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Psychotria nuda (Cham. & Schltdl.) Wawra: ROOTING OF STOCK PLANTS IN DIFFERENT PHENOPHASES AND ENVIRONMENTS

Psychotria nuda (Cham. & Schltdl.) Wawra: ENRAIZAMENTO DE MATRIZES EM DIFERENTES FENOFASES E AMBIENTES

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ABSTRACT

Psychotria nuda (Cham. & Schltdl.) Wawra (Rubiaceae) is a native species from the Dense Ombrophylous Forest (dense tropical rain forest), typical in the low altitude areas of the forest, with occurrence in the states of Minas Gerais, Rio de Janeiro, São Paulo, Paraná and Santa Catarina. Due to the presence of yellow flowers with red receptacle and anise colored fruits, the plant has an ornamental potential, such as a pharmaceutical potential, thanks to the variety of alkaloids that have already been isolated demonstrating structural diversity of its active components. This work aimed to verify the effect on stem cuttings rooting of different collection environments and of different phenophases of *Psychotria nuda* stock plants at the time of collection, and to verify what is the correlation between variables defined as percentage of rooted cuttings, number of roots per cutting and average length of the three longest roots per each cutting (cm) with permanence of two half leaves and formation of callus. Phenophases are intended as different times of the year in which stock plants presented distinct phenological characteristics. In the two phenophases studied (phenophases 1 and 2) canopy sprouts were collected in Antonina, Paraná state, from two different environments (plain and hillside) of the Dense Ombrophylous Forest (dense tropical rain forest). Cuttings were prepared with 10 to 13 cm length and two halved leaves. After 60 days in a greenhouse, the percentage of cuttings with roots, with callus, alive, dead, the number of roots per cutting and the length of the three longer roots per each rooted cutting were evaluated. The collection environments and the phenophases at the time of collection influenced the percentage of rooting, such that the phenophase 1 and the hillside environment were the conditions that resulted in the best rooting percentage (90%). The permanence of leaves during rooting period helped, in most cases, the formation of adventitious roots. Anticipated formation of callus supported rooting in phenophase 2, while in phenophase 1 the formation of adventitious roots was independent from callus formation.
Keywords: grandiúva-d´anta; cutting; phenology.

RESUMO

Psychotria nuda (Cham. & Schltdl.) Wawra (Rubiaceae) é uma espécie nativa da Floresta Ombrófila Densa, restrita às florestas de baixa altitude, com ocorrência nos estados de Minas Gerais, Rio de Janeiro,

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São Paulo, Paraná e Santa Catarina. Possui potencial ornamental, devido à presença de flores amarelas, cálice vermelho e frutos cor anis, e farmacêutico, visto que vários alcaloides já foram isolados, demonstrando diversidade estrutural de compostos ativos. O presente trabalho teve como objetivo verificar o efeito de diferentes ambientes de coleta e da fenofase em que se encontravam as plantas matrizes de *Psychotria nuda* no enraizamento de estacas caulinares, além de verificar a correlação entre as variáveis porcentagem de estacas enraizadas, número de raízes por estaca e comprimento médio das três maiores raízes por estaca (cm) com a permanência de duas meias folhas e formação de calos. Entende-se por fenofases, as épocas com características fenológicas distintas em que se encontravam as plantas matrizes. Nas duas fenofases estudadas (Fenofases 1 e 2), brotações de copa foram coletadas em Antonina-PR em dois ambientes distintos (planície e encosta) da Floresta Ombrófila Densa. Estacas foram confeccionadas com 10-13 cm de comprimento e duas folhas reduzidas à metade. Após 60 dias em casa de vegetação, foram avaliadas a porcentagem de estacas enraizadas, com calos, vivas, mortas, número de raízes/estaca e comprimento das três maiores raízes/estaca. Os ambientes de coleta e as fenofases influenciaram na porcentagem de enraizamento, onde a fenofase 1 e o ambiente encosta foram as condições que apresentaram o melhor enraizamento (90%). A manutenção das folhas nas estacas durante o período de enraizamento, na maioria das situações, favoreceu a formação de raízes adventícias, e a formação antecipada de calos favoreceu o enraizamento na fenofase 2. Já na fenofase 1, a formação de raízes adventícias foi independente da formação de calos.
Palavras-chave: grandiúva-d'anta; estaquia; fenologia.

INTRODUCTION

Psychotria nuda (Cham. & Schltdl.) Wawra (Rubiaceae) is a native species from the Dense Ombrophylous Forest that is common from the states of Minas Gerais and Rio de Janeiro down to Santa Catarina, with a wide geographical distribution in Brazil, with ornamental (DELPRETE et al., 2005) ecologic (ALMEIDA and ALVES, 2000), medicinal and pharmacological importance (FRAGOSO, 2007). It is well known that this species reacts to rooting, with and without application of synthetic auxins, in different percentages, presenting variations according to the environments and the times of stem cuttings collection (WITT and ZUFFELLATO-RIBAS, 2009; NERY, 2010).

Cutting propagation is not only to remove a sprout from a stock plant, put it into a substrate and then wait and see the root formation. This description can generally define the technique, but there are various factors involved in this process, giving positive or negative results to rooting induction. This stated, the level of success obtained in vegetative propagation by cuttings is determined by the relations between different factors such as physiological conditions of the stock plant, variations in the climatic environment, type and the position of collected sprouts on the stock plant, and more (ZUFFELLATO-RIBAS and RODRIGUES, 2001; HARTMANN et al., 2002).

Physiological conditions of the stock plant, like water content, level of reserve, nutrients and hormonal balance, are directly influenced by the period of the year and by the environments the plant is growing in, such that formation of adventitious roots is influenced (HARTMANN et al, 2002). Following a literature review about adventitious rhizogenesis in native plants, divergent results were encountered in the same species, and these results could be due to the fact that the material collection was made from stock plants located in different environments (FERREIRA et al., 2001; KNAPIK, et al., 2003; PIMENTA et al., 2005; BORTOLINI, 2006; FERRIANI, 2006; FERREIRA, 2008). According to these authors, the main intent of their works was to evaluate isolated rooting reaction for each single species, meanwhile, they realized that it was of fundamental importance to give greater attention to environments influences and to phenophases at the time of collection from the stock plant, because these two factors were deeply influencing the rhizogenetic reaction of vegetative propagation.

Permanence of leaves during rooting period and anticipated callus formation on cuttings base are factors to be considered when rhizogenesis process is studied. Presence and permanence of leaves on cuttings are helping factors for the formation of adventitious roots, since they are source of auxins and co-factors and decisive in the rooting process of some species (HARTMANN et al., 2002). Cuttings with leaves, in general, respond with greater success to rooting, presenting bigger root induction and superior number and length of the formed roots (XAVIER et al., 2003; LIMA et al., 2003). Formation of adventitious roots can originate directly from differentiation of cells close to the vascular system, or indirectly, when the cells with not oriented division create callus that remain for a period and then lately starts an organized division, creating the primary root (HARTMANN et al., 2002). Meanwhile, for some species, anticipated formation of callus prevents rooting (QUADROS, 2009).

In front of the importance of environment and phenophases in which the stock plant is found, in concern of the adventitious rhizogenesis process, the intention of this research was to study the effect of different collection environments and phenophases of the stock plants on *Psychotria nuda* cuttings rooting, and to verify the influence of callus formation and permanence of leaves on formation and development of adventitious roots.

METHODOLOGY

Vegetal material of *Psychotria nuda* used for cuttings experiments was collected in two distinct environments of the Dense Ombrophylous Forest (D.O.F). The first one is located in a part of the plain D.O.F in the lower lands, which presents a soil classified as “Neossolo Fluvico”, or a poorly developed soil, formed by alluvial deposits, not presenting any type of B diagnostic horizon (EMBRAPA, 2006). The second environment is located in a submontane part of the D.O.F. (hillside), presenting a soil classified as “Cambissolo Haplico”, or a soil formed by mineral material with incipient B horizon that does not present A humic horizon and lacks alluvial character within 120 cm from surface (EMBRAPA, 2006). The two collection environments are characterized by native vegetation with low human presence. They are surviving areas of the original D.O.F.

Collection was made in the Natural Reserve of Morro da Mina, located close to coordinates UTM 7189000 e 72300 (DATUM SAD 69), in the territory of Antonina, in Paraná state, belonging to SPVS (Sociedade de Pesquisa em Vida Selvagem e Educação Ambiental), which covers an area of 3,300 ha (8,151 acres). In this property, hillside areas of ‘Serra do Mar’ and plain areas of the coastline forest can be found, all way down to Antonina’s bay (Sociedade de Pesquisa em Vida Selvagem e Educação Ambiental-SPVS, 2010

Vegetal material for cuttings was collected in two distinct phenophases of the species *Psychotria nuda* (Tab 1); the first named Phenopahase 1 in November/2009, corresponding to spring season and the second named phenophase 2 in June 2010, corresponding to the end of autumn. The main difference between the two phenophases is presence or absence of leaves sprouts. In each environment and phenophase, 20 mature stock plants were identified, with similar diameter and height (average 9.9 cm diameter and 4.7 cm height), giving a total number of 80 stock plants, that were previously marked on field to ease further identification.

Canopy sprouts from 20 mature stock plants from each collection environment were used to obtain the cuttings. Collecting expeditions always took place early in the morning, and the experiment installations were held in two days, when each day was dedicated to vegetal material preparation of one of the studied environments (plain and hillside). After collection, cuttings were stored into plastic bags marked with stock plant identification, and taken to the Botanic Department of UFPR (Curitiba, PR state) for the installation procedure.

From each stock plant, 20 semi-hardwood 10 to 13-cm length cuttings were prepared, all with bevel cut in the base and straight cut on the top, where two leaves were kept on the apical portion of the cuttings after reducing in half their area with the intent to reduce water loss from leave transpiration. During the preparation process all cuttings were kept in buckets with water to prevent dehydration. After preparation, cuttings received a phytosanitary treatment consisting in a sodium hypoclorite 0.5% immersion for 10 minutes (bactericidal action), followed by rinsing in clean running water for 5 minutes. Later, cuttings were planted into 53 cm^3 polypropylene tubes with substrate formed by medium size vermiculite and carbonized rice shell in a 1:1 proportion (v/v). The greenhouse was kept under controlled temperature and humidity (24± 2°C, about 90% of relative humidity) until the time of evaluation that took place 60 days after the experiment installation. Permanence time in the rooting bed was determined according to NERY (2002).

After this period the following variables were evaluated: percentage of rooting (alive cuttings presenting roots with at least 1 mm length, with or without callus), total number of roots per cutting, length of the three longest roots in each cutting (cm),

TABLE 1: Phenologic behavior of *Psychotria nuda* during collection period. Antonina, PR state, 2009-2010.
TABELA 1: Comportamento fenológico de *Psychotria nuda* nas épocas de coleta do material vegetal. Antonina, PR, 2009-2010.

Month/Year	Phenologic description	Phenophase
November/2009 (spring)	Stock plants in full vegetative conditions, with presence of young sprouts, green and mature fruits and flowers budding.	1
June/2010 (autumn)	Stock plants in reduced vegetative activity, with presence of flowers, mature fruits and absence of young sprouts.	2

TABLE 2: Monthly weather data from Antonina,PR state, during the period of September/2009 to August/2010.
TABELA 2: Dados meteorológicos mensais de Antonina-PR durante o período de Setembro/2009 a Agosto/2010.

Month/Year	Rainfall (mm)	Temperature (Cº)		
		Average	Average of lower	Average of higher
Sep/2009	366.4	18.5	15.7	23.0
Oct/2009	146.2	19.5	16.1	24.6
Nov/2009	334.2	24.2	20.2	34.5
Dec/2009	396.0	24.5	20.7	30.7
Jan/2010	692.8	24.7	21.6	31.1
Feb/2010	521.8	25.8	21.8	33.1
Mar/2010	281.6	24.1	20.8	29.5
Apr/2010	374.6	21.2	17.4	26.8
May/2010	137.8	18.9	15.0	24.5
Jun/2010	163.4	17.1	13.5	23.4
Jul/2010	217.2	17.3	14.0	22.7
Aug/2010	112.6	16.2	12.5	22.7

Source : SIMEPAR (Sistema Meteorológico do Paraná), 2010

percentage of cuttings with callus (alive cuttings, without roots, with formation of undifferentiated cellular mass on the base), percentage of alive cuttings (alive cuttings with no rooting induction and no callus formation) and percentage of mortality (cuttings found with necrotic tissues).

Data were analysed according to an entirely randomized pattern with factorial arrangement of 2x2 (phenophases x collection environments) of the treatments, with 20 replications, where each repetition consisted in cuttings collected from one stock plant, and 20 cuttings per experimental unit. Variations of treatments were tested concerning homogeneity according to Bartlett test. Variables that presented homogeneous variations were submitted to variance analysis and the ones that presented significant variations on test F were compared according to Tukey test at a 5% level of probability.

Besides the variables above described, at the time of evaluation the number of cuttings that remained with two half leaves on during the rooting period was also quantified, and the number of rooted cuttings that presented callus formation. With these variables, *Pearson* correlation coefficient was calculated and evaluated according to F statistic, at 5% and 1% of significance.

RESULTS AND DISCUSSION

Concerning the variables percentage of rooting and average length of the three longest formed roots per cutting, interaction between collection environments and phenophases was statistically significant, indicating that these factors are dependent such that the effect of collection environment depends on the phenophase of the stock plant at the moment of collection. On the other hand for the variable number of roots formed per cutting, the double interaction between the collection environment factor and phenophases was not statistically significant, indicating that these factors are independent, only phenophases presented a significant effect on the rooting variable (Table 3)

Results indicate that formation of adventitious roots was favored when cuttings were prepared with material collected from stock plants in phenophase 1, not depending on the collection environment, statistically differing from phenophase 2. Hillside was the best collection environment, in phenophase 1 with leaves, giving 90% of rooting, statistically differing from plain environment. Concerning phenophase 2 there was no statistical difference between the environments.

Phenophase 1 is characterized by great vegetative growth of stock plants (Table 1), in other words presenting leaves, fruits and young flowers budding, which are sources of auxins and rooting co-factors. (BENINCASA and LEITE, 2004; TAIZ and ZEIGER, 2004). This way it is possible to conclude that in phenophase 1, the quantity of endogenous auxin was enough to induce adventitious roots formation. General averages of rooting percentages in the two considered periods (Table 4) were similar to the ones obtained by NERY (2010), that is 77.08% in spring time (phenophase 1) and 54.17% in autumn (phenophase 2). Results encountered by the author confirm the ones encountered in this work. In spring time, which corresponds to the leaf sprouting period, was the best period for vegetal material collection.

In phenophase 1 (November 2009) a high level of rainfall was registered and also higher average temperatures, being the opposite of what happened during the time stock plants in phenophase 2 (June 2010) (Table 2). According to ALMEIDA and ALVES (2000), the growth period (leaf budding) of the species *Psychotria nuda* is during the humid season and the hotter months with longer photoperiod, resulting in an increase of the rooting, reasonably due to the presence of young leaves and flowers encountered in phenophase 1, and also due to the adequate physiological conditions of the stock plants in this period like for example water quantity contained. Works realized with the species *Coffea canephora, Tibouchina sellowiana* and *Psychotria nuda* demonstrate the relation between high rainfall levels and the rooting increase (PURUSHOTHAM et al. 1984; BORTOLINI et al., 2008; NERY, 2010).

TABLE 3: Variance analysis (test F) for percentage of rooted cuttings, number of roots and average length of the three longest roots of each cutting (cm), for two collection environments, coming from canopy sprouts collected during phenophases 1 and 2.

TABELA 3: Análise de variância (teste F) para porcentagens de estacas enraizadas, número de raízes e comprimento médio das três maiores raízes por estaca (cm), para dois ambientes de coleta, provenientes de brotações de copa coletadas nas fenofases 1 e 2.

Variation sources	G.L.	Q.M.		
		Rooted	Number	Length
Environments (A)	1	577.813^{ns}	9.385 ^{ns}	0.780 **
Phenophases (B)	1	20320.313**	187.885 **	81.406 **
Interaction AxB	1	5865.313**	9.385 ^{ns}	1.326 **
Error	76	278.010	3.357	0.065
Total	79			
CV (%)		26.55	43.76	17.67
Bartlett test (x^2)		6.989 ^{ns}	5.36 ^{ns}	5.942 ^{ns}

Where in: *= significant at 5%, **= significant at 1%, ^{ns} = not significant at 5%

TABLE 4: Average of percentage of rooted cuttings, number of roots per cutting and average length of the three longest roots per cutting (cm) of *Psychotria nuda*, considering two collection environments, from canopy sprouts collected during phenophases 1 and 2.

TABELA 4: Médias das porcentagens de estacas enraizadas, número de raízes por estaca e comprimento médio das três maiores raízes por estaca (cm) de *Psychotria nuda*, para dois ambientes de coleta, provenientes de brotações de copa coletadas nas fenofases 1 e 2.

Rooted (%)			
Phenophases	Environments		Averages
	Plain	Hillside	
Phenophase 1	67.5 Ba	90.0 Aa	78.8
Phenophase 2	52.8 Ab	41.0 Ab	46.9
Averages	60.1	65.5	
N. of Roots			
Phenophases	Environments		Averages
	Plain	Hillside	
Phenophase 1	5.0	6.4	5.7 a
Phenophase 2	2.7	2.7	2.7 b
Averages	3.9	4.5	
Length of Roots (cm)			
Phenophases	Environments		Averages
	Plain	Hillside	
Phenophase 1	2.2 Ba	2.7 Aa	2.5
Phenophase 2	0.5 Ab	0.4 Ab	0.4
Averages	1.4	1.6	

Where in: Averages followed by the same uppercase letter on the horizontal and from the same lowercase letter on the vertical do not differ significantly among them at 5% level of probability according to Tukey test.

The statistical difference verified among the two environments in phenophase 1 where the hillside gave a superior result, possibly occurred because of water accumulation in the plain soil, since there is a flat relief. As it was previously described, the month of collection referred to phenophase 1 (November 2009) had a high level of rainfall, and this could have prevented from rooting the cuttings collected from stock plants in this area, due to excessive presence of water in the soil. Floodplains are characterized by low and relatively flat grounds, situated close to rivers, small streams, lakes and swamps, being areas where floods periodically occur during rain season due to the small depth of their ground water. In case of water in excess, soil conditions are frequently adverse for two reasons: reduction of gases quantity and reduction of nutritive substance presence (CURI et al., 1988).

Variation of cuttings rooting percentage occurring in the same species was verified for *Sapium glandulatum,* where different researches with the same experimental conditions but collecting from stock plants in different environments, presented rooting variations due to this last variable (FERREIRA et al., 2001; CUNHA et al., 2004; FERREIRA, 2008). Results obtained by these authors and in the present work point out the environment influence in cuttings rooting.

Accordingly, phenological phases of the stock plants and the environment where they developed, modified the percentage of cuttings rooting of *Psychotria nuda*. This indicates that, even if the species is presenting a high percentage of rooting, choice of the stock plant is of fundamental importance. The high percentage of rooted cuttings obtained in this research and the conclusions described by NERY (2010), that vegetative propagation by cutting is feasible, not being necessary the use of vegetal regulators to increase rooting, demonstrating that *Psychotria nuda* easily forms roots from stem cuttings.

Phenophase 1 presented the higher average roots percentage per cutting, being 5.7, and this number is statistically superior to the same average in phenophase 2 (2.7). Numerically, the highest average number of roots was encountered in cuttings collected from stock plants in phenophase 1, located on hillside (6.4). Experiments realized by NERY (2010), with the same species, obtained similar results (7.73 roots per cutting).

For the average length of the three longest roots per cutting, in phenophase 1, the hillside environment was the one that gave statistically better results (2.7cm) than the plain environment; on the other hand considering phenophase 2, there was no significant difference among the environments. For both the collection environments was phenophase 1, when stock plants were in full vegetative growth, that gave best results, differing statistically from phenophase 2 (Table 4). Results obtained in this work, considering the variable average length of the three longest roots per cutting are similar to the ones obtained by NERY (2010), who obtained 2.37cm of length from spring time collection, for the species *Psychotria nuda.*

The best results for the variables number of roots and average length of the three longest roots per cutting obtained in phenophase 1 are due to the positive effect of the season on formation of the radical system. Besides the rooting percentage, the number and length of formed roots per cutting are the most relevant variables in seedlings production (ANTUNES et al.,1996). A better result for these variables indicates that the seedlings obtained will have a better development, since seedlings with a better radicial system will have better surviving rate once planted in pot or field.

For the variable percentage of cuttings with callus, interaction between environments and phenophases was not statistically significant, indicating that these factors are independent; in any case the factors presented a significant though not interactive effect. While for the variable percentage of alive cuttings the factors were dependent, such that there was statistical significance between collection environments and phenophases (Table 5)

Due to the high rooting performances in phenophase 1, the best result for the variable cuttings with callus was observed in phenophase 2 (38.8%). Phenophase 2 could present a higher rooting percentage if the cuttings had remained in rooting bed for a longer period of time, since callus is a mass of undifferentiated cells which could start differentiation process, forming the young roots sprouts (HARTMANN et al., 2002). The period in rooting bed is variable according to the treated species, there is not a common optimum time period. OLIVEIRA et al. (2001), working with native species from gallery forest, found a variation from 2 to 4 months for the root system formation. For some species, the necessary time in rooting bed is less or equal to 30 days (CUNHA et al., 2003; WENDLING et al., 2005).

The higher percentage of alive cuttings, 2.3%, occurred in the period of low vegetative activity in the hillside environment, being statistically different from the leaf sprouting season and plain environment (Table 6). NERY (2010) obtained 6.26% of alive cuttings of *Psychotria nuda* in spring and 22.91% in autumn (phenophase 2).

TABLE 5: Variance analysis (test F) for percentage of cuttings with callus and alive cuttings, for two collection environments coming from canopy sprouts in phenophases 1 and 2.

TABELA 5: Análise de variância (teste F) para porcentagens de estacas com calos e estacas vivas, para dois ambientes de coleta, provenientes de brotação de copa coletadas nas fenofases 1 e 2.

Variation sources	G.L.	Q.M.	
		Callus	ALIVE
Environments (A)	1	720.000*	300.313 *
Phenophases (B)	1	21451.250**	812.813 **
Interaction AxB	1	80.000^{ns}	2702.813 **
Erro	76	138.783	71.727
Total	79		
CV (%)		52.65	82.13
Bartlett test (x^2)		6.650 ^{ns}	2.395 ^{ns}

Where in: *= significant at 5%, **= significant at 1%, ^{ns} = not significant at 5%

TABLE 6: Average percentage of cuttings with callus and alive cuttings of *Psychotria nuda* for two collection environments, coming from canopy sprouts collected during phenophases 1 and 2.
TABELA 6: Médias das porcentagens de estacas com calos e vivas de *Psychotria nuda*, para dois ambientes de coleta, provenientes de brotação de copa, coletadas nas fenofases 1 e 2.

With Callus (%)			
Phenophases	Environments		Averages
	Plain	Hillside	
Phenophase 1	10.0	2.0	6.0 b
Phenophase 2	40.8	36.8	38.8 a
Averages	25.4 A	19.4 A	

Alive (%)			
Phenophases	Environments		Averages
	Plain	Hillside	
Phenophase 1	11.0 A a	3.3 Bb	7.1
Phenophase 2	5.8 B a	21.3 Aa	13.5
Averages	8.4	12.3	

Where in: Averages followed by the same uppercase letter on the horizontal and by the same lowercase letter on the vertical do not significantly differ among them at level of 5% of significance according to Tukey test.

TABLE 7: *Pearson* correlation coefficients between callus formation in rooted cuttings and variable percentage of rooted cuttings, number of roots per cutting and average length of the tree longest roots per cutting (cm) for the species *Psychotria nuda.*
TABELA 7: Coeficientes de correlação de Pearson entre a formação de calos em estacas enraizadas e as variáveis porcentagem de estacas enraizadas, número de raízes por estaca e comprimento médio das três maiores raízes por estaca (cm), para a espécie *Psychotria nuda*.

Rooted		
Phenophases	Environments	
	Plain	Hillside
Phenophase 1	0.396^{ns}	0.139^{ns}
Phenophase 2	0.829**	0.791**

N. of Roots		
Phenophases	Environments	
	Plain	Hillside
Phenophase 1	-0.067^{ns}	0.191^{ns}
Phenophase 2	0.480*	0.519*

Length of Roots		
Phenophases	Environments	
	Plain	Hillside
Phenophase 1	-0.061^{ns}	0.175^{ns}
Phenophase 2	0.175^{ns}	0.552*

Where in:* = significant at 5%, **= significant at 1%,^{ns} = not significant at 5%

For the percentage of dead cuttings, no statistical analysis was possible because of low mortality rate (11.5%), independent from the collection period of the cuttings and from the environment where the stock plants were located, thus demonstrating the high potential of survival for this species. Percentage of dead cuttings obtained in this work, in the two phenophases, was lower than those obtained by NERY (2010), 14.58% in spring time and 18.75% in autumn.

Results obtained through *Pearson* correlations (Table 7), which tested the degree of association between presence of callus in rooted cuttings base and the variable percentage of rooted cuttings, number of roots per cutting and average length of the three longest roots per cutting (cm), indicate that in phenophase 1 formation and development of the roots are not dependent from the anticipated callus formation, showing that the differentiation and development of adventitious roots are not related to callus formation on cuttings base.

In this phenophase, possibly, auxin quantity is bigger due to the presence of young sproutings, thus overcoming endogenous cytokinins concentrations and causing a meristematic cellular division preferably oriented to form adventitious roots. A meristema can induce various types of differentiation; regulation of the process will depend on auxins and cytokinins. When quantity of auxins is higher than cytokinins, aventitious roots are formed, while callus is formed when quantities are comparable. If auxins quantity is small, callus will evolve into leaf gems (HARTMANN et al., 2002).

When phenophase 2 is considered, correlations were significant and positive in most situations, showing that root formation is preceded by callus formation. Since in this phenophase stock plants did not have young sprouts, it is possible that endogenous concentration of auxins was not enough to differentiate the meristematic tissues into roots at first time, giving as first only callus formations. Passing a time in rooting bed it is possible that auxins located in leaves were transferred to base of cuttings, changing the relation between auxins and cytokinins quantities thus promoting cellular differentiation into roots formation. This answer emphasizes the need to keep leaves on the cuttings

TABLE 8: *Pearson* correlation coefficients between two half leaves permanence percentage and the variable percentage of rooted cuttings and average length of the three longest roots per cutting (cm) of *Psychotria nuda*.

TABELA 8: Coeficientes de correlação de Pearson entre a permanência de duas meias-folhas e as porcentagens de variáveis estacas enraizadas, número de raízes por estaca e comprimento médio das três maiores raízes por estaca (cm), para a espécie *Psychotria nuda*.

Phenophases	Environments	
	Plain	Hillside
Rooted		
Phenophase 1	0.609**	0.222 ns
Phenophase 2	0.995**	0.998**
N. of Roots		
Phenophase 1	0.523**	0.466*
Phenophase 2	0.772**	0.664**
Length of Roots		
Phenophase 1	0.023 ns	0.237 ns
Phenophase 2	0.264 ns	0.690**

Where in: * = significant at 5%, **= significant at 1%, ns = not significant at 5%

during the rooting period, mostly in dormant phenophase (phenophase 2). Leaves play a key role in cuttings rooting, because they supply auxins and co-factors, among other substances, stimulating meristematic activity and cellular differentiation (PAIVA and GOMES, 1995; HARTMANN et al., 2002).

For *Pearson* correlations (Table 8), realized between the permanence of two halved leaves on the cuttings and the variables percentage of rooted cuttings and number of roots per cutting, on most of the situations of the stock plants, presented positive and significant correlation, showing that keeping two halved leaves on cuttings during the rooting period helps the birth and the number of adventitious roots. Only for hillside collection environment and phenophase 1 there was no significant correlation between the variables permanence of two halved leaves and percentage of rooted cuttings. Cuttings coming from stock plants under these conditions gave the higher rooting percentage (Table 4). This answer indicates that rooting induction takes place even with early leaves loss in the rooting bed, being their maintenance unnecessary, thus showing that stock plants under these conditions can present auxins and co-factors levels high enough for radicial system induction.

In the low vegetative activity period of the species (phenophase 2) an association between variables is observed as being bigger, considering rooted cuttings and number of roots per cutting, showing that in this season maintenance of two half leaves is fundamental to have a good rooting result. In this phenophase, stock plants were having low physiological activity in their branches, consequently auxins and rooting co-factors transportation from leaves to base of cuttings was fundamental to start adventitious roots formation. Permanence of leaves on cuttings helps rooting process due to the continuity of photosyntetic process which is responsible for carbohydrates synthesis giving the necessary energy source to roots formation (HAISSIG, 1984).

The variable average length of the three longest roots per cutting (cm) presented an opposite behaviour towards the one previously described, since the majority of conditions did not present significant correlation, showing that maintenance of two halved leaves during the rooting period does not optimize adventitious roots growth.

CONCLUSIONS

Under the development conditions of this work, it was possible to conclude that:

- Collection environments and phenophases of stock plants have an influence on stem cuttings rooting percentage of *Psychotria nuda.*
- Phenophase 1 and hillside environment are the conditions which are better supporting adventitious roots formation in *Psychotria nuda.*
- Early callus formation in cuttings bases is independent from adventitious roots formation in phenophase 1 (high physiological activity season); for phenophase 2 (low physiological activity season), formation of roots is anticipated by callus formation.
- The maintenance of leaves on cuttings during rooting period, in most situations, helps induction and number of adventitious roots per cutting.

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