GROWTH AND PHYSIOLOGY OF *Annona squamosa* L. UNDER DIFFERENT IRRIGATION DEPTHS AND PHOSPHATE FERTILIZATION

CRESCIMENTO E FISIOLOGIA DE MUDAS DE Annona squamosa *L. SOB DIFERENTES LÂMINAS DE IRRIGAÇÃO E ADUBAÇÃO FOSFATADA*

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ABTRACT: This study aimed to evaluate the growth and physiology of *Annona squamosa* seedlings under increasing irrigation depths and phosphorus doses. The experiment was conducted in protected environment, evaluating five irrigation depths and four $P_2O_5^-$ doses, arranged in 5 x 4 factorial scheme, in randomized blocks, with four replicates and one plant per plot. Treatments were irrigation depths of 60, 80, 100, 120 and 140% of the real evapotranspiration of the seedlings – ETr and phosphorus doses of 0, 350, 700 and 1050 mg dm^{-3} of $P_2O_5^-$. Plants were evaluated at 120 days after transplanting with respect to growth, gas exchanges, leaf water potential and total dry matter accumulation. Phosphorus dose of 350 mg dm^{-3} promotes satisfactory dry matter accumulation for *A. squamosa* seedlings, especially under 100% ETr irrigation. Leaf water potential and gas exchanges of *A. squamosa* are affected by water stress, through both lack and excess of water. Stomatal conductance is the variable most sensitive to the lack of water, whereas photosynthesis and water use efficiency are the most sensitive to the excess of water. Irrigation depth of 100% ETr is ideal to produce *A. squamosa* seedlings, but the irrigation depth of 80% ETr can be used to produce these seedlings, disregarding small losses of growth.

KEYWORDS: Annonaceae. Water availability. Gas exchanges. Soil fertility

INTRODUCTION

Annona squamosa L., whose fruit is popularly known as 'pinha', 'fruta do conde', 'ateira' or 'anona', is a fruit species belonging to the Annonaceae family with great socioeconomic potential in Brazil and worldwide (LEMOS, 2014). This crop is in full expansion in the country due to the great attractiveness of its fruits, and the Northeast region is the main producer, especially the states of Alagoas, Bahia, Ceará and Pernambuco, where there may be two harvests per year if cultivation practices such as fertilization and irrigation are adopted (PEREIRA et al., 2010; MEDEIROS et al, 2014).

In addition, Annonaceae species are considered as of easy adaptability to different edaphoclimatic conditions, and some species grow well under hot and humid climates of typically tropical zones, showing good levels of yield, being commercially cultivated in several parts of the world due to the profitability achieved (SÃO JOSÉ et al., 2014).

In semi-arid regions, climate conditions play a decisive role in crop irrigation management, to the point of causing restriction in the yield, compromising the efficiency of the agricultural production system (AZEVEDO et al., 2014). Given the existing scarcity of water in these regions, in drought periods it is important to consider the efficiency in the use of this resource, either in selecting the amount of water to be applied or in the management adopted (VICENTE et al., 2015). In response to the lack of water, plants trigger various physiological events among which the osmotic adjustment is the most common, aiming to maintain water potential and cell turgor close to adequate levels (PEREIRA et al., 2012; ASHRAF; HARRIS, 2013).

Besides adequate irrigation management, fertilization is another decisive practice to increase crop yield. In a study evaluating phosphate

Received: 25/04/18
Accepted: 10/10/18

fertilization on the production of *A. squamosa* seedlings, Freitas et al. (2013) observed that this nutrient is crucial for seedling development, due to its function in root growth, thus allowing for greater exploration of the soil and water absorption by the plant.

In semi-arid regions, there is a predominance of tropical soils with low natural availability of phosphorus and such lack requires greater caution with respect to phosphate fertilization management in these regions because the low availability of the nutrient associated with the water limitations found negatively affect crop development (LIMA et al., 2017; SÁ et al., 2017).

Given the above, this study aimed to evaluate the growth and physiology of *A. squamosa* seedlings under increasing irrigation depths and phosphorus doses.

MATERIAL AND METHODS

The experiment was conducted from October 2015 to February 2016 for 120 days in a protected environment at the Center of Sciences and Agri-Food Technology (CCTA) of the Federal University of Campina Grande, municipality of Pombal – PB, Brazil, located at geographic coordinates 6º48'16'' S and 37º49'15" W at altitude of 174 m.

Temperature and rainfall were monitored along the experimental period; maximum temperature varied from 30 to 39 ºC, whereas minimum temperature varied from 15 to 26 ºC, and their mean values were respectively equal to 34.1±1.3 and 21.4±2.0 ºC, recorded from beginning to the end of the experiment.

The experimental design was randomized blocks in a 5 x 4 factorial scheme, comprising four blocks and one plant per plot, corresponding to five irrigation depths (60, 80, 100, 120 and 140% of real evapotranspiration – ETr) and four phosphorus doses (D1 = 0; D2 = 350; D3 = 700 and D4 = 1050 mg dm^{-3} of P_2O_5), in the form of single superphosphate (18% P_2O_5, 20% Ca^{2+}, 12% S), ground, applied 30 days before transplanting the seedlings.

Sowing was carried out on polyethylene trays containing 162 cells with capacity for 0.05 dm^3 each, by planting one seed per cell, which was filled with commercial substrate based on pine bark, humus and vermiculite, at 1:1:1 proportion. Seedlings remained in tray cells until the appearance of the third true leaf, at 30 days after sowing (DAS). Then, they were transplanted to 3.78 dm^3 lysimeters containing substrate at 2:1:1 proportion: two parts of soil, one part of washed sand and one part of bovine manure. The chemical characteristics of this substrate are presented in Table 1. Plants were acclimated for seven days, maintaining soil moisture close to field capacity through daily irrigations, determined based on drainage lysimetry (BERNARDO et al., 2008), and the irrigation depths began to be applied 8 days after transplanting (DAT).

Table 1. Physical-chemical characterization of the substrate used to produce *A. squamosa* seedlings

Textural classification	Soil Density g cm^{-3}	Total porosity %	Organic matter g kg^{-1}	P mg dm^{-3}	Sortive complex Ca^{2+}	Mg^{2+}	Na^+	K^+
					--------- cmol$_c$ dm^{-3} --------			
Sandy franc	1.38	47.00	32	17	5.4	4.1	2.21	0.28

Saturation extract

pHse	ECse dS m^{-1}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Cl^-	SO_4^{2-}	CO_3^{2-}	HCO_3^-	Saturation %
		----------	----------	----------	mmol$_c$dm^{-3}	----------	----------	----------	----------	
7.41	1.21	2.50	3.75	4.74	3.02	7.50	3.10	0.00	5.63	27.00

Organic matter: Walkley-Black wet digestion; Ca^{2+} and Mg^{2+} extracted with 1 mol L^{-1} KCl at pH 7.0; Na^+ and K^+ extracted with 1 mol L^{-1} NH_4OAc at pH 7.0; ECse – electrical conductivity of the soil saturation extract.

Drained water volume was collected by zinc gutters to determine plant water consumption in the treatments of 120% field capacity, obtained by the difference between applied and drained volumes, resulting in the consumed volume as the 100% water depth is multiplied by the factors 0.6, 0.8, 1.0, 1.4 to obtain water depths of 60, 80, 100 and 140% field capacity, respectively.

At 120 DAT, when seedlings were ready to be taken to the field, the following parameters were determined: plant height (PH), measured with a graduated ruler as the main stem length from the base to the tip; number of leaves (NF), determined by counting the photosynthetically active leaves; and stem diameter (SD), measured using a digital caliper and expressed in mm.

Leaf water potential was determined at 120 DAT by collecting one sample in each plot from 4 to 6 a.m. and placing it in a Scholander pressure bomb to measure the negative hydrostatic pressure (tension) existing in the xylem of many plants, according to Scholander et al. (1965).

At 120 DAT, gas exchanges were measured in the plants using a portable photosynthesis meter (LCPro+ - ADC BioScientific Ltd), operating with controlled temperature of 25 °C, irradiation of 1200 µmol photons m^{-2} s^{-1} and CO_2 from the environment at 3 m height from soil surface, to obtain CO_2 assimilation rate (*A*) (µmol m^{-2} s^{-1}), transpiration (*E*) (mol of H_2O m^{-2} s^{-1}), stomatal conductance (gs) (mmol of H_2Om^{-2} s^{-1}) and internal CO_2 concentration (Ci), in the third leaf from the apex. These data were used to calculate intrinsic water use efficiency (WUEi) (A/E) [(µmol m^{-2} s^{-1}) (mol H_2O m^{-2} $s^{-1})^{-1}$] and instantaneous carboxylation efficiency (EiCi) (SÁ et al., 2017).

Total dry matter (TDM) corresponded to the sum of leaf dry matter, stem dry matter and root dry matter, which were obtained by drying the material from these plant parts in a forced-air oven at 65 ºC until constant weight.

The data were subjected to analysis of variance by F test and means were compared by regression using the program Sisvar, version 5.1 (FERREIRA, 2011).

RESULTS AND DISCUSSION

The interaction between irrigation depths and phosphate fertilization had significant ($p < 0.05$) effect on total dry matter. For leaf water potential, internal CO_2 concentration, transpiration, CO_2 assimilation rate, stomatal conductance, intrinsic water use efficiency, plant height, number of leaves and stem diameter, there was only significant ($p < 0.05$) effect of irrigation depths. Instantaneous carboxylation efficiency was not influenced by the studied factors (Table 2).

Table 2. Significance of F test for leaf water potential (Ψf), internal CO_2 concentration (Ci), transpiration (E), CO_2 assimilation rate (A), stomatal conductance (gs), intrinsic water use efficiency (WUEi) (A/E), instantaneous carboxylation efficiency (EiCi), plant height (PH), number of leaves (NL), stem diameter (SD) and total dry matter (TDM) of the *A. squamosa* under irrigation depths and phosphate fertilizer doses at 120 days after treatments began to be applied.

FV	GL	Significância do teste ‘F’										
		Ψf	Ci	E	A	Gs	WAEi	EiCi	PH	NL	SD	TDM
Bloco	3	*	*	*	*	*	*	ns	ns	ns	ns	*
Lâm (L)	4	*	*	*	*	*	*	ns	*	*	*	*
Dose (D)	3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
L x D	12	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Resíduo	57	-	-	-	-	-	-	-	-	-	-	-
C. V. (%)		18.64	7.22	9.35	13.84	13.19	11.33	17.81	5.80	5.48	7.39	7.39
Média Geral		4.28	260.11	2.43	10.57	0.19	4.39	0.04	98.82	39.78	9.81	9.81

[NS] and * = not significant and significant at 0.05 probability level ($p < 0.01$ and $p < 0.05$), respectively; C.V.= coefficient of variation.

Increase in irrigation depths up to the maximum estimated value of 94% increased CO_2 assimilation rate to 10.775 µmol m^{-2} s^{-1}, but irrigations using higher water depths, especially from 120% ETr, reduced the net photosynthesis of the *A. squamosa* seedlings. Such reduction, due to irrigation depths above 94% ETr, is an indication of sensitivity of the *A. squamosa* plants to excess water because the CO_2 assimilation of plants subjected to 140% ETr is 5.51% lower than that of plants under 60% ETr (Figure 1A).

Stomatal conductance (*gs*) showed a quadratic response similar to that of CO_2 assimilation rate, in which the increase in irrigation depths up to 108% ETr led to highest stomatal conductance, 0.2008 mmol of H_2O $m^{-2}s^{-1}$. From 108% ETr on, there was a reduction in stomatal conductance (Figure 1B).

In response to water stress by lack (60 and 80% ETr) or excess (120 and 140% ETr), there was a reduction in the photosynthesis of the *A. squamosa* seedlings caused by the increase in the resistance to CO_2 diffusion through the stomata and mesophyll (BENLLOCH-GONZÁLEZ et al., 2010; GALMÉS et al., 2011). Increment in the resistance to CO_2 diffusion may be due to stomatal closure in response to the decrease in leaf water potential (ARAÚJO; DEMINICIS, 2009; ENDRES et al., 2010).

Transpiration was stimulated by irrigation depths, reaching highest value of 2.85 mmol of H_2O $m^{-2}s^{-1}$ at the maximum estimated water depth of 135% ETr (Figure 1C). Water tends to change from liquid to gaseous state due to the difference in water vapor concentration between leaf intercellular spaces and the external atmospheric mass (TAIZ et al., 2015). Silva et al. (2015a) reported a behavior usually exhibited by plants, which show high transpiration rates so that, as water becomes scarce in the soil, the plant begins to reduce its transpiration rate to minimize water loss and save the water available in the soil.

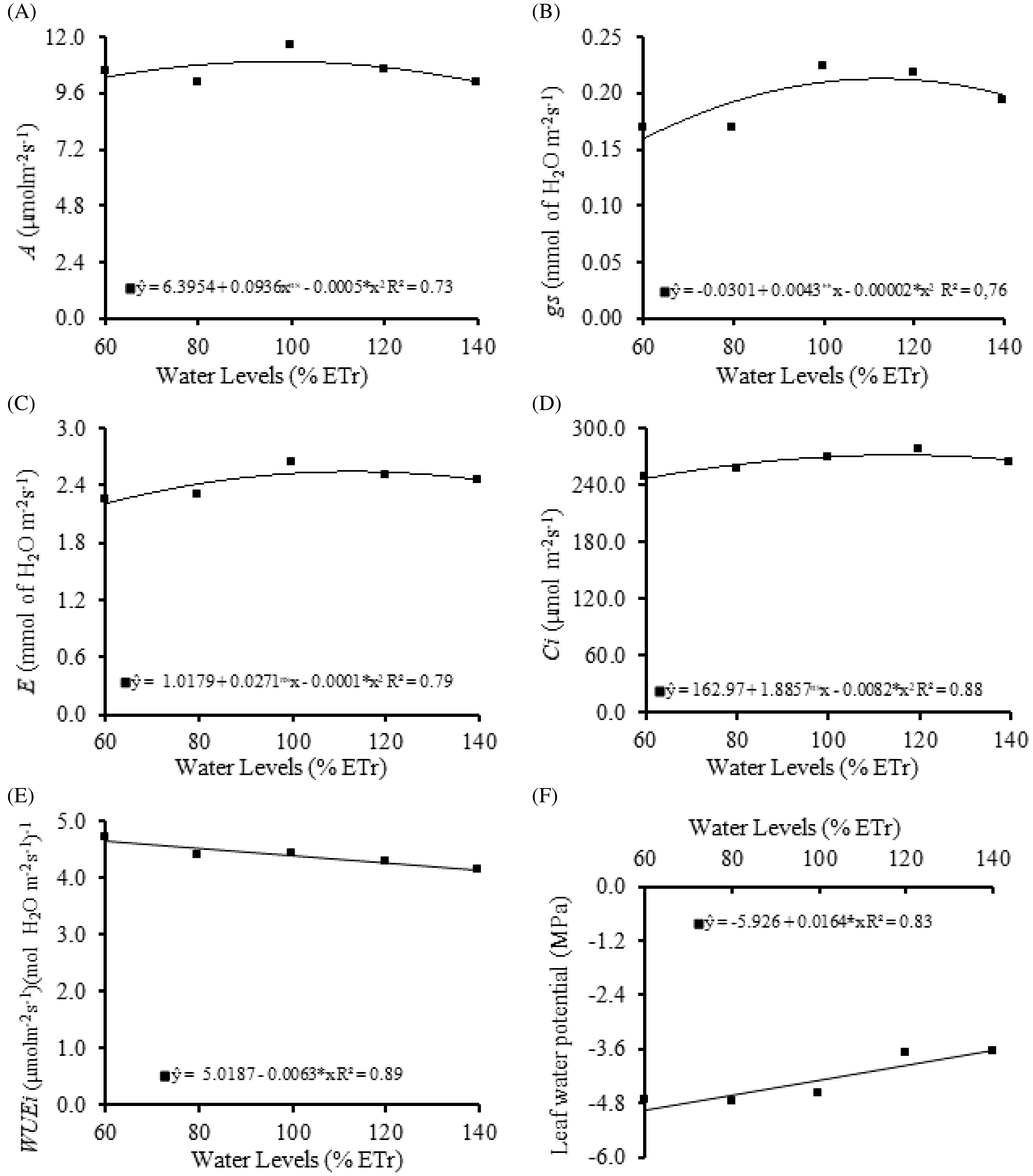

Figure 1. CO_2 assimilation rate - *A* (A), stomatal conductance - *gs* (B), transpiration - *E* (C), internal CO_2 concentration - *Ci* (D), intrinsic water use efficiency - *WUEi* (E) and leaf water potential (F) of *Annona squamosa* L. seedlings as a function of irrigation depths at 120 days after treatments began to be applied.

Increase in irrigation depths led to increments in internal CO_2 concentration (*Ci*) up to a maximum of 271.38 μmol m^{-2} s^{-1}, obtained in seedlings irrigated with the estimated water depth of 115% ETr (Figure 1D). Such behavior is common because the internal CO_2 concentration follows the same trend of stomatal conductance and, consequently, a limitation in the stomata would be the limiting factor for adequate development, since the larger the stomatal opening, the greater CO_2 diffusion to the substomatal chamber (TAIZ et al., 2015). According to Bosco et al. (2009), stomatal closure and the consequent reduction in the normal CO_2 flux towards the site of carboxylation are the main responsible for the reduction in photosynthesis, as observed in the present study.

Intrinsic water use efficiency decreased linearly at a rate of 0.006 ($\mu molm^{-2}s^{-1}$) (mol H_2O $m^{-2}s^{-1})^{-1}$ per unit increase in irrigation depths, with losses of 10.86% between seedlings irrigated with 60 and 140% ETr (Figure 1E). In response to the lack of water, plants trigger several physiological events, such as the stomatal closure observed in plants irrigated with 60 and 80% ETr, probably trying to obtain better osmotic adjustment to maintain water potential and cell turgor close to adequate levels (PEREIRA et al., 2012). Despite that, under excess water conditions, stomatal closure did not contribute to increasing water use efficiency, as observed under conditions of water stress by lack of water.

Excess of water was more harmful to photosynthesis, consequently limiting water use efficiency. Batista et al. (2008) studying *Cecropia pachystachya*, and Oliveira & Gualtieri (2017), studying *Tabebuia aurea*, found reductions in stomatal conductance of plants subjected to flood, which also led to reduction in their photosynthetic activity, corroborating the result observed in the present study. Although the period of saturation in our experiment was short, because of the drainage system, it was sufficient to reduce the photosynthetic activity of *A. squamosa* plants, but it did not affect carboxylation efficiency, indicating that this stress was not able to cause more severe alterations, for instance on the supply of ATP and NADPH to the functioning of the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (MACHADO et al., 2010; SÁ et al., 2017). The effects mainly occurred on the stomata, and the reduction in photosynthesis followed stomatal closure and the decrease in CO_2 influx (Figures 1A, B and D).

Leaf water potential (Ψf) increased linearly as a function of the increase in irrigation depths (Figure 1F), with increments of 0.33 KPa for every 20% increase in irrigation depth. Lowest water potentials occurred in the leaves of plants irrigated with 60% ETr, coinciding with the low levels of transpiration, stomatal conductance and photosynthesis, denoting limiting conditions of water availability to the plants. Such reduction in leaf water potential, according to Martins et al. (2010), results in a short water deficit, caused by the high evaporative demand of the atmosphere, and can be related to the capacity of the transpiration to exceed water absorption by roots.

Increase in irrigation depths (% ETr) linearly stimulated seedlings growth in height, number of leaves and stem diameter, which increased respectively by 3.27 cm, 0.7 leaves and 0.29 mm for every 20% increase in irrigation depth (Figure 2A, B and C). Plants favored by greater water availability tend to have higher potential for cell turgor, distension of the protoplasmic layer and the wall of a plant cell due to its liquid content, compared with those under water deficit (TAIZ et al., 2015), and consequently greater growth. It is important to point out that, despite the reduction in photosynthetic activity, the growth of the *A. squamosa* seedlings was not negatively affected by the increase in irrigation depths, during the stage of seedling production. Similar results were observed by Silva et al. (2015b) and Queiroz et al. (2017) in seedlings of Surinam cherry and *Eucalyptus globulus* under water stress, respectively, whose growth was also reduced by the reduction in irrigation depths.

The highest phosphorus doses tested, 700 and 1050 mg dm^{-3} of $P_2O_5^-$, only led to satisfactory results when plants were under irrigation depths above 100% ETr, whereas plants subjected to 0 and 350 mg dm^{-3} of $P_2O_5^-$ showed superior dry matter accumulation when under irrigation depths of up to 100% ETr. It should be pointed out that the dry matter accumulation in plants irrigated with 80% ETr and cultivated in the substrate with addition of 0 and 350 mg dm^{-3} of P_2O_5 was similar to that of plants subjected to 100% ETr (Figure 2D).

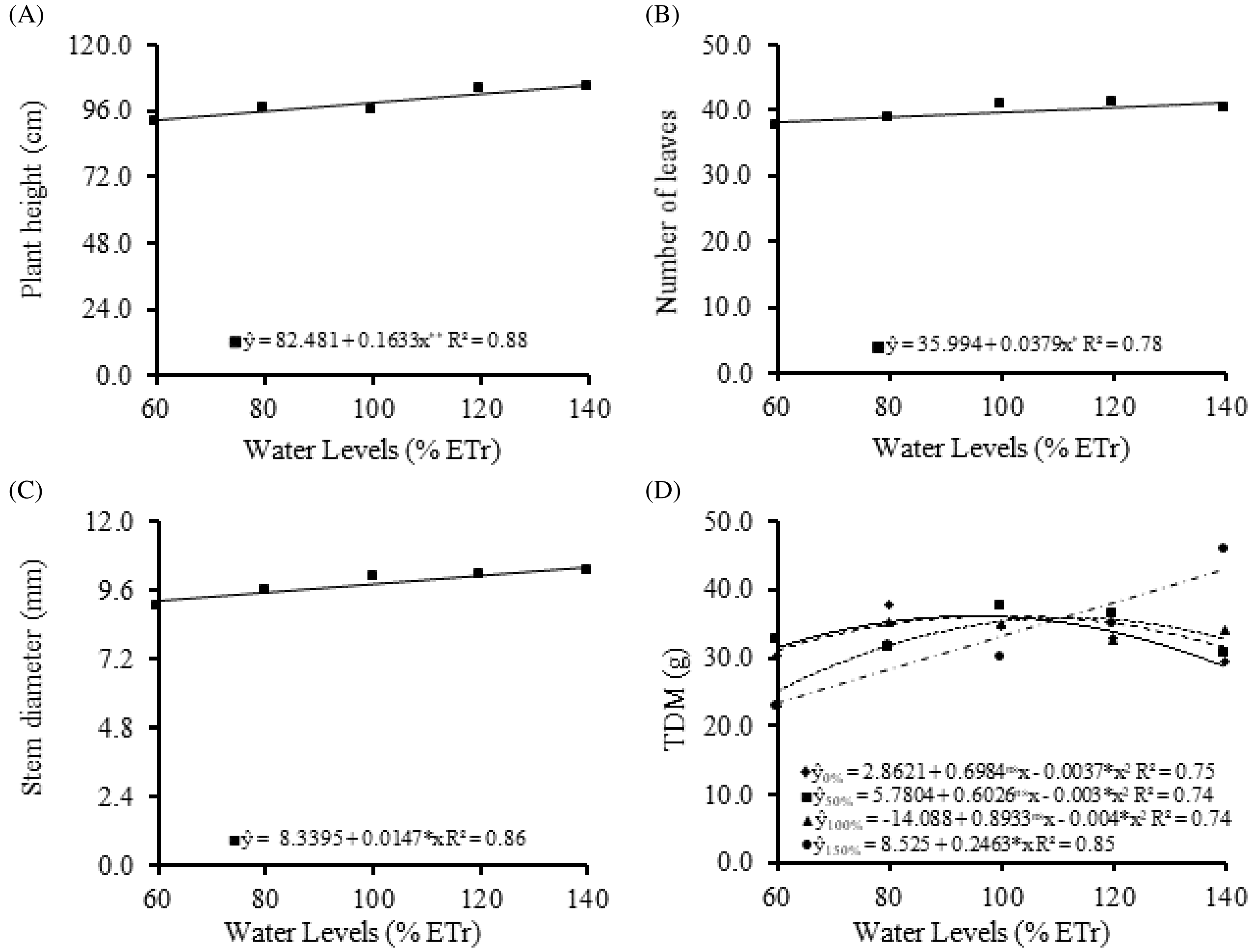

Figure 2. Plant height (A), number of leaves (B) and stem diameter (C) and total dry matter, TDM (D) of *Annona squamosa* L. seedlings as a function of the interaction between irrigation depths and phosphorus doses (♦D1 = 0; ■D2 = 350; ▲D3 = 700 and ●D4 = 1050 mg dm^{-3} of P_2O_5), at 120 days after treatments began to be applied.

According to Silva & Farnezi (2009), the nutrients which most compromised the production of soursop (*A. muricata*) seedlings were P, N, Ca and Mg. Considering the great demand for nutrients by Annonaceae plants, the substrates with higher contents of nutrients such as the phosphorus tested in the present study, are probably more promising for their growth. Nevertheless, very high doses of phosphorus in the form of single superphosphate maximized the effect of water stress on plants at the lowest water depths, probably due to the release of calcium sulfate salts and, consequently, reduction in osmotic potential. In addition, the positive responses at the highest doses of phosphorus associated with the highest irrigation depths may be related to the leaching of salts (SÁ et al., 2015; LIMA et al., 2017), especially calcium sulfate salts present in the phosphate fertilizer.

CONCLUSIONS

Phosphorus dose of 350 mg dm^{-3} leads to satisfactory dry matter accumulation in *A. squamosa* seedlings, especially under irrigation with 100% ETr.

Leaf water potential and gas exchanges of the *A. squamosa* are affected by water stress, through both lack and excess of water; stomatal conductance is the variable most sensitive to the lack of water, whereas photosynthesis and water use efficiency are the most sensitive to the excess of water.

Irrigation depth of 100% ETr is ideal to produce *A. squamosa* seedlings, but the irrigation depth of 80% ETr can be used to produce these seedlings, disregarding small losses in growth.

RESUMO: Objetivou-se avaliar o crescimento e a fisiologia de mudas de *A. squamosa* sob lâminas crescentes de irrigação e doses de fósforo. O experimento foi conduzido em ambiente protegido, avaliando cinco lâminas de irrigação e quatro doses P_2O_5, arranjados em esquema fatorial 5 x 4, em blocos casualizados, com quatro repetições e uma planta por parcela. Os tratamentos das lâminas foram 60, 80, 100, 120 e 140% da evapotranspiração real das mudas – ETr e as quatro doses de fósforo 0, 350, 700 e 1050 mg dm^{-3} de P_2O_5. As plantas foram avaliadas aos 120 dias após o transplantio, quanto ao crescimento, trocas gasosas, potencial hídrico foliar e acúmulo de matéria seca total. A dose de fósforo de 350 mg dm^{-3} proporciona acúmulo de matéria seca satisfatório para as mudas de *A. squamosa*, principalmente sob irrigação com 100% da ETr. O potencial hídrico foliar e as trocas gasosas da *A. squamosa* são afetadas pelo estresse hídrico, tanto por falta como por excesso de água, sendo a condutância estomática a variável mais sensível a falta de água e a fotossíntese e eficiência do uso da água as mais sensíveis ao excesso de água. A lâmina de 100% da ETr é a ideal para a produção de mudas de *A. squamosa*, porém a lamina de 80% da ETr pode ser utilizada para a produção de mudas de *A. squamosa*, admitindo-se pequenas perdas no crescimento.

PALAVRAS-CHAVE: Annonaceae: Disponibilidade hídrica: Trocas gasosas: Fertilidade do solo

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