



## Fruit and seed biometry and germination of *Victoria amazonica* (Poepp.) J.C. Sowerby (Nymphaeaceae) from the Pantanal floodplain

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**ABSTRACT.** Numerous plant species are easily established in the wide flood plains of the Pantanal wetlands due to the environmental heterogeneity. The aquatic macrophytes excel in permanently flooded areas, particularly Nymphaeaceae species. *Victoria amazonica*, popularly known as the vitória-régia, is hallmarked for its beauty. However, the biology and conditions necessary for the seed germination of this flowering plant remain unknown. In the present study, the fruit and seed morphology and biometry were described and the seed germination was evaluated under different abiotic conditions. To this end, mature fruits of *V. amazonica* were collected from the bays near Paraguay River in the south Pantanal floodplain. The fruits and seeds were described and measured using digital caliper. Intact and mechanically scarified seeds were germinated under different temperature, light and substratum conditions, and the initial development was described. The fruits measured 67.5 x 119.7 mm in size and contained 100-700 seeds. The average seed measured 10.6 mm in length and 9.8 mm in width. The highest germination occurred at 25°C, independent of the light condition. The seeds were considered neutral photoblastic. The seedlings showed heterophylly, and the main root was degenerated, forming adventitious roots. Morphological differences were observed in seedlings developed under different light conditions.

**Keywords:** aquatic macrophyte, hydrophytes, water lilies, vitória-régia.

### Biometria do fruto e da semente e germinação de *Victoria amazonica* (Poepp.) J.C. Sowerby (Nymphaeaceae) do Pantanal

**RESUMO.** O Pantanal, ampla planície inundável, facilita o estabelecimento de inúmeras espécies vegetais por sua heterogeneidade ambiental. As macrófitas aquáticas se destacam em áreas permanentemente alagadas, com destaque para a família Nymphaeaceae. A espécie *Victoria amazonica*, conhecida popularmente como vitória-régia, é marcada por sua beleza. Entretanto, sua biologia e as condições necessárias à germinação de suas sementes permanecem desconhecidas. Nesse estudo foram descritas a biometria e a morfologia do fruto e da semente, e avaliada a germinação em diferentes condições abióticas. Para isso, frutos de *V. amazonica* foram coletados em baías próximas ao rio Paraguai no Pantanal sul. Os frutos e as sementes foram descritos e mensurados usando paquímetro digital. Sementes íntegras e escarificadas mecanicamente foram submetidas a testes de germinação em diferentes condições de temperatura, luz e substrato, e o desenvolvimento inicial das plântulas foi descrito. Os frutos mediram 67,5 x 119,7 mm, e portavam 100-700 sementes. As sementes mediram 10,6 mm de comprimento e 9,8 mm de largura em média. A maior porcentagem de germinação ocorreu a 25°C independente da condição de luz. As sementes foram consideradas fotoblásticas neutras, e as plântulas possuem heterofilia, degeneração da raiz principal e formação de raízes adventícias. Foram observadas diferenças morfológicas nas plântulas desenvolvidas na presença e na ausência de luz.

**Palavras-chave:** macrófita aquática, hidrófitas, vitória-régia.

#### Introduction

The Pantanal floodplain shows diverse aquatic vegetation, which occupies periodically or permanently flooded areas (Allem & Valls, 1987). The lakes receive water from rivers through drainage areas (Bacani & Sakamoto, 2007), similar to all wet areas on the planet. These water dynamics

lead to the significant accumulation of sediment and seeds from several plant species (Van Der Valk & Davis, 1976).

The macrophyte populations, as determined by seed banks, are efficient in recovering communities after flooding or drought (Ferreira, Mormul, Thomaz, Pott, & Pott, 2011). Seasonal flooding

favors the occurrence of species adapted to flooding during the wet season and drought during the dry season (Scremin-Dias, Lorenz-Lemke, & Oliveira, 2011). In particular, macrophytes stand out in permanently flooded areas because these plants show peculiar adaptations that facilitate growth at different moisture gradients (Esteves, 1998).

The Nymphaeaceae is quite representative among aquatic macrophytes occurring in Pantanal areas, with seven *Nymphaea* species and one *Victoria* species (Pott & Pott, 2000). *Victoria amazonica* (Poepp.) JC Sowerby is a floating fixed hydrophyte with peltate and circular leaves of up to 2 meters in diameter that are connected to the main stem through long flexible secondary stems (Sculthorpe, 1967; Cronk & Fennessy, 2001). Previous studies have focused on the floral morphology (Corrêa, 1952), blooming and pollination processes (Seymour & Matthews, 2006), stamen morphology (Heinsbroek & Heel, 1969), and ovary pseudosyncarpia (Weberling, 1992) of these plant species. In addition, specific information about the flower, fruits, seeds and seedlings morphology of plants collected from the Brazilian Amazon has been reported (Rosa-Osman, Rodrigues, Mendonça, Souza, & Piedade, 2011). However, little is known about the conditions for the seed germination of *V. amazonica* and the morphological characteristics of the initial development of these plants. Does the occurrence of this species in the Pantanal floodplain (Pott & Pott, 2000), where the climatic characteristics differ from the Amazon, might reflect distinct seed and seedling peculiarities and the adaptation of these plants to this environment?

Successful seedling establishment in this environment depends on the seed germination process. Germination is the stage in the plant life cycle that directly influences plant distribution in the environment (Souza, Pacheco, Matos, & Ferreira, 2007). The maximum germination percentage was observed in germination tests performed under optimum conditions for each species (*Ministério da Agricultura, Pecuária e Abastecimento* [MAPA], 2009), and several factors affect germination. Thus, identifying the optimum temperature and light conditions for maximum germination is essential for propagation of certain species. In addition, this knowledge provides information concerning species biology and adaptation to the environment and provides subsidies for preservation of these plants.

In the present study, we described the fruit and seed morphology and biometry, and evaluated the seed germination of *Victoria amazonica* under different abiotic conditions. This study provides the

first information about the fruit, seeds and seedlings of *V. amazonica* population occurring in the Pantanal floodplain.

## Material and methods

### Study area and seeds processing

The Pantanal floodplain in Mato Grosso do Sul show a tropical climate 'Aw', according to Köppen, with a 1.070 mm average annual rainfall. The rainy season occurs from October to March, and the dry season occurs from April to September (Soriano, 1997). The average annual temperature is 26°C. In addition, frost might sporadically occur (Cadavid García, 1984).

The fruit samples were collected in October 2010 from three bays under Paraguay River influence (17° 58' 19.8" S and 57° 29' 28.8" W; 17° 54' 41.8" S and 57° 27' 37.5" W; 18° 0.2' 40.0" S and 57° 29' 33.0" W) in Corumbá, Mato Grosso do Sul State, Brazil. In the total, 23 mature fruits were collected, and vouchers were deposited in the Herbarium CGMS, *Universidade Federal de Mato Grosso do Sul* (UFMS), Campo Grande, Brazil, under registration 29788.

The fruits were transported in Styrofoam boxes to the Seed Ecophysiology Laboratory. To determine the initial water content, the seeds were immediately extracted from the fruits, and the aryl was removed.

### Fruit and seed morphology and biometry

The fruit and seed morphology were analyzed in field and in laboratory with stereomicroscope Leica, and described according to Hoehne (1948), Judd, Campbell, Kellogg, Stevens and Donoghue (2002) and Vidal and Vidal (2003) terminology.

The biometrics data and number of seeds per fruit were determined for the 23 fruits collected. Biometrics data were determined for 400 seed samples. The fruits and seeds were measured using a digital caliper. The fruit length was considered from the peduncle insertion to the base of the perianth, and fruit width was obtained perpendicular to this area. The seed length was considered from the hilo to the opposite side, and seed width was obtained perpendicular to this area. The seed moisture content was determined through oven drying at 105°C for 24 hours, according to MAPA (2009). The 1000 seeds weight was obtained from.

### Germination and seedling morphology

The seeds were distributed into paper towel rolls moistened with 3.0 times its weight in water and maintained in a growth chamber BOD equipped

with four fluorescents light of 250 v until stable germination was obtained. The temperatures conditions included 20, 25, 30 and 20-30°C (12 hours at 20°C and 12 hours at 30°C). At all temperatures, the experiment was conducted in the absence and presence of light to evaluate the effect of this factor on germination. The seeds were considered germinated upon primary root emission of at least 3 mm. The seeds were submitted to mechanical scarification of the seed integument using sandpaper number 3 in the hilum opposite area. These seeds were placed on paper towel moistened with 3.0 times its weight in water, kept at 25, 30 and 20-30°C (12 hours at 20°C and 12 hours at 30°C). All treatments were performed using four repetitions with 40 seeds each.

To establish conditions similar to the natural environment of the species occurrence, 40 seeds were distributed into four trays filled with washed sand and 40 seeds in four trays containing soil collected at the area of the species occurrence. The seeds were placed at 2 cm deep. The set was moistened at a water depth of approximately 1 cm and maintained at room temperature ( $25 \pm 2^\circ\text{C}$ ) under laboratory conditions and constant light.

The results were expressed as a germination percentage, and efficient germination was considered to be a higher germination percentage obtained in a shorter period of time.

The experiment was designed in a factorial 4 x 2 (temperature and light condition) and 3 x 2 (temperature and scarification). We used a completely randomized experimental design, and the data were analyzed using ANOVA, followed by Tukey's test at 5% probability.

The seedling morphology was described according to Souza (2009) and Wheeler Haines (1975).

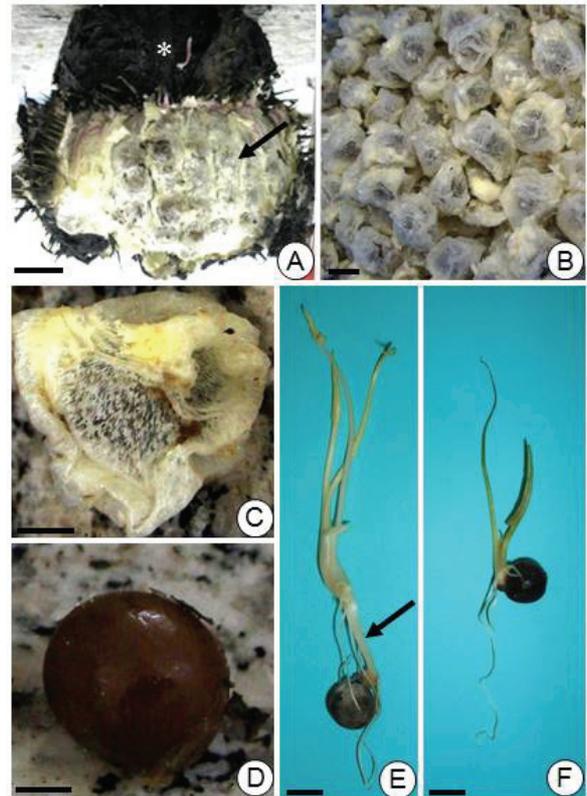
## Results

### Fruit and seed morphology

The fruits are covered by spine, and have greenish color when immature and brown color at maturity. They are polismermic, fleshy, indehiscent, apocarpic, multicarpellate, plurilocular and berry type. In the apical portion is placed the perianth in decomposition (Figure 1A). They develop underwater and at maturity the fruit rots releasing the seeds. The fruit average size was  $67.0 (\pm 13.1)$  mm in length and  $119.7 (\pm 19.5)$  mm in width. The number of seeds per fruit ranged 100 - 700, with an average of 300 to 400 seeds per fruit.

The seeds are hard, globular shape and surrounded by a white bag, probably aryl (Figure

1B-C); without the white bag the seed shows dark brown color (Figure 1D). They are placed along the main axis, featuring axial placentation, and to develop involved in pulp. The embryo has two cotyledons, one developed and the second stunted. The embryo is placed in the hilar region, surrounded by whitish reserve tissue. The length of the seeds was  $10.6 (\pm 4.0)$  mm, and the width was  $9.8 (\pm 0.6)$  mm. The thousand seed weight was  $392.9 (\pm 11.5)$  g. The water content in the seeds immediately after extraction from the fruits was 44.8%.



**Figure 1.** Fruit, seed and seedling morphology of *Victoria amazonica* (Poepp.) JC Sowerby. A. General aspects of fruit and perianth in decomposition (asterisk). Arrow indicates the seed and the white pulp. B. General aspects of seeds. C. Detail of the seed surrounded by a white bag, probably aryl. D. Mature seed without aryl. E. Seedlings developed in the absence of light. Arrow indicates the developed mesocotyl. F. Seedlings developed in the presence of light. Scale bars: A = 2 cm; B, E-F = 1 cm; C = 5 mm; D = 3 mm.

### Germination

Germination was slow and non-uniform, once the first seeds germinated at 60 days after treatment was initiated, and germination was observed for approximately 170 days. At 25°C promoted higher percentage germination, regardless of the lighting conditions. At 30°C, germination ranged from 16.25 to 18.75%, with statistically similar results to those

obtained for the best germination temperature. Temperatures of 20 and 20-30°C were inadequate, showing no success in germination (Table 1).

**Table 1.** Germination percentage (%) of *Victoria amazonica* (Poepp.) JC Sowerby seeds under different lighting conditions (light and dark) and temperatures.

Temperature	Germination percentage	
	Light	Dark
20°C	0.00 ± 0.00 Ba	0.00 ± 0.00 Ca
25°C	25.60 ± 5.90 Aa	25.62 ± 2.37 Aa
30°C	18.75 ± 5.95 Aa	16.25 ± 5.95 Ba
20-30°C	1.00 ± 0.00 Ba	1.00 ± 0.00 Ca
C.V.	17.1	

Capital letters indicate comparisons between lines, and lowercase letters indicate comparisons between columns. Means followed by the same letter do not differ according to Tukey test at 5% significance. C.V.: coefficient of variation.

The germination of the scarified seeds was slow and non-uniform, once the first seeds germinated at 70 days after experiment was initiated, and stabilized at 150 days after. Compared with non-scarified seeds, scarified seeds showed optimized germination at 20-30°C in the dark, but low germination was observed at the other temperatures at 25 and 30°C in both light condition (Table 2).

After 170 days, seedling emergence from non-scarified and scarified seeds was 24.00% ( $\pm$  5.00) and 22.64% ( $\pm$  4.32), respectively, in soil obtained from the collection site. Washed sand was not

**Table 2.** Germination percentage (%) of scarified and non-scarified *Victoria amazonica* (Poepp.) JC Sowerby seeds under different lighting conditions (light and dark) and temperatures.

Temperature	Light		Dark	
	Non-scarified	Scarified	Non-scarified	Scarified
25°C	25.60 ± 5.90 Aa	16 ± 4.50 Ab	25.65 ± 2.37 Aa	12 ± 2.55 Ab
30°C	18.75 ± 5.95 Aa	2 ± 0.50 Bb	16.25 ± 5.95 Ba	13 ± 2.30 Aa
20-30°C	1.00 ± 0.00 Bb	8 ± 1.50 ABa	1.00 ± 0.00 Cb	13 ± 3.45 Aa
C.V.	24.9		14.6	

Capital letters indicate comparisons between lines, and lowercase letters indicate comparisons between columns, Means followed by the same letter do not differ according to Tukey test at 5% significance. C.V.: coefficient of variation.



**Figure 2.** Evolution of germination process of *Victoria amazonica* (Poepp.) JC Sowerby in the presence of light. Scale bar = 2 cm.

successful for seedling emergence from both non-scarified and scarified seeds.

### Seedling morphology

The seedling development was irregular, occurring for 170 days. The embryos showed two cotyledons, developed and stunted, in the hilar region surrounded by a whitish reserve tissue. The first step in the seed germination process is the embryonic axis protrusion, starting the seedling stage. At this stage, the protrusion of the acicular cotyledon occurs simultaneously with hypocotyl emergence. The primary root emerges from the basal region of the hypocotyl and is subsequently replaced with adventitious roots arising from the cotyledon node. The acicular cotyledon gradually degenerates after the onset of the first sagittate eophyll. Morphological differences between seedlings developed in the absence (Figure 1E) and presence of light (Figure 1F) were observed. In seedlings emerging in the absence of light, the mesocotyl extends below the cotyledonary nodes of up to 30 mm in length, connecting seedling to seed reserves (Figure 1E). In seedlings obtained in the presence of light, this structure is a maximum of 3 mm in length (Figure 1F). The Figure 2 shows the evolution of germination process in the presence of light.

## Discussion

The germination percentage of *V. amazonica* seeds was low, and this process was irregular and slow. The first seeds germinated after two months, and germination continued for approximately six months, suggesting the presence of dormancy. In addition, germination occurred independent of the light conditions, but the temperature and substrate affected germination and were limiting under some conditions.

The fruit develops underwater environment due to flower stalk curved in spiral-shaped after pollinated (Sculthorpe, 1967). The fruit permanence underwater, allows the pericarp decomposition, and the organic material can be used as food by underwater fauna. This process makes seeds exposure on the water environment; and the presence of airtight bag shaped allow the seeds float at the water surface and be dispersed. The funicle airtight has already been described as efficient on the seeds dispersion, since it involves all the seed, allowing its fluctuation (Barroso, Amorim, Peixoto, & Ichaso, 1999). The seeds can penetrate the adjacent sediment and germinate under ideal light, oxygen and space for growth situation (Cronk & Fennessy, 2001). The fruit and seed morphology of *V. amazonica* occurring in the Pantanal floodplain do not differ from individual occurring in the Amazon (Rosa-Osman et al., 2011).

Low germination percentage described here does not represent a standard for this plant family. Indeed, *Nymphaea odorata* showed 100% germination (Richards & Cao, 2012). However, for *V. amazonica* seeds from the Amazon, the germination percentage was also low and only occurred under hypoxic conditions, total light absence and mechanical scarification (Rosa-Osman, 2010). Mitra, Basu, & Mukhopadhyay (1982) placed *V. amazonica* seeds on moistened cotton in a greenhouse with temperatures ranging from 22.8 to 35.9°C, and observed 22% germination after 4 months. The data obtained in the present study suggest that among the evaluated conditions, 25°C is the best condition for obtaining *V. amazonica* seedlings in the Pantanal floodplain, regardless of the light conditions, thereby extending the results of a previous study (Mitra et al., 1982).

Mechanical scarification was not efficient to increase the percentage of *V. amazonica* seed germination from the Pantanal floodplain, as the highest percentage germination was observed for non-scarified seeds. However, *V. amazonica* seeds from the Amazon showed successful germination only under conditions scarification (Rosa-Osman, 2010). Moreover, seeds maintained at 20-30°C

germinated only when scarified, indicating the importance of this in nature, when seeds are subjected to temperature variations between day and night. Frequently, hard impermeable seed tegument is typical of physical dormancy, and scarification methods may be used to overcome this dormancy and accelerate germination (Marcos Filho, 2005). Scarification can occur in nature when the seed passes through the digestive tract of an animal or is naturally roughened by rain and substrate friction. Although *V. amazonica* seeds present a hard tegument, scarification did not promote higher germination rates, suggesting that the seed has another type of combined dormancy that is likely associated with the immature embryo and/or the presence of inhibitor hormones. Notably, failed germination of *V. amazonica* seeds in washed sand observed in the present study requires further examination.

In the present study, *V. amazonica* seeds were considered neutral photoblastic and germinated independent of the light conditions. However, seeds of the same species from Amazon populations were negative photoblastic, germinating only in the total absence of light (Rosa-Osman, 2010). Ferreira and Borghetti (2004) considered *V. amazonica* to be recalcitrant and dormant seeds because they have inhibitory substances in the airtight besides hard tegument. Crawford (1992) reported that *Victoria* seeds lose viability when subjected to drying. Preliminary tests performed in the present study, using clean seed samples, scarified and soaked in tetrazolium solution at 1%, the embryo showed red staining after 8 hours of soaking (data not shown), indicating that 100% of seeds were viable.

The degeneration of the main root during the first week of seedling formation and the emergence of adventitious roots in the cotyledon node are characteristics described for monocot embryos and seedlings (Pereira, Pereira, Rodrigues, & Andrade, 2008; Nakamura & Scatena, 2009). Although this species belongs to the basal angiosperms, this family is closely related to the monocot group, where this feature is common (Angiosperm Phylogeny Group [APG] III, 2009). Mitra et al. (1982) also described the occurrence of taproot degeneration for *V. amazonica* seedlings obtained from the Botanical Garden lakes in India.

Four distinct eophylls have been described for the *V. amazonica* seedlings from the Amazon (Rosa-Osman et al., 2011), but this work was possible to describe until the formation of second eophyll. Slightly more than one week after the protrusion of the embryonic axis, we observed seedlings with the

first eophyll formed and the second eophyll in formation. The heterophylly occurs in Nymphaeaceae species only in the seedling stage (Hoehne, 1948; Sculthorpe, 1967); however, in the laboratory, it was not possible to verify the emergence of all eophylls because seedlings did not develop until the next adulthood. The continued development would only be possible with seedling transplanted under ideal conditions of vegetative growth and development, in soil with nutrients and enough light for photosynthesis. The development of the seeds on paper towels was not continued because the reserve tissue was not sufficient to support the development and training of other leaves. Although germination was performed in the laboratory, we attempted to cultivate the seedlings obtained in the germination test, but this endeavor was not successful, reflecting a lack of basic information on the needs of this species for vegetative growth and the small number of seedlings and appropriate structures to maintain these plants under different experimental conditions.

The presence of the mesocotyl in seedlings obtained in the dark has been previously described as a type of underground stem (Rosa-Osman, 2010). However, Wheeler Haines (1975) identified this structure in other Nymphaeaceae species as a mesocotyl. The mesocotyl is the first internode connection between the coleoptile and the plumule. This characteristic is common in Poaceae and Cyperaceae species, and although it has evolved independently in each group, this feature would be important to characterize these families as monocots (Wheeler Haines, 1975).

The presence of a functional mesocotyl, in the absence of light, might reflect an important adaptive character for *V. amazonica* because this structure facilitates successful seedling establishment and germination below the soil and water surface. Mesocotyl elongation facilitates the elevation of the apical meristem to the soil surface, and with the emergence of photosynthetically active eophylls above the surface, assimilation is initiated with a completely independent seed reserve thereafter.

Despite the few studies available on *V. amazonica* seeds, these data are consistent with the indication that the low percentage of germination obtained for this species is a feature reflecting high seed production, given the predominance of the number of seeds, ranging from 300 to 400 seeds, with large sizes. However, because the non-germinated seeds showed no fungal contamination or morphological changes, it is likely that germination continues after the 170 days evaluated in this study. This slow germination could represent an important contribution to the successful

establishment of this species, using dormancy as a germination guarantee over time, increase the possibility of seedling recruitment under different environmental conditions, and increase its environmental occurrence in areas where water seasonality is intense and unpredictable (Scremin-Dias, 2000; Pott, Oliveira, Damasceno-Junior, & Silva, 2011; Scremin-Dias et al., 2011). Moreover, long mesocotyl formation in seedlings germinated in the dark might represent an important role under natural conditions because the substrate on which the seeds germinate under natural conditions is dark and muddy, preventing the entry of light.

## Conclusion

The germination percentage of *V. amazonica* seeds was low, and this process was irregular and slow. The highest germination occurred at 25°C, independent of the light condition. The first seeds germinated after two months, and germination continued for approximately six months, suggesting the presence of dormancy. The seedlings showed heterophylly, and the main root was degenerated, forming adventitious roots. Morphological differences were observed in seedlings developed under different light conditions.

This is the first experimental approach on the seed germination of *V. amazonica* occurring in the Pantanal floodplain. From this study new questions were asked, as how substrate and oxygenation affect in the seed germination, and the factors leading to break dormancy. Further studies are being conducted to increase knowledge about these aspects.

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