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Effects of Substrate Salinity and Nutrient Levels on Physiological Response, Yield, and Fruit Quality of Habanero Pepper

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***Abstract*. Although habanero peppers (*Capsicum chinense*, Jacq.) are highly appreciated as a result of their organoleptic and pungency properties, the crop faces edaphic stresses throughout Mexico. A study was conducted to determine how the photosynthetic parameters, vegetative growth, yield, and fruit quality of the plant change in response to suboptimal conditions in the substrate. Habanero plants were grown in an inert substrate (perlite) and exposed to increased salinity levels (4 and 7 dS·m^{-1}) and reduced nitrogen and phosphorus conditions. Plants grown with a Hoagland-based solution were used as controls. High salinity conditions reduced the light-saturated photosynthetic rates (64% of the control) but did not compromise yield or fruit quality. This effect was possibly the result of the addition of Ca^{2+}, which reduced salinity-induced calcium deficiency. Although comparable low nitrogen levels in previous studies were shown to cause a severe reduction in plant viability, in our study, low nitrogen reduced the light-saturated photosynthetic rates (47% of the control) and shoot:root ratio (67% of the control) but did not significantly affect yield or fruit quality. Low nitrogen and 7-dS·m^{-1} treatments increased fructose and glucose content (increases of 27% and 21%, respectively). Low phosphorus significantly affected plant growth and yield and reduced fructose content (73% of the control). Plants were not sensitive to low nitrogen and high salinity, possibly as a result of the use of nitrate-based fertilizers and the addition of calcium, respectively. These results provide guidelines for habanero pepper production under suboptimal edaphic conditions.**

Habanero pepper plants belong to the *Capsicum* genus, which contains ≈27 domesticated species and 2000 cultivars (Eshbaugh, 1993). These cultivated species originate from three to five wild relatives (Andrews, 1995; DeWitt and Bosland, 1996) and their phytogenetic center of origin is probably Peru or Bolivia (Basu and De, 2003). Although habanero peppers were first introduced into Mexico from Cuba (Laborde and Pozo, 1984), currently the Yucatan peninsula is considered a genetic reservoir for this species. Habanero peppers are highly appreciated as a result of their flavor and high pungency; however, their widespread production is limited by a lack of highly productive cultivars that show good performance when facing suboptimal biotic and abiotic factors (Santana-Buzzy, 2010).

High salinity conditions in agricultural soils constitute one of the most serious challenges faced by horticultural crops in Mexico. It is estimated that by 2002, more than 1 million hectares were affected by salinity problems (SEMARNAT, 2012), causing a reduction in agricultural productivity between 30% and 50% (Umali-deininger, 1993). This problem may continue to worsen as a result of deficient irrigation practices, the wide use of well water and fertilizers, high evaporative conditions, and the recurrence of droughts. Plants of the *Capsicum* genus show wide genetic heterogeneity (Loaiza-Figueroa et al., 1989), which is evident by the presence of sensitive and tolerant genotypes in their response to salinity (Aktas et al., 2006; Niu et al., 2010b). In sensitive cultivars, salinity affects growth, yield, and fruit quality (Ayers and Westcot, 1985; Lycoskoufis et al., 2005; Navarro et al., 2002). Bell pepper (*C. annuum* L. 'California Wonder') is an example of a sensitive cultivar in which salinity primarily affects plant–water relations. At low salt levels (30 mM NaCl or ≈3.8 dS·m^{-1}), this response is related to osmotic stress, whereas at higher levels (60 mM NaCl or ≈7.5 dS·m^{-1}), the plant–water effects are related to ionic stress (Silva et al., 2008). The detrimental impact of salinity on chile ancho plants (*C. annuum* 'Caballero') varies for each tissue; fruits are more sensitive than leaves and stems (Azuma et al., 2010). On the other hand, *C. annuum* 'NMCA10652' has shown more tolerance to salinity treatments, having a 100% survival rate and showing no significant changes in plant growth and fruit yield up to a soil electrical conductivity (EC) of 4.1 dS·m^{-1} (Niu et al., 2010a).

Another important limiting factor for plant development that affects *Capsicum* plants is soil nutrient deficiency. In modern agriculture, nitrogen (N) is considered the most commonly deficient nutrient followed by phosphorus (P) (Halvin et al., 2005). In *Capsicum,* N deficiency affects plant growth, yield, and the accumulation of secondary metabolites (e.g., capsaicin) (Johnson and Decoteau, 1996; Medina-Lara et al., 2008). Similarly, P deficiency reduces the light-saturated photosynthetic rate, stomatal conductance, and leaf internal CO_2 concentration of chile ancho 'San Luis' and bell pepper 'Jupiter' (Davies et al., 1999). P concentration of 0.25 mM in a hydroponic system has been found to be insufficient for pepper production of *C. frutescens* (Aldana, 2005). A deficit of both macronutrients (N and P) severely reduces yield and fruit size (weight and length) of 'California Wonder' (Roy et al., 2011).

On the other hand, plant growth conditions also affect the accumulation of primary and secondary metabolites within the pepper fruit. Changes in metabolite levels have an impact on the plants' organoleptic and nutritious properties. The metabolites involved in protection or detoxification change at high salinity levels (Mansour, 2000) and conditions of macronutrient deficiency (Dixon and Paiva, 1995). These metabolites also change as a result of the plants' adaptation to high salinity or macronutrient deficiency, affecting fruit quality parameters (Wang and Frei, 2011).

The objective of this study was to characterize the effects of long-term exposure to common suboptimal substrate conditions on a full range of parameters in habanero pepper plants. These parameters include yield and photosynthetic response and the impact on fruit quality by measuring capsaicinoids, total phenolics, ascorbic acid, carotenoids, and sugars in the pericarp of ripe fruit.

Materials and Methods

Plant materials. The study was conducted under greenhouse conditions at ITESM facilities in Monterrey, N.L., Mexico (lat. 25°40′ N, long. 100°18′ W, altitude 430 m). Habanero pepper 'Orange' seeds (Seminis, St. Louis, MO) were established in 128-cavity trays

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filled with 2 sphagnum peatmoss:1 perlite (by volume). At 45 d after planting (Oct. 2012), the seedlings were transplanted to 7.6-L containers filled with perlite; plants were irrigated daily with well water (EC of 0.9 $dS \cdot m^{-1}$) and twice a week with a Hoagland-based solution (see below). The study was ended 9 months later (July 2013).

Experimental treatments were initiated at the onset of flowering and consisted of the application of different nutrient solutions (Table 1) twice a week on seven individual plants per treatment (total number of plants was 35). Control treatments consisted of Hoagland-based nutrient solution (hereinafter referred to as Hoagland) at pH 6.0 with an EC of 2.6 $dS \cdot m^{-1}$. This solution was modified from the basic Hoagland solution (Hoagland and Arnon, 1950) by adjusting to 15 mM N concentration, recommended for Jalapeño pepper (Johnson and Decoteau, 1996) and to 2.5 mM P concentration recommended for Tabasco pepper (Aldana, 2005). Increased salinity levels (EC of 4 and 7 $dS \cdot m^{-1}$) were achieved by the addition of sodium chloride to the Hoagland solution. Sodium chloride was gradually increased with each application of Hoagland solution at a rate of 2 $dS \cdot m^{-1}$ per week to avoid possible salt shock. Additionally, to prevent sodium-induced calcium deficiency, each salinity treatment was supplemented with 10 mM Ca^{2+} as $CaCl_2$ (Cabañero et al., 2004). Low N and low P solutions were prepared based on the Hoagland solution by reducing the concentration from 15 to 5 mM for N and from 2.5 to 0.25 mM for P. $CaCl_2$ was added to the low N treatment for calcium balance (Table 1).

Photosynthetic response. Light response curves were measured at the end of the study on healthy mature leaves from four different plants under each treatment condition using a portable photosynthesis system (LI-6400XT; LI-COR, Lincoln, NE). Leaves were exposed to irradiance levels from 0 to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and allowed to equilibrate. In the leaf chamber, the flow rate of CO_2 was fixed at 400 $\mu mol \cdot s^{-1}$, air temperature was set at 25 °C, and relative humidity at 50% to 70%. The photosynthetic parameters evaluated included light compensation point, photosynthetic efficiency, and light-saturated photosynthetic rate. Photosynthetic efficiency was estimated using the slope of the response curve at the five lowest photosynthetic photon flux densities (from 0 to 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Bassman and Zwier, 1991; Björkman, 1981).

Biomass analysis. General plant growth and partition were assessed using three representative plants from each treatment condition to complete a biomass analysis. Yield data included the cumulative number of fruits and their corresponding weights for each plant. Foliar area was calculated by scanning all leaves from each plant using a portable area meter (LI-3000C; LI-COR). Fresh and dry weight biomasses were calculated separately for shoots and roots.

Metabolite extraction. Fruit samples were obtained by tagging fruits at the breaker ripening stage and collecting them 3 weeks later. On harvesting, calix and peduncle were removed from the fruits, and the pericarp tissue was separated from the placenta. Pericarp was frozen in liquid N and preserved at –80 °C until metabolite analysis.

Frozen pericarps from six fruits per plant replicate were pooled and grinded in a frozen mortar with liquid N_2; for the low P case, one fruit per plant was analyzed as a result of low fruit production. The grinded material was divided into aliquots of ≈0.2 g for each metabolite extraction. In general, grinded samples were sonicated for 5 min in an appropriate solvent depending on the extraction protocol (details below). Then, the liquid phase was separated by centrifugation (14,000 *g* at 4 °C) for 10 min; the supernatant was recovered and kept on ice while the extraction was repeated at least another two times on the pellet. Pooled extracts were filtered using syringe filters (0.2 μm) and collected in amber glass vials for measurement.

Ascorbic acid. Ascorbic acid analysis was performed using the technique described by Gökmen et al. (2000) with modifications. Extractions were made in deionized water. Polyphenols were eliminated from the sample using a solid-phase extraction (SPE) cartridge (Oasis MAX, Milford, MA). Extracts were passed through an equilibrated SPE (6 mL methanol with 0.1% HCl followed by 6 mL water with 0.1% HCl) from which the flow-through was recovered. Cleaned extracts were filtered and incubated with 1 $mg \cdot mL^{-1}$ dithiothreitol at room temperature for 75 min under N_2 atmosphere. Sample separation and detection were conducted on an high-performance liquid chromatography (HPLC) photo diode array system (HPLC-PDA 2996, Milford, MA) using an Atlantis dC18 column (Catalog 186001344; Milford, MA) with an isocratic elution using a phosphate buffer (200 mM; pH 2.4) as the mobile phase. Ascorbic acid quantification was estimated at 244 nm using calibration curves made with an ascorbic acid standard (Sigma-Aldrich, St. Louis, MO).

Sugars. Sugars were extracted with deionized water and the extracts were filtered and injected into an HPLC-evaporative light-scattering detection system (ELSD 1200 series, Santa Clara, CA). Separation was achieved at 35 °C using an Xbridge column (Catalog 186004870, Milford, MA) with a gradient of acetonitrile:water (80:20, v/v) with 0.2% triethylamine (Phase A) to acetonitrile: water (30:70, v/v) containing 0.2% triethylamine (Phase B) with a flow rate of 1 $mL \cdot min^{-1}$. The gradient consisted of 10% Phase B at Time 0, increasing until 70% in 16 min. For the ELSD, the drift tube temperature was set at 50 °C. Fructose, glucose, and sucrose were identified and quantified by comparison with an external calibration mixture made with commercial standards (Merck, Darmstadt, Germany).

Carotenoids. Carotenoids were measured by extracting samples with 10 mL of acetone followed by sonication and filtration through filter paper (Whatman number 1, Little Chalfont, U.K.) under dim light. The permeated liquid was flushed with N_2 and kept on ice while samples were re-extracted until the solid was colorless. The concentration of the pooled extracts was measured at 450 nm in a spectrophotometer (DU 800, Pasadena, CA). Total carotenoid content was expressed as β-carotene equivalents calculated using a β-carotene extinction coefficient in acetone ($\varepsilon = 140663\ L \cdot cm^{-1} \cdot mol^{-1}$).

Phenolics. Total phenolic contents were determined using the Folin-Ciocalteu (FC) assay (Folin and Ciocalteu, 1927). Aliquots were extracted in accordance with Marinova et al. (2005) using methanol:water (80:20 v/v). FC reagent (Sigma-Aldrich) was added to the extracts and the total phenolic concentration was measured at 765 nm with a multimode microplate reader Synergy HT (BioTek, VT) and expressed as gallic acid equivalents according to a calibration curve made with gallic acid (Sigma-Aldrich).

Capsaicinoids. Capsaicinoids were extracted using the procedure described by Wahyuni et al. (2011). Briefly, samples were subjected to methanol extraction and the extracts were separated using liquid chromatography (HPLC-PDA 2996) using a Luna C18(2) column (Catalog 00F-4251-B0, Torrance, CA) maintained at 40 °C with a 28-min gradient of water:formic acid 0.1% (Phase A) and acetonitrile:formic acid 0.1% (Phase B) starting with 50% Phase B that increased to 63% in 15 min. Then, the column was washed for 3 min with 90% B and equilibrated for 10 min before the next injection with a flow rate of 0.19 $mL \cdot min^{-1}$. Detection was achieved at 280 nm by a PDA detector. Capsaicin and dihydrocapsaicin were identified and quantified

Table 1. Hoagland-based nutrient solution components in different treatments.[z]

Component (mM)	Ctrl	4 $dS \cdot m^{-1}$	7 $dS \cdot m^{-1}$	Low nitrogen	Low phosphorus
KNO_3	3.5	3.5	3.5	1	5
KH_2PO_4	2.5	2.5	2.5	2.5	0.25
$MgSO_4$	2	2	2	2	2
Iron	0.1	0.1	0.1	0.1	0.1
$Ca(NO_3)_2$	5	5	5	2	5
$CaCl_2$	0	5	5	3	0
KCl	2.5	2.5	2.5	2.5	2.5
$NaNO_3$	1.5	1.5	1.5	0	0
NaCl	0	5	15	0	0
Electrical conductivity	2.6	4	7	2.6	2.6

[z]Seven individual plants were used for each treatment. Microelements (μM) H_3BO_3, 46.0; $MnCl_2 \cdot 4\ H_2O$, 9.0; $ZnSO_4 \cdot 7\ H_2O$, 0.76; $CuSO_4 \cdot 5\ H_2O$, 0.32; $H_2MoO_4 \cdot H_2O$, 0.11.

using an external calibration curve obtained using commercial standards (Sigma, St. Louis, MO).

Statistical analysis. Treatments were conducted for seven independent plant replicates. The results show the calculated mean and SD values. Data were analyzed using analysis of variance (SPSS Version 15, Chicago, IL). Tukey's honestly significant difference post hoc tests and homogeneous subset analyses were conducted to determine which treatments significantly differed from each other. Variations were considered significant at the $P \leq 0.05$ significance level. Light response curves were evaluated using repeated measures analysis.

Results

Photosynthesis. The impact of nutrient levels and salinity conditions on photosynthetic efficiency was measured in four independently treated plants and compared with controls grown with Hoagland solution irrigation. Light response curves show how each treatment affected the capacity of the plant to fixate CO_2 (Fig. 1). Control plants had a photosynthetic efficiency comparable to values obtained in previous studies on *C. annuum* 'Zhongjiao' and 'Niujiao' (Fu et al., 2010). The light-saturated photosynthetic rate was reduced significantly at low N and 7 dS·m^{-1} salinity levels to 47% and 64%, respectively, of the observed control rate (Table 2; Fig. 1). Light compensation point and photosynthetic efficiency were unaffected by any of the treatments.

Yield and biomass. Three plants per treatment and control were subjected to complete biomass analysis. Foliar area and leaf biomass were reduced significantly in the low P treatment. On the other hand, the shoot:root ratio showed a significant reduction in the low N and low P treatments (Table 3). Fruit yield, measured as the number of fruits and biomass per plant, was only significantly reduced when plants were exposed to low P. Individual plants that were subjected to this treatment produced just one to six fruits per plant in comparison with the average of 34 produced by the control plants (Table 3). Interestingly, these few fruits reached a similar weight as the controls and matured normally. The other treatments did not produce significant changes in the other fruit parameters measured. Nonetheless, although not significantly different, plants exposed to low N conditions presented a slight increase in yield.

Metabolites in fruit. Metabolites related to fruit quality (pungency, flavor, and antioxidants) were quantified in pericarp tissue: capsaicinoids (capsaicin and dihydrocapsaicin), sugars (fructose, glucose, and sucrose), and antioxidants (ascorbic acids, carotenoids, and phenolics). Capsaicin and dihydrocapsaicin levels were measured as indicators of pungency (Fig. 2). Capsaicinoid accumulation varies greatly among habanero cultivars; the levels found in 'Orange' are comparable to the low content end of those found in the pericarp of other habanero cultivars (Canto-Flick et al., 2008). Capsaicin was the most abundant capsaicinoid in all treatments, averaging 70% of total capsaicin levels; dihydrocapsaicin was the second-most abundant capsaicinoid detected in all pericarp samples. Although not significantly different from the controls, capsaicin and dihydrocapsaicin levels were highest for the high salinity treatments (179.9 mg/100 g at 7 dS·m^{-1}) and reduced at the low P concentration (66.4 mg/100 g) (Fig. 2).

Sugars contribute considerably to the flavor and nutritional value of *Capsicum*

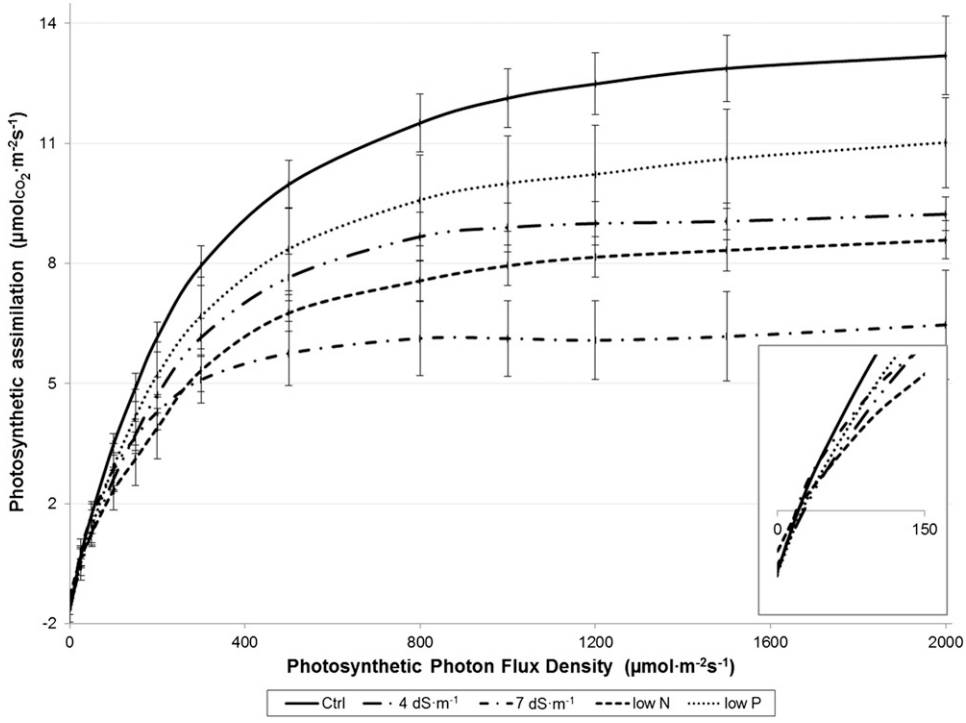

Fig. 1. Light response curve in habanero 'Orange' exposed to suboptimal nutrient levels and increased salinity conditions. Each point represents the average of at least four biological replicates; vertical lines indicate ± SE.

Table 2. Comparison of photosynthetic characteristics of mature leaves of habanero 'Orange' grown under suboptimal substrate conditions.[z]

Parameters	Ctrl	4 dS·m^{-1}	7 dS·m^{-1}	Low nitrogen	Low phosphorus
LCP (µmol·m^{-2}·s^{-1})	22.7 (6.9)	30.6 (8.7)	23.6 (11.9)	30.5 (13.1)	29.9 (11.9)
*P*max (µmol CO_2 m^{-2}·s^{-1})	12.7 (1.0) c[y]	8.7 (0.4) a,b,c	6.0 (1.4) a*	8.1 (0.5) a,b*	10.5 (1.1) b,c
PE	0.036 (0.002)	0.028 (0.002)	0.028 (0.003)	0.022 (0.004)	0.032 (0.005)

[z]LCP = light compensation point; *P*max = light-saturated photosynthetic rate; PE = photosynthetic efficiency (± SE).
[y]Different letter indicates significant difference from homogeneous subset Tukey's test when the main effect was significant.
*Statistically significant difference, $P \leq 0.05$ from analysis of variance compared with control Tukey's post hoc test.

Table 3. Influence of treatments on yield of habanero 'Orange' pepper plants.[z]

Parameters	Ctrl	4 dS·m^{-1}	7 dS·m^{-1}	Low nitrogen	Low phosphorus
Leaf area (cm^2)	4244.0 (746.9) a[y]	3394.0 (636.6) a, b	2893.2 (582.1) a, b	3015.0 (931) a, b	1920.0 (708.4) b*
Dry shoot (g/plant)	97.2 (24.7) a	80.3 (3.4) a	65.6 (5.3) a, b	63.1 (18.1) a, b	36.2 (10.3) b*
Dry root (g/plant)	24.8 (3.1)	20.2 (1.6)	20.9 (5.0)	24.6 (9.2)	15.4 (3.4)
Shoot:root ratio	3.9 (0.7) a	4.0 (0.2) a	3.2 (0.6) a, b	2.6 (0.3) a, b*	2.3 (0.8) b*
Fruit number (g/plant)	34 (7) a, b	24 (10) a	36 (18) a, b	45 (16) b	2 (2) c *
Fruit biomass (g/plant)	150.9 (28.7) a, b	97.8 (18.3) a	103.6 (36.5) a	189.7 (53.8) b	8.3 (8.7) c*
Average fruit weight (g)	4.4 (0.4)	4.4 (1.0)	3.3 (1.1)	4.3 (0.4)	3.7 (0.6)
Fruit moisture (%)	87.2 (1.3)	86.6 (0.5)	85.8 (1.3)	86.1 (0.5)	86.7 (0.4)

[z]Values represent the average of at least three biological replicates (± SD).
[y]Different letter indicates significant difference from homogeneous subset Tukey's test when the main effect was significant.
*Statistically significant difference, $P \leq 0.05$ from analysis of variance compared with control Tukey's post hoc test.

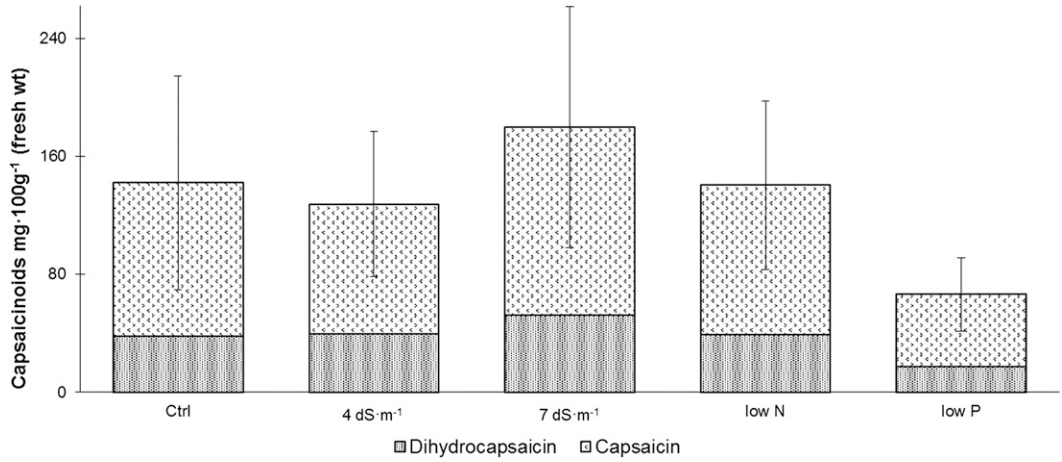

Fig. 2. Levels of capsaicin and dihydrocapsaicin in pericarp of ripe fruits of habanero 'Orange' grown under suboptimal nutrient levels and increased salinity conditions. Each bar represents the average of at least five biological replicates; vertical lines indicate ± SD of total capsaicinoids.

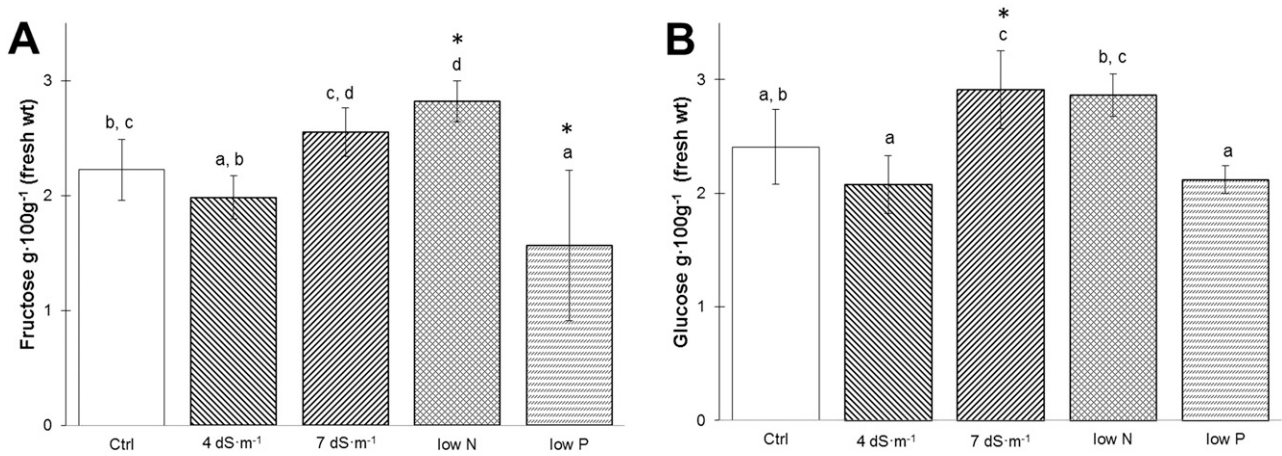

Fig. 3. Levels of fructose (**A**) and glucose (**B**) in pericarp of ripe fruits of habanero 'Orange' grown under suboptimal nutrient levels and increased salinity conditions. Each bar represents the average of at least five biological replicates; vertical lines indicate ± SD. *Statistically significant difference, $P \leq 0.05$ from analysis of variance compared with control Tukey's post hoc test. Different letter indicates significant difference from homogeneous subset Tukey's test when the main effect was significant.

fruits (Luning et al., 1994; Navarro et al., 2006). In all treatments studied, fruit accumulated glucose and fructose; sucrose accumulation was below our detection limits in all samples. Significant differences were found in some treatments regarding both sugars in mature pepper pericarp. Low N and the salinity treatment 7 dS·m^{-1} significantly increased fructose and glucose levels by 27% and 21%, respectively (Fig. 3). Conversely, low P significantly reduced fructose accumulation in the fruit (73% of the control).

Capsicum fruits are considered a good source of provitamin A (carotenoids) and vitamin C (ascorbate); these groups of metabolites have well-recognized antioxidant properties. In addition, the fruits also accumulate phenolic compounds that can have an antioxidant effect when consumed (Antonious et al., 2006). Surprisingly, the conditions tested in this study did not significantly affect the accumulation of these metabolites in mature habanero peppers (Fig. 4). Phenolic compounds accumulated to the same extent throughout all samples analyzed (Fig. 4C). However, we did observe an increasing trend in the accumulation of carotenoids and ascorbic acid in the low P condition, the treatment that caused the highest impact, lowering fruit production to just a couple of fruits per plant (Fig. 4A–B).

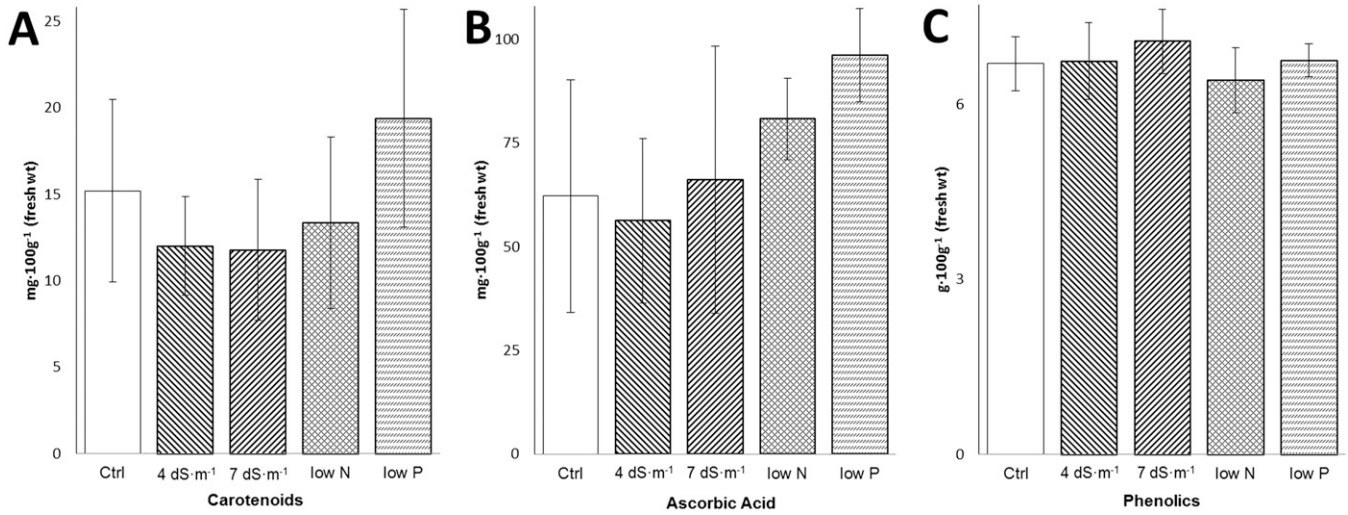

Fig. 4. Levels of carotenoids (**A**), ascorbic acid (**B**), and phenolics (**C**) in pericarp of ripe fruits of habanero 'Orange' grown under suboptimal nutrient levels and increased salinity conditions. Each bar represents the average of at least five biological replicates; vertical lines indicate ± SD.

Discussion

Horticultural crops present a wide variety of responses to long-term exposure to suboptimal substrate conditions. In the case of *Capsicum*, different cultivars have shown a wide variety of responses to nutritional and abiotic stresses. Thus, it is important to characterize cultivars based on the most common edaphic problems faced by horticultural crops. Habanero pepper is considered an economically important cultivar in southern Mexico, where soil conditions are not optimal (e.g., high salinity, low N and P). In this article, we present an integrated analysis that investigated these effects on several aspects of plants' physiological parameters, including photosynthetic response, yield, biomass, and fruit quality. All experiments were conducted using an inert substrate (perlite). Although the use of substrates may not necessarily reflect an actual plant–soil interaction, they constitute a suitable means to evaluate plant responses to controlled conditions in the substrate (Poorter et al., 2012). This knowledge can be promptly applied for habanero pepper greenhouse production and/or serve as the basis for field determinations.

Increased salinity. Suboptimal substrate conditions had an inhibitory effect on the light-saturated photosynthetic rate. High salinity levels have been documented as reducing photosynthetic response (see Sudhir and Murthy, 2004), and in our study, only the high salinity treatment (7 dS·m^{-1}) significantly reduced the light-saturated photosynthetic rates (47.2% of the control) (Table 3). These results are in agreement with those of Azuma et al. (2010), who grew chile ancho peppers with 50 and 100 mM of salt (≈7 and 11 dS·m^{-1}, respectively) and observed reductions in the light-saturated photosynthetic rate of *Capsicum* plants of 18% and 32%, respectively. Reduction of the photosynthetic response caused by salinity treatments was more evident in habanero pepper plants than chile ancho; however, light-saturated photosynthetic rate measurements for these plants were performed earlier in their development, which could have impacted the effects of the treatments. In our case, photosynthetic measurements were carried out at the end of the study; therefore, the reduction in photosynthetic response could be related to an intensified effect of sodium and/or chlorine in plant tissue over time.

Although the increased salinity treatment (7 dS·m^{-1}) reduced the photosynthetic response, it did not significantly affect any of the measured fruit yield parameters (i.e., total and average fruit weight, number of fruits, or moisture content). Our results this time contrast with the changes observed in chile ancho plants, in which the fruit was the only organ that changed biomass in response to high salinity treatments (a reduction of 66% of the control).

On the other hand, Niu et al. (2010a) reported that comparable salinity levels (2.5 and 4.1 dS·m^{-1}) severely damaged habanero plants grown in the field, whereas all plants in this group of greenhouse-grown habanero peppers survived throughout this study. The contrasting responses could have been caused by differences in experimental conditions. Plants grown in the field were probably exposed to higher salinity levels than the applied treatments as a result of intrinsic soil salinity, interaction with soil particles, and variations in soil–water availability. In addition, the timing of the treatments was different; although we started at the onset of flowering, in their work, salinity treatments were applied to younger plants. Furthermore, we added Ca^{2+} to the nutrient solution (Table 1), which possibly reduced salinity-induced calcium deficiency. All this could contribute to the resilience to higher salinity conditions observed in this work. Our results also show that even the high salinity levels used in our study were not sufficiently elevated to affect fruit yield when supplemented with calcium. Therefore, we suggest that, for habanero pepper plants, the addition of calcium could ameliorate the effects of high salinity on fruit yield, as suggested by Cabañero et al. (2004) for 'California'.

High salinity treatments (7 dS·m^{-1}) also caused a significant increase in fruit glucose levels. Accordingly, in 'Caballero' peppers, a 20% increase in soluble sugars was observed in fruit grown at high salinity levels (Azuma et al., 2010). Salinity in soil can be responsible for changes in fruit metabolism and physiology (Saito et al., 2008). Also, in another study on Solanaceae, tomato plants that were exposed to moderate salinity levels presented an increase in starch biosynthesis in developing fruits, which is believed to increase sink strength. During maturation, subsequent starch hydrolysis could increase soluble sugar levels (Petreikov et al., 2009). In the 'Momotaro Fight' tomato cultivar, increased sugar accumulation from NaCl treatment (5 dS·m^{-1}) was sufficient to be perceived by consumers, which could have a positive impact on its organoleptic properties. It appears that tomato cultivars can be more susceptible to salinity treatments (including reduction in yield, fruit number, and weight) than habanero 'Orange'. Other fruits that have presented increased sugar levels after salinity treatment include: melons, grapes, and oranges (Botía et al., 2005; Grieve et al., 2007; Li et al., 2013).

In regard to pungency, increased salinity levels did not have a significant effect on capsaicinoid accumulation in pericarp (capsaicin and dihydrocapsaicin). Although pungency is related to environmental conditions and increases in response to stressful conditions (Harvell and Bosland, 1997), for habanero 'Orange', high salinity conditions (7 dS·m^{-1}) marginally increased pericarp capsaicin content (not statistically significant at $P \leq 0.05$). Similar trends in capsaicin concentration were found in 'Jalapeño' peppers under salt treatments (Arrowsmith et al., 2012).

High salinity conditions (7 dS·m^{-1}) reduced light-saturated photosynthetic rates but did not compromise fruit production or

quality (determined by fruit number and weight and content of key metabolites, i.e., capsaicinoids, phenolics, ascorbic acid, and carotenoid levels). The addition of calcium under high salinity conditions could be used to mitigate salinity effects on *Capsicum* plants.

Low nitrogen. Although low N (5 mM·L^{-1}) treatments caused a significant reduction in the light-saturated photosynthetic rate, and plant leaves were chlorotic (a characteristic symptom of low N), this treatment did not cause a reduction in yield when compared with controls (15 mM·L^{-1}). Low N conditions significantly reduced the shoot:root ratio in habanero plants (67% of the control) but did not significantly affect dry shoot, dry root, or yield parameters (Table 3). Our results contrast with those reported by Medina-Lara et al. (2008), who grew plants of the same habanero cultivar in iron-rich (luvisol) soils; when exposed to comparable N levels (0, 1, 7.5 mM urea), the plants showed severe inhibition of fruit production. In their study, the effect of low N could have been aggravated by its retention by the metal ions in the iron-rich soils (Baker, 1987) or possible sequestration by the organic fraction of their soil (Soon, 1998). Furthermore, their use of a non-readily available N source (urea) in comparison with our use of KNO_3 and $Ca(NO_3)_2$ in the Hoagland solution could have generated even lower accessible amounts of N.

Low N treatments caused a significant increase in soluble sugar contents, particularly fructose ($P \leq 0.05$) (Fig. 3A). These results may be related to the tendency of plants to accumulate carbohydrates when exposed to low N conditions (Davies and Winsor, 1967). In tomato plants grown in the field, the sugar:total solids ratio and glucose and fructose concentrations decreased slightly with the increase of N fertilization (Parisi et al., 2006). In a comparable study, also using greenhouse-grown tomato plants, a decrease in nitrate fertilization (from 12 to 4 mM) caused a reduction in vegetative growth but did not affect commercial yield and increased the soluble sugar levels in the fruit (Benard et al., 2009).

Capsaicinoid levels were not significantly affected by N availability (Fig. 2). A previous study on the same habanero cultivar supports this observation (Medina-Lara et al., 2008). Low N treatments slightly increased ascorbic acid content (Fig. 4B), similar to the values observed in tomato fruit (Benard et al., 2009), but our results were not statistically significant ($P \leq 0.05$). These results agree with a recent study that reported that bioactive compounds with antioxidant activity and the total polyphenols of habanero were not significantly affected by changes in N fertilization (32, 80, 160, 320 kg N/ha) (Núñez-Ramírez et al., 2011).

Reduction of N levels in the Hoagland solution (to 5 mM·L^{-1} of N) using nitrate-based fertilizers caused relatively minor effects on overall plant productivity (yield, pungency, and other fruit metabolites). Comparable rates of N fertilization using urea as the main N source in the same cultivar caused severe damage to the plants. Therefore, it is recommended that supplemental N fertilization for habanero plants should use nitrate-based fertilizers, because they are more efficient for supplemental N fertilization for habanero pepper production.

Low phosphorus. From all the evaluated treatments, low P conditions caused the least amount of reduction in photosynthetic parameters. Low P had no significant effect on the light-saturated photosynthetic rate, photosynthetic efficiency, and light compensation point (Fig. 1; Table 2). The observed effect of the low P treatment diverged with that observed by Davies et al. (1999), who found that low P treatments significantly affected the photosynthetic response.

Nevertheless, at the whole-plant level, low P levels caused the poorest aerial development and reduced fruit yield (Table 3). In this study, dry-root biomass was reduced to 62% of the control, whereas dry-shoot biomass was significantly reduced to 37% of the control. The effect of low P on aerial development could be attributable in part to the reduction in photosynthesis (Davies et al., 1999), as is evidenced in this work (Fig. 1), but mainly because plants in P-deficient conditions typically tend to increase root mass (reduced shoot:root ratio) as an adaptive strategy to access P (given its relative soil immobility). Although similar responses have been documented for 'Long Slim Cayenne' peppers, this study constitutes the first evidence of this type of response for habanero pepper.

Low P conditions also caused a very significant reduction in the number of fruits and the fruit biomass. However, once the fruits were set, they had the same weight and water content as the controls (Table 3). Soluble sugar contents (particularly fructose) were reduced by low P conditions, most likely caused by a reduction of available photo assimilates (Fig. 3A). Other metabolites (carotenoids, ascorbic acid, and phenolics) were not significantly affected. Although not statistically significant, ascorbic acid content was higher than the controls (Fig. 4). Low P seems to affect capsaicin accumulation; also, although not significant, the low-P-treated plants produced the least capsaicin across all treatments (Fig. 2), which may have an impact on pungency.

Previous work with 'Long Slim Cayenne' pepper plants treated with higher levels of P showed consistent effects to those obtained in this work (increased plant height, leaf area, shoot, and root dry matter, number of fruit per plant, and yield compared with control plants) (Emongor and Mabe, 2012). The remarkable response to the P conditions shows that P is a critical nutrient for habanero pepper plants and proper fertilization has a significant impact on plant growth and yield.

Conclusion

This work describes the responses of habanero pepper to a number of common abiotic conditions in the soil. Low P conditions were more devastating to habanero pepper plant development and yield than either low N or moderate salinity. Reduced N and increased salinity had a relatively minor effect on overall plant productivity. The effects of salinity conditions on habanero pepper production were moderated with the inclusion of calcium chloride to reduce the effect of salinity-induced calcium deficiency. Results from this work can aid in the formulation of guidelines for fertilization and the management of salinity conditions in horticultural production areas.

Literature Cited

Aktas, H., K. Abak, and I. Cakmak. 2006. Genotypic variation in the response of pepper to salinity. Sci. Hort. 110:260–266.

Aldana, M.E. 2005. Effect of phosphorus and potassium fertility on fruit quality and growth of tabasco pepper (*Capsicum frutescens*) in hydroponic culture. MS thesis, Louisiana State University, LA.

Andrews, J. 1995. Peppers, the domesticated capsicums. University of Texas Press, Austin, TX.

Antonious, G.F., T.S. Kochhar, R.L. Jarret, and J.C. Snyder. 2006. Antioxidants in hot pepper: Variation among accessions. J. Environ. Sci. Health B 41:1237–1243.

Arrowsmith, S., T.P. Egan, J.F. Meekins, D. Powers, and M. Metcalfe. 2012. Effects of salt stress on capsaicin content, growth, and fluorescence in a Jalapeño cultivar of *Capsicum annuum* (Solanaceae). BIOS 83:1–7.

Ayers, R. and D.W. Westcot. 1985. Water quality for agriculture. FAO Irrigation and Drainage Paper. 20 June 2013. <http://www.fao.org/docrep/003/t0234e/t0234e00.htm>.

Azuma, R., N. Ito, N. Nakayama, R. Suwa, N.T. Nguyen, J.A. Larrinaga-Mayoral, M. Esaka, H. Fujiyama, and H. Saneoka. 2010. Fruits are more sensitive to salinity than leaves and stems in pepper plants (*Capsicum annuum* L.). Sci. Hort. 125:171–178.

Baker, J. 1987. Distribution of N in a simulated profile of a podsolic gray luvisol following urea fertilization. Can. J. Soil Sci. 67:271–280.

Bassman, J.H. and J.C. Zwier. 1991. Gas exchange characteristics of *Populus trichocarpa, Populus deltoides and Populus trichocarpa × P. deltoides* clones. Tree Physiol. 8:145–159.

Basu, S.K. and A.K. De. 2003. Capsicum: Historical and botanical perspective, p. 1–15. In: De, A.K. (ed.). Capsicum: The genus Capsicum. Taylor & Francis, London, UK.

Benard, C., H. Gautier, F. Bourgaud, D. Grasselly, B. Navez, C. Caris-Veyrat, M. Weiss, and M. Génard. 2009. Effects of low nitrogen supply on tomato (*Solanum lycopersicum*) fruit yield and quality with special emphasis on sugars, acids, ascorbate, carotenoids, and phenolic compounds. J. Agr. Food Chem. 57:4112–4123.

Björkman, O. 1981. Responses to different quantum flux densities, p. 57–107. In: Lange, O.L., P.S. Nobel, C.B. Osmod, and H. Ziegler (eds.). Physiological plant ecology I. Springer, Berlin, Germany.

Botía, P., J.M. Navarro, A. Cerdá, and V. Martínez. 2005. Yield and fruit quality of two melon cultivars irrigated with saline water at different stages of development. Eur. J. Agron. 23:243–253.

Cabañero, F.J., V. Martínez, and M. Carvajal. 2004. Does calcium determine water uptake

under saline conditions in pepper plants, or is it water flux which determines calcium uptake? Plant Sci. 166:443–450.
Canto-Flick, A., L.G. Iglesias-Andreu, E. Balam-Uc, J.J. Bello-Bello, C. Lecona-Guzmán, D. Solís-Marroquín, S. Avilés-Viñas, E. Gómez-Uc, G. López-Puc, and N. Santana-Buzzy. 2008. Capsaicinoids content in habanero pepper (*Capsicum chinense* Jacq.): Hottest known cultivars. HortScience 43:1344–1349.
Davies, F.T., S.A. Duray, L. Phavaphutanon, and R.S. Stahl. 1999. Influence of phosphorus on gas exchange and plant growth of two morphologically distinct types of *Capsicum annuum*. Photosynthetica 36:99–106.
Davies, J.N. and G.W. Winsor. 1967. Effect of nitrogen, phosphorus, potassium, magnesium and liming on the composition of tomato fruit. J. Sci. Food Agr. 18:459–466.
DeWitt, D. and P.W. Bosland. 1996. Peppers of the world: An identification guide. Ten Speed Press, Berkeley, CA.
Dixon, R.A. and N.L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097.
Emongor, V.E. and O. Mabe. 2012. Effects of phosphorus on growth, yield and yield components of chilli pepper (*Capsicum annuum* L.). Proc. XXVIII IHC-IS on Quality-Chain Mgt. of Fresh Veg.: From Fork to Farm 936:327–333.
Eshbaugh, W.H. 1993. Peppers: History and exploitation of a serendipitous new crop discovery, p. 132–139. In: Janick, J. and J.E. Simon (eds.). New crops. Wiley, New York, NY.
Folin, O. and V. Ciocalteu. 1927. On tyrosine and tryptophane determinations in proteins. J. Biol. Chem. 73:627–650.
Fu, Q.S., B. Zhao, Y.J. Wang, S. Ren, and Y.D. Guo. 2010. Stomatal development and associated photosynthetic performance of capsicum in response to differential light availabilities. Photosynthetica 48:189–198.
Gökmen, V., N. Kahraman, N. Demir, and J. Acar. 2000. Enzymatically validated liquid chromatographic method for the determination of ascorbic and dehydroascorbic acids in fruit and vegetables. J. Chromatography 881:309–316.
Grieve, A.M., L.D. Prior, and K.B. Bevington. 2007. Long-term effects of saline irrigation water on growth, yield, and fruit quality of 'Valencia' orange trees. Aust. J. Agr. Res. 58:342–348.
Halvin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 2005. Soil fertility and fertilizers. 7th Ed. Pearson Education, Upper Saddle River, NJ.
Harvell, K.P. and P.W. Bosland. 1997. The environment produces a significant effect on pungency of chiles. HortScience 32:1292.
Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station. Circular 347:1–32.
Johnson, C.D. and D.R. Decoteau. 1996. Nitrogen and potassium fertility affects jalapeño pepper plant growth, pod yield, and pungency. HortScience 31:1119–1123.
Laborde, C. and C. Pozo. 1984. Presente y Pasado del Chile en México. Secretaría de Agricultura y Recursos Hidráulicos, México.
Li, X.L., C.R. Wang, X.Y. Li, Y.X. Yao, and Y.J. Hao. 2013. Modifications of Kyoho grape berry quality under long-term NaCl treatment. Food Chem. 139:931–937.
Loaiza-Figueroa, F., K. Ritland, J.A. Laborde-Cancino, and S.D. Tanksley. 1989. Patterns of genetic variation of the genus Capsicum (Solanaceae) in Mexico. Plant Syst. Evol. 165:159–188.
Luning, P.A., R. van der Vuurst de Vries, D. Yuksel, T. Ebbenhorst-Seller, H.J. Wichers, and J.P. Roozen. 1994. Combined instrumental and sensory evaluation of flavor of fresh bell peppers (*Capsicum annuum*) harvested at three maturation stages. J. Agr. Food Chem. 42:2855–2861.
Lycoskoufis, I.H., D. Savvas, and G. Mavrogianopoulos. 2005. Growth, gas exchange, and nutrient status in pepper (*Capsicum annuum* L.) grown in recirculating nutrient solution as affected by salinity imposed to half of the root system. Sci. Hort. 106:147–161.
Mansour, M.M.F. 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. Biol. Plant. 43:491–500.
Marinova, D., F. Ribarova, and M. Atanassova. 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J. of the Univ. of Chem. Technol. and Metallurgy 40:255–260.
Medina-Lara, F., I. Echevarría-Machado, R. Pacheco-Arjona, N. Ruiz-Lau, A. Guzmán-Antonio, and M. Martinez-Estevez. 2008. Influence of nitrogen and potassium fertilization on fruiting and capsaicin content in habanero pepper (*Capsicum chinense* Jacq.). HortScience 43:1549–1554.
Navarro, J.M., P. Flores, C. Garrido, and V. Martinez. 2006. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chem. 96:66–73.
Navarro, J.M., C. Garrido, M. Carvajal, and V. Martinez. 2002. Yield and fruit quality of pepper plants under sulphate and chloride salinity. J. Hort. Sci. Biotechnol. 77:52–57.
Niu, G., D.S. Rodriguez, E. Call, P.W. Bosland, A. Ulery, and E. Acosta. 2010a. Responses of eight chile peppers to saline water irrigation. Sci. Hort. 126:215–222.
Niu, G., D.S. Rodriguez, K. Crosby, D. Leskovar, and J. Jifon. 2010b. Rapid screening for relative salt tolerance among chile pepper genotypes. HortScience 45:1192–1195.
Núñez-Ramírez, F., D. González-Mendoza, O. Grimaldo-Juárez, and L. Cervantes Díaz. 2011. Nitrogen fertilization effect on antioxidants compounds in fruits of habanero chili pepper (*Capsicum chinense*). Intl. J. of Agr. and Biol. 13:827–830.
Parisi, M., I. Giordano, A. Pentangelo, and G. Villari. 2006. Effects of different levels of nitrogen fertilization on yield and fruit quality in processing tomato. Proc. IS. Towards Ecol. Sound Fert. Strategies for Field Veg. Prod. 700:129–132.
Petreikov, M., L. Yeselson, S. Shen, I. Levin, A.A. Schaffer, A. Efrati, and M. Bar. 2009. Carbohydrate balance and accumulation during development of near-isogenic tomato lines differing in the *AGPase-L1* allele. J. Amer. Soc. Hort. Sci. 134:134–140.
Poorter, H., F. Fiorani, M. Stitt, U. Schurr, A. Finck, Y. Gibon, B. Usadel, R. Munns, O.K. Atkin, F. Tardieu, and T.L. Pons. 2012. The art of growing plants for experimental purposes: A practical guide for the plant biologist. Funct. Plant Biol. 39:821–838.
Roy, S.S., M.S.I. Khan, and K.K. Pall. 2011. Nitrogen and phosphorus efficiency on the fruit size and yield of Capsicum. J. of Expt. Sci. 2:32–37.
Saito, T., C. Matsukura, Y. Ban, K. Shoji, M. Sugiyama, N. Fukuda, and S. Nishimura. 2008. Salinity stress affects assimilate metabolism at the gene-expression level during fruit development and improves fruit quality in tomato (*Solanum lycopersicum* L.). J. Jpn. Soc. Hort. Sci. 77:61–68.
Santana-Buzzy, N. 2010. Chile habanero (*Capsicum chinense* Jacq.), p. 269–279. In: del Castillo, L., M.L. Robert, A. Larqué, and I. Higuera (eds.). CICY treinta años de labor científica y educativa. Centro de Investgación Científica de Yucatán, Mérida.
SEMARNAT. 2012. La degradación de los suelos en México, p. 122–142. In: SEMARNAT (ed.). Informe de la situación del medio ambiente en México. Compendio de estadísticas ambientales. Indicadores clave y de desempeño ambiental. SEMARNAT, México.
Silva, C., V. Martínez, and M. Carvajal. 2008. Osmotic versus toxic effects of NaCl on pepper plants. Biol. Plant. 52:72–79.
Soon, Y.K. 1998. Nitrogen cycling involving non-exchangeable ammonium in a gray luvisol. Biol. Fertil. Soils 27:425–429.
Sudhir, P. and S.D.S. Murthy. 2004. Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42:481–486.
Umali-deininger, D. 1993. Irrigation-induced salinity: A growing problem for development and the environment. The World Bank, Washington, DC.
Wahyuni, Y., A.R. Ballester, E. Sudarmonowati, R.J. Bino, and A.G. Bovy. 2011. Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. Phytochemistry 72:1358–1370.
Wang, Y. and M. Frei. 2011. Stressed food—The impact of abiotic environmental stresses on crop quality. Agr. Ecosyst. Environ. 141:271–286.