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Dry heat exposure increases hydrogen peroxide levels and breaks physiological seed coat-imposed dormancy in *Mesembryanthemum crystallinum* (Aizoaceae) seeds

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ABSTRACT

Background and aims: Seeds from many Aizoaceae species are characterized by their ability to survive extremely high temperatures. In addition, physiological seed dormancy has been described for some members of the family. In this study, we investigated whether we could identify Aizoaceae species with physiological seed dormancy that can be broken by heat exposure. Furthermore, we explored the morphological and biochemical mechanisms through which heat may break this dormancy.
Methods: For 24 Aizoaceae species, germination percentages were compared following exposure to heat treatment (103 °C for 17 h) and control conditions (18 °C and 15% RH). Seeds from *Mesembryanthemum crystallinum* were also exposed to ultra-drying treatment (3.93 and 2.51% MC). Morphology of the outer seed coat was characterized by SEM, whereas permeability of the seeds was assessed through seed mass increases in water, as well as through analysis of dye infiltration using seed sectioning and light microscopy. Seed oil content was determined by TD-NMR, whilst H_2O_2 levels were quantified using a spectrophotometric DMAB-MBTH protocol.
Key results: Three Aizoaceae species showed an increase in total germination following dry heat treatment. The physiological seed coat-imposed dormancy of *Mesembryanthemum crystallinum* seeds was found to be broken completely by dry heat exposure, which imposes both high temperature and ultra-drying stress, but not by high temperature or ultra-drying alone. Although no structural changes of the outer seed surface were detected using SEM, imbibition of water increased after dry heat treatment, whilst the seed coats remained impermeable to high-molecular-weight compounds. Analysis of H_2O_2 levels indicated that exposure to dry heat increased levels of this reactive oxygen species in *M. crystallinum* seeds relative to control conditions.
Conclusions: Here we describe for the first time in a species of the Aizoaceae family that exposure of seeds to dry heat can break physiological seed coat-imposed dormancy completely. Although *M. crystallinum* seeds became more permeable to water following dry heat treatment, this was not the case for methylene blue dye, suggesting that dry heat does not remove a potential barrier to diffusion of high-molecular-weight compounds. However, the observed elevation in H_2O_2 levels following dry heat treatment, possibly enabled by enhanced oxygen uptake through increased seed permeability, indicates a potential mechanism by which physiological dormancy is released in *M. crystallinum* seeds.

1. Introduction

Although several studies have reported that heat exposure (80 °C or higher) can promote germination of seeds with permeable coats (Baskin and Baskin, 2014), the mechanisms by which heat can break physiological seed dormancy are relatively unknown. For example, various hypotheses have been tested regarding the observation that germination of *Grevillea linearifolia* seeds increased from 23% to 50% following

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exposure to 80 °C for 10 min (Briggs and Morris, 2008). Whilst removing seed coats could break physiological dormancy in this species completely, no evidence was found to suggest that heat exposure led to the breakdown of potential chemical germination inhibitors in the coat (Morris et al., 2000). In addition, multiple barriers to the diffusion of high-molecular-weight compounds in the seed coat were not removed by the imposed heat treatment and therefore did not explain the effect of heat on germination for this species (Briggs et al., 2005; Briggs and Morris, 2008). For example, leaching of high-molecular-weight compounds such as abscisic acid (ABA), which is an inhibitor of embryo elongation, may be important for overcoming coat-imposed dormancy (Steinbrecher and Leubner-Metzger, 2017).

For the current study we aimed to identify plant species with seeds characterized by physiological dormancy that can be broken completely by heat exposure in order to increase understanding of this phenomenon. To this end, we decided to test a selection of Aizoaceae species, as both physiological dormancy and extreme heat tolerance have been reported for this family. The majority of Aizoaceae species are native of the arid (summer-dry) Succulent Karoo region in southern Africa (Ihlenfeldt, 1994). Based on the scientific literature to date, more than 20 Aizoaceae species are known to have seeds that can survive extremely high temperature of 103 °C for 17 h (Daws et al., 2007). In addition, approximately 30 species are reported to have physiological seed dormancy, which is the only type of dormancy confirmed for Aizoaceae so far (Baskin and Baskin, 2014). Although Liede (1990a, b) suggested that species in the *Erepsia* genus of the Aizoaceae family have impermeable seed coats and therefore physical dormancy, no evidence was presented in the form of water uptake curves, leaving the possibility that the observed effectiveness of mechanical or chemical scarification and heat treatment in promoting germination was due to their breaking of physiological seed coat-imposed dormancy. In *Erepsia*, exposure to extreme heat of 300–550 °C for 5–15 sec increased germination to approximately 3.4–12.5% depending on the species tested (Liede, 1990b).

The focus of our study was on the > 20 Aizoaceae species known to survive heat treatment of > 100 °C for 17 h (Daws et al., 2007). At least one of the species in this set (*Mesembryanthemum crystallinum*) is also listed as having physiological dormancy, whilst the seed dormancy status of the other species is unknown (Baskin and Baskin, 2014). Dormancy of *M. crystallinum* seeds has been confirmed by various authors (Esler and Cowling, 1995; Adams et al., 1998; Fukuhara et al., 1999; Fukuhara and Bohnert, 2000) and seeds from different locations in the seed capsules are known to have varying degrees of dormancy, with darker coloured seeds at the base of the capsules being most dormant and therefore late germinating (Adams et al., 1998; Fukuhara et al., 1999; Fukuhara and Bohnert, 2000). Experimental results seem to indicate that the dormancy is indeed of a physiological nature. For example, late germinating seeds were found to take up between 10–12% of water in 12 h, a finding that rules out physical dormancy (Fukuhara et al., 1999).

Some hypotheses have been put forward regarding the type of physiological seed dormancy that is characteristic of *M. crystallinum* seeds. Fukuhara et al. (1999) suggest that the early to late range in germination times of *M. crystallinum* seeds could be due to a maternal effect on seed germination through the seed coat. This hypothesis seems to be supported by data from the Seed Information Database, which reports that partial removal of *M. crystallinum* seed coats prior to germination led to high final germination levels (Royal Botanic Gardens Kew, 2017). Together, these observations seem to fit the definition of Finch-Savage and Leubner-Metzger (2006), who describe physiological seed coat dormancy as being characterized by a block conferred by the covering layers.

Given the possible presence of physiological seed (coat) dormancy in Aizoaceae species and the tolerance of many Aizoaceae species to extremely high temperatures, we hypothesized that physiological seed (coat) dormancy in Aizoaceae species may be broken completely by heat. In addition, since heat exposure can lead to further water loss of dry (15% equilibrium relative humidity) seeds, we hypothesized that one of the ways in which heat may break seed dormancy is through ultra-drying. For example, storage of seeds under ultra-dry conditions (1.5% MC) for 38 years was found to break dormancy in the Brassicaceae species *Alyssum scutigerum* (Pérez-García et al., 2007). Finally, we hypothesized that seed structural (e.g. changes in permeability) and biochemical mechanisms (such as levels of reactive oxygen species) may be involved in the breaking of dormancy by dry heat exposure (Egerton-Warburton, 1998; Keeley and Fotheringham, 1998; El-Maarouf-Bouteau and Bailly, 2008). In order to test our hypotheses, we first documented the germination responses of heat-exposed seeds relative to untreated controls from > 20 Aizoaceae species known to survive 103 °C for 17 h.

2. Materials and methods

2.1. Identification of Aizoaceae species for which dry heat promotes seed germination

Survival of Aizoaceae species following seed exposure to dry heat treatment was re-tested according to previously published methods (Daws et al., 2007). This was done in order to identify the level of seed survival relative to control conditions that is particular to each species, which was not presented by Daws et al. (2007). Briefly, seedlots stored in RBG Kew's Millennium Seed Bank (-20 °C and 15% RH) from the same 24 Aizoaceae species used by Daws et al. (2007) were acclimatized to dry room conditions (18 °C and 15% RH). Following this, dry heat-exposed (103 °C for 17 h in open dishes) and control (18 °C and 15% RH) seeds were sown on 1% water agar and placed at 20 °C for germination testing. Each group (heat-exposed or control) consisted of four replicates of 25 seeds per replicate. Total germination percentage (number of germinated seeds relative to the total number sown) per group was determined after 19 days and the data were analysed statistically using t-tests in Excel 2013 (two-tailed distribution, two-sample equal variance). In addition, viability of the *Prenia tetragona* seedlot was checked by tetrazolium staining (International Seed Testing Association (ISTA, 2003). 74 dry seeds were placed over water for 1 day at 20 °C prior to being sown on 1% agar for 3 days at the same temperature. Subsequently, the seeds were scarified and exposed for 2 days to 1% buffered 2,3,5-triphenyl tetrazolium chloride at 30 °C in the dark (International Seed Testing Association (ISTA, 2003). An assessment of seed viability was performed after rinsing the seeds with water and sectioning them longitudinally. Seeds that had germinated or that contained fully-stained, bright-red embryos were deemed viable, whereas seeds containing embryos that did not stain, or that stained only partially, were considered non-viable. Seeds without embryo (empty) or infested with fungal growth were also noted.

2.2. Assessment of water uptake by M. crystallinum seeds

The remainder of the experiments were performed on seeds from *M. crystallinum* seedlot 0071336 (Table 1), which consisted of dark-coloured, highly dormant seeds. In order to determine whether dry heat

Table 1
Details of the main *M. crystallinum* collection used for this study.

Species	*M. crystallinum*
Family	Aizoaceae
Seed coat colour	Dark
MSB seedlot	0071336
Country of origin	USA
Collection location	33°40′N 117°′W
MSB storage conditions	− 20 °C and 15% RH
Germination temperature	20 °C