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Soil Water Supply during Dry Weather Constraint of Sweet Orange (*Citrus sinensis* L. Osb.) Seedlings in Response to Gas Exchanges, Photosynthetic Functions, Leaf Carbohydrate Contents and Biomass Production

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ABSTRACT

Regarding the influences and consequences of abiotic stress, dry weather is one of the major causes of crop losses worldwide. The aim of this study was to test the adaptation potential of sweet orange (*Citrus sinensis* L. Osb.) in dry environmental conditions with a high vapor pressure deficit. Two different soil moisture levels were used in a pot trial for sweet orange seedlings in greenhouse conditions. One year old citrus seedlings, which are grown as exotic plants in semi-arid regions, were studied under moist soil conditions and during mild soil water stress. Higher leaf gas exchange and physiological response values were found for the well-watered soil condition. However, no difference in the total chlorophyll content was observed. In addition, photosynthetic functions were very different in response to soil water status. However, no significant differences were found between the soil water levels for thylakoid light harvesting and leaf carbohydrate content. In addition, there were significant differences in biomass partitioning between the well-watered seedlings and the mildly stressed seedlings. Consequently, the total dry mass difference in the entire citrus seedlings was approximately 40% for both soil water levels. This study indicates that citrus seedling could adapt to survive and grow in dry weather conditions with a sufficient soil water supply. The drought stress tolerance of citrus varieties and their adaptation and growth in semi-arid regions are discussed.

Key words: Abiotic stress, drought stress, vapor pressure deficit, plant adaptation, semi-arid regions, photosynthetic nitrogen-use efficiency, dry mass partitioning

INTRODUCTION

Currently, climate change is expected to cause variations in precipitation patterns. Specifically drier, warmer and higher Vapor Pressure Deficit (VPD) conditions are expected to occur in many

regions around the world (Venkateswarlu and Shanker, 2012). Plants possess advanced features for adapting to life and growing in harsh environments (Ahmad *et al.*, 2010). The adaptation potential of plants to survive in different environmental conditions may vary between plant varieties (Vinod, 2012).

Under natural conditions, plant physiological responses are strongly associated with abiotic environmental factors, particularly those of climate and water stress (Roy and Basu, 2009). In addition, abiotic stress conditions constrain and limit plant growth, development, yield and biomass production (Nagarajan and Nagarajan, 2010). For many crops, drought is one of the most limiting factors, especially during soil water deficits. Soil water is a substrate for nutrient transport from soil to living plants and for other photosynthesis activity responses. Furthermore, plant water stress responses are not only represented in plant water status by a reduction in leaf water potential or relative water content but also by stomatal conductance, CO₂ assimilation, transpiration, specific leaf weight, carbohydrate accumulation, nutrient metabolism and plant growth and biomass distribution (Sanchez-Rodriguez *et al.*, 1999; Ashraf *et al.*, 2007; Leakey *et al.*, 2009). Moreover, it was reported that the selective drought tolerance of some crops can be determined by their photosynthetic capacity and chlorophyll functions (Kausar *et al.*, 2006; Xu and Zhou, 2011). In addition, there is a relationship between photosynthetic functions and drought stress levels in Norway spruce seedlings (Ditmarova *et al.*, 2010). Similarly, physiological mechanisms can be affected by high VPD or low Relative Humidity (RH). Specifically, high VPD or low RH conditions have been linked to stomatal conductance and leaf transpiration (Arve *et al.*, 2011).

Citrus production may be impacted by dry weather conditions that result from climate change. In citrus orchards, a water deficit usually results in retarded growth during each citrus tree development stage (Davies and Albrigo, 1994). Because soil water stress decreases stomatal conductance in citrus trees (Poggi *et al.*, 2007), reduced CO₂ uptake is more sensitive to high VPD when citrus is grown under soil water deficit conditions (Brakke and Allen, 1995). During seasonal changes, the percentage of stomatal limitation to photosynthesis in citrus trees may be limited to approximately 40-60% as a result of a high VPD (especially for the summer season) (Ribeiro *et al.*, 2009). Although a high VPD affect leaf stomatal conductance, it usually leads to decreased net photosynthesis, transpiration rate and water use efficiency in citrus plants (Habermann *et al.*, 2003). Furthermore, the photosynthetic apparatus in citrus may be inhibited by carbohydrate accumulation (Nebauer *et al.*, 2011). However, positive responses to sufficient water supplies during the dry season are observed in the form of gas exchange and young citrus tree growth (Aiyelaagbe and Orodele, 2007).

Thus, the availability of adequate water to prevent water deficit conditions is necessary for the water management of citrus seedlings in orchards or nurseries (Boman *et al.*, 1999). In this study, citrus seedlings (as exotic plant samples) were grown in semi-arid regions at two different soil moisture levels. The objective of this study was to test the adaptation potential of sweet orange for surviving and growing in dry environmental conditions. To accomplish this objective, the gas exchange processes, the physiological responses of leaf, the photosynthetic functions and the leaf carbohydrate and biomass accumulations were measured.

MATERIALS AND METHODS

Plant material and experimental conditions: The experiment was conducted in the Beijing region of China. The sweet orange (*Citrus sinensis* L. Osb.) seedlings were scion grafted onto Trifoliate orange (*Poncirus trifoliata* Raf.) rootstocks, which were used as an exotic plant material

from June to October of 2011. Nine healthy one-year-old seedlings of each treatment were selected and grown individually in 4-L plastic pots in greenhouse conditions. A growing media of clay loam soil, sand and composted manure was mixed at a ratio of 2:1:1 (v/v). Subsequently, the pH of the mixed soil was measured and was approximately 7.82 ± 0.09 . Meanwhile, the average monthly mean microclimate inside of the greenhouse was recorded. The VPD, air temperature, RH and Photosynthetically Active Radiation (PAR) were 3.22 ± 0.53 kPa, $27.68 \pm 3.27^\circ\text{C}$, $52.87 \pm 9.82\%$ and $811.20 \pm 129.54 \mu\text{mol m}^{-2} \text{sec}^{-1}$, respectively. Two watering regimes were set up with drip irrigation in a block design to control soil moisture status. Two different soil moisture statuses were used throughout this study period, including a Well-Watered (WW) soil moisture status at 90% of the field capacity ($27.36 \pm 0.97\%$ or -0.19 ± 0.07 bar) and a Mild Soil (MS) moisture status at 60% of the field capacity ($18.12 \pm 1.30\%$ or -4.63 ± 1.92 bar). The soil moisture measurements that were used to determine the amount of water available for seedlings were conducted with a portable soil moisture meter (TRIME-FM TDR, IMKO, Germany) and were collected three times per week. In addition, a fertigation system was set up to supply plant nutrients (as required) once a month.

Physiological response and photosynthetic capacity measurements: Two fully exposed and expanded mature leaves were randomly selected from each citrus seedling to measure their physiological responses on selected clear days between 10.00-14.00 h at approximately 2-week intervals. Leaf gas-exchange parameters were collected with a portable gas-exchange system (LI-6400XT, Li-Cor Inc., USA) that determined net photosynthesis rate (P_n), stomatal conductance (g_s), transpiration rate (E) and photosynthetic Water-Use Efficiency (WUE). In addition, the midday leaf water potential (Ψ_l) was determined with a plant water status console (model 3005, Soil Moisture Equipment Corp., USA). Furthermore, photosynthetic response to atmospheric CO_2 partial pressure (an A/C_i curve) was analyzed to measure the maximum rate of carboxylation ($V_{c,\text{max}}$) and electron transport (J_{max}) by fitting curves with the photosynthesis model equations (Von Caemmerer and Farquhar, 1981; Pimentel *et al.*, 2007). To determine the maximum carbon assimilation rate (A_{max}), the leaves were exposed to a saturated Photosynthetic Photon Flux Density (PPFD) of $1,200 \mu\text{mol m}^{-2} \text{sec}^{-1}$ under an altered ambient LED light source (6400-02B). The leaf chamber was constantly controlled at 25°C and at 50% relative humidity. The ambient CO_2 partial pressure was set to 400, 200, 100, 75, 50, 25 and 0 and then increased stepwise to 370, 600, 800, 1,000 and $1,200 \mu\text{mol CO}_2 \text{mol}^{-1}$. During the A/C_i responses, the mesophyll conductance (g_m) was estimated with the fitting curve method (Harley *et al.*, 1992). In addition, the fluorescence parameters were measured with a portable fluorometer (PAM-2000, Walz, Germany) to determine the maximum PSII photochemistry quantum yield (F_v/F_m) and the PSII electron transport quantum yield (Φ_{PSII}).

Nitrogen partitioning parameters for photosynthetic functions: The proportion of nitrogen (N) that was invested in photosynthetic functions was determined in terms of Rubisco (P_r), bioenergetics (P_b) and thylakoid light-harvesting components (P_l) (Niinemets and Tenhunen, 1997). All of these values were calculated from the $V_{c,\text{max}}$, J_{max} , leaf total N and total chlorophyll content (Chl) values. The partitioning of N per leaf area (N_A) and leaf dry mass (N_M) were used to express the relative leaf N content. Photosynthetic Nitrogen-Use Efficiency (PNUE) was calculated as the ratio of A_{max} to N_A .

Leaf traits, biochemical parameters and biomass partitioning: Each shoot was selected for a leaf area measurement. Leaf samples were monitored without damaging the leaves by using a portable area meter (LI-3000C, Li-Cor Inc., USA). In the laboratory, some leaf samples were immediately cut into 1 cm² leaf blade discs and used to record the fresh weight. The fully turgid weight of the leaf discs was collected after soaking them in distilled water for 16 h in the dark. The dry weight was obtained by drying the leaf disks at 60°C for 24 h before reweighing. Therefore, the leaf Relative Water Content (%RWC) was calculated according to Turner (1981) as follows:

$$\text{RWC (\%)} = \frac{\text{Fresh weight-Dry weight}}{\text{Turgid weight-Dry weight}} \times 100$$

Next, the Specific Leaf Weight (SLW) was calculated from the leaf dry mass per unit area of the leaf disc. After all of the fresh and dry leaf disc weights were recorded, the SLW values could represent the projected leaf area values. The total Chl of each leaf sample was extracted with N,N-dimethylformamide (DMF). The absorbance of the extracts was performed at wavelengths of 647 and 664 nm with a UV spectrophotometer (UV-2550, Shimadzu, USA). The leaf total Chl was calculated by using the equation found in Inskeep and Bloom (1985). In addition, the fresh leaf sample residues were oven dried at 65°C for 48 h. Ground and dried tissues were used to analyze the amount of leaf N by the Kjeldahl method and the leaf carbohydrate content (soluble sugars and starch) by the anthrone method (Sivaci, 2006). Five months after the experiment was started, all citrus seedling individuals (six plants total per treatment) were destructively harvested to determine their biomass partitioning. Plant parts (leaves, branches, stems, roots) were separated and placed in an oven at 105°C to dry until a constant weight was achieved.

Statistical analysis: Statistical analyses were performed in SPSS 15.0 (SPSS Inc., Chicago, USA). A t-test was used to compare the mean values of the different physiological parameters from the two citrus seedling soil water treatment levels. The Least Significant Difference (LSD) at a probability level of $p \leq 0.05$ was determined between the physiological variables.

RESULTS

Leaf gas exchanges and photosynthesis responses: Leaf gas exchanges and physiological responses were significantly different between the two different soil water statuses during high VPD conditions. The Well-Watered (WW) seedlings had significantly higher P_n (Fig. 1a), g_s (Fig. 1b), E (Fig. 1c), WUE (Fig. 1d), Ψ_1 (Fig. 1e), RWC (Fig. 1f) and SLW (Fig. 1g) values than the Mildly Stressed (MS) seedlings. However, the total Chl (Fig. 1h) was not significantly different between these treatments.

A large difference was found between the WW and MS seedling treatments for P_n , g_s , E and Ψ_1 after the first 2 weeks of water regime treatment. Although, the WUE and SLW slightly increased for the WW seedlings during the experimental treatment, they were markedly different than those of the MS seedlings after 6 and 8 weeks, respectively. For the RWC parameter, the MS seedlings had a lower stable value during soil water stress conditions. Although the total Chl in the MS seedlings was lower than in the WW seedlings, this difference was not significant.

Nitrogen investments in photosynthetic and PSII functions: Soil water level significantly affected some N parameters that were allocated to photosynthetic functions (Table 1). Highly

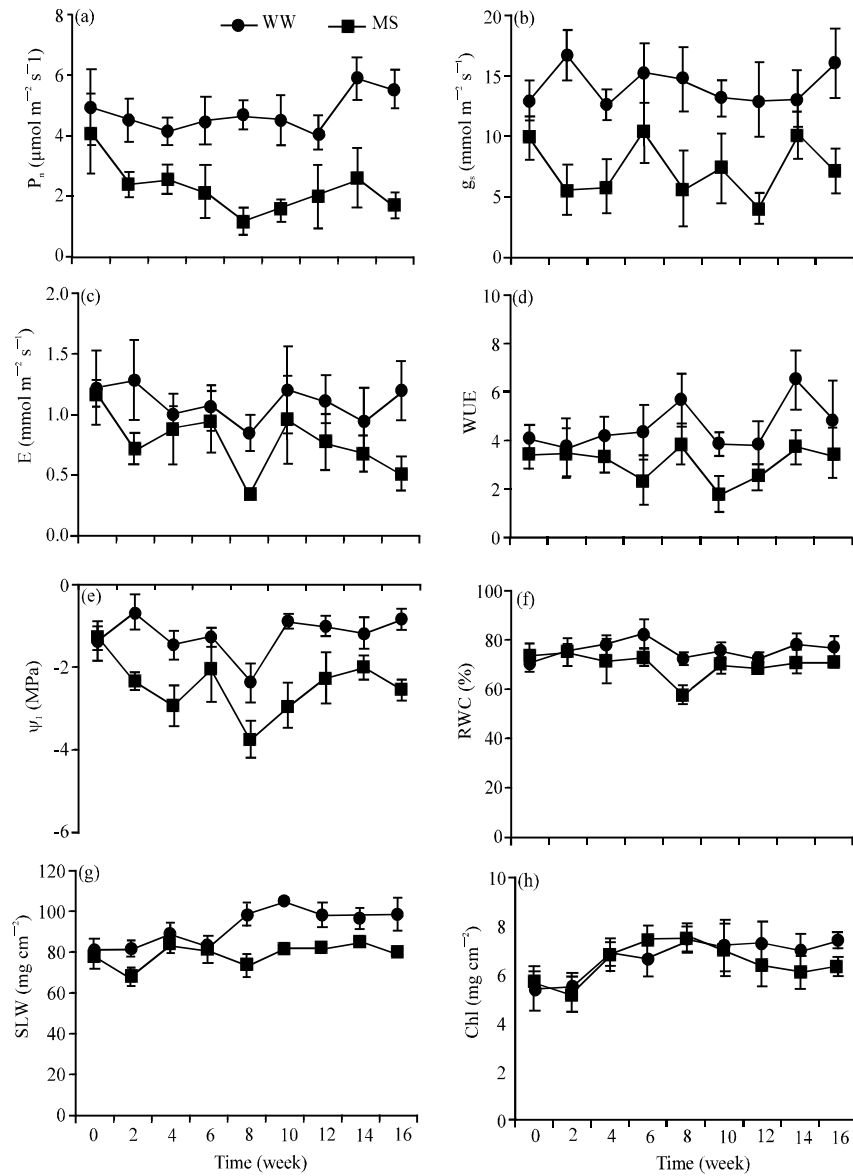


Fig. 1(a-h): Variations in (a) Net photosynthesis rate (P_n), (b) Stomatal conductance (g_s), (c) Transpiration rate (E), (d) Photosynthetic water-use efficiency (WUE), (e) Leaf water potential (Ψ_l), (f) Leaf relative water content (RWC), (g) Specific leaf weight (SLW) and (h) Chlorophyll content (Chl) in citrus seedlings to well-watered soil (WW) and mild soil water stress (MS) treatments, Values are Mean \pm SD

significant differences ($p \leq 0.001$) were found for A_{max} (7.00 ± 0.92 and $3.86 \pm 0.77 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$), J_{max} (93.71 ± 9.18 and $40.80 \pm 8.71 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$) and P_r (0.12 ± 0.05 and $0.05 \pm 0.01 \text{ g N}_{rubisco} \text{ g N}_{total}^{-1}$) between the WW and MS treatments, respectively. Mean values for g_m (0.09 ± 0.01 and $0.03 \pm 0.01 \text{ mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$), PNUE (2.53 ± 0.83 and 1.53 ± 0.22), $V_{c,max}$ (42.51 ± 13.59 and $17.83 \pm 5.34 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$) and P_b (0.03 ± 0.01 and $0.01 \pm 0.01 \text{ g N}_{bioenergetics} \text{ g N}_{total}^{-1}$) of the WW and MS treatments were also significant differences ($p \leq 0.01$) as represented by their respective values. However, no significant differences were found for LMA (109.84 ± 18.42 and

Table 1: Physiological parameters of leaf traits, photosynthetic functions, PSII functions, carbohydrate content and dry mass partitioning in citrus seedlings for well-watered soil (WW) and mild soil water stress (MS) treatments

Physiological parameters	WW	MS	p-values
Leaf traits			
LMA (g m ⁻²)	109.84±18.42	99.40±22.41	0.400
N _A (g m ⁻²)	3.11±0.41	2.58±0.41	0.440
N _M (g N/100 g dry mass)	2.43±0.22	2.58±0.72	0.220
Photosynthetic functions			
A _{m, max} (μmol CO ₂ m ⁻² sec ⁻¹)	7.00±0.92	3.86±0.77	≤0.010
g _m (mol CO ₂ m ⁻² sec ⁻¹)	0.09±0.01	0.03±0.01	≤0.010
PNUE	2.53±0.83	1.53±0.22	≤0.001
V _{c, max} (μmol CO ₂ m ⁻² sec ⁻¹)	42.51±13.59	17.83±5.34	≤0.010
J _{m, max} (μmol CO ₂ m ⁻² sec ⁻¹)	93.71±9.18	40.80±8.71	≤0.010
P _r (g N _{rubisco} /g N _{total})	0.12±0.05	0.05±0.01	≤0.001
P _b (g N _{bioenergetics} g/N _{total})	0.03±0.01	0.01±0.01	≤0.010
P _l	0.83±0.18	1.04±0.19	0.070
PSII functions			
F _v /F _m	0.79±0.02	0.72±0.02	≤0.010
Φ _{PSII}	0.62±0.05	0.53±0.02	≤0.050
Carbohydrate content			
Soluble sugars (mg g ⁻¹ DW)	64.76±14.53	67.81±9.62	0.830
Starch (mg g ⁻¹ DW)	64.41±8.76	66.28±9.25	0.730
Dry mass partitioning			
Leaves (g tree ⁻¹)	23.50±2.39	13.53±2.45	≤0.001
Branches (g tree ⁻¹)	7.91±3.00	2.41±0.81	≤0.010
Stems (g tree ⁻¹)	33.54±8.13	19.52±1.89	≤0.010
Roots (g tree ⁻¹)	58.62±8.92	35.46±1.59	≤0.001

Values are Mean±SD (n = 6) from a t-test analysis

99.40±22.41 g m⁻²), N_A (3.11±0.41 and 2.58±0.41 g m⁻²), N_M (2.43±0.22 and 2.58±0.72 g N/ 100 g dry mass) and P_l (0.83±0.18 and 1.04±0.19) which were shown as the WW and MS treatments, respectively. In addition, the significantly lower for either F_v/F_m (0.72±0.02) (p≤0.01) or Φ_{PSII} (0.53±0.02) (p≤0.05) of the MS treatment than the WW treatment (0.79±0.02 and 0.62±0.05, respectively) was observed (Table 1).

Carbohydrate content and biomass partitioning of citrus seedlings: The leaf carbohydrate contents were not significantly affected by soil water levels (Table 1). The total soluble sugar (64.76±14.53 and 67.81±9.62 mg g⁻¹ DW) and starch (64.41±8.76 and 66.28±9.25 mg g⁻¹ DW) values were similar between the two soil water treatments, which were compared respectively. In addition, the soluble sugar values increased more than the starch values.

The biomass partitioning in citrus seedlings was significantly higher in the WW seedling treatment, which were found in leaves (23.50±2.39 and 13.53±2.45 g tree⁻¹) (p≤0.001), stems (33.54±8.13 and 19.52±1.89 g tree⁻¹) (p≤0.01) and roots (58.62±8.92 and 35.46±1.59 g tree⁻¹) (p≤0.001) (Table 1), respectively, as compared to the MS seedling treatment. Moreover, the largest difference was observed in the branch dry mass (7.91±3.00 and 2.41±0.81 g tree⁻¹) (p≤0.01), which was 68.83% higher in the WW treatment than in the MS treatment. Consequently, the average total dry mass of the entire citrus seedling plants was 42.56% higher in the WW treatment than in the MS treatment.

DISCUSSION

High VPD environments usually limit the g_s (Katul *et al.*, 2009) and lead to reduced P_n , E (Day, 2000) and WUE values in citrus leaves (Habermann *et al.*, 2003). However, citrus seedlings clearly adapted for survival and growth during the dry weather conditions based on their various physiological responses, including their stomatal limitation during the 16 weeks of water supply. These results indicated that the water supplied in the WW treatment can mitigate the effects of a high VPD. This mitigation allowed the citrus seedlings to adapt to the semi-arid regions in northern China. Furthermore, citrus seedlings were able to maintain a high water status by maintaining high Ψ_1 and RWC levels when soil moisture was not limiting. However, it has been reported that g_s is highly sensitive to high VPD rather than to water status, which leads to photosynthesis limitations in plants (Rosati *et al.*, 2006). This relationship was most likely a causal one that directly limited the leaf gas exchange potentials, especially for P_n , g_s and E , as a consequence of the well-watered citrus seedlings. Therefore, it is likely that the gas exchange responses of citrus seedlings in semi-arid regions with unrestricted water supply were constrained and were lower than those in other citrus growing regions, such as in subtropical and tropical climates (Syvertsen and Lloyd, 1994; Ribeiro and Machado, 2007).

Although, the LMA, N_A and N_M of citrus leaves did not differ between the two soil water treatments, the well-watered seedlings had higher photosynthetic functions, particularly for A_{max} , PNUE, $V_{c,max}$, J_{max} , P_r and P_b (Table 1). However, the inhibition of these functions in the mildly stressed seedlings was not accompanied by a parallel decreases in P_1 . The variations in A_{max} , $V_{c,max}$ and J_{max} could be caused by changes in N_A or N_M (Han *et al.*, 2004; Warren *et al.*, 2004). However, the P_r and P_b functions were derived from the $V_{c,max}$ and J_{max} values (Delagrange, 2011). This result showed that soil water stress limits photosynthetic functions in citrus seedlings. Similarly, photosynthetic functions are lower in almond (Egea *et al.*, 2011), olive (Diaz-Espejo *et al.*, 2007) and grape (De Souza *et al.*, 2005) leaves during drought and in ash and oak (Grassi *et al.*, 2005) leaves during low soil water potential conditions.

Furthermore, the PSII functions (F_v/F_m and Φ_{PSII}) slightly decreased during soil water deficit conditions. In contrast, the PSII in wheat and okra leaves was not affected by water stress (Lu and Zhang, 1999; Dkhil and Denden, 2012). The A_{max} limitation could potentially be eliminated by increasing the VPD (Bunce, 2003). However, the PSII functions were slightly affected by a high VPD (Shirke and Pathre, 2004) and could be affected more during severe stress conditions (Xu *et al.*, 2009). Therefore, it is likely that the reduced photosynthetic functions that were observed in citrus seedlings at the high VPD could be increased by maintaining a well-watered soil status.

In addition, the leaf carbohydrate content of the citrus seedlings was similar for both soil water levels. However, the total soluble sugars in the leaves during severe stress were higher than in normal or mild stress conditions. This finding was observed previously in apple leaves (Li and Li, 2005), in which the osmotic adjustment played a role in cell protection during dehydration (Ma *et al.*, 2009). However, these conditions also resulted in decreased starch contents (Naschitz *et al.*, 2010). In this study, mild stress did not affect the soluble sugar content of seedlings or deplete the starch concentration relative to the well-watered seedlings.

Soil water status appeared to play a highly important role in the partitioning of whole dry mass. This role was demonstrated by a difference of approximately 40% between the water treatments. Mildly stressed seedlings were completely limited by long-term soil water stress. Consequently, the Specific Leaf Weight (SLW) allocation in mildly stressed leaves remained constant at the beginning

of the experiment (Fig. 1g). These results showed that seedlings could recover, survive and resume growth when watered following a period of limited biomass production during high VPD and mild soil water stress conditions (Aiyelaagbe and Orodele, 2007; Xu *et al.*, 2009).

CONCLUSION

In conclusion, citrus seedlings responded differently to dry weather conditions between water treatments. During the WW supply, leaf gas exchanges or physiological responses were likely altered to adapt to the drier environment in this study period. However, the soil water deficit became a limiting factor for physiological responses during the MS supply conditions. Consequently, well-watered citrus seedlings demonstrated greater photosynthetic function and dry mass partitioning of all living parts at the end of the experiment. Accordingly, the physiological parameter limitations at high VPD may be reduced by providing an adequate soil water supply. Although the physiological function responses are more sensitive to high VPD conditions, the soil water supply can be used to generate physiological responses and biomass production in citrus seedlings. Therefore, in a dry weather scenario, the physiological responses and photosynthetic capacities are limited by a soil water deficit and a high VPD, which constrains carbohydrate accumulation and biomass production in citrus seedlings. However, drought tolerant citrus varieties should be considered to obtain a more complete understanding of the adaptation potential of citrus trees for survival and growth during dry weather conditions.

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