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Comparative Analysis of Morphological and Physiological Traits in Four Citrus Cultivars

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Abstract

This study considers citrus rootstocks as autonomous biological entities and examines whether, and to what extent, they differently regulate plant–water relations and biomass allocation as well as how such physiological variations translate into differences in vegetative vigor. To address these questions, four citrus genotypes—Sour Orange (SO), Volkamer Lemon (VL), Swingle Citrumelo (CTR), and Troyer Citrange (TC)—were compared with respect to their morphological traits, biomass distribution, and hydraulic properties. These four rootstocks were selected as they represent contrasting genetic backgrounds and well-documented differences in vigor, stress tolerance, and hydraulic behavior, providing an effective model for assessing intrinsic physiological variability. The findings reveal pronounced rootstock-specific differences in water acquisition, transport, and utilization, with direct implications for the hydraulic architecture, leaf water status, and partitioning of biomass between above- and belowground organs. CTR exhibited a highly integrated hydraulic strategy, characterized by elevated conductance across both aerial and root systems and accompanied by greater biomass allocation to the canopy and absorptive roots, resulting in an enhanced overall vigor. SO and VL displayed an intermediate physiological performance, whereas TC demonstrated a restricted hydraulic transport capacity, which is associated with lower biomass allocation, reduced leaf water potential, and diminished vigor. By assessing rootstocks independently of scion influences, this work demonstrates that variations between citrus rootstocks cannot be explained solely by morphological traits but instead reflect contrasting physiological strategies governing the coordinated management of water and carbon resources. These results highlight the rootstock as a central determinant of hydraulic functioning, biomass partitioning, and plant vigor and provide a conceptual basis for selecting rootstocks that are better suited to water-limited environments.

Keywords: hydraulic conductance; leaf water potential; rootstock; water relation

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1. Introduction

Rootstocks play a central role in modern citrus cultivation, yet they have largely been studied as components of the graft–scion system rather than as autonomous biological entities with their own structural and physiological traits [1–3]. While this integrated approach has practical value, it complicates the distinction between intrinsic rootstock characteristics and those arising from scion interactions. Consequently, significant gaps remain in our understanding of the physiological differences between citrus rootstocks when evaluated as independent plants.

In light of these gaps and by considering rootstocks as autonomous plants, the central scientific questions of this study are whether, and to what extent, different citrus rootstocks differ in their hydraulic conductance and response to water stress as well as how these differences relate to overall vegetative vigor.

Historically, the use of rootstocks has been crucial for shaping the structure and productivity of the global citrus industry. In citrus cultivation, rapid rootstock replacement, often driven by the need to cope with emerging biotic stresses, has repeatedly reshaped propagation practices, whereas substitutions aimed at improving fruit yield or quality have generally induced more gradual changes [4]. The development of the Italian citrus nursery sector is closely linked to the severe outbreak of collar rot (gummosis) in the late 19th century, a destructive root disease caused by *Phytophthora* spp. Before the spread of this pathogen, propagation relied mainly on cuttings in species naturally predisposed to rooting, such as lemon and citron, while sweet orange, mandarin, and Sour Orange were typically propagated by seeds [5].

The high susceptibility of seed-propagated citrus species to *Phytophthora* prompted a shift toward grafting, with Sour Orange emerging as the predominant rootstock due to its rusticity, adaptability, longevity, and, critically, disease resistance. Later, the global citrus industry faced a new and severe threat with the emergence of the citrus tristeza virus (CTV), first reported in South Africa in 1910. Initially attributed to graft incompatibility between sweet orange and Sour Orange, CTV soon came to have a major economic influence, spurring extensive research aimed at identifying alternative, resistant rootstocks. In regions where tristeza is endemic, Sour Orange has been largely replaced by tolerant genotypes, whereas in areas where the disease is absent or only recently reported, such as Texas, Morocco, and Italy, it continues to be widely used [6].

Within this context, understanding the physiological mechanisms by which rootstocks influence the growth and performance of grafted plants is essential. Among the processes most affected by rootstock selection, water relations play a central role, particularly in the face of climate change, which demands increasingly water-efficient irrigation management. Hydraulic conductance (K), defined as the capacity of a system to transport water, is a key determinant of a plant's ability to supply leaves and tissues with water and nutrients [7]. The plant–water balance depends not only on the amount of water absorbed by the roots but also on the efficiency of water transport along the soil–plant continuum, which is strongly influenced by the anatomical organization of the vascular system, including the xylem vessel diameter and density [8]. Environmental factors, particularly prolonged water stress, can induce marked morpho-anatomical modifications in the xylem, directly affecting the hydraulic conductance of stems, leaves, and roots [9].

Root hydraulic conductance is considered one of the principal regulators of water movement along the soil–plant continuum [10], and limitations at this level can significantly influence whole-plant–water relations. Most water flow resistance occurs at the root level [11]. Plants dynamically modulate hydraulic conductance in response to environmental cues; beyond stomatal regulation, aquaporins, membrane channels facilitating transcellular water flow, play a key role, especially under water deficits [12]. Recent studies have also demonstrated that plants can restore the functionality of embolized xylem vessels via refilling mechanisms, thereby recovering their hydraulic capacity [13]. Another potential mechanism is the so-called ionic effect, whereby changes in the xylem sap ion concentration may enhance hydraulic conductance [14].

Numerous fruit crop studies have demonstrated that rootstocks differ considerably in the vigor they confer to the scion, leading to the selection of clonal materials with specific growth-modulating properties. The East Malling breeding program in the UK exemplifies this approach, having produced apple rootstocks with a pronounced dwarfing capacity [15].

Graft combinations of genotypes with differing vigor profiles alter the metabolite distribution and overall hydraulic architecture. Low-vigor rootstocks generally exhibit reduced hydraulic conductance in both roots and stems, as observed in grapevine [16], peach [17] and citrus [18]. Dwarf cherry rootstocks have been shown to possess smaller-diameter xylem vessels relative to vigorous rootstocks [19].

These traits make low-vigor plants potentially more susceptible to water deficits, reduced gas exchange, and lower assimilation rates, ultimately affecting dry matter production and partitioning. Another factor influencing water relations in grafted plants is the hydraulic resistance at the graft union site. Several studies have demonstrated that the graft junction represents a significant barrier to water flow [1], although such resistance may diminish over time once vascular continuity is fully re-established. Successful grafting depends on callus formation, followed by cambial differentiation and functional vascular reconnection between the scion and rootstock [20].

The aim of this work was to analyze differences between citrus rootstocks in regard to their hydraulic conductance, response to water stress, and vegetative vigor, evaluating them as autonomous biological entities. We hypothesized that (i) rootstocks possess intrinsic differences in hydraulic conductance; (ii) these differences determine varying capacities to respond to water stress; and (iii) lower hydraulic conductance is associated with reduced vegetative vigor and altered dry matter partitioning. The results of this study aim to provide practical guidance for the selection and management of rootstocks in sustainable and climate-resilient citrus systems.

2. Materials and Methods

2.1. Experimental Sites, Plant Cultivation Conditions, and Seedling Transplanting

This experiment was conducted during 2024 across two distinct environments. Genotype seedlings were initially grown at the Serratore plant nursery, located in southern Italy (Lamezia Terme), where the experiment was initiated. Subsequently, from summer until the end of the trial, the plants were transferred to the greenhouse of the AGRARIA Department at the Mediterranean University of Reggio Calabria, where the second phase of the experiment was conducted. Sour Orange (*Citrus aurantium*), Volkamer Lemon (*Citrus volkameriana*), Swingle Citrumelo, and Troyer Citrange seeds were collected from certified mother tree orchards and routinely subjected to genetic and sanitary monitoring to ensure the absence of viruses and other infectious pathogens. After pulp removal, seeds underwent a thermal sanitization treatment (52 °C for 10 min), aimed at inactivating *Phytophthora* spp., followed by a fungicidal application. Sanitized seeds were stored at 4–5 °C, maintaining high germination potential for up to one year (4000–5000 seeds kg^{-1}), and maintained in cold storage (4 °C) in the nursery until sowing. Sowing was carried out in spring 2023, when environmental temperatures supported rapid germination, thus reducing the duration of the seedbed residence and lowering pathogen incidence rates. Seed emergence occurred within 30–40 days, and full germination was completed in 3–4 weeks, with noticeable variations between genotypes due to differences in polyembryony and germination vigor. Previously sanitized substrates consisted of blends of agriperlite, peat, compost, and coconut coir residues. Because of polyembryony, multiple seedlings frequently emerged from a single container; excess seedlings were removed during transplanting. Seedlings were transplanted into 8 L containers in June 2024, one year after sowing. Transplanting was performed once nucellar embryos could be clearly distinguished from hybrid ones, enabling the selection of genetically uniform nucellar seedlings with characteristics consistent with the mother plants. The substrate preparation was mechanized: a first hopper combined sandy soil and blond peat at a 1:2 (v/v) ratio, while a second hopper ensured that the mixture was steam sterilized. Containers were filled and perforated using a drill

to create the planting hole, after which seedlings were manually transplanted. Following transplanting, plants were irrigated to ensure proper root–substrate adherence and transferred to the growth area.

2.2. Experimental Design

At the Agraria Department's nursery and greenhouse, the plants were arranged in a randomized complete block design. For each block, three replicates were used, each consisting of 3 plants, for a total of 36 plants (9 per genotype) (Figure 1).

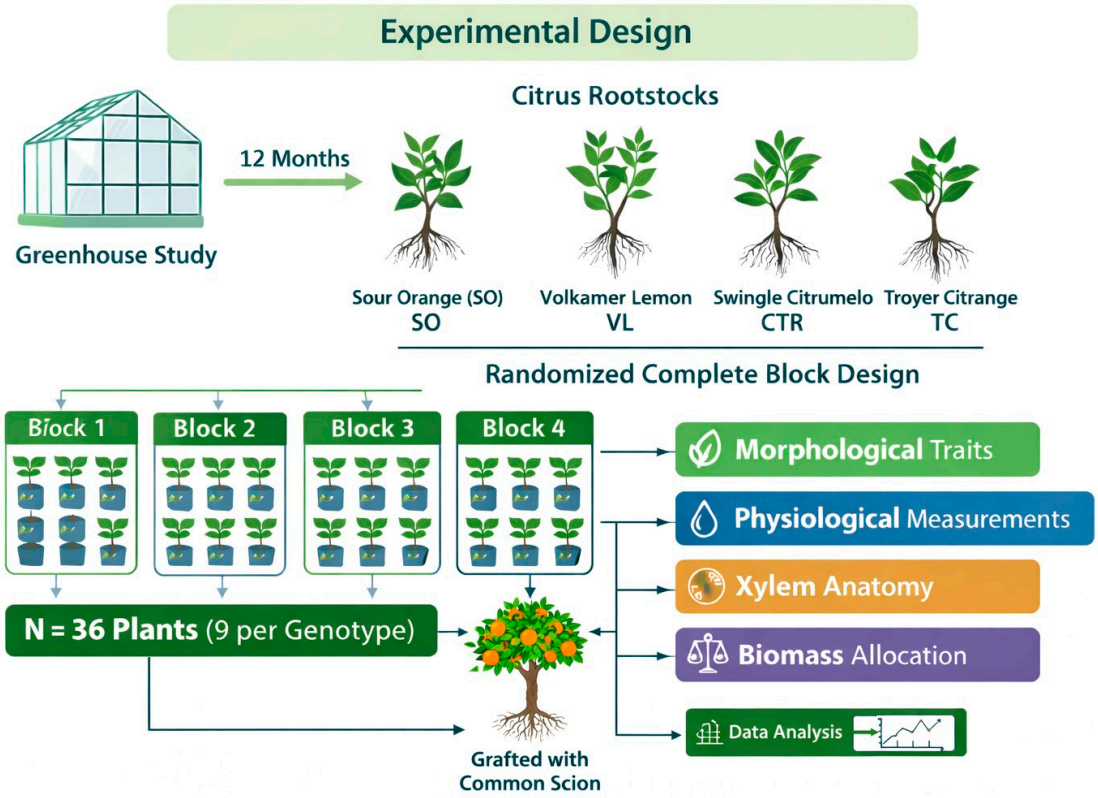

Figure 1. Experimental design of the greenhouse study showing citrus plants grafted on four rootstocks (SO, VL, CTR, TC) grown for 12 months in a randomized complete block design (36 plants total), with assessment of morphological, physiological, and biomass traits.

2.3. Agronomic Management During the Trial

Plants were maintained inside a greenhouse at the Department of Agriculture (Reggio Calabria) on a surface covered with an anti-algal ground covering and a shade net that reduced PPFD by approximately 40%. Standard phytosanitary and agronomic practices were utilized throughout the experiment. The soil water potential was maintained between 0 and 20 centibars from May to September, using two daily irrigation events (07:00 and 15:00) delivered via microtube irrigation, and it was measured via Watermark sensors placed in the pots (one sensor per genotype per block). Plants received a modified nutrient solution (3 mM $Ca(NO_3)_2$, 3 mM KNO_3, 2 mM $MgSO_4$, 2.3 mM H_3PO_4, and 17.9 µM Fe-EDDHA) for one of the two daily irrigations [21]. The pH of the nutrient solution was adjusted to 6.0 using 1 M KOH or 1 M H_2SO_4.

2.4. Eco-Physiological Measurements

During the last ten days of June, July, and August, gas exchange, water potential, and SPAD measurements were conducted.

2.4.1. Gas Exchange and Fluorometric Measurements

For each experimental treatment, 3 mature leaves per plant were selected, totaling 27 leaves per treatment within each block. Gas exchange, leaf temperature, and chlorophyll fluorescence were measured using a portable open-flow gas exchange system (LI-COR 6400XT, Lincoln, NE, USA) equipped with a standard measuring chamber and an integrated fluorometer (Leaf Chamber Fluorometer 6400-40, Lincoln, NE, USA).

2.4.2. Leaf Water Potential Determination

The leaf water potential (Ψleaf) was measured as an indicator of plant–water status, reflecting the balance between soil water availability and leaf transpiration. Measurements were performed using a pressure chamber (Scholander-type, PMS Instrument Company, Albany, OR, USA). Mid-distal leaf segments were excised and immediately wrapped in plastic film to minimize dehydration and then inserted into the chamber with the cut surface exposed. The chamber was pressurized using compressed nitrogen until xylem sap emerged from the cut surface; the corresponding pressure was recorded as Ψleaf (MPa).

2.5. Morphological Measurements and Destructive Sampling

At the beginning of October, collar diameter, plant height, and leaf number measurements were performed on all plants. The leaf number was determined via direct counting, and the total leaf area (TLA) was measured using a leaf area meter (LI-COR 3100, LI-COR Biosciences, Lincoln, NE, USA), which calculated the mean leaf area. Subsequently, the shoot and root systems were separated; roots were gently washed with low-pressure water to remove substrates. They were then classified into absorbing roots and structural roots. The genotype axis was separated from both the root and scion (if present). The fresh mass of the absorbing roots was recorded, and 10% subsamples were scanned using WinRhizo to determine the total root length, which was extrapolated to the full sample. All plant components (shoot and root fractions) were oven-dried at 70 °C to a constant weight to assess the dry biomass partitioning.

2.6. Root System Imaging and Analysis

For each plant, five samples of absorbing roots were randomly collected. Root morphological characteristics were quantified using WinRhizo (Regent Instruments Inc., Québec, QC, Canada). Washed root samples were submerged in a transparent tray placed on a root-scanning unit, and high-resolution images were acquired. An image analysis classified roots according to predefined diameter classes and the quantified total root length, root length per class, and root volume.

2.7. Hydraulic Conductance Measurements

Before the destructive step, three plants per treatment were used for hydraulic conductance measurements using a Hydraulic Conductance Flow Meter (HCFM-XP Gen 3; Dynamax, San Francisco, CA, USA). The instrument generates variable flow rates by rapidly altering the applied pressure while simultaneously measuring resulting fluxes. The dynamic relationship between pressure and flow, displayed on a PC via proprietary software, enables the calculation of hydraulic conductance (K) as the slope of the linear regression of flow (F) versus pressure (ΔP), following the relation $K = F/\Delta P$. This system includes a pressurized water source, capillary tubing connected to pressure transducers, and connectors adapted to plant organs. The HCFM enables both "quasi-steady-state" measurements, in which small fluctuations of pressure and flow are recorded by the software, and "transient" measurements, characterized by stepwise increases in pressure (up to 0.3 MPa) with continuous monitoring (one measurement every 3 s). Transient measure-

ments were used to determine the root systems' hydraulic resistance. At the end of the hydraulic conductance measurements, morphological measurements were performed, following the same procedure used for all the other plants.

2.8. Correlation

Relationships between the morphological, physiological, and hydraulic traits of the four citrus rootstocks were assessed using Pearson's correlation analysis, considering all genotypes jointly. Correlation coefficients (r) were used to quantify the strength and direction of associations among the measured variables.

2.9. Statistical Analyses

Data were analyzed using a one-way ANOVA in SPSS v.22.0. Differences between means were evaluated using Tukey's HSD test when the ANOVA indicated significant effects. Additionally, the dataset was subjected to a multivariate Principal Component Analysis (PCA) using XLSTAT v. 2014 (Addinsoft, New York, NY, USA) to explore relationships between rootstocks and measured traits.

3. Results

3.1. Vegetative Growth, Leaf Area, and Canopy Traits

The four genotypes exhibited clear morphological divergence in both above- and belowground traits, as summarized in Table 1. CTR displayed the greatest vigor, with a mean plant height of 173.23 cm, which is approximately 9% taller than VL and SO and 23% taller than TC. Therefore, this taller height was associated with a larger collar diameter. In addition, CTR exhibited the greatest basal stem thickness, which was 10%, 15%, and 18% larger than in VL, SO, and TC, respectively (Table 1).

Table 1. Average plant height (H), stem base diameter (D), and absorptive root length (LR) measured in the four citrus genotypes: Sour Orange (SO), Volkamer Lemon (VL), Swingle Citrumelo (CTR), and Troyer Citrange (TC).

Genotype	H cm	D mm	LR cm
SO	158.72 ± 1.78 b	190.11 ± 1.11 c	439.6 ± 3.55 b
VL	163.34 ± 1.56 b	200.01 ± 0.99 b	497.1 ± 4.71 b
CTR	173.23 ± 2.34 a	220.13 ± 1.22 a	668.2 ± 3.13 a
TC	140.78 ± 2.89 c	182.98 ± 0.76 d	406.7 ± 2.79 c

Different letters indicate statistically significant differences at $p < 0.05$ (Tukey's test).

Another distinguishing characteristic of CTR was its larger total leaf area (TLA), which exceeded those of SO, VL, and TC by approximately 30%, 50%, and 100%, respectively (Table 2). This greater TLA was associated with a higher number of leaves per plant. The leaf number was significantly higher in CTR compared with SO (−13%) and VL (−30%), which were statistically similar to each other, while TC (−51%) exhibited the lowest leaf count (Table 2). These results suggest that differences in canopy sizes between citrus genotypes are primarily driven by leaf density rather than by individual leaf expansion. This pattern is further influenced by differences in leaf morphology, as CTR and TC are trifoliate citrus plants in contrast to VL and SO. Consistently, the mean single leaf area (LA) did not differ significantly across genotypes, despite slight numerical increases observed in CTR and TC.

Table 2. Total leaf area (TLA), mean leaf area (LA), and leaf number (LN) measured in four citrus genotypes during destructive sampling: Sour Orange (SO), Volkamer Lemon (VL), Swingle Citrumelo (CTR), and Troyer Citrange (TC).

Genotype	TLA cm^2	LN	LA cm^2
SO	5203.27 ± 4.53 b	178.5 ± 3.15 b	29.15 ± 0.15 ns
VL	4405.68 ± 8.75 c	144 ± 9.45 c	30.595 ± 0.73
CTR	6785.64 ± 4.78 a	206 ± 4.70 a	32.94 ± 0.87
TC	3277.33 ± 7.81 d	100 ± 8.90 d	32.77 ± 0.45

Different letters indicate statistically significant differences at $p < 0.05$ (Tukey's test); ns = not significant.

3.2. Biomass Partitioning Between Above- and Belowground Compartments

As shown in Table 3, the distribution of dry matter between the different plant compartments varied markedly across the evaluated citrus genotypes. With respect to the aboveground compartment, CTR allocated the lowest proportion of dry matter (36%) compared with the other genotypes, indicating a relatively greater investment in belowground biomass. SO and VL formed an intermediate group, with 51% and 58% of dry matter allocated to aboveground organs, respectively, whereas TC allocated a significantly higher proportion of dry matter to aboveground organs (88%), consistent with its generally lower biomass allocation to the root system. Overall, the data presented in Table 3 also highlight substantial rootstock-dependent differences in the biomass partitioning between above- and belowground compartments, including structural and absorptive roots. The amount of root system biomass was significantly greater in CTR. The proportion of absorptive roots accounted for 60% and 59% of the total root biomass in CTR and VL, respectively, whereas in SO this value decreased to 40%. The lowest proportion of absorptive roots was recorded in TC, at 36%. The root dry mass per unit root length (Table 3) was highest in CTR, was intermediate and statistically similar in VL and SO, and was lowest in TC. Overall, the data in Table 3 highlight substantial differences in biomass partitioning, reflecting divergent morphological or physiological growth patterns that may influence whole-plant performance.

Table 3. Biomass partitioning in structural roots, absorbing roots, aboveground tissues, leaves, and total plant dry weight in four citrus genotypes: Sour Orange (SO), Volkamer Lemon (VL), Swingle Citrumelo (CTR), and Troyer Citrange (TC).

Genotype	Structural Roots g	Absorbing Roots g	Aboveground Tissues g	Leaves g	Total Dry Weight Plant
SO	28.84 ± 1.88 a	19.397 ± 2.75 b	61.81 ± 3.71 a	20.66 ± 2.11 b	130.71 ± 3.50 b
VL	16.94 ± 2.33 b	24.591 ± 3.58 b	62.33 ± 3.11 a	25.11 ± 4.72 a	128.97 ± 2.78 b
CTR	27.44 ± 1.66 a	36.337 ± 4.77 a	65.89 ± 5.11 a	28.99 ± 3.90 a	158.65 ± 5.11 a
TC	7.259 ± 2.20 c	4.039 ± 3.15 c	50.11 ± 4.77 b	10.19 ± 3.78 c	71.59 ± 6.33 c

Different letters indicate statistically significant differences at $p < 0.05$ (Tukey's test).

3.3. Photosynthetic Performance, Water Relations, and Chlorophyll Fluorescence

The physiological measurements revealed clear differences between the genotypes' photosynthetic performances. CTR exhibited the highest photosynthetic rate, whereas TC had the lowest value, which was more than 30% lower than CTR (Table 4); SO and VL displayed intermediate rates. The stomatal conductance (gs) exhibited the most pronounced differences among the four citrus genotypes, with all treatments differing significantly from one another (Table 4). The highest gs was recorded in CTR, with values 23%, 43%, and 78% higher than those observed in VL, SO, and TC, respectively (Table 4). A similar pattern was observed for the intercellular CO_2 concentration. Water use efficiency (WUE) highlighted clear trade-offs among genotypes (Table 4). TC achieved WUE values up to

50% higher than those of CTR, whereas genotypes characterized by higher photosynthetic activity, such as CTR and VL, exhibited lower WUE, reflecting a balance between carbon assimilation and water loss. An intermediate response was observed in SO (Table 4). Clear differences also emerged in the leaf water potential (Ψleaf), further highlighting the distinct hydraulic behavior of each genotype (Table 4). CTR and VL exhibited the least negative Ψleaf values, indicative of superior leaf hydration and more efficient water transport within the soil–plant continuum. In quantitative terms, the Ψleaf in SO was approximately 41% more negative than in CTR, while TR exhibited values 37% more negative, suggesting that both SO and TC operated under comparatively higher water tension conditions. The similar behavior of CTR and VL, differing by only 4%, reflects their well-documented hydraulic efficiency and capacity to sustain transpiration without experiencing substantial declines in water status. VL, although less vigorous in terms of leaf area development, also maintained a favorable water potential, consistent with its robust root system. In contrast, SO exhibited moderate vigor but showed signs of greater hydraulic stress, while TC demonstrated the weakest vegetative performance coupled with a more negative water potential, characteristics that are typically associated with dwarfing or semi-dwarfing behavior. Overall, the traits observed in Table 4 indicate that the examined genotypes differ substantially in their capacity to support canopy development and maintain adequate leaf hydration. These contrasting behaviors have important implications for orchard management and genotype selection, particularly under conditions where water availability may limit growth or productivity.

Table 4. Gas exchange parameters, water use efficiency, and leaf water potential (Ψleaf) of different citrus genotypes [Sour Orange (SO); Volkamerian Lemon (VL); Swingle Citrumelo (CTR); Troyer Citrange (TC)].

Genotype	An µmol CO_2 m^{-2} s^{-1}	gs µmol H_2O m^{-2} s^{-1}	Ci ppm	E mmol H_2O m^{-2} s^{-1}	WUE	Ψleaf Bar
SO	9.350 ± 0.59 ab	0.068 ± 0.001 c	150.00 ± 24.67 ns	1.341 ± 0.107 c	7.151 ± 0.83 ab	8.31 ± 1.15 a
CTR	12.201 ± 0.82 a	0.117 ± 0.004 d	102.56 ± 17.54	2.416 ± 0.89 d	5.036 ± 0.21 c	6.12 ± 1.33 b
TC	6.256 ± 0.53 c	0.033 ± 0.003 a	98.62 ± 18.18	0.806 ± 0.05 a	7.764 ± 0.41 a	5.88 ± 1.34 b
VL	10.686 ± 0.53 ab	0.088 ± 0.002 b	114.17 ± 22.13	1.946 ± 0.041 b	5.499 ± 0.31 bc	8.07 ± 1.89 a

Different letters indicate statistically significant differences at $p < 0.05$ (Tukey's test); ns = not significant.

Table 5 presents the chlorophyll fluorescence parameters and electron transport rates (ETRs) of the citrus genotypes. CTR exhibited the highest maximum PSII efficiency in the light-adapted state (Fv'/Fm'), which was approximately 18% higher than TC's values, which were the lowest, while SO and VL demonstrated an intermediate efficiency (Table 6). The photochemical quenching (qP) followed a similar pattern, with TC and VL displaying a 15–20% higher qP than SO, indicating a more effective utilization of absorbed light for photochemistry (Table 5). The effective quantum yield of PSII (PhiPS2) was slightly higher in CTR and VL, around 15% greater than TC, reflecting more efficient electron transport per absorbed photon. Non-photochemical quenching (NPQ) demonstrated minimal variation across the genotypes, suggesting a similar engagement of photoprotective mechanisms under the experimental conditions (Table 5). In contrast, ETR differed markedly, with TC displaying an approximately 35% lower ETR compared to SO, CTR, and VL, indicating reduced electron transport capacity in this genotype (Table 5). Overall, the results in Table 5 indicate that citrus genotypes employ distinct strategies for light energy management: CTR and VL favor higher photochemical efficiency and electron transport, while TC exhibits a lower PSII efficiency and ETR, potentially as a trade-off between light use and stress protection. SO exhibits intermediate values, balancing photochemical activity and electron transport.

Table 5. Chlorophyll fluorescence parameters and electron transport rates (ETRs) of different citrus genotypes [Sour Orange (SO); Volkamerian Lemon (VL); Swingle Citrumelo (CTR); Troyer Citrange (TC)]. Measurements include the maximum efficiency of PSII in the light-adapted state (Fv′/Fm′), photochemical quenching (qP), effective quantum yield of PSII (PhiPS2), non-photochemical quenching (NPQ), and electron transport rate (ETR).

Genotype	Fv′/Fm′	qP	PhiPS2	NPQ	ETR $\mu mol\ m^{-2}\ s^{-1}$
SO	0.595 ± 0.02 c	0.221 ± 0.01 c	0.155 ± 0.11 a	2.110 ± 0.33 ns	152.333 ± 1.33 a
CTR	0.655 ± 0.03 a	0.241 ± 0.04 b	0.158 ± 0.11 a	2.098 ± 0.56	151.331 ± 1.78 a
TC	0.555 ± 0.04 d	0.256 ± 0.03 a	0.136 ± 0.13 b	2.117 ± 0.65	99.981 ± 2.15 b
VL	0.611 ± 0.03 b	0.259 ± 0.03 a	0.153 ± 0.06 a	2.101 ± 0.61	149.321 ± 2.39 a

Different letters indicate statistically significant differences at $p < 0.05$ (Tukey's test); ns = not significant.

Table 6. Specific hydraulic conductance of shoot (hypogeal portion) and root in four citrus genotypes [Sour Orange (SO); Volkamerian Lemon (VL); Swingle Citrumelo (CTR); Troyer Citrange (TC)].

Genotype	Specific Epigeal Hydraulic Conductance $kg\ s^{-1}$	Specific Hypogeal Hydraulic Conductance $kg\ s^{-1}$
SO	$9.60 \times 10^{-7} \pm 0.25 \times 10^{-7}$ a	$5.15 \times 10^{-7} \pm 0.12 \times 10^{-7}$ c
VL	$7.518 \times 10^{-7} \pm 0.18 \times 10^{-7}$ b	$9.15 \times 10^{-7} \pm 0.22 \times 10^{-7}$ b
CTR	$8.500 \times 10^{-7} \pm 0.20 \times 10^{-7}$ a	$1.12 \times 10^{-6} \pm 0.24 \times 10^{-7}$ a
TC	$2.441 \times 10^{-7} \pm 0.10 \times 10^{-7}$ c	$4.58 \times 10^{-7} \pm 0.11 \times 10^{-7}$ c

Different letters indicate statistically significant differences at $p < 0.05$ (Tukey's test).

As reported in Table 6, the specific hydraulic conductance varied significantly between the epigeal and hypogeal portions across the four citrus genotypes. In the epigeal portion, SO and CTR exhibited the highest specific hydraulic conductance, and no significant difference was detected between them (Table 6). Both genotypes exhibited values approximately three- to fourfold higher than TC, while VL displayed intermediate conductance. These results indicate that SO and CTR have markedly superior hydraulic efficiency in the aboveground portion compared to TC. In contrast, the hypogeal portion exhibited a different pattern. CTR demonstrated the highest root-specific conductance, followed by VL, whereas SO and TC displayed lower values. Relative to TC, CTR, VL, and SO presented an increase of approximately 144%, 100%, and 12%, respectively (Table 6).

3.4. Functional Linkages Across Structural and Hydraulic Traits

The correlation analysis highlighted consistent linkages between plant structure, gas exchange, and hydraulic performance. The plant height, stem diameter, and length of absorptive roots were strongly and positively related to the specific hydraulic conductance in both above- and belowground compartments. The net CO_2 assimilation (An) closely tracked the stomatal conductance (gs) and was positively associated with transpiration and electron transport rates, indicating the coordinated control of carbon fixation and water vapor fluxes. In contrast, the WUE declined with increasing An, gs, and transpiration, reflecting the inherent trade-off between carbon gain and water consumption. Hydraulic variables followed distinct trends: belowground-specific hydraulic conductance was negatively associated with leaf water potential, positively related to canopy size (TLA, LN), and inversely related to non-photochemical quenching (NPQ).

3.5. Multivariate Analysis of Morphological and Physiological Traits

The PCA biplot, which captures over 83% of the data variance, effectively illustrates the main physiological and morphological strategies of the tested genotypes (Figure 2). The

first principal component (F1) establishes a clear trade-off between high photosynthetic activity and structural biomass accumulation. The VL and TC genotypes are clustered on the positive side of F1, correlating strongly with variables associated with high gas exchange performance and photochemical efficiency (Figure 2). This group adopts a high-capacity, efficient physiological strategy. The CTR genotype is strongly associated with the negative side of F1, aligning with the high biomass traits (aboveground organs, root categories) and water use efficiency (WUE) (Figure 2). This suggests that CTR allocates more resources to structural growth and exhibits a more conservative water-use strategy, potentially sacrificing peak instantaneous photosynthetic rates compared to the VL/TC group. SO is distinctly separated along the negative F2 axis (Figure 2). This position indicates a unique physiological profile defined by its relationship with specific epigeal hydraulic conductance and leaf water potential, setting it apart from the main F1 strategies (Figure 2). In conclusion, this analysis reveals two primary, contrasting strategies: one maximizes the photosynthetic output (VL/TC), and the other prioritizes structural development and water conservation (CTR). However, SO exhibits a distinct hydraulic response (Figure 2).

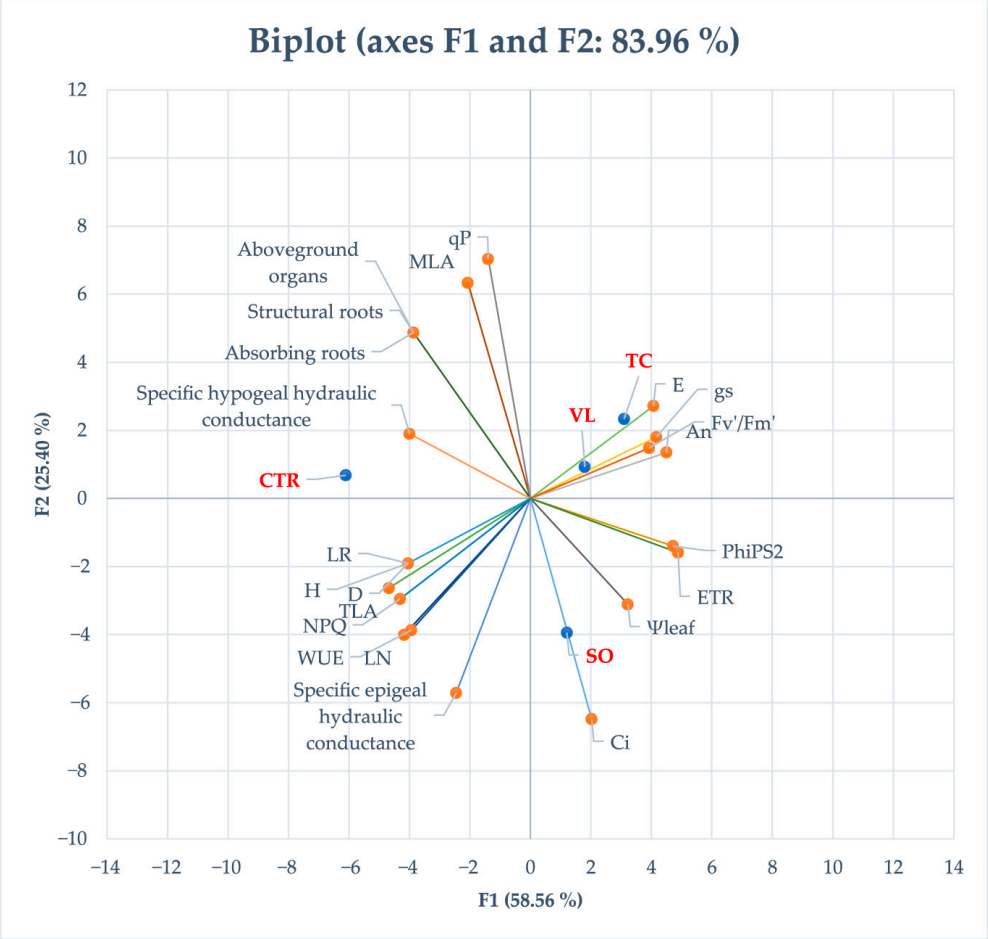

Figure 2. Principal Component Analysis (PCA) biplot of physiological, morphological, and hydraulic characteristics in different citrus genotypes [Sour Orange (SO); Volkamerian Lemon (VL); Swingle Citrumelo (CTR); Troyer Citrange (TC)].

4. Discussion

This research reveals substantial genotype-driven variation in the functional coordination between epigeal and hypogeal plant compartments, demonstrating that citrus genotypes employ distinct integrated functional strategies to balance carbon gain, water transport efficiency, and overall physiological performance.

4.1. Genotype-Driven Differences in Growth and Biomass Allocation

CTR consistently emerged as the most vigorous genotype, exhibiting superior shoot growth, extensive leaf area expansion, and the highest total biomass. These traits were underpinned by a proportionally large, well-developed root system. Such tight coupling between canopy growth and root investment typifies vigorous, non-dwarfing genotypes and reflects a highly effective regulation of carbon allocation and water movement along the root–shoot continuum. Conversely, TC was characterized by a markedly reduced canopy and significantly lower total biomass, linked to the constrained development of the belowground compartment, particularly the absorptive root fraction. This growth pattern aligns with dwarfing or semi-dwarfing behavior and leads to an overall reduction in both hydraulic capacity and photosynthetic potential [22]. VL and SO occupied intermediate positions but adopted physiologically distinct strategies. VL exhibited a well-balanced allocation between shoot and root systems, supported by a substantial investment in absorptive roots and elevated root hydraulic conductance, which translated into comparatively favorable leaf water potential values. This configuration suggests an efficient hydraulic strategy capable of sustaining adequate water flow under the conditions tested. In contrast, SO displayed moderate shoot development accompanied by relatively limited root investment, a combination that may perform adequately under optimal conditions but could become limiting under soil water deficits.

4.2. Root System Architecture and Functional Implications

One of the most notable outcomes of this study is the clear differentiation between genotypes in relation to the partitioning of the root biomass between absorptive and structural components. CTR devoted the largest share of its belowground biomass to fine absorptive roots, whereas TC and SO allocated significantly smaller proportions. VL again exhibited an intermediate pattern, maintaining a sufficiently developed absorptive root system to support effective water uptake. Given that absorptive roots constitute the primary interface for water and nutrient acquisition and exert a strong influence on whole-plant hydraulic conductance [23,24], these differences have important functional implications. The elevated root dry mass per unit length observed in CTR points to a mechanically robust and hydraulically efficient root system capable of sustaining high water fluxes while preserving hydraulic continuity [25].

4.3. Hydraulic Conductance and Water Transport Coordination

The variation in the hydraulic conductance between aboveground and belowground compartments further elucidates the contrasting strategies of the genotypes. CTR combined a high conductance in both the canopy and the root system with less negative leaf water potential values, indicating a well-integrated and efficient hydraulic system along the soil–plant continuum under adequate water availability. This configuration allows for the maintenance of high transpiration and photosynthetic rates without generating excessive xylem tension. VL exhibited a similar profile, although with slightly lower values than CTR, suggesting a balanced strategy in terms of the water transport capacity between the two compartments. In contrast, SO displayed a relatively high aboveground conductance associated with more limited root conductance. This hydraulic asymmetry may increase vulnerability under soil water deficits, as a highly conductive canopy imposes a transpirational demand that the root system may not be able to fully meet, thereby increasing xylem tension and the risk of cavitation [26,27]. TC, with low conductance in both compartments, represents a hydraulically conservative system characterized by reduced water fluxes. However, without direct measurements of vulnerability to cavitation or hydraulic safety margins, no inference can be made regarding its hydraulic safety [28,29].

4.4. Physiological Performance and Integrated Functional Strategies

Physiological measurements confirm that the vigor, hydraulic architecture, and gas exchange behavior are tightly interconnected [30]. Accordingly, CTR and VL exhibited relatively high photosynthetic rates and stomatal conductance, consistent with a favorable plant–water status and an efficient transport system. SO exhibited intermediate values, whereas TC exhibited the lowest assimilation and transpiration rates but the highest water use efficiency, indicative of a strongly conservative strategy [31]. Chlorophyll fluorescence data further support this interpretation: CTR and VL maintained high PSII efficiency and sustained electron transport rates, whereas TC and, to a lesser extent, SO exhibited signs of photochemical limitations consistent with hydraulic constraints [32]. Overall, the four genotypes displayed distinct functional profiles. CTR exhibited traits associated with high resource acquisition; VL exhibited an intermediate and balanced configuration; TC expressed a conservative strategy characterized by reduced fluxes; and SO combined a moderate shoot performance with a comparatively limited root capacity. These patterns reflect differences in functional coordination under the conditions tested; however, positioning genotypes along a full acquisitive–conservative spectrum would require additional information on hydraulic safety thresholds and performance under resource limitations [32].

4.5. Multivariate Interpretation (PCA) and Implications for Rootstock Selection

The PCA highlights consistent covariation patterns among structural, hydraulic, and physiological traits, revealing a clear separation between genotypes based on their trait combinations. While the PCA does not provide a mechanistic validation of functional strategies, it supports the assertion that root–shoot coordination plays a central role in determining whole-plant performance. Therefore, genotype selection should carefully consider the hydraulic and physiological relationships between the scion and genotype. These interactions should be the focus of targeted studies aimed at predicting the performance of different grafting combinations under specific pedoclimatic conditions, thereby optimizing the sustainable management of citrus orchards.

5. Conclusions

This research reveals that citrus genotypes exert a profound impact on overall plant performance by shaping the functional interplay between epigeal and hypogeal belowground compartments. Substantial variation was observed across genotypes in terms of growth patterns, hydraulic conductance, and physiological traits, reflecting distinct strategies for managing water transport, carbon assimilation, and hydraulic safety. CTR displayed the highest vigor and hydraulic efficiency, combining elevated conductance in both shoots and roots with a superior leaf water status and enhanced biomass accumulation. VL adopted a moderately vigorous yet balanced approach, demonstrating a sufficient root hydraulic capacity and favorable leaf water potential despite only intermediate canopy development. SO exhibited similar intermediate vigor but with a comparatively underdeveloped root hydraulic network, potentially limiting its performance under soil water deficits. TC, in contrast, followed a conservative strategy, characterized by reduced hydraulic conductance, restrained growth, and elevated water use efficiency. A critical factor underlying these differences was the distribution of belowground biomass, especially the allocation to absorptive roots, which directly influenced the water uptake efficiency and the overall plant hydraulic function. Collectively, these findings underscore the pivotal role of coordinated root–shoot hydraulics in determining citrus rootstock performance. In the context of an increasing drought frequency and climate change, a mechanistic understanding of rootstock hydraulic and physiological traits is essential. Therefore, the selection of rootstocks should involve a careful consideration of their hydraulic and physiological

compatibility with the scion to optimize water use, enhance drought resilience, and support the long-term sustainability of citrus orchards.

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