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Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution

II. Iron-stress response mechanisms

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Abstract

The response mechanisms to Fe chlorosis have been widely studied in herbaceous plants and qualitative and quantitative differences have been found among species and among genotypes within the same species. Similar studies on woody plants are scarce. The objective of this work was to characterize specific response mechanisms in peach rootstocks with differing tolerances to Fe chlorosis in calcareous soils and to determine where acidification and reduction occur along the root system.

Plants were grown individually, in growth chamber, in flasks with 700 mL of aerated nutrient solution with or without Fe. Without Fe, the peach Nemaguard was the most chlorotic, developed the least reducing capacity and mobilized less Fe and Mn from roots to young leaves compared to the other rootstocks. The pH decrease of the nutrient solution with Fe-deficiency was significant only in the plum rootstocks. Using an agar technique, both responses, reducing capacity and acidification, were located at the subapical zones of the roots, although no swollen root tips were apparent.

Introduction

Iron-deficiency responses have been widely studied and two different strategies have been established. Plants with strategy I may respond by increasing the capacity of the root to reduce ferric chelates, by rhizosphere acidification and by excretion of phenolic compounds. These responses are located in the subapical root zone and are frequently associated with morphological changes. The strategy II occurs in grasses and involved release of phytosiderophores. The mechanisms involved in these responses and their ecological significance have been reviewed recently by Römheld (1987) and Bienfait (1988).

Most of the work on this subject has been carried out with herbaceous plants. There are very few reports, however, on woody species. Kannan (1985) showed that two papaya cultivars decreased the pH of a nutrient solution in the absence of Fe. Ao *et al.* (1985) found that apple roots under Fe stress reduced ferric chelates at a greater level than unstressed plants, and they studied the effect of several factors on this response. Varanini and Maggioni (1982) studied some characteristics of Fe reduction and uptake by grapevine roots.

In the first part of this work, six peach rootstocks with different tolerances to lime-induced Fe chlorosis were classified for their tolerance to Fe chlorosis induced by bicarbonate in nutrient solution (Romera *et al.*, 1989). In this second part the objective was to characterize these same rootstocks by their response mechanisms (reducing capacity and acidification) and by identifying the location of these responses along the root.

Materials and methods

Plant material, culture and treatments

The plant material used and the growth conditions were the same as reported in the companion paper (Romera *et al.*, 1989). Two methods of propagating the plants were used: rooted cuttings and *in vitro* culture. Three separate experiments were conducted at the same time as those reported in the companion paper. The composition of the basic nutrient solution (BNS) was in m*M*): $Ca(NO_3)_2$, 2; K_2SO_4, 0.75; $MgSO_4$, 0.65; KH_2PO_4, 0.5 and (in μM): KCl, 25; H_3BO_3, 10; $MnSO_4$, 1; $CuSO_4$, 0.5; $ZnSO_4$, 0.5; $(NH_4)_6Mo_7O_{24}$, 0.05. Two treatments were compared: −Fe (BNS, without Fe, pH 6) and Control (BNS with 10 μM FeEDDHA (ferric ethylenediaminedi (o-hydroxyphenylacetate), pH 6).

Determinations

The chlorosis score was visually determined according to a scale from 0 (green) to 5 (severe chlorosis with necrosis). The pH of the nutrient solution was daily measured. The root reducing capacity was determined in intact plants. After 30 minutes of pretreatment in the BNS without microelements at pH 6, roots were submerged in 200 mL of the BNS without microelements and with 3×10^{-4} *M* Ferrozine (3- (2-pyridyl)-5,6-bis (4-phenylsulfonic acid)-1,2,4-triazine) and 10^{-4} *M* FeEDTA (ferric ethylene diamine-tetraacetate) at pH 5. The absorbance was determined at 562 nm after 6 hours in the growth chamber. This determination was repeated for each plant several times on different days during the experiment. Once a determination was finished, plants were returned to the original nutrient solution overnight for washing and then changed to a new solution. The location of the responses was determined by the agar technique (Marschner *et al.*, 1982). Excised roots were submerged in a fluid agar medium which contained 0.75% agar, 0.006% bromocresol purple, and the BNS, pH 6, for location of pH response and 0.75% agar, 3×10^{-4} *M* Ferrozine, 10^{-4} *M* FeEDTA and the BNS without microelements at pH 5, for location of reducing capacity response. The Fe and Mn concentration was determined in fully expanded young leaves and root system, by the method of acid digetion and atomic absorption spectrophotometry. Roots were previously washed in 0.05 *N* HCl for 3 minutes.

Three plant replications were used in each treatment and analysis of variance, Duncan multiple range test and F-test were performed to analyze the results.

Results

In treatment without Fe, the peach Nemaguard developed more severe chlorosis and sooner than the other rootstocks (Table 1). Results from

Table 1. Effect of the treatment without Fe on chlorosis score (0 = no chlorosis, 5 = severe chlorosis plus necrosis), reducing capacity (nmol Fe^{2+} $g FW^{-1} h^{-1}$) and solution pH. Each value is the mean of the three plant replications

Rootstock	Chlorosis score		Reducing capacity		Minimum pH
	12 d	20 d	Control	−Fe	
Experiment 2					
Nemaguard	5.0a	5.0a	93a	61a*	6.6a
Brompton	0.1b	1.2b	46b	168b**	4.5b
San Julian A	0.6b	0.4b	47b	250bc**	4.4b
GF677	1.0b	0.5b	61b	299c**	6.2c
Experiment 3					
Adafuel	0.8a	3.3a	38a	186a**	6.2a
P. de Soto 101	0.3a	4.7a	37a	271a**	5.2b
GF677	0.2a	3.7a	38a	216a**	6.3a

Within each rootstock * ($P = 0.05$) and ** ($P = 0.01$) indicate significant differences compared to the control according to the F-test. Within each experiment, values of the same column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan multiple range test.

Exp. 1, comparing Nemaguard with the hybrid Adafuel, were similar and are not presented.

Not always the maximum reducing capacity was attained the same day, even for plant replications of the same rootstock. The same was true for the minimum pH. So, in Table 1 means of the maximum reducing capacity and minimum pH attained by each plant replication are presented, in spite of the day that these values were attained. The reducing capacity increased in the Fe-stressed treatments (−Fe) as compared to the control in all rootstocks except Nemaguard. The acidification response of the treatment (−Fe) compared to the control was important only for the three plums (Brompton, San Julian A and Puebla de Soto 101). In the other rootstocks the pH in the treatment (−Fe) was always below that of the control (pH above 7), the difference being greater in the hybrid rootstocks (GF 677 and Adafuel) than in the peach (Nemaguard).

The development of reduction and acidification under Fe stress varied among rootstocks as illustrated in Figures 1 and 2. The reducing capacity of Nemaguard decreased with time, whereas that of GF677 and Adafuel increased to a maximum and then decreased (Fig. 1). In these three rootstocks the response of the three plant replications occurred at the same time and mean

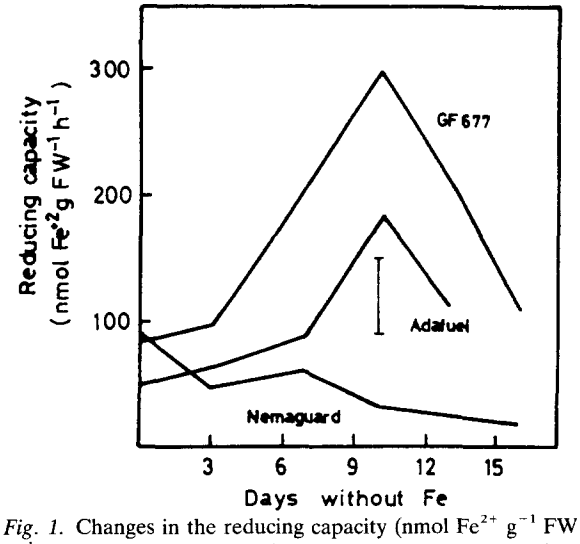

Fig. 1. Changes in the reducing capacity (nmol Fe^{2+} g^{-1} FW h^{-1}) of GF677, Nemaguard (Exp. 2) and Adafuel (Exp. 3) in the treatment (−Fe). Each value is the mean of three plant replications. Vertical bar represents LSD ($P = 0.05$) at 10 days without Fe.

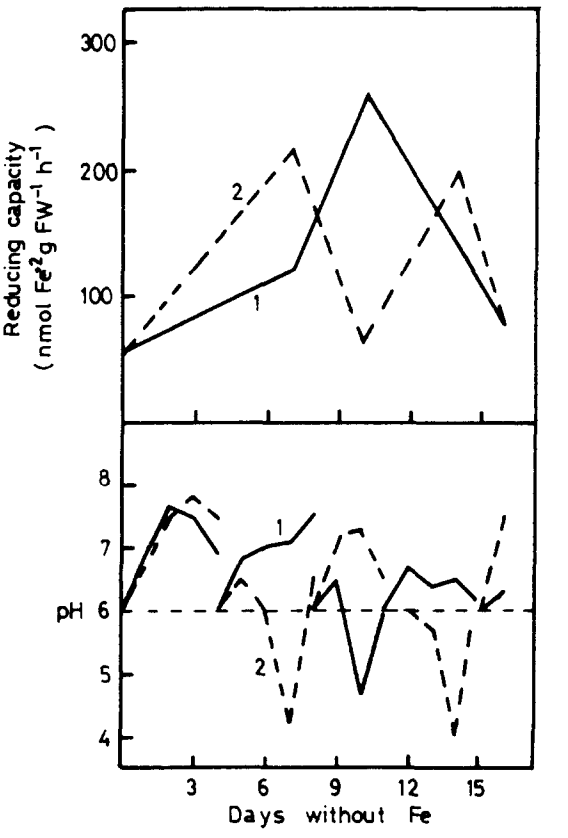

Fig. 2. Changes in the reducing capacity (nmol Fe^{2+} g^{-1} FW h^{-1}) and solution pH of two plants (1 and 2) of the plum San Julian A in the treatment (−Fe).

values are shown in Figure 1. Figure 2 shows the reducing capacity and pH over time for two plant replications of San Julian A. These two plants are representative of what was observed with plum rootstocks. Both responses were rhythmic with the maximum reducing capacity often attained at the same time as acidification was strong.

According to the agar technique, both responses were located at the subapical root zones in all the rootstocks studied. The intensities of the responses were in agreement with those observed in nutrient solution.

Leaf Fe was consistently lower in the treatment (−Fe) than in the control, but this effect was only significant for Nemaguard, Brompton and Adafuel (Table 2). In Exp. 2, leaf Mn was significantly higher in the treatment (−Fe) than in the control for the Fe-chlorosis tolerant rootstocks, but not for the susceptible Nemaguard.

Table 2. Iron and Mn contents in young leaves of the different rootstocks (leaves were collected at 12 days of the treatments). Each value is the mean of three plant replications

Rootstock	Fe ($\mu g\, g^{-1}$ DW)		Mn ($\mu g\, g^{-1}$ DW)	
	Control	−Fe	Control	−Fe
Experiment 2				
Nemaguard	56a	32a**	28a	31a
Brompton	64a	41a**	32a	85c**
San Julian A	59a	46a	31a	84c**
GF677	61a	50a	21b	56b**
Experiment 3				
Adafuel	76a	24a**	84a	101a
P. de Soto 101	58a	45a	62a	76a*
GF677	58a	42a	81a	98a

Within each rootstock * ($P = 0.05$) and ** ($P = 0.01$) indicate significant differences compared to the control according to the F-test. Within each experiment, values of the same column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan multiple range test.

In Exp. 3, this effect was less important (Table 2). In roots (data not shown), no differences in Fe were found among rootstocks. The average root Fe was about 200 to 300 $\mu g\, g^{-1}$ DW in the treatment (−Fe) and 500 to 600 $\mu g\, g^{-1}$ DW in the control. However, Nemaguard accumulated more root Mn (130 to 150 $\mu g\, g^{-1}$ DW average) than the other rootstocks (60 to 80 $\mu g\, g^{-1}$ DW average) in both control and treatment (−Fe). No differences were found in root Mn between these treatments in any of the rootstocks.

Discussion

The mechanisms of response to Fe-deficiency stress, acidification and reducing capacity, have been widely described for herbaceous plants with strategy I responses (Bienfait, 1988; Römheld, 1987). Our work also shows the activity of similar mechanisms in tree species used as peach rootstocks. The responses were found to be located at the subapical root zone, as has been reported in herbaceous plants (Marschner *et al.*, 1982), but no swollen root tips were apparent in roots. Furthermore, the lower reducing capacity of the susceptible peach Nemaguard and the higher acidification response of the three tolerant plums tested, suggest differences between susceptible and tolerant rootstocks are associated with these factors. These mechanisms have also been studied in a few woody species, such as the reducing capacity in apple (Ao *et al.*, 1985) and the acidification response in papaya (Kannan, 1985), but these responses observed have not necessarily been linked to Fe-efficiency in these species.

The responses to Fe-deficiency have been shown to increase not only the uptake and translocation of Fe but also that of Mn and Zn (Römheld *et al.*, 1982). Our results are in agreement with those and show that Mn accumulated in young leaves of Fe stressed (−Fe) tolerant rootstocks, but did not in the Fe-chlorosis susceptible Nemaguard.

The differences in the intensity of the responses obtained in this work may partially explain the differences in tolerance to calcareous soils. The use as a screening technique of the reducing capacity presents several problems derived from its rhythmic pattern (Römheld and Marschner, 1981) and from expressing results on root fresh weight basis (when comparing species with different root systems). The possible Fe contamination during the determination of the reducing capacity could have affected later measurements but this does not seem to be very important in this work because reducing capacity consistently increased in GF677 and Adafuel after several determinations (Fig. 1). Furthermore, plant material from similarly treated origins should be used, since this may affect results. This is illustrated by the more severe chlorosis developed in plants from *in vitro* culture (Exp. 3) than in plants from rooted cuttings (Exp. 2).

This work and the preceding one also show the possibility of working with very young trees, in the same way as with herbaceous plants, especially by the use of *in vitro* culture techniques. By these techniques, very small plants can be obtained in a few months and handled in small containers with nutrient solution for screening or for studying physiological parameters.

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