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Short communication

Under ground: what pH favors germination and the root growth of *Zeyheria tuberculosa* (Vell.) Bureau ex Verl.

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ABSTRACT

This work aimed to examine the germination and initial growth of Zeyheria tuberculosa (Vell.) Bureau ex Verl. seedlings at different pH values. pH of the deionized water was adjusted with hydrochloric acid and/or sodium hydroxide to 4.7, 5.7, 6.7, 7.7 and 8.7. The following parameters were evaluated: final germination percentage (GP), germination speed index, time required for 50% germination, germination rate, viability of the remaining seeds and length of the radicle. All parameters were reduced in alkaline pH. Maximum germination of 94% was reached in the treatment control, followed by pH 4.7. At alkaline pH (7.7 - 8.7), GP was 43 and 31%, respectively. Therefore, at these pHs, germination was reduced. We provided direct evidence that pH is a limiting factor for germination and for the radicle growth of Z. tuberculosa seedlings. In addition, we show experimental evidence of why this species is widely distributed in the Cerrado biome, where the soils are more acidic, and the relationship between soil composition and high germination in acidic medium was corroborated with this study. We suggest that the germination test at different pHs be included in reforestation programs with native species to predict the establishment of seedlings in soils with different pH.

Keywords: acidic pH, alkaline pH, Bignoniaceae, ipê-felpudo, reforestation, seedling establishment.

The loss of Brazilian plant cover has intensified, reducing biodiversity, threatening native species and maintaining plant genetic resources (Mapbiomas 2020). *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae) is a pioneer species that occurs in a wide latitudinal range in Brazilian and Bolivian forests, but it is threatened due to agricultural expansion and intense logging (IUCN 1998; CNC Flora 2012). It is currently classified as vulnerable and has been included in the Red List of Threatened Species of Brazilian Flora (Lohmann *et al.* 2013) and on the International Union for Conservation of Nature's Red List of Threatened Species (Souza *et al.* 2017).

To reverse this scenario, the production of *Z. tuberculosa* seedlings for use in silviculture, reforestation or recovery of degraded areas programs becomes necessary. Thus, more specific information that helps to understand the physiological aspects of propagation is essential for the success of these programs and the maintenance of natural populations. Despite the potential use of these species, their propagation aspects have not been well described in

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the literature. The seed is the main form of propagation of forest species (Roveri Neto & Paula 2017; Sasaya *et al.* 2021), with germination and seedling establishment being the two critical stages in the life cycle of plants (Pérez-Corona *et al.* 2013; El-Maarouf-Bouteau *et al.* 2015).

Seed germination is regulated by internal and external factors, such as the concentration of hydroxyl (OH^-) and hydrogen (H^+) ions (Mahmood *et al.* 2016; Vahabinia *et al.* 2019; Sasaya *et al.* 2021), which act on the structure, solubility and activity of most enzymes (Orij *et al.* 2009). Furthermore, this factor can induce signaling events that lead to adaptive responses in plants (Tsai & Schmidt 2021) or damage compounds, organelles and/or the physical structure of the seed during the germination process (Bastos *et al*. 2013). However, this aspect has been neglected in the installation of germination tests and even at the time of planting, which can culminate in little developed or abnormal seedlings. Thus, this work hypothesizes that pH 6.7 favors seed germination and initial growth of *Z. tuberculosa* seedlings. The objective was to examine the germinal responses and initial growth of *Z. tuberculosa* seedlings at different pH values.

Fruits at the beginning of dehiscence of *Z. tuberculosa* (Vell.) Bur. were collected from 10 mother trees in the municipality of Nepomuceno, Minas Gerais, Brazil (21°13'50"S; 45°10'50" WGR) in August 2020. After harvesting, the fruits were dried in the shade and kept in full sun for two days to facilitate manual removal of the seeds without causing mechanical damage (Carvalho 2005). After obtaining the seeds, the experiment was installed. The average initial degree of seed moisture was 3.51%.

The effect of pH on seed germination was evaluated using four pH solutions: 4.7 and 5.7, simulating acidic pH, 7.7 and 8.7, simulating alkaline pH and 6.7, values close to that of deionized water. Deionized water was adjusted with hydrochloric acid (HCl - 1 M) or sodium hydroxide (NaOH - 1 M) to obtain the desired solution. The final pH of the solution was measured with a GEHAKA model PG1000 benchtop pH.

Five replications with 16 seeds each were used; previously, the seed wings were removed and disinfected by immersion in a sodium hypochlorite solution (1.0% v/v) and detergent (three drops) for 10 minutes, followed by three washes in deionized water. Then, in a gerbox recipient, the seeds were sown on two sheets of germination paper previously sterilized in an oven at 105 °C for 2 h and moistened with deionized water in each pH range in a volume equivalent to 2.5 times the dry mass of paper (Brasil 2009). The gerbox were kept in a germination chamber (SOLAB) with a constant temperature of 30 °C and a photoperiod of 12 h of white light (40 µmol photons $m^{-2}s^{-1}$) (Lima 2003). Seeds were considered germinated after radicle protrusion (≥ 2 mm). Germination was evaluated daily for 20 days. Seeds remaining from the germination test were evaluated for viability according to the methodology described by Abbade and Takaki, 2014, with modifications. Seeds were cut longitudinally with tweezers and a scalpel, placed in dark bottles, totally submerged in a tetrazolium solution at a concentration of 1% (pH 7.0) and kept in a germination chamber (SOLAB) in the dark at a temperature of 30 °C for 1 h. The two halves of the seed were individually evaluated with the naked eye, and the location of the coloration in relation to the essential areas for growth was considered. After the test, we assessed the percentage of final germination (GP) (Ranal *et al.* 2009), germination speed index (GSI) (Maguire, 1962), time required for 50% of the seeds to germinate (T_{50}) (Farooq *et al.* 2005) and germination rate (GR), calculated as the reciprocal of T_{50} (Romano *et al.* 2019). After 18 days of sowing, the length of the radicle was measured with the aid of a caliper (Mitutoyo), but it was performed only in replicates that had at least five germinated seeds.

We used a completely randomized design with five treatments (pH values). A pH of 6.7 was used as a control due to the proximity of the pH of the deionized water. Data normality and variance homogeneity were verified through Shapiro–Wilk and Bartlett tests, respectively. Data were submitted for analysis of variance, and averages were compared by *Tukey's post hoc* test, considering $p < 0.05$. Data analysis was performed using the package ExpDes.pt (Ferreira *et al.* 2021) of the statistical software R, version 4.2.2 (R Core Team 2021).

At alkaline pH (8.7), the beginning of the germination process occurred 12 days after the germination test, which was not observed at the other pH values (Fig. 1). The maximum GP of *Z. tuberculosa* seeds was reached in the control treatment, which was not significantly different from the acidic pH (4.7). However, both alkaline pH values (7.7 and 8.7) reduced the GP by 54% and 67%, respectively, differing from the control treatment (Fig. 1). For the GSI (Fig. 2A), the seeds submitted to solutions with pH values of 4.7 and 6.7 (control) presented higher averages. However, pH 8.7 reduced the germination speed of *Z. tuberculosa* by approximately 75% compared to the control, providing an increase of approximately 20% in the T_{50} (Fig. 2B) of these seeds. Concerning GR, it can be seen that at acidic pH (4.7), there was an increase of approximately 16% of seeds germinated in relation to the control treatment (Fig. 2C). Regarding the viability of the remaining seeds, it was observed that in alkaline pH values (7.7 - 8.7), the average number of viable seeds was higher compared to the control treatment, presenting viability of 49 to 64% (Fig. 2D).

For the initial radicle length, at acidic pH (4.7), there was an increase of 9%, with no significant difference from the control treatment. On the other hand, alkaline pH values (7.7 and 8.7) reduced the root length of seedlings by 88 and 96%, respectively, significantly differing from the control treatment (Fig. 3).

The characterization of the germination process and the establishment of native tree seedlings is important for the survival of forest species, in addition to providing subsidies

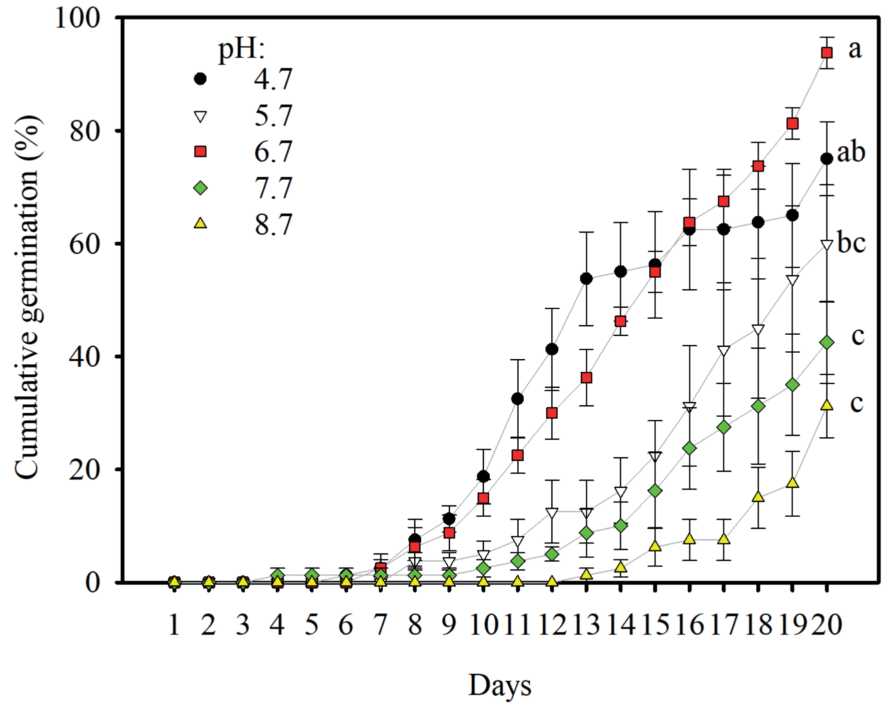

Figure 1. The germination percentage of *Z. tuberculosa* seeds at different pH values. Averages ± standard error. Averages followed by the same letter do not differ significantly from each other by the Tukey *post hoc* test ($P < 0.05$).

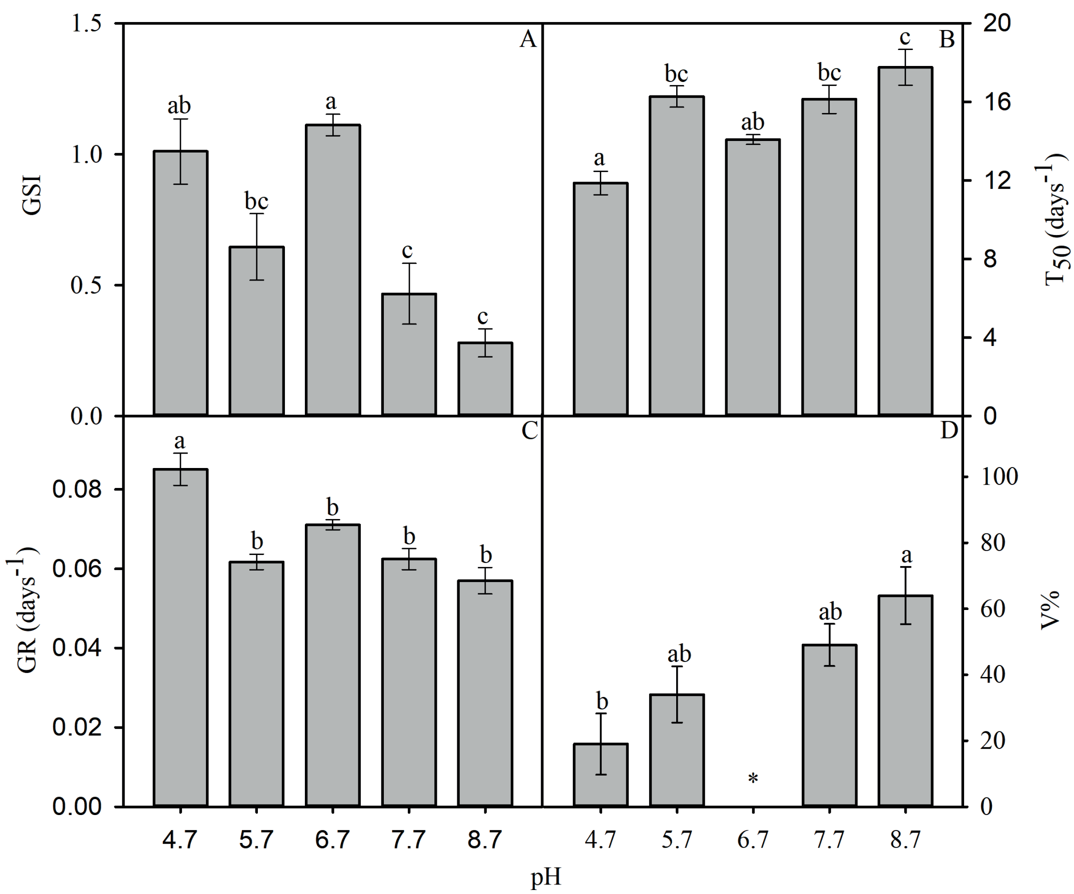

Figure 2. (**A**) Germination speed index - GSI, (**B**) the time required for germination of 50% of seeds - T_{50}, (**C**) germination rate - GR and (**D**) percentage of remaining seed viability - %V of *Z. tuberculosa* seeds at different pH values. Averages ± standard error. Averages followed by the same letter do not differ significantly from each other by the Tukey *post hoc* test ($P < 0.05$).

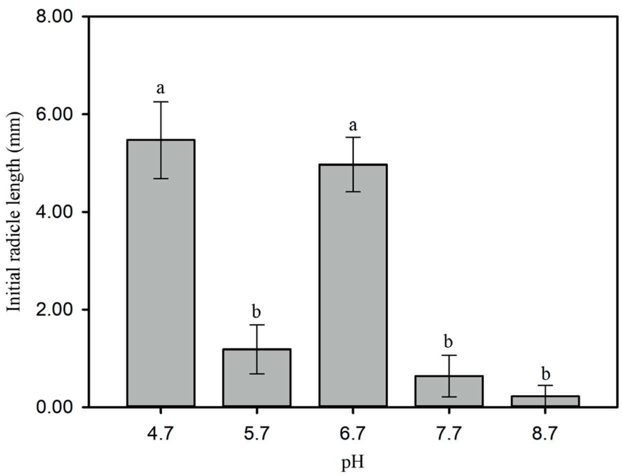

Figure 3. Initial length of the radicle of *Z. tuberculosa* seedlings at different pH values. Averages ± standard error. Averages followed by the same letter do not differ significantly from each other by the Tukey *post hoc* test ($P < 0.05$).

for projects of silviculture, reforestation or recovery of degraded areas. We provided direct evidence that pH is a limiting factor for seed germination, as well as for the initial radicle growth of *Z. tuberculosa* seedlings, which is a relevant aspect to be verified in the propagation of this species. However, the germinal responses showed that *Z. tuberculosa* seeds can germinate in all pH ranges tested in this study. This germination ability can give this native tree species greater plasticity to variations in soil pH (germinative niche), which can increase its competitive capacity with other species. It may also be related to its dissemination in different regions of Brazil and Bolivia (CNC Flora 2012; Lohmann *et al.* 2013).

During the germination process, different enzymes are involved in the mobilization and use of seed reserves (Abbade & Takaki 2012; Bettey & Finch-Savage 1996), and their activity is directly affected by the pH of the medium since this factor acts on the kinetics of most enzymes (Orij *et al.* 2009). Thus, seeds soaked in acidic (5.7) and alkaline (7.7 and 8.7) pH in the experiment may have reduced the synthesis and action of enzymes necessary for the germination of *Z. tuberculosa* seeds, which caused a delay in the process. As observed in the viability test of the remaining seeds, these pH values did not cause death or loss of seed viability.

Similarly, the initial radicle growth of *Z. tuberculosa* seedlings was drastically affected at alkaline pH values (7.7 and 8.7). At these pH values, the proton level was possibly not maintained in the apoplast, reducing the available energy, in the form of ATP, for membrane transport processes (Moreau *et al.* 2021). Among these processes, apoplast acidification is necessary for cell expansion and, consequently, root growth (Barbez *et al.* 2017), in addition to being the main mechanism of cell elongation by auxin. Moreover, pH modifies the activity of cell wall enzymes important for the loosening of these structures, including expansins, xyloglucan hydrolases, and pectin methylesterases (Moreau *et al.* 2021). We emphasize that, as far as we know, this is the first study that evaluated the effect of pH on seed germination and initial growth of ipe-felpudo seedlings. Thus, this information will help in the construction of knowledge.

When dispersed from the mother plant, *Z. tuberculosa* seeds are subject to different environmental conditions that directly affect the germination of their seeds and consequently the initial growth of the radicles (Tudela-Isanta *et al.* 2018). Among these soil conditions, pH is not always favorable for the seeds to express their germination potential, as well as the formation of well-developed roots to support the initial growth of the seedlings (Basto *et al.* 2015; Tudela-Isanta *et al.* 2018). Thus, this work shows that pH is an important factor to be considered in the propagation of these species.

Germination and initial growth of *Z. tuberculosa* occur at different pH values, but these processes are favored at pH 4.7 and 6.7. The results of this work corroborate that *Z. tuberculosa* can be distributed in regions with more acidic pH, such as the Cerrado biome, where most soil has low nutrient availability and high aluminum saturation and is naturally acidic (pH 4.8-5.1) (Lopes & Guilherme 2016). It is important to emphasize that although the pH did not impede germination, the establishment of seedlings can be a bottleneck for species in regions with more alkaline soils or those subject to alkalization. Thus, we suggest that the germination test at different pH values be included in reforestation programs using native species as a prediction for their establishment in soils with different pH values.

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