PLANT GROWTH-PROMOTING MICROBIAL INOCULANT FOR *Schizolobium parahyba* pv. *parahyba*[1]

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ABSTRACT – *Schizolobium parahyba* pv. *amazonicum* (Huber ex Ducke) Barneby (*paricá*) occurs naturally in the Amazon and is significant commercial importance due to its rapid growth and excellent performance on cropping systems. The aim of this paper was to evaluate a microbial inoculants such as arbuscular mycorrhiza fungi (AMF) and *Rhizobium* sp. that promote plant growth. The inocula was 10 g of root colonized and spores of *Glomus clarum* and/or 1 mL of cell suspension (10^7 CFU/mL) of *Rhizobium* sp. and/or 100 g of chemical fertilizer NPK 20-05-20 per planting hole. The experimental design was complete randomized blocks with five replications and eight treatments (n = 800). Plant height, stem diameter and plant survival were measured. The results were tested for normality and homogeneity of variances and analyzed by ANOVA and Tukey test ($p < 0.05$). *Rhizobium* sp and AM fungi showed no effect on plant growth. Environmental factors probably influenced the effectiveness of symbiosis of both microorganisms and plant growth. The chemical fertilizer increased *S. parahyba* growth. During the first 120 days plants suffered with drought and frost, and at 180 days plants inoculated with microorganism plus chemical fertilizer showed higher survival when compared with control. The results showed that the microbial inoculants used showed an important role on plant survival after high stress conditions, but not in plant growth. Also was concluded that the planting time should be between November to December to avoid the presence of young plants during winter time that is dry and cold.

Keywords: *Rhizobium* sp.; *Glomus*; Rhizosphere.

INÓCULO DE MICROGANISMOS PROMOTORES DO CRESCIMENTO PARA Schizolobium parahyba var. parahyba

*RESUMO – Schizolobium parahyba var. amazonicum (Huber ex Ducke) Barneby (*paricá*) ocorre naturalmente na Amazônia e tem grande importância comercial devido ao seu rápido crescimento e excelente performance no sistema de silvicultura extensiva. O objetivo deste trabalho foi avaliar a inoculação de inoculantes microbianos como fungo micorrízico arbuscular (FMA) e* ***Rhizobium*** *sp. que promovem o crescimento da planta. O inóculo foi composto de 10 g de raízes colonizadas e esporos de* Glomus clarum *e, ou, 1 mL da suspensão de células de* ***Rhizobium*** *sp. (10^7UFC/mL) e, ou, 100 g de fertilizante químico NPK 20-05-20 por cova. O desenho experimental foi em bloco completamente ao acaso, com cinco repetições e oito tratamentos (n = 800). Foram*

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*avaliados o comprimento da Planta, o diâmetro do caule e a sobrevivência da planta. Os resultados foram testados quanto à normalidade e homogeneidade da variância e as diferenças significativas, determinadas pela ANOVA e teste de Tukey ($p < 0,05$). A inoculação do **Rhizobium** sp e **G. clarum** não tiveram diferenças significativas em relação ao crescimento das plantas-controle. Fatores ambientais provavelmente influenciaram a efetividade da simbiose de ambos os microrganismos e o crescimento da planta. O fertilizante aumentou o crescimento de **S. parahyba**. Durante os primeiros 120 dias, as plantas sofreram condições de estresse com períodos de seca e geada; aos 180 dias, as plantas inoculadas com microrganismos e adubadas tiveram maior crescimento quando comparada com o controle. Os inoculantes tiveram importação em função do controle da mortalidade das plantas durante os períodos de estresse, mas não foi observado efeito no crescimento da planta. Também, concluiu-se que o período de plantio deve ser entre novembro e dezembro, para evitar que as plantas com porte pequeno entrem no período de inverno, que é seco e frio.*

*Palavras-chave: **Rhizobium** sp.; **Glomus**; Rhizosphere.*

1. INTRODUCTION

The drastic losses of original native forests in the Northwest region of Parana state in Southern Brazil occurred along the last century during the process of colonization. From 100% of forest cover in the 19th century, only 5% of the Atlantic Forest was remnants and many reforestation projects in degraded areas were carried with native trees (IPARDES, 2010).

Leguminous plants have great potential to restore degraded areas, and in addition increase soil quality and fertility (ALVINO-RAYOL et al., 2011). *Schizolobium parahyba* (Vell.) S.F. Blake var. *parahyba* (LEWIS, 2010), commonly known as guapuruvu, is a leguminous with fast grow that is used in a reforestation project (PIETROBOM; OLIVEIRA, 2004; SEREDA et al., 2008; CALLADO; GUIMARÃES, 2010). *S. parahyba* is a pioneer tree that occurs naturally in Atlantic Forest (LORENZI, 1992) in the states of Bahia, Espírito Santo, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo (CARVALHO, 2005). This is considered an ecologically and economically important species due to its significant wood yield potential (BORTOLETTO JÚNIOR; BELINI, 2002).

The use of growth-promoters microorganism inoculum should be considered as a technological improvement of intensive forest cultivation using N-fixing bacteria and arbuscular mycorrhizal fungi (AMF) to increase wood production (SCHIAVO; MARTINS, 2003; SIVIERO et al., 2008). In the rhizosphere soil there are several species of microorganisms composing the microbial community that act in biogeochemical cycles with an important role on soil fertility and plant growth (ANDRADE, 2004; HERNANDÉZ-ORTEGA et al., 2011).

Pioneer leguminous woody plants establish symbiosis with AM fungi, which result in benefit for both, transferring P for plant and carbohydrates to fungi (ZANGARO et al., 2003). Many bacteria from rhizosphere soil can promote plant growth and named plant growth promoting rhizobacteria (PGPR) (ARTUSOON et al., 2006). In many cases, *Rhizobium* sp. strains, a symbiotic bacteria, can act as PGPR, mainly in non-nodule formation plant as *S. parahyba* (SIVIERO et al., 2008). The inoculation of legume plants with PGPR and AM fungi can increase plant growth (ABD-ALLA et al., 2000; MARIN et al., 2010), and there are several reports that show a beneficial effects in the interaction of AMF and diazotrophic bacteria (BAREA et al., 2005; RAIMAM et al.,2007; SALA et al., 2007; MIYAUCHI et al., 2008).The aim of this work was to evaluate the contribution of the microbial inoculants *Glomus clarum* and *Rhizobium* sp. in promote plant growth of *S. parahyba* under field conditions.

2. MATERIAL AND METHODS

2.1. Experimental Design

The experiment was carried out at Xambrê, PR, in southern Brazil (23° 47' 27" S and long. 53° 35' 45" W) from March to November 2011. The climate is humid subtropical, the mean of rainfall around 1.4 m year^{-1} (IAPAR, 2000) and the soil was a Rhodic Ferralsol (FAO-UNESCO, 1989).The study area (120 m X 60 m) previously was covered with *Brachiaria decumbens* and 60 days before planting was sprayed glyphosate to desiccate the grass.

The experimental design was a randomized complete block arranged in a factorial treatment combination with five replications and three factors: N-fixing bacteria

(*Rhizobium* sp.), AM fungi (*G. clarum*) and fertilize NPK (20:5:20), resulting in the following treatments: 1. Control; 2. *Rhizobium* sp.; 3. *G. clarum*; 4. Fertilizer; 5. *Rhizobium* sp. + fertilizer; 6. *Rhizobium* sp.+ *G. clarum*; 7. *G. clarum* + fertilizer; and 8. *Rhizobium* sp.+ *G. clarum* + fertilizer). Each block was composed by eight plots corresponding to the eight treatments and each plot had twenty plants arranged in spacemen of 3 m X 3 m.

2.2. Soil and plant

Soil chemical characterization was made from a composite sample collected before experiment installation at 0-20 cmof depth. The chemical soil analysis was: pH ($CaCl_2$) 4.5; Al^{3+} 0.26 $cmol_c$ dm^{-3}; H + Al 4.60 $cmol_c$ dm^{-3}; Ca^{2+} 0.86 $cmol_c$ dm^{-3}; Mg^{2+} 0.35 $cmol_c$ dm^{-3}; K^+ 0.26 $cmol_c$ dm^{-3}; P 24.0 mg dm^{-3}; C 7.37 g dm^{-3}. Seeds of *S. parahyba* were collected at the campus of the State University of Londrina, Londrina, PR, Brazil, selected, mechanically scarified and sown in tubettes in substrate Rhodic Ferralsol (FAO-UNESCO, 1989) mixed with vermiculite 4:1. After 30 days, the seedlings were taken to the experimental area and planted.

2.3. Bacteria inoculum

The bacterial strain used as inoculum was *Rhizobium* sp. isolated from nodules of *Cassia* sp. provided by our own collection. To prepare the inocula, the strain was cultivated in Petri dishes with YMA media (VINCENT, 1970) plus Congo red (0.25%) and incubated at 28 °C 48 hÉ¹. The cells were suspended in sterile saline solution (NaCl 0.85%) at 10^7 colony forming unit (CFU $mL^{É¹}$) according to $CaCO_3$ solution standard and 1 mL of bacterial suspension was dropped around the seedling when the first pair of leaves appeared.

2.4. AM Fungi inoculum

The inoculum of *G. clarum* was from our own collection and is keeping in pots with *Brachiaria decumbens*. Ten grams of crude inocula (spores, colonized root and mycelia) containing 53 spores g^{-1} of soil was added in the tubettes, after inoculation a thin layer of soil was covered (around 2 cm) and then the seed was sowed. The number of AMF spores and root colonization of *B. decumbens* which was present in the experimental area before the establishment of the experiment was determined and the number of spores and root colonization of *S. parahyba* were evaluated at the end of experiment. Treatment with chemical fertilization occurred with addition of 100 g of NPK fertilizer 20-5-20 per plant.The percentage of plants roots infected with AM fungi was estimated on stained samples (PHILLIPS; HAYMAN, 1970) by the grid-line intersect method (GIOVANETTI; MOSSE, 1980) by microscopic examination.

2.5. Data analysis

The variables evaluated were total height and survival at 30, 60, 120,180 and 240 days, and stem diameter (10 cm above the soil) at 180 and 240 days after planting seedlings. Data sets were tested for normality and homogeneity of variance and were evaluated by analyses of variance (ANOVA). The Tukey's Honest significant difference test was performed at *p* d" 0.05.

3. RESULTS

After 30 days no significative differences were observed in the variables analyzed. After 60 days and during the all experiment the plants treated with fertilizer showed higher height when compared with others treatments (Table 1).

The same results were observed in stem diameter (10 cm above the soil) after 180 and 240 days, plants treated with fertilizer showed larger stem diameter when compared with others treatments (Table 2).

Plants survival showed different response. After 30 to 120 days the survival of control plants was the same of more effective treatments, except for Rhi. After 120 days, all treatments showed differences when compared with control except for Rhi and Gc. The survival of control plant was very low after 240 days (16.7%) and plants inoculated with *Rhizobium* sp. and *G. clarum* presented 40 and 36.7% of plants survival, respectively. However, the treatment Fert showed 73.3% of survival, but differences were observed only for control, Rhi and Gc. In 180 and 240 days, the dual inoculation (Gc + Rhi) showed good response of survival against stress conditions (around 50%) as well as when the microorganisms were combined with chemical fertilizer as *Rhizobium* sp. and *G. clarum* with 50 to 60% of survival (Figure1).

During the experiment before 120 days the plants suffered a high stress conditions, first at all there was drought time for 60 days. After 120 days, in June occurred a frost for two days, in this conditions the tropical woody plant suffered an intensive stress with low temperature (Table 3).

Table 1 – Total height (cm) of *Schizolobium parahyba* pv. *parahyba* treated with AM fungi (*Glomus clarum*), PGPR *Rhizobium* sp., and fertilizer NPK 20-5-20 at 30, 60, 120, 180 and 240 days after seedling planting. (Gc) *Glomus clarum*; (Rhi) *Rhizobium* sp.; and (Fert) Fertilizer. Means in the column with the same letter are not significantly different according to Tukey test ($p< 0.05$).

Tabela 1 *– Altura total (cm) do* ***Schizolobium parahyba*** *var.* ***parahyba*** *tratada com fungo MA (****Glomusclarum****), PGPR* ***Rhizobium*** *sp. e fertilizante NPK 20-5-20 aos 30, 60, 120, 180 e 240 dias após o plantio das mudas. (Gc)* ***Glomusclarum****; (Rhi)****Rhizobium*** sp.*; e (Fert) Fertilizante. Médias nas colunas com a mesma letra não apresentam diferenças significativas pelo teste de Tukey (p<0,05).*

Treatment	30 days	60 days	120 days	180 days	240 days
Gc	23.94 a	31.15 a	37.32 a	44.51 a	78.73 a
Gc control	25.21 a	32.79 a	39.03 a	46.04 a	75.99 a
Rhi	24.20 a	32.05 a	38.21 a	42.94 a	75.27 a
Rhi control	24.96 a	31.89 a	38.14 a	47.61 a	80.01 a
Fertilizer	24.78 a	33.97 b	41.82 b	50.06 b	84.60 b
Fert. control	24.38 a	29.97 a	34.53 a	39.51 a	68.65 a
ANOVA (*p* values)					
Gc	0.0567	0.1183	0.1986	0.8616	0.3166
Rhi	0.2442	0.8804	0.9605	0.2159	0.5997
Fertilizer	0.5363	0.0005	0.0001	0.0053	0.0049
Gc*Rhi	0.5465	0.5201	0.9303	0.6658	0.8595
Gc*Fert.	0.6758	0.6316	0.9061	0.4208	0.1484
Rhi*Fert.	0.2098	0.3429	0.0825	0.1650	0.5934
Gc*Rhi*Fert.	0.6758	0.5078	0.2063	0.7645	0.8865

Table 2 – Stem diameter of *Schizolobium parahyba* pv. *parahyba* after 180 and 240 days after seedling planting. (Gc) *Glomus clarum*; (Rhi) *Rhizobium* sp.; and (Fert) Fertilizer. Means in the column with the same letter are not significantly different according to Tukey test ($p< 0.05$).

Tabela 2 *– Diâmetro do caule de* ***Schizolobium parahyba*** *var. parahyba após 180 e 240 dias após o plantio das mudas. (Gc)* ***Glomus clarum****; (Rhi)* ***Rhizobium*** sp.*; e (Fert) Fertilizante. Médias nas colunas com a mesma letra não apresentam diferenças significativas pelo teste de Tukey (p< 0,05).*

Treatment	180 days	240 days
Gc	1.22 a	2.27 a
Gc control	1.18 a	2.30 a
Rhi	1.16 a	2.18 a
Rhi control	1.24 a	2.40 a
Fertilizer	1.38 b	2.60 b
Fert. Control	1.00 a	1.90 a
ANOVA (*p* values)		
Gc	0.5537	0.7631
Rhi	0.4850	0.3496
Fertilizer	0.0005	0.0011
Gc*Rhi	0.6919	0.6312
Gc*Fert.	0.8392	0.2757
Rhi*Fert.	0.3367	0.9691
Gc*Rhi*Fert.	0.6351	0.7519

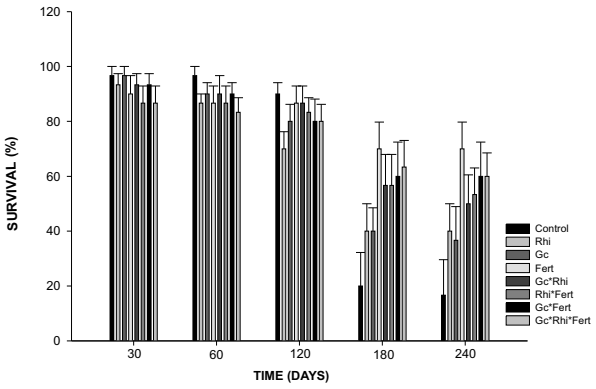

Figure 1 – Effect of AM fungi (*Glomus clarum*), PGPR *Rhizobium* sp strain, and fertilizer NPK 20-5-20 on survival (%) on *Schizolobium parahyba* pv. *parahyba* at 30, 60, 120, 180 and 240 days after seedling planting. Bars represent the standard error of mean. Gc: *Glomus clarum*; Rhi: *Rhizobium* sp; and Fert.: Fertilizer.

Figura 1 *– Efeito do fungo MA (****Glomus clarum****), da cepa PGPR de* ***Rhizobium*** *sp, e fertilizante NPK 20-5-20 na sobrevivência (%) de* ***Schizolobium parahyba*** *var.* ***parahyba****, durante 30, 60, 120, 180 e 240 dias após o plantio da muda. Barras representam o erro-padrão. Gc:* ***Glomus clarum****; Rhi:* ***Rhizobium*** *sp; e Fert.: Fertilizante.*

Table 3 –Monthly temperature and pluviometric index during the experimental time (March to November, 2011).
***Table 3** –Temperatura mensal e índice pluviométrico durante o experimento (março a novembro de 2011).*

Days	Month	Temperature (ºC)	Precipitation (m)
Planting	Mar.	20.5	1.031
30	Apr.	15.7	1.682
60	May	14.9	0.092
90	Jun.	11.0	1.389
120	Jul.	14.8	1.418
150	Aug.	15.1	1.081
180	Sep.	16.4	0.692
210	Oct.	18.4	192.0
240	Nov.	19.1	205.4

In the soil samples collected before establishment were found 28 spores g^{-1} of rhizosphere soil, and root samples of *B. decumbens* showed 83% of AM root colonization. At the end of the experiment were found 28 spores of AM fungi g-1 of rhizosphere soil from inoculated plants of *S. parahyba* and roots showed 50% of AM colonization, in non-inoculated plants were found 19 spores g^{-1} of soil and 30% of AM root colonization.

4. DISCUSSION

Plants from *Brachiaria* genus are largely used as AMF host, because grass as *B. brizantha* increase the potential of AM inocula in the soil due to its high level of colonization by AM fungi (SANTOS et al., 2000; CAPRONI et al., 2003; CORDEIRO et al., 2005; MELLO et al., 2006). The roots from *B. decumbens* showed high level of AM colonization before *S. parahyba* was planted in the experimental area.

The colonization rate of *Brachiaria* roots by native AM present in the experimental area did not influence the colonization root of *S. parahyba* by *G. clarum*, because the non-inoculated plants showed very low AM colonization when compared with inoculated plant with *G. clarum*. Is large known that our own inoculum of *G. clarum* showed high infectivity and effectivity on plant host. The performance of introduced AMF depends of many factors to compete with native AMF such as fast infectivity and establishment and ability to maintain root colonization (WILSON; TOMMERUP, 1992).

On the other hand, Siviero et al. (2008) found that *S. parahyba* pv. *amazonicum* inoculated with *G. clarum* increased plant growth in Amazon area, and agree with Santos et al. (2000) where *G. clarum* showed high level of root colonization of *Cryptomeria japonicum* and success to compete with indigenous community of AM fungi.

The low effectiveness of *G. clarum* on plant growth probably was influenced by high level of P of soil (24.0 mg dm^{-3}). Is large known that the high concentration P may influence negatively the AM fungi (BRESSAN; VASCONCELLOS, 2002; KIRIACHEK et al., 2009), also Costa et al. (2005) found that the inoculation of AMF in seedlings of *Ancornia speciosa* and *Malpighia ermaginata* increases plant growth only in soil with low P around 3.0 and 4.0 mg dm^{-3} .

G. clarum prefer pH up to 6.0 (SIQUEIRA; FRANCO, 1988), and the low pH 4.5 in the experimental soil may also influenced the establishment of the symbiosis between *G. clarum* and plant root. Also the interaction between *Rhizobium* sp. strain under field conditions is influenced by temperature, soil acidity, nutrient concentration and plant host. These factors can promote low response of inoculation, decreasing efficiency of plant to establish a symbiotic relationship with diazotrophic bacteria (MORAES et al., 2010). In spite of *Schizolobium* spp is non-nodule forming plant Siviero et al. (2008) found response of *Rhizobium* sp inoculation acting as free living bacteria in the rhizosphere of *S. parahyba* pv *amazonicum*.

The presence of *G. clarum* and *Rhizobium* sp. did not promote *S. parahyba* growth but protected against stress conditions occurred during the experiment time. After planting the seedlings suffered with dry weeks and a freeze conditions for two days. The non-inoculated plants showed a high level of mortality when compared with inoculated plants with microorganisms or fertilized. Some studies demonstrated that *S. parahyba* did not support low temperatures and high decrease plant growth or death (CARVALHO, 2003; SOUZA et al., 2011). *S. parahyba* showed sensibility for temperature variation (CALLADO; GUIMARÃES, 2010) and low capacity of osmotic adaptation when compared with *S. parahyba pv amazonicum* during water stress (CARVALHO, 2005).

The high mortality observed in non-inoculated plants suggested that low temperatures affected endogenous mycorrhizal symbiosis (HEINEMEYER;

FITTER, 2004), but not inoloculated AM fungi who might increase the tolerance of plants to drought keeping water potential gradient in the root protecting against oxidative stress increasing plant tolerance to drought (PORCEL; RUIZ-LOZANO, 2004). The effects of arbuscular mycorrhizal symbiosis on host plant tolerance against the water deficit caused by drought is related with the increase of stomatal conductance that consequently enhances the water use efficiency of plants (RUIZ-LOZANO; AROCA, 2010).

S. *parahyba* var. *parahyba* responded by chemical fertilize increased plant growth but not survival when compared with inoculated plants with *G. clarum* and/or *Rhizobium* sp. suggest that inoculation of microorganisms conferred climate stress resistance. The mortality occurred between 120 and 180 days especially in control plants, the results suggested that the nutrient deficiency associated with environmental factors caused plant stress and death.

5. CONCLUSIONS

The high level of P soil in the experimental area probably decreased the effectiveness of *G. clarum* and *Rhizobium* sp. on plant growth when compared with fertilized plants. Also the environmental conditions during winter time (dry season and freeze) increased plant mortality especially of control plants. Otherwise AM fungi and *Rhizobium* sp. protected plants against drought and freeze. Otherwise, the planting time must be between November to December to avoid the presence of young plants during winter time.

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