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Behaviour of soybean seedlings in situations involving cotyledon removal

Allan de Marcos Lapaz[1*], Luiz Felipe de Melo Santos[2], Gabriel Leonardi Antonio[1], João Henrique Silva Vera[1], Lucas Aparecido Manzani Lisboa[1] and Paulo Alexandre Monteiro de Figueiredo[1]

[1]*São Paulo State University (UNESP), School of Natural Sciences and Engineering, 15385-000 Ilha Solteira, SP – Brazil*
[2]*São Paulo State University (UNESP), College of Technology and Agricultural Sciences, 17900-000 Dracena, SP – Brazil*
[*]*Corresponding author:* allanlapaz60@gmail.com

Abstract

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Cotyledons have photosynthetic and reserve functions. In initial development, cotyledon herbivory makes the majority of species more susceptible to the environment. This study aimed to reproduce herbivory situations occurring in the field, caused by insect pests, through manual partial and total removal of the cotyledons, in order to evaluate the deleterious effects on the initial development of soybean [*Glycine max* (L.) Merrill]. The experimental design was completely randomized and arranged in a 2 × 3 factorial scheme, with two soybean cultivars (BMX Potência RR and Syngenta 1562 IPRO) and three situations involving removal of cotyledons (0%, 50% and 100%) in four replications, totalling 24 experimental plots. The partial or total removal of cotyledons caused a reduction in parameters related to the growth and initial development of soybean (stalk diameter, shoot length, root primary length, shoot dry mass and root dry mass), which is probably due to the nutritional and morphological changes documented in this study. In general, the Syngenta 1562 IPRO cultivar was more sensitive to cotyledon removal than the BMX Potência RR cultivar.

Keywords: Glycine max (L.) Merrill; initial development; morphophysiology; nutrients

Introduction

Germination is a complex process that occurs under suitable abiotic conditions through imbibition of water. Once the imbibition is done, carbohydrates are mobilized as reduced sugars, marking an increase in the intensity of cellular respiration. In sequence, the accumulated starch and the storage proteins in the embryo are used as sources for growth but they are quickly exhausted (Verma et al., 2015).

After germination, growth is highly dependent on the organic and inorganic sources provided by the cotyledons. The cotyledons have photosynthetic and reserve functions, aiming to supply the demand of the seedling until its leaves establish photosynthetic processes (Kitajima, 1992; Sakpere et al., 2015).

The soybean has cotyledons that remain above ground (epigeal) after germination and present a flat-type appearance, with a coriaceous or fleshy texture after little expansion (Kitajima, 1992).The main functionality of coriaceous cotyledons is to provide reserves for seedlings. In this way, they present a small photosynthetic contribution. However, before full expansion of the first leaf, photosynthesis in these cotyledons may represent 75% of the carbon export, supplying most of the respiratory needs of the seedlings (Kitajima, 1992; Ibarra Manríquez et al., 2002).

In initial development, cotyledon herbivory makes the majority of species more susceptible to the environment (Mancilla-Leytón et al., 2013; Lapaz et al., 2017). Therefore, understanding the tolerance of soybean to herbivory in cotyledons is of great importance for the sizing and impact of the damage caused to this crop.

Herbivory of the soybean is provoked in the majority of cases by insect pests (Costa et al., 2003) damaging the mass of the cotyledons partially or totally, which can compromise the initial development of the seedling (Hayashi et al., 2012).

The deleterious effect of herbivory on cotyledons has been observed in previous studies with leguminous plants, such as: *Canavaliaensi formis* (L.) DC. and *Phaseolus vulgaris* L. (Hayashi et al., 2012); *Glycine max* (L.) Merrill (Moscardi et al., 2012); *Lespedeza potaninii* V. N. Vassil., *Ammopiptanthus mongolicus* (Maxim.) S. H. Cheng and *Sophora alopecuroides* L. (Hu et al., 2016); and *Phaseolus vulgaris* L. (Lapaz et al., 2017).

This study aimed to reproduce situations of herbivory in cotyledons occurring in the field, caused by insect pests, through manual partial and total removal of the cotyledons, in order to evaluate the deleterious effects on the initial development of soybean [*Glycine max* (L.) Merrill].

Material and Methods

Experimental Site

The experiment was carried out under greenhouse conditions in the city of Dracena, São Paulo State, Brazil (21° 29′ LS and 51° 2′ LW; 396 m altitude), from May to July 2018.

The greenhouse used was an arch type, with 6 m sides, covered with a transparent plastic film light diffuser with a thickness of 1000 microns, the sides covered by a Sombrite screen with 50% light-holding capacity.

Experimental design and treatments

The experimental design was completely randomized, arranged in a 2 × 3 factorial scheme with two soybean cultivars (BMX Potência RR and Syngenta 1562 IPRO) and three situations involving removal of cotyledons (0%, 50% and 100%) in four replications, totalling 24 experimental plots. In the 0% treatment (control), the cotyledons were kept intact; in the 50% treatment, 50% (one) of the cotyledons was removed; and in the 100% treatment, all (two) cotyledons were removed.

Experimental installation and cultivation

Polypropylene vessels with a capacity of 2 dm^{-3} were used. The substrate used was Carolina Soil®, composed of sphagnum peat, expanded vermiculite, dolomitic limestone, gypsum and NPK fertilizer traces, with pH 5.5 ± 0.5, CE 0.4 ± 0.3 mS cm^{-1} and a density of 155 kg m^{-3}.

In each experimental plot, 10 seeds of uniform size separated with the aid of a sieve of 5.5 m were sown covered with a thin layer of substrate. Four days after sowing, the majority of the seeds were germinated. On the fifth day after sowing, seedlings were thinned to two per vessel, the seedlings that presented greater vigour, homogeneity of size and health among themselves being chosen. Shortly thereafter, the cotyledons were removed with the aid of a scalpel according to the treatments.

During the experimental period, irrigation of the experiment was carried out manually with deionized water, maintaining the humidity at 80% of field capacity, determined by the method of weighing five vessels.

At the end of the experiment, that is, 26 days after sowing, the following parameters were measured:

Growth and initial development

The following parameters were determined: stalk diameter, measured 1 cm from the collar of the seedling by means of a digital calliper, with the result expressed in cm $seedling^{-1}$; shoot and root primary length, both measured with the aid of a ruler, the results expressed in cm $seedling^{-1}$.

Then, the seedlings were separated into shoots and roots. The material was identified, packaged in paper bags and dried in an oven at 65°C for 2 days. Then the shoot and root dry mass was measured, and the results expressed in mg $seedling^{-1}$.

Nutritional parameters

Before disassembling the experiment, chlorophyll index was determined using model CCM-200 apparatus. Evaluation was done at noon on sunny days, in the first expanded trefoil (counting from the base) from each experimental plot.

Posterior, the shoot dry mass of each experimental plot was ground in a Wiley mill to determine the concentration of nitrogen (N) in the shoot. For this, 0.1 g was weighed in the digestion tube, and 3 mL of sulphuric acid (H_2SO_4) and catalyst salts were added. The samples were put into a digester block in a ventilated flue for 4 h in order to obtain the ammonium sulphate ($(NH_4)_2SO_4$) compound. In this condition, the temperature was gradually increased up to 350°C.

Sodium hydroxide (NaOH) (10 N, 25 mL) was added to the samples; they were transferred to a microdistiller where they were distilled and fixed in 10 mL of 2% boric acid (H_3BO_3) solution in the presence of green bromocresol and methyl red indicators, until 40 mL of the ammonium borate solution ($NH_4H_2BO_3$) was obtained. The samples were then titrated with 0.1 N hydrochloric acid solutions (HCl) until the colour turned. The nitrogen concentration was expressed in g kg^{-1} of shoot dry mass by the equation described below (Malavolta et al., 1997).

$$N = ((V \times f \times 0.014)/P) \times 100, \quad (1)$$

where: V = spent volume of HCl in the titration, f = normality of HCl, 0.014 = milliequivalent of N in grams (g), P = weight

Ultrastructure parameters

Before disassembling the experiment, leaf fragments were cut from the samples in the medial region of the limbos of the first expanded trefoil (counting from the base), and root fragments from the samples were cut 1 cm below the seedling's collar for histological analysis. The leaf and root fragments were fixed in F.A.A. 70 solution (37% formaldehyde and acetic acid and 70% ethanol at a ratio of 1.0 : 1.0 : 18.0, V/V) for 48 h.

Subsequently, the seedling tissue fragments were subjected to the following steps: dehydration, diaphanization, inclusion and fixation in paraffin, according to the methodology described by Kraus and Arduim (1997).

With the aid of a microtome table containing a steel blade, 8-μm sections were cut from each embedded fragment. Each embedded fragment corresponds to an experimental plot. The sections of samples were fixed with Mayer adhesive, stained with 1% safranin and mounted on slides and cover slips with Entellan® adhesive (Kraus & Arduim, 1997).

The slides were observed under an Olympus® optical microscope model BX43 with a coupled camera to measure the ultrastructural parameters using the CellSens Standard® image analysis program, which was calibrated with a microscopic rule at the same zoom level as the photographs.

In the midrib region of the leaves and roots in the cross-sections, the following ultrastructural parameters were observed: mesophyll thickness, palisade parenchyma length, leaf xylem diameter, leaf phloem diameter, epidermal thickness of the abaxial face, epidermal thickness of the adaxial face, root xylem diameter and root phloem diameter (Carlquist, 1975). Each experimental plot was represented by the average of 10 measurements.

Data analysis

The data were subjected to analysis of variance using the F test, at $p \leq 0.05$. When significant, the parameters were submitted to the Tukey test ($p < 0.05$). All statistical analysis of the data was performed using protocols developed in the R software (R Development Core Team, 2018).

Results

Growth and initial development

Stalk diameter and shoot length presented an isolated effect only for the factor cotyledon removal. Root primary length, shoot dry mass and root dry mass presented interaction between factors, as shown in Table 1.

Table 1. Significance of F test for values of stalk diameter (SD), shoot length (SL), root primary length (RPL), shoot dry mass (SDM) and root dry mass (RDM)

SV	F test				
	SD	SL	PRL	DMS	DMR
Cultivar	ns	ns	ns	ns	ns
Cotyledon r.	***	***	***	***	***
Interaction	ns	ns	***	**	*
CV (%)	14.03	13.20	16.04	13.09	15.95

Source of variation (SV); coefficient of variation (CV); ^{ns} – not significant; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$

The stalk diameter decreased with cotyledon removal. However, for removal of 50% and 100% of the cotyledons, stalk diameter did not differ, being lower, on average, by 48% in relation to the control (Figure 1A).

The removal of 50% of the cotyledons did not affect the shoot length, while the removal of 100% of the cotyledons caused a deleterious effect on growth, registering a decrease of 34% in relation to the control (Figure 1B).

For the parameter of root primary length, the BMX Potência RR cultivar was not affected by the removal of 50% of the cotyledons, when compared to the control. On the other hand, the Syngenta 1562 IPRO cultivar, when 50% of cotyledons were removed, showed greater sensitivity to this situation (Figure 1C), presenting a decrease of 43% in relation to the control. When 100% of the cotyledons were removed, once again, the Syngenta 1562 IPRO cultivar was more sensitive (Figure 1C). The decreases registered were 31% for BMX Potência RR and 71% for the Syngenta 1562 IPRO cultivar in relation to the control. It was verified that the Syngenta 1562 IPRO cultivar had higher values than the BMX Potência RR cultivar only in the control, with variation of 27%. When 50% and 100% of the cotyledons were removed, the BMX Potência RR cultivar had higher values than the Syngenta 1562 IPRO cultivar, registering a variation of 30% and 42%, respectively (Figure 1C).

The reduction in shoot dry mass with cotyledon removal was drastic. On average, the removal of 50% and 100% of the cotyledons resulted, respectively, in a reduction of 62% and 88% for the cultivars (Figure 1D). The Syngenta 1562 IPRO cultivar was more sensitive to the removal of 50% of the cotyledons, by 31% when compared to the cultivar BMX Potência RR. On the other hand, without cotyledon removal (control), the Syngenta 1562 IPRO cultivar showed a greater increase than the BMX Potência RR cultivar, being superior by 13% (Figure 1D).

With cotyledon removal, the cultivars also presented a drastic reduction in root dry mass (Figure 1E). However, the BMX Potência RR cultivar showed a statistical difference between the treatments which removed 50% and 100% of the cotyledons, while the Syngenta 1562 IPRO cultivar presented no difference (Figure 1E). The Syngenta 1562 IPRO cultivar was more sensitive to removal of 50% of the cotyledons, registering a variation of 14% (Figure 1E).

Chlorophyll index and concentration of N in the shoots

The chlorophyll index presented an isolated effect for cotyledon removal and cultivar factors. The concentration of

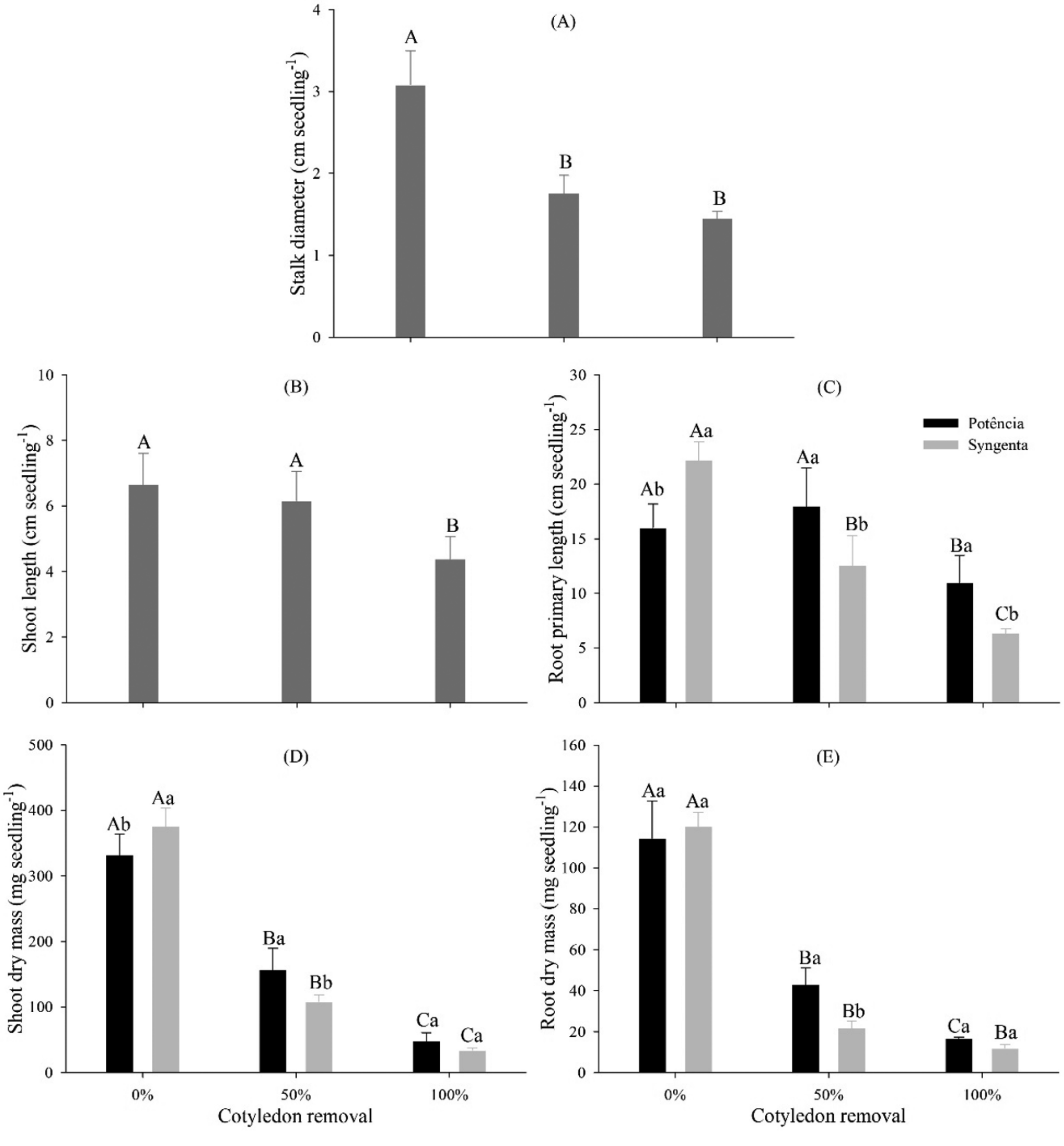

Fig. 1. Stalk diameter (*A*), shoot length (*B*), root primary length (*C*), shoot dry mass (*D*) and root dry mass (*E*) as a function of partial and total removal of cotyledons of soybean cultivars BMX Potência RR and Syngenta 1562 IPRO. Capital letters compare the behaviour of each cultivar under situations of cotyledon removal, while lowercase letters compare situations of cotyledon removal among cultivars; isolated lowercase letters compare the cultivars regardless of cotyledon removal situation, according to Tukey's test. Vertical bars represent the standard deviation

N in the shoots presented interaction between factors. The data are contained in Table 2.

Table 2. Significance of F test for values of chlorophyll index (SPAD) and concentration of nitrogen in the shoot (N)

SV	F test	
	SPAD	N
Cultivar	**	***
Cotyledon r.	***	***
Interaction	ns	*
CV (%)	6.77	1.46

Source of variation (SV); coefficient of variation (CV); ns – not significant; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$

The chlorophyll index was significantly lower when the cotyledons were removed (Figure 2A). There was a decrease in the chlorophyll index with the removal of 50% and 100% of the cotyledons, at a rate of 20% and 38% in relation to the control, respectively. Among the cultivars, the chlorophyll index exhibited by the Syngenta 1562 IPRO cultivar was higher than that of the BMX Potência RR cultivar, presenting a variation of 10% (Figure 2A).

The removal of 100% of the cotyledons caused a higher concentration of N in the shoots, respectively 7.5% and 11% for the cultivars BMX Potência RR and Syngenta 1562 IPRO, when compared to the control (Figure 2B). For this parameter, the Syngenta 1562 IPRO cultivar demonstrated higher values in all situations, that is, with or without cotyledon removal, as shown in Figure 2B.

Ultrastructural parameters of the leaf

Mesophyll thickness, palisade parenchyma length and leaf xylem diameter presented an isolated effect only for the factor of cotyledon removal (Table 3). Leaf phloem diameter presented an isolated effect for the factors cotyledon removal and cultivar (Table 3). The epidermal thickness of the abaxial face and epidermal thickness of the adaxial face presented interaction between factors (Table 3).

The values obtained for the parameters of mesophyll thickness, palisade parenchyma length and leaf xylem diameter decreased with cotyledon removal, as shown in Figures

Table 3. Significance of F test for values of mesophyll thickness (MT), palisade parenchyma length (PPL), leaf xylem diameter (LXD), leaf phloem diameter (LPD), epidermal thickness of the abaxial face (ABET) and epidermal thickness of the adaxial face (ADET)

SV	F test					
	MT	LPP	LXD	LPD	ABET	ADET
Cultivar	ns	ns	ns	*	ns	ns
Cotyledon r.	***	***	***	***	***	***
Interaction	ns	ns	ns	ns	***	*
CV (%)	12.62	11.50	9.71	7.97	5.48	7.65

Source of variation (SV); coefficient of variation (CV); ns – not significant; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$

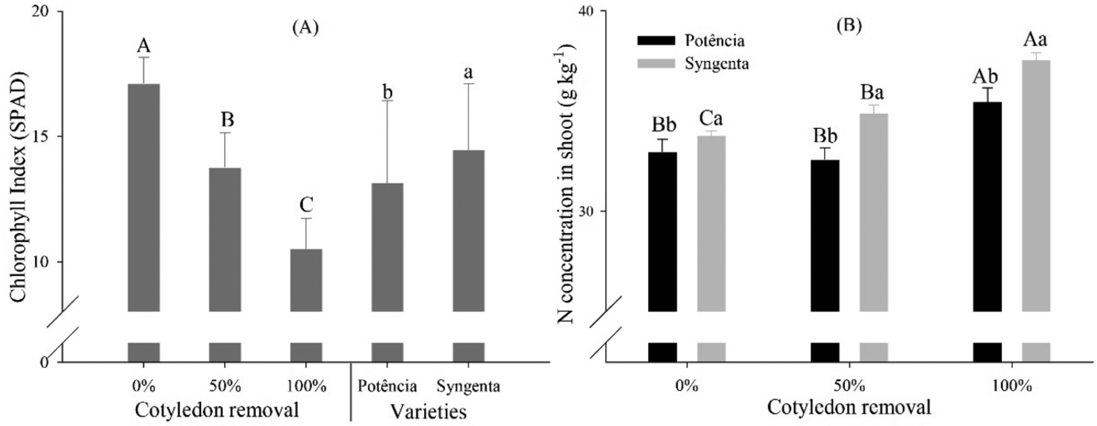

Fig. 2. Chlorophyll index (*A*) and concentration of N in the shoots (*B*) as a function of partial and total removal of cotyledons in soybean cultivars BMX Potência RR and Syngenta 1562 IPRO. Capital letters compare the behaviour of each cultivar under situations of cotyledon removal, while lowercase letters compare situations of cotyledon removal among cultivars; isolated uppercase letters compare the cotyledon removal situations independently of the cultivar, while isolated lowercase letters compare the cultivars independently of cotyledon removal situation, according to Tukey's test. Vertical bars represent the standard deviation

3A–C. The decreases were, respectively, 17%, 23% and 23% for the removal of 50% of the cotyledons; and 35%, 57% and 52% for the removal of 100% of cotyledons when compared to the control.

Leaf phloem diameter was not affected by the removal of 50% of the cotyledons. However, the removal of 100% of the cotyledons presented a lower value, registering a reduction of 36% in relation to the control (Figure 3D). In addition, it was verified that the BMX Potência RR cultivar had a larger leaf phloem diameter than the cultivar Syngenta 1562 IPRO, presenting a variation of 7%, as shown in Figure 3D.

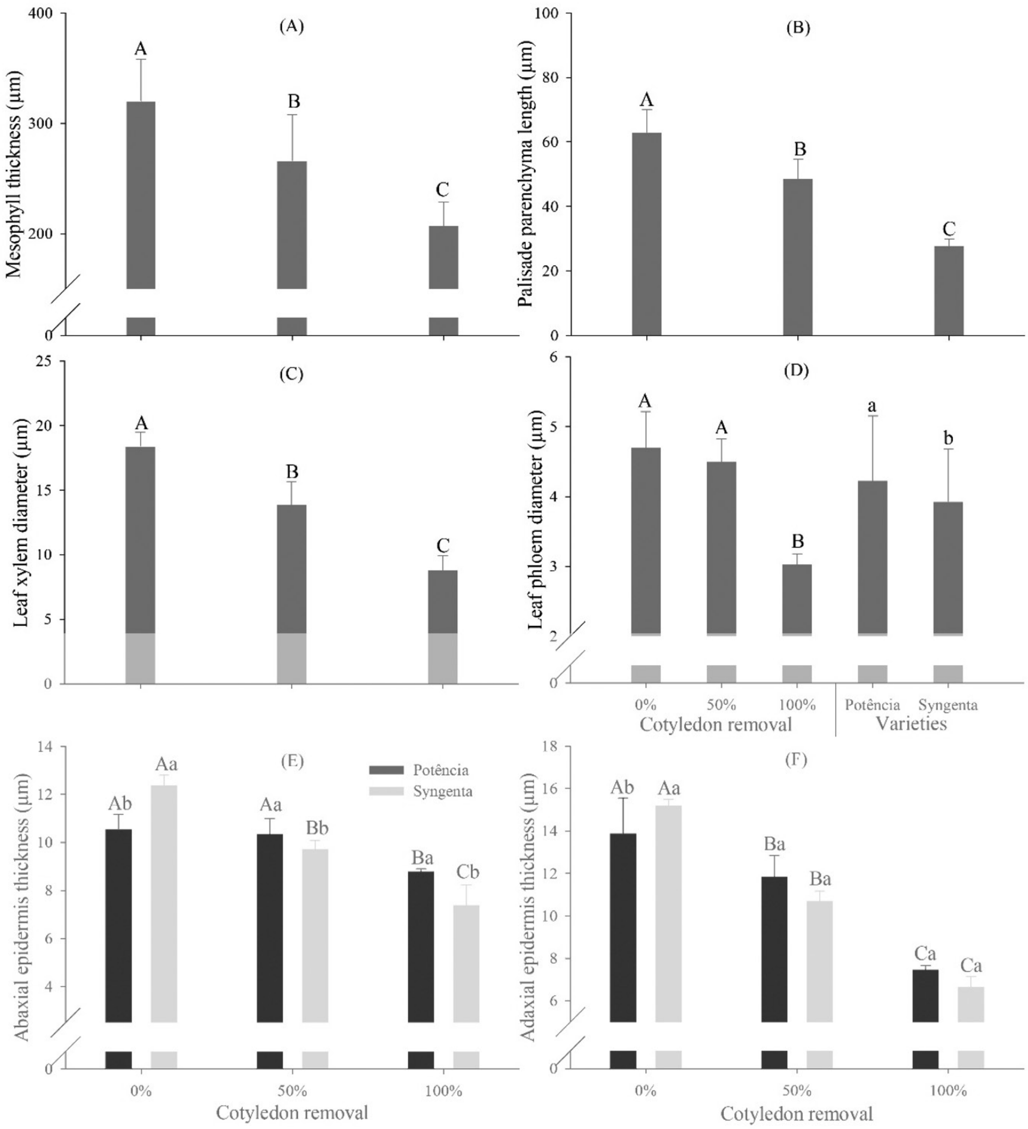

Fig. 3. Mesophyll thickness (*A*), palisade parenchyma length (*B*), leaf xylem diameter (*C*), leaf phloem diameter (*D*), epidermal thickness of the abaxial face (*E*) and epidermal thickness of the adaxial face as a function of partial and total removal of cotyledons in soybean cultivars BMX Potência RR and Syngenta 1562 IPRO. Capital letters compare the behaviour of each cultivar under situations of cotyledon removal, while lowercase letters compare situations of cotyledon removal among cultivars; isolated uppercase letters compare the cotyledon removal situations independently of the cultivar, while isolated lowercase letters compare the cultivars independently of cotyledon removal situation, according to Tukey's test. Vertical bars represent the standard deviation

The epidermal thickness of the abaxial face was not affected by the removal of 50% of cotyledons for the cultivar BMX Potência RR. On the other hand, the cultivar Syngenta 1562 IPRO was sensitive to this situation, showing a decrease of 21% in relation to the control (Figure 1E). The removal of 100% of the cotyledons presented the greatest reduction for both cultivars; the decreases were 16% for the cultivar BMX Potência RR and 40% for the cultivar Syngenta 1562 IPRO in relation to the control (Figure 1E). It was verified that the cultivar Syngenta 1562 IPRO had a higher value than the cultivar BMX Potência RR only in the control, registering a variation of 15%, while for the removal of 50% and 100% of cotyledons, the cultivar BMX Potência RR had a higher value than the cultivar Syngenta 1562 IPRO, registering a variation of 6% and 16%, respectively (Figure 1E).

The epidermal thickness of the adaxial face was lower with cotyledon removal. The removal of 50% and 100% of the cotyledons resulted in reductions of 14% and 46% (BMX Potência RR) and 30% and 56% (Syngenta 1562 IPRO) when compared to the control, respectively (Figure 1F). There was no difference within cultivars between the removal of 50% and 100% of the cotyledons. On the other hand, the control presented a statistical difference: the cultivar Syngenta 1562 IPRO had a higher value than the cultivar BMX Potência RR, presenting a variation of 9.5%.

Ultrastructural parameters of the root

The root xylem diameter presented an isolated effect for the factors cotyledon removal and cultivar. The root phloem diameter presented an isolated effect only for the factor cotyledon removal, as shown in Table 4.

Table 4. Significance of F test for values of root xylem diameter (RXD) and root phloem diameter (RPD)

SV	F test	
	RXD	RPD
Cultivar	**	ns
Cotyledon r.	***	***
Interaction	ns	ns
CV (%)	14.33	8.56

Source of variation (SV); coefficient of variation (CV); ^{ns} – not significant; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$

The root xylem diameter decreased with removal of the cotyledons, but there was no difference between removal of 50% and 100% of the cotyledons, the diameter being, on average, 30% lower than in the control (Figure 4A). Among the cultivars, the root xylem diameter of the cultivar BMX Potência RR presented a higher value than the Syngenta 1562 IPRO cultivar, presenting a variation of 30% (Figure 4A).

The root phloem diameter decreased with cotyledon removal, but there was no difference between removal of 50% and 100% of the cotyledons, the diameter being, on average, 18% lower than in the control (Figure 4B).

Discussion

Growth and initial development

The results obtained in this work show the importance of the role of cotyledonary reserves in the initial development of soybean seedlings. As expected, partial or total cotyledon removal was deleterious to the growth and initial development of this species. The extent of the impact was dramati-

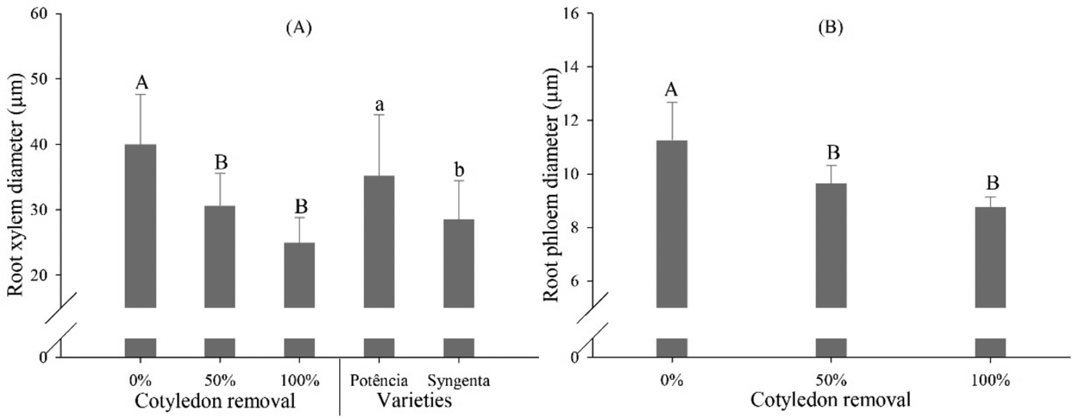

Fig. 4. Root xylem diameter (*A*) and root phloem diameter (*B*) as a function of partial and total cotyledon removal in BMX Potência RR and Syngenta 1562 IPRO soybean cultivars. Capital letters compare the cotyledon removal situations independently of the cultivar, while isolated lowercase letters compare the cultivars independently of cotyledon removal situation, according to Tukey's test. Vertical bars represent the standard deviation

cally higher in the situation where the cotyledons were completely removed, making seedlings more susceptible to the environment and delaying their establishment.

The negative effect provided in soybean by cotyledon removal can be related to less availability of reserves, including nutrients, carbon, energy, hormones and photosynthetic activity (Oliveira & Morais, 1999; El-Amhir et al., 2017). In this way, it is inferred that the cotyledonary reserves destined for sink regions, where the tissues are in full growth, are limiting, which impairs the mobilization and compound synthesis necessary for initial seedling development.

As the seedling establishment stage is the most vulnerable period of the crop's cycle (Hayashi et al., 2012), the lower values observed for root primary length and root dry mass on cotyledon removal make the establishment of seedlings even more difficult and influence their nutritional quality. This is because larger and bulkier roots provide seedlings with better performance in absorbing water and nutrients and hormone synthesis, promoting higher above-ground biomass and ensuring the self-sustainability of the seedling (El-Amhir et al., 2017; Shi et al., 2017).

Hu et al. (2016) obtained results similar to those in this study with the species *Lespedeza potaninii* V. N. Vassil., *Ammopiptanthus mongolicus* (Maxim.) S. H. Cheng and *Sophora alopecuroides* L.; they verified a reduction in relative growth rate with cotyledon removal, and the survival rate was also reduced in the last two species. Hayashi et al. (2012), studying the influence of cotyledon removal on the initial development of the species *Canavaliaensi formis* (L.) DC. and *Phaseolus vulgaris* L. verified that both species that remained for a shorter period with cotyledons presented lower values for mass of dry matter in relation to those that retained cotyledons for longer. Moscardi et al. (2012) observed that total cotyledon removal in *Glycine max* (L.) Merrill, in stage R8, reduced plant height and the height of insertion of the first pod, which reveals that even after establishment, certain damage from cotyledon removal is reflected at the end of the crop's cycle.

Chlorophyll index and concentration of N in the shoots

Cotyledons have advantageous metabolic resources for the initial development of seedlings (Garcíia-Cebriáan et al., 2003). Consequently, cotyledon removal affects the chlorophyll index, mainly with 100% cotyledon removal; in this situation, the concentration of nitrogen in the shoots was highest, which suggests that the nitrogen in non-protein form was the main factor for the decline of the chlorophyll index.

The observed effect on the concentration of nitrogen in the shoots can be explained by the effect of dilution. The increase in development of the seedlings (Figure 1), associated with a lower concentration of nitrogen in the shoots, resulted in its dilution (Figure2B); the control of the BMX Potência RR cultivar registered the highest dilution. On the other hand, 100% cotyledon removal in the Syngenta 1562 IPRO cultivar registered the lowest dilution.

The dilution effect in the concentration of nitrogen in the shoots was similar between cultivars; however, the cultivar Syngenta 1562 IPRO presented lower dilution in situations of cotyledon removal, which probably is due to the lower growth observed in these situations (Figure 1).

Ultrastructural parameters of the leaf and root

The cotyledons were revealed to be indispensable for leaf and root ultrastructural constitution, forming organs capable of performing their respective roles. Cotyledon removal caused a deleterious effect on the ultrastructural parameters of the leaf and root and was consequently reflected in the smallest spacing of the cells of the tissues studied (Figures 3 and 4), which could affect the performance of the seedlings' organs. Lapaz et al. (2017) obtained similar results with the species *Phaseolus vulgaris* L.

The smallest thickness observed in conductor tissues (Figures 3C, 3D, 4A and 4B) could influence the transport of xylem sap and phloem sap, making transportation of the sap inefficient. In this way, photosynthetic performance and photoassimilate distribution are compromised (Figueiredo et al., 2013).

The difference observed in palisade parenchyma length between treatments (Figure 3B) may have triggered the smallest amount of chloroplasts and consequent lower values recorded for chlorophyll index (Figure 2A), which is corroborated by the fact that these parameters had the same behaviour. That is, a smaller amount of chloroplasts was associated with the palisade parenchyma length.

Seedlings with the smallest coating tissues are more prone to mechanical damage and herbivory caused by insect pests because these tissues provide a physical barrier to the leaves. Therefore, coating tissues increase the chances of seedling survival during initial development (Viana et al., 2015).

As a result, the understanding of macro-and micromorphological modifications of plant organs and tissues, as well as the effects caused to these, should be increasingly studied, in order to improve the understanding and direction of research pertinent to the theme (Lisboa et al., 2018). Symptomatology is widely used to evaluate the damage caused by biotic or abiotic factors. In this case, structural aspects aid in understanding the mechanisms that cause injuries in plants (Moreira & Isaias, 2008).

Conclusion

Partial or total cotyledon removal caused a reduction in parameters related to the growth and initial development of soybean, which is probably due to the nutritional and morphological changes documented in this study.

In general, the Syngenta 1562 IPRO cultivar was more sensitive to the cotyledon removal than the BMX Potência RR cultivar.

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