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Bacillus sp. RZ2MS9 AND THE BACTERIA-FREE FILTRATE IN THE SEED GERMINATION AND GROWTH OF MAIZE SEEDLINGS

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ABSTRACT

Seed vigor and seedling growth directly impact the early stages of maize production. These traits might be improved with the use of bioinoculants. This work aimed to evaluate the influence of *Bacillus* sp. RZ2MS9 and its bacteria-free filtrate in the seeds germination rate (G) and speed (GSI) and seedlings length (SL) and dry mass (SDM) of two maize hybrids. After receiving experimental treatments, seeds of maize hybrids DKB390 and 30A37PW® were grown in a germinator at 25ºC and 90% humidity. GSI was evaluated daily while G (%), SL (cm) and SDM (mg.10 seeds^{-1}) were evaluated after 7 days. GSI and SDM were higher in both hybrids treated with *Bacillus* sp. RZ2MS9. The bacteria-free filtrate produced higher GSI in the 30A37PW® hybrid only in comparison to the control treatments. Thus, the *Bacillus* sp. RZ2MS9 and its extracellular secreted compounds might comprise alternative tools to improve development and production of maize plants.

Keywords: *Zea mays*, biostimulant, inoculation, seed vigor, extracellular compounds

Bacillus sp. RZ2MS9 E O FILTRADO LIVRE DE BACTÉRIAS NA GERMINAÇÃO DE SEMENTES E CRESCIMENTO DE PLÂNTULAS DE MILHO

RESUMO

O vigor das sementes e o crescimento das plântulas impactam diretamente os estágios iniciais da produção de milho. Essas características podem ser melhoradas com o uso de bioinoculantes. Este trabalho teve como objetivo avaliar a influência de *Bacillus* sp. RZ2MS9 e seu filtrado livre de bactérias sobre a taxa (G) e velocidade (GSI) de germinação de sementes e sobre o comprimento (SL) e massa seca (SDM) de plântulas de dois híbridos de milho. Após receberem os tratamentos experimentais, as sementes dos híbridos DKB390 e 30A37PW® foram cultivadas em uma germinadora a 25ºC e 90% de umidade. A GSI foi avaliada diariamente enquanto G (%), SL (cm) e SDM (mg.10 seeds^{-1}) foram avaliadas após 7 dias. GSI e SDM foram maiores em ambos os híbridos tratados com *Bacillus* sp. RZ2MS9. O filtrado livre de bactérias produziu maior GSI no híbrido 30A37PW® somente em comparação aos tratamentos controle. Portanto, *Bacillus* sp. RZ2MS9 e seus compostos secretados podem compreender ferramentas alternativas para melhorar o desenvolvimento e a produção de plantas de milho.

Palavras-chave: *Zea mays*, bioestimulante, inoculação, vigor de semente, compostos extracelulares

INTRODUCTION

Among the maize production-chain, seed germination and seedling growth promotion are defined as one of the main stages for plant growth and stabilization in the field (SCHLINDWEIN et al., 2008).

The promotion of seed germination associated with inoculation of biological compounds has been reported to be an important cue in research works and field experiments (NOUMAVO et al., 2013). In fact, the pursuit for a greater uniformity of seed germination, have led many growers to use biostimulants (SILVA et al., 2016; YAKHIN et al., 2017).

Biostimulants are defined as the mixture of two or more substances or microorganisms able to stimulate plant growth by means of enhanced nutrient uptake or phytohormones supplying (NARDI et al., 2016). Some commercially available biostimulants are composed of bacterial hormones that act as mediators of plant physiological processes, promoting plant growth and development since its early stages and increasing their potential of water and nutrients absorption (MOTERLE et al., 2011; YAKHIN et al., 2017). These products might be applied directly to plants or in seed treatments as a way of favoring the expression of the genetic potential of the hybrid (SILVA et al., 2016).

Biostimulants often account for more than one plant growth-promoting trait, such as the case of the diazotrophic auxin-producing bacteria studied by Lee et al. (2006), which improved rice seed germination and vigor along with seedling dry weight by balancing phytohormones production. In the same manner, phosphate-solubilizing and nitrogen-fixing bacteria were co-inoculated on tamarind seeds and improved seeds germination speed and percentage (DAS et al., 2018).

During the last decade, many studies have prospected plant growth promoting microorganisms with the potential to influence plant physiology by means of several mechanisms (BATISTA et al., 2018). Along with the most studied microbial plant growth promoting traits, there is an increasing interest to evaluate those potential microorganisms to enhance seed germination and vigor (TAVANTI et al., 2020).

It has been demonstrated that maize cultivars inoculated with combined bacterial strains (*Pseudomonas* spp. and *Azospirillum lipoferum*) had improved seed germination and seedling growth (NOUMAVO et al., 2013). Moreover, Cassán et al. (2009) demonstrated that the inoculation of maize and soybean with *Azospirillum brasilense* AZ39 and *Bradyrrhizobium japonicum* E109 improved seed germination and promoted early seedling growth by means of bacterial produced phytohormones such as indole 3-acetic acid (IAA).

Among the various known bacterial genera, *Bacillus* spp. has a crucial role in agriculture. According to Li et al. (2015), their physiological and morphological characteristics, such as heat resistance, spore production and its Gram + characteristic, makes it an important material for plant growth promotion as an inoculant source, especially in the modern agriculture. Hu et al. (2019) demonstrated the beneficial effects of *Bacillus subtillis* QM3 and its antioxidant enzymatic activities (peroxidase and superoxide dismutase) on the wheat seeds germination under salt stress. Another strains of *Bacillus subtilis* also increased seed vigor and seedling emergence in soybean by means of biological nitrogen fixation, leading to an enhanced content of storage proteins (TAVANTI et al., 2020). Moreover, Figueira et al. (2019) demonstrated the improved germination of *Salicornia ramosissima* seeds inoculated with *Bacillus aryabhattai* SP1016-20.

In this context, the RZ2MS9 strain (*Bacillus* sp.), a rhizobacteria isolated from the rhizosphere of guarana (*Paulinia cupana*) plants from the Amazonian rainforest, presents a real potential to colonize and promote maize and soybean growth (BATISTA et al., 2018). This strains ability to promote plant growth was closely linked to its capacity of biological nitrogen fixation, IAA and siderophores production and phosphate solubilization (BATISTA et al., 2018).

Bacterial inoculants can promote plant growth by means of a close association with the plant or simply by the release of extracellular compounds from the bacterial cell, such as antibiotics, phytohormones or siderophores. Nevertheless, many studies about the bacterial mechanisms affecting germination only evaluate inoculated seeds, but do not investigate the isolate role of the bacterial extracellular secreted compounds (Li et al., 2015). This knowledge might provide important insights for future products development, since bacterial survival in the environment is an often constraint. This alternative practice might have the potential to improve seed germination and crop yield of many important crop productions such as maize, rice, soybean and cotton (RAVEN et al., 2007).

Therefore, the aim of this study was to understand the influence of the *Bacillus* sp. RZ2MS9 strain and its bacteria cell-free filtrate in the germination and seedlings vigor on tropical maize seeds. It is mainly related to the pursuit of new strategies in the maize growth promotion in order to improve its sustainable production.

MATERIAL AND METHODS

The bacterial strain used in this study was *Bacillus* sp. RZ2MS9, which was previously isolated from the rhizosphere of *Paulinia cupana* [(Mark.) Ducke] (BATISTA et al., 2018). The strain has been maintained in culture media Luria-Bertani (LB). *Bacillus* sp. RZ2MS9 and its extracellular products were inoculated in the maize hybrids DKB 390 (Dekalb Brazil Seeds) with a mass of one thousand seeds of 36.7 ± 0.6 g, and 30A37PW® (Morgan Seeds and Biotechnology) with a mass of one thousand seeds of 25.8 ± 0.3 g. Both hybrids moisture were corrected to 130 g kg^{-1}.

To evaluate the influence of RZ2MS9 strain and its extracellular products on germination of maize seeds and seedling development, four experimental treatments were used: 1 – RZ2MS9 (*Bacillus* sp. cultivated in LB medium); 2- Filtered (Bacteria-free LB medium only with RZ2MS9 extracellular products; 3 - LB Medium (Control); 4 - Water (Control).

To obtain the treatments, the RZ2MS9 strain was multiplied in test tubes containing 5 mL of 100% LB medium, under agitation of 150 rpm at 28 °C for 24 hours, until an optical density of 1,508 at 600 nm, comprising a bacterial suspension of approximately 2.5 x 10^9 CFU.mL^{-1}.

The above-mentioned bacterial suspension was used as the RZ2MS9 treatment. The Filtered treatment was obtained by filtering the same bacterial suspension with a 0.22 μm membrane to retain the bacterial cells and maintain only its extracellular products. Sterilized LB medium and distilled water were used as control treatments.

For both maize hybrids, a 1 mL aliquot of each treatment was added to sterilized plastic bags containing 300 maize seeds. Therefore, a rate of about 8.3 x 10^6 CFU per seed was obtained during inoculation in the RZ2MS9 treatment.

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Subsequently, the moisture content of the seeds (H%) was measured, where two replicates with 4 g of seeds were kept in an oven at 105ºC ± 3ºC for 24 hours (NAKAGAWA, 1999) until constant weight.

The seeds germination speed index (GSI) and germination rate (G) were evaluated with 8 replicates (n = 25). The seedling length (SL) and seedling dry mass (SDM) were also evaluated with 8 replicates (n = 10). In all evaluations, the seeds were arranged in rolls composed of three germitest sheets moistened in 2.5 times their dry weight with distilled water, and then they were maintained in a germinator at 25ºC and saturated atmosphere at 90% humidity.

The GSI evaluations were performed daily and calculated by the formula GSI = Σ (ni / ti), where: ni = number of seeds germinating at time 'i'; ti = time after test installation, following the 24-hour time scale. On the seventh day, seed germination (G) was verified, accounting for the number of normal plants, which presented the essential structures of root system (primary root and seminal roots) and aerial part (complete and developed epicotyl). The seeds that did not have such structures were considered as abnormal plants or unviable seeds.

The SL and SDM were evaluated after 7 days of incubation. Then, the length of the aerial part and the primary root of the normal seedlings were measured (in centimeters) for determination of the total length. In addition, the seedlings obtained from the SL test were subjected to cotyledon removal and the seedlings were packed in paper bags and dried at 65 ± 5 ºC until constant weight, the results were expressed as milligram dry mass per 10 seedlings (NAKAGAWA, 1999).

The obtained data were treated for outliers and subjected to the Shapiro-Wilk and Kolmogorov-Smirnov tests, for data normality and homogeneity of variances, respectively. Subsequently, they were submitted to analysis of variance (ANOVA), identifying significant differences at the 95% probability level. Finally, the Scott-Knott test were applied for mean averages comparison under a p-value < 0.05. The analyses were performed using the software RStudio (version 1.2.5033).

RESULTS AND DISCUSSION

According to the results, no difference was observed in the moisture content (H%) of the seeds, which could be assumed as a bias after the treatments (Table 1). The seeds of the maize hybrid DKB 390 (36.7 ± 0.6 g) presented a higher value for mass of a thousand seeds in comparison to the hybrid 30A37PW® (25.8 ± 0.3 g). This factor may influence the speed of germination of maize because larger seeds need a higher period to reach the hydration pattern (approximately 55% moisture) that favors germination, delaying the protrusion of the radicle (VINHAL-FREITAS et al., 2011). In all treatments, it was possible to observe that the hybrid 30A37PW® presented higher GSI ($p < 0.05$) in comparison to the hybrid DKB 390 (Table 1), also presenting higher G (%) values, which points to a difference of seed vigor among the maize hybrids.

Table 1. Mean values and standard deviation of the moisture content (H%), germination (G%) and germination speed index (GSI) of the seeds of maize hybrids DKB 390 and 30A37PW®, Piracicaba, São Paulo State, Brazil, March, 2018.

Treatments	DKB 390			30A37PW®		
	H	G	GSI	H	G	GSI
	-------------%--------		Adimensional	-------------%--------		Adimensional
RZ2MS9	11,9±(0,13)^{ns}	96^{a}	0.89±(0.03) Ba	13,6±(0,09)^{ns}	100^{a}	1.33±(0.01) Aa
Filtered	12,9±(0,13)^{ns}	90^{b}	0.83±(0.03) Bb	12,8±(0,15)^{ns}	100^{a}	1.27±(0.01) Ab
LB medium	12,6±(0,00)^{ns}	89^{b}	0.77±(0.04) Bc	13,0±(0,05)^{ns}	99^{a}	1.20±(0.03) Ac
Water	12,4±(0,09)^{ns}	90^{b}	0.81±(0.05) Bb	12,5±(0,07)^{ns}	97^{a}	1.22±(0.02) Ac

Averages followed by the same lowercase letter in the columns or uppercase letter in the lines do not differ from each other at the 5% error level by the Scott-Knott test.
^{ns} = Not significant by the F test ($p < 0.05$).

Despite those differences among the maize hybrids, data indicates that in both of them the inoculation with RZ2MS9 produced a GSI significantly higher ($p < 0.05$) in comparison to the other treatments (Table 1). In comparison to the controls, the RZ2MS9 treatment increased the GSI from 10-15% in DKB 390 and 9-11% in 30A37PW®. In the DKB 390 hybrid, the RZ2MS9 treatment was also related to a higher G%, while no significant difference was observed for 30A37PW® hybrid (Table 1).

Interestingly, the Filtered treatment also produced a higher GSI in comparison to the control treatments, except for the Water treatment in the DKB 390 hybrid (Table 1). In this treatment, the GSI increases were from 2-8% in DKB 390 and 4-5% in 30A37PW®. Although the Filtered treatment does not match the means obtained with RZ2MS9, this result indicate that the bacterial extracellular compounds can influence seed germination speed in some degree, similarly

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to the effects of plant growth regulators or biostimulants (MOTERLE et al., 2011). Noteworthy, in the DKB 390 hybrid, there was a significant statistical difference between the controls, with the Water treatment presenting a superior average in comparison to the LB medium treatment, which is a positive result, since it demonstrated that the nutrients contained in the LB medium did not influenced the GSI.

Other parameters evaluated to determine the influence of *Bacillus* sp. RZ2MS9 and its secreted compounds were seedling length (SL) and seedling dry mass (SDM). The results showed that the SDM values followed the same statistical probability presented for the GSI, due to the faster growth of the seedlings that received the RZ2MS9 treatment; consequently, they presented higher SDM (Table 2).

Table 2. Mean values and standard deviation of seedling length (SL) and seedling dry mass (SDM) for maize seeds of hybrids DKB 390 and 30A37PW®. Piracicaba, São Paulo State, Brazil, April, 2018.

Treatments	DKB 390		30A37PW®	
	SDM	SL	SDM	SL
	$mg.10\ seeds^{-1}$	cm	$mg.10\ seeds^{-1}$	cm
RZ2MS9	516.83±(12.77) Ba	25.12±(1.13) ^{ns}	560.36±(17.67) Aa	24.94±(0.70) ^{ns}
Filtered	476.16±(7.97) Ab	23.17±(2.51) ^{ns}	490.55±(17.67) Ab	20.92±(3.51) ^{ns}
LB medium	408.50±(13.44) Ac	21.26±(3.19) ^{ns}	365.62±(42.42) Ac	23.17±(2.51) ^{ns}
Water	480.33±(14.43) Ab	23.97±(2.00) ^{ns}	410.33±(28.28) Bc	24.76±(1.06) ^{ns}

Averages followed by the same lowercase letter in the columns or uppercase letter in the lines do not differ from each other at the 5% error level by the Scott-Knott test.
^{ns} = Not significant by the F test ($p < 0.05$)

Results for the 30A37PW® hybrid demonstrated that the RZ2MS9 treatment had SDM 14% higher than the Filtered treatment and both were superior to the controls, being 34-53% higher than the LB Medium and 20-37% higher comparing to the Water treatment (Table 2). These results highlight that both bacterial cells and extracellular products were capable of benefiting the growth of the seedlings of this hybrid. Regarding the DKB 390 hybrid, the RZ2MS9 treatment increased SDM from 8-27% in comparison to the other treatments. In this hybrid, the Filtered treatment was statistically equivalent to the control Water treatment, being both superior ($p < 0.05$) to the LB medium, which again indicates that the nutrients present in the LB medium did not influence the results.

Several studies have demonstrated the potential impact of seeds bioinoculants containing endophytic or epiphytic bacteria on plant growth promotion and also on seed germination and seedling dry matter accumulation (BASHAN et al., 2014). Ramamoorthy et al. (2000) showed that seed germination increased in rice lots inoculated with *Azospirillum lipoferum* and *A. brasilense*, 14 days after sowing. Similarly, Karthikeyan et al. (2007) observed that seeds of *Catharanthus roseus* (L.) inoculated with diazotrophic bacteria (*Azospirillum* and *Azotobacter*), isolated from the rhizosphere and root of this plant, increased the germination percentage, as well as vigor and dry mass accumulation of seedlings grown under gnotobiotic conditions.

In fact, applying those microbes in seeds can improve germination through nitrogen fixation, phosphate solubilization, organic acid and indole-3-acetic acid (IAA) production, siderophore production, fungal antagonism or induction of systemic resistance (BASHAN et al., 2014; DAS et al., 2018). In addition, it can be assumed that the production of phytohormones is one of the main factors responsible for the increases in germination and biomass accumulation in the seedlings (BARAZANI & FRIEDMAN, 2000).

Among the known phytohormones, auxins are responsible for the growth of plants, acting directly on the mechanisms of cell expansion and differentiation. Auxins are considered as one of the main hormones involved in seed germination processes (RAVEN et al., 2007), as they are linked to cell growth by increasing cell wall plasticity and irreversible stretching.

Batista et al. (2018) demonstrated that *Bacillus* sp. RZ2MS9 presents high IAA production. In the present study, data analysis suggest that bacterial produced IAA might play a crucial role in the improvement of seed and seedlings development, since the seeds treated with *Bacillus* sp. RZ2MS9 or with its filtered suspension showed greater germination speed and growth of the seedlings (Tables 1 and 2). Finally, along with the RZ2MS9 IAA production, other effects that improve seed germination might also be present (YAKHIN et al., 2017) and need further exploration.

CONCLUSION

Bacillus sp. RZ2MS9 strain and its secreted compounds, such as IAA present in the bacteria–free filtrate, has the potential to increase the germination rate and speed of germination of maize seeds and also increase dry mass of maize seedlings.

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REFERENCES

BARAZANI, O.; FRIEDMAN, E. 2000. Effect of exogenously applied L-tryptophan on allelochemical activity of plant growth-promoting rhizobacteria (PGPR). **Journal of Chemical Ecology**, v.26, n.2, p.343-349. https://doi.org/10.1023/A:1005449119884.

BASHAN, Y.; DE-BASHAN, L. E.; PRABHU, S. R.; HERNANDEZ, J.P. 2014. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). **Plant and Soil**, v.378, p.1–33. https://doi.org/10.1007/s11104-013-1956-x.

BATISTA, B. D.; LACAVA, P. T.; FERRARI, A.; TEIXEIRA-SILVA, N. S.; BONATELLI, M. L.; TSUI, S.; MONDIN, M.; KITAJIMA, E.W.; PEREIRA, J.O.; AZEVEDO, J.L.; QUECINE, M.C. 2018. Screening of tropically derived, multi-trait plant growth-promoting rhizobacteria and evaluation of corn and soybean colonization ability. **Microbiological Research**, v.206, p.33–42. https://doi.org/10.1016/j.micres.2017.09.007.

CASSÁN, F.; PERRIG, D.; SGROY, V.; MASCIARELLI, O.; PENNA, C.; LUNA, V. 2009. *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). **European Journal of Soil Biology**, v. 45, n.1, p.28–35. https://doi.org/10.1016/j.ejsobi.2008.08.005.

DAS, S.; DASH, D.; GUPTA, S. B.; DEOLE, S. 2018. Study of characterization of tamarind associated Rhizobium spp. and phosphate solubilizing bacteria and their potency for germination of tamarind seeds. **The Pharma Innovation Journal**, v.7, n.11, p.282–286. Available at: <http://www.thepharmajournal.com/archives/2018/vol7issue11/PartE/7-10-110-931.pdf> Accessed on: Apr. 15, 2020

FIGUEIRA, C; FERREIRA, M. J.; SILVA, H.; CUNHA, A. 2019. Improved germination efficiency of *Salicornia ramosissima* seeds inoculated with *Bacillus aryabhattai* SP1016-20. **Annals of Applied Biology**, v.174, n.3, p.319–328. https://doi.org/10.1111/aab.12495.

HU, Q.; LIU, R.; LIU, J. 2019. Effects of *Bacillus subtilis* QM3 on Germination and Antioxidant Enzymes Activities of Wheat Seeds under Salt Stress. **Open Access Library Journal**, v.6, n.3, p.1–9. https://doi.org/10.4236/oalib.1105218.

KARTHIKEYAN, B.; JALEEL, C. A.; GOPI, R.; DEIVEEKASUNDARAM, M. 2007. Alterations in seedling vigour and antioxidant enzyme activities in *Catharanthus roseus* under seed priming with native diazotrophs. **Journal of Zhejiang University – SCIENCE B**, v.8, n.7, p.453-457. https://doi.org/10.1631/jzus.2007.B0453.

LEE, H.S.; MADHAIYAN, M.; KIM, C.W.; CHOI, S.J.; CHUNG, K.Y. 2006. Physiological enhancement of early growth of rice seedlings (*Oryza sativa* L.) by production of phytohormone of N2-fixing methylotrophic isolates. **Biology and Fertility of Soils**, Berlin, v.42, p.402-408. https://doi.org/10.1007/s00374-006-0083-8.

LI, Z.; GUO, B.; WAN, K.; CONG, M.; HUANG, H.; GE, Y. 2015. Effects of bacteria-free filtrate from *Bacillus megaterium* strain L2 on the mycelium growth and spore germination of *Alternaria alternata*. **Biotechnology and Biotechnological Equipment**, v.29, n.6, p.1062–1068. https://doi.org/10.1080/13102818.2015.1068135.

MOTERLE, L. M.; SANTOS, R. F.; SCAPIM, C. A.; de LUCCA e BRACCINI, A.; BONATO, C. M.; CONRADO, T. 2011. Effect of plant growth regulator on germination and vigor of soybean seeds. **Revista Ceres**, v.58, n.5, p.651–660. https://doi.org/10.1590/S0034-737X2011000500017.

NAKAGAWA, J. Testes de vigor baseados no desempenho das plântulas. In: Krzyzanowski, F. C.; Vieira, R. D.; França Neto, J. B. (Ed.). **Vigor de sementes: conceitos e testes**. Londrina: ABRATES, 1999, cap.2.1, p.2-24.

NARDI, S.; PIZZEGHELLO, D.; SCHIAVON, M.; ERTANI, A. 2016. Plant biostimulants: Physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. **Scientia Agricola**, Piracicaba, Brazil, v.73, n.1, p.18–23. https://doi.org/10.1590/0103-9016-2015-0006.

NOUMAVO, P. A.; KOCHONI, E.; DIDAGBÉ, Y. O.; ADJANOHOUN, A.; ALLAGBÉ, M.; SIKIROU, R.; GACHOMO, E.W.; KOTCHONI, S.O.; BABA-MOUSSA, L. 2013. Effect of Different Plant Growth Promoting Rhizobacteria on Maize Seed Germination and Seedling Development. **American Journal of Plant Sciences**, v.4, n.5, p.1013–1021. http://dx.doi.org/10.4236/ajps.2013.45125.

RAMAMOORTHY, K.; NATARAJAN, N.; KILLIKULAM, T. N. A. U.; VALLANAD, P.O. 2000. Seed biofortification with *Azospirillum* spp. for improvement of seedling vigour and productivity in rice (*Oryza sativa* L.). **Seed Science and Technology**, Bassersdorf, Switzerland, v.28, n.3, p. 809-815. Available at: <https://www.cabdirect.org/cabdirect/abstract/20013009232> Accessed on: Mar. 13, 2020.

RAVEN, P. H.; EVERT, R. F.; EICHHORN, S.E. 2007. **Biologia vegetal** 7a ed. Rio de Janeiro, Guanabara Koogan, 856p.

SCHLINDWEIN, G.; VARGAS, L. K.; LISBOA, B. B.; AZAMBUJA, A.C.; GRANADA, C. E.; GABIATTI, N.C.; PRATES, F.; STUMPF, R. 2008. Influence of rhizobial inoculation on seedling vigor and germination of lettuce. **Ciência Rural**, v.38, n.3, p.658-664. https://doi.org/10.1590/S0103-84782008000300010.

SILVA, R. D. A.; SANTOS, J.L.; OLIVEIRA, L. S.; SOARES, M. R. S.; SANTOS, S. M. S. 2016. Biostimulants on mineral nutrition and fiber quality of coton crop. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.20, n.12, p.1062–1066. https://doi.org/10.1590/1807-1929/agriambi.v20n12p1062-1066.

TAVANTI, T.R.; TAVANTI, R.F.R.; GALINDO, F.S.; SIMÕES, I.; DAMETO, L.S.; de SÁ, M.E. 2020. Yield and quality of soybean seeds inoculated with *Bacillus subtilis* strains.

Revista Brasileira de Engenharia Agrícola e Ambiental, v. 24, n.1, p. 65-71. https://doi.org/10.1590/1807-1929/agriambi.v24n1p65-71.

VINHAL-FREITAS, I. C.; SEGUNDO, J. P.; SILVA, M.; ITUIUTABA, E. 2011. Germinação e vigor de sementes de soja classificadas em diferentes tamanhos. **Agropecuária Técnica**, v.32, n.1, p.108–114. https://doi.org/10.25066/agrotec.v32i1.9567.

YAKHIN, O. I.; LUBYANOV, A. A.; YAKHIN, I. A.; BROWN, P. H. 2017. Biostimulants in plant science: A global perspective. **Frontiers in Plant Science**, v.7, 2049 p. https://doi.org/10.3389/fpls.2016.02049.

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