

## Selection of olive varieties for tolerance to iron chlorosis

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### Summary

Under certain conditions, olive trees grown on calcareous soils suffer from iron chlorosis. In the present study several olive varieties and scion-rootstock combinations were evaluated for their tolerance to iron chlorosis. Plants were grown over several months in pots with a calcareous soil, under two fertilization treatments. These consisted of periodic applications of nutrient solutions containing either, 30  $\mu\text{mol/L}$  FeEDDHA or not Fe. Tolerance was assessed by the chlorosis and growth parameters of plants grown without Fe, compared to those plants grown with Fe. Results show that there are differences in tolerance among olive varieties and that tolerance is mainly determined by the genotype of the rootstock. These results open the way to use tolerant varieties for those conditions where iron chlorosis could become a problem.

**Key words:** iron-chlorosis – olive varieties – scion-rootstock

### Introduction

Iron chlorosis is a nutrient disorder caused by iron (Fe) deficiency. The typical symptoms consist of an interveinal yellowing or chlorosis of young leaves. Iron is an essential micronutrient that linked to proteins participates in different metabolic process such as chlorophyll synthesis or electron transport through the respiratory and the photosynthetic chains (see Chaney 1984 and Korcak 1987 for general reviews).

Plants growing on calcareous soils are faced with characteristic problems in relation with Fe acquisition. The total Fe content in these soils is high but the available fraction for the plants is insufficient. This is caused by the very low solubility of iron oxides at the alkaline pH conditions that are buffered by the presence of bicarbonate in these soils (Lindsay and

Schwab 1982). The incidence of iron chlorosis depends on numerous factors, such as the properties of the iron oxides and carbonates present in the soil, high water or compacted soil conditions, high or low temperatures, and plant genotype (Perret and Koblet 1984, Inskeep and Bloom 1986, Loeppert 1986, Wei et al. 1994). Tolerant plants can adapt to these conditions by developing different strategies that allow them to solubilise and absorb the required Fe (Römheld and Marschner 1986, Guerinot and Yi 1994).

One of the best alternatives to prevent iron chlorosis problems is the use of tolerant plant species or genotypes. Differences in tolerance have been found in many herbaceous and woody species (Hamzé et al. 1986, Kolesch et al. 1987, Zaiter et al. 1988, Chaney et al. 1992, Tagliavini et al. 1993, Cianzio 1995, Socias i Company et al. 1995). With fruit trees it is common to use the commercial varieties grafted on tolerant rootstocks, as the plant tolerance is mainly determined by the

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rootstock genotype (Byrne et al. 1989, Almaliotis et al. 1995, Socias i Company et al. 1995).

For selecting tolerant plant material different methods have been used. The most general are field experiments, growth in pots with calcareous soil and hydroponic culture with high bicarbonate and low Fe concentrations. Field experiments have been successfully used for herbaceous species such as soybean (Cianzio et al. 1979) or chickpea (Singh et al. 1986) and for woody species such as grape-vine (Kolesch et al. 1987) or peach rootstocks (Byrne 1988). In pots and hydroponics culture the plants are grown under controlled and uniform conditions, and so avoid the variability of soil and environmental conditions characteristic of field experiments, and in general the experimental period required is shorter than in the field. Pot experiments have been used, for example, with soybean (Fairbanks et al. 1987), subclover (Wei et al. 1994) and pear rootstocks (Tagliavini et al. 1993). Examples of the

hydroponic culture method can be found for soybean (Coulombe et al. 1984), chickpea (Chaney et al. 1992) and prunus rootstocks (Romera et al. 1991, Shi and Byrne 1995).

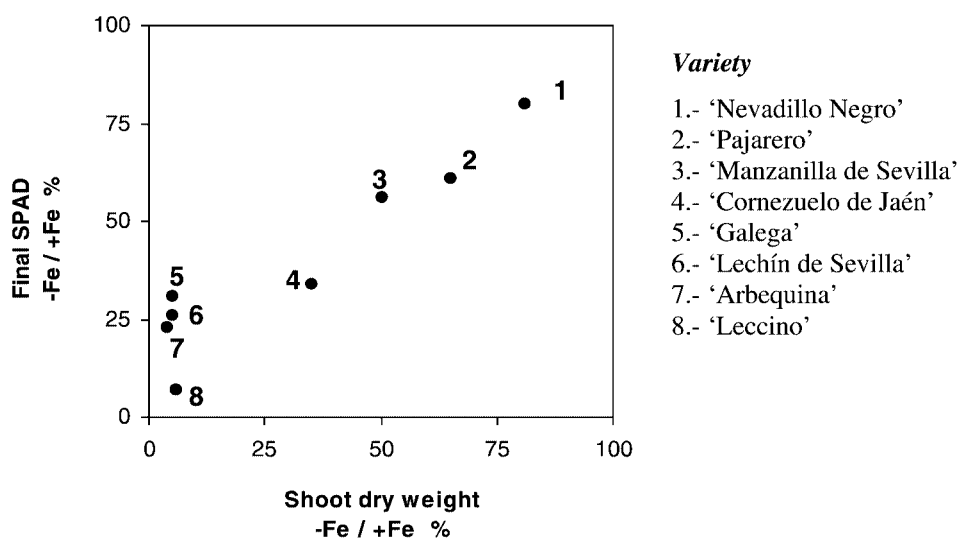
There is very little information about genotypic differences in tolerance to iron chlorosis regarding olive trees. Therefore, the objective of this study was to evaluate the tolerance of different olive varieties and scion-rootstock combinations, by growing plants in pots with a calcareous soil.

## Materials and Methods

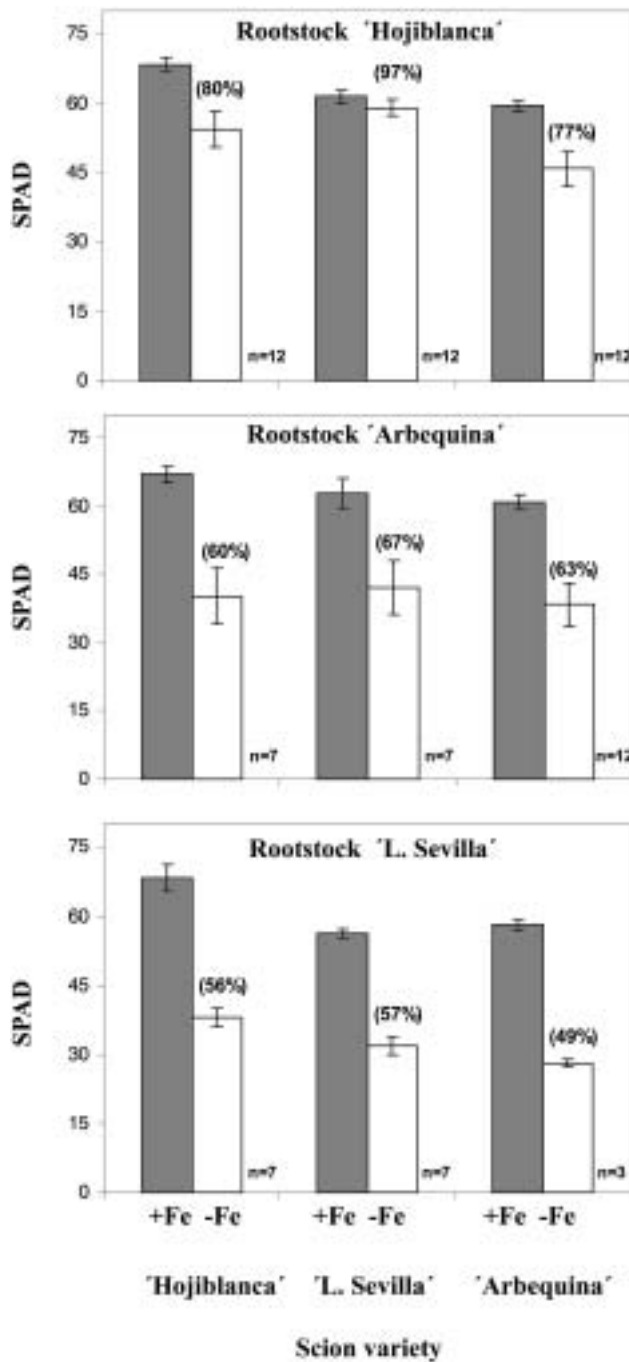
Three independent experiments were carried out, the first to compare varieties and the remaining to compare scion-rootstock combinations. Young olive (*Olea europaea* L.) plants were provided by the «Banco Mundial de Variedades de Olivo de Córdoba», produced by vegetative propagation from softwood cuttings. For the experiments with scion-rootstock combinations, plants were grafted by chip budding.

**Table 1.** Final SPAD and shoot dry weight of eight olive varieties grown in pots with calcareous soil fertilized with nutrient solution with Fe (+ Fe) or without Fe (–Fe). Values are mean  $\pm$  SE (n = 12). The relative values in the (–Fe) treatment with respect to the (+Fe) treatment, expressed as percentage, are also included.

Variety	Final SPAD			Shoot dry weight (g/plant)		
	+Fe	–Fe	–Fe/+Fe (%)	+Fe	–Fe	–Fe/+Fe (%)
Arbequina	51.2 $\pm$ 1.1	11.7 $\pm$ 0.8	23	12.8 $\pm$ 1.6	0.5 $\pm$ 0.1	4
Cornezuelo de Jaén	57.8 $\pm$ 0.8	19.5 $\pm$ 3.7	34	12.2 $\pm$ 2.3	4.3 $\pm$ 0.9	35
Galega	52.3 $\pm$ 1.1	16.6 $\pm$ 1.8	31	6.2 $\pm$ 1.5	0.3 $\pm$ 0.1	5
Leccino	48.3 $\pm$ 2.9	3.2 $\pm$ 1.3	7	10.8 $\pm$ 4.0	0.6 $\pm$ 0.3	6
Lechín de Sevilla	50.1 $\pm$ 1.5	12.8 $\pm$ 3.4	26	19.0 $\pm$ 1.8	0.9 $\pm$ 0.2	5
Manzanilla de Sevilla	63.1 $\pm$ 0.4	35.1 $\pm$ 1.6	56	15.0 $\pm$ 2.8	7.5 $\pm$ 0.5	50
Nevadillo Negro	58.5 $\pm$ 0.4	46.8 $\pm$ 3.7	80	15.7 $\pm$ 2.7	12.7 $\pm$ 0.6	81
Pajarero	62.3 $\pm$ 1.1	37.7 $\pm$ 3.0	61	18.4 $\pm$ 2.9	12.0 $\pm$ 1.6	65

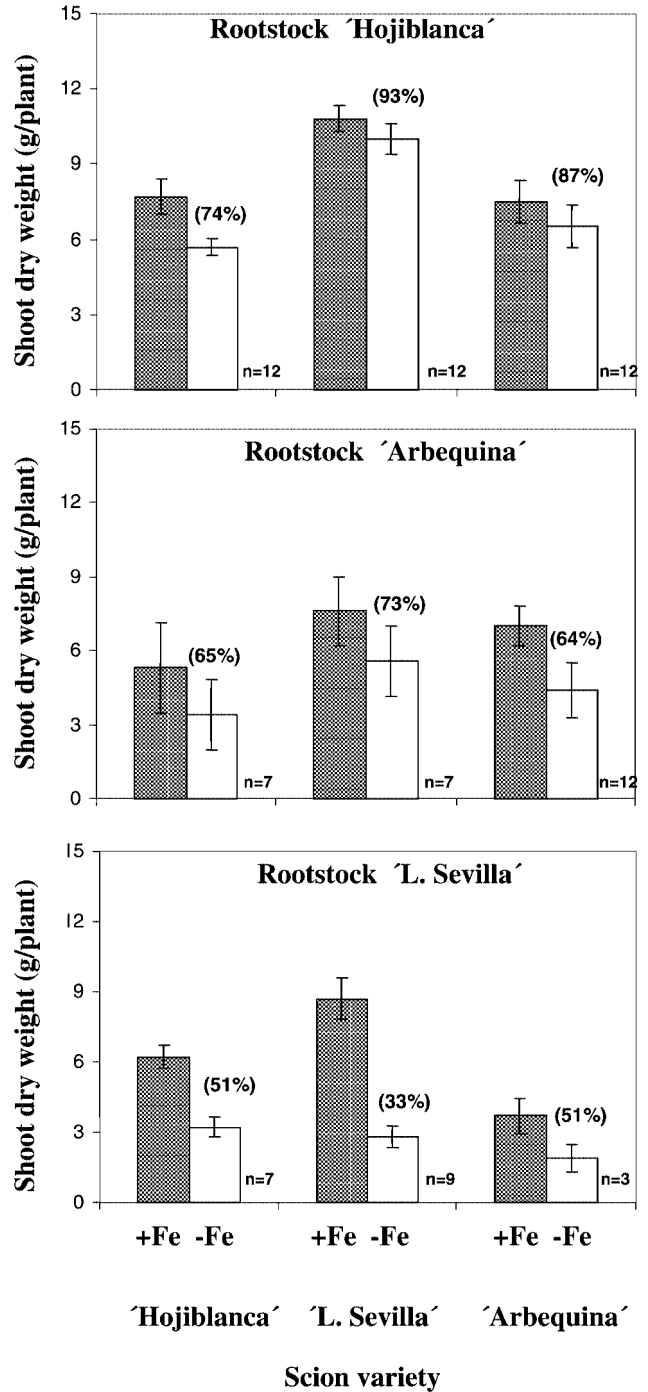


**Figure 1.** Relationship between the relative final SPAD and shoot dry weight values from Table 1.



**Figure 2.** Final SPAD of the nine scion-rootstock combinations obtained by combining three olive varieties, ‘Hojiblanca’, ‘Arbequina’ and ‘Lechín de Sevilla’, used either as scion or rootstock. Plants were grown in pots with calcareous soil fertilized with nutrient solution with Fe (+Fe) or without Fe (–Fe). Values are mean ± SE (the number of replications for each combination is indicated in the graphs). The relative values, as percentages, are given in brackets.

In all experiments plants were grown in a greenhouse where the minimum temperature in winter did not descend below 10 °C and the maximum in summer did not exceed 32 °C. Each plant grew individually in



**Figure 3.** Shoot dry weight of the same nine scion-rootstock combinations presented in Figure 2. Other indications are as in Figure 2.

a 3 L pot containing a calcareous soil mixed with sand in a 3:1 (v:v) proportion. Two fertilization treatments, (+Fe) and (–Fe), were applied. The number of plant replications depended on plant availability and was 12 for the experiments with non-grafted plants and 3 to 12 for the experiments with grafted plants.

Fertilization treatments consisted of the weekly irrigation with 200 mL per plant of the corresponding nutrient solution. The composi-

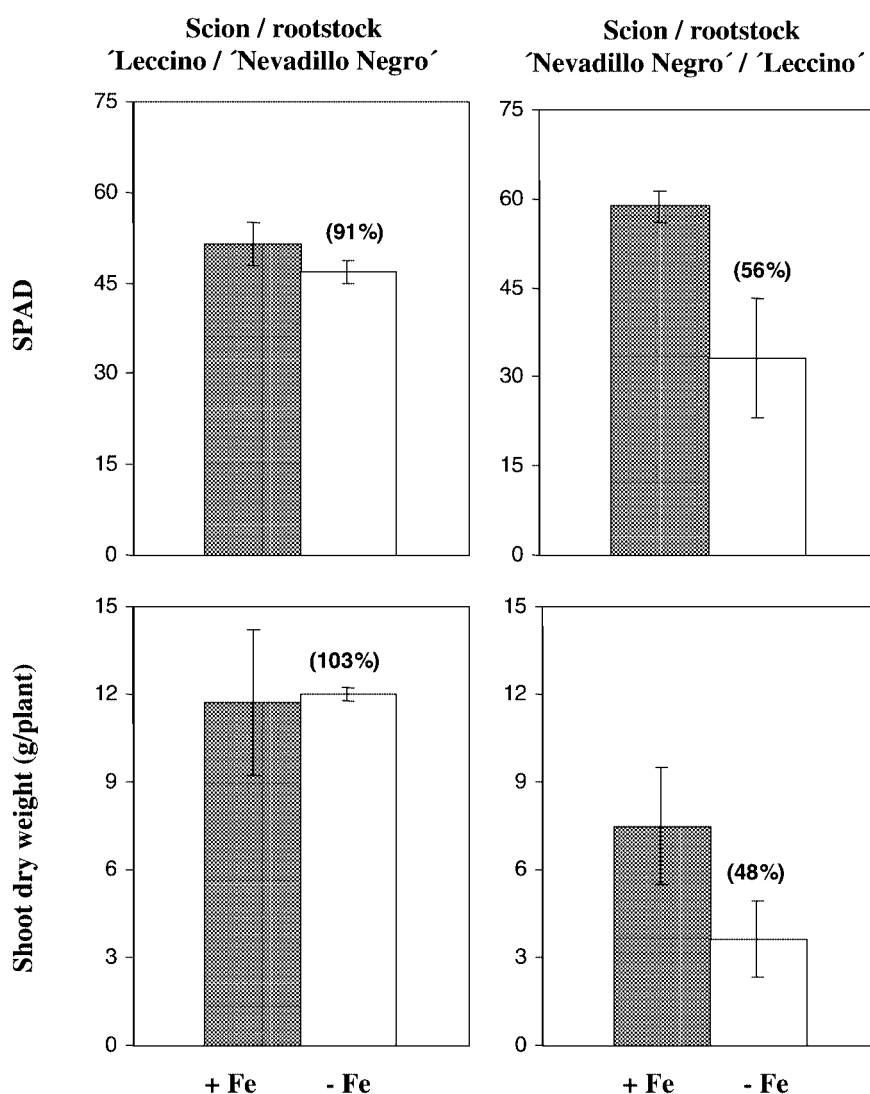
tion of the nutrient solution was (in mmol/L): 2, Ca(NO<sub>3</sub>)<sub>2</sub>; 0.75, K<sub>2</sub>SO<sub>4</sub>; 0.65, MgSO<sub>4</sub>; 0.5, KH<sub>2</sub>PO<sub>4</sub>; and (in μmol/L): 50, KCl; 10, H<sub>3</sub>BO<sub>3</sub>; 1, MnSO<sub>4</sub>; 0.5, CuSO<sub>4</sub>; 0.5, ZnSO<sub>4</sub>; 0.05, (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>. It also contained 30 μmol/L FeEDDHA for the (+Fe) treatment and no Fe for the (-Fe) treatment. The first experiment started at the beginning of November and lasted for 203 days. The second experiment started at the beginning of May and lasted for 92 days. The third experiment started in the middle of June and lasted for 58 days. Though the first experiment was longer it should be considered that growth of plants during the first half of the period was negligible, so the effective period of growth was around 100 days.

During the experimental periods chlorosis and growth parameters were periodically determined. Chlorosis was measured in young leaves by means of a portable chlorophyll-meter (SPAD-502, Minolta) that gives SPAD values related to the chlorophyll content, so that low SPAD values are an indication of low chlorophyll content. Growth was

measured by the increase in length of shoots developed. At the end of the experiments the dry weight of this shoot growth was obtained. For simplicity, only final data are presented as they were representative of the entire experimental period.

## Results

In the first experiment, eight varieties were compared and clear differences in tolerance were observed. The final SPAD values and the shoot dry weight are presented in Table 1. In the (+Fe) treatment the SPAD values were high, between 48.3 for ‘Leccino’ and 63.1 for ‘Manzanilla de Sevilla’. In the (-Fe) treatment the SPAD values were lower for all the varieties, with values below 20 for five varieties and above 35 for three va-



**Figure 4.** Final SPAD and shoot dry weight of the two reciprocal scion-rootstock combinations obtained with the olive varieties, ‘Leccino’ and ‘Nevadillo Negro’. Plants were grown in pots with calcareous soil fertilized with nutrient solution with Fe (+Fe) or without Fe (-Fe). Values are mean ± SE (n = 4 for ‘Leccino’/‘Nevadillo Negro’ and n = 3 for ‘Nevadillo Negro’/‘Leccino’). The relative values, as percentages, are given in brackets.

rieties. The shoot dry weight in the (+Fe) treatment ranged from 6.2 g for 'Galega' to 19.0 g for 'Lechín de Sevilla'. In the (-Fe) treatment growth was lower and again there were differences among varieties. Tolerance can be assessed by the relative chlorosis and growth in the (-Fe) treatment with respect to the (+Fe) treatment. These values, expressed as percentages, are shown in Table 1 and they are also represented as SPAD against shoot dry weight in Figure 1. Consequently, the varieties that were most susceptible were 'Leccino', 'Arbequina', 'Lechín de Sevilla' and 'Galega', most tolerant were 'Nevadillo Negro', 'Pajarero' and 'Manzanilla de Sevilla', and intermediate was 'Cornezuelo de Jaén'.

In the second experiment, nine scion-rootstock combinations were compared. These were obtained by combining three varieties, 'Hojiblanca', 'Arbequina' and 'Lechín de Sevilla', used either as scion or as rootstock. Final SPAD and shoot dry weight are shown in Figures 2 and 3, respectively, with the ratios of the values of (-Fe)/(+Fe) expressed as percentages in brackets. Results show that tolerance was mainly determined by the variety used as rootstock. So, combinations with 'Hojiblanca' as rootstock were the most tolerant, followed by those with 'Arbequina' as rootstock as intermediate and those with 'Lechín de Sevilla' as rootstock as most susceptible.

In the third experiment, the two reciprocal combinations between 'Leccino' and 'Nevadillo Negro' were compared. Results of final SPAD and shoot dry weight show again that tolerance was higher in the combinations that had the tolerant variety, 'Nevadillo Negro', as rootstock (Fig. 4).

## Discussion

Olive trees are widely grown on calcareous soils of the Mediterranean area without showing, in general, problems of iron chlorosis. In consequence, this species is considered more tolerant than other fruit species such as peach or quince. Nonetheless, iron chlorosis has been observed in some olive orchards (Matar et al. 1977, Fernández-Escobar et al. 1993). This could be related to different causes such as: location specific poor soil or environmental conditions; use of irrigation, fertilization or higher plantation density, aimed to increase production; or the introduction of less tolerant varieties.

While genotypic differences in tolerance have been described for many species (Hamzé et al. 1986, Kolesch et al. 1987, Zaiter et al. 1988, Chaney et al. 1992, Tagliavini et al. 1993, Cianzio 1995, Socias i Company et al. 1995), the only information for olive trees comes from field observations, with the difficulties of interpretation that this involves. For this reason, the aim of this study was to evaluate the tolerance of several olive varieties under controlled conditions.

In previous investigations we tried the hydroponic method developed by the Chaney group (Coulombe et al. 1984) for herbaceous species. This method gave good results with

peach rootstocks (Romera et al. 1991) but with olive there were several problems. Some varieties did not grow well in this system, fungal infections were frequent and in general the effects on chlorosis and growth were not well related. The growth in pots with calcareous soil is an alternative method used by others (Fairbanks et al. 1987, Ocumpaugh et al. 1992, Tagliavini et al. 1993, Wei et al. 1994) and as shown in the present study it has produced good results for olive. Wide differences in tolerance among olive varieties were identified based on the effects on chlorosis and growth that otherwise were well related. In the experiments with grafted plants it was also shown that tolerance was mainly determined by the rootstock genotype as occurs in other species (Byrne et al. 1989, Almaliotis et al. 1995, Socias i Company et al. 1995).

The results obtained in this study open the way to the use of olive varieties tolerant to those conditions in which iron chlorosis could become a problem. Nonetheless, it would be convenient to confirm the behaviour of the most tolerant varieties under field conditions.

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