

Automatically controlled deficit irrigation of lettuce in “organic potponics”

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ABSTRACT: Concerns with water crisis involve all sectors of society and irrigated agriculture remains the main water consumer. This study evaluated an agricultural production system for lettuce cultivation in greenhouse, “organic potponics”, to economize water and manure use, using a Simplified Irrigation Controller (SIC), based on soil matric potential monitoring. Five irrigation volumes were evaluated in pots with 4.8 L, fertilized with 200 g of vermicompost. One of the volumes was controlled with the SIC. The other volumes represented 130, 80, 60 and 33 % of that controlled by the SIC and all treatments received water at the same time. Shoot fresh weight, head diameter and stomatal conductance (gs) increased linearly with irrigation volumes. For shoot dry weight, number of leaves and water use efficiency (WUE), the regression was quadratic with maximum values at 126, 114 and 83 %, respectively. Leaf relative water content did not show variation among treatments and changes in some fluorescence parameters (Reo/RC, Sm, N and ϕ RO) were much more remarkable to drought compared with the F_v/F_m ratio, one of the most commonly used stress indicators. The data indicated a tradeoff between WUE and plant growth thus the economic values of water and lettuce should be taken into account to indicate the best SIC irrigation volume. Organic potponics is promising and should be further improved to save on water, labor and fertilizer use.

Keywords: *Lactuca sativa*, water use efficiency, production system, water management, automation

Introduction

The economy in water and nutrients for plant cultivation will depend on the efficiency of the sensor-based controllers for irrigation to avoid either drainage or drought, which causes waste of water or growth limitation, respectively (Cia et al., 2012; Boaretto et al., 2014; Medici et al., 2014). The lack of such controllers at a low cost and ready to be used in commercial conditions may explain why precise irrigation is rarely used for growing plants in pots, as most use timers or manual control (Montesano et al., 2016).

Our research group developed a low-cost automatic controller for irrigation, sensor-based, which has been proved robust, efficient and can be assembled by farmers themselves (Medici et al., 2010). This controller has the advantage of being built with low cost materials, even though it requires trained personnel for installation and periodic maintenance. We used and tested the Simplified Irrigation Controller (SIC, Medici et al., 2010) in the field, in pot-plant studies and in small vials for seedlings (Batista et al., 2013; Dias et al., 2013; Gomes et al., 2014; Gonçalves et al., 2014).

We have now developed an alternative plant cultivation system, named “organic potponics”, in which the plants receive water and manure according to their requirements. This cultivation system is different from hydroponics and fertigation, where nutrients are supplied through the irrigation system. The organic potponics irrigation delivers water only, using SIC, while solid manure is directly applied to the substrate in the pots.

SIC is limited to a range of adjustment of soil water tension between 4 and 12 kPa. For papaya seedlings

this range was sufficient to cause water stress (Dias et al., 2013), but not for lettuce plants (Gonçalves et al., 2014). Therefore, in this study, we are testing volumes lower than the those supplied by SIC to impose controlled stress, which can improve water use efficiency in organic potponics system for lettuce. This system was evaluated using agronomic and physiological traits, such as stomatal conductance, chlorophyll content and chlorophyll *a* fluorescence, which are drought-sensitive traits (Martinazzo et al., 2011; Dias et al., 2013). This study sets the ground for a widespread use of SIC for the cultivation of plants in pots for commercial and research purposes.

Materials and Methods

Site description

Greenhouse experiments were conducted in Seropédica, RJ/Brazil (22°48'00" S; 43°41'00" W; 33.0 m). Lettuce seedlings (*Lactuca sativa* L. cv. Regina) were transplanted 30 days after sowing in polyethylene trays to pots and placed in the center of the pots. Each 4.8-L pot was filled with A horizon of a Planosol, and received 200 g of cow manure vermicompost placed into two holes (diameter: 5 cm and depth: 10 cm), 25 cm distant from the center of the pot. The chemical analysis of the cow manure vermicompost showed 22, 6, 12, 16 and 8 g kg⁻¹ dry weight for N, P, K, Ca and Mg, respectively.

Treatments, experimental design and irrigation management

Five irrigation volumes using low-cost SIC were evaluated; a volume automatically controlled (100 %

SIC), a volume larger than this (130 %) and three smaller volumes (80, 60 and 33 %). These treatments were chosen based on our previous works with SIC (Batista et al., 2013; Gomes et al., 2014), which indicated that this device supplies the whole plant water demand and on the current literature for deficit irrigation in which these levels of irrigations are used (Medici et al., 2014). SIC-sensor is composed of a ceramic capsule used in common domestic water filters placed into the plant substrate. The capsule keeps close relationship with the soil water tension (Medici et al., 2010).

The experimental design was randomized blocks with six blocks and five treatments, totaling 30 plots. Each pot with one lettuce plant represented a plot.

In each block, SIC was installed (100 % SIC) and adjusted to start irrigation when soil water tension reached 6 kPa. Other pots within the block received irrigation water at the same time. The different discharges were achieved by combining drippers of 2 and 4 L h⁻¹ (nominal discharge). The administered nominal discharges were as follows: 12, 8, 6, 4 and 2 L h⁻¹ in each pot within the block (Figure 1). The exact applied discharges from each treatment were determined by an in-situ test, which showed 130, 100, 80, 60 and 33 % volume of SIC. All plots received an irrigation volume of 200 mL at planting.

Soil moisture and climate

The soil moisture was monitored daily by the time-domain reflectometry (TDR) technique, using a handmade probe in each pot installed near the roots of lettuce plants. The dielectric constant (Ka) data were collected with TDR 100 (Campbell Scientific, Logan, Utah) and transformed into volumetric water content (θ) through a calibration curve ($\theta = 0.0263Ka - 0.0807$). The calibration curve was obtained by laboratory test using the same soil used in the experiment. The samples were placed in PVC columns and monitored by TDR probe and weighting.

A USB temperature and humidity data logger (Impac, São Paulo, Brazil) recorded greenhouse climate data.

Evaluated traits

Agronomic and physiological traits were evaluated and the amount of water used in the cultivation was monitored.

Fluorescence transients were measured on the 15th (morning), 31st (morning) and 32nd (afternoon) day after transplanting (DAT) using a portable Plant Efficiency Analyzer, Handy PEA (Hansatech, Norfolk, UK). Leaves were dark-adapted for 20 min and maximum fluorescence was induced with a saturating pulse of 3 mE m⁻² s⁻¹ for 0.8 seconds. The fluorescence intensities were obtained at the following time points: 50 μ s (minimum fluorescence, F_0), 100 μ s, 300 μ s, 2 ms (F_J), 30 ms (F_I) and at maximum fluorescence (F_M). The F_v is the difference between F_M and F_0 . These time points were fed into the JIP-Test (Tsimilli-Michael and Strasser, 2008)

to calculate the fluorescence parameters: Absorption flux (of antenna Chls) per RC (ABS/RC), Trapped energy flux (leading to QA reduction) per RC (TR_0 /RC), Electron transport flux (further than QA⁻) per RC (ET_0 /RC), Electron flux reducing end electron acceptors at the PSI acceptor side per RC (RE_0 /RC), Dissipated energy flux per RC (DI_0 /RC), Quantum yield for electron transport (ϕE_0), Quantum yield for reduction of end electron acceptors at the PSI acceptor side (ϕR_0), Quantum yield for dissipated energy (ϕD_0), F_v/F_M : Maximum quantum yield of primary photochemistry ($\phi P_0 = F_v/F_M$), Efficiency for electron transport, i.e., efficiency/probability that an electron

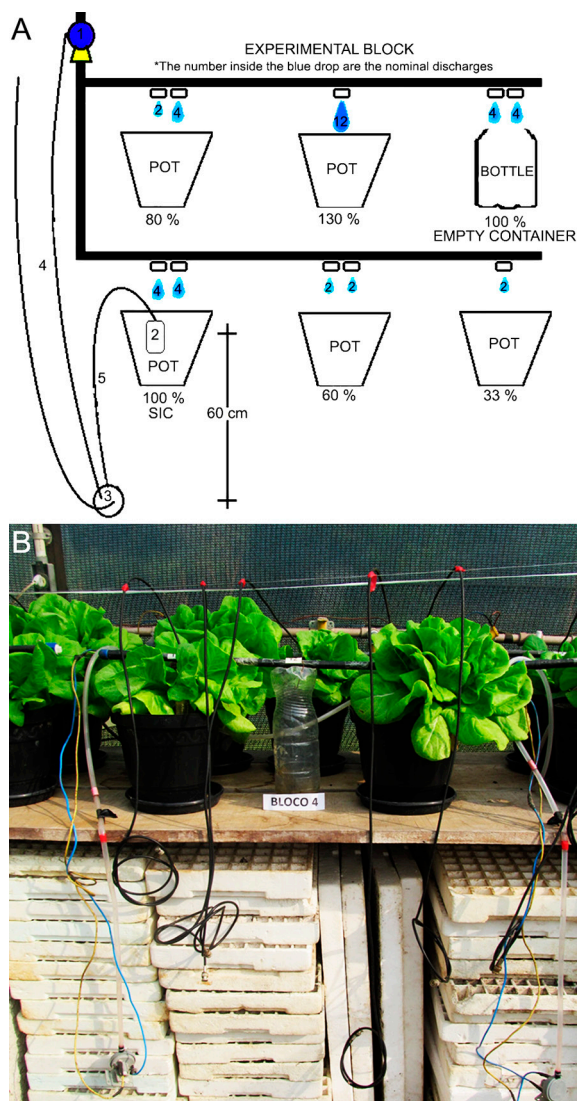


Figure 1 – Performed experiment: example of one experimental block (A) and photograph of block 4 on experimental site (B). Components of Simplified Irrigation Controller – SIC [1 - electromagnetic valve; 2 - ceramic capsule filter (tension sensor); 3 - pressostat from a washing machine (switcher); 4 - electric wires; and 5 - flexible tube] adapted from Medici et al. (2010).

moves further than QA^- (ψ_{EO}). Efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors of PSI (δ_{RO}), Efficiency with which a trapped exciton move an electron into the electron transport chain from QA^- to the PSI end electron acceptors (ρ_o), Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors (PI_{ABS}) and Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors (PI_{Total}).

Chlorophyll content was monitored on the 15th and 30th DAT, using an electronic chlorophyll meter, ClorofiLOG (Falker - Porto Alegre, Brazil). The readings were carried out in the middle of young fully expanded leaves in each plot.

Stomatal conductance (gs) was measured on the 28th, 31st, 32nd and 38th DAT using a SC-1 Leaf Porometer (Decagon Devices, Washington, USA). Measurements were taken close to 12h00 on the first days and at 09h00, 12h00 and 15h00 on the last day.

Relative water content (RWC) was analyzed on the 31st DAT at 12h00, following the methodology of Cia et al. (2012). One foliar segment (2 cm length \times 2 cm width) was collected from the middle portion of young fully expanded leaves from each plot, properly stored in plastic bags and cooled to avoid tissue dehydration until analysis in the laboratory. The RWC was calculated using the formula:

$$RWC = (FW - DW) / (TW - DW) \times 100$$

where: FW is fresh weight, DW is dry weight and TW is turgid weight.

To obtain FW, the foliar segment of known area (4.0 cm²) was weighed and immersed in water for 24 h for subsequent TW determination. Foliar segments were dried in a forced-air oven at 65 °C until a constant weight was reached (approx. 72 h).

At end of the experiment, total fresh and dried weights of each plant, leaf number and head diameter were obtained.

The applied volume was estimated using an empty container placed in each block, which received water directly from drippers equal to those in the pot of 100 % SIC. Water use efficiency (WUE) was calculated by the ratio between the shoot fresh weight and the applied volume of water.

Regression analysis

All data were analyzed by ANOVA for regressions with 0.05 significance through the software Sisvar (Universidade Federal de Lavras, Lavras, Brazil).

Results

Soil moisture

The lowest irrigation volume applied provided, on average, approximately 63 % reduction in soil water content compared to the highest irrigation treatment, which provided the highest moisture throughout the experiment.

Agronomic traits

The application of different irrigation volumes induced a linear shoot fresh weight gain and the same behavior happened to the diameter of lettuce heads, while for shoot dry weight and leaf number, the quadratic regression model revealed significance by the analysis of variance (Table 1). The maximum points of the regressions for shoot dry weight and number of leaves were 126 and 114 % SIC, respectively. The quadratic model was the only that showed significance to describe the behavior of WUE (Table 1). The volume of 80 % of SIC provided the highest WUE.

Physiological traits

The total content of leaf chlorophyll (Chl a + b) measured on the 15th DAT (Figure 2) exhibited a linear decrease as the water volume increased, whereas the chlorophyll b content on the 30th DAT (Figure 3) showed a quadratic response.

Table 1 – Regression analysis for the effect of different volumes of applied irrigation water using SIC on soil water content; Shoot fresh weight; Shoot dry weight; Diameter; Leaves numbers; and Water use efficiency of *Lactuca sativa*.

Agronomic Traits	Percentage Volumes of SIC (Simplified Irrigation Controller)				
	130 %	100 %	80 %	60 %	33 %
Soil Water Content (cm ³ cm ⁻³) y: 0.0187+0.001x (p < 0.0001) R ² = 0.9953	0.36	0.33	0.32	0.29	0.27
Shoot Fresh Weight (g per plant) y: 7.5693+1.9522x (p < 0.0001) R ² = 0.9309	244.27	207.45	195.70	118.93	58.25
Shoot Dry Weight (g per plant) y: -4.1551+0.2784x-0.0011x ² (p < 0.0109) R ² = 0.9904	13.64	13.42	11.22	8.15	4.09
Diameter (cm) y: 25.46+0.1399x (p < 0.0003) R ² = 0.8415	42	39	41	33	29
Number of leaves (units) y: 0.2412+0.591x-0.0026x ² (p < 0.0013) R ² = 0.9816	34	34	33	25	17
Water Use Efficiency (g L ⁻¹) y: 13.933+0.3982x-0.0024x ² (p < 0.0372) R ² = 0.7184	24.76	27.59	32.83	27.59	24.63

The gs measured on the 28th and 38th DAT, increased linearly according to the irrigation depths tested (Figures 4 and 5). The gs values on the 31st and 32nd DAT were the same for plants with and without water stress (data not shown).

On the 15th DAT, the reception of electrons by PSI reaction center (Reo/RC) was affected by volumes above and below 100 % of SIC and the highest value was recorded for the treatment with 100 % SIC (Figure 6). A linear reduction in the total number of electrons transferred to the electron transport chain (N) was observed for the lowest volumes applied (Figure 7). The normalized total area above the OJIP curve - kinetic steps to fluorescence induction - (Sm) showed a quadratic model for the 31st DAT, decreasing from irrigation volume of 33 % up to 100 % and showing a linear reduction as the applied volume of water increased for 32nd DAT (Figures 8 and 9). This parameter reflects multiple events of re-

duction in the Q_A^- pool. On the 31st DAT, N showed a quadratic behavior, decreasing from irrigation volume of 33 % up to 100 % (Figure 10). The quantum yield of electron transport Q_A^- to the final acceptor of electrons PSI (ϕR_0) exhibited linear reduction as the applied volume of water increased (Figure 11).

No effect on the maximum quantum yield of PSII primary photochemistry ($\phi P_0 = F_v/F_M$) was observed in any treatments. RWC was not different among treatments, with an overall average of 83 % (data not shown).

Discussion

Soil moisture

Soil moisture data (Table 1) obtained in this study for the volume of 100 % SIC adjusted to 6 kPa were between the values presented for the tensions of 3 and 9 kPa obtained with the use of SIC in the production of

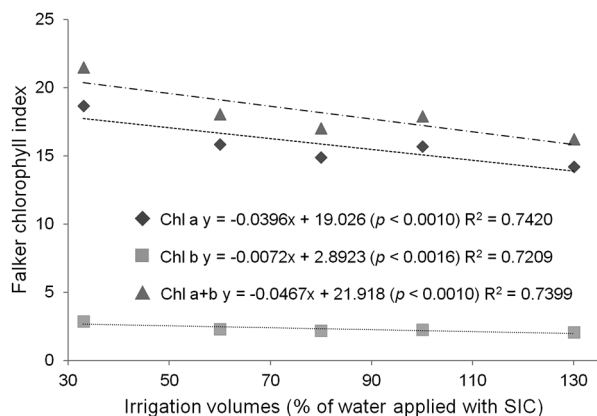


Figure 2 – The effect of different volumes of applied irrigation water using SIC on chlorophyll content measured on the 15th day after transplanting.

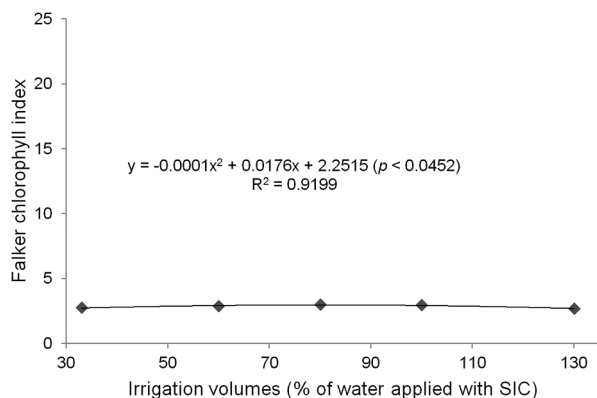


Figure 3 – The effect of different volumes of applied irrigation water using SIC on chlorophyll b measured on the 30th day after transplanting.

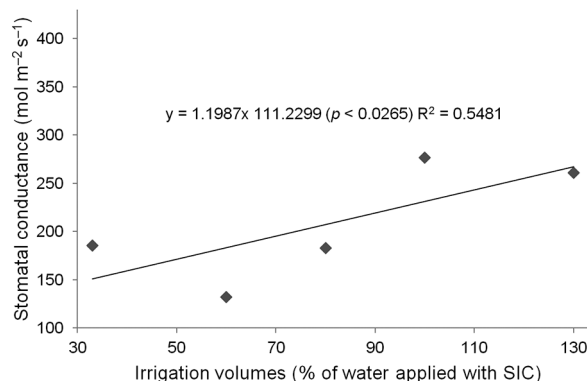


Figure 4 – The effect of different volumes of applied irrigation water using SIC on stomatal conductance values obtained on the 28th day after transplanting at 12h00.

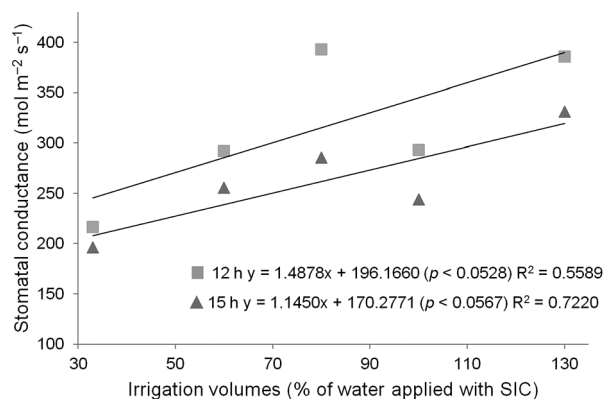


Figure 5 – The effect of different volumes of applied irrigation water using SIC on stomatal conductance values obtained at two different periods of the day –12h00 and 15h00 on the 38th day after transplanting.

lettuce in pots with Planosol (Batista et al., 2013). Thus, the data obtained showed consistency, since the tension used in this study is an average of tensions evaluated

by the previously mentioned authors. Soil water content had a direct influence on the fresh weight gain of the lettuce plants.

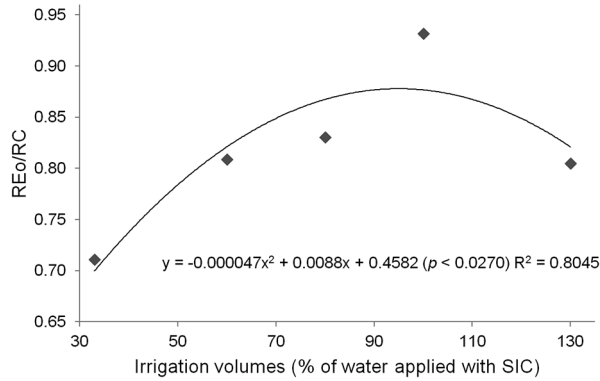


Figure 6 – The effect of different volumes of applied irrigation water using SIC on reception of electrons by PSI reaction center (Reo/RC) on the 15th day after transplanting.

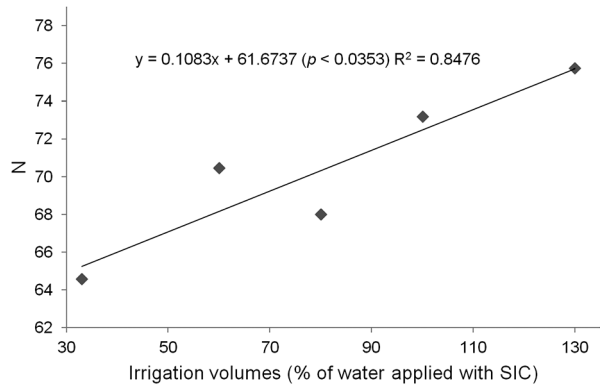


Figure 7 – The effect of different volumes of applied irrigation water using SIC on total number of electrons transferred to the electron transport chain (N) on the 15th day after transplanting.

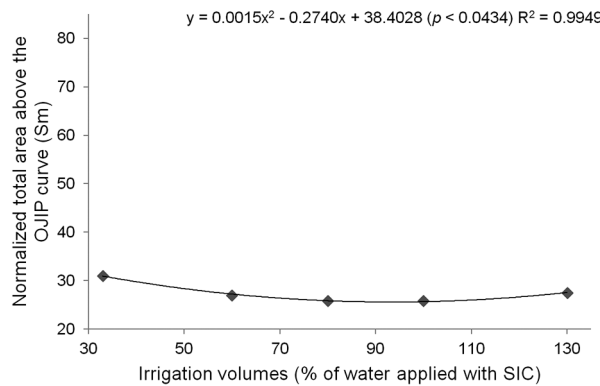


Figure 8 – The effect of different volumes of applied irrigation water using SIC on normalized total area above the OJIP curve (Sm) on the 31th day after transplanting in the morning.

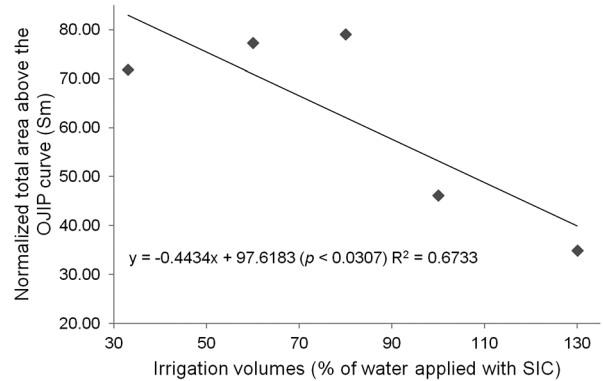


Figure 9 – The effect of different volumes of applied irrigation water using SIC on normalized total area above the OJIP curve (Sm) on the 32th day after transplanting in the afternoon.

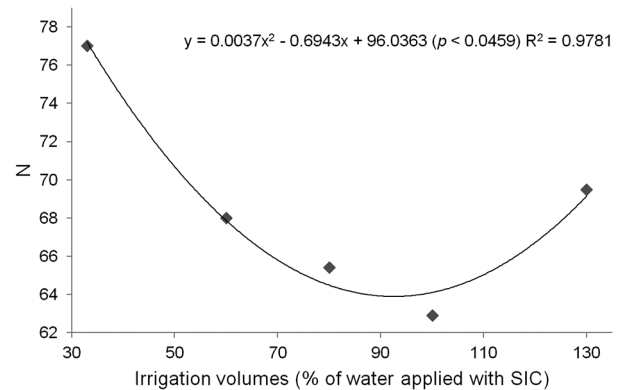


Figure 10 – The effect of different volumes of applied irrigation water using SIC on total number of electrons transferred to the electron transport chain (N) on the 31th day after transplanting in the morning.

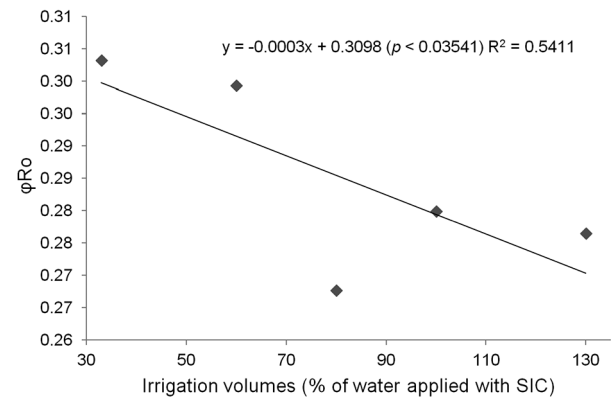


Figure 11 – The effect of different volumes of applied irrigation water using SIC on the quantum yield of electron transport Q_A to the final acceptor of electrons of PSI (ϕ_{Ro}) on the 31th day after transplanting in the morning.

Agronomic traits

Shoot fresh weight values (Table 1) observed in this study were close to those reported by Batista et al. (2013), who evaluated SIC efficiency in two soil types and at two tension levels (3.0 and 9.0 kPa).

WUE values (Table 1) obtained in this study are close to those reported in the literature (Unlukara et al., 2010; Alkhader and Rayyan, 2013; Batista et al., 2013) all with lettuce in the pot system. The values were also similar to the best values observed in a conventional farming field by Gomes et al. (2014) and Gonçalves et al. (2014). The WUE obtained in this study with the purpose of commercial lettuce cultivation system is close to the WUE from a very recent report with the same purpose, but using another sensor-based irrigation controller (Montesano et al., 2016).

It can be seen that the calculated volume for the maximum shoot dry weight (126 % SIC) is greater than the volume for the maximum WUE (83 % SIC); hence, there is a difference between the best volume for WUE and for dry weight gain. However, soil moisture values for these two treatments were very similar, which suggests that the application of severe drought becomes unnecessary to achieve water use efficiency. Water economy in agriculture can be achieved by keeping low soil water tension (Medici et al., 2014). Higher WUE without deficit irrigation was reported for *Pelagornium × hotorum* commonly used as an ornamental plant (Boyle et al., 2016). The authors also observed that the lowest water use due to stomatal closure resulted in a greater reduction of plant growth, leading to a lower WUE under deficit irrigation compared to well-watered plants. Montesano et al. (2016) reported the same behavior observed in this study, i.e., lower lettuce growth and higher WUE with light deficit irrigation. In this study, we recommend the use of irrigation volume of 80 % SIC, which provides a commercial production of lettuce plants with better WUE.

The agronomic data indicate the potential of the organic potponic system because the values are close to those reported in the literature, both for protected or field cultivations (Gomes et al., 2014; Gonçalves et al., 2014; Montesano et al., 2016). Commercial lettuce production spends about 250 L kg⁻¹ of water demands (Barbosa et al., 2015). Here, it was used only 30.45 L kg⁻¹, which is close to other studies conducted under precise control of irrigation conditions (Montesano et al., 2016).

Physiological traits

The chlorophyll content data measured on the 15th DAT (Figure 2) are consistent with other studies that showed increased chlorophyll content with drought (Weih et al., 2011; Rahimi et al., 2013), which is possibly the effect of concentration due to the lower growth observed for the leaves. Nevertheless, the literature reports the opposite, i.e., drought adversely affecting the chlorophyll content (Kiani et al., 2008; Massacci et al., 2008; Jaleel et al., 2009), and in these cases the drought is generally more severe than that imposed in the pres-

ent study. Mild drought can cause increased chlorophyll content due to lower leaf growth, while severe drought can lead to chlorophyll degradation.

The chlorophyll content on the 30th DAT (Figure 3) exhibited a similar behavior as mentioned for the 15th DAT. Therefore, as drought becomes more severe (irrigation lower than 88 % of that applied with SIC), the chlorophyll content begins to decrease, which is driven by some loss caused by a more severe deficit in water availability. Possibly, the fact that this is only evident at this stage of development indicates a greater sensitivity of adult plants to drought.

The gs decreased with the reduction of water applied and, consequently, when lower water contents were reached in the soil (minimum of 0.02 cm³ cm⁻³). This negative association between gs and soil water content is in agreement with the work of Kato and Okami (2011). A low gs value is an important diffusive limitation to photosynthesis (Flexas et al., 2004), and this explains, at least in part, the lowest dry weight values observed in less irrigated treatments. The gs on the 28th DAT was lower than on the 38th DAT for all treatments. Perhaps this was due to higher water vapor pressure deficit (VPD) values (approx. 4.6 kPa) on the 28th DAT (Klein et al., 2013; Hsie et al., 2015). The values of gs recorded here were close to those obtained for lettuce by Kim et al. (2004). The decrease in gs from 12h00 to 15h00 (Figure 5) was probably due to a small reduction in VPD and a sharp drop in global radiation, as observed for papaya seedlings from 12h00 to 15h00 (Dias et al., 2013).

Plant growth, photosynthesis and stomatal opening can be limited under water deficit due to regulation by physical and chemical signals (Xu et al., 2010). Moreover, the recovery of plants after a drought episode due to irrigation reestablishment has been well documented (Lloret et al., 2004; Gallé et al., 2007; Xu et al., 2010). The management of irrigation used in this study by the SIC installed in the pot with drippers of 8 L h⁻¹ allowed irrigation to be controlled by the amount water needed by the plants. This fact initially caused a deficit to the plants receiving less water, negatively affecting their growth. However, as time passed and better irrigated plants demanded more water due to their higher growth rate, smaller plants continued to proportionally receive irrigation according to the needs of the larger plants. Meaning that, 33 % SIC was temporally a well water treatment for the smaller plants allowing them to grow further. As smaller plants accelerate growth, the rate of 33 % SIC once again becomes a limiting factor, taking them back to the water stress scenario, in a cyclical behavior. Equivalent gs for all treatments on the 31st and 32nd DAT would support this interpretation, showing a recovery of lettuce plants after a drought episode followed by proportionally more irrigation, i.e., smaller plants effectively experienced cycles of drought and well water conditions despite the fact that during the duration of the whole experiment, the proportions of irrigation remained constant.

The drought and well-watered cycles experienced by smaller plants (33 % SIC) can be observed in the behavior of fluorescence parameters (S_m , N and ϕR_0) on the 31st and 32nd DAT. The fact that smaller plants showed the highest fluorescence values on those dates suggests that these plants were receiving plenty of water in comparison to their demand allowing them to further invest in the photosynthetic performance. Plants subjected to water stress, followed by hydration, showed that growth recovery, photosynthesis and stomatal opening depend on drought intensity and duration. These parameters may even surpass that of the control plants (Xu et al., 2010). In this study, there was no imposition of drought followed by rehydration on plants, different from Xu et al. (2010).

The maximum quantum yield of PSII primary photochemistry ($\phi P_0 = F_v/F_m$) is not necessarily an effective indicator for stress situations caused by water restriction, although this trait is commonly used as a stress indicator (Paoli et al., 2010; Bussotti et al., 2011). Yet, the lack of effect of irrigation volumes on this parameter on F_v/F_m may suggest that, even the most water-limited treatment tested, 33 % of SIC, was not a severe drought condition. The maintenance in the F_v/F_m ratio after the applied stress revealed that the maximum photochemical quantum yield remained high under drought, indicating that the electron transport chain was resistant to dehydration (Nar et al., 2009). Likewise, a situation of slight decrease in the quantum yield was observed during moderate drought for some of the tested varieties of *Hordeum vulgare* L., but with a severe drought, a decrease for all varieties analyzed was observed (Oukarroum et al., 2007).

In this study, water conservation in the leaves may have been at least partly due to stomatal closure. The RWC maintenance associated with g_s reduction and osmotic adjustment was reported for *Nicotiana glauca* (González et al., 2012). The high values of RWC exhibited in plants with low irrigation volumes here could also be due to the osmotic adjustment, which has been reported for lettuce (Lucini et al., 2015).

Further studies should be performed on organic potponics aiming to achieve the best levels of irrigation volume and organic manure for other lettuce and plant species. This kind of research is important for urban agriculture, where high efficiency is demanded for the use of water, manure, land and labor (Maheshwari et al., 2016).

Conclusions

We have explored a new cultivation system, named organic potponics, for lettuce using the SIC device to irrigation automation, adjusted to a tension of 6 kPa in the soil. Lettuce plants exhibited the greatest WUE for the irrigation volume of 83 % SIC, while provided a shoot dry weight in accordance to commercial plant standards. RWC was similar among the different treatments, showing that lettuce plants used stomatal

closure to reduce water losses and possibly osmotic adjustment. The level of water limitation imposed in this study caused a reduction of approximately 70 % in the shoot dry weight, while there was no serious loss in photosynthetic performance. The organic potponics is a promising system for plant cultivation with economy of water, manure and labor.

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