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Original Article

Germination and seedling development in *Pistia stratiotes* L.

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Abstract: This study aimed to describe the post-seminal development, evaluate how the available water, light and temperature influence seed germination and to describe the vegetative propagation by selecting one of the collected plants of *Pistia stratiotes*. The plant material was collected in a tributary of the Rio Igarapu, Parnaíba, Piauí State, Brazil. A botanical description of the species was made using individuals collected in the study area. For the germination tests, a total of 1400 seeds were harvested and three experiments were conducted, varying the amount of available water, light and temperature. The results showed that the highest percentage of germination is occurred with seeds pre-washed with distilled water, sown on filter paper with abundant water (enough to form a sheet of water) and exposed to alternating light at an average temperature of 37°C. Germination began on the fourth day after sowing, marked by the appearance of the cataphyll. After 22 days, the seedling stage was completed. Vegetative propagation of this species is efficient and fast. In 50 days, a total of 134 new vegetative shoots were recorded in just one individual. The results showed that the germination rate is related to the amount of light and temperature.

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Introduction

Pistia stratiotes L., the only species of the genus *Pistia*, is an aquatic macrophyte of the family Araceae, known by the vernacular names of water lettuce (Howard and Harley, 1998). Recent studies using molecular and fossil evidence (Renner and Zhang, 2004) concluded that *Pistia* is part of an ancient clade originating in the Cretaceous in the Tethys Sea region of the Old World. Its present-day pantropical distribution is probably the result of dispersion of seed and plant parts by birds (Holm et al., 1977; Cabi 2016), but in more recent times its foliage has made it attractive as an ornamental aquatic plant and this may also have contributed to its dispersal in modern times (Cícero et al., 2007). The species is recorded in all five major regions of Brazil (Coelho et al., 2015). In Piauí, where the present study was carried out, *P. stratiotes* occurs in annual or perennial ponds and eutrophicated lowland creeks and canals (igarapés).

Pistia stratiotes is ecologically important in watersheds as it is a bioindicator of pollution and is characterized by its rapid vegetative multiplication. It is an invasive species whose proliferation may rapidly cover the whole surface of a water body, impeding the penetration of sunlight and thus affecting the efficiency of transmission and intensity of light within the water. The dense carpets of *Pistia* thus formed result in the diminution of oxygen content in the water body and hence reduction of both animal and plant biodiversity (Langeland and Burks, 2008).

Studies of this species have focussed especially on the ecology of its control and proliferation (Tucker, 1983; Sommaruga et al., 1993; Noelli, 1996; Camargo and Biudes, 2006; Zimmels et al., 2006; Lu et al., 2010; Wang, 2010; Sánchez-Galván et al., 2013; Khan et al., 2014; Chen et al., 2014; Das et al., 2014; Yang et al., 2014; Ugya et al., 2015). Various studies of its germination and reproductive biology have been

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carried out since the 1980s, for example those of Piertese et al. (1981), Sharma and Sridhar (1981), Tucker (1983), Dray-Jr and Center (1989), Harley (1990), Dewald and Lounibos (1990), Sajna et al. (2007), Kan and Song (2008), and Eid et al. (2016). However only a few such studies have been published based on research in Brazil, e.g. Silva (1981), Teixeira et al. (2007), Henry-Silva et al. (2008) and Cancian et al. (2009).

The identification of seedlings and study of their biology is fundamentally important for a better knowledge of plants in general, but also because of their practical importance for horticulture and agriculture, and for understanding how plants colonize and establish themselves in their natural habitat (Guerra et al., 2006). Morphological studies of seeds are also important for species identification both in the laboratory and the field, and as aids to ecological and developmental studies (Oliveira and Pereira, 1984). Knowledge of the life cycle of species is in turn indispensable because basic knowledge of the morphology and germination of species makes possible understanding of the natural mechanisms existing in ecosystems, such as the renewal of their resources and how species behave at different stages of development (Kuniyoshi, 1983).

The aim of this study was to describe the morphology of *P. stratiotes*, investigate the effect on germination of available water content, light levels and temperature, and study the post-seminal development with the aim of understanding the population dynamics of this species in its natural habitat.

Materials and methods

The materials were collected in a stretch of the Rio Igarapu (02°55'39.73"S and 41°46'21.21"W) in the Bebedouro District, Parnaíba, Piauí State, Brazil. The altitude of sampling station was 5 m above sea level, with a mean annual minimum and maximum temperatures of 20 and 32°C, respectively. Mean annual rainfall is 1200 mm (in Parnaíba), varying between 800 and 1600 mm, designated as an Equatorial Maritime Regime, with five to six

consecutive months of higher rainfall with a dry season for the rest of year (Aguiar, 2004).

The botanical description was made using randomly collected individuals. Voucher specimens are deposited in the Parnaíba Delta Herbarium (HDELTA) of the Federal University of Piauí.

Seed germination was studied using a sample of 1400 seeds divided into three experiments assays to observe germination under different conditions of light, temperature and water availability. The petri dishes had a diameter of 12 cm and plastic trays with 46 x 29 cm comp and 6.5 cm height. The design of the experiments is described in Table 1. The germination study was carried out in the Plant Cell and Molecular Laboratory of the Federal University of Piauí (UFPI) at Parnaíba.

The emergence of the cataphyll was used as the criterion to mark the initiation of germination. Observations of the germination were done daily, the analysed variables were the percentage germination and germination rate index (IVG), which were calculated according to Maguire (1962). The percentage germination follows standard norms for seed analysis (Brasil, 1992). The data were analysed using Analysis of Variance (ANOVA) followed by the Tukey Test for multiple comparisons.

For the study of post-seminal development, seedlings from the third germination assay were used. The illustrations were prepared using an optical stereomicroscope equipped with a drawing tube and the terminology adopted for the description based on Mayo et al. (1997).

Every day, the leaf number, leaf length and leaf width were recorded in the seedlings. The representative plants of each germination phase were illustrated and described in a chronological sequence from germination to the attainment of the young plant stage. Observation of the vegetative propagation was made by selecting one of the collected plants, and placing it in a 30L plastic vessel with water collected from the plant's habitat. Every day, the evaporated water was replaced from the tape water (AGESPISA). This experiment was carried out for 50 days.

Table 1. Experimental design for seed germination in *Pistia stratiotes*.

Experiment	Experiment conditions	Treatment	Wash	N°. seeds	Photoperiod	Exposure to light	Light intensity	Temperature
1	600 seeds	1	Pre-wash with distilled water	2 plates, 100 seeds per plate	12h light/ 12h dark	shade	50%	35°C
		2			12h light/ 12h dark	Sun	100%	37°C
		3			continuous light	laboratory	100%	24°C
2	800 seeds	1 (control)	Absence of Prewash		continuous light	laboratory	100%	24°C
		2	Pre-wash with distilled water		12h light/ 12h dark	Sun	100%	37°C
		3			12h light/ 12h dark	shade	50%	35°C
4	continuous light	laboratory		100%	24 °C			
3	600 seeds *	2 group 1	Pre-wash with distilled water and watered with collection water	100 seeds per plate	12h light/ 12h dark	Sun	100%	37°C
		2 group 2	Pre-wash with distilled water **			shade	50%	35°C
		3 group 1	Pre-wash with distilled water and watered with collection water					
		3 group 2	Prewash with distilled water **		shade			
		4 group 1			continuous light	laboratory	100%	24 °C
		4 group 2						

* Seeds were used which did not germinate in the second experiment (from treatments 2, 3 and 4).

** Water from city supply network.

Results and Discussion

Taxonomic description

Pistia stratiotes L. Sp. Pl. 963 (1753)

(Fig. 1A-E)

Floating aquatic macrophyte, free or fixed, 3.4-8.4 cm long, acaulous; stolons green, pilose, lacking foliar structures, 3-7.5 × 1-1.7 cm. Roots adventitious, brown and white, pilose, 16-20 cm long. Leaves simple, rosulate, subsessile to petiolate; petiole white, glabrous, 0.1-0.3 cm long; leaf blade green, spongy, obovate-oblong, base attenuate, apex retuse, 2-9 × 1.5-4.5 cm; major veins parallel, 5-8, prominent on abaxial surface, pale green, with abundant trichomes on both surfaces. Inflorescence solitary per leaf, 5-7 per plant, axillary, composed of unisexual flowers, 1-1.4 cm long; perigon absent; peduncle 0.2-0.4 cm long. Spathe green at base, white at apex, adnate to ovary in basal portion, margins between stigma and androecium forming a lower, tubular feminine chamber, upper part more expanded forming an open male chamber with a blade, densely pilose externally, 0.2-0.5 × 0.2-0.3 cm. Male flowers forming a verticel

near or at spadix apex; stamens connate in pairs to form 5-7 yellow synandria in verticillate male zone. Female flower at spadix base; pistil 5-7 mm long, ovary ovoid, oblique to spadix axis, trichomes present around ovary, 3-4 × 2-3 mm, unilocular, ovules numerous, placenta basal, stigma short but distinct. Fruit a berry, green, pilose, ellipsoid, irregular, 5-7 × 3-3.1 cm. Seed barrel-shaped, brown, 3-9 per fruit, 1.2-1.5 mm long, micropyle with an operculum, testa reticulate, thick.

In the area of collection, the species occurs at the margins of creeks, in the polluted localities, and usually associated with other species such as *Eichhornia crassipes* (C. Mart.) Solms, *Lemna* sp., and *Wolffiella* sp.

Germination of *P. stratiotes*

First germination experiment: Germination of the seeds in the first experiment took place four days after sowing, with rupture of the operculum. During this period, the germination index for treatment 1 was 18%; treatment 2, 19%; treatment 3, 2% (Table 2). The results also showed a greater germination



Figure 1. *Pistia stratiotes* L. a. Habit; b. Detail of the inflorescence insertion; c. Detail of the stolons; d. Inflorescence; e. Seed.

Table 2. The results of the experiment 1. Germination (%) and germination velocity index (GVI) of seeds of *Pistia stratiotes*. Pairs of values with the same letter indicate non-significant differences between means as tested by ANOVA and Tukey's multiple comparison post-test; those with different letters indicate significant difference.

Treatments	Germination	GVI
1	76%	7.06 a
2	89%	8.18 a
3	64%	0.7 b

percentage and germination rate index (IVG) in treatment 2. However, there was no significant difference between treatments 1 and 2 in the percentage germination. For IVG, a significant difference was found between treatment 3 (exposure to laboratory conditions), with a value of 0.7, and others, concluding that seeds of *P. stratiotes* germinate when pre-washed with distilled water and then sown

in Petri dishes lined with filter paper and kept wet with distilled water under continuous light in the laboratory, or in the open with light alternating between exposure to sunlight and shade. Speed of the germination was also greater with the alternating sun/shade regime.

Second germination experiment: There was no germination in any treatments in this experiment using

Table 3. The results of the experiment 3. Germination (%) and germination velocity index (GVI) of seeds of *Pistia stratiotes*. Pairs of values with the same letter indicate non-significant differences between means as tested by ANOVA and Tukey's multiple comparison post-test; those with different letters indicate significant difference.

Treatments	Germination	GVI
2 group watered with collection water	98% a	98a
2 group 2 watered with AGESPISA tape water	92% a	92a
3 group 1 watered with collection water	55% b	47b
3 group 2 watered with AGESPISA water	64% b	48b
4 groups 1 and 2 watered with AGESPISA water	67.5% b	75.5c

substrate irrigated with distilled water. The result shows that *Pistia* seeds are sensitive to desiccation. This species is a floating of fixed aquatic plant (depending on the water level of the habitat) and it is therefore to be expected that its seeds germinate only in the presence of abundant water. According to Teixeira et al. (2007), the species invests more in sexual reproduction in dry periods when the water level is lower; the seeds are able to resist the drier conditions through dormancy and later germinate in the rainy season.

Third germination experiment: In the third experiment, seeds which did not germinate in the second experiment were used. The seeds were watered abundantly so that they were covered by a layer of water derived either from their natural habitat, or from tape water. The results showed that water availability has an effect on seed germination. Based on Table 3, the percentage germination of seeds exposed to the sun (direct daylight, for 12h) was significantly different in three light regimes: direct sunlight for 12h, 50% sunlight (shade) for 12h, and laboratory artificial light for 24h. However, within each light regime, there was no significant difference in percentage germination or germination speed (IVG) between water treatments (habitat vs. mains water supply). The seeds germinate better with distilled water, sown on filter paper with sufficient water to form a water layer, and exposed to the sun for 12h at a mean temperature of 37°C. Cancian et al. (2009) showed that *P. stratiotes* is sensitive to low temperatures, showing less growth, leaf yellowing and leaf death at 15°C, whereas at 25°C with a 12h photoperiod higher growth rates were observed.

Kan and Song (2008) studied the germination of

P. stratiotes in relation to potential seed storage. They showed that there was no germination in darkness, or in far red light, but white light and red light and alternating 12h photoperiods of red light and darkness promoted germination. Percentage germination of seeds that had been rapidly dehydrated was 73.3%, and in white light, seed germination changed from zero to 68%. Addition of nitric oxide brought about germination even in darkness. Water is the factor with the most influence on the process of germination. After inhibition, the tissues of the seed undergo rehydration and as a consequence, an intensification of respiration and other metabolic activities, which result in the provision of energy and necessary nutrients for the resumption of growth by the embryonic axis (Kramer and Kozlowski, 1972). The speed of water absorption varies among species with the number of pores in the tegument surface, the availability of water, temperature, hydrostatic pressure, area of contact between seed and water, intermolecular forces, chemical composition and physiological condition of the seed (Nassif et al., 1998).

The water layer can enhance germination in *P. stratiotes*. Cassol et al. (2008) studied the effect of flood conditions (saturated soil at 5, 10 and 20 cm submersion depth) on the germination of seeds of *Sagittaria montevidensis*, a weedy aquatic plant, and verified that a water layer favoured germination. Rego et al. (2009) showed that there was an interaction between temperature and water quantity in the substrate in germination of seeds of *Blepharocalyx salicifolius*. Silva (1981) collected seeds of *P. stratiotes* in three different environments (Lago Castanho, Rio Solimões and Lago Baixio) and

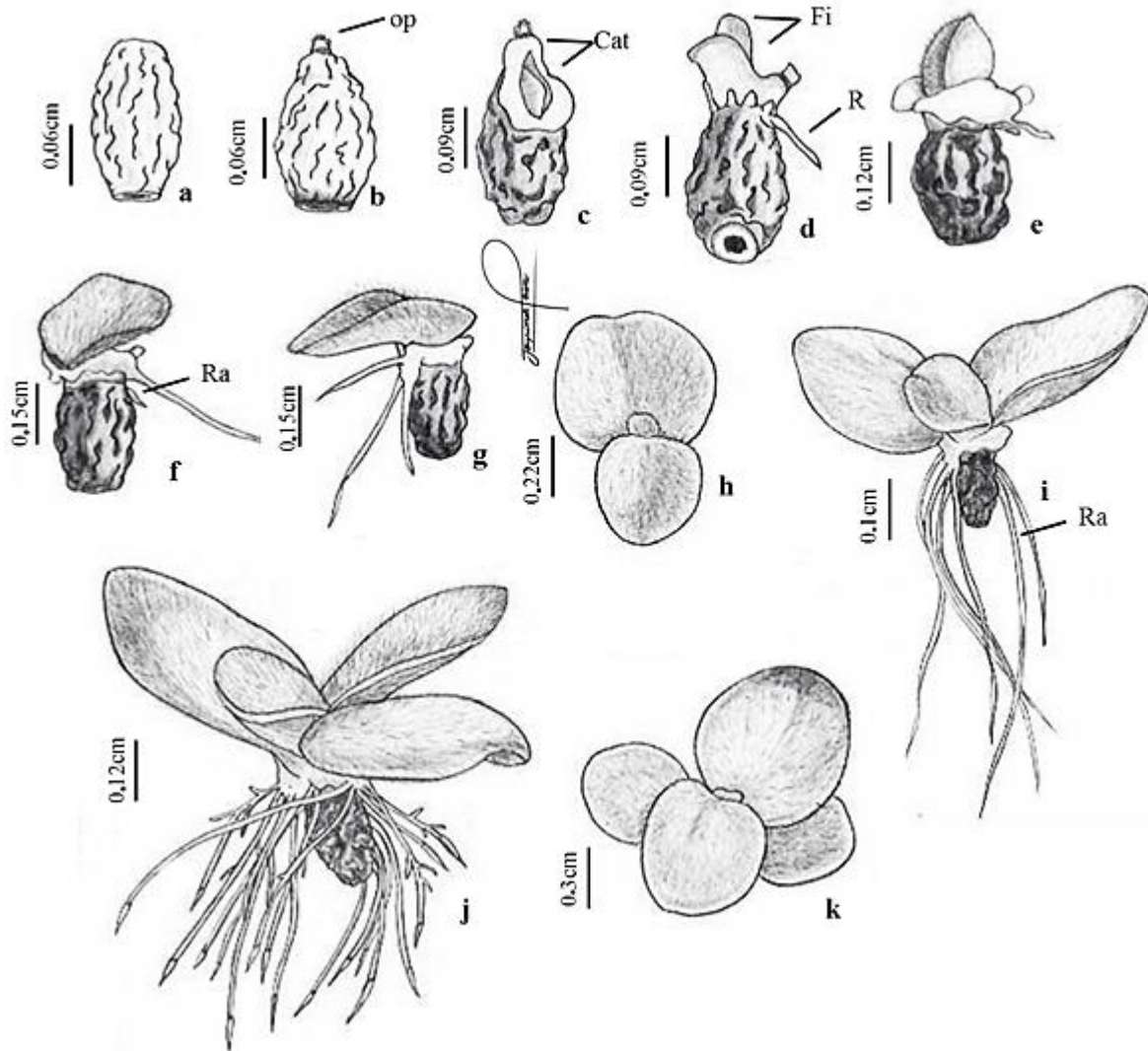


Figure 2. Post-seminal development of *Pistia stratiotes*. a. Seed; b. Turgid seed; c. Emergence of the cataphyll (Cat); d. First true leaf (Fi), and the emergence of the radicle (R); e. First leaf begins to open; f. First leaf opens completely, showing trichomes and adventitious roots (Ra); g. Presence of the 3rd root, death of the radicle; h. Appearance of the second true leaf; i. Appearance of the third true leaf; j. Roots now numerous; k. End of seedling phase.

germinated them in beakers with water up to a level of 10 cm. With this method, this author obtained a low index of seed germination per fruit (24.2, 21.3 and 10.8%) in the three environments. When they were placed in the germination vessel, the seeds floated at first and then sank two to six days later, or sank as soon as they touched the water, and then in both cases germinated at the bottom and after about seven days rose to the surface as seedlings. Silva (1981) carried out a second experiment testing the seed germination of *P. stratiotes* at depths of 10 and 30 cm (in beakers with water), obtaining 40 and 32% mean germination, respectively. At 10 cm, all seedlings floated to the top

after germination but at 30 cm depth none did so. These results showed that depth could be a factor which influences propagation by seed (Silva, 1981).

The seeds of *P. stratiotes* are small, with an average length of 1.2-1.5 mm, and thus the water layer need be no more than 2 cm in depth to ensure that the water completely surrounds it to facilitate its penetration into the seed. In the present study, we suggest that the pre-washing with distilled water provides a first contact between seed and water which predisposes it to further water penetration and thus initiates imbibition. In addition, the water washes the seed tegument and thus may remove any germination inhibitor substances



Figure 3. Vegetative propagation of *Pistia stratiotes* L., days after the beginning of the experiment. (a) day 8, (b) day 12, (c) day 39, and (d) day 50.

which may be present. In addition to water, it is essential that other conditions are optimal for the seed to achieve maximum germination capacity (Figliolia et al., 1993), among which are the light intensity and temperature to trigger germination.

In the present study, the seeds of *P. stratiotes* were exposed to continuous light (in the laboratory) or alternating light and dark photoperiods of 12 hours, and either in shade (50% intensity) or in full sun (100% intensity). The results showed that exposure to full sun favoured germination. In the locality where the seeds were collected, the light intensity was high from September to January. During this period, the temperature is also high with little variation from night to day. In their natural habitat the seeds probably germinate soon after dispersal.

Post-seminal development in *P. stratiotes*: Seed production is high in its natural habitats and the

collected seeds showed 98% viability of germination. At the beginning of germination, the seed becomes turgid by absorption of water. On the second day, the cataphyll pushes open the seed operculum (Fig. 2b). On the third day, the cataphyll, ca. 2 × 1 mm in size, emerges completely and in some seeds the first leaf appeared (Fig. 2c). The radicle, 0.5-1 mm long, and first leaf occur on the fourth day, making it possible for the seedling now to float. In this phase, the cataphyll measures 2 × 1 mm and first leaf 2×1 mm (Fig. 2d). On the fifth day, the first leaf shows the presence of trichomes (Fig. 2e). On the sixth day, the radicle measures 5.5 mm, the first leaf, now completely unfolded with 2 mm long, the trichomes are clearly evident and numerous, and a second root was appeared (Fig. 2f). On the seventh day, the third root was appeared (Fig. 2g) and on thirteenth day, the radicle was disappeared and the seedlings are now

free-floating (Figs. 2g-h). On the fourteenth day, other leaves appeared (Fig. 2i) and on the eighteenth day, the roots were numerous (Fig. 2j). On the twentieth day, the tegument falls off and seedling stage was completed (Fig. 2k).

Vegetative propagation: In addition to an efficient reproduction by seed germination, *P. stratiotes* propagates itself vegetative. We observed the formation of approximately 28 stoloniferous shoots by eighth day of the experiment, 36 by twelfth day, and 134 by thirty ninth day. After 50 days, 146 new vegetative produced rosettes were observed (Fig. 3a-d). Thus an intense asexual reproduction by means of stolons in a short time was observed, having an IVG of 4.4% individuals per day. After 50 days, we observed that 24 individuals were in flower, although we did not verify the presence of fruits. Teixeira et al. (2007) studied the reproductive strategy of *P. stratiotes* and verified that 54% of individuals produced asexually did not flower in wet localities, and in flooded localities more than 85% failed to flower. They observed less than 34% of individuals with fruits.

The vegetative multiplication of *P. stratiotes* is considered important for its propagation. Normally an individual produces from 1-7 short stolons arising in the axils of inconspicuous prophylls that surround the base of each foliage leaf and they develop as the plant grows to its natural full size (Lemon and Posluszny, 2000). Temperature, photoperiod and nutrient availability affect gaining biomass and production of shoots in *P. stratiotes*, although details of how this takes place remain little understood (Cancian et al., 2009). The reproduction of aquatic plants consists in both sexual and asexual mechanisms (Harper, 1977). Although sexual reproduction introduces new genetic individuals into the population, vegetative propagation produces modules with the potential for physiological independence which contribute to the survival and reproductive success of the plant, as well as diminishing the risk of mortality of the species (Cook, 1979), allowing it to explore wide areas and new localities, facilitating a greater dispersion of the propagules (Lovett- Doust, 1981).

The present study shows that seed germination in *P. stratiotes* is influenced by temperature, abundance of water, and light levels. In the scarcity water, the seeds fail to germinate. Knowledge of the germination of weedy species is of fundamental importance for the development of management practices. *Pistia stratiotes*, a weedy aquatic species, can interfere with the life cycle of plant and animal species which share its environment.

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