et 0 °C par les racines coupées de boutures des trois espèces de vigne étudiées.

Table 1

The influence of 1 mM BPDS on the absorption rate of iron from 10 μM FeEDTA solutions at 30 and 0 °C, pH 5.5, by excised grapevine roots

Effet du BPDS 1 mM sur la vitesse d'absorption du fer de solutions 10 μM à 30 et 0 °C, pH 5,5. par des racines coupées de boutures de vigne

	Temperature (°C)	Iron absorption rate (nmol × g^{-1} × h^{-1}) Control	1 mM BPDS	Inhibition (%)
1103 P.	30	51.4	4.3	91.7
	0	34.1	3.0	87.4
420 A	30	19.4	2.0	89.6
	0	5.0	0.9	81.7
Verduzzo	30	14.3	2.7	80.9
	0	4.4	1.0	76.7

Table 2

Kinetic constants of iron absorption at 30 °C and pH 5.5 by excised grapevine roots

Constantes cinétiques de l'absorption fer à 30 °C et pH 5,5 par des racines coupées de vigne

	V_{max} (nmol × g^{-1} × h^{-1})	K_m (μM)
Verduzzo	129.2	62.8
420 A	44.7	23.1
1103 P.	444.4	110.4

duzzo showed the smallest changes and its rate, even though doubled going from pH 5 to 8, did not reach more than 30 nmol × g^{-1} × h^{-1}. At 0—2 °C a similar pattern was observed though at lower levels and inhibition varied between 50 and 60 % at pH 8.

Increases of concentration of available iron were followed by increases of initial uptake rate as measured over the first 10 min period, as shown in Fig. 3. The highest values were reached by 1103 P. roots, the lowest ones by 420 A, and Verduzzo was on an intermediate position. By the double reciprocal method (Lineweaver-Burk plot)

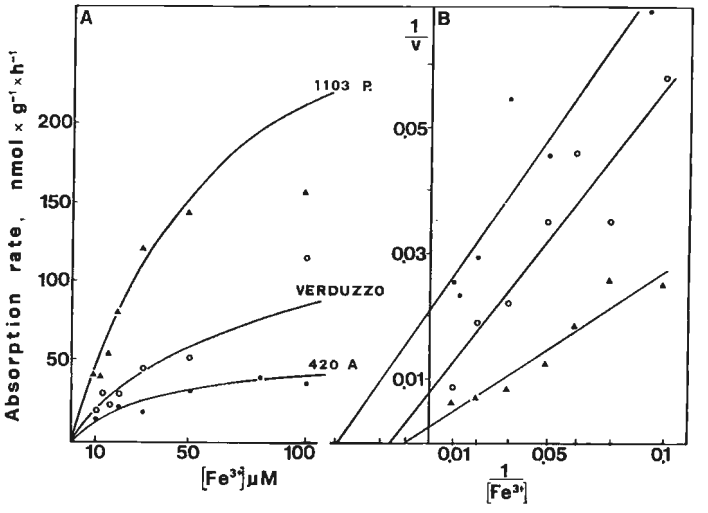

Fig. 3: A) The influence of iron concentration on the rates of absorption from FeEDTA solutions at 30 °C and pH 5.5 by roots excised from cuttings of the three grapevines studied. — B) Double reciprocal plot of data shown in A) to obtain the kinetic constants reported in Table 2.

A) Effet de la concentration du fer sur la vitesse d'absorption des solutions de FeEDTA à 30 °C et pH 5,5 par les racines coupées de boutures des trois espéces de vigne étudiées. — B) Diagramme doublement réciproque des données montrées en A), pour obtenir les constantes cinétiques présentées dans le Tableau 2.

applied to iron concentrations and uptake rates, the V_{max} and K_m of uptake for each type of grapevine root were calculated (Table 2). V_{max} of 1103 P. was 10 fold that of 420 A while Verduzzo was on intermediate values. K_m values followed a similar pattern showing that affinities for iron were in the order 420 A > Verduzzo > 1103 P.

Discussion

From these results it is possible to conclude that iron uptake by grapevine roots displays some peculiar features together with characteristics common to usually investigated plants.

First of all, an obligatory reductive step from Fe^{3+} to Fe^{2+} is shown by the effect of BPDS; therefore, Fe^{2+} and not Fe^{3+} is taken up by roots. This is in accordance with findings of CHANEY *et al.* (1972) and the general scheme outlined by BROWN (1978). Furthermore, passive phenomena of Fe^{3+} fixation or Fe^{2+} exchange could explain the rapid initial accumulation (10—20 min) followed by lower absorption rates (compare CLARKSON and SANDERSON 1978). But the effect of the Fe^{2+}-specific chelator BPDS which prevents absorption proves that Fe^{3+} fixation is not involved. Fe^{2+} exchange should be excluded since even washing periods longer than 30 min do not modify the time course of absorption.

On the other hand, decreases of uptake rates with time could indicate that saturation of an iron pool occurs quickly, the internal concentration acting as regulator of uptake rate. Such a mechanism could be similar to those proposed by GLASS (1976) for potassium uptake by barley roots, and for sulfate uptake by sulfur starved *Chlorella* cells by PASSERA and FERRARI (1975). With regard to the conspicuous absorption capacity still operating at 0—2 °C, the following considerations support the view of an active phenomenon: BPDS inhibits this low-temperature uptake showing that Fe^{2+} is involved and that the reducing activity is operating. Moreover, the time course of uptake does not show any tendency to a plateau shape as a consequence of saturation of cell wall exchange sites. Furthermore, at increasing pH uptake rates change paralleling at lower level those at 30 °C; as Fe^{3+} fixation can be excluded root tissues of grapevine species and varieties should differ markedly in cell wall structures to justify such strong differences as due to passive not exchangeable Fe^{2+} absorption. Lastly, a similar behaviour is displayed also when potassium is taken up (MAGGIONI 1979 a). On the contrary, the absence of appreciable oxygen consumption excludes that absorption can be driven by energy simultaneously supplied by respiratory metabolism and supports the hypothesis of passive absorption.

Since ordinary air (not CO_2-free) was bubbled in all the experiments the effect of the pH of the medium is not distinguishable from the effects of bicarbonate ions present at increasing concentration in the solutions as pH increases (NG and POEL 1978). However, a noticeable result is that changes of absorption rates at varying pH are very different for the three grapevines. They represent characteristics of the species and, therefore, they could be seen as indications related to the different behaviour of grapevines in soils of different pH.

Uptake efficiency evaluated in terms of velocity (V_{max}) and affinity (K_m) is also diversified considerably for the three grapevines, values varying by about one order of magnitude. It is important to note that higher V_{max} is shown by roots characterized by lower affinity, and vice versa. This result agrees with findings from other plant systems, e.g. potassium uptake by maize roots (MAGGIONI 1979 b). It could mean that plants can face nutritional shortages either by increasing absorption rates or by

increasing affinity for ions, with an option determined by the evolutive development (Crowley 1975, Clarke and Allendorf 1979). Kinetic constants can represent quantitative indexes for characterization of species and varieties in order to determine their effectiveness in absorbing ions from the soil and their capacity of adaptation to different pedo-climatic environments. The two rootstocks studied are not very different as to resistance to highly calcareous soils causing chlorosis (Pastena 1974). However, the data shown demonstrate that such a similar level of resistance is reached by different expedients. 420 A displays binding sites characterized by high affinity for iron and low uptake velocity while 1103 P. shows opposite characteristics: it aims at facing nutritional shortages by higher uptake velocity even though using lower affinity absorption sites.

These indications, even though partly conjectural since the results obtained impose further studies, suggest that a systematic survey of these parameters may be worth. They may allow a better understanding of root performances in relation to nutritional and environmental requirements.

Summary

Excised roots from cuttings of *Vitis vinifera* cv. Verduzzo and of two rootstocks *V. berlandieri* × *V. riparia* 420 A and *V. berlandieri* × *V. rupestris* 1103 P. were tested for iron absorbed from FeEDTA solutions.

Absorption rates decreased with time. At 0—2 °C iron absorption was only 50—60 % inhibited. BPDS, a specific chelator for Fe^{2+}, inhibited almost completely absorption showing that reduction from Fe^{3+} to Fe^{2+} was needed both at 30 °C and at 0—2 °C. As pH increased from 5 to 8 absorption rate doubled for the cv. Verduzzo, increased about 5 times for 1103 P. and about 10 times for 420 A.

Also the apparent kinetic constants of absorption were very different in the three grapevines: both V_{max} and K_m were in the order: 1103 P. > Verduzzo > 420 A.

The presence of a conspicuous part of not exchangeable absorption apparently not dependent on metabolism and the possibility of characterizing cultivars and rootstocks as to nutritional requirements through ion absorption measurements are discussed.

Acknowledgement

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