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CROP PRODUCTION

Physiological alterations in 'Rubinela' lettuce (*Lactuca sativa* L.) cultivated in conventional and hydroponic systems

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ABSTRACT. Lettuce (*Lactuca sativa* L.) is the most consumed leafy vegetable in Brazil. It is cultivated using at least four distinct systems, the most common of which are conventional and hydroponic systems. These systems provide different cultivation conditions for plants, causing physiological changes that are important for commercial production, such as nutrient uptake and biomass accumulation. However, only a few studies have compared the physiological aspects of these two cultivation systems. The objective of this study was to evaluate the physiological behavior of 'Rubinela' lettuce plants grown in hydroponic and conventional pot systems, by comparing dry mass (DM) and fresh mass (FM) production, number of leaves (NF), stomatal density, and contents of chlorophyll, carotenoids, anthocyanin, sugars, and starch. Plants cultivated in hydroponic systems presented significant differences in chlorophyll content, producing more biomass than plants cultivated in conventional pot systems, probably because of better nutritional conditions, primarily with respect to macronutrients, provided by the nutrient solution of the hydroponic system. The lower water availability encountered by plants cultivated in conventional pot systems influenced the increased sugar and starch concentrations, as well as the anthocyanin content, which may be a strategy to mitigate the possible damage caused by hydric stress conditions.

Keywords: anthocyanin; biomass; foliar pigments; plant physiology.

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Introduction

The increasing consumption of leafy vegetables has been justified by the remarkable global demand for healthier and low-calorie diets. Lettuce (*Lactuca sativa* L.) is undoubtedly the most consumed leafy vegetable in Brazil, with an estimated annual production of 908,186 tons per year (IBGE, 2017). Among the different varieties developed by the Federal University of São Carlos (São Paulo State, Brazil), Rubinela is the first crispy lettuce cultivar with a red color. The Rubinela cultivar has some noteworthy characteristics for production, such as adaptation to tropical conditions, color, large size, resistance to diseases, such as downy mildew (*Bremia lactucae*), and tolerance to premature bolting (Rossi et al., 2020).

Conventional and organic farming, performed in open fields, along with protected cultivation using hydroponic systems or growing directly in the soil, are the four systems frequently used to cultivate lettuce. These systems differ from each other in several aspects, such as crop management, postharvest handling, yield, and quality. The broad open cultivation areas and adverse climate conditions of the conventional system, such as temperature and pluviosity, contribute to the emergence of plant pathogens and pests, compromising the yield and quality of the product (Savvas, Gianquinto, Tuzel, & Gruda, 2013).

Hydroponics present a wide range of advantages, such as production feasibility in smaller areas, reduced water demand, nutrient use efficiency, reduction of operational handling and management during the crop cycle, and harvest anticipation, in addition to the reduced incidence of soil pathogens and total control of nutrients and water supply (Savvas et al., 2013). Thus, the hydroponic system in protected cultivations provides a flexible and intensified production alternative, with higher quality crop yield, even in areas that encounter adverse cultivation conditions (Martinez-Mate, Martin-Gorriz, Martínez-Alvarez, Soto-Garcia, & Maestre-Valero, 2018).

Lettuce hydroponic cultivation systems mainly use the nutrient film technique (NFT). In this technique, the nutritive solution flows through the channels where plant roots are located, providing nutrients, water, and oxygen required for plant development (Furlani, Silveira, Bolonhesi, & Faquin, 1999). Under normal cultivation conditions, crop development is invariably influenced by the lowest water and nutrient supply along with other soil and climate restrictions (Fuentes & King, 1989). However, few studies have focused on plant physiological aspects with respect to distinct cultivation systems (Rosa et al., 2014; Souza et al., 2019; Thomas, Biradar, Chimmad, & Janagoudar, 2021).

The objective of this study was to evaluate the physiological behavior of Rubinela lettuce cultivated in hydroponic systems and in pots, with the latter being considered a conventional system.

Material and methods

We evaluated lettuce plants (*Lactuca sativa* L.) of the cultivar Rubinela grown in two cultivation systems: hydroponics and pots (conventional). Seedlings were obtained by germination in phenolic foam trays maintained under hydroponic conditions. On the 10^{th} day, seedlings were transferred to individual cells and maintained for another 14 days in a hydroponic nursery, and those measuring 5 ± 1 cm were selected for the experiment. The experiment was carried out in a greenhouse at *Centro de Ciências Agrárias* (CCA) of the Federal University of Santa Catarina (UFSC), Florianópolis, Santa Catarina State, Brazil, with temperatures ranging from 18.8°C (minimum) to 26.4°C (maximum).

A completely randomized experimental design was employed, with two treatments (hydroponic and pot cultivation systems) evaluated in experimental units containing six replicates (six plants per experimental unit) and a boundary line. Plants were distributed in the hydroponic channels spaced 0.25 m apart from each other with a 0.25 m distance between holes and pots, and a planting density of 16 plants m^{-2} in both systems.

Cultivation conditions

The electrical conductivity of the cultivation solution was maintained at 1.85 to 2.00 dS m^{-1} (deciSiemens m^{-1}), with corrections every two days. The hydroponic nutrient solution was adapted from Furlani et al. (1999), following Barcelos-Oliveira (2008), and encompassing the following nutrients: special Hydro® calcium nitrate (750 ppm), potassium nitrate (500 ppm), purified MAP (monoammonium phosphate; 150 ppm), magnesium sulfate (400 ppm), copper sulfate (concentration of 13%; 0.15 ppm), zinc sulfate (concentration of 22%; 0.50 ppm), manganese sulfate (concentration of 26%; 1.50 ppm), boric acid (concentration of 17%; 1.50 ppm), sodium molybdate (concentration of 39%; 0.15 ppm), and HydroFerro® (30 ppm). For pot cultivation (soil), 2 dm^3 vessels were filled with substrate in a 1:1:1 proportion of peat, sand, and organic matter originating from vegetable residues (Table 1). Field capacity of the vessels was estimated by the gravimetric method after complete saturation (Schmugge, Jackso, & McKim, 1980), maintaining the value at approximately 70% in the first irrigation. The vessels were then irrigated manually twice a day to ensure complete substrate saturation.

Table 1. Chemical composition of the substrate (peat: sand: organic matter in a 1:1:1 proportion) used in conventional cultivation of Rubinela lettuce.

Clay % (m/v)	OM % (m/v)	Water (pH 1.1)	SMP index	P	K	Al	Ca	Mg
				(mg dm^{-3})			($cmol_c$ dm^{-3})	
13	3.9	5.6	6.2	157.5	474.7	0.0	11.2	6.3
H + Al	Al	CTC pH 7.0	Bases	K	Ca		Mg	
($cmol_c$ dm^{-3})			(% saturationat CTC)					
3.47	0.0	22.18	84.37	5.47	50.50		28.40	

OM: Organic matter; m/v: mass/volume; SMP index: Shoemaker, Mac Lean, and Pratt index.

Analyzed parameters

Plants were harvested 59 days after sowing, which was equivalent to 37 days after transplanting in both cultivation systems. Parameter evaluations were performed on two plants per replicate, totaling 12 plants per treatment.

Growth parameters were based on the length of the longest root (RL), aerial height (AH), total plant height (TPH, equal to RL + AH), leaf number (LN), fresh root mass (FRM), fresh aerial mass (FAM), total fresh mass (TFM, equal to FRM + FAM), root dry mass (RDM), aerial dry mass (ADM), total dry mass (TDM, equal to RDM + ADM), and the TFM/TDM ratio. For dry mass quantification, the samples were kept in a ventilation oven at 60°C until they reached a constant weight. Total chlorophyll *a* and *b*, and carotenoid contents were determined based on the procedure described by Hiscox and Israeltam (1979) using fresh leaves of the middle portion of the plants, collected immediately after harvesting. Leaf central veins were removed to obtain only leaf blade samples, and foliar pigment content was estimated following the method described by Wellburn (1994).

Anthocyanin content was measured using the pH differential method (Giusti & Wrolstad, 2001), with modifications. Samples of 100 g of leaves, comprising mature and non-senescent representatives and excluding major veins, were mixed with 50 mL of methanol extractor solvent acidified (1% HCl) and kept in the dark for 24h. Thereafter, samples were diluted in two buffer solutions, one in 0.025 M potassium chloride (pH 1.0) and the other in 0.4 M sodium acetate (pH 4.5). For both buffer solutions, the absorbance values at wavelengths of 530 and 700 nm were recorded using spectrophotometry (Bel Photonics 2000 UV), as described by Rosa et al. (2014). The anthocyanin content was measured using equation (1), and the total anthocyanin content was expressed in milligrams of anthocyanin per 100 g of fresh leaves (mg 100 g^{-1}), calculated as cyanidin 3-glucoside (Molecular Weight = MW = 449.2) using equation (2).

$$A = (A530 - A700) solution\ pH\ 1.0 - (A530 - A700) solution\ pH\ 4.5 \quad (1)$$

$$C\left(\frac{mg}{100g}\right) = \frac{(A * MW * dilution\ factor)}{\varepsilon * 1} \quad (2)$$

*where ε = molar absorptivity (26900 mol L^{-1}) and 1 = cuvette thickness (cm).

The total soluble sugar content, expressed in mg g^{-1} of fresh mass (mg g^{-1} FM), was determined separately in leaves, roots, and the whole plant (or total, meaning sugar content in leaves + sugar content in roots) using the phenol-sulfuric spectrophotometric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The total sugar content was measured from the standard curve (y = 0.0174x + 0.0156; r^2 = 0.9983) using glucose as the standard. The total starch content in the leaves and roots was determined as described by Rosa et al. (2014).

Stomatal density was determined in two leaves per replicate, using the synthetic enamel fixation method applied to both leaf surfaces, abaxial and adaxial. After gentle removal, the enamel sheets were placed on microscope slides for subsequent observation. For a total of 20 readings per treatment, stomata densities were recorded in five visible frames per slide using an optical microscope (CH30 Olympus and BX 40 Olympus) at 200x magnification with a total visible area of 0.16 mm^2.

Statistical analysis

Data on aerial plant height (AH), aerial fresh mass (AFM), and total fresh mass (TFM) were transformed by $\log_{10}$ for variance homogenization. All data obtained were submitted to the Cochran test and subsequent analysis of variance (ANOVA), and when significant, subjected to means separation using Tukey's test ($p < 0.05$).

Results and discussion

Statistical significance was observed for RL, AH, TPH, LN, FRM, FAM, TFM, ADM, TDM, and TFM/TDM ratios (Table 2). The RL, AH, TPH, and LN values of hydroponic plants were at least 40% higher than those plants cultivated in pots (Table 2). In addition, the means of FRM, FAM, TFM, ADM, TDM, and TFM/TDM ratios of hydroponically cultivated plants were 159, 193.7, 162.2, 20.9, 19.75, and 117.7% higher, respectively, compared with pot-cultivated plants (Table 2).

Table 2. Production and growth parameters for both cultivation systems. Length of longest root (RL), aerial plant height (AH), total plant height (TPH), leaves number (LN), fresh root mass (FRM), fresh aerial mass (FAM), total fresh mass (TFM), root dry mass (RDM), aerial dry mass (ADM), total dry mass (TDM), and TFM/TDM ratio of Rubinela lettuce (*Lactuca sativa* cv. Rubinela) plants cultivated in pots (conventional) and in the hydroponic system.

	Pots (Conventional)	Hydroponic
RL (cm)	37.75 b	53.33 a
AH (cm)	17.67 b	24.83 a
TPH (cm)	55.42 b	78.17 a
LN	9.67 b	13.58 a
FRM (g)	7.22 b	18.70 a
FAM (g)	34.05 b	100.00 a
TFM (g)	45.27 b	118.71 a
RDM (g)	0.5625 a	0.5350 a
ADM (g)	11.08 b	13.40 a
TDM (g)	11.64 b	13.94 a
TFM/TDM	3.84 b	8.36 a

Means followed by the same letters in the columns do not differ statistically from each other by Tukey test ($p < 0.05$).

The greatest leaf number observed in plants cultivated in hydroponic systems is a remarkable characteristic for commercialization. Commercialization of mostly leafy vegetables, such as lettuce, is determined on a unit-basis rather than weight-basis, considering the appearance, volume, and number of leaves per plant (Diamante, Santino Junior, Inagaki, Silva, & Dallacort, 2013). Greater accumulation of dry and fresh mass in hydroponically cultivated lettuce, compared to the conventional system, was also observed by Rossi et al. (2020) for the same cultivar, and by Souza et al. (2019) for the cultivar 'Crocantela'. This increase is probably due to the greater assimilation and nutrient uptake in hydroponic systems because they encounter the highest nutrient availability by nutritive solution and less hydric stress (Souza et al. 2019; Santos Filho et al. 2009). Nonetheless, Rosa et al. (2014) reported decreased dry mass content due to greater hydration of the leaves of plants of lettuce cv. 'Mimosa verde' and 'Mimosa roxa' grown in hydroponic systems.

The mean root dry mass did not differ between the two cultivation systems (Table 2). Souza et al. (2019) also reported similar results and stated that although plants cultivated in pots showed lower growth of aerial height, their root systems may have developed to optimize nutrient uptake from the substrate. However, the greater FRM observed in hydroponic plants may be due to considerable root hydration in this cultivation system (Table 2).

Regarding leaf pigment content, differences were observed between the two cultivation systems in terms of chlorophyll *a*, total chlorophyll content, and chlorophyll *a/b* ratio. Mean values of these parameters were 42.8, 28, and 45.4% lower, respectively, in lettuce plants cultivated in pots (Table 3). Contents of these pigments are influenced by water and nutrients availability, especially nitrogen (Soratto, Carvalho, & Arf, 2004; Grzesiak, Rzepka, Hura, Hura, & Skoczowski, 2007; Rosa et al., 2014; Souza et al., 2019). Lettuce plants cultivated in pots (conventional system) might experience hydric stress conditions related to the manual cultivation process and frequency of watering (twice a day). According to Long, Humphries, and Falkowski (1994), a reduction in the contents of chlorophyll *a* and *b* is a consequence of water deficiency, that may lead to photoinhibition and reduced photosynthetic efficiency and chlorophyll synthesis. Reduced chlorophyll content in plants cultivated under hydric stress has been reported for *Lactuca sativa* (Rosa et al., 2014; Souza et al., 2019), *Gossypium hirsutum* L. (Parida, Dagaonkar, & Phalak, 2007; Massacci, Nabiev, & Pietrosanti, 2008), *Catharanthus roseus* (Jaleel, Gopi, Manivannan, & Panneerselvam, 2008), *Saccharum officinarum* (Jangpromma et al., 2010; Silva, Santos, Vitorino, & Rhein, 2014), *Hordeum vulgare* (Anjum, Yaseen, Rasool, Wahid, & Anjum, 2003), and *Zea mays* (Holá et al., 2010). In addition, hydroponics provide better nutritional conditions than soil cultivation systems, primarily with respect to macronutrients, resulting in plants with significant differences in foliar pigment content (Souza et al., 2019).

Table 3. Physiological parameters for both cultivation systems. Contents of chlorophyll *a*, *b*, total chlorophyll, chlorophyll *a/b* ratio, total carotenoids (expressed in mg g^{-1} FM) and anthocyanins (expressed in mg 100 g^{-1}), mean content of leaf (LSu), root (RSu), and total (TSu) soluble sugars and leaf (LSt) and root (RSt) starch of Rubinela lettuce (*Lactuca sativa* cv. Rubinela) cultivated in pots (conventional) and hydroponic systems.

	Pots (Conventional)	Hydroponics
Chl*a*	0.3223 b	0.5634 a
Chl*b*	0.2673 a	0.2556 a
Total Chl	0.5897 b	0.8191 a
Chl*a/b* ratio	1.2096 b	2.2174 a
Carot	0.1733 a	0.1297 a
Anthoc	154.0555 a	52.6632 b
The following data are in µg equivalent to glucose.g of fresh $mass^{-1}$		
LSu	0.0159 a	0.0119 b
RSu	0.0221 a	0.0099 b
TSu	0.0389 a	0.0219 b
LSt	0.0097 b	0.0117 a
RSt	0.0123 a	0.0106 b

Means followed by the same letters in the columns do not differ statistically from each other by Tukey test ($p < 0.05$).

Anthocyanin content was 65.8% lower in plants cultivated in hydroponic system compared with plants cultivated in pots (Table 3). Commonly, leaves synthesize anthocyanins during growth and/or senescent developmental stages, or after exposure to environmental stresses such as drought, high light intensity, or low temperatures (Chalker-Scott, 1999; Hoch, Zeldin, & McCown,2001; Steyn, Wand, Holcroft, & Jacobs, 2002). Anthocyanins play a crucial role in mitigating photo inhibitory or photooxidative injuries in the leaves by reducing the incidence of high-energy *quanta* in the chloroplasts and by eliminating free radicals before they cause structural damage to chloroplast or cell membranes (Neill & Gould, 2003).

Differences were observed in the soluble sugar content in the leaves, roots, and in the whole plant, and in starch content in the leaves and roots (Table 3). The means encountered for soluble sugar content in leaves (LSu), roots (RSu), and whole plant (TSu), and for starch content in the roots (RSt), were lower at 25.2, 55.2, 43.7, and 13.8%, respectively, in plants cultivated in the hydroponic system compared to those cultivated in the conventional system. However, the starch content in the leaves (LSt) was 17.1% lower in plants cultivated in the conventional system (Table 3). The increase in sugar and starch content in plants cultivated in pots might be due to osmotic adjustment, since these plants were subjected to inconstant water availability. Because of the low water availability, starch is degraded, and an increase in soluble reducing sugar content occurs. This mechanism of intracellular accumulation of osmotically active solutes is critical for the maintenance of cellular turgidity, primarily allowing the maintenance of stomatal opening and photosynthesis under conditions of low water availability (Kramer & Boyer, 1995). Souza et al. (2019) also reported an increase in total soluble sugars in the leaves and roots of *L. sativa* cv. Crocantela in response to hydric stress.

Leaves of Rubinela lettuce presented stomata on both surfaces, characterizing them as amphistomatic. In contrast to Souza et al. (2019), no statistical differences were observed in stomatal density between the cultivation systems (Table 4). In a similar experiment with lettuce plants of the Crocantela cultivar, a higher stomatal density was reported in plants cultivated in pots compared to those cultivated in hydroponic systems (Souza et al., 2019).

Table 4. Post-harvest parameters for both cultivation systems. Stomata density (mm^2) per leaf surface (adaxial and abaxial) of Rubinela lettuce (*Lactuca sativa* cv. Rubinela) plants cultivated in pots (conventional) and hydroponic systems.

	Adaxial surface	Abaxial surface
	--------------------- stomata mm^{-2} --------------------	
Pots	22.57 a	34.38 a
Hydroponics	20.83 a	31.51 a

Means followed by the same letters in the columns do not differ statistically from each other by Tukey test ($p < 0.05$).

Conclusion

Plants cultivated in the hydroponic system presented the lowest nutritional and hydric stress, enabling greater physiological activities that resulted in plants with a larger accumulation of biomass. Plants cultivated in pots, which are considered conventional systems, suffered from higher hydric stress, resulting in reduced photosynthetic activity. This indicates that, the accumulation of sugars, starch and anthocyanins in potted plants occurs as a mechanism to mitigate the damage caused by such stress.

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