



Growth trend and tolerance of Swingle citrumelo in Mazandaran calcareous soils, Iran

Ali Asadi Kangarshahi¹ and Negin Akhlaghi Amiri^{2*}

1, Soil and Water Research Department, Mazandaran Agricultural and Natural Resources Research and Education Center, AREEO, Sari, Iran

2, Horticulture Crops Research Department, Mazandaran Agricultural and Natural Resources Research and Education Center, AREEO, Sari, Iran

ARTICLE INFO

Original Article

Article history:

Received 11 October 2020

Revised 9 December 2020

Accepted 27 December 2020

Available online 10 April 2021

Keywords:

Calcium carbonate

Chlorosis

Citrus

Rootstock

Vegetative growth

DOI: [10.22077/jhpr.2020.3801.1175](https://doi.org/10.22077/jhpr.2020.3801.1175)

P-ISSN: 2588-4883

E-ISSN: 2588-6169

*Corresponding author:

Horticulture Crops Research Department,
Mazandaran Agricultural and Natural
Resources Research and Education Center,
AREEO, Sari, Iran.

Email: n.akhlaghi@areeo.ac.ir

© This article is open access and licensed under the terms of the Creative Commons Attribution License <http://creativecommons.org/licenses/by/4.0/> which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

ABSTRACT

Purpose: In this study, the response of Miyagawa Satsuma mandarin (*Citrus unshiu*) on Swingle citrumelo rootstock to calcareous soils of the east of Mazandaran in Iran was investigated. **Research method:** The experiment was done by seven soils with different calcium carbonate (2-45%) for two years. **Findings:** Soils with 14% total lime and 5% active lime had the highest shoot dry weight. Soils with 30 and 45% total lime, and 14 and 16% active lime had the highest chlorosis and soils with no lime or 3% active lime and 2 to 9% total lime had the least chlorosis. Soils with 2 and 9% total lime had the highest Fe concentration in root and the least chlorosis. Fe concentration in the roots was about 7.5 times that of the leaves, which show Fe accumulation and inactivation in the root. Mn concentration in leaves in most soils was lower than optimum, while available Mn in most soils was more than optimum (2.5 mg kg^{-1}). Mn concentration at the roots was about 3.2 times of that in the leaves. In contrast, although available Zn of some soils was lower than optimum, in most soils the Zn concentration of leaves was in the optimum range. **Research limitations:** No limitations were founded. **Originality/Value:** Mn, due to low uptake and transmission efficiency from roots to shoots and severe deficiency in leaves is the most limiting microelement in this rootstock-scion combination. According to this research, Swingle citrumelo rootstock is appropriate in soils with total and active lime less than 14% and 5%, respectively.

INTRODUCTION

Calcium carbonate that exists in more than 30% of the world's land is one of the most critical soil chemical properties that limit Fe availability for plants (Chen & Barak, 1982; Loeppert et al., 1994). Mazandaran province is one of the most critical horticultural areas of Iran with 120,000 hectares citrus cultivation area and more than two million tons annual production (Asadi Kangarshahi & Akhlaghi Amiri, 2014a). Figure 1, has shown the map of Mazandaran province and the lands under cultivation of all its crops. Cross-sectional studies of the soils of Mazandaran province (Fig. 2) have shown that the amount of calcium carbonate in the orchards of this region gradually increases from west to east (Asadi Kangarshahi & Akhlaghi Amiri, 2014a). Currently, Swingle citrumelo rootstock for citrus in these areas is expanding rapidly. Whereas this rootstock has been reported sensitive to soil calcium carbonate, and possibility of leaf chlorosis and decline of citrus trees on it is very high (Castle & Nunnallee, 2009; Pestana et al., 2005; Sudahono et al., 1994). Field observations of authors in citrus gardens in the east of Mazandaran have shown that this rootstock in soils with high calcium carbonate, often suffered from lime chlorosis. Hence, non-scientific promotion of this rootstock could affect fruit yield and quality.

Fe availability for plants in most calcareous soils is low and leads to chlorosis, decrease yield, and fruit quality in sensitive crops like some citrus rootstocks and varieties. In these soils, citrus trees on susceptible rootstocks often show severe deficiency symptoms or Fe chlorosis, due to, high pH, and bicarbonate concentration in soil solution. However, Fe deficiency in calcareous soils of Mediterranean regions is for the high concentration of Ca and bicarbonate in soil solution (Mengel, 1995). High bicarbonate concentration in the root zone usually affects metabolic processes in leaves and roots, and causes to develop of Fe chlorosis symptoms in leaves (Mengel, 1995). Active lime represents a part of soil lime that has a high specific surface and is very reactive. This active lime, indirectly, is responsible for growth reduction and physiological disorders like chlorosis (Castle & Nunnallee, 2009; Yang et al., 2010). Active Fe in the leaf and root represents a part of Fe that is divalent and active metabolically (Katyal & Sharma, 1980; Neaman & Aguirre, 2007).

Using Swingle citrumelo rootstock in calcareous soils because of its inability to uptake adequate microelements like Fe is limited. Most citrus varieties, especially blood oranges on this rootstock in soils with high calcium carbonate, don't have sufficient yield and quality, they also, have many problems for gardeners (Manthey et al., 1994). A few studies have done for appropriate rootstocks in calcareous soils (Asadi Kasngarshahi & Akhlaghi Amiri, 2020; Martinez Cuenca et al., 2017). Most of these reports indicate that Swingle citrumelo in calcareous soils with heavy texture shows chlorosis symptoms and Fe deficiency (Albrecht et al., 2018). Several reports show that Poncirus (*Poncirus trifoliata* L.) and its commercial hybrids are sensitive or semi sensitive to calcareous soils (Albrecht et al., 2018; Fu et al., 2016; Levy & Shalheret, 1990).

Lime-induced chlorosis can reduce yield and quality and delay citrus fruit maturity (Pestana et al., 2001; Qrtiz et al., 2007). Several reports indicate that the most appropriate way to solve this problem is the use of rootstocks with Fe chlorosis tolerant (Davies & Albrigo, 1994; Pestana et al., 2001). Some research indicates that Swingle citrumelo is not a suitable rootstock for calcareous soil (Castle & Stover, 2001; Pestana et al., 2001; 2005). Evaluate of vegetative growth of grapefruit trees on Swingle citrumelo rootstock showed that as the soil calcium carbonate increased to 2%, vegetative growth reduced strongly, but, grapefruits on Swingle citrumelo in high pH soils of Texas and Florida had relatively high yield (Castle & Stover, 2001; Wutscher et al., 1975). Lemon trees on Swingle citrumelo in Arizona indicated severe chlorosis and low vegetative growth due to Fe deficiency (Qrtiz et al., 2007; Wright et

al., 1999); Louzada et al. (2008) reported that Swingle citrumelo is not a suitable rootstock to replace sour orange in the south of Texas.

Predict of Fe chlorosis possibility in fruit trees during orchard planting is very important, and any mistake in this stage makes it impossible to gain desirable following yield (Loeppert et al., 1994). In Mazandaran province (in the north of Iran), from the center to the east of the flat area, and, in foothill, also in the margin of the forests (south) toward flat area (north), lime amount increases gradually (0-40%). Also, Swingle citrumelo rootstock is expanding in this area (Asadi Kangarshahi & Akhlaghi Amiri, 2014a). So, response of this rootstock to the different amount of lime in this region is essential. The purpose of this study is to investigate the growth trend, nutritional responses, and tolerance of Miyagawa Satsuma mandarin on Swingle citrumelo rootstock to chlorosis in calcareous soils of the east of Mazandaran province in the north of Iran.

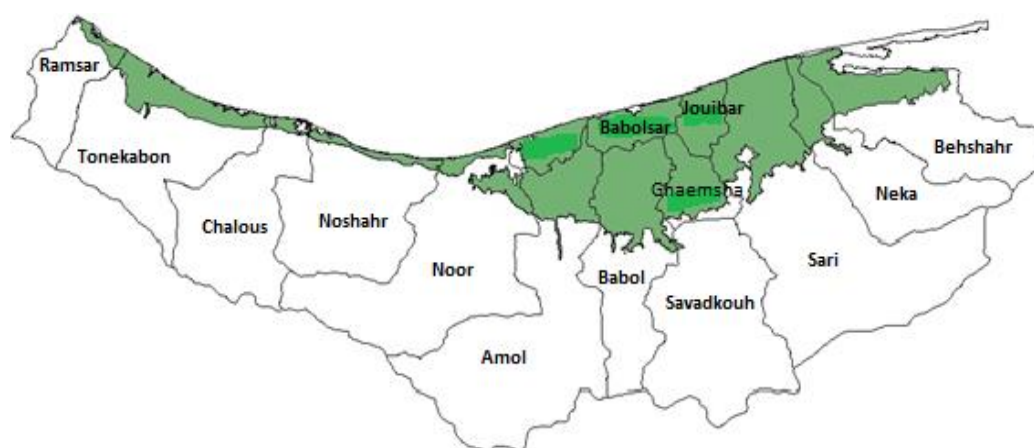


Fig. 1. Agricultural lands (green areas) and non-agricultural lands (white areas) in different cities of Mazandaran province, Iran.



Fig. 2. Distribution of calcium carbonate in soils of Mazandaran province. Yellow areas, 0-5%; light blue, 5-10%; brown, 10-15%; green, 15-20%; pink, 20-30%; bold blue, 30-30% and gray, moreover 50% of calcium carbonates are equivalent (Asadi Kangarshahi & Akhlaghi Amiri, 2014a).

MATERIALS AND METHODS

The experiment was performed for two years in a potted form in a randomized complete block design (RCBD) by seven soils and four replications. According to soil maps and several studies in the east of Mazandaran gardens (Asadi Kangarshahi & Akhlaghi Amiri, 2014a), seven soil samples was selected with a wide range of calcium carbonate (2-45%). Soil samples were collected from an extensive geographic area of the region (major regions in citrus cultivation) in the east of Mazandaran (Babol, GhaemShahr, Sari, and Neka). After drying samples in the air and crossing from two-millimeter filter, some physical and chemical analysis were performed: equivalent calcium carbonate by titration with acid (Bashour & Sayegh, 2007), active calcium carbonate by titration with potassium permanganate (Bashour & Sayegh, 2007), clay, silt and sand by hydrometer method (Gee & Bauder, 1986), soil reaction in saturated paste (McClean, 1982), organic matter by Walkley-Black method (Nelson & Sommers, 1982), manganese, iron, and zinc by DTPA (Lindsay & Norvel, 1978) were measured.

30 kg of soil samples was poured into big plastic pots. Nitrogen fertilizer (60 mg nitrogen per kg of soil), in the form of ammonium sulfate was added to each pot (Asadi Kangarshahi & Akhlaghi Amiri, 2014b). Before planting, phosphorus (triple superphosphate) and potassium (potassium sulfate) fertilizer was added only to soils that had respectively less than 15 mg kg⁻¹ available phosphorous (Olsen & Sommers, 1982) and 300 mg kg⁻¹ available potassium (ammonium acetate method). 20 mg kg⁻¹ phosphorous and 200 mg kg⁻¹ potassium were added to soil 5, and 25 mg kg⁻¹ phosphorous, and 100 mg kg⁻¹ potassium to soil 7 (Asadi Kangarshahi & Akhlaghi Amiri, 2014b). Then uniform seedlings of Satsuma mandarin (*Citrus unshiu*) cv. Miyagawa on Swingle citrumelo rootstock with a height of about 50 cm and one cm diameter were planted in each pot.

After planting of seedlings, during the growth period, fertigation was done with potassium nitrate (1.4 mmol L⁻¹), potassium sulfate (0.6 mmol L⁻¹), magnesium sulfate (1 mmol L⁻¹), mono ammonium phosphate (0.6 mmol L⁻¹), ammonium sulfate (3 mmol L⁻¹), and ammonium molybdate (1 μmol L⁻¹) once every two weeks and irrigation done with the random weighing of pots, regularly (Asadi Kangarshahi & Akhlaghi Amiri, 2018). Leaf samples were prepared in August from the middle leaves of the current shoot around each seedling (Embleton et al., 1973; Marchal, 1984). Plant samples first were oxidized by dry method, then Fe, Mn, Zn, and Cu concentration were measured by atomic absorption spectrometry. To estimate the severity of chlorosis, we used a rating scale in the second year from one to five (Table 1), which in it, one was leaves showing no sign of Fe chlorosis and five was leaves showing total chlorosis with some defoliation (Byrne et al., 1995).

Table 1. Leaf chlorosis grading guide for newly expanded leaves of seedlings

Leaf chlorosis rate	Symptoms
1	Healthy green leaves
2	Yellowish-green interveinal areas, green veins
3	Greenish-yellow interveinal areas, green veins
4	Yellow-interveinal areas, green veins
5	Yellow-white interveinal areas, pale green veins, some defoliation

Table 2. Some physical and chemical properties of experimental soils

Properties	Soils and region						
	1	2	3	4	5	6	7
	South of Babol	West of GhaemShahr	South of Sari	West of Neka	North of Neka	West of Sari	East of Sari
Clay (%)	23	29	19	41	13	37	23
Silt (%)	30	26	35	18	29	29	37
Sand (%)	47	45	46	41	58	34	40
CCE (%)	2	9	14	30	40	25	45
ACCE (%)	0	3	5	14	7	10	16
O.M. (%)	1.17	0.95	1.80	1.60	0.65	1.52	1.10
pH	6.8	7.45	7.86	7.60	7.77	7.78	7.76
P (mg kg ⁻¹)	26	22	15	17	11.20	18.30	9.87
K (mg kg ⁻¹)	404	380	360	460	221	325	265
Fe (mg kg ⁻¹)	7.20	6.40	8.80	8.90	4.40	8.22	6.80
Mn (mg kg ⁻¹)	3.10	4.20	3.96	5.40	3.20	7.71	3.40
Zn (mg kg ⁻¹)	2.40	2.50	0.70	0.70	0.91	1.60	1.50

Active Fe in the leaf and root was read with a spectrophotometer at 510 nm (Katyál & Sharma, 1980; Neaman & Aguirre, 2007). Table 2 have indicated some soil physical and chemical characteristics. Soils equivalent lime range was variable from two to 45, active lime from zero to 16, clay from 13 to 41, silt from 18 to 37, sand from 34 to 58, and organic carbon from 0.65 to 1.8 percent.

Finally, all data were analyzed with statistical software, and the means were compared with Duncan's test, and recommendations presented. Plant responses contain: the trend of vegetative growth, root and shoot dry weight, leaves chlorosis degree, active and total Fe, Mn, Zn, and Cu in leaves and root also the relation of active lime with active Fe in leaves.

RESULTS AND DISCUSSION

Trends of vegetative growth and dry weight

The results showed that during the first two months, the highest stem diameter of Miyagawa satsuma mandarin on Swingle citrumelo rootstock was in the soil with 9% lime and the least was in the soil with 25 and 30% lime; then, over time, the growth trend changed in different soils (Fig. 3). At the end of the experiment, seedlings in the soil with 2% lime had the highest stem diameter. Stem diameter of seedlings in soils with 45, 9, 14, 25, and 40% lime placed after the soil with 2% lime (Fig. 3). According to Table 3 the highest and lowest dry weight of aerial parts of *Citrus unshiu* cv. Miyagawa on Swingle citrumelo rootstock were obtained from soils with 14, and 45% lime, respectively and aerial parts dry weight in soils with 2, 9, 25, 30, and 40% lime placed after soil with 14% lime, respectively. The highest root dry weight was obtained from 14% lime soils, following by 25, 2, 45, 9, 30, and 40% lime. However, root dry weight was similar in 2, 9, and 14% lime soils and did not have any statistically significant difference.

Leaf chlorosis degree index

According to Table 3, the highest active lime and chlorosis degree was obtained from soils with 30 and 45% lime and the lowest chlorosis degree obtained from soils with 2 and 9% lime.

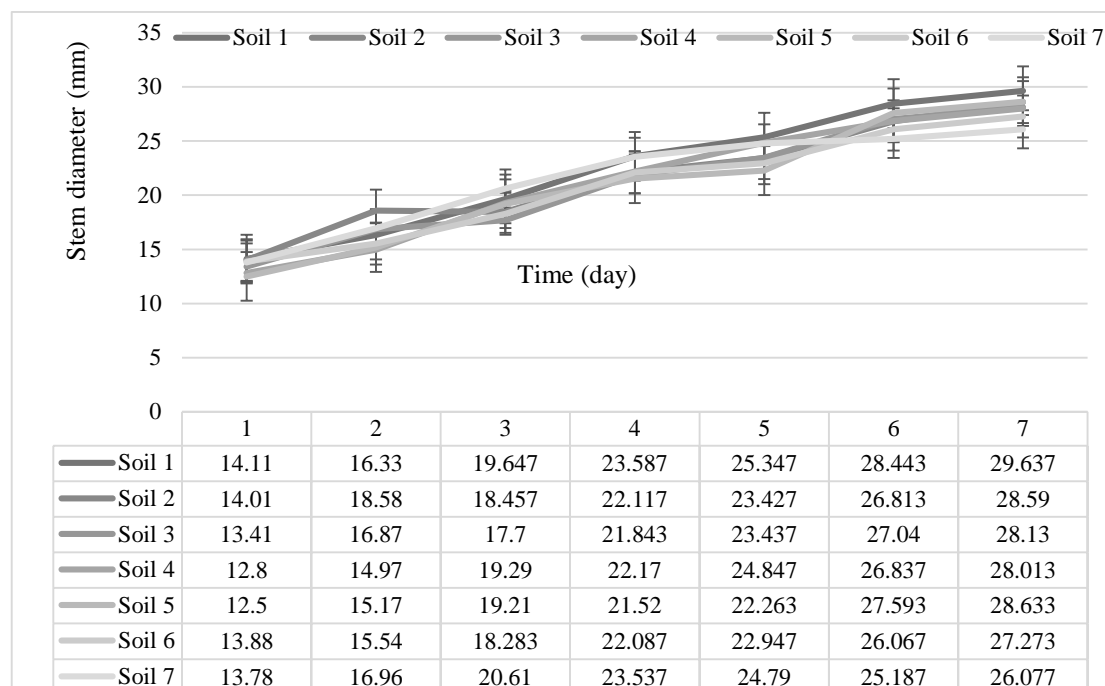


Fig. 3. The stem diameter growth trend of Miyagawa satsuma mandarin on Swingle citrumelo rootstock in different soils.

Table 3. Growth, chlorosis degree and micro-elements concentration of leaves and root of Swingle citrumelo in experimental soils

		Soils						
		1	2	3	4	5	6	7
Dry weight (g seedling ⁻¹)	Roots	201 c	195 c	285 a	164 d	135 e	243 b	201 c
	Leaves	457 b	452 b	500 a	278 cd	265 d	204 c	214 e
Chlorosis degree	Leaves	1.2 d	1.5 d	2.3 c	4 a	2.6 bc	3.1 b	4 a
	Roots	1118 a	1121 a	836 c	837 c	914 bc	830 c	967 b
Fe concentration (mg kg ⁻¹)	Leaves	142 a	94 c	145 a	149 a	119 b	97 c	147 a
	Roots	43	37	40	41	61	22	42
Active Fe concentration (mg kg ⁻¹)	Leaves	50 a	46 a	36 b	43 ab	18 c	33 b	46 a
	Roots	47 a	48 a	38 b	34 b	41 ab	39 b	52 a
Mn concentration (mg kg ⁻¹)	Leaves	8 cd	6 d	7 cd	10 c	13 bc	15 b	35 a
	Roots	64 c	115 b	108 b	102 b	161 a	97 bc	75 c
Zn concentration (mg kg ⁻¹)	Leaves	25 ab	22 ab	29 a	26 ab	31 a	20 b	19 b
	Roots	15 d	39 a	20 c	31 b	33 b	20 c	20 c
Cu concentration (mg kg ⁻¹)	Leaves	5 c	7 bc	4 c	12 a	9 b	5 c	7 bc

Total Fe concentration in roots and leaves

Results indicate that total Fe concentration in the root is much more than the leaves, as, Fe concentration in root was 946 and in leaves was 127 mg kg⁻¹. Root Fe concentration in soils with 2 and 9% lime was 1118 and 1121 mg kg⁻¹ root dry weight respectively, while leaf Fe concentration in the same soils was 142 and 94 mg kg⁻¹ leaf dry weight. So, total Fe concentration in roots is respectively 7.87 and 11.92 times of leaves Fe concentration. In general, total Fe concentration in roots in all soils was about 7.45 times of total Fe concentration in leaves. It shows that the most Fe uptake, accumulates and precipitates in the root. It corresponds with other results in calcareous soils that reported the most absorbed Fe, deposited, and stored in the root apoplastic cells (Kosegarten et al., 1999; Mengel, 1995).

Active Fe concentration in roots and leaves

Miyagawa satsuma mandarin on Swingle citrumelo in the soils with 2, 9 and 45% lime had the highest active Fe concentration in leaves followed by the soils with 30, 14, 25, and 40% lime (Table 3). However, the highest root active Fe concentration was obtained from the soil with 40% lime about 61 mg kg⁻¹. In contrast, the least root active Fe concentration was obtained from the soil with 25% lime. Generally, in soils with 2, 9, 25, 30, and 45% lime, leaf active Fe concentration was more than root active Fe concentration, but in the soils with 14, and 40% lime, root active Fe concentration was more than leaf active Fe concentration. It shows that active Fe concentration in the root and leaves is affected by soil properties. In total, the mean active Fe concentration in the root and leaves in different soils was 41, and 38 mg kg⁻¹, which there was no significant difference between active Fe concentration in roots and leaves (Table 3).

Mn concentration in roots and leaves

Roots in soils with 45, 9, and 2% lime, had the highest Mn concentration: 52, 48, and 47 mg kg⁻¹ (Table 3). Also, leaf Mn concentration in the soil with 45% lime was more than other soils. Leaf Mn concentration in the soil with 45% lime was 34.9 mg kg⁻¹ and in the soils with 25, 40, 30, 2, 14, and 9% lime was 14.7, 12.9, 10, 7.4, 5.9, and 7.6 mg kg⁻¹, which was much less than Mn optimum concentration in leaves of Satsuma mandarin. In general, the mean Mn concentration in the root of Miyagawa Satsuma mandarin on Swingle citrumelo rootstock in different soil was 42.71 and in the leaves was 13.34 mg kg⁻¹. The mean of Mn concentration in the root was 3.2 times of that in the leaves.

Zn concentration in roots and leaves

The means of Zn concentration in roots and leaves (Table 3) indicated that the highest and the least root Zn concentration was obtained from soils with 40, and 2% lime. Root Zn concentration in the soil 5 was 161 and in the soil 1 was 64.4 mg kg⁻¹. The highest leaf Zn concentration was obtained from the soils with 40 and 14% lime. The average Zn concentration in the roots and leaves in different soils was about 103 and 24.6 mg kg⁻¹. The mean of Zn concentration in the roots was about 4.2 times of that in the leaves. Also, in this research, there is a significant linear relation ($R^2 = 0.40^*$) between the root and leaves Zn concentration (Fig. 4).

Cu concentration in roots and leaves

The means of Cu concentration in roots and leaves (Table 3) indicate that roots in the soil with 9% lime had the highest Cu concentration (39.3 mg kg⁻¹). Root Cu concentration in the soils with 40, 30, 14, 25, 45, and 2% placed after soil with 9% lime. The least Cu concentration in the root was 15.13 mg kg⁻¹, which was obtained in the soil with 2% lime. However, the highest and the least Cu concentration in the leaves were 11.9 and 3.8 mg kg⁻¹, which were obtained in the soils with 30 and 14% lime. The average root Cu concentration in all soils was 25.53 and in the leaves was 6.88 mg kg⁻¹. The mean root Cu concentration was about 3.7 times of that in the leaves. The relation between the mean of leaf Cu concentration was shown in Figure 5. This relationship was statistically significant at the 5% level, and with increasing Cu concentration in the roots, Cu concentration increased, too.

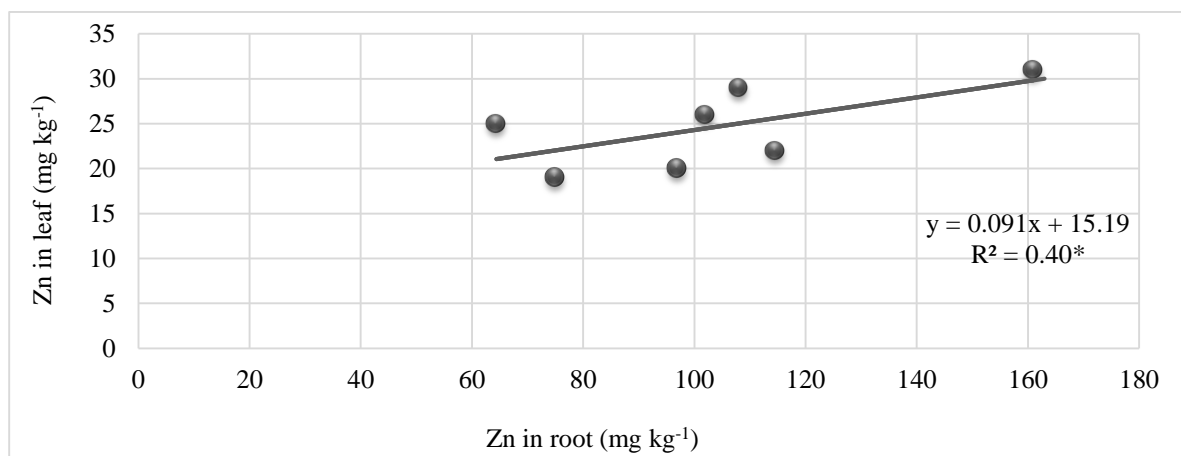


Fig. 4. The relation between Zn concentration in roots and leaves of Miyagawa satsuma mandarin on Swingle citrumelo rootstock.

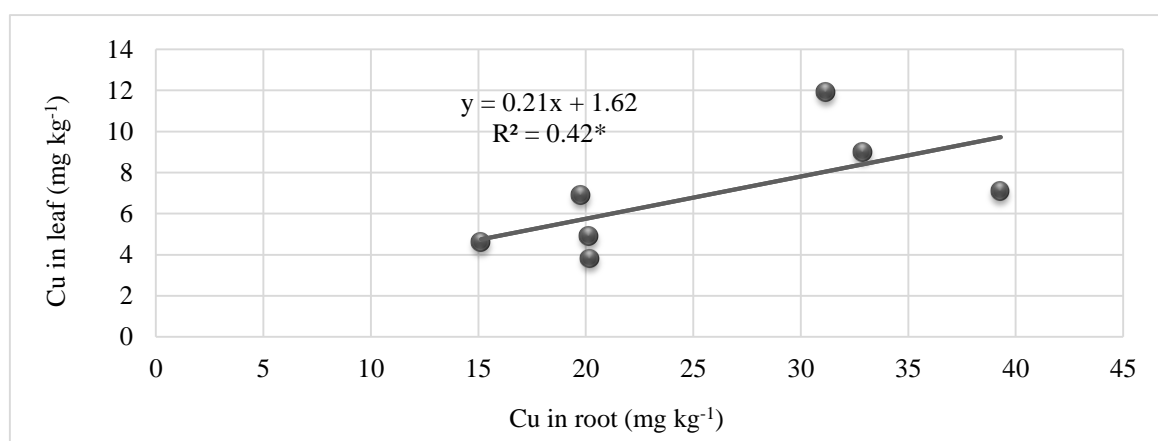


Fig. 5. The relation between Cu concentration in leaves and roots of Miyagawa satsuma mandarin on Swingle citrumelo rootstock.

The relation of the soil active Fe with the leaf active Fe

In Miyagawa satsuma mandarin on Swingle citrumelo rootstock, with increasing active lime of soils, active Fe concentration of leaves decreased (Fig. 6). Regarding slope of the regression line, its intensity was equal to a 2.04 unit reduction in leaf active Fe concentration per each unit increase in soil active lime concentration, which was statistically significant at a 1% level ($R^2 = 0.96^{**}$). The effect of increasing active lime on leaf active Fe in Swingle citrumelo showed that with growing soil active lime up to about 5%, leaf active Fe concentration was almost constant. More increasing of active lime, intensified the trend of reducing active Fe concentration. According to this study, the tolerable active lime for Swingle citrumelo is by 5%.

Overall, this research showed that the highest shoot dry weight was obtained from the soils with maximum 14% lime or with 5% active lime. By increasing total lime or active lime, shoot average dry weight was reduced, severely. Also, the least leaf chlorosis was in the soils 1 and 2, and the active Fe in the leaves was also higher in these soils.

Sufficient available Fe in the soil (DTPA extractable) for citrus trees is 4-5 mg kg⁻¹ (Asadi Kangarshahi & Akhlaghi Amiri, 2014a; 2020). The range of available Fe in experimental soils was 4.40-8.90 mg kg⁻¹, so the amount of available Fe was more than adequate. In the soils 1 and 2, which had the lowest leaf chlorosis, available Fe content was

7.20 and 6.40 mg kg⁻¹, respectively. However, the highest degree of leaf chlorosis was observed in the soils with 30 and 40% lime, which their available Fe was 8.90 to 6.80 mg kg⁻¹. Therefore, the leaf chlorosis is depends on other factors than the amount of available Fe. These yellowish symptoms are not only due to reduced useable Fe, but also, soil lime, soil solution bicarbonate, soil biological and physical properties are major factors to controlling Fe concentration in soil solution and play a significant role in preparing Fe for trees in calcareous soils (Asadi Kangarshahi & Akhlaghi Amiri, 2014b; 2018). There was only a significant relationship between active lime amounts and leaf active Fe. Therefore, the amount of active lime is the most critical soil characteristic to predict chlorosis degree for Swingle citrumelo. This study indicated that Satsuma mandarin trees on Swingle citrumelo rootstock in calcareous soils with more than 9% total lime or with more than 3% active lime, showed chlorosis while had more available Fe (Fig. 7).

There are two forms of Fe in the soil solution: Fe²⁺ and Fe³⁺. The relative amount of them depends on pH and pe of the soil solution. Plants, including citrus trees, mainly absorb Fe in the form of Fe²⁺. In the outer membrane (plasmalemma) of the root tip, Fe³⁺ reductase enzyme allows reduction of Fe³⁺ to Fe²⁺, then Fe²⁺ uptake by root cells. The activity of this enzyme depends on pH, and high pH reduces its activity (Ammari & Mengel, 2006; Kosegarten et al., 2004). The high pH of soil solution and root apoplasts in calcareous soils and its high buffer strength reduce the activity of Fe reductase enzyme and precipitate it in the wall of root cells. In this case, it is possible Fe usability, availability and concentration in the soil solution, and apoplast of the root cell wall be high, but plants suffer from Fe deficiency (Ammari & Mengel, 2006).

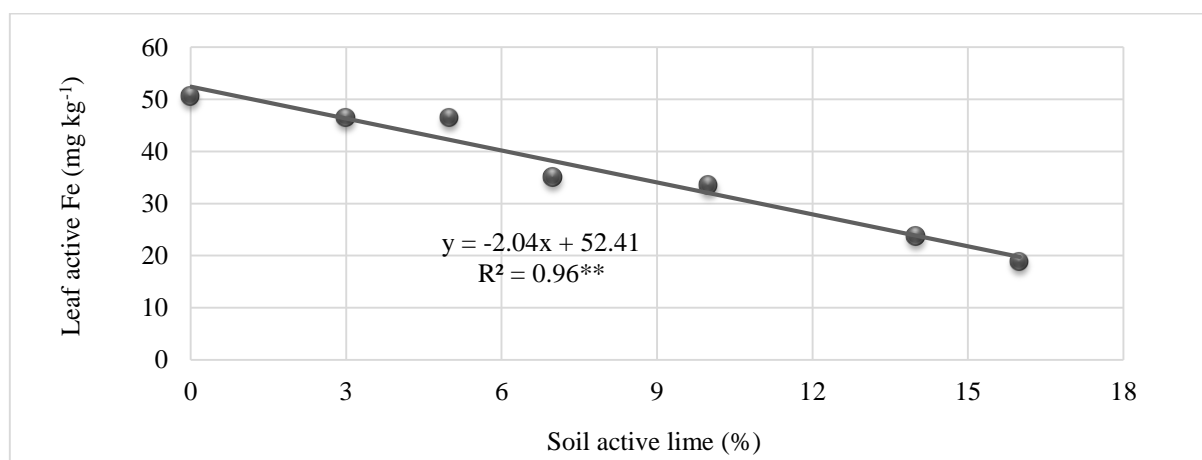


Fig. 6. The effect of the increasing soil active lime on the leaf active Fe concentration in Miyagawa satsuma mandarin on Swingle citrumelo rootstock.

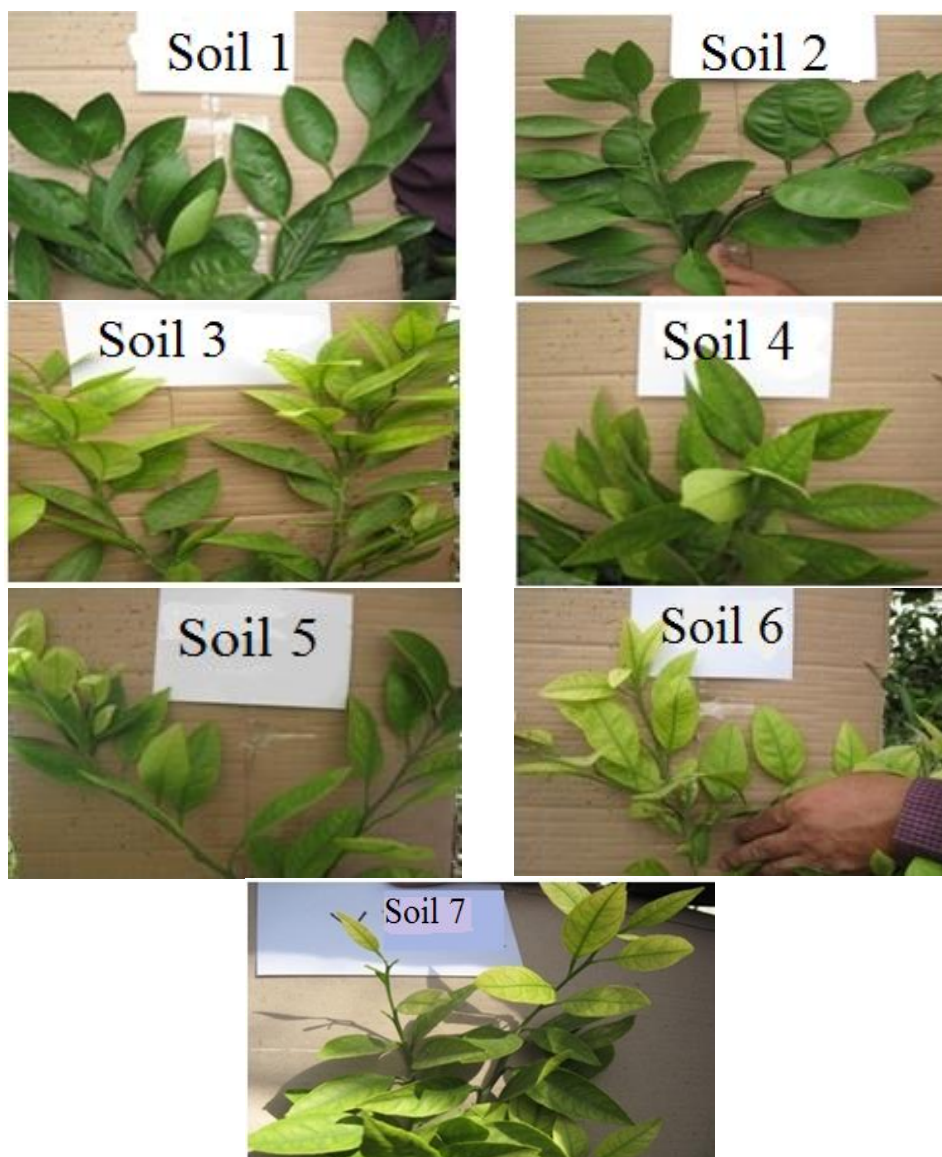


Fig. 7. Symptoms of leaf chlorosis degree of Miyagawa satsuma mandarin on Swingle citrumelo rootstock in calcareous soils (soils characteristics are describe in [Table 2](#)).

According to this study, in the soils with more than 9% total lime, even if available Fe is optimal, leaf chlorosis happens. Also, the root total Fe was about 7.45 times of that in the leaf, which indicates Fe accumulation and deposition in the apoplast of root cells ([Ammari & Mengel, 2006](#); [Kosegarten et al., 2004](#)). These results correspond with other researches in calcareous soils ([Asadi Kangarshahi & Akhlaghi Amiri, 2020](#); [Fu et al., 2016](#); [Kosegarten et al., 1999](#); [Mengel & Kirkby, 2001](#)). In general, this research showed that Satsuma mandarin trees on Swingle citrumelo rootstock showed leaf chlorosis in calcareous soils with more than 14% lime or 5% active lime. So, using this rootstock for Satsuma mandarin in soils with more than 14% lime is not recommended that correspond with [Albrecht et al. \(2018\)](#), [Donnini et al. \(2009\)](#) and [Castle and Nunnallee \(2009\)](#).

Reports of [Asadi Kangarshahi and Akhlaghi Amiri \(2014a; 2018\)](#) show that critical available Mn and Zn in the soil (DTPA extractable) for citrus trees is 2.5 and 1.5-2 mg kg⁻¹, respectively. The range of available Mn in tested soils was 3.10-7.71 mg kg⁻¹ which was more than sufficient. Also, Mn and Zn critical level in leaves of Satsuma mandarin trees are 25-80

and 25-50 mg kg⁻¹, respectively (Asadi Kangarshahi & Akhlaghi Amiri, 2014a; 2018; Asadi Kangarshahi et al., 2011). Leaf analysis in this experiment showed that the range of leaf Mn concentration in soils 1 to 6 was 14.7 to 15.9 mg kg⁻¹, but in the soil 7, the leaf Mn concentration was 34.9 mg kg⁻¹. Mn deficiency is widespread in calcareous soils with high organic matter and in silt clay soils with high lime and organic matter and poor drainage (Asadi Kangarshahi & Akhlaghi Amiri, 2014a; 2018; Asadi Kangarshahi et al., 2006; Mortvedt et al., 1991). According to critical available Zn in the soil for citrus trees, in this study, available Zn in the soils with 14, 30, and 40% lime was less than sufficient, in the soils with 25 and 45% lime was sufficient and in the soils with 2 and 9% lime was more than sufficient. Results showed that leaf Zn concentration in the soils with 2, 14, 30, and 40% lime was sufficient and in the soils with 9, 25, and 45% lime was less than sufficient which indicates that there was not a significant relation between available Zn and leaf Zn concentration in this research. However, soils with 25 and 45% lime and high organic matter had the lowest leaf Zn concentration. In soils with high organic matter, Zn reaction with humic acids and humin may result in the formation of Zn stable complexes with high molecular weight which can reduce the usability of Zn (Asadi Kangarshahi & Akhlaghi Amiri, 2014a; Asadi Kangarshahi & Malakouti, 2007; Mortvedt et al., 1991). Also, an increase in soil active lime and pH of soil solution, generally increase Zn uptake by soil structural components, sedimentation, also decrease Zn transfer from soil solution to root surface, which could reduce the usability of Zn (Asadi Kangarshahi & Akhlaghi Amiri, 2014a; Asadi Kangarshahi & Malakouti, 2007; Mortvedt et al., 1991).

CONCLUSION

Results showed that there is no significant correlation between soils available Fe and Fe concentration in the roots and leaves and also between Fe of the roots and leaves. Soils with the highest chlorosis had a lower ratio of the root Fe to the leaf Fe concentration. So, measuring available Fe in the soil is not a suitable criterion for predicting leaf chlorosis degree in citrus trees or at least in this scion and rootstock. Fe deficiency in the early stages of growth when spring flashes occur leads to slower growth of new leaves and a decrease in leaf size. If Fe deficiency occurs during leaf development, it reduces chlorophyll concentration and leads to chlorosis. According to the results, in calcareous soils, Fe deficiency is due to its precipitate in the apoplast of the leaf and the root cells and decrease its physiological efficiency; as only 42% of Fe in the root and 30% of Fe in the leaf was in the active form.

Results also indicated that Mn deficiency in the leaves of citrus trees in the north of Iran is not because of Mn deficiency in the soil, indeed it is due to its low transfer efficiency from the root to the shoot.

According to this research, it is recommended that in soils with more than 14% total lime and 5% active lime, Swingle citrumelo rootstock should not be used. It is suggested that similar studies be carried out on growth trends and responses of developing rootstocks in the east of Mazandaran like Troyer citrange, Carrizo citrange, and C-35 in the soils of this region.

Conflict of interest

The authors have no conflict of interest to report.

REFERENCES

- Albrecht, U., Alferez, F., & Zekri, M. (2018). *Rootstock and scion selection*. Florida Citrus Production Guide, University of Florida Publications.
- Ammari, T., & Mengel, K. (2006). Total soluble Fe in soil solution of chemically different soils. *Geoderma*, 136, 876-885. <https://doi.org/10.1016/j.geoderma.2006.06.013>
- Asadi Kangarshahi, A., & Akhlaghi Amiri, N. (Ed.). (2014a). *Advanced and applied citrus nutrition (1)*. Tehran: Education and Agricultural Promotion Publications.
- Asadi Kangarshahi, A., & Akhlaghi Amiri, N. (Ed.). (2014b). *Advanced and applied citrus nutrition (2)*. Tehran: Education and Agricultural Promotion Publications.
- Asadi Kangarshahi, A., & Akhlaghi Amiri, N. (Ed.). (2018). *Establishment of sustainable citrus orchard*. Tehran: Education and Agricultural Promotion Publications.
- Asadi Kangarshahi, A., & Akhlaghi Amiri, N. (2020). Evaluation of growth rate and vegetative and physiological characteristics of Satsuma mandarin on C-35 rootstock in some calcareous soils. *Journal of Soil Research*, 34(2), 215-234.
- Asadi Kangarshahi, A., & Malakouti, M.J. (2007). Effect of Zn application on growth and Zn absorption and concentration in soybean. *Iranian Journal of Agricultural Science*, 38(2), 321-328.
- Asadi Kangarshahi, A., Akhlaghi Amiri, N., & Malakouti, M.J. (2011). Effect of 4 years using of Zn on yield and quality of Sangin orange. *Iranian Journal of Soil and Water Research*, 42(1), 77-86.
- Asadi Kangarshahi, A., Malakouti, M.J., & Charati, A. (2006). Mn calibration under field condition and its role in yield of soybean. *Iranian Journal of Agricultural Science*, 37(5), 869-845.
- Bashour, I., & Sayegh, A. A. (Ed.). (2007). *Methods of analysis for soils of arid and semi-arid regions*. Rome: Food and Agriculture Organization of the United Nations.
- Byrne, D. H., Rouse, R. E., & Sudahono, D. H. (1995). Tolerance to citrus rootstocks to lime induced iron chlorosis. *Subtropical Plant Science*, 47, 7-11.
- Castle, B., & Stover, E. (2001). *Update on use of Swingle citrumelo rootstock*. Florida: Institute of Food and Agricultural Sciences.
- Castle, W. S., & Nunnallee, J. (2009). Screening citrus rootstocks and related selections in soil and solution culture for tolerance to low-iron stress. *Horticultural Science*, 44, 638-645. <https://doi.org/10.21273/hortsci.44.3.638>
- Chen, Y., & Barak, P. (1982). Iron nutrition of plants in calcareous soils. *Advances in Agronomy*, 35, 217-240. [https://doi.org/10.1016/s0065-2113\(08\)60326-0](https://doi.org/10.1016/s0065-2113(08)60326-0)
- Davies, F. S., & Albrigo, L. G. (1994). *Citrus*. Wallingford: CAB International.
- Donnini, S., Castagna, A., Ranieri, A., & Zocchi, G. (2009). Differential responses in pear and quince genotypes induced by Fe deficiency and bicarbonate. *Journal of Plant Physiology*, 166, 1181-1193. <https://doi.org/10.1016/j.jplph.2009.01.007>
- Embleton, T. W., Jones, W. W., Labanauskas, C. K., & Reuther, W. (1973). Leaf analysis as a diagnostic tool and guide to fertilization. In *The Citrus Industry* (Reuther W ed.). University of California Press, California, USA, 3, 183-210.
- Fu, L., Chai, L., Ding, D., Pan, Zh., & Peng, Sh. (2016). A novel citrus rootstock tolerance to iron deficiency in calcareous soil. *Journal of the American Society for Horticultural Science*, 141(2), 112-118. <https://doi.org/10.21273/JASHS.141.2.112>
- Gee, G. W., & Bauder, J. W. (1986). Particle size analysis. In *Methods of Soil Analysis*, Part1. (Klute A ed.). SSSA, Madison, WI. 383-411. <https://doi.org/10.17660/actahortic.2000.531.15>
- Katyal, J. C., & Sharma, B. D. (1980). A new technique of plant analysis to resolve iron chlorosis. *Plant and Soil*, 55, 105-119. <https://doi.org/10.1007/bf02149714>
- Kosegarten, H., Hoffman, B., Rocco, E., Grolig, F., Gluesenkamp, K., & Mengel, K. (2004). Apoplastic pH and FeIII reduction in young sunflower (*Helianthus annuus*) roots. *Physiologia Plantarum*, 122, 95-106. <https://doi.org/10.1111/j.1399-3054.2004.00377.x>
- Kosegarten, H., Hoffmann, B., & Mengel, K. (1999). Apoplastic pH and Fe³⁺ reduction in intact sunflower leaves. *Plant Physiology*, 121, 1069-1079. <https://doi.org/10.1104/pp.121.4.1069>
- Levy, Y., & Shalheret, J. (1990). Ranking the salt tolerance of citrus rootstocks by juice analysis. *Scientia Horticulturae*, 45, 89-98. [https://doi.org/10.1016/0304-4238\(90\)90071-1](https://doi.org/10.1016/0304-4238(90)90071-1)

- Lindsay, W. L., & Norvel, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal*, 42, 421-428. <https://doi.org/10.2136/sssaj1978.03615995004200030009x>
- Loeppert, R. H., Wei, L. C., & Ocumpaugh, W. R. (1994). Soil factors influencing the mobilization of iron in calcareous soils. In *Biochemistry of Metal Micronutrients in the Rhizosphere* (Manthey J A, Crowley D A and Luster D G eds.). Lewis Publishers, Boca Raton, United States, 343-360.
- Louzada, E. S., Del Rio, H. S., Setamou, M., Watson, J. W., & Swietlik, D. M. (2008). Evaluation of citrus rootstocks for the high pH, calcareous soils of South Texas. *Euhytica*, 164, 13-18. <https://doi.org/10.1007/s10681-008-9701-x>
- Manthey, J. A., McCoy, D. L., & Crowley, D. E. (1994). Stimulation of rhizosphere iron reduction and uptake in response to iron deficiency in citrus rootstocks. *Plant Physiology Biochemistry*, 32, 211- 215.
- Marchal, J. (1984). Citrus. In *Plant analysis as a guide to the nutrient requirements of temperate and tropical crops* (Martin-Prevel P, Gagnard J and Gautier P eds.). Lavoisier Publishing INC, New York, USA, 320-354.
- Martinez Cuenca, M. R., Primo Capella, A., Quinones, A., Bermejo, A., & Forner Giner, M. A. (2017). Rootstock influence on iron uptake responses in citrus leaves and their regulation under the Fe paradox effect. *Peer Reviewed Journal*, 5, 1-25. <https://doi.org/10.7717/peerj.3553>
- Mclean, E. O. (1982). Soil pH and lime requirement. In *Methods of soil analysis*, Part 2 (Page A L ed.). Madison publisher, Wisconsin, USA, 199- 224. <https://doi.org/10.2134/agronmonogr9.2.2ed.c12>
- Mengel, K., & Kirkby, E. (Ed.). (2001). *Principles of plant nutrition*, 5th edition. Dordrecht, the Netherlands: Kluwer Academic Publisher.
- Mengel, K. (1995). Iron availability in plant tissues-iron chlorosis in calcareous soils. In *Iron Nutrition in Soils and Plant* (Abadia J ed.). Kluwer Academic Publishers, Dordrecht, the Netherlands, 389-397. https://doi.org/10.1007/978-94-011-0503-3_53
- Mortvedt, J. J., Cox, F. R., Shuman, L. M., & Welch, R. M. (Ed.). (1991). *Micronutrients in Agriculture*. Wisconsin, USA: SSSA Book Series, Soil Science Society of America, Madison publisher.
- Neaman, A., & Aguirre, L. (2007). Comparison of different methods for diagnosis of iron deficiency in avocado. *Journal Plant Nutrition*, 30, 1098-1108. <https://doi.org/10.1080/01904160701394550>
- Nelson, D. W., & Sommers, L. E. (1982). Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis*, Part 2 (Page A L ed.). Soil Science Society of America, Madison publisher, Wisconsin, USA.
- Olsen, S. R., & Sommers, L. E. (1982). Phosphorus. In *Methods of Soil Analysis*, Part 2 (Page A L Ed.). Soil Science Society of America, Madison Publisher, Wisconsin, USA, 403-430.
- Pestana, M., David, M., de Varennes, A., Abadia, J., & Faria, E. A. (2001). Responses of Newhall orange trees to iron deficiency in hydroponics: effects on leaf chlorophyll, photosynthetic efficiency and root ferric chelate reductase activity. *Journal of Plant Nutrition*, 24, 1609-1620. <https://doi.org/10.1081/pln-100106024>
- Pestana, M., Varrnnes, A., Abadia, J., & Araujo Faria, E. (2005). Differential tolerance to iron deficiency of citrus rootstocks grown in nutrient solution. *Scientia Horticulturae*, 104, 25-36. <https://doi.org/10.1016/j.scienta.2004.07.007>
- Qrtiz, P. R., Meza, B. J. C., Garza Requena, F. R., Flores, G. M., & Etchevers Barra, J. D. (2007). Evaluation of different iron compound in chlorotic Italian lemon. *Plant Physiology and Biochemistry*, 45, 330-334. <https://doi.org/10.1016/j.plaphy.2007.03.015>
- Sudahono, Byrne, D. H., & Rouse, R. E. (1994). Greenhouse screening of citrus rootstocks for tolerance to bicarbonate-induced iron chlorosis. *Horticultural Science*, 29, 113-116. <https://doi.org/10.21273/hortsci.29.2.113>
- Wright, G. C., Tilt, P. A., & Pena, M. A. (1999). *Results of scion and rootstock trials for citrus in Arizona*. Final report for project 98-12. University of Arizona, College of Agriculture.

- Wutscher, H. K., Maxwell, N. P., & Shull, A. V. (1975). Performance of nucellar grapefruit (*Citrus Paradisi Macf.*) on 13 rootstocks in south Texas. *Journal of the American Society for Horticultural Science*, 100, 48-51.
- Yang, L., Li, G., Lin, Q., & Zhao, X. (2010). Active carbonate of chestnut soils in different lands. *Ecology Environmental Science*, 19, 428-432.