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Germinative capacity of native plant species with forage potential under tropical rainforest conditions at the mountain-foot

Capacidade germinativa de plantas nativas com potencial forrageiro em condições de floresta tropical

Faver Álvarez [1] (ORCID 0000-0003-4282-1782), Armando Sterling[2] (ORCID 0000-0002-9955-9175), Paula Andrea Rios Parra[1] (0000-0002-7436-4013)

[1] Forage Evaluation and Animal Nutrition Laboratory, University of the Amazon, Caquetá, Colômbia. * Author for correspondence: p.rios@udla.edu.co
[2] Amazonian Institute of Scientific Research Sinchi, Faculty of Basic Sciences, University of the Amazon, Caquetá, Colombia.

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ABSTRACT

The humid tropics harbor great biological diversity, in addition to native species with forage potential as alternatives for animal feed. However, using trees and shrubs and having plantations are limited by the absence of information on their growth and seed germination. Therefore, the goal was to evaluate the germination capacity of native plant species with forage potential in tropical rainforest conditions, selecting eight species with important nutritional value: *Bauhinia tarapotensis, Crescentia cujete, Zygia longifolia, Cecropia ficifolia, Piptocoma discolor, Piper bredemeyeri, Calathea lutea and Heliconia rostrata.* Two treatments were evaluated: T1: Seeds (sexual and asexual) immersed for 12 hours in water, and T2: Seeds (sexual and asexual) without any pre-germination treatment (control). Significant differences were found in the germination percentage among species, treatments and the interaction of the two factors ($p < 0.01$). The species *C. cujete, C. ficifolia* and *P. discolor* presented better germination percentages with T1, in contrast to *P. bredemeyeri*, which adapted better to T2. In the same sense, *H. rostrata* (93%), *C. cujete* (91%) and *Z. longifolia* (89%) with T1, were the species with the highest germination capacity when compared to the other species, being desirable for use as fodder in agricultural production systems, due to their high germination power. In general, the pre-germinative treatment with water was very effective in increasing the permeability of the seed coat and, therefore, favored the breaking of endogenous physiological dormancy.

KEYWORDS: Feeding; latency; wooded ecosystems; dormancy; restoration; reproduction; propagation.

RESUMO

Os trópicos úmidos abrigam uma grande diversidade biológica, bem como espécies nativas com potencial forrageiro como alternativas para a alimentação animal. No entanto, a implementação de árvores e arbustos e o estabelecimento de plantações são limitados pela falta de informação sobre o seu crescimento e germinação de sementes. Por conseguinte, o objetivo era avaliar a capacidade germinativa das espécies vegetais nativas com potencial forrageiro em condições de floresta tropical, selecionando oito espécies com importante valor nutricional: *Bauhinia tarapotensis, Crescentia cujete, Zygia longifolia, Cecropia ficifolia, Piptocoma discolor, Piper bredemeyeri, Calathea lutea e Heliconia rostrata.* Foram avaliados dois tratamentos: T1: Sementes (sexuais e assexuais) imersas durante 12 horas em água, e T2: Sementes (sexuais e assexuais) sem qualquer tratamento pré-germinativo (controle). Foram encontradas diferenças significativas na percentagem de germinação entre espécies, tratamentos e a interação dos dois fatores ($p < 0,01$). As espécies *C. cujete, C. ficifolia e P. discolor* apresentaram melhores percentagens de germinação com o T_1, em contraste com *P. bredemeyeri* que melhor se adequou ao T_2. No mesmo sentido, *H. rostrata* (93%), *C. cujete* (91%) e *Z. longifolia* (89%) com T_1, foram as espécies com maior capacidade germinativa quando comparadas com as outras espécies, sendo desejáveis para utilização como forragens em sistemas de produção agrícola, devido ao seu elevado poder germinativo. Em geral, o tratamento pré-germinativo com água foi muito eficaz para aumentar a permeabilidade do tegumento da semente, e por isso favoreceu a superação da dormência fisiológica endógena.

PALAVRAS-CHAVE: Alimentação; latência; ecossistemas florestais; dormência; restauração; reprodução; propagação

INTRODUCTION

The humid tropics harbor great biological diversity worldwide (WILSON 2005). The Colombian Amazon region has one of the largest natural wooded ecosystems in the country, with a great variety of flora and a plethora of useful species that provide raw materials for industry, as well as tree species with forage potential for animal feeding. This could contribute to the nutritional diet of livestock production systems that currently exist in the Amazon region. In this sense, one of the most relevant agricultural production proposals for the tropics is including multipurpose trees and shrubs in livestock farming as a viable alternative to achieve ecological and productive stability in livestock areas (SUÁREZ & ORJUELA 2011).

Livestock systems in the Colombian Amazon piedmont, particularly in Caquetá, have been based on the inclusion of fodder tree species due to how easy it is to obtain seeds, their adaptation methods, the fodder's yield and the nutritional quality reported in varied research for the region (CIPAGAUTA HERNÁNDEZ & ORJUELA CHAVES 2003, SUÁREZ et al. 2008b, SUÁREZ et al. 2008a). Moreover, the knowledge generated on native trees and shrubs with forage potential has been based on nutritional quality (CIPAGAUTA HERNÁNDEZ & ORJUELA CHAVES 2003, SUÁREZ & ORJUELA 2011) and the selectivity of native trees and shrubs in stubble areas (ORJUELA et al. 2015). However, the knowledge generated on these species in terms of propagation (reproduction and germination) is limited or nonexistent. Therefore, there is limited knowledge on the conditions required for successful germination and for establishing plantlets of diverse species of wild trees with an agricultural interest (ALVARADO-LÓPEZ et al. 2014).

Seed germination is a complex process that leads to lengthening a seed's embryonic axis, allowing the seedlings' subsequent emergence. Furthermore, finalizing germination requires activating a complex regulation system that is affected by intrinsic factors (i.e., the vigor of the embryo) and extrinsic factors (i.e, environmental conditions, such as temperature, oxygen and the availability of water) (SU et al. 2016). The germination characteristics of especially valuable tree species have significant implications for conservation, introduction to production systems and establishing plantations (LIU et al. 2017). Nevertheless, the germination requirements or patterns of the seeds within a species often vary in their response to small differences in environmental conditions (ABE & MATSUNAGA 2011, BEVINGTON 1986, HONĚK & MARTINKOVÁ 1996, HUMARA et al. 2000, SHIMONO & KUDO 2003); this is considered the result of the integration of genetic and environmental factors (KELLER & KOLLMANN 1999, MEYER et al. 1989). Likewise, the processes associated with germination, establishing seedlings and their survival are important factors that determine the dynamics of plant regeneration (HARPER 1977, WILLSON & TRAVESET 2000, LEVINE & MURRELL 2003, BONJORNE DE ALMEIDA & GALETTI 2007).

Knowing the forms of germination of plant species with forage potential in the Amazon region would allow improving the diets of animals in livestock production systems. In addition, including these species will favor transforming these systems towards sustainability and resilience against climate change. Therefore, the objective of this study was to evaluate the germinative capacity of eight native plant species with forage potential in response to two pre-germination treatments in tropical rainforest conditions.

MATERIAL AND METHODS

Study area

This work was carried out at the Amazonian Research Center CIMAZ – Macagual "Cesar Augusto Estrada González" (1°37' N and 75°36' W) at 300 m.a.s.l located 22 km from Florencia, in the south of the department of Caquetá (Colombian Amazon), with about 380 ha dedicated to livestock farming and some agroforestry arrangements. The study area has an annual average precipitation of 3,793 mm, sunshine of 1,707 hours/ year^{-1}, an average temperature of 25.5 °C and a relative humidity of 84% (ESTRADA & ROSAS 2007). This research center is in a Tropical Rainforest life zone (TR) defined by HOLDRIDGE (1987). It has the characteristics given to it in the Afm group (Warm Tropical Rainforest) in the climate classification according to KÖPPEN & GEIGER (1936).

Plant material

In accordance with CIPAGAUTA HERNÁNDEZ & VELÁSQUEZ (2004), the eight plant species with forage potential selected for this study were: *Bauhinia tarapotensis, Crescentia cujete, Zygia longifolia, Cecropia ficifolia, Piptocoma discolor, Piper bredemeyeri, Calathea lutea* and *Heliconia rostrata*. These species have desirable traits when it comes to their percentage of Crude Protein (CP) and In Vitro Dry Matter Digestibility (IVDMD) (nutritional variables). Moreover, they have two growth habits: perennial ligneous (native trees and shrubs) and native herbaceous plants (Table 1).

Table 1. Studied plant species with forage potential. Source: prepared by author.

Serial #	Names		Growth habit	Nutritional Variable		Type of Seed	Source
	Common	Scientific		CP	IVDMD		
1	Pate vaca	*Bauhinia tarapotensis* Benth. ex JF Macbr	Tree	21.6	47.9	Sexual	CIPAGAUTA HERNÁNDEZ & VELÁSQUEZ (2004)
2	Totumo	*Crescentia cujete* Bureau & K. Schum.	Tree	10.6	80.4	Sexual	CIPAGAUTA HERNÁNDEZ & VELÁSQUEZ (2004)
3	Carbon	*Zygia Longifolia* (Humb. & Bonpl. ex Willd.) Britton & Rose	Tree	14.1	17.3	Sexual	CIPAGAUTA HERNÁNDEZ & VELÁSQUEZ (2004)
4	Yarumo	*Cecropia ficifolia* Warb. ex Snethl.	Tree	30.6	53.8	Asexual	SUÀREZ & ORJUELA (2011)
5	Boca de Indio	*Piptocoma discolor* (Kunth) Pruski	Tree	12.4	***N/A	Sexual	ÁLVAREZ *et al.* (Unpublished)
6	Cordoncillo	*Piper bredemeyeri* (J. Jacq.) Trel. y Yunck.	Shrub	13.7	50.2	Asexual	CIPAGAUTA HERNÁNDEZ & VELÁSQUEZ (2004)
7	Bijao	*Calathea lutea* (Aubl.) Schult.	Herbaceous	24.2	64.6	Asexual	CIPAGAUTA HERNÁNDEZ & VELÁSQUEZ (2004)
8	Heliconia	*Heliconia rostrata* WJ Kress	Herbaceous	21.5	60.7	Asexual	CIPAGAUTA HERNÁNDEZ & VELÁSQUEZ (2004)

*CP%: Crude Protein; **IVDMD%: *In Vitro* Dry Mater Digestibility; ***N/A: Not Applicable.

Seeds of the eight species were collected in their natural habitat (secondary forests) in CIMAZ – MACAGUAL “Cesar Augusto Estrada González” conservation areas, where seeds of sexual and asexual reproduction were obtained due to the lack of plant material in some plant species with forage potential, being selected as follows:

i) sexual seeds

were collected directly from the ground and from branches using shears, through careful cuts to avoid losing the seed due to inadequate handling

ii) the asexual reproduction seeds (stumps)

were collected by bevel cuts, which were then packed in properly labeled paper bags. The collected seeds (sexual and asexual) were transferred to the plant nursery’s seedbeds (which are 25 cm tall and 10 m long, made of cement blocks and with fine sand substrates) located at the Amazonian Research Center CIMAZ-MACAGUAL.

The seeds (sexual and asexual) of the eight species placed in the seedbeds were initially prepared, cleaned (organic material extraction) and submitted to solar radiation reduction with a polyshadow net at 75% semi-darkness.

Experimental design

The experiment was carried out in a completely random design, with an 8×2 factorial arrangement (factor 1: species, and factor 2: pre-germination treatment) and three repetitions per each factorial combination. The treatments were T_1: seeds (sexual and asexual) submerged for 12 hours in water at room temperature, and T_2: seeds (sexual and asexual) without any pre-germination treatment (control) (Figure 1).

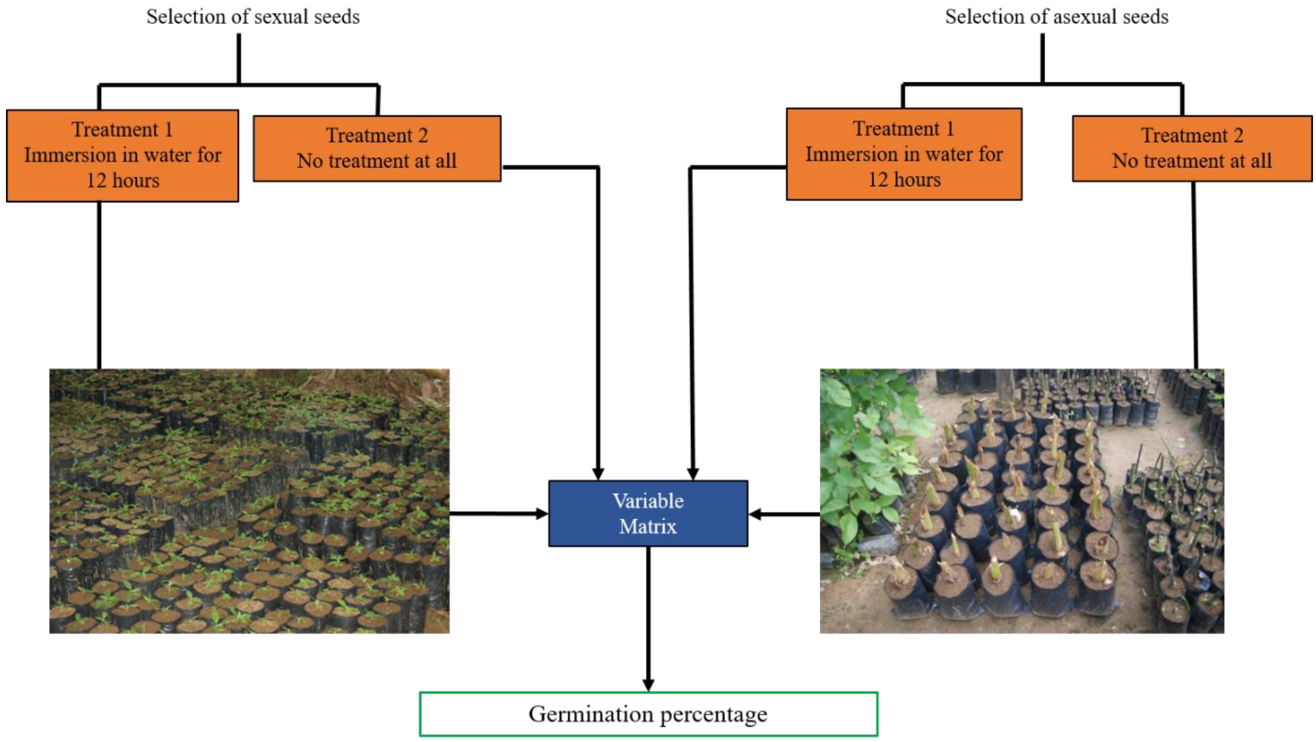


Figure 1. Conceptual model of germination percentage based on treatments applied to sexual and asexual seeds of plants with forage potential.

Evaluation of germination capacity

The germination capacity of the eight species was evaluated during the 45 days (February to March 2018) from the germination percentage of seeds treated in seedbeds with the double analysis method according to the International Standards for Seed Testing (ISTA) (ISTA 2016). Thus, 4,800 seeds of eight different species were taken across two treatments, with three replicates, where each experimental unit was a sample of 100 seeds. Then, each sample was placed in a seedbed for evaluation. The days of germination per species and per pre-germination treatment were recorded. The germination percentage was calculated with the following equation (Equation 1):

$$\%G. = \frac{\text{Number of germinated seeds}}{\text{Number of planted seeds}} \times 100 \quad (1)$$

where:
% G = germination percentage

Data analysis

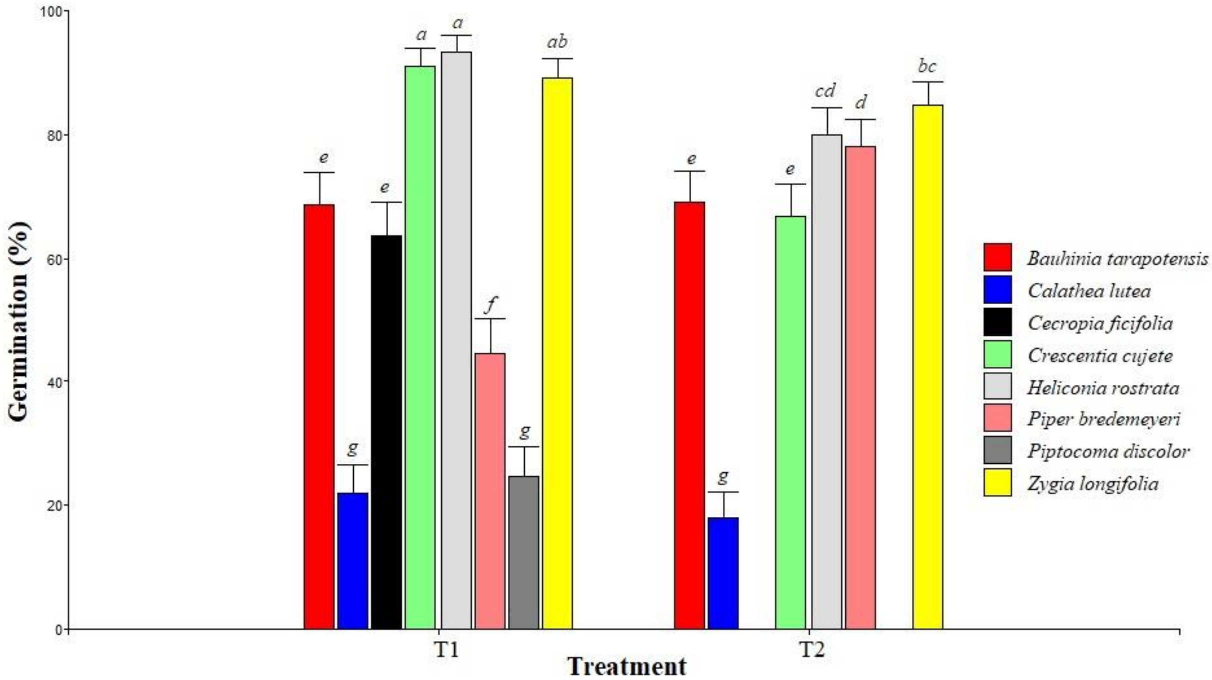
A Generalized Linear Model (GLM) was fitted to evaluate the fixed effect of species, treatment and the interaction between them. The binomial distribution with a logit link function was used in the GLM. A Fisher's LSD test with a significance level of 5% was used to compare the means of the fixed effects in the model.

A hierarchical cluster analysis using the Euclidean distance and Ward's method applied on the first two axes of a principal components analysis (PCA) of the germination and days to germination variables combined by treatment for the eight species allowed classifying the species according to their germinative capacity. Finally, a principal component analysis (PCA) with a Monte Carlo test (1,000 simulations) at a 5% significance allowed organizing and exploring the relationships between species and studies variables according to the applied treatment. The GLM was adjusted with the *nlme* package (PINHEIRO et al. 2018) in the R 4.0.3 software (R CORE TEAM. 2020) using the interface of InfoStat v. 2020 (DI RIENZO et al. 2020). The cluster analysis on PCA was performed with the *FactoMineR* package (HUSSON et al. 2020) and the PCA with the Monte Carlo test was performed with the *ade4* package (DRAY et al. 2020). using R 4.0.3.

RESULTS AND DISCUSSION

Significant differences were found in the mean germination percentage between species, treatments and interaction between both factors (p<0.01). In this sense, the species with the highest germination capacity regardless of the pre-germination treatment were: sexual seeds *Z. longifolia* (86%), *C. cujete* (65%), *B. tarapotensi* (68%) and asexual seeds *H. rostrata* (81%) and *P. bredemeyeri* (78%) (Figure 2). Regardless of the species, the T1 treatment (seeds submerged in water for 12 hours) showed a higher germination percentage effectiveness (62%) compared to T2 (seeds without any pre-germination treatment), which showed a germination percentage of 49%. This demonstrates the efficiency of water on the speed of seed growth, without forgetting that species such as *B. tarapotensi* tend to generate a condition of tegumentary dormancy, something very common in leguminous plants (YÉPEZ & ARBOLEDA 2009). This was attributed to the controlled hydration process, which allows recovering viability and increasing longevity, accelerating germination and preparing the seeds to adapt to the environmental conditions (SÁNCHEZ et al. 2001). It is in turn promoted by priming treatments, favoring the activation of many biochemical and physiological processes related to germination, including tolerance to stress and self-repairing cell membranes (BEWLEY & BLACK 1982, HEYDECKER & COOLBEAR 1977).

The species*treatment interaction (Figure 2) showed that the differences in the means of germination percentages between pre-germination treatments did not differ between seed types but were influenced by the species (p<0.05) since by activating the phytochrome protein that activates the endosperm and, in turn, regulates temperature, it generates a positive germination rate (BEWLEY & BLACK 1982).



Values correspond to the means and standard error. Means followed by the same letter do not differ statistically (Fisher's LSD test, p> 0.05).

Figure 2. Germination percentage (%) of eight native plants with forage potential under two pregermination treatments with (+) and without (-) water.

The species *H. rostrata, C. cujete* and *Z. Longifolia* had the highest germination capacity with T1 (seeds submerged in water for 12 hours), with germination percentages of 93%; 91% and 89%, respectively (Figure 1). However, the germination capacity of *H. rostrata* and *Z. Longifolia* did not differ significantly from that observed for T2 (seeds without any pregerminative treatment), with germination percentages of 80% and 85%.

In the same way, *B. tarapotensis* and *C. lutea* did not present significant differences between the two pre-germination treatments (p<0.05), while *C. cujete, C. ficifolia* and *P. discolor* showed a significantly higher germination percentage with T_1 (P<0.05), unlike *P. bredemeyeri,* which adapted better to T_2 (P < 0.05) with a higher germination percentage (77%) compared to T_1 (44%). (It is the only report for the moment).

VALLEJOS et al*.* (2009) propose that asexual seeds depend on the tree, since the cuttings and their sections must be appropriate for the germination process. Another influencing factor in this species' germination may have been excess humidity (treatment with water), acting as a cleanse from substances that could inhibit germination (PADILLA et al*.* 2012) and delay the germination process, keeping the radicle from emerging. Adequate rooting releases biological processes, such as water intake and the emergence of radicles, where it grows, extends and penetrates the structures around it (MORENO et al. 2006).

Germination of *P. discolor* seeds under natural conditions requires almost a quarter of a year for seedlings to emerge (STIMM et al. 2008). However, *P. discolor* was the only species that presented dormancy until day 30, unlike the other species, but showed reliability in the germination process with pregerminative treatments. ABRIL-SALTOS et al. (2017) evaluated the germination percentage of *P. discolor* under pre-germination treatments with gibberellic acid, in which they submerged *P. discolor* seeds for one hour. The result was a germination percentage of 6.%. This percentage is much lower than the one in our work, which was 24%. The work by MENDOZA & LEÓN (2012) reported a *P. discolor* germination percentage of 95%. This result differs from the result obtained in our experiment. It can be inferred that two factorswo factors cause this difference cause this difference: *i*) immediately planting the seeds after obtaining them and *ii*) environmental factors, such as humidity, temperature, oxygen and light.

The hierarchical cluster analysis (Euclidean distance and Ward's method) performed on the first two principal components of the germination and days to germination variables combined by treatment generated three groups of species (Figure 3). Group 1 was comprised of the species *C. ficifolia, P. bredemeyen, Z. longifolia, C. cujete* and *B. tarapotensis,* which had intermediate germination percentages (31%; 61%; 86%; 78% and 68%, respectively). Group 2 included species *H. rostrate,* with the highest germinative capacity (86 %), and group 3 was comprised of the species with the lowest germinative capacity, *P. discolor* and *C. lutea* (12%; 19%) (Fig. 3).

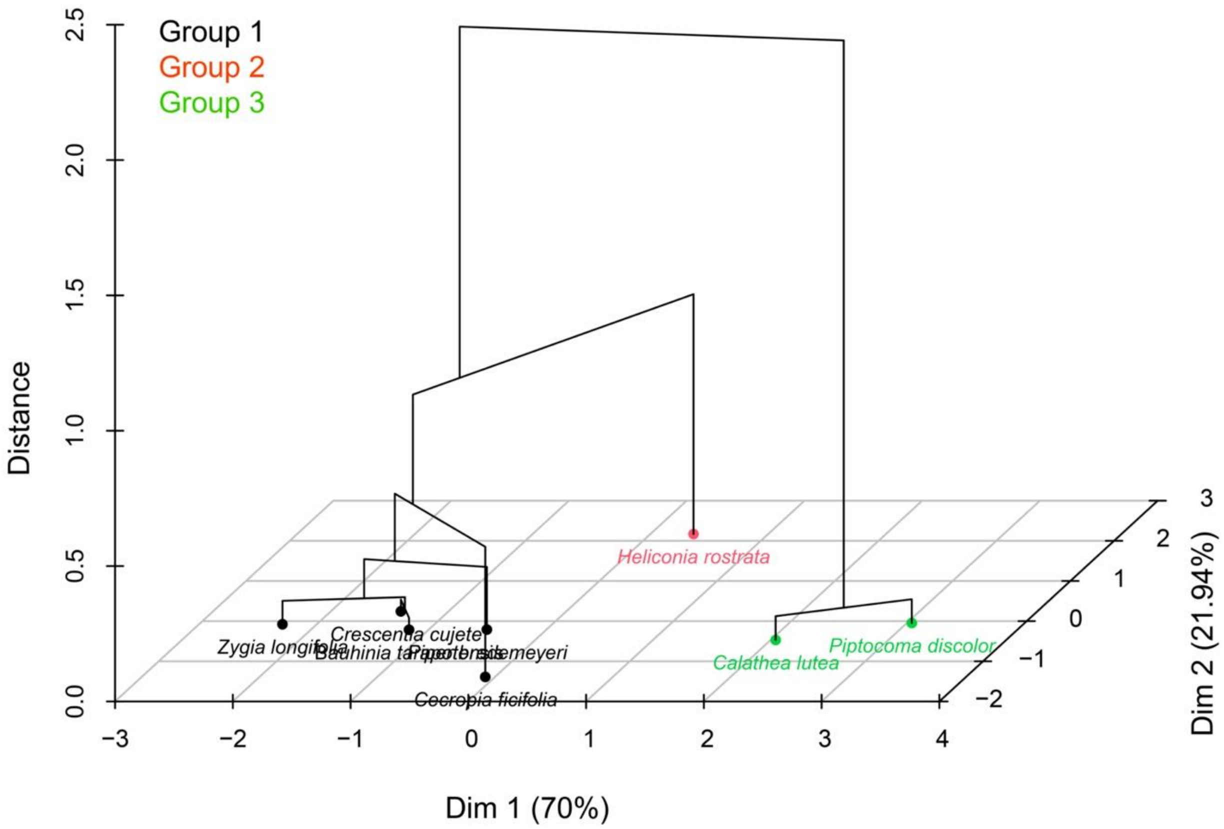
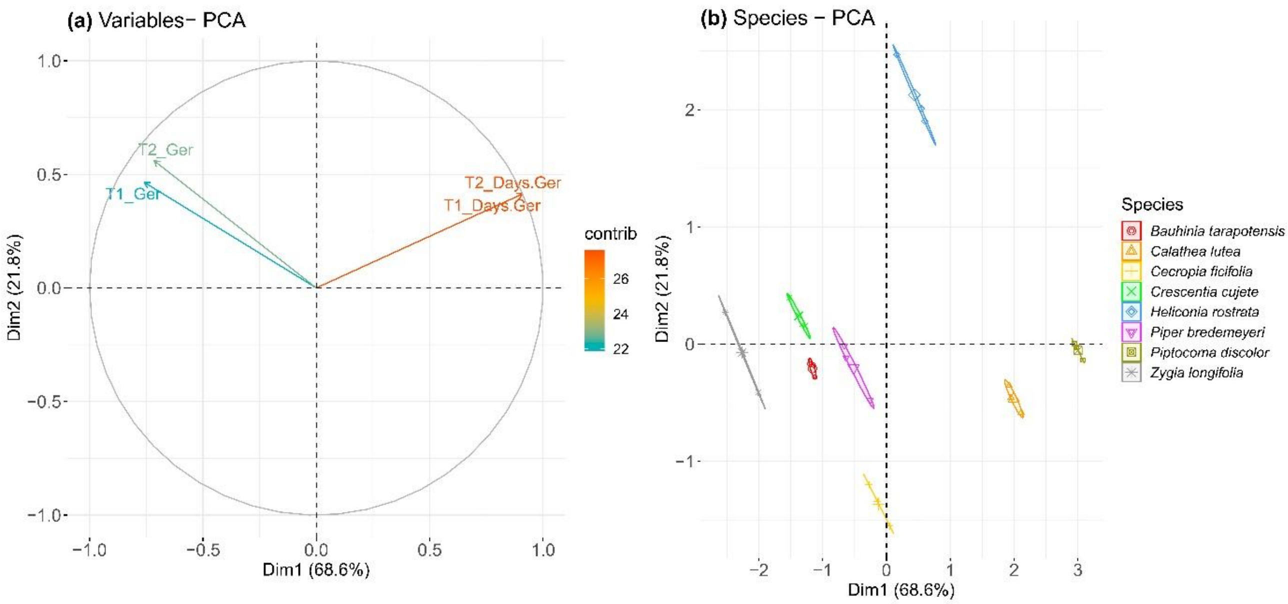


Figure 3. Hierarchical clustering on principal component analysis (PCA) of eight native plants species with forage potential based on germination percentage and days to germination variables.

The principal component analysis (PCA) performed with the germination and days to germination variables combined with the two pre-germination treatments accounted for 90 % of total variability for the first two components. The first principal component accounted for 68% of variability and separated the species with the highest germination times in both treatments (*C. lutea* and *P. discolor*), the species that germinated in the least amount of time (*Z. longifolia*, *C. cujete*, *B. tarapotensis* and *P. bredemeyeri*) and those that had a favorable germinative capacity. The second principal component accounted for 22% of variability and separated the species *H. rostrata* as the highest in terms of germination percentage for both treatments, in contrast to the species *C. ficifolia,* which had a lower germinative potential. High significant differences were observed in terms of germination percentages and times between the eight species (p<0.01) (Figure 4).

The results showed the efficiency of the *Z. longifolia* species, taking into account the ratio percentage*days of germination, since for T1 (89%) and T2 (85%) it only needed 3 days to break dormancy and initiate its development. Unlike the species *P. discolor*, which needed 30 days for germination, ending with a percentage of 25% for T1, however, for T2, together with the species *C. ficifolia*, did not germinate (0%) being omitted from the analysis.

However, *B. tarapotensis* showed germination with or without treatment and an activation time of 8 days, results similar to those reported by VARGAS et al. (2015) with an imbibition for day 8 and control treatment as the best germination percentage.



a) Correlations between the germination percentage and days to germination variables combined with treatment.
b) Ordination of plant species.

Figure 4. Projection of the first two axes on a principal component analysis of the germination percentage and days to germination variables on the factorial plane, combined with treatments to organize the plant species.

The conditions for germination processes are variable and depend on biological factors. However, according to the obtained results, pre-germination treatments in water have effects on the germination percentage of asexual and sexual seeds, as demonstrated (62%), in contrast to treatment two, in which no type of leaching was performed (49%). This yielded a positive result for implementation in forage systems aimed at improving animal consumption.

The usefulness of the seeds of native species, turn out to be alternatives in agricultural scenarios such as traditional animal production systems, since, by improving the speed of growth of these, will provide a fast and effective propagation (VARGAS et al. 2015). Generating feed that will be of great benefit to the nutritional requirements of the animal. In addition to counteracting environmental impacts by reducing the rate of deforestation and the loss of biodiversity, soil compaction and erosion (ALONSO 2011).

CONCLUSION

This study concluded that the pre-germinative treatment with water was the most effective in increasing seed germination capacity. This effect was more evident in *C. cujete* (91%), *C. ficifolia* (64%) and *P. discolor* (24%). compared to the treatment without water (67%, 0% and 0%, respectively). The species *H. rostrata* (93%), *C. cujete* (91%) and *Z. longifolia* (89%) under the water treatment had the highest germination capacity and their germination days were few to break dormancy. This indicates that these three species have a high germination level to be used as forage species in agroforestry systems for livestock production in the conditions of the Andean-Amazonian foothills of Colombia. The species P discolor presented 30 days to initiate germination. However, this species is very promising because of its good nutritional value as a source of feed for cattle in the region.

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