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Production and photosynthetic activity of Mimosa Verde and Mimosa Roxa lettuce in two farming systems[1]

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ABSTRACT

Lettuce (*Lactuca sativa* L.) is the most commonly consumed leaf vegetable in the Brazilian diet, and it is a good source of vitamins and minerals. It is widely grown in the conventional farming system. However, the hydroponic farming system has been gaining importance in the market, wining confidence from consumers, who are becoming increasingly more demanding on food quality. The objective of this study was to evaluate the performance of two lettuce cultivars on hydroponic and conventional farming systems for the production of fresh mass (FM) and dry mass (DM), photosynthesis, contents of chlorophyll and anthocyanin. The following two experiments were carried out: hydroponics farming (HF) and conventional farming (CF), performed in protect and unprotect environments, respectively, in Florianópolis, SC. Mimosa Verde cultivar (MV) showed greater fresh mass than Mimosa Roxa (MR), in both farming systems and the two cultivars presented better performance in the hydroponic system (287.7 g MV and 139.1 g MR) than the conventional system (129.7 g MV and 111.8 g MR). Mimosa Verde cultivar presented lower average contents of total chlorophyll (7.7 mg g^{-1} FM) than Mimosa Roxa (11.8 mg g^{-1}FM), and both cultivars displayed higher means for this variable in the hydroponic farming system. Mimosa Roxa presented higher contents of anthocyanin in the conventional system (88.24 mg g^{-1} FM) than the ones in the hydroponic system (36.89 mg g^{-1} FM). The best results for CO_2 net assimilation rate regarded to photosyntheticaly active photon flux density were found in the hydroponic system, for both lettuce cultivars. Variation in the contents of chlorophyll were also found. Those variations were higher in the protected system than in the hydroponic system and contents of anthocyanin were higher in the conventional system.

Key words: biomass, *Lactuca sativa*, photosynthesis, anthocianin.

RESUMO

Produção e atividade fotossintética de alface Mimosa Verde e Roxa em dois sistemas de cultivo

A alface (*Lactuca sativa* L.) é a hortaliça folhosa mais consumida na alimentação do brasileiro, sendo boa fonte de vitaminas e de sais minerais. É largamente produzida no sistema convencional. No entanto, o cultivo hidropônico vem ganhando espaço no mercado, conquistando a confiança dos consumidores, cada vez mais exigentes com a qualidade dos alimentos. O objetivo deste trabalho foi avaliar o desempenho de duas cultivares de alface, nos sistemas de cultivo hidropônico e convencional, para a produção de massa fresca (MF) e massa seca (MS), fotossíntese, teores de

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antocianinas e de clorofilas. Foram conduzidos dois experimentos: um no sistema de cultivo hidropônico (CH) e outro em cultivo convencional (no solo) (CC), realizados em ambientes protegido e desprotegido, respectivamente, no município de Florianópolis, SC. A cultivar Mimosa Verde (MV) apresentou maior peso de massa fresca, em comparação com Mimosa Roxa (MR), em ambos os sistemas de produção, e ambas as cultivares apresentaram melhor desempenho no sistema hidropônico (287,7 g MV e 139,1 g MR), em relação ao convencional (129,7 g MV e 111,8 g MR). A cultivar MV apresentou menores teores médios de clorofila total (7,7 mg g^{-1} MF), quando comparada com MR (11,8 mg g^{-1} MF) e ambas apresentaram teores médios mais elevados dessa variável no cultivo hidropônico. Para a cultivar MR, os teores de antocianinas, no cultivo convencional, foram superiores (88,24 mg g^{-1} MF) aos do cultivo hidropônico (36,89 mg g^{-1} MF). Os melhores resultados para a taxa de assimilação líquida de CO_2, com relação à densidade de fluxo de fótons fotossinteticamente ativos, foram observados no cultivo hidropônico, para ambas as cultivares. Também se observou variação nos teores de clorofila, que foram maiores no ambiente protegido do sistema hidropônico, e antocianinas, que foram maiores no cultivo convencional.

Palavras-chave: antocianina, *Lactuca sativa*, fotossíntese, biomassa.

INTRODUCTION

The acknowledge of quality of life by the world population has increased the demand for healthy products (Neto *et al*., 2011). Lettuce (*Lactuca sativa* L.) is the leafy vegetable most consumed in the Brazilian diet because of its pleasant flavor, low price and for being a source of vitamins and minerals (Soares, 2002).

Lettuce is most grown in the conventional farming system, where this vegetable is grown directly in the soil, in a limited period of the year, with no restrictions on the use of chemical fertilizers and pesticides (Campos, 2005). However, demands by the consumers have increased, requiring the production of good quality lettuce all over the year. Therefore, the hydroponic farming, usually associated with the protected environment, have gained ground in the productive sector and conquered the preference of the consumer.

In addition to the reasons associated with the consumer profile, the hydroponic farming in protected environment is becoming more important due to the following factors: better use of the cultivation area, early harvest, more efficient nutrient use, better sanitary quality of the product and the possibility of controlling abiotic factors that restrict farming in certain seasons (Santos *et al*., 2008). Another positive aspect of such farming system is its potential contribution to the development of urban agriculture and household food production.

However, it is known that the photosynthetic process and the contraction of pigments in plants is directly affected by the quality of light, CO_2 concentration, mineral nutrition and temperature (Taiz & Zeiger, 2009). Thus, studies on farming systems and the understanding of the physiological and biochemical issues related to the quality and yield performance of crops are important aspects to consider for the adoption of the best suited farming system for each market segment.

The intake of chlorophyll as a component of the human diet contributes to tissue growth, acting as a substance promoting the multiplication of fibroblasts, which are connective tissue cells, responsible for the healing process (Tanaka, 1997). According to Hagiwara *et al*. (2001), anthocyanins have important biological activities for the human health, distinguishing themselves as being anticarcinogenic, antioxidant and antiviral. However, there are few studies relating the levels of anthocyanins and chlorophylls with morphophysiological performance of lettuce.

The objective of this study was to evaluate the performance of two lettuce cultivars in hydroponic and conventional farming systems for biomass production (dry mass and fresh mass), photosynthesis, contents of anthocyanins and chlorophylls.

MATERIAL AND METHODS

Two experiments were conducted to evaluate the performance of two lettuce cultivars (Mimosa Rosa Grenadine of Vilmorin®; Mimosa Verde Salad Bowl, Sakata ®) in hydroponic (HF) and conventional (soil) (CF) farming systems, carried out in protected and unprotected environments, respectively, in Florianópolis, SC.

Regarding the conventional farming (CF), an experimental area was used in conditions of open-field (unprotected) farming, located at the Centro de Treinamento (CETRE) of the Empresa de PesquisaAgropecuária e Extensão Rural de Santa Catarina –EPAGRI. It was used a complete random block experimental design with two treatments (lettuce cultivars) and four replications. The experimental plot consisted of four 2.4 m long rows, spaced by 0.40 m from each other. Each row consisted of seven plants, 0.40 m apart, setting a density of 6.25 plants m². The useful area of the plot (1.6 m^2) was formed by the two inner rows, excluding a plant from each end. Fertilization was performed according to the recommendation of the Manual of Fertilization and Liming for the states of Rio Grande do Sul and Santa Catarina for lettuce crop (CQFS-RS/SC, 2004).

The hydroponic farming was carried out using the technique of the Laminar Flow of Nutrients (LFT) in the area assigned for the protected farming in the Hydroponics Laboratory (LabHibro) of the Agricultural Sciences Center (CCA) of UFSC, from September to October, 2011. The protected environment was provided by a bow-shaped greenhouse, with metal frame, with a ceiling height of 2.70 m and covered with transparent, diffusor type low density polyethylene film (LDPE), with 150 microns of thickness and UV treatment, with no gadgets to control humidity and temperature. It was used a completely randomized block experimental design with two treatments (lettuce cultivars) evaluated in experimental units with four plants and four replications. Plants were distributed in the final production bench, comprised of cultivation channels spaced by 0.25 m between each other and 0.25 m between holes. Planting density in this system was 16 plants m^{-2}. The electrical conductivity of the cultivation solution was kept between 1.85 and 2.00 dS/m (deciSiemens per meter), being corrected every two days. The nutrient solution used was adapted from Furlani (1999), according to Barcelos-Oliveira (2008), comprised by the following nutrients: special Hydro® calcium nitrate (750 ppm), potassium nitrate (500 ppm), purified MAP (mono-ammonium phosphate) (150 ppm), magnesiumsulphate (400 ppm), copper sulphateat a concentration of 13% (0.15 ppm), zinc sulfate at a concentration of 22% (0.50 ppm) manganese sulfate at a concentration of 26% (1.50 ppm), boric acid at a concentration of 17% (1.50 ppm), sodium molybdateat a concentration of 39% (0.15 ppm) and HydroFerro® (30 ppm).

Mimosa Roxa (MR) and Mimosa Verde (MV) cultivars commercial pelleted seeds with 100% purity and 96% germination were used in both trials. The seeds were germinated in phenolic foam and maintained in a hydroponics system for 29 days, until transplantation to their respective farming systems. During this period, the seedlings received the nutrient solution formulated by Furlani (1999) and the electrical conductivity of the solution was maintained between 0.90 and 1.40 dS/m.

After lettuce growing period (63 days after sowing in the hydroponics system; 77 days after sowing in the conventional system), it was carried out the determination of total fresh mass (FM) and dry mass (DS), fresh root mass (FRM) and fresh leaf mass (FLM) and the number of leaves per plant (NL) was counted. For the determination of total fresh mass (FM) and dry mass (DM), a sample of two plants of the useful area of the plot of each treatment was harvested and weighed on a precision scale before and after drying. Drying was performed in an oven at 60°C for 72 hours, and weighing was made when the weight was constant. The percentage of DM was obtained by the relationship between the dry mass and fresh mass (Schena *et al*., 2007). The number of leaves per plant was counted manually in two plants sampled from each treatment.

Total concentration of chlorophyll was determined in healthy and complete leaves, obtained from eight plants sampled from the useful area of the plot of each cultivar, immediately after harvest. Samples of 100 mg of fresh leaves were incubated in a water bath with 7 ml of dimethylsulfoxide (DMSO) for two hours at 65°C and no maceration was done. After filtering, the total volume was adjusted to 10 ml. The values were obtained by spectrophotometry, considering the optical density measured at 645 nm and 663 nm, according to the methodology described by Borghezan *et al*. (2003).

The content of anthocyanin was measured by the pH differential method, adapted from the method described by Giusti & Wrolstad (2001). Thus, 100 g of sample were weighed to 50 mL of methanol extractor solvent acidified with 1% HCl to obtain a higher concentration in the extract. The values of absorbance at wave lengths of 530 and 700 nm were obtained by spectrophotometry (Bel Photonics 2000 UV).The total contents of anthocyanin were expressed in milligrams of anthocyanins per 100 grams of fresh leaves (mg g^{-1} FL).

The curves of net CO_2, assimilation rates in response to the photosynthetically active photon flux density (PPFD) of 0; 50; 100; 500; 1000; 1500 and 2000 µmol $m^{-2} s^{-1}$, were determined with the infrared gas analyzer (Licor Li 6400 XT Portable Photosynthesis System). The apparent quantum efficiency (χ_a; µmol CO_2/µmol photons) was estimated by setting a linear equation in the range in which the variation of *A* as a function of PPFD was linear, i.e., $A = c + (\chi_a \times \text{PPFD})$, where c and χ_a are adjustment parameters according to the methodology described by Mota *et al*. (2009). Evaluations were performed at the harvest point, on a fully expanded leaf, per plant, on the two central plants of each useful area of the plot, between 10 a.m. and 1 p.m. (Siebeneichler *et al*, 1998).

The results of production of fresh and dry mass of the plants (total fresh and dry mass and fresh mass of leaf and root) as well as the contents of chlorophyll and anthocyanins, were submitted to analysis of variance and Tukey's test ($p < 0.05$) with the Statistica 6.0 (StatSoft, 2001) program. The results of photosynthetic assimilation were evaluated from the polynomial regression analysis.

RESULTS AND DISCUSSION

The cultivation periods of lettuce in hydroponic (HF) and conventional (CF) farming systems were 63 and 77 days, respectively. The longest cultivation periods of the plants in the CF may be due to the development of new roots, replacing those existing in the plant at the time of transplantation, and are degraded after being transferred to the field, in contact with the soil (Beninni *et al*., 2005). This behavior does not occur in the HF, possibly because the water and all the nutrients are available in adequate amounts over the growing season. It is also important to consider that higher temperatures are generally reached in the protected cultivation, which tends to reduce the crop cycle.

The values of fresh weight (total, roots and leaves) were higher in the HF (Table 1). Differences for this trait among cultivars were found only in the HF. The MV cultivar presented, in the HF, statistically superior performance in all characteristics associated with fresh weight than the MR cultivar. The MV cultivar displayed a higher number of leaves in HF than MR cultivar. This performance differs from that obtained by Radin *et al*. (2004), who found a greater number of leaves on lettuce grown in a greenhouse and submitted to drip irrigation than those grown in soil at field conditions. According to these authors, the environment influences the development of plants and those grown in protected environments display a higher production of fresh mass of the leaf, but lower percentage of dry mass. The lower content of the dry mass of hydroponic lettuce is due to its greater hydration. Furthermore, the effect of the additional supply of nitrogen on production (Callegari *et al.*, 2001) would also explain the variations between farming systems, regarding to the components of the biomass. This situation is an advantage to plants submitted to HF because the water and all the nutrients are in controlled amounts and proportions over the growing period, unlike the CF, which is submitted to greater environmental changes.

Since this study was conducted during the rainy season, there may have been environmental variations in the conventional farming, which in turn may have affected the productive capacity of the two cultivars. Rainfall during the 77 farming days corresponded to 264.10 mm, distributed in 23 days, equivalent to 29.8% of the expected rainfall in this period. The relative humidity reached a mean of 78% ± 2.7% (September: 79.3%; October: 80.1% and November: 75%), while the average temperature was 19.4°C in CF and 24.7°C in HF.

The total amount of chlorophyll varied significantly among cultivars and even more among the farming environments. The MR cultivar showed higher mean values of total chlorophyll than those achieved by MV cultivar in both farming environments (Figure 1). Paulus *et al*. (2010), who showed that the total amount of chlorophyll of the purple cultivar was 50% higher than the green cultivar, obtained a similar result. Besides the genetic factors, these authors explain that these variations may also be due to the destruction of the structure of the chloroplast by the action of chlorophyllase, under conditions of salt stress. In this study, it is believed that the differences found in the content of chlorophyll between the two farming systems could be explained by the genetic constitution of the cultivars and by the less control of irrigation and fertilization in the field cultivation. However, it was not used any methodological procedures that would evidence these hypotheses in this study.

In both HF and protected environment, the plants showed higher amounts of total chlorophyll (Figure 1). This result can be explained by the control of the nutrients available in the HF, especially nitrogen (N) in the leaves, as a significant part of this macroelement takes part in the synthesis and structure of chlorophyll molecules (Taiz & Zeiger, 2009). Similar results were found by Manzocco *et al.* (2011), who found a relationship between contents of chlorophyll and the highest concentration of nitrate in plants grown in the hydroponics system than in plants grown in the conventional farming system (CF).

Table 1. Total fresh (FM) and dry mass (DM) accumulation, root fresh mass (RFM), leaf fresh mass (LFM), and number of leaves per plant (NL) at harvest, in two lettuce cultivars, Mimosa Verde (MV) and Mimosa Roxa (MR) in hydroponic (HF) and conventional farming systems (CF)

Cultivar	FM (g)		DM (%)		RFM (g)		LFM (g)		NL	
	CF ^{NS}	HF	CF ^{NS}	HF	CF ^{NS}	HF	CF	HF	CF	HF
MV	129.68	287.69 a	14.06	11.91 a	11.71	38.34 a	13.61	33.50 a	38.63	27.31 a
MR	111.79	139.07 b	9.83	5.36 b	8.60	19.60 b	15.94	18.89 b	32.00	17.81 b
Mean	120.74	213.38	11.95	8.63	10.16	28.97	14.77	26.20	35.31	22.56
CV (%)	30.91	5.26	7.11	6.77	30.43	11.23	34.47	12.49	8.20	10.73
P value	0.2695	0.0000	0.7620	0.0001	0.5066	0.0000	0.0249	0.0001	0.2531	0.0003

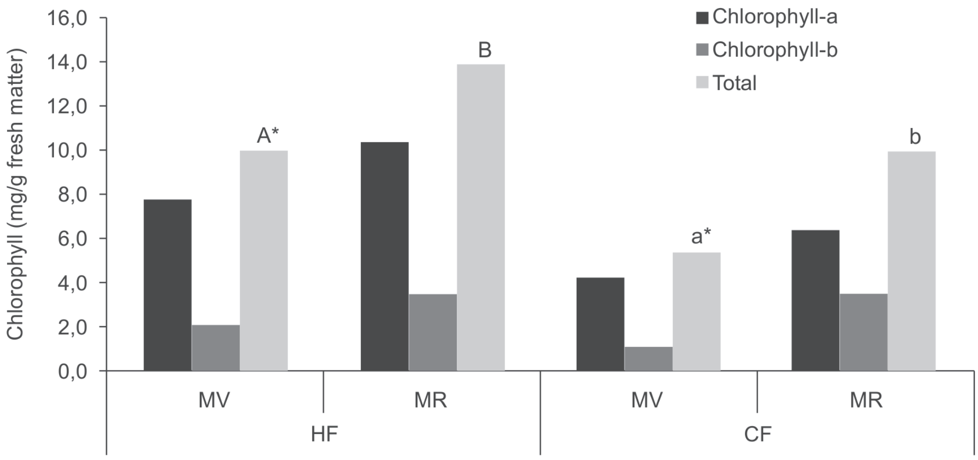
Means followed by different letter in the same column differ from each other by the Tuckey's test at 5% of probability.^{NS} Non-significant at 5% of probability by the F test.

The contents of chlorophyll-a were more abundant than chlorophyll-b for the two cultivars and in both cultivation systems (Figure 1). This result is consistent with that described by Gross (1991), who confirms the higher abundance of chlorophyll-a, which corresponds to approximately 75% of green pigments found in plants.

In addition to supply controlling of water and nutrients, HF interferes in the solar radiation due to the covered environment provided by the protected farming. Galvani *et al.* (2001) emphasize that for the conditions of polyethylene covered greenhouse, the values of internal radiation during the day are smaller than the external ones because of the absorption and reflection of a fraction of direct radiation by polyethylene. As sunlight induces the synthesis of anthocyanins (Couto, 2009), this may explain the difference in the contents of this secondary metabolite among plants from the HF and CF (Figure 2). Moreover, according to Gobbo-Neto & Lopes (2007), anthocyanin is an antioxidant flavonoid accumulated in superficial tissues, with protective action of chlorophyll against photo-destruction through the absorption of UVB rays. Therefore, lettuce grown in unprotected farming may present higher contents of anthocyanins in their leaves owed to the protective response against solar radiation.

The curves of net CO_2 assimilation rates in response to photosynthetic photon flux density (PPFD) can be seen in Figure 3. For all treatments, as PPFD increases, the rate of CO_2 assimilation increases up to a maximum point when photosynthesis tends to decrease from this value. The highest results for the net CO_2 assimilation rates were found in MV and MR cultivars, in the HF system in which the maximum rate was achieved with photosynthetic photon flux density of 1758 and 1685 µmol m^{-2} s^{-1}, respectively (Figure 3). Regarding MV and MR cultivars, the maximum photosynthetic activity was achieved with the photon flux density of 1740 and 1636 µmol m^{-2} s^{-1}, respectively, in the CF system (Figure 3).

However, since photosynthetic efficiency is assessed per unit of area and the number of plants per unit of area may affect this variable, it should be considered the higher



(*)Means followed by the same letters, upper case letter for hydroponic farming system (HF) and lower case letter for conventional farming system (CF) do not differ from each other by the Tukey's test at 5% of probability. Values of CV(%) = 6,2 for CF and CV(%) = 9,6 for HF.

Figure 1. Contents of chlorophyll-a and chlorophyll-b and total (mg $g^{-1}$of leaf fresh mass) and the relationship between chlorophyll-a and chlorophyll-b in two lettuce cultivars, Mimosa Verde (MV) and Mimosa Roxa (MR), in hydroponic (HF) and conventional farming system (CF).

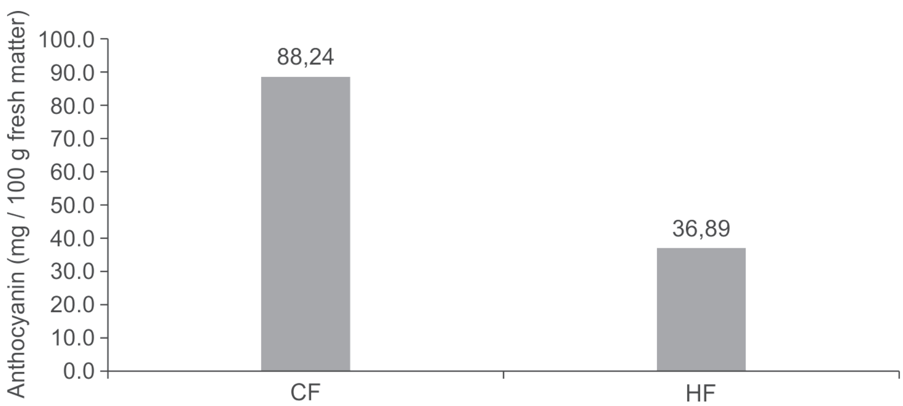


Figure 2. Contents of anthocyanin in mg 100 g^{-1} of lettuce (Mimosa Roxa cultivar) leaf fresh mass in hydroponic and conventional farming system.

plant density used in the HF (16 plants m^{-2}) than in the CF (6.25 plants m^{-2}). Thus, if the same analysis is made based on the individual plant, an increased photosynthetic efficiency is achieved in CF (mean of 241.1 µmol PPFD $plant^{-1}$) compared to that achieved in HF (mean of 107.6 µmol PPFD $plant^{-1}$). For this reason, it is suggested that the intensity of light absorbed by the plant depends on the density of plants and on the environment where they are located.

The excess of light activates a mechanism that decreases its absorption to inhibit photosynthesis mainly by the photoinhibition process (Streit *et al*., 2005). The maximum rate of CO_2 assimilation was achieved with PPFD by MV and MR cultivars, 1704 µmol m^{-2} s^{-1} on average, showing that the values higher than those may affect the process of photoinhibition in plants. Kim & Hori (1989) comment that the photosynthetic rate correlates directly with the content of chlorophyll in the leaves. It was found in this study that regardless of the system used, MR cultivar showed a lower maximum CO_2 assimilation rate in relation to PPFD compared with MV, even with higher concentrations of chlorophyll contents. This decrease in photosynthetic activity by MR may be related to a typical response to radiation by the purple lettuce. This group of lettuce has a high metabolic cost of photoprotection, so that the plants divert the energy produced by photosynthesis to synthesize phenolic compounds, such as anthocyanins (Garcia-Macias *et al*., 2007; Tsormpatsidis *et al*., 2008; Tsormpatsidis *et al*., 2010).

The maximum CO_2assimilation rates were 15.53 and 14.18 µmol m^{-2} s^{-1} for MV and MR cultivars, respectively, grown in HF; these rates were 9.62 and 7.61 µmol m^{-2} s^{1} in MV and MR treatments in CF, respectively. The highest rates of photosynthetic activity found in HF can be explained by the features of the system, in which the availability of nutrient and water absorption are kept constant by the nutrient solution, favoring the stomatal opening and $CO_{2,}$ capture which increases efficiency of net $CO_{2,}$assimilation as a consequence, as well as the higher average temperature, which has a positive effect on the net photosynthesis (Costa *et al*., 2001). On the other hand, these factors are poorly controlled in CF, and they can be linked to issues related to soil, fertilizer and water and thermal fluctuations. Additionally, the lower energy deviation, derived from photosynthesis for photoprotection of greenhouse grown plants may also be an explanation for the higher maximum rates of CO_2 assimilation obtained for the MV and MR cultivars, in the HF.

The higher photosynthetic rates found in hydroponic crops are evidenced by increase in the concentration of CO_2 and temperature in greenhouses as well as the availability of water and mineral nutrients.Thus, the metabolism is stimulated and the rate of net CO_2 assimilation is increased. Thus, the levels of CO_2 rise because photorespiration decreases, as observed in the physiology of other C3-type species (Taiz & Zeiger, 2009).

As the concentration of internal carbon increased, the rate of photosynthesis increased in all treatments. The highest values were found for MV and MR cultivars in the HF, in which the maximum net assimilation rates were 25 and 24.6 µmol m^{-2} s^{-1}, respectively (Figure 4). MV and MR treatments achieved the values of 21.5 and 20.3 µmol m^{-2} s^{-1}in CF. According to Zhou & Han (2005), photosynthesis can be stimulated by the greater availability of CO_2 and light. Thus, the increase in photosynthesis may be related to a greater availability of CO_2 in the leaf. This can be confirmed by the increase in the concentration of internal carbon and by the consequent increase in the net assimilation rate.

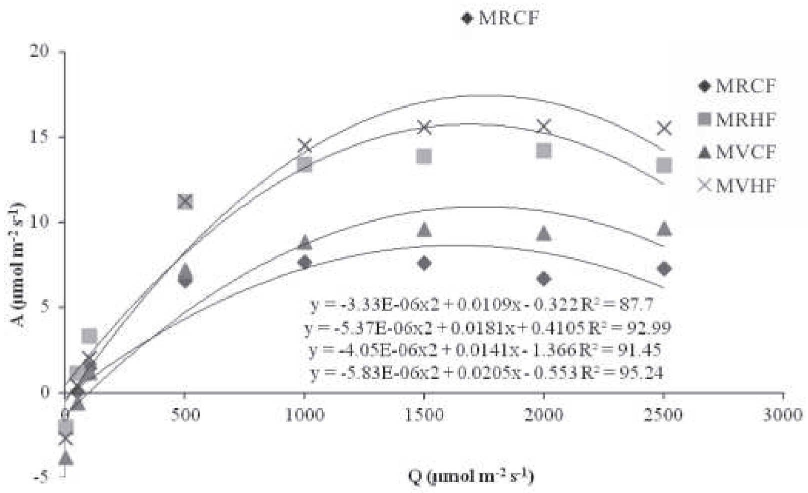


Figure 3. Net CO_2 leaf assimilation rate (A) according to the photosynthetically photon flux density (Q), on two lettuce cultivars, Mimosa Verde, in conventional (MVCF) and hydroponic farming system (MVHF) and, Mimosa Roxa, in conventional (MRCF) and hydroponic farming system (MRHF).

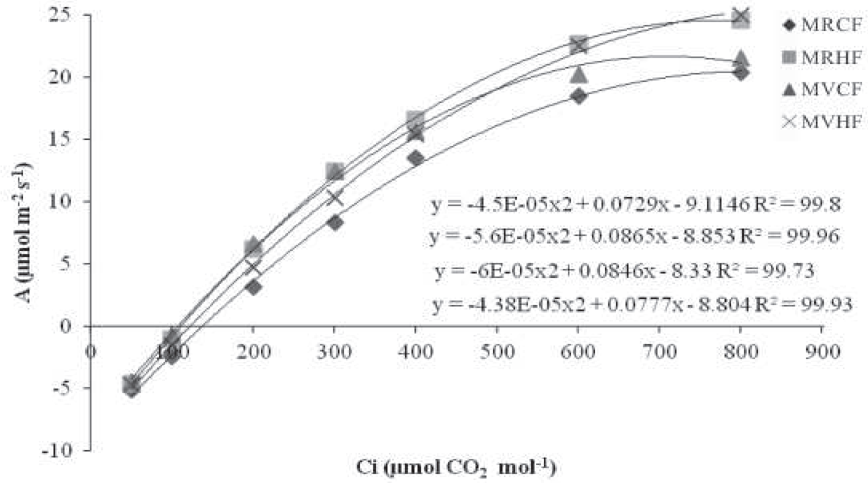


Figure 4. Net CO_2 leaf assimilation rate (A) according to internal CO_2 concentration (Ci), in two lettuce cultivars, Mimosa Verde, in conventional (MVCF) and hydroponic farming system (MVHF) and, Mimosa Roxa, in conventional (MRCF) and hydroponic farming system (MRHF).

The rate of photosynthesis, measured per unit of area (m²) showed greater efficiency in HF than in the CF system. However, the specifications of the spacing between rows and among plants varied for each culture system. Based on the difference in planting density between the two systems, it may be seen that there were greater individual photosynthetic performances in the CF than in the HF in accordance with the results of the dry mass production, which was also higher in CF when the level of individual plant is compared. Similar results were obtained by Romano (2001).

In both systems evaluated, the increase of these components (chlorophyll and anthocyanins) can have a direct effect on the composition of the product and on the quality of the final product. Even though the relationship between quantity and quality of the consumed product is not much exploited in the context of agricultural studies, these data show the importance of understanding the physiological effects of the cultivation systems in order to manage the production according to the preferences of the consumer.

CONCLUSIONS

Protected environment associated with hydroponic farming system provided larger amounts of chlorophyll and higher rates of CO_2 net assimilation for Mimosa Verde and Mimosa Roxa cultivars.

For Mimosa Roxa cultivar, conventional farming (plants grown in the soil in unprotected environment), provided greater amount of anthocyanins and a higher number of leaves per plant.

Despite the lower production of fresh and dry weight by Mimosa Roxa cultivar in both systems, a qualitative gain was found due to a higher content of anthocyanin, provided by conventional farming.

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