Screening citrus rootstocks for iron-deficiency tolerance

William S. Castle[a*]
John A. Manthey[b]

[a] University of Florida, IFAS,
Citrus Research and Education
Center, 700 Experiment Station
Road, Lake Alfred, FL 33850, USA

[b] U.S. Citrus and Subtropical
Products Laboratory, USDA, ARS,
600 Avenue S, NW, Winter Haven,
FL 33881, USA

* *Correspondence and reprints*

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Abstract — Introduction. High-carbonate soils are a serious limiting factor for citrus growth because they limit the availability of several micronutrients, particularly iron (Fe). The usual solutions to micronutrient problems are to either not plant in such soils or use expensive chelates or use low-Fe stress tolerant rootstocks. The objective of the study was to screen a broad range of citrus genotypes for tolerance to low-Fe stress. **Materials and methods**. Plants of 26 *Citrus* and closely related genotypes were assayed for their ability to reduce Fe^{3+} as a measure of their tolerance to Fe-deficiency stress. Three-month-old seedlings were grown for 2 to 3 months in nutrient solution without Fe. White root tips were periodically harvested and tested for their Fe reduction capacity. **Results and discussion**. Genotypes with the largest Fe reduction responses were Volkamer and Eureka lemon, Etrog citron, sour orange, and Rangpur. Several genotypes showed low to intermediate Fe reduction responses. The poorest responses were from Swingle citrumelo, Duncan grapefruit, Thong Dee pummelo, Ridge Pineapple sweet orange, trifoliate orange, and a number of papeda selections. The results complement previous rankings of low-Fe stress tolerance, and suggest that the high tolerance among certain *Citrus* genotypes may originate with *C. medica*. Also, Rangpur plants acidified the unbuffered nutrient solution to pH values below 5, and had lower Fe reduction rates than the plants in buffered solution. (© Elsevier, Paris)

***Citrus* / rootstocks / variety trials / calcareous soils / chlorosis / iron**

Recherche de porte-greffes d'agrumes tolérants à une déficience en fer.

Résumé — Introduction. Des sols très calcaires limitent très fortement le développement des agrumes parce qu'ils diminuent la disponibilité en certains microéléments, en fer particulièrement. Pour pallier le manque de microéléments, plusieurs solutions sont habituellement utilisées : absence de plantation dans de tels sols, utilisation de coûteux chelates ou plantation de porte-greffes tolérants aux stress dus à de faibles teneurs en fer. L'étude a permis d'évaluer une large gamme de génotypes d'agrumes vis-à-vis de ce caractère de tolérance. **Matériel et méthodes**. Les plants de 26 espèces de *Citrus* et de génotypes proches ont été testés pour leur capacité à réduire l'ion Fe^{3+} afin de mesurer leur tolérance vis-à-vis d'une déficience en fer. Des plantules de 3 mois ont été placées pendant 2 à 3 mois dans une solution nutritive sans fer. Des pointes de racines blanches ont régulièrement été prélevées et leur aptitude à réduire le fer a été testée. **Résultats et discussion**. Les génotypes donnant la meilleure réponse pour la réduction du fer ont été les citronniers Volkamer et Eureka, le cédratier Etrog, ainsi que le bigaradier et la lime Rangpur. D'autres génotypes ont donné des réponses faibles ou modérées. Les moins bonnes réponses ont été celles du citrumelo Swingle, du pomélo Duncan, du pamplemoussier Thong Dee, des orangers Ridge Pineapple et trifolié, ainsi que de certaines sélections de papeda. Les résultats complètent de précédents classements de tolérance aux stress et suggèrent que les fortes tolérances trouvées chez certains génotypes de *Citrus* pourraient provenir de *C. medica*. Par ailleurs, les plants de Rangpur ont acidifié une solution nutritive non tamponnée jusqu'à des valeurs de pH 5 et ont eu de plus faibles taux de réduction que les plants placés en solutions tamponnées. (© Elsevier, Paris)

***Citrus* / porte greffe / essai de variété / sol calcaire / chlorose / fer**

1. introduction

A cursory survey of the literature quickly shows the worldwide significance of micronutrient deficiencies associated with high-carbonate soils [1–5]. Problems occur with many crop plants including perennial tree crops like citrus [6]. High-carbonate soils are a serious limiting factor in many areas where citrus is grown because they limit the availability of several micronutrients, particularly iron (Fe) [3]. In situations where the deficiency is left uncorrected, citrus and other plants become unproductive [7]. The usual solutions to micronutrient problems are to either not plant in such soils or use expensive soil-applied chelates, or foliar applications. Another solution is to use low-Fe stress tolerant rootstocks. Unfortunately, the number of such citrus rootstocks that are also satisfactory for other important horticultural traits is limited [6, 8–10].

The search for suitable citrus rootstocks for high pH / high-carbonate soils has generally been based on screening tests [11] and rootstock trials [12] to determine resistance to 'lime-induced chlorosis.' Many of these germplasm evaluations have been conducted in local high-carbonate soils either in natural field sites [13–15] or in containers filled with the local soil [1, 4]. Some tests have involved solution culture [5, 11]. The experimental conditions and procedures vary considerably among the different evaluations. Tests have been run with native soils that range from 0% to > 80% total $CaCO_3$, and with nutrient solutions that do or do not contain Fe. Many trials are based on the presence of bicarbonate as a presumed critical component in any chemical system causing lime-induced chlorosis [2]. The final evaluation of the plant material is often based on chlorosis ratings, and measurements of plant Fe concentration.

Plants have evolved several mechanisms to tolerate low-Fe stress [3]. In dicots, there are a number of inducible root responses including elevated rates of electron release at root surfaces that enable Fe^{3+} reduction reactions to occur, and acidification that helps liberate Fe from unavailable sources in the rhizosphere environment, and possibly within the plant itself.

There is evidence for these responses occurring in citrus [16]; therefore, our objectives were to screen a broad range of citrus genotypes for tolerance to low-Fe stress including representatives from various taxonomic groups within the orange subfamily, and to characterize the physiological changes in citrus roots during Fe stress. In this report, we present results associated primarily with the first objective.

2. materials and methods

Screening runs were conducted in 1994 at the U.S. Department of Agriculture facility at Pasadena, CA, and in 1995 and 1996 at the University of Florida, CREC, Lake Alfred, FL. Seeds were obtained from arboreta in California and Florida. Seeds were sown generally in the spring in temperature-controlled greenhouses with natural lighting. When the plants were about 10 cm tall, they were removed from their containers, washed free of the potting soil, and transferred to 40-liter plastic tubs filled with nutrient solution. Each tub held 50 plants of one genotype. The plants were suspended in the lid of the tub and secured with a soft foam stopper in 3-cm diameter holes spaced equally over the lid. Each tub was filled with about 30 L of solution containing 1.3 mM $Ca(NO_3)_2$, 1.0 mM KNO_3, 0.8 mM $MgSO_4$, 0.1 mM K_2HPO_4, 0.56 µM $ZnSO_4$, 6.7 µM $MnSO_4$, 0.24 µM $CuSO_4$, 0.2 µM Na_2MoO_4, and 33.0 µM boric acid. The solutions contained no added Fe, but about 0.15 ppm (2.7 µM) were present in the tap water used to make the solutions. All solutions were aerated and maintained in the pH range 7.5 to 8.0. The solutions were changed about every 3 weeks; pH was monitored regularly with a standard electrode and adjusted as needed with dilute KOH or H_2SO_4.

Relative root Fe^{3+} reduction rates were measured, as described by Manthey et al. [16, 17], over a 3- to 4-month period beginning usually in early summer. The assays involved collecting 20 to 30 white root tips, 1.0 to 1.5 cm long, from among the 50 plants of each genotype. The root tips were placed in a solution containing nutrients, 0.3 mM FeHEDTA, and 0.2 mM bathophenanthrolinedisulfonic acid (BPDS). Duplicate sets of root tips were collected every 7 to 10 d and assayed under darkened conditions at 33 °C in a shaker water bath. The amount of Fe^{2+} $(BPDS)_3$ formed was measured colorimetrically at 536 nm in 2 h intervals for a 6 h period. These data were combined with root dry weights to calculate Fe reduction rates. The assays were continued until it was clear that a genotype peak value had been obtained. The control reduction rates were determined from the first new white root tips that appeared after the plants were transferred. The subsequent assays occurred as the plants became chlorotic and are labelled as such in *table I*. Interpretation of the results is based on the chlorotic rates.

The 1996 screening run included two sets of Rangpur plants. Previous runs showed that Rangpur responded to low-Fe stress by acidifying the solution; thus, plants in one tub were allowed to grow without any solution pH adjustment while the solution in the second tub was maintained between 7.5 and 8.0.

The control Fe reduction rates and their associated standard deviations, and the peak rates are presented. If a genotype was included in more than one run, a typical value was chosen for statistical analysis. The data were tested for significant differences using a modified Moses procedure specifically developed for 'best' or peak values that are not normally distributed, thus rendering analysis of variance inappropriate [18]. The procedure involves a nonparametric test based on maximum ranks assigned to each genotype in a joint ranking across all assays.

3. results and discussion

Shoot and root growth generally commenced within a few days after the seedlings were transferred to the minus-Fe solutions, and mild leaf-vein symptoms of Fe-deficiency chlorosis appeared in some genotypes soon afterwards. New roots were produced continuously throughout the assay period. New leaves developed typical chlorosis symptoms ranging from those that were small and white, to normal-sized leaves with mild interveinal chlorosis. The more severe symptoms were generally associated with those genotypes that had the lowest Fe reduction rates (*table I*).

The control Fe reduction rates ranged from 1.7 to 5.1, but most values were clustered around the mean of 3.0. Volkamer lemon, Etrog citron, Eureka lemon, Rangpur (in buffered nutrient solution), and sour orange plants had the highest 'chlorotic' rates. The mandarin plants had mostly intermediate rates. The genotypes from the papeda subgenus as well as the pummelo, grapefruit, sweet orange, and trifoliate orange cultivars had the lowest rates which were not greatly above their respective control rates. In the various horticultural or taxonomic groups examined, round orange, grapefruit, and pummelo do not appear to be useful sources of low-Fe stress tolerance. Similar results were obtained in several screening trials conducted in Texas with high-carbonate soil [7].

Trifoliate orange is well known for poor tolerance to low-Fe stress associated with high-carbonate soils. This trait is transmitted to the citrange and citrumelo hybrids of trifoliate orange, but not apparently to the same extent in each kind of hybrid. The Morton and C-35 citrange plants had greater responses in our trial than Swingle citrumelo plants, a difference reported previously [16]. In a field trial with soil, Morton plants showed only mild chlorosis symptoms after 10 months [15].

Changsha (data not given) and Nasnaran were the poorest within the mandarin group. Shekwasha mandarin plants had lower rates than those of Cleopatra and Sun Chu Sha although each of these three rootstocks is generally reported to be suitable for use in calcareous soils [6–8]. Shekwasha and Cleopatra have been field tested under virtual limerock conditions in Florida [13]. They were rated highly for their adaptation to the soil, but neither were horticulturally acceptable when budded with Tahiti lime [13]. Shekwasha plants had no chlorosis in a Texas

Table I.
Control rates (± SD) and peak chlorotic rates (μmol $Fe^{3+}\cdot h^{-1}\cdot g^{-1}$ dry weight) of Fe^{3+} (FeHEDTA) reduction by white root tips of citrus rootstocks and other genotypes.

Plant	Fe^{3+} reduction rate	
	Control	Chlorotic
Volkamer lemon, *C. volkameriana* Ten. and Pasq.	3.7 ± 0.2	23.0 a[v]
‘Eureka‘ lemon, *C. limon* L.	3.8 ± 0.6	21.3 a
Sour orange, *C. aurantium* L.	4.5 + 0.5	17.5 a
‘Etrog‘ citron, *C. medica.* L.	3.9 ± 0.7	16.8 ab
Rangpur (buffered soln.), *C. limonia* Osbeck	2.8 ± 0.7	13.4 ab
Sun Chu Sha mandarin,[w] *C. reticulata* Blanco	3.6 ± 0.7	9.5 bc
Cleopatra mandarin	4.7 ± 1.4	8.4 bc
Morton citrange [*C. sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.]	2.9 ± 0.3	7.9 c
Mexican lime, *C. aurantifolia* (Christm.) Swing.	5.1 ± 0.2	7.8 c
Shekwasha mandarin, *C. depressa* Hayata	2.3 ± 0.3	6.6 cd
Rangpur (buffered solution)	2.5 ± 04	6.3 cd
C-35 citrange	2.5 ± 0.1	6.3 cd
C. ichangensis, Swing.	2.9 ± 0.3	6.3 cd
Moli kurikuri (*C. macroptera* hybrid)[x]	(not avail.)	5.8 d
C. latipes Hook. f. and Thomas	2.7 ± 0.2	5.6 d
‘Thong Dee‘ pummelo, *C. grandis* L.	3.1 ± 0.1	5.1 d
Gou Tou (putative hybrid)	2.4 ± 0.1	4.7 de
Swingle citrumelo[y] [*C. paradisi* Macf. × *P. trifoliata*]	3.4 ± 0.7	4.4 de
Smooth Flat Seville[z] (putative hybrid)	3.1 ± 0.5	4.4 de
‘Duncan‘ grapefruit, *C. paradisi*	2.6 ± 0.6	4.0 de
Kinkoji, *C. obovoidea* Hort. ex Tak.	2.7 ± 0.1	3.8 de
C. hanaju Hort. ex Shirai	2.7 ± 0.3	3.4 e
C. micrantha Wester	2.7 ± 0.4	3.3 e
Nasnaran, C. *amblycarpa* (Hassk.) Ochse	1.7 ± 0.2	3.2 e
‘Mark’ trifoliate orange, *P. trifoliata*	2.4 ± 0.1	3.1 e
‘Ridge Pineapple‘ sweet orange, *C. sinensis*	1.9 ± 0.3	2.9 e
Ichang lemon (*C. ichangensis* × *C. grandis*)	2.2 ± 0.2	2.6 e

[v] Significant differences are based on a nonparametric analysis [18].
[w] Sun Chu Sha was included in three screening runs. For two runs, the Fe-deficient plants reduced Fe^{3+} at rates similar to the one listed in this table; however, in the third assay, the rates for the Fe-deficient plants were as high as 17.5 with control rates of 3.3 ±0.2. This would indicate a higher tolerance for Sun Chu Sha.
[x] Possibly a hybrid of *C. macroptera* and *C. grandis* [23].
[y] Two previous analyses showed a rate for the Fe-deficient Swingle plants to be approximately twice the control rate [16].
[z] In one of the screening runs, the Fe-deficient Smooth Flat Seville plants reduced Fe^{3+} at rates as high as 8.7 with control rates of 4.4 ± 0.4.

study involving calcareous soil [15], but recent trials in Florida (being conducted by Castle) confirm the previous horticultural conclusions of Campbell [13]. Sun Chu Sha was released by the U.S. Department of Agriculture as a rootstock tolerant to calcareous soils. This has not been fully demonstrated to date, but our screening results are encouraging and suggest that Sun Chu Sha may be superior to Cleopatra.

Smooth Flat Seville is a natural hybrid of unknown taxonomic status that apparently originated in Australia [19]. It has attracted interest in Florida as a possible substitute for sour orange [20]. Its likely parents include pummelo (poorly tolerant to low-Fe stress) and sour orange (highly tolerant) [19, 21]. Because of this parental mixture, and the large variation that occurs among its seedlings [20], the response of Smooth Flat Seville to low-Fe stress can be expected to vary at it did in our screening runs (*table I* and footnote). Nevertheless, the Smooth Flat Seville plants typically had relatively low responses in our study, thus, it is interesting that a Smooth Flat Seville hybrid with poorly tolerant trifoliate orange performed relatively well in another screening study [11].

Gou Tou and Kinkoji are two additional rootstocks being evaluated in Florida as possible sour orange replacements [20]. Gou Tou has performed well when infected with some of the world's severest isolates of citrus tristeza virus. Smooth Flat Seville and Kinkoji are growing satisfactorily in Florida with Hamlin sweet orange scion infected with local severe tristeza isolates. Smooth Flat Seville, Gou Tou, and Kinkoji have not been adequately tested in field situations for tolerance to low-Fe stress, but our results indicate that it is unlikely that they will perform similar to sour orange.

An unexpected result of our study were the large Fe reduction rates of the citron plants. Citron is considered to be one of the three true *Citrus* species and taxonomically related to lemon and lime types [21]. This genetic link may explain the high reduction rate of, e.g., Volkamer lemon, and will be useful information in future germplasm improvement efforts.

During the 1996 run, the Rangpur plants acidified the unbuffered solution to below pH 4.3, and were twice the height of those in the buffered solution. The plants in the unbuffered solution had visibly less Fe chlorosis. A similar response was observed for sour orange plants in a different run. These results indicate that *Citrus* genotypes can respond in different ways to low-Fe stress. Moreover, different environments may stimulate different responses within one genotype and possibly explain certain inconsistencies in the literature. For example, among the two sets of Rangpur plants, the buffered ones had higher Fe reduction rates. Buffering may have interfered with acidification, and thus, enhanced the Fe reduction response.

Overall, there is a reasonable match between our results and those obtained from other studies involving different methods. We expanded the scope of genotypes characterized, and determined that Fe reduction responses range from high, to moderate, to low, and to essentially no response. Such a range has been found in only a few other plant species. Most plants studied so far have shown either no response, or a full response; few plant species have such well-characterized low to moderate responses. Our study also demonstrated the possible genetic association between citron and other genotypes. Additional study in soil to define any practical thresholds of tolerance to $CaCO_3$ [22] seems appropriate.

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note

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Búsqueda de porta-injertos de agrios tolerantes a una deficiencia de hierro.

Resumen — Introducción. Suelos muy calcáreos limitan muy fuertemente el desarrollo de los agrios porque reducen la disponibilidad de ciertos micro elementos, de hierro particularmente. Para paliar la falta de micro elementos, se acostumbra utilizar varias soluciones: no sembrar en tales suelos, utilizar costosos chelatos o sembrar porta-injertos tolerantes a los estreses causados por bajos contenidos de hierro. El estudio ha permitido evaluar una amplia gama de genotipos de agrios frente a este carácter de tolerancia. **Material y métodos**. Se sometieron a prueba las plantas de 26 especies de *Citrus* y de genotipos parecidos por su capacidad a reducir el ion Fe^{3+} a fin de medir su tolerancia frente a una deficiencia de hierro. Plántulas de 3 meses fueron colocadas durante 2 a 3 meses en una solución nutritiva sin hierro. Regularmente se tomaron puntas de raíces blancas y su aptitud a reducir el hierro fue sometida a prueba. **Resultados y discusión**. Los genotipos dando la mejor respuesta para la reducción del hierro fueron los limoneros Volkamer y Eureka, el cidro Etrog, así como el naranjo amargo y la lima Rangpur. Otros genotipos dieron respuestas bajas o moderadas. Las peores respuestas fueron las del citrumelo Swingle, del pomelo Duncan, del pomelo Thong Dee, de los naranjos Ridge Pineapple y trifoliado, así como de ciertas selecciones de papeda. Los resultados completan clasificaciones anteriores de tolerancia a los estreses y sugieren que las fuertes tolerancias encontradas en ciertos genotipos de *Citrus* podrían provenir de *C. medica*. Por otro lado, las plantas de Rangpur acidificaron una solución nutritiva no tamponada hasta valores de pH 5 y tuvieron tasa más bajas de reducción que las plantas colocadas en soluciones tamponadas. (© Elsevier, Paris)

***Citrus* / portainjertos / ensayos de variedades / suelo calcáreo / clorosis / hierro**