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# BIOINPUTS IN THE GERMINATION AND INITIAL DEVELOPMENT OF *Vachellia caven* (Molina) Seigler & Ebinger

## *BIOINSUMOS NA GERMINAÇÃO E DESENVOLVIMENTO INICIAL DE Vachellia caven (Molina) Seigler & Ebinger*

**Gabriela Neves Righi Corrêa[1], Marciéli Quatrin[2], Antonio Carlos Ferreira da Silva[3], André Ebone[4], Raquel Stefanello[5] e Silvane Vestena[6]**

## ABSTRACT

*Vachellia caven* (Molina) Seigler & Ebinger, a species found in Rio Grande do Sul, is considered a precursor species, and when it comes to reforestation, organisms like this are of the utmost importance. The use of compounds with bacteria can help with the germination and initial growth of plants and control phytopathogens, while also reducing the need for fungicides and fertilizers, which are often harmful to the soil and the environment. Organisms such as *Bacillus subtilis*, *B. megaterium*, *B. amyloliquefaciens*, *Azospirillum brasilense*, *Pseudomonas fluorescens* and *Priestia megaterium* can be used for this purpose. The aim of this study was to evaluate the results of different bioinputs on the germination and initial growth of *V. caven*. To this end, seed dormancy was overcome with concentrated sulfuric acid for two hours, and seven treatments were used, two of which were control treatments (one with water and the other with mineral oil) and five treatments with bioinputs. Each treatment had four replicates with 50 seeds each. The seeds were sown on *germitest* paper and kept in a growth chamber. Parameters such as first germination count, germination, shoot and root length, and shoot and root dry mass were assessed. After analysis, it was possible to see that under the conditions tested, there was no increase or decrease in the germination rate of *V. caven*. This infers that the bioinputs used, as they have a neutral action on the germination and initial growth of *V. caven*, may be safe if they are used in biological control of phytopathogens, nitrogen fixation, and other nutrients, with a view to producing seedlings of this plant species.

**Keywords:** bioproducts; seeds; *Vachellia caven;* germination; initial growth.

## *RESUMO*

*Vachellia caven (Molina) Seigler & Ebinger, uma espécie presente no Rio Grande do Sul, é considerada uma espécie precursora, quando se trata de reflorestamento, organismos assim são de suma importância.*

1 Aluna do Curso de Ciências Biológicas, Universidade Federal de Santa Maria (UFSM), RS. E-mail: gabrielanevesrighi@gmail.com. ORCID: https://orcid.org/0009-0001-3443-4443

2 Aluna do Curso de Ciências Biológicas, Universidade Federal de Santa Maria (UFSM), RS. E-mail: marcieli.quatrin@acad.ufsm.br. ORCID: https://orcid.org/0009-0007-3160-5810

3 Doutorado em Ciências; Professor na Universidade Federal de Santa Maria (UFSM). E-mail: acfsilva2@uol.com.br. ORCID: https://orcid.org/0000-0002-1050-1656

4 Mestrado em Agronomia pela Universidade Federal de Santa Maria (UFSM). E-mail: andreebone@biogreen.com.br. ORCID: https://orcid.org/0000-0002-3773-4564

5 Doutorado em Agronomia; Bióloga; Universidade Federal de Santa Maria (UFSM). E-mail: raquelstefanello@yahoo.com.br. ORCID: https://orcid.org/0000-0003-3079-2099

6 Doutorado em Fisiologia Vegetal; Professora na Universidade Federal do Pampa e em exercício provisório na Universidade Federal de Santa Maria (UFSM). E-mail: silvanevestena@gmail.com. ORCID: https://orcid.org/0000-0002-9797-1211

*A utilização de compostos com bactérias pode auxiliar na germinação e crescimento inicial de plantas e controle de fitopatógenos por sua vez, também diminui a necessidade da inserção de fungicidas e fertilizantes, que muitas vezes são maléficos para o solo e o ambiente. Organismos como Bacillus subtilis, B. megaterium, B. amyloliquefaciens, Azospirillum brasilense, Pseudomonas fluorescens e Priestia megaterium podem ser utilizados com este viés. Deste modo, o objetivo deste trabalho foi avaliar o resultado da inserção de diferentes bioinsumos na germinação e crescimento inicial do V. caven. Para tanto, a dormência das sementes foi superada com ácido sulfúrico concentrado por duas horas e foram utilizados sete tratamentos, sendo dois tratamentos controle (um com água e outro com óleo mineral) e cinco tratamentos com bioinsumos. Cada tratamento possuía quatro repetições com 50 sementes cada. As sementes foram semeadas sobre papel germitest e os mesmos foram mantidos em uma câmara de crescimento. Foram avaliados parâmetros como primeira contagem de germinação, germinação, comprimento de parte aérea e raiz, massa seca de parte aérea e raiz. Após a análise, foi possível visualizar que nas condições testadas em nenhum tratamento houve aumento ou diminuição da taxa de germinação do V. caven, inferindo-se que os bioinsumos utilizados, por apresentarem ação neutra na germinação e crescimento inicial de V. caven, possam ser seguros se forem utilizados em ações de controle biológico de fitopatógenos, fixação de nitrogênio e outros nutrientes, visando a produção de mudas desta espécie vegetal.*

***Palavras-chave:*** *bioprodutos; sementes; Vachellia caven; germinação; crescimento inicial.*

## INTRODUCTION

The species *Vachellia caven* (Molina) Seigler & Ebinger is native to the southwest of the State of Rio Grande do Sul and belongs to the Fabaceae family; it is a medium-sized, deciduous, and thorny shrub (ESCOBAR *et al*., 2010). It is considered a pioneer species, which means that it has a positive effect on the establishment of other species in the region. It creates ecological conditions that trigger regeneration and recovery processes of native herbaceous and woody species, improving the abiotic microenvironment established in the region. In situations of drought and deforestation, such as the current one, such species are even more valuable to cultivate. As with most forest seeds, dormancy must be overcome. There are several ways to overcome seed dormancy, including mechanical scarification, chemical scarification, normal washing, stratification, and others (ESCOBAR, 2010; KERBAUY, 2019; OLIVEIRA *et al*., 2021; TAIZ *et al*., 2024).

The use of bioinputs in the initial growth of plants is currently increasing. Through various mechanisms, populations of microorganisms can contribute to seed germination. This makes it possible to increase the percentage of germination and growth (FRANÇA *et al*., 2017; CADORE *et al*., 2020).

Bioinputs are biological products composed of bacterial or fungal microorganisms, or of animal or plant origin, that can satisfactorily support the germination process of seeds and the development of seedlings (MARCHESE; FILIPPONE, 2018), and are probably one of the most important tactics in the world today. This is due to the emerging demand to reduce dependence on chemical fertilizers and the need to develop sustainable practices (MACHADO *et al*., 2012; CHAGAS JUNIOR *et al*., 2022). In addition, the production of low-cost inoculants with plant growth-promoting microorganisms is an alternative to reduce environmental risks caused by inadequate and sometimes excessive use of inputs and pesticides (BRAGA JUNIOR *et al*., 2018; DIAZ *et al*., 2019). These include *Bacillus subtilis*, *B. megaterium*, *B. amyloliquefaciens*, *Azospirillum brasilense*, *Pseudomonas fluorescens* and *Priestia megaterium*.

The *Bacillus subtilis* species found naturally in the soil is a rhizobacterium, which means that it can live in the roots of a plant (CHAGAS JUNIOR, 2022; OLIVEIRA; BOIAGO, 2022); it can often act as a plant growth promoter, helping to fight phytopathogens, fix nitrogen, and solubilize nutrients. The *B. megaterium* species has a high capacity to solubilize phosphates, allowing plants to use the phosphorus. *Bacillus*-based bioproducts have great potential to aid seed germination (OLIVEIRA; BOIAGO, 2022; SILVA *et al*., 2022). The *Bacillus amyloliquefaciens* species has great potential to produce secondary metabolites that can typically control phytopathogenic fungi (SZILAGYI-ZECCHIN *et al*., 2015). Bacteria of the genus *Azospirillum* act in nitrogen fixation, a critical process for plant development, and also help root growth by releasing amino acids (GAIOTTO, 2021).

Bioinputs also contribute to root development, which will increase the absorption of water and nutrients (FRANÇA *et al*., 2017; CADORE *et al*., 2020). Furthermore, they can serve as an alternative technology in the development not only of sustainable agriculture, but also in the cultivation of forest species such as *V. caven*. By promoting seed germination, these bioinputs are also more economically viable options and beneficial to the environment. Since the introduction of chemical agents, whether pesticides or fertilizers, often leads to environmental problems and can be a source of water and soil contamination, or even cause the death of animals. Organic waste can be a raw material for bioinputs, generating products that are more affordable and less harmful to the environment (MARCHESE; FILIPPONE, 2018; GUIMARÃES *et al*., 2021; SANTOS *et al*., 2021; AGUADO *et al*., 2023). Few studies exist with the use of these bioinputs in tree species, emphasizing the importance of this work with the use of these microorganisms in research involving tree species like the one treated in this work. Although studies related to plant growth promoting microorganisms have been known for decades, it is necessary to deepen studies on forest species, since most studies focus on agricultural species. Considering the above, the aim of this work was to evaluate the neutral, positive, or negative effects of different bioinputs on the germination and initial development of *Vachellia caven* (Molina) Seigler & Ebinger.

## MATERIAL AND METHODS

The work was carried out at the Seed and Plant Tissue Culture Laboratory of the Federal University of Santa Maria (UFSM), Santa Maria, RS, with seeds collected at the main campus. Before setting up the experiment, the seed dormancy was overcome with concentrated sulfuric acid ($H_2SO_4$) for 120 minutes (two hours), and the seeds were immersed in distilled water for three hours.

Five bioinputs were used (Doubac Meta®, Doubac Sigma®, AZ Platinum®, Greenphos® and Valentia®) at a concentration of 1 x $10^{-8}$ CFU $mL^{-1}$ according to the methodology of Cadore *et al.* (2016), all formulated with bacterial isolates, distilled water as a control treatment, and a mineral oil-based treatment (Table 1).

**Table 1 -** Commercial name and composition of bioinput treatments.

| Treatment | Trade name | Composition |
|---|---|---|
| A | - | Distilled water |
| B | - | Mineral oil |
| C | Doubac Meta® 2 x $10^{-8}$ UFC $mL^{-1}$ | *Bacillus amyloliquefaciens* |
| D | Doubac Sigma® 1 x $10^{-8}$ UFC $mL^{-1}$ | *Bacillus subtilis* |
| E | AZ Platino® 2 x $10^{-8}$ UFC $mL^{-1}$ | *Azospirillum brasilense* |
| F | Greenphos® 1 x $10^{-8}$ UFC $mL^{-1}$ | *B. megaterium, Pseudomonas fluorescens* e *Azospirillum brasilense* |
| G | Valentia® 1 x $10^{-8}$ UFC $mL^{-1}$ | *Priestia megaterium* e *P. fluorescens* |

Each treatment consisted of four replicates of fifty seeds sown on germination paper moistened with 2.5 times the weight of the paper in distilled water (BRASIL, 2009), with sufficient humidity to maintain the treatments during the experiment period. The paper rolls were placed in sealed plastic bags and placed in a growth chamber with controlled temperature (25 ± 2 ºC) and light (12 h photoperiod).

The effect of the products on seed germination was assessed using the following tests:

First germination count - The number of normal seedlings that emerged seven days after the start of the experiment was evaluated, with normal seedlings being those with developed roots and shoot (BRASIL, 2009).

Germination - After fourteen days, the number of normal seedlings that had germinated was assessed, with the results expressed as a percentage (BRASIL, 2009).

Shoot and root length of seedling - Ten normal seedlings were selected from each replicate, and the average length of the shoot and root was measured in centimeters (KRZYZANOWSKI *et al*., 2020).

Shoot and root dry mass of seedling - ten seedlings from each replicate were dried in an oven (60 ± 5 °C for 48 h) and the dry mass of shoot and root was determined on a precision balance (KRZYZANOWSKI *et al*., 2020).

The design used was completely randomized and consisted of different biological inputs. The data were analyzed using Sisvar software (version 5.6). The significance level was set at $p < 0.05$.

## RESULTS AND DISCUSSION

Analysis of the data (Table 2) showed that there was no increase or decrease in germination percentage in any of the bioinput treatments compared to the control treatments (A and B).

The treatment with the highest germination percentage (95%) and the one with the lowest (83%) were statistically similar, showing that there was no statistical significance between treatments. It can be concluded that the bacteria were not harmful and did not prevent the germination of *V. caven* seeds.

**Table 2 -** First count (FC), germination (G), shoot length (SL), root length (RL), shoot dry mass (SDM) and root dry mass (RDM) of *Vachellia caven* (Molina) Seigler & Ebinger seedlings subjected to different bioinput treatments.

| Treat. | FC (%)$^{ns}$ | G (%)$^{ns}$ | SL (cm)$^{ns}$ | RL (cm)$^{ns}$ | SDM (mg)$^{ns}$ | RDM (mg)$^{ns}$ |
|---|---|---|---|---|---|---|
| A | 87 a | 89 a | 2,90 a | 6,53 a | 5,28 a | 3,53 a |
| B | 83 a | 86 a | 2,89 a | 5,93 a | 5,42 a | 3,50 a |
| C | 92 a | 94 a | 2,80 a | 6,19 a | 5,49 a | 3,12 a |
| D | 87 a | 87 a | 2,70 a | 6,39 a | 5,06 a | 3,40 a |
| E | 87 a | 88 a | 2,92 a | 5,85 a | 5,50 a | 3,19 a |
| F | 82 a | 83 a | 2,55 a | 6,25 a | 5,21 a | 3,19 a |
| G | 94 a | 95 a | 2,24 a | 6,23 a | 4,33 a | 2,97 a |
| C.V.(%) | 7,32 | 6,72 | 14,30 | 5,06 | 11,66 | 8,37 |

ns = not significant. C.V. = coefficient of variation.

In everyday life, bacteria are often treated only as organisms that are harmful to other organisms, but in fact many bacteria pose a risk to public health by causing disease in humans. When it comes to the plant kingdom, there are bacteria that are harmful to plants, but the interaction between plants and microorganisms is extremely complex and, in most cases, very beneficial. Bacteria can fix nitrogen and receive carbohydrates in return; this process is of great benefit to both the plant and the bacteria (BARBOSA *et al.*, 2014; TAIZ *et al.*, 2024). Another process in which microorganisms act is to increase the availability of phosphorus to the root system, with bacteria of the Bacillus genus being the most efficient at solubilizing phosphorus (GUIMARÃES *et al.*, 2021). Although the bioinputs used in this study did not influence the initial development of *V. caven*, several authors consider that these bacteria are important and can be beneficial to agricultural crops and forest species. Oliveira and Boiago (2022) tested the effect of using bioproducts on the initial development of bean plants (*Phaseolus vulgaris* L.). Unlike Clemente *et al.* (2016), who observed a significant increase in yield of carrots following the application of compost fermented with selected strains of *Bacillus* sp.

Many bacterial species aid seed germination and also control phytopathogens (MARQUES *et al.*, 2014). There are reports on the benefits of using *Bacillus* spp. in seed germination and in controlling undesirable microorganisms (FERREIRA *et al.*, 2021). In many soils where heavy metals and fertilizer residues are present, some bacteria, such as the genus *Pseudomonas*, have the ability to adapt to these environments and can contribute to environmental detoxification and plant growth (RUIZ-HERNANDEZ *et al.*, 2024).

Chagas Junior *et al.* (2022) observed that there were no statistical differences in the parameters evaluated at 30 and 52 days after sowing, and that the cultivation period was decisive for the results. They noted that *B. subtilis* showed the ability to provide a greater increase in biomass in soybean plants at 52 days. It should be noted that in this study the cultivation period was 15 days, following the methodology of Brazil (2009) for this species.

The satisfactory results of bioinputs, especially with *Bacillus*, may be related to the different mechanisms by which this bacterium acts, such as the production of hydrogen cyanide, phytohormones, enzymes, and the availability of nutrients (phosphorus and nitrogen) (BRAGA JUNIOR *et al*., 2018; GUIMARÃES *et al*., 2021; SANTOS *et al*., 2021), being essential for nutrient recycling and having potential as biofertilizers to increase productivity, as well as being able to benefit the plant in times of stress (BRAGA JUNIOR *et al*., 2018; DIAZ *et al*., 2019). In addition, studies by Jain *et al.* (2016) showed that isolates of *Bacillus* sp. can increase the fresh weight of the aerial part and root in soybean, as well as increase the number of lateral roots.

The use of *Bacillus* species as growth promoters and biological control agents for various agricultural crops provides an attractive, efficient, and less environmentally aggressive method than pesticides and chemical fertilizers. This makes the practice of using bioagents a more sustainable alternative in economic and environmental terms (SHAFI *et al*., 2017). According to Oliveira *et al.* (2016) and Oliveira and Boiago (2022), bacteria possess a rapid action potential on germination and seedling emergence, aerial and root growth, crop development, and yield due to the production of growth-promoting substances.

Agriculture and its productivity have increased significantly in recent times, which has led to an increase in the use of chemical fertilizers and the contamination of natural resources. The solution to these problems can come from nature itself, through the implementation of bioinputs that can help reduce the use of these fertilizers and, in turn, contribute to the preservation of the environment (RODRÍGUEZ-SAHAGÚN *et al*., 2020). Corroborating the results of the present study, other studies emphasize the satisfactory effects of the use of bioinputs on the germination and initial development of plant species (OLIVEIRA *et al*., 2016; CADORE *et al*., 2020; FLORENCIO *et al*., 2022; PORTO *et al*., 2022); furthermore, the use of these bioinputs can play a crucial role in increasing crop adaptation when exposed to abiotic stresses, promoting more sustainable and productive agriculture (PORTO *et al*., 2022; OLIVEIRA *et al.,* 2024).

## CONCLUSION

Under the conditions of the test, it was possible to verify that all bioinputs, regardless of the formulation of the isolated, gave neutral results in the parameters evaluated, that is, for the germination percentage, initial development and dry mass of the shoot and root, the use of bioinputs did not have a negative effect; thus, showing prospects for future studies.

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