

Physiological performance and productivity of Tahiti lime under irrigation and rootstocks in the Amazon

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Abstract

The aim of this research was to study the physiological and productive performance of five rootstocks cultivated in the lemon 'Tahiti CNPMF-02' under irrigated and non-irrigated conditions in the Amazon in the sixth year of dense planting. In a completely randomized design, in a 2x5 factorial scheme (irrigation x rootstock) evaluated in the dry and rainy season, applied to the rootstocks citrumelo 'Swingle' (CTSW), lemon tree 'Cravo Santa Cruz' (LCSC), citrandarim 'San Diego' (CTDI), and 2 hybrids crossing tangerine 'Sunki Tropical' and *Poncirus trifoliata* 'Flying Dragon' called TSKCxTRFD-003 (003), TSKCxTRFD-006 (006), being evaluated water relations, gas exchange, chlorophyll fluorescence, among other physiological characteristics and also the production components were. Rootstock 006 has a more efficient physiological metabolism, with and without irrigation in the face of water deficit, being more productive in the Amazon. Rootstock 003, although with good metabolic performance and adequate plant architecture, even under water deficit conditions, did not demonstrate changes in productivity. The CTDI rootstock, even when irrigated, did not demonstrate gains in productivity in the dry season, even with efficient physiological mechanisms of drought tolerance. The LCSC rootstock has less tolerance to water deficit, and even under irrigation in the dry season, it does not show improvements in its performance. However, this rootstock has high productivity in the rainy season regardless of irrigation. The CTSW rootstock must be used in restricted plantings only in management that can be irrigated, associated with annual crown adjustment pruning, avoiding densification without densification and mandatory annual crown adjustment pruning.

Keywords: dense planting, drought tolerance, gas exchange, irrigation strategies water deficit

Introduction

The estimated area of lemon cultivation in Brazil reached 51,809 hectares in 2022 (FUNDECITRUS, 2022), especially the acid lime variety known as 'Tahiti' [*Citrus latifolia* (Yu. Tanaka) Tanaka], which has shown increasing participation in foreign markets, ranking as the third most exported fruit. The production of 'Tahiti' limes reached 1.5 million tons in 2021, placing Brazil in the fifth position in the world production of limes and lemon (KIST; BELING, 2023).

In the Amazon region, the state of Pará stands out as the third-largest lemon-producing state in Brazil, with a yield of 84.7 thousand tons in 2021, behind the traditional citrus-producing regions of São Paulo and Minas Gerais (KIST; BELING, 2023).

Maximizing commercial yields and ensuring the economic sustainability of 'Tahiti' lime production in the Amazon depends on careful selection of the scion/rootstock combinations. Additionally, irrigation is one of

the technologies that has demonstrated the most positive outcomes across different regions of Brazil, contributing to increased fruit production both during and outside the main harvest season (BREMER NETO et al., 2013; ESPINOZA-NÚÑEZ et al., 2011; PINTO et al., 2023).

The choice of rootstock is one of the most critical factors in establishing a productive 'Tahiti' lime orchard, as it is responsible for providing plant support, absorbing water and nutrients from the soil, promoting vigor and early fruiting, and synthesizing certain plant hormones. Furthermore, it also influences the precocity and fruit maturation timing, fruit weight, juice yield, peel and juice color, and the levels of sugar and acid. The most suitable rootstocks also enhance fruit retention on the tree, postharvest shelf life, and tolerance to salinity, drought, frost, diseases, nematodes, among other stressors (AZEVEDO et al., 2021).

Rootstocks confer different survival strategies to

the scion under water deficit conditions (SANTANA-VIEIRA et al., 2016), such as reduced transpiration and increased water use efficiency, often associated with partial stomatal closure and/or reduced plant growth, as well as the preferential allocation of energy toward root system development (LYNCH; WOJCIECHOWSKI, 2015).

Brazilian citrus production is currently dominated by two main rootstocks: 'Rangpur' lime (27%) and 'Swingle' citrumelo (55%) (FUNDECITRUS, 2022). However, rootstock diversification is of great importance for the sustainability of the citrus industry, particularly in mitigating risks associated with abiotic factors—mainly related to drought. Even in the Amazon region, seasonal water scarcity and the high costs of irrigation system implementation drive the ongoing search for rootstocks that are either more water-use efficient under irrigated systems or more drought-tolerant in rainfed orchards (SAMPAIO et al., 2021).

In this study, five rootstocks were evaluated: 'Swingle' citrumelo (*C. paradisi* Macfad. × *P. trifoliata*) (CTSW) and 'Rangpur' lime – 'Santa Cruz' clone (LCSC). The three remaining 'new' rootstocks had previously shown superior agronomic performance in Guarantã do Norte, Mato Grosso (RONCATTO et al., 2021): 'San Diego' citrandarin (*C. sunki* × *P. trifoliata* 'Swingle') (CTDI), and two hybrids from the cross between 'Sunki Tropical' tangerine (*C. sunki*) and *Poncirus trifoliata* 'Flying Dragon', designated TSKC × TRFD – 003 (003) and TSKC × TRFD – 006 (006), recently registered as 'CNPMF Donadio' and 'CNPMF Matta', respectively. These hybrids were developed through the Citrus Breeding Program (PMG Citros) of Embrapa Mandioca e Fruticultura, located in Cruz das Almas, Recôncavo Baiano (CARAVINA et al., 2022).

The objective of the present study was to evaluate the physiological and agronomic performance of 'Tahiti' lime grafted onto five different rootstocks under varying water availability during the dry and rainy seasons of the Amazon biome.

Materials and Methods

Study Area

The experiment was established using a collection of 280 citrus plants from 14 different rootstocks, all grafted with the same scion variety: the acid lime 'Tahiti CNPMF-02'. The lime trees have been planted since December 2016 at the experimental farm of the Federal Institute of Mato Grosso (IFMT), in Guarantã do Norte – MT, located at 09°47'15" S and 54°54'36" W, at an altitude of 340 m, within the Amazon biome. According to the Köppen-Geiger classification, the local climate is

Aw (tropical with a dry winter), with an annual average temperature of 25°C, precipitation of 2,000 mm, and a dry season from May to September (CARAVINA et al., 2021).

The soil was classified as a typic dystrophic Hapludox, and for the soil water balance calculations, the final available water capacity (AWC) was estimated at 90 mm (SOIL SURVEY STAFF, 2014).

Evapotranspiration was estimated using remote meteorological data from the Guarantã do Norte-MT weather station of the National Institute of Meteorology (INMET), supported by forecasts from the COSMO (Consortium for Small-scale Modeling) numerical weather prediction model with a horizontal resolution of 7 km. Variables included relative humidity, wind speed and direction, and solar radiation, accessed via the Sistema de Suporte à Decisão na Agropecuária (SISDAGRO) (INMET, 2023).

For calculating the crop water balance (CWB), data from SISDAGRO were used, which estimates a crop coefficient (Kc) of 0.85 for lime. Precipitation was recorded locally using a rain gauge (Boca Grande São Izidro®), while temperature and relative humidity were monitored with a DataLogger (Skill-Tec®).

Experimental Conditions and Treatments

For the first six years, all plants were irrigated during the dry season and were not pruned to fit the dense planting layout (6.5 m × 3.0 m). Canopy pruning was carried out only in March 2022, 63 months after planting.

The experiment began in April 2022, using a split-plot factorial design with five rootstocks and two irrigation levels (with and without irrigation), totaling six replicates per treatment and 60 experimental units. Evaluations were conducted during two distinct periods: the dry season (May to September), with fruit harvested in November 2022, and the rainy season (October to March), with harvest in March 2023.

Irrigation started in April, at the onset of the dry season, using micro-sprinklers under the canopy, applying a fixed daily water depth of 1.52 mm. Evapotranspiration was corrected using a KL factor following the methodology of Fereres (1981), considering an average wetted area percentage (WAP) of 45% (MANTOVANI; BERNARDO; PALARETTI, 2007).

During the 170-day dry season, from May to October 2022, irrigation replenished on average 28% of the crop evapotranspiration (ETc). The highest decadal ETc was recorded in the first ten days of September, at 72.52 mm, representing 19.56% of daily ETc (see **Figure 1**).

Local minimum, maximum, and average temperatures over 12 months are shown in **Figure 2**.

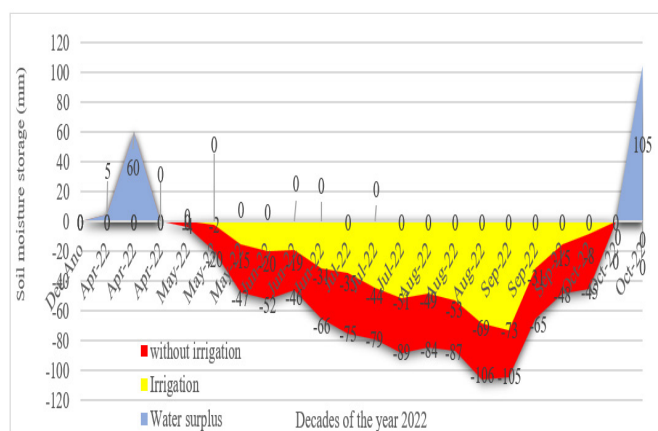


Figure 1 – Decadal crop water balance (CWB) of 'Tahiti' lime with and without irrigation during the 2022 dry season at the Federal Institute of Mato Grosso – Campus Guarantã do Norte-MT.

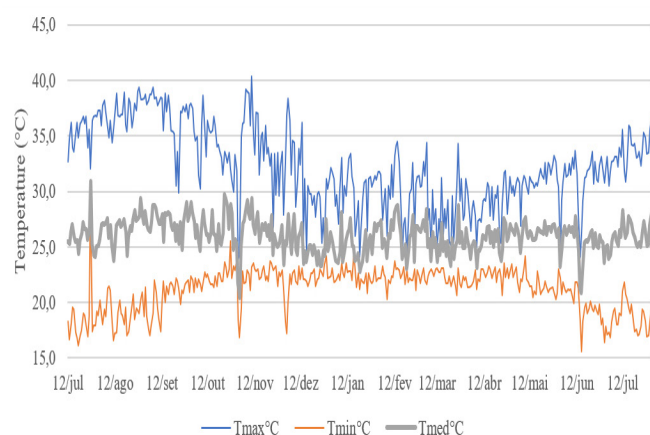


Figure 2 – Maximum, minimum, and average temperatures recorded at IFMT – Campus Guarantã do Norte-MT from July 2022 to July 2023.

Plant Evaluations

Physiological evaluations of the plants in their respective treatments, as detailed below, were carried out at the beginning June 2022, during a period clearly characterized as the dry season, and again in March 2023, during a period clearly characterized as the rainy season. Accumulated rainfall during the rainy season was 1,407 mm with an excess water availability of 1,098 mm (CWB).

Evaluations and sample collections were always performed on leaves at breast height, fully expanded, without apparent signs of morphological damage, and positioned in the canopy on the sun-facing side of the canopy.

Water Relations Evaluations

Leaf water potential (Ψ_w) measurements were taken using a Scholander-type pressure pump (SCHOLANDER et al., 1965) by pressurizing the leaf blade with nitrogen gas in a sealed chamber until the xylem sap was expelled through the petiole. The required pressure was recorded by a digital pressure gauge attached to the equipment. Leaf collection was carried out in the morning between 4:00 and 6:00 a.m.

To determine the osmotic potential (Ψ_o), fully expanded leaves were collected and subsequently stored in a freezer at -20°C until evaluation, when they were allowed to thaw, with subsequent sap extraction by compression of the thawed sample. The extracted sap was centrifuged for 10 minutes at 5,000 rpm, and then 10 μL samples of the supernatant were used to read the osmolality in a vapor pressure osmometer (Vapro 5520 - Wescor®). The osmotic potential (ψ_o) values were calculated using the Van't Hoff equation, and the results converted to MPa.

Relative water content (RWC) was determined by collecting three leaf discs, each 1.0 cm in diameter, and weighing them on a precision scale to obtain fresh mass (FM). Subsequently, the discs were submerged in distilled water for 24 hours and then weighed to obtain turgid mass (TM). Finally, the discs were left to dry in an oven at 65°C for 48 hours to obtain dry mass (DM). RWC (%) was calculated using Equation (1):

$$\text{RWC} = ((\text{FM} - \text{DM}) / (\text{TM} - \text{DM})) \times 100 \quad (1)$$

Gas Exchange Evaluations

Gas exchange was evaluated with an infrared gas analyzer (IRGA) equipped with a fluorometer (model LI-6800xt, LI-COR Inc.®, Lincoln, USA). The following parameters were obtained: photosynthetic rate (A), transpiration rate (E), stomatal water vapor conductance (gs), carboxylative efficiency in terms of substomatal/ambient CO_2 ratio (C_i/C_a ratio). Measurements were performed between 8:00 and 11:00 a.m., using a constant photosynthetically active radiation (PAR) of 1500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, with a constant CO_2 concentration of 400 ppm, temperature of $25\text{--}27^\circ\text{C}$, and relative humidity of 50%–60%.

Chlorophyll a Fluorescence Evaluation

The transient chlorophyll a fluorescence variables were performed on the same leaf as the photosynthesis measurements, using a portable fluorometer (FluorPen FP100, Photon Systems Instruments®; Drasov, Czech Republic), on a fully expanded leaf, which was previously adapted to the dark for 30 minutes for complete

oxidation of the photosynthetic electron transport system. Subsequently, a pulse of 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of blue light was emitted, measuring the minimum fluorescence (F_0) at 50 μs , when all PSII reaction centers (photosystem II) are open and defined as the O step, followed by the J step (at 2 μs), the I step (at 30 μs) and the maximum fluorescence (F_m) when all PSII reaction centers are closed, known as the P step. These values were used to estimate several bioenergetic indices of PSII, according to (STRASSER; SRIVASTAVA; TSIMILLI-MICHAEL, 2000). The values related to the effective quantum yield of PSII (ϕE_0), energy absorption flux per reaction center (ABS/RC), and the dissipated energy flux per reaction center (DI_0/RC) were obtained.

Falker® Chlorophyll Index Evaluation

The non-destructive determination of chlorophylls (Falker® Chlorophyll Index – ICF®) was performed using a portable electronic chlorophyll meter (ClorofilOG, model CFL 1030 – FALKER®, Porto Alegre, Brazil). The characteristics evaluated were the chlorophyll a index (Clor a), chlorophyll b index (Clor b) and total chlorophyll index (ClorTotal), avoiding the main vein of the leaves.

Fruit Harvest and Yield Assessment

Fruits with commercial standards were harvested weekly throughout the year. For phytosanitary control purposes, all non-commercial and non-standard fruits, whether fallen or not, were removed from the experiment. The weekly commercial harvest was interrupted 30 days before the two harvests, with individual weighing of the sample units statistically analyzed in the study—one in the dry season and the other in the rainy season.

The off-season occurs annually from May to November, with reduced production associated with water deficits in the plants, even with the use of irrigation (Figure 1). This increased the commercial value of the fruit (Figure 3). This fact determined the two dates required for the fruit sampling in this study. Consequently, the dry season sampling occurred on November 29, 2022 (Figure 3).

The main harvest occurs in the rainy season, starting in December and lasting until April. Sampling for this harvest took place on March 7, 2023 (Figure 3)

Canopy Volume Analysis and Productive Efficiency

Crown volume (VCopa) was calculated biannually after canopy adjustment pruning in March 2022, using the formula $V = 2 \cdot 3 \cdot 1 \cdot [(\pi \cdot D^4 \cdot 1) \cdot H]$, where:

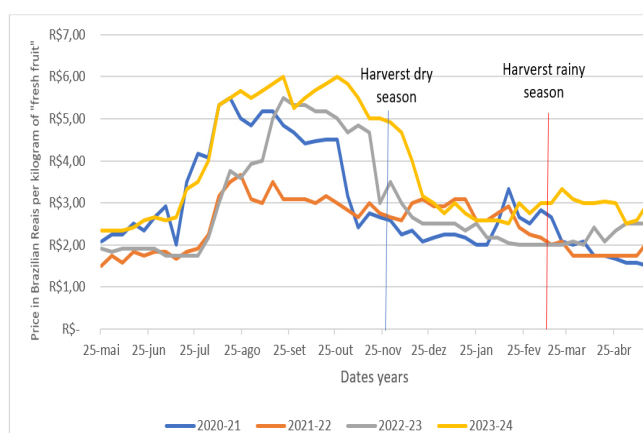


Figure 3 - Weekly market prices of 'Tahiti' lime at the Cuiabá Wholesale Supply Center – MT (2020–2024), and dates of samples harvest during of production in the dry and rainy season in 2022–2023 crop in Guarantã do Norte – MT.

where V is the crown volume, D is the average crown diameter (m), and H is the plant height (m); Vegetative Vigor Index (VVI), calculated by the formula $VVI = [H + D + (DPE \times 10)] / 100$, H is the plant height (cm), D is the crown diameter (cm), and DPE is the rootstock trunk diameter (cm) BORDIGNON et al., 2003.

Productive efficiency (EfProd) was calculated as the ratio between fruit yield ($\text{kg} \cdot \text{plant}^{-1}$) and canopy volume (m^3), and results were compared with historical data obtained from the same plants in previous evaluations (CARAVINA et al., 2023a; RONCATTO et al., 2023b).

Experimental Design and Statistical Analyses

The experiment followed a completely randomized design in a 2×5 factorial scheme, consisting of two water availability conditions (irrigated and non-irrigated) and five rootstocks, with six plants per treatment, totaling 60 experimental units. Evaluations were conducted during two periods: the dry season and the rainy season. Data were first tested for normality using the Shapiro-Wilk test at a 5% significance level, followed by analysis of variance (ANOVA) and F-test. Means were compared using Tukey's test at a 5% significance level, with statistical analyses performed using R software.

The original data for the variables number of fruits (nFrutos), total yield in grams (PTotal), and productive efficiency per plant (EfProd) did not present normality, so they were subjected to logarithmic transformation to present a normal distribution.

Results and Discussion

Regardless of rootstock, and as expected, irrigated plants exhibited improved values for water relation traits compared to non-irrigated plants. However,

no significant differences were observed. Non-irrigated ones presented lower water potential values (Figure 4C), a result similar to that reported for the LCSC rootstock grafted with Pera Orange during the dry season (SAMPAIO et al., 2021), as well as lower relative water content (RWC) values (Figure 4E). Nevertheless, during the rainy season, a recovery in these water relation traits was observed in the non-irrigated plants across all rootstocks evaluated (Figures 4B, D, and F). Interestingly, non-irrigated plants grafted onto rootstocks LCSC and 003 during the dry season exhibited higher RWC values in the rainy season than the irrigated plants (Figure 4F), indicating a strong recovery capacity in these genotypes. For all other water relation traits, no significant differences were observed among the rootstocks, regardless of the water availability regime imposed

In general, water restriction during the dry season led to a reduction in gas exchange parameters across all

evaluated rootstocks (Figures 5A, C, and E). During this period, although a decrease in photosynthetic rate was observed in the non-irrigated rootstocks, the rootstock CTDI maintained photosynthetic rates equivalent to the irrigated control plants (Figure 5A). Interestingly, among the irrigated plants, the lowest photosynthetic rates were observed precisely in CTDI and 006 (Figure 5A). Similarly, to photosynthetic rate, the dry season induced significant reductions in stomatal conductance (Figure 5C) and transpiration rate (Figure 5D) in all non-irrigated rootstocks.

Among irrigated plants during the dry season, lower photosynthetic rates were again observed in rootstocks CTDI and 006. However, notably higher stomatal conductance and, consequently, higher transpiration rates were observed only in irrigated plants grafted onto LCSC during the dry season, without translating into higher photosynthetic rates compared to the other rootstocks.

During the rainy season, in general, no significant

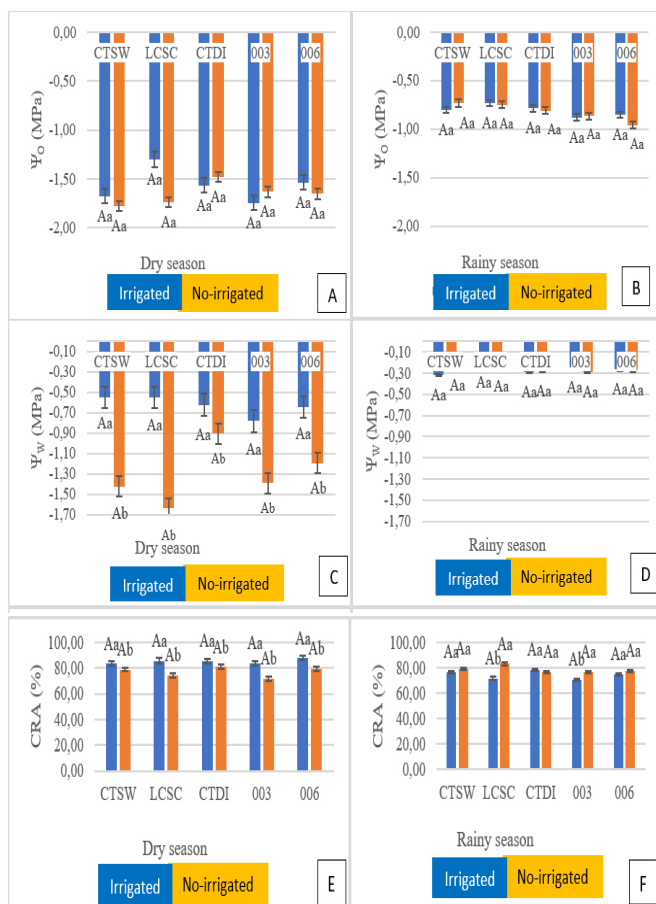


Figure 4 – Osmotic potential (Ψ_o) (A and B), leaf water potential (Ψ_w) (C and D), and relative water content (RWC) (E and F) of 'Tahiti' acid lime trees grafted onto five different rootstocks, under irrigated and non-irrigated conditions, subjected to water stress during the dry and rainy seasons (2022–2023), at six years of age in Guarantã do Norte, MT, Brazil. Means followed by the same uppercase letter (among rootstocks) or lowercase letter (within the same rootstock) do not differ statistically according to Tukey's test at a 5% probability level.

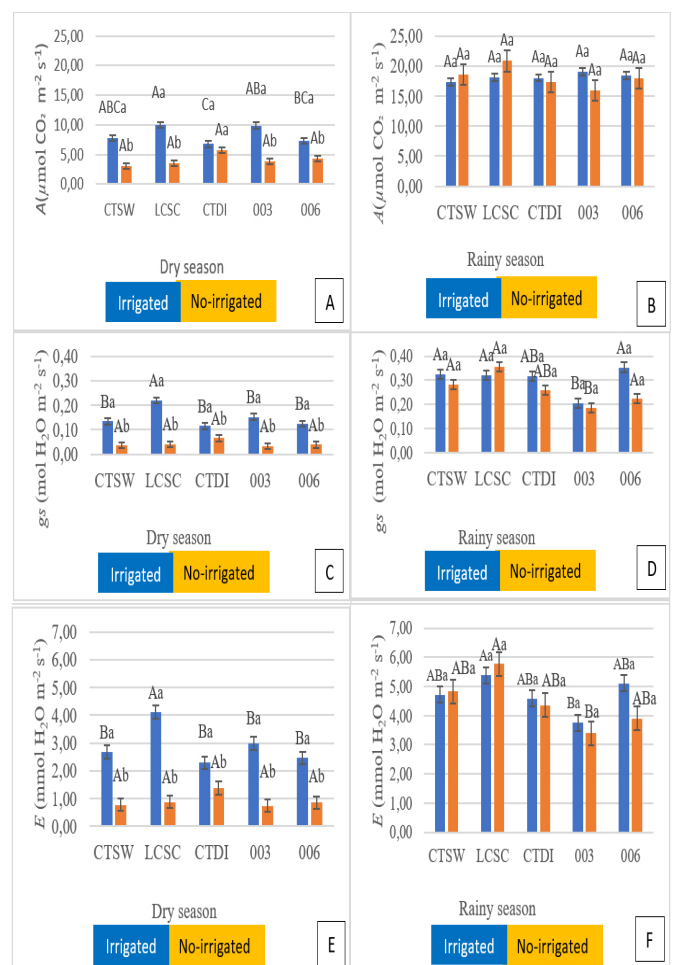


Figure 5 – Photosynthetic rate (A) (A and B), stomatal conductance (g_s) (C and D), and transpiration rate (E) (E and F) of 'Tahiti' acid lime trees grafted onto five rootstocks, under irrigated and non-irrigated conditions, subjected to water deficit during the dry and rainy seasons (2022–2023), at six years of age in Guarantã do Norte, MT.

differences were observed between irrigated and non-irrigated plants, regardless of rootstock. The only exception was rootstock 003, which exhibited lower stomatal conductance (Figure 5D) and transpiration rate (Figure 5F) under both water regimes, without affecting the photosynthetic rate (Figure 5B).

Brazil. Means followed by the same uppercase letter (among rootstocks) and lowercase letter (within the same rootstock) do not differ significantly according to Tukey's test at a 5% probability level.

Water restriction also reduced the C_i/C_a ratio across all non-irrigated rootstocks during the dry season (Figure 6A). However, regardless of water regime, rootstock 003 consistently exhibited the lowest values for this parameter (Figure 6A). In the same season, Rubisco carboxylation efficiency (A/C_i) remained stable only in the non-irrigated CTDI and 006 rootstocks (Figure 6C),

while all other rootstocks showed significant reductions in this trait under water deficit. Under irrigated conditions, no major variation in these parameters was observed among rootstocks, except for CTDI, which showed a lower A/C_i compared to the others (Figure 6C). As expected, water use efficiency (A/E) was significantly increased in all non-irrigated rootstocks during the dry season (Figure 6E). Among irrigated plants, however, a lower A/E was observed only in the LCSC rootstock.

In general, during the rainy season, these physiological traits recovered, and differences between irrigated and non-irrigated plants were no longer observed (Figures 6B, D, and F). The only exception was rootstock 003, which consistently exhibited a lower C_i/C_a and higher A/E ratio regardless of water regime.

The low C_i/C_a ratio observed during the dry season (Figure 6A), with significant differences among all rootstocks under irrigation, can be explained by stomatal closure, which limits carbon entry. However, all the carbon that enters is effectively fixed, indicating that drought does not cause metabolic damage severe enough to inhibit carbon fixation. The reduced photosynthetic rate (A) observed (Figure 5A) is therefore attributed to stomatal limitations. This demonstrates a good agronomic response of the scion-rootstock combination, even under non-irrigated conditions facing the regional water deficit. Furthermore, with the return of rains, all rootstock materials quickly recover full metabolic function (Figure 6B).

Regarding instantaneous Rubisco carboxylation efficiency (Figure 6C) during the dry period, the irrigated 003 rootstock showed higher efficiency than CTDI and the highest absolute value among all rootstocks. This indicates greater efficiency in fixing the carbon entering the stomatal cavity, maintaining low internal CO_2 concentration and achieving high carbon fixation rates. However, under non-irrigated conditions, no differences were observed among rootstocks. It is noteworthy that the CTDI rootstock exhibited low efficiency even with irrigation, showing no significant responsiveness to irrigation in this parameter.

For the 006 rootstock, no significant differences in instantaneous Rubisco carboxylation efficiency (A/C_i) (Figure 6C) were found between irrigation treatments. When correlated with the minor statistical difference in photosynthetic rate (Figure 5A) and the second-lowest C_i/C_a ratio among rootstocks (Figure 6A), this suggests a higher tolerance to water deficit.

The Falker® chlorophyll indices (chlorophyll *a*, chlorophyll *b*, and total chlorophyll) did not show differences at the two physiological assessment times.

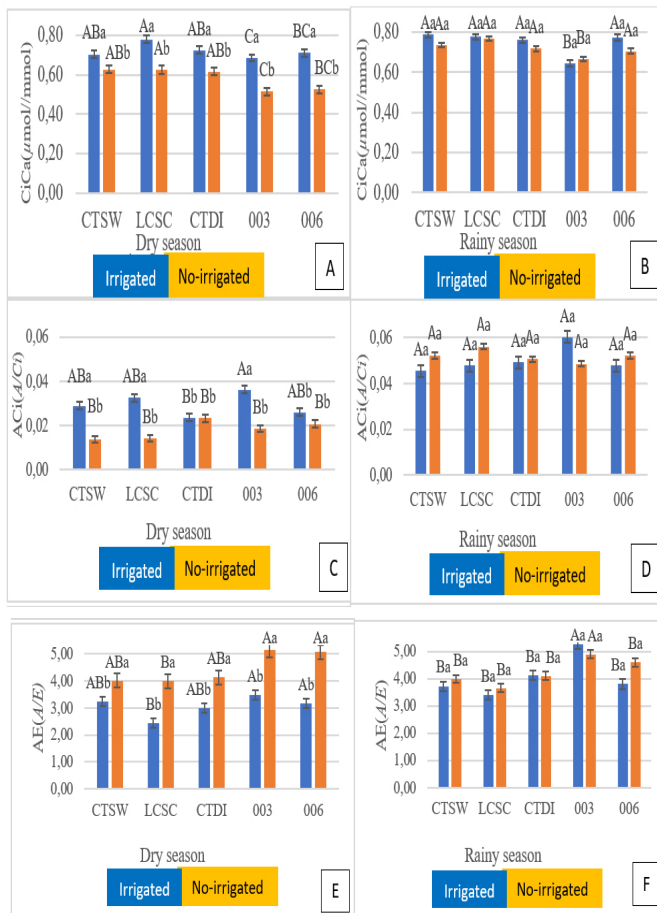


Figure 6 – Intercellular to ambient CO_2 concentration ratio C_i/C_a (A e B), instantaneous Rubisco carboxylation efficiency (A/C_i) (C and D), and instantaneous water use efficiency (A/E) (E and F) of 'Tahiti' acid lime trees grafted onto five rootstocks, under irrigated and non-irrigated conditions, subjected to water stress during the dry and rainy seasons (2022–2023), at six years of age in Guarantã do Norte, MT, Brazil. Means followed by the same uppercase letter (among rootstocks) and lowercase letter (within the same rootstock) do not differ significantly according to Tukey's test at a 5% probability level.



Figure 7 – Maximum quantum yield of PSII (F_v/F_m) (A and B) and effective quantum yield of PSII (ϕE_0) (C and D) in 'Tahiti' acid lime grafted onto five rootstocks under irrigated and non-irrigated conditions subjected to water deficit during the dry and rainy seasons (2022–2023) at six years of age in Guarantã do Norte-MT, Brazil. Means followed by the same uppercase letter among rootstocks and lowercase letter within the same rootstock do not differ significantly by Tukey's test at 5% probability.

rootstocks during the dry season (Figures 7A and 7C). However, regardless of irrigation, the CTSW rootstock consistently showed the lowest F_v/F_m values. During the rainy season, F_v/F_m and ϕE_0 showed no differences among rootstocks or irrigation treatments (Figures 7B and 7D).

We observed that under water deficit conditions (Figures 8A and 8C), there was an increase in the energy absorption flux per reaction center (ABS/RC) and energy dissipation flux per reaction center (D_i/RC), regardless of the rootstock. However, independent of the water regime, rootstock 006 exhibited the lowest values for these parameters compared to rootstock CTSW. Nevertheless, during the rainy season, recovery of ABS/RC and D_i/RC fluxes was observed in plants across all rootstocks studied, with no significant differences (Figures 8B and 8D).

Throughout the evaluated year, the vegetative development parameters, including canopy volume (VCopa) and vegetative vigor index (IVV), followed the continuous growth of the plants during the dry season (Figures 9A and 9C) and rainy season (Figures 9B and 9D). As expected, the canopy volume of all irrigated rootstocks was higher in both seasons. However, rootstock 006 stood out as the most vigorous, followed by 003 at the end of the crop cycle (Figure 9B). In the IVV, this trend was repeated and accentuated when compared with CTSW as the control (Figure 9D).

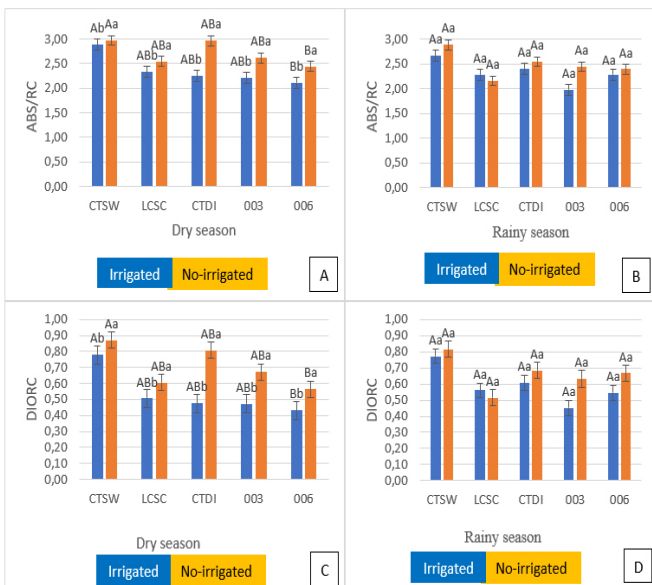


Figure 8 – Energy absorption flux per reaction center (ABS/RC) (A and B), energy dissipation flux per reaction center (D_i/RC) (C and D) of 'Tahiti' acid lime grafted onto five rootstocks, irrigated and non-irrigated under water deficit during the dry and rainy seasons (2022–2023) at six years of age in Guarantã do Norte-MT, Brazil. Means followed by the same uppercase letter among rootstocks, and lowercase letters within the same rootstock, do not differ by Tukey's test at 5% probability.

Overall, water deficit caused minimal changes in chlorophyll fluorescence characteristics. The maximum quantum yield of PSII (F_v/F_m) and the effective quantum yield of PSII (ϕE_0) remained stable in all non-irrigated

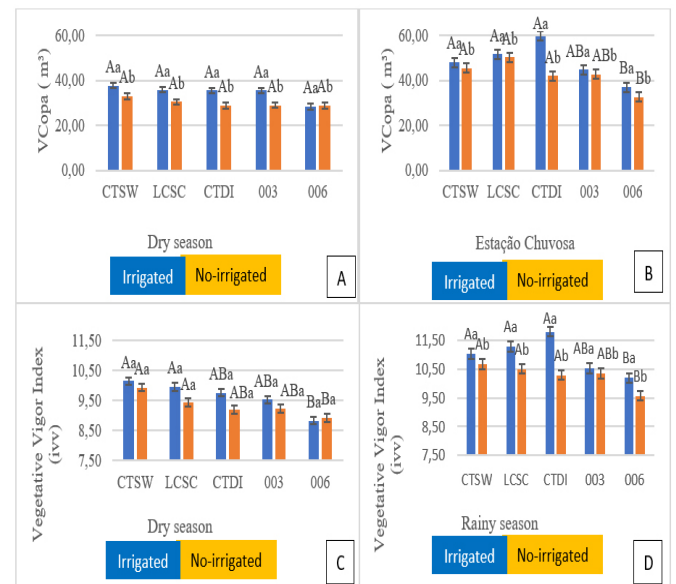


Figure 9 – Canopy volume in m^3 (VCopa) (A and B) and vegetative vigor index (IVV) (C and D) of 'Tahiti' acid lime grafted onto five rootstocks, irrigated and non-irrigated under water deficit during the dry and rainy seasons (2022–2023) at six years of age in Guarantã do Norte-MT, Brazil. Means followed by the same uppercase letter among rootstocks, and lowercase letters within the same rootstock, do not differ by Tukey's test at 5% probability.

Rootstock 006, regardless of the imposed water regime, showed superior performance during water deficit (Figures 10A, C, and E) in all productive components: number of fruits per plant (nFrutos), total production in grams per plant (PTotal), and relative productive efficiency (EfProd), presenting consistent behavior across parameters.

In the rainy season, an increase in all productive components was observed regardless of rootstock (Figures 10B, D, and F). Irrigation during the dry season still positively influenced the CTSW and CTDI rootstocks for nFrutos and PTotal (Figures 10B and D). Interestingly, the non-irrigated LCSC rootstock was more productive than the irrigated plants (Figures 10B, D, and F).

In this same rainy season, among the rootstocks,

006 was the most productive and efficient when irrigated (Figures 10B, D, and F), and together with LCSC for the non-irrigated plants. It is important to highlight the poor performance of the non-irrigated CTSW rootstock for nFrutos and EfProd.

The results obtained demonstrated better performance of the new rootstocks in both physiological and productive aspects when compared to CTSW and LCSC. The use of irrigation enabled improvements in water relations, with increased water availability in all rootstocks for the parameters Ψ_o , Ψ_w , and RWC.

The rootstock CTDI exhibits physiological mechanisms of drought tolerance, such as maintaining higher leaf water content (RWC) and better photosynthetic rate (A compared to CTSW, the most widely used rootstock in Brazilian citriculture and in the Tahiti lime-producing region studied in the Amazon. However, the productive performance of CTDI was similar to CTSW in nearly all evaluated parameters, including gas exchange, stomatal conductance (g_s), transpiration rate (E), chlorophyll fluorescence (Ci/CA , A/E, $e\phi E_o$), as well as production components.

The rootstock LCSC showed the lowest tolerance to water deficit, did not respond to irrigation, and stood out in productivity during the rainy season without irrigation. Due to its lower productive efficiency, it is not recommended for high-density planting. In other regions, LCSC is considered a reference for drought tolerance (BREMER NETO et al., 2013; CANTUARIAS-AVILÉS et al., 2012; DE CARVALHO et al., 2019; ESPINOZA-NÚÑEZ et al., 2011), but our data contradict this.

Among irrigated plants during the dry season, lower photosynthetic rates were observed in those grafted onto CTDI and 006 rootstocks. Interestingly, the highest stomatal conductance and consequently higher transpiration rates were found in irrigated plants on LCSC during the dry season, without resulting in higher photosynthetic rates compared to other rootstocks. The higher g_s in LCSC during this period likely allows greater water loss through stomata and is probably related to its well-known drought survival mechanism through sustained water extraction via root architecture and function, contrasting with CTDI, which reduces water use during the most critical drought period (SAMPAIO et al., 2021).

Non-irrigated LCSC plants produced more fruits (nFrutos) and total yield (PTotal) than irrigated plants during the rainy season, reinforcing its drought tolerance and low irrigation response. This behavior aligns with expected drought tolerance reported in other studies

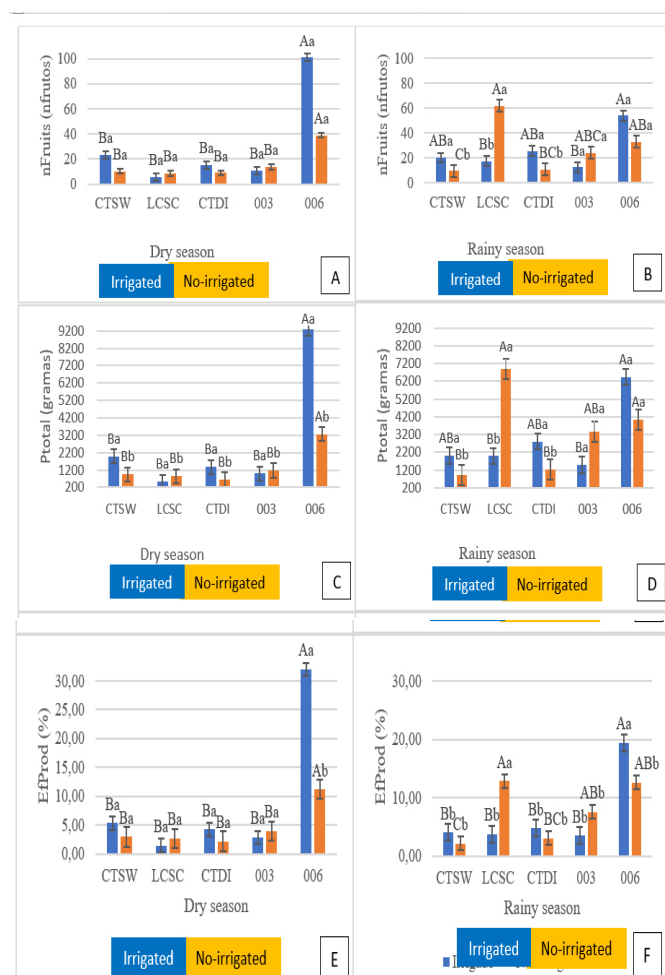


Figure 10 – Number of fruits per plant (nFrutos) (A and B), total production in grams per plant (PTotal) (C and D), and relative productive efficiency (EfProd) (E and F) of 'Tahiti' acid lime grafted onto five rootstocks, irrigated and non-irrigated under water stress during the dry and rainy seasons (2022–2023) at six years of age in Guarantã do Norte-MT, Brazil. Means followed by the same uppercase letter among rootstocks, and lowercase letters within the same rootstock, do not differ by Tukey's test at 5% probability. Original data were log10 transformed to meet normality assumptions.

(AMORIM et al., 2018; CANTUARIAS-AVILÉS et al., 2012; ESPINOZA-NÚÑEZ et al., 2011). However, under Amazonian conditions, its accelerated canopy volume (V_{copa}) growth reduces productive efficiency ($EfProd$).

For rootstock 003, its efficient metabolism and plant architecture that confer drought tolerance did not translate into total productivity or productive efficiency. Throughout the water deficit period, the amounts of $Clor$ and $ClorTot$ in 003 were higher than in CTSW, possibly associated with higher photosynthesis A and lower transpiration E , along with greater instantaneous water use efficiency (A/E) and lower Ci/Ca ratio, enabling rapid recovery with the return of rainfall.

The rapid response to increased water availability is evident as non-irrigated 003 plants in the dry season exhibited better RWC values during the rainy season than irrigated plants. They also maintained lower g_s and E regardless of irrigation, without affecting photosynthesis A . This is an interesting trait indicating better water economy and water use efficiency, as reduced stomatal conductance decreases water loss by transpiration without reducing carbon assimilation rate.

Analyzing vegetative development (V_{copa} and IVV) at the end of the crop cycle, rootstock 003 can be considered "semi-dwarf," a desirable trait for high-density planting in the Amazon. While its productivity is similar to CTSW, it compensates with higher productive efficiency, though still below 006.T

Rootstock 006 has a more efficient physiological metabolism with and without irrigation under water deficit, explaining its higher productivity during both dry and rainy seasons in the Amazon. Throughout the drought period, 006 maintained higher leaf water content (RWC) and chlorophyll a compared to CTSW. Additionally, A/Ci did not differ with irrigation, and combined with the small statistical difference in photosynthetic rate (Figure 5A) and the second lowest Ci/Ca ratio among rootstocks (Figure 6A), this indicates greater drought tolerance.

During the dry season, ABS/RC and $(DI_o)/RC$ in rootstock 006 were significantly lower than in CTSW, further indicating higher drought tolerance.

The superior productivity of rootstock 006 can also be explained by its vegetative development at the end of the cycle, where it stood out as dwarf-type, and due to its size at the end of the season, no additional pruning was necessary. This suggests that metabolites were directed towards fruit production in both dry and rainy seasons, irrigated or not. A similar but less pronounced behavior was observed in rootstock 003. Plants exhibiting dwarfing characteristics are considered agronomically superior as

reduced rejuvenation pruning enhances their use in high-density orchards.

For V_{copa} and IVV, rootstocks showed similar behaviors to the first three crop cycles in the same experiment, which was irrigated year-round without pruning (Roncatto et al., 2023a).

During the dry season, $nFrutos$, $PTotal$, and $EfProd$ of rootstock 006, both irrigated and non-irrigated, exceeded those of other rootstocks, confirming its agronomic superiority. This also coincides with the highest market value of fruits over four consecutive off-seasons in Mato Grosso (Figure 3). Rootstock 006 demonstrated the best overall productive component performance among rootstocks evaluated in both seasons. Thus, in high-density plantings, its dwarf canopy volume does not compromise total production or productive efficiency, corroborating results from the first three irrigated crop cycles (CARAVINA et al., 2023b).

Rootstock CTSW exhibits high vegetative vigor and low drought tolerance; therefore, its use in the Amazon should be restricted to irrigated, non-dense plantings with mandatory annual pruning for canopy management. One factor compromising its performance is the high DIO/RC flux, as CTSW dissipates energy non-photochemically as heat or reactive oxygen species (ROS), likely linked to its greater water demand needed to sustain larger canopy growth compared to the dwarfing rootstock 006.

When choosing rootstocks, the consequence of increased canopy volume (V_{copa}) without proportional increases in total yield ($PTotal$) is higher costs for cultural practices and pruning, along with greater difficulty in mechanization and harvest (Stuchi et al., 2012), thus not recommended for dense planting due to reduced productive efficiency. In our study, CTSW showed negative results when non-irrigated for $nFrutos$ and $EfProd$.

The increased $EfProd$ of CTSW in the rainy season (Figure 10F) is conditioned by irrigation during the dry season, as observed in other citrus-growing regions (BREMER NETO et al., 2013). In Amazonian conditions, accelerated canopy growth reduces productive gains during the rainy season; therefore, its use should be limited to non-dense plantings with mandatory annual canopy pruning.

Conclusions

Rootstock 006 exhibits a more efficient physiological metabolism, both with and without irrigation under water deficit, which explains its higher productivity during the dry and rainy seasons in the Amazon.

For rootstock 003, its efficient drought tolerance metabolism and plant architecture do not translate into higher total productivity or productive efficiency.

Rootstock CTDI shows physiological mechanisms of drought tolerance and responds to irrigation, but does not provide significant productive gains compared to currently used rootstocks in the Amazon.

Rootstock LCSC presents the lowest tolerance to water deficit, does not respond to irrigation, and stands out in productivity during the rainy season without irrigation; however, it is not recommended for high-density plantings due to lower productive efficiency.

Rootstock CTSW has high vegetative vigor and low drought tolerance; therefore, its use in the Amazon should be limited to irrigated, non-dense plantings with mandatory annual canopy pruning.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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