

Scarification and gibberellic acid in the germination and initial development of pindaíba (*Duguetia lanceolata* ST Hil)

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Abstract

Duguetia lanceolata St. Hill is a species native to the Atlantic Forest. Its seeds contain substances that have a germination inhibiting effect. In view of the above, the present work aimed to evaluate the influence of gibberellic acid and mechanical scarification on germination and seedling development of the *D. lanceolata*. This work was developed at IFSULDEMINAS - Campus Muzambinho, MG, Brazil. The seeds were obtained from fruits collected from adult plants. The experimental design was in randomized blocks, in a 5x2 factorial scheme with three replications with eighteen plants per plot. The treatments consisted of different concentrations of gibberellic acid (0, 5, 10, 15 and 20 mg L⁻¹) and mechanical scarification (presence and absence). Leaf number, stem diameter and germination percentage were evaluated. The data were subjected to analysis of variance, the significant difference between treatments being determined by the F test and later, the qualitative variables were analyzed by the Scott-Knott test, and the quantitative variables by regression, both at the 5% level. The gibberellic acid and scarification do not influence the stem diameter and the number of seedling leaves. There was a linear increase in the percentage of germination with increasing doses of gibberellic acid up to the concentration 20.0 mg L⁻¹ when scarification did not occur (35.19%). Quadratic behavior occurred with gibberellin doses when the seeds undergo mechanical scarification with a maximum dosage of 10.0 mg L⁻¹ and corresponding germination of 42.59%. Mechanical scarification promoted an increase in germination when GA₃ was not applied, and at the dosage of 10.0 mg L⁻¹.

Keywords: Annonaceae; GA₃; Radicle protrusion; Break dormancy.

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Introduction

According to Carvalho (2006), native species can be an alternative to solve social problems. In this way, native plants must occupy new market niches, diversifying income for farming families, combining economic development with environmental conservation.

The plant species of the *Annonaceae* family was described in 1789 and are plants generally distributed among tropical areas of the American, African and Asian continents. This family has about 2,500 species distributed in approximately 135 genera (CHATROU et al., 2012). Of the genera that make up the *Annonaceae* family, 34 can be found in South America, with predominance of the genera *Annona* L., *Duguetia* St. Hil., *Guatteria* Ruiz et Pavon, *Rollinia* St. Hil and *Xylopia* L. In Brazil, there are 29 genera, including *Duguetia* spp. with 50 species out of the 70 cataloged (RIBEIRO et al., 2002).

Duguetia lanceolata St. Hill is a native species of the Brazilian closed Atlantic Forest, popularly known as pindaíba, pindaíva, pindaúva and perovana (RODRIGUES; CARVALHO, 2001). The *D. lanceolata* is in the official list of Brazilian flora species threatened with extinction in the state of São Paulo, and is considered to be in danger in Rio Grande do Sul (NCFLOA, 2012).

The species is arboreal with 15.0 to 20.0 meters in height and 0.4 to 0.6 m in stem diameter, its trunk is straight and has a grayish-brown shell. The leaves are simple, glabrous, whitish, and scaly at the bottom; lanceolate, with smooth margin and cardboard texture, they are 2.5 to 3.0 cm wide and 6.0 to 12.0 cm long. The flowers are large and purple in color, the fruits are bacaceous, red, and very showy (MANICA et al., 2003).

The use of forest species presents many difficulties, that are caused by the lack of information about their cultivation, being necessary to expand the works in the propagation and production of seedlings (FERREIRA, 2000).

Seed germination is a biological phenomenon that can be considered by botanists as the resumption of embryo growth, with the subsequent rupture of the tegument by the radicle (ANDRADE et al., 1995). In this case, obtaining seed is, indispensably, the most important phase in the production process of seedlings of native species for reforestation, since native species are usually propagated via seminal (SENA, 2008).

Some native species have seminal dormancy mechanisms that prevent the germination process, even though the conditions are favorable. In these cases, for germination to occur, techniques are needed to stimulate germination. This blockage can occur at any stage of germination, by mechanisms related to the seed itself, or be induced by environmental or genetic effects (BENECH-ARNOLD et al., 2013).

The seeds of the *Annonaceae* family are orthodox, i.e., they tolerate long-term storage; however, they contain substances that have a germination-inhibiting effect, consequently causing dormancy, which, together with the impermeability and resistance of the tegument, provide antagonistic factors to the uniform and rapid germination (JOSE; SILVA; DAVIDE, 2007). According to Borghetti (2004), the embryo contained in the seeds of the species of the *Annonaceae* family has incomplete maturation and, in this case, the seeds do not germinate soon after dispersion, requiring a post-maturation time.

According to Taiz et al., (2017), the embryo's dormancy loss is often associated with a sharp drop in the ratio between the abscisic acid (ABA) and gibberellic acid (GA) phytohormones. Cunha and Casali (1989) point out that the inhibitory effect of ABA on germination can be reversed by

GA₃ when used in concentrations that exceed its content. One of the methods used to overcome physiological dormancy is the exogenous application of these phytohormones

Phytohormones are chemical messengers, produced in a cell, that modulate cellular processes interacting with specific proteins that function as receptors linked to signal transduction routes. Even at low concentrations, these phytohormones can activate responses in target cells (TAIZ et al., 2017).

According to Manica (2003), in addition to the physiological factors that prevent or hinder the germination of *Annonaceae* seeds, there is still physical dormancy. Among the physical characteristics, this dormancy is related to the density of the tegument, which causes the impermeability of the bark, making gas exchange difficult. The seed tegument has a high lignin content, then, some species of the family can take up to 180 days to germinate.

Scarification is the recommended method for overcoming physical dormancy, which aims to make the seed envelope more permeable to water absorption and gas exchange, as well as to facilitate the emergence of the root and plumule. Mechanical scarification consists of the friction of the seeds against an abrasive surface or by scraping a small part or section of the tegument (FACHINELLO; HOFFMANN; NACHTIGAL, 2000; CARVALHO, 2003).

Results of overcoming dormancy in *Annonaceae* seeds were observed by Campos et al. (2015), who studied the combination of physical scarification and gibberellic acid concentrations in biribá pre-germinative treatment (*Rollinia mucosa* (Jacq.) Baill). The authors observed a significant increase in the percentage of seed germination and in the initial growth of seedlings when physical scarification was used associated with GA₃ up to a maximum concentration of 1000.0 mg L⁻¹. Complementary results were obtained by Matias, Vilar, and Dantas (2018), who found better results in the germination of the *Annona cf. Montana* (Macfad) with the application of gibberellin and with the chemical scarification with Potassium Nitrate (KNO₃).

For nurseries and producers, the dormancy mechanism of *Duguetia lanceolata* it is a disadvantage, inducing the unevenness of seedlings and greater demand for production time, besides running a greater risk of loss of seeds due to deterioration.

In this context, this study aimed to evaluate the influence of the gibberellic acid and mechanical scarification on seed germination and the development of *Duguetia lanceolata* St. Hill seedlings.

Material and methods

Characterization of the experimental area

The experiment was carried out at the Forest Species Seedling Production Laboratory of the Federal Institute of Education, Science, and Technology of Southern Minas Gerais - Campus Muzambinho, MG, from July 2017 to July 2018.

The municipality of Muzambinho, that belongs to the southern region of the state of Minas Gerais, has geographical latitude coordinates: 21°20'59,94"S and longitude: 46°31'34.82"W, with an average altitude of 1,013.0 meters.

The region's climate is humid temperate with dry winter and moderately hot summer (Cwb), according to Köppen's classification (SÁ JUNIOR et al. 2012).

Treatments and experimental design

The experimental design was in randomized blocks (DBC), in a 5 x 2 factorial scheme containing three replicates with eighteen plants per plot. The experimental factors consisted of different concentrations of gibberellic acid (0, 5, 10, 15 and 20 mg L⁻¹) and mechanical scarification (presence and absence).

The seeds were divided according to the pre-established treatments. Mechanical scarification consisted of vigorous friction of the tegument with sandpaper No. 150 on the ends of the seeds. In the treatment with growth regulator, the seeds were kept soaked for 24 hours in gibberellic acid solutions, in the concentrations of each treatment. The seeds without the growth regulator were soaked in distilled water.

Experiment installation and conduction

The seeds used were obtained from fruits collected randomly from five adult plants of *Duguetia lanceolata* ST Hill, located on a rural property in the municipality of Muzambinho – MG.

The fully ripe fruits were manually pulped using a sieve and running water and, after extraction, the seeds were dried on paper and kept in a shaded place until the time of the experimental processes.

The seeds were soaked in the corresponding treatment for 24 hours. After imbibition, the seeds were sown in plastic bags of 1.5 liters, using as soil substrate ravine and bovine manure tanned in a 3 x 1 proportion enriched with 3.0 kg of simple superphosphate for each 1m³ of the substrate. The plastic bags were kept in nursery conditions with 50.0% shading and irrigation when necessary.

Characteristics evaluated

After 150 days, were evaluated the percentage of germination, number of leaves, and stem diameter. The seeds were considered germinated after the radicle protrusion. No destructive analysis was performed because the species is at high risk of extinction.

Statistical analysis

The data obtained were subjected to analysis of variance with the use of the SISVAR statistical software (FERREIRA, 2011), the significant difference between treatments being determined by the F test. Detecting differences between treatments, the means under the scarification factor were grouped by the Scott-Knott test at the level of 5% of significance, and the means referring to the GA dosage₃ were submitted to regression analysis.

Results and discussion

From the analysis of variance, it was found that there was an interaction between the factors studied for the variable percentage of germination. For the variables stem diameter and number of leaves, there was also no statistical difference between treatments for the isolated factors.

Data regarding the influence of the gibberellic acid and mechanical scarification on seed germination and seedling development of the *Duguetia lanceolata* ST Hill are shown in Table 1.

Table 1 – Percentage of seed germination, stem diameter (mm), and leaf number of *Duguetia lanceolata* ST. Hill subjected to different concentrations of gibberellic acid and mechanical scarification. Muzambinho/MG, 2018.

GA ₃ (mg L ^{-first})	Scarification					
	Presence		Absence		Absence	
	Germination (%)		Diameter (mm)		Number of leaves	
0	35.18 a*	27.77 b	2.06 a	2.13 a	4.86 a	5.13 a
5	36.33 a	31.48 a	2.28 a	1.97 a	4.33 a	4.53 a
10	42.59 a	29.63 b	1.82 a	2.16 a	5.06 a	5.13 a
15	37.04 a	33.33 a	2.00 a	2.47 a	5.73 a	5.57 a
20	29.63 a	35.18 a	1.86 a	2.04 a	4.40 a	6.73 a
CV (%)	16.36		18.64		17.65	

(*) Means followed by the same lowercase letter on the line do not differ significantly at the 5% level by the Scott-Knott test.

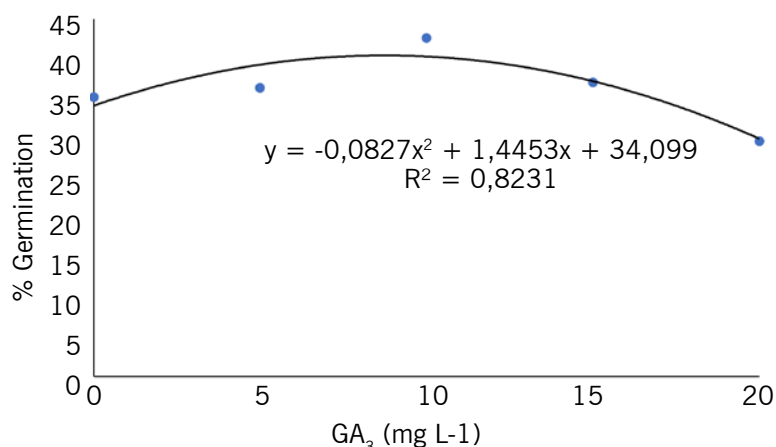
Source: Elaborated by the authors (2018).

At concentrations of 0.0 and 10.0 mg L⁻¹ from GA₃, the presence of mechanical scarification promoted a significant increase in the germination percentage of *D. lanceolata* when compared to the absence of the method (Table 1). This fact may be related to mechanical scarification that favors the weakening of the rigid seminal envelope, making it more porous, thus facilitating the development of the embryo.

These results corroborate those found by Ferreira et al. (2002) in *Annona squamosa* and Pereira et al. (2004) in *Annona crassiflora* Mart who obtained higher average germination in seeds that were scarified mechanically before imbibition in gibberellic acid. Lorenzi (2002) also noted that mechanical and chemical scarification in *A. crassiflora* Mart. provided an increase in the germination rate, reaching rates above 50.0 %. However, Campos et al. (2015) found no significant difference for the scarification of (*Rollinia mucosa* (Jacq.) Baill) after 85 days after sowing (DAS).

Figure 1 shows the regression analysis regarding the gibberellic acid dosage in the presence of scarification. The regression obtained quadratic behavior with a high coefficient of determination (82.3%). This result proves that the plants responded positively to the acid increase in the presence of scarification up to the maximum dosage of 8.27 mg L⁻¹. In higher dosages, however, there was a decrease in the germination percentage. This fact corroborates the hypothesis that plant hormones act only when present in low concentrations.

Figure 1 – Germination percentage of *Duguetia lanceolata* ST Hill subjected to mechanical physical scarification with sandpaper nº 150 and different dosages of GA₃ (Muzambinho, 2018).



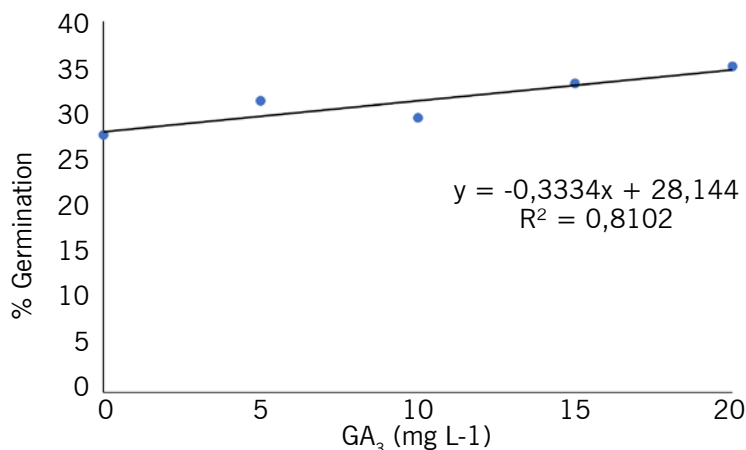
Source: Elaborated by the authors (2018).

Within plant cells, signal transduction routes never work in isolation, but operate as part of a complex network of signaling interactions (TAIZ et al., 2017). In this sense, it is worth mentioning that the hormone increases germination when the balance between ABA and GA₃ occurs. At higher dosages, hormonal imbalance and inhibition of the action site and consequently germination may occur.

Similar results were obtained by Oliveira et al. (2010) who, by testing the commercial gibberellic acid (0.1 ia) in the germination of atemoya seeds (*Annona cherimola* Mill. x *A. squamosa* L.) cv 'GEFNER,' obtained a decrease in germination at dosages above 750.0 mg L⁻¹ in soaking time of 36 hours.

Figure 2 shows the germination percentages in the seeds that were not scarified. Unlike the scarified ones, the regression found showed linear behavior with a high coefficient of determination (81.02%). There was an increase in germination compared to the control, regardless of the tested dose. In this case, the dose absorbed by the embryo did not cause any antagonistic reaction or hormonal imbalance.

Figure 2 – Germination percentage of *Duguetia lanceolata* ST Hill subjected to different dosages of GA₃ (Muzambinho, 2018).



Source: Elaborated by the authors (2018).

Gibberellin may be responsible for mobilizing the energy reserves present in the endosperm of seeds of various species (TAIZ et al., 2017). This may indicate that the exogenous application of gibberellic acid may alter the metabolism of the seed, prompting a premature supply of energy necessary for the development of the embryo. Since the ABA x GA ratio plays a decisive role in maintaining seed dormancy, it is believed that the environmental conditions that break dormancy fundamentally operate at the level of the genetic networks that affect balance. This affirmation is consistent with the fact that seed treatment with GA, in general, can replace a positive sign in breaking dormancy.

Different results were found by Vasconcellos (2015), who tested the increment of 1,000.0 mg L⁻¹ from GA₃ with the aid of a spray bottle on the *Annona squamosa* but did not obtain germination without physical scarification.

Both understanding and knowing the dormancy mechanism and the dormancy overcoming in seeds are relevant factors in the seedling production process (SILVA et al., 2011). According to the results obtained, gibberellic acid increased the percentage of germination regardless of whether scarification occurred or not. In the absence of application and with a dosage of 10.0 mg L⁻¹, scarification also boosted an increase in the germination rate. The germination percentages obtained with the hormonal application are still considered low from an agronomic point of view. Gómez-Castañeda et al. (2003) point out that the embryos of *Annonaceae* seeds are immature, and it takes at least six months for embryonic maturation to occur or for growth and differentiation promoting hormones such as auxins and cytokinin to be applied.

Conclusions

Gibberellic acid and mechanical scarification do not influence the stem diameter and the number of seedling leaves of *D. lanceolata*. However, germination is positively responsive to the implementation of both techniques as long as the plant's limit to hormonal increase is respected.

There was a linear increase in the germination percentage with increasing doses of gibberellic acid up to the maximum concentration tested 20.0 mg L⁻¹ when scarification did not occur (35.19%).

There is a quadratic behavior related to the doses of gibberellin when the seeds undergo mechanical scarification with a maximum point at a dosage of 8.27 mg L⁻¹, and corresponding germination of 40.39%.

Mechanical scarification boosted an increase in germination when GA₃ was not applied, and at a 10.0 mg L⁻¹ dosage.

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Escarificação e ácido giberélico na germinação e desenvolvimento inicial de pindaíba (*Duguetia lanceolata* ST Hil)

Resumo

A *Duguetia lanceolata* St. Hill é uma espécie nativa da Floresta Atlântica. Suas sementes contêm substâncias que possuem efeito inibidor de germinação. Diante do exposto, o trabalho teve como objetivo avaliar a influência do ácido giberélico e da escarificação mecânica na germinação e no desenvolvimento de plântulas de *D. lanceolata*. O trabalho foi desenvolvido no IFSULDEMINAS – Campus Muzambinho/MG. As sementes foram obtidas de frutos coletados de plantas adultas. O delineamento experimental foi em blocos casualizados, em esquema fatorial 5 x 2, com 3 repetições 18 plantas por parcela. Os tratamentos consistiram em diferentes concentrações de ácido giberélico (0, 5, 10, 15 e 20 mg L⁻¹) e escarificação mecânica (presença e ausência). Foram avaliados número de folhas, diâmetro de caule e porcentagem de germinação. Os dados foram submetidos à análise de variância, sendo a diferença significativa entre tratamentos determinada pelo Teste F e, posteriormente, analisadas pelo Teste de Scott-Knott as variáveis qualitativas e por regressão as quantitativas, ambas ao nível de 5 %. O diâmetro de caule e o número de folhas de plântulas não são influenciados pelo ácido giberélico e pela escarificação. Houve incremento linear na porcentagem de germinação com o aumento das doses de ácido giberélico até a concentração 20,0 mg L⁻¹, quando não ocorreu escarificação (35,19%). Registrou-se comportamento quadrático em relação às doses de giberelina quando as sementes passam por escarificação mecânica com máximo de uma dosagem de 10,0 mg L⁻¹ e germinação correspondente de 42,59 %. A escarificação mecânica promoveu incremento na germinação quando não houve aplicação de GA₃ e na dosagem de 10,0 mg L⁻¹.

Palavras-chave: Annonaceae; GA₃. Protusão da radícula. Quebra de dormência.

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