Morphophysiological and developmental parameters of soybean cultivars

Parâmetros morfofisiológicos e de desenvolvimento de cultivares de soja

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Abstract
The choice of cultivars is an important factor for greater productivity in agriculture. The objective of this work is to know the morphophysiological and developmental parameters of soybean cultivars. The experiment was conducted in August 2021, at Faculdades Integradas Stella Maris (FISMA), located in the Municipality of Andradina, State of São Paulo. The design was completely randomized, where five soybean varieties were grown: 8579R5F; 84I86; M8644IPRO; DM80I79; 81I81 and with four replicates, totaling 20 plots or pots. The cultivar 8579R5F stood out in the development parameters in number of trifoliate (NT), shoot dry mass (DMAP) and also in the concentrations of chlorophylls A and B. The cultivar M8644IPRO showed the highest internal leaf morphology parameters for phloem diameter (PD) and palisade parenchyma thickness (PPT). Chlorophyll A and B concentrations present correlations with positive linear responses with shoot dry mass. Stomatal functionality (SF) and phloem diameter (PD) present correlations with positive linear responses with adaxial epidermis thickness (ADET).
Keywords: *Glycine max* (L) Merrill; Cultivars; Plant morphology; Plant physiology.

Resumo
A escolha de cultivares é um fator importante para uma maior produtividade na agricultura. O objetivo desse trabalho é conhecer os parâmetros morfofisiológicos e de desenvolvimento de cultivares de soja. O experimento foi realizado em agosto de 2021, nas Faculdades Integradas Stella Maris (FISMA), localizadas no Município de Andradina, Estado de São Paulo. O desenho foi completamente aleatório, onde foram cultivadas cinco variedades de soja: 8579R5F; 84I86; M8644IPRO; DM80I79; 81I81 e com quatro réplicas, totalizando 20 parcelas ou vasos. A cultivar 8579R5F se destacou nos parâmetros de desenvolvimento em número de trifólios (NT), massa seca da parte aérea (DMAP) e também nas concentrações das clorofilas A e B. A cultivar M8644IPRO apresentou maiores parâmetros de morfologia interna da folha para o diâmetro de floema (PD) e espessura do parênquima paliçádico (PPT). As concentrações de clorofilas A e B apresentam correlações com respostas lineares positivas com a massa seca da parte aérea. A funcionalidade estomática (SF) e diâmetro de floema (PD) apresentam correlações com respostas lineares positivas com a espessura da epiderme adaxial (ADET).
Palavras-chave: *Glycine max* (L) Merrill; Cultivares; Morfologia vegetal; Fisiologia vegetal.

Resumen
La elección de los cultivares es un factor importante para una mayor productividad en la agricultura. El objetivo de este trabajo es conocer los parámetros morfofisiológicos y de desarrollo de cultivares de soja. El experimento se

realizó en agosto de 2021, en las Faculdades Integradas Stella Maris (FISMA), ubicadas en el Municipio de Andradina, Estado de São Paulo. El diseño fue completamente al azar, donde se sembraron cinco variedades de soya: 8579R5F; 84I86; M8644IPRO; DM80I79; 81I81 y con cuatro repeticiones, totalizando 20 parcelas o macetas. El cultivar 8579R5F sobresalió en los parámetros de desarrollo en número de trifoliados (NT), masa seca aérea (DMAP) y también en las concentraciones de clorofilas A y B. El cultivar M8644IPRO presentó mayores parámetros de morfología interna de hoja para el diámetro de floema (PD) y espesor del parénquima en empalizada (PPT). Las concentraciones de clorofila A y B presentan correlaciones con respuestas lineales positivas con la masa seca de los brotes. La funcionalidad estomática (SF) y el diámetro del floema (PD) presentan correlaciones con respuestas lineales positivas con el espesor de la epidermis adaxial (ADET).
Palabras clave: *Glycine max* (L) Merrill; Cultivares; Morfología vegetal; Fisiología.

1. Introduction

Soybean (*Glycine max* L. Merrill) is an oilseed originally from Asia and introduced in Brazil in 1882 in the state of Bahia. The increase in its production is linked to technological advances, cultural management and improved efficiency of rural producers. Several genetic improvements in agricultural crops aim to increase herbicide tolerance, insect resistance, agricultural and technological productivity, with the possibility of expanding to other areas of agriculture (Gazzoni, 2018; Barrozo & Rosa, 2018).

Its importance stems from the fact that it is one of the most important and accessible sources of vegetable proteins and fatty acids for food security, as they serve as the basis for poultry, swine and cattle rations. Furthermore, as a result of advances in the human food industry, they are used in the formulation of many food products (Gazzoni & Dall'agnol, 2018).

A combination of molecular biology techniques and exogenous gene transfer represents a powerful tool to introduce new traits into a specific plant. In this sense, understanding the anatomical and morphological changes in plant species as a result of certain programs or genetic alterations can help researchers to improve and develop plant-based technologies to maximize their impact on plants (Teles & Fuck, 2018).

It is important to note that the changes visible to the naked eye are caused by changes in plant tissues in their basic structures and where it can be well observed in their vascular tissues. As a result, it is necessary to determine whether these changes that are caused by the environment or the nature of the cultivar (Castro et al., 2009).

The objective of this work is to know the morphophysiological and developmental parameters of soybean cultivars.

2. Material and Methods

2.1 Installation and Conduct of the Experiment

The experiment was carried out in August 2021, at Faculdades Integradas Stella Maris (FISMA), located in the Municipality of Andradina, State of São Paulo. The design was completely randomized, where five soybean varieties were grown: 8579R5F; 84I86; M8644IPRO; DM80I79; 81I81 and with four replications, totaling 20 plots or pots.

The pots had a volumetric capacity of five dm^{-3} and were filled with soil originating from the 0-0.3 m layer classified as Hypoferric Red Latosol (Embrapa, 2013) and present the following chemical attributes as shown in Table 1.

Table 1. Chemical attributes of the soil at the time of installation of the experiment. Andradina, 2021.

pH	OM	P	K	Ca	Mg	H+Al	Al	SB	CTC	V%	m%
$CaCl_2$	g dm^{-3}	mg dm^{-3}	---------------------- mmol$_c$ dm^{-3} ---------------------								
3.9	18	2.0	1.6	5.0	3.0	42	11	9.6	51.6	19	53

OM: Organic matter; SB: Sum of bases; V%: Base saturation; m%: Saturation by aluminum.
Source: Authors.

The soil was fertilized according to the requirements of the corn crop according to Raij et al. (1996). And six viable seeds of the cultivar were planted five centimeters deep. During the experiment, all vessels were irrigated until reaching field capacity and all cultural treatments were carried out.

2.2 Development parameters

At 30 days after planting, the following variables were determined: plant height (PH) determined using a ruler graduated in millimeters; stem diameter (SD) determined using a caliper graduated in millimeters; number of trefoils (NT) determined by direct counting on the plant. The dry mass of aerial part and dry mass of root (DMAP and MSR) were determined by drying in a circulation oven and air renewal at a constant temperature of 65°C until reaching constant weight.

2.3 Chlorophyll Parameters

The concentrations of chlorophylls A and B (Chloro A and B) were determined by direct reading with the use of the ClorofiLog® device (Falker), given the values in SPAD index (Parry et al. 2014) and later converted into absolute values of the pigments as described by Chang &Troughton (1972).

2.4 Morphological parameters

A fragment of the middle third of the first fully expanded leaf was collected from the apex of the plant. All the fragments received the relevant procedures for dehydration, diaphanization, inclusion and blocking, and with the aid of a microtome, cross sections of 10.0 µm were carried out in each tissue fragment, where they were stained with safranin. The slides were observed under an optical microscope with a camera attached to perform measurements of histological variables through an image program, calibrated with a microscopic ruler at the same magnification, where the following tissues were measured: leaf phloem diameter (PD); leaf xylem diameter (XD), Palisade parenchyma thickness (PPT) and adaxial and abaxial epidermis thickness (ADET and ABET) (Kraus & Arduim, 1997). For all variables, ten measurements were performed per slide, totaling 40 measurements per treatment.

The impression was also performed on the inferior or abaxial epidermal surface of the collected leaf fragments using cyanoacrylate ester, to determine the stomatal functionality of the inferior or abaxial surface (SF) and stomatal density of the inferior or abaxial surface (DEN) (Carlquist, 1975; Castro et al., 2009). For all variables, 10 measurements were performed per slide. The plots were represented by the average value obtained from the measurements of each characteristic.

2.5 Statistical analysis

For statistical evaluation, the variables were submitted to normality tests using the Shapiro-Wilk and test Tukey at 5% probability (Banzatto & Kronka, 2013), a Pearson correlation was also performed using the statistical program R (R Core Team, 2015).

3. Results

No significant difference was found between soybean cultivars for plant height (PH) and stem diameter (SD) parameters, as shown in Table 2.

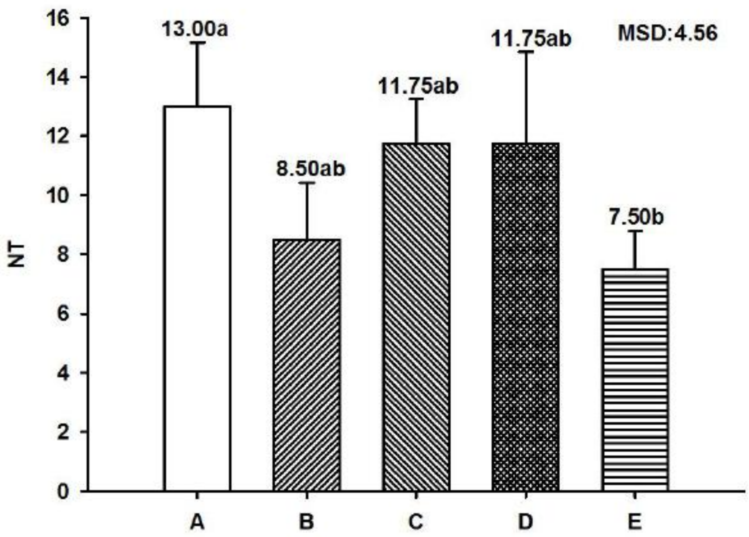
Table 2. Statistical analysis of plant height (PH); stem diameter (SD); number of trefoils (NT); dry mass of aerial part (DMAP) and dry mass of root (DMR) of soybean varieties. Andradina, 2021.

	PH(cm)	SD(cm)	NT	DMAP(g)	DMR(g)
p value	0.1881ns	0.6117ns	0.0083**	0.0020**	0.0001**
OM	75.91	0.95	10.50	1.28	1.86
SD	10.25	0.20	2.08	0.40	0.40
SEM	5.12	0.10	1.04	0.20	0.20
CV (%)	13.50	21.05	19.90	31.43	21.51

** – significant at the 1% probability level ($p<0.01$); * – significant at the 5% probability level ($0.01=<p<0.05$); ns – not significant ($p>=0.05$); OA: overall average; SD: standard deviation; SEM: standard error of the mean; CV: coefficient of variation. Source: Authors.

However, a difference was observed for the number of trifoliate (NT), where the cultivar 8579R5F had the highest average, which implied 42.30% higher than 81I81, which showed the lowest average, as shown in Figure 1.

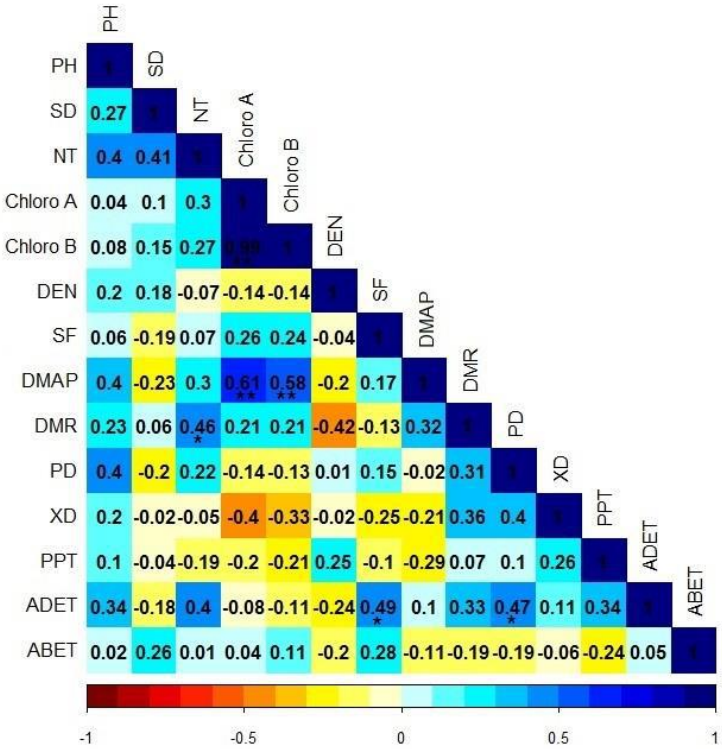
Figure 1. Mean values of number of trefoils (NT) of soybean varieties. A – 8579R5F; B – 84I86; C – M8644IPRO; D – DM80I79 and E – 81I81. MSD: Minimal significant difference. The means followed by the same letter do not differ statistically from each other. The Tukey Test was applied at the level of 5% probability. Andradina, 2021.



Source: Authors.

A significant correlation was found between the number of trifoliate (NT) and the root dry mass (DMR) as shown in Figure 2.

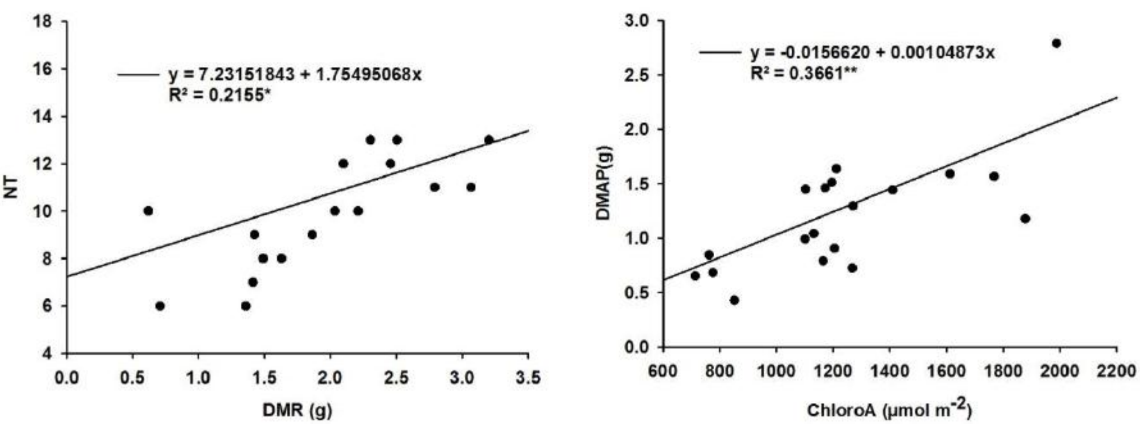
Figure 2. Pearson's correlation between the variables analyzed in the soybean cultivars. Andradina, 2022. SP. PH - Plant height; SD – Stem diameter; NT – Number of trefoils; Chloro A and B – Chlorophylls; DEN – Stomatal density; SF – Stomatal functionality; DMAP – Dry mass of aerial part; DMR – Dry mass of root; PD – Phloem diameter; XD – Xylem diameter; ADET and ABET – Abaxial Abaxial epidermis thickness. Andradina, SP, 2020. ** – significant at the 1% probability level (p<0.01); * – significant at the 5% probability level (0.01=<p<0.05).

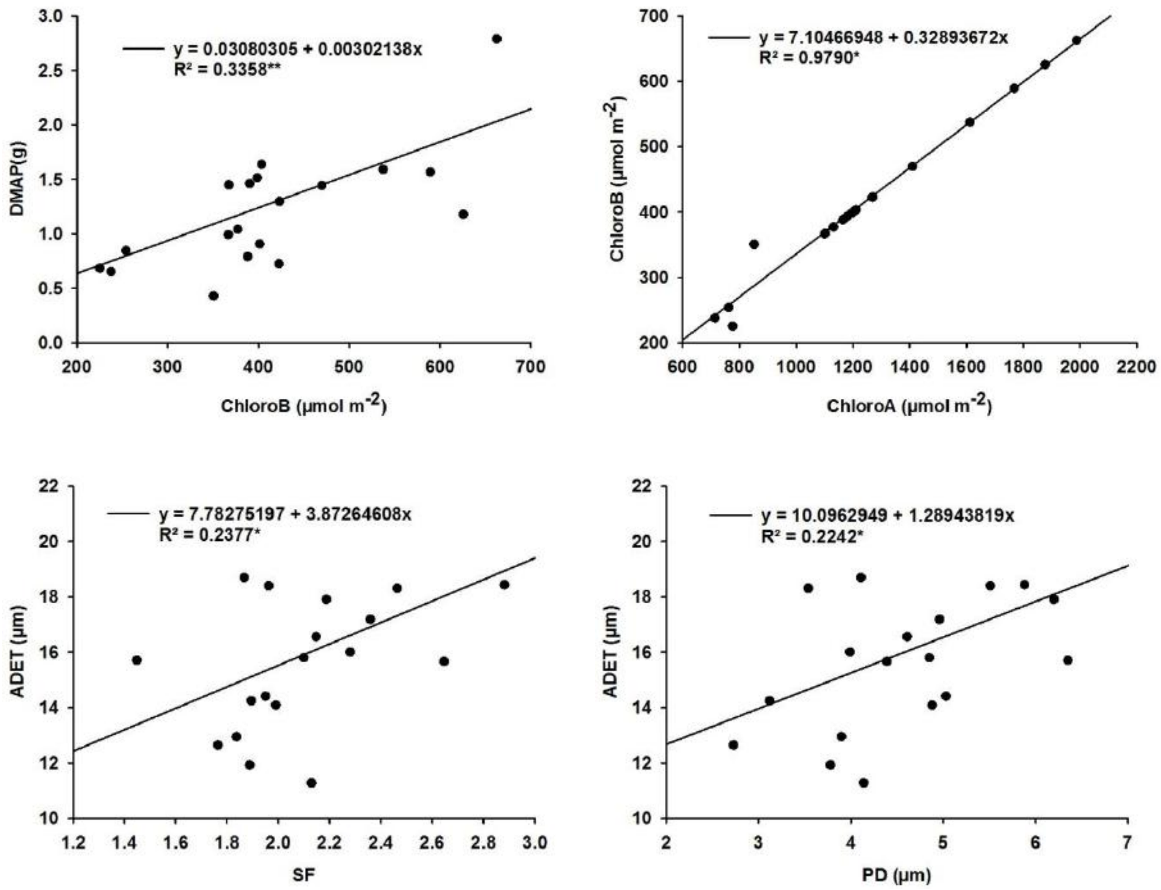


Source: Authors.

Thus, with the increase in root dry mass (DMR), it provides an increase in the number of trifoliate (NT) as shown in Figure 3.

Figure 3. Significant linear regressions of Pearson's interactions of the variables analyzed in soybean cultivars. Andradina, 2021.

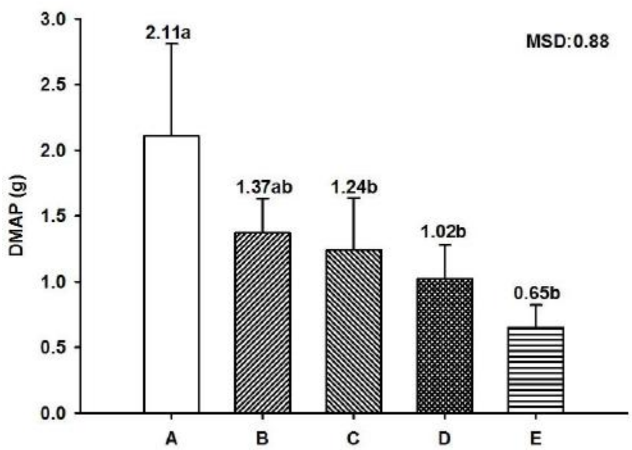




Source: Authors.

A difference was also observed between the soybean cultivars for the parameter dry mass of the aerial part (DMAP), where again the cultivar 8579R5F stood out, presenting the best results, which showed a difference 69.19% higher in relation to 81I81 as shown in Figure 4.

Figure 4: Mean values of dry mass of aerial part (DMAP) of soybean varieties. A – 8579R5F; B – 84I86; C – M8644IPRO; D – DM80I79 and E – 81I81. MSD: Minimal significant difference. The means followed by the same letter do not differ statistically from each other. The Tukey Test was applied at the level of 5% probability. Andradina, 2021.

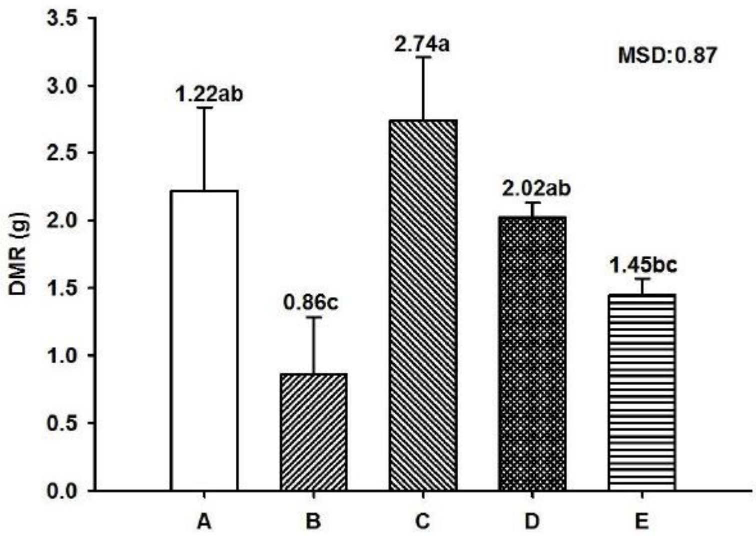


Source: Authors.

The aerial part dry mass (DMAP) presented a positive correlation between the chlorophylls, with the increase of its concentration it provides an increase in the dry mass of the soybean as shown in Figure 3, as the chlorophyll provides a greater efficiency in the photosynthetic rate.

A statistical difference was found for the root dry mass (DMR) between the cultivars, where again the 8579R5F cultivar was superior by 29.50% in relation to the 84I86 cultivar, which presented the lowest average as seen in Figure 5.

Figure 5. Mean values of dry mass of root (DMR) of soybean varieties. A – 8579R5F; B – 84I86; C – M8644IPRO; D – DM80I79 and E – 81I81. MSD: Minimal significant difference. The means followed by the same letter do not differ statistically from each other. The Tukey Test was applied at the level of 5% probability. Andradina, 2021.



Source: Authors.

A statistical difference was observed between the cultivars for the levels of Chlorophylls A and B, as shown in Table 3.

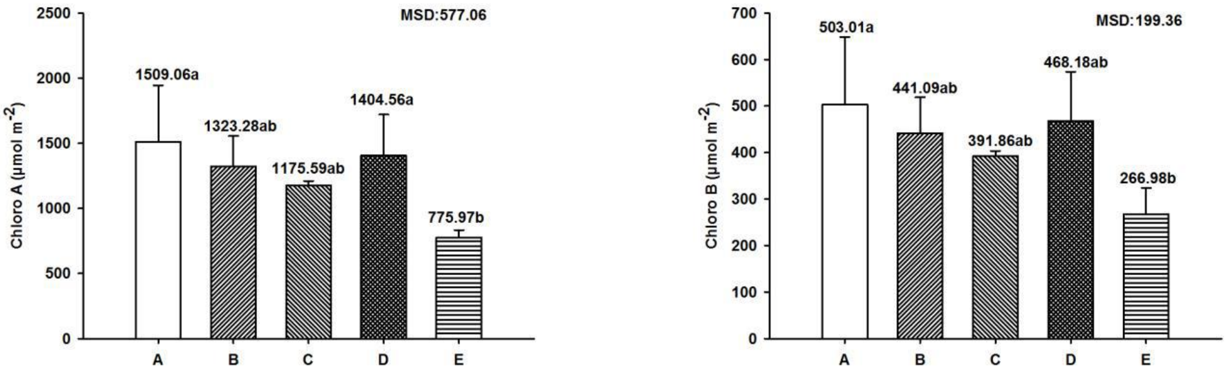
Table 3. Statistical analysis of chlorophylls (Chloro A and B), stomatal functionality (SF) and stomatal density (DEN) of the soybean varieties. Andradina, 2021.

	Chloro A	Chloro B	SF	DEN
p value	0.0120*	0.0203*	0.1289ns	0.2072ns
OA	1237.69	414.22	2.12	208.75
SD	264.28	91.30	0.30	22.47
SEM	132.14	45.65	0.15	11.23
CV (%)	21.35	22.04	14.08	10.76

** – significant at the 1% probability level (p<0.01); * – significant at the 5% probability level (0.01=<p<0.05); ns – not significant (p>=0.05); OA: overall average; SD: standard deviation; SEM: standard error of the mean; CV: coefficient of variation. Source: Authors.

Again, cultivar 8579R5F showed higher averages for chlorophyll A and B contents, where this difference was 48.38 and 46.92%, respectively, higher in relation to 81I81, as shown in Figure 6. Due to the same physiological origin, chlorophylls showed a significant correlation as seen in Figures 2 and 3.

Figure 6: Mean values of chlorophylls A and B (Chloro A and B) of soybean varieties. A – 8579R5F; B – 84I86; C – M8644IPRO; D – DM80I79 and E – 81I81. MSD: Minimal significant difference. The means followed by the same letter do not differ statistically from each other. The Tukey Test was applied at the level of 5% probability. Andradina, 2021.



Source: Authors.

There was no statistical difference between soybean cultivars for the parameters of stomatal functionality (SF) and stomatal density (DEN) as observed in Table 3. However, a significant correlation was found between stomatal functionality (SF) with the thickness of the stomata. adaxial epidermis (ADET), with the positive linear increase in functionality provides a greater thickness of the epidermis as seen in Figure 3.

However, a statistical difference was observed between soybean cultivars for phloem diameter (PD), where M8644IPRO had the highest average, showing 36.82% higher than cultivar DM80I79, as shown in Table 4.

Table 4. Statistical analysis of leaf phloem diameter (PD); leaf xylem diameter (XD), Palisade parenchyma thickness (PPT) and adaxial and abaxial epidermis thickness (ADET and ABET) of the corn varieties. Andradina, 2021.

	PD(µm)	XD(µm)	PPT(µm)	ADET(µm)	ABET(µm)
8579R5F	5.01ab	14.19	47.47b	17.51	11.73
84I86	4.28ab	11.68	62.44ab	15.10	11.27
M8644IPRO	5.54a	16.46	68.83a	17.68	9.98
DM80I79	3.50b	12.58	57.28ab	15.44	12.41
81I81	4.66ab	16.74	57.08ab	14.40	11.58
MSD	1.64	7.28	16.95	5.58	5.04
p value	0.0176*	0.1755ns	0.0193*	0.3018ns	0.6703ns
OA	4.60	14.33	58.62	16.02	11.39
SD	0.75	3.33	7.76	2.56	2.31
SEM	0.37	1.66	3.88	1.28	1.15
CV (%)	16.33	23.29	13.24	15.97	20.29

Equal means in the column do not differ statistically, Tukey's test was applied at 5% probability of the event occurring. ** – significant at the 1% probability level ($p<0.01$); * – significant at the 5% probability level ($0.01=<p<0.05$); ns – not significant ($p>=0.05$); MSD: Minimum significant difference; OA: overall average; SD: standard deviation; SEM: standard error of the mean; CV: coefficient of variation. Source: Authors.

Soybean cultivars did not differ statistically for xylem diameter, adaxial and abaxial epidermis, while a difference was found between soybean cultivars for the parameter of palisade parenchyma thickness, where again the cultivar M8644IPRO was the one that stood out the most. , with approximately 31.03% higher than the 8579R5F, which showed lower mean values, as seen in Table 4.

4. Discussion

The number of leaves ensures a higher rate of photosynthesis of plants, which leads to a greater absorption of atmospheric CO_2 and which is fixed in its dry mass, in this way, the number of leaf trifoliate can be influenced with the availability of absorbed nutrients by the root system, where it allows a greater exploration in volume of soil (Ramakrishna & Barberon, 2019). With the linear increase of the mass, it can reflect in a greater exploration in the deeper regions of the soil, which starts to provide a greater amount of nutrients and water for the leaves of the soybean (Müller et al., 2021). The soybean genotype factor may be a limiting factor in this relationship between these two traits (NTxDMR) due to their genetic variations (Hossain et al., 2014).

The increase in the dry mass of a plant is directly linked to the photosynthesis rate, where the concentration of chlorophyll A and B pigments is a limiting factor, since the photosynthetic apparatus demands molecules with stable integrity, which thus guarantees its maximum performance energy, which can result in a higher rate of atmospheric CO_2 fixation in its dry mass (Wang et al., 2021) this fact can be well elucidated with the positive interaction found as shown in Figures 2 and 3. The absorption action sites of each wavelength of each chlorophyll may present differences between species and even in cultivars, as demonstrated by studies by Hossain et al. (2014) where they still report these phenotypic variations of responses to water stress where they respond differently between them.

These morphophysiological differences between soybean cultivars raise the need for further studies to ensure better development of the culture in different production environments. The internal morphology of the organs may show changes from the initial phase of establishment to the productive phase of the culture, as more developed tissues can guarantee greater final productivity (Wallner et al., 2017; López-Salmerón et al., 2019). The phloem vessels guarantee a better distribution of metabolized sap to the other regions of the plant organism, mainly in the relationship between source and drain of metabolites for the grain filling phase in the reproductive phase of the plant (Pfautsch et al., 2015). It is important to highlight the correlation between the phloem vessels (Figures 2 and 3) with the thickness of the adaxial epidermis, which demonstrates the importance of more developed vessels that provide larger tissues, the phloem may even favor the exchange of water with the xylem (López-Salmerón et al., 2019; Mehdi et al., 2019).

Plants can produce these metabolites in greater quantities when they have a more developed palisade parenchyma, as this tissue has the largest number of chloroplasts in its cells, which guarantees a better photosynthetic rate (Almeida et al., 2021; Carrera et al., 2021; Giaquinta et al., 1985). It is worth mentioning that this tissue has a high density and resistance, due to the toothpick shape of its cells that are arranged in the adaxial region of the leaf (Castro et al., 2009).

5. Final Considerations

The cultivar 8579R5F stood out in the development parameters in number of trifoliate (NT), shoot dry mass (DMAP) and also in the concentrations of chlorophylls A and B.

The cultivar M8644IPRO showed the highest internal leaf morphology parameters for phloem diameter (PD) and palisade parenchyma thickness (PPT).

Chlorophyll A and B concentrations present correlations with positive linear responses with shoot dry mass.

Stomatal functionality (SF) and phloem diameter (PD) present correlations with positive linear responses with adaxial epidermis thickness (ADET).

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