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Short communication

Seed storage and germination in *Kumara plicatilis*, a tree aloe endemic to mountain fynbos in the Boland, south-western Cape, South Africa

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ABSTRACT

Seed storage under appropriate conditions is a relatively inexpensive means of safeguarding plant genetic material for *ex situ* conservation. Post-storage germination trials are used to determine the viability of stored seeds, and hence the efficacy of the particular storage treatment. *Kumara plicatilis* (=*Aloe plicatilis*) is a tree aloe endemic to mountain fynbos in the Boland, south-western Cape. The viability and germination behaviour of *K. plicatilis* seeds were assessed for seeds stored for four and nine months at −80 °C, 4 °C, 25 °C and under ambient conditions in a laboratory. Seeds were germinated under controlled conditions and germination rates and percentages determined. Ungerminated seeds were tested for viability using tetrazolium salt. Seed viability was not significantly reduced during storage. Seeds stored at −80 °C for four and nine months exhibited the fastest germination rate overall (both 5.9 ± 0.3 weeks, mean ± S.E.), and slowest was for seeds stored under ambient conditions for four and nine months (both 7.8 ± 0.4 weeks). All seed lots showed similar percentage germination after four months of storage (78.0–90.4%). The highest percentage germination overall was for seeds stored at −80 °C for four months (90.4%) and the lowest was for seeds kept at 4 °C and −80 °C for nine months (39.2 and 39.6%, respectively). Respective percentage viability for ungerminated seeds in these two treatments was 82% and 87%, respectively, indicating the induction of secondary dormancy. Induced dormancy triggered by protracted cold temperatures may be an adaptation that enables seeds to survive prolonged extreme conditions that are unfavourable for germination. Further research on the long-term storage of aloe seeds would be beneficial for developing long-term seed storage and germination testing protocols for *ex situ* conservation.

1. Introduction

Seed storage is vital for the long-term preservation of germplasm, and the maintenance of genetic diversity for the conservation of threatened and commercially important plant species, which has become a global concern over recent decades (Bonner, 1990; Vertucci and Roos, 1990). During storage, all seeds undergo deterioration, the rate of which is dependent on storage temperature, seed moisture content and the species concerned (Tang et al., 1999). Hence, germination tests are important for determining the viability of a seed lot, the efficacy of a particular pre-sowing treatment (e.g. storage), or the number of seedlings that may be expected from sowing a certain number of seeds in the field or nursery (Czabator, 1962).

Seed germination may be immediate, but usually there is a delay, either as a result of quiescence, or dormancy, which may be morphological, physical, or physiological (Fenner and Thompson, 2006). Quiescent seeds will germinate as soon as conditions conducive to germination are present, while dormant seeds preclude germination when conditions are favourable, but the probability of seedling survival and growth is low (Fenner and Thompson, 2006; Finch-Savage and Leubner-Metzger, 2006). Hence, germination of dormant seeds occurs only when conditions for establishing a new plant generation are likely to be suitable (Fenner and Thompson, 2006; Finch-Savage and Leubner-Metzger, 2006). During dry storage, seeds of certain species undergo physiological changes in a process known as dry after-ripening, which is often reflected in a decline in the level of innate dormancy, and decreased specificity of germination requirements (Probert, 2000). Importantly, for species adapted to regions of seasonal drought and dry soils, physiological changes recorded during dry storage reflect a natural mechanism, which governs the timing of germination in the wild (Probert, 2000). Thus, studying a species' dormancy and germination characteristics following seed storage, may provide insight into its regeneration strategies *in situ*.

The Alooideae is the largest subfamily in the Asphodelaceae, a family of succulent-leaved, petaloid monocots, primarily found on the African continent (Smith and Van Wyk, 2009). The subfamily comprises the genus *Aloe* (±350 species), the newly established genera *Aloidendron* (five species) and *Aloeampelos* (six species) and the reinstated monotypic genus *Kumara* (Grace et al., 2013), collectively termed 'aloes' herein. With approximately 140 aloe taxa, South Africa has the highest aloe

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species richness in Africa (Klopper et al., 2009). Aloes flower primarily during winter and produce large quantities of small, double-winged, air-borne seeds (Smith and Van Wyk, 2009). Although there has been a recent increase in research on aloe seed biology and ecology (see Bairu et al., 2009; Mukonyi et al., 2011; Symes, 2012; Arena et al., 2013; Cousins et al., 2013; Kulkarni et al., 2013), knowledge on the subject, including dispersal, germination, seed bank dynamics, and especially dormancy, longevity and storage, is still limited (Cousins and Witkowski, 2012). Hence, in an effort to build on our currently narrow understanding of aloe seed biology, this study investigates the effects of various seed storage treatments on the viability and germination behaviour of the seeds of *Kumara plicatilis*, a charismatic tree aloe endemic to mountain fynbos in the Boland, south-western Cape.

2. Methods

2.1. Study species and area

K. plicatilis (L.) G.D. Rowley (Asphodelaceae: Alooideae), recently segregated from *Aloe* s.s. (Daru et al., 2013; Grace et al., 2013; Rowley, 2013), is one of six tree aloes indigenous to South Africa, and the only one that occurs in the Cape fynbos (Van Wyk and Smith, 2008). *K. plicatilis* has a restricted distribution in mountainous parts of the Boland in the south-western Cape. It occurs from the Groot Winterhoek Mountains near Tulbagh in the north to the Franschhoek Mountains in the south. From east to west it occurs in the Du Toit's Kloof Mountains near Rawsonville/Worcester to the Paardeberg between Malmesbury and Wellington. *K. plicatilis* grows in well-drained, acidic soils on steep rocky slopes and outcrops in winter rainfall areas (400–2000 mm/year) (Cousins et al., 2014). The species is relatively long-lived and slow-growing, reaching a maximum height of 5 m, with dichotomously branching stems, each ending in a set of 12–16 strap-shaped succulent leaves. (Fig. 1) (Van Wyk and Smith, 2008). Its flowering season is from August to early November, with dispersal of the small, double-winged, wind-dispersed seeds commencing in December/January (Van Wyk and Smith, 2008).

Fig. 1. A *Kumara plicatilis* individual in habitat. Photographer: Stephen Cousins.

2.2. Seed collection and storage

Fresh seeds were collected from 2 to 3 inflorescences on 40 *K. plicatilis* individuals at a population near Rawsonville/Worcester in December 2010. The seeds were initially kept under ambient conditions in a laboratory in brown paper bags for three months. All the seeds were sorted by hand to separate intact from empty seeds. Only the intact and therefore potentially viable seeds were used for the experiment. Initial germinability of the seeds was determined using 300 seeds, the results of which are presented in Cousins et al. (2013). Two-thousand-four-hundred seeds from the original batch were then separated into eight sets of 300. The seeds were placed into 20 ml centrifuge vials, 20 seeds/vial, giving a total of 15 vials for each set. The vials were placed in sealed brown paper bags to maintain dark conditions for the duration of the storage period. The eight seed sets were spilt into two groups of four: one group to be stored for four months (4-month stored seed henceforth) and the other for nine months (9-month stored seed). A set from each of the two groups of four was then stored using the following four temperature treatments: (a) in a freezer at −80 °C, (b) in a cold room at 4 °C, (c) in an incubator at 25 °C and (d) under ambient conditions (typical December to February (summer) temperatures and humidity) in a laboratory at the University of the Witwatersrand, Johannesburg.

2.3. Seed water content calculation

Seed water content was calculated on a fresh mass basis pre-storage using 100 fresh seeds, and again after four and nine months of storage using 50 seeds/treatment. The samples of 50 seeds were taken from each set of 300 seeds, leaving 250 seeds/treatment for the germination trials. Seeds were weighed on a *Precisa 92SM-202A* scale correct to 0.0001 g, then oven-dried at 80 °C for 24 h and reweighed to determine their water content.

2.4. Germination trials

Seeds were germinated in a phytotron chamber with a 12 hour day/night cycle and light provided by fluorescent bulbs at ~650 nm. Temperatures were set at 25 °C during the day and 15 °C at night and relative humidity at a constant 50%. For each treatment, the 250 seeds were divided equally among ten 90 mm Petri dishes (25 seeds/dish), with two layers of sterilized filter paper under them and one on top. They were supplied with sterile distilled water until the filter paper was saturated. The germination trials were conducted for 18 weeks for all four treatments and for both storage durations. Monitoring for germination took place once a week, and watering twice a week. Germinated seeds were removed from the dishes and planted in seedling trays to be grown for *ex situ* conservation. Seeds that had not germinated by the end of the trials were tested for viability using standard tetrazolium tests, with a 1% solution of 2,3,5-Triphenyl-tetrazolium chloride salt under ambient, dark conditions for 24 h (Savonen, 1999).

The peak value (PV) and germination value (GV) were calculated according to the methods described in Czabator (1962). To obtain the PV, which is an index of seed vigour, the *T* value (percentage germination) / (number of weeks) is calculated for each week, and the highest *T* value is equal to the PV. The GV is then calculated as (PV) × (mean weekly germination), and gives a composite index of percentage germination and germination rate (germination index). Mean weeks to germination was calculated as another indicator of seed vigour.