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Research Article

Influence of cotyledons on phenotypic characteristics in soybean seedlings

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Abstract

Different factors can interfere with the success of crop establishment, such as cotyledon destruction in the early stages of soybean cultivation. In this context, the influence of cotyledons on phenotypic characteristics in soybean seedlings was evaluated. The experiment was conducted in a greenhouse, and seeds of the TMG 803 cultivar, previously classified by size using sieves with circular holes (P5.5, P6.0, P6.5, P7.0, and P7.5), were used. After germination, cotyledons were removed at stages VE, VC, V1, and V2, and for each stage, three types of cotyledon removal were performed: no cotyledon removal, removal of one cotyledon, and removal of two cotyledons. The experiment followed a 5×4×3 factorial design in a randomized block layout with four repetitions, and the experimental unit was the average of two seedlings grown in a pot. The epicotyl length, internode length, petiole length of the first trifoliate leaf, and seedling height were evaluated at development stages V2 and V3. The data were analyzed by analysis of variance and subjected to the Tukey test at a 5% significance level. The influence of seed size on epicotyl length (evaluated at V2 and V3) and internode length (evaluated at V2) was more pronounced when cotyledons were removed at stages VE and VC. Internode length (evaluated at V3), petiole length (evaluated at V2), and seedling height (evaluated at V2 and V3) were influenced by seed size, independent of other factors. The number of cotyledons removed influenced the length of the evaluated traits.

Keywords: Breeding; additional descriptors; biostatistics; morphological descriptors; early soybean evaluation; effect of seed size; soybean sowing.

Introduction

In Brazil, soybean cultivation yielded a total production of 147.38 million tons over an area of approximately 46.03 million hectares, with an average yield of 3,202 kg/ha (Companhia Nacional de Abastecimento, [CONAB], 2024). Soybean has a grain rich in proteins and oil, used mainly for human and animal consumption, and in the production of biofuels and vegetable oil (Foleto et al., 2024; Prauchner et al., 2024; Schünemann et al., 2024; Zuse et al., 2024)

Soybean genetic improvement programs through cultivar development have undeniably contributed to the agricultural advancement of Brazil (Matsuo, Borém, & Sediyama, 2021). For cultivar registration, several procedures are required to demonstrate that the cultivar is distinct, uniform, and stable (Campos, Machado, Viana, & Azevedo, 2009; Viana, 2013). Several studies have proposed new morphological descriptors for soybean, including epicotyl length and other traits as potential descriptors for early soybean evaluation (Nogueira et al., 2008; Matsuo, Sediyama, Cruz, & Oliveira, 2012; Matsuo, Sediyama, Cruz, Oliveira, & Cadore, 2012; Alves et al., 2019; Camargos, Campos, Alves, Ferreira, & Matsuo, 2019; Hanyu, Ferreira, Cecon, & Matsuo, 2020; Gontijo et al., 2021).

Seed size is one of the factors that can influence seed germination and vigor (Carvalho & Nakagawa, 2012). According to Krzyzanowski, França Neto and Costa (1991), seed size positively affects soybean yield by improving sowing precision and crop establishment. Furthermore, seed size variations within the same cultivar could impact yield, especially during dry periods in the grain-filling stage (Empresa Brasileira de Pesquisa Agropecuária, [EMBRAPA], 2005).

Losses in soybean yield can be caused by biotic stress caused, for example, by insect pests, especially the initial pests that occur during the first stages of crop development (Ávila & Grigolli, 2014). These pests can cause the death of seeds and/or seedlings, reduce root growth, destroy the cotyledons or unifoliolate leaves, cut off the tips or shoots of trifoliate leaves, damage which can affect the crop's grain yield (Hoffmann-Campo, Oliveira, Moscardi, Corrêa-Ferreira, & Corso, 2012; Ribeiro & Costa, 2000). It is also reported that the *Helicoverpa caterpillar* can attack soybean seedlings, feeding on the cotyledons (Czepak, Vivan, & Albernaz, 2013), which could affect the initial development of the crop, interfering with the recommended plant population for each cultivar (Guazina, Degrande, Souza, & Gauer, 2019). The assessment of damage to the early stages of soybean seedlings has been studied by different researchers (Bueno et al., 2010; Moscardi, Bueno, Bueno, & Garcia, 2012; Fernandes & Ávila, 2016), but with a focus on yield or agronomic characters measured mainly at the end of the crop cycle.

Given the above, there was a need for further studies to verify the effect of removing cotyledons in the early stages of the seedling, in addition to analyzing the effect of seed size on phenotypic characters evaluated in the early stages of soybeans. Therefore, the iam of this study was to evaluate the influence of cotyledons on phenotypic characters in soybean seedlings.

Materials and Methods

The experiment was conducted in a greenhouse located at the Federal University of Viçosa, Rio Paranaíba Campus, in the municipality of Rio Paranaíba, MG, Brazil. The coordinates are Latitude 19° 11’ 37”, Longitude 46°14’50”, Altitude 1,067 m. The recorded average minimum temperature was 19.1ºC, and the average maximum temperature was 43.8ºC. Management practices, fertilization, cultural treatments, and phytosanitary controls were carried out according to technical recommendations for the crop (Sediyama, 2009).

Soil with organic matter was used to fill the 3 dm³ pots. Seeds of the TMG 803 cultivar were previously classified by size using sieves with circular holes (P5.5, P6.0, P6.5, P7.0, and P7.5). According to EMBRAPA (2020), a seed classified as P5.0 is one that has a diameter between 5.0 and 6.0 mm, meaning the seeds will pass through 6.0 mm round holes and be retained on the sieve with 5.0 mm round holes. After the pots, labels, and seeds were prepared. Sowing was done at a depth of 1.5 cm. Cotyledon removal was performed at the VE, VC, V1, and V2 development stages (Fehr & Caviness, 1977), and for each development stage, three types of cotyledon removal were carried out: no cotyledon removal, removal of one cotyledon (randomly selected between the two existing cotyledons – Figure 1), and removal of two cotyledons. Cotyledon removals occurred at the VE stage with 4.6 days after planting, at VC stage with 7.0 days after planting, at V1 stage with 11.3 days after planting, and at V2 stage with 16.2 days after planting.

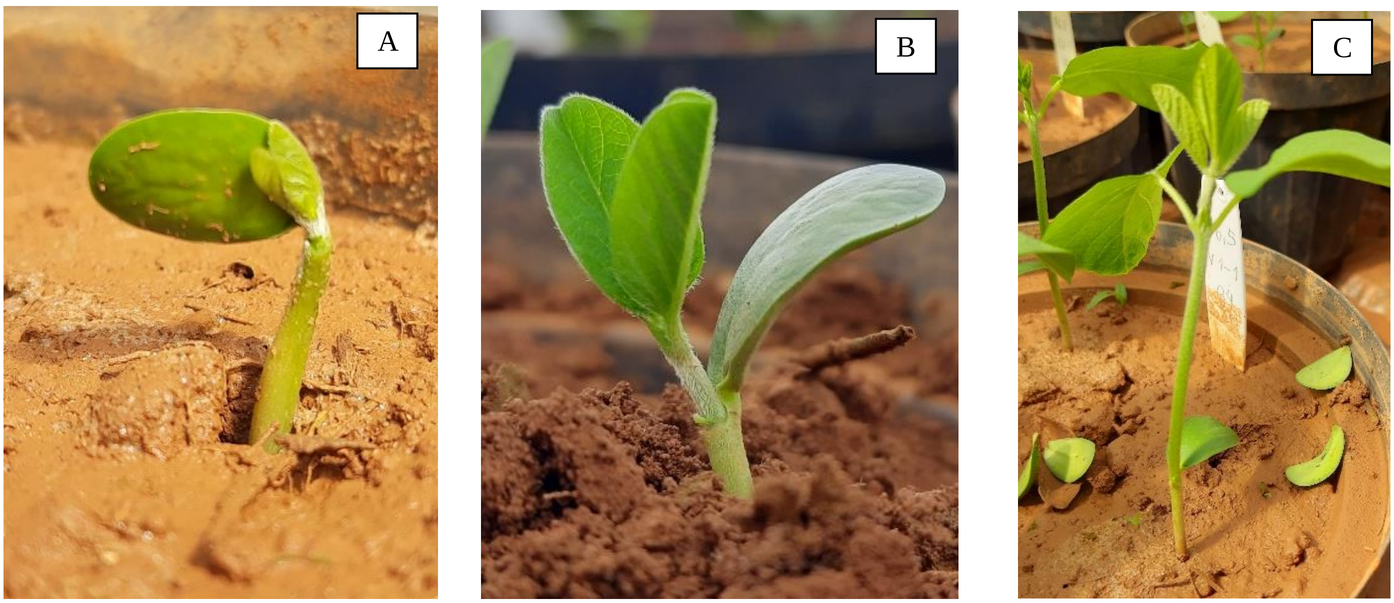

Figure 1. Seedlings with the removal of one cotyledon. (A) VE development stage, (B) VC development stage, and (C) V1 development stage.
Photos by: Éder Matsuo.

The experiment was conducted in a 5×4×3 factorial scheme, in a randomized block design with 4 replications. Factor A was seed size (Seeds) with 5 levels, Factor B was development stages when cotyledon removals were performed (Stages) with 4 levels, and Factor C was the number of cotyledons removed with 3 levels. The experimental unit was the average of two seedlings cultivated in a pot. The response variables were: epicotyl length – EL – (on the main stem, the distance between the cotyledonary node and the node of insertion of the unifoliate leaves); internode length – IL – (on the main stem, the distance between the node of insertion of the unifoliate leaves and the node of insertion of the first trifoliate leaf); petiole length of the first trifoliate leaf – PL; and seedling height – SH. The evaluations were carried out at the V2 and V3 development stages (referred to in this work as V2 and V3, respectively) using a millimeter ruler, with the unit of measurement in centimeters.

The collected and tabulated data were analyzed using analysis of variance with a triple factorial arrangement, adopting a 5% probability level, according to the following mathematical model:

$$Y_{ijkl} = m + B_i + Se_j + St_k + R_l + SeSt_{jk} + SeR_{jl} + StR_{kl} + SeStR_{jkl} + e_{ijkl}$$

Where:

Y_{ijkl}: is the observed value related to the plot that received the seed size j in Stage k with removal l in repetition i
m: is the general average of the experiment
B_i : is the effect of the i-th Block
Se_j: is the effect of the j-th Seed
St_k: is the effect of the k-th Stage
R_l: is the effect of the l-th
$SeSt_{jk}$: is the effect of the interaction between the j-th Seed and k-th Stage
SeR_{jl}: is the effect of the interaction between the j-th Seed and l-th
StR_{kl}: is the effect of the interaction between the k-th Stage and l-th
$SeStR_{jkl}$: is the effect of the interaction between the j-th Seed, k-th Stage, and l-th
e_{ijkl}: is the random error associated with observation ijkl

For comparisons, when necessary, the Tukey test at a 5% probability level was applied. Statistical analyses were performed using the R Program (R CORE TEAM, 2024) with the ExpDes.pt package (Ferreira, Cavalcanti, Nogueira, & Ferreira, 2018).

Results and Discussion

No significant effect ($p > 0.05$) was observed for the triple interaction across all variables studied. However, significant effects were found for Seeds, Stages, and Cotyledons for all variables, except PL_V3 (Table 1). For the variables IL_V3, PL_V2, PL_V3, and SH_V3, there was no significant effect ($p > 0.05$) of the Se×St, Se×R, and St×R interactions, while for EL_V2, a significant effect ($p < 0.05$) was observed for all cited interactions. For EL_V3, IL_V2, and SH_V2, no significant interaction effects were found for Se×R, Se×St, and Se×R across the respective variables (Table 1).

The coefficients of variation were 11.67%, 11.32%, 18.33%, 14.27%, 22.75%, 12.02%, 10.95%, and 17.27% for EL_V2, EL_V3, IL_V2, IL_V3, PL_V2, PL_V3, SH_V2, and SH_V3, respectively. The epicotyl length values are consistent with the studies by and Gontijo et al. (2021) who reported variation coefficients of 7.96% and, Nogueira et al. (2008) reported coefficients of variation values of 15.76% for sowing in December, 16.54% for sowing in February, 10.52% for sowing in May and 9.96% for sowing in June.

When analyzing the Seed×Stage interaction for epicotyl length measured in V2, it was found that cotyledon removal at the VE, VC, V1, and V2 stages influenced its length (Table 2). Smaller seeds resulted in seedlings with shorter epicotyls compared to seedlings from larger seeds. Fixing each Seed level, it was observed that cotyledon removal in seedlings from P5.5, P6.0, and P7.0 seeds during early development stages led to a reduced epicotyl length.

Analyzing the Seed×Cotyledon interaction for epicotyl length in V2 (Table 2), it was found that, when cotyledon levels were fixed, seedlings from smaller seeds had shorter epicotyls. The removal of no cotyledon led to greater epicotyl length compared to the removal of 2 cotyledons. The removal of 1 cotyledon sometimes resulted in a length similar to 0 or 2 cotyledons removals, showing that its behavior varies according to seed size. Thus, epicotyl length in V2 was shorter in seedlings of smaller seeds that underwent cotyledon removal (1 or 2 units).

Analyzing the Cotyledon × Stage interaction for epicotyl length in V2 (Table 2), no difference in epicotyl length was observed when 0, 1, or 2 cotyledons were removed at V1 or V2. For VE or VC stages, the removal of 1 or 2 cotyledons resulted in shorter epicotyls compared to seedlings with no cotyledon removal. Therefore, epicotyl length in V2 was negatively affected in seedlings with 1 or 2 cotyledons removed at VE or

VC, resulting in shorter epicotyls.

Table 1. Summary of analysis of variance of the epicotyl length (EL), internode (IL), petiole of the first trifoliate leaf (PL) and seedling height (SH) measured at the V2 and V3 development stages, Rio Paranaíba – MG[1].

S.V.	df	EL_V2	EL_V3	IL_V2	IL_V3
Block	3	4.1077** [1]	3.1779**	2.0936**	1.5486**
Seed (Se)	4	14.6388**	14.8365**	6.3378**	3.1404**
Stage (St)	3	9.8376**	10.7163**	1.1743**	1.4003**
Removal (R)	2	16.0900**	15.6199**	1.6506**	1.7746**
Se*St	12	0.9781*	1.3564**	0.2415 ns	0.3561 ns
Se*R	8	0.9718*	0.7197ns	0.2524 ns	0.2528 ns
St*R	6	4.8946**	4.3495**	0.8671**	0.2210 ns
Se*St*R	24	0.5352ns	0.5172ns	0.3070 ns	0.2176 ns
Residue	177	0.4833	0.4590	0.2573	0.2435
Averages (cm)		5.96	5.99	2.76	3.45
C.V. (%)		11.67	11.32	18.33	14.27
S.V.	**df**	**PL_V2**	**PL_V3**	**SH_V2**	**SH_V3**
Block	3	3.2827 ns	10.6870**	14.3517**	30.3379*
Seed (Se)	4	13.1118**	2.0567 ns	57.5132**	92.1963**
Stage (St)	3	4.8931*	1.7868 ns	24.9446**	80.8712**
Removal (R)	2	6.5432**	2.8838 ns	38.6226**	139.5557**
Se*St	12	0.6601 ns	1.1900 ns	2.0568 ns	8.9517 ns
Se*R	8	1.3329 ns	0.5401 ns	1.3663 ns	10.7372 ns
St*R	6	2.0033 ns	0.6026 ns	9.1697**	14.3434 ns
Se*St*R	24	1.6084 ns	1.5753 ns	1.1827 ns	10.1490 ns
Residue	177	1.2861	1.4920	1.3166	30.3379
Averages (cm)		4.98	10.16	10.47	16.95
C.V. (%)		22.75	12.02	10.95	17.27

[1] **, *, ns Significant at 1%, 5% and not significant, respectively, by F-test. Stage: Developmental stage at which cotyledon removal was performed. Removal: Number of cotyledons removed. Seed: Size of round-hole sieve.

Table 2. Average of the epicotyl lengths according to the Stage×Seed, Removal×Seed and Removal×Stage evaluated in V2 development stages in a greenhouse, Rio Paranaíba – MG[1].

Stage	Seed									
	P5.5		P6.0		P6.5		P7.0		P7.5	
VE	4.65	Bc[1]	4.77	Bc	5.80	Aa	5.75	Ac	6.37	Aa
VC	5.05	Cbc	5.30	BCbc	6.11	Aa	6.02	ABbc	6.43	Aa
V1	5.85	Ba	5.87	Bab	5.87	Ba	7.00	Aa	6.71	Aa
V2	5.49	Bab	6.29	Aa	6.35	Aa	6.56	Aab	6.92	Aa
Removal	**Seed**									
	P5.5		**P6.0**		**P6.5**		**P7.0**		**P7.5**	
0	5.63	Ba	5.73	Ba	6.83	Aa	6.77	Aa	6.96	Aa
1	5.21	Cab	5.61	BCa	6.12	ABb	6.34	Aab	6.75	Aa
2	4.94	Cb	5.33	BCa	5.16	Cc	5.90	ABb	6.11	Ab

Removal	Stage							
	VE		VC		V1		V2	
0	6.49	Aa	6.23	Aa	6.36	Aa	6.45	Aa
1	5.62	Bb	5.82	ABb	6.25	Aa	6.35	Aa
2	4.35	Cc	5.17	Cb	6.05	Aa	6.39	Aa

[1]Within each interaction (Stage×Seed, Removal×Seed and Removal×Stage) the means followed by the same uppercase letters horizontally and lowercase letters vertically do not differ significantly from each other by the Tukey test, at 5% probability. Stage: Developmental stage at which cotyledon removal was performed. Removal: Number of cotyledons removed. Seed: Size of round-hole sieve.

The length of the epicotyl when assessed at V3 in seedlings from seeds of size P7.5 showed the same averages regardless of the stage at which the cotyledons were removed (Table 3). For the other seed levels (P5.5, P6.0, P6.5 and P7.0), cotyledon removal at Stage VE had the lowest average, while removal at V2 resulted in seedlings with the highest average epicotyl length. And removal at VC and V1 resulted in epicotyl lengths of intermediate size.

Table 3. Average of epicotyl lengths assessed at V3 development stages according to the Stage×Seed and Stage×Removal splits, in a greenhouse, Rio Paranaíba – MG[1].

Stage	Seed									
	P5.5		P6.0		P6.5		P7.0		P7.5	
VE	4.81	Cb	4.73	Cc	5.60	Bb	5.67	Bc	6.52	Aa
VC	4.99	Bb	5.27	Bbc	6.31	Aab	6.05	Abc	6.49	Aa
V1	5.87	Ba	5.94	Bab	5.84	Bab	7.03	Aa	6.71	Aa
V2	5.47	Ba	6.42	Aa	6.46	Aa	6.57	Aa	6.95	Aa

Stage	Removal					
	0		1		2	
VE	6.44	Aa	5.57	Bb	4.38	Cc
VC	6.32	Aa	5.89	Aab	5.255	Bb
V1	6.56	Aa	6.25	ABa	6.02	Ba
V2	6.32	Aa	6.35	Aa	6.455	Aa

[1]Within each interaction (Stage×Seed and Stage×Removal) the means followed by the same uppercase letters horizontally and lowercase letters vertically do not differ significantly from each other by the Tukey test, at 5% probability. Stage: Developmental stage at which cotyledon removal was performed. Removal: Number of cotyledons removed. Seed: Size of round-hole sieve.

When analyzing cotyledon removal within each Stages (Table 3), it was found that in the VE stage, the removal of 0, 1, or 2 cotyledons resulted in statistically distinct epicotyl lengths. In VC, the removal of 0 and 1 cotyledons did not differ, but the removal of 2 cotyledons resulted in shorter epicotyls. In V1, removing 1 cotyledon led to an intermediate epicotyl length between 0 and 2 removals, with significant differences between 0 and 2 removals, the longest length being observed with 0 removal. At V2, cotyledon removal did not influence epicotyl length, regardless of the number of cotyledons removed.

Internode length in V2 was influenced by the removal of 2 cotyledons at the VC stage and by the removal of 1 or 2 cotyledons at VE (Table 4). When 0 cotyledon were removed, internode length did not differ between removal stages (VE, VC, V1, and V2). However, with the removal of 1 cotyledon, the internode length differed, with shorter lengths observed in VE compared to V1 and V2. With 2 cotyledons removed, seedlings from VC had significantly shorter internodes compared to those from V2. Additionally, internode length was affected by seed size, with smaller seeds (5.5) producing shorter seedlings compared to larger seeds (P6.5, P7.0, and P7.5). This indicates that cotyledon removal at VE or VC affects internode length and that smaller seeds result in seedlings with shorter internodes when evaluated in V2.

In V3, analyzing the simple effects revealed that smaller seeds (P5.5 and P6.0) produced seedlings with shorter internodes, while seeds classified as P6.5 produced intermediate lengths, and seeds of P7.0 and P7.5 resulted in the longest internodes. Cotyledon removal at VE and VC stages led to shorter internodes compared to removal at V2, and seedlings without cotyledon removal were taller than those with 1 or 2 cotyledons removed. This indicates that the removal of 1 or 2 cotyledons at early stages and smaller seed sizes independently resulted in seedlings with shorter internodes when evaluated in V3.

Table 4. Average of internode lengths evaluated at V2 and V3 development stages (IL_V2 and IL_V3) as a function of the Stage×Removal and the simple effects Seed, Stage and Removal, in a greenhouse, Rio Paranaíba – MG[1].

Stage	**Removal – IL_V2**					
	0		**1**		**2**	
VE	3.01	Aa[1]	2.49	Bb	2.53	Bab
VC	2.83	Aa	2.73	Aab	2.33	Bb
V1	2.95	Aa	3.11	Aa	2.66	Aab
V2	2.75	Aa	2.91	Aa	2.92	Aa

Seed	**IL_V2**		**IL_V3**		**Stage**	**CI_V3**		**Removal**	**CI_V3**	
P5.5	2.2	c	3.13	d	VE	3.32	b	0	3.62	a
P6.0	2.6	b	3.31	cd	VC	3.39	b	1	3.41	b
P6.5	2.8	ab	3.44	bc	V1	3.46	ab	2	3.34	b
P7.0	3.1	a	3.60	ab	V2	3.67	a			
P7.5	3.1	a	3.80	a						

[1]Within the interaction (Stage × Removal) and each isolated effects of Seed, Stage and Removal, the means followed by the same uppercase letters horizontally and lowercase letters vertically do not differ significantly from each other by the Tukey test, at 5% probability. Stage: Developmental stage at which cotyledon removal was performed. Removal: Number of cotyledons removed. Seed: Size of round-hole sieve.

Petiole length was influenced by seed size, with P5.5-sized seeds resulting in shorter petioles, while P7.5-sized seeds produced longer petioles (Table 5). Cotyledon removal at VC resulted in shorter petioles compared to removal at V2.

Table 5. Average length of the petiole of the first trifoliate leaf (PL_V2) evaluated at the V2 development stage through the simple effects Seed, Stage and Removal, in a greenhouse, Rio Paranaíba – MG[1].

Seed	**PL_V2**		**Stage**	**PL_V2**		**Removal**	**PL_V2**	
P5.5	4.22	c[1]	VE	4.78	ab	0	5.08	ab
P6.0	4.80	bc	VC	4.70	b	1	5.21	a
P6.5	5.06	ab	V1	5.20	ab	2	4.66	b
P7.0	5.22	ab	V2	5.26	a			
P7.5	5.63	a						

[1]Means followed by the same lowercase letters vertically do not differ significantly from each other by the Tukey test, at 5% probability. Stage: Developmental stage at which cotyledon removal was performed. Removal: Number of cotyledons removed. Seed: Size of round-hole sieve.

The Seedling Height by interaction of Stage×Cotyledon for seedlings evaluated in V2 (Table 6) showed that cotyledon removal (1 or 2 cotyledons) at early development stages (VE or VC) resulted in reduced seedling height. In the isolated effects of Seed (Table 6), observed that seed size influenced seedling height when evaluated at the V2 and V3 stages. Cotyledon removal at early stages (VE and VC) resulted in smaller seedlings when evaluated at V3, and the removal of 2 cotyledons significantly affected seedling growth (evaluated at V3), differing from seedlings with 0 or 1 cotyledon removed.

Cotyledons are living tissues that possess the enzymatic machinery necessary to break down and transport their own reserves to nourish the growth of the embryonic axis during germination (Carvalho & Nakagawa, 2012). During seedling emergence, cotyledons turn green due to the formation of photosynthetically active pigments in their plastids, stimulated by light (Müller, 1981a). Thus, photosynthesis begins in the cotyledons a few days after germination (Müller, 1981b), and net photosynthesis (accumulation of products after subtracting the amount used in respiration) can only be detected 9 to 12 days after sowing (Ogren & Rinne, 1973). Moreover, differences in photosynthetic capacity exist from plant to plant within the same soybean cultivar

(Dreger, Brun, & Cooper, 1969) and among cultivars (Curtis, Ogren, & Hageman, 1969; Dornhoff & Shibles, 1970; Hanway & Weber, 1971). Therefore, cotyledons are essential for the early development of seedlings, as they provide nutrient reserves, ATP, amino acids, and sucrose through the consumption of their reserves. Once exhausted, they wilt, shrivel, and fall off (Sediyama, Pereira, Sediyama, & Gomes, 1985).

Table 6. Average of seedling heights evaluated in V2 and V3 (SL_V2 and SL_V3) according to the Stage×Removal and the simple effects Seed, Stage and Removal, in a greenhouse, Rio Paranaíba – MG[1].

Stages	Removal – SH_V2					
	0		1		2	
VE	11.33	Aa[1]	9.70	Bc	8.41	Cb
VC	10.86	Aa	10.31	Abc	8.96	Bb
V1	11.21	Aa	11.15	Aab	10.55	Aa
V2	10.92	Aa	11.41	Aa	10.95	Aa

Seed	SH_V2		SH_V3		Stage	SH_V3		Removal	SH_V3	
P5.5	9.11	c	14.80	b	VE	15.69	c	0	17.95	a
P6.0	9.74	c	16.53	a	VC	16.30	bc	1	17.46	a
P6.5	10.50	b	17.14	a	V1	18.22	a	2	15.46	b
P7.0	11.22	a	18.16	a	V2	17.61	ab			
P7.5	11.82	a	18.14	a						

[1]Within the interaction (Stage×Removal) and each isolated effects of Seed, Stage and Removal, the means followed by the same uppercase letters horizontally and lowercase letters vertically do not differ significantly from each other by the Tukey test, at 5% probability. Stage: Developmental stage at which cotyledon removal was performed. Removal: Number of cotyledons removed. Seed: Size of round-hole sieve.

Artificial destruction of cotyledons during early seedling development can significantly reduce plant growth due to the loss of their reserve compounds and the photosynthesis carried out by this seedling organ (Amarante, Bisognin, & Canci, 1995). However, when cotyledons are destroyed at later stages, no such growth reduction occurs, likely because by that stage, unifoliate leaves and other photosynthetic tissues in soybean seedlings have developed sufficient photosynthetic capacity to sustain seedling growth (Hanley & May, 2006; Hanley & Fegan, 2007). This information corroborates the findings of Thomas & Costa (1993), who concluded that cotyledons contribute to soybean seedling growth up to the eleventh day after sowing. In intact seedlings, cotyledon reserves are depleted by the fifth day after emergence, and the presence of at least one cotyledon influences seedling survival. This may explain the impact of removing 1 or 2 cotyledons at VE and VC stages on epicotyl length, internode length, petiole length, and seedling height, as cotyledon removal in this study occurred at 4.6 days after planting (VE stage), at 7.0 days after planting (VC stage), at 11.3 days after planting (V1 stage), and at 16.2 days after planting (V2 stage).

Larger seeds result in larger and more vigorous seedlings because larger seeds contain more reserves, which may contribute to their growth (Finch-Savage & Bassel, 2015). Additionally, seed size can vary within a species depending on the production environment and can influence seed germination success, seedling establishment, and thus seed vigor (Fenner, 1991). Silva (2013) reported that seed size influenced epicotyl length, with smaller seeds producing shorter epicotyls when measured at the V3 stage. According to this author, smaller seeds have fewer reserves, which may inhibit the plant's potential growth during the early stages of development.

Conclusion

For epicotyl length (assessed at V2 and V3) and internode length (assessed at V2), the influence of seed size was greater when cotyledons were removed at Stages VE and VC. Internode length (assessed at V3), petiole length (assessed at V2) and seedling height (assessed at V2 and V3) were influenced by seed size in isolation.

The number of cotyledons removed influenced the length of the characters assessed. For the epicotyl (assessed at V2 and V3), the greater the number of cotyledons removed associated with a smaller seed size or early stage of removal, the shorter was its length. For internode, petiole and seedling height (assessed at V3), the number of cotyledons removed influenced their length. For seedling height (assessed at V2), the greater number of cotyledons removed associated with an early stage of removal resulted in shorter seedlings.

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