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Seed germination and post-seminal development of *Victoria amazonica* (Poepp.) J. C. Sowerby (Nymphaeaceae) in Central Amazon

Sônia Maciel da Rosa[a], Maria Teresa Fernandez Piedade[b], Luiz Antonio de Souza[c*]

[a] State University of the State of Amazonas, Brazil
[b] National Institute of Amazonian Research, Brazil
[c] State University of Maringa, Brazil
[*] Autor correspondente (lasouza@uem.br)

INFO

Keyworks
rhizomatous stem
acicular cotyledon
eophyll
metaphyll
floodplain

ABSTRACT

The Amazon floodplains are specific ecosystems with high biodiversity and endemism, divided in várzeas, white fertile muddy waters, and igapós with poor and acid waters, black or clear, and mixed waters between the two typologies. The main purpose of this investigation was to characterize the seed germination and post-seminal development of *Victoria amazonica* (Poepp.) J.C. Sowerby, one of the few hydrophytes occurring in várzea and mixed waters in the region. In addition, it was evaluated the influence of environmental characteristics on the growth of the plants. Germination was monitored in seeds with and without scarification, in control, and treatments in water with low oxygen and in the dark. The plant development was monitored in field and laboratory; material from juvenile and adult plants was collected at six sampling sites near Manaus. Germination is favored in the dark, in hypoxic conditions, in water and seed-coat shaved. The seedling has two cotyledons, one inside the seed and the other with an acicular shape, eophyll deltoid-hastate, abortive primary root and adventitious roots. New eophylls and the first metaphyll appear in twenty-day-old plants. The stem is rhizomatous. The mixed water environment was not very different from the várzea which is reflected in a similar chemical composition of the plants of both systems. The rhizomes of the várzea environment have caloric value three times higher than that of mixed water. This condition and the presence of larger leaves suggest the várzea floodplain to be more favorable environment for growth of *Victoria amazonica*.

RESUMO

Germinação da semente e desenvolvimento pós-seminal de Victoria amazonica (Poepp.) J. C. Sowerby (Nymphaeaceae) na Amazônia Central

As várzeas amazônicas são ecossistemas específicos de alta biodiversidade e endemismo, que consistem em várzeas, com águas barremtas férteis em nutrientes, e igapós com água pobre e ácida, escura ou clara, e águas mistas entre os dois tipos referidos. O principal objetivo dessa investigação foi caracterizar a germinação das sementes e o desenvolvimento pós-seminal de *Victoria amazonica* (Poepp.) J.C. Sowerby, que é considerada uma das poucas hidrófitas de várzea e águas mistas da região. Além disso, foi avaliada a influência das características ambientais no crescimento das plantas. A germinação foi monitorada em sementes com e sem escarificação, no controle e tratamentos com água de baixo teor de oxigênio e no escuro. O desenvolvimento da planta foi acompanhado no campo e laboratório; material de plantas juvenis e adultas foi coletado em seis amostras de locais próximos de Manaus. A germinação é favorecida no escuro, em condições hipóxicas, água e semente escarificada. A plântula tem dois cotilédones, um que permanece no interior da semente e outro acicular, eofilo deltoide-hastado, raiz primária abortiva e raízes adventícias. Novos eofilos e primeiro metafilo formam-se em plantas de vinte dias. O caule é rizomatoso. Ambiente de água mista não foi diferente da várzea, comprovado pela composição química similar das plantas de ambos sistemas. Os rizomas da várzea têm valores calóricos três vezes maiores do que os de água mista. Esta condição e a presença de folhas maiores sugerem que a várzea é ambiente mais favorável para o crescimento de *Victoria amazonica*.

Palavras-chaves
caule rizomatoso
cotilédone acicular
eofilo
metafilo
várzea

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INTRODUCTION

The Amazon is located in the north of South America, and approximately 67% of its area belongs to Brazil, with the remainder distributed between Venezuela, Suriname, Guyana, Bolivia, Colombia, Peru and Ecuador. The Amazon basin is located in the circumglobal belt of evergreen tropical forests, with 68% of its area located in Brazil; the main tributaries of the Amazon River are followed along of its courses, through extensive flood plains called “várzeas” and/or “igapós” (Belúcio et al., 2018). The Amazon is the region with the greatest biodiversity in the world, but there are few scientific studies of the native species belonging to this vegetation (Ribeiro et al., 1999).

Vegetation formations in the Amazon are determined by climate and edaphic factors (Schubart, 1983). The variability of the soil and the hydrological conditions (Salati, 1983) determine the appearance of different vegetation coverings. Surveys reveal that 70% of the vegetation formations are terra-firme forests and the remaining 30% are represented by different formations characterized by the flooded forests of várzea and igapó, the meadows, the campinaranas, the várzea fields, the ground vegetation, savanna vegetation and mangroves (Braga, 1979).

Among these Amazonian vegetation formations, wetlands cover about 750.000 km², 60% of whitch correspond to the muddy waters of the várzea and 40% to the black or clear water igapó (Wittmann and unk, 2016). There is approximately 20% coincidence between the woody flora of the floodplain and the igapó. In addition to the woody flora, aquatic macrophytes are also found in the flooded Amazonian areas. With the increase in the exploitation of resources in areas flooded by man, these environments are being impacted, putting their resident flora and fauna at risk (Junk et al., 1989).

The floodplains with white or muddy waters are highly productive areas and draw attention for the fertility of their soils and the richness of aquatic macrophytes. The igapós, with black and clear waters, have soils that are poor in nutrients and acidic waters, presenting less richness of species of herbaceous plants (Junk, 1993; Piedade et al., 2010). Among the species of aquatic plants, which occur in both floodplains and igapós, notably in mixed areas between the two types of water, *Victoria amazonica* (Poepp) Sowerby (Nymphaeaceae) stands out, which is the plant selected for this study.

Water, oxygen, temperature and light are basic conditions required for seed germination (Fowler and Bianchetti, 2000). Therefore, knowing how these environmental factors influence germination is very important. Germination of mature seeds, when it occurs under appropriate environmental conditions, is understood as the process of reactivating the growth of the embryo, reaching the highest point, that is the rupture of the integument, radicle protrusion and subsequent formation of the seedling (Fowler and Bianchetti, 2000).

Some seeds have dormancy, which is one of the main problems for the production of seedlings of many species, notably those of forest interest. Seed dormancy is a process characterized by delayed germination, when seeds, even under favorable environmental conditions (humidity, temperature, light and oxygen), do not germinate. This phenomenon can last for a few days or even months, and can last for several years (Sert et al., 2009). Therefore, when faced with this phenomenon, it is necessary to know how the species overcome the state of dormancy, so that through it is possible to search for alternatives for a fast and homogeneous germination.

The seed-coat impermeability to water is the most common mechanism of seed dormancy in tropical legumes (Nascimento et al., 2003). The mechanical and chemical scarifying treatments on the seeds favored the overcoming of dormancy when compared to seeds without treatment. *Victoria amazonica* seeds have resistant seed-coat, requiring scarification for a more accelerated germination (Rosa-Osman et al., 2011; Tozin et al., 2016).

According to Rosa-Osman et al. (2011), the germination of *Victoria amazonica* occurs in hypoxic conditions and in the dark, with only 4.7% of dissolved oxygen. These conditions correspond to germination in a natural environment, where the seeds, after being disseminated by the water and having the aryl that keeps them floating disintegrated, sink, and if the water conditions are favorable, they germinate. The authors complement that if at the moment of seed germination, the place is dry, they remain dormant until the rivers flood to germinate and form the adult plant that is fixed on the substrate, with floating leaves.

So, the aim of this paper was to complement the investigation on seed germination and post-seminal development of *Victoria amazonica*. In addition, it was evaluated the influence of environmental characteristics on the growth of the plants.

MATERIAL AND METHODS

The seeds collected in the field were packaged in plastic bottles and sent to the Ecophysiology laboratory of the INPA/Max-Planck Project, INPA, Manaus. In the laboratory, the seeds were placed in closed pots of water under hypoxia conditions and in the dark for later germination treatments. To test

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the best germination conditions of *Victoria amazonica*, ten types of treatments (T) were initially carried out with ten seeds in each one, confronting oscillations of light or dark, water or substrate, presence or absence of oxygen. The treatments were (n=10): T_1 – Light + water + oxygen (made with aquarium pumps); T_2 – Dark + water+ oxygen (container covered with aluminum foil); T_3 – Light + water; T_4 – Dark + water; T_5 – Light + substrate; T_6 – Dark + substrate; T_7 – Light + water – without oxygen; T_8 – Dark + water – without oxygen; T_9 – Light + substrate + water + scraped integument in the hilum region; and T_{10} - Dark + substrate + water + scraped integument in the hilum region.

After these pilot experiments, in which the observations were carried out for a period of 90 days, the best conditions for seed germination were defined. Therefore, a new experiment was carried out with the most appropriate treatments, and other treatments were also improved, for example, with or without the presence of aryl in the seed. In sequence, the following treatments were performed (n=25): T_{11} – Dark + substrate + water + shaved integument + without aryl; T_{12} – Dark + substrate + water + without aryl; and T_{13} – Dark + substrate + water + with aryl in the seeds.

In the last group of treatments, the seeds, in 25 repetitions per treatment, were followed for a period of 40 days, until germination ceased. For the dark treatments, aluminum foil was used to seal the container, distilled water was used and as a floodplain soil substrate. The seed hilum was removed with a stylus. The observations on germination were daily, with notes of the results.

The development of *Victoria amazonica* has been established and documented combining monitoring and collection of plants in the field and germination treatments, as described at the beginning of this topic. Field collections to document the plant development were made in the month of November, when the rainy season begins and sprouts and germination occur.

Seedling was considered the phase that covers the plant from the consummated germination of the seed to the formation of the first leaf or eophyll. After this phase, the next stage is the "tirodendro" (called by other authors as young plant or juvenile plant), which comprises the end of the development of the first seedling eophyll until the moment when the first metaphylls appear (Souza, 2009; Souza et al., 2009). The terminology adopted for the morphological study of the seedling and "tirodendro" was based on Souza et al. (2009).

The seedlings used to describe the plant development were the result of germination experiments. Ten days after germination, the seedlings were transferred to ponds with water and floodplain substrate and also to a 30 L aquarium to complete their development. From the seedlings, young and adult *Victoria amazonica* plants, the leaves, cotyledon and juvenile deltoid-stemmed leaves, lanceolate to the orbicular-peltate were described morphologically.

RESULTS AND DISCUSSION

Germination conditions

In the first experiment carried out to test the seeds germination (pilot experiment, T_1 to T_{10}, 10 seeds per treatment) only T_{10} showed germination in the 90 days of observation. This treatment corresponded to the following variables: dark, lowland substrate, water, and scraped seed-coat. In the other treatments in the presence of light, oxygen, with and without substrate, germination was not observed. Another treatment in which germination showed relative success was floodplain substrate, water, scratched seed-coat in the hilum region and without lighting; however, in this treatment, the fungal infestation around the seeds impaired the germination and the establishment of young plants.

In the second experiment, three treatments were followed (T_{11}, T_{12} and T_{13}), all with dark, substrate and water variables, adding a shaved seed-coat in T_{11} and maintaining the aryl in T_{13}. The treatment that showed the greatest germination success was also the one in which seed-coat was shaved, which showed a number of seeds about three times greater than in the other treatments, even though, in this one, a greater propensity to contamination by fungi was also observed, which, however, did not reduce germination or the rate of seedling and young plant formation (Table 1).

The seeds were completely dormant for long time that is imposed by the seed-coat and a very long seed aryl. Therefore statistical analysis of the data was impossible, since it would require a very long monitoring of germination and a higher number of seeds to carry out the experiments than that obtained during the collections in the study environments. It was also observed that many of the seeds that did not germinate had unviable embryos.

Most of the seedlings transferred either to tanks with shade or to an aquarium remained only 30 days in this condition, dying after this period. This is probably due to the reduction in the supply of nutrients in the environment, and the fact that many seedlings were attacked by phytophagous, which ingested the leaf lamina, which weakened the plants

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and culminated in their death. Those that managed to reach the young plant stage developed until the fifth eophyll.

Plant development

The development was accompanied concomitantly with the germination of the seeds, where each morphological phase from the emergence to the "tirodendro" (young plant) was analyzed and illustrated, as soon as this material was removed from the experiments.

The seedling initially has a small primary root (Figure 1A) that quickly atrophies, giving rise to the development of adventitious roots (Figure 1D). The cotyledon (Figure 1) has an acyclic shape, is chlorophyllous, and curves and dries when the first leaf (eophyll) appears completely. Although the embryo has two cotyledons, only one develops in the seedling. The seedling is considered hypogeal, as it has no hypocotyl (excessively reduced hypocotyl), with a unilateral cotyledon (Figure 1).

Table 1 - Total emergencies, seedlings and "tirodendros" (young plants) of *Victoria amazonica* in experiment II, with three treatments (n=25 seeds).

Phases/time	**Treatments**		
	T_{11}	**T_{12}**	**T_{13}**
Emergencies	15	05	07
Seedlings	15	05	07
Tirodendros	08	03	04
Time (days)	40	40	40

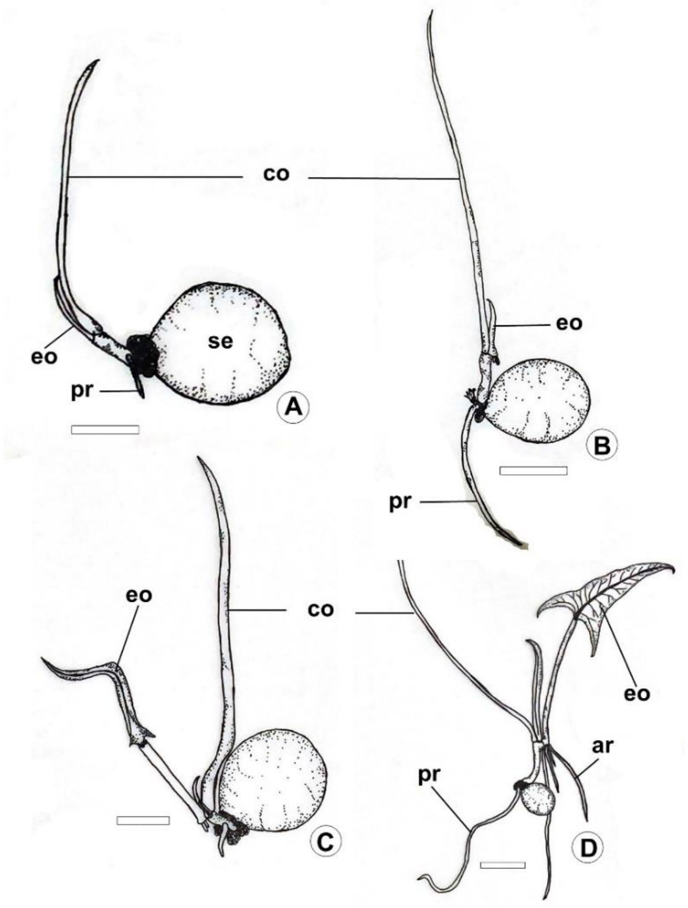

Figure 1 - Seedling morphology of *Victoria amazonica*. A - Seedlings two/five/seven/ten days old after germination, respectively. (ar=adventitious root; co=cotyledon; eo=eophyll; pr=primary root; se=seed). Scale bars: 5mm (A-C), 10mm (D).

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After two days of life, the seedling (Figure 1A) consists of the primary root, the cylindrical stem that emerges from the seed and under it the acicular cotyledon and the first eophyll still adhered to the cotyledon. On the fifth day after germination (Figure 1B), the primary root and cotyledon develop, and the eophyll detaches becoming fully developed in the plant with ten days of life (Figure 1C,D). In this phase, the seedling also has adventitious roots (Figure 1D) originating from the base of the first eophyll, still remaining with the primary root, the stem, the cotyledon that begins to bend and the beginning of the development of the second eophyll of the plant.

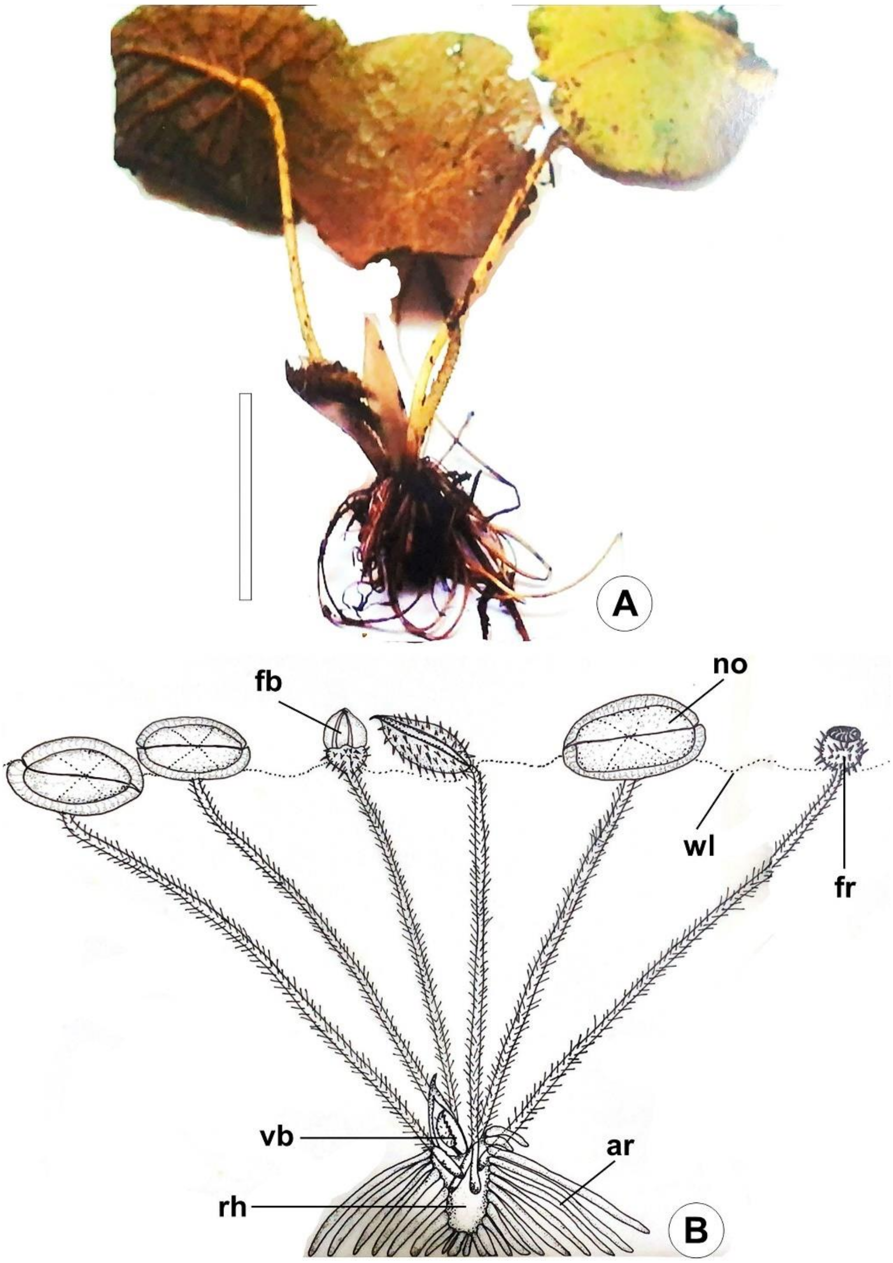

Figure 2 - “Tirodendro” (young plant) (A) and schematic drawing of adult plant of *Victoria amazonica*. (ar=adventitious roots; fb=flower bud; fr=fruit; no=nomophyll; rh=rhizome; vb=vegetative bud; wl=water level). Scale bar: 10cm.

Normally, the adventitious roots develop into the main roots of the plant, at the age of twelfth day of development. The primary root still remains in this phase, however, soon it will lose its function, atrophying. The stem remains and in the following stages it begins to develop and form the rhizome that will be present in the adult plant. The apical bud appears at this stage and will prevail

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throughout the rest of the life cycle, allowing the appearance of young leaves (Figure 2A).

The second deltoid-stemmed eophyll begins its formation on the tenth day after germination and on the twelfth day it is fully expanded, presenting a short petiole devoid of spines, wedged base and acuminated apex, with parallel striations, while the first eophyll has petiole with fragile spines and reticulate streaks in the leaf blade (Figure 2A). The third eophyll appears when the seedling is twelve days old and persists until the twentieth day, it has long petiole with thorns, cordiform base, acuminate apex and reticulated streaks (Figure 2A). From this stage, a new phase begins, in which the leaves will constantly renew and will acquire the format that prevails in the adult plant, viz. orbicular peltated shape.

On the twentieth day of seedling development, the fourth and fifth eophylls are formed, which are characterized by presenting reticulated venation and long petiole with spines. The former eophyll is peltate (petiole in the center of leaf blade), and consists of auricular base and aute apex, whereas the fifth eophyll or metaphyll resembles the nomophyll (Figure 2A). The well-know nomophylls (Figure 2B) have peltate orbicular shape, all rolled up at the beginning of development, stretching after twenty days, culminating in a leaf of approximately 2m in diameter, with edge up to 12cm high and petiole reaching 8m in length.

After eighty days of life, the small germinated seed turns into a beautiful adult plant, which is already fertile, with flower buds, fruits, young and adult leaves (Figure 2B). The plant fixation is done by the adventitious roots and the rhizomatous stem, the petiole is long with a maximum average value of 6.85 m, coated with resistant spines that help in the protection of the plant against aquatic predators. The pelleted orbicular leaf has dimensions of leaf area of up to 3.27 m^2. With such development, an adult plant presents rhizome on average 65 cm in length ($n = 6$), without the presence of thorns, being that it is totally buried in the substrate, and from it the young leaves and flower buds that will originate flowers and fruits.

Our results showed that *Victoria amazonica* has seed-coat that is impermeable to water entry, which is characterized as a physical dormancy. According to Ferreira and Borghetti (2004) the *Victoria amazonica* seeds are recalcitrant and dormant, showing the aryl with inhibitory substances and resistant seed-coat. It is possible for a seed to be recalcitrant and dormant at the same time, depending on the ideal conditions necessary for its establishment (Ferraz and Sampaio, 1996). Dormancy is not necessarily a disadvantage; it can minimize competition and prevent germination in unfavorable conditions for the establishment of seedlings (Sert et al., 2009). Tozin et al. (2016) investigated the germination of *Victoria amazonica* in the “Pantanal Matogrossense”, Brazil, and found that the highest germination occurred at 25º C, independent of the light condition; for the authors the seeds were considered neutral photoblastic.

After the seedling stage, the formation of several other forms of eophylls and metaphylls occurs until the plant reaches the adult stage, characterized by the orbicular leaves (nomophylls). *Victoria amazonica* presents heterophylly in its development (it was also verified in seedlings of *Victoria amazonica* from plants collected in the Pantanal of Mato Grosso, Brazil, by Tozin et al., 2016), with the following morphological sequence of the leaves: acicular (cotyledon), lanceolate-stemmed, deltoid-stemmed, peltate and orbicular leaf (Gwynne-Vaughan, 1897; Hoehne, 1948; Sculthorpe, 1985; Rosa-Osman, 2005).

The seedling stage of *Victoria amazonica* begins with seed germination and extends to the age of ten days, when the plant shows the first expanded eophyll. The main characteristic of the seedling is the presence of the acicular cotyledon, a condition that deserved special attention from Rosa-Osman et al. (2011). It must be pointed out here that *Nelumbium* Juss., a genus of Nymphaeaceae, is devoid of acicular leaf (Gwynne-Vaughan, 1897); moreover, the author mistakenly considered the cotyledon to be the first leaf or eophyll of the seedling. It must be pointed out here that “tirodendro” (young plant) phase of *Victoria amazonica* is reached with the formation and expansion of the first metaphyll, an observation also reported by Rosa-Osman et al. (2011). The first metaphyll appears in the plant at twenty days of age.

Two morphological features of the *Victoria amazonica* plants proved to be important for them to have better development in varzea floodplain, the rhizomatous stem and the presence of larger leaves. It was found that the rhizomes of the várzea environment have caloric value three times higher than that of mixed water.

CONCLUSIONS

The *Victoria amazonica* seeds have physical dormancy (hard seed-coat), and the aril compounds also inhibit the germination. The most favorable conditions for germination include dark, lowland substrate, water and scraped seed-coat. Plant development revealed that the seedling is hypogeal with acicular cotyledon, the primary root is replaced entirely by adventitious roots, the stem is

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rhizomatous, the first eophyll is lanceolate-shaped, and peltate orbicular metaphyll forms in a 20-day-old plant. The conditions of the rhizomes of the várzea environment have caloric value three times higher than that of mixed water, and the presence of larger leaves suggest the várzea floodplain to be more favorable environment for growth of *Victoria amazonica*.

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