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Germination of *Aristolochia elegans* Mast. seeds at different temperatures and concentrations of gibberellin

Hugo Roldi Guariz[1]*, Gabriel Danilo Shimizu[1], Ítala Menegon Castilho[1], Jean Carlo Baudraz de Paula[1], Walter Aparecido Ribeiro Junior[1], Huezer Viganô Sperandio[2]

[1]State University of Londrina, Londrina, Brazil
[2]Federal University of the Jequitinhonha and Mucuri Valleys, Diamantina, Brazil
*Corresponding author, e-mail: hugo.guariz@gmail.com

Abstract

Aristolochia elegans is a plant widely appreciated for its attractiveness, being used as an ornamental species. However, information on the germination behavior of its seeds is scarce. Thus, the objective of the current work is to evaluate the germination of seeds of *A. elegans* under varying conditions of temperature and concentrations of gibberellin. The seeds were immersed in gibberellin solution at concentrations of 100, 200, and 300 mg L^{-1}, before being sown on blotting paper and transferred to germinators at constant temperatures of 15, 20, 25, 30, and 35°C. The percentage, speed index, mean time, and germination speed were evaluated. There was no interaction effect or isolated effect of gibberellin, only an isolated effect of temperature on all parameters evaluated. For germination, a maximum response was obtained at the estimated temperature of 29.14˚C, with 75% germination, and the minimum temperature was estimated at 16.9˚C. The mean germination speed increased with increases in temperature, up to a maximum of 29°C, and at temperatures above this value, the speed decreased. It is concluded that the temperature of 29.4˚C leads to the best germination, while at temperatures below 16.9˚C, the estimated minimum temperature, there is no seed germination. In addition, there is no effect of gibberellin on germination when doses are applied between 100 and 300 mg L^{-1}.

Keywords: *Aristolochiaceae*, gibberellic acid, soaking, optimum temperature, landscaping

Introduction

Aristolochiaceae is a family of the order *Piperales*, which comprises approximately 550 species and four genera, one of the most recognized being the *Aristolochia,* which encompasses shrubs, herbaceous plants, and gigantic lianas that are found in tropical and subtropical regions of the world (Melo et al., 2013). In the Brazilian territory, *Aristolochiaceae sensu stricto* is represented only by the genus *Aristolochia* L. with 93 species, 38 of which are endemic (Freitas & Araújo, 2017; Rebouças et al., 2020). In the Northeast region there are 30 species, with the Atlantic Forest as the center of richness of the genus in the country, with 49 recorded species (Rebouças et al., 2020).

The representatives of *Aristolochia* occur preferentially in drier environments and in shallow soils, such as in steppic savanna (open shrubby Caatinga, arboreal Caatinga, and carrasco), savanna (Cerrado), although, they have also been recorded in humid environments, such as dense rainforest (humid forest), seasonal deciduous forest (dry forest), and lowland semideciduous seasonal forest (plateau forest) (Maekawa et al., 2010; Rebouças et al., 2020).

As described by Nakonechnaya et al. (2018), the genus *Aristolochia* is a promising source of new pharmaceutical agents, as these plants contain a wide range of biologically active components, such as fargesin and cubebin (Arellanes et al., 2012) and methanol extracts of antitoxin activity (Izquierdo et al., 2010), and for having ornamental species for the formation of landscape compositions and interior gardening.

The *Aristolochia elegans* plant, popularly known as cipó-de-mil-homens and papo-de-turu, is used as an ornamental, mainly because of the curious appearance of its flowers and because it does not present an unpleasant odor given off by other flowers of species of

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the same genus. In cultivation, *A. elegans* can be used on pergolas, arbors, fences, or beside trees, whose branches will intertwine with those of the vine, which, if not pruned periodically, will suffocate the supporting tree (Capellari Junior, 2005). It is a perennial, fast-growing climbing herb that can reach three meters in height. The plant gives off a subtle odor, with alternating heart-shaped leaves, purple flowers with white dots. It reproduces by seeds, and is cultivated worldwide as an ornamental species.

It is recognized as an invasive species in Hawaii, New Caledonia, Coco Island, and Polynesia, also generating social, economic, and environmental impacts in Australia and is potentially invasive, with recommended eradication in Santa Cruz Island, in the Galápagos (Rentería et al., 2007). It is also known that in other parts of the world, such as Mexico, its wild populations are declining, and in some regions it has become extinct due to predatory collection, motivated by its pharmacological properties used in traditional Mexican medicine (Torres et al., 2007).

Capellari Junior (2005) describe Aristolochiaceae as plants that present a differentiated appearance, aggregating primitive characteristics in relation to stem morphology and evolved characteristics in relation to floral morphology. Also according to this author, they are little known, and could be used in landscaping, as they arouse people's interest. However, there are scarce literature data on these species regarding the biological properties of their seeds and the germination process in many species has not been fully studied (Nakonechnaya et al., 2018). Thus, the objective of the work is to evaluate the germination of seeds of *A. elegans* under varying conditions of temperature and concentrations of gibberellin.

Material and Methods

The seeds were collected in the area of the Laboratory of Biodiversity and Ecosystem Restoration (LABRE) (23°19'29.15"S, 51°11'53.57"W, elevation 604m), of the Campus of the State University of Londrina (UEL) and the study was conducted in the Seed Analysis Laboratory of the Agricultural Sciences Center belonging to the same institution. The plants were grown outdoors, composing the local landscaping and the seeds were harvested when the fruit was fully ripe and already open for the release of the seeds. The region's climate is classified according to Köppen as humid subtropical Cfa, with an average annual temperature of 22.1 °C and precipitation of 1290 mm.

The gibberellic acid - GA_3 (90%) powder was dissolved at concentrations of 100, 200, and 300 mg L^{-1}. Therefore, it was necessary to convert it into liquid form, with 70% alcohol. The necessary amounts of GA_3 were separated and placed in beakers, drops of alcohol were added until the complete dilution of the gibberellic acid. Afterwards, the solution was incorporated into 1 L of distilled water. The imbibition took place in the dark for 1h, in 200 ml beakers, lined with aluminum foil. For the germination test, the seeds were sown in colorless and transparent plastic boxes with a lid (Gerbox®), measuring 11 x 11 x 3.5 cm, on two sheets of blotting paper, with an added volume of distilled water of 2.5 times the weight of the paper to ensure maintenance of moisture (Brasil, 2009). The seeds were kept in a BOD germinator under a white fluorescent lamp with a photoperiod of 12 hours of light. The experiment lasted for 20 days, carried out between the months of October and November 2020, with daily counting of the germinated seeds. The weight of a thousand seeds and the moisture content of the seeds were determined as recommended by the Seed Analysis Rules (Brasil, 2009), and the results expressed in grams and percentages, respectively.

The design used was completely randomized with a 4 X 5 factorial arrangement, with four concentrations of gibberellin (0, 100, 200, and 300 mg L^{-1}) and five constant temperatures (15, 20, 25, 30, and 35 °C), distributed in 04 repetitions with 25 seeds each, kept in germinators.

Seeds were considered germinated when the radicle protrusion reached a size greater than 2 mm. At the end of the test, from daily data on the number of germinated seeds, the following variables were calculated: % of Germination (G) or Germinability, calculated using the formula of Labouriau and Valadares (1976); germination speed index (GSI), calculated using the formula of Maguire (1962); mean germination time (*t*) in days, calculated using the formula suggested by Ferreira and Borghetti (2013); and mean germination speed (MGS) in $days^{-1}$, calculated using the formula suggested by Ferreira & Borghetti (2013). Data were checked for normality of errors and homoscedasticity of variances using the Shapiro-Wilk and Bartlett tests, respectively. If the assumptions were met, the analysis of variance was performed at the 5% probability level. If significance was found for both isolated factors or interaction, multiple linear regression was performed, on the other hand, if significance was found for only one of the factors, polynomial regression or modified four-parameter Brain-Cousens log-logistic regression was performed, obtained by the BC.4 function of the drc package (Ritz et al., 2016).

The Brain-Cousens model comprises the following equation:

$$Y = \frac{d - fx}{1 + \exp\left(b(\log(x) - \log(e)\right)}$$

where: b: uninterpretable constant coefficient; c: inferior horizontal asymptote; d: superior horizontal asymptote; e: uninterpretable constant coefficient; f: Hormesis effect size: the higher the value, the greater the hormesis effect. f = 0 corresponds to no hormesis effect and the resulting model is the four-parameter log-logistic model.

Based on the log-logistic regressions, the maximum points of the curve were estimated, considered to be the optimal temperature for germination to occur, and estimated by the *predict* function of R software (R Core Team, 2020), simulating 10,000 observations within the range studied. This same procedure was performed for minimum temperature, in the case of the germination variable. In order to facilitate understanding of the effects of the factors, although not significant for one of them, it was represented as a response surface plot. All analyses were performed using R software (R Core Team, 2020).

Results and Discussion

Figure 1 (a, b, and c) shows the differentiated morphology of the flowers of *A. elegans* with a red-purple color corolla with white streaks, very ornamented, which makes the flower attractive for landscapers as well as for its pollinators, and differently from other aristolochiaceae, does not have a strong or nauseating odor, a fact that makes some species unsuitable for landscape use. The fruits in the form of hanging baskets contribute to the ornamental character of the species, with capsules filled with heart-shaped seeds dispersed by the wind (Capellari Junior, 2002; Capellari Junior, 2005; Freitas and Araújo, 2017).

The weight of a thousand seeds was 1.5 g and the moisture content was 7.43%. The F test of the analysis of variance did not find any interaction effect or isolated effect of gibberellin, only an isolated effect of temperature in all parameters evaluated (Table 1).

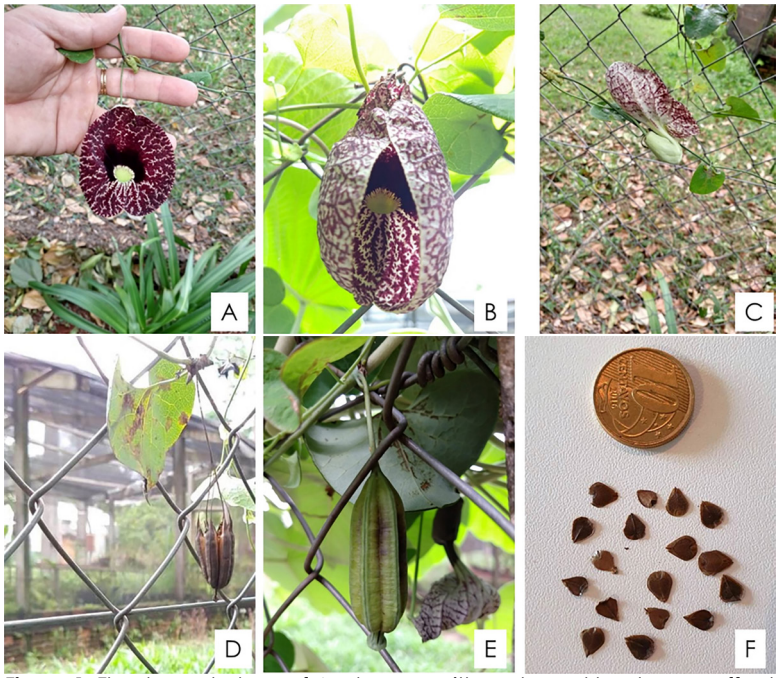

Figure 1. Floral morphology of *A. elegans*, with exuberant landscape effect of the corolla, blossomed and not expanded (A and B), the constituents of the peritoneum (C), ripe fruit (D), green fruit (E), and seeds with anemochoric dispersion (F).

Table 1. F-test of the fixed effects analysis of variance of the factors temperature, gibberellin, and interaction of the parameters germination, germination speed index (GSI), mean germination time (t), and mean germination speed (MGS).

Source of variation	Germination	GSI	t	MGS
Temperature (A)	219.76**	297.2501**	40.659**	85.7003**
Gibberellin (B)	3.2610^{ns}	2.2266^{ns}	0.469^{ns}	0.0659^{ns}
A x B	1.300^{ns}	1.8554^{ns}	0.286^{ns}	0.6203^{ns}
CV	21.07	18.97	36.90	28.78

*, **, ns significant at 5%, 1%, and non-significant by Fisher's F test, respectively.

This lack of a gibberellin effect could be explained by the fact that the concentrations used were insufficient to promote any alteration in the germination percentage of seeds, a fact also evidenced by Nobrega et al. (2018) with *Psidium guineense* SW., in which the authors suggest that the hormone concentrations within the seed are

sufficient for it to carry out its germination process, with an adequate endogenous level of gibberellin in the seeds, so that its addition did not influence the germination process. As envisioned by Paixão et al. (2020), in which the author concludes that the treatment with gibberellic acid in seeds of *Amburana cearensis* A.C. Smith was not efficient to improve the emergence and development of seedlings, and pre-germinative treatments with this hormone was unnecessary, as also pointed out by Shahvand et al. (2015) with wild oat seeds.

Almeida et al. (2020) mention that gibberellic acid had no effect on germination of tomato seeds, justifying that these seeds already have high natural germination, which may have contributed to the non-effect of the application, as well as a possible insufficient soaking time in the gibberellic acid solution, hypotheses which also elucidate the results presented herein. In contrast, Bhat et al. (2020) reported an increase in the germination of *A. tagala* with increasing dosages of gibberellic acid (100, 200, and 300 ppm), reaching a maximum germination percentage of 62.10%.

For *A. elegans*, the GA_3 did not interfere in the germination potential of the seeds a positive or negative way for, whereas Ferreira et al. (2002) in a study with seeds of *Annona squamosa* L., found a significant effect on germination, with increased germination percentage at the dosage of 1000 mg L^{-1} of GA_3, however, a high percentage of abnormal seedlings was also observed in relation to the control. Negative effects are also pointed out by Dourado et al. (2020), who found that gibberellin impaired the germination and germination speed index of seeds of *Cedrella fissilis* Vell.

Germination data, germination speed index, mean germination time, and germination speed were analyzed according to the log-logistic model proposed by Brain-Cousens (Figure 2). For germination, the curve obtained a maximum response at the estimated temperature of approximately 29˚C, with 75% germination. On the other hand, the minimum temperature for germination to occur was estimated at 16.9˚C, below this value, germination did not occur (Table 2).

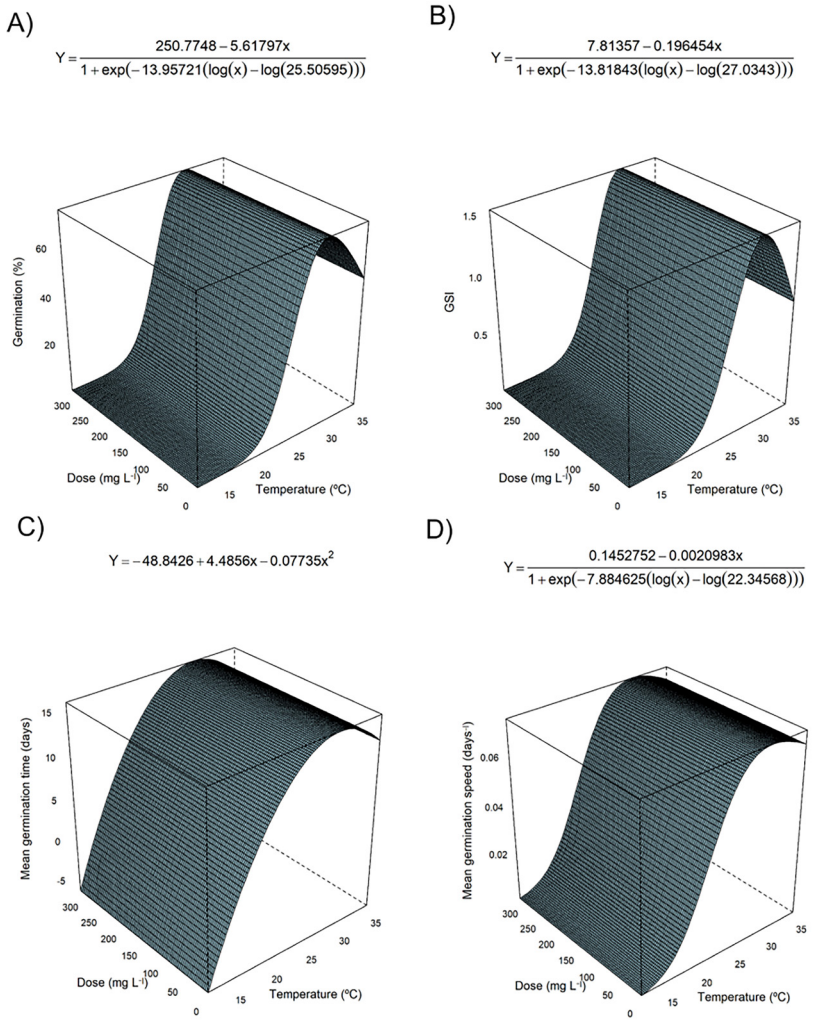

Figure 2. Behavior of Germinability variables (A), Germination Speed Index (B), Mean Germination Time (C) and Mean Speed (D) of seeds from *A. elegans* as a function of temperature and dosages of gibberellin according to the Brain-Cousens log-logistic model.

Table 2. Optimal temperature and maximum response of the germination process variables of *Aristolochia elegans* M.

Variable	Regression model	Optimum temperature	Minimum temperature	Response at optimal temperature
Germination (%)	BC.4	29.14	16.90	75.33
GSI	BC.4	29.71	-	1.55
t (days)	Quadratic	29.00	-	16.18
MGS	BC.4	29.72	-	0.07

where: BC.4: Four-parameter modified Brain-Cousens log-logistic model.

Temperature regulates germination in several ways: it determines germination capacity (final percentage), germination speed (time for first count and final count), and in some species, it can overcome primary or secondary dormancy (Lima Junior, 2011).

The temperature significantly influenced the germination process of seeds of *A. elegans* M., highlighting that germination did not occur at temperatures of 15ºC and there was a gradual increase in germination from 20ºC to the estimated temperature of 29.14ºC. At temperatures of 20 and 25ºC, the percentages of germinated seeds were 4.5 and 48% respectively. In the treatment at 30ºC the percentage reached was 75% and at 35ºC there was a decrease in the percentage of germinated seeds (54%). This behavior characterizes the species as stenothermic, that is, one that presents narrow temperature limits for germination. It is probable that at a temperature of 30ºC, there was more efficient degradation of the reserves present in the seeds, which favored germination and, consequently, the development of the seedlings (Berto et al., 2020).

These results corroborate Maekawa et al. (2010), who verified the non-occurrence of germination at temperatures of 15 and 40ºC in seeds of *Aristolochia esperanzae* O. Kuntze, a decrease in germination from 35ºC, and also recorded temperatures of 25 and 30ºC as the most favorable for germination. However, Ribeiro et al. (2013) found that seeds of *A. galeata*, a climbing species that occurs predominantly on forest edges, demonstrated high tolerance to high temperatures.

The low values found for all variables of analysis of the germination process at the extreme temperatures of 15 and 35ºC are justified, according to Gualtieri & Fanti (2015), as germination would be limited by thermo-denaturation processes of proteins, inactivation of enzymes, and phase transition processes of the lipid bilayer of membranes (varying from high fluidity, resulting from high temperatures to the gel-crystalline state), promoting alterations in the organization of lipids in cell membranes and consequently changing the diffusion barrier imposed by the seed coating and loss of function.

Oliveira & Barbosa (2014), testing *Cedrella fissilis* seeds, reported that as temperature increases, germination is negatively affected, as also seen by the authors at a temperature of 35ºC.

At a temperature of 20ºC, the beginning of germination occurred on the 17th day after sowing, at a temperature of 25ºC the germination process started on the 13th day, and for temperatures of 30 and 35ºC root protrusion occurred on the 9th and 11th days, respectively, after planting.

Based on the maximum points obtained in the equations of the four parameters evaluated (Figure 2 and Table 2), an optimal mean temperature of 29.4˚C (29-29.72˚C) was obtained, which is similar to that reported by Maekawa et al. (2010), who verified that for seeds of *A. esperanzae*, the optimal temperature was 30ºC, being the most suitable for conducting the germination test. Hakemi et al. (2020) found that the immersion of seeds from *A. baetica* in gibberellin solution (125 and 200 ppm) was favorable to overcome dormancy and also reported that temperature significantly affected seed germination as it had an inhibitory effect at 0, 5, 10, 25, 30, and 35ºC and a favorable effect at 15 and 20ºC.

Nakonechnaya et al. (2018) studied the germination of 17 species of the genus *Aristolochia* and reported that the duration of the germination period differs between species, ranging from 1 month to 3.5 years, with species occurring in tropical zones beginning to germinate between 15-30 days after sowing, with germination rates of between 84-100% and temperature between 22-24ºC. The results found in the current work fall within the mentioned time and temperature intervals. The variability of temperatures conducive to germination of species of the genus *Aristolochia* was also reported by Adams et al. (2005), with temperatures between 15–25ºC for *A. macrophylla* and *A. manshuriensis*, between 20-35ºC for *A. tomentosa,* and 10ºC for *A. californica*. These findings are explained by Lima Junior (2011), in that tropical species generally require higher optimal temperatures for germination than temperate zone species.

These differences in optimal temperatures presented by different authors corroborate Brancalion (2010), who relates the different germination requirements to the characteristic temperatures of the biomes in which the species are inserted, constituting a physiological adaptation of the seeds to these environmental conditions. Figliolia (2015) points out that temperature

is the most active factor in the resumption of embryo growth in the seeds of some species, acting directly on the speed of biochemical processes and on the capacity of the seeds to complete the germination process, noting that each species requires specific conditions for this process to occur more intensely.

The germination speed (Figure 2 D) increased as a function of temperature increase, decreasing from the estimated temperature of 29.72 °C, with an MGS of 0.07 days-1, a fact also evidenced by Carvalho & Nakagawa (2000), who report that germination will be faster and the process more efficient with increasing temperatures, up to a certain limit. The limit found for *A. elegans* was 29.14ºC and germination of 75.33%, as temperatures above this limit promoted a decrease in germination. The effects of temperature on germination speed differ from those observed for total germination, in which the optimum temperature for germination is different from germination speed, that is, the optimum speed is always greater than the total germination (Carvalho & Nakagawa, 2000; Marcos Filho, 2015), as pointed out in this work, with temperatures of 29.71 and 29.14 °C.

The mean germination time (t) corresponds to the mean time required for a set of seeds to germinate, giving the process a kinetic character (Ferreira & Borghetti, 2013).

For the mean germination time (*t)* the temperature that showed the best results was 29°C, expressing a drop from the ideal temperature, following the germination behavior. This fact was also described by Brito et al. (2020) with seeds from *Schinopsis brasiliensis* ENGLER, in which a temperature of 35 °C showed the best result for the mean germination time, pointing out that temperatures lower than the this reduce the germination speed, resulting in a change in the uniformity of emergence.

As observed, the mean germination speed increased with increasing temperature to a maximum of 29°C (Figure 2C). As reported by Paim et al. (2018), the mean germination time is a parameter of great importance for the determination of seed vigor, as it assumes that the most vigorous seeds germinate in a shorter time, a fact not demonstrated in this work, since the treatments with the lowest mean germination time do not necessarily present the *highest* germination percentages, attesting that the mean time alone is not a parameter to determine seed vigor.

Conclusions

The estimated temperature of 29.4˚C allows the best germination for the species *Aristolochia elegans* M., while below 16.9˚C, seed germination does not occur.

There is no effect of gibberellin on the germination parameters of seeds of *Aristolochia elegans* M. when applied at doses between 100 and 300 mg L^{-1}.

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