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## Substrate and seed sowing position on the production of *Plukenetia volubilis* L. seedlings

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### Key words:

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### ABSTRACT

*Plukenetia volubilis* is a species native to the Amazonia and has economic potential due to the high contents of polyunsaturated fatty acids and vitamins of the seeds; however, information about production of seedlings in nursery is scarce. Factors that contribute to fast emergence and generation of vigorous seedlings are desirable for plant production in the nursery. Therefore, this study aimed to identify the most favorable substrate and seed position for the production of *P. volubilis* seedlings. The seeds were sown in the following four positions: hilum facing up, hilum facing down, seed lying on its cotyledon suture, and seed lying flat on one of its cotyledon faces. The substrates were sand, vermiculite and commercial substrate. Treatments were distributed according to a completely randomized design in a 4 × 3 factorial arrangement (seed position and substrate) with three replicates of 25 seeds each. The following variables were evaluated: emergence percentage; first count of seedlings; number, width and length of leaves; plant height; stem diameter; dry matter of shoots and roots. It was concluded that the production of *P. volubilis* seedlings is not affected by either substrate or seed position at sowing. The substrates most favorable to the quality of *P. volubilis* seedlings were the commercial substrate and vermiculite, always for the positions of seed lying on its cotyledon suture, with faces parallel to tube walls, and seed lying flat on one of its faces.

### Palavras-chave:

emergência  
*Sacha inchi*  
plântulas  
vigor

## Substrato e posição de semente na produção de mudas de *Plukenetia volubilis* L.

### RESUMO

*Plukenetia volubilis* é uma espécie nativa da Amazônia e apresenta potencial econômico devido aos elevados teores de ácidos graxos poli-insaturados e vitaminas das sementes, mas, as informações para a produção de mudas em viveiros são escassas. Fatores que contribuam para rápida emergência das plântulas e a formação de mudas vigorosas são desejáveis na produção de plantas em viveiros. Assim, objetivou-se identificar o substrato e a posição de semente mais favoráveis à produção e à qualidade de mudas de *P. volubilis*. As sementes foram colocadas para germinar em quatro posições: com o hilo voltado para cima, com o hilo voltado para baixo, semente de lado sobre a espessura e de lado sobre uma das faces. Os substratos utilizados foram: areia, vermiculita e substrato comercial. O delineamento utilizado foi inteiramente casualizado em arranjo fatorial 4 x 3 (posições da semente x substratos) com quatro repetições de 25 sementes. Foram avaliados: a porcentagem de emergência; primeira contagem de plântulas; número; largura e comprimento de folha; altura; diâmetro do colo; massa seca de parte aérea e de raiz da muda. Concluiu-se que a produção de mudas de *P. volubilis* não foi afetada pelo substrato nem pela posição da semente na sementeira no tubete. Os substratos mais favoráveis à qualidade das mudas de *P. volubilis* foram o comercial e a vermiculita, sempre para as posições de semente de lado sobre a espessura com faces paralelas às paredes do tubete ou sobre uma das faces da semente.



## INTRODUCTION

*Plukenetia volubilis* L. belongs to the Euphorbiaceae family and produces seeds with high levels of proteins and lipids (Ruiz et al., 2013). The oil extracted from the seeds has high nutritional quality, because it contains polyunsaturated fatty acids omega 3, 6 and 9, besides vitamins A and E (Follegatti-Romero et al., 2009; Gutiérrez et al., 2011; Ruiz et al., 2013). Its cultivation in Brazil is represented by a small portion in its native environment and does not have high production (Bordignon et al., 2012).

Developing techniques favorable to seedling production would represent an advancement in the domestication and rational exploitation of the economic potential of *P. volubilis*, which is mainly obtained by extractivism (Rivera et al., 2009). Information on temperature, light and substrate to produce seedlings of this species in nursery is scarce (Oliveira et al., 2013; Cardoso et al., 2015).

The most utilized substrates for Euphorbiaceae seeds include sand, as used for *Cnidocolus quercifolius* (Brasil, 2013) and *Alchornea castaneifolia* (Paula et al., 2013), vermiculite as used for *Alchornea triplinervia* and *Cnidocolus floribundus* (Brasil, 2013), and commercial mixtures as used for *Sebastiania commersoniana* (Boene et al., 2013). For *P. volubilis*, there were only reports on the initial growth and survival of seedlings in vermiculite (Cardoso et al., 2015) and fine moss (Rosa & Quijada, 2013), and germination in mixtures of substrates (Lequepi, 2010) and vermiculite (Silva et al., 2016).

Seed position in the substrate at sowing is a factor that can facilitate the emergence of seedlings and reduce their time of permanence in the nursery, as observed for *Amburana cearensis* (Guedes et al., 2010) and *Schizolobium amazonicum* (Pinto et al., 2014). Increase in germination speed can guarantee lower deterioration in seeds with high oil contents such as sacha inchi, because seeds of oilseed crops are known to have lower viability (Joker & Jepsen, 2003). Thus, this study aimed to identify the most favorable substrate and seed position at sowing for the production and quality of *P. volubilis* seedlings.

## MATERIAL AND METHODS

*P. volubilis* seeds, from the germplasm bank of Embrapa Western Amazon, AM, Brazil, were brought to the Laboratory of Seed Analysis, of the Department of Plant Production – Plant Science, of the Faculty of Agrarian and Veterinary Sciences, São Paulo State University (UNESP), in Jaboticabal, SP, Brazil (21° 15' 18" S; 48° 19' 19" W).

Seeds were sown in the substrates fine sand (particles between 0.10 and 0.25 mm), medium vermiculite (particles between 1.19 and 0.50 mm) and commercial substrate, in small tubes (35 x 140 mm). The commercial substrate was Basaplant Florestais, which is formulated based on pine bark, fibrous peat, coconut fiber, vermiculite, NPK and micronutrients (Baseagro, 2016).

Four seed positions in relation to substrate surface were evaluated: A) hilum facing up; B) hilum facing down; C) seed lying on its edge with faces parallel to tube walls; D) seed lying flat on one of its faces (Figure 1). Three replicates of 25 seeds were used for each treatment.



Figure 1. Seed positions at sowing: hilum (h) facing up (A), hilum facing down (B), seed lying on its edge (C) and seed lying flat on one of its faces (D)

After sowing, the tubes were arranged on polypropylene trays (620 x 420 x 165 mm), maintained in greenhouse from May to April 2014 at the Department of Plant Production – Plant Science, of the Faculty of Agrarian and Veterinary Sciences, São Paulo State University (UNESP), in Jaboticabal-SP, Brazil, and irrigated to maintain the substrates at 60% field capacity (Brasil, 2013). Temperature and relative air humidity along the experimental period were  $28 \pm 3$  °C and  $68 \pm 3\%$ , respectively. To evaluate treatment effects on emergence and initial growth of seedlings, the following quality parameters were evaluated:

a) seedling emergence in substrate – determined by counting normal seedlings emerged at 35 days after sowing (DAS), considering those with part of the hypocotyl visible, outside the substrate, and apparently complete and healthy (Brasil, 2013). The results were expressed in percentage (Brasil, 2013).

b) first count – determined by recording the percentage of normal seedlings emerged at 12 DAS (Silva et al., 2016).

c) number of expanded leaves per plant, at 35 DAS, which were immediately removed from the substrate and washed in running water.

d) width and length of the second leaf – leaf counted from plant base and variables measured with a caliper. The results were expressed in centimeters.

e) seedling height – measured as the distance between the base and last leaf insertion height, with a millimeter ruler, and the results were expressed in centimeters.

f) stem diameter – measured close to the substrate with a digital caliper and the results were expressed in millimeter.

g) shoot and root dry matter – shoots and roots were collected, separately placed in Kraft paper bags and dried in forced-air oven at 65 °C until constant weight. Then, the parts were weighed on precision scale (0.001 g).

Analysis of variance was conducted in a completely randomized design, in a 4 x 3 factorial scheme (seed positions x substrates), with four replicates of 25 seeds. Treatment means were compared by Tukey test ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

Table 1 shows the results of the analysis of variance for the parameters evaluated regarding seed position at sowing and type of substrate used in seedling production.

Substrates and seed positions did not affect emergence percentage of *P. volubilis* seedlings, which was on average 86%. These results were similar to those obtained in the substrates

Table 1. Results of the analysis of variance for emergence, first count, number, width and length of leaves, seedling height, stem diameter, shoot dry matter and root dry matter of *Plukenetia volubilis* seedlings sown in different substrates (S) and positions (P)

Parameters evaluated	Factor of variation			Mean	CV (%)
	F (S)	F (P)	F (S x P)		
Emergence (%)	0.70 <sup>ns</sup>	2.57 <sup>ns</sup>	0.39 <sup>ns</sup>	86.00	11.46
First count (%)	9.28**	1.31 <sup>ns</sup>	1.25 <sup>ns</sup>	43.00	45.90
Number	35.17**	5.94**	0.94 <sup>ns</sup>	3.00	12.35
Leaves Width (cm)	197.77**	3.64*	2.15 <sup>ns</sup>	5.67	6.25
Length (cm)	42.69**	3.48**	2.77*	11.89	15.48
Seedling height (cm)	289.11**	4.86**	5.39**	13.98	4.05
Stem diameter (mm)	47.89**	0.82 <sup>ns</sup>	5.71**	4.92	2.71
Dry Shoots (g)	134.73**	15.20**	4.62**	0.78	8.45
Roots (g)	70.69**	20.68**	8.91**	0.25	13.40

\*, \*\* and <sup>ns</sup>Value significant at  $p \leq 0.05$ ,  $p \leq 0.01$  and not significant by F test; CV – Coefficient of variation

more favorable to the germination of this species: vermiculite (Silva et al., 2016), paper roll (Cardoso et al., 2015) and fine moss (Rosa & Quijada, 2013); which led to maximum germination of 95, 88 and 70%, respectively. Nonetheless, Oliveira et al. (2013) observed that the germinative performance of *P. volubilis* seeds depends on progeny and environmental temperature during the sowing period, and seedling emergence may vary between 75 and 100%.

There was no effect of interaction between substrate and seed position on the first count of seedlings or number and width of leaves. Interaction effect was found only on leaf length, seedling height, stem diameter, shoot dry matter and root dry matter.

Seedling emergence speed was evaluated by the first count test, which revealed that the type of substrate had effect on this variable (Table 2). However, the same fact was not observed for seed position at sowing. This test identified faster seedling emergence in sand and vermiculite, with 56 and 53% of seedlings emerged at 12 DAS, respectively.

These values were statistically higher than that obtained in the commercial substrate, 24%. Lighter substrates such as sand and vermiculite allow greater gas exchange and better drainage, reducing the physical barrier for the initial development of the seedling (Maggioni et al., 2014; Silva et al., 2016).

Although sand allowed maximum emergence speed, seedlings produced in this substrate showed lower number of leaves and lower values of leaf width compared with those grown in commercial substrate and vermiculite. Comparing these latter substrates, commercial substrate was superior to

vermiculite for leaf width, but similar to it for the number of leaves. Thus, in general, the commercial substrate was responsible for maximum leaf expansion, probably due to the supply of macro- and micronutrients after emergence and their mobilization to the shoots, as observed in *Eugenia uniflora* seedlings (Antunes et al., 2012).

Regarding the effect of seed position on leaf growth, seed planted with hilum facing down, on its edge and on one of its faces resulted in seedlings with maximum number and width of leaves, whereas seed planted with hilum facing up was unfavorable to leaf development, because it led to seedlings with lower number and width of leaves. This result can be explained by the higher energy demand of the seed for root growth and movement of cotyledons in the soil when the seed is planted with hilum facing up, compared with other positions.

*P. volubilis* have epigeal germination and the radicle is produced in the hilum and micropyle region (Oliveira et al., 2013). Therefore, when seeds are planted with hilum facing up, the primary root is produced close to the surface and grows around the seed toward the tube bottom, due to geotropism. In other seed positions, this was not observed because the radicle in the seed was facing down or lying on its side, and it favored seedling emergence at these positions.

In the sowing with hilum facing up, only after the root grew, became fixed and a strap-shaped structure was formed in the hypocotyl, the seedling was able to have support to rotate the cotyledons in the substrate and push them above the surface. In the sowing with hilum facing up, moving the cotyledons inside the tube may have been more difficult, due to the physical space, compared with the other positions.

Comparing the effect of substrates on leaf length, in general, the commercial substrate was superior to the others. Vermiculite showed an intermediate performance, whereas sand was the worst substrate for leaf length, regardless of seed position at sowing, except for seeds with hilum facing up or lying flat on one of the faces, because in these cases the substrate sand did not differ from vermiculite (Table 3).

The effect of the substrates on the height of *P. volubilis* seedlings was similar to that observed on leaf length and width because, for all seed positions at sowing, the commercial substrate, vermiculite and sand produced tall (~16.7 cm), intermediate (~13.9 cm) and short (~11.3 cm) seedlings, respectively.

Commercial substrate and vermiculite probably provided more favorable conditions to plant growth than the sand, due to the aeration, greater moisture retention, chemical composition

Table 2. First count, number and width of leaves of *Plukenetia volubilis* sown in different substrates and at the following seed positions: hilum facing up (A), hilum facing down (B), seed lying on its edge (C) and seed lying flat on one of its faces (D)

Parameters evaluated	Substrate			Position			
	Sand	Vermiculite	Commercial	A.	B.	C.	D.
First count (%)	56 A	53 A	24 B				
Number of leaves	2.50 B	3.70 A	3.60 A	2.80 B	3.40 A	3.20 AB	3.60 A
Leaf width (cm)	4.11 C	5.98 B	6.94 A	5.48 B	5.54 AB	5.98 A	5.71 AB

Means followed by the same lowercase letter in the row do not differ by Tukey test at 0.05 probability level

Table 3. Leaf length, seedling height, stem diameter, shoot dry matter and root dry matter of *Plukenetia volubilis* seedlings from seeds sown in different substrates and in the following positions: hilum facing up (A), hilum facing down (B), seed lying on its edge (C) and seed lying flat on one of its faces (D)

Parameters evaluated	Substrate	Position			
		A. 	B. 	C. 	D. 
Leaf length (cm)	Sand	8.1 bA	7.5 cA	8.6 bA	9.4 bA
	Vermiculite	10.8 abA	12.8 bA	12.5 aA	11.5 abA
	Commercial	12.8 aB	19.3 aA	15.8 aAB	13.4 aB
Seedling height (cm)	Sand	10.8 cB	10.6 cB	11.5 cAB	12.2 cA
	Vermiculite	12.5 bC	15.1 bA	13.7 bBC	14.2 bAB
	Commercial	16.8 aA	17.2 aA	16.7 aA	16.2 aA
Stem diameter (mm)	Sand	5.0 aA	5.1 aA	5.3 aA	5.3 aA
	Vermiculite	4.9 aA	4.9 aA	4.9 bA	5.0 bA
	Commercial	4.9 aA	4.8 aAB	4.6 cBC	4.4 cC
Shoot dry matter (g)	Sand	0.31 bA	0.29 cA	0.33 bA	0.36 bA
	Vermiculite	0.43 aB	0.64 aA	0.59 aA	0.59 aA
	Commercial	0.46 aB	0.54 bAB	0.60 aA	0.59 aA
Root dry matter (g)	Sand	0.45 aA	0.20 bB	0.25 abB	0.26 bB
	Vermiculite	0.35 bA	0.28 aA	0.27 aA	0.34 aA
	Commercial	0.17 cAB	0.12 cB	0.20 bA	0.15 cAB

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ by Tukey test at 0.05 probability level

and temperature maintenance, as reported for *Schizolobium parahyba* by Martins et al. (2012).

Additionally, the commercial substrate was better than vermiculite and sand because it contains macro- and micronutrients in its formulation (Baseagro, 2016). The nutrients of the substrate are usually assimilated by plant roots, allowing for greater development of leaves and seedling height, as observed for *Sicana odorifera* (Lima et al., 2010), *E. uniflora* (Antunes et al., 2012) and *S. parahyba* (Martins et al., 2012).

Unlike the results for height, the greatest diameter of *P. volubilis* was found in seedlings produced in the sand, followed by those grown in vermiculite and commercial substrate, which showed mean values of 5.2, 4.9 and 4.6 mm, respectively (Table 3). Nonetheless, this result alone does not express the quality of the seedlings, as they had greater diameter and lower height. It is important to highlight that, according to Gomes et al. (2002), despite the difficult measurement of this parameter, the contribution of diameter and height/diameter ratio to seedling quality is low in comparison to other parameters, such as total dry matter.

Seed position had effect on the diameter only in the commercial substrate, because seeds planted with hilum facing up and with hilum facing down originated *P. volubilis* seedlings with greater diameter (4.9 and 4.8 mm, respectively), compared with the positions of seed lying on its edge and seed lying flat on one of its faces, which originated seedlings with lower diameter, 4.6 and 4.4 mm, respectively (Table 3).

Commercial substrate and vermiculite, which caused maximum leaf length and seedling height, also led to maximum shoot dry matter accumulation (~0.55 g) in the seedlings compared with those grown in sand (0.32 g), in all positions of sowing. All seed positions at sowing were favorable to seedling dry matter accumulation, except for seed planted with hilum facing up, which led to the lowest values (Tables 2 and 3).

The obtained results can be explained by the fact that the length and number of leaves in the shoots are related to the mass produced, as observed by Antunes et al. (2012) in

*E. uniflora* seedlings and by Alves et al. (2008) in *Erythrina velutina* seedlings produced in commercial substrate.

For root dry matter accumulation, maximum values were found in *P. volubilis* seedlings produced in sand, followed by those grown in vermiculite and commercial substrate (Table 3). These results are related to the plant need for root expansion to assimilate nutrients when they were deficient or even absent (Silva & Delatorre, 2009), as in the case of sand and vermiculite.

Conversely, the commercial substrate has readily available nutrients and such root growth was not observed. This greater expansion in the roots of seedlings grown in substrates with absence or unavailability of nutrients was also found in seedlings of *Mimosa caesalpinifolia* (Nogueira et al., 2012) and *Senegalia tenuifolia* (Araújo et al., 2016).

Dry matter is an important parameter to evaluate seedling quality and is decisive to select the best treatments, because higher dry matter may represent a temporary reserve of assimilates, which can guarantee plant survival after transplantation to the field.

## CONCLUSIONS

1. Production of *Plukenetia volubilis* seedlings was not affected by substrate or seed position at sowing in the tube.
2. The substrates most favorable to the quality of *Plukenetia volubilis* seedlings were commercial substrate and vermiculite, always for the positions of seed lying on its edge, with faces parallel to tube walls, or seed lying flat on one of its faces.

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