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Silicon alters early development of bean seedlings from seeds infected by Anthracnose

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Abstract
The present study aimed to evaluate the effect of seed coating with Si in response to infection in a cultivar susceptible and resistant to *Colletotrichum lindemuthianum*, on the physiological and health potential of seeds and seedlings, during the initial development of bean culture. The experiment was carried out in a completely randomized design, in a 2 × 2 × 2 factorial scheme, with four replications, where the factors were: cultivar (BRS Esplendor and IPR Tuiuiú), silicate application (with and without Agrosilício® coating) and inoculation (seeds inoculated or not with C. *lindemuthianum*). In the evaluation of the initial development of the culture, the following variables were measured: germination, first count of the germination test, emergence, emergence speed index (IVE), total seedling length, shoot and root length, total dry mass, aerial part dry matter, root dry matter and aerial part dry matter at 15 days after sowing (DAS). The incidence of C. *lindemuthianum* in beans was determined by the paper roll method. Covering the seeds with Si reduces the incidence and severity of C. *lindemuthianum* and provides greater growth of bean seedlings. The effect of responses potentiated by Si is more expressive in the cultivar of greater susceptibility.
Keywords: germination; mineral nutrition; *Phaseolus vulgaris* L.; seed coating, *Colletotrichum lindemuthianum*.

Silício altera desenvolvimento inicial de plântulas de feijão oriundas de sementes infectadas por Antracnose

Resumo
O presente trabalho teve por objetivo, avaliar o efeito do recobrimento de sementes com Si em resposta à infecção em uma cultivar suscetível e resistente ao *Colletotrichum lindemuthianum*, sobre o potencial fisiológico e sanitário de sementes e plântulas, durante o desenvolvimento inicial da cultura do feijão. O experimento foi realizado em delineamento inteiramente casualizado, em esquema fatorial 2 × 2 × 2, com quatro repetições, onde os fatores foram: cultivar (BRS Esplendor e a IPR Tuiuiú), aplicação de silicato (com e sem recobrimento com Agrosilício®) e inoculação (sementes inoculadas ou não com *C. lindemuthianum*). Na avaliação do desenvolvimento inicial da cultura foram mensuradas as seguintes variáveis: germinação, primeira contagem do teste de germinação, emergência, índice da velocidade de emergência (IVE), comprimento total da plântula, comprimento de parte aérea e de raiz, massa seca total, massa seca de parte aérea, massa seca de raiz e massa seca de parte aérea aos 15 dias após semeadura (DAS). A incidência de *C. lindemuthianum* em feijão foi determinada pelo método do rolo de papel. O recobrimento das sementes com Si diminui a incidência e a severidade de *C. lindemuthianum* e proporciona maior crescimento das plântulas de feijão. O efeito de respostas potencializadas pelo Si tem maior expressividade na cultivar de maior suscetibilidade.
Palavras-chave: germinação; nutrição mineral; *Phaseolus vulgaris* L.; revestimento de sementes, *Colletotrichum lindemuthianum*.

Introduction

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc & Magnus) Lams. Scrib is the main fungal disease of bean crop. It is a devastating disease in regions with moderate temperature and high relative humidity causing up to 100% damage to grain yield in highly susceptible cultivars and compromising seed quality (PADDER *et al.*, 2017).

Seeds contaminated by the fungus are the main form of survival and dissemination of *C. lindemuthianum* to undamaged areas, representing a great threat during the establishment of the crop. The pathogen can also survive in cultural remains. Secondary dissemination occurs mainly through water splashes and wind (SINGH; SCHWARTZ, 2010; SILVA *et al,.* 2013). The disease can occur at any stage of development, but it is during the seedling phase that the greatest susceptibility to the pathogen occurs, as the tissues are poorly lignified. Anthracnose lesions can occur in cotyledons, hypocotyl, leaves, stem, branches, pods and seeds (CAMPA *et al.*, 2015).

The management of the disease must involve a set of measures such as crop rotation, use of resistant cultivars, pathogen-free seeds, and fungicides for the treatment of seeds and for spraying on the aerial part. However, the sequential use of fungicide can lead to the selection of individuals resistant to certain chemical groups (SINGH; SCHWARTZ, 2010; CRUZ *et al.*, 2014; MARINGON, 2002I; TRABANCO *et al.*, 2015). Thus, other strategies with the use of alternative measures are important to reduce the damage caused by the disease. Among the potential alternatives is silicon.

Silicon (Si) is considered a non-essential element, but it plays a significant role, especially in reducing biotic and abiotic damage to plants (DEBONA *et al.*, 2017). In many cultures the effect of Si is reported to reduce disease intensity (DALLAGNOL *et al.*, 2015; FORTUNATO *et al.*, 2012, 2014; POLANCO *et al.*; DORNELES *et al.*, 2017). The mechanisms by which Si acts, range from a physical barrier to the penetration of pathogens by deposition of Si and polymerization in the cuticle and cell wall (HUANG *et al.*, 2011; GUERRIERO *et al.*, 2016); as well as by activating plant defense biochemical responses by activating defense enzymes (POLANCO *et al.*, 2014; DORNELES *et al.*, 2017), production of phenolic compounds (TATAGIBA *et al.*, 2014), phytoalexins (HAYNES, 2017) and lignin (POLANCO *et al.*, 2012); and at the molecular level by regulating the expression of genes involved in the defense and response to stress (GHAREEB *et al.*, 2011; WANG *et al.*, 2015).

The high leaf concentration of Si reduced the symptoms of anthracnose in the ribs of bean leaves (CRUZ *et al.*, 2014), increased the activities of antioxidant enzymes (POLANCO *et al.*, 2014) and the concentration of lignin (POLANCO *et al.*, 2012), important events for increasing the plant's resistance against fungus infection. Rice plants supplied with Si produced vigorous seeds with a lower incidence of symptoms of *Bipolar oryzae* in seedlings (DALLAGNOL *et al.*, 2013). In tomato, the increase in the concentration of silicon in the root correlated positively with the reduction in the severity of the disease caused by *Fusarium oxysporum* f. sp. radicis-lycopersici (HUANG *et al.*, 2011). Tunes *et al.* (2014), found that seed coating with CAC and kaolin reduced the incidence of fungi in rice seeds. The application of Si-containing products in the treatment of rice and soybean seeds has resulted in greater emergence and development of seedlings with greater vigor (TUNES *et al.*, 2014; OLIVEIRA *et al.*, 2016). Thus, it is believed that the covering of bean seeds with products containing Si, can be potentially beneficial for the development of seedlings, in addition to strengthening the defense against the attack of pathogens in the early stages of the crop.

In this study, the effect of silicon seed coating in response to infection in a cultivar susceptible and resistant to *C. lindemuthianum* was evaluated on the physiological and health potential of seeds and seedlings during the initial development of beans.

Material and Methods

The experiment was carried out at the Seed Analysis Laboratory and at the Seed Pathology Laboratory of the Faculty of Agronomy Eliseu Maciel, of the Federal University of Pelotas, Campão do Leão - RS. The cultivars BRS Esplendor, characterized as resistant, and IPR Tuiuiú, characterized as susceptible to *C. lindemuthianum* (COSTA *et al.*, 2011; IAPAR, 2010), were used in the experiments. The superficial disinfestation of the seeds was carried out through baths, of 60 seconds each, with 70% alcohol (v /v), followed by sodium hypochlorite 1% (v/v) and three baths of distilled and sterilized

water (JUNGES *et al.*, 2017). Then, the seeds were put to dry under filter paper at room temperature for 48 hours, and subsequently subjected to the inoculation process.

Inoculation in bean seeds: The isolate CL01 from *C. lindemuthianum*, belonging to the library of the Plant Bacteriology Laboratory of the Federal University of Pelotas was used for inoculation. To this end, the fungus was seeded in petri dishes containing BDA culture medium (potato, dextrose and agar) modified in order to restrict water to -0.6Mpa using sucrose solute. Immediately after the fungus was harvested, the plates were incubated in a BOD-type growth chamber at 22 ± 1ºC and a 12-hour photoperiod for 25 days (CARVALHO, 1999). After this period, the bean seeds were randomly distributed on the plates and lightly pressed on the medium completely colonized by the fungus, the treatment containing seeds not artificially inoculated with the fungus was conditioned only on the modified culture medium, for 48 hours, at a temperature of 22ºC and a photoperiod of 12h. Then the seeds were removed and distributed on filter paper, at a controlled temperature of 20ºC, for 48 hours, so that they could return to the initial water content. After this period, the seeds were submitted to the Si coating process.

Silicon Application: The source of Si used was Agrosilício® (calcium and magnesium silicate), composed of 10.5% Si, 25% calcium and 7% magnesium. The form of application of Si was by covering the seeds, at a dose of 90g of Si for 100kg of seed (+ Si), equivalent to 854.7g of the commercial product, plus polymer (Collor Seed®) and water totaling a volume of syrup from 1L to $100kg^{-1}$ of seeds. The coating process was carried out manually in plastic polyethylene bags. For this, 90g of Si, 300mL of polymer and 700mL of water were homogenized, and then 200g of seeds were added, followed by manual agitation of the bags for 2 minutes, until the product was completely adhered to the seed. Then, the bags containing the covered seeds were opened and the seeds were placed to dry at 20ºC for 48 hours. In the treatment without silicate coating (-Si), the seeds only received 300mL of polymer and 700mL of water.

Initial performance of bean seedlings: In the evaluation of the initial culture development, the following variables were measured: germination, first count of the germination test, emergence, emergence speed index (IVE), total seedling length, shoot length and of root, total dry matter, dry matter of aerial part, dry matter of root and dry matter of aerial part at 15 days after sowing (DAS).

The germination evaluation was carried out with four replications of 50 seeds per treatment, sown in a germitest® paper roll, moistened with distilled water, in the proportion of 2.5 times the mass of the paper and stored in a germinator at 25 ± 2ºC. The count of normal seedlings was carried out at five and nine days after the installation of the test and the results are expressed in percentages (BRASIL, 2009).

The first germination count test was carried out in conjunction with the germination test, constituting the record of the percentage of normal seedlings verified on the fifth day after sowing, the results being expressed in percentages of normal seedlings (BRASIL, 2009).

The seedling emergence evaluation was carried out with 200 seeds per treatment, distributed in four replications of 50 seeds. Each repetition was composed of 10 plastic cups of 500mL, containing sand washed, sieved and styled in an autoclave twice (120ºC and 1atm for an hour with a 24h interval). Five seeds were sown in each cup at a depth of 2 cm. The sand was moistened with water, obeying the field capacity of 60% of the retention and kept moist whenever necessary. The treatments were kept in a controlled environment with a temperature of 20 ± 1ºC and a photoperiod of 12h. The evaluation was carried out daily up to 15 days after sowing (DAS), considering normal emerged seedlings as those that had the primary leaves expanded in the final emergency, with results expressed in percentages. The daily emergency assessment data was used to determine the IVE. For this, for each repetition, the formula of Maguire (1962) was applied:

IVE: (E1 ÷ N1) + (E2 ÷ N2) + ... + (En ÷ Nn).

Where: IVE = emergency speed index; E1, E2, En: number of normal seedlings computed in the first, second and last count and N1, N2, Nn: number of days between sowing and the first, second and last count.

The determination of the length of the aerial part, of the root and the total length of the seedling was carried out in a similar way to the one used in the germination test, being evaluated on the fifth day after the test assembly, in four subsamples of 10 seedlings for each treatment. The seedlings were randomly sampled from the sowing of 20 seeds by repetition in the upper

third of the germitest® paper sheet, moistened with distilled water with 2.5 times the mass of the paper. The rolls were placed in a germinator at 25 ± 2ºC. Measurements were determined with the aid of a ruler graduated in millimeters, the root length being obtained by subtracting the total length by the length of the aerial part. The results were expressed in centimeters (NAKAGAWA, 1999).

The determination of aerial part dry mass, root dry mass and total dry mass was carried out in the normal seedlings evaluated in the length test. The root and the aerial part of the seedlings of each repetition were separated and dried in an oven at 70ºC, until the constant weight, obtained in 72h. The total dry mass was obtained by adding the weight of the dry mass of root and dry mass of the aerial part, the results being expressed in grams (NAKAGAWA, 1999).

Dry mass of aerial part at 15 DAS was determined after the final count of the emergency test, aerial part of the normal seedlings of each repetition of the treatments were collected, placed in paper bags and dried in a regulated oven at 60ºC until reaching constant weight, obtained in 72h. The results were expressed in grams per seedling.

Evaluation of the incidence and severity of *C. lindemuthianum* in beans: The incidence of *C. lindemuthianum* in beans was determined by the paper roll method, using four repetitions of 50 seeds (BRASIL, 2009). The seeds were distributed on sheets of germitest® paper, moistened with distilled water in the proportion of 2.5 times the mass of the paper. The rolls were packed in black plastic bags with small perforations, where they were then placed inside a BOD-type camera, with a controlled temperature of 22ºC in the dark and remaining for a period of 7 days. The evaluation was performed on the seventh day after incubation, by observing dark necrotic lesions in the cotyledons and the hypocotyl. The results are expressed as a percentage of incidence.

In the plants of the emergency test, from the fifth day after sowing, evaluations of the symptoms of the disease in the primary leaves of the seedlings began. The evaluations were carried out every 48 hours using a diagrammatic scale described in Appendix B, by Dalla Pria and Amorim (2010). With the data of severity as a function of time, the area under the disease progress curve was calculated (CAMPBELL; MADDEN, 1990), according to the following formula: AACPD: $\Sigma[(Y1+Y2/2)*(t_2-t_1)]$. Where: Y1: evaluation score at time t1; Y2: grade of consecutive evaluation at time t2; t1-t2: interval in days between two consecutive evaluations.

The severity of the disease assessed at 15 DAS was determined by McKinney's disease index (MCKINNEY, 1923), according to the formula: ID (%): ((Σ (Y * Xy)) / (Xt * h) * 100. Where: Y: note; Xy: number of plants with this grade; Xt: total number of plants; h: maximum scale value.

Subsequently, at the end of the emergency test, the incidence of disease damage in the hypocotyl and leaf was determined. In this evaluation, damage characteristic of the disease was observed in all seedlings of the repetitions of treatments, the results being expressed as a percentage of disease incidence (MAFFIA *et al.*, 2007).

Quantification of Phaseolin: To quantify phaseolin in bean hypocotyls, the methodology proposed by Dixon *et al.* (1983) with modifications. For that, the bean seeds were distributed evenly, in four repetitions of 10 seeds per treatment, in a germitest® paper roll and moistened with distilled water in the proportion of 2.5 times the mass of the paper. The paper rolls were placed in plastic bags with small perforations and deposited upright in a BOD camera at 25ºC, in the dark. After seven days, four 4 cm segments from the central hypocotyl region were detached from the seedlings and weighed on a precision scale (0.001g), after being transferred to test tubes, containing 10mL of 98% ethanol. The test tubes were kept at 4ºC for 48h, and then shaken on a shaking table at 150rpm at 25ºC for one hour, to extract the phaseolin. The concentration of the phaseolin formed was measured in a spectrophotometer at 280nm. The results were expressed in units of absorbance per gram per fresh mass.

Determination of the silicon and calcium concentration in the aerial part of the seedling: At the end of the experiment, the aerial part of seedlings (hypocotyl + leaf) from each repetition of the treatments was collected, washed with deionized water, dried at 70ºC for 72h, then crushed in a mill with a 40 mesh sieve. The Si concentration was determined based on the colorimetric analysis of alkaline digestion of 0.1 g of dry tissue (ELLIOTT; SNYDER, 1991). The Ca concentration in the aerial part was determined by nitro-perchloric digestion of 0.2 g of dry tissue according to the methodology of Malavolta *et al.* (1987).

Statistical analysis: The experiment was carried out in a completely randomized design, in a 2 × 2 × 2 factorial scheme, with four replications, where the factors were: cultivar (BRS Esplendor and IPR Tuiuiú), silicate application (with and without Agrosilício coating)®) and inoculation (seeds inoculated or not with *C. lindemuthianum*).

The data were submitted to the tests of normality of the residues by Shapiro-Wilk and homogeneity of the variances by Bartlett, and when meeting the assumptions, the analysis of variance and the comparison of means by the Tukey test was made, at the level of 5% of probability. The variables that did not meet the assumptions were transformed using the Box-Cox system, making it possible to adjust the data for the ANOVA.

Results and Discussion

In this study, the presence of *C. lindemuthianum* in bean seeds negatively influenced the performance of normal seedlings in the PCG test, germination, IVE and final emergence, in addition to obtaining a higher percentage of disease incidence in seedlings (HF), index of disease and incidence of leaf damage and hypocotyl.

Initial performance of bean seedlings: In the first count of the germination test (PCG), there was no significant interaction between the factors, only for inoculation and cultivar (Table 1). Inoculation of seeds with *C. lindemuthianum* reduced the percentage of normal seedlings by 11%, compared to uninoculated seeds. Among the cultivars cv. IPR Tuiuiú obtained the lowest percentage of PCG in relation to BRS Esplendor.

Table 1. Percentage of normal seedlings from the first germination count test, from bean seeds of two cultivars, which were inoculated (I) or not inoculated (NI) with *C. lindemuthianum*.

Treatments	First germination test count (%)
Inoculação	
I	78 b
NI	89 a
Grow crops	
BRS Esplendor	94 a
IPR Tuiuiú	72 b

* Means followed by the same letter do not differ significantly by Tukey's test at 5% probability.

Significant interaction between cultivar and inoculation (Table 2) and inoculation and cover (Table 4) occurred for germination. The presence of the fungus in the seeds reduced the germination of the seeds independent of the covering (Table 4), being in 13% for the cv. IPU Tuiuiú and 6% at BRS Esplendor (Table 2). The cv. BRS Esplendor presented the highest germination percentages in relation to cv. IPU Tuiuiú, about 21% for inoculated seeds and 14% for non-inoculated seeds (Table 2).

Table 2. Percentage of germination as a function of bean cultivars inoculated (I) or not (NI) with *C. lindemuthianum.*

Inoculation	**Grow crops**	
	IPR Tuiuiú	BRS Esplendor
	Germination (%)	
I	72 Bb	93 Ab
NI	85 Ba	99 Aa

* Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly by Tukey's test at 5% probability.

The cv. BRS Esplendor was significantly more vigorous than the IPR Tuiuiú, verified by the emergency tests, dry mass of root (MSR) (Table 3), total length of seedlings (CTP), length of aerial part (CPA), length of root (CR), seedling dry mass at 15 DAS (MSP15) and emergence speed index (IVE).

Table 3. Averages of seedling emergence and dry root weight (MSR) as a function of bean cultivars.

Grow crops	Emergency (%)	MSR (g seedling^{-1})
BRS Esplendor	87 a	0,16 a
IPR Tuiuiú	62 b	0,13 b

* Means followed by the same letter in the column do not differ significantly by Tukey's test at 5% probability.

Seedling emergence was negatively influenced in the presence of the fungus, regardless of seed covering and when comparing inoculated seeds within the covering, we found that when in the presence of the fungus, the seeds covered with Si, provided an increase of 7% of normal seedlings emerged when compared to the absence of Si (Table 4).

Table 4. Seedling germination and emergence, depending on the covering (+ Si) or not (-Si) of the seeds with Si and the inoculation (I) or not (NI) with *C. lindemuthianum.*

Si (90 g 100kg^{-1} of seeds)	Inoculation			
	I	NI	I	NI
	Germination (%)		Emergency (%)	
+Si	80 Ba	93 Aa	70 Ba	82 Aa
-Si	84 Ba	91 Aa	65 Bb	81 Aa

* Means followed by the same letter, lower case in the column and upper case in the line do not differ significantly by the Tukey test at 5% probability.

The dry root mass (MSR) was 35.3% higher in seedlings from seeds covered with Si, compared to those not covered. However, the opposite result was observed for dry mass of aerial parts, in the absence of Si there was an increase of 13.5% in the content of MSPA, in relation to those covered with Si (Table 5).

Table 5. Root dry matter (MSR) and aerial part dry matter (MSPA) of seedlings from seed covered (+ Si) or not with Si (-Si).

Si (90 g 100kg^{-1} of seeds)	MSR	MSPA
	----------(g seedling^{-1})----------	
+Si	0,17 a	1,15 b
-Si	0,11 b	1,33 a

* Means followed by the same letter in the column do not differ significantly by Tukey's test at 5% probability.

Significant interaction between the factors cultivar, inoculation and seed covering, were verified for the tests of total seedling length (CTP), shoot length (CPA), root length (CR), seedling dry mass at 15 DAS (MSP15) and emergency speed index (IVE) (Table 6). Different behavior between cultivars in relation to the inoculated seeds was observed when the seeds were covered with Si. The cv. IPR Tuiuiú (susceptible) inoculated, showed higher values of CTP, CR and MSP15 in relation to those not inoculated, corresponding to an increase of approximately 15, 19, 13% respectively. As for the cv. BRS Esplendor (resistant), there was a reduction of CPA in 8.4% when in the presence of the fungus compared to the non-inoculated ones, while for the other variables (CTP, CR and MSP15) there were no significant differences (Table 6).

Table 6. Total seedling length (CTP), shoot length (CPA), root length (CR), seedling dry mass at 15 DAS and emergence speed index (IVE), depending on the seed coverage with Si (+ Si) or not (-Si) and inoculation (i) or not (NI) with *C. lindemuthianum* in two bean cultivars.

	Total seedling length (cm seedling^{-1}**)**			
Inoculation	+Si		-Si	
	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor
I	22.95 Baα	27.32 Aaα	14.02 Baβ	20.54 Aaβ
NI	19.60 Bbα	28.92 Aaα	13.53 Baβ	19.39 Aaβ
	Shoot length (cm seedling^{-1}**)**			
Inoculation	+Si		-Si	
	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor
I	10.20 Baα	12.59 Abα	6.05 Baβ	7.79 Aaβ
NI	9.25 Baα	13.75 Aaα	5.81 Baβ	7.31 Aaβ
	Root Length (cm Seedling^{-1}**)**			
Inoculation	+Si		-Si	
	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor
I	12.75 Baα	14.73 Aaα	7.97 Baβ	12.76 Aaβ
NI	10.35 Bbα	15.17 Aaα	7.72 Baβ	12.08 Aaβ
	Seedling dry mass at 15 DAS (g seedling^{-1}**)**			
Inoculation	+Si		-Si	
	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor
I	0.08 Baα	0.10 Aaα	0.06 Bbβ	0.01 Aaα
NI	0.07 Bbα	0.11 Aaα	0.07 Baα	0.11 Aaα
	IVE			
Inoculation	+Si		-Si	
	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor
I	1.63 Bbα	2.13 Abα	1.44 Abβ	1.52 Abβ
NI	1.98 Baα	3.53 Aaα	1.98 Baα	2.62 Aaβ

* Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly from each other by the Tukey test at 5% probability. Means followed by equal symbols (β or α) do not differ significantly for silicon (with + Si and without -Si) within each inoculated (I) or uninoculated (NI) cultivar.

When in the absence of Si, there were no statistically significant differences for the growth variables (CTP, CPA, CR and MSP15) in relation to the inoculation within each cultivar, except for MSP15 which the fungus provided a reduction of 5.9%, in compared to those not inoculated (Table 6).

Inoculated seeds *C. lindemuthianum* provided a reduction in IVE regardless of cultivar and seed covering, negatively affecting the rapid emergence of seedlings (Table 6).

Seeds covered with Si, regardless of cultivar and inoculation, provided seedlings with higher CTP, CPA, CR and IVE, except in this last variable, where the supply of Si in cv. IPU Tuiuiú not inoculated, had no significant effect. While for MSP15, the significant effect of Si was only observed for cv. IPU Tuiuiú inoculated with the fungus, which presented an increase of 17% in comparison with the uncovered seeds (Table 6).

Incidence and severity of anthracnose: A lower incidence of symptoms of *C. lindemuthianum* (CI) in cotyledons and in the hypocotyl of bean seedlings was observed in cv. BRS Esplendor when compared to cv. IPU Tuiuiú (Tables 7 and 8), and in uninoculated seeds, in relation to the inoculated seeds regardless of the cultivar (Table 7). In cv. IPU Tuiuiú, the seeds covered with Si showed an incidence of 17.25% lower in relation to the seeds not covered. However, there was no significant difference regarding the effect of Si for cv. BRS Esplendor (Table 7).

Table 7. Incidence of *C. lindemuthianum* in beans (CI), depending on the seed coverage with Si (+ Si) or not (-Si) and cultivars.

Si	**Incidence of *C. lindemuthianum* (%)**	
(90 g 100kg^{-1} of seeds)	IPR Tuiuiú	BRS Esplendor
+Si	44,50 Ab	17,25 Ba
-Si	61,75 Aa	21,25 Ba

* Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly by Tukey's test at 5% probability.

The area under the disease progress curve (AACPD) was 88% smaller in seedlings originating from uninoculated seeds, compared to those inoculated in cv. BRS Esplendor (Table 8).

Table 8. Incidence of *C. lindemuthianum* in beans (CI), area under the disease progress curve (AACPD) and induction of phaseolin in hypocotyls, depending on cultivars and inoculation (I) or not (NI) of *C. lindemuthianum* in bean seeds.

Inoculation	**Grow crops**					
	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor
	IC (%)		**AACPD**		**Phaseolin** (ABS(280nm)/g.p.f)	
I	84 Aa	35 Ba	5,63 Aa	6,71 Aa	0,81 Aa	0,60 Ba
NI	22,3 Ab	3,5 Bb	5,21 Aa	0,81 Bb	0,52 Ab	0,58 Aa

* Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly by Tukey's test at 5% probability.

There was no significant difference in AACPD between cultivars for inoculated seeds, but for seedlings of non-inoculated seeds, AACPD was 84.4% lower in cv. BRS Esplendor in relation to IPR Tuiuiú (Table 8). Therefore, for AACPD it was 75% lower in seedlings from seeds covered with Si compared to seedlings of seeds not covered (Table 9).

Table 9. Area under the disease progress curve (AACPD) of seeds covered (+ Si) or not with Si (-Si).

Si (90 g 100kg^{-1} of seeds)	**AACPD**
+Si	2,07 b
-Si	8,25 a

* Means followed by the same letter in the column do not differ significantly by Tukey's test at 5% probability.

Significant interaction between inoculation and cultivar occurred for the accumulation of phaseolin in bean hypocotyl. The highest absorbance, up to 36%, was observed for cv. IPR Tuiuiú inoculated, compared to the same non-inoculated cultivar, and with cv. BRS Esplendor (Table 8). The accumulation of phaseolin in bean hypocotyl showed a significant interaction between inoculation and the Si coating. Inoculated seeds that did not receive the Si coating, obtained greater absorbance, compared to the non-inoculated ones, and with the seedlings covered with Si (Table 10).

Table 10. Induction of phaseolin in bean hypocotyls as a function of seed coating with Si (+ Si) or not (-Si) and inoculation (i) or not (NI) with *C. lindemuthianum*.

Si (90 g.100kg^{-1} of seeds)	**Inoculation**	
	I	NI
	Phaseolin (ABS (280nm)/g.p.f)	
+Si	0,59 Ab	0,59 Aa
-Si	0,82 Aa	0,52 Ba

* Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly by Tukey's test at 5% probability.

The cv. IPR Tuiuiú, regardless of inoculation and cover, showed the highest percentages of the disease index (ID), leaflet incidence (IF) and symptoms in the hypocotyl (IH) compared to cv. BRS Esplendor, except for ID in covered and inoculated seeds, where the cultivars did not show significant differences (Table 11).

As for inoculation, the presence of the fungus in the seeds of cv. BRS Esplendor provided the highest percentages of ID, IF and IH in relation to the non-inoculated ones, regardless of the seed covering, while for cv. IPU Tuiuiú, this behavior of increased damage was verified only for seeds covered with Si, in the variables of ID and IF (Table 11).

Table 11. Disease index (ID), incidence of disease damage in leaves (IF), incidence of disease damage in the hypocotyl (IH) caused by inoculation (I) or not (NI) of *C. lindemuthianum* depending on the coverage of the seeds with Si (+ Si) or not (-Si) in two bean cultivars.

Inoculation	**Disease index (ID)** (%)			
	+Si		-Si	
	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor
I	17,29 Aaα	15,07 Aaα	33,58 Aaβ	22,44 Baβ
NI	12,81 Abα	10,49 Bbα	32,13 Aaβ	10,87 Bbα
Inoculation	**Incidence of disease damage in leaves (IF) (%)**			
	+Si		-Si	
	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor
I	21,79 Aaα	6,25 Baα	49,51 Aaβ	33,59 Baβ
NI	4,74 Abα	0 Bbα	52,42 Aaβ	5,19 Bbβ
Inoculation	**Incidence of disease damage in the hypocotyl (IH) (%)**			
	+Si		-Si	
	IPR Tuiuiú	BRS Esplendor	IPR Tuiuiú	BRS Esplendor
I	9,57 Aaα	2,41 Baα	7,32 Aaα	3,51 Baα
NI	6,70 Aaα	0,71 Bbα	6,19 Aaα	1,39 Bbα

* Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly by Tukey's test at 5% probability. Means followed by the same symbols (β or α) do not differ significantly from each other, for silicon (with + Si and without -Si) within each inoculated (I) or uninoculated (NI) cultivar.

The covering of the seeds with Si significantly reduced the ID and IF, in comparison with the absence of Si, regardless of the inoculation or cultivar, however this same difference was not observed between the covering of the seeds for the HI (Table 11).

Shoot concentration of silicon and calcium: There were no significant differences for the concentration of Si in aerial parts from seeds covered or not with Si. Significant interaction occurred between cultivar and inoculation. An increase of 18.8% in the Si concentration in the aerial part was observed for cv. BRS Splendor uninoculated compared to inoculated. Si concentration was 20.7% higher at BRS Esplendor compared to cv. IPU Tuiuiú, both not inoculated (Table 12).

Table 12. Concentration of silicon (Si) in the aerial part of seedlings, depending on cultivars and inoculation (I) or not (NI) with *C. lindemuthianum* in bean seeds.

Inoculation	**Si** (g kg^{-1})	
	IPR Tuiuiú	BRS Esplendor
I	3,82 Aa	3,53 Ab
NI	3,45 Ba	4,35 Aa

* Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly by Tukey's test at 5% probability.

For calcium concentration in the aerial part, there was a significant interaction between inoculation and Si coating (Table 13).

Table 13. Concentration of calcium (Ca) in the aerial part of seedlings, depending on the covering of the seeds with Si (+ Si) or not (-Si) and of the inoculation (I) or not (NI) with *C. lindemuthianum* in seeds of bean.

Inoculation	**Ca** (g.kg^{-1})	
	+Si	-Si
I	2,00 Bb	2,47 Aa
NI	2,49 Aa	2,41 Aa

* Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly by Tukey's test at 5% probability.

In the inoculated seeds, the Ca concentration was 19% lower in seedlings from seeds covered with Si in relation to seedlings of seeds not covered. For seedlings from covered seeds, the Ca concentration was 19.7% higher in seedlings of uninoculated seeds compared to inoculated ones (Table 13).

The incidence of anthracnose symptoms in seedlings evaluated on a germitest paper roll (CI), varied from 35 to 84%, while in leaves they reached 52.4% and in hypocotyl in 9.6% among cultivars, however note It is believed that the non-inoculated seeds also obtained high incidences of the disease in the seedlings, this fact is probably due to the fungus being infecting the seeds internally, since the superficial disinfestation of the seeds was carried out initially. Symptomatic seedlings showed small brown lesions in the cotyledons, and dark colored lesions in the hypocotyl, in an elongated and depressed shape. In the leaves, on the bottom, the dark brown necrotic lesions appeared on the ribs, while on the upper part, chlorotic lesions appeared next to the necrotic ones, and often causing deformation of the leaf. According to Silva *et al.* (2013), the greater the amount of inoculum in the seeds, under favorable environmental conditions for the development of the fungus, the greater the incidence and severity of the disease in the field. Rey *et al.* (2009), verified that the transmission of *C. lindemuthianum* seed-seedling can reach up to 80%.

Among the cultivars, the best initial seedling performance was for cv. BRS Esplendor, which originated vigorous seedlings with high germination, a fact that may be related to a better initial seed quality. Lower incidence and severity of the disease was also observed in this cultivar, when compared to cv. IPR Tuiuiú. BRS Esplendor is considered a cultivar with a higher level of resistance among recent cultivars (PEREIRA *et al.*, 2017).

In this work, covering the seeds with Si helped in the growth of bean seedlings, as well as in reducing the severity of anthracnose in the early stages of development. The reduction in the intensity of the disease was observed for both cultivars studied, IPR Tuiuiú (susceptible) and BRS Esplendor (resistant), indicating a possible potentiation of the resistance of the bean to *C. lindemuthianum* during the establishment of the seedlings.

Seeds of the susceptible cultivar (IPR Tuiuiú) inoculated with fungus and supplied with Si had a better response when compared to cv. resistant, it was possible to observe a greater growth, in relation to the non-inoculated ones (Table 6). According to Cooke and Leishman (2016), Si has little effect on the metabolism of

plants under normal conditions, but when exposed to some type of stress they can adapt efficiently, being beneficial in these conditions, as it has an important role in activities physiological or structural survival of plants. One of the probable ways in which Si provides resistance is through the induction of the priming state, which results in the anticipation and increase in the intensity of defense responses after infection (DALLAGNOL *et al.*, 2015), such as the recruitment of defenses and in the absence of pathogens, metabolic costs are reduced (CONRATH *et al.*, 2002).

The bean is a plant species that has the capacity to accumulate low concentrations of Si in the leaf tissues, reaching values of less than 5 g kg-1 in dry matter of the aerial part, which it classifies as a non-accumulating species of Si, however these depend on the availability of Si in the medium, age of the plant, type and location of the tissues involved in the absorption, which directly affects the accumulated quantity (JONES; HANDRECH, 1987; DATNOFF *et al.*, 2001). The highest concentration of Si in the aerial part of beans was observed in cv. BRS Splendor from uninoculated seeds, which may be related to greater production of dry matter, since the deposition and accumulation of Si is directly related to the production of biomass in bean seedlings (OLIVEIRA, 2009).

The deposition of the element can help in the rigidity of the plant, but it can also potentiate the host's defense enzymes and compounds (DALLAGNOL *et al.*, 2015). The beneficial effects verified by the supply of Si in tomato seedlings, were involved in extracellular biochemical functions in the apoplast, even though no Si accumulation was found in the cells (SHI *et al.*, 2014). According to Katz (2014), the beneficial effects of Si are not always proportional to its accumulation and the low concentration in the tissues does not mean that it has a limited function.

For the concentration of Ca in the aerial part of beans, a higher content was observed in seeds inoculated and without the covering with Si, and in the seeds covered with Si and not inoculated. These results indicate that this element did not participate in the benefits generated by the covering of the seeds in the control of the disease, since effects occurred through the covering of the seeds are conferred by Si. Zambolim and Ventura (2012) report that the severity of the disease is inversely proportional to the content of Ca in plant tissue, however it was not possible to observe this relationship at work.

The supply of nutrients through the covering of seeds, provides benefits for the growth of seedlings, in addition to reducing the incidence and severity of the disease in the early stages of development in the bean culture. Regarding cultivar successive to *C. lindemuthianum*, Si contributed by mitigating the damage caused by the fungus, causing the seedlings to respond favorably to the supply of the element, through the technique of covering the seeds and thus being a promising measure in the system of integrated disease control management.

Conclusions

Covering the seeds with Si reduces the incidence and severity of *Colletotrichum lindemuthianum* and provides greater growth of bean seedlings.

The effect of responses potentiated by Si is more expressive in the cultivar of greater susceptibility.

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