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Behavior of new citrus hybrid rootstocks under water stress in greenhouse conditions

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ABSTRACT

Citrus, an important fruit crop in the Mediterranean region is significantly affected by climate change, which affects plant morphology and physiology. Grafting has improved the citrus industry. In this study, we studied the impact of water stress, 100%, 75%, and 50% substrate field capacity on seedlings of new hybrid citrus rootstocks. Growth rates, stomatal conductance, and fresh and dry weights were assessed, and proline, soluble sugar, and chlorophyll contents were estimated. Water stress affected all citrus rootstock seedlings. The growth parameters decreased with increasing stress levels. According to growth parameters, *Poncirus trifoliata×Citrus Volkameriana* seedlings were the least affected, followed by *Poncirus Trifoliata×Citrus reshni Hort. ex Tan*. (H1) seedlings, whereas those of *Poncirus trifoliata×Citrus reshni Hort. ex Tan.* (H2) were the most affected. Seedlings of *Poncirus trifoliata×Citrus reshni Hort. ex Tan*. (H5) accumulated less proline, whereas *Poncirus Trifoliata×Citrus reshni Hort. ex Tan*. (H3) had the highest content. The chlorophyll content and stomatal conductance decreased with increasing drought stress. Overall, the *Poncirus trifoliata×Citrus volkameriana* rootstock was more tolerant to drought stress, and the *Poncirus trifoliata×Citrus reshni Hort. ex Tan*. (H2) appeared to be sensitive. Future citrus breeding programs should prioritize the development of rootstocks that can tolerate dry conditions.

1. INTRODUCTION

Citrus trees are perennials that are commonly subjected to soil and atmospheric droughts. Citrus groves typically require irrigation in arid and semi-arid areas. Periods of water deficit harm yield and vegetative growth and minimize fruit size and occasionally quality, resulting in considerable economic losses [1-3]. In addition, stomatal conductance, transpiration, and CO_2 assimilation are decreased by water stress [4-6]. The root system signals the leaves to induce stomatal closure [7]. Different drought-resistant strategies have been developed by plants, such as decreased leaf mass or enhanced root development [8,9]. Osmotic adjustment helps plants support growth and photosynthesis by maintaining the leaf turgor required to open the stomata [4]. Drought-affected plants experience significant damage owing to the secondary effects of oxidative stress. This stress is a consequence of the downregulation of the photosynthetic process, which in turn causes alterations in the electron transport chain during photosynthetic reactions. Consequently, reactive oxygen species (ROS) are generated when the photosynthetic process is insufficiently active [10]. Excessive ROS production may damage nucleic acids, proteins, and lipids. These substances can then be oxidized and undergo detrimental effects such as enzyme inhibition, chlorophyll degradation, membrane disruption, loss of organelle function, decreased metabolic efficiency, and carbon fixation [11]. Plants prevent cell dehydration by either promoting water inflow through the accumulation of active solutes that decrease osmotic potential or limiting water efflux through the hardness of cell walls [12]. Plants produce several essential solutes, such as sugars, polyols, polyamines, proline, glycine, and betaine [13]. Proline is the most studied amino acid among the previously listed osmolytes [14]. Soluble sugars are important osmoprotectants, which means that they are essential for cellular osmotic adjustment and shield cellular structures from external stress. Rangpur lime is one of the most drought-tolerant rootstocks, while Poncirus trifoliata (trifoliate orange) is considered susceptible to drought [15]. The present experiment aims to study the behavior of six new hybrid rootstocks under three different water stress conditions in a greenhouse based on morphological, physiological, and biochemical indicators.

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2. MATERIAL AND METHODS

2.1 Plant Material and Growing Conditions

The experiment was conducted during the summer of 2022 in a greenhouse of the National Institute for Agricultural Research (INRA),

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Kenitra, Morocco, at an average temperature of 28°C, and a relative humidity of 60%. Healthy mature fruits of new hybrid rootstocks [Table 1] were harvested in the El Menzeh experimental field. The seeds were extracted, rinsed, and dried in the shade. The seeds were sown in plastic basins filled with peat. Once the seedlings were three months old, uniform seedlings with 4–6 leaves and a height of approximately 10 cm were transplanted into 1 L plastic pots (14 cm high, 13 cm top diameter, and 10 cm bottom diameter) filled with the same amount of a 1:1 mixture of peat and sand. Plants were regularly watered twice a week with a half-concentrated solution of Hoagland [16] for a one-month acclimatization period.

2.2 Stress Application

Water stress was applied after the acclimatization period to the new environment for two months by applying two different proportions of the maximum water-holding capacity. To find the maximum water-holding capacity, the seedlings were irrigated until saturation, and excess water was allowed to drain for 24 h. After draining, the pots were weighed to determine their weight at field capacity (FC) [17]. 100% FC for control rootstocks, 75% FC for moderate stress, and 50% FC for severe stress [18]. The substrate moisture was determined by measuring each pot twice a week on an electronic scale, and water was supplied when needed to preserve the moisture content within the desired levels [19].

2.3 Stem Growth, Stem Diameter, Number of Leaves, Stomatal Conductance

Stem height, stem diameter, and number of leaves were measured at the beginning and end of the treatment. Height was measured from the soil surface to the top of the stem. Stem diameter was measured using a digital caliper 2 cm from the soil surface. Stomatal conductance was recorded using an SC-1 Leaf Porometer (Decagon Devices, Pullman, WA, USA) on fully developed leaves in the upper section of the stem from 9:00 to 11:00 am [20].

2.4 Fresh and Dry Matter of Stems, Leaves, and Roots

After harvesting, rootstocks were divided into leaves, stems, and roots. Each plant part was placed in a bag and weighed before and after drying at 80°C for 72 h to determine the fresh and dry matter [21].

Table 1: List of new citrus hybrid rootstocks used in the experiment.

Rootstock	Code
Poncirus Trifoliata × *Citrus Volkameriana*	H1
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (H1)*	H2
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (H2)*	H3
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (H3)*	H4
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (H4)*	H5
Poncirus Trifoliata × *Citrus reshni Hort. ex Tan. (H5)*	H6
Citrus limonia Osbeck (Lime Rangpur)	LR
Poncirus Trifoliata	PT

2.5 Determination of Proline Content in Leaves

The leaf proline content was determined according to the method described by Bates et al. [22]. In screw-cap tubes, 50 mg of dried and ground leaves were combined with 3 ml of 3% aqueous sulfosalicylic acid. The resulting mixture was heated for 1 h at 80°C in a water bath. The obtained extract (1 mL) was mixed with 1 ml of glacial acetic acid and 1 ml of ninhydrin acid. The mixture was reheated at 100°C for 30 min. 5 ml of toluene was added to each tube, and the optical density of the mixture was measured at 520 nm using a spectrophotometer. The values obtained were expressed in mg/g dry matter (DM) using the equation of the standard curve prepared with L-proline.

2.6 Determination of Leaf Sugar Content

Leaf sugar content was estimated according to the method described by DuBois [23]. In screw-cap tubes, 3 ml of 80% ethanol was combined with 50 mg of dried ground leaves. The mixture was then incubated in the dark for 48 h. The extract (1 mL) was mixed with 1 ml of 5% phenol and 5 ml of sulfuric acid. The reaction was stopped by placing the tubes in a water bath for 30 min, and the optical density of the mixture was measured at 485 nm using a spectrophotometer. The values obtained were expressed in mg/g DM using the equation of the standard curve prepared with glucose.

2.7 Estimation of Total Chlorophyll

The total chlorophyll content of the leaves was determined using the method of Arnon [24]. 100 mg of fresh leaves were ground and transferred to stoppered tubes containing 10 ml of 80% acetone. The tubes were incubated for 48 h in the dark at 4°C, and the supernatant obtained was filtered. The optical densities were measured at 645 and 663 nm. The results were expressed in mg/g of fresh matter (FM).

2.8 Statistical Analysis

The experiment was conducted using a split-plot model with two factors: the rootstock and irrigation rate. The irrigation dose factor was designated as the main factor, whereas the rootstock factor was designated as the sub-factor. The analysis of variance of the collected data was performed using the general linear models (GLM) of the Statistical Analysis System (SAS) software. Data presented as percentages underwent angular transformation $arcsin\sqrt{p}$ [25]. Significant differences between means were determined using Duncan's test at the 95% confidence level.

3. RESULTS

3.1 Effect of Water Stress on the Stem Height, Diameter, and Number of Leaves

Water stress caused a reduction in all the growth parameters monitored, with the reduction being most noticeable at the 50% FC level. The variation in the percentage reduction in stem height [Figure 1], stem diameter [Figure 2], and the number of leaves [Table 2]. For stem height, at 75% FC, rootstock H2 had the highest percentage reduction (66%), and rootstock H6 had the lowest (24%). At 50% FC, the H6 rootstock had the lowest percentage reduction (48%), and the H2 hybrid had the highest reduction (76%). For stem diameter, at 75% FC, the H5 rootstock had the lowest percentage reduction (12%), and the H2 hybrid had the highest reduction (57%). At 50% FC, the highest percentage of reduction was recorded for H2 (74%), and the lowest for H5 (40%). In terms of leaf number, for the controls, hybrid H4 had the

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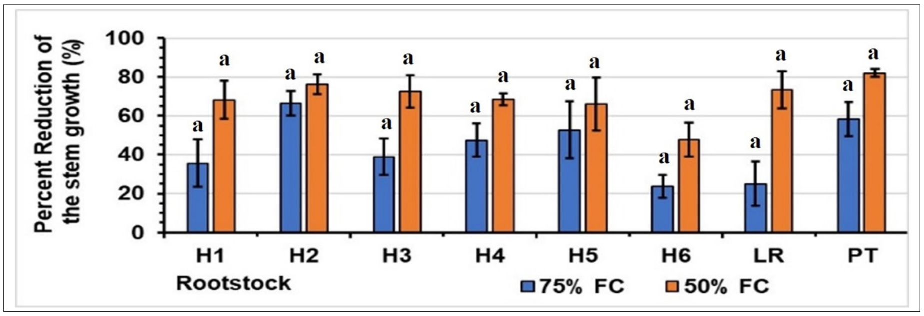

Figure 1: Effect of water stress on the stem growth Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), vertical bars represent the standard error ($n = 6$).

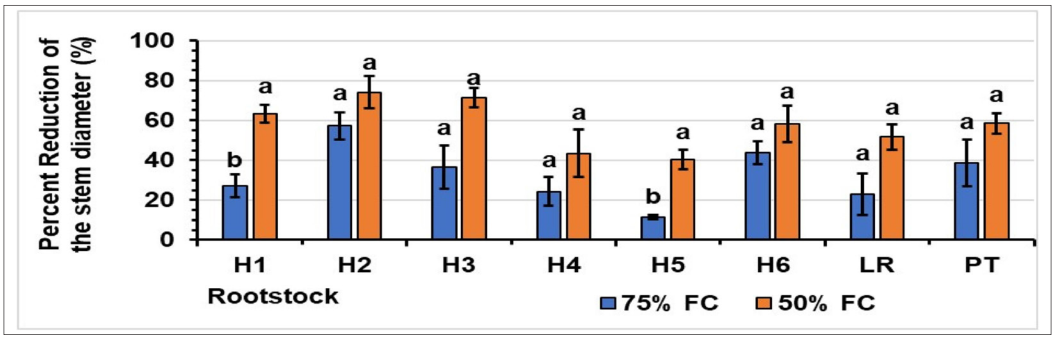

Figure 2: Effect of water stress on the stem diameter Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), vertical bars represent the standard error (n = 6).

Table 2: Effect of water stress on number of leaves.

Rootstock	Control	75% FC	50% FC
H1	17 ± 0.67^{a}	11 ± 1.21^{b}	7 ± 1.54^{c}
H2	22 ± 3.31^{a}	14 ± 5.06^{ab}	8 ± 2.92^{b}
H3	19 ± 3.31^{a}	13 ± 1.00^{a}	1 ± 0.43^{b}
H4	25 ± 2.52^{a}	13 ± 2.23^{b}	2 ± 2.08^{c}
H5	17 ± 2.12^{a}	14 ± 1.00^{b}	7 ± 1.02^{c}
H6	19 ± 3.18^{a}	12 ± 2.11^{ab}	3 ± 1.29^{b}
LR	36 ± 7.79^{a}	17 ± 2.88^{ab}	9 ± 2.05^{b}
PT	6 ± 2.00^{a}	2 ± 0.79^{ab}	–3 ± 1.35^{b}

Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), mean values ± standard error (n = 6).

highest number of 25 leaves, and hybrids H1 and H5 had the lowest number (17). At 75% FC, hybrids H1 and H5 had the highest number of 14 leaves, and hybrid H1 had the lowest. At 50% FC, hybrid H2 had the highest number of 8 leaves, and hybrid H3 had the lower of 1 leaf [Figure 3].

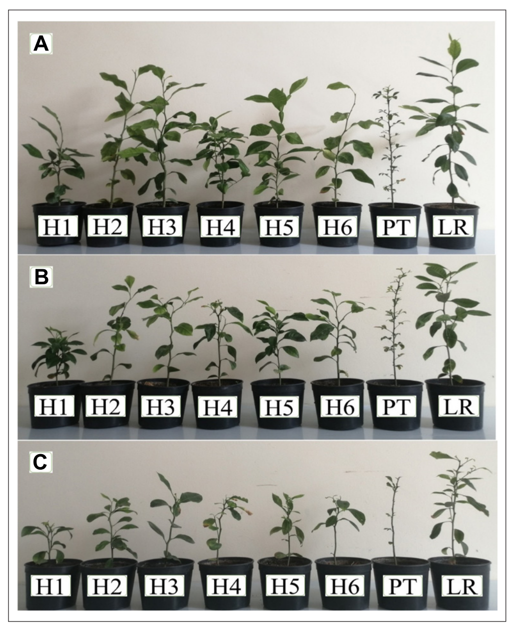

Figure 3: Effect of water stress on the appearance of the hybrid citrus rootstocks tested: **(A)** 100% of the field capacity, **(B)** 75% of the field capacity, **(C)** 50% of the field capacity.

3.2 Effect of Water Stress on the Stomatal Conductance of Leaves

According to the results in Figure 4, stomatal conductance varies according to the rootstock and irrigation dose. The stomatal conductance of control rootstocks was high, whereas that of rootstocks irrigated with 50% FC was low. For the controls, hybrid H2 had the highest value of 19.70 mmol/m^2/s, and hybrid H5 had the lowest value of 15.75. For rootstocks irrigated with 75% FC, the highest stomatal conductance (14.53) was observed for hybrid H2, and the lowest

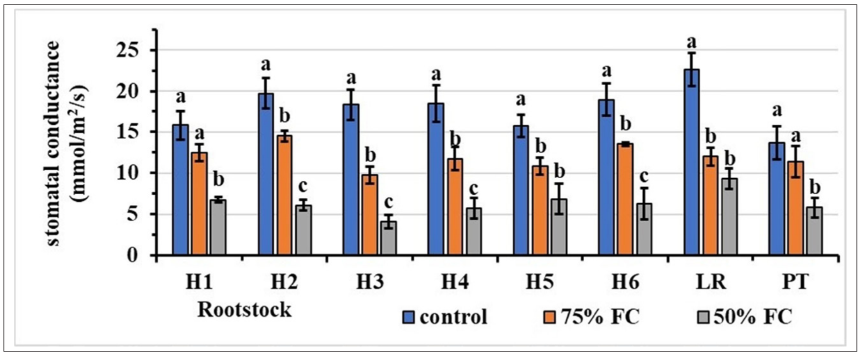

Figure 4: Effect of water stress on stomatal conductance (mmol/m2/s) Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), vertical bars represent the standard error (n = 6).

value of 9.78 was recorded for hybrid H3. For the 50% FC level, rootstock H3 recorded the lowest stomatal conductance of 4.13 mmol/m^2/s, while genotype H5 had the highest value of 6.85 mmol/m^2/s.

3.3 Effect of Water Stress on the FM of Leaves, Stems, and Roots

The water stress reduced the FM of different parts of the seedlings for all rootstocks: leaves [Table 3], stems [Table 4], and roots [Table 5]. For leaves, the mass of FM varied between 12.89 g and 2.56 g. For controls, the H2 rootstock had the highest mass, and the H1 rootstock had the lowest mass. For stems and roots, rootstock H3 had the highest mass (9.37 g), and hybrid H5 had the lowest mass (1.84 g). For roots, the root mass of hybrid H5 at 75% FC is higher than that of control, 10.71–10.40 g, respectively, and hybrid H6 has the same mass for control and 75% FC.

3.4 Effect of Water Stress on the Dry Matter of Leaves, Stems, and Roots

The water stress reduced the dry matter of the different parts of the seedlings: leaves [Table 6], stems [Table 7], and roots [Table 8]. The variation in dry matter was relatively same as that of FM, except for roots, where the root mass of H2, H4, and H6 hybrids at 75% FC was greater than that of the controls.

3.5 Effect of Water Stress on the Chlorophyll Content of Leaves

Figure 5 shows that the irrigation dose reduced the chlorophyll content of rootstocks. For the controls, chlorophyll content was high, with the H3 rootstock having the highest value of 3.53 mg/g FM and the H4 hybrid having the lowest at 2.78. At 75% FC, rootstock H3 had the highest chlorophyll content of 3.09, rootstock H4 had 3.53 mg/g FM, and the lowest content of 2.01. In contrast, at 50% FC, the highest chlorophyll content of 2.67 mg/g FM was recorded for hybrid H3, and hybrid H5 had the lowest content of 1.47 mg/g FM.

3.6 Effect of Water Stress on Leaf Proline Content

The results in Figure 6 show that water stress triggers proline accumulation in the leaves. For the controls, the highest proline content (49.2) was observed for hybrid H4, and the lowest was 34.9 for hybrid H1. At 75% FC, rootstock H4 had the highest content (85.8), and rootstock H1 had the lowest content (54.6). At the 50% level, hybrid H4 had the highest proline content of 122.1 µg/g DM, while hybrid H6 had the lowest at 85.3 µg/g.

Table 3: Effect of water stress on the fresh matter of leaves (g).

Rootstock	Control	75% FC	50% FC
H1	7.18 ± 0.73^{a}	5.34 ± 0.12^{b}	4.00 ± 0.21^{b}
H2	12.89 ± 2.60^{a}	7.31 ± 0.57^{ab}	4.27 ± 0.90^{b}
H3	12.75 ± 1.73^{a}	7.02 ± 0.50^{b}	3.43 ± 0.45^{b}
H4	9.90 ± 0.44^{a}	7.04 ± 0.20^{b}	2.98 ± 0.96^{c}
H5	9.38 ± 0.87^{a}	6.19 ± 0.67^{b}	3.39 ± 0.54^{c}
H6	9.24 ± 0.87^{a}	6.13 ± 0.75^{b}	2.56 ± 0.38^{c}
LR	12.68 ± 0.95^{a}	7.09 ± 0.33^{b}	3.40 ± 0.80^{c}
PT	1.89 ± 0.17^{a}	1.65 ± 0.13^{a}	1.11 ± 0.40^{a}

Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), mean values ± standard error (n = 6).

Table 4: Effect of water stress on the fresh matter of stems (g).

Rootstock	Control	75% FC	50% FC
H1	3.93 ± 0.78^{a}	2.67 ± 0.23^{ab}	2.07 ± 0.08^{b}
H2	9.28 ± 1.56^{a}	5.29 ± 0.26^{b}	3.35 ± 0.38^{b}
H3	9.37 ± 0.43^{a}	4.12 ± 0.31^{b}	3.01 ± 0.45^{b}
H4	5.99 ± 0.20^{a}	4.30 ± 0.08^{b}	2.95 ± 0.40^{c}
H5	3.96 ± 0.88^{a}	2.20 ± 0.29^{ab}	1.84 ± 0.18^{b}
H6	5.38 ± 0.01^{a}	3.52 ± 0.12^{ab}	2.11 ± 0.34^{c}
LR	6.57 ± 0.35^{a}	4.39 ± 0.11^{b}	3.04 ± 0.57^{b}
PT	4.11 ± 0.54^{a}	3.28 ± 0.33^{a}	2.79 ± 0.15^{a}

Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), mean values ± standard error (n = 6).

3.7 Effect of Water Stress on the Soluble Sugar Content of Leaves

As shown in Figure 7, water stress increased the leaf sugar content of the rootstocks tested. For the controls, the sugar content was high, with the H3 rootstock at 2.36 mg/g DM and the lowest content of 1.53 observed for the H4 hybrid. At 75% FC, rootstock H3 had the highest

Table 5: Effect of water stress on fresh matter of roots (g).

Rootstock	Control	75% FC	50% FC
H1	7.93 ± 2.25^{a}	6.12 ± 1.04^{a}	5.30 ± 0.7^{a}
H2	13.21 ± 3.25^{a}	9.77 ± 2.16^{a}	6.52 ± 0.82^{a}
H3	14.27 ± 3.97^{a}	9.45 ± 2.16^{a}	7.43 ± 0.26^{a}
H4	10.4 ± 1.67^{a}	10.71 ± 3.00^{a}	5.63 ± 1.72^{a}
H5	7.72 ± 2.15^{a}	5.74 ± 1.54^{a}	3.98 ± 0.70^{a}
H6	7.70 ± 1.73^{a}	7.71 ± 1.34^{a}	4.48 ± 0.65^{a}
LR	8.5 ± 0.90^{ab}	12.67 ± 3.52^{a}	4.88 ± 0.59^{b}
PT	6.12 ± 1.37^{a}	6.23 ± 1.65^{a}	5.64 ± 1.80^{a}

Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), mean values ± standard error (n = 6).

Table 6: Effect of water stress on the dry matter of leaves (g).

Rootstock	Control	75% FC	50% FC
H1	2.34 ± 0.24^{a}	1.76 ± 0.10^{b}	1.43 ± 0.06^{c}
H2	4.38 ± 0.42^{a}	2.49 ± 0.15^{b}	1.47 ± 0.16^{c}
H3	4.07 ± 0.37^{a}	2.51 ± 0.25^{b}	1.29 ± 0.13^{c}
H4	3.21 ± 0.15^{a}	2.46 ± 0.13^{a}	1.12 ± 0.35^{b}
H5	3.00 ± 0.39a	2.04 ± 0.24ab	1.28 ± 0.22b
H6	2.95 ± 0.11^{a}	2.15 ± 0.22^{b}	0.96 ± 0.17^{c}
LR	4.02 ± 0.45^{a}	2.89 ± 0.23^{a}	1.27 ± 0.33^{b}
PT	0.68 ± 0.07^{a}	0.65 ± 0.06^{a}	0.46 ± 0.13^{a}

Stress levels with the same letter do not differ significantly for the same rootstock; at $P \leq 0.05$ (one-way ANOVA, separated by Duncan's test), mean values ± standard error (n = 6).

Table 7: Effect of water stress on the dry matter of stems (g)

Rootstock	Control	75% FC	50% FC
H1	1.31 ± 0.17^{a}	1.01 ± 0.04^{b}	0.98 ± 0.04^{b}
H2	3.84 ± 0.49^{a}	2.30 ± 0.08^{b}	1.64 ± 0.21^{b}
H3	3.43 ± 0.29^{a}	1.94 ± 0.12^{b}	1.45 ± 0.19^{b}
H4	2.43 ± 0.11^{a}	1.77 ± 0.36^{b}	1.42 ± 0.16^{c}
H5	1.60 ± 0.31^{a}	0.91 ± 0.14^{a}	0.98 ± 0.09^{a}
H6	2.02 ± 0.04^{a}	1.40 ± 0.21^{b}	1.01 ± 0.22^{b}
LR	2.94 ± 0.28^{a}	2.42 ± 0.32^{b}	1.54 ± 0.37^{b}
PT	1.65 ± 0.21^{a}	1.36 ± 0.07^{a}	1.11 ± 0.20^{a}

Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), mean values ± standard error (n = 6).

sugar content (3.36), and rootstock H5 had the lowest (2.12). At 50% FC, the highest sugar content of 4.24 mg/g DM was recorded for hybrid H3, whereas hybrids H4 and H5 had the lowest content (2.60 mg/g).

Table 8: Effect of water stress on the dry matter of roots (g).

Rootstock	Control	75% FC	50% FC
H1	2.86 ± 0.39^{a}	2.01 ± 0.24^{a}	2.27 ± 0.20^{a}
H2	4.14 ± 0.49^{a}	4.41 ± 0.76^{a}	3.27 ± 0.60^{a}
H3	5.15 ± 0.71^{a}	4.37 ± 0.87^{a}	3.69 ± 0.18^{a}
H4	3.63 ± 0.60^{a}	4.57 ± 1.21^{a}	2.81 ± 0.76^{a}
H5	2.52 ± 0.33^{a}	2.04 ± 0.58^{a}	1.89 ± 0.39^{a}
H6	2.46 ± 0.09^{b}	3.42 ± 0.32^{a}	2.12 ± 0.32^{b}
LR	2.92 ± 0.20^{b}	6.81 ± 1.54^{a}	2.43 ± 0.34^{b}
PT	2.09 ± 0.13^{a}	2.25 ± 0.60^{a}	2.51 ± 0.85^{a}

Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), mean values ± standard error (n = 6).

4. DISCUSSION

Water stress is one of the main environmental factors that affect the development, growth, and productivity of plants. Citrus is often exposed to water shortages, particularly in arid and semiarid areas. The choice of rootstock is among the most important decisions a grower makes. Citrus rootstock influences the morphological, biochemical, and physiological characteristics of the grafted scion. A rootstock with drought tolerance is of great importance to remedy this climatic hazard and reduce production losses. In the present study, we evaluated the behavior of new hybrid citrus rootstocks under water-stress conditions in a greenhouse. The effects of 50% FC and 75% FC on stem height, stem diameter, number of leaves, stomatal conductance, fresh and dry biomass, total chlorophyll, soluble sugars, and proline were studied. The results showed that stem height, number of leaves, and stem diameter were affected by water stress, with a significant reduction in these parameters at the 50% FC level. The hybrid H6 has the lowest stem growth reduction; in contrast, the hybrid H2 has the highest stem growth reduction. In terms of leaves, hybrid H2 had the highest number of leaves, whereas hybrid H3 had the lowest. Indeed, the application of water stress to seedlings caused a reduction in stem diameter. Similar findings have been reported by Wu et al. [26] and Rodriguez-Gamir et al. [27]. Water stress changes the water relationship within the plants and decreases turgor, gas exchange, and growth [28]. Shafqat et al. [29] revealed that the ability of plants to maintain growth under limited water indicates their tolerance ability. Beniken et al. [18] reported that water stress caused leaf drop and a reduction in stem height. In the case of severe 50% FC water stress, variability between rootstocks was observed in growth, which could be explained by a reduction in photosynthetic and metabolic reactions that are limited to biosynthetic pathways. Similar results have been reported by Wu et al. [26] and Rodriguez-Gamir et al. [27]. The fresh and dry biomass of various plant parts was affected by stress. The lowest fresh matter of leaves was observed in the hybrid H6, compared to the hybrid H1, which showed the greatest tolerance. The reduction of fresh and dry matter shown in all hybrids and more pronounced in 50% FC may be the result of physiological and biochemical disturbance, which may be accentuated with the reduction of leaf area and leaf drop [30,31]. Craine [30] and Munns et al. [32] stated that practically all rootstocks examined showed inhibitory impacts of water stress on biomass. Our study also demonstrated the effect of water stress on stomatal conductance, since stomatal closure lowers photosynthesis and water loss. Our results are in agreement with those reported by

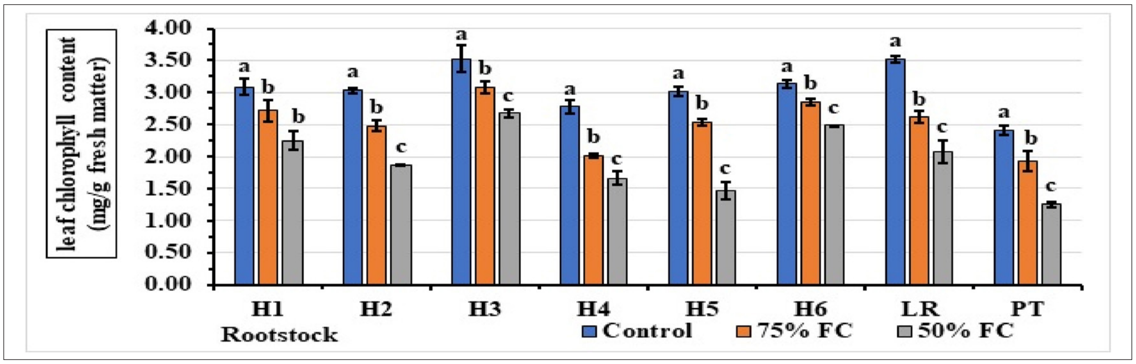

Figure 5: Effect of water stress on leaf chlorophyll content. Stress levels with the same letter do not differ significantly for the same rootstock; at $p \leq 0.05$ (one-way ANOVA, separated by Duncan's test), vertical bars represent the standard error (n = 6).

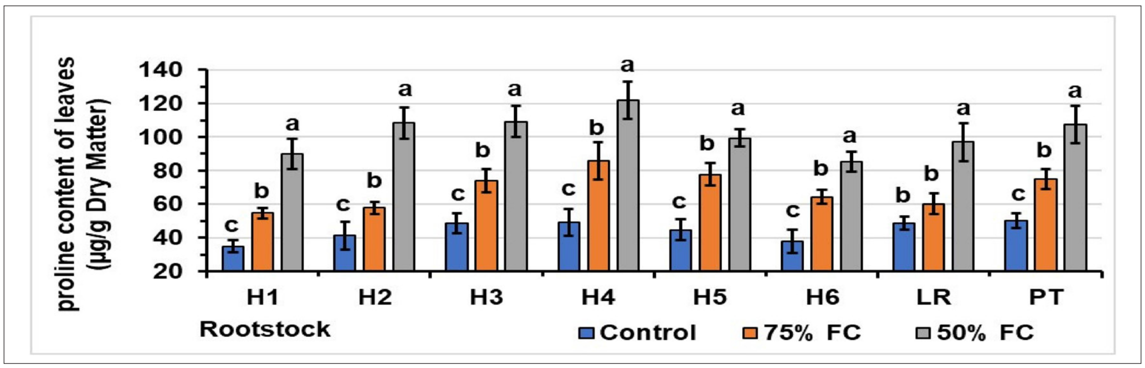

Figure 6: Effect of water stress on proline content of leaves. Stress levels with the same letter do not differ significantly for the same rootstock; at $P \leq 0.05$ (one-way ANOVA, separated by Duncan's test), vertical bars represent the standard error (n = 6).

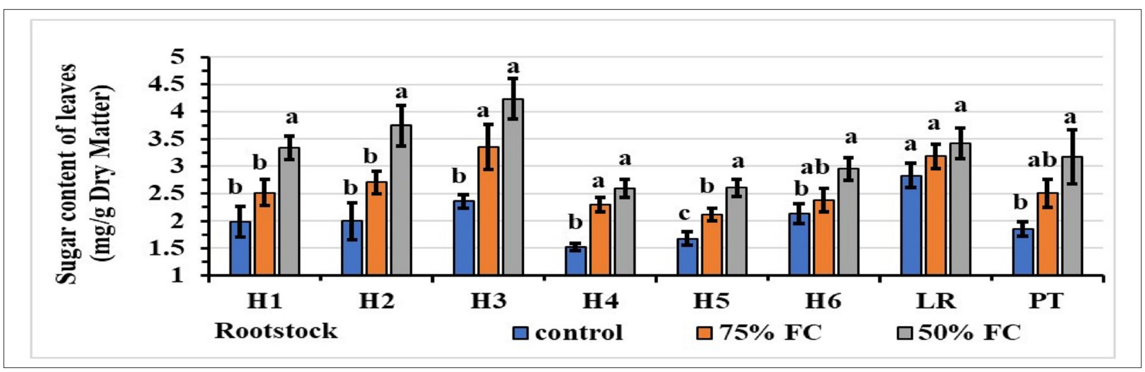

Figure 7: Effect of water stress on soluble sugar content of leaves. Stress levels with the same letter do not differ significantly for the same rootstock; at $P \leq 0.05$ (one-way ANOVA, separated by Duncan's test), vertical bars represent the standard error (n = 6).

Rodriguez-Gamir et al. [27], Hutton and Loveys [33], and De Campos et al. [34], who reported that this decrease in stomatal conductance is due to stomatal closure and a consequent reduction in transpiration, which also leads to a reduction in photosynthetic responses. Boyer and Kramer [5] reported that when water stress is experienced by plants, reducing stomatal conductance is the first short-term response to prevent water loss through transpiration. In the medium term, increasing root development maximizes water intake. García-Sánchez et al. [4] and Beniken et al. [18] reported that the tolerance capacity of these rootstocks is linked to their high osmotic adjustment capacity via osmolyte accumulation, which enables them to maintain their vital photosynthetic activities under water-stress conditions. Results show that the chlorophyll content decreased with the increase in stress. At 50% FC, the highest chlorophyll content was recorded for the hybrid H3 and the lowest for H5. Hussain et al. [35] reported that water stress decreases the chlorophyll content of citrus rootstock. Citrus rootstock with high chlorophyll content in water stress conditions is considered tolerant [36]. Water stress affects the normal functioning of photosynthetic machinery; degradation and photooxidation of chlorophyll caused by transpiration imbalance disturb the plant's capacity to harvest light and reduce total photosynthetic output, leading to a reduction in carbohydrate transport and growth [37]. Several authors, Cohen and Naor [38], Weibel et al. [39], and Gijón et al. [40] have reported that the drop in chlorophyll content is the result of reduced stomatal opening. This stomatal closure limits water loss through evapotranspiration while simultaneously reducing the inflow of atmospheric CO_2 required for photosynthesis. Proline content increased with the increase in stress levels. Among the hybrids tested, the highest content was recorded in the hybrid H4, whereas the lowest content was observed in the hybrid H6. Hussain et al. [35] observed an increase in proline content in citrus rootstock when subjected to water stress. Proline, as an osmoprotectant compound, prevents macromolecules from dehydration, adjusts osmotic pressure, protects proteins from denaturation, detoxifies reactive oxygen species, and protects membranes against lipid peroxidation [28,41]. Shekafandeh et al. [42] observed that tolerant genotypes accumulate more proline than sensible genotypes. Other authors, Khedr et al. [43], Demiral and Türkan [44], and Ma et al. [45], have reported that proline strengthens the antioxidant system and combats stress damage. It may also play a role in regulating the cytoplasmic pH or constitute a nitrogen reserve used by the plant after the stress period. Sugar content increased with the increase in stress levels. Among the hybrids tested, the highest content was recorded in the hybrid H3, whereas the lowest content was observed in the hybrids H4 and H5. Plants develop other tolerance mechanisms consisting of the accumulation of solutes such as sugars, which reduce osmotic potential at the cellular level by osmotic adjustment in the sense of keeping water in the cell. Similar results have been reported in citrus fruits by Molinari et al. [46] and Beniken et al. [18]. These researchers observed that the total soluble sugar content of rootstock leaves increases with the severity of water stress. This increase could be a parameter of adaptation to water-deficit conditions, as it helps maintain high cellular integrity at the tissue level.

5. CONCLUSION

We studied the effect of water stress on six new hybrid citrus rootstocks under greenhouse conditions. Water stress expressively affected the physiological, morphological, and biochemical parameters of the rootstocks tested and induced different behaviors under stress conditions. The results showed that, as the applied stress increased, the stem growth and stem diameter were negatively affected, and the number of leaves, as well as their relative water content and stomatal conductance, decreased. In addition, the leaf chlorophyll content, the fresh matter, and the dry matter decrease with an increase in water stress. The greatest reductions were recorded at 50% FC, whereas the control had the maximum values. The results indicate that leaf proline and soluble sugar contents increased with stress intensity. Among the rootstocks tested, hybrid H1 proved to be more drought-tolerant than the other rootstocks in terms of growth parameters, having low stem growth reduction associated with a high number of leaves and having a high fresh matter quantity when subjected to severe stress (50% FC). while hybrid H3 was considered sensitive, with high stem growth reduction combined with the drop of leaves, leading to a minor quantity of fresh matter.

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7. AUTHORS' CONTRIBUTIONS

All of the authors significantly contributed to the article's conception and design, data acquisition, analysis, and interpretation; they also collaborated in its drafting or critical revision for valuable intellectual content; they agreed to submit the work to the current journal; they approved the final version that would be published; and they agreed to take responsibility for all aspects of the work. According to the guidelines/requirements of the International Committee of Medical Journal Editors (ICMJE), all authors are eligible to be authors.

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9. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All data generated and analyzed are included within this article.

12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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