

BIOFERTILIZERS AND BIOCONTROLLERS AS AN ALTERNATIVE TO THE USE OF CHEMICAL FERTILIZERS AND FUNGICIDES IN THE PROPAGATION OF YERBA MATE BY MINI-CUTTINGS

Fermin Gortari^{2*}, Maximo Ivan Petruk Nowosad³, Margarita Esther Laczeski⁴, Andrea Onetto⁴, Iliana Julieta Cortese⁴, Maria Lorena Castrillo⁴, Gustavo Angel Bich⁴, Adriana Elizabeth Alvarenga⁴, Ana Clara Lopez⁴, Laura Villalba⁴, Pedro Dario Zapata⁴, Patricia Rocha³ and Fernando Niella³

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² Universidad Nacional de Misiones, Facultad de Ciencias Forestales, Argentina. E-mail: <fergortari@yahoo.es>.

³ Universidad Nacional de Misiones, Laboratorio de Propagación Vegetativa, Facultad de Ciencias Forestales, Argentina. E-mail: <maximoivanpetruk@gmail.com>, <procha910@gmail.com> and <fermandoniella@gmail.com>.

⁴ Universidad Nacional de Misiones, Instituto de Biotecnología Misiones, Facultad de Ciencias Exactas, Químicas y Naturales, Argentina. E-mail: <mlaczeski@gmail.com>, <onettoandrea@gmail.com>, <cortesejulieta@gmail.com>, <mlc_827@hotmail.com>, <gustavo_buch@hotmail.com>, <adrianaealvarenga@gmail.com>, <anaclara_lopez@yahoo.com.ar>, <lavilla65@gmail.com> and <pdr_dario@yahoo.com>.

*Corresponding author.

ABSTRACT – The production of yerba mate seedlings through seeds has several limitations, which can be overcome by *ex vitro* vegetative propagation techniques such as the mini-cuttings, in which it is usually necessary to use synthetic chemical fertilizers and fungicides. However, there is a tendency towards sustainable agriculture, using biofertilizers (growth-promoting bacteria) and biocontrollers (*Trichoderma* sp.). Therefore, the objectives of this work were to evaluate the effect of biofertilizers on the production of mini-cuttings from yerba mate mini-stumps; as well as the effect, of biocontrollers on survival and rooting capacity of mini-cuttings. Strains of *Bacillus* sp. and *Trichoderma asperelloides* of yerba mate were used under two radiation conditions. There was a positive relationship between the availability of radiation and the production of mini-cuttings and the rooting capacity. All the mini-stumps sprouted regardless of treatments. The largest production of viable mini-cuttings occurred in a situation of high radiation and fertilization; while the treatments with growth-promoting bacteria and high radiation had intermediate values. The mini-cuttings inoculated with *Trichoderma asperelloides* had higher rooting percentage, greater number and length of roots than the mini-cuttings treated with fungicide. Therefore, we demonstrated that the use of chemical products can be replaced by biological ones and achieves acceptable yields.

Keywords: *Ilex paraguariensis*; PGPR; *Trichoderma*.

BIOFERTILIZANTES E BIOCONTROLADORES COMO ALTERNATIVA AO USO DE FERTILIZANTES E FUNGICIDAS QUÍMICOS NA PROPAGAÇÃO DO ERVA-MATE POR MINIESTACAS

RESUMO – A produção de mudas de erva-mate através de sementes tem várias limitações, que podem ser superadas por técnicas de propagação vegetativa *ex vitro*, como miniestacas, nas quais geralmente é necessário o uso de fertilizantes químicos sintéticos e fungicidas. Portanto, há uma tendência para a agricultura sustentável, usando biofertilizantes (bactérias promotoras de crescimento) e biocontroladores (*Trichoderma* sp.). Portanto, os objetivos deste trabalho foram avaliar o efeito dos biofertilizantes na produção de miniestacas a partir de minicepas de erva-mate; bem como o efeito dos biocontroladores na sobrevivência e na capacidade de enraizamento de miniestacas. *Bacillus* sp. e *Trichoderma asperelloides* de erva-mate foram utilizados sob duas condições de radiação. Houve uma relação positiva entre a disponibilidade de radiação e a produção de miniestacas e a capacidade de enraizamento. Todos os minicepas brotaram independentemente dos tratamentos. A maior produção de miniestacas viáveis ocorreu em uma situação de alta radiação e fertilização; enquanto



os tratamentos com bactérias promotoras de crescimento e alta radiação apresentaram valores intermediários. As miniestacas inoculadas com *Trichoderma asperelloides* apresentaram maior porcentagem de enraizamento, maior número e longitude de raízes que as miniestacas tratadas com fungicida. Portanto, provamos que o uso de produtos químicos pode ser substituído por produtos biológicos e esse fato atingem alcança rendimentos aceitáveis.

Palavras-Chave: *Ilex paraguariensis*. PGPR. *Trichoderma*.

1. INTRODUCTION

The *Ilex* genus (Aquifoliaceae) is distributed around the world, particularly in temperate, tropical and subtropical regions. One of the most important species, within the genus, is *Ilex paraguariensis* Saint Hilaire, known as yerba mate, typical of northeastern Argentina, southern Brazil and eastern Paraguay. This species constitutes one of the main crops in the province of Misiones, with approximately 165,327 hectares cultivated (INYM, 2016).

Although yerba mate plantations are abundant in the area, the generation of seedlings through seeds has several limitations, including the reduced germination and long periods of stratification (Wendling, 2004). Consequently, vegetative propagation, through mini-stumps and mini-cuttings, allows to overcome the limitations of sexual multiplication. Plant propagation using this technology was successfully developed for this species (Nauman et al., 2017; Stuepp et al., 2017; Sá et al., 2018; Pimentel et al., 2019) and others, mainly *Pinus* sp. (Rocha and Niella, 2004; Niella et al., 2010; Majada et al., 2011; Martínez-Alonso et al., 2012) and *Eucalyptus* sp. (Wendling et al., 2003; Assis and Mafia, 2007); in which agrochemicals are applied.

Nowadays, there is a tendency towards sustainable agriculture, avoiding the indiscriminate use of chemical products. In this sense, the use of biological products that enhance growth of crops in the area is one of the most promising alternatives (Alarcón and Ferrera-Cerrato, 2012). Biofertilizers and biocontrollers are products obtained from microorganisms found in the soil that can improve the physiological response of plants. In addition, it will contribute to microorganism biodiversity conservation, and a more stable production in the long term (Adesemoye et al., 2009; Grageda-Cabrera et al., 2012; Santos et al., 2012).

Among these microorganisms are plant growth promoting bacteria (PGPR), which can stimulate the growth and development of agricultural crops due to

their activity as biofertilizers (Çakmakçı and Tingir, 2001; Glick, 2012). In the rhizosphere and roots of yerba mate a wide diversity of bacteria has been found with the capacity to promote plant growth (Bergottini et al., 2015; Bergottini et al., 2017). On the other hand, among the most widely studied species, as biocontrollers, are those of the genus *Trichoderma* due to their efficiency, reproductive capacity, ecological plasticity, stimulating effect on crops and their action as inducer of systemic resistance to different pathogens (Vega, 2001; Woo et al., 2014). They have antagonistic action against a wide range of phytopathogenic fungi, such as: *Fusarium oxysporum*, *Fusarium roseum*, *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotium* sp., *Sclerotinia* sp., *Pythium* sp., *Phytophthora* sp., *Alternaria* sp., among others (Montealegre et al., 2014; Woo et al., 2014). Although there are some examples of the use of PGPR bacteria (Teixeira et al., 2007; Peralta et al., 2012) and of *Trichoderma* sp. (Zaldúa and Sanfuentes, 2010), in the production of mini-cuttings for different *Eucalyptus* sp., there is no precedent for the use of these microorganisms in *Ilex paraguariensis* production.

The combination of modern vegetative propagation techniques associated with the application of biofertilizers and biocontrollers would allow us to obtain homogeneous plant material in a friendly environment. Therefore, the objectives of this work were to evaluate the effect of biofertilizers on the production of mini-cuttings from yerba mate mini-stumps; as well as the effect of biocontrollers on survival and rooting capacity of mini-cuttings.

2. MATERIALS AND METHODS

2.1. Biofertilizer effect on sprout capacity and mini-cuttings production from young yerba mate mini-stumps (Experiment 1).

This experiment was carried out with 6-month-old yerba mate seedlings, grown in 1-liter pots with composted pine bark substrate (110 g of substrate

per pot). The mini-stumps production and growth was developed according to Rocha et al. (2019). The nutrient source treatments were applied two weeks after the detopping of the seedlings as follow: PGPR (inoculation with the selected combination of PGPR, 3 applications of 5 ml each, every 15 days of a suspension of 1.5×10^8 CFU / ml) and Fertilization (F, slow release fertilizer application to the substrate, Plantacote® Plus 6M, 3 kg / m³). A combination of native bacteria strains belonging to *Bacillus* genus with plant growth promoting capacity isolated from yerba mate roots from the Province of Misiones and available in the internal strain repository of the Misiones Biotechnology Institute (UNaM) were used. At the same time, two radiation conditions were evaluated: low radiation (L, 25% of photosynthetically active radiation (PAR)) and high radiation (H, 50% of PAR). The measurements of PAR were made with a Ceptometer (Cavadevices) during the day and several days during the experiment for a correct characterization inside the greenhouse, over the mini-stumps, and at the same time directly to the sun. Three measurements were made at 70-day intervals (September, December and February) after decapitation, where mini-cuttings were harvested. The variables measured were sprout capacity, shoots number and number of viable mini-cuttings (diameter greater than 2mm, 4 to 6 cm long and at least two internodes) per mini-stumps. Therefore, this experiment consists of three factors: Evaluation date (September, December or February), nutrient source (F or PGPR) and radiation (L or H).

2.2. Effect on rooting capacity of mini-cuttings from yerba mate mini-stumps (experiments 2.1 and 2.2).

The rooting capacity assessment was carried out with mini-cuttings obtained from experiment 1, for the September (experiment 2.1) and February (experiment 2.2) harvests. The same treatments of experiment 1 were maintained (Nutrient source and Radiation). Though, in this case, fungal control factor with 2 levels were added. The first was the application of commercial fungicide (Z, Zineb) and the second was the inoculation with *Trichoderma asperelloides* IBM193 (T). This strain was isolated from roots of yerba mate plants in the Province of Misiones and is available in the internal strain repository of Misiones Biotechnology Institute (UNaM). This strain was selected given its biological control abilities. Three inoculations of IBM193 of 5 ml

each were performed every 15 days, from a suspension of 1.5×10^6 spores / ml. Rooting was performed under controlled conditions of humidity and temperature in greenhouse with micro-irrigation. After 60 days of installation, the following variables were evaluated: survival and rooting percentage (alive mini-cuttings with roots of at least 5 mm in length); number of roots/mini-cuttings and maximum root length/mini-cuttings (mm). These experiments consisted of three factors: nutrient source (F or PGPR), radiation (L or H) and fungal control (T or Z).

2.3. Statistical analysis.

The experiments were carried out in a completely randomized design, with a minimum of 10 replications per treatment, the experimental unit was the mini-stump or the mini-cutting. In experiment 1, ANOVA was performed considering evaluation date (September, December or February), nutrient source (F or PGPR) and radiation (L or H) as factors. Complete interactions between the three factors were analyzed. If any interaction was significant (more than two levels), means were compared by Tukey test ($p < 0.05$). In experiments 2.1 and 2.2, ANOVA was performed considering nutrient source (F or PGPR), radiation (L or H) and fungal control (T or Z) as factors. The same post hoc comparison of means was done, if the interaction was significant. Presumptions of independency, normality and variance homogeneity were proven for the variables (regrowth percentage, shoots number, viable mini-cuttings number, survival percentage, rooting percentage, roots number and roots maximum length).

3. RESULTS

Regardless of the treatments, all the mini-stumps sprouted (data non show). The number of shoots/mini-stumps, showed interaction between evaluation date and source of nutrients; September showed no significant differences between fertilizers or PGPR treatment, with 2.55 and 2.95 shoots/mini-stumps respectively. However, on the following dates, fertilizer treatment showed higher number of shoots, 3.95 shoots/mini-stumps for F and 3.25 shoots/mini-stumps for PGPR treatments in December; and 4.35 shoots/mini-stumps for F and 3.50 shoots/mini-stumps for PGPR treatments in February (Table 1, Figure 1.A). Interaction between evaluation date

Table 1 – ANOVA tests for shoots number and viable mini-cuttings number per mini-stump. Significant factors and interactions p-value are in bold letter ($p \leq 0.05$).

Tabela 1 – Teste ANOVA para número de brotes e número de mini-estacas viável por mini-cepa. p-value significativos para fatores e interações estão em negrita ($p \leq 0.05$).

ANOVA test for shoots number per mini-stump					
	SS	D. of Freedom	MS	F	p
Intercept	2473,01	1	2473,01	1902,16	$\leq 0,01$
Nutrient source (N)	7,10	1	7,10	5,46	0,02
Radiation (R)	50,02	1	50,02	38,47	$\leq 0,01$
Evaluation Date (D)	51,52	2	25,76	19,81	$\leq 0,01$
N x R	0,14	1	0,14	0,10	0,74
N x D	14,67	2	7,33	5,64	$\leq 0,01$
R x D	13,16	2	6,58	5,06	$\leq 0,01$
N x R x D	7,78	2	3,89	2,99	0,06
Error	266,52	205	1,30		

ANOVA test for viable mini-cuttings number per mini-stump					
	SS	D. of Freedom	MS	F	p
Intercept	2590.01	1	2590.01	828.62	$\leq 0,01$
Nutrient source (N)	185.41	1	185.41	59.31	$\leq 0,01$
Radiation (R)	84.21	1	84.21	26.94	$\leq 0,01$
Evaluation Date (D)	107.01	2	53.50	17.11	$\leq 0,01$
N x R	86.45	1	86.45	27.66	$\leq 0,01$
N x D	47.45	2	23.72	7.59	$\leq 0,01$
R x D	1.52	2	0.75	0.24	0.78
N x R x D	56.93	2	28.46	9.10	$\leq 0,01$
Error	637.63	204	3.12		

(Fonte: Times New Roman, 10).

and radiation was observed; shoots production was always higher at high radiation intensities. However, differences in number of shoots/mini-stumps grown at high radiation and low radiation increased depending on the evaluation dates; the difference between low and high radiation

treatments were 0.61, 0.67 and 1.69 in September, December and February respectively (Table 1, Figure 1.A).

Viable mini-cuttings production/mini-stumps was modified by triple interaction (D, N and R). The lowest production of viable mini-cuttings/

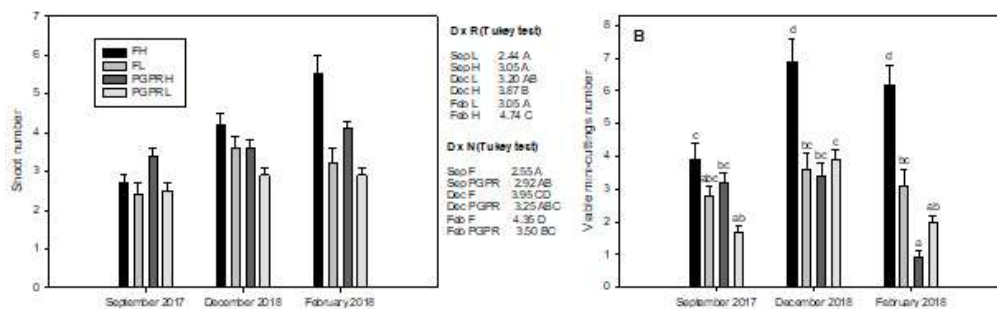


Figure 1 – Shoots number (A) and viable mini-cuttings number (B) per mini-stump. Evaluation date (D): September (Sep), December (Dec) or February (Feb); Nutrient source (N): Fertilization (F) or (PGPR); Radiation (R): high radiation (H, 50% of PAR) or low radiation (L, 25% of PAR). Tukey test is included when interactions are significant, different letters indicate significant differences.

Figura 1 – Número de brotes (A) e número de mini-estacas (B) por mini-cepa. data da avaliação (D): Setembro (Sep), Dezembro (Dec) ou Fevereiro (Feb); Fonte de nutrientes (N): Fertilização (F) ou PGPR; Radiação (R): alta radiação (H, 50% PAR) ou baixa radiação (L, 25% PAR). Teste Tukey é incluído quando as interações são significativas. Letras diferentes indicam diferenças significativas.

mini-stumps, both with low and high radiation, was observed in February with PGPR treatment, and in December with low radiation; not exceeding 2 viable mini-cuttings/ministumps. While the highest production was achieved, with fertilizer and high radiation treatments, in December and February, with 6 or more viable mini-cuttings/mini-stumps. The rest of the treatments presented intermediate values between 3 and 4 viable mini-cuttings/mini-stumps (Table 1, Figure 1.B).

Mini-cuttings survival rate for experiment 2.1 varied depending on radiation and the type of fungal control. The higher the intensity of radiation in the mini-stumps, higher the survival rate, 70% and 50% for high and low radiation respectively; and higher survival rate in fungicide-treated mini-cuttings compared to inoculated ones, 72% and 47% respectively (Table 2, Figure 2.A). However, for experiment 2.2, mini-cuttings survival rate was only modified by intensity of radiation. Mini-cuttings obtained from mini-stumps growing with high radiation presented the highest values, more than 96% compared to 70% for low radiation. There were no interactions between factors for this variable in both experiments (Table 2, Figure 2.B).

Rooting percentage for experiment 2.1 was not modified by any of the factors and there were no interactions between them (Table 2, Figure 2.C). However, in experiment 2.2 there was an effect of nutrient source and radiation. Mini-cuttings from mini-stumps inoculated with PGPR presented a value of 67% while for mini-cuttings obtained from fertilized mini-stumps was 41%. Regarding radiation, mini-cuttings from mini-stumps growing with high radiation had a value of 79%, while those from mini-stumps growing in low radiation were 30% (Table 2, Figure 2.D).

Roots number/mini-cutting variations, in experiment 2.1, were observed according to the fungal control method. Mini-cuttings inoculated with *Trichoderma asperelloides* IBM193 presented higher number of roots than those that received fungicide application, 7.8 and 4.7 respectively (Table 3, Figure 2.E). In experiment 2.2, roots number was modified by the conditions of the mini-stumps, source of nutrient and intensity of radiation. Mini-cuttings from mini-stumps growing with high radiation had greater

roots number compared to mini-cuttings obtained from mini-stumps with low radiation, 3.9 and 2.0 respectively. Also, mini-cuttings from mini-stumps inoculated with PGPR had greater number of roots than those from fertilized mini-stumps, 3.9 and 2.0 respectively (Table 3, Figure 2.F). There were no interactions between factors for this variable in both experiments (Table 3).

Maximum root length in experiment 2.1 was modified by intensity of radiation and by type of fungal control. Mini-cuttings inoculated with *Trichoderma* sp. presented a value of 1.9 mm while mini-cuttings with fungicide application approximately 1.0 mm (Table 3, Figure 2.G). In experiment 2.2 there was only effect of radiation intensity. Mini-cuttings from mini-stumps growing at greater intensity showed higher values of root length than those from lower radiation, 1.2 mm and 0.5 mm respectively (Table 3, Figure 2.H). There were no interactions between factors for this variable in both experiments (Table 3).

4. DISCUSSION

Yerba mate vegetative propagation is a technique that will help to mitigate problems regarding sexual propagation. Nevertheless, it is still necessary to adjust protocols for its application on a commercial scale, taking into account certain limitations such as environmental management and nutrition (Wendling et al., 2007). However, its technical viability has been demonstrated, with an average production of 291 mini-cuttings/m² of garden, 95% survival of mini-stumps and 85% of mini-cuttings rooting capacity (Wendling et al., 2007). In our experiment, all yerba mate mini-stumps sprouted, in accordance with results observed for this species in similar systems in Brazil (Wendling et al., 2010; Pimentel et al., 2019).

Production of viable mini-cuttings per *I. paraguariensis* mini-stumps varies depending on the mini-garden system adopted (Wendling et al., 2010). For our system, viable mini-cuttings production varied depending on date of collection, source of nutrients and radiation level, with interaction between three factors. The highest production was observed in December and February for fertilization and high radiation treatments with more than 6 viable mini-cuttings/mini-stumps; while the lowest production was between 1-2 viable mini-cuttings for treatments

Table 2 – ANOVA tests for survival percentage and rooting percentage for mini-cuttings in experiments 2.1 and 2.2. Significant factors and interactions p-value are in bold letter ($p \leq 0.05$).

Tabela 2 – Teste ANOVA para porcentagem de sobrevivência e porcentagem de enraizamento para as mini-estacas nos experimentos 2.1 e 2.2. p-value significativos para fatores e interações estão em negrito ($p \leq 0.05$).

ANOVA test for survival percentage in experiment 2.1					
	SS	D. of Freedom	MS	F	p
Intercept	36.86	1	36.86	170.91	≤ 0.01
Nutrient source (N)	0.15	1	0.15	0.73	0.39
Radiation (R)	1.06	1	1.06	4.94	0.02
Fungal control (C)	1.65	1	1.65	7.67	≤ 0.01
N x R	0.13	1	0.13	0.60	0.43
N x C	0.75	1	0.75	3.50	0.06
R x C	0.60	1	0.60	2.81	0.09
N x R x C	0.82	1	0.82	3.80	0.06
Error	22.85	106	0.21		

ANOVA test for survival percentage in experiment 2.2					
	SS	D. of Freedom	MS	F	p
Intercept	72.34	1	72.34	537.67	≤ 0.01
Nutrient source (N)	0.52	1	0.52	3.89	0.06
Radiation (R)	3.96	1	3.96	29.45	≤ 0.01
Fungal control (C)	0.29	1	0.29	2.19	0.14
N x R	0.13	1	0.13	0.97	0.32
N x C	0.13	1	0.13	0.97	0.32
R x C	0.29	1	0.29	2.20	0.14
N x R x C	0.14	1	0.14	0.97	0.33
Error	14.80	110	0.13		

ANOVA test for rooting percentage in experiment 2.1					
	SS	D. of Freedom	MS	F	p
Intercept	10.40	1	10.40	49.20	≤ 0.01
Nutrient source (N)	0.20	1	0.20	0.97	0.32
Radiation (R)	0.69	1	0.69	3.28	0.07
Fungal control (C)	0.70	1	0.69	3.29	0.07
N x R	0.65	1	0.65	3.14	0.08
N x C	0.01	1	0.01	0.01	0.90
R x C	0.10	1	0.10	0.50	0.47
N x R x C	0.03	1	0.03	0.18	0.66
Error	22.41	106	0.21		

ANOVA test for rooting percentage in experiment 2.2					
	SS	D. of Freedom	MS	F	p
Intercept	35.43	1	35.43	203.24	≤ 0.01
Nutrient source (N)	2.04	1	2.04	11.70	≤ 0.01
Radiation (R)	7.26	1	7.26	41.66	≤ 0.01
Fungal control (C)	0.12	1	0.12	0.72	0.39
N x R	0.14	1	0.14	0.84	0.36
N x C	0.03	1	0.03	0.17	0.67
R x C	0.53	1	0.53	3.05	0.80
N x R x C	0.03	1	0.03	0.20	0.65
Error	19.17	110	0.17		

(Fonte: Times New Roman, 10).

inoculated with PGPR and low radiation (Table 1, Figure 1.B). The production values obtained for fertilization and high radiation treatments are similar

to those obtained in Colombo (Brazil), although in that case there were 6 collections dates (Wendling et al., 2010).

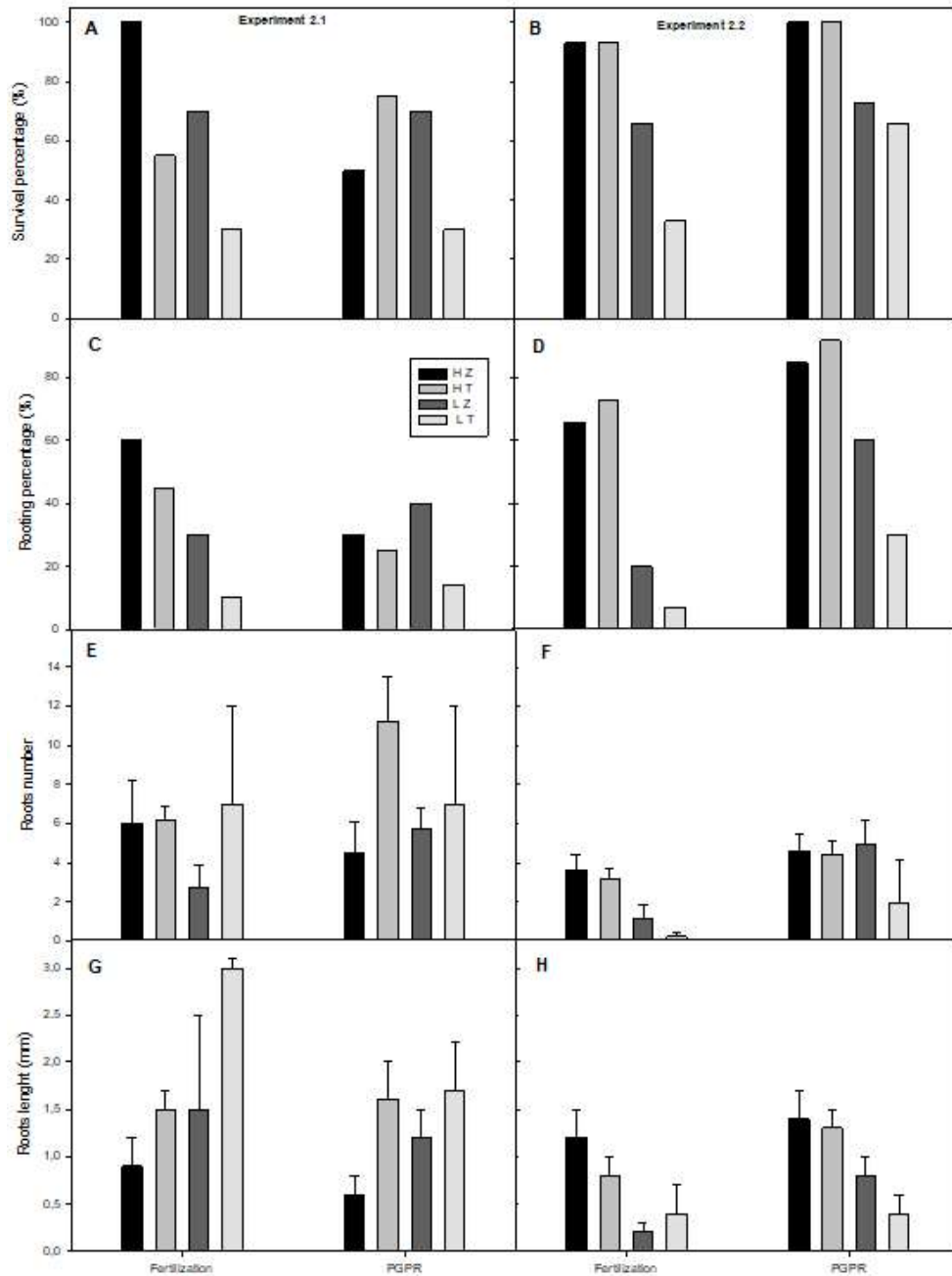


Figure 2 – Survival percentage (%), rooting percentage (%), roots number and roots length (mm) per mini-cutting in experiment 2.1 (September 2017; A, C, E y G) and experiment 2.2 (February 2018; B, D, F y H). Fertilization or inoculation (PGPR); high radiation (H, 50% of PAR) or low radiation (L, 25% of PAR); Zineb application (Z) or Trichoderma sp. inoculation (T).

Figura 2 – Porcentagem de sobrevivência (%), porcentagem de enraizamento (%), número de raízes e longitude de raízes (mm) por mini-cepas nos experimento 2.1 (Setembro 2017; A, C, E e G) e experimento 2.2 (Fevereiro 2018; B, D, F e H). Fertilização ou inoculação (PGPR); alta radiação (H, 50% PAR) ou baixa radiação (L, 25% PAR); aplicação zineb (Z) ou inoculação com Trichoderma sp. (T).

Table 3 – ANOVA tests for roots number and roots length per mother plant. Significant factors and interactions p-value are in bold letter ($p \leq 0.05$).

Tabela 3 – TESTE ANOVA PARA NÚMERO E LONGITUDE DE RAÍZES AS MINI-ESTACAS NOS EXPERIMENTOS 2.1 E 2.2. P-VALUE SIGNIFICATIVOS PARA FATORES E INTERAÇÕES ESTÃO EM NEGRITA ($P \leq 0.05$).

ANOVA test for roots number in experiment 2.1					
	SS	D. of Freedom	MS	F	p
Intercept	1562.76	1	1562.76	93.76	≤ 0.01
Nutrient source (N)	38.08	1	38.08	2.28	0.13
Radiation (R)	26.28	1	26.28	1.57	0.21
Fungal control (C)	83.60	1	83.60	5.01	0.03
N x R	0.15	1	0.15	0.01	0.92
N x C	13.16	1	13.16	0.78	0.37
R x C	3.17	1	3.17	0.19	0.66
N x R x C	48.64	1	48.64	2.91	0.10
Error	683.36	73	9.36		
ANOVA test for roots number in experiment 2.2					
	SS	D. of Freedom	MS	F	p
Intercept	739.58	1	739.58	80.77	≤ 0.01
Nutrient source (N)	78.77	1	78.77	8.54	≤ 0.01
Radiation (R)	77.29	1	77.29	8.44	≤ 0.01
Fungal control (C)	27.52	1	27.52	3.01	0.08
N x R	13.37	1	13.37	1.46	0.23
N x C	4.28	1	4.28	0.46	0.49
R x C	13.14	1	13.14	1.43	0.23
N x R x C	7.38	1	7.38	0.80	0.37
Error	769.08	84	9.15		
ANOVA test for roots length in experiment 2.1					
	SS	D. of Freedom	MS	F	p
Intercept	67.30	1	67.30	73.39	≤ 0.01
Nutrient source (N)	1.09	1	1.09	1.18	0.27
Radiation (R)	5.63	1	5.63	6.14	≤ 0.01
Fungal control (C)	7.36	1	7.36	8.02	≤ 0.01
N x R	1.04	1	1.04	1.13	0.29
N x C	0.01	1	0.01	0.01	0.90
R x C	0.60	1	0.60	0.66	0.41
N x R x C	1.02	1	1.02	1.12	0.29
Error	66.95	73	0.91		
ANOVA test for roots length in experiment 2.2					
	SS	D. of Freedom	MS	F	p
Intercept	52.63	1	52.63	80.33	≤ 0.01
Nutrient source (N)	2.53	1	2.53	3.86	0.52
Radiation (R)	11.52	1	11.52	17.56	≤ 0.01
Fungal control (C)	0.70	1	0.70	1.07	0.30
N x R	0.02	1	0.02	0.01	0.95
N x C	0.20	1	0.20	0.31	0.57
R x C	0.06	1	0.06	0.09	0.76
N x R x C	0.53	1	0.53	0.81	0.37
Error	55.04	84	0.65		

(Fonte: Times New Roman, 10)

Fertilization affects production of mini-cuttings, greater nitrogen availability is associated with greater production (Martínez-Alonso et al., 2012). Although

composted pine bark is widely used as substrate in forest nurseries in Misiones province, due to its abundance and low price, it is characterized by high

cation exchange capacity and mainly low nitrogen concentration, making fertilization necessary (Jerez, 2007). The traditional way is the application of chemical fertilizers; however, use of PGPR bacteria is possible. One of the physiological mechanisms that might explain the positive interaction between PGPR and plants is the fact that microorganisms improve the nutritional status of plants. The nutrients directly involved, could be explained by nitrogen fixation, iron chelation by microorganism releasing substances that made it available to the roots (Pii et al., 2015), and the release of organic acids that solubilize phosphorus (Know et al., 2011). However, the positive effect of PGPR may also be due to the release of auxins and other hormones (Ramos et al., 2003) that stimulate the proliferation of fine roots, and consequently the ability to absorb nutrients present in the soil (Domínguez-Núñez et al., 2012). In experiment 1, inoculated mini-stumps produced low or intermediate values of viable mini-cuttings compared to chemical fertilization. The mechanisms previously described, in the present work, could only be related to nitrogen fixation and hormone production, due to the substrate (composted pine bark) used for the mini-stumps production.

The other factor analyzed in experiment 1 was the intensity of radiation received by mini-stumps. Yerba mate is a plant that grows under canopy in natural conditions (Eibl et al., 2000), where intensity of radiation is regulated by the upper canopy. Therefore, under direct sunlight, the excess of radiation, being unable to dissipate it, causes photoinhibition of photosystem II and the consequent loss of growth and yield (Nishiyama and Murata, 2014). The greatest development of leaves and accumulation of biomass in yerba mate plants occurs when radiation is 50% of direct radiation, being less under full sun or very low radiation intensities (Sansberro et al., 2002; Sansberro et al., 2004). Our results agree with this idea, the highest values of production of viable mini-cuttings were produced in high intensity radiation treatments, which in our case corresponded to 50% of direct solar radiation (Table 1, Figure 1.B).

At the same time, radiation intensity was also the main factor that influenced the performance of the mini-cuttings; higher survival rates, rooting percentages, roots number and roots length were achieved in high radiation treatments (Table 2 and 3, Figure 2). In *Corylus avellanda* mini-cuttings, survival

and rooting are positively affected by availability of radiation, whereas higher the available radiation, greater the concentration of carbon reserves in stems (Tombesi et al., 2015).

Optimal conditions for mini-cuttings growth are characterized by high relative humidity and high temperature (Zaldúa and Sanfuentes, 2010); conditions that also favor diseases development, making necessary to control or prevent such development. When we compared use of chemical fungicide and inoculation with *Trichoderma asperelloides* IBM193, differences in mini-cuttings survival were slightly higher in fungicide treatment in experiment 2.1. Meanwhile in experiment 2.2, there were no differences between treatments, only higher percentage of rooting was observed (Table 2, Figure 2). We do not strictly measure diseases incidence, but higher mini-cuttings survival could be related to a better phytosanitary status. On the other hand, inoculation of IBM193 strain has a positive effect on mini-cuttings rooting (number and length of roots). Inoculation of mini-cuttings of *Eucalyptus globulus* with strains of *Trichoderma* sp. and *Clonostachys* sp. reduced infection levels of *Botrytis cinerea* and at the same time, increased rooting percentage (Zaldúa and Sanfuentes, 2010). In mini-cuttings of *Passiflora edulis* it was demonstrated that the application of *Trichoderma* sp. increased root production and that this increase was strongly influenced by the inoculation form (Pereira, 2012). This radical stimulation is possibly due to the ability of *Trichoderma* sp. to produce auxins or their precursors (López-Bucio et al., 2015). The application of PGPR could enhance rooting in addition to its proven capacity to stimulate plant growth, also associated with the production of auxins (Teixeira et al., 2007).

5. CONCLUSIONS

We have studied yerba mate vegetative propagation by mini-stumps and mini-cuttings propagation system, under conditions of conventional use of fertilizers and fungicides; and under the paradigm of a more sustainable and environmentally friendly production, using biological products (PGPR and *Trichoderma* sp.); and two radiation conditions.

There is a positive relationship between availability of radiation and production of viable mini-cuttings as well as rooting capacity.

The highest production of viable mini-cuttings was achieved in fertilization treatments compared to inoculated with PGPR ones. However, treatments inoculated with PGPR give acceptable yields.

In the comparison of fungicide and inoculation with *Trichoderma* sp. treatments, there were no differences, or they were minimal. Differences on rooting percentage, roots number and roots length/mini-cutting were greater in inoculated plants.

Therefore, it is demonstrated that the use of chemical products can be replaced by biological ones and achieve acceptable yields.

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