Desiccation tolerance of *Tapirira obtusa* seeds collected from different environments[1]

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ABSTRACT – This study was aimed at evaluating the desiccation sensitivity in seeds of the tree *Tapirira obtusa* (Benth.) J. D. Mitchell collected from three different environments and subjected to two distinct drying speeds. Seeds were collected from a rocky area, in the "Cerrado", and in a riparian forest area, in the region of municipality of Lavras, State of Minas Gerais. The seeds were subjected to drying with magnesium chloride (slow drying) or silica gel (fast drying), into closed environment, until moisture contents of 40%, 30%, 20% and 10%, considering as control, the percentage of germination at the initial moisture content in each environment, which varied from 47% to 50%. Percentages of germination and normal seedlings as well as germination speed index were assessed. For the three environments studied, there was no effect of slow drying on seed germination. Seeds from area of Cerrado, however, have shown a slight reduction on germination when subjected to fast drying. Oppositely, seeds from rocky area had germination increased when subjected to fast drying. Seeds from riparian forest area had no reduction on germination percentage, independent of drying speed. Results suggest that seeds of *T. obtusa* are not sensitive to desiccation.

Index terms: drying, germination, sensitivity, dehydration.

Tolerância à dessecação em sementes de *Tapirira obtusa* procedentes de diferentes ambientes

RESUMO – O objetivo deste trabalho foi avaliar a sensibilidade à dessecação de sementes de *Tapirira obtusa* (Benth.) J. D. Mitchell provenientes de três ambientes e submetidas a duas velocidades de secagem. As sementes foram coletadas em área de campo rupestre, Cerrado e em mata ciliar, da região de Lavras, MG. As sementes foram submetidas à secagem com cloreto de magnésio (secagem lenta) ou sílica gel (secagem rápida), em ambiente fechado, até os graus de umidade de 40%, 30%, 20% e 10%, considerando como controle o percentual de germinação na umidade inicial em cada ambiente, a qual variou de 47% a 50%. Foram avaliados os percentuais de germinação e de plântulas normais e também o índice de velocidade de germinação. Nos três ambientes analisados, não houve efeito da secagem lenta sobre a germinação das sementes. Sementes oriundas do Cerrado apresentaram pequena redução da germinação, quando submetidas à secagem rápida. Sementes oriundas de áreas rupestres apresentaram aumento na germinação quando submetidas à secagem rápida. Sementes oriundas de mata ciliar não apresentaram redução na germinação, independente da velocidade de secagem. Os resultados sugerem que sementes de *T. obtusa* não apresentam sensibilidade à dessecação.

Termos para indexação: secagem, germinação, sensibilidade, desidratação.

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Introduction

Desiccation tolerance can be defined as capacity of a given organism in resisting loss of water and reestablish their normal metabolism functions after rehydration (Alpert, 2000). In relation to tolerance to desiccation and storage, seeds are sorted as: orthodox (tolerant); recalcitrant (sensitive) (Roberts, 1973); and intermediate (Ellis et al., 1990). Due to sensitiveness to drying and to low temperatures, the recalcitrant seeds are difficult of being studied, besides their susceptibility to fungal contamination and fast deterioration (Pammenter and Berjak, 1999).

The knowledge of seed response to drying is essential in order to establish strategies and methodologies for their storage. This way, Hong and Ellis (1996) have proposed a basic methodology in determining behavior of seeds in relation to drying and storage. One of the parameters evaluated by this methodology is their drying until low levels of moisture and then evaluating their survival capacity under these conditions.

The environmental condition in which the seed develops influences several characteristics, including its vigor and its tolerance to desiccation (Daws et al., 2004; Delph et al., 1997; Weiner et al., 1997). Among the conditions influencing seed development are hydric availability, time and intensity of light exposure, and availability of nutrients (Fenner, 1991). Daws et al. (2004) have found that tolerance to desiccation is also influenced by environmental conditions.

The tree *Tapirira obtusa* (Benth.) J. D. Mitchell, previously known as *Tapirira marchandii* Engl. (Lorenzi, 2000; Silva-Luz and Pirani, 2010) occurs in Brazil, in the North Region (states of Amazonas, Tocantins, and Acre), Northeast Region (Bahia), West Central Region (Mato Grosso, Goiás, Distrito Federal and Mato Grosso do Sul), and Southeast Region (Minas Gerais, Espírito Santo, São Paulo, and Rio de Janeiro). The species has a wood easy to work and is moderately durable under adverse conditions, and can be used for shipbuilding. The fruits are suitable to feed the avifauna (mainly wild pigeons – the reason for its common name "Pigeon Tree") and the flowers are attractive to insects, being hence useful for environmental reforestation (Lorenzi, 2000).

Studies on different species within the genus *Tapirira* are concentrated in the presence and use of their chemical compounds; but studies referring to their propagation via seeds are scarce (Correia et al., 2006; 2008). Therefore, considering the importance of this species as well as the lack and importance of studies related to its tolerance to desiccation, this research work was performed with the objective of assessing tolerance to desiccation of seeds of *Tapirira obtusa* collected from different environments.

Material and Methods

The experimental work was carried out in the Laboratory of Forest Seeds, Federal University of Lavras, State of Minas Gerais, from January to April, 2011. Seeds of *Tapirira obtusa* were collected between January and February, 2010, in a rocky area, a Cerrado area, and in a riparian forest area, in the region of municipality of Lavras, State of Minas Gerais, Southeast Brazil. Immediately after harvesting, seed were processed by manual maceration of fruits in a sieve under tap water, for removing aril and residues of fruits. After that, seeds from each environment were separately dried for removal of superficial water, and immediately subjected to initial test of moisture content and germination.

Two drying speeds were assessed: the slow drying, performed with magnesium chloride ($MgCl_2 \cdot 6H_2O$), at 36% RH; and fast drying, performed with silica gel at 21% RH. Both tests of drying speeds were performed into "hygrostat" type boxes, at 20 ºC. Seeds were dried until moisture contents of 40±2%, 30%±2, 20%±2, and 10%±2. To obtain such units, periodical weighing of seed were performed until reaching the desired mass, according to equation (Hong and Ellis, 1996):

$$Target\ mass = \frac{(100 - \text{Initial moisture})}{(100 - \text{Target moisture})} \text{ x Initial mass}$$

When the expected seed mass was reached, four subsamples of 25 seeds each were removed from the main sample for each collecting site, which were then subjected to the germination test. For this, seeds of each subsample were evenly distributed on top of two sheets of paper towels, covered with another sheet of the same paper and then moistened with sterile distilled water, in a proportion of 2.5 times the mass of dry paper, made into roll and placed into a seed germinator, at 25 ºC, under constant light. After a daily counting of percentage germination was performed, using as assessment parameters: the germination speed index (GSI); the final germination; and the percentage of normal seedlings. The experiment was carried out in a completely randomized experimental design, with treatments arranged into a 3 x 2 x 4 triple factorial scheme (3 environments x 2 drying speeds x 4 moisture contents). Three additional treatments (initial germination for each environment) were also used.

Percentage data were subjected to Shapiro-Wilk's normality test. When non-normal distribution of data

($p < 0.05$) was detected, these were transformed into arc sine $\sqrt{x/100}$. After that, when data were normally distributed, these were subjected to ANOVA, and when statistically significant differences among treatments were found by the F-test, the Tukey test, at 5% probability, was applied. Data with distribution non-normal, even after transformation, were analyzed by the Generalized Linear Model (GLM) method, through Binomial Distribution, and when statistically significant differences among treatments were found by the Chi-squared test, the Tukey test, at 5% probability, was applied. These analyses were performed using the software R for Windows, version 2.12.0 (R Development Core Team, 2010).

Results and Discussion

On Table 1 are shown values of initial moisture content of seeds and values of the moisture contents that were reached after each assessment test. The drying process using magnesium chloride (slow drying) took more than 10 h to reach moisture content of 10%, which was the highest period observed for seeds of the Cerrado area (14 h) (Figure 2A). The drying process in silica gel (fast drying) occurred in 7 h in seeds from areas of Cerrado and riparian forest; and took 4 h for seeds collected in rocky area reach 10% moisture content (Figure 2B).

Table 1. Moisture contents (± 2%; wet basis) reached during drying of seeds of *Tapirira obtusa* collected in three different environments and assessed by using two different drying speeds, at 20 ºC.

Collection environment	Drying speed	Initial moisture content (%)	Moisture content (±2%)			
			40	30	20	10
Rocky area	Slow	47.00	40.00	30.50	21.00	11.80
	Fast	47.00	38.77	31.06	21.96	10.65
Cerrado area	Slow	50.30	40.00	31.00	19.00	11.00
	Fast	50.30	38.76	30.79	20.24	11.56
Riparian forest area	Slow	48.78	41.50	30.00	20.00	11.90
	Fast	48.78	41.96	29.52	21.36	11.40

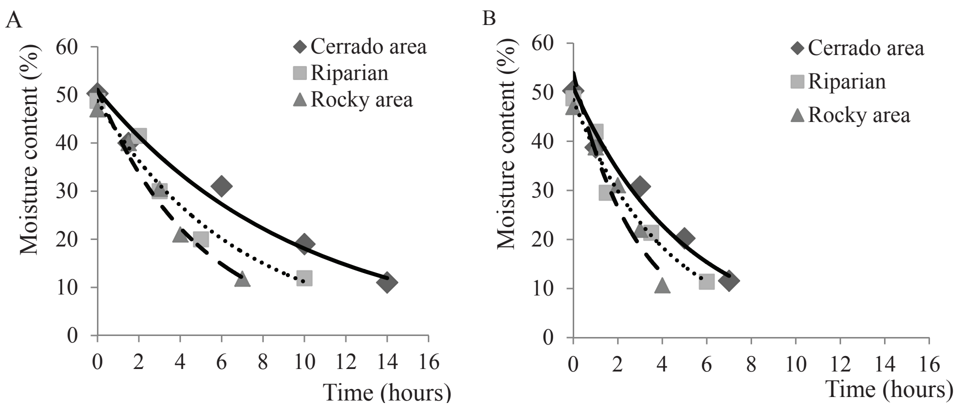

Figure 2. Curves of seed drying of *Tapirira obtusa* subjected to slow drying (A) and fast drying (B).

Seeds from the rocky area have had their germination significantly influenced by the drying speed ($p = 2.2 \times 10^{-16}$) and by moisture content ($p = 0.0004$), with interaction between the two factors ($p = 7.5 \times 10^{-5}$). Moisture content did not influence germination of seeds subjected to slow drying process. Nevertheless, it can be observed an increase on germination percentage for the seeds subjected to the fast drying process (Figure 3A).

Germination of seeds from the Cerrado area was significantly influenced by the drying speed ($p = 2.2 \times 10^{-5}$) and by moisture content ($p = 0.0154$), but without interaction between the two factors ($p = 0.2505$). The reduction on germination percent along the drying process was explained by linear equation for both slow and fast drying procedures (Figure 3B), with higher reduction in these values when seed were subjected to fast drying until 10% moisture content. There was not influence of drying speed ($p = 0.1406$) or moisture content ($p = 0.0613$) on germination of seeds collected in the riparian forest area; and without interaction between the two factors ($p = 0.7556$) (Figure 3C).

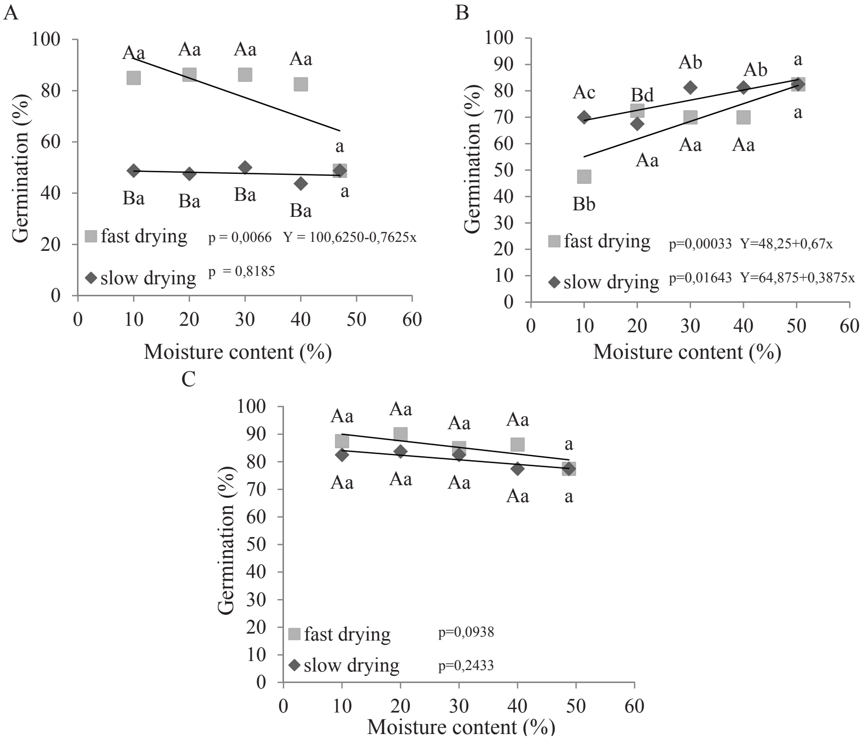

Figure 3. Germination of seeds of *Tapirira obtusa* collected in three different environments and subjected to two different drying speeds. (A) Seeds from a rocky area; (B) seeds from a Cerrado area; and (C) seeds from a riparian forest area. Capital letters compare means between drying speed within the same moisture content. Small letters compare means between moisture contents within the same during speed. Non-significant equations were not shown.

Seeds freshly harvested in the rocky area had significantly lower initial germination percentage ($p = 0.0018$) as well as for the other percentage moisture contents of 40% ($p = 0.0004$), 30% ($p = 2.87x10^{-6}$), 20% ($p = 0.0049$) and 10% ($p = 0.0025$) when compared to those seeds harvested in other environments (Table 2). Seeds from areas of Cerrado and riparian forest did not present statistically significant differences between each other within their germination percentages, independent of moisture content to which they were induced. When subjected to the fast drying process, however, seeds from the Cerrado area presented the lowest germination percentages in moisture contents of 40% ($p = 0.0304$), 20% ($p = 3.92x10^{6}$) and 10% ($p = 0.0004$) (Table 3).

Germination speed index (GSI) of seeds from the rocky area was significantly influenced by the slow drying process with linear reduction on the GSI ($p = 0.0014$) and by moisture content ($p = 0.0128$), but without interaction between these two factors ($p = 0.1622$) (Figure 4A). Seeds subjected to fast drying, however, did not have their GSI influenced.

Table 2. Germination percentage of seeds of *Tapirira obtusa* collected in three different environments and subjected to slow drying process until reaching different moisture contents.

Collection environment	Moisture content (%; wet basis)				
	Initial	40	30	20	10
Rocky area	48.7 B*	43.7 B*	50.0 B*	47.5 B*	48.7 B*
Cerrado area	82.5 A	81.2 A	81.2 A	67.5 AB	70.0 A
Riparian Forest area	77.5 A	77.5 A	82.5 A	83.7 A	82.5 A

*Means followed by the same capital letter in the columns are not statistically different by the Tukey test at 5% probability.

Table 3. Germination percentage of seeds of *Tapirira obtusa* collected in three different environments and subjected to fast drying process until reaching different moisture contents.

Collection environment	Moisture content (%; wet basis)				
	Initial	40	30	20	10
Rocky area	48.7 B*	82.5 B*	86.2 A*	86.2 AB*	85.0 B*
Cerrado area	82.5 A	70.5 C	70.0 A	72.5 B	47.5 C
Riparian Forest area	77.5 A	86.2 A	85.0 A	90.0 A	87.5 A

*Means followed by the same capital letter in the columns are not statistically different by the Tukey test at 5% probability.

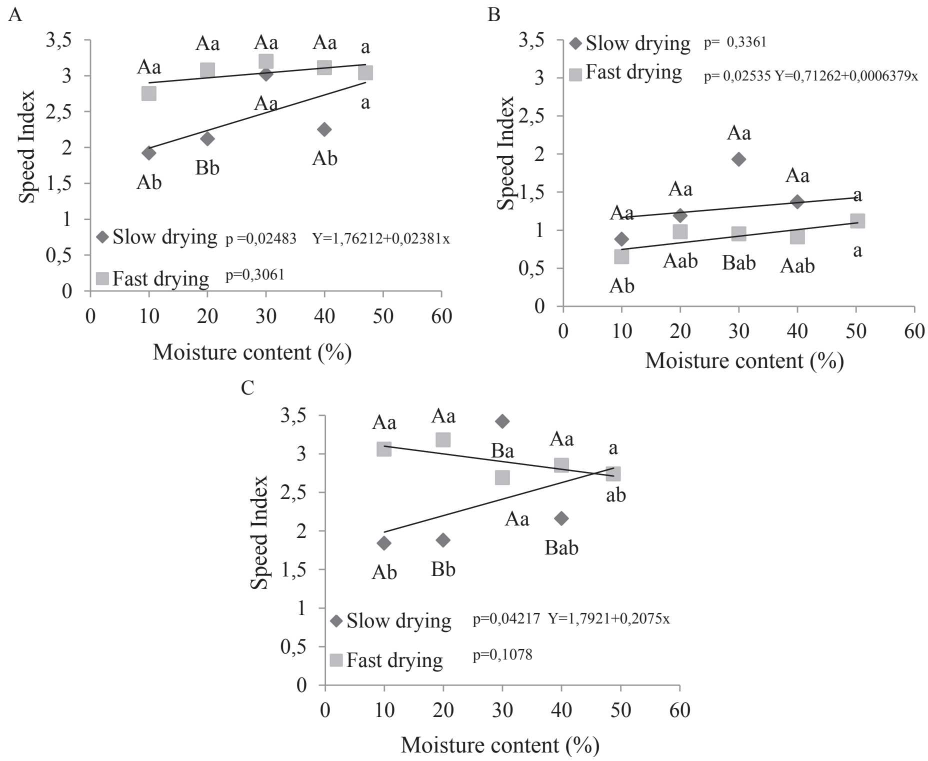

Figure 4. Germination speed index of seeds of *Tapirira obtusa* collected in three different environments and subjected to two different drying speeds. (A) Seeds from a rocky area; (B) seeds from a Cerrado area; and (C) seeds from a riparian forest area. Capital letters compare means between drying speeds within the same moisture content. Small letters compare means between moisture contents within the same during speed. Non-significant equations were not shown.

The GSI of seeds from Cerrado area was influenced by speed of drying (p = 0.0002) and by moisture content (p = 0.0389), without interaction between the two factors (p = 0.9517). When subjected fast drying, however, seed from that location have presented linear reduction of germination speed (Figure 4B). Notwithstanding, it was not observed influence of moisture content on the GSI of these seeds when subjected to the slow drying process.

The GSI of seeds from the riparian forest area was not influenced by drying speed (p = 0.3188), but there have been influence of moisture content (p = 0.0031) and interaction between these two factors (p = 0.0093). Influence of moisture content on germination speed was not verified when seed were subjected to fast drying, while seeds subjected to slow drying have presented linear reduction on the GSI values when the moisture content was reduced (Figure 4C).

Seed from the Cerrado area have presented the lowest values for germination speed when compared to seeds from the rocky and riparian forest area ($p = 7.069 \times 10^{-6}$), seeds freshly harvested and slowly dried until moisture contents of 40% (p = 0.0098), 30% (p = 0.0031), 20% (p = 0.0058) and

10% (p = 0.0222) (Table 4). The same behavior was observed in the seeds of the fast drying process until moisture contents of 40% ($p = 5.709 \times 10^{-7}$), 30% (p = 0.0001), 20% ($p = 3.923 \times 10^{-6}$) e 10% ($p = 1.75 \times 10^{-5}$) (Table 5).

Table 4. Germination speed index of seeds of *Tapirira obtusa* collected in three different environments and subjected to slow drying process until reaching different moisture contents.

Collection environment	Moisture content (%; wet basis)				
	Initial	40	30	20	10
Rocky area	3.04 A*	2.25 A*	3.02 A*	2.12 A*	1.92 A*
Cerrado area	1.12 B	1.37 B	1.93 B	1.19 B	0.88 B
Riparian forest area	2.74 A	2.16 A	3.42 A	1.88 A	1.84 A

*Means followed by the same capital letter in the columns are not statistically different by the Tukey test at 5% probability.

Table 5. Germination speed index of seeds of *Tapirira obtusa* collected in three different environments and subjected to fast drying process until reaching different moisture contents.

Collection environment	Moisture content (%; wet basis)				
	Initial	40	30	20	10
Rocky area	3.04 A*	3.11 A*	3.20 A*	3.08 A*	2.75 A*
Cerrado area	1.12 B	0.91 B	0.95 B	0.98 B	0.65 B
Riparian forest area	2.74 A	2.85 A	2.69 A	3.18 A	3.06 A

*Means followed by the same capital letter in the columns are not statistically different by the Tukey test at 5% probability.

Percentage of normal seedlings of seeds from the rocky area was influenced by drying speed ($p = 2.2 \times 10^{-16}$) and moisture content (p = 0.0004), with interaction between the factors ($p = 9.458 \times 10^{5}$). The seeds subjected to the fast drying process have presented increase in percent normal seedlings, which was lower for freshly harvested seeds (Figure 5A). No influence of moisture content on percentage of normal seedlings was observed for the seeds subjected to slow drying process; and the percentage of normal seedlings from seeds subjected to this procedure were lower than the fig ures observed in the fast drying process (Figure 5A).

In seed samples from the Cerrado area, the percentage of normal seedlings was influenced by the drying speed (p = 0.0102) and moisture content (p = 0.0002), with no interaction between the factors (p = 0.0865). There was no influence of moisture content when seeds were subjected to the slow drying procedure. There was a linear reduction, however, for data of percentage of normal seedlings when the fast drying process was performed (Figure 5B). Concerning percentage of normal seedling, the seeds from the riparian forest area were not influenced by germination speed (p = 0.3192) and moisture content (p = 0.0963) and interaction between the factors also has not occurred (p = 0.8238) (Figure 5C).

The percentage of normal seedlings starting from seeds of the rocky area and subjected to the slow drying process was lower when compared to values observed for the seeds from areas of Cerrado and riparian forest (p = 0.0033) either for freshly harvested seeds or seeds subjected to the drying process until temperatures of 40% (p = 0.0002), 30% (p = 0.0031), 20% (p = 0.0222) and 10% (p = 0.0049) (Table 6). When subjected to the fast drying process until 10% moisture content (p= 0.0002), however, the seeds from the Cerrado area have presented the lowest percentage of normal seedlings when compared to the remaining environments. When seed were subjected to fast drying until moisture contents of 40% (p = 0.1352), 30% (p = 0.1695) and 20% (p = 0.0831), no influence of the original environment was found for the percentage of normal seedlings (Table 7).

The increase on germination percentage of seeds harvested in the rocky area and subjected to the initial drying process may be an indication of overcoming dormancy (Figure 3A). The same might have occurred in relation to the GSI increase for seeds from the riparian forest area when subjected to slow drying process, once the seeds when were dried until 30% moisture content have presented increases on germination speed (Figure 4C). Besides, the seeds from the rocky area, when subjected to the slow drying, maintained their low germination capacity, independent of the moisture content, with values statistically equal to those of the other seed collection areas. The same behavior was observed when seeds were subjected to the fast drying procedure until moisture contents of 40%, 30%, 20% and 10%.

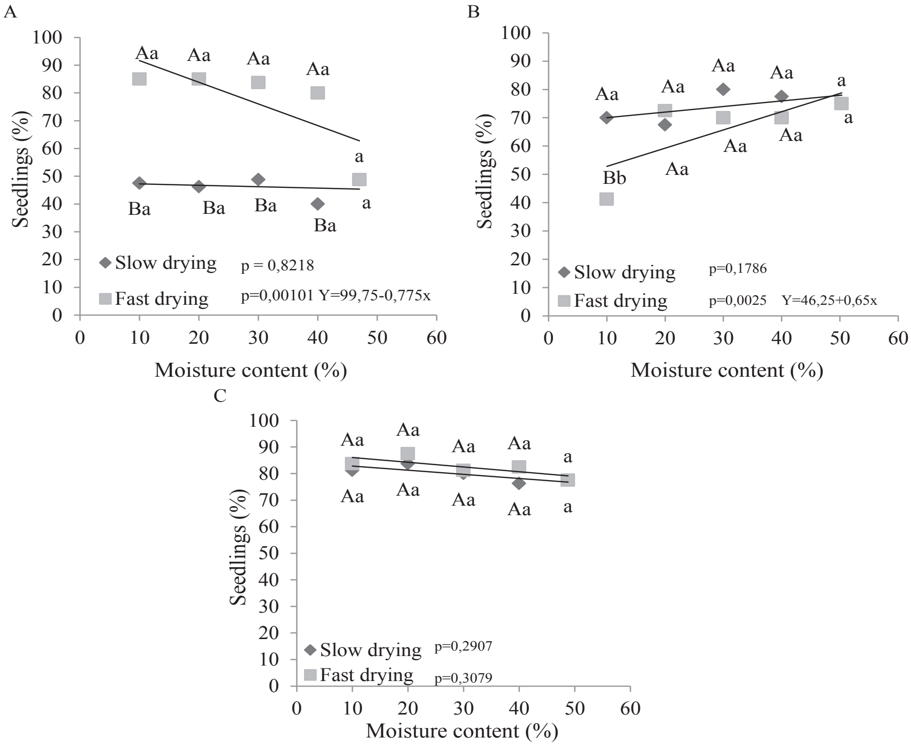

Figure 5. Percentage of normal seedlings obtained from seeds of *Tapirira obtusa* collected in three different environments and subjected to two different drying speeds. (A) Seeds from a rocky area; (B) seeds from a Cerrado area; and (C) seeds from a riparian forest area. Capital letters compare means between drying speed within the same moisture content. Small letters compare means between moisture contents within the same drying speed. Non-significant equations were not shown.

Table 6. Percentage of normal seedlings grown from seeds of *Tapirira obtusa* collected in three different environments and subjected to slow drying process until reaching different moisture contents.

Collection environment	Moisture content (%; wet basis)				
	Initial	40	30	20	10
Rocky area	48.7 B*	40.0 B*	48.7 B*	46.2 B*	47.5 B*
Cerrado area	75.0 A	77.5 A	80.0 A	67.5 AB	70.0 A
Riparian forest area	77.5 A	76.2 A	80.0 A	83.7 A	81.2 A

*Means followed by the same capital letter in the columns are not statistically different by the Tukey test at 5% probability.

Table 7. Percentage of normal seedlings grown from seeds of *Tapirira obtusa* collected in three different environments and subjected to fast drying process until reaching different moisture contents.

Collection environment	Moisture content (%; wet basis)				
	Initial	40	30	20	10
Rocky area	48.7 B*	80.0 A*	83.7 A*	85.0 A*	85.0 A*
Cerrado area	75.0 A	70.0 A	70.0 A	72.5 A	41.2 B
Riparian forest area	77.5 A	82.5 A	81.2 A	87.5 A	83.7 A

*Means followed by the same capital letter in the columns are not statistically different by the Tukey test at 5% probability.

Davide et al. (2003) have reported that after the drying process, there was an increase on germination of seeds of *Cryptocarya ascherosiana* Mez. Likewise, within this study, it was verified an increase on germination of *T. obtusa* seeds, suggesting that the drying procedure might have induced overcoming of seed dormancy, as observed along the fast drying period in seeds collected in the rocky area (Figure 3A).

Despite reduction of values observed for seeds harvested in the Cerrado area, when these seeds were dehydrated until 10% moisture content as well as reductions on the GSI values found for the seeds harvested in rocky area and riparian forest area, results have indicated that seeds of *T. obtusa* tolerate desiccation. Such fact can be verified mainly when the behavior of seeds harvested in the riparian forest area is considered, since no influence of drying process was found as well as by the fact that there has been no reduction of this performance for the seeds harvested in the rocky area. It was possible to observe, however, that seeds from all the three environments have presented different responses to the drying process.

Oliveira et al. (2009) have observed that seeds of *Talisia subalbens* (Mart.) Radlk. have presented different behavior for drying speed. By subjecting seeds of this species to oven drying method, at 35 ºC, the authors found increases on germination percentage as compared to the germination obtained for seeds dried under normal environmental conditions. According to the same authors, this behavior is common for recalcitrant seeds. In this study, there was statistically significant reduction for seeds harvested in the Cerrado area when the moisture content was reduced from 20% to 10%. In the remaining environments, however, such behavior was not detected.

The tolerance to desiccation is a defense mechanism so that the seeds are able to survive low hydric availability until favorable conditions for germination occur. This way, plants producing seeds with such capacity must have been naturally selected to colonize areas where frequent drought periods take place (Alpert, 2005; Barbedo and Bilia, 1998). Daws et al. (2006) have found differences for this characteristic in experiments with seeds of the Sycamore Maple tree (*Acer pseudoplatanus* L.), which are tolerant to desiccation. The authors suggest that characteristics of the seed may undergo changes in response to adaptation to different environmental conditions.

The period of *T. obtusa* seed dispersal coincides with rainy period (Lorenzi, 2000). This manner, favorable conditions for their germination occur immediately after dispersal. Besides, under natural environmental conditions it is expected that seeds are naturally subjected to a slow drying process. According to results obtained in this study, loss of germination does not occur when seeds are subjected to the slow drying procedure artificially induced.

Kageyama and Viana (1989), cited by Davide et al. (2003), have reported that is more likely that seed of climax species will present recalcitrant behavior. According to them, seeds of this group germinate immediately after dispersion, forming hence a grouping of seedlings around the mother-plant. Such behavior has already been reported for various species within this ecological group (Davide et al., 2003; José et al., 2007) and *T. obtusa* is classified as climax species in an ecological succession (Souza et al., 2007; Silva et al., 2004).

As far as tolerance to desiccation is concerned the behavior of seeds is very much variable in function of plant species, and sometimes within the same species, variable levels of tolerance/sensibility may occur as a function of the environment or even within the same seed lot (Gaff, 1997, cited by Alpert, 2000). In this study, seeds from the Cerrado area reacted to desiccation in a more sensitive manner than those seeds harvested in the rocky and riparian forest areas. However, there has not been a total loss of germination for the seeds from the Cerrado area during the drying process, what suggests that seeds of this environment present little sensitivity to desiccation, but part of these seeds still germinate, even if they have been subjected to the fast drying process until 10% moisture content.

Conclusions

The slow drying process does not influence germination of seeds of *Tapirira obtusa*, independently of the environment in which they were produced.

The fast drying process results in small reduction on germination of seeds of *Tapirira obtusa* produced in the Cerrados.

Seeds of *Tapirira obtusa* do not present sensitivity to desiccation.

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