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Photosynthesis, Water Use Efficiency, and Growth Responses of Two Lettuce Cultivars to Different Cover Crop Species

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ABSTRACT

A high demand for lettuce, combined with a growing appeal for the sustainability of production processes, requires changes in production techniques. In such a context, no-till practices appear as a conservation alternative. The current work evaluated the development and productivity of lettuce crops in a no-tillage system. The experiment had four treatments, i.e., conventional management, millet, sunn hemp, and a combination of covers, comprising millet and sunn hemp. Two lettuce cultivars, Pira Roxa and Valentina, were used in this research. The experimental design was set in randomized blocks, with three replications. Physiological, biometric, and productive characteristics were considered, including net photosynthetic rate, stomatal conductance, CO_2 concentration, transpiration, water use efficiency, relative chlorophyll content, head diameter, fresh weight, and productivity. In the Valentina cultivar, there was an increase in the photosynthetic rate when using straw, regardless of the cover plant species. In the cultivar Pira Roxa, the photosynthetic rate was higher without the presence of straw, causing an increase in water use efficiency in the presence of straw. Higher transpiration and head diameter were found in treatments that included all cover plant species in the soil. In addition, fresh weight, leaf count, and productivity increased when using millet straw and the combination for the Valentina cultivar. Regardless of the cover crop or the arrangement between cover plants, the presence of straw on the soil increased the gas exchange capacity in plants, resulting in productive gains.

Introduction

Domesticated lettuce (*Lactuca sativa* L.) originates from wild lettuce species in Asian territories with a mild climate. The heterogeneity of vegetable species and varieties provides opportunities for cultivation throughout Brazil (Beling et al., 2016), arousing the interest of producers, mainly due to the economic return of the activity, which can be developed in small agricultural areas, contributing to social development and economic growth (Girardello et al., 2017).

Lettuce is a leafy vegetable of great importance in diets, proving to be a source of vitamins and

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mineral salts (Monteiro Neto et al., 2014).
The high demand for this vegetable has made considerable changes in production techniques, concerning management systems (Salomão et al., 2020). In this way, there has been a growing search for the use of agricultural practices that conserve the soil and natural resources, contributing to an economically practicable production (Gerlach et al., 2019), resulting in regional growth and providing greater well-being to the population (Girardello et al., 2017).
No-tillage systems have emerged as one of the alternatives to mitigate the effects caused by conventional cultivation. No-tillage involves farmers leaving the soil vulnerable and exposed to natural elements, such as wind and solar radiation, making it difficult to protect against natural weather (Kochann; Denardin, 2000). No-tillage reduces soil profile loss by five times more than the conventional system, stabilizing soil aggregates through crop rotation (Salomão et al., 2020; Castro Filho et al., 1998). It allows for successive soil cover from plant remains in crop rotation (Firmiano et al., 2022). It ensures countless benefits to the soil, such as barriers to erosion processes, limits moisture loss, decreases the thermal range in the soil, development of the physical condition of the soil, and enhanced soil fertility (Gerlach et al., 2019; Cecconello et al., 2020), in addition to reducing phytosanitary problems in the crop (Melo et al., 2010).
A study on the effects of different soil covers on the growth and yield of lettuce plants and soil temperature showed that the use of soil covers with organic material (elephant grass straw) provided superior results when compared to synthetic materials used in research (black polyethylene, silver polyethylene, white polyethylene, transparent polyethylene, and uncovered soil) and even bare soil (Meneses et al., 2016). Regarding the type of mulch used, some studies considered grass and leguminous residues (Meneses et al., 2016). Silva et al. (2017) argued for strategies to increase mulch efficiency, using species with slower decomposition, such as grasses. Also, Vendruscolo et al. (2017) pointed to some potential ground cover species, such as millet, forage sorghum, and jack bean.
Research on jack beans and mulch showed high potential for use as a cover crop due to its biomass production, above 6.0 Mg ha-1, as its residues provided adequate soil cover and contributed to the cycling of large amounts of nutrients (Teixeira et al., 2014). In another study, the use of sunn hemp and millet intercropped in different proportions provided a good amount of soil cover while improving the evaluated characteristics of lettuce (Bertolino et al., 2021) and pepper (Cunha et al., 2023). Therefore, an alternative to reduce the speed of decomposition of legumes and prevent the occurrence of high N immobilization in the soil is to introduce intercropping between grasses and legumes. This technique benefits from the high yield of dry matter compared to the solitary cultivation of the species (Gerlach et al., 2019).
The benefits of covering the soil with either organic or synthetic material for growing lettuce have been reported by several authors and also for other crops such as peppers, garlic, carrots, tomatoes, and melons, demonstrating increased yields. In this context, it is essential to develop research on the viability of different cover crops to guide farmers.
The work was performed to evaluate the development and productivity of two lettuce cultivars (Pira Roxa and Valentina) in a no-tillage system under different vegetation covers in sandy soils of the Brazilian Cerrado.

Material and Methods

The experiment was developed and conducted at the Experimental Farm of the State University of Mato Grosso do Sul, Cassilândia Unit, located at geographic coordinates 19º05'46” S and 51º48'50” W and an altitude of 521 m. According to the Köppen's classification, the climate in the region is predominantly rainy tropical (Aw), with an average annual precipitation of 1,520 mm and a temperature of 24.1 °C, respectively. During the experiment, climatological data were recorded by equipment installed next to the cultivation area (Fig. 1).
The study area was situated in a no-tillage farm. In 2019, the soil was amended for chemical characteristics, according to Silva (2009) and Teixeira et al. (2017). Subsequently, two cycles of cover crops were planted. Before planting the seedlings, the following physical and chemical characteristics of the soil were verified: pH (CaCl2): 5,0, Ca^{2+}: 2,4 cmolc dm^{-3}, Mg^{2+}: 1,4 cmolc dm^{-3} e K^{+}: 0,3 cmolc dm^{-3}, P (resin): 14,0 mg dm^{-3}, Organic matter: 13,0 g dm^{-3}, Al^{3+}: 0,0 cmolc dm^{-3}, H+Al: 1,7 cmolc dm^{-3}, Cation exchange capacity: 5,8 cmolc dm^{-3}, Base saturation: 71%, Sand: 83,0%, Silt: 2,5% e Clay: 14,5%.
The design was in randomized blocks and in a 4 x 2 factorial scheme, with four replications. Treatments consisted of different cover crops: control (conventional management), millet, sunn hemp, a combination of the covers (millet and sunn hemp) and two lettuce cultivars (Pira Roxa and Valentina). The choice of plant materials was based on their characteristics. The Pira Roxa cultivar is curly and has an intense red,

tropicalized color, suitable for annual cultivation, at mild or high temperatures (Sala and Costa, 2005). Its color and red intensity depend on the chlorophyll content and anthocyanin content, which give it greater resistance (Ryder, 1999). The Valentina cultivar has exceptional qualities such as hardiness in any situation and adaptation to tropical growing conditions, as well as resistance to edge blight. Indicated for year-round planting in the main lettuce-producing regions, Valentina has an early production cycle (Oliveira et al., 2021).

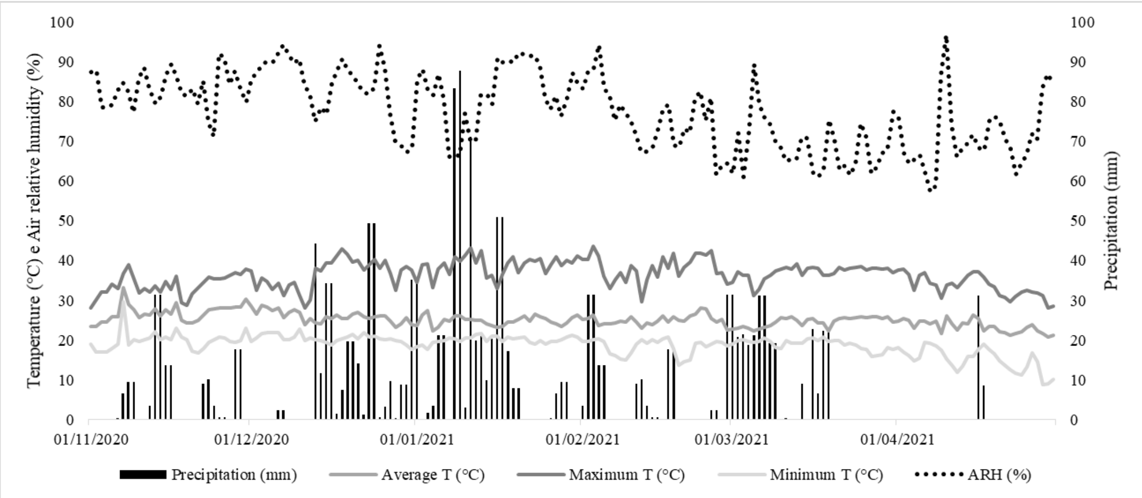

Fig. 1. Climatic conditions of precipitation, relative air humidity and maximum, average, and minimum temperature during the study.

The sowing of cover crops was carried out in November, and the plants grew for 60 days. Then, the plants were killed with herbicide (glyphosate), cut by mowing, and deposited homogeneously on the beds. During this period, lettuce seedlings (cv. Pira Roxa and Valentina) were also obtained and made ready for transplanting in March.

Each plot measured 1.00 x 1.25 m (1.25 m^2) and consisted of 26 lettuce plants, spaced 0.15 m between plants and 0.25 m between rows. Furthermore, to obtain a useful plot, eight central plants of the two internal lines were evaluated, excluding the two external lines. The plants at the ends served as borders. During the lettuce growth period, there was no need to apply chemical pesticides. In addition, topdressing fertilizers consisted of 90 kg of N ha^{-1} and 60 kg of K2O ha^{-1}, divided into three applications at 25, 45, and 60 days after transplanting. Urea was a nitrogen source (46% N) and KCl was a potassium source (60% K2O).

Irrigation was performed using microsprinklers, keeping the soil at field capacity. Before transplanting the seedlings, the straws were counted on the beds using a template with a known area, under which all materials from the cover plants were collected. The material was taken to a forced air circulation oven at 65 °C until reaching a constant mass. They were subsequently weighed on a semi-analytical scale, and the value was extrapolated to an area of one hectare (10,000 m^2).

Forty days after transplanting, four plants were randomly selected and evaluated for net photosynthesis (A), stomatal conductance (gs), intracellular CO_2 concentration (Ci), and transpiration (E) in the morning, when the plants were in full gas exchange activity, between 8 and 10 AM. Disease- and pest-free leaf samples were selected using a portable photosynthesis meter (LCi, ADC Bioscientific, Hertfordshire, United Kingdom) and water use efficiency (WUE) was calculated using a ratio between net photosynthesis and transpiration (A/E) and the efficiency of carboxylation (EICI) using a ratio between net photosynthesis and intracellular CO_2 concentration (A/Ci).

The relative chlorophyll content was evaluated using a digital chlorophyll meter (CCM-200, Opti-Sciences, Hudson, USA). The head diameter was positioned with a graduated ruler just above the plant canopy. After harvest, the leaf count and aerial fresh weight were evaluated, followed by stipulating the productivity by multiplying the fresh weight by the population in one hectare (208 thousand plants). Data were subjected to preliminary tests of normality and homoscedasticity, followed by analysis of variance and the comparison of means via the t test (LSD) ($P \leq 0.05$). Microsoft Excel (2013) executed Pearson's linear correlation.

Results

Whole cover crop treatments allowed the presence of a significant layer of cover on the soil. No significant difference occurred between species or treatment with the intercropped planting of sunn hemp and millet (Fig. 2).

We found that significant effects of the straws occurred on photosynthesis (A) in the cultivar Valentina, for which there was preeminence in treatments containing straw on the soil surface, on average 66% compared to the control treatment. Also, in cultivation conditions without straw, the cultivar Pira Roxa had 66% higher A than the Valentina (Fig. 3A).

Regarding intracellular CO_2 concentrations, no differences occurred between treatments (Fig. 3B). However, higher transpiration rates (E) occurred in lettuce cultivars using soil coverings, 52% higher than the control treatment. In addition, the E of the Pira Roxa cultivar was 15% higher than the Valentina (Fig. 3C). According to the results of stomatal conductance (gs), a difference occurred only between cultivars, and the Pira Roxa cultivar was superior to Valentina by 35% (Fig. 3D).

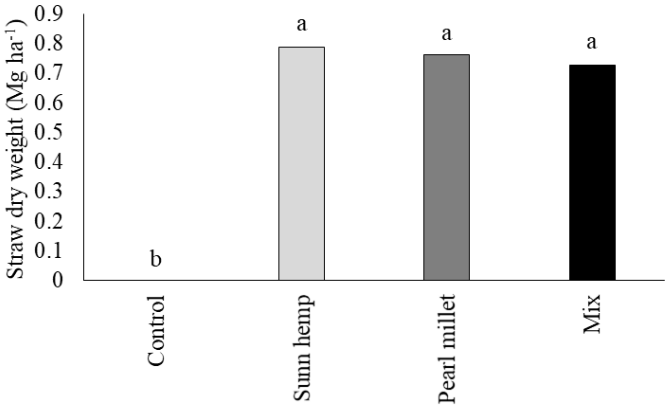

Fig. 2. Dry weight of straw from different cover crops. Bars with similar lowercase letters do not differ from each other by the LSD test (P≤0.05).

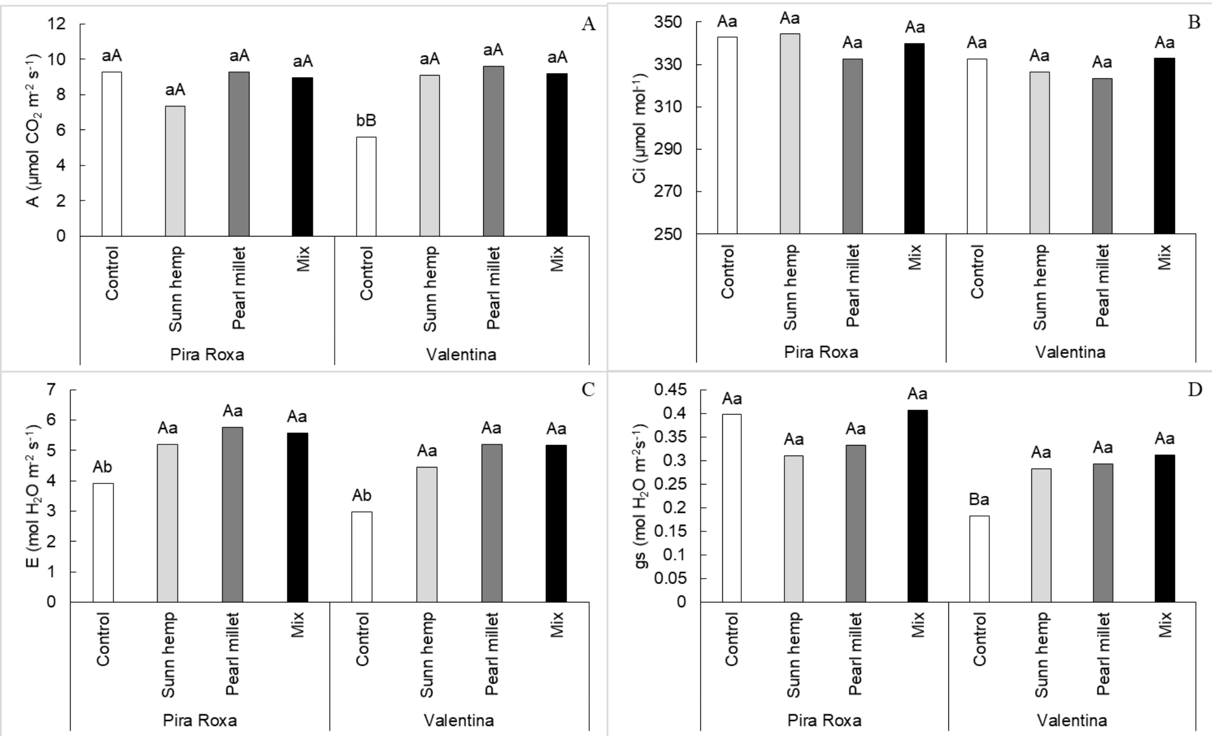

Fig. 3. Net photosynthesis (A) (A), intracellular CO2 concentration (Ci) (B), transpiration (E) (C), and stomatal conductance (gs) (D) of two lettuce cultivars grown on different vegetation covers. Mean values followed by the same lowercase letter within each variety and uppercase within the same treatments do not differ from each other by the LSD test (P≤0.05).

Regarding variations in water use efficiency (WUE), the straws affected the Pira Roxa cultivar (Fig. 4A), with the control treatment having its WUE 52% higher than the others. When checking the effect of the cultivars on each soil cover, the WUE value of the Pira Roxa cultivar was 28% higher in the control. In contrast, sunn hemp provided a WUE value that was 43% higher in the Valentina cultivar. Carboxylation efficiency was not affected in lettuce plants of the Pira Roxa cultivar. However, the Valentina cultivar showed significant increases (65.4%) in response to the straws compared to the control treatment (Fig. 4B).

Regarding the head diameter trait, the interaction between soil cover and cultivars was not significant. Analyzing the soil cover, this trait increased in all treatment groups containing straw as soil cover (Fig. 5). Accordingly, an average gain of 16.55% was obtained when straws were used.

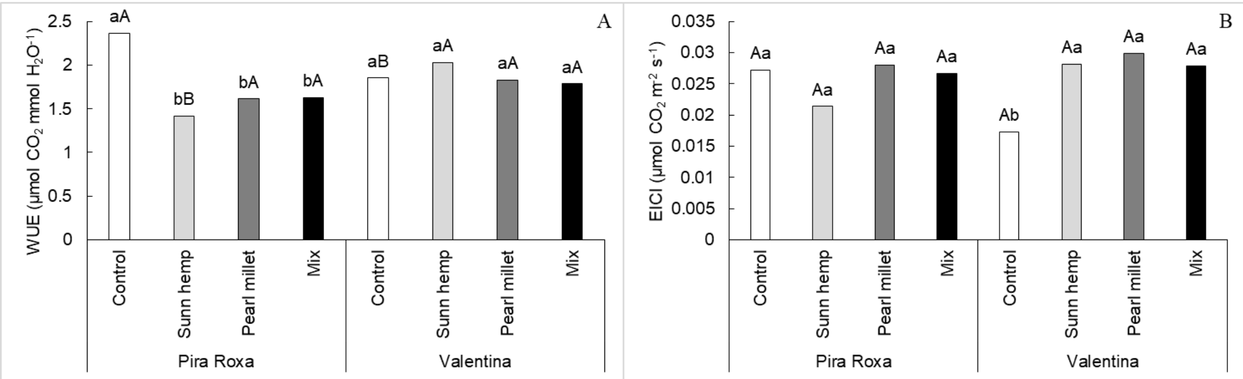

Fig. 4. Water use efficiency (WUE) (A), and carboxylation efficiency (EICI) (B) of Pira Roxa and Valentina as two lettuce cultivars grown on different vegetation covers. Mean values followed by the same lowercase letter within each variety and uppercase within the same treatments do not differ from each other by the LSD test (P≤0.05).

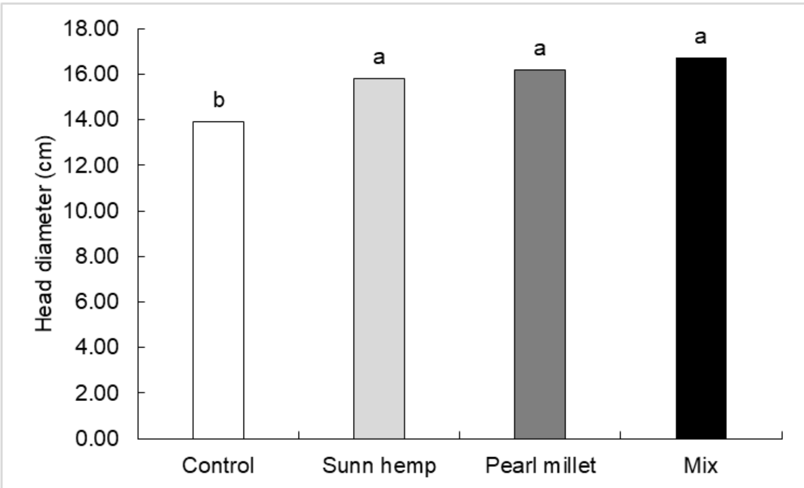

Fig. 5. Head diameter of lettuce grown on different vegetable coverings. Mean values followed by the same letter do not differ statistically by the LSD test (P≤0.05).

Regarding the relative chlorophyll content, positive responses occurred in the treatment containing millet straw for both cultivars, and superiority of the Pira Roxa cultivar in the control, millet, and mixed treatments (Fig. 6A), with increases of 25%, 52%, and 45%, respectively.

The leaf count increased in the Pira Roxa by the presence of sunn hemp and millet straws, with no significant difference from the mixture treatments in the cultivation of Valentina with all straws, compared to the control treatment. Among the cultivars, Pira Roxa was superior in the control treatment by 30%, while Valentina was superior in the treatments containing millet and the mixture covers, on average 19% and 54%, respectively (Fig. 6B).

The results referring to the characteristics of fresh weight and productivity for the Pira Roxa cultivar were the same since they are related. Nevertheless, all straws benefited Valentina plants in cultivation, highlighting the usefulness of the millet cover and the mixture. Among the cultivars, Pira Roxa was 97% superior in the control treatment, while Valentina was superior in the treatments containing millet and the mixture, on average 27% and 61%, respectively (Figs. 6C, D). Also, for Valentina, an average gain of 100% was estimated for both traits when using the soil covers.

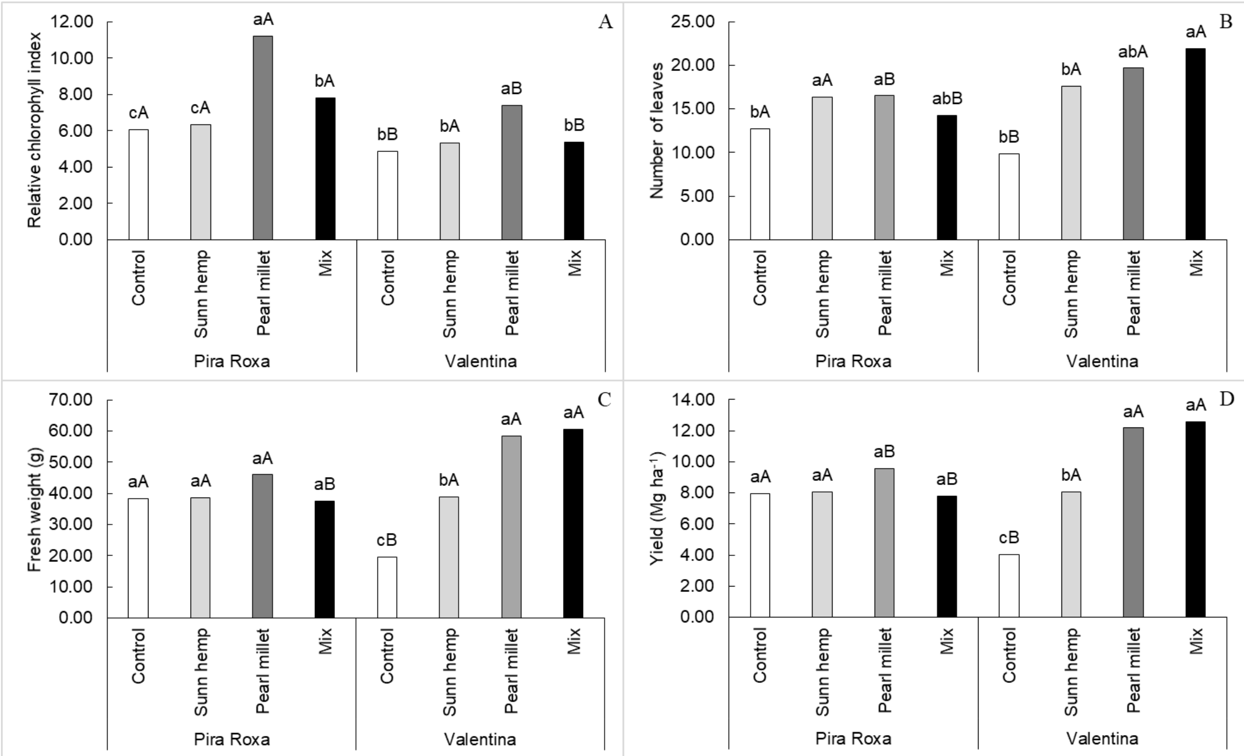

Fig. 6. Relative chlorophyll content (A), number of leaves (B), fresh weight (C), and yield (D) of the two lettuce cultivars grown with different cover crops. Mean values followed by the same lowercase letters within each variety and uppercase within the same treatments do not differ from each other by the LSD test (P≤0.05).

Pearson's linear correlation revealed a positive correlation between most biometric characteristics. Regarding the relative chlorophyll index, there was a positive correlation with fresh weight, productivity, and transpiration. A positive correlation was also verified between net photosynthesis and transpiration. However, net photosynthesis negatively correlated with intracellular CO_2 content, while water use efficiency negatively correlated with leaf count and transpiration (Table 1). Using straw on the soil surface correlated positively with the head diameter, fresh weight, number of leaves, yield, transpiration, and water use efficiency (Table 1).

Discussion

Some factors can limit photosynthetic rates, such as low amounts of light or CO_2, water-deficit stress, or high temperatures (Taiz et al., 2015). However, we found that the control treatment of the Pira Roxa cultivar had better performance (Fig. 3A), assuming that this result is related to the amount of anthocyanins present in the cultivar, as anthocyanins have antioxidant potential and act by reducing plant stress (Hinojosa et al., 2020).

The no-tillage system brings several advantages for the subsequent cultures, according to Melo et al. (2010), such as not disturbing the soil, avoiding erosion, reducing phytosanitary problems due to crop rotation, soil thermal stability, and mild temperatures, which benefit the root system of some vegetables (Cecconello et al., 2020). It also has the potential to improve the physical and water conditions of the soil, keeping the soil moist and increasing water availability (Cardoso et al., 2012). With the straw present on the soil surface (Fig. 2), the availability of minerals is also verified since it does not decrease by leaching (Collier et al., 2011), which results in the gains observed concerning photosynthesis and transpiration (Figs. 3A, C).

The higher WUE in the control treatment is due to the lower transpiration rate (Pazzagli et al., 2016) and lower stomatal conductance, which may be related to water-deficit stress caused by evaporation when there is no vegetation cover (Buesa et al., 2021). Maintaining straw as a physical barrier on the soil also allows for greater water availability. According to Fietz et al. (2015), higher leaf area indices can increase crop demand for water, thus reducing water use efficiency, as observed in the present study (Fig. 4A) through a negative linear correlation between the number of leaves and WUE. This correlation resulted from lower water availability, in which plants tend to close their stomata, reducing water loss through direct exchange with the atmosphere (Taiz et al., 2015).

Table 1. Estimations of Pearson's linear correlation coefficient between characteristics of lettuce plants cultivated in a no-tillage system with different soil coverings.

	HD	RCI	FW	NL	Yield	ci	E	gs	A	WUE	EICI	Straw
HD		0.360	0.635	0.621	0.654	-0.337	0.594	0.001	0.330	-0.433	-0.016	0.604
RCI	ns		0.614	0.496	0.587	-0.245	0.508	0.002	0.313	-0.362	0.224	0.448
FW	**	*		0.857	0.990	-0.314	0.668	0.126	0.478	-0.354	0.395	0.668
NL	*	ns	**		0.858	-0.127	0.803	0.276	0.473	-0.579	0.456	0.926
Yield	**	*	**	**		-0.301	0.656	0.116	0.475	-0.356	0.366	0.672
ci	ns	ns	ns	ns	ns		-0.144	0.527	-0.510	-0.470	-0.053	-0.029
E	*	*	**	**	**	ns		0.561	0.746	-0.509	0.481	0.772
gs	ns	ns	ns	ns	ns	*	*		0.415	-0.279	0.213	0.231
A	ns	ns	ns	ns	ns	*	**	ns		0.178	0.314	0.346
WUE	ns	ns	ns	*	ns	ns	*	ns	ns		0.210	0.710
EICI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		0.422
Straw	*	ns	**	**	**	ns	**	ns	ns	**	ns	
					p≤0.01	p≤0.05	p≤0.05	p≤0.01				

HD = head diameter; RCI = relative chlorophyll index; FM = fresh weight; ci = intracellular CO_2 concentration; E = perspiration; gs = stomatal conductance; A = net photosynthesis; WUE = water use efficiency; **, *, and ns = significant at 1%, 5% probability, and not significant, respectively, by the t-test.

The transpiration rates obtained for the treatments have positive linear correlations with vegetative development and productivity (Table 1). The decrease in transpiration correlates with water deficiency (Tufail et al., 2023). The results demonstrated that cultivars can be better adapted to the environment, opening their stomata and maintaining their transpiration or indicating a greater availability of resources for physiological activities when cultivated with soil covers.

The physiological characteristics correlated directly or indirectly with the productive traits of curly lettuce (Table 1), demonstrating that its maintenance or promotion during the productive cycle is essential to obtain more productive plants with quality for the market. Results obtained in studies on other species of vegetables confirm this fact. Research on beet cultivars under different soil covers showed a greater weight and yield gain when cultivating with mulch and plant residues (Souza et al., 2020). Similarly, Madeira and Oliveira (2004) studied cover crops and the formation of straw in an onion no-tillage system, showing the best productivity and the highest average bulb weight when using millet and sorghum straw.

Covers crops have distinct characteristics for nutrient cycling and their decomposition rate (Vendruscolo et al., 2018) and may serve as an extra source of nutrients for the crop (Kader et al., 2017). Thus, grasses stand out in the no-tillage system for slow decomposition (Mingotte et al., 2014). For the production of vegetables, weed control, increased water, and nutrient availability are indispensable characteristics of great importance (Vendruscolo et al., 2017). In addition, legumes, such as sunn hemp, tend to improve the nutrition of the subsequent crop, as they provide plant residue production and rapid nutrient cycling, in addition to the ability to fix atmospheric N (Leal et al., 2013).

Notably, the no-tillage system has remarkable influence on improving soil characteristics, as well as the interaction between soil and plant, allowing better development of plants, enhancing economic gains (Vendruscolo et al., 2017), and improving the use of nutritional resources such as nitrogen (Gomides et al., 2023). In addition, this system is beneficial for growing vegetables in tropical regions, where soil and climate conditions are less conducive to the growth of plant species from milder climates.

Conclusion

Regardless of the cover plant or the arrangement among cover plants, the presence of straw on the soil increases the gas exchange capacity of lettuce plants, resulting in productive gains. Among the cover crops, pearl millet provided the best results for the parameters relative to chlorophyll content, leaf count, fresh weight, and productivity. Lettuce genotypes responded differently to the species cultivated as cover crops but had a positive response to the presence of straws.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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