ISSN 0103-9954

PHYSIOLOGICAL IMMATURITY AND HYDROPRIMING OF *Parkia nitida* Miq. SEEDS WITH PHYSICAL DORMANCY

IMATURIDADE FISIOLÓGICA E CONDICIONAMENTO HÍDRICO DE SEMENTES DE *Parkia nitida* Miq COM DORMÊNCIA FÍSICA

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ABSTRACT

Parkia nitida (Fabaceae Mimosoideae) is a neotropical timber tree of economic e ecological importance due to its wood and the possibility of rapid growth in degraded areas. is a neotropical tree species of economic and ecological importance. That study aimed to verify if the freshly harvested seeds exhibit physiological immaturity and apply hydropriming on seeds with different degrees of imbibition to enhance the germination performance. The same seed lot was tested immediately after collection and after one year of storage. The treatments were 0, 20, 40 and 60% of partial imbibition in water at 15°C, followed by a drying period of 7 days. The seeds were sown in washed sand in the nursery (4 x 25 seeds / treatment). Seed moisture was assessed before and after imbibition, and after drying. Statistical tests ANOVA and Kruskal-Wallis showed significant difference between treatments for final germination percentage and mean germination time (p> 0.05). Fresh seeds of *Parkia nitida* showed 35% of normal seedling development, this value increased after one year of storage to 76%. Hydropriming of the fresh seeds was able to increase the germination to the same value as the stored seeds and reduced the mean emergence time from 7 to 4 days. The positive action of hydropriming was observed only after a partial imbibition of 20% (29 hours), longer imbibition periods significantly reduced the germination. The results indicate on a “critical imbibition level” between 20 and 40% of turgency. The need for a post-harvest maturation for *Parkia nitida* seeds could be overcome with hydropriming of the fresh seeds.

Keywords: physical dormancy; tropical forest seed; priming; Fabaceae.

RESUMO

Parkia nitida (Fabaceae Mimosoideae) é uma árvore madeireira neotropical de importância econômica e ecológica devido à sua madeira e à possibilidade de crescimento rápido em áreas degradadas. Esse estudo teve como objetivo verificar se as sementes recém-colhidas apresentam imaturidade fisiológica e aplicar hidrocondicionamento com diferentes graus de embebição para melhorar o desempenho germinativo. O mesmo lote de sementes foi avaliado imediatamente após a colheita e após um ano de armazenamento. Os tratamentos foram 0, 20, 40 e 60% de embebição parcial em água a 15°C, seguida de um dessecamento por 7 dias. A semeadura ocorreu em areia lavada no viveiro (4 x 25 sementes/tratamento). O teor de água das sementes foi determinado antes e após a embebição, e após o dessecamento. Os testes estatísticos ANOVA e Kruskal-Wallis mostraram que existe diferença significativa entre os tratamentos para a percentagem final e o tempo médio de germinação (p>0.05). Sementes recém-colhidas de *Parkia nitida* apresentaram 35% de desenvolvimento de plântulas normais, este valor incrementou para 76% após um ano de armazenamento

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Recebido para publicação em 30/08/2012 e aceito em 7/02/2014

das sementes. As sementes frescas conseguiram alcançar o mesmo valor de germinação das sementes armazenadas por um ano e ainda reduziu o tempo médio de emergência de 7 para 4 dias. O efeito positivo do hidrocondicionamento foi observado somente após uma embebição parcial de 20% (29 horas). Períodos de embebição mais prolongada reduziram significativamente a germinação. Os resultados indicam um "nível crítico" de embebição entre 20 e 40% de turgência. A necessidade de um período de maturação pós-colheita, para sementes de *Parkia nitida*, pode ser superada com hidrocondicionamento das sementes frescas.
Palavras-chave: dormência fisiológica; semente florestal tropical; condicionamento; Fabaceae.

INTRODUCTION

Parkia nitida (Leguminosae, Mimosoideae) is a medium- to large-sized tree up to 40 m high, widely distributed from southern Panama to the central region of Amazonia (HOPKINS, 1986). Its timber is sold in Brazil under the name of faveira, faveira-grande, faveira-benguê, fava-arara-tucupi among others (HOPKINS, 1986). Due to its economic and ecological interests it is recommended for reforestation programs and recuperation of degraded areas (MARUYAMA and UGAMOTO, 1989). The seeds are large with an average of 0.7 g (range 0.4 to 1.0 g), in the region of Manaus (CAMARGO et al., 2002) and 1.0 g in the region of Paragominas (CRUZ et al., 2001). The physical dormancy, due to water-impermeable seed coat, can be overcome with chemical (sulfuric acid) or mechanical scarification (CRUZ et al., 2001). Germination is epigeal-phanerocotyledonar and field emergence needs about 7 days (CAMARGO et al., 2002).

Good quality seeds should reach a high final germination in a relatively short time. However, seed germination of native forestry species is often irregular and may need a long time, even when exposed to favorable environmental conditions (MURDOCH and ELLIS, 2000). Heterogeneity of the seed lot, when not all seeds have reached maturity at seed collection may result in delayed and poor germination. In this case, after ripening of the seeds during storage may increase seed quality (BLACK et al., 2006). Priming may reduce the need of seed storage, accelerate and increase seed germination. Hydropriming consists of a pre-imbibitions of seeds, enough to activate metabolism, but insufficient to allow radicle protrusion (HEYDECKER and COOLBEAR, 1977; BRADFORD, 1995). The technique is simple, however, the scientific understanding is complex as it involves several physiological processes from metabolic activation to the repair of membranes and DNA (CASTRO et al., 2004). These processes occur mainly after the seeds have imbibed, when metabolism is activated (phase II of imbibition curve), however before the protrusion of the radical (BRADFORD, 1995). It is recommended that the imbibition (phase I of the imbibition curve) is performed at low temperatures (15°C), considering that under these conditions the process is slow and homogeneous, and metabolic activity of seeds is reduced, avoiding possible damage from lack of oxygen (NASCIMENTO, 2004). After hydropriming the seeds can be dehydrated and stored for a short time (days or weeks) before sowing (NASCIMENTO, 1998).

Hydropriming is being successfully applied to agricultural crop seeds, and especially in vegetables and flowers that generally have small seeds with permeable seed coats (NASCIMENTO, 2004; AFZAL et al., 2004; DEMIR and MAVI, 2004; BASRA et al., 2005; MAVI et al., 2006; FAROOQ et al., 2006, 2007). Studies with forestry species are limited, for example: *Anadenanthera peregrina* L. (PINHO et al., 2010), *Cecropia schreberiana* Miq. (SÁNCHEZ et al., 2003), *Dalbergia nigra* (Vell.) Fr. All. (BORGES et al., 1996), *Miconia candolleana* Trian. (BORGES et al., 1994), *Platymiscium pubescens* Micheli (BORGES et al., 2002) and *Ateleia glazioviana* BAILL (ROSA et al., 2005). Few species were studied with physical dormancy: *Hibiscus elatus* Sw. (SÁNCHEZ et al., 2003), *Cassia excelsa* Schrad. (JELLER et al., 2003), *Parkia multijuga* Benth. (CALVI et al., 2008) and *Parkia pendula* (Willd.) Benth ex Walp. (VARGAS and FERRAZ 2008). The two species of the genus *Parkia* showed positive results after hydropriming, both the percentage of emergency as the speed of the process was significantly increased. In *Parkia pendula* the positive action of hydropriming was observed primarily in fresh seeds, which indicates that a post-maturation period may be reduced (VARGAS and FERRAZ 2008). Interestingly, in both species the positive action of hydropriming was observed after a partial imbibition, at the first part of the typical triphasic imbibition curve.

This study aimed to verify if the freshly harvested seeds exhibit physiological immaturity and apply hydropriming on seeds with different degrees of imbibition to enhance the germination performance.

MATERIALS AND METHODS

The climate near Manaus (Amazonas) is rainy tropical and type "afi" in the Köppen classification. Annual rainfall is approximately 2,325 mm and monthly temperature average at 26.6 º C with relative humidity between 84 and 90% (INPE, 2003).

Fruits of *Parkia nitida* were harvested from seven trees on 21 and 22 October 2004, when the natural detachment of the indehiscent fruits had begun. The trees had the following latitudinal and longitudinal coordinates: -3.09586 S, -59.98671 E; -3.09717 S, -59.9880 E; -2.9840 S, -60.07570 E; -2.9838 S, -60.0751 E; -2.9621 S, -60.0744 E; 2.883 S, -59.918 E; -2.842 S, -59.843 E and were distant from each other to avoid sib seeds and about the same number of fruits were collected from the trees. The fruits were opened manually with knifes and scissors to remove the seeds. The resin surrounding the seeds was dissolved by immersion in water for 24 hours. After drying in an airy and shaded environment, the lot was homogenized and seeds were kept for 24 hours in a freezer (-18ºC) in airtight pots, to avoid possible spread of insects, and stored in an air-conditioned room (25±3°C).

Hydropriming was applied to freshly harvested seeds after 7 days of storage (31±5°C, 85±10% RH). Mechanical scarification with an electric sharpening stone on the opposite side from the radicle protrusion was applied before hydropriming.

An imbibition curve in distilled water at 15ºC was elaborated with 12 freshly harvested seeds, individually weighed every 24 hours until turgency. Based on the final weight, the time required to reach a partial imbibition of 10%, 20%, 40%, 60% , 80% and 90% of the fully imbibed seeds was determined graphically (Figure 1).

The hydropriming study consisted of five treatments, with three levels of imbibition, plus the controls of freshly harvested and one year stored seeds. (Table 1).

Experimental design was randomized and consisted of four treatments with different degrees of imbibition (control, 20, 40 and 60%) with four replicates of 25 seeds for each germination assay. Each replicate was placed in a nylon net (10 x 13 cm) for imbibition and subsequent drying for seven days above wire meshes in an air conditioned room with a ventilator (31±5°C, 85±10% RH). Seeds collected in 2002 were stored until 2004 in an air conditioned room (24 ± 2 ° C, 68% ± 3% RH).
The moisture content was obtained for each treatment with two samples of 30 seeds and expressed in percentage of the fresh weight (BRASIL, 2009) after drying the samples at 105±2°C until stability in repeated measurements of 24 hours.

The seeds were sown in washed sand at

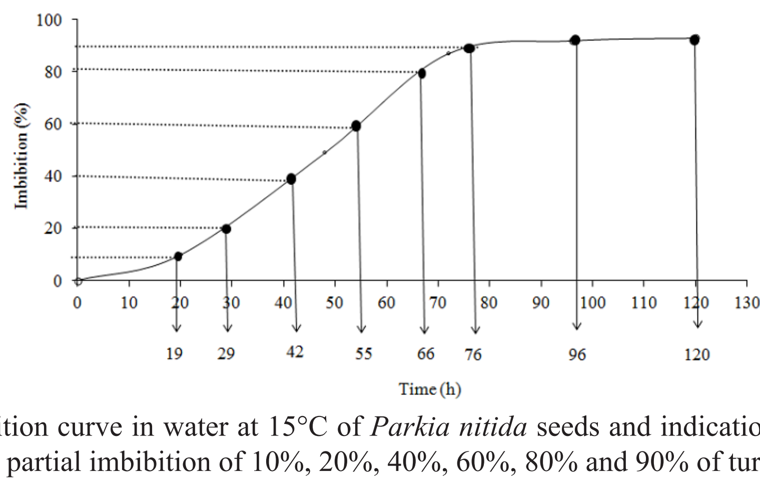

FIGURE 1: Imbibition curve in water at 15°C of *Parkia nitida* seeds and indication of required time to reach a partial imbibition of 10%, 20%, 40%, 60%, 80% and 90% of turgency.

FIGURA 1: Curva de embebição em água a 15°C das sementes de *Parkia nitida* e determinação do tempo necessário para alcançar a embebição parcial de 10%, 20%, 40%, 60%, 80% e 90% da turgência.

TABLE 1: Hydropriming treatments and moisture content (fresh weight basis) of *Parkia nitida* seeds.
TABELA 1: Tratamentos de hidrocondicionamento e teor de água (base úmida) das sementes de *Parkia nitida.*

Seed condition	Required degree of imbibition	Imbibition time at 15ºC	Drying time after imbibition	Moisture content after imbibition and drying
	(%)	(h)	(d)	(%)
Freshly harvested	0	0	0	9,4
One year stored	0	0	0	9,0
Freshly harvested	20	29	7	14,1
Freshly harvested	40	42	7	15,1
Freshly harvested	60	55	7	15,7

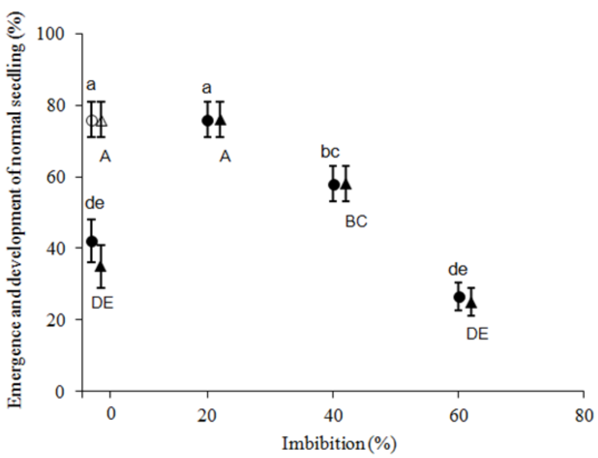

FIGURE 2: Emergence (circle) and development of normal seedling (triangle) of *Parkia nitida*, after seed hydropriming with different degrees of partial imbibition of freshly harvested seeds (closed symbols) and of one year stored seeds (open symbols). Different letters indicate significant differences of emergence (lowercase) and normal seedling development (uppercase).

FIGURA 2: Emergência (círculo) e formação de plântula normal (triângulo) de *Parkia nitida,* após condicionamento hídrico das sementes com diferentes graus de embebição parcial de sementes recém-colhidas (símbolos fechados) e após 1 ano de armazenamento (símbolos abertos). Letras diferentes indicam diferenças significativas, para emergência (letras minúsculas) e formação de plântula normal (letra maiúscula).

1 cm depth in plastic boxes (36 x 55 x 18 cm) in the nursery. Daily temperature ranged between 26 and 36°C (average of minimum and maximum, respectively) with relative humidity of 85±10%. Two germination criteria were observed: the "emergence", assessed after the appearance of the hypocotyl hook above the substrate, and the "normal seedling", assessed after starting of the unfolding process of the first leaf leaflets and the perfect state of development of other parts of the plant. For both germination criteria it was calculated: final germination percentage and mean germination time (SANTANA and RANAL, 2004).

The data were analyzed with analysis of variance (F test) and by comparison of the averages at 5%, using the Tukey test, after the assertion of normality (Shapiro - Wilk) and homogeneity (Levene's test). The values that did not show a normal distribution were transformed into natural logarithm values of ln (x + 0.05). When transformation did not normalize the data, we applied the non-parametric Kruskal-Wallis test (SANTANA and RANAL, 2004).

RESULTS AND DISCUSSION

Freshly harvested seeds of *Parkia nitida* reached 42% emergence, of which 35% resulted in normal seedlings. The same lot after one year of storage had 76% of emergence and all seedlings showed normal development. The results indicate that *Parkia nitida* seeds needed a maturation period after harvest (Figure 2).

Physiological maturity generally coincides with the time of natural dispersal, however, seeds of some species require a further maturation time to reach maximum germination and vigor, for example: *Carica papaya* (LINN.) (AROUCHA et al., 2005), *Salvia splendens* Sellow (CARVALHO and NAKAGAWA, 2000), *Sesamum indicum* (LINN.) (AZEVEDO et al., 2003), *Cassia excelsa* Schrad. (JELLER et al., 2003), *Cecropia schreberiana* Miq., *Hibiscus elatus* Sw. (SANCHEZ et al., 2003), *Platymiscium pubescens* Micheli (BORGES et al., 2002) and *Ateleia glazioviana* BAILL (ROSA et al., 2005).

Hydropriming of the freshly harvested seeds was able to increase the germination percentage to the same value (76%) as of the seeds stored for one year (Figure 2). No difference in the time for emergence and for seedling development of freshly harvested seeds in comparison to stored seeds was observed. However the time for emergence was reduced from 7 to 4 days after hydropriming (Figure 3).

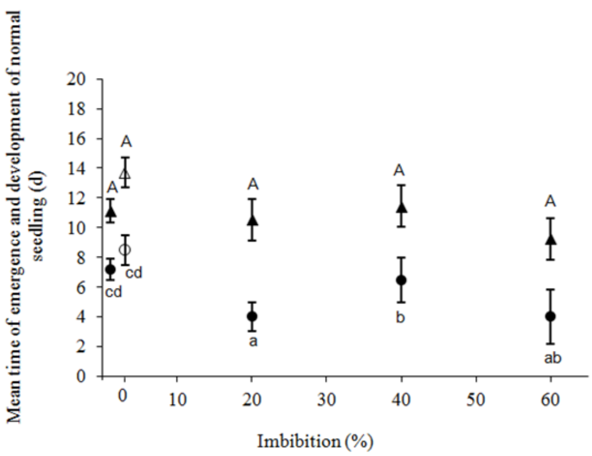

FIGURE 3: Mean time of emergence (circle) and development of normal seedlings (triangle) of *Parkia nitida*, after seeds with different degrees of partial imbibition of freshly harvested seeds (closed symbols) and of seeds stored for one year (open symbols). Different letters indicate significant differences of emergence (lowercase) and normal seedling development (uppercase).

FIGURA 3: Tempo médio da emergência (círculo) e formação de plântula normal (triangulo) de *Parkia nitida,* após condicionamento hídrico das sementes com diferentes graus de embebição parcial de sementes recém-colhidas (símbolos fechados) e após 1 ano de armazenamento (símbolos abertos). Letras diferentes indicam diferenças significativas, para emergência (letras minúsculas) e formação de plântula normal (letras maiúsculas).

The positive action of hydropriming was observed only after a partial imbibition of 20% (29 hours). Longer imbibition periods of 42 and 55 hours, which reached a partial imbibition of 40% and 60%, respectively, significantly reduced the germination (Figure 2). The results indicate a "critical imbibition level" of between 20 and 40% of turgency, for both emergence and development of normal seedlings. Looking at the imbibition curve, which is generally described to have a triphasic pattern (BLACK et al., 2006), the critical level for the *Parkia nitida* seeds is reached in the first half of the first phase (Figure 1).

A similar result was previously found after hydropriming of *Parkia pendula* seeds; here the critical level was between 40 and 60% of turgency, reached after 8 and 13 hours of imbibition respectively (VARGAS and FERRAZ, 2008). With *Parkia multijuga* seeds, positive actions of hydropriming were observed before the seeds reached the second phase of the imbibition curve with ≤70% of turgency, with a moisture content of ≤45.4% (CALVI et al., 2008).

The positive action of priming occurs normally when the imbibition process has reached saturation, during the second phase of the imbibition curve, by the metabolic activation of the seeds (BRADFORD, 1995). However in the three species of *Parkia* the advantage of seed priming occurred only in the first phase of the imbibition curve. The seeds of these species show physical dormancy with a water-impermeable seed coat. Specific cells, located near the micropyle and functioning as a valve, permit the seeds to dehydrate and enter into equilibrium in a dry environment, and do not allow the absorption of water under humid conditions (BLACK et al., 2006). Physical dormancy prevents the seeds from following the oscillations of humidity in their natural environment, different from seeds with permeable seed coats, which gain or lose moisture according to the relative humidity.

The hypothesis that seeds with physical dormancy may respond differently to priming was raised earlier (CALVI et al., 2008, VARGAS and FERRAZ, 2008). However only a few results are known of priming studies with physical dormant seeds. In *Cassia excelsa* seeds, after chemical scarification, no increase in germination percentage and speed was observed after osmopriming (-0,2; -0,4 and -0,6 Mpa) at 20°C in comparison to the control (JELLER et al. 2003). In studies with *Parkia multijuga* (CALVI et al., 2008) and *Parkia pendula* (VARGAS and FERRAZ, 2008) hydropriming was efficient only at a partial imbibition, with lower moisture contents than in seeds which do not have physical dormancy.

CONCLUSIONS

At fruit maturity, *Parkia nitida* seeds are physiologically immature. Hydropriming improved germination performance of fresh seed to the same value of stored seeds. The beneficial results were only achieved during the first phase of the typically triphasic pattern of the imbibition curve. The results add another species with physiological dormant seeds on the list, which respond to seed hydropriming, at the beginning of imbibition.

ACKNOWLEDGEMENTS

CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - National Counsel of Technological and Scientific Development), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), and FAPEAM (Fundação de Amparo à Pesquisa do Estado do Amazonas - Amazonas Research Foundation) are gratefully acknowledged for their financial support.

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