International Journal of Botany

ISSN: 1811-9700

science alert

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International Journal of Botany 5 (2): 186-189, 2009
ISSN 1811-9700

Management of Iron Deficiency Stress in Citrus through Soil Application of Vivianite to a Calcareous Soil

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Abstract: Iron deficiency is a common abiotic stress in citrus trees grown on calcareous soils, where considerable reduction in yield is expected if not treated. In this study the effectiveness of synthetic vivianite [($Fe_3(PO_4)_2.8H_2O$)] to prevent Fe chlorosis in citrumelo Swingle (*Citrus paradisi* Macf. x *Poncirus trifoliata*) a susceptible rootstock to Fe deficiency stress, was investigated. One-year old citrumelo plants were grown on calcareous soil-sand mixture under greenhouse conditions and treated with: (1) no Fe (as control); (2) 1.6 g FeEDDHA plant^{-1} (3) 5.4 g vivianite plant^{-1}. Chlorophyll measurements were performed on the youngest fully expanded leaves in terms of SPAD index and at the end of the experiment leaf chlorophyll and Fe concentrations and growth vigor (young shoot dry weight) were determined. Vivianite was as effective as the FeEDDHA. Vivianite significantly prevented the development of Fe chlorosis. Chlorophyll concentration of plants treated with vivianite was significantly higher than those of control plants although vivianite-treated plants had almost equal leaf Fe concentration as control plants, vivianite significantly improved the vigor of citrus plants similar to the FeEDDHA compared to the control treatment. These results suggest that vivianite is an effective alternative to the environmental-unfriendly and expensive Fe-chelates for preventing Fe deficiency in citrus orchards.

Key words: Iron chlorosis, vivianite, synthetic Fe-chelate, citrumelo swingle, spad index

INTRODUCTION

The adverse impact of iron (Fe) deficiency stress on yield of citrus trees grown on calcareous soils has stimulated the search for ecological agronomic means by which Fe is made available to these fruit trees. The conventional approach for solving the problem by Fe supplementation is characterized by high cost and environmental implications (Pestana *et al*., 2005). Applying inorganic Fe fertilizers to soil is generally ineffective due to the formation of highly insoluble compounds (such as ferric hydroxide) under alkaline pH conditions. However, combining Fe with chelates can reduce the rate of formation of these insoluble Fe compounds (Lucena, 2003). Unfortunately, Fe-chelates may also be chemically or microbially degraded, strongly retained by soil minerals or organic matter, or inactivated through the exchange of Fe with other cations (Alvarez-Fernandez *et al*., 2005; Lucena, 2003). In addition, the effectiveness of FeEDDHA may be limited due to leaching to deep soil layers contiguous to the water table, which might impose environmental and health hazards (Abadia *et al*., 2004).

The need for a sustainable agriculture calls for long-term, low-input and cost-effective strategies to overcome Fe deficiency stress. Recently, a long-lasting prevention of chlorosis has been achieved in field-grown pear (Iglesias *et al*., 2000) and olive (Rosado *et al*., 2002) by soil application of $Fe_3(PO_4)_2.8H_2O$; a synthetic iron(II)-phosphate analogous to the mineral vivianite. This research was aimed to investigate the effectiveness of synthetic vivianite, compared to the mineral one, to prevent Fe chlorosis in citrumelo Swingle (*Citrus paradisi* Macf. x *Poncirus trifoliata*) a highly susceptible citrus rootstock to Fe deficiency stress.

MATERIALS AND METHODS

Homogeneous one year-old citrumelo Swingle plants were transferred into plastic pots in early March-2006 and grown under plastic house conditions on a 25 kg of calcareous loamy soil (pH 8.1, active carbonate 115 g kg^{-1},

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DTPA-extractable Fe 7.6 mg kg^{-1}, sand 51.0%, silt 22.5% and clay 26.5%). The soil has been previously sieved through a 1 cm sieve. Plants were adequately irrigated and fertilized. Each plant received 2.6 g N, 1.2 g P and 1.3 g K during the period of the experiment.

Citrumelo plants were grown with the following treatments:

- No Fe addition as a control
- Fertilized with FeEDDHA
- Fertilized with vivianite

The FeEDDHA treatment was applied as FeEDDHA solution at a rate of 18.6 mg Fe $plant^{-1}$ (1.6 g FeEDDHA $plant^{-1}$). Vivianite was injected into the soil in 3-4 points around the root system of each plant (15 cm deep) at a rate of 5.4 g vivianite $plant^{-1}$ (Rosado *et al*., 2002). Both FeEDDHA and vivianite were applied at the beginning of the experiment. Vivianite ($Fe^{II}_3(PO_4)_2.8H_2O$) was prepared according to Rosado *et al*. (2002) as follows: 25 g of MAP ($NH_4H_2PO_4$) was dissolved in 1 L then 75 g of $FeSO_4.7H_2O$ were gradually added while stirring. The resulted suspension contains 90 g vivianite suspension L^{-1}. A completely randomized blocks design was used. Each treatment was replicated 5 times (one plant each).

The parameters that have been measured through out the experiment were:

- Chlorophyll concentration of fully expanded young leaves (when developed) was measured by chlorophyll meter (SPAD 502 Minolta Corp., Osaka, Japan) and expressed as SPAD index of citrumelo plants

At the end of the experiment (end of July) the following parameters were determined:

- Leaf chlorophyll concentration according the method described by Abadia *et al*. (2004)
- Leaf Fe concentration was measured by the spectrophotometer according the method described by Dominik and Kaupeniohann (2000)
- Growth vigor expressed as dry weight of young shoots (those developed during the last 2 months of the experiment)

The data were subjected to analysis of variance and, whenever the F test showed significance, the means were compared using Least Significance Difference (LSD) at $p \leq 0.05$ using SAS version 9.

RESULTS AND DISCUSSION

In this experiment, the citrus rootstock revealed visible symptoms of Fe deficiency (leaf interveinal chlorosis) starting from early May, which was effectively prevented by a supply of FeEDDHA equivalent to 18.6 mg Fe $plant^{-1}$ (Table 1). Thus, the positive impact imposed by vivianite is supposed to result from improving the Fe nutrition of the citrus rootstock.

The application of vivianite effectively improved the Fe nutritional status of the citrus rootstock compared to the control treatment as indicated by the SPAD index (Table 1) and leaf chlorophyll concentration (Table 2), which confirms previously obtained results (Iglesias *et al*., 2000; Rosado *et al*., 2002). Likewise, vivianite significantly improved the vigor of citrus plants similar to the FeEDDHA compared to the control treatment (Table 3).

Noteworthy, the increase in chlorophyll concentration induced by vivianite occurred without increasing leaf Fe concentration similar to the FeEDDHA treatment. Leaf Fe concentrations of the vivianite- and FeEDDHA-treated plants were almost similar to that of the chlorotic leaves of the control plants (Fig. 1). Current results corroborate those obtained on kiwi fruit (Barton and Abadia, 2006). Various soil factors resulting in severe inhibition of root growth might be responsible for triggering a restriction in shoot growth (Table 3), which elevates the Fe concentration in chlorotic leaves as a consequence of the diminished dilution of Fe concentration.

Citrus rootstocks differ in their susceptibility to Fe deficiency, which causes economic losses through persistent leaf chlorosis and progressive necrosis of young shoots (Chapman, 1968). The use of Fe efficient rootstocks such as *Citrus macrophylla*, *Citrus jambhiri* and several other rough lemon varieties is the best approach to preventing Fe availability problems. Yet these rootstocks are highly susceptible to other citrus diseases and are, therefore, used less frequently than the

Table 1: Leaf chlorophyll concentration (SPAD index) of citrumelo plants

Treatments	05/04	25/04	15/05	15/06	30/06	15/07	25/07
Control	38.0±2.7a	33.0±2.7a	29.6±2.3b	28.6±2.6b	27.2±1.8b	25.6±2.6b	23.8±1.3b
Fe-EDDHA	38.4±2.6a	35.8±4.0a	35.6±1.5a	36.6±1.1a	37.6±1.1a	38.2±1.6a	40.2±2.0a
Vivianite	39.8±1.9a	32.8±2.4a	35.0±2.0a	35.8±1.1a	36.8±1.5a	37.0±2.0a	39.2±0.8a

Values are expressed as Mean±SE. Within each column values followed by different letter(s) are significantly different (LSD test at $p \leq 0.05$)

Table 2: Leaf chlorophyll concentration (μg cm^{-2}) of citrumelo plants at the end of the experiment (July, 25th)

Treatments	Chl. Concentration
Control	11.2±2.2b
Fe-EDDHA	21.2±3.2a
Vivianite	17.8±2.6a

Values are expressed as Mean±SE. Within each column values followed by different letters are significantly different (LSD test at $p \le 0.05$)

Table 3: Young shoot dry weight (g) of citrumelo plants at the end of the experiment (July, 25th)

Treatments	Young shoot dry weight
Control	19.8±2.7c
Fe-EDDHA	40.8±4.1a
Vivianite	32.2±2.6b

Values are Mean±SE. Within each column values followed by different letters are significantly different (LSD test at $p \le 0.05$)

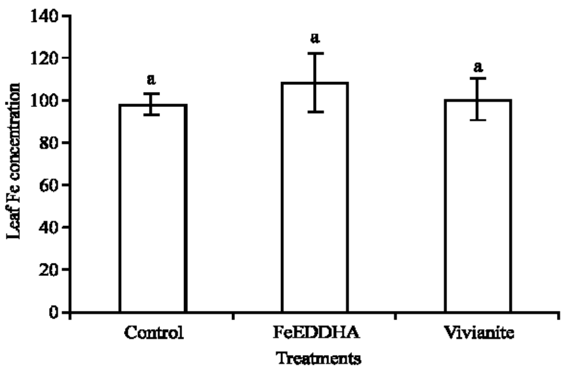

Fig. 1: Leaf Fe concentration (μg g^{-1} DW) of citrumelo plants at the end of the experiment (July, 25th). Bars with same letter(s) are not significantly different (LSD test at $p \le 0.05$)

commonly used Fe inefficient citranges and related rootstocks derived from the trifoliate orange (*Poncirus trifoliata*) (Gogorcena *et al*., 2004). According to Jolley and Brown (1994), Goos and Johnson (2000) and Naeve and Rehm (2006) various fertilizer and cultural management practices are available to cure/prevent Fe deficiency. Application of Fe in chelating forms represents the most widespread technique. Although, Fe chelates are effective, they provide a temporary remedy for Fe deficiency chlorosis and are not considered environmental-friendly (Barton and Abadia, 2006).

The effectiveness of the vivianite as a source of Fe for a citrus rootstock is proved for the first time in this research. Vivianite particles range between 2-10 μm in length and are hardly mobile through the soil profile, thus remain at the depth of application. Moreover, the effectiveness of vivianite lies on the poorly crystalline Fe oxides (ferrihydrite and lepidocrocite) resulting from the oxidation and incongruent dissolution of vivianite. The formation of these oxides mainly depends on the continuous removal of phosphate from vivianite (Rosado *et al*., 2002). It is assumed that under the experimental conditions of this research precipitation and/or adsorption of phosphate on active soil surfaces and root uptake likely represent the main mechanisms of phosphate removal.

CONCLUSION

It is concluded that citrus trees growing on calcareous soils can potentially benefit, in terms of Fe nutrition, from vivianite as an effective alternative to the environmentally-unfriendly and expensive FeEDDHA for preventing Fe deficiency. Citrus trees growth vigor was also significantly improved by vivianite compared to the control.

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