Initial Development of Hymeneae courbaril (Linnaeus.) (FABACEAE) under inoculation of edophytic bacteria from Bacillus and Herbaspirillum genus

Desenvolvimento inicial de Hymeneae courbaril (Linnaeus.) (FABACEAE) sob inoculação de bactérias endofíticas dos gêneros Bacillus e Herbaspirillum

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ABSTRACT
The rise of environmental problems and the need for recovery of degraded areas have caused interest in the knowledge of native Brazilian species. One of the major barriers to replanting native forests is the production of seedlings of species that can fuel reforestation programs. Hymenaea courbaril is a forest species, has great forest and environmental importance and is very suitable for plants in degraded areas, which can be restored by vegetation, recommended for silvipastoral, pasture afforestation and others. One of the major barriers to replanting native forests is the production of seedlings of species that can fuel reforestation programs. One solution is how growth promoting bacteria that are part of the plant's resident population and are not phytopathogenic. These bacteria can be used to treat micropropagated seeds, explants and seedlings incorporated into the plant substrate. Thus, due to the lack of studies with growth-promoting bacteria in tree species, the aim of this work is to evaluate or development of H. courbaril seedlings. Inoculants are inoculated with growth-promoting endophytes using biometric parameters such as plant size. collection, leaf number, plant height, root system length, biomass and dry matter of shoot and root system. In general the bacteria Herbaspirillum sp. the best result was obtained when an inoculation directly in the soil, presenting greater development of biometric characters. For the genus Bacillus sp. seed inoculation provided further development of the plants. This study contributes to future research and recommends the use of microbial agents to promote H. courbaril growth.

Keywords: Herbaspirillum sp., Bacillus sp., biometry; vegetal development, Jatobá.

RESUMO
Hymenaea courbaril é uma espécie florestal, apresenta grande importância florestal e ambiental, sendo muito indicada para plantios em áreas degradadas destinadas à restabelecimento da vegetação, recomendada para sistema silvipastoril, na arborização de pastos e dentre outros. Uma das grandes barreiras na recomposição de florestas nativas é a produção de mudas de espécies que possam abastecer programas de reflorestamento. Uma solução são as bactérias promotoras de crescimento que fazem parte da população residente das plantas, não são fitopatogênicas. Essas bactérias podem ser utilizadas para tratamento de sementes, explantes e mudas micropropagadas e incorporadas ao substrato de plantio. Dessa maneira, em virtude da carência de trabalhos com bactérias promotoras de crescimento em espécies arbóreas florestais objetivou-se por meio desse trabalho avaliar o desenvolvimento de mudas de H. courbaril inoculadas com bactérias endofíticas promotoras de crescimento utilizando parametros biométricos como diâmetro do coleto, número de folhas, altura da planta, comprimento do sistema radicular, biomassa e matéria seca da parte aérea e sistema radicular. No geral a bactéria Herbaspirillum sp. obteve melhor resultado quando a inoculação se procedeu no solo, apresentando maior desenvolvimento dos caracteres biométricos. Para o gênero Bacillus sp. a inoculação feita na semente proporcionou maior desenvolvimento das plantas. Esse estudo colabora para futuras pesquisas e recomenda-se o uso de agentes microbianos para promover o crescimento de H. courbaril.

Palavras-chave: Herbaspirillum sp., Bacillus sp., biometria, desenvolvimento vegetal, Jatobá.

1 INTRODUCTION

The rise of environmental problems and the necessity to recover degraded areas have aroused interest in the knowledge of native Brazilian species. One of the major barriers in the restoration of native forests is the production of seedlings of species that can supply reforestation programs (Moraes 1998; Carvalho 2000). The growth-promoting bacteria are part of the resident population of plants, they are not phytopathogenic.

These bacteria can be used to treat micropropagated seeds, explants and seedlings and incorporated into the planting substrate (Mariano 2004). Currently, studies are found in the literature that report the use of endophytic bacteria with vegetable species, and there is a shortage in work with forest species, thus the importance of this work in observing the interaction of these bacteria with a tree species, such as sugarcane (Silva et al. 2015), comom bean (Oliveira et al. 2018), cabbage (Araújo et al. 2019) amog others.

Hymenaea courbaril (Linnaeus) (FABACEAE) has great forest and environmental importance, being very suitable for planting in degraded areas intended for the restoration of vegetation, recommended for silvopastoral system, in the afforestation of pastures (Souza and Lima 2012), it is also considered promising for the recovery of areas with soil contaminated with heavy metals (Marques et al. 2000), in addition to having the potential to fix and store carbon (Nesi et al. 2016).

One of the difficulties in obtaining jatobá seedlings is their low percentage of germination that goes from 20 to 40 days, due to the presence of dormancy in their seeds, which can be broken by manual scarification methods on the side opposite the hilum, followed by immersion in water, (Nascimento and Oliveira 1999)

Aiming at promoting growth, there is currently a lot of research focused on the use of endophytic bacteria that comprise a group of microorganisms that can act to stimulate the growth and development of plants through direct and / or indirect mechanisms, which can be found on surfaces roots, rhizosphere and phyllosphere, and in the internal tissues of different plant species (Hungary et al. 2010) which has the ability to fix nitrogen, solubilize phosphorus (Silva et al. 2018) and or produce hormones such as auxins, gibberellins, cytokinins and ethylene (Nascimento et al. 2018), the other form of action of these bacteria is in the control of pathogens, causing growth by allowing the

plant to express its full potential, which could be limited if a pathogen was present (Baschan and De-Bashan 2005; Silva et al. 2015).

The introduction of bacteria can be done directly in the soil, as well as inside the plants (Paz 2009), or even, in the rhizoplaneum (Lemos 2009). According to Romeiro (2007), several parameters can be analyzed to evaluate growth promotion, such as percentage of seed germination, germination time, plant height, number of leaves, dry matter weight of the aerial part and roots, in addition to the weight of dry matter from the entire plant. Few studies exist with the use of these bacteria 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 Mafia et al. (2005) evaluated the effect of promoter bacteria incorporated into the substrate of clonal eucalyptus mini gardens, and observed an increase in root biomass. Thus, the objective of this study was to evaluate the development of Hymenaea courbaril seedlings inoculated with growth-promoting endophytic bacteria using biometric parameters.

2 MATERIAL AND METHODS

2.1 STUDY LOCATION

The study was carried out at in the Center for Agricultural Sciences of the Federal University of Alagoas, in Rio Largo, with latitude 9 ° 29 ’45’ ’S longitude 35 ° 49’ 54’’ W, altitude of 127 meters. The climate of the region according to Koppen is rainy tropical, with dry summer and average annual precipitation of 1,150.2 mm, with the driest months from November to December and the rainiest months from July to August.

2.2 COLLECTION OF SOIL, SEEDS AND BREAKING DORMANCY

For the production of Hymenaea courbaril seedlings, the soil used was collected at the Center for Agricultural Sciences and autoclaved at 121 °C for 2 hours, after 6 days of soil rest, the seed dormancy was broken using the mechanical scarification method in sandpaper in the region of the seed opposite the hilum, the seeds were subsequently disinfected in alcohol (70%) for 1 min, sodium hypochlorite (2%) for 1min and then three consecutive washes were made with distilled and sterile water. At the end of the process, the seeds were soaked in distilled water for 24 hours (Souza and Segato, 2016).

2.3 TREATMENTS

Four strains of bacteria were used, three of the Bacillus genus and one of the Herbaspirillum genus and all are deposited at Micoorganisms Collection of the Laboratory of Microbiology from the University. The endophytic bacteria were cultured in LB medium (Lúria-Bertani), and left in a shaker for 24 hours. After this period, the experiment was set up using polyethylene bags with a capacity of 3 liters of soil and organized innoculations forms (Table 1).

Table 1. Experimental design.

Seeds treatment			Soil treatment		
Endophyte	Treatment	Specie	Endophyte	Treatment	Specie
I4	T1	*Bacillus* sp.	I4	T5	*Bacillus* sp.
I2	T2	*Herbaspirillum* sp.	I2	T6	*Herbaspirillum* sp.
ISO34	T3	*Bacillus* sp.	ISO34	T7	*Bacillus* sp.
ISO64	T4	*Bacillus* sp.	ISO64	T8	*Bacillus* sp.

For the inoculation of the bacteria in the seeds, the bacterial solution was added to a container where the seeds were left immersed for 1 hour. For inoculation into the soil, 5 ml of the bacterial solution was injected with the aid of a disposable syringe into the experimental bags (Melo 2015). The endophytic bacterial isolates belong to and are deposited in the collection of microorganisms of the Microbiology Laboratory of the Agricultural Sciences Center of the Federal University of Alagoas.

2.4 BIOMETRIC PARAMETERS EVALUATED

The evaluations were carried out seven days after germination, being carried out with the aid of a digital caliper, and the variables evaluated were: stem diameter, number of leaves, plant height, root length, green mass (on a scale of precision) and dry matter of the aerial part and the root after 48 hours in a forced ventilation oven with a temperature of 65-70 ° C.

2.5 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experimental design was completely randomized with 5 replications and arranged in a 4x2 factorial scheme, with four endophytic bacteria and two forms of inoculation (incorporation in seed by immersion and directly on soil with aid of a syringe) and the control treatment, which did not receive inoculation. The experiments were conducted during 60 days under protected environment. The data on the biometric characteristics of the seedlings were subjected to analysis of variance (F test), at the level

of 5% probability. In the characteristics in which the effect of the treatments was observed, the Tukey test was applied at 5% probability using the Sisvar software (Ferreira 2014).

3 RESULTS

Based on the statistical analysis, for the variable colle diameter it is observed that the treatments that received the inoculation of the bacteria in the seeds by immersion present higher values regarding the biometric parameters. Within the treatments that received this inoculation, treatment 1 (inoculated with the bacteria of the genus Bacillus) shows better development in the colle diameter. In general, for this variable, the best form of inoculation was with the injection of the bacterial solution directly into the soil, with better performance for the isolate I2. For ISO64 and ISO34 isolates, the best form of inoculation was when done on seeds. Which shows that the factors have dependent interaction.

For inoculations made in the soil, treatment 8 inoculated with bacteria of the genus Bacillus stands out with higher values and differing statistically from other treatments with the same inoculation ($p \leq 0.05$), and without differing from the control.

Regarding the plant height variable, the treatment with inoculation in the soil, presents better results. Treatment 6, which received the endophytic bacteria Herbaspirillum sp., Has higher values for plant height. This same bacteria, when inoculated in the seed, presents lower values, considering that its effectiveness is better in this inoculation condition. In the inoculation made in the seed, it is observed that the isolates ISO64 and ISO34 present better performance in comparison to the others (Table 2).

Table 2. Biometric parameters in H. Courbaril seedling under inoculation of endophytic bacteria.

Inoculation	Endophyte	Plant height (cm)	Colle diameter(mm)
Soil	I2	28,46 b	3,16 b
	I4	3,10 a	0,00 a
	ISO64	22,20 ab	1,26 a
	ISO34	12,42 ab	2,66 b
Seed	I2	11,78 a	1,22 a
	I4	17,64 ab	2,10 a
	ISO64	24,34 a	2,54 b
	ISO34	24,50 a	2,38 b
Control	----------	28,10 b	3,56 b
----------		Leaf number	Root length (cm)
Soil	I2	6,80 b	38,00 b
	I4	1,00 a	7,00 a
	ISO64	5,60 ab	38,00 b

	ISO34	2,80 ab	15,00 a
Seed	I2	2,40 a	19,00 a
	I4	4,00 a	33,60 b
	ISO64	4,80 a	26,80 b
	ISO34	5,20 b	31,20 b
Control	----------	6,00 a	25,80 b

*Means followed by the same letter do not differ each others by Tukey test (p≤0,05).

For the root length variable, it is possible to observe that bacteria provide an increase in their development for some treatments. The one that received inoculation in the soil with Herbaspirillum sp. it presents greater development when compared with other endophytes in the same inoculation condition. However, this same endophytic bacteria has the opposite behavior when inoculated in the seed, with values reduced by half, where the ISO64 and ISO34 isolates, both of the Bacillus genus, stand out.

In general, Herbaspirillum sp. denotes the best result when the inoculation was carried out directly in the soil, showing greater development of the root system. For the genus Bacillus sp. inoculation in the seed provides greater growth of the root system of H. Courbaril seedlings.

Analyzing the biomass (Figure 1) and dry matter (Figure 2) variables of the aerial part and the root system, consecutivelly, it is observed that the majority of treatments have a better supply of biomass in the aerial part. For, although the root system has shown full development, it is known that biomass is influenced by the accumulation of water in the tissues, as it is also known that the roots of this species do not function as a water reservoir, but only as absorption and translocation of water and nutrients.

Fig 1. Biomass of H. courbaril under innoculation of endophytic bactéria.

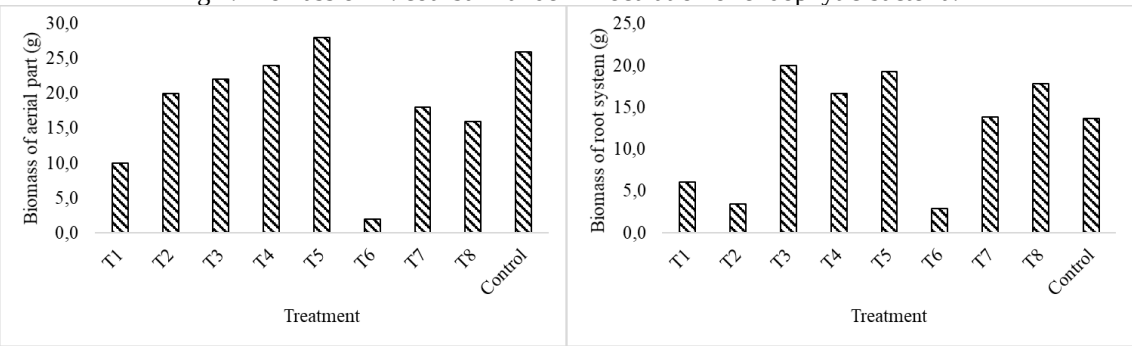
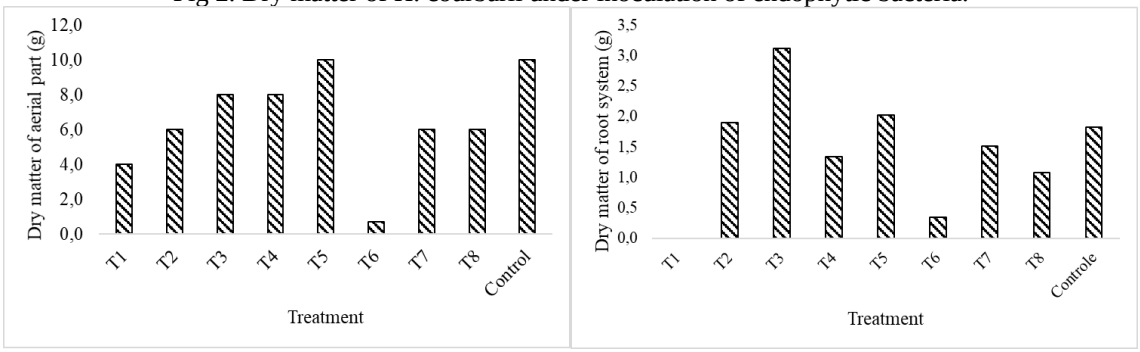


Fig 2. Dry matter of H. courbaril under inoculation of endophytic bactéria.



4 DISCUSSION

Several mechanisms of action have been suggested to explain the phenomenon of growth promotion caused by endophytic bacteria. Among the benefits promoted by them is the increase in nitrogen fixation, production of auxin, gibberellins, cytokinins and control of ethylene levels, solubilization of phosphates and sulfur oxidation, increase in the availability of nitrate, extracellular production of antibiotics, lytic enzymes and hydrocyanic acid, increased root permeability and competition for nutrients at root sites, as well as induction of systemic resistance (Silva et al. 2015; Silva et al. 2018; Nascimento et al. 2018; Araújo et al. 2019). Despite all these benefits, research on the use of growth-promoting bacteria in the forest area, when compared to the agricultural area, is practically incipient.

However, the use of these bacteria has emerged as a promising technology, being studied until then as an alternative for the addition of biomass in different forest species, which can provide average gains of 15 to 30% and, in special cases, even double the biomass produced (Chanway 1997). In this study, it was found that bacteria of the genera Bacillus and Herbaspirillum promote an increase in plant height, in addition to providing greater development of the root system, which, possibly, may be related to changes in the hormonal balance of the vegetative propagule, induced by the tested bacteria.

In this work it is observed that, the treatment that received inoculation in the soil with Herbaspirillum sp. obtained higher values for all variables. This genus is currently composed of 10 species, and depending on the strain, some species of Herbaspirillum can fix 19 to 54% of the nitrogen required by this culture (Kennedy et al. 2004). Herbaspirillum species can also contribute to plant growth through other factors, in addition to biological nitrogen fixation. In the literature there are reports of the detection of the production of AIA and gibberellins A1 and A3 (Bastián et al. 1998; Silva et al.

2015). Radwan et al. (2002) also found the production of indoles by strains of Herbaspirillum, including H. rubrisubalbicans.

The greater development of plants that received bacteria of this genus, may be related to these mechanisms, since AIA and gibberellins are hormones that promote plant growth. The same bacteria with inoculation in the seeds did not show such satisfactory results when compared to those inoculated in the soil, this may be related to the fact that H. courbaril seeds have exogenous dormancy caused by the impermeability of the tegument and may have caused difficulty for the bacteria to penetrate. inside.

The difference observed in the results between plants and bacterial isolates may be related to differences in the environment, in addition to the genetic makeup of the species under study. Many studies have reported that plant growth-promoting bacteria, especially those belonging to the Bacillus genera, proliferate not only around the root system, but can also colonize the internal tissues of various plant species (Hallmann et al. 1997).

In another study, the genus Bacillus is described as capable of acting as biocontrol agents, by inhibiting pathogenic microorganisms such as Phytophtora spp. (Silva et al. 2019), which is considered one of the growth promotion mechanisms, by suppressing the growth of the pathogen.

The data presented here are subsidies for the development of future research related to the production of seedlings of forest species, especially those that have the potential to recover degraded areas. Although studies related to plant growth promoting microorganisms have been known for decades, it is necessary to deepen studies on forest species, since these studies are concentrated on agricultural species aimed at food purposes.

5 CONCLUSIONS

The use of endophytic bacteria is an alternative in promoting growth in H. Courbaril seedlings. In this study it was possible to observe that the endophytic bacteria have specificities such as the inoculation mode, which showed that there is a dependency between the factors.

H. courbaril shows symbiosis with endophytic bactéria and it can be applicable to seedling production to reforesting programs.

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