ROOTING POTENTIAL OF *ILEX PARAGUARIENSIS* CUTTINGS OF TWO POPULATIONS OF SOUTHERN BRAZIL IN TWO ROOTING ENVIRONMENTS

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Resumo

Potencial de enraizamento de estacas de Ilex paraguariensis de duas populações do Sul do Brasil em dois ambientes de enraizamento. O sucesso da estaquia de *Ilex paraguariensis* pode ser afetada pela população de origem, assim como o genótipo e o ambiente de enraizamento. Desta forma, o objetivo desta pesquisa foi testar a propagação vegetativa de *I. paraguariensis* por estaquia em genótipos de duas populações inseridos em dois ambientes de enraizamento. As populações testadas foram Três Barras (TB) e Urupema (UR), ambas do estado de Santa Catarina, selecionando sete genótipos de cada local. Após a coleta e estaqueamento, as populações e genótipos foram igualmente distribuídas em dois ambientes de enraizamento, um estufim (MT) com irrigação por microaspersão e outro em uma estufa com nebulização intermitente (GWIN) e controle de temperatura e umidade. A sobrevivência foi consideravelmente baixa para ambas populações, mas com resultados singulares, em que TB obteve melhor adaptação ao GWIN (36,5%) e UR para o MT (22,6%). O enraizamento de estacas variou grandemente em todas as perspectivas. A população TB obteve os melhores resultados de enraizamento em GWIN (21,7%), enquanto UR não obteve nem 5% de enraizamento no MT (2,4%) ou GWIN (3,2%). Os genótipos TB6, TB3 e TB1 apresentaram os melhores resultados de enraizamento para TB em GWIN (todos acima de 30,0%), enquanto somente UR3 apresentou resultados significativos em GWIN (18,8%). Estes resultados, principalmente quanto à sobrevivência e enraizamento, confirmam que há uma forte relação envolvendo o local de origem do material, os indivíduos dentro de uma população e os ambientes de enraizamento.
Palavras-chave: erva-mate, propagação vegetativa, ambiente de enraizamento.

Abstract

The success of *Ilex paraguariensis* cutting can be affected by the population of origin, as well as the genotype and the rooting environment. Therefore, the objective of this search was to test the vegetative propagation of *I. paraguariensis* cuttings from two populations and their genotypes inserted in two rooting environments. The populations tested were from Três Barras (TB) and Urupema (UR), both from Santa Catarina state, by selecting seven genotypes in each. After collecting and cutting, the populations and genotypes were evenly distributed in two rooting environments, one being a mini tunnel (MT) with irrigation by micro sprinklers and the other in a greenhouse with intermittent nebulization (GWIN) with temperature and humidity control. Survival was considerably low for both populations but with singular results, as TB was better adapted to GWIN (36.5%) and UR to MT (22.6%). The rooting of cuttings varied greatly in all perspectives. The TB population obtained the best rooting results inside GWIN (21.7%), while UR did not achieve 5% of rooting in MT (2.4%) nor in GWIN (3.2%). The genotypes TB6, TB3 and TB1 obtained the best rooting results for TB in GWIN (all above 30.0%), while only UR3 obtained significant results in GWIN (18.8%). These results, mainly regarding survival and rooting, confirm that there is a strong relation involving the origin place of the material, the individuals of a population and the rooting environments.
Keyword: mate herb, vegetative propagation, rooting environment.

INTRODUCTION

Ilex paraguariensis Saint-Hilaire is a South American tree species of great economic importance in Southern Brazil, as it is vastly produced, exported and consumed in this region. However, the total production of this species have been undergoing fluctuations in relation to past years, approximately 5.5% less, comparing the last couple of years. Furthermore, the total value has also decreased in almost 17.0% in this comparison (IBGE, 2021). Aside from market variables, several plant factors could have been promoting this decrease, such as excessive exploration, an elevated degree of maturation, the depletion of energetic resources and genetic heterogeneity between individuals (XAVIER *et al.*, 2013; WENDLING & BRONDANI, 2015).

A logical solution to these difficulties resides on the establishment of new and selected juvenile plants, aiming on not only maintaining productivity, but also improving it. Thus, the appropriate selection of individuals is of extreme importance, an activity that can be exceedingly difficult due to two main situations: the use of seeds to produce seedlings and the genetic heterogeneity present on them. Firstly, *I. paraguariensis* seeds are known for having germination difficulties, specially due to the presence of dormancies, mainly physical and morphological, as well as the possibility of physiological, and a great presence of empty seeds (SOUZA *et al.*, 2020), forcing the use of seeds with unknown genetic quality. Moreover, the genetic heterogeneity itself, caused by sexual breeding, which is beneficial for the processes of natural selection, can often hamper *I. paraguariensis* vegetative improvement protocols (WENDLING & BRONANDI, 2015). The possible solution to the production of high quality seedlings may relies on the vegetative propagation of this species through direct selection of superior materials (VIEIRA *et al.*, 2021).

A great number of searches regarding the vegetative improvement of this species have been performed in the last years, mostly taking the vegetative propagation as the main technic for this objective. However, there are still many challenges regarding this technic, mainly aiming to overcome the ontogenetic age (WENDLING *et al.*, 2013; STUEPP *et al.*, 2015; 2017a, 2017b; NASCIMENTO *et al.*, 2018) as well as regarding the nutrition of the mother tree (NASCIMENTO *et al.*, 2019), the use of growth regulators (BITENCOURT *et al.*, 2009; NAGAOKA *et al.*, 2013; PIMENTEL *et al.*, 2019; NASCIMENTO *et al.*, 2020), testing established clones (BRONDANI *et al.*, 2008; 2009; BISOGNIN *et al*., 2018; PIMENTEL *et al.*, 2019; GAZZANA *et al*., 2020; VIEIRA *et al.*, 2021) and rooting environments (BRONDANI *et al.*, 2008; 2009; NASCIMENTO *et al.*, 2020). In this manner, it is important to take in consideration the possibility of different vegetative propagation potentials of *I. paraguariensis* from contrasting natural populations, whereas specific conditions such as maturation, for example, may not be a determinant factor. Therefore, the vegetative rescue step of this species at natural environments, with the correct selection of individuals, might be also a determinant factor on the success of the vegetative propagation.

Different populations of this species may possess unique individuals with singular characteristics (WENDLING & BRONANDI, 2015; VIEIRA *et al.*, 2021), which is a result of a long-term process of natural selection over unique environments. There might be not only differences between populations, but also at individual level inside the same population, whereas the genotypes can take an important part in the success of rooting of cuttings (XAVIER *et al.*, 2013; NASCIMENTO *et al.*, 2020). In a correlation of importance, the materials may have different responses to different rooting environments, taking into consideration singular structures of different technological advancements (BRONDANI *et al.*, 2008; 2009). Additionally and in contrast, it is possible for some genotypes to present better adaptation beyond these individual circumstances, in a correlation between both material origin and rooting environment (NASCIMENTO *et al.*, 2020).

The objective of this search was to evaluate *I. paraguariensis* rooting of cuttings, comparing seven different genotypes of two populations, totaling fourteen individuals, put to root in two rooting environments under different technological capacities. Thus, this work was conducted hypothesizing that rooting is dependent not only at population level, but also at genotype level, which can be improved or hampered according to variables such as temperature and humidity of the rooting environment.

MATERIALS AND METHODS

Vegetative material of *I. paraguariensis* was collected (September 2020) from two different regions of Santa Catarina State, in Southern Brazil. Both of these locations belonged to private native production areas, being: Três Barras (TB) (26°12’19” S; 50°21’62” W) and Urupema (UR) (27°96’52” S; 49°83’92” W). All locations belong to a *Cfb* climate with well-distributed rainfall throughout the year. TB has an annual average temperature of 17.0 °C, while UR is known for its low temperatures during winter, with an annual average of 14.1 °C.

The mother plants were randomly selected according to its sanity and phenotypic similarity, totaling seven individuals in each location. The individuals were chosen with at least 50.0 m apart from each other, aiming to ensure greater genetic diversity. As a criterion for consideration of the native area, only mother plants under active shading from other plants species were chosen, such as *Araucaria angustifolia* (Bertol.) Kuntze. Mother plants with similar diameters were selected to reduce the effect of physiological age. The averages of diameter at chest height (DCH) obtained were 7.5 cm (± 1.4 cm) for TB and 6.8 (± 1.1 cm) cm for UR.

Canopy sprouts produced in the year of seven mother plants in each location were collected after their selection. Therefore, 14 genotypes were obtained, seven from TB and seven from UR. The sprouts were collected with pruning shears, always above 1.30 m (DCH) and stored in styrofoam with cooled water to avoid its excessive dehydration during the transport to the Forest Nursery at the University of the State of Santa Catarina (UDESC) in Lages (SC). The transportation process occurred immediately after the material collection, whereas the trip took approximately four hours for TB and less than one hour for UR.

Right after the transportation, the sprouts were cut into cuttings (approximately 7 cm long) with at least one leaf with a reduced area (50%) to avoid its excessive transpiration. Afterwards, the cutting were put to root into 110 cm^3 tubes containing commercial substrate (mixture of turf, vermiculite, class "A" organic waste and limestone; pH 5.5; density 130.0 kg/m^3) with the addition of 6 g L^{-1} of controlled release fertilizer in the proportion 15-9-12 (NPK). The purpose of the controlled release fertilizer was to guarantee the minimum nutritional values for effectively rooted cuttings.

The trays containing the tubes and cuttings were conditioned into two different rooting environments (Figure 1), being: a mini tunnel (MT) and a greenhouse with intermittent nebulization (GWIN). MT is a simplified structure inserted in a shade house, located in the Forest Nursery of UDESC, in Lages (SC), with automatic irrigation by micro sprinklers composed of four daily irrigations (9:00 AM; 12:00 AM; 3:00 PM; 6:00 PM) of ten minutes each (PEREIRA *et al.*, 2019). GWIN is a more technological structure belonging to a forestry company, in the municipality of Otacílio Costa (SC), with automatic temperature control (between 20 °C and 30 °C) by thermostat and humidity (always above 70.0%) (NASCIMENTO *et al.*, 2020).

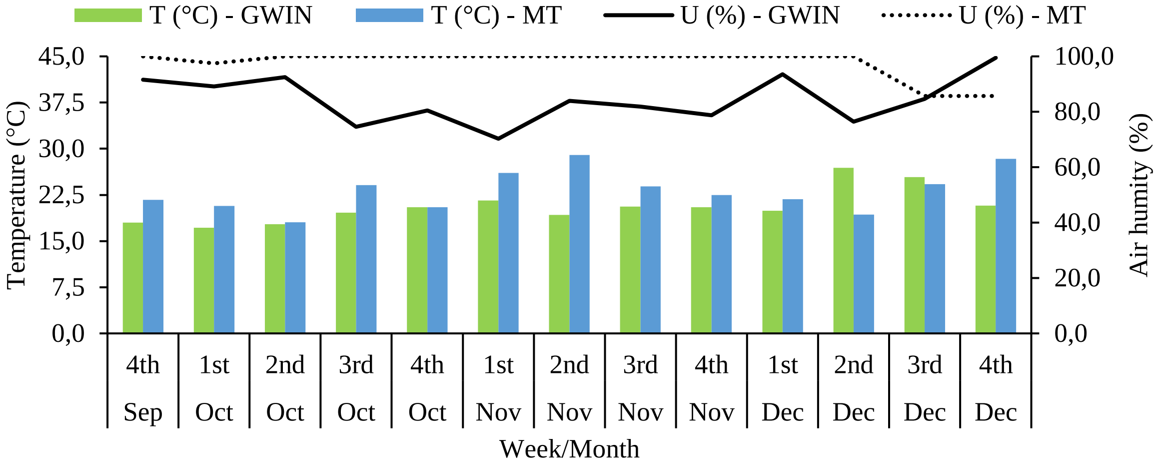


Figure 1. Weekly data of temperature (°C) and air relative humidity (%) inside GWIN and MT for the duration of the experiment of *I. paraguariensis* cuttings.

Figura 1. Dados semanais de temperatura (°C) e umidade relativa do ar (%) dentro de GWIN e MT para a duração do experimento de estaquia de *I. paraguariensis*.

After 100 days (December 2020) the survival (%), callus (%), rooting (%), average number of roots and average length of roots (cm) were evaluated from the cuttings. The percentage of all variables was evaluated based on the total cuttings quantity. Survivors were cuttings with live wood, old leaves or young sprouts, rooted or not. For calluses, were considered alive cuttings with undifferentiated cell mass at the base, rooted or not. Those with induction of root primordia of at least 5.0 mm in length were considered as rooted. Cuttings with new sprouts were those with the presence of new leaves after the process of cutting. The original leaves (the ones kept during the process of cutting) were evaluated regarding if they were still present on the cuttings at the evaluation moment.

The experiment was conducted in a completely randomized design with three different factorial arrangements. The first one being: 2x2, with factor A consisting of two populations, composed of seven replicates of 48 cuttings each, and B the two rooting environments. As for the second and third: 7x2, with factor A consisting of the seven genotypes for each population, composed of four replicates of 12 cuttings each, and B the two rooting environments. Therefore, three experiments were evaluated: i) the cutting of two populations (TB and UR) according to two rooting environments (MT and GWIN); ii) the cutting of seven genotypes from TB in the same two rooting environments, and; iii) the cutting of seven genotypes from UR in the same two rooting environments.

Due to the occurrence of non-normality, the data were firstly transformed through the application of boxcox. After confirming normality (Shapiro-Wilk test; $P > 0.05$), the data were submitted to ANOVA ($P < 0.05$), followed by the Scott-Knott test ($P < 0.05$). The statistics was performed using the R statistical software (R CORE TEAM, 2020). It was also performed an Experimental Accuracy (EA%) calculation for significant treatments by ANOVA. This aims to evaluate the degree of confidence in the experiment, observing values for selection purposes, considering the ranges: less than or equal to 50.0% low; between 50.0% and 70.0% moderate; between

70.0% and 90.0% high and; greater than 90.0% very high (RESENDE & DUARTE, 2007; NAVROSKI *et al.*, 2013), being evaluated by the formula:

$$Experimental\ Accuracy\ (\%) = \sqrt{1 - \left(\frac{1}{F}\right)} \times 100$$

For: F = F value obtained in the ratio of the mean squares of the treatment and waste from analysis of variance (ANOVA).

RESULTS

Considering only the populations and the rooting environments, all variables achieved factorial significance between these treatments with a very high EA% condition (> 90.0%) (Table 1). As for the genotypes, the populations tested obtained different relations for the vegetative variables. For TB, only the survival, rooting and number of roots were factorial significant, with EA% varying between high (> 70.0% ≤ 90.0%) and very high. For UR, only the survival and callus obtained the same factorial significance with a very high EA%.

Table 1. Statistical factors evaluated in ANOVA for the vegetative variables of *I. paraguariensis* cutting according to two populations and their genotypes in two rooting environments.
Tabela 1. Fatores estatísticos avaliados na ANOVA para as variáveis vegetativas para a estaquia de *I. paraguariensis* de acordo com duas populações e seus genótipos em dois ambientes de enraizamento.

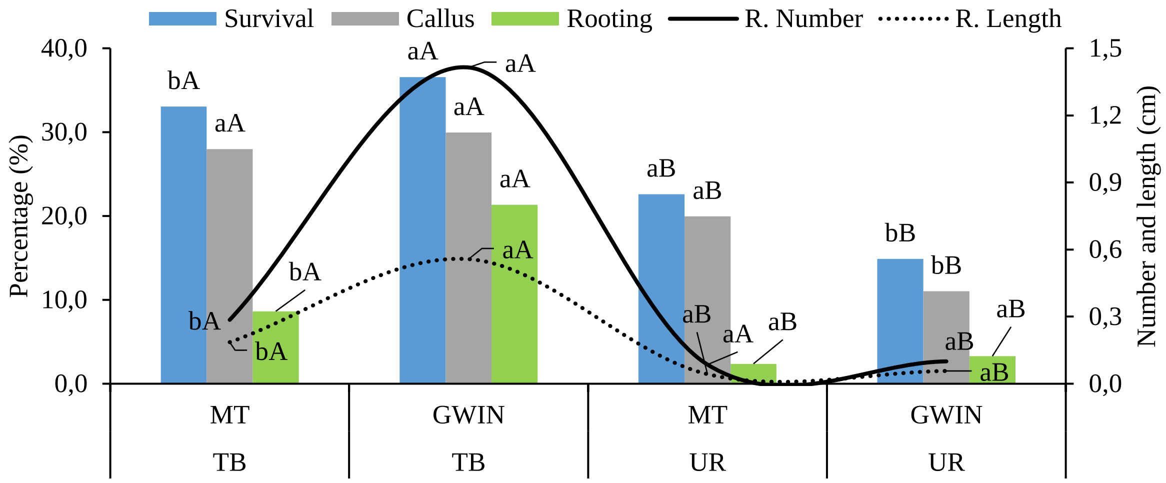
Source	Factor	Survival	Callus	Rooting	Roots number	Roots length
Pop.	PF	< 0.0001	0.0001	< 0.0001	< 0.0001	0.0001
	PP	-	-	-	-	-
	PE	-	-	-	-	-
	F	35.46	16.9	26.22	35.97	17.33
	EA%	98.6	97.0	98.1	98.6	97.1
TB	PF	< 0.0001	0.4222	0.0022	0.0016	0.1362
	PG	-	< 0.0001	-	-	0.0011
	PE	-	0.3546	-	-	0.0001
	F	8.45	NS	4.17	4.36	NS
	EA%	93.9	-	87.2	87.8	-
UR	PF	< 0.0001	< 0.0001	0.6489	0.3037	0.2862
	PG	-	-	< 0.0001	< 0.0001	< 0.0001
	PE	-	-	0.5199	0.4677	0.4074
	F	7.12	7.29	NS	NS	NS
	EA%	92.7	92.9	-	-	-

Considered significance for ANOVA of 0.05; PF: P value for the factorial interaction between treatments; PP: P value for the population treatment; PE: P value for the environment treatment; PG: P value for the genotype treatment; F: F value considering only the factorial interaction; EA%: Experimental Accuracy (%) considering F; NS: non-significant.

In a general perspective of the populations (Figure 2), the survival of cuttings was better for TB than UR, but the rooting environments acted differently on each population. The best results for TB were obtained in GWIN (36.5%), and even though MT achieved approximate results (33.0%), there was not statistical equality. As for UR, it obtained considerable results in MT (22.6%), while in GWIN the result was almost 8.0% lower (14.9%).

Callus formation was proportional to survival for all populations, regardless of the environment (between 3.0% and 5.0% less than survival). The highest callus rate was found by TB in GWIN (29.9%), followed by TB in MT (27.8%), UR in MT (19.9%) and UR in GWIN (11.0%).

Rooting was superior in TB, especially inside GWIN (21.3%), almost three times when compared to MT (8.6%). UR obtained low percentages of rooting (less than 3.5%) independently the environment. The variables of average root number and length followed the same perspective as rooting, with TB inside GWIN obtaining the best results (1.4 roots of 0.6 cm), with more than four times the number of roots and three times the length when compared to MT (0.3 roots and 0.2 cm). As for UR, due to the low rooting, all the variables associated obtained values lower than 0.1.



Averages followed by the same letter do not differ according to the Scott-Knott average test (P < 0.05). Lowercase letters for populations and uppercase letter for rooting environments.

Figure 2. Survival (%), callus formation (%), rooting (%), average root number and average root length (cm) of *I. paraguariensis* cuttings according to two populations in two rooting environments.

Figura 2. Sobrevivência (%), formação de calos (%), enraizamento (%), número médio de raízes e comprimento médio de raízes (cm) de estacas de *I. paraguariensis* de acordo com duas populações em dois ambientes de enraizamento.

Regarding the survival of the genotypes from the TB population, a high variance between individuals was found, but not between rooting environments (Table 2). Therefore, TB6 (66.7%) in both environments, followed by TB3 (47.9%; 43.8%) also in both environments and TB4 (43.8%) in GWIN can be highlighted with the best survival rates.

Callus formation was not significant for the environments, following a similar perspective of the survival rate, with TB6 obtaining the highest difference between both variables (more than 12.0%). The presence of callus was not proportionally followed by rooting for most of the genotypes from MT, while in GWIN these results were close.

Rooting itself was considerably superior in GWIN, highlighting TB6 (39.6%), TB3 (39.6%) and TB1 (32.5%). Comparing with MT, TB6 (18.8%), TB3 (8.3%) and TB1 (14.6%) were also similar to TB2 (10.4%), all of the with less than 10.0% of rooting. Root number and length were similar in proportion of rooting, but with finer results for TB1 (3.3 roots and 0.7 cm) when compared to the other better-rooted genotypes.

Table 2. Survival (%), callus formation (%), rooting (%), average root number and average root length (cm) of *I. paraguariensis* cuttings from Três Barras (TB) according to the genotype and rooting environment.

Tabela 2. Sobrevivência (%), formação de calos (%), enraizamento (%), número médio de raízes e comprimento médio de raízes (cm) de estacas de *I. paraguariensis* de Três Barras (TB) de acordo com o genótipo de ambiente de enraizamento.

Genotype	TB1	TB2	TB3	TB4	TB5	TB6	TB7	Aver.
Survival (%)								
MT	25,0 cA	29.2 cA	47.9 bA	25,0 cB	25,0 cA	66.7 aA	12.5 dA	33.0
GWIN	35.0 cB	35.4 cA	43.8 bA	43.8 bA	18.8 dA	66.7 aA	12.5 eA	36.6
Averages	30.0	32.3	45.9	34.4	21.9	66.7	12.5	34.8
Callus (%)								
MT	22.9	29.2	33.3	25.0	22.9	52.1	10.4	28.0
GWIN	32.5	22.9	35.4	33.3	18.8	54.2	12.5	29.9
Averages	27.7 b	26.1 b	34.4 b	29.2 b	20.9 c	53.2 a	11.5 d	29.0

Rooting (%)								
MT	14.6 aB	10.4 aA	8.3 aB	2.1 bA	4.2 bA	18.8 aB	2.1 bA	8.6
GWIN	32.5 aA	12.5 bA	39.6 aA	6.3 bA	14.6 bA	39.6 aA	4.2 bA	21.3
Averages	23.6	11.5	24.0	4.2	9.4	29.2	3.2	15.0
Number of roots								
MT	0.8 aB	0.3 aA	0.1 aB	< 0.1 aA	0.1 aA	0.5 aB	< 0.1 aA	0.3
GWIN	3.3 aA	0.6 bA	2.3 aA	0.3 bA	0.7 bA	2.5 aA	0.2 bA	1.4
Averages	2.1	0.5	1.2	0.2	0.4	1.5	0.2	0.9
Length of roots (cm)								
MT	0.4	0.2	0.1	< 0.1	0.2	0.2	< 0.1	0.2 b
GWIN	1.0	0.5	1.0	0.2	0.3	0.8	< 0.1	0.6 a
Averages	0.7 a	0.4 a	0.6 a	0.2 b	0.3 b	0.5 a	< 0.1 b	0.4

Averages followed by the same letter do not differ according to the Scott-Knott average test ($P < 0.05$). Lowercase letters for rooting environments (rows) and uppercase letter for genotypes (columns).

Taking in perspective the survival of the UR population, a great variance between genotypes and rooting environments was obtained. It was moderately superior inside MT for UR3 (56.2%) and UR2 (52.1%), with average results for UR1 (20.8%). A significant result was also obtained for in GWIN for UR3 (52.1%) and moderate for UR2 (31.2%), while all other genotypes presented survival rates lower than 10.0%.

Callus, as previously stated, also followed a similar standard to the survival rate, highlighting UR2 in GWIN, with a difference higher than 12.0% between these variables. Similarly, to the TB genotypes, the presence of callus was not followed by rooting for the UR population, as low percentages of it were also found.

In total, only four genotypes obtained rooted cuttings, featuring UR3 (15.7%), while the others (UR2, UR4 and UR6) achieved less than 3.0%. Due to that, the variables of root number and length were only notable for UR3 (0.5 roots and 0.2 cm).

Table 3. Survival (%), callus formation (%), rooting (%), average root number and average root length (cm) of *I. paraguariensis* cuttings from Urupema (UR) according to the genotype and rooting environment.

Tabela 3. Sobrevivência (%), formação de calos (%), enraizamento (%), número médio de raízes e comprimento médio de raízes (cm) de estacas de *I. paraguariensis* de Urupema (UR) de acordo com o genótipo de ambiente de enraizamento.

Genotype	UR1	UR2	UR3	UR4	UR5	UR6	UR7	Aver.
Survival (%)								
MT	20.8 bA	52.1 aA	56.2 aA	10.4 cA	4.2 cA	8.3 cA	6.2 cA	22.6
GWIN	6.2 cB	31.2 bB	52.1 aA	8.3 cA	4.2 dA	2.1 dB	0.0 dB	14.9
Averages	13.5	41.7	54.2	9.4	4.2	5.2	3.1	18.7
Callus (%)								
MT	18.8 bA	47.9 aA	54.2 aA	10.4 cA	0.0 dA	8.3 cA	0.0 dA	19.9
GWIN	6.2 cB	18.8 bB	45.8 aB	4.2 cA	2.1 cA	0.0 cB	0.0 cA	11.0
Averages	12.5	33.4	50.0	7.3	1.1	4.2	0.0	15.5
Rooting (%)								
MT	0.0	2.1	12.5	0.0	0.0	2.1	0.0	2.4
GWIN	0.0	2.1	18.8	0.0	2.1	0.0	0.0	3.3
Averages	0.0 b	2.1 b	15.7 a	0.0 b	1.1 b	1.1 b	0.0 b	2.8
Number of roots								
MT	NR	< 0.1	0.4	NR	NR	0.1	NR	0.1
GWIN	NR	0.1	0.5	NR	< 0.1	NR	NR	0.1
Averages	0.0 b	< 0.1 b	0.5 a	0.0 b	< 0.1 b	< 0.1 b	0.0 b	0.1

Length of roots (cm)								
MT	NR	< 0.1	0.1	NR	0.0	< 0.1	NR	< 0.1
GWIN	NR	< 0.1	0.2	NR	< 0.1	NR	NR	0.1
Averages	0.0 b	< 0.1 b	0.2 a	0.0 b	< 0.1 b	< 0.1 b	0.0 b	< 0.1

Averages followed by the same letter do not differ according to the Scott-Knott average test ($P < 0.05$). Lowercase letters for rooting environments (rows) and uppercase letter for genotypes (columns). NR: not rooted.

DISCUSSION

In all conditions, the EA% was considered at least high (> 70.0%). This can guarantee a high possibility of achieving similar results considering future analysis with the material of same origin (RESENDE & DUARTE, 2007; NAVROSKI *et al.*, 2013).

The maintenance of survival can be strictly related to the variables of temperature and humidity, as well as possible adapted genotypes and populations. Rooting environments with high temperature and humidity fluctuation tend to accelerate the cuttings metabolism, quickly diminishing their energy resources and eventually going through fully oxidization (XAVIER *et al.*, 2013; NASCIMENTO *et al.*, 2020). This, however, was not verified for the UR population, allowing a different interpretation.

Temperature was considerably similar between both rooting environments for the experiment period (Figure 1), with MT being approximately 5.0 °C hotter than GWIN in most of the evaluations. The variance between lowest and highest temperatures was also similar between environments, ranging from approximately 5.0 °C, the lowest in October, and 39.0 °C, the highest in December. Naturally, the relative air humidity is also important, and it varied greatly between rooting environments. In a general perspective about humidity, MT obtained the maximum humidity in almost every evaluation with exception of the last two, while GWIN varied considerably between evaluations.

This variation in humidity between environments can be explained by their structures. MT is a small and simplified system with irrigation by micro aspersion, whereas the quantity of water is enough to maintain not only air but also the substrate with high humidity. However, GWIN is a larger structure, optimized for the rooting of cuttings from *Eycalyptus* sp., which do not demand high humidity, whereas the nebulization system is programed to maintain only at leaf-level moisture. The survival of *I. paraguariensis* cuttings may not be deeply dependent on the infrastructure of the rooting environment, with positive results also found in simpler structures (BRONDANI *et al.*, 2008; 2009). This was not observed in the present search, since survival varied greatly comparing both rooting environments. An environment with high humidity is crucial for the maintenance of propagules, as this material does not have the capability of absorbing water (XAVIER *et al.*, 2013).

However, with the results obtained in this search, it is possible that survival, and therefore all the other variables associated to rooting, are affected by the rooting environments as much as the population of origin, i.e., different correlations between both. The population of UR is from a region known to have the lowest temperatures during winter in Brazil, as well as a lower sunlight incidence and high humidity (MARTINS-RAMOS *et al.*, 2011), and these factors are crucial to determine the species development and adaptation to new environments, especially cuttings of *I. paraguariensis* (NASCIMENTO *et al.*, 2018, 2019, 2020). Therefore, this population probably obtained a better survival response to MT due to the higher humidity inside the rooting environment itself, promoting better conditions.

As for genotypes and populations, some UR *I. paraguariensis* genotypes can be considerably more adapted than others, regardless of the rooting environment, as tested in a previous work. The difference of survival can vary up to 50.0% in this population, highlighting genotypes such as G6 (72.9%) as one of the bests, and G2 (23.9%) as the one with the lowest results (NASCIMENTO *et al*., 2020). However, this variance may not be as drastic and with higher survival rates when using well adapted genotypes, especially with mini-cuttings, with rates higher than 40,0% and up to 100,0% (BISOGNIN *et al*., 2018; VIEIRA *et al*., 2021). Furthermore, half-sibling genotypes, i.e. mini-cuttings from mini-stumps of the same progeny, tend to have similar survival rates varying only by the decimal numbers, no more than 0.5% (GAZZANA *et al*., 2020).

Only small differences between the rates of survival and callus formation occurred, which is easily seen observing both populations. Considering only the total averages of the factorial treatments, survival reached 26.7%, while the callus formation obtained only 4.0% less. According to previous studies on the cutting of *I. paraguariensis* from the population UR (NASCIMENTO *et al.*, 2018; 2019; 2020), non-reinvigorated material obtained the highest calluses rates (approximately 10.0% less than survival rate). Furthermore, even though mini-stumps sustain reinvigorated materials, mini-cuttings may still present high rates of calluses, up to 60.0% in some genotypes (VIEIRA *et al*., 2021). The presence of calluses indicates an elevated degree of maturation of the material used for propagation (WENDLING *et al.*, 2014; STUEPP *et al*., 2015; 2017a). However, the rooting of cuttings with calluses is still possible if they are maintained for longer periods inside the rooting environment (STUEPP *et al.*, 2017b).

In a relation between place of origin, considering environmental conditions, and rooting environment, it is possible to state that TB obtained a faster response for all variables, even though with also high rates of calluses. This is easily seen by the variable rooting, whereas TB was superior to UR (almost three times in total averages), and even better inside GWIN (more than two times for TB itself). In a similar previous experiment, the UR population obtained better rooting results inside GWIN (11.1%) when compared to MT (4.9%) (NASCIMENTO *et al.,* 2020). These results corroborates perfectly with the ones of the present search, as low results were also obtained (less than 3.5%), but GWIN was still superior (3.3%). Additionally, the present search confirms the findings of the previous cited work, reinforcing the hypothesis of a strong environmental origin influence over the capacity of *I. paraguariensis* vegetative propagation of this region.

Although some genotypes obtained a considerable rooting rate, such as TB1, TB3, TB6 and UR3, it is important to consider that these results would possibly be higher with a reinvigorated material (WENDLING *et al.,* 2013; 2014). Well-established clones of *I. paraguariensis* can have considerable rooting rates, with more than 70.0% of mini-cuttings rooted, while inferior materials less than 5.0% (PIMENTEL *et al.*, 2019; VIEIRA *et al.*, 2021), considering the mini-stumps as a material with juvenile characteristics, suitable for vegetative propagation. However, it is also possible to obtain materials of high rooting capacity from native areas, directly from canopy sprouts, with some genotypes reaching more than 45.0% of rooted cuttings (NASCIMENTO *et al*., 2020). Therefore, it is possible to consider that the rooting variation may not be only intrinsic to the species, relying on variables such as vigor, which may not be easy to determine at the first moment, but also on the genotypic level, since some same aged materials from same populations appear to have superior rooting rates.

The rooting of genotypes according to the rooting environments may present a complicated relationship, as some previous studies present that there might not be significant differences for well-established clones (BRONDANI *et al.,* 2008; 2009). Meanwhile, the same rescued vegetative material obtained significant differences when testing the MT and GWIN environments, with rooting achieving more than 20.0% of difference between them (NASCIMENTO *et al.,* 2020). In the present search a stronger similarity was found to the last cited work, being easily seeing for the genotype TB3, as GWIN (39.6%) obtained a rate higher than 30.0% of more rooted cuttings than MT (8.6%). This variation was lower for the UR population, but still observable for UR3, as GWIN (18.8%) obtained more rooted cuttings than MT (12.5%), but not statistically different (15.7% on average).

The variables of root number and length were a consequence of rooting, which can be easily seen at population and genotype level. It is also important to state that the number of roots can be affected by the level of maturation of the propagule, as previously tested by the UR population, with an average of 0.8 roots per cutting from canopy material (NASCIMENTO *et al.,* 2018). In the present search, the results were similar with the cited work for the same population (0.5 for UR3). However, in a more recent work with the material from the same population (NASCIMENTO *et al.,* 2020), it was found that it is possible to find better rooting individuals, achieving better results for the rooting variables (4.3 roots of 1.3 cm). As for the TB population, TB1 obtained the most singular results, even though it did not achieved the best rooting rate, approximately 7.0% less than TB3 and TB6. This reinstates that there can be a strong and yet sensible relation between the origin of the material, the individuals inside each and the rooting environments.

CONCLUSION

- The cutting of *I. paraguariensis* is dependent on the relation between the populations of origin, as well as their genotypes, and the rooting environments.
- The correct *I. paraguariensis* genotype inserted in a specific rooting environment may guarantee substantial rooting results.
- *I. paraguariensis* cuttings seem to be strongly dependent on humidity for survival, favored by micro sprinkler irrigation, but with inferior rooting results.

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