RESEARCH

Growth, chlorophyll fluorescence and gas exchange of pepper (*Capsicum chinense* Jacq.) plants in response to uptake and partitioning of nutrients

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ABSTRACT

Habanero pepper (*Capsicum chinense* Jacq.) does not have a specific fertilization. Therefore, the aim of this study was to evaluate growth, photosynthesis and accumulation of macro and micronutrients of habanero pepper plants. Plants were established in nutrient film technique (NFT) hydroponic systems. Two universal balanced solutions (Steiner and Hoagland) were compared versus a conventional solution (control: Soria solution). The concentration of macro and micronutrients in roots, stems, and leaves, photosynthetic activity of plants, total nitrate, amino acid and protein contents, and growth parameters were evaluated. According to the results, concentrations of K, Mg, Ca, Cu and Zn in plant tissues were higher in the Steiner and Hoagland treatments than control. In addition, the Steiner and Hoagland treatments increased the maximum photosynthetic rate (A_{max}) (81% and 80% respectively), light-saturated CO_2 assimilation rate (A_{sat}) (3.8-fold and 3-fold, respectively) and maximum catalytic activities of Rubisco (V_{cmax}) (51% and 30% respectively) with respect to the control. Hoagland treatment increased total nitrate content (3.66 mg g^{-1} FW), but Steiner treatment increased amino acids in leaves (169.97 mg g^{-1} FW) and control increased total proteins (1.49 mg g^{-1} FW). Steiner and Hoagland solutions significantly increased plant height (59% and 41% respectively), leaf area (1.26-fold and 1.02-fold, respectively), and total dry mass (80% and 69% respectively) with respect to the control solution. The results suggest that Steiner and Hoagland nutrient solutions provided nutrients that improve growth and photosynthetic activity. Moreover, total nitrate, amino acid and protein accumulation depend on the N source employed in nutrient solutions.

Key words: Growth, macronutrients, micronutrients, photosynthesis.

INTRODUCTION

In recent years, worldwide interest in the habanero pepper (*Capsicum chinense* Jacq.) crop has increased, due to the use of its fruits as one of the spiciest condiments in the world (Meneses-Lazo and Garruña, 2020). The Yucatan peninsula has the denomination of origin of habanero pepper and this is one of the areas with the highest yield in the world (Meneses-Lazo and Garruña, 2020). However, the fertilization used by producers is not based on balanced nutrient solutions that allow maximum nutrient utilization. Producers usually fertilize crops based on empirical knowledge (Alejo-Santiago et al., 2015). In habanero pepper, there are numerous nutrient solutions that were formulated empirically, without analyzing

both concentrations and nutrient sources (Meneses-Lazo and Garruña, 2020). One of the formulations employed most is Soria et al. (2002) (125-100-150 kg ha^{-1} N-P-K). However, this solution does not include Ca, Mg, and S macronutrients, which are assumed to be found in adequate quantities in the soil. Also, this formulation uses urea as the main N source, which is highly polluting because its degradation products can be volatilized (between 40% and 50%), in addition to requiring microorganisms so that N can be assimilated by plants (Staley et al., 2018).

On the other hand, one of the main problems in tropical vegetables is the heterogeneous growth of plants in the growth phase, the stage where producers suffer the greatest economic losses due to low quality plants that do not tolerate transplantation to the field (Garruña-Hernández et al., 2014). Poor nutrition is one of the issues to consider to solve this problem. Applying an appropriate amount of each nutrient allows plants to perform basic metabolic functions (Nieves-González et al., 2015), which gives plants greater vigor during their growth and development. In this sense, the use of nutrient solutions with an ionic balance allows the efficient uptake of both water and nutrients by plants and their distribution to where they are required (Barker and Pilbeam, 2015). Therefore, it is necessary to use balanced nutrient solutions that supply the mineral demand that the plant requires. Nutrient film technique (NFT) hydroponic systems are ideal for conducting studies of nutrient uptake, distribution and accumulation in plants, because they allow the efficient evaluation of both nutrient source and plant demand (Asao, 2012).

Studies have been performed on the habanero pepper crop investigating the effect of some mineral elements on specific responses of plants (Pacheco-Arjona et al., 2011; Nieves-González et al., 2015). However, there are no studies on the effect of balanced nutrient solutions during the growth stage of habanero pepper plants. Therefore, the aim of this study was to evaluate both macro and micronutrient accumulation, growth and photosynthesis of habanero pepper plants cultivated in nutrient solutions.

MATERIALS AND METHODS

Plant material and hydroponic systems

The experiment was conducted at the Instituto Tecnológico de Conkal, Yucatan, Mexico. Habanero pepper (*Capsicum chinense* Jacq.) seeds were used (Geneseeds, Jalisco, Mexico). They were planted in 200-cavity polystyrene seedbeds using Canadian moss as substrate. Foliar fertilization (19:19:19 NPK, 1 g L^{-1}, biweekly) started 20 d after sowing (das). At 50 das the plants were transplanted to nutrient film technique (NFT) hydroponic systems that were in a growth room (4 × 4 × 2.2 m) with controlled conditions: photosynthetic photon flux density (PPFD) was 450 μmol m^{-2} s^{-1}, 16:8 h photoperiod, 22 °C and 60% RH. In each NFT system a data logger (HOBO U12-012, Onset Computer Corporation, Bourne, Massachusetts, USA) was used to measure environmental conditions. Each system had a 280 L h^{-1} submergible pump (4.5 W, Aquakril, Queretaro, Mexico) to supply the corresponding nutrient solution with a film thickness of 1.5 cm, gutters used were of polyvinyl chloride (PVC) with a diameter of 7.62 cm, a 20 L bucket was connected to gutters to collect 12 L nutrient solution that were used in each system.

Nutrient solutions

Three nutrient solutions were used: Steiner (Steiner, 1984), Hoagland (Hoagland and Arnon, 1950), and Soria (Soria et al., 2002). Each solution was a treatment and each treatment was established in an independent NFT system. Steiner and Hoagland solutions were chosen as they are universal balanced solutions that include all essential macronutrients (N, P, K, Ca, Mg, and S), whereas Soria solution was chosen as a control due to its use by habanero pepper producers in Yucatan. However, N and P sources were modified for this solution: ammonium nitrate was used instead of urea (to avoid the use of microorganisms that transform urea to ammonium) and monopotassium phosphate was used instead of phosphoric acid (to avoid low pH). The three solutions were supplemented with micronutrients. For the Steiner and Hoagland solutions the recommended concentration for hydroponic crops was used, whereas for Soria solution the recommended concentration for fertigation crops was used. Calcium nitrate, potassium nitrate, ammonium nitrate, magnesium sulfate, potassium sulfate, monopotassium phosphate and micronutrients (Fe, Mn, Cu, Zn, B and Mo) were used as fertilizer sources (Table 1). Every third day the nutrient solution of the systems was changed, the pH was adjusted (from 5.5-6.0), and the dissolved oxygen (> 4 mg L^{-1}) and electrical conductivity (EC; ≤ 2 dS cm^{-1}) were measured in all treatments.

Table 1. Formulation of nutrient solutions used in the growth stage of habanero pepper plants cultivated in nutrient film technique (NFT) hydroponic systems.

Treatments	Macronutrients							Micronutrients
	NO_3^-	$H_2PO_4^-$	SO_4^{-2}	K^+	Ca^{+2}	Mg^{+2}	NH_4^+	
	meq L^{-1}							Mg L^{-1}
Steiner	12.0	1.0	7.0	7.0	9.0	4.0	-	20-30
Hoagland	14.0	1.0	4.0	6.0	8.0	4.0	1.0	20-30
Soria (control)	10.5	4.9	-	7.5	-	-	7.9	35-45

Micronutrients content (% w/w): Fe-EDTA 7.5%, Mn-EDTA 3.5%, Cu-EDTA 0.28%, Zn-EDTA 0.7%, B 0.65%, Mo 0.26%.

Nutrient content of plants

Thirty days after beginning the treatments, five plants per treatment were dried in a forced air oven at 70 °C for 72 h. Then P, K, Ca, Mg, Fe, Mn, Cu and Zn contents were determined with the X-ray microfluorescence technique (Bruker M4 Tornado 100; Berlin, Germany) at 50 kV and 200 μA, without filters, under vacuum conditions at 20 mbar. Values in mass percentage were obtained and the element quantity in mg g^{-1} dry mass was calculated based on the dry mass of the analyzed sample. On the other hand, total N determination (organic and inorganic) was performed with an N and C analyzer (Thermo Fisher, Waltham, Massachusetts, USA) using 10 mg powdered sample per replicate.

For elementary analyses, five plants per treatment were used and they were analyzed per organ (roots, stems and leaves). With this data, total accumulation per plant was calculated.

Chlorophyll contents and photosynthetic parameters

To quantify chlorophylls a and b and total chlorophyll, 2 g fresh leaf tissue were used, which was macerated with liquid nitrogen. Then 3 mL acetone at 80% (v/v) were added and shaken in a vortex for 30 s. Samples were allowed to stand for 30 min at 4 °C in darkness and were centrifuged at 14000 rpm for 30 min. Supernatant volume was quantified and readings of acetone extracts at three wavelengths were performed (645, 652 and 662 nm). Then chlorophylls a and b and total chlorophyll contents were determined following the equations according to Val et al. (1985).

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured in five plants per treatment with a pulse modulated amplitude fluorometer (PAM, Walz, Effeltrich, Germany). Measurements were taken at 60 das on the third leaf from the apex. Prior to measurement, the plants were acclimated in total darkness for 60 min. Maximum quantum efficiency (F_v/F_m), photochemical (qP) and non-photochemical (NPQ) quenching, electron transport rate (ETR) and quantum yield of photosystem II (PSII) (Φ_{PSII}) were determined. The saturating light pulse and those used for ETR_{PSII} and Φ_{PSII} curves were recommended by Samaniego-Gámez et al. (2016).

Gas exchange

An infrared gas analyzer (LI6400xt, LI-COR, Lincoln, Nebraska, USA) was used to measure the response of photosynthesis (CO_2 assimilation rate; A_N) to the CO_2 concentration inside leaf air spaces (intercellular CO_2 concentration; C_i) and photosynthetic photon flux density (PPFD). Thus, photosynthetic CO_2 response curves (A_N/C_i) and photosynthetic light response curves (A_N/PPFD) were calculated. CO_2 concentrations and PPFD used in the curves were recommended by Meneses-Lazo et al. (2018). The light-saturated CO_2 assimilation rate (A_{sat}) and the maximum photosynthetic rate (A_{max}) were calculated through von Caemmerer and Farquhar (1981) equations. The maximum catalytic activities of Rubisco (V_{cmax}), maximum rate of electron transport demand for RuBP regeneration (J_{max}) and stomatal limitation were calculated according to Ethier and Livingston (2004). Five plants were evaluated per treatment.

Total nitrate, amino acid and protein contents

The protocol described by Santiago-Antonio et al. (2014) was followed for the extraction, starting from 1 g fresh leaf and root tissue. Total nitrate determination was performed according to Cawse (1967) using a KNO_3 standard curve (Sigma Aldrich, St. Louis, Missouri, USA). Total amino acid analysis was performed according to Yemm and Cocking (1955) and total protein analysis was performed according to the method of Bradford (1976).

Dry mass and growth parameters

To determine dry mass per organ and total dry mass, five plants per treatment were used in a forced air oven at 70 °C for 72 h, 30 d after beginning the experiment. To determine growth parameters, five plants per treatment were used. The number of leaves was recorded and leaf area was estimated with a leaf area meter (LI-3100, LICOR). Plant height and stem diameter were measured with a digital caliper (General Ultra-Tech, New York, USA).

Experimental design and statistical analysis

A completely randomized design with four replicates per treatment was used, each replicate with an independent NFT system. Three treatments (T1 = Steiner's nutrient solution; T2 = Hoagland's nutrient solution; T3 = Control, Soria's nutrient solution) were evaluated. Experimental unit was 60 plants (20 cm between plants). All parameters were evaluated in growth stage. The data were analyzed with ANOVA ($p \leq 0.05$) and Tukey's test ($\alpha = 0.05$). STATISTICA version 7 software (StatSoft, Tulsa, Oklahoma, USA) was used.

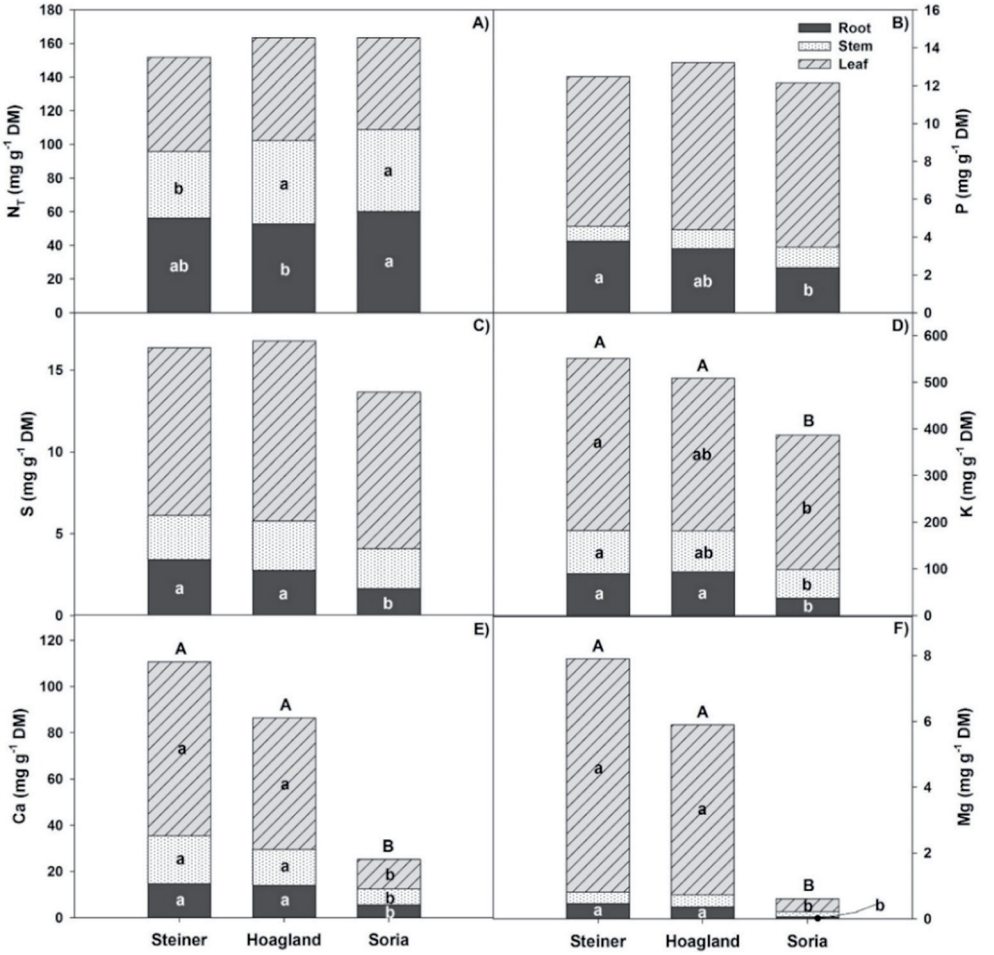
RESULTS AND DISCUSSION

Macronutrients in habanero pepper plants

In total macronutrient accumulation per plant, there were nonsignificant differences among treatments for N, P and S (Figures 1A, 1B and 1C), whereas plants with the Steiner and Hoagland solutions increased total K (551 and 508 mg g^{-1} respectively), Ca (110 and 86 mg g^{-1} respectively), and Mg (7.44 and 5.37 mg g^{-1} respectively) contents compared to plants with the control solution (K: 386.49; Ca: 25.24; Mg: 0.61 mg g^{-1}) (Figures 1D, 1E and 1F). In regard to macronutrient accumulation in leaves (Figures 1A, 1B, 1C, 1D, 1E and 1F), the trend was similar to total accumulation per plant. It is likely that because this was the organ with the greatest nutrient accumulation, it influenced total accumulation per plant more than other organs such as roots and stems. In the stems, significant differences were only observed in N (Steiner: 39.63, Hoagland: 49.31 and Control: 48.72 mg g^{-1}), K (Steiner: 92.63, Hoagland: 86.8 and Control: 60.5 mg g^{-1}) and Ca (Steiner: 20.73, Hoagland: 15.67 and Control: 6.9 mg g^{-1}) (Figures 1A, 1D and 1E). The plant roots in Steiner and Hoagland solutions significantly exceeded those of the control solution in the accumulation of P (3.8, 3.39 and 2.38 mg g^{-1} respectively), S (3.41, 2.35 and 1.64 mg g^{-1} respectively), K (89.81, 94.03 and 37.47 mg g^{-1} respectively), Ca (14.71, 13.9 and 5.5 mg g^{-1} respectively) and Mg (0.46, 0.36 and 0.06 mg g^{-1} respectively) (Figures 1B, 1C, 1D, 1E and 1F). However, N accumulation in roots was greater with control solution than Hoagland solution (60.1 and 52.93 mg g^{-1} respectively) (Figure 1A).

During the growth stage, it is possible to use N sources based on NO_3^- and switch to NH_4^+ and NO_3^- (20%/80%, respectively) in yield stages (Tucuch-Haas et al., 2012). Zhang et al. (2019) mention that plants prefer N in the form of NH_4^+ because its assimilation to amino acids within the plant is immediate, whereas NO_3^- requires more energy for its transformation to NH_4^+. In this sense, Steiner solution only uses NO_3^- as an available N source, whereas NO_3^- and NH_4^+ were used in the Hoagland and control solutions, so the N source in these solutions was high. However, when there is a higher NH_4^+ concentration, uptake of other nutrients such as K, Ca, Mg becomes less efficient (Barker and Pilbeam, 2015), this was observed with K^+, its accumulation in control plants was lower, probably due to competition with NH_4^+ for high-affinity transport systems (Pacheco-Arjona et al., 2011). Similarly, the control solution does not include S, Ca and Mg in its formulation, although low concentrations of these macronutrients were found. According to Gayosso-Rodríguez et al. (2018), moss of the genus *Sphagnum* may contain Na, Ca, Mg, K and phosphates in its nutrient composition. In this sense, during the seedbed stage (before transplanting) Canadian moss was used for both germination and growth of habanero pepper seedlings, and therefore Ca and Mg concentrations were probably obtained in this period of time. On the other hand, Na et al. (2014) mention that NO_3^- can increase Ca^{+2} uptake, whereas NH_4^+ can limit it. This possibly increased Ca^{+2} accumulation in plants with the Steiner and Hoagland solutions. In the case of S, its presence in plants with the control solution is possibly due to small concentrations of potassium sulfate (1.8%) in the commercial fertilizer used (potassium nitrate, Ultrasol NKS, Mexico). The difference among SO_4^{-2} concentrations in roots is possibly due to the presence of NH_4^+, which can limit its uptake (Barker and Pilbeam, 2015).

Figure 1. Total N (A), P (B), S (C), K (D), Ca (E) and Mg (F) contents in habanero pepper plants with three nutrient solutions.



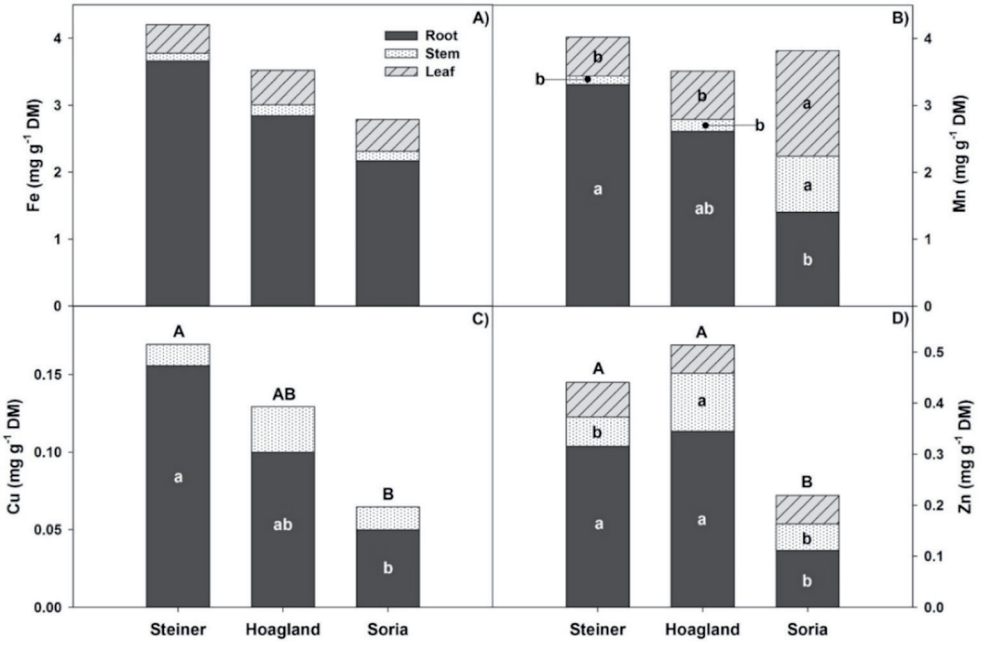
Data are means ± SE; different letters (lowercase letter: in root, stem and leaf; uppercase letter: in complete plant) show significant differences among treatments according to Tukey's test ($\alpha = 0.05$); n = 5.

Micronutrients in habanero pepper plants

In accumulated micronutrient concentrations per plant, for both total Fe and Mn per plant there were nonsignificant differences among treatments (Figures 2A and 2B). However, the Steiner and Hoagland solutions increased Cu (0.17 and 0.13 mg g^{-1} respectively) and Zn (0.44 and 0.51 mg g^{-1} respectively) accumulation compared to the control solution (Cu: 0.065; Zn: 0.22 mg g^{-1}) (Figures 2C and 2D). In regard to Fe and Zn concentration in leaves, there were nonsignificant differences among treatments, whereas Cu was not detected in leaves by X-ray microfluorescence equipment (Figures 2A, 2C and 2D), possibly due to low or zero Cu concentrations in the leaves. In the roots, Steiner and Hoagland solutions increased Mn (3.32 and 2.61 mg g^{-1} respectively), Cu (0.156 and 0.1 mg g^{-1} respectively) and Zn (0.56 and 0.52 mg g^{-1} respectively) accumulation compared to the control solution (Mn: 1.41; Cu: 0.05; Zn: 0.11 mg g^{-1}) (Figures 2B, 2C and 2D). In the stems, Mn was higher with the control solution (0.83 mg g^{-1}) (Figure 2B), whereas Zn accumulated more with the Hoagland solution (0.11 mg g^{-1}) (Figure 2D). In the leaves, Mn was higher with the control solution (1.58 mg g^{-1}) compared to Steiner and Hoagland solutions (0.58 and 0.73 mg g^{-1} respectively). In the case of Mo, concentrations could not be quantified in any plant organ.

In contrast to macronutrients, all micronutrients accumulated more in the roots than the aerial part, due to their low mobility within the plant (Marschner, 2012). In case of Mn, its uptake in plants increases when the nutrient solution is acidified by the presence of H^+ protons (Aftab and Hakeem, 2020). In this sense, the pH of the control solution was acidified with NH_4^+ (pH between 4.9 and 5.5), and this probably favored a higher Mn uptake and its distribution in the

Figure 2. Iron (A), Mn (B), Cu (C) and Zn (D) contents in habanero pepper plants with three nutrient solutions.



Data are means ± SE; different letters (lowercase letter: in root, stem and leaf; uppercase letter: in complete plant) show significant differences among treatments according to Tukey's test ($\alpha = 0.05$); n = 5.

plant (Figure 2B). On the other hand, Cu was the only micronutrient which concentrations in habanero pepper leaves were not recorded. In this regard, Marschner (2012) showed that Cu is accumulated in the roots and has low translocation to the aerial part. This element can modify both root growth and morphology. In the case of Zn, its uptake can be limited when NH_4^+ is used as the main N source (Barker and Pilbeam, 2015), which could explain its low concentrations in habanero pepper plants with the control solution.

Chlorophyll contents

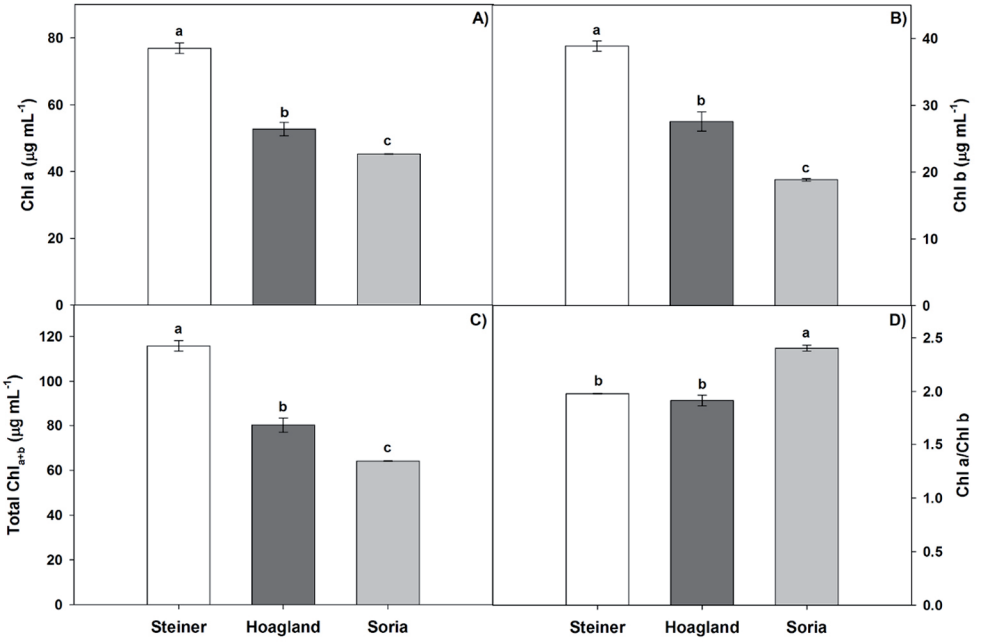
In habanero pepper plants with Steiner solution there were higher Chl *a* (76.93 μg mL^{-1}), Chl *b* (38.87 μg mL^{-1}) and total Chl_{a+b} (115.8 μg mL^{-1}) contents compared to plants with the Hoagland (Chl *a*: 52.71 μg mL^{-1}; Chl *b*: 27.58 μg mL^{-1}; total Chl_{a+b}: 80.29 μg mL^{-1}) and control (Chl *a*: 45.28 μg mL^{-1}; Chl *b*: 18.85 μg mL^{-1}; total Chl_{a+b}: 64.13 μg mL^{-1}) solutions (Figures 3A, 3B, and 3C). On the other hand, the Chl *a*/Chl *b* ratio in plants with the control solution (2.4) showed higher values than the other treatments (Steiner: 1.98, Hoagland: 1.91) (Figure 3D).

Chlorophyll's structure has a porphyrin ring containing Mg, so the presence of this element is essential for synthesis of chlorophylls (Marschner, 2012). In this context, is possible that Mg supply in the Steiner and Hoagland solutions increased chlorophyll concentration. Nevertheless, the Chl *a*/Chl *b* ratio was higher in plants with the control solution. Albanese et al. (2016) remark that the increase in values of the Chl *a*/Chl *b* ratio is due to an increase in reaction centers when plants grow under high light intensities. However, light intensity was the same in the three treatments, but it is possible that plants with the control solution invested the little Mg they had to generate more Chl *a* than Chl *b* and guarantee electron transport in the photosystems. On the other hand, Chen et al. (2010) mention that both Chl *a* and Chl *b* are involved in the harvesting of light energy, but only Chl *a* is indispensable in energy transduction at reaction centers of the photosystems.

Chlorophyll fluorescence

Maximum quantum efficiency (F_v/F_m), photochemical (qP) and non-photochemical (NPQ) quenching (Table 2) and maximum quantum yield of PSII (Φ_{PSII}) (Figure 4B) did not show significant differences among treatments. However, plants with the Steiner and Hoagland solutions had a greater electron transport rate (ETR) than plants with the control solution from 1200 μmol photons m^{-2} s^{-1} upwards (Figure 4A).

Figure 3. Chlorophyll a (Chl *a*) (A), chlorophyll b (Chl *b*) (B), total chlorophylls (Chl_{a+b}) (C), and Chl *a*/Chl *b* ratio (D) in habanero pepper plants with three nutrient solutions.



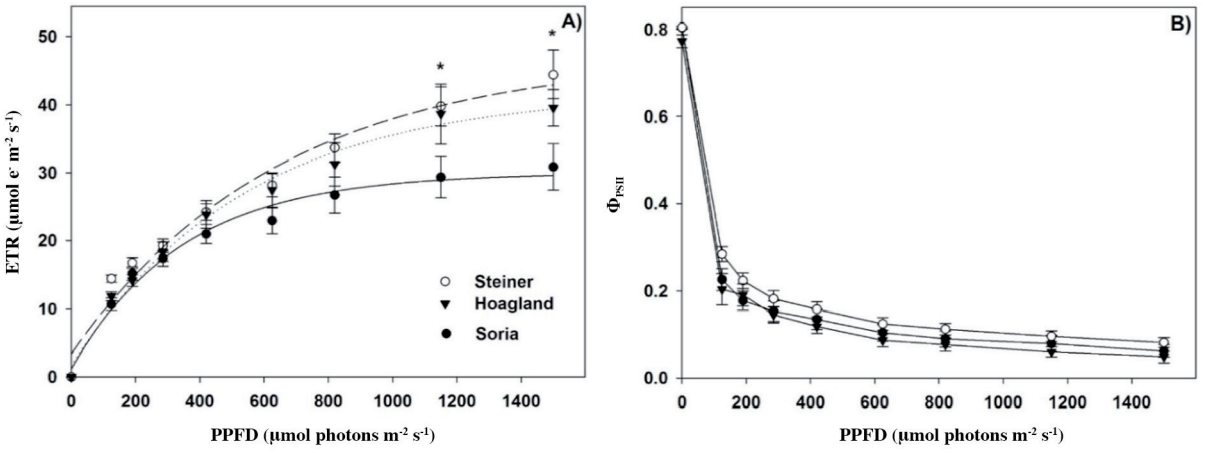
Data are means ± SE; different letters show significant differences among treatments according to Tukey's test (α = 0.05); n = 5.

Table 2. Maximum quantum efficiency (F_v/F_m), photochemical (qP) and non-photochemical (NPQ) quenching in habanero pepper plants with three nutrient solutions.

Treatments	F_v/F_m	qP	NPQ
Steiner	0.79 ± 0.03	0.25 ± 0.02	0.14 ± 0.02
Hoagland	0.80 ± 0.05	0.26 ± 0.04	0.23 ± 0.08
Soria (control)	0.77 ± 0.01	0.24 ± 0.02	0.24 ± 0.02

Data are means ± SE; n = 15.

Figure 4. Electron transport rate (ETR) (A) and maximum quantum yield of photosystem II (Φ_{PSII}) (B).



Data are means ± SE; *Significant differences (ANOVA, $p \leq 0.05$); n = 15; PPFD: photosynthetic photon flux density.

Despite not finding differences in chlorophyll fluorescence, a higher electron transport rate (ETR) was recorded in habanero pepper plants with the Steiner and Hoagland solutions. According to Fu et al. (2014), extreme light and temperature conditions can cause the closure of reaction centers affecting leaf photochemistry. However, light and temperature conditions were appropriate for plants in all treatments, so it is likely that no differences were observed. On the other hand, it is very likely that the differences observed in the ETR were due to increased Chl *a* concentration, as discussed previously.

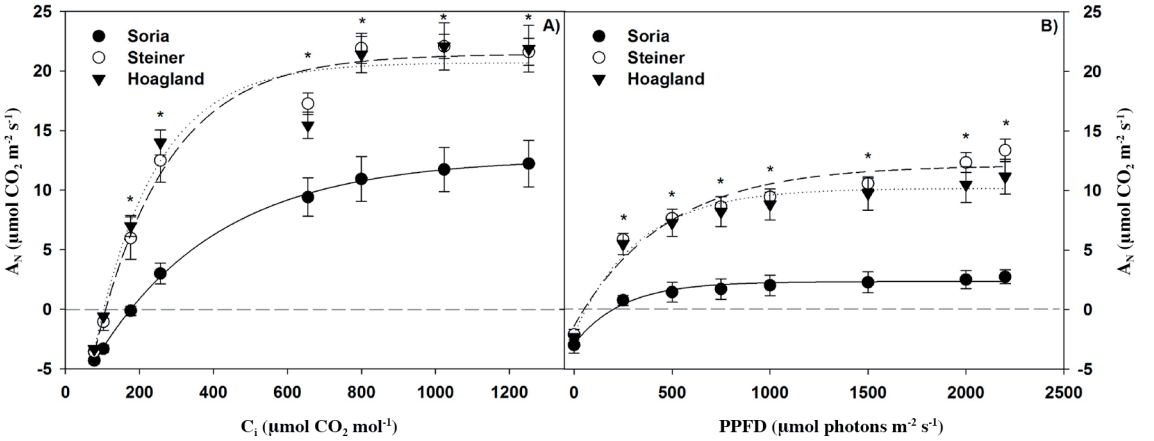
Gas exchange

In the A_N/C_i response curves, plants with the Steiner and Hoagland solutions reached their compensation point with lower intercellular CO_2 concentration (109 and 105 µmol CO_2 mol^{-1} respectively) and had higher saturation points (Steiner: 22.1 µmol CO_2 m^{-2} s^{-1} and Hoagland: 22 µmol CO_2 m^{-2} s^{-1}) than plants with the control solution (177 µmol CO_2 mol^{-1} and 12.2 µmol CO_2 m^{-2} s^{-1} respectively) (Figure 5A). In the A_N/PPFD response curves, plants with the Steiner and Hoagland solutions reached their compensation points with a lower amount of light photons (52 and 61 µmol photons m^{-2} s^{-1} respectively) and reached higher saturation points (Steiner: 13.4 and Hoagland: 11.2 µmol CO_2 m^{-2} s^{-1}) than plants with the control solution (209 µmol photons m^{-2} s^{-1} and 2.8 µmol CO_2 m^{-2} s^{-1} respectively) (Figure 5B).

Considering that P is directly involved in the Calvin-Benson-Bassham cycle and P concentrations were similar among treatments, this element is ruled out as the cause of the increase in photosynthesis in plants with the Steiner and Hoagland solutions. However, increased C assimilation is a consequence of the rise in electron transport rate (ETR) from PSII to PSI (Figure 4A). This increases the availability of ATP and NADPH in the Calvin-Benson-Bassham cycle and, at the same time, carboxylation of intercellular CO_2 (Sekhar et al., 2014).

In the Steiner and Hoagland treatments, maximum photosynthetic rate (A_{max}) (22.1 and 22 µmol CO_2 m^{-2} s^{-1} respectively), light-saturated CO_2 assimilation rate (A_{sat}) (13.4 and 11.2 µmol CO_2 m^{-2} s^{-1} respectively), maximum catalytic activities of Rubisco (V_{cmax}) (65 and 56 µmol CO_2 m^{-2} s^{-1} respectively), and maximum rate of electron transport demand for RuBP regeneration (J_{max}) (125 and 122 µmol e^- m^{-2} s^{-1} respectively) were significantly higher than plants with the control solution (A_{sat}: 2.8 µmol CO_2 m^{-2} s^{-1}, A_{max}: 12.2 µmol CO_2 m^{-2} s^{-1}, V_{cmax}: 43 µmol CO_2 m^{-2} s^{-1} and J_{max}: 110 µmol e^- m^{-2} s^{-1}) (Table 3). Also, plants with the control solution had greater stomatal limitation (50%) than those with the Steiner and Hoagland solutions (14% and 6% respectively).

Figure 5. Photosynthetic CO_2 response curves (A_N/C_i) (A) and photosynthetic light response curves (A_N/PPFD) (B) in habanero pepper plants with three nutrient solutions.



Data are means ± SE plotted with a defined fitting equation (R^2 = 0.97 and 0.98 respectively); *Significant differences (ANOVA, $p \leq 0.05$); n = 15. A_N: CO_2 assimilation rate; C_i: intercellular CO_2 concentration; PPFD: photosynthetic photon flux density.

Table 3. Maximum photosynthetic rate (A_{max}), light-saturated CO_2 assimilation rate (A_{sat}), maximum catalytic activities of Rubisco (V_{cmax}), maximum rate of electron transport demand to RuBP regeneration (J_{max}) and stomatal limitation (l) in habanero pepper plants with three nutrient solutions.

Treatments	A_{max}	A_{sat}	V_{cmax}	J_{max}	l
	———	μmol CO_2 m^{-2} s^{-1}	———	μmol e^- m^{-2} s^{-1}	%
Steiner	22.1 ± 1.0a	13.4 ± 0.9a	65 ± 4.2a	125 ± 4.7a	14 ± 1.3b
Hoagland	22.0 ± 1.9a	11.2 ± 1.4a	56 ± 4.4a	122 ± 5.1a	6 ± 0.8c
Soria (control)	12.2 ± 1.9b	2.8 ± 0.5b	43 ± 3.1b	110 ± 5.5b	50 ± 4.8a

Data are means ± SE; different letters in columns show significant differences according to Tukey's test (α = 0.05); n = 15.

It is evident that the stomatal limitation presented by plants with the control solution affected all photosynthetic parameters. Some authors (Marschner, 2012; Barker and Pilbeam, 2015) suggest that K^+ is closely involved in opening and closing stomata, and a decrease in K^+ concentration can affect proper stomatal function. In this study, the plants with Steiner solution had the highest K concentration in both the whole plant (551.23 mg g^{-1}) and leaves (368.8 mg g^{-1}) (Figure 1D) and were the ones that presented the lowest stomatal limitation, in contrast to plants with the control solution. In this sense, NH_4^+ concentrations in the control solution possibly limited K^+ uptake in habanero pepper plants, due to great competitiveness for high-affinity transporters required for K (Pacheco-Arjona et al., 2011; Barker and Pilbeam, 2015).

Total nitrate, amino acid and protein contents

Plants with the Hoagland solution recorded greater total nitrate accumulation in fresh leaf (2.07 mg g^{-1} FW) and root (1.6 mg g^{-1} FW) tissues compared to those with the Steiner solution (1.8 and 1.5 mg g^{-1} FW respectively) (Figures 6A and 6B). Plant leaves with the Steiner solution recorded greater total amino acid accumulation (170 mg g^{-1} FW) (Figure 6C), whereas in the roots no differences were found among treatments (Figure 6D). In total protein content, it was observed that plants with the control solution had higher values in leaves (0.921 mg g^{-1} FW) and roots (0.57 mg g^{-1} FW) compared to plants with the Steiner (leaves: 0.79, roots: 0.49 mg g^{-1} FW) and Hoagland (leaves: 0.57, roots: 0.44 mg g^{-1} FW) solutions (Figures 6E and 6F).

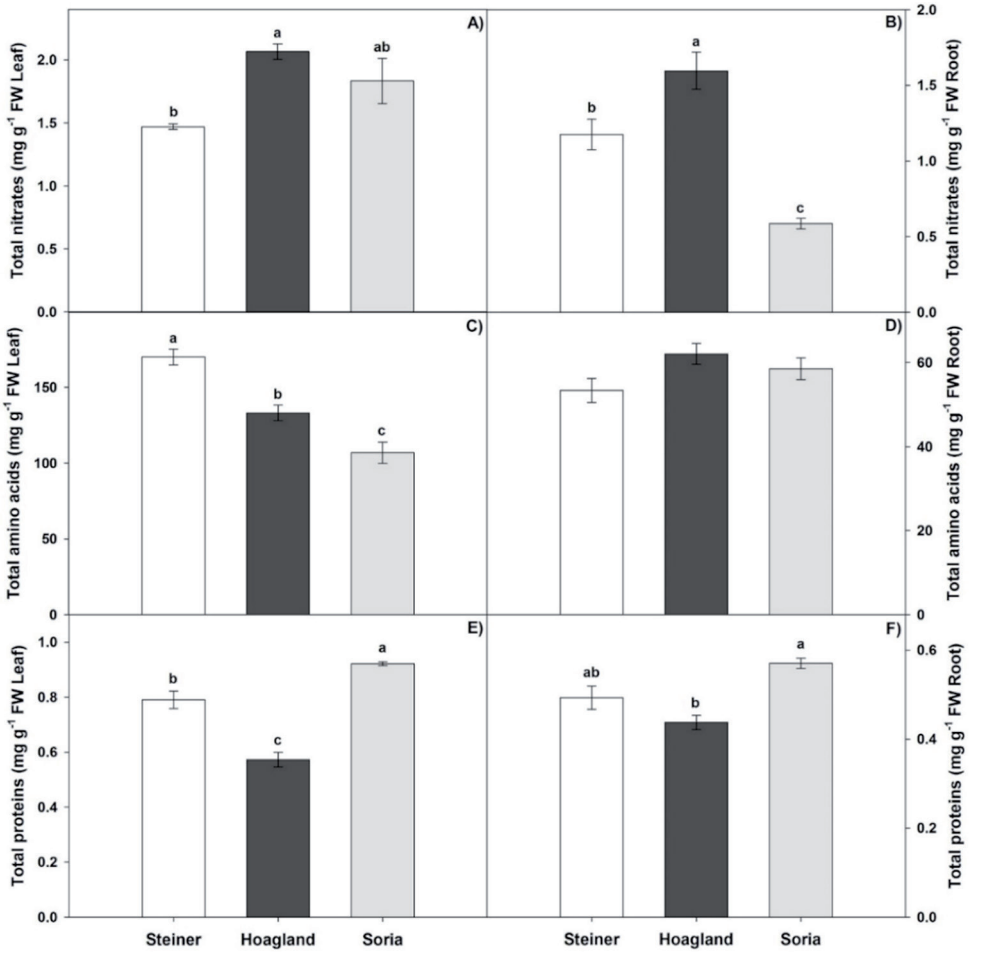
The use of N in habanero pepper plants probably depends on available N sources and the concentration in which they are applied. Nitrogen accumulation in the form of total nitrates is related to an increase in dry biomass, because it increases root growth and regulates foliar expansion by implementing NO_3^- as the main N source (Santiago-Antonio et al., 2014). In this sense, the control solution contributed N in the form of NO_3^- and NH_4^+ in concentrations of 10.5 and 7.9 meq L^{-1}, and this probably generated a preferential uptake of NH_4^+ over NO_3^- because it does not represent a higher energy cost for plants (Zhang et al., 2019). On the other hand, amino acids accumulate in greater quantity in the aerial part (Santiago-Antonio et al., 2014). In this sense, both NH_4^+ and NO_3^- can be incorporated into organic forms through the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle to produce amino acids or proteins. Considering this occurs when NH_4^+ concentrations are high (Barker and Pilbeam, 2015), it could be the case with the control solution.

Growth parameters in habanero pepper plants

Nonsignificant differences were observed between Steiner and Hoagland treatments. However, both plant height and leaf area in plants with the Steiner (17.28 cm and 351.26 cm^2 respectively) and Hoagland (15.32 cm and 314.53 cm^2 respectively) solutions were greater than plants with the control solution (10.9 cm and 155.66 cm^2 respectively). Plants with the Hoagland solution (70 leaves) had a greater number of leaves than those with the control solution (39 leaves) and there were no differences in stem diameter among treatments (Figure 7). The same trend was observed in dry mass accumulation as in the growth parameters, with no differences between the Steiner and Hoagland treatments. However, plants with the Steiner and Hoagland solutions increased dry mass accumulation in the leaves (1.12 and 1.13 g respectively), roots (0.52 and 0.49 g respectively) and in total (2.16 and 2.04 g respectively) compared to those with the control solution (0.69, 0.22 and 1.02 g respectively) (Figure 8).

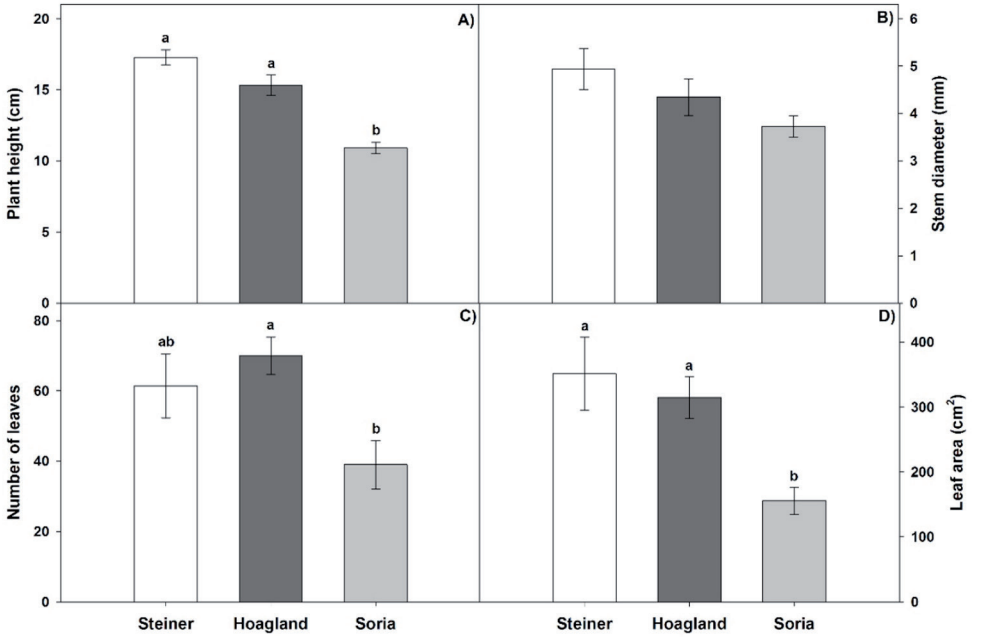
In this study, the Steiner and Hoagland solutions increased dry biomass, plant height and leaf area. These treatments had higher Ca and Mg concentrations that promoted both cellular division and chlorophyll synthesis respectively (Barker and Pilbeam, 2015), which helped to increase both leaf area and photosynthetic rate. Therefore, the growth of habanero pepper plants in Steiner and Hoagland solutions is a consequence of the accumulation of macro and micronutrients, and their physiological and biochemical activities.

Figure 6. Total nitrates in leaves (A) and roots (B), total amino acids in leaves (C) and roots (D), and total proteins in leaves (E) and roots (B) in habanero pepper plants with three nutrient solutions.



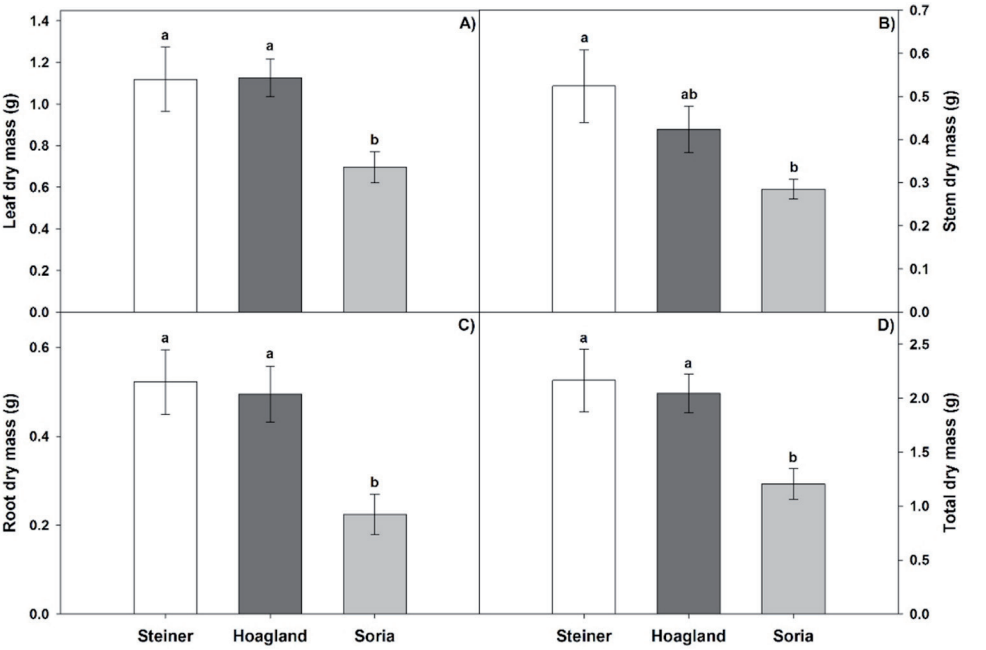
Data are means ± SE; different letters show significant differences according to Tukey's test (α = 0.05); n = 5.

Figure 7. Plant height (A), stem diameter (B), number of leaves (C) and leaf area (D) of habanero pepper plants with three nutrient solutions.



Data are means ± SE; different letters show significant differences according to Tukey's test (α = 0.05); n = 5.

Figure 8. Leaf (A), stem (B), root (C) and total (D) dry mass in habanero pepper plants with three nutrient solutions.



Data are means ± SE; different letters show significant differences according to Tukey's test (α = 0.05); n = 5.

CONCLUSIONS

Steiner and Hoagland solutions provide greater K, Ca, Mg, Cu and Zn concentrations to habanero pepper plants. Macronutrient accumulation formed a gradient from least to greatest from roots to leaves, whereas micronutrient accumulation was the opposite. Also, these treatments increase chlorophyll synthesis (*a*, *b* and total), electron transport rate, light-saturated CO_2 assimilation rate, maximum photosynthetic rate, maximum catalytic activities of Rubisco and maximum rate of electron transport demand for RuBP regeneration, saturation points in photosynthetic CO_2 response curves and photosynthetic light response curves, and growth parameters (plant height, number of leaves, leaf area, dry mass). Plants with Hoagland solution uptake nitrate and store it in the cells of leaves and roots. Nitrogen assimilation in plants with Steiner solution is used for amino acids, whereas in the control solution it is used for proteins.

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