Seed germination and seedling growth of two *Pseudobombax* species (Malvaceae) with contrasting habitats from Brazilian Cerrado

Clesnan Mendes-Rodrigues, Paulo Eugênio Oliveira & Marli Aparecida Ranal

Programa de Pós-graduação em Ecologia e Conservação de Recursos Naturais, Instituto de Biologia, Universidade Federal de Uberlândia, Caixa Postal 593, 38400-902 Uberlândia, Minas Gerais, Brazil; clesnan@hotmail.com, poliveira@ufu.br, ranal@ufu.br

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Abstract: Pseudobombax tomentosum and P. longiflorum are common trees in the Cerrado region, but the former species is more common in forest edges while the later is present in open cerrado areas. This work aimed to investigate differences in seed germination and seedling growth in these species, from seed collected from Cerrado areas in Central Brazil. For this, a seed germination experiment was designed and included four replicates with 25 seeds per species; seeds were randomly distributed in the germination chamber. To evaluate initial seedling growth, seedlings height was measured up to 67 days after seedling emergence; besides, some of these seedlings were grown for biomass evaluation during nine months. Results showed that seeds of the two species had the same germinability (near 100%) and mean germination time (ca. 12 days). However, P. longiflorum showed a more spread seed germination through time, with higher values of coefficient of variation in germination time and uncertainty index; and lower values of synchronization than P. tomentosum. The two species showed basically the same growth pattern, but lower values for height of apical meristem, diameter of underground structures (mostly roots), dry mass of shoots, underground structure and total mass of seedlings in P. tomentosum were obtained, compared to P. longiflorum. Both species allocated more dry mass to underground structures in detriment of shoot. This probably allows resprouting behavior which prevents hydric stress and detrimental fire action typical of the open Cerrado areas. Rev. Biol. Trop. 59 (4): 1915-1925. Epub 2011 December 01.

Key words: Cerrado, seed germination measurements, Neotropical savannas, plant growth, seedling growth, *Pseudobombax longiflorum*, *Pseudobombax tomentosum*.

Seed germination patterns differ in the same family, genus and even for the same species (Baskin & Baskin 1998). Inside the Bombacaceae, now the subfamily Bombacoideae, Malvaceae sensu lato (APG 2003), there are species with dormant and non-dormant seeds (Vazquez-Yanes 1974, Joly & Crawford 1983, Danthu *et al.* 1995), but information about seed germination patterns is not available yet for most taxa of this group.

Germination within the large Neotropical genus *Pseudobombax* is still poorly studied and the few published works are restricted to the germinability assessment, and sometimes to the speed of the process, based on the first and the last seed germinated in experimental conditions (Souza & Válio 2001, Souza-Silva *et al.* 2001, Zamith & Scarano 2004, Wittmann *et al.* 2007, Lopes *et al.* 2008). Other studies for the family focused on the ratio between dry mass of roots and shoots or on seedling growing under different light and soil conditions, without records for *Pseudobombax* (Moreira & Klink 2000, Scalon *et al.* 2003).

Among the species of Bombacoideae from cerrado, *P. longiflorum* and *P. tomentosum* are species that occur in open fire-prone plant formations, but also at the edge or inside gallery, deciduous and mesophyllous forests (Gribel 1988, Silva & Scariot 2004). *P. longiflorum* is much more common in open savanna formations and has hanging bat pollinated flowers (Coelho & Marinho Filho 2002). P. tomentosum is much more common in forest habitats where its sturdy flowers depend on non-flying mammals passing from crown to crown for pollination (Gribel 1988). Except from the differences in habitat, the species are similar in relation to the distribution, phenology and seed dispersion period (Lorenzi 1992, Silva Júnior et al. 2005). The absence of exclusive habitats between the two species reflect the presence of physiognomic gradients and transitions among savanna and forest areas in the Cerrado Biome (Ribeiro & Walter 1998). The water stress and the occurrence of seasonal fire in the Cerrado biome select species with ability to resprout after these events, leading to the allocation of available resources to the roots or other underground structures in detriment of the shoots in open formation species while forest species show allocation to aerial parts in detriment of the roots (Hoffmann & Franco 2003). Inside mesophyllous forest and gallery forest formations, light is the limiting factor but habitat shows less oscillations in water regime and temperature, favoring fast and synchronized seed germination whenever gaps or leaf fall cycles improve seedling survival chances as discussed by Garwood (1983). Meanwhile, in the savanna habitats, the limiting factors are water and nutrients, and seed germination spread in time, and even dormancy, may favor seedling survival (Moreira & Klink 2000, Hoffmann & Franco 2003, Oliveira 2008, Vieira et al. 2008).

In this context, vicariant species as the ones studied here provide the opportunity to see how habitat specialization would be reflected in germination and seedling growth (Hoffmann & Franco 2003). The objective of this paper was to test, on the one hand, if seed germination of *P. longiflorum* would be more asynchronous and seedling mass allocated predominantly in the underground structures, favoring seedling survival in the open savanna areas. On the other hand, for *P. tomentosum*, typical of forest formation with less water stress, with an expected more synchronized germination process and seedlings would have more mass allocated to the stems, favoring light absorption and continuous growth.

MATERIAL AND METHODS

Studied species: Pseudobombax longiflorum (Mart. & Zucc.) Robyns (Bombacoideae, Malvaceae) is found in most Cerrado areas. It is fairly common in Minas Gerais and down to the North of Paraná State. It is a deciduous tree, which occurs sparsely in open Cerrado areas (Lorenzi 1992). Flowering occurs from July to November and fruits mature during the next dry season, from July to November (Silva Júnior et al. 2005). P. tomentosum (Mart. & Zucc.) Robyns (Bombacoideae, Malvaceae) is usually a larger tree, found in Cerradão (dense cerrado with almost closed woodland) and Cerrado (closed vegetation dominated by trees and shrubs with herbaceous vegetation between them), but mainly in the edges and sometimes inside mesophyllous and gallery forests. It occurs in most Cerrado region down to Southern areas of São Paulo and Mato Grosso do Sul. It flowers from July to August and the fruits mature from August to October. It is a deciduous and heliophyllous plant, also appearing less frequently in open vegetation, usually on sandy or humic clay soils, but also on better drained oxisoils common in the Cerrado region (Lorenzi 1992, P.E. Oliveira, pers. observ.). The voucher specimens can be found in Herbarium Uberlandense.

Studied area: About 200 seeds of both species were collected in the Cerrado areas, Neotropical savannas region in Central Brazil (*sensu* Ribeiro & Walter 1998). The seeds of *P. tomentosum* were collected in forest areas, near to Uberlândia city, Minas Gerais state (19°07' W - 48°22' S) and the seeds of *P. longiflorum* were collected in adjacent open pasture and areas along the BR050 highway (17°06' W - 47°45' S), between Uberlândia and Brasília. Germination and seedling growth experiments were carried out at the Campus

of the Federal University of Uberlândia. The region climate is characterized as Aw according to the Köppen scale (Köppen 1948), tropical humid climate with a dry winter (April to September) and rainy summer (October to March) (Rosa *et al.* 1991).

Seed germination: Mature fruits of both species were collected in September and October 2003. The fruits were collected directly from the trees and placed to dry at room temperature in order to facilitate the extraction of the seeds. Seeds were stored in paper bags at room temperature (between 25 and 30°C), in a dry chamber containing silica gel with humidity indicator, and kept until the installation of the experiment, 30 days later. The seeds were sown on fine vermiculite (expansion volume of 0.1m³), inside transparent germination boxes, moistened as necessary with distilled water. Boxes were kept in a germination chamber (Seedburo Company, model MDG2000) under continuous light (mean=11.90, SD=6.52µmol/ m²/s of photosynthetically active radiation), at 25°C. The experimental units were randomly distributed in the germination chamber, four replicates for each species with 25 seeds per replicate. The seed sample size was somewhat limited as a consequence of the high level of predation of fruits and seeds during the collection year. Although seed samples used for the experiments were rather small, the data was still sufficient for consistent statistical tests.

The number of germinated seeds was observed daily and protrusion of the radicle or any part of the embryo was used as germination criterion. Germinability (G), represented by the percentage of germination in the experimental conditions (Labouriau 1983), mean germination time (MGT) (\bar{t} , Labouriau 1970), germination time to 50% germination (GT50), germination time of the first germinated seed (GTFS), germination time of the last germinated seed (GTLS), coefficient of variation of the germination time (CVGT) (CV_t, Ranal & Santana 2006), mean germination rate (MGR) (\bar{v} , Labouriau 1970), uncertainty of germination (UG) (U, Labouriau & Valadares 1976) and synchronization index of germination (ZG) (Z, Ranal & Santana 2006 adapted from Primack 1980) were used to describe the germination process. Further details on mathematical expressions, authorship, intermediate calculus, the sense and the applications of these germination measurements can be found in Ranal & Santana (2006) and Ranal *et al.* (2009). All germinated seeds were checked for the presence of more than one embryo per seed, known as polyembryony and common in Bombacoideae (Mendes-Rodrigues *et al.* 2005).

Seedling morphology and height: Seeds germinated in laboratory conditions were transplanted to black plastic bags (30cm high per 13cm diameter), filled with previously sieved open Cerrado oxisol. Seedlings were kept in a greenhouse covered with black plastic net with 50% shading and moistened when necessary. Starting from emergence date, periodic measurements of the maximum height of each plant, considered from ground to the apex of the last leaf were carried out (Fig. 1 H-1). The data were adjusted to a linear regression model, as a function of the days from emergence. Some 20 P. longiflorum seedlings were evaluated up to 67 days after emergence and 15 P. tomentosum seedlings up to 63 days after emergence, when initial growth appeared to stabilize (seedlings showed no further increment in height). The seedling functional morphology was classified based on Miquel (1987). These seedlings were kept for nine months under the same conditions to asses a biomass allocation evaluation, as described below.

Biomass allocation: Seedlings for biomass evaluation were analyzed nine months after sowing, before foliar abscission started. Seven plants of each species were removed from the soil and analyzed for the leaf number per plant, length of the shoot (considered from the soil to the apex of the last leaf) (Fig. 1 H-1), height of the apical meristem (from soil to the apical meristem) (Fig. 1 H-2), height of insertion of the first leaf from soil (Fig. 1 H-3), diameter at the shoot base, the largest

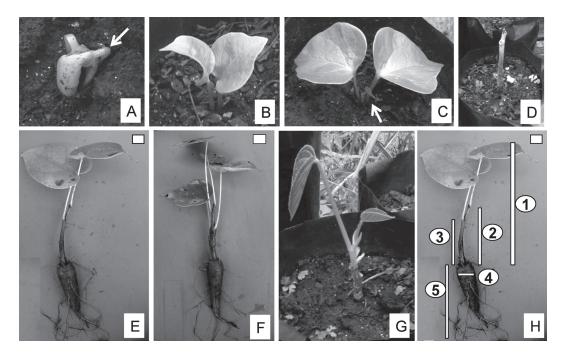


Fig. 1. Development phases of *Pseudobombax* seedlings, (A-E, G) Development phases of *P. longiflorum*. (F and H) Development phases of *P. tomentosum*. (A) Epygeal seedling emergence due to petiole elongation (see the arrow). (B) Cotyledon leaves with both petioles. (C). Aerial part emission (see the arrow). (D) Seedling with about 9-10 months, without leaves. (E) Seedling with 9 months; see the massive underground structure. (F) Seedling with 9 months; see the massive underground structure. (H) Seedling measurements: 1. Plant height, 2. Apical meristem height, 3. Height of the insertion of the first leaf, 4. Underground structure diameter, 5. Underground structure length. Scale bars = 2cm.

diameter of the underground structure (Fig. 1 H-4) and the length of the underground structure (Fig. 1 H-5). The seedlings were divided into aerial (stems and leaves) and underground parts (mostly roots), and dried to constant mass at 70°C (eight days). The dry mass ratio between underground and aerial parts and between underground and total seedling dry mass were calculated (Hunt 1990). Non-quantitative observations continued up to 18 months after sowing.

Germination measurements and seedling biometry data were evaluated for normality with the Shapiro-Wilk test. Data with normal distribution were compared using Student's *t*-test (all seedling biometry measurements, except underground structure length) and data without normal distribution were compared by Mann-Whitney test (all germination measurements, plus underground structure length). The relative frequency of seed germination was compared between species using the Kolmogorov-Smirnov test. The seedling height collected during the first 70 days was adjusted to a simple linear regression model as a function of the time from emergence and the significance of the model tested with ANOVA. The slope and intercept of the linear regression were compared between species using Student's *t*-test. For all the statistical analysis 0.05 of significance was adopted and procedures were based on Sokal & Rolf (1995).

RESULTS

Seed germination: Seeds of *P. longi*florum and *P. tomentosum* did not present significant differences in germinability, mean germination time and mean germination rate (Table 1). On the other hand, seeds of *P. tomentosum* presented greater homogeneity (lower value of CVGT) and higher synchrony of germination (lower value of UG and higher value of ZG) than *P. longiflorum*. The first germination was registered for *P. longiflorum* four days after the beginning of the experiment and the last germination occurred after 20 days. For *P. tomentosum* germination started after seven days and continued up to the 18th day after sowing (Fig. 2). The distribution of the relative frequency of germination differs between species (D=0.1980, p<0.05) and demonstrated that in *P. longiflorum*, this process was more spread out through time, with some peaks of germination in days 11, 13, 14 and 16, while in *P. tomentosum*, germination was less spread out in time and a strong germination peak occurred at the 11th day after sowing (Fig. 2). After 20 days, the remaining seeds were already degenerating and no further germination occurred. The two species presented only one embryo per seed and no polyembryony was recorded.

 TABLE 1

 Mean germination measurements (±SD) of P. longiflorum and P. tomentosum (Bombacoideae, Malvaceae) seeds

Measurement (unity) ¹	P. longiflorum	P. tomentosum	U^2	p ³
G (%)	99.00 ± 2.00	97.00 ± 2.00	4.00	0.2482
MGT (day)	12.17 ± 0.32	11.68 ± 0.48	4.30	0.1489
GT50 (day)	13.25 ± 0.50	13.25 ± 0.50	8.00	1.0000
GTFS (day)	4.50 ± 1.00	7.75 ± 0.50	0.00	0.0209
GTLS (day)	18.25 ± 1.26	16.75 ± 0.96	2.50	0.1124
CVGT (%)	30.17 ± 1.78	22.25 ± 4.83	4.00	0.0209
MGR (day-1)	0.08 ± 0.02	0.09 ± 0.003	4.30	0.1489
UG (bit)	3.29 ± 0.09	2.81 ± 0.24	4.00	0.0209
ZG	0.08 ± 0.004	0.14 ± 0.04	4.00	0.0209

CVGT=coefficient of variation of the germination time, G=germinability, MGT=mean germination time, MGR=mean
germination rate, GT50=germination time to 50% germination, GTFS=germination time of the first seed,
GTLS=germination time of the last seed, UG=uncertainty of germination, ZG=synchronization index of germination.

2. U=statistics of the Mann Whitney test.

3. p=probability.

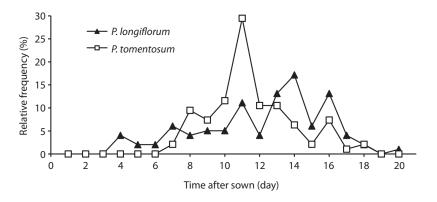


Fig. 2. Relative frequency (%) of seed germination of two Pseudobombax species (Bombacoideae, Malvaceae).

Seedling morphology and height: The seedlings of both species presented photosynthetically active cotyledons exposed above ground (Fig. 1 A-C) and were classified as phanero-epigeal-foliaceous (PEF sensu Miquel 1987). In classical PEF type the cotyledons are suspended from the ground by elongation of hypocotyl, while in these two species of Pseudobombax the elongation is caused by elongation of cotyledon petiole (Fig. 1 A). Although cotyledons are hold above ground, their fixation point remains below ground (Fig. 1 A-C). The seedling height of both species was well adjusted to a simple linear regression model (r²=0.8091, F_{1.105}=445.02, p<0.0001 for *P. longiflorum* and r²=0.8087, F_{1.105}=393.21, p<0.0001 for *P. tomentosum*) (Fig. 3A, 3B). The two species showed no significant differences between the slopes of

the equation (t=0.136, d.f.=198, p>0.05), nor between intercepts (t=0.832, d.f.=199, p>0.05), presenting basically the same pattern of continuous growth during the first 70 days.

Biomass allocation of dry mass: Both species presented an underground structure (mostly roots) which represented about 80% of the total mass of seedlings (Fig. 1 E-F). Among the analyzed characteristics, the height of the apical meristem, diameter and the dry mass of underground structure, shoot and whole seedling dry mass were statistically higher in P. longiflorum in comparison with P. tomentosum (Table 2). For the other characteristics there were no significant differences. Both species cessed growth between 10 and 12 months, followed by leaf loss and resprout after the cool dry period (Fig. 1 D-G).

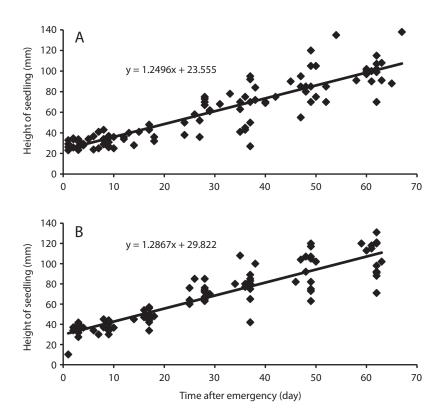


Fig. 3. Growth in height of two *Pseudobombax* species (Bombacoideae, Malvaceae) seedlings cultivated under 50% shading in Uberlândia, Minas Gerais state, Brazil. (A) *P. longiflorum*. (B) *P. tomentosum*.

TABLE 2

Mean of biometry and allocation of dry matter in seedlings (±SD) of *P. longiflorum* and *P. tomentosum* (Bombacoideae, Malvaceae), cultivated by nine months under 50% of natural light in Uberlândia, Minas Gerais state, Brazil

Measurement (unity)	P. longiflorum	P. tomentosum	Statistics1	p ²
Number of leaves	4.00±0.67	4.28±0.90	t=0.60	0.5577
Seedling height (mm)	128.29±13.41	118.30±17.79	t=1.19	0.2585
Apical meristem height (mm)	59.07±9.33	41.06±8.49	t=3.78	0.0026
Shoot diameter (mm)	6.56±0.98	6.94±1.27	t=0.63	0.5373
Underground structure length (mm)	113.95±18.23	109.19±13.51	U=29.00	0.6200
Underground structure diameter (mm)	24.54±1.14	20.60±1.33	t=5.96	0.0001
Height of the first leaf insertion (mm)	29.30±11.44	23.07±6.98	t=1.23	0.2425
Aerial dry mass (mg)	1 179.76±156.52	981.67±181.15	t=2.19	0.0490
Underground dry mass (mg)	6 145.10±277.16	4 310.81±1142.06	t=4.13	0.0061
Seedling dry mass (mg)	7 324.86±331.90	5 292.48±1200.35	t=4.32	0.0050
Underground/aerial dry mass ratio	5.09±0.64	4.27±1.06	t=1.76	0.1030
Aerial/seedling mass ratio	0.16±0.02	0.20±0.001	t=1.98	0.0707

1. t=statistics of the t "Student" test, U=statistics of the Mann Whitney test.

2. p=probability.

DISCUSSION

Both species of Pseudobombax presented high germinability and similar growth patterns, but there were some differences in germination time and synchrony, which may be associated to their preferential habitat. The high germinability of the seeds of both Pseudobombax studied species is similar to the one observed in other Bombacoideae (Melo et al. 1979, Sousa-Silva et al. 2001, Scalon et al. 2003, Zamith & Scarano 2004, Fanti & Perez 2005, Maia et al. 2005, Wittmann et al. 2007, Lopes et al. 2008, Zamora-Cornelio et al. 2010), and contrast with lower germinability recorded for other species of Pseudobombax (Sánchez & Zepeda 2004, Sautu et al. 2006). This also indicates the absence of marked dormancy in Pseudobombax, although species with mechanical dormancy occur in Bombacoideae (Danthu et al. 1995, Barbosa et al. 2004, Pinto et al. 2004).

The germination process of both species was relatively slower; with mean germination times higher than the 5.1 to 7.1 days recorded for other Bombacoideae (Souza & Válio 2001, Ranal *et al.* 2010), but was similar to seedling emergence times described for the group (Mendes-Rodrigues *et al.* 2005). The germination time to 50% of germination (GT50) was also higher than the 5 to 7 days recorded for other species in the group (Maia *et al.* 2005, Zamora-Cornelio *et al.* 2010).

The duration of germination process was distributed along 4.50 to 18.25 days in the studied species (Germination time of the first seed-GTFS and of the last seed-GTLS), which is inside the ample variation recorded for species in the group, which ranged from 3 (Zamora-Cornelio *et al.* 2010) to 19 days (Sautu *et al.* 2006) to GTFS and from 6 (Zamora-Cornelio *et al.* 2010) to 284 days (Sautu *et al.* 2006) to GTLS.

The gradual germination process for both studied species of *Pseudobombax* can be considered a type of relative dormancy (*sensu* Labouriau 1983). It is not a mechanical dormancy as observed for *Ochroma pyramidale* (Cav. ex Lam.) Urb. (Zamora-Cornelio *et al.* 2010), but it results in a germination process spread through time in opposition to a single germination peak observed in e.g. *Ceiba speciosa* (A.St.-Hil., A.Juss. & Cambess.) Ravenna (Ranal *et al.* 2010). This strategy of spreading the germination through time is present in other species occurring in the Cerrado region (Carvalho *et al.* 2005, Pereira *et al.* 2009, Ranal *et al.* 2010, Mendes-Rodrigues *et al.* 2010).

P. tomentosum showed significantly higher synchronization of germination than that observed for *P. longiflorum* seeds. The more asynchronous and heterogeneous germination observed for *P. longiflorum* seeds would increase survival ability at the beginning of the rains, which may vary in intensity and frequency in the Cerrado region (Oliveira 2008).

The seedlings of the two species of *Pseudobombax* differed from others species within the subfamily as *Bombacopsis glabra* (Pasq.) A. Robyns (Baker 1960) and *Eriotheca pubescens* Schott & Endl. (Mendes-Rodrigues *et al.* 2005) which are polyembrionic. The functional morphology type of seedlings of *P. longiflorum* and *P. tomentosum* differed from *P. ellipticum* (Kunth) Dugand and *P. munguba* (Mart. & Zucc.) Dugand, which present the cotyledons suspended by hypocotyl elongation (Sánchez & Zepeda 2004, Maia *et al.* 2005), a pattern commonly observed in classical PEF type (Ressel *et al.* 2004).

P. longiflorum and *P. tomentosum* presented similar growth pattern during the first 70 days. This trend of linear initial growth has been also observed for other Bombacoideae such as *Ceiba pentandra* (L.) Gaertn. (Pedroso & Varela 1995) and *B. glabra* (Scalon *et al.* 2003). The leaf fall and resprout after the cooler part of the dry season were not previously recorded for *Pseudobombax*, but this brevideciduous leafing behavior is common among seedlings of other Cerrado species (Oliveira & Silva 1993, Oliveira 2008).

Larger seedlings observed in *P. longiflorum* in relation to *P. tomentosum* could also be viewed as a way to increase chances of survival, since open Cerrado plants have a limited growing season (Oliveira 2008). Nevertheless, both species presented seasonal growth and dry matter allocation ratios similar to other Cerrado trees (Oliveira & Silva 1993, Moreira & Klink 2000). Despite their difference in predominant habitat, the two species of *Pseudobombax* allocated most dry mass to underground structures

in detriment of the shoots. This root biased allocation would allow greater resistance to water and temperature stress during the dry period in open Cerrado plant formations, but would hinder competition and growth in forest environments (Hoffmann & Franco 2003, Vieira et al. 2008). The preferential investment to underground structures has been also recorded for other species from open Cerrado formations (Moreira & Klink 2000, Hoffmann & Franco 2003), including some species of Bombacoideae (Moreira & Klink 2000, Ronquim et al. 2003, Ressel et al. 2004). But even for vicariant pairs of species restricted to either the savanna or forest, the physiological and developmental features were not easy to contrast (Hoffmann & Franco 2003). It is important to notice here that the differences in seedling size may be a result of a better adjustment of P. longiflorum seeds to the Cerrado soil used in the experiment.

Although the two *Pseudobombax* species differ in synchrony of germination and in some aspects of growth pattern, those differences are not clearcut and may be explained by the distribution overlap between species. New and more refined experiments will be necessary to see whether these differences, despite being significant, would really favour habitat specialization in the studied species.

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RESUMEN

Pseudobombax tomentosum y P. longiflorum son árboles comunes en la región del Cerrado, pero la primera especie es más común en los bordes del bosque mientras que el segundo está presente en áreas abiertas del cerrado. Este trabajo tuvo como objetivo investigar las diferencias en la germinación de las semillas y el crecimiento de ambas especies. Los individuos fueron recolectados en áreas del Cerrado, la región de savanas neotropicales en el centro de Brasil. Fueron utilizados para cada especie cuatro repeticiones con 25 semillas cada una para el experimento de germinación, distribuidas al azar en la cámara de germinación a 25°C. Las plántulas fueron evaluadas en cuanto al crecimiento inicial hasta 67 días después de la emergencia de la plántula. Algunas plantas se mantuvieron para la evaluación de la biomasa durante nueve meses, v luego se mideron para evaluar diferencias en el crecimiento y distribución de masas entre especies. Ambas presentaron la misma capacidad germinativa (cerca de 100%) y tiempo medio de germinación (alrededor de 12 días). Sin embargo, P. longiflorum presentó una germinación más dispersa en el tiempo, con valores más altos de coeficiente de variación de tiempo de germinación y el índice de incertidumbre, y valores más bajos de la sincronización que P. tomentosum. Las dos especies demostraron básicamente el mismo patrón de crecimiento, pero P. tomentosum presentó valores más pequeños en la altura del meristemo apical, diámetro de las estructuras subterráneas (sobre todo las raíces), masa seca de la estructura aérea, estructura subterránea y plántulas en comparación con P. longiflorum. Ambas especies asimilaron más masa seca en las estructuras subterráneas en detrimento de las aéreas. Esto probablemente permite una conducta de rebrote que evita el estrés hídrico y la acción perjudicial de incendios típicos de las zonas del cerrado abierto.

Palabras clave: Bombacoideae, Cerrado, germinación de semillas, savanas neotropicales, crecimiento de las plantas, *Pseudobombax tomentosum*, *Pseudobombax longiflorum*.

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