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Environmental factors on seed germination, seedling survival and initial growth of sacha inchi (*Plukenetia volubilis* L.)[1]

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ABSTRACT – Sacha inchi (*Plukenetia volubilis* L.) is an Amazon species of elevated agro-industrial potential due the high content of omega-3 and omega-6 in its seeds. Despite of it, little information about its propagation by seeds is currently available. Thus, the aim of this study was to assess seed germination, seedling survival and growth of this species under different conditions of substrate (on paper, between papers and paper roll), light (continuous darkness, 12-h photoperiod and continuous light) and temperature (continuous temperature at 20, 25, 30, 35 and 40 °C). Germination is stimulated by substrates with increased surface contact with the seeds, presence of light and temperatures between 25 and 35 °C. Survival and initial growth of seedlings are favored by vermiculite, continuous light and 30 °C temperature. These conditions allow rapid and uniform germination of seeds and better establishment and development of seedlings. We encourage the propagation of sacha inchi by seeds, since we consider it a feasible technique.

Index terms: *Plukenetia volubilis*, substrate, light, temperature, seedling production.

Fatores ambientais na germinação de sementes e na sobrevivência e crescimento inicial de plântulas de sacha inchi (*Plukenetia volubilis* L.)

RESUMO – A sacha inchi (*Plukenetia volubilis* L.) é uma espécie amazônica de elevado potencial agroindustrial devido ao alto teor de ômega-3 e ômega-6 em suas sementes. Apesar disto, pouca informação acerca de sua propagação seminífera é encontrada. Dessa forma, o objetivo do trabalho foi avaliar a germinação das sementes, sobrevivência e crescimento das plântulas da espécie em diferentes condições de substrato (sobre papel, entre papel e em rolos de papel), luz (escuro contínuo, fotoperíodo de 12 horas e luz contínua) e temperatura (constante nas temperaturas de 20, 25, 30, 35 e 40 °C). A germinação é estimulada por substratos que possuam maior superfície de contato com a semente, pela presença de luz e por temperaturas entre 25 e 35 °C. A sobrevivência e o crescimento inicial das plântulas são favorecidos pela vermiculita, luz contínua e temperatura de 30 °C. Estas condições permitem uma germinação rápida e uniforme e um bom estabelecimento e desenvolvimento das plântulas. Nós encorajamos a propagação seminífera de sacha inchi, uma vez que ela se mostra uma técnica viável.

Termos para indexação: *Plukenetia volubilis*, substrato, luz, temperatura, produção de mudas.

Introduction

Information about the influence of abiotic factors on germination is essential for the study of seeds. Temperature, light and substrate affect seed germination in a species-dependent manner and may, in many cases, inhibit the germination process (Carvalho and Nakagawa, 2000). The same environmental factors also influence the seedlings survival and growth and the better understanding of these elements is essential in order to produce high-quality seedlings (Nogueira et al., 2003). Studies indicate a wide variability in the requirements of these components for the best growth and development of seedlings. This fact justifies this type of analysis for lesser known species and especially the native ones (Zamith and Scarano, 2004).

Sacha inchi (*Plukenetia volubilis* L. – Euphorbiaceae)

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is native to the Amazon region, occurring in Brazil, Peru, Colombia, and Venezuela (Céspedes, 2006). Its seeds have high agro-industrial potential due their elevated content of fatty acids, such as α-linolenic acid (omega-3) and linoleic acid (omega-6) (Follegatti-Romero et al., 2009). The species also has desirable features for reforestation and slope protection and it is appointed as an alternative to the recovery of degraded areas and to family farming programs (Bordignon et al., 2012).

Although studies for *in vitro* propagation of sacha inchi can be found (Bordignon et al., 2012; Rodrigues et al., 2014), information about seed germination and seedling growth is incipient (Rosa and Quijada, 2013). Therefore, the objective of this study was to evaluate the substrate, light and temperature requirements for seed germination and for seedlings survival and initial growth of sacha inchi.

Material and Methods

Sacha inchi seeds were acquired from Germplasm Bank of Sacha inchi of Embrapa Amazônia Ocidental, Manaus, AM, Brazil (they were collected in November 2012) and stored at 20 °C until the beginning of the experiments. Before implementing the tests, seeds were treated with Captan 0.5%.

Seeds were eliminated when they were deformed, shriveled and visibly attacked by insects. Since little is known about sacha inchi seeds, prior characterization was performed on water content (%), fresh weight (g), length (mm), width (mm) and thickness (mm). Water content was obtained from four replications of 10 seeds by the standard method in an oven at 105 °C for 24 hours (Brasil, 2009). Mass, length, width and thickness data were obtained with the aid of analytical balance and digital caliper. For this purpose, 50 randomly selected seeds were used from the total seeds.

Seed germination

To assess the effect of the substrate on germination, seeds were sown on paper, between papers and in paper rolls as recommended by the Regras para Análise de Sementes – Brazilian Rules for Seed Analysis (Brasil, 2009). Germitest papers were placed in petri dishes and the seeds distributed on or between the sheets. The substrates were moistened with an equivalent volume of water at 2.5 times its dry weight. The dishes and rolls were sealed to prevent excessive water losses and they were maintained in B.O.D. chambers under continuous light and at 30 °C.

The effect of different light conditions (continuous darkness, 12-h photoperiod and continuous light) on seed germination was also evaluated. Seeds were distributed in germitest paper rolls and maintained in B.O.D. chambers at 30 °C. The assessment of the germination rate in the absence of light was carried out in a dark room with the aid of green light.

In order to evaluate the effect of temperature on seed germination, seeds were distributed in germitest paper rolls and maintained in B.O.D. chambers under continuous light, and at the continuous temperatures of 20, 25, 30, 35 and 40 °C.

In each germination test, four replicates of 25 seeds per treatment were used. Germination tests lasted seven days and at the end, germination percentage and germination speed index (GSI) were assessed (Maguire, 1962). Seeds with primary root longer than 1 mm were considered germinated. The light of the germination tests was provided by four 20-W lamps.

Seedling survival and initial growth

Survival and growth of seedlings were assessed in different substrate, light and temperature conditions at the end of 15 days, since this species has a short vegetative phase. Sacha inchi seeds were sown in germitest paper rolls and maintained in B.O.D. chambers under continuous light and at 30 °C for five days. The seedlings obtained were transferred to plastic trays and placed in B.O.D. in the conditions described below.

To check the influence of the substrate, 200 seedlings were transplanted to plastic trays containing similar amounts of sand or fine vermiculite, wet to field capacity. The sand used was prior washed and sifted in a square mesh sieve number 4. The experiment was conducted under continuous light and at 30 °C for 15 days.

To evaluate the influence of light, another sample of 200 seedlings was transplanted to plastic trays containing vermiculite wet to field capacity. The trays were stored at 30 °C, under a 12-h photoperiod or continuous light for 15 days.

The effect of temperatures was also assessed. For this, a sample of 300 seedlings was transplanted to plastic trays containing vermiculite wet to field capacity. The trays remained in continuous light and at 25, 30 or 35 °C for 15 days.

In the survival tests 100 seedlings distributed in four replicates of 25 per treatment were assessed and in the growth tests 40 seedlings distributed in four replicates of 10 per treatment were used. At the end of 15 days, survival rates and seedlings growth parameters were evaluated. The number of leaves (N_L), seedling dry weight (W_S), leaves dry weight (W_L), roots dry weight (W_R), the leaf mass ratio ($LMR=W_L/W_S$), and root mass ratio ($RMR=W_R/W_S$) were estimated. Measurements were performed using an analytical balance. To determine the dry weight, plant material was dried in an oven at 70 °C for 72 hours. The light of the survival and growth tests was provided by four 20-W lamps.

Experimental design and statistical analysis

The statistical design was completely randomized and the data were submitted to analysis of variance (ANOVA). For variables with significant F ($P < 0.05$) and treatment's degree of freedom bigger than one, a grouping of averages was performed by the Scott-Knott test ($P < 0.05$). For variables with significant F and treatment's degree of freedom equal to one, the F test was conclusive. Both germination and survival percentage were transformed to arcsin $(x/100)^{1/2}$ prior to analysis and all data were checked for normality and homoscedasticity.

Results and Discussion

Seed characteristics and germination

Sacha inchi seeds had a 7.09 ± 0.25% water content and the following biometric characteristics: 0.97 ± 0.2 g weight, 1.8 ± 0.1 cm length, 1.5 ± 0.1 cm width, and 0.8 ± 0.1 cm thickness. Unlike many Amazonian species seeds, which have high humidity (O'Neill et al., 2001), sacha inchi seeds exhibited low water content in this study. The low moisture content of seeds and its large size indicate that sacha inchi seeds may be orthodox (Roberts, 1973). In order to confirm this classification desiccation and cold storage tests should be performed.

Seed germination started on the 3rd day after sowing and it varied depending on the substrate, light and temperature (Figure 1). About the substrates, both between paper and paper roll promoted significantly higher germination percentages (80 and 88%) relative to the substrate on paper (21%). In addition, the paper roll promoted higher GSI (4.5) compared to other substrates (Figure 1A). The substrates between paper and paper roll have greater contact surface with the seeds and therefore may favor their hydration and germination.

Assessing sacha inchi germination under different substrates, Rosa and Quijada (2013) found 70% and 3.0 as maximum germination rate and GSI, when moss was used as substrate. These values are low compared with values obtained in our study, when paper roll (88% and 4.5) and between papers (80% and 3.6) were used. Thus, these substrates show up as more suitable for sacha inchi seed germination.

The presence of light has not shown to be an essential factor for sacha inchi germination and its seeds may be considered as neutral photoblastic (Vázquez-Yanes and Orozco-Segovia, 1990). However, sacha inchi seed germination was improved in the presence of light (88% in continuous light and 83% in 12-h photoperiod) regarding its absence (69%) (Figure 1B). Similarly, higher GSIs were found in the presence of light (4.5 in continuous light and 4.4 in 12-h photoperiod) compared to the continuous darkness (3.8). In resume, although the presence of light is not a necessary factor for sacha inchi germination to occur, it allows greater speed and percentage of seed germination.

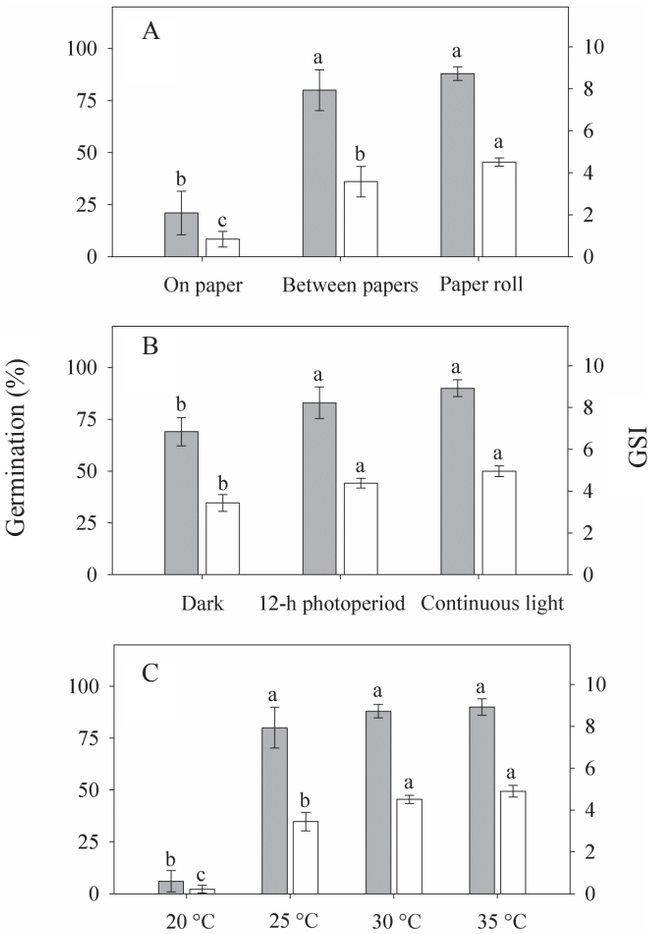

Figure 1. Germination (%) (gray bars) and GSI (white bars) of sacha inchi seeds under different conditions of substrate (A), light (B) and temperature (C). Bars with the same letter do not differ by the Scott-Knott test ($P < 0.05$). Mean ± SD, n = 4.

The highest germination percentages were found at 25, 30 and 35 °C (80, 88 and 90%, respectively), and they did not differ statistically from each other. However, GSIs at 30 and 35 °C (4.5 and 4.9) were superior to GSI at 25 °C (3.5). The 20 ºC temperature negatively affected the germination rate and GSI (6% and 0.2); and the 40 °C temperature completely inhibited germination during the evaluation period (Figure 1C). It can be inferred that the minimum temperature for the sacha inchi seed germination is lower than 20 °C and the maximum temperature is between 35 and 40 °C. Although

sacha inchi is native and occurs in Amazon biome, typical of elevated temperatures, its germination was inhibited at 40 °C. This result is in line with the information from Brancalion et al. (2010) that suggest temperatures around 30 °C for germination of species from that biome.

Survival and growth of seedlings

Sacha inchi seedlings survival was affected by the substrate and temperature, but not by the light. Seedling survival was higher in vermiculite (98%) than in the sand (79%). The temperature of 35 °C significantly reduced seedling survival (52%) compared to temperatures of 25 and 30 °C (94 and 97%), which did not differ significantly from each other (Figure 2).

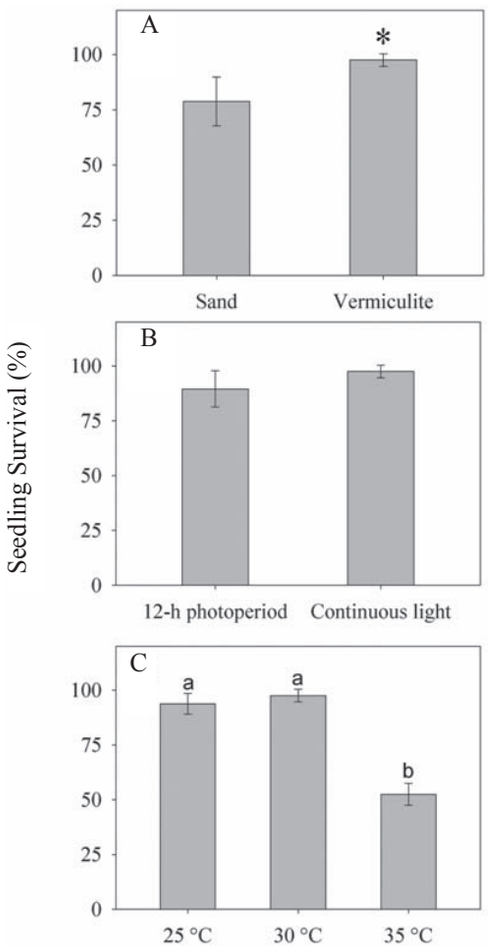

Figure 2. Seedling survival (%) of sacha inchi under different substrate (A), light (B) and temperature (C) conditions. Bars with an asterisk indicate that the F test was significant ($P < 0.05$) and bars with the same letter do not differ by the Scott-Knott test ($P < 0.05$). Mean ± SD, n = 4.

Although all evaluated factors have promoted changes in sacha inchi seedling growth, temperature was the component that most affected the assessed parameters (Table 1 and Figure 3). Substrate did not show significant difference between sand and vermiculite for N_L and W_S (Table 1). However, significant differences between both substrates were observed on the other parameters. The development of roots from seedlings grown in sand was higher (larger W_R and RMR) relative to the seedlings grown in vermiculite. On the other hand, seedlings grown in vermiculite exhibited greater development of the leaves compared to seedlings maintained in sand (higher W_L and LMR).

Sand has higher aeration in relation to vermiculite (Sodré et al., 2007), which may have allowed the roots to develop better in that substrate. Furthermore, sand has lower water retention, which may have stimulated greater root growth, aiming to favor the process of water uptake.

Fine vermiculite has a low particle size (0.3 to 0.5 mm) and, therefore, higher water retention and low aeration (Martins et al., 2012). Thus, root growth was disfavored and the leaves became the main sink of the plant.

The light condition affected only the leaves development (Table 1). The continuous light allowed to obtain higher W_L and LMR compared to the 12-h photoperiod. This fact indicates that seedlings under continuous light invest more on leaves, the photosynthetic organ of higher interest. It is possible that the seedlings maintained in continuous light are the most promising during the initial establishment.

Temperature was the factor with higher interference on seedlings development and it affected all evaluated parameters (Table 1 and Figure 3). At 30 °C seedlings developed well and showed the high N_L. Seedlings maintained at 25 and 30 °C exhibited similar W_S, but at 30 °C they showed higher W_L and W_R (due to increased LMR and RMR) than at 25 °C. Although the total biomass of seedlings maintained at 25 and 30 °C were similar, the seedlings maintained at 30 °C had more developed leaves (photosynthetic organ) and roots (organ responsible for the water and nutrients uptake), which characterizes them as better established.

The temperature at 35 °C resulted in high mortality of seedlings and dramatically affected the growth of the surviving seedlings, reducing the parameters: N_F, W_T, W_F and W_R (Table 1). High temperatures negatively affect important physiological mechanisms such as photosynthesis, photorespiration and transpiration, which are directly and indirectly related to the carbon balance of plants (Machado et al., 2002). It is likely that the constant temperature at 35 °C occasions an intense stress, enhancing the transpiration and photorespiration events, and reducing photosynthesis, which disfavors seedling establishment and development.

The propagation of sacha inchi by seeds is presented as a feasible alternative, since under suitable conditions of substrate, light and temperature the species shows rapid and uniform germination. Furthermore, both seedling survival and growth are satisfactory when appropriate environmental conditions are given.

Table 1. Number of leaves (N_L), seedling dry weight (W_S) (g), leaves dry weight (W_L) (g), roots dry weight (W_R) (g), leaf mass ratio (LMR) and root mass ratio (RMR) of sacha inchi seedlings maintained under different substrate, light and temperature conditions.

	Substrate		Light		Temperature		
	Sand	Vermiculite	Photoperiod	Continuous light	25 °C	30 °C	35 °C
N_L	4.15	3.75	3.75	3.75	1.85 b	3.75 a	0.20 c
W_S	0.51	0.46	0.40	0.46	0.49 a	0.46 a	0.22 b
W_L	0.11*	0.15	0.10*	0.15	0.09 b	0.15 a	0.01 b
W_R	0.23*	0.15	0.15	0.15	0.12 b	0.15 a	0.07 c
LMR	0.24*	0.33	0.27*	0.33	0.18 b	0.33 a	0.03 c
RMR	0.46*	0.33	0.37	0.34	0.24 b	0.34 a	0.33 a

Means followed by an asterisk indicate that the F test was significant ($P < 0.05$) and means followed by the same letter do not differ by the Scott-Knott test ($P < 0.05$). Mean, n = 4.

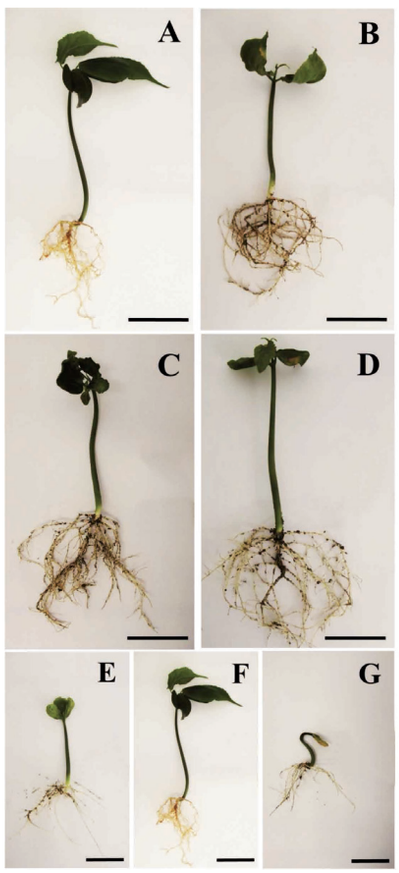

Figure 3. Seedlings of sacha inchi maintained in different substrate (sand – A and vermiculite – B), light (continuous light – C and 12-h photoperiod – D) and temperature (25 – E, 30 – F and 35 °C – G) conditions. Bars = 5 cm.

Conclusions

The sacha inchi seed germination is improved by substrates with higher surface contact, presence of light and by temperatures between 25 and 35 °C. Both survival and growth of sacha inchi seedlings are favored by vermiculite, continuous light and temperature at 30 °C. We consider the propagation of sacha inchi by seeds a feasible technique.

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References

BORDIGNON, S.R.; AMBROSANO, G.M.B.; RODRIGUES, P.H.V. Propagação *in vitro* de Sacha inchi. *Ciência Rural*, v.42, n.7, p.1168-1172, 2012. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782012000700005

BRANCALION, P.H.S.; NOVEMBRE, A.D.L.C.; RODRIGUES, R.R. Temperatura ótima de germinação de sementes de espécies arbóreas brasileiras. *Revista Brasileira de Sementes*, v.32, n.4, p.15-21, 2010. http://www.scielo.br/scielo.php?pid=S0101-31222010000400002&script=sci_arttext

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS, 2009. 395p. http://www.agricultura.gov.br/arq_editor/file/2946_regras_analise__sementes.pdf

CARVALHO, N.M.; NAKAGAWA, J. *Sementes*: ciência, tecnologia e produção. 4 ed. Jaboticabal: FUNEP, 2000. 424p.

CÉSPEDES, E.I.M. Cultivo de Sacha Inchi. Tarapoto, San Martin, Peru: INIIA, Subdirección De Recursos Geneticos Y Biotecnología, 2006. 11p.

FOLLEGATTI-ROMERO, L.A.; PIANTINO, C.R.; GRIMALDI, R.; CABRAL, F.A. Supercritical CO_2 extraction of omega-3 rich oil from Sacha inchi (*Plukenetia volubilis* L.) seeds. *Journal of Supercritical Fluids*, v.49, n.3, p.323-329, 2009. http://www.sciencedirect.com/science/article/pii/S0896844609001119

MACHADO, E.C.; MEDINA, C.L.; GOMES, M.M.A.; HABERMANN, G. Variação sazonal da fotossíntese, condutância estomática e potencial da água na folha de laranjeira 'valência'. *Scientia Agricola*, v.59, n.1, p.53-58, 2002. http://www.scielo.br/pdf/sa/v59n1/8073.pdf

MAGUIRE, J.D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, v.2, n.1, p.176-177, 1962.

MARTINS, C.C.; MACHADO, C.G.; SANTANA, D.G.; ZUCARELI, C. Vermiculita como substrato para o teste de germinação de sementes de ipê-amarelo. *Semina: Ciências Agrárias*, v.33, n.2, p.533-540, 2012. http://www.uel.br/revistas/uel/index.php/semagrarias/article/view/6370

NOGUEIRA, R.J.M.C.; ALBUQUERQUE, M.B.; SILVA JUNIOR, JF. Efeito do substrato na emergência, crescimento e comportamento estomático em plântulas de mangabeira. *Revista Brasileira de Fruticultura*, v.25, n.1, p.15-18, 2003. http://www.scielo.br/pdf/rbf/v25n1/a06v25n1

O'NEILL, G.A; DAWSON, I.; SOTELO-MONTES, C.; GUARINO, L.; GUARIGUATA, M.; CURRENT, D.; WEBER, J.C. Strategies for genetic conservation of trees in the Peruvian Amazon. *Biodiversity & Conservation*, v.10, n.6, p.837-850, 2001. http://link.springer.com/article/10.1023%2FA%3A1016644706237

ROBERTS, E.H. Predicting the storage life of seeds. *Seed Science and Technology*, v.1, n.4, p.499-514, 1973. http://www.scielo.br/scielo.php?script=sci_nlinks&ref=000079&pid=S0101-31222007000300024000018&lng=en

RODRIGUES, P.H.V.; BORDIGNON, S.V.; AMBROSANO, G.M.B. Desempenho horticultural de plantas propagadas *in vitro* de Sacha inchi. *Ciência Rural*, v.44, n.6, p.1050-1053, 2014. http://www.scielo.br/scielo.php?pid=S0103-84782014000600016&script=sci_arttext

ROSA, R.L.; QUIJADA, J. Germinación del sacha inchi, *Plukenetia volubilis* L. (McBride, 1951) (Malpighiales, Euphorbiaceae) bajo cuatro diferentes condiciones. *The Biologist*, v.11, n.1, p.9-14, 2013. http://sisbib.unmsm.edu.pe/bvrevistas/biologist/v11_n1/pdf/a2v11n1.pdf

SODRÉ, G.A.; CORÁ, J.E.; SOUZA-JÚNIOR, J.O. Caracterização física de substratos à base de serragem e recipientes para crescimento de mudas de cacaueiro. *Revista Brasileira de Fruticultura*, v.29, n.2, p.339-344, 2007. http://www.scielo.br/pdf/rbf/v29n2/27.pdf

VÁZQUEZ-YANES, C.; OROZCO-SEGOVIA, A. Ecological significance of light controlled seed germination in two contrasting tropical habitats. *Oecologia*, v.83, n.2, p.171-175, 1990. http://link.springer.com/article/10.1007%2FBF00317748

ZAMITH, L.R.; SCARANO, F.R. Produção de mudas de espécies das restingas do município do Rio de Janeiro, RJ, Brasil. *Acta Botanica Brasilica*, v.18, p.161-176, 2004. http://www.scielo.br/pdf/abb/v18n1/v18n1a14.pdf