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Growth and gas exchange of okra under irrigation, organic fertilization and cover of soil

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The objective was to evaluate leaf area and gas exchange of okra plants for different irrigation depths, organic matter content and mulch. The activities were conducted in an experimental area of the State University of Paraíba, Campus IV, Catolé do Rocha-PB. An experimental design with randomized blocks, treatments distributed in a factorial 2 × 5 × 2 related to two irrigation levels, 100 and 50% of crop evapotranspiration (ETc), five levels of organic matter in the soil (1.8, 2.8, 3.8, 4.8 and 5.8%) and soil with and without mulch, totaling 20 treatments replicated in four blocks, was used. The experimental unit consisted of 27 plants. The increase of soil organic matter stimulated growth in leaf area and the gas exchange of okra plants. The irrigation with the smallest depth of water inhibited the expansion of leaf area, but provided a greater efficiency in okra's gas exchange. Mulch alleviated the reduced effects of irrigation water depth and stimulated the activity of okra gas exchanges.

Key words: *Abelmoschus esculentus*, physiology, leaf area.

INTRODUCTION

The okra (*Abelmoschus esculentus* (L.) Moench) is a very popular vegetable in tropical and subtropical regions. It belongs to the Malvaceae family due to the hardiness of plants and especially to the tolerance to heat, and it is a culture of easy handling (Oliveira et al., 2003).

This culture is a popular food with a high nutritional value and a great acceptance in the market. Small producers are the responsible for all Brazilian production (Paes et al., 2012). It is estimated that the world production of okra is about 5 to 6 million tons per year. This represents about 1.5% of total world plant production, with India as the largest producer of okra (Muralidharan and Rajendran, 2013). Brazil is characterized as a country with appropriate conditions for the cultivation of most vegetables with economic interest, including okra. However, this culture, like many others, depends on irrigation to obtain an economically viable production for all production regions of Brazil (Nascimento et al., 2013).

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According to Al-Harbi et al. (2008), with high temperatures and low relative humidity, the rational use of irrigation is an essential factor to achieve a high fruit yield, considering that water stress, either by deficit or excess, may compromise okra's production capacity (Abd El-Kader et al., 2010). Due to the growing preference by consumers, a significant increase in okra crop in Brazil has been reported, mainly in the states of Rio de Janeiro, São Paulo and Sergipe (Cavalcante et al., 2010). This expansion expresses the need for studies that report irrigation managements of okra, as well as the use of technologies that minimize the effects of water stress in order to promote yields with economic viability.

As to irrigation management, the basis for the quantification of water to be applied in a given culture is commonly associated with the field capacity of soil and vegetation surfaces to lose water to the atmosphere. The usual way to quantify the volume of water to be applied throughout the cycle is to consider the processes of evaporation and plant transpiration together. This is called evapotranspiration (Rao and Smith, 2006). It can be altered with the use of practices aiming to reduce water loss by evaporation. Mulch in soil surface with vegetable or plastic material keeps the soil wet and less heated and reduces the effect of water losses by evaporation (Teófilo et al., 2012). It reduces thereby the effects of water deficit on plants. It also adds organic material to the soil, given that it acts on soil's water retention and contributes to the nutrition of the plant.

Cavalcante et al. (2010), working with an organic fertilizer with okra, observed that chicken manure provides a larger foliar calcium, phosphorus and magnesium content; goat manure increased potassium accumulation; and bovine manure increased nitrogen in okra leaves. This information agrees with that of Oliveira et al. (2013), who conclude that bovine manure can be used as an alternative organic fertilizer for okra crop. The authors report satisfactory responses of crop production with the use of bovine manure at the dosage of 60 $t.ha^{-1}$. However, the increase in soil organic matter content has not been the research focus for this culture.

One of the main ways of evaluating responses of plants under different water availability conditions are the parameters of gas exchange, because 90% of organic production of plants occurs in response to photosynthetic activity (Floss, 2004) and is directly related to stomatal resistance, which may cause a decrease in net photosynthesis (Silva et al., 2014). Thus, gas exchanges constitute an important tool in determining adaptation and stability of plants to certain environmental conditions. Considering the above, the objective was to evaluate leaf area and gas exchange of okra plants for different irrigation depths, organic matter content and mulch.

MATERIALS AND METHODS

The study was conducted from November 2013 to April 2014 in Paraíba State University, Campus IV, sector of Agroecology, in the municipality of Catolé do Rocha (6°20'38" S, 37°44'48" W and altitude of 270 m), Paraíba, Brazil.

The climate is BSw'h', according to the Koppen classification, characterized by a hot semi-arid climate, with two distinct seasons, one rainy with irregular rainfall and one without precipitation. The average annual rainfall is 800 mm and the average temperature is 27°C, with the rainy season concentrated between the months of February and April. The climatic conditions on site during the experiment obtained from the meteorological station of UEPB, Campus IV, were air temperature: 31°C, temperature of soil protected with mulch: 28°C, and soil in the open: 35°C, relative humidity: 80%, and precipitation: 416 mm.

The soil was classified as Eutrophic Fluvic Neosol (EMBRAPA, 2011) and, in the first 20 cm of depth, it had 661, 213, 126 and 42 $g.kg^{-1}$ of sand, silt, total clay and clay, respectively, dispersed in water or natural clay, soil density and with particles 1.51 and 2.76 $g.cm^{-3}$, respectively, with a total porosity of 0.45 $m^3 m^{-3}$. Humidity values at the level of field capacity, permanent wilting point and available water are 23.52, 7.35 and 16.17%, respectively. As for the chemical composition, the soil, at the same depth, had pH = 7.02; P and K = 53 and 297 $mg.dm^{-3}$; Na^+ = 0.3; Ca^{2+} = 4.63; Mg^{2+} = 2.39; Al^{3+}= 0.0, H^+= 0.0 and CEC = 8.08 $cmol_c\ dm^{-3}$; base saturation V = 100% and OM = 1.8%. Values were obtained using the methods suggested by EMBRAPA (2011).

The sowing was made on November 15, 2013, with five seeds of cultivar Santa Cruz 47 per hill; the thinning was made when the plants had three true leaves, which took place on December 04, 2013, resulting in one plant per hill. The adopted spacing was 1 meter between rows and 0.4 m between plants. The experimental design was randomized complete blocks with a factorial 2 × 5 × 2 related to two water depths [100 and 50% of crop evapotranspiration (ETc)], five levels of soil organic matter (1.8, 2.8, 3.8, 4.8 and 5.8%) with and without mulch, using parsley plant residues (*Ipomoea asarifolia*), with a 5 cm thick layer, resulting in 20 treatments, replicated in four blocks, totaling 80 plots. Thus, each plot is a treatment that consisted of three rows 3.2 m long and 2 m wide, with rows spaced by 1 m, making a total area of 6.4 m^2. Each line with nine plants totals 27 per plot.

The plants were planted in hills with 30 cm × 30 cm × 30 cm, prepared with ground material in its first 30 cm with bovine manure, which had the following chemical characteristics: N = 14.29 $g.Kg^{-1}$; P = 2.57 $g.Kg^{-1}$; K = 16.79 $g.Kg^{-1}$; Na^+ = 5.59 $g.Kg^{-1}$; Ca^{2+} = 15.55 $g.Kg^{-1}$; Mg^{2+} = 4.02 $g.Kg^{-1}$; OM = 39.60%. This was applied incorporated to the soil considering the treatments that is, considering the values needed to raise the content of organic matter in the soil from 1.8 to 2.8, 3.8, 4.8 and 5.8% of the content of soil organic matter. A dosage of 16 $g.pit^{-1}$ of superphosphate was added, calculated according to the content existing in the soil and considering the recommendation of Ribeiro et al. (1999).

The amount of organic matter incorporated into the soil with a density (ds) of 1.51 $g.dm^{-1}$ in pits with 27 L (27,000 cm^3) and content of organic matter in bovine manure is of 506 $g.kg^{-1}$. It was calculated based on Equation 1, corresponding to quantities of 1,700.00, 3,400.00, 5,100.00 and 6,800.00 $g.pit^{-1}$ to raise the content of organic matter in the soil of 1.8 to 2.8, 3.8 4.8 and 5.8%, respectively.

$$M = (DOMI - DOMES)\ Pv*ds/OMCBM \qquad (1)$$

Where: M = amount of organic matter to be applied per hill ($g.kg^{-1}$); DOMI = dosage of organic matter to be increased ($g.kg^{-1}$); DOMES = dosage of organic matter in soil ($g.kg^{-1}$); OMCBM = organic matter content in bovine manure ($g.kg^{-1}$) and Pv = Pit volume (dm^3)

The formation and production fertilization of the culture was made based on soil analysis, with nitrogen and potassium applications plotted at twenty, forty and sixty days after sowing. Nitrogen was supplied at a dosage of 4 g per plant using

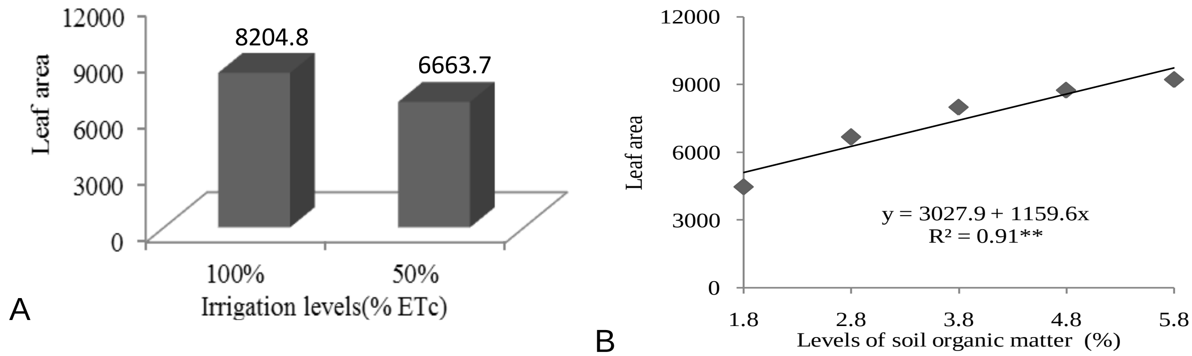

Figure 1. Leaf area (LA) (cm^2) of okra plants at 60 days after planting, under irrigation levels (A) and Levels of soil organic matter (B).

ammonium sulphate (21% N) as a source, and potassium derived from KCl was applied at a dosage of 3 g of potassium chloride per plant.

Irrigation of plants was carried out with a drip irrigation system. The irrigation water depth was calculated considering the evapotranspiration (ETc) of the culture, calculated by multiplying reference evapotranspiration, obtained by the method of Class A tank, and crop coefficient (Kc), considering the development stage of the plant. Thus, the consumptive use (Cu) was obtained, which was multiplied by the percent of wet area (P) = 40% in order to determine the daily irrigation depth (DID) used for the calculation of the depths in each treatment, i.e., for L1, 100% DID was applied, and for L2, 50% DID was applied.

At 60 days after planting (DAP), gas exchange in fully developed leaves were determined from 8:00 to 10:00 am, moment when the internal concentration of CO_2 (*Ci)* (µmol $m^{-2}.s^{-1}$), transpiration (*E*) (mmol of H_2O $m^{-2}.s^{-1}$), stomatal conductance (*gs*) (mmol of H_2O $m^{-2}.s^{-1}$) and assimilation rate of CO_2 (*A*) (µmol $m^{-2}.s^{-1}$) were determined. With these data, water use efficiency (*WUE*) (*A*/*T*) [(µmol $m^{-2}.s^{-1}$) (mol H_2O $m^{-2}.s^{-1}$)$^{-1}$] and instantaneous efficiency of carboxylation Φc (*A*/*Ci*) (Brito et al., 2012) were quantified. For the measurements, portable photosynthesis measurement equipment, model "LCPro+" from ADC BioScientific Ltda., was used with a constant light source of 1,200 µmol photons $m^{-2}.s^{-1}$.

The leaf area was estimated in six photosynthetically active leaves from three central plants of each of the three lines at 60 DAP through a nondestructive method, multiplying length (L) by the greater width (W) (Santos et al., 2005). From each plant, the most expanded leaf of the six evaluated was collected to obtain actual area with a staining and divided by the estimated area (L x W). The obtained value is the correction factor that was multiplied by the estimated area to obtain the most likely area of plants.

Data were evaluated by analysis of variance; the qualitative factors were compared by 'T' test at 5% probability, and the quantitative factor was evaluated by regression analysis. Analyses were performed using the computer software SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

The interactions between irrigation depth, organic matter content, soil mulch coverage and organic matter added to the soil did not exert significant effects on any analyzed variable. The interactions between organic matter and soil cover interfered significantly with internal concentration of CO_2 (*Ci*), plant transpiration (*E*), stomatal conductance (*gs*), water use efficiency (WUE) and instantaneous carboxylation efficiency (Φc) (Figures 2, 4 and 5). All variables responded to the isolated effect of irrigation depths, except CO_2 assimilation rates and water use efficiency (WUE) (Figures 2, 4 and 5). The leaf area (LA) and CO_2 assimilation rate (*A*) responded to the increase in levels of soil organic matter (Figures 1 and 2).

Leaf area increased with increased water depths in plants and soil organic matter content in the soil (Figure 1). The reduction of irrigation depths decreased leaf area expansion from 8.204.8 to 6.663.7 cm^2, equivalent to an 18.78% loss in plants irrigated with 100 and 50% ETc (Figure 1A). This reduction is the effect of soil water deficit, reducing the availability of water and compromising plant's physiological activity, as shown in Figure 2.

The increase in organic matter levels caused a linear increase in okra's leaf area of up to 1,159.6 cm^2 for each 1% increase in soil organic matter content (Figure 1B). As observed for net assimilation rate (Figure 2), the increase in organic matter content in the soil favored a higher synthesis of photosynthates or assimilates, possibly because it keeps the soil with better physicochemical and water conditions, resulting in a higher availability of nutrients to plants, stimulating vegetative growth such as okra's leaf area (Figure 1B).

As for CO_2 assimilation rate, there is a significant influence only for soil organic matter content, a linear increase influence with a unit increase of 1,315 µmol $m^{-2}.s^{-1}$ for each 1% increase in soil organic matter content (Figure 3). Possibly, these results occur due to a higher supply of nutrients provided by organic matter, especially nitrogen and potassium, favoring the synthesis of photoassimilates by okra plants and thereby providing a greater plant growth due to a linear increase behavior of leaf area of okra plants in function of soil organic matter content (Figure 1B). It is possible to observe that the values of okra's CO_2 assimilation rate stood at 16.06 and 21.43 µmol $m^{-2}.s^{-1}$, normal values for C3 plants (Taiz and

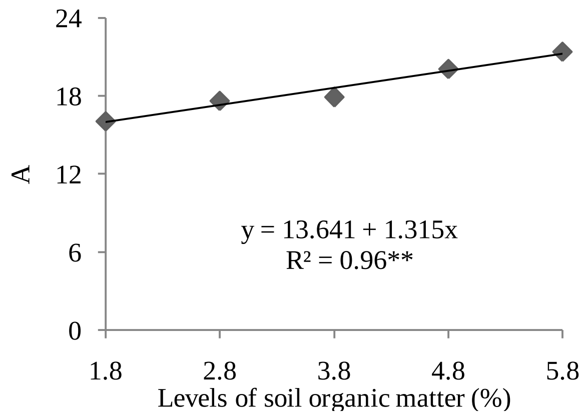

Figure 2. CO_2 assimilation rate (*A*) (µmol m^{-2} s^{-1}) of the okra plants at 60 days after planting under levels of soil organic matter.

Zeiger, 2013).

The reduction of water depth from 100 to 50% ETc reduced the internal concentration of CO_2 by 10.2% (Figure 3A).

This decline is the response of stomatal closure as a protective measure, resulting consequently in the reduction of CO_2 influx to the substomatic camera, since a significant reduction in stomatal conductance of plants grown under this water depth was reported (Figure 3E).

This also happened to the increase of activity of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) (Machado et al., 2005), thereby increasing the consumption of CO_2, since an increased efficiency in plants carboxylation grown under 50% ETc depth was observed. Thus, it is believed that the stomatal closure associated with an increased activity of RuBisCO directly reduces the internal concentration of CO_2. Soares et al. (2012a) observed a similar behavior with tomato plants in the vegetative phase, where plants with lower water availability had lower internal concentrations of CO_2.

Figure 3B shows an increased internal concentration of CO_2 in plants grown without mulch. It was also observed that, in plants grown without mulch, when grown in a soil with organic matter content exceeding the estimated value of 2.96% (Figure 3B), there were reductions in internal concentrations of CO_2, possibly due to the increase in the photosynthetic activity of plants. This can be verified by studying instantaneous efficiency of carboxylation.

For okra plants grown with mulch, reductions in the internal concentration of CO_2 up to 3.65% of organic matter were observed (Figure 3B). However, in plants grown in a soil containing 3.65% more organic matter, there was an increase in the internal concentration of CO_2, possibly due to increased water availability in the soil, which favored the increase of stomatal activity and, due to the opening of the stomata, a larger influx of CO_2 and consequently a greater *Ci* (Figure 3B).

It is observed that, when grown with mulch, plants have the lowest levels of organic matter in the soil and a higher consumption of CO_2. It denotes, therefore, that mulch is a good alternative for okra crop in semi-arid regions, reducing initial spending on fertilizer application and contributing to a greater physiological efficiency due to the increase in CO_2 assimilation (Figure 2).

The reduction of water depth from 100 to 50% ETc caused an increased plant transpiration, from 3.26 to 3.43 mmol H_2O $m^{-2}.s^{-1}$, with a 5.2% superiority (Figure 3C). This happens despite the higher opening of stomata expressed by the stomatal conductance observed in plants irrigated with 100% ETc in relation to those irrigated with 50% ETc (Figure 3E), possibly due to a lower soil water availability, limiting the absorption of water and nutrients. Otherwise, increased transpiration is related to the reduction of heat stress in the plant (Brito et al., 2012; Taiz and Zeiger, 2013).

Data from transpiration of plants grown in soil without mulch did not adjust to any regression model. Therefore, they were represented by the average of 3.386 mmol of H_2O $m^{-2}.s^{-1}$, independent of the organic matter content. On the other hand, in soil with mulch, the addition of organic matter stimulated an linear increase in transpiration of plants of 0.177 mmol of H_2O $m^{-2}.s^{-1}$ per increase unit of organic feedstock (Figure 2D). This increase is the response of greater soil water availability due to reduced losses by evaporation related to soil cover.

The reduction of irrigation water depth inhibited stomatal conductance from 0.39 to 0.31 mmol H_2O $m^{-2}.s^{-1}$, corresponding to a 20.5% decrease among plants irrigated with 100 and 50% of okra evapotranspiration (Figure 2C). Perhaps this result is related to the fact that plants under the depth of 100% ETc have higher water availability, not causing limitation to stomatal opening, as observed by Melo et al. (2010) with watermelons.

However, these results are related to a smaller leaf

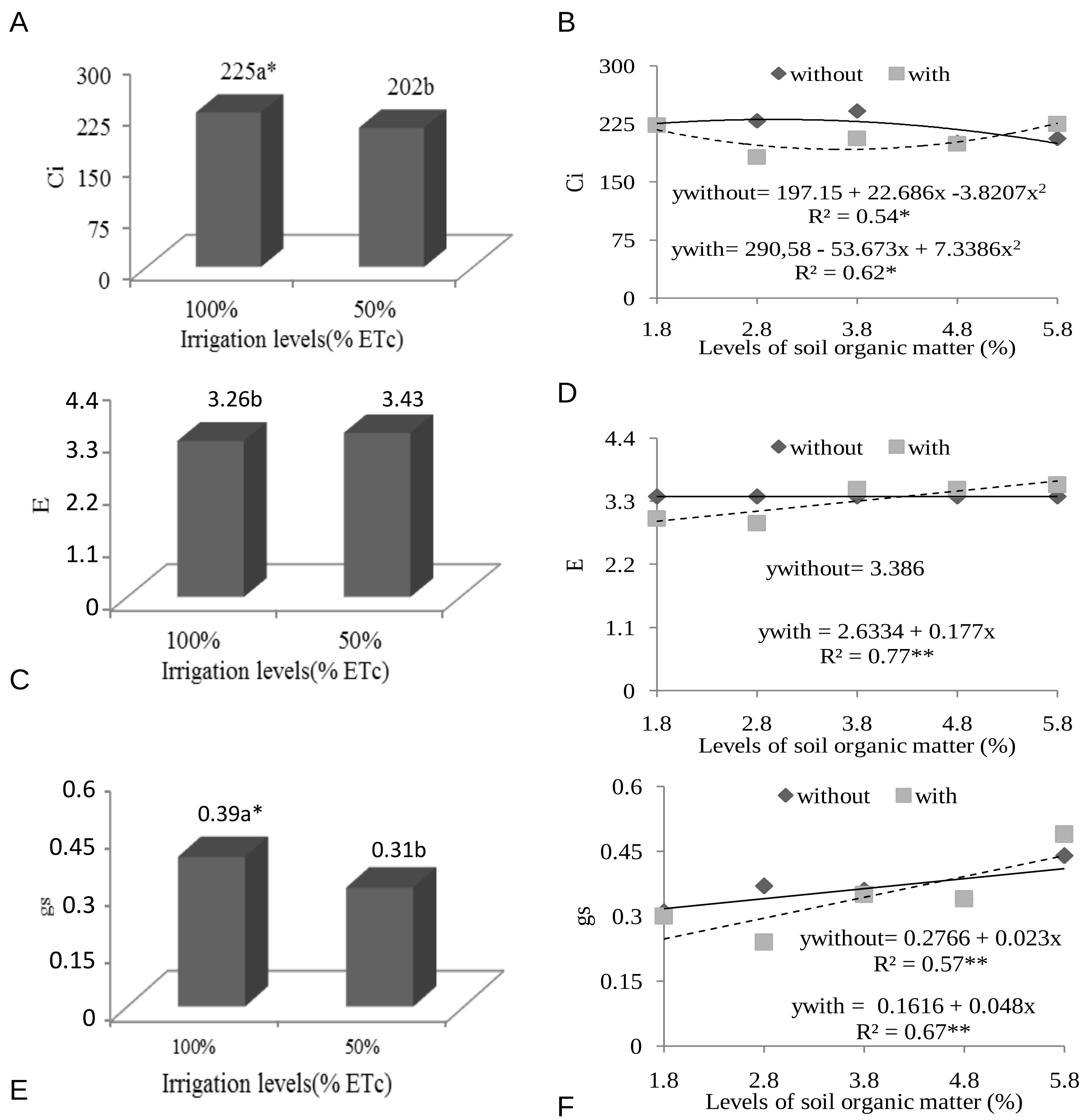

Figure 3. Internal CO_2 concentration (*Ci*) (µmol m^{-2} s^{-1}) (A and B), transpiration (*E*) (mmol de H_2O m^{-2} s^{-1}) (C and D) and stomatal conductance (*gs*) (mmol de H_2O m^{-2} s^{-1}) (E and F) in okra at 60 days after planting, under irrigation levels (A, C and E), ground cover and levels of soil organic matter (B, D and F).

area observed in plants grown under the water depth of 50% ETc, which can be regarded as a species mechanism, even under low water availability conditions, to reduce the aggressiveness of water stress (Figure 1A). Considering that stomata are responsible for regulating the activity of gas exchange (Silva et al., 2014), it is believed that there was no water restriction because no significant effects of the water depth factor were observed neither for CO_2 assimilation rates nor for efficient use of water (Figures 3 and 4).

In plants grown in soil without mulch, there was an increase in stomatal conductance due to the increase of organic matter in the soil. A linear and increasing behavior was noted with the increase of 0,023 mmol H_2O $m^{-2}.s^{-1}$ in response to a unit increase of organic matter content in the soil (Figure 2D). This response may be related to increased availability of water to plants due to the retention of organic matter in the soil, thus favoring stomatal opening and increase of photosynthetic activity and water use efficiency (Figure 3 and 4).

In relation to plants grown with crop cover, there is a linear and increasing behavior of stomatal conductance. A

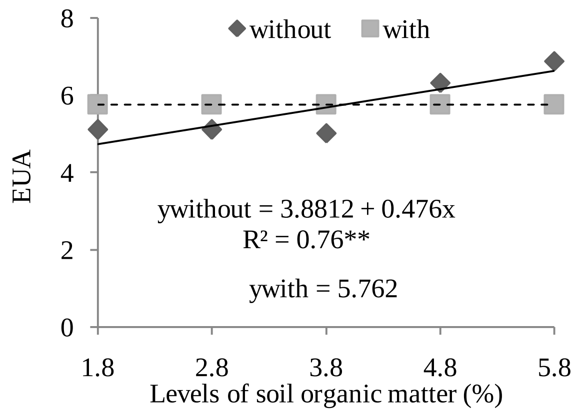

Figure 4. Water use efficiency (*WUE*) [(µmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$] in okra at 60 days after planting, under ground cover and levels of soil organic matter.

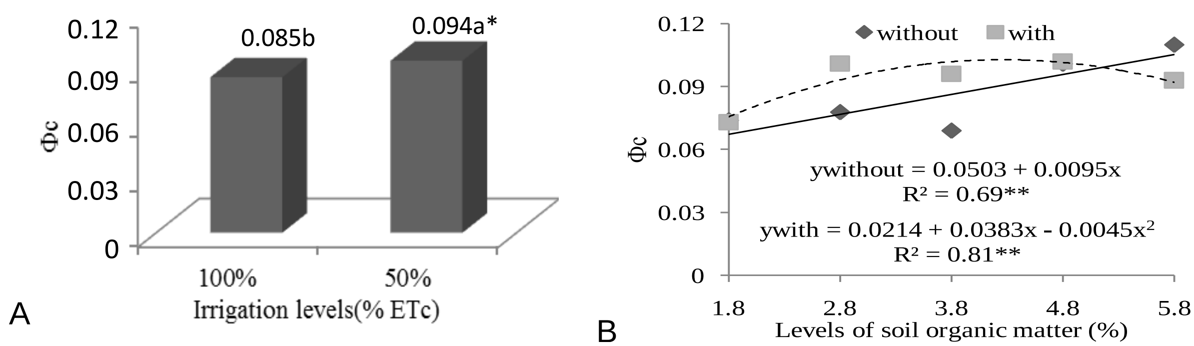

Figure 5. Instantaneous efficiency of carboxylation (Φc) [(µmol m^{-2} s^{-1}) (µmol m^{-2} s^{-1})$^{-1}$] in okra at 60 days after planting, under irrigation levels (A), ground cover and levels of soil organic matter (B).

verified unit increase of 0.048 mmol of H_2O $m^{-2}.s^{-1}$ was due to organic matter content in the soil. However, the results obtained with this cropping system were lower than those observed for the uncovered crop in the first three levels of organic matter (1.8, 2.8 and 3.8%), then exceeding from the fourth level on (4.8) (Figure 2D). Possibly, the increase of organic matter content has served as a stimulus for the plants in the treatment without coverage due to the greater availability of water and nutrients in three organic matter contents, that is, 1.8, 2.8 and 3.8%. This does not occur with plants grown in soil with a cover crop possibly due to the abundant availability of water in the soil in relation to the ground without a cover crop. Thus, the increased stomatal conductance of these treatments was due to the need of absorption of nutrients in order to increase photosynthetic activity of plants (Figure 2D), since there was a positive response of CO_2 assimilation rate due to the increase in soil organic matter (Figure 3).

It is observed that plants grown with mulch did not differ regarding water use efficiency due to the content of organic matter in the soil, with an average of 5.762 (µmol $m^{-2}.s^{-1}$) (mol H_2O $m^{-2}.s^{-1}$)$^{-1}$ (Figure 4). These results show the lack of water stress over okra plants with a crop cover, and the increase of stomatal conductance was due to the need for a greater CO_2 influx to regulate the photosynthetic process, since there were no changes in water use efficiency (Figures 2 to 4).

In soil without cover, a linear and increasing behavior for efficient use of water was observed. This was due to the content of organic matter in the soil, with a unit increase of 0.476 (µmol $m^{-2}.s^{-1}$) (mol H_2O $m^{-2}.s^{-1}$)$^{-1}$ for each 1% increase in the content of soil organic matter (Figure 4). This is due to the increase of photosynthetic activity observed with increased soil organic matter, contributing to okra nutrition and water retention in the soil, favoring the increase of CO_2 assimilation rates. This favored water use efficiency, given that no statistical difference for transpiration in function of organic matter was observed for okra plants grown in this culture system (Figure 2 to 4). Soares et al. (2012b) observed a divergent behavior with tomato plants during the blooming phase, where, despite the linear behavior of CO_2 assimilation rates, it was not enough to offset the losses by transpiration. This reflects the adaptive capacity of okra to losses by transpiration. Okra is more efficient regarding water use if compared to tomato.

The instantaneous carboxylation efficiency explains the CO_2 inflow process occurring in the substomatic chamber in relation to the amount of CO_2 absorbed in the photosynthetic process (Taiz and Zeiger, 2013). Plants irrigated with the lowest water level (50% ETc) were more efficient regarding the carboxylation process than plants grown under a 100% ETc (Figure 5). Considering that the instantaneous carboxylation efficiency expresses the efficiency of CO_2 inflow process occurring in the substomatic chamber in relation to the amount of CO_2 assimilated in the photosynthetic process, the lower concentration of CO_2 observed in the 50% ETc depth is related to a greater consumption by the RuBisCO enzyme, increasing photosynthetic activity and instantaneous carboxylation efficiency (Figure 5). Perhaps the increased instantaneous carboxylation efficiency in plants grown under 50% ETc depth is influenced by a smaller leaf area obtained by okra plants grown under this water depth. An increase of photosynthetic activity of each photosynthetic active area is necessary to maintain okra plant vegetative growth (Figure 5A).

Soares et al. (2012a), studying gas exchanges of tomato during the vegetative stage of culture, found that the carboxylation efficiency of these plants reduced as they approached a 100% real evapotranspiration. This corroborates what was observed in this study, that is, this may be related to the reduction of photosystem II efficiency, following Suassuna et al. (2011), who verified reductions in efficiency of photosystem II greater than 80% considering evapotranspiration in melons.

It is also observed that okra plants grown without mulch showed a linear and increasing response to the increase of organic matter content in the soil (Figure 5B). This fact can be related to the linear response of CO_2 assimilation rate, possibly due to a higher nutritional intake provided by organic matter. As for plants grown under mulch, it is observed that they showed a quadratic response to the increase of organic matter in the soil, reaching an instantaneous efficiency of carboxylation peak when grown in a soil with organic matter content at 4.25%, with 10.3 [(μmol m^{-2} s^{-1}) [(μmol m^{-2} s^{-1})$^{-1}$], which decreased thereafter (Figure 5B).

Conclusions

The increase of soil organic matter levels caused a growth in gas exchange and a growth of okra plants. The reduction of irrigation water depth from 100 to 50% of crop evapotranspiration inhibited the expansion of leaf area, but stimulated okra's gas exchanges, especially when combined with a cover crop, although smaller plants irrigated with 50% ETc were more efficient regarding water use.

Conflict of Interest

The authors have not declared any conflict of interest.

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