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Seed tolerance to environmental stressors in two species of *Xyris* from Brazilian *campo rupestre*: Effects of heat shock and desiccation

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ABSTRACT

Campo rupestre is a fire-prone ecosystem with marked seasonality. In this work, we evaluated the effects of two recurrent stressors in *campo rupestre* on seed germination of two typical perennial herbs – *Xyris asperula* and *X. trachyphylla*. Heat shock at different temperatures and exposure times were used to simulate fire passage, and desiccation of germinating (imbibed) seeds was used to simulate drought. The aim of this study was to evaluate the tolerance (based on germination) of seeds subjected to these stressors. Seeds of both species showed a significant tolerance to heat shock. Exposure at 100 °C for any amount of exposure time does not affect seed germination. Decrease in germinability (seed death) became more evident only at 180 °C. Water uptake by seeds was fast, and slow drying for 48 h does not affect the germination of seeds imbibed for 24, 48, 72 or 96 h. Similarly, slow or fast drying for up to eight days did not cause changes in seed germination. Our results demonstrated that seeds of *X. asperula* and *X. trachyphylla* are tolerant to high temperatures caused by fire passage and to drought. These characteristics are important for these species to maintain reproductive success in the harsh environment of *campo rupestre*.

1. Introduction

Campo rupestre comprises megadiverse grasslands distributed among rocky outcrops (usually at altitudes exceeding 900 m), predominantly of the Espinhaço Mountain Range in Brazil (Silveira et al., 2016). This ecosystem is currently included in the Cerrado domain (Brazilian savanna) although it has several unique characteristics, including high species endemism (Alves and Kolbek, 1994; Alves et al., 2014; Silveira et al., 2016). *Campo rupestre* is fire-prone environment with a marked seasonality – a rainy and a dry period, which can last for up to five months during the autumn/winter seasons (Alves et al., 2014).

High temperatures caused by passage of fire, and the occurrence of drought, are recurrent stressors to plants in *campo rupestre* (Silveira et al., 2016; Morales et al., 2015). According to Crawford (1989), stress is considered "any environmental factor which restricts growth and reproduction of an organism or population". Specifically, for seeds, high temperatures caused by fire passage and drought could negatively affect germination and survival in the soil, thereby hindering regeneration from seed for some species of plants (Keeley and Fotheringham, 2000; Kranner et al., 2010; Daws et al., 2007). In fire-prone environments, however, it is expected that seeds have mechanisms to tolerate the high temperatures caused by the passage of fire (Bell and Williams, 1998; Ribeiro et al., 2013; Ribeiro and Borghetti, 2014). In general, seeds subjected to high temperatures respond in three ways: (1) death (non-tolerant seeds); (2) germination at the same percentage (tolerant seeds) or (3) increased germination – including cases of dormancy break (tolerant seeds with increased germination) (Luna et al., 2007; Jaureguiberry and Díaz, 2015). These responses, however, vary according to the temperature and exposure time (Fichino et al., 2016; Ribeiro et al., 2013). Regarding plant species of *campo rupestre*, there have been few studies evaluating seed tolerance of high temperatures (Le Stradic et al., 2015).

Due to the seasonality found in *campo rupestre*, seeds in the soil seed bank are subjected to changes in water availability. Thus, seeds could imbibe and dry periodically, following fluctuations in soil moisture. The ability of a seed to dry until it reaches equilibrium with the air and then recover its function after rehydration is called desiccation tolerance (DT) (Hoekstra et al., 2001; Alpert, 2005). Seeds with DT usually lose this ability during the germina-

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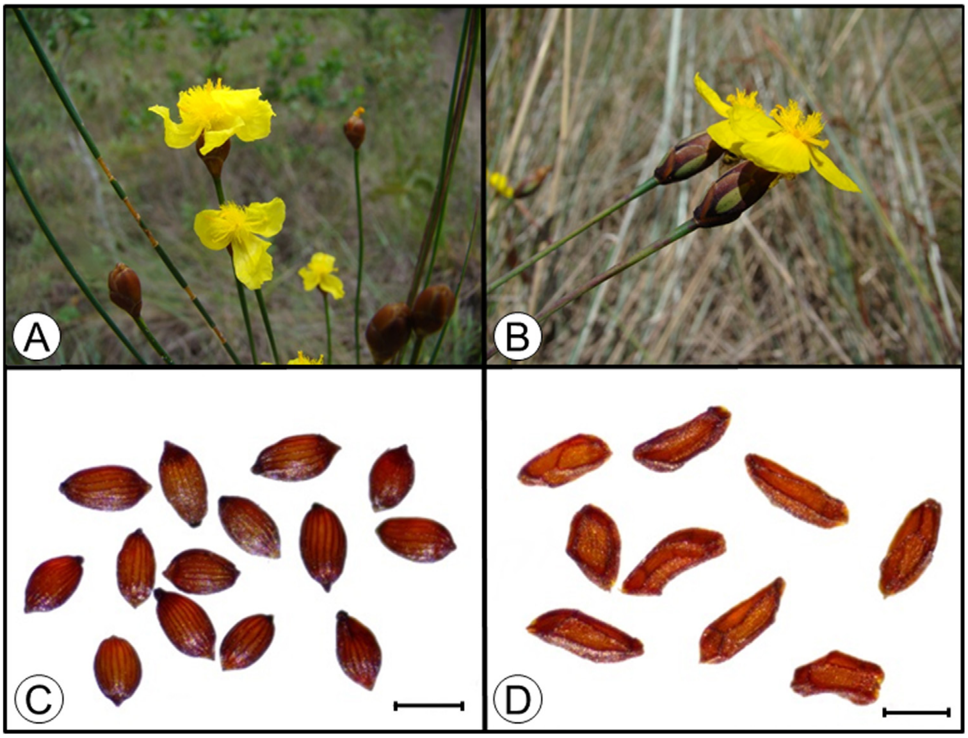

Fig. 1. Detail of *Xyris asperula* flowers (A) and seeds (C). Detail of *Xyris trachyphylla* flowers (B) and seeds (D). Scale bar, 200 μm. Photos: Nara Mota (A and B), Fábio Vieira (C and D).

tion (imbibition) process, implying that at a certain time, the drying of an imbibed seed is lethal (Hong and Ellis, 1992; Reisdorph and Koster, 1999). Thus, short dry spells within the rainy season, or an occasional rain event during the dry season, could cause death to imbibed or germinating seeds by desiccation (Blain and Kellman, 1991; Engelbrecht et al., 2006). The loss of DT in germinating seeds is a source of seed mortality in seasonal environments, although it has been poorly studied (Daws et al., 2007).

In this work, we studied the seeds of two typical, and widely distributed, species of *Xyris* of *campo rupestre* – *Xyris asperula* Mart. and *X. trachyphylla* Mart. These species produce small seeds (Abreu and Garcia, 2005), which are dispersed during the dry season. We subjected these seeds to recurrent stressors of their natural habitat: (1) heat shock at various temperatures and for various exposure times; and (2) drying of seeds imbibed for different lengths of time. The effects of these stressors were evaluated on germination percentage (for all experiments) and mean germination time (only for heat shock treatments). We aimed to assess how tolerant the seeds of these species are to these stressors, and to discuss the results from an ecological perspective.

2. Material and methods

2.1. Seed collection and germination tests

Mature fruits of *Xyris asperula* and *X. trachyphylla* (Fig. 1) were collected from at least 20 individuals between June and August 2015, in *campo rupestre* of Serra do Cipó, Minas Gerais, Brazil. The seeds were removed manually from the fruits, and stored in amber bottle for one month until the experiments. For all germination tests, were used six replicates of 25 seeds for each treatment. Seeds were placed in Petri dishes containing a double-layer of filter paper moistened with nystatin solution (0.5%). Germination tests were performed in germination chambers with a 12h-photoperiod (~40 μmol m^{-2} s^{-1}) and at optimum temperature for each species, 20 °C for *X. trachyphylla* (Abreu and Garcia, 2005) and 30 °C for *X. asperula* (unpublished data). Germination was evaluated daily for 30 days in order to calculate germination percentage (%G). For the seeds submitted to heat shock treatments was also calculated the mean germination time (MGT). The emergence of the vegetative axis was used as a criterion for germination (Abreu and Garcia, 2005).

2.2. Heat shock treatments

Dry heat shock treatments were performed to simulate the high temperatures caused by fire passage. The seeds (four replicates of 25 seeds) were placed into a Petri dishes and exposed to temperatures of 100 °C, 120 °C, 150 °C and 180 °C for 1, 2 and 5 min in a forced circulating air-drying oven. The experiment was conducted in a factorial design with 4 (temperatures) and 3 (exposure times). Seeds not subjected to heat shock were the control treatment. After exposure to the treatments, the seeds were placed to germinate, as described in 2.1.

2.3. Desiccation tolerance of imbibed seeds

The imbibition curve was determined from seed water content. Non-imbibed seeds and seeds imbibed at 24, 48, 72 and 96 h were weighed before (fresh mass) and after (dry mass) drying in an oven at 105 ± 2 °C for 24 h (Brasil, 2009) in order to calculate seed water content during imbibition. Seeds (four replicates of approximately 120 seeds) were placed in Petri dishes with double layer of filter paper moistened with deionized water and imbibed in a germination chamber at 25 °C with continuous light (~40 μmol m^{-2} s^{-1}). After completing each imbibition period, seeds were transferred to dry filter paper to remove surface water, before determining mass.

After imbibition, seeds (only non-germinated) were transferred to Petri dishes with dry filter paper and kept in a germination chamber under continuous light at 25 °C for 48 h for slow dry-

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