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RESEARCH ARTICLE

# SEED GERMINATION OF *Aristolochia esperanzae* O. KUNTZE IN DIFFERENT SUBSTRATES AND WATER POTENTIAL

**Maekawa,L[1]., Albuquerque,MC[2]., Coelho, MFB[3] *., Guimarães, SC[4] and Camili EC[5]**

[1]Instituto Federal de Mato Grosso, Rua J. Quadra 08,SetorChácara CEP: 78320-000, Juína-MT – Brazil
[2,3,4,5]Universidade Federal de Mato Grosso, Av. Fernando Correa da Costa, 2.367,
Boa Esperança, CEP 78060-900 - Cuiabá, MT - Brazil

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**ABSTRACT**

The aim of this study is to identify the most suitable substrates and the critical limits of water potential for germination of *Aristolochia esperanzae* seeds. Two experiments were conducted. At the first, were evaluated the substrates sand, sand and vermiculite at a ratio 1:1,roll of paper, on paper and between paper. In the second, the seeds were germinated in plastic boxes on paper substrate, moistened with water or polyethylene glycol solutions (PEG 6000), the following water potentials: zero (control); -0.2; -0.4; -0.8; -1.0 and -1.2 MPa. The experimental design was completely randomized with four replications. Seed germination occurs in water potential of zero to -0.8 MPa and the formation of normal seedlings at zero to -0.4 MPa. The substrate on paper provide higher percentage and speed of germination and are more suitable for the *Aristolochia esperanzae* seed germination test.



## INTRODUCTION

The exploitation of genetic resources of medicinal plants in Brazil is related in large part with the extraction of wild material. Despite the considerable volume of export of various medicinal species in raw form or its by-products, very few species came to be cultivated even in small scale (Vieira & Alves, 2004).

Among the native species of medicinal interest is *Aristolochia esperanzae* O. Kuntze which has an active role in the dynamics of forest communities and is of great ecological importance (Rezende & Ranga, 2005). The species occurs in the Cerrado ofMato Grosso State and is among the 509 species of medicinal flora (GuarimNeto & Moraes, 2003).

The species belongs to the genus Aristolochia, largest and most diverse genre of Aristolochiaceae family, which contains more than 400 species in tropical and temperate regions (Ohi-Toma *et al,* 2006). These plants are sources of aristolochic acid, used in traditional Chinese medicine and dietary supplements, with action related to kidney failure and is a potential carcinogen (Lewis & Alpert, 2000). In folk medicine has antiseptic properties, sedative, antipyretic, hypertension and against snake venom (GuarimNeto & Morais, 2003). Pacheco *et al*, (2010) found in antibacterial tests with extracts, fractions and isolated compounds *Aristolochia esperanzae* and the use of the species population in the cases of infection/inflammation caused by rheumatoid arthritis has scientific support.

Research involving genetic resources of native medicinal species will only be applied if they are ensured the survival and availability of this genetic material (Albuquerque *et al*, 2003). So are relevant studies of factors affecting the conservation of germplasm, seed germination and plant development.

For germination test it is necessary to choose the substrate to provide the environment in which the seed can germinate and develop. This choice depends on the size of the seed, your requirement with respect to moisture and aeration, light sensitivity, and ease that the material provides for the development and evaluation of seedlings (Brasil, 2009). Scalon *et al* (2007) evaluated the effect of substrates on the germination of seeds *Aristolochia triangular*, but the focus was the production of seedlings in the nursery and not to conduct germination tests under controlled and reproducible conditions.

The choice of substrate interferes with the amount of water that must be placed for providing seed germination. Water must be available for the seeds in the appropriate amount, since each species has a critical level for germination to occur (Marcos Filho, 2015). Many studies have attempted to evaluate seed imbibition by determining the osmotic pressure in saline

*✉ ***Corresponding author:*** **Maekawa,L**
Instituto Federal de Mato Grosso, Rua J. Quadra 08,SetorChácara CEP: 78320-000, Juína-MT – Brazil

solution capable of halting the water uptake by the seed (Labouriau, 1983) in order to determine critical levels of germination.

Water stress normally decreases the percentage and the germination rate, but there is great variation among species, from those very sensitive to the toughest. Water stress can act positively in the establishment of the species. As these are quite heterogeneous in response to this stress condition, germination is distributed in time and space, thus increasing the likelihood that the seedlings meet environmental conditions favorable to the establishment and development (Marcos Filho, 2015). Low availability of water causes a reduction in growth, by decreasing the expansion and cell elongation, due to the decrease of turgor (Taiz & Zeiger, 2013).

Thus, the objective of this study was to determine the most appropriate substrates and the critical limits of water potential for germination of *Aristolochia esperanzae* seeds.

## MATERIALS AND METHODS

The fruits of *Aristolochia esperanzae* were collected in Cuiabá, in the period from July to September 2011. After collection, the seeds were removed from the fruit, dry, clean and manually homogenized and submitted the following preliminary tests: water content by the method of oven at 105 °C ± 3 °C for 24 hours and weight of a thousand seeds, using eight sub-samples of 100 seeds (Brasil, 2009). By the beginning of the experiment, in November 2011, the seeds were kept in plastic boxes in a refrigerated chamber (15 °C ± 2 °C and 80%± 4% air humidity).

Two experiments were conducted. At first, the experimental design was completely randomized with five treatments and four replications of 25 seeds, and the seeds disposed in the following substrates: sand, sand + vermiculite in the ratio 1: 1, paper roll, on paper and between paper.

The substrates sand (S) and sand + vermiculite (SV) were sterilized at 105 °C for 5 hours, then moistened up to 60% of its water holding capacity. The seeds were coated with a layer of sand approximately 2 mm. The substrates on paper (SP) between the paper (BP) and roll of paper (RP) were moistened with distilled water in the amount of 2.5 times the mass of each substrate.

In treatments S, SV, SP and BP were used transparent plastic boxes, type "gerbox" kept closed during the experiment with transparent plastic film. Treatment RP was packed in transparent plastic bag.Evaluations were performed daily and calculated the percentage the average time and the normal seedlings formation rate index (Labouriau, 1983; Maguire, 1962), and counted the firm seeds and damaged seeds. In assessing the normal seedling were considered the criteria of RAS (Brasil, 2009).

At the second experiment we used a completely randomized design with six treatments and four replications of 25 seeds. The seeds were germinated in transparent plastic box, substrate on paper (SP), moistened with water or polyethylene glycol solutions (PEG 6000) in the following water potentials: zero (control); - 0.2; - 0.4; - 0.8; -1.0 and -1.2 MPa. Daily evaluations were performed, calculating percentages and average time of germination and formation of normal seedlings. Germinated seeds were considered which delivered roots 2 mm in length. The amount of PEG 6000 to obtain the necessary potential was obtained based on Villela *et al*. (1991).

Every five days was made there wetting of the samples with PEG 6000 solution in the corresponding concentration. At the end of the germination test (30 days), the seed shad not germinated in treatments with lower potential were washed with distilled water. These seeds were germinated in transparent plastic boxes on paper soaked with water at 25 °C, to see if there germination.

In both experiments, treatments were placed in a growth chamber at 25 °C and photo period of eight hours for 30 days. Data were analyzed by F test, after being subjected to the tests of normality / homogeneity and changed when necessary. Means were compared by Tukey test at 5% probability. In the data analysis we used the Statistical Programme SAEG (Ribeiro Jr .& Melo, 2009).

## RESULTS

### *Effect of substrates*

The seeds showed water content of 9.8% and thousand seed mass 4.14 g. The percentage of normal seedlings and speed index were lower in the substrate sand + vermiculite (SV) and the average time did not vary between the substrates (Table 1). The coefficient of variation for the formation of normal seedlings was 16.88%, considered reasonable, because it is not domesticated species.

**Table 1.** Average percentage (% PN), average times (TM) and normal seedlings formation rate indices (IV), blemished seed percentages (SD%) and firm seeds (FS%) of *A. esperanzae* in different substrates. SP = on paper, BP = between paper, PR = paper roll, S = sand and SV = sand + vermiculite 1: 1.

| Substrate | PN% | TM (days) | IV | SD% | SF% |
|---|---|---|---|---|---|
| SP | 89 a | 21,7 a | 1,06 a | 4 | 7 |
| BP | 79 a | 21,8 a | 0,94 a | 8 | 13 |
| RP | 96 a | 19,6 a | 1,28 a | 2 | 0 |
| S | 88 a | 20,5 a | 1,09 a | 0 | 0 |
| SV | 32 b | 16,9 a | 0,36 b | 0 | 0 |
| CV(%) | 16,88 | 18,66 | 18,55 | | |

Means followed by the same letter in columns do not differ by Tukey test at 5% probability

In the SP substrate, the firm seeds (7%) had apparently viable to the cutting test. The formed seedlings showed good development and there was no presence of fungus. BP treatment, also the firm seeds were apparently viable and able to germinate. In this treatment there was the appearance of fungi on the outside in 8% of non-germinated seeds and were deteriorated.

In the SV substrate was observed the lowest percentage of normal seedlings, probably due to more water and less oxygen, resulting in drastic reduction of seed germination. Bezerra *et al*. (2002) found that the vermiculite reduced the percentage of germination of *Momordicacharantia* L. The percentage of *Ochroma pyramidal* seed germination was lower in the substrate sand + vermiculite 1: 1 (39.8%) than in vermiculite (50.9%) (Alvino & Rayol, 2007). The addition of vermiculite in the sand substrate reduced the percentage of germination of this species in the same way as seen with seeds of *A. esperanzae*. Probably this experiment, the amount of water calculated on 60% of the mixture of sand, vermiculite retention capacity was excessive due to the porosity and greater holding capacity provided by the vermiculite.

In Figure 1 are the curves germination (normal seedlings) built up of different treatments where variations are observed in germination during the 30 days of the experiment.

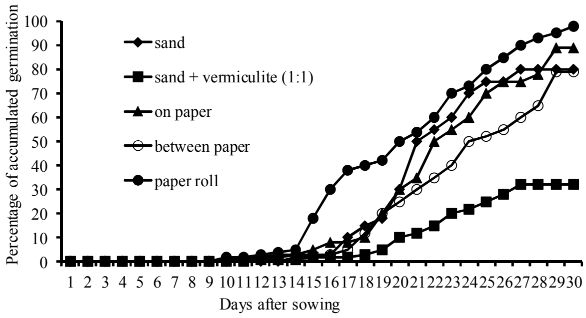


**Figure 1** Accumulated germination (%) of *Aristolochia esperanzae* O. Kuntze seeds on different substrates.

The issue of roots in substrates occurred at different times. In paper roll and on paper substrates, the first roots were observed after 10 days. In the substrate between paper issuing roots occurred on the 11th day after sowing. The formation of seedlings in paper roll substrate, started on the 13th day (2%); in other substrates occurred later, 14 days in on paper, sand and sand + vermiculite substrates and 15th in the between paper. These variations are expressed in time for the formation of seedlings, so the germination rate, and confirmed as a suitable period of 30 days and the paper roll and on paper substrates for driving the *A. esperanzae* germination test.

Moreover, the seedlings were developed and found better ease of handling in paper roll and on paper substrates. The seeds of *A. esperanzae*, which have flattened and are medium in size, has its favored development in these substrates, as well as checked with *Cedrela fissilis* seed, *Tabebuia spp.* and *Eucalyptus spp.* (Figliolia & Pina-Rodrigues, 1993). For other species, the paper substrate was also considered better, as *Operculina macrocarpa* (France *et al.*, 2002) and *Basella rubra*, vine species characteristics (Lopes *et al.*, 2005) as *Aristolochia esperanzae*.

***Effect of water stress with PEG 6000***

The germination and the formation of normal seedlings of *A. esperanzae* decreased as decreased water availability on the substrate (Table 2). In -0.8 MPa water potential, germination was 1% and potential -1.0 and -1.2 MPa not germinated. In the potential -0.2 and -0.4 MPa, even germination to occur, the formation of seedlings was reduced by 43% and potential -0.8, -1.0 and -1.2 MPa there was the formation of normal seedlings. These results are similar to those obtained Rosa *et al.* (2005) studied the stress induced by PEG in *Ateleia glazioviana* Baill., checking that from -0.4 MPa existed decrease for germination, more pronounced for the potential to -0.6 and -0.8 MPa.

**Table 2** Percentages mean germination (PG) and normal seedlings (PN) and average time of germination (TMG) and seedling formation (TMP) of *Aristolochia esperanzae* O. Kuntze in different water potentials.

| water potential (MPa) | PG | PN | TMG (dias)[1] | TMP (dias)[1] |
|---|---|---|---|---|
| 0 | 98 a | 98 a | 13,9 b | 18,5 b |
| - 0,2 | 78 a | 42 b | 22,7 a | 28,3 a |
| - 0,4 | 70 a | 11 c | 24,5 a | 27,9 a |
| - 0,8 | 1 b | 0 c | 27,0 a | 30,0 a |
| - 1,0 | 0b | 0c | 0c | 0c |
| - 1,2 | 0b | 0c | 0c | 0c |
| CV (%) | 23,04 | 24,23 | 30,02 | 30,31 |

Means followed by the same capital letter in the column do not differ by Tukey test at 5% probability. 1The means were transformed to root (x + 0.5)

Other species show significant reductions in the percentage of germination, as from -0.5 MPa in *Bowdichia virgiloides* Kunth seeds (Silva *et al.*, 2001) to -0.1 MPa in *Schizolobium amazonicum* seeds Huber ex Ducke (Braga *et al.*, 2008), between -0.1 and -0.4 MPa in *Adenanthera pavonina* L. seed (Fanti & Perez, 1998) and -0.4 MPa in *Erythrina falcata* Benth seeds (Pelegrini *et al.* 2013). This low threshold of tolerance to water stress gives *P. alliacea* adaptive character, providing high-capacity establishment on the field in low water regime conditions.

The reduction of water potential was more damaging to the formation of normal seedling for germination. In potential -0.4 MPa was found 70% of emission of roots and only 11% of normal seedlings. Whereas the formation of seedling expressed seed vigor, it can be considered that the water reduction in the substrate considerably affected the force, and to a lesser intensity, *A. esperanzae* seed germination.

Avila *et al.* (2007) also observed a reduction in germination (normal seedlings) canola seeds when subjected to successive reduction of the osmotic potential. The germination values were satisfactory up to -0.25 MPa, while in the lower osmotic potential of -1.0 MPa, germination showed values close to zero. Lopes & Macedo (2008) determined maximum germination of Chinese cabbage (*Brassica pekinensis*) in the presence of osmotic solution to -0.2 MPa, did not differ from control (0 MPa), with decrease in the percentage of germination under the influence osmotic potential of -0.8 MPa.

Rosa *et al.* (2005) obtained similar results in *Ateleia glazioviana* which is osmotically affected by PEG 6000, by lowering the osmotic potential of the substrate, and the percentage germination rate were reduced, with potential less than -0.4 MPa may be considered critical the germination of the species. Stefanello *et al.* (2006) found that behavior in seed *Pimpinella anisum* L. and Teixeira *et al.* (2011) in *Crambe abyssinica* Hochst seeds.

The reduction in the percentage of germination as the osmotic potential becomes more negative can be explained by the decrease in the speed of metabolic and biochemical processes, delaying or reducing the germination of seeds and interfering with the soaking and cell elongation of the embryo (Marcos Filho, 2015).

In this study the seeds when not emitted roots after 30 days on a substrate containing PEG 6000 were washed and placed on moistened substrate with water, it was observed that the same seedlings germinated and formed (Table 3).

**TABLE 3.** Percentages of germination (PG) and normal seedlings (PN) of *Aristolochia esperanzae* O. Kuntze, after the seeds were removed from treatment with PEG 6000.

| Previous treatment (MPa) | PG | PN |
|---|---|---|
| -0,2 | 92 | 92 |
| -0,4 | 91 | 91 |
| -0,8 | 87 | 76 |
| -1,0 | 88 | 78 |
| -1,2 | 87 | 56 |

The fact occur seed germination and formation of normal seedlings after the seeds have been removed treatments can be stated that there was prolongation of stationary phase (Phase 2) of the soaking process, when the seeds were in a substrate with PEG 6000 because of reduction of enzyme activity and, consequently, lower meristem development and delay in root protrusion. However, it was noted that at higher water cuts (-0.8, -1.0 and -1.2 MPa) formation of normal seedlings was most affected, probably due to increased stress caused to the seeds.

In the water potential of zero (control), germination was initiated earlier and faster. In this water potential, the issue of roots began on the 13th day and the seedling training on the 18th day after sowing. After 23 days reached 93% of normal seedlings, stabilizing at 98% at 26 days after sowing.The reduction in osmotic potential and consequent reduction in water availability to the metabolic processes of germination also affect the speed at which this process occurs. The reduction in the germination speed was observed when used parameter mean germination time (Table 2), since the seeds 13,9dias slow to germinate and the seedlings 18.5 days in control and potential of -0.2 to - 0.8 mis that took 22 days and from -0.1 no germination. Pereira & Lopes (2011) observed similar behavior in *Jatropha curcas* seeds.

The reduction in germination rate, increasing the time between sowing and start of the primary root issue is probably due to the lower water uptake by the seeds, because the increase in osmotic concentration causes a decrease in water gradient substrate seed system (Marcos Filho, 2015).Water stress normally decreases the percentage and the germination rate, but there is great variation among species, from those very sensitive to the toughest (Marcos Filho, 2015). For seed *Aristolochia esperanzae* tolerance for germination was the potential equal to -0.8 MPa and formation of normal seedlings, -0.4 MPa, although this potential the percentage of normal seedlings was very low, 11%.

These limits were also observed in *Pterogyne nitens* seed with reduced germination rate from -0.4 MPa (Nassif & Perez, 1997) and *Ateleia gazioviana* seeds potential below -0.4 MPa may be considered critical germination (Rosa *et al*. 2005). Pereira and Lopes (2011) found that reducing the osmotic potential of the substrate significantly reduced the germination of *Jatropha curcas* seeds, and the germination rate and seedling performance. Greater reduction in germination and early seedling development was observed in the osmotic potential -0,2Mpa and from -1.2MPa there was inhibition of germination.

## CONCLUSION

The paper substrate in the form roll and on paper is more suitable for *Aristolochia esperanzae* seed germination test.

Germination of *Aristolochia esperanzae* seed is very sensitive to water stress and occurs in water potential of zero to -0.8 MPa and the formation of normal seedlings at zero to -0.4 MPa.

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