RESPONSE MECHANISMS OF GRAPEVINE ROOTSTOCKS TO SEVERE IRON DEFICIENCY

MÉCANISMES DE RÉPONSE DE PORTE-GREFFES DE VIGNE AU SEVERE CARENCE EN FER

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SUMMARY

In many important viticultural areas of the Mediterranean basin, plants often face prolonged periods of scarce iron (Fe) availability in the soil. The main objective of the present work was to compare physiological and biochemical response mechanisms to severe Fe-deficiency in *Vitis* genotypes. A hydroponic experiment was conducted, in which three rootstocks characterized by a different susceptibility degree to Fe chlorosis have been submitted to two Fe levels. The Fe chlorosis susceptible rootstock 101-14 (*Vitis riparia x Vitis rupestris*) reacted to a prolonged Fe-deficiency reducing the root activity of phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH). Noteworthy, it accumulated high levels of citric acid in roots. In contrast, the Fe chlorosis tolerant rootstock 110 Richter (*Vitis berlandieri x Vitis rupestris*) was capable of maintaining an active metabolism of organic acids in roots, accumulating them to a lesser extent than 101-14. Similarly to 101-14, SO4 genotype (*Vitis berlandieri x Vitis riparia*) displayed a strong decrease of PEPC and MDH enzyme activities. Nevertheless it was able to avoid excessive accumulation of citric acid in roots, similarly to 110 Richter. In conclusion, root PEPC and MDH activities represent an important tool for screening Fe chlorosis tolerance. After a prolonged exposure to Fe-deficiency, the accumulation of organic acids in the roots may not represent a reliable indicator of Fe chlorosis tolerance.

RÉSUMÉ

Parmi d'importantes zones viticoles du basin méditerranéen, les plantes sont souvent confrontées à des périodes prolongées de faible disponibilité en fer (Fe) dans le sol. L'objectif principal de ce travail a été de comparer les mécanismes de réponse physiologiques et biochimiques à la sévère carence en fer dans de génotypes de *Vitis*. Une expérience hydroponique a été menée, dans lequel trois porte-greffes caractérisés par un degré de sensibilité différente à la chlorose en Fe ont été soumis à deux niveaux de Fe. Le porte-greffe 101-14 (*Vitis riparia x Vitis rupestris*), susceptible à la chlorose ferrique, a réagi à une carence en fer prolongée en réduisant l'activité racinaire de la phosphoenolpyruvate carboxilase (PEPC) et de la malate dehydrogenase (MDH). Il convient de noter la présence d'un haut niveau d'accumulation d'acide citrique dans les racines. Par contre, le porte-greffe 110 Richter (*Vitis berlandieri x Vitis rupestris*), tolérant à la chlorose ferrique, a été capable de maintenir une activité métabolique des acides organiques des racines en les accumulant à un niveau plus faible que 101-14. De façon similaire á 101-14, le genotype SO4 (*Vitis berlandieri x Vitis riparia*) a montré une forte décroissance de l'activité enzymatique de PEPC et de MDH. Néanmoins, il a été capable d'éviter une accumulation excessive d'acide citrique dans les racines, tout comme 110 Richter. En conclusion, l'activité des PEPC et MDH racinaires représentent un outil important dans la détection de la tolérance de la chlorose ferrique. Après une exposition prolongée à une carence en Fe, l'accumulation d'acides organiques dans les racines peuvent ne pas représenter un indicateur fiable de la tolérance á la chlorose en Fe.

Keywords: Iron chlorosis, phosphoenolpyruvate carboxylase, organic acids, Krebs cycle enzymes, grapevine genotypes.
Mots-clés : Chlorose ferrique, phosphoenolpyruvate carboxylase, acides organiques, cycle enzimatique de Krebs, genotype de vigne.

INTRODUCTION

In many important viticultural areas of the Mediterranean basin, characterized by soils with high concentration of active lime and alkaline pH, Fe availability for plants is extremely low due to its scarce solubility in the soil, causing Fe deficiency in susceptible genotypes. This nutritional disorder can cause a dramatic reduction of vineyard economical life cycle as well as grape yield (Bavaresco et al., 2003).

Grapevine belongs to Strategy I plants, therefore, under Fe deficiency, it is able to increase Fe reductase activity and enhance net excretion of protons and root organic compounds (eg. organic acids, phenols), lowering pH and increasing Fe solubility of the rhizosphere (Brancadoro et al., 1995; Jimenez et al., 2007). Iron chelates are usually effective, however, they are not economically and environmentally sustainable (Rombolà and Tagliavini, 2006). The use of tolerant rootstocks may represent an economical and efficient method for preventing Fe chlorosis. It is well known that the susceptibility degree to Fe chlorosis in grapevine is highly variable for different genotypes (Tagliavini and Rombolà, 2001). Studies focalized on response mechanisms to Fe deficiency in grapevine rootstocks reported that genotypes from *Vitis vinifera* and *Vitis berlandieri* display an enhanced ability to reduce Fe

at root level by Fe-chelate reductase (FCR) enzyme, and release protons into the rhizosphere under low external availability (Brancadoro et al., 1995; Covarrubias and Rombolà, 2013; Dell'Orto et al., 2000; Jimenez et al., 2007; Ksouri et al., 2006; Rombolà and Tagliavini, 2006). In addition, in various species it has been demonstrated that tolerant genotypes may increase the activity of PEPC enzyme and the concentration of organic acids (particularly citric acid) in roots (Covarrubias and Rombolà, 2013; Jimenez et al., 2007; Ollat et al., 2003; Rombolà and Tagliavini, 2006). Therefore, these parameters have been used as biochemical markers for screening Fe chlorosis tolerant genotypes (Rombolà and Tagliavini, 2006). Under field conditions, plants often face prolonged periods of scarce Fe availability, which are successfully overcome without adverse effects on leaf chlorophyll content and yield. Nevertheless, most of the experiments on grapevine have studied biochemical response mechanisms to Fe shortage during short periods (2 weeks), therefore, information concerning the behavior of grapevine genotypes under prolonged Fe deficiency is scarce. The main objective of the present work was to compare physiological and biochemical response mechanisms to a severe Fe deficiency in *Vitis* genotypes with different tolerance degree to Fe chlorosis.

MATERIALS AND METHODS

Micropropagated plants of rootstocks 101-14 (*Vitis riparia* x *Vitis rupestris*), 110R (*Vitis berlandieri* x *Vitis rupestris*) and SO4 (*Vitis berlandieri* x *Vitis riparia*) were grown in a greenhouse in 10 l plastic containers filled with 8 L of a half Hoagland nutrient solution (Covarrubias and Rombolà, 2013) with 6 plants for each container. The three grapevine genotypes were grown with Fe (+Fe; 10 µM of Fe-EDDHA; control treatments) and without Fe (-Fe). The nutrient solution was renewed twice a week, the pH was monitored daily at 9:00 am and was adjusted to 6.0 after every renewal with HCl 0.1 M.

Leaf chlorophyll content was periodically monitored on the first completely expanded leaf by the SPAD MINOLTA 502 (Osaka, Japan). When apical leaves of -Fe plants showed extremely severe Fe-chlorosis symptoms (SPAD value < 6), the experiment was concluded and the plants were divided into roots, main shoot and leaves for dry weight determinations and following analysis.

At the end of the experiment, root tip (20–30 mm long) samples were collected from each plant and kept at –80°C for enzyme activity analysis. The activity of phosphoenolpyruvate carboxylase (PEPC), malate dehydrogenase (MDH), citrate synthase (CS) and isocitrate dehydrogenase ($NADP^+$-IDH) were determined. The extraction and enzyme assays were performed as described by Jimenez et al. (2007), and references therein. Protein concentration was determinate by the Bradford method (Bradford, 1976). The organic acid concentrations in root tip samples were determined by HPLC according to Neumann (2006).

Data were analyzed by a two-way analysis of variance with SAS software (SAS Institute, Cary, NC). A factorial statistical design with two factors (Fe and genotype) and two levels of Fe (+Fe and −Fe) and three levels of genotype (101-14, 110R and SO4) were adopted.

RESULTS AND DISCUSSION

Until 14 days from treatments imposition, Fe deficiency decreased leaf chlorophyll content regardless to the genotype (Tab. I). During the following period, an interaction between Fe level and genotype was detected (Tab. I). At the end of the experiment, Fe-deficiency decreased the chlorophyll content of genotypes by 99,6% in 101-14, 92% in SO4 and 72% in 110 Richter. Interactions between genotype and Fe level were also recorded on organ biomass data (data not shown). Iron deficiency decreased the biomass of roots, shoots, leaves and total weight of plants. The highest decrease has been recorded in 101-14 genotype and the lowest one in 110 Richter (data not shown). For SO4 rootstock, the effect of Fe deficiency on dry biomass was intermediate (data not shown). Data suggest, that when plants were submitted to a prolonged Fe deficiency (32 days), 110 Richter rootstock exhibited the lowest reduction of chlorophyll content and biomass as compared to 110 Richter rootstock grown in presence of Fe in the nutrient solution, indicating the highest tolerance to severe Fe deficiency. In contrast, a dramatic reduction of chlorophyll content and biomass has been observed in 101-14 genotype. The rootstock SO4 exhibited an intermediate behavior. The prolonged Fe deprivation period resulted into severe Fe deficiency symptom even in the Fe chlorosis tolerant genotype. The degree of Fe chlorosis severity showed by genotypes is in line with tolerance levels to Fe chlorosis reported in literature (Tagliavini and Rombolà, 2001). The rootstock 110 Richter is a hybrid originated from the Fe chlorosis tolerant species *Vitis berlandieri* and the Fe chlorosis slightly susceptible species *Vitis rupestris*. In contrast, 101-14 rootstock was originated from *Vitis rupestris* and the Fe-chlorosis highly susceptible species *Vitis riparia* (Brancadoro et al., 1995; Jimenez et al., 2007; Ollat et al., 2003). The tolerance level of the originating species may also explain the intermediate Fe chlorosis symptoms

Table I. Time course of chlorophyll content (SPAD index) for three grapevine genotypes (101-14; 110 Richter; SO4) grown in a nutrient solution containing 0 µM and 10 µM of Fe-EDDHA.

Évolution dans le temps de la teneur en chlorophylle (SPAD) pour trois génotypes de vigne (101-14, 110 Richter; SO4) cultivées dans une solution nutritive contenant 0 µM et 10 µM de Fe-EDDHA.

	Days of treatment										
	7	11	14	18		21		26		29	
				+ Fe	- Fe	+ Fe	- Fe	+ Fe	- Fe	+ Fe	- Fe
Genotype (G)											
101-14	14.6	16.8	16.9	23.9	6.5	24.8	0.9	25.1	0.4	26.3	0.1
110 Richter	14.9	16.3	16.3	19.1	11.6	21.2	7.6	22.4	6.5	21.9	6.1
SO4	15.9	17.6	15.7	22.0	4.5	22.7	3.0	22.6	1.8	21.3	1.7
Significance	n.s.	n.s.	n.s.								
Iron (Fe)											
+ Fe	16.8	19.1	19.5								
- Fe	13.5	14.7	13.0								
Significance	**	**	***								
G x Fe interaction	n.s.	n.s.	n.s.	***		***		*		**	
SEM				1.14		1.02		1.60		1.55	

[a]Abbreviation and symbols: ns, *, **, *** = not significant and significant at p≤0.05, p≤0.01 and p≤0.001 levels, respectively. [b]SEM = standard error of the interaction means.

Table II. Activities (nmol mg^{-1} protein min^{-1}) of PEPC, MDH, CS, $NADP^+$-IDH enzymes and protein concentration (mg g^{-1} FW) measured in root tip extracts for three grapevine genotypes (101-14; 110 Richter; SO4) grown in a nutrient solution containing 0 µM and 10 µM of Fe-EDDHA.

Activités (nmol mg^{-1} protein min^{-1}) des enzymes PEPC, MDH, CS, NADP +-IDH et de la concentration en protéines (mg g^{-1} FW), mesurée dans des extraits d'apex de la racine de trois génotypes de vigne (101-14; 110 Richter; SO4) cultivées dans une solution nutritive contenant 0 µM et 10 µM de Fe-EDDHA.

	PEPC		MDH		CS	$NADP^+$-IDH	Protein
	+ Fe	- Fe	+ Fe	- Fe			
Genotype (G)							
101-14	10.6	3.4	367.4	233.7	7.2	4.0 a	40.0
110 Richter	3.0	3.5	252,5	283.5	6.1	0.7 c	37.3
SO4	9.8	1.9	373.2	199.7	6.0	1.7 b	38.0
Significance					n.s.	***	n.s.
Iron (Fe)							
+ Fe					6.7	1.9	38.5
- Fe					6.7	2.4	38.4
Significance					n.s.	n.s.	n.s.
G x Fe interaction	*		**		n.s.	n.s.	n.s.
SEM	1.15		37.5				

[a]Abbreviation and symbols: ns, *, ** = not significant and significant at p≤0.05 and p≤0.01 levels, respectively. Means followed by the same letter in each column were not significantly different as per the SNK test. [b]SEM = standard error of the interaction means.

exhibited by SO4 genotype, a hybrid from *Vitis*

berlandieri x Vitis riparia.

The interactions obtained in PEPC and MDH enzymes activity of roots indicate differences regarding the response to a prolonged Fe-deficiency between genotypes (Tab. II). The severe Fe deficiency did not modify the activity of PEPC and MDH in 110 Richter rootstock, whereas in 101-14 and SO4 genotypes Fe deficiency decreased these enzymes activities (Tab. II). $NADP^+$-IDH and CS enzyme activities in roots did not change according to Fe deficiency, whereas $NADP^+$-IDH showed differences between genotypes (Tab. II). The enzyme PEPC catalyzes the incorporation of bicarbonate into a C_3 organic acid, phosphoenolpyruvate (PEP), generating oxalacetate, which is converted to malate by malate dehydrogenase. In grapevine, Jimenez et al. (2007) reported an increase in roots PEPC activity in the Fe chlorosis tolerant genotype Cabernet Sauvignon submitted to a short period of Fe depletion (7 days), whereas a lower increase has been observed in the sensitive cv Gloire de Montpellier. Moreover, in the Fe chlorosis tolerant rootstock 140 Ruggeri, Fe deficiency increased the activity of root PEPC enzyme (Covarrubias and Rombolà, 2013). In our experiment, the observed reduction in the activity of PEPC and MDH enzymes in roots of 101-14 and SO4 rootstocks submitted to 32 days of Fe shortage, reveals a high susceptibility to a severe Fe deficiency in the substrate. In addition, this result may be the consequence of a general metabolic reprogramming associated to protein turnover caused by Fe deficiency (Donnini et al., 2010; Rodríguez-Celma et al., 2011). In contrast, data concerning the activity of these enzymes for 110 Richter, suggest a relatively higher tolerance to Fe deficiency. These results indicate that the duration and severity of Fe deficiency stress can modulate biochemical responses at root level and the intensity changes according to the genotype behavior.

At the end of the experiment, significant interactions between Fe level and genotype were recorded in citric and malic acids concentration in roots (Tab. III). Iron deficiency increased citric acid concentration in roots of the three genotypes. A highly pronounced accumulation has been recorded in 101-14 genotype (27-fold), followed by 110 Richter and SO4 (5-fold and 2-fold respectively) (Tab. III). Severe Fe deficiency increased the concentration of malic acid in roots of 101-14 rootstock, whereas for 110 Richter and SO4 the malic acid concentration in roots did not change.

The strong accumulation of citric acid and, at lower extent, of malic acid recorded in roots of 101-14 – Fe vines contrasts with the low activity of PEPC and MDH enzymes. It is possible that the high accumulation of citric and malic acids in roots submitted to a prolonged Fe-deficiency in 101-14 genotype, contributed to slow down the activity of PEPC and MDH due to an inhibitory effect (previously reported by Chollet et al., 1996; López-Millán et al., 2000; Wong and Davies, 1973) caused by these acids. In addition, the strong accumulation of malic and particularly of citric acid, recorded in 101-14 -Fe roots, suggests a scarce ability of this rootstock to avoid high levels of organic acids by degradation, xylem loading or exudation into the

Table III. Organic acids in root tissue (mg g^{-1} FW) determined at the end of experiment for three grapevine genotypes (101-14; 110 Richter; SO4) grown in a nutrient solution containing 0 µM and 10 µM of Fe-EDDHA.

Concentrations des acides organiques dans les tissus racinaires (mg g^{-1} FW) déterminées à la fin de l'expérience pour les trois génotypes de vigne (101-14; 110 Richter; SO4) cultivées dans une solution nutritive contenant 0 µM et 10 µM de Fe-EDDHA.

	Citrate		Malate		Ascorbate	Total	
	+ Fe	- Fe	+ Fe	- Fe		+ Fe	- Fe
Genotype (G)							
101-14	0.03	0.81	0.61	0.94	0.0067	0.65	1.76
110 Richter	0.07	0.35	0.60	0.39	0.0012	0.68	0.73
SO4	0.21	0.47	0.60	0.44	0.0004	0.81	0.91
Significance					n.s.		
Iron (Fe)							
+ Fe					0.003		
- Fe					0.003		
Significance					n.s.		
G x Fe interaction	***		*		n.s.	***	
SEM	0.045		0.110			0.114	

[a]Abbreviation and symbols: *, *** = significant at p≤0.05 and p≤0.001 levels, respectively. [b]SEM = standard error of the interaction means.

rhizosphere. In 110 Richter rootstock, the moderate increases of citric acid concentration (Tab. III) and the activity of PEPC and MDH enzymes (Tab. II) in roots of Fe-deficient plants indicate that the organic acids metabolism was still active after a prolonged Fe-shortage. The moderate accumulation of citric acid and tendentially lower level of malate induced by Fe-deficiency in roots of 110 Richter indicate a marked capability to avoid an excessive accumulation of organic acids in roots. Regarding SO4 genotype, a different behavior has been found. Similarly to 101-14 genotype, lower PEPC and MDH activity were recorded in SO4 Fe-deficient plants, clearly indicating a slowing down of the organic acids metabolism. In contrast, organic acids data suggest a high capability of SO4 rootstock to avoid high levels of organic acids in roots. These results indicate that SO4 submitted to a severe Fe-deficiency behaves as 110 Richter for certain tolerance responses to Fe chlorosis (avoiding a high accumulation of organic acids in roots), and simultaneously behaves as 101-14 displaying susceptibility responses to Fe-depletion (slowing down the activity of PEPC and MDH activity in roots). These physiological observations are in line with the intermediate level of Fe-deficiency symptoms exhibited in leaf chlorophyll content and plants biomass production as compared with 110 Richter and 101-14 genotypes. Additional physiological response mechanisms to a severe Fe-deficiency, related to the reduction capacity of roots and exudation of organic compounds for different genotypes, could contribute to explain the different Fe chlorosis tolerance of these grapevine rootstocks.

CONCLUSIONS

Data show that root PEPC and MDH enzymes activities represent an important tool for screening Fe chlorosis tolerance among genotypes. However, the high levels of organic acid accumulation recorded in 101-14 and SO4 genotypes after a severe exposure to Fe-deficiency suggest caution in using this parameter for screening Fe chlorosis tolerance, because this trait may be a symptom of the scarce capability of roots to use, transport or exudate these compounds, rather than a reliable indicator of Fe chlorosis tolerance.

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