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Water retention of substrates potentiates the quality of lettuce seedlings

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Key words: chemical properties, *Lactuca sativa* L., physical properties, root system morphology, shoot morphology.

Abstract: A difficulty in the production of lettuce seedlings in containers is to assure the production of shoot biomass with limited portion of roots, restricted to a small volume of substrate. Therefore, we investigate if substrates associated to lettuce cultivars interfere in the seedling quality. The treatments, outlined in a two-factorial scheme, were two cultivars of lettuce and four substrates, arranged in a randomized complete block design, with three replications. The results showed that seedlings produced in the substrate with higher water retention capacity had higher performance in relation to shoot morphology and root system morphology. In conclusion, the data show that the seedlings quality of lettuce cultivars associate with the types of substrates studied and that seedlings produced in substrate with higher water retention have better quality.

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1. Introduction

In horticultural crops the supply of quality seedlings to the producers is important to obtain high production after the establishment of the plants in their growth medium. Such quality is related to the plants resistance to biotic and abiotic stresses (Zhao *et al.,* 2016). Among vegetables, lettuce (*Lactuca sativa* L.) is one of the most cultivated (Kim *et al.,* 2016), with a world production of approximately 25 million tons (FAO, 2014). In order to maintain this production in an upward manner, quality seedlings must be provided to producers. Thus, the production of seedlings is one of the most important stages in lettuce cultivation, because this process reflects on the productive performance of the plants (Auler *et al.,* 2015). However, a difficulty in the production of seedlings in containers is to ensure the production of shoot biomass with limited portion of root (Lemaire, 1995), restricted to a small volume of substrate, in response to the species/cultivars used.

As the substrates have a wide variation in their physicochemical properties (Fermino and Kämpf, 2012), choosing a suitable material is essential to the development of the plants (Mondragón-Valero *et al.,* 2017). The substrates used must be low cost and easy to handle (Noya *et al.,* 2017), to have porosity around 85% (Kämpf *et al.,* 2009) and water retention capacity (Graceson *et al.,* 2013). For lettuce, it is necessary to choose material that ensures these physical characteristics, since it is a species with high water demand (Nunes *et al.,* 2017). Moreover, the choice of the cultivar is also important, because the genotypes can interact with biotic and abiotic factors, influencing the quality of the seedlings produced (Martins *et al.,* 2017).

Knowing that substrates used in seedlings production are essential to plants germination and establishment (Auler *et al.,* 2015) and that crop productivity is linked to this input (Smiderle *et al.,* 2001), this question arises: how substrates associated to lettuce cultivars affect the seedlings quality?

Therefore, based on the hypothesis that the quality of lettuce cultivars is dependent of the water retention capacity of the substrate, the objective of the present study was to evaluate if substrates associated to lettuce cultivars interfere in the seedlings quality. This study provides a view of the development of lettuce seedlings using different substrates to improve the seedlings quality (e.g., increase the growth of shoot biomass and root system) grown in greenhouses.

2. Materials and Methods

Plant material, treatments description and experiment site

The seeds of lettuce used in the work were of the cultivars Mimosa Roxa Salad Bowl (Purple), of bright greenish purple color, and Mônica SF 31 (Green), of medium green color, both of the group crisphead.

The materials used as substrates were carbonized rice husk (CRH), Horta 2® (HOR), TN Gold® (TNG) and a mixture (MIX) composed of 40% CRH, 40% HOR and 20% TNG. The composition of HOR consists of pine bark, vermiculite, acid correction and fertilizers (nitrogen, phosphorus and potassium) in quantities not supplied by the manufacturer. The composition of TNG consists of sphagnum peat, expanded vermiculite, dolomitic limestone, agricultural gypsum and fertilizers (nitrogen, phosphorus and potassium) in quantities not supplied by the manufacturer. No fertilizer was added to the substrates. The rice husk used in the work was carbonized (Kämpf *et al.,* 2006).

The experiment was developed in the Brazilian subtropics, in the city of Passo Fundo/RS (28° 15’ 46” S, 52° 24’ 24” W), from April to May (Fall) of 2017. The trial took place on trays kept on metal benches, 1.2 m above the soil surface, in a agricultural greenhouse of 90 m², with semicircular roof, installed in the northeast-southeast direction. The galvanized steel frame was covered with low density polyethylene film, with anti-ultraviolet additive and with a thickness of 150 microns, and the sides were covered with anti-aphid screen.

The irrigation used was with sprinklers, in the mechanized system, with a flow rate of 2 l min^{-1} per unit. The irrigation regime consisted of four sprinklers per day, with total wetting of seven minutes. The water blade supplied to the seedlings was 4.35 mm day^{-1}. During the execution of the experiment, the photosynthetically active radiation (PAR) and the mean air temperature inside the greenhouse were monitored, with mean values of 110.5 µmol m^{-2} s^{-1} and 17.4°C, respectively.

Experimental design

The treatments, outlined in a two-factorial scheme, consisted of two lettuce cultivars (Purple and Green) and four substrates (CRH, HOR, TNG and MIX). The production of the seedlings was carried out in trays of expanded polystyrene, with dimensions of 0.34 m of width and 0.68 m of length. Each tray had 128 cells, with a volume of 35 cm³. The experimental design was randomized blocks with three replicates (n= 3; one replicate per tray, i.e. three trays were used in the experiment).

On April 20, the trays were filled with the substrates CRH, HOR, TNG and MIX, and after that, five seeds of the lettuce cultivars were sown in each cell. In each tray each treatment was composed of 16 seedlings, that is, a total of 48 seedlings per treatment (16 seedlings/treatment x 3 replicates). Considering that we used 8 treatments, our experiment consisted of a total of 384 seedlings (16 seedlings/treatment x 3 replicates x 8 treatments).

Determination of physicochemical properties of substrates

A sample of 1 l of each substrate was collected and analyzed to obtain physicochemical attributes of the materials.

The physical attributes determined in the substrates were: density (D), total porosity (TP), aeration space (AS), readily available water (RAW) and buffer water (BW).

The chemical attributes determined in the substrates were nitrogen (N), phosphorus pentoxide (P_2O_5), potassium oxide (K_2O), organic carbon (OC), hydrogenionic potential (pH), electrical conductivity (EC) and cation exchange capacity (CEC) (MAPA, 2014).

Regarding the seedlings, the evaluations began one week after sowing. Morphological attributes of the shoot and the root system of the seedlings were evaluated.

Determination of shoot morphology

In relation to the shoot morphology, forty-eight seedlings per treatment were evaluated. Four days after sowing the percentage of seed germination was evaluated by means of the equation:

$$\text{Germination (\%)} = (\text{seed germinated}/\text{total number of seeds}) \times 100 \quad (1)$$

After the germination, thinning was performed, leaving one plant per cell in each tray. In addition, the date of emergence of the cotyledons and the issuance of the first, second and third leaves were noted. Thirty-three days after sowing, the stem base diameter (SBD) and the shoot height (SH) of the seedlings were measured with a digital caliper. The fresh (SFW) and dry (SDW) weight of the shoot was also evaluated. In order to obtain the dry weight, the plants were kept in a drying oven with forced air circulation, at 65°C for 48 hours, until constant weight, and weighed in an electronic analytical balance.

Determination of root system morphology

Regarding to the root system morphology, forty-eight seedlings per treatment were evaluated. The roots were collected and washed in water to eliminate the substrate fragments. Thus, the roots were scanned and then the images obtained were analyzed by WinRHIZO® software. The attributes evaluated were the total root length (TL, cm), root surface area (SA, cm²) and root volume (RV, cm³). The roots were grouped by software in different diameter classes in relation to their total length (Böhm, 1979): very thin roots (VTR, Ø <0.5 mm), fine roots (FR, Ø 0.5 to 2 mm) and thick roots (TR, Ø > 2 mm). The fresh (RFW) and dry (RDW) weight of the root system was also evaluated, following the methodology described previously.

Determination of seedlings quality

The seedlings quality was obtained by models of plant development. These development models are mathematical models that consider plant growth variables (Cournède *et al.,* 2013), such as shoot morphology and root system morphology. Thus, the seedling vigor index (SVI) was determined according to Abdul-Baki and Anderson (1973), by the equation:

$$SVI = \text{germination (\%)} \times (\text{shoot length} + \text{root length}) \quad (2)$$

It was determined, also, the Dickson quality index (DQI), proposed by Dickson *et al.* (1960), by the equation:

$$DQI = (TDW) / (H/SBD + SDW/RDW) \quad (3)$$

where TDW = total dry weight (g); H = shoot height (cm); SBD = stem base diameter (cm); SDW = shoot dry weight (g); RDW = root dry weight (g).

In addition, dry matter accumulation (DMA) of the shoot and of the root system was determined, according to Atif *et al.* (2016), by the equation:

$$DMA = (DW/FW) \times 100 \quad (4)$$

where DW = dry weight (g); FW = fresh weight (g).

Statistical analysis

The data were submitted to analysis of variance and the means of the treatments were compared by Tukey test, at 5% probability of error, with the aid of the Assistat® program (Silva and Azevedo, 2016).

3. Results

Physicochemical properties of substrates

The results of the physical characterization of the substrates used in this experiment (Table 1) showed

Table 1 - Physical properties of the substrates used in the study

Substrates	Density ($kg\ m^{-3}$)	Total porosity ($m^3\ m^{-3}$)	Aeration space ($m^3\ m^{-3}$)	Readily available water ($m^3\ m^{-3}$)	Buffer water ($m^3\ m^{-3}$)
Carbonized rice husk	170±12.33 c	0.879±0.11 b	0.365±0.02 b	0.395±0.10 a	0.009±0.003
Horta 2®	241±05.26 a	0.837±0.10 d	0.303±0.01 d	0.149±0.02 d	0.020±0.001
TN Gold®	088±10.98 d	0.916±0.13 a	0.519±0.11 a	0.202±0.08 c	0.007±0.002
MIX	183±07.47 b	0.869±0.14 c	0.325±0.08 c	0.259±0.09 b	0.030±0.001
Mean	170.50	0.87	0.378	0.251	0.016
Coefficient of variation (%)	13.58	14.11	15.26	14.45	12.25

MIX= mixture composed of 40% Caborized rice husk, 40% Horta 2® and 20% TN Gold®. Data presented as mean ± standard deviation. Means followed by the same letter in the column did not differ significantly by the Tukey test (P≤0.05, n= 3).

that, considering the density of the materials, the TNG substrate is the lightest. With the values of TP, AS, RAW and BW of Table 1 we elaborated a graph to visualize the relation between air and water in each substrate (Fig. 1). We observed that TNG material showed an unbalanced air-water relation (Fig. 1). In addition, we observed a better balance between air-water in the MIX substrate, that is, when the other materials were combined (40% CRH, 40% HOR and 20% TNG).

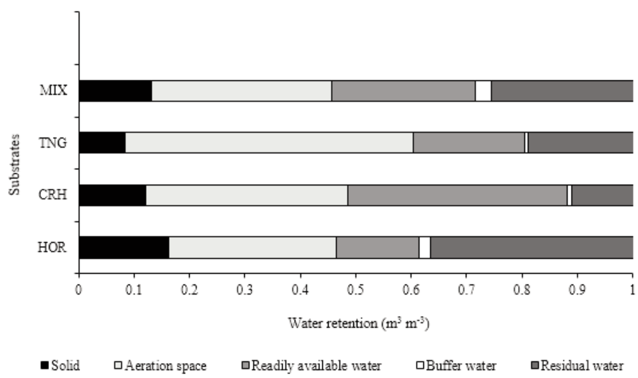

Fig. 1 - Physical characterization of the substrates used in the study; n= 3 CRH= Carbonized rice hull; HOR= Horta 2®; TNG= TN Gold®; MIX= mixture.

In addition, also with the values of TP, AS, RAW and BW of Table 1 we elaborated a graph to visualize the water retention curve of each substrate (Fig. 2), according to De Boodt and Verdonck (1972). The HOR and MIX substrates presented higher water retention, requiring volumes of 0.385 $m^3\ m^{-3}$ and 0.285 $m^3\ m^{-3}$, respectively, to remain in the range of water easily available to plants (10-50 -cm H_2O) (Fig. 2). On the other hand, the CRH substrate had greater drainage of water (Fig. 2).

The four materials showed availability of nutrients, except for K_2O. Among the substrates, CRH presented 23% more pH than TNG. The opposite was obtained for EC and CEC, with CRH being 58% lower than the HOR for EC and 85% lower than TNG for CEC (Table 2).

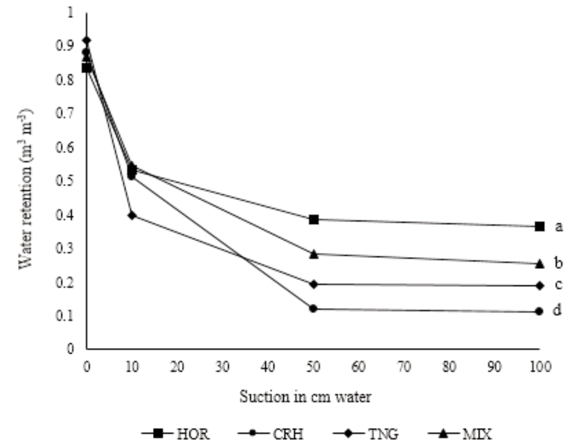

Fig. 2 - Water retention curve of the substrates. CRH: Carbonized rice hull; HOR: Horta 2®; TNG: TN Gold®; MIX: mixture. Different letters indicate significant differences by the Tukey test (P≤0.05, n = 3).

Shoot morphology

In all treatments there was 100% germination of the seeds. Up to the first true leaf, the two cultivars took the same period (11 days) to differentiate. The differences began to be identified from the second true leaf. For this attribute, the two cultivars produced in the CRH and the Green cultivar produced in the TNG took longer to emit the third true leaf (Fig. 3). As a consequence, at the time of transplantation the third leaf was poorly expanded.

We did not observe effects of the cultivars on the shoot morphology of the seedlings. This means that the seedlings of both cultivars can be produced on any of the substrates. However, we observed only effect in relation to the substrates for the SH, SFW and SDW.

The seedlings produced on the HOR substrate had higher SH (5.86 cm ± 1.82) than those produced on the CRH substrate (3.24 cm ± 0.77), but did not differ

Table 2 - Chemical properties of four substrates

Substrates	N % (m/m)	P_2O_5 % (m/m)	K_2O % (m/m)	OC % (m/m)	pH	EC (mS cm^{-1})	CEC (mmolc kg^{-1})
Carbonized rice husk	0.69±0.01 a	1.71±0.10 a	0	07.21±03.03 d	7.2±1.0 a	0.19±0.01 c	134.60±12.2 d
Horta 2®	0.36±0.09 c	0.39±0.06 d	0	12.60±06.22 b	6.1±2.2 c	0.45±0.01 a	278.60±15.4 b
TN Gold®	0.65±0.01 b	1.37±0.11 c	0	31.16±11.02 a	5.6±1.3 d	0.36±.0.2 b	892.98±13.7 a
MIX	0.35±0.02 c	1.48±0.03 b	0	10.38±04.33 c	6.4±3.1 b	0.37±0.03 b	230.05±11.2 c
Mean	0.51	1.23	0	15.33	6.32	0.34	384.05
Cofficient of variation (%)	12.25	15.90	0	16.30	11.51	17.91	14.15

MIX= mixture composed of 40% Caborized rice husk, 40% Horta 2® and 20% TN Gold®. Data presented as mean ± standard deviation. Means followed by the same letter in the column did not differ significantly by the Tukey test (P≤0.05, n= 3).

statistically from the seedlings produced in TNG and MIX (Fig. 4 A). In addition, the seedlings produced on the HOR substrate showed higher SFW (0.27 g ± 0.17) and SDW (0.012 g ± 0.0033) than those produced on other substrates (Fig. 4 B and C, respectively). In general, seedlings obtained on the HOR substrate produced 71% more shoot biomass than those grown in CRH (Fig. 4).

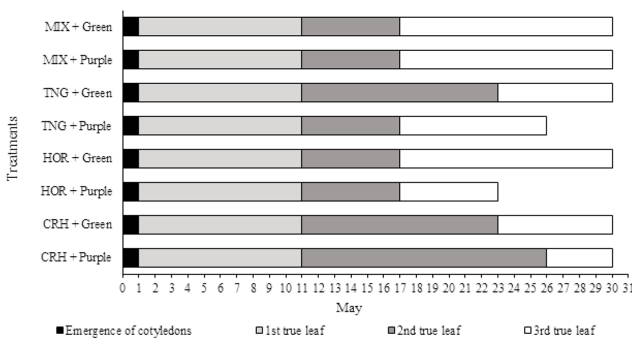

Fig. 3 - Combination of two lettuce cultivars produced in four substrates in relation to the period of emergence and expansion of the first true leaves. CRH= Carbonized rice hull; HOR= Horta 2®; TNG= TN Gold®; MIX= mixture; Purple=Mimosa Roxa Salad Bowl cultivar; Green= Mônica SF 31 cultivar.

Root system morphology

Regarding the root system morphology, we observed significant differences for the interaction between substrates and cultivars in relation to RV and TR (Table 3). For the interaction between the factors, the best combination corresponded to the seedlings of the Green cultivar produced on the HOR substrate, both for root volume and for thick roots (Table 3).

We also observed significant differences for substrates only for RFW, RDW, SA and FR (Table 4). In this respect, we observed that the seedlings produced in HOR presented a larger SA, but did not differ statistically from the seedlings produced in MIX (Table 4). The seedlings developed in CRH had smaller amount of FR in relation to those obtained in the other substrates (Table 4). Regarding the RFW, seedlings produced in HOR were superior to those obtained in CRH, but did not differ from the seedlings produced in TNG and MIX (Table 4). In addition, the seedlings obtained from HOR and MIX had a higher RDW in comparison to those developed in CRH and TNG (Table 4).

In relation to lettuce cultivars the significant differences occurred for RFW, RDW, TL, SA and FR (Table 4). Thus, we observed that the seedlings of the Green cultivar presented superior performance of the root

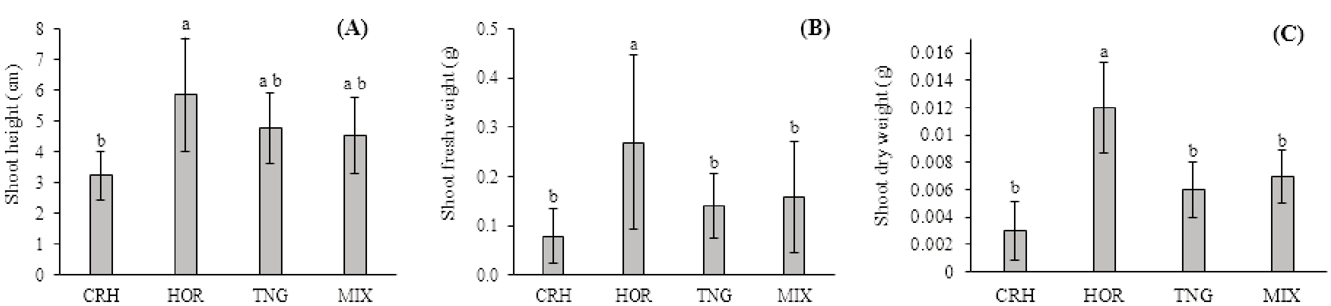

Fig. 4 - Shoot morphology of lettuce seedlings produced in substrates. (A) Shoot height (SH, cm); (B) Shoot fresh weight (SFW, g); (C) Shoot dry weight (SDW, g). Values are means ± standard deviation. Different letters above columns indicate significant differences by the Tukey test (P≤0.05, n = 3). CRH= Carbonized rice hull; HOR= Horta 2®; TNG= TN Gold®; MIX= mixture.

Table 3 - Root system morphology of two lettuce cultivars (*Lactuca sativa* L.) produced in four substrates

Substrates	Root volume (cm³)		Thick root (cm)	
	Purple cv.	Green cv.	Purple cv.	Green cv.
Carbonized rice husk	0.019 ±0.004 A b	0.043±0.017 A c	0.090±0.15 A a	0.078±0.10 A c
Horta 2®	0.074 ±0.029 B a	0.185±0.023 A a	0.501±0.41 B a	3.021±0.51 A a
TN Gold®	0.053 ±0.014 A ab	0.079±0.027 A bc	0.209±0.17 A a	0.660±0.61 A bc
MIX	0.066 ±0.007 B a	0.111±0.019 A b	0.420±0.22 B a	1.440±0.78 A b
Mean	0.07		0.80	
Cofficient of variation (%)	21.77		53.88	

MIX= mixture composed of 40% Caborized rice husk, 40% Horta 2® and 20% TN Gold®. Data presented as mean ± standard deviation. Means followed by the same letter in the column did not differ significantly by the Tukey test (P≤0.05, n= 3).

Table 4 - Root system morphology of two lettuce cultivars (*Lactuca sativa* L.) produced in four substrates

	Total root length (cm)	Root surface area (cm²)	Fine root (cm)	Root fresh weight (g)	Root dry weight (g)
Substrates					
Carbonized rice husk	21.18±05.89	2.81±1.13 c	5.01±2.52 b	0.030±0.01 b	0.0017±0.0008 b
Horta 2®	28.42±10.55	6.54±2.48 a	9.87±2.30 a	0.087±0.04 a	0.0036±0.0010 a
TN Gold®	24.44±13.36	4.39±1.78 bc	8.61±2.58 a	0.060±0.04 ab	0.0023±0.0006 ab
MIX	28.73±09.55	5.54±1.53 ab	9.09±1.93 a	0.061±0.02 ab	0.0032±0.0006 ab
Cultivars					
Purple	21.92±08.13 b	3.70±1.54 b	7.20±3.07 b	0.038±0.01 b	0.0021±0.0008 b
Green	29.46±10.54 a	5.94±2.23 a	9.55±2.52 a	0.080±0.04 a	0.0033±0.0010 a
Mean	25.69	04.82	08.37	00.05	0.0027
CV (%)	26.28	21.32	20.07	46.77	35.41

MIX= mixture composed of 40% Caborized rice husk, 40% Horta 2® and 20% TN Gold®. Data presented as mean ± standard deviation. Means followed by the same letter in the column did not differ significantly by the Tukey test (P≤0.05, n= 3).

system in relation to the seedlings of the Purple cultivar (Table 4).

Seedlings quality

Regarding the seedlings quality, we observed statistical diferences for the substrates and for the cultivars. Analyzing only the substrates, there were significant differences for the DQI. As for the cultivars, the significant differences were observed regarding the SVI and DQI.

Seedlings produced on the HOR substrate presented higher DQI (0.000249 ± 0.000089) than those produced in CHR (0.00011 ± 0.000071), but did not differ statistically from seedlings produced in MIX (Fig. 5). This higher quality of the seedlings produced on HOR increased by 56% in relation to those produced on CHR material (Fig. 5).

The Green cultivar, regardless of the substrate, presented superiority regarding the SVI (Fig. 6 A) and DQI (Fig. 6 B) in relation to the Purple cultivar.

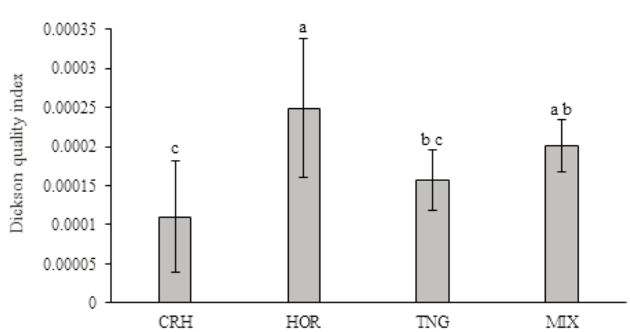

Fig. 5 - Quality of the development of lettuce seedlings produced in four substrates. Values are means ± standard deviation. Different letters above columns indicate significant differences by the Tukey test (P≤0.05, n= 3). CRH= Carbonized rice hull; HOR= Horta 2®; TNG= TN Gold®; MIX= mixture.

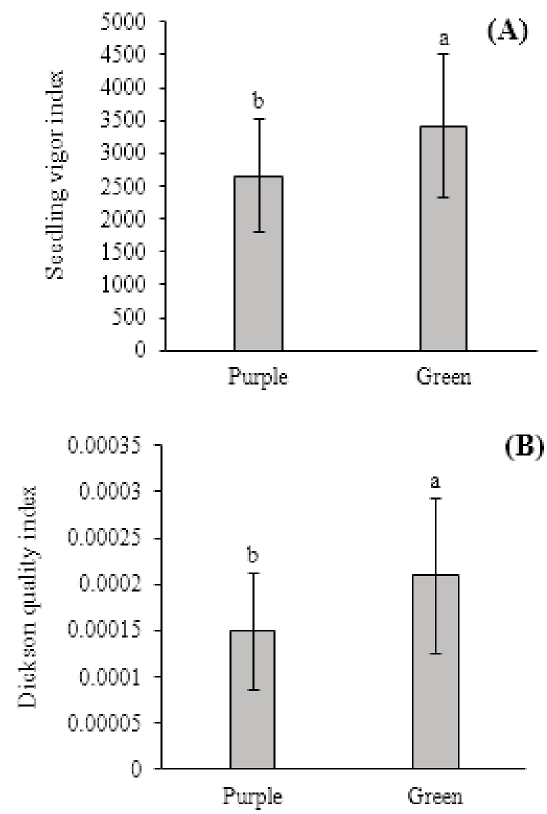

Fig. 6 - Development of seedlings of two lettuce cultivars produced in four substrates. (A) Seedling vigor index (SVI); (B) Dickson quality index (DQI). Values are means ± standard deviation. Different letters above columns indicate significant differences by the Tukey test (P≤0.05, n = 3). Purple: Mimosa Roxa Salad Bowl cultivar; Green: Mônica SF 31 cultivar.

4. Discussion and Conclusions

In general, the research showed that the quality of lettuce cultivars was associated with the types of substrates studied. However, our study showed that substrates with higher water retention promoted greater development of seedlings, through models of plant development. In this way, the physical characterization of the substrates used in the production of seedlings allows to select materials with greater water availability, in order to increase the seedlings quality.

The higher quality of the seedlings produced in the HOR substrate was attribute to the higher availability of water of this material, because the water retention capacity of the substrates influences the growth and development of the seedlings (Graceson *et al.,* 2013), covering the shoot morphology (Prevedello and Armindo, 2015) and the root system morphology (Ferraz *et al.,* 2005). In practice, the data referring to the root system morphology indicated that seedlings of the Green cultivar produced in the HOR substrate have a more structured lump, which improves seedling sustainability after transplanting and increases plant survival.

As in CRH there is predominance of large particle sizes, this impairs water retention by the material (Zorzeto *et al.,* 2014), which explained the lower quality of the seedlings produced in this substrate. In practice, the expansion data of the first true leaves showed that seedlings produced in the CRH would not be suitable for transplant because, in addition to lower water retention, this substrate has pH above the ideal range (5.0 to 6.5) (Bunt, 1988), which reduces the availability of nutrients to plants (Lemaire, 1995).

The higher the seedlings quality delivered to the producers the better the development of the plants in their growth medium and the lower their susceptibility to stresses after transplantation. Through the DQI, we verified that more robust seedlings were produced in the HOR substrate. During the production of the seedlings we observed that the PAR mean (110.5 µmol m^{-2} s^{-1}) was below ideal for the lettuce, corresponding to 196.7 µmol m^{-2} s^{-1} (Ferentinos *et al.,* 2000). This may have reduced the photosynthetic rate of the plants, reducing accumulations of biomass and, therefore, DQI values.

As in our study, other studies showed higher growth and development of seedlings produced in substrates with higher water retention (Smiderle *et al.,* 2001; Costa *et al.,* 2007), as verified in the HOR, and lower performance for seedlings produced in substrates with low water availability (Freitas *et al.,* 2013), as observed in the CRH.

Considering the physicochemical characterization of the substrates, the chemical properties are less relevant than the physical properties (Belda *et al.,* 2016). This is because fertirrigation is provided to the plants, according to the need of the cultivated species. However, the physical quality of the substrates must still be weighed in the choice of materials. The density of the substrates, for example, is linked to plant stability (Noya *et al.,* 2017; Wisdom *et al.,* 2017). Very light substrates (< 100 kg m^{-3}) do not sustain plants and very dense substrates (>300 kg m^{-3}) impair the root growth of seedlings due to mechanical impediment (De Boodt and Verdonck, 1972; Fermino and Kämpf, 2012). In addition, a common problem in substrates is insufficient aeration (Nemati *et al.,* 2002) and, therefore, the nurseryman should choose materials with higher aeration levels to improve root growth and increase the acquisition of water and nutrients by seedlings (Jones and Dolan, 2012).

Thus, in order to maximize the quality of the seedlings produced, nurserymen must obtain the physical characterization of the substrates, selecting materials with greater water retention capacity. In addition, seedling development models, such as SVI and DQI, can be used as indicators of the quality of seedlings produced, as we have verified in our study.

In conclusion, the data show that the seedlings quality of lettuce cultivars associate with the types of substrates studied. In addition, we proves that substrates with greater water retention promote greater development of the seedlings. We emphasize that the use of development models can be an alternative to analyze the seedlings quality provided to producers in order to increase lettuce production. We suggest to substrate producers to sell materials with a clear label informing the physicochemical characteristics of the substrate so that nurserymen and producers can establish an adequate management to potentiate the lettuce production chain.

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