GERMINATION STUDIES ON *Tabebuia impetiginosa* Mart. SEEDS

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ABSTRACT: *Seed germination and seedling production of native forest tree species are an important step in ex situ conservation programs and in the reforestation with ecological purposes. Therefore, understanding seed germination and its regulation is mandatory for the complete success of the conservation programs and revegetation techniques. Thus, morphological studies, temperature requirements for seed germination and its control by gibberellins (GAs) were studied in Tabebuia impetiginosa ("ipê-roxo") seeds. The best temperature for germination under constant light was 30°C. The imbibition of T. impetiginosa seeds followed the common triphasic pattern, with most of the seeds attaining phase II at 24 hours and phase III at 72 hours of imbibition. Visible germination, as radicle elongation, started at 30 hours in water-imbibed seeds and at 24 hours in GA-imbibed seeds. Seeds imbibed in Paclobutrazol, an inhibitor of GA biosynthesis, failed to germinate. However, application of exogenous gibberellins overcame inhibition and allowed germination, suggesting that GAs are regulators of Tabebuia impetiginosa seed germination. The results suggested that germination in Tabebuia impetiginosa seeds is controlled by elongation of the radicle and gibberellins may play an important role in regulating it. The possible role of gibberellins is discussed.*

Keywords: Tabebuia impetiginosa, "ipê-roxo", seed, germination, gibberellins, ex situ conservation, ecological reforestation.

ESTUDOS DE GERMINAÇÃO EM SEMENTES DE *Tabebuia impetiginosa* Mart.

RESUMO: A germinação de sementes e a produção de mudas de espécies nativas são etapas importantes da conservação *ex situ* e dos reflorestamentos com finalidade ambiental. Conseqüentemente, o entendimento do processo germinativo e da sua regulação é imperativo para o sucesso de programas de recuperação e conservação ambientais. Assim, foram estudados, em sementes de *Tabebuia impetiginosa* ("ipê-roxo"), aspectos morfológicos e a fisiologia da germinação, com ênfase na temperatura e o controle de giberelinas (GA). A máxima germinação ocorreu na temperatura de 30°C na presença de luz. A embebição das sementes seguiu um padrão trifásico comum para a maioria das espécies, tendo a fase II da curva de embebição sido alcançada com 24 horas e a fase III com 72 horas. A germinação visível, considerada como elongação da radícula, iniciou-se após 30 horas para sementes embebidas em água e 24 horas para sementes embebidas em giberelina. Sementes embebidas em Paclobutrazol, um inibidor da biosíntese endógena de giberelina, não apresentaram germinação. Todavia, a aplicação de giberelina exógena aliviou a inibição e permitiu que as

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sementes germinassem, sugerindo que giberelinas endógenas regulam a germinação em sementes de *Tabebuia impetiginosa*. Os resultados mostraram ainda que a germinação ocorre apenas por meio da elongação da radícula e que giberelinas endógenas podem controlar este crescimento. A possível função de giberelinas é discutida.

Palavras chave: *Tabebuia impetiginosa*, ipê-roxo, semente, germinação, giberelinas, conservação *ex situ* e reflorestamentos ambientais.

1 INTRODUCTION

Tabebuia impetiginosa Mart ("ipê-roxo"), belonging to Bignoniaceae family is a native tree species found in the Atlantic Forest, "Cerrado" and "Caatinga" biomes of Brazil (Lorenzi, 1992). Its stem bark has pharmaceutical interests because of its anti-inflammatory and antimicrobial properties (Koyama et al., 2000; Anesini & Perez, 1993). The species is also a very important timber producer (Carvalho, 1994) and is largely used in landscape gardening and in reclamation of disturbed lands (Lorenzi, 1992).

However, *Tabebuia impetiginosa* has been under severe threat because of the increasing human activities in the biomes where the species grows, including agricultural practices and grazing. This has led Siqueira & Nogueira (1992) to include *Tabebuia impetiginosa* in the list of species under threat, calling attention for the importance of this species and the need of its conservation. Thus, during the last years the demand for seeds and seedlings with high quality has increased to assist conservation programs aiming to diminish imminent risks of extinction.

Therefore, studying morphology and physiological aspects during seed germination, as well as its regulation, is mandatory for the complete success of conservation program and for further use in revegetation techniques. This knowledge is a prerequisite in order to develop satisfactory protocols for regeneration of the species at intervals during long term storage for maintenance of genetic variation and to accommodate seedling supply for reforestation programs.

Germination and its regulation have never been studied in *Tabebuia impetiginosa* seeds. Various physiological and biochemical studies conducted in a numerous species showed that seed germination initiates with water uptake by the seed and ends with the start of elongation of the embryonic axis (Bewley & Black, 1994). Radicle protrusion occurs when the expansive force of the embryo exceeds the mechanical restraint of the endosperm and seed coat. In endospermic seeds, weakening of the endosperm allows radicle protrusion (Groot & Karssen, 1987; Hilhorst & Toorop, 1997), while in non-endospermic seeds the mechanism is unknown.

Gibberellins (GAs) play an important role in promoting seed germination, as shown by Koornneef and van der Veen (1980) and Groot & Karssen (1987) in GA-deficient mutants of *Arabidopsis thaliana* and tomato seeds. The use of Paclobutrazol, an inhibitor of GAs biosynthesis, showed that GAs are synthesized *de novo* during germination in *Arabidopsis* seeds (Debeaujon & Koornneef, 2000).

During germination of endospermic seeds, gibberellins act promoting weakening of the endosperm cap that surrounds the radicle tip and also promotes embryo growth (Karssen et al., 1989). Cowling et al. (1999) showed that GA regulates hypocotyl growth by altering the extent of hypocotyl cell elongation in *Arabidopsis thaliana*. GA treatment induces

the mRNA levels of *CsAGP1*, a gibberellin-responsive gene in cucumber hypocotyls related to stem elongation (Park et al., 2003). Albeit the importance of GA has been shown in model plants, it is still scant the information about the role of GA during germination on forest tree seeds such as *Tabebuia impetiginosa*.

Thus, this work studied the seed morphology, temperature requirement for seed germination and germination *sensu stricto* and its control by endogenous gibberellins in *Tabebuia impetiginosa* seeds.

2 MATERIALS AND METHODS

Seed source. Fruits of *Tabebuia impetiginosa* were collected in Lavras, MG, Brazil in 2002 during seed dispersion. Fruits were let to dry until complete dehiscence, seeds were extracted and dried to 7% of moisture content. Afterwards, the seed coat (wing) was manually removed and seeds were stored at 5^{o} C until the start of the experiment.

Germination conditions. Seeds without coat (Figure 1D and E), were surface sterilized by washing with 1% sodium hypochlorite for 10 minutes, rinsed in water and placed in 8.5 cm Petri dishes on 2 filter papers imbibed with 10 ml of demineralized water. Seeds were incubated at temperatures of 5, 10, 15, 20, 25, 30, 35 and 40^{o} C at continuous light. Seeds were also incubated in GA_{4+7} solution (100mM) or Paclobutrazol solution (100mM). GA_{4+7} 10^{-3} M stock solutions were prepared in 1 N KOH, followed by adjusting the pH to 7.0 with 1 N HCl. Paclobutrazol 10^{-3} M stock solutions were prepared by dissolving in acetone (0.1% v/v) and stirring overnight. The germination (radicle elongation >2 mm) (Figure 1E) was recorded daily until the percentage of germinated seeds were constant.

Imbibition curve. Intact seeds were imbibed in water as described above and the fresh weight was measured every six hours.

Radicle length. The length of twenty radicles from water-, GA- and Paclobutrazol-imbibed seeds was measured daily using calipers.

Morphological studies. To better characterize their morphology, *Tabebuia impetiginosa* seeds were photographed with a digital camera **(**Canon, Power Shot 540) and a binocular (Leica MZ75). Images were also recorded using a scanning electron microscope (Nijsse et al., 1998).

Statistical analyses: The statistical analyses were performed by using the statistical program SISVAR (UFLA).

3 RESULTS AND DISCUSSION

Seeds of the Bignoniaceae family such as *Tabebuia impetiginosa*, have a structure called wing that assists their wind-dispersion (anemochory) from the mother plant. The wings are a flattened extension of the layers of the seed coat with a relatively large surface area (Werker, 1997) (Figure 1A and B). A cross section of the wings (Figures A and B) reveals the presence of empty spaces which helps the seeds to be wind-transported. At the dry stage of the seed the external surface of the cotyledons resembles a “root system” (Figure 1F).

Seeds of *Tabebuia impetiginosa* are named endospermic or exalbuminous since in this species, the endosperm is consumed during embryogenesis and is absent at the mature stage (Figure 1C). In exalbuminous seeds, reserves such as carbohydrates, proteins, oils are located in the embryo (Werker, 1997) and are used during seed

germination until the seedling becomes photosynthetically active.

To study the requirements of temperature on germination of *Tabebuia impetiginosa* seeds, they were incubated at constant light and different temperatures (5, 10, 15, 20, 25, 30, 35 and 40°C). The results showed that the temperature that allowed fastest germination was 30°C (Figure 2). Under such temperature radicle elongation started at 30 hours of imbibition. After 36 hours of imbibition, 50% of the seeds germinated and at 72 hours of imbibition, 100% of the seeds had shown radicle elongation (Figure 2). Water uptake during imbibition followed a triphasic pattern, with most of the seeds achieving phase II of imbibition at 24 hours (Figure 3). Thus, phase I of germination corresponded to a rapid imbibition aiming to begin the resumption of metabolism blocked when seeds are dried at the maturation drying phase of the seed development. Phase II of germination was only achieved when the fresh weight increased about 40% (Figure 3). After that, the hydration level remained constant for 48 hours. Many cellular and metabolic events are triggered by water uptake during germination such as activation of respiration, synthesis of proteins and RNA, and amino-acid metabolism. (Bewley, 1997; Bove et al., 2001). In average the phase III of germination was attained after 72 hours of imbibition when the radicle started to elongate (Figure 3). In *Brassica napus* seeds, which are also non-endospermic, cell wall loosening and decline in turgor in the embryo cells control germination (Schopfer & Plachy, 1985). In *Tabebuia impetiginosa* seeds, germination is probably controlled by extensibility of the embryo cells, indicating that a similar mechanism as in *Brassica napus* is controlling germination, since both species do not have endosperm that needs to weaken allowing radicle protrusion.

Gibberellins are a class of phyto-hormones that regulate various aspects of plant development including seed germination (Bewley & Black, 1994). Our results showed that exogenous gibberellins enhanced radicle elongation during germination of *Tabebuia impetiginosa* seeds (Figure 5). In water-imbibed seeds, germination started at 30 hours whereas in GA-imbibed seeds, the first seed showed radicle elongation (> 2mm) at 24 hours of imbibition (Figures 4 and 5). Germination was inhibited completely when the seeds were imbibed in Paclobutrazol, an inhibitor of GA biosynthesis (Figure 4). Exogenous gibberellins overcame the inhibition of germination imposed by Paclobutrazol (Figures 4 and 5), showing that gibberellins are synthesized *de novo* during germination. The results indicated that gibberellins are important regulator of germination in *Tabebuia impetiginosa* seeds, as already shown in *Arabidopsis* and tomato (Koornneef & van der Veen, 1980; Karssen et al., 1989).

The site of GA action has been suggested by Karssen et al. (1989) as being in the endosperm and in the embryo. In the endosperm of tomato seed, gibberellins regulate endosperm degradation by inducing enzymes activity such endo-ß-mannanase (Groot et al., 1988). Our results showed that in *Tabebuia impetiginosa* seeds, gibberellins induced radicle elongation. Probably, GAs induce the increase of the embryo pressure potential during imbibition as showed in coffee seeds by Silva (2002). As observed in others species, the increase in the pressure potential leads to radicle cell wall extension causing radicle cell expansion, embryo growth and germination (Bewley & Black, 1994).

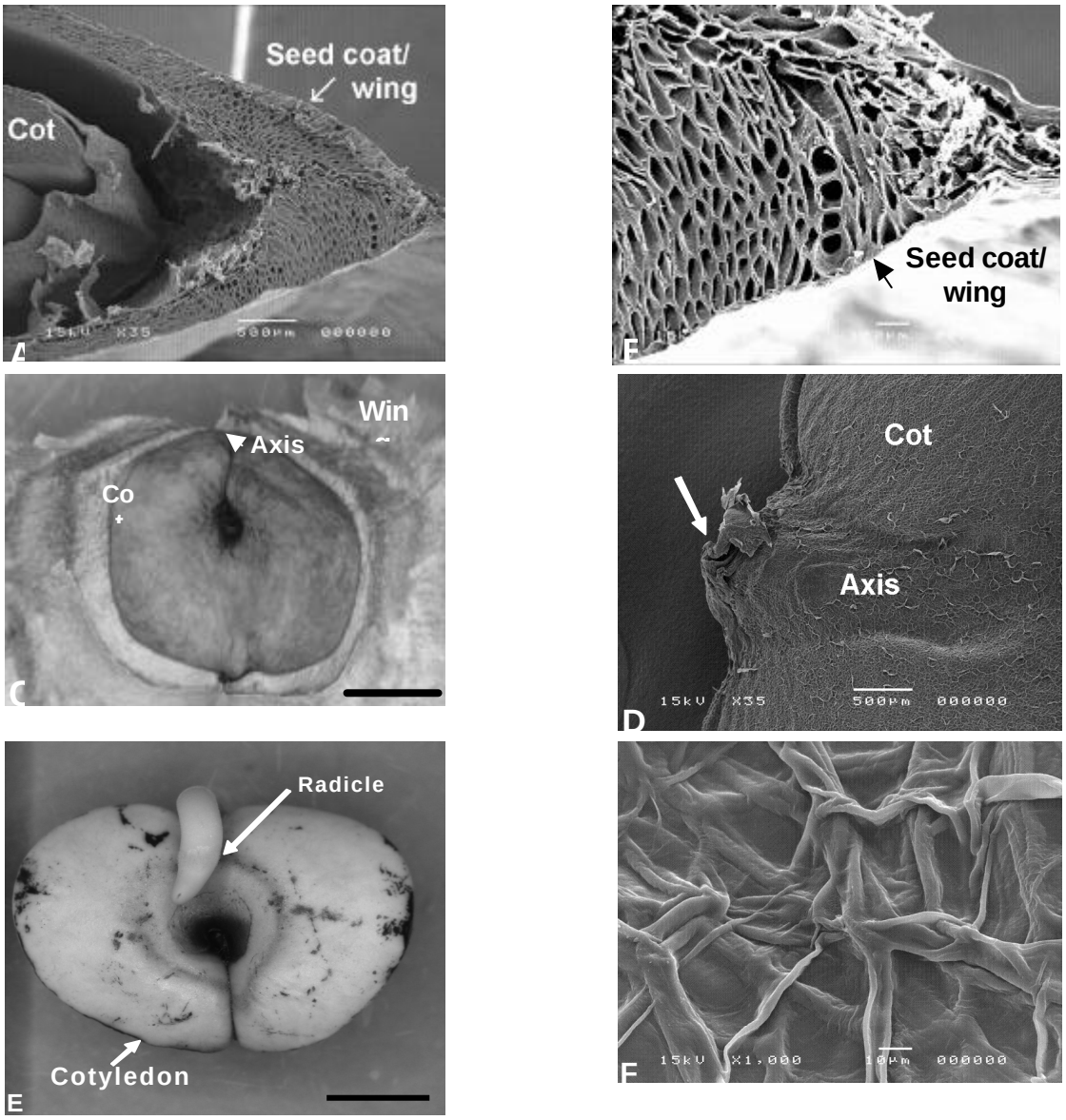

Figure 1. Dry and germinated seeds of *T. impetiginosa*. (A) Dry seed with the seed coat, showing the presence of the wings and cotyledons; (B) Internal vision of the seed coat (wing); (C) Dry seed with part of the seed coat removed; (D) Seed without seed coat. Observe the localization of the cotyledons and embryonic axis. Arrow indicates where the radicle will elongate; (E) Germinated seed without seed coat after 72 hours of imbibition; (F) External surface of the embryo from dry seed. Bars indicate 500µm in (A) and (D) ; 100 µm in (B) and (F) and 5 mm in (C) and (E).

Figura 1. *Sementes secas e germinadas de T. impetiginosa. (A) semente seca com o tegumento. Observe a presença das asas e cotilédones; (B) visão interna do tegumento (asas); (C) semente seca com parte do tegumento removido; (D) visão aproximada da semente sem tegumento. Observe a localização dos cotilédones e eixo embrionário. A seta indica o local onde haverá elongação da radícula; (E) semente germinada sem tegumento após 72 horas de embebição; (F) superfície externa do embrião de semente seca. Barras indicam 500µm em (A) e (D); 100 µm em (B) e (F) e 5 mm em (C) e (E).*

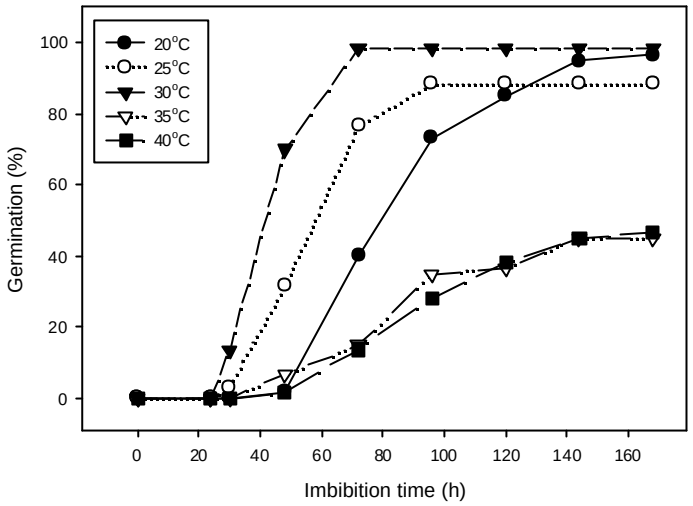

Figure 2. Germination of *T. impetiginosa* seeds in water at different temperatures. Data points are the average of 4 replications of 25 seeds.

Figura 2. *Germinação de sementes de T. Impetiginosa em água em diferentes temperaturas. Pontos representam a média de 4 repetições de 25 sementes.*

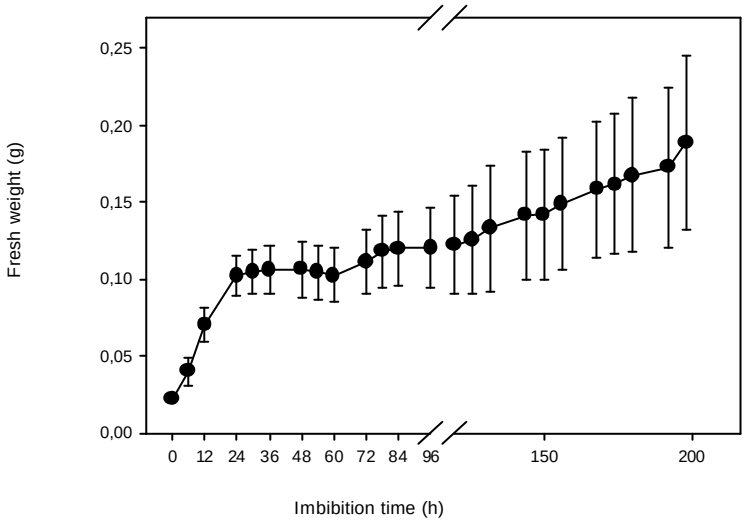

Figure 3. Imbibition curve during *T. impetiginosa* seed germination. Error bars indicate standard deviations.

Figura 3. *Curva de embebição de sementes de T. impetiginosa. Barras indicam desvio padrão.*

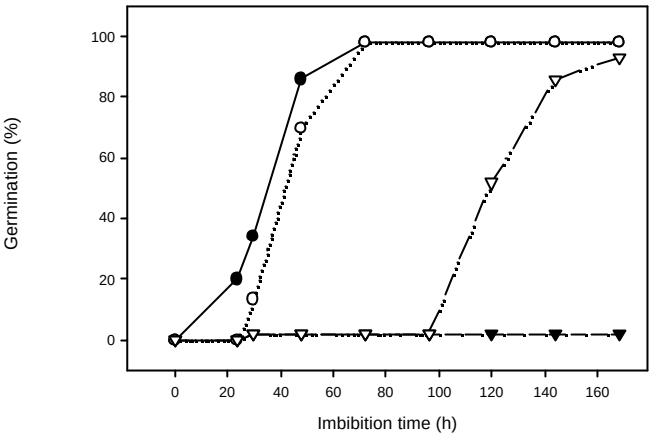

Figure 4. Germination of *T. impetiginosa* seeds in 100 µm GA_{4+7}(•), water (o), 100µm of Paclobutrazol (?) and 100µm of Paclobutrazol followed by 100µm GA_{4+7}(∇). Data points are the average of 4 replications of 25 seeds.

Figura 4. *Germinação de sementes de T. impetiginosa em 100 mm GA_{4+7} (·), água (o), 100mm de Paclobutrazol (?) e 100 mm de Paclobutrazol seguido de 100mm GA_{4+7} (Ñ). Pontos representam média de 4 repetições de 25 sementes.*

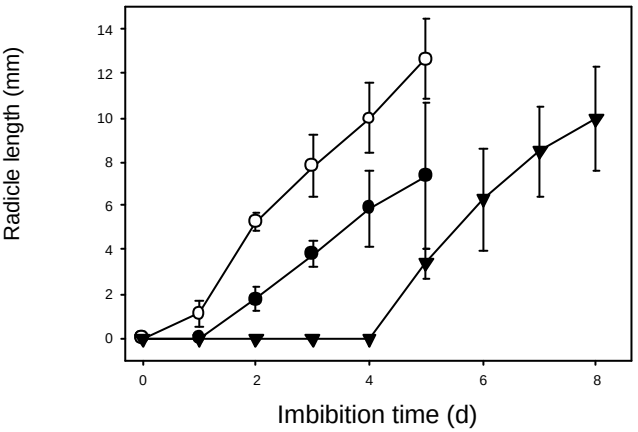

Figure 5. Radicle length (mm) of *T. impetiginosa* seeds during imbibition in water (•),100 µm GA_{4+7} (o) and 100 µm Paclobutrazol followed by 100 µm GA_{4+7} (?). Data points are the average of 20 radicles.

Figura 5. *Comprimento da radícula (mm) de T. impetiginosa durante embebição em água (·), 100 mm GA_{4+7} (o) e 100 mm Paclobutrazol seguido de 100 mm GA_{4+7} (?). Pontos representam a média de 20 radículas.*

4 CONCLUSION

The results reveal that the ideal temperature under constant light to germinate *Tabebuia impetiginosa* seeds is 30° C, with germination occuring through radicle elongation. Gibberellins are synthesized *de novo* during imbibition and are involved in the regulation of germination by controlling radicle elongation.

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