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Seed germination and dormancy break in *Eragrostis polytricha*, a native Brazilian grass species with potential for recovery of degraded lands

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ABSTRACT

Ferruginous Rocky Outcrops have high levels of species richness and endemism, but have been threatened by several anthropic actions, especially mining. *Eragrostis polytricha*, a common grass species in the vegetation of these outcrops, has shown promising features for use in the recovery of mining areas. However, in order to fully understand the species' potential for such use, its requirements for germination, seed dormancy break and seedling development must be determined. Thus, we aimed to: (1) assess the temperature conditions needed for seeds of *E. polytricha* to germinate; (2) evaluate the effects of KNO_3 in breaking seed dormancy; and (3) analyze the germination efficiency of seeds that are still in spikelets. The experiment included seven treatments: 15–35 °C with KNO_3, 20–30 °C with KNO_3, 15 °C with KNO_3, 25 °C with KNO_3, 35 °C with KNO_3, 20–30 °C with water, and 20–30 °C with KNO_3 using spikelets. The treatments with alternating temperatures associated with KNO_3 yielded the highest germination rates, suggesting that these two factors combined can break seed dormancy. Seeds inside spikelets exhibited a high germination percentage, and thus represent an interesting alternative for seedling production.

Keywords: alternating temperatures, hairy sheath lovegrass, KNO_3, mining areas, native species

Introduction

Environmental degradation is a global process, being caused by diverse factors and affecting people in many different ways according to their economic, political and social circumstances (Mortimore 1998). Although it usually takes place on arid soils (UNEP 1997), environmental degradation can also occur in other areas. Overall, ca. 98.8 % of degraded lands are associated with extractivism, while 1.2 % are related to mining activity, road construction, incorrect urban waste disposal, and other causes (Neto *et al.* 2004). Mining activity seen considerable growth in Brazil over the last years, yet the expansion of the sector has been followed by major impacts such as vegetation loss, which has greatly contributed to the occurrence of soil erosion (Mechi & Sanches 2010).

Concomitantly with the ongoing degradation, concern over the conservation and recovery of natural resources has been growing worldwide (Durigan *et al.* 2011). In that sense, studies on seed germination are essential for the recovery of degraded lands, insofar as they may help characterize the germination niche of different species and obtain information on the natural conditions under which their seeds are likely to germinate (Albrecht & Penagos 2012). Germination is one of the most important steps in the plant life cycle, encompassing a series of events associated with the development of the plant reproductive

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structure (Kerbauy 2004; Bewley *et al.* 2013). The timing and percentage of seed germination are influenced by a set of factors which act collectively, namely: light, temperature, water potential, gases, and chemical and biotic factors (extrinsic or environmental factors); and seed viability, dormancy and morphology (intrinsic or internal factors) (Kerbauy 2004; Bewley *et al.* 2013).

Rocky Outcrops have been subjected to severe impacts due to ever-increasing environmental degradation by anthropic activity (Nunes *et al.* 2015), all that while hosting one of the highest species richnesses in the world (Morellato & Silveira 2018). Recent studies have shown that these ecosystems are also an important center of biological diversity and plant endemism (ca. 40 % of its species are endemic) (Pontara *et al.* 2018). The Ferruginous Rocky Outcrops, which form one of such ecosystems, develop over ironstone (*i.e.* itabirites and cuirasses known as “canga”) (Silveira *et al.* 2016). These areas have been threatened by climate change associated with intense mining activity (Jacobi & Carmo 2008; Fernandes *et al.* 2018), a problem which is compounded by ineffective conservation policies. In the face of such a scenario, a strategy that might contribute to implementing conservation and restoration practices in these ecosystems would be knowing the biology of their species.

According to Silva & Mielniczuck (1997), grass species have high developmental capacity and high potential for soil recovery in degraded areas such as mining sites, since their roots are uniformly distributed in the soil, have high density and are periodically renewed. *Eragrostis polytricha*, a species that occurs in the Brazilian Cerrados (Boechat & Longhi-Wagner 2000) and also in the Ferruginous Rocky Outcrops (Viana & Lombardi 2007; Vincent & Meguro 2008), has shown promising potential to be used for such finality, and thus knowing its germination demands will provide us with the information needed to taking the next steps in the devising and implementation of revegetation programs. *Eragrostis polytricha* is a pioneer grass species (Boechat & Longhi-Wagner 2000) and has great potential for the early stages of vegetation recovery, as it plays a major role in increasing the contents of organic material in the soil while also helping stabilize the substrate (Martins 1996). Furthermore, the species is native to Brazil, and according to Carmona (1998) native species adapt more easily and more rapidly to the edaphic and climatic conditions of the area undergoing restoration.

The occurrence of seed dormancy in *E. polytricha* has been confirmed by Ramos *et al.* (2016) and Ramos *et al.* (2017), yet the temperature conditions that propitiate highest germination success in the species remains unknown. Temperature is an environmental factor of extreme importance for seed germination (Oliveira *et al.* 2015). For native Brazilian plant species, seed germination may occur at a certain temperature range, depending on the region and the biome where the given species naturally occurs (Brancalion *et al.* 2010). Temperature also plays a role in controlling seed dormancy (Offord & Meagher 2001), which is often interpreted as an adaptive response of a particular species to stress conditions (Garwood 1983; Mathias & Kisdi 2002), by means of increased adaptability and decreased germinability during an unfavorable time for seedling establishment (Keya 1997). Along with temperature, chemicals like KNO_3 have been revealed to be able to break seed dormancy, contributing to accelerate germination not only in grasses (Akamine 1944; Garber *et al.* 1974; Eira 1983; Gazziero *et al.* 1991; Frank & Nabinger 1996; Figueiredo *et al.* 2012; Baličević *et al.* 2016; Batista *et al.* 2016; Kreuser *et al.* 2016; Richard *et al.* 2016; Libório *et al.* 2017) but also in species from other plant groups (Wei *et al.* 2010; Bian *et al.* 2013; Cárdenas *et al.* 2013; Lay *et al.* 2015).

Another noteworthy feature of *E. polytricha* is that it has spikelets with 3–5 florets, which produce very small caryopses. Separating caryopses from spikelets is a difficult process, which renders unfeasible the production of seedlings through this method. Thus, in order to use the species in revegetation programs, which themselves require large-scale seedling production, an interesting alternative would be to assess the feasibility of obtaining germinated seeds from caryopses that are still inside spikelets, since collecting spikelets is a much easier and faster procedure.

In view of the above, we aimed to: (1) assess the effects of different temperatures in seed germination; (2) evaluate the effects of KNO_3 on germination speed; and (3) verify whether there is any difference in germination efficiency between isolated caryopses and seeds still in spikelets.

Materials and methods

Study site

The study was conducted at Gerdau’s Unidade de Pesquisa e Inovação em Campos Rupestres Ferruginosos at Ouro Branco municipality, Minas Gerais state, southeastern Brazil, where different plant species from the Ferruginous Rocky Outcrops are cultivated. The study area is located at coordinates 20°31’17.43” S and 43°44’18.89” W. Mean annual rainfall in the region is 2056 mm and mean temperature is 25.5 °C. Climate in the region is type Aw (tropical) according to Köppen’s classification (Climate-Data 2020).

Seed collection and preparation

In May 2017, panicles of *E. polytricha* Ness were collected from 40 randomly selected individuals that were cultivated at the Gerdau’s Unidade de Pesquisa e Inovação em Campos Rupestres Ferruginosos. Panicles were collected when their spikelets had a high number of mature caryopses. In grasses, the seed coat is adhered to the pericarp, and therefore the caryopsis (fruit) was the unit used in the germination test, since it is impossible to separate one structure from the other. A schematic model of panicle development in *E. polytricha* is shown in Figure 1.

The collected material was stored in paper bags and kept in laboratory environment at a controlled temperature of 25 °C for three months until the beginning of the germination experiment. A seed blower (De Leo) was used to separate seeds from inert material and from spikelets with empty florets. Florets are structures in which the caryopses develop, being present in the spikelets. Blower-segregated material was then manually separated in "naked seeds" and spikelets. These procedures and the germination tests were all performed at the Laboratório de Análise de Sementes of the Departamento de Fitotecnia at Universidade Federal de Viçosa, Brazil.

Germination experiments

Germinability was tested at different temperatures with a fixed concentration of KNO_3. Four replicates of 50 seeds were used per treatment. Treatments were applied on Gerboxes covered with two filter-paper sheets imbibed with a 0.2 % KNO_3 solution at 15–35 °C (treatment 1), 20–30 °C (treatment 2), 35 °C (treatment 3), 25 °C (treatment 4) and 15 °C (treatment 5). All these treatments were applied to "naked seeds". An additional treatment of 20–30 °C was applied to spikelets (also on filter-paper sheets imbibed with 0.2 % KNO_3) (treatment 6) to test the germination efficiency at that condition. In parallel, another treatment was applied using only water (*i.e.*, no KNO_3 solution) at 20–30 °C (treatment 7). Seeds were kept in BODs (Eletrolab, model EL 202/4) under light (8-h photoperiod) and were evaluated on a daily-basis. Substrate was wetted whenever necessary, until germination stabilized. Seedlings were counted on a daily-basis until the count stabilized. The criterion adopted to consider seeds germinated was when the whole seedling (shoot and primary root) had developed.

Statistical analysis

The germination percentage and germination speed index (GSI) were calculated and a cumulative germination curve was constructed for each treatment. The experimental design was completely randomized with four replicates. Data on germination percentage and GSI at different temperatures were subjected to analysis of variance and means were compared by Tukey's test at 5 % probability (Zar 1984). GSI was calculated using the following equation (Souza & Varela 1989):

$$GSI=\frac{N_1}{D_1}+\frac{N_2}{D_2}+...+\frac{N_n}{D_n}$$

where N_1, N_2 and N_n are the number of germinated seeds on the first, second and nth day of counting; and D_1, D_2 and D_n are the first, second and nth day of counting.

Results

Seeds subjected to treatment 1 (15–35 °C with KNO_3) started to germinate on the fifth day of experiment, and thereafter germination rate increased, reaching 98.5 %

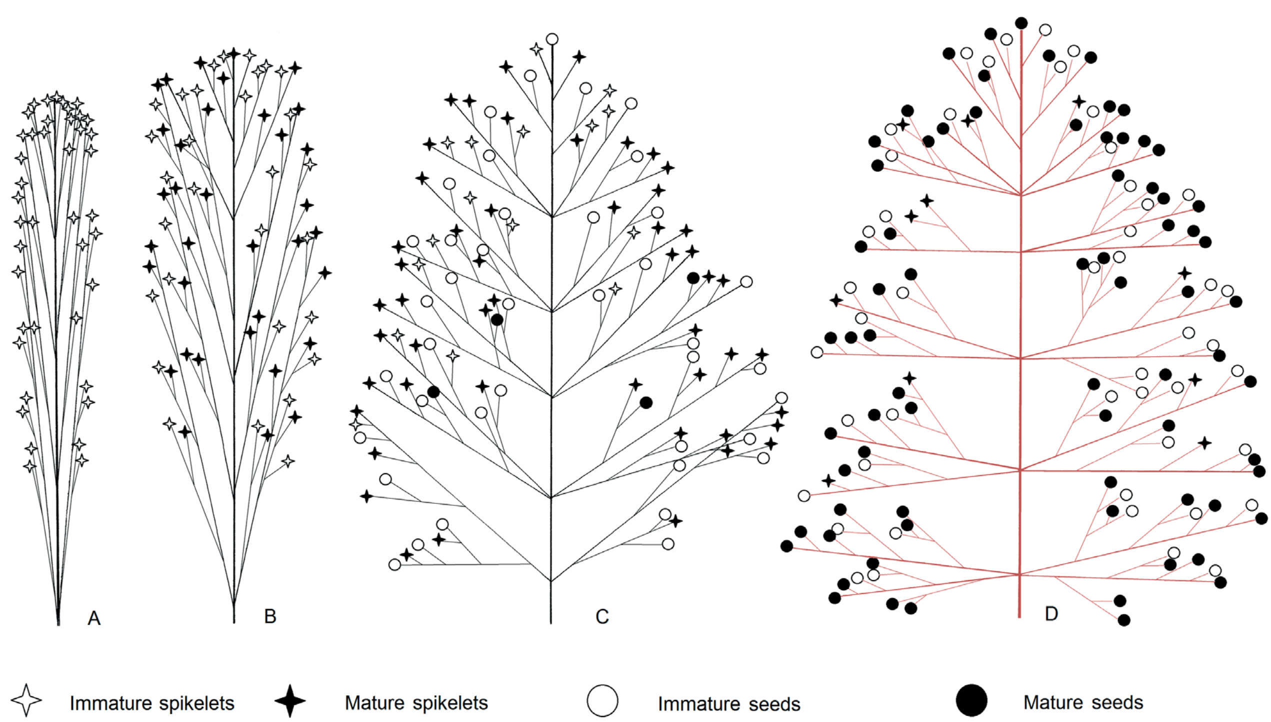

Figure 1. Schematic model of panicle development in *Eragrostis polytricha*.

on the fifteenth day of evaluation and stabilizing until the eighteenth day; seeds from this treatment showed a GSI of 6.6. Seeds from treatment 2 (20–30 °C with KNO_3) germinated from the eighth day, with germination percentage reaching 93 % on the sixteenth day and then stabilizing until the eighteenth day of evaluation; seeds from this treatment showed a GSI of 4.62. Despite differing significantly ($P < 0.05$) in their GSI, seeds from these two treatments showed no significant difference in germination percentage (Figs. 2, 3).

Treatments with constant temperatures yielded low seed germination percentages. Germination at treatment 3 (35 °C with KNO_3) started on the fourth day, increasing thereafter until reaching a maximum 33 % on the fourteenth day and then stabilizing up until the eighteenth day of experiment; seeds from this treatment showed a GSI of 2.39. Seeds subjected to treatment 4 (25 °C with KNO_3) started to germinate on the seventh day, after which the germination rate increased and reached 33 % on the fourteenth day, stabilizing until the eighteenth day of experiment; seeds from this treatment showed a GSI of 0.78. At treatment 5 (15 °C with KNO_3), no seed germinated (0 %). Thus, data on treatment 5 was not used in the means test since the germination rate of all its replicates was null. Treatments 3 and 4 (constant temperatures) differed significantly ($P < 0.05$) in both germination percentage and GSI (Figs. 2, 3). It is worth noting that treatments with alternating temperatures (1 and 2) also differed significantly ($P < 0.05$) in those same parameters in comparison with treatments with constant temperatures (3, 4 and 5) (Figs. 2, 3).

Treatment 7 (20–30 °C with water) differed significantly ($P < 0.05$) from treatment 2 (20–30 °C with KNO_3) in both germination percentage and GSI (Figs. 2, 3). These findings suggest that KNO_3 may contribute to breaking dormancy of the species seeds, since in the comparison between treatments with the same temperature range we found significantly higher values of germination percentage and GSI (93.5 % and 4.62, respectively) at the treatment with KNO_3 than at the treatment with only water (46.5 % and 2.44, respectively) (Figs. 2, 3). Since treatment 1 (15–35 °C with KNO_3) also yielded high germination percentage and GSI (95.5% and 6.6, respectively), dormancy break in *E. polytricha* seeds is probably related to the joint action of KNO_3 and alternating temperatures.

Treatment 6 (20–30 °C with KNO_3 using spikelets) yielded a high germination percentage (80 %) and a GSI of 4.4. These values did not differ significantly ($P < 0.05$) from those obtained at treatment 2 (20–30 °C with KNO_3), of 93.5 % germination percentage and 4.62 GSI. These findings indicate that spikelets of *E. polytricha* may be used directly to obtain seedlings, thereby eliminating the need to separate individual caryopses (Figs. 2, 3).

Discussion

The use of KNO_3 in association with alternating temperatures yielded the highest germination rates for *E. polytricha* seeds, conversely to the use of KNO_3 in association with constant temperatures, which in turn showed significantly lower germinability. Treatments using spikelets and those using isolated caryopses did not differ significantly when subjected to the same temperatures and same KNO_3 concentrations, thus indicating that using spikelets to obtain germinated seeds does not interfere with germination success. The developmental stages of *E. polytricha* seeds are shown in Figure 4.

Seed dormancy in *E. polytricha*, which had already been reported by Ramos *et al.* (2016) and Ramos *et al.* (2017), probably represents a strategy that ensures germination on the rainy season and consequently increases the chances of seedling establishment in the Brazilian savannas. This

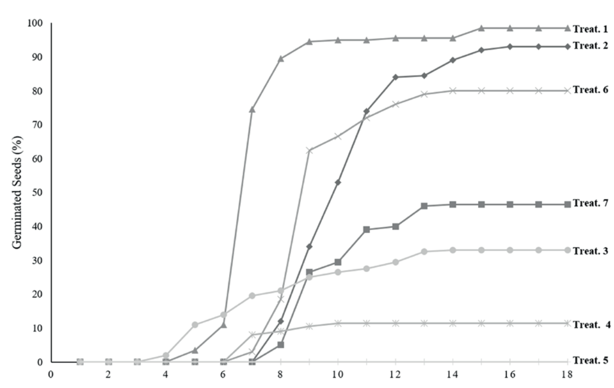

Figure 2. Cumulative germination percentages of *Eragrostis polytricha* seeds subjected to different treatments. Treatment 1: 15–35 °C with KNO_3; treatment 2: 20–30 °C with KNO_3; treatment 3: 35 °C with KNO_3; treatment 4: 25 °C with KNO_3; treatment 5: 15 °C with KNO_3; treatment 6: 20–30 °C with KNO_3 using spikelets; treatment 7: 20–30 °C with water.

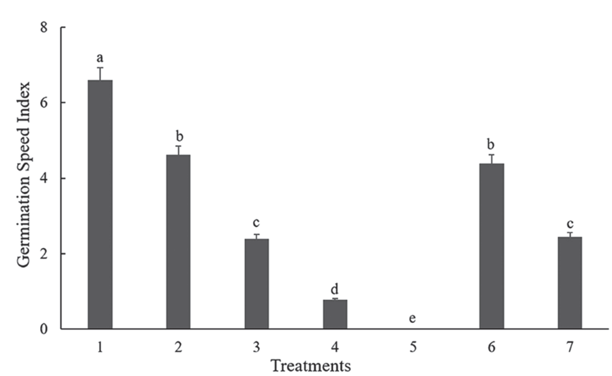

Figure 3. Germination speed index (GSI) of *Eragrostis polytricha* seeds subjected to different treatments. Means followed by the same letter do not differ by Tukey's test at 5 % probability. Treatment 1: 15–35 °C with KNO_3; treatment 2: 20–30 °C with KNO_3; treatment 3: 35 °C with KNO_3; treatment 4: 25 °C with KNO_3; treatment 5: 15 °C with KNO_3; treatment 6: 20–30 °C with KNO_3 using spikelets; treatment 7: 20–30 °C with water.

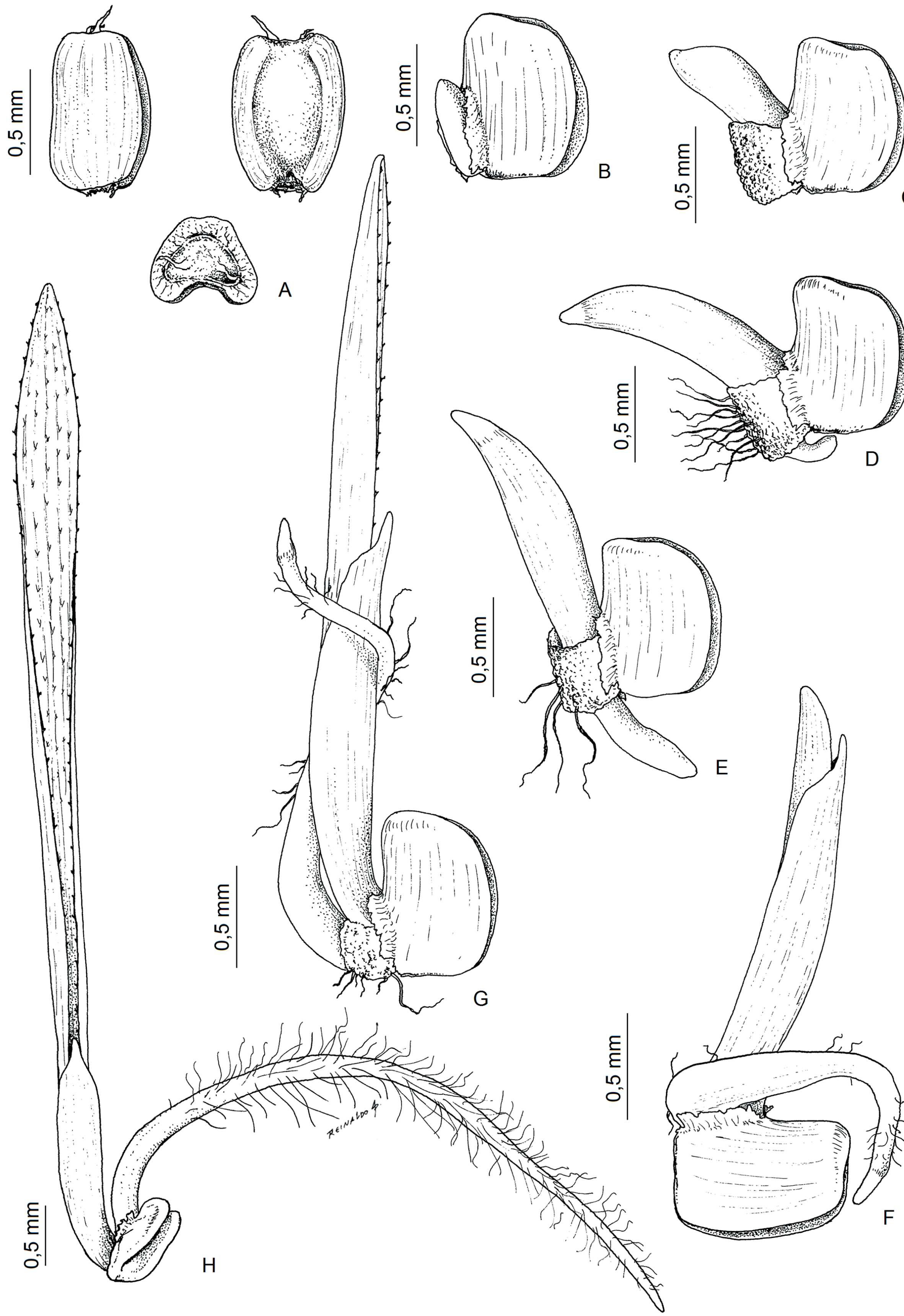

Figure 4. Developmental stages of *Eragrostis polytricha* seeds. (**A**) Seed in three views. (**B**–**H**) Germination until development of the first leaf.

strategy might well be the same one adopted by species from Rocky Outcrops, since these ecosystems are also characterized by the presence of a dry season, high solar radiation incidence and low water retention capacity (Benites *et al.* 2007; Le Stradic *et al.* 2015; Silveira *et al.* 2016). Seed dormancy has also been reported to other Rocky Outcrop species (Mendes-Rodrigues *et al.* 2010; Albrecht & Penagos 2012).

Treatments of alternating temperatures (15–35 ºC and 20–30 ºC) associated with KNO_3 yielded higher germination rates than constant-temperature treatments. Other studies on *Eragrostis* species have reported similar results, with alternating temperatures promoting seed germination (Carmona 1998; Chauhan 2013; Bittencourt *et al.* 2016; Bittencourt *et al.* 2017). In general, seeds of native Brazilian species have been reported to show high germination success at the temperature range of 20 to 35 ºC (Ferreira & Borghetti 2004; Brancalion *et al.* 2010). According to Holdsworth *et al.* (2008), the temperature oscillation that occurs between day and night is associated with the regulation of enzymes that are responsible for the synthesis and degradation of abscisic acid and gibberellins. Accordingly, Penfield & Hall (2009) observed that an essential condition for germination to occur is the balance between those two hormones, which itself is probably affected by temperature oscillation.

Alternating temperatures along with KNO_3 probably act in breaking seed dormancy and accelerating germination. Seed germination studies conducted on other grass species using KNO_3 have also reported positive results, with increased germination rates being detected for *Triticum aestivum* Matus-Cádiz & Hucl 2003), *Sorghum hapelense* (Baličević *et al.* 2016), *Buchloë dactyloides* (Kreuser *et al.* 2016), *Brachiaria brizantha* (Batista *et al.* 2016), and *Brachiaria humidicola* (Libório *et al.* 2017). According to Roberts (1972) and Ellis *et al.* (1983), the effect of potassium nitrate as a dormancy-breaking agent may derive from its oxidative and electron-acceptor features, which contribute to stimulating the pentose phosphate pathway, which in turn is probably associated with the neutralization or decrease of seed dormancy (Roberts 1972; Ellis *et al.* 1983). Sarath *et al.* (2006), in a study on *Panicum virgatum*, found that dormancy break and acceleration of germination are both promoted by imbibition with NO. Similar results were found with *Arabidopsis* and *Hordeum vulgare* (Bethke *et al.* 2004; Libourel *et al.* 2005), suggesting that NO acts as an endogenous regulator of those processes. KNO_3 probably has a similar function, and further studies may help better clarify its mechanism of action on seed germination in different species, including *E. polytricha*.

The percentage of germination (80 %) obtained with seeds inside spikelets (treatment 6) shows that bracts (glumes, lemma and palea) do not represent a barrier against germination in *E. polytricha*. Studies on other grass species have demonstrated that the presence of bracts in spikelets can decrease seed germination (Andersen 1953; Gallart *et al.* 2008; Ma *et al.* 2010a; Silva *et al.* 2016). The reason for this is not that bracts block or even decrease water absorption by seeds, but rather that they impose a mechanical barrier against germination, which thus leads to a lower rate of germinated seeds in comparison with the rate obtained with naked seeds (Ma *et al.* 2010b). Accordingly, the germination percentage we obtained with seeds inside spikelets was lower than those obtained at treatments 1 and 2, in both of which naked seeds were used. Still, germination rate of seeds inside spikelets was high nonetheless, which is a major result since separating caryopses from spikelets is quite a laborious procedure. Should large-scale seedling production be considered in the future, the use of spikelets instead of naked caryopses to obtain germinated seeds may represent an interesting alternative.

Eragrostis polytricha is a pioneer species with high seed yield and fast seedling development (pers. obs.), all of which are features that suggest a potential for its use in recovery programs at mining areas. Additionally, the fact that the species is native to Brazil further supports that potential, since according to Andrade *et al.* (2002) native species are indicated to that use as they have higher chances of restoring a degraded area to a condition more similar to the original one, due to the higher affinity that these species have with the soil, local weather and other species in the region. Our findings may subsidize further studies focusing on identifying the best substrate conditions for seed germination and seedling development of the analyzed species. Those steps are essential to reaching a high level of success in revegetation programs using *E. polytricha*.

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