Tolerance of Citrus Rootstocks to Lime-induced Iron Chlorosis

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Additional index words: Calcareous soil, bicarbonate, high pH, chlorophyll meter, Poncirus.

ABSTRACT

Seedlings of 26 citrus genotypes used for rootstocks were field-tested for their tolerance to lime-induced chlorosis at two locations in south Texas. The soil pH ranged from 7.8 to 8.3 and soil HCO_3^- - ranged from 275 ppm to 336 ppm. A block of 'Ray Ruby' grapefruit (*Citrus paradisi* Macf.) grafted on 16 rootstocks was also evaluated for tolerance to lime-induced chlorosis. In general, non-trifoliate rootstocks were tolerant, while pure trifoliate (*Poncirus trifoliata* L. Raf) rootstocks were very susceptible. Trifoliate hybrids showed tolerance levels that ranged from very susceptible to tolerant. The most tolerant rootstocks were *Citrus obovoidea* Hort. ex *Takahashi* (Kinkoji), *C. canaliculata* Tan, Texas sour orange (*C. aurantium* L.), Tosu sour orange (*C. neo-aurantium*. Tan.), Cleopatra mandarin (*C. reticulata* Blanco), Schaub rough lemon, standard rough lemon, Vangasay lemon (*C. limon* L. Burm.), 1578-201 (*C. sinensis* L. Osbeck x *C. jambhiri* Lush.), Sunki mandarin x Swingle trifoliate (*C. reticulata* x *P. trifoliata*), and Shaddock x Rubidoux trifoliate (*C. grandis* Osbeck x *P. trifoliata*). The most susceptible rootstocks were Rangpur lime x Swingle trifoliate (*C. limonia* Osbeck x *P. trifoliata*), Cleopatra mandarin x Rubidoux trifoliate (*C. reticulata* x *P. trifoliata*), Sunki mandarin x Benecke trifoliate (*C. reticulata* x *P. trifoliata*), Benton citrange (*C. sinensis* L. Osbeck x *P. trifoliata*), and the three trifoliates (Flying Dragon, Pomeroy, and Argentine).

RESUMEN

Se realizó un estudio de campo en dos localidades en el sur de Texas para evaluar la tolerancia a la clorosis inducida por cal presentada por plántulas de 26 genotipos de cítricos usados como portainjertos, El pH del suelo varió de 7.8 a 8.3 y el HCO_3^- varió de 275 ppm a 336 ppm. También se evaluó la tolerancia inducida por cal en un bloque de toronjo variedad 'Ray Ruby' (*Citrus paradisi* Macf.) injertado en 16 portainjertos. En general, los portainjertos no trifoliados fueron tolerantes, mientras que los portainjertos trifoliados puros (*Poncirus trifoliata* L. Raf) fueron muy susceptibles. Los híbridos trifoliados mostraron niveles de tolerancia que variaron de muy susceptibles a tolerantes. Los portainjertos mas tolerantes fueron *Citrus obovoidea* Hort ex *Takahashi* (Kinkoji), *C. canaliculata* Tan, narango agrio de Texas (*C. aurantium* L.), naranjo agrio Tosu (*C. neo-aurantium* Tan.), mandarino Cleopatra (*C. reticulata* Blanco), limón rugoso Schaub, limon rugoso estandard, limón Vangasay (*C. limon* L. Burm.), 1578-201 (*C. sinensis* L. Osbeck x *C. jambhiri* Lush.), mandarino Sunki x trifoliado Swingle (*C. reticulata* x *P. trifoliata*), y trifoliado Shaddock x trifoliado Rubidou (*C. grandis* Osbeck x *P. trifoliata*). Los portainjertos más susceptibles fueron lima Rangpur x trifoliado Swingle (*C. limonia* Osbeck x *P. trifoliata*), mandarino Cleopatra x trifoliado Rubidou (*C. reticulata* x *P. trifoliata*), mandarino Sunki x trifoliado Benecke (*C. reticulata* x *P. trifoliata*), citrange Benton (*C. sinensis* L. Osbeck x *P. trifoliata*), y los tres trifoliados (Flying Dragon, Pomeroy, y Argentina).

On calcareous soils, iron (Fe) chlorosis has been attributed to low Fe availability, high pH and high bicarbonate content and is referred to as lime-induced iron chlorosis. Low Fe availability is caused by the oxidation of Fe^{2+} to Fe^{3+} and the precipitation of $Fe(OH)_3$ which is favored by increasing pH. In general, the solubility of Fe decreases 1000 fold for every unit increase in pH above pH 4 and is minimal between pH 7.4 and 8.5 (Bohn, 1967). High levels of bicarbonate in the soil solution have also been reported to decrease iron uptake and translocation in the plant (Alcantara et al., 1988; Coulombe et al., 1984). Iron deficiency in citrus leaves occurs in many of the citrus growing areas of the world (Vose, 1982). It is easily identified by visible symptoms of interveinal chlorosis in the leaves resulting from reduced chlorophyll content (Wallihan, 1955; Wallihan et al., 1969). The intensity of leaf Fe chlorosis can be quantified by total-Fe, active-Fe, leaf chlorophyll concentration, readings of a chlorophyll meter, or by visual ratings of leaf chlorosis. The most common approach is the use of visual ratings (Coulombe et al., 1984; Hamze et al., 1986; Maxwell and Wutscher, 1976; Sudahono et al., 1994). In work with citrus, all the above indicators of chlorosis were well correlated with each other except for total-Fe. Thus, active-Fe, leaf chlorophyll concentration, readings of portable chlorophyll meter and visual ratings of chlorosis are useful, although given the time involved in measuring active-Fe and chlorophyll concentration, the use of visual ratings and readings of a portable chlorophyll meter are the most efficient approaches (Sudahono et al., 1994).

Table 1. Leaf chlorosis ratings and SPAD 501 meter readings of citrus seedlings grown at two sites in southern Texas (Edinburg and Weslaco) on calcareous soils.

	Edinburg		Weslaco	
Rootstock	Chlorosis[z]	SPAD	Chlorosis[z]	SPAD
Non-trifoliate				
China sour orange	2.5	40	2.5	48
Sour orange mutation	2.0	34	1.7	50
Texas sour orange	1.3	47	2.0	50
Cleopatra mandarin	2.0	38	1.7	46
Parson's special mandarin	3.3	32	—	—
Sun Chu Sha mandarin	2.3	26	3.0	42
Standard rough lemon	1.3	35	1.5	49
Vangasay lemon	2.0	34	1.7	51
1578-201[y]	2.0	41	1.7	49
1578-173[y]	2.7	39	2.0	42
Trifoliate hybrids				
F-80-18 citrumelo	2.7	40	3.0	47
F-81-17 citrumelo	3.0	37	3.3	42
F-80-8 citrumelo	2.3	44	3.3	42
F-80-3 citrumelo	2.3	30	3.5	45
F-80-5 citrumelo	2.0	34	3.0	42
Swingle citrumelo	3.3	33	3.3	40
Troyer citrange	2.5	32	3.0	44
Benton citrange	4.0	15	3.7	34
F-81-12 citrange	2.5	35	3.0	42
1573-26 citrange[y]	2.3	40	3.0	44
Cleopatra mandarin x Flying Dragon trifoliate	3.3	24	3.0	46
Cleopatra mandarin x Rubidoux trifoliate	3.5	20	3.3	40
Sunki mandarin x Benecke trifoliate	3.3	15	3.3	35
Rangpur lime x Swingle trifoliate	3.3	26	4.0	26
Smooth Flat Seville x Swingle trifoliate	2.7	35	3.0	46
Trifoliate				
Flying Dragon trifoliate	5.0	16	5.0	16
LSD (0.05)	1.1	8	1.3	7

[z]Chlorosis rating scale of fully developed new growth: 1 = healthy green leaves, 3 = greenish-yellow interveinal area, green veins, 5 = yellow-white interveinal areas, pale green veins, some defoliation.

[y]1578-201 and 1578-173 = *Citrus sinensis* x *C. Jambhiri*; 1573-26 = *C. Sinensis* x *Poncirus trifoliata*.

Several screening techniques for determining tolerance to calcareous conditions in citrus rootstocks have been used. Cooper et al. (1954) conducted field screening of citrus by using prepared seedbeds of calcareous soils containing 2.15% $CaCO_3$ and 0.20% $CaCO_3$. They found that seedlings of all sweet orange (*Citrus sinensis* (L.) Osbeck) cultivars showed moderate or severe iron chlorosis, while seedlings of trifoliate hybrids showed mild to severe chlorosis. The tolerant cultivars were Suen Kat mandarin (*C. reticulata* Blanco), Kunembo mandarin (*C. nobilis* Lour.), Shekwasha (*C. depressa* Hay.), Rangpur lime (*C. limonia* Osbech), sour orange (*C. aurantium*), and rough lemon (*C. jambhiri* Lush.). In experiments using plants in containers with calcareous soils and sand culture watered with nutrient solution, Hamze et al. (1982; 1986) reported that *Citrus jambhiri* (rough lemon) and *C. macrophylla* Wester were highly resistant, *C. volkameriana* (Pasq.) Tan., *C. aurantium*, *C. reticulata* and *C. limonia* were moderately tolerant, *C taiwanica* (Tan.) Shim., *C. sinensis* (L.)

Table 2. Leaf chlorosis ratings and SPAD 501 meter readings of 'Ray Ruby' grapefruit scion on 16 citrus rootstocks at Sharyland Orchard Block 385 S in Mission, Texas.

Rootstock	Chlorosis[z]	SPAD
Non-trifoliate		
Texas sour orange	1.2	69
C. canaliculata	1.3	68
Tosu sour orange	1.5	65
C. obovoidea	1.0	77
Schaub rough lemon	1.0	68
Trifoliate hybrids		
Carrizo citrange	2.3	37
Morton citrange	2.5	44
Troyer citrange	2.2	57
C-32 citrange	3.0	45
C-35 citrange	2.3	39
Swingle citrumelo	2.5	52
Sunki mandarin x Swingle trifoliate	1.2	68
Shaddock x Rubidoux trifoliate	1.7	61
Trifoliate		
Flying Dragon trifoliate	3.7	29
Pomeroy trifoliate	3.7	26
Argentine trifoliate	3.8	28
LSD (0.05)	0.7	24

[z]Chlorosis rating scale of fully developed new growth: 1 = healthy green leaves, 3 = greenish-yellow interveinal area, green veins, 5 = yellow-white interveinal areas, pale green veins, some defoliation.

Osbeck, Troyer, and Carrizo citranges, (*C. sinensis* x *P. trifoliata*) were moderately susceptible, while *P. Trifoliata* and *Swingle citrumelo* (*P. trifoliata* x *C. paradisi* Macf.) were susceptible. More recently, Sudahono et al. (1994) using sand culture and nutrient solutions with high bicarbonate levels, showed that Texas sour orange has a high level of tolerance to bicarbonate-induced chlorosis as did Smooth Seville x Argentive *trifoliate* and F-81-12 citrange. Their results with standard rootstocks (Texas sour orange, Cleopatra mandarin, Vangasay lemon, Troyer citrange, Benton citrange, and Flying Dragon triloliate) agreed with previous reports on their relative tolerance to calcareous soils.

Sour orange is the most widely used commercial rootstock in South Texas because it is tolerant to salinity, soil borne diseases, waterlogging and soils that are calcareous and high in clay content (Rouse et al., 1986). Unfortunately, sour orange is susceptible to the citrus tristeza virus (CTV). This devastating virus is now in Mexico and is spreading north towards Texas (Rouse et al., 1986; Wutscher, 1979). Alternative rootstocks tolerant to calcareous soils and CTV are needed.

The objective of this study was to evaluate with chlorosis ratings and relative leaf chlorophyll contents the tolerance to lime-induced iron chlorosis of seedlings of 26 citrus cultivars used as rootstocks and of 16 citrus rootstocks grafted with 'Ray Rudy' grapefruit grown in calcareous soils.

MATERIALS AND METHOD

Seedlings of 26 citrus genotypes (Table 1) were planted at two sites with calcareous soils in the Lower Rio Grande Valley of Texas: Weslaco (Texas Agricultural Research and Extension Center) and Edinburg (Eubanks Nursery). There were three one-plant replications of each genotype at each location in a randomized block design. The plots were flood irrigated as needed. The soil at both locations is fine-loamy, mixed, hyperthermic family of topic calciustolls. The Weslaco site is caracterized by Willacy fine sandy loam soil containing 17% to 20% clay in the suface 43cm and a pH range of 7.3 to 7.8. The Edinburg site is characterized by Hidalgo sandy clay loam containing 20% to 30% clay and a pH range of 7.9 to 8.4. The A horizon of both of these soils is reported to contain 5% to 35% calcium carbonate with weak concreations and soft bodies (Jacobs et al., 1981). Soil samples from the top 25 cm were taken at each location and analyzed for carbonate ($CaCO_3$) and bicarbonate (HCO_3-) contents with the procedure described by Allison and Moore (1965) and Soil Conservation Service (1972), respectively. Observations of foliar chlorosis were made in Sept. 1990, 18 months after planting. The seedlings were rated for chlorosis based on their fully expanded new leaves as follows: 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, palegreen veins, some defoliation. Leaf chlorophyll levels of five fully expanded new leaves were measured with a SPAD-501 chlorophyll meter (Minolta Company, 101 Williams Drive, NJ 07446, USA and by extraction with DMSO (dimthyl sufoxide) followed by spectrophotometry (Hiscox and Isrealstam, 1979).

An additional field observation was conducted in Sept. 1990 on an established young citrus block 20 months from planting at Sharyland Orchard (Mission, TX) to observe the performance of 'Ray Ruby' grapefruit (*C. paradise* Macf.) on 16 rootstocks (Table 2) in a calcareous soil. This soil has an A horizon (0-33) with a dark sandy-loam soil (13-21% clay, pH 6.6-7.8) and a B horizon (33-165cm) with brown sandy clay loam (18-30% clay, pH 7.4-8.4) (Jacobs et al., 1981). Each rootstock was replicated three times as single-plant plots in a randomized complete block design. The data collected included general appearance based on the visual scoring method mentioned above and relative chlorophyll level as measured by the SPAD-501 chlorophyll meter. Five fully developed young leaves (3 to 7 leaves from apex) were measured per plant.

Data were subjected to SAS General Linear Model (GLM) Procedure and the means were separated by Least Significant Difference (LSD) test (SAS Institute Inc., 1988). Data for chlorosis and SPAD readings were analyzed for correlations

RESULTS AND DISCUSSION

The soil at the Edinburg and Weslaco sites had high pH (8.3 and 7.8, respectively), $CaCO_3$ content (5.0% and 3.8%, respectively) and HCO_3-level (336 ppm and 275ppm, respectively). These factors combined to create soils in which many citrus rootstocks developed lime-induced Fe chlorosis. The rootstocks that showed few or no chlorosis symptoms across both sites were Texas sour orange, standard rough lemon, sour orange mutation, Cleopatra mandarin, Vangasay lemon and the lemon hybrid 1578-201 (Table 1). Flying Dragon trifoliate exhibited severe chlorosis with considerable defoliation and was more chlorotic than the other genotypes. Trifoliate hybrid groups (citrange, citrumelo, other trifoliate) were intermediate between the non-trifoliate and trifoliate groups and ranged from chlorotic (3.7 - 4.0, Benton citrange) to moderately chlorotic (2.0-3.0, F-80-5).

Leaf chlorophyll content was well correlated with the SPAD-501 chlorophyll meter readings (r=0.99***). The SPAD chlorophyll meter (Tabel 1) followed the chlorosis data as indicated by significant correlations between these parameters (Edinburg and Weslaco were r=0.60**, and 0.77**, respectively), but nevertheless the SPAD readings were not as consistent between sites as was the chlorosis data. The highest and most consistent SPAD readings were seen in Texas sour orange, China sour orange, 1578-201 and 1578-173. This group is followed by standard rough lemon, sour orange mutation, Vangasay lemon, Cleopatra mandarin, F-80-18, F-80-8, and 1573-26. In both sites, Flying Dragon trifoliate was in the lowest group for SPAD 501 readings (Table 1).

The Sharyland site has calcareous soil with a pH, HCO_3-level and $CaCO_3$ content of 7.1, 275 ppm and 3.8% respectively. 'Ray Rudy' grapefruit scions grafted on lemon and *C. obovoidea* were free of chlorosis (Table 2). Those on sour orange types (Sunki mandarin x Swingle trifoliate and Shaddock x Rubidoux trifoliate) showed slight chlorosis, while those on citrange and citrumelo showed moderate chlorosis. Trees on trifoliate rootstocks showed severe chlorosis and had the lowest SPAD readings.

The level of tolerance to lime-induced chlorosis agrees well between the seedlings and scion field trials when one considers the tolerance of the various groups as well as the few rootstocks (Texas sour orange, Swingle citrumelo, Troyer citrange, Flying Dragon trifoliate) the two trials had in common (Tables 1 and 2). This agrees with Cooper and Peynado (1956) who stated that except for one case, the citrus rootstock rather than the scion controlled the development of iron chlorosis.

Non-trifoliate rootstocks (sour orange, manadin, and lemon) were generally moderately tolerant to tolerant, as has been reported for non-trifoliate rootstocks such as rough lemon, sour orange and mandarin (Cooper and Paynado, 1956; Hamze et al., 1986; Khadr et al., 1966; Newcombe, 1978; Sudahono, 1994; Wutscher el al., 1970). It shoud be noted that some madarins such as Parson's Special (this study) and Sanguine (Maxwell and Watscher, 1976) as well as mandarin hybrid (Sunki mandarin x Benecke trifoliate and the two Cleopatra mandarin x trifoliate hybrids) are susceptible to lime-induced chlorosis.

The three pure trifoliates were very susceptible to calcareous soil conditions as expected from previous reports (Armstrong and Furr, 1956; Hamze and Nimah, 1982; Hamze et al., 1986; Khadr et al., 1966; Sudahono, 1994). However, the trifoliate hybrids ranged from very susceptible (Sunki mandarin x Benecke trifoliate, Cleopatra mandarin x Rubidoux trifoliate, Cleopatra mandarin x Flying Dragon trifoliate, Benton citrange) to moderately tolerant or tolerant (1573-26, Sunki mandarin x Swingle trifoliate, Shaddock x Rubidoux trifoliate).

Non-trifoliate rootstocks generally showed higher tolerance than trifoliate rootstocks, while trifoliate hybrids showed a range of tolerance from very susceptible to tolerant to lime-induced chlorosis. The most tolerant rootstocks were *Citrus obovoidea*, *C. canaliculata*, Texas sour orange, Tosu sour orange, Cleopatra mandarin, Schaub rough lemon, Standard rough lemon, Vangasay lemon, 1578-201, Sunki mandarin x Swingle trifoliate, and Shaddock x Rubidoux trifoliate. These rootstocks need to be tested further in a wider range of soils to better assess their productivity and their adaptability to other factors such as salinity, soil borne diseases, cold hardiness, water logging, heavy soil and resistance to CTV. These may offer a step in the direction of developing rootstock alternatives which are resistant to CTV for the Lower Rio Grande Valley.

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