

Rootstock Effects on Leaf Photosynthesis in ‘Navelina’ Trees Grown in Calcareous Soil

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Abstract. Citrus crops in the Mediterranean region are often grown in high pH calcareous soils, which promote nutrient imbalances, especially iron deficiency. ‘Navelina’ trees grafted on eight different citrus rootstocks were assessed in terms of their relative tolerance to these soils. To do so, leaf gas exchange and chlorophyll *a* fluorescence parameters were measured for 2 consecutive years in leaves without visible injury symptoms. Trees were grown on the rootstocks Carrizo citrange, hybrids of Cleopatra mandarin × *Poncirus trifoliata* no. 5 (F-A 5), 13 (F-A 13), and 16 (030116), Troyer citrange × common mandarin no. 18 (F-A 418), King mandarin × *P. trifoliata* no. 7 (F-A 517), 020324 (Troyer citrange × Cleopatra mandarin), and Volkamer lemon × *P. trifoliata* no. 64 (230164). g_s and transpiration rate were clearly lower in leaves of shoots grafted on Carrizo citrange than in those on the other rootstocks assayed, but net photosynthetic flux did not differ. However, leaves of shoots on Carrizo citrange displayed a decline in their maximum quantum yield of PSII photochemistry [variable fluorescence/maximum fluorescence (F_v/F_m) ratio] and other chlorophyll parameters in the steady state such as photochemical quenching (q_p) and the quantum efficiency of PSII photochemistry (Φ_{PSII}) as well as an increase in nonphotochemical quenching (q_N). Other rootstocks such as 030116, F-A 517 and, especially, F-A 5 showed the highest Φ_{PSII} and F_v/F_m values, whereas F-A 5 had the lowest q_N in the steady state. The photosynthetic characteristics observed in leaves on Carrizo citrange showed them to be the least tolerant to these calcareous conditions, whereas photosynthesis of ‘Navelina’ trees budded on F-A 5 were the most tolerant.

Citrus is the major fruit crop grown in the Mediterranean area, especially in the Valencia region in Spain, and is one of the most important fruit crops in the world. It is estimated that between 20% and 50% of fruit trees grown in the Mediterranean basin suffer from iron (Fe) deficiency (Jaegger et al., 2000). The most prevalent cause of Fe deficiency in this region is the presence of high levels of carbonate ions in calcareous soils, leading to a high pH and low availability of Fe and the condition known as lime-induced chlorosis (Pestana et al., 2005). The citrus trees planted in these soils often show signs of severe Fe deficiency or Fe chlorosis because of low Fe availability (Wallace, 1986). Iron deficiency

affects the biochemistry, morphology, and physiology of the whole plant because Fe is an important cofactor of many enzymes, including those involved in the biosynthetic pathway of chlorophylls (Abadia, 1992; Larbi et al., 2006; Molassiotis et al., 2006).

In calcareous soils, citrus-growing success depends on availability of suitable rootstock that is tolerant of low Fe. Trifoliate orange [*Poncirus trifoliata* (L.) Raf.], sweet orange [*C. sinensis* (L.) Osb.], and Carrizo citrange (*C. sinensis* × *P. trifoliata*) are all susceptible to lime-induced chlorosis, whereas sour orange (*C. aurantium* L.) and Cleopatra mandarin (*C. reshni* Hort. ex Tan.) are more lime-tolerant (Castle, 1987; Hamzé et al., 1986; Pestana et al., 2005; Treeby and Uren, 1993). Within this context, a breeding program is being carried out at the Instituto Valenciano de Investigaciones Agrarias (IVIA; Valencia, Spain) in the search for better-performing citrus rootstocks in alkaline soils. As a result of this program, four new hybrid rootstocks are now available in Spanish citrus nurseries: Forner-Alcaide 5 (F-A 5) and F-A 13 (both *C. reshni* Hort. ex Tan. × *P. trifoliata*), F-A 418 [Troyer citrange (*C. sinensis* × *P. trifoliata*) × *C. deliciosa* Ten.], and F-A 517 (*C. nobilis* Lour. × *P. trifoliata*). These rootstocks have

been tested in calcareous soils. ‘Navelina’ trees grafted on F-A 5 or F-A 13 yielded ≈40% more than trees on Carrizo citrange, whereas trees on F-A 5 or F-A 13 produced smaller but similar quality fruit than those on Carrizo (Forner et al., 2003; Forner-Giner et al., 2003).

Evaluation of growth and yield parameters may not be sufficient to rank citrus rootstocks according to their tolerance to Fe chlorosis (Pestana et al., 2003, 2005). In this respect, physiological parameters like leaf chlorophyll, CO₂ gas exchange, and chlorophyll *a* fluorescence emission are known to be affected in leaves of trees that are not tolerant to Fe chlorosis or calcareous soils (Baveresco et al., 2006; Chouliaras et al., 2004; Larbi et al., 2006; Molassiotis et al., 2006; Morales et al., 2000).

The main objective of this study was to evaluate calcareous soil tolerance of ‘Navelina’ orange trees grafted on Carrizo citrange and seven new citrus rootstocks by assessing gas exchange and chlorophyll *a* fluorescence.

Materials and Methods

Plant material. Measurements were taken for leaves of 13-year-old ‘Navelina’ orange trees, *Citrus sinensis* (L.) Osb. (selection Iniasel 7), grafted on Carrizo citrange and seven new citrus rootstocks obtained by J. Forner at the IVIA: F-A 5, F-A 13, F-A 418, F-A 517, 030116 (Cleopatra mandarin × *P. trifoliata*), 020324 (Troyer citrange × Cleopatra mandarin), and 230164 (*C. volkameriana* Ten. & Pasq. × *P. trifoliata*). The trees were planted in 1993 with nursery plants (1 year after grafting) and arranged in a complete randomized experimental plot. There were six trees for each rootstock type in the plot. Tree spacing was 2.5 × 6 m. The soil was sandy loam with 40% of CaCO₃, 8% deactive calcium carbonate, and pH 8. A relative level of carbonate between 5% and 10% is considered high. Standard cultural practices were followed with drip irrigation and fertilizing without iron chelates, mechanical weed control between rows, and chemical control between trees. Drip irrigation frequency was adjusted according to the season of the year, from once per week in winter to 5 d per week in summer, with 40 L/tree per irrigation. Water pH was 7.6, electrical conductivity 1.4 dS·m⁻¹, Cl⁻ 134.8 mg·L⁻¹, and NO₃⁻ 158.0 mg·L⁻¹. Fertilization was applied from March to September in the following amounts: ammonium nitrate (33%) 2 kg/tree, monoammonium phosphate 0.5 kg/tree, and potassium nitrate 0.8 kg/tree. During these months, the percentage distribution was 10, 12, 15, 18, 20, 15, and 10 for ammonium nitrate; 10, 20, 15, 15, 15, 15, and 10 for monoammonium phosphate; and 7, 10, 13, 15, 25, 20, and 10 for potassium nitrate.

Gas exchange measurements. Measurements were taken at the end of October during 2 consecutive years (2005 to 2006) on eight expanded spring leaves for each rootstock type in the field. A portable gas exchange system (CIRAS-2; PP Systems,

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Hitchin, U.K.) was used with a PLC 6(U) universal leaf autocuvette in closed-circuit mode (Larbi et al., 2006). All measurements were taken on attached mature leaves that were ≈ 6 months old without visible injury symptoms and with an apparent homogeneous green color. All measurements were performed at saturation irradiance ($1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) between 1030 HR and 1130 HR, whereas leaf temperature ranged between 24 and 26 °C. The infrared method was used to determine leaf temperature. The gas exchange parameters determined at saturation irradiance were: transpiration rate (E) [$\text{mmol}(\text{H}_2\text{O})\cdot\text{m}^{-2}\cdot\text{s}^{-1}$], net photosynthetic flux (P_n) [$\mu\text{mol}(\text{CO}_2)\cdot\text{m}^{-2}\cdot\text{s}^{-1}$], g_s to water vapor [$\text{mmol}(\text{H}_2\text{O})\cdot\text{m}^{-2}\cdot\text{s}^{-1}$], and CO_2 substomatal concentration (C_i) [$\mu\text{mol}(\text{CO}_2)\cdot\text{mol}^{-1}$ (air)]. The enclosed portion of the leaf was 2.50 cm^2 and the cuvette flow was $200 \text{ mL}\cdot\text{min}^{-1}$. The ambient CO_2 concentration was maintained at 375 ppm and citrus leaves were chosen according to Larbi et al. (2006).

The environmental conditions during measurements were: temperature means, 24.1 °C (2005) and 24.9 °C (2006). Air relative humidity means were 65.8% (2005) and 64.2% (2006). No rainfall was recorded on the measurement days. These environmental conditions were similar on all measurement days.

Pigment analysis. The concentration of Chl per area was estimated in the same attached leaves as those for which gas exchange measurements were taken using a SPAD portable apparatus (Minolta Co., Osaka, Japan). Strong positive correlations between SPAD readings and chlorophyll concentration were found in leaves on all rootstocks ($r^2 > 0.8$).

Chlorophyll *a* fluorescence measurements. Like in the case of gas exchange, chlorophyll *a* fluorescence (Chl F) was measured at ambient temperature (24 to 26 °C) in the field during the same time period on 2 consecutive years using a portable fluorometer (PAM-2001; Walz, Effeltrich, Germany). The same eight spring flush leaves for each rootstock used for previous measurements (four leaves per year) were darkened for 30 min before fluorescence measurement. The minimum (dark) fluorescence (F_o) was obtained after chlorophyll excitation with a weak beam from a light-emitting diode. Maximum fluorescence (F_m) was determined following a 600-ms pulse of saturating “white light.” The yield of variable fluorescence (F_v) was calculated as $F_m - F_o$. After 2 min of dark readaptation, actinic “white light” ($430 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was switched on and a saturating pulse ($14,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was applied at 60-s intervals for 13 min to determine: 1) maximum fluorescence yield during actinic irradiation (F_m'); 2) the level of modulated fluorescence during a brief interruption of actinic irradiation in the presence of far-red radiation (F_o'); and 3) the Chl F yield of fluorescence during the actinic illumination (F_i). Quenching resulting from non-photochemical dissipation of absorbed light energy (q_N) was calculated at each saturating pulse according to the equation $q_N = (F_m' - F_m)/(F_m' - F_o')$ (van Kooten and Snell, 1990).

Table 1. Total chlorophyll and gas exchange parameters of leaves from ‘Navelina’ trees grafted on eight rootstocks grown in calcareous soil.^z

Rootstocks	SPAD readings ^y	P_n [$\mu\text{mol}(\text{CO}_2)\cdot\text{m}^{-2}\cdot\text{s}^{-1}$]	C_i [$\mu\text{mol}(\text{CO}_2)\cdot\text{mol}^{-1}$ (air)]	g_s [$\text{mmol}(\text{H}_2\text{O})\cdot\text{m}^{-2}\cdot\text{s}^{-1}$]	EVAP (E) [$\text{mmol}(\text{H}_2\text{O})\cdot\text{m}^{-2}\cdot\text{s}^{-1}$]
F-A 5	65.3 a	10.6 a	487 b	30 a	0.73 a
030116	55.9 b	7.0 abc	506 ab	27 a	0.72 a
F-A 517	56.3 b	9.4 ab	369 c	27 a	0.68 a
F-A 13	57.9 ab	7.8 abc	449 bc	26 a	0.67 a
020324	63.1 ab	7.2 abc	486 b	30 a	0.68 a
230164	41.4 c	5.3 c	599 a	20 a	0.67 a
F-A 418	55.8 b	7.4 abc	525 ab	29 a	0.77 a
Carrizo					
citrange	38.0 d	6.3 bc	543 ab	6 b	0.13 b

^zFor each parameter, one-way analysis of variance was performed with rootstocks as the independent variable. Values represent means ($n = 8$). Data from both years are pooled. Values in columns followed by the different letters are statistically different at $P < 0.05$ (least significant difference test).

^ySPAD reading in the leaves to estimate the content of chlorophyll; P_n = net photosynthetic flux; C_i = CO_2 substomatal concentration; g_s = g_s to water vapor; E = transpiration rate.

The coefficient for photochemical quenching, q_p , which represents the fraction of open PSII reaction centers, was calculated as $(F_m' - F_i)/(F_m' - F_o')$ (Schreiber et al., 1989). The quantum efficiency of PSII photochemistry, Φ_{PSII} , closely associated with the quantum yield of noncyclic electron transport, was estimated from $(F_m' - F_i)/F_m'$ (Genty et al., 1989). The fraction of photons absorbed by PSII, which was not used in photochemistry or dissipated in the PSII antenna (%X), was estimated from (F_v'/F_m') $(1 - q_p) \times 100$ (Demmig-Adams et al., 1996).

Statistical analysis. Data collected in the 2 consecutive years were tested for significance using analysis of variance using time and rootstocks as independent variables (statistical significance, $P \leq 0.05$), but the time variable (year) did not account for a significant amount of variation. Means were compared using the least significant differences method at a 95% confidence level. Statistical analyses were performed using StatGraphics Plus for Windows, Version 2.1 (Statistical Graphics, Englewood Cliffs, NJ).

Results

Leaf chlorophyll. The SPAD measurements were used as an equated estimate of leaf chlorophyll concentration, because there was a linear relationship between these two parameters ($r^2 > 0.8$; Jifon et al., 2005). Leaves of shoots grafted on F-A 5, F-A 13, and 020324 had the highest Chl concentration. Leaves of shoots budded on 230164 and Carrizo citrange showed the lowest Chl concentration (Table 1).

Gas exchange measurements. The leaves of shoots grafted on F-A 5 had the highest net photosynthetic flux (P_n), which differed significantly from leaves on 230164 and Carrizo citrange but not from those grafted on the other rootstocks (Table 1). Leaves of shoots budded on F-A 517 had the lowest C_i , whereas leaves of shoots grafted on the other rootstocks had significantly higher C_i with the exception of leaves of shoots budded on F-A 13. g_s and transpiration (E) were significantly lower in Carrizo citrange than in the other rootstocks.

Changes in chlorophyll *a* fluorescence parameters. Table 2 shows the Chl *a* fluorescence of dark-adapted parameters for the ‘Navelina’ spring flush leaves. The maximum quantum yield of PSII photochemistry estimated using the F_v/F_m ratio was the smallest in leaves on Carrizo citrange. The decline in this ratio was mostly the result of a decrease in the fluorescence parameter F_m , not of an increase in the minimum level of fluorescence, F_o . The higher F_v/F_m values from leaves on F-A 5 and 030116 than on the other rootstocks was the result of an increase in F_m .

An apparently steady state was reached after ≈ 4 min of actinic illumination for quantum yield (Φ_{PSII}) of the PSII electron transport and the fraction of open PSII centers (q_p) in leaves on all rootstocks (Fig. 1A–B). Two minutes into the kinetics study, the values of Φ_{PSII} and q_p for leaves on Carrizo citrange were the lowest. The steady state of the Φ_{PSII} value for leaves on Carrizo citrange was $\approx 27\%$ lower than Φ_{PSII} value for leaves on F-A 5, 030116, and F-A 517.

Immediately after actinic irradiation, q_N rose quickly and reached maximum values

Table 2. Changes in F_o , F_m , and F_v/F_m at the steady state in dark-adapted leaves from ‘Navelina’ trees grafted on eight rootstocks grown in calcareous soil.^z

Rootstocks	F_o	F_m	F_v/F_m
F-A 5	0.214 abc	1.163 a	0.816 a
030116	0.211 abc	1.162 a	0.816 a
F-A 517	0.203 bc	1.082 ab	0.802 ab
F-A 13	0.226 ab	0.962 bc	0.761 c
020324	0.207 abc	1.059 bc	0.795 ab
230164	0.232 a	0.981 bc	0.776 bc
F-A 418	0.190 c	0.917 c	0.789 abc
Carrizo			
citrange	0.218 ab	0.769 d	0.720 d

^zFor each parameter, one-way analysis of variance was performed with rootstocks as independent variable. Values represent means ($n = 8$). Values in columns followed by the different letters are statistically different at $P < 0.05$ (least significant difference test).

F_o = the minimum (dark) fluorescence; F_m = the maximum fluorescence; F_v = the yield of variable fluorescence ($F_m - F_o$); F_v/F_m = the maximum quantum yield of PSII photochemistry.

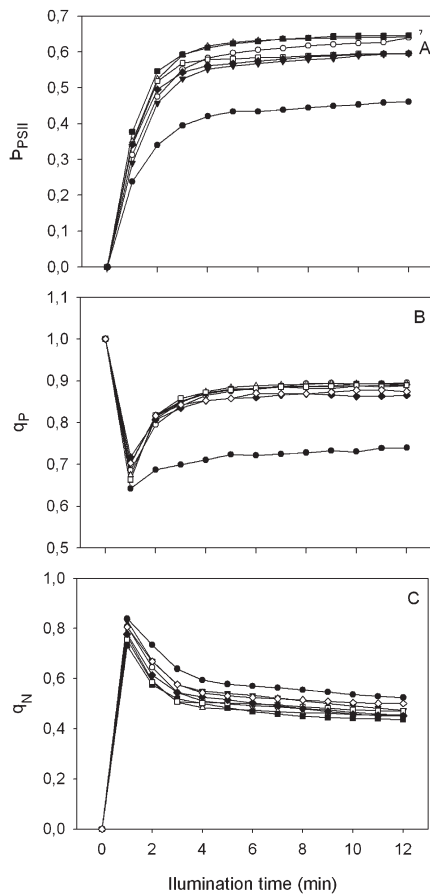


Fig. 1. Effects of rootstock on the kinetics of fluorescence parameters Φ_{PSII} , the quantum efficiency of PSII photochemistry (A), q_P , photochemical quenching (B), and q_N , non-photochemical quenching (C) of 'Navelina' trees grown in calcareous soil. Data are mean values and SD for $n = 8$. For all of measurements, SD of Φ_{PSII} , q_P , and q_N was lower than 0.03, 0.04, and 0.02, respectively. Symbols denote Carrizo citrange (●), F-A 517 (○), F-A 13 (▼), 030116 (△), F-A 5 (■), 020324 (□), 230164 (◆), and F-A 418 (◇).

within 1 min (Fig. 1C). The maximum value was obtained for leaves of shoots grafted on Carrizo citrange. Nonphotochemical quenching declined until reaching steady-state values after 8 min of actinic irradiation with higher values for leaves on Carrizo citrange.

Discussion

In this article, we describe how rootstock affected the photosynthetic process in leaves from 'Navelina' trees grown on calcareous soil. In this respect, net photosynthetic values for leaves of shoots grafted on the eight different citrus rootstocks under study were similar to those reported in the literature (Calatayud et al., 2006; Syvertsen, 1987; Vu et al., 2002). Only leaves on F-A 5 rootstocks showed a higher P_n rate than those on Carrizo citrange, whereas the intercellular CO_2 concentration (C_i) in leaves on Carrizo citrange was similar to the majority of other rootstocks. However, the g_s and the E for

leaves on Carrizo citrange were the lowest, as was chlorophyll content, when compared with the other citrus rootstocks assayed under the same conditions. Therefore, the lowest g_s and E values for leaves of shoots grafted on Carrizo citrange did not decrease in P_n or C_i . This can be explained by the fact that, as occurs under water stress, only very critically low levels of g_s affect P_n and C_i , which is in agreement with Flexas et al. (2004).

On the other hand, although P_n for leaves of shoots grafted on Carrizo citrange was not lower than for those grafted on the other rootstocks under study, fluorescence was impaired because leaves of shoots grafted on Carrizo citrange had a lower F_m , F_v/F_m ratio, Φ_{PSII} , and q_P , but higher q_N than those on the other rootstocks. The same behavior has been reported for Troyer citrange, in which the F_v/F_m ratio decreased significantly with low Fe availability in calcareous soils (Pestana et al., 2005).

Maximum efficiency of photosystem II (F_v/F_m) is only slightly affected by Fe deficiency in orange tree leaves (Pestana et al., 2001). However, we show that the variable part of the fluorescence was clearly affected by Carrizo citrange rootstocks on citrus trees grown on calcareous soils. The F_v/F_m ratio was much lower in leaves of shoots grafted on this rootstock without changes in F_o as compared with leaves on the other rootstocks, especially F-A 5 and 030116, for which F_v/F_m values were the highest (Table 2). This decrease in the value of F_v/F_m in Carrizo citrange was the result of a decrease in F_m (maximum fluorescence yield in the dark-adapted leaves), suggesting that the photochemistry of PSII was affected (Calatayud et al., 2004). By contrast, under the same conditions, F_m leaves of shoots budded on F-A 5 and on 030116 remained unaffected. Similar changes have been observed in Fe-deficient leaves of peach and pear trees and grapevine (Baveresco et al., 2006; Morales et al., 2000; Nedunchezian et al., 1997). Furthermore, 'Navelina' leaves of shoots grafted on Carrizo citrange displayed the lowest fraction of open PSII centers, q_P , in the steady state (Fig. 1B). This would indicate an incomplete reoxidation of the Q_A acceptor and an increase in closed PSII centers during actinic irradiation (Calatayud et al., 2002). Leaves of shoots grafted on the other rootstocks had significantly higher q_P values than on Carrizo citrange. The greater rate of closing of PSII centers in leaves on Carrizo citrange reduced the possibility of electron transport to photosystem I (Calatayud et al., 2004; Seaton and Walker, 1990). According to these q_P values, efficiency of PSII centers (Φ_{PSII}) value in the steady state was lowest in leaves of shoots grafted on Carrizo citrange. In addition, the incomplete reoxidation of the Q_A acceptor led to a greater fraction of reduced Q_A in Carrizo citrange and, consequently, an increase in nonphotochemical quenching at the expense of photochemical use of excitation energy, as shown by our results for q_N values. Leaves of shoots grafted on Carrizo citrange and F-A 418

showed the highest photoprotective q_N in the steady state (Fig. 1C). 'Navelina' leaves of shoots budded on the other rootstocks presented q_N values that were significantly minor to Carrizo citrange. The complementary study of the fraction neither used in photochemistry nor dissipated in the antennae (%X) showed that Carrizo citrange had the highest %X as compared with the other rootstocks (data not shown). This fraction is the least desirable pathway because it may lead to oxidative damage (Calatayud et al., 2003; Demmig-Adams et al., 1996).

On the other hand, the study of non-photochemical quenching (q_N) during fluorescence kinetics showed that q_N reached their maximum value within 1 min for leaves of shoots grafted on all rootstocks and leaves on Carrizo citrange had the highest q_N value (Fig. 1C). Subsequently, q_N values underwent a fast relaxation apart from Carrizo citrange, which had a slower ramp (Fig. 1C). This would be related to the fact that 'Navelina' leaves of shoots grafted on Carrizo citrange rootstocks grown in calcareous soil had lower stomatal opening and transpiration (g_s and E values) than those budded on all the other rootstocks. This limitation also correlated with a reduction in energy conversion efficiency of PSII (Φ_{PSII}) for Carrizo citrange during fluorescence kinetics, because its yield was significantly lower compared with the other rootstocks 2 min into the kinetics study (Fig. 1A). Moreover, the fraction of open PSII centers, q_P , decreased, reaching $\approx 70\%$ for all rootstocks after the first saturation. However, this situation quickly reversed and all rootstocks recovered, reaching 90% of open PSII centers in the following saturations, apart from Carrizo citrange, which could not surpass 73% (Fig. 1B). This could be the result of the incomplete reoxidation of Q_A , leading to the downregulation of photosynthesis in comparison with the other rootstocks under assay.

In conclusion, based on chlorophyll content, gas exchange, and the Chl *a* fluorescence characteristics, trees grafted on F-A 5 performed best under these calcareous soil conditions, whereas those on Carrizo citrange were poorly adapted. Trees on the other six hybrid rootstocks performed well. These results are consistent with previous field evaluations on F-A 5 and F-A 13 rootstocks.

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