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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

S.R. Cousins *, E.T.F. Witkowski, D.J. Mycock

School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, Wits 2050, 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

* Corresponding author at: P.O. Box 425, Glenvista, Johannesburg 2058, South Africa. Tel.: +27 735021425 (mobile).
E-mail address: steevyc@gmail.com (S.R. Cousins).

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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.

2.5. Statistical analyses

A one-way ANOVA tested for the difference between the water content of stored seeds and initial water content, while two-way ANOVAs tested for an interaction between storage duration and temperature treatments for (a) seed water content and (b) mean weeks to germination. Chi-square contingency tables were used for 4-month and 9-month stored seeds to test for associations for (a) percentage germination and (b) post-storage viability (germinated plus ungerminated viable seeds).

3. Results

3.1. Seed water content

There were no differences between the water content of stored seeds and the initial water content of 6.91 ± 0.18% (mean ± S.E.) except for 9-month stored seeds kept at 25 °C, which were significantly lower (4.63 ± 0.60%) ($F_{4,\ 294} = 4.51$, $p = 0.0014$) (Table 1). There was no interaction between storage temperature and duration for seed water content ($F_{3,\ 392} = 0.537$; $p = 0.657$), however, the water content of 9-month stored seed at 25 °C was significantly lower than that of 4-month stored seed at 4 °C ($p = 0.015$) (Table 1).

3.2. Germination of stored seeds

Seeds stored at −80 °C for four and nine months exhibited the fastest germination rate overall (both 5.9 ± 0.3 weeks), while the slowest was for seeds stored under ambient conditions for four and nine months (both 7.8 ± 0.4 weeks).

For percentage germination, there was an association between length of seed storage (4-months versus 9-months) and storage treatment, with decreased germination for the −80 °C and 4 °C treatments after 9 months compared with those stored for only 4 months ($\chi^2_3 = 60.56$; $p < 0.001$). Percentage germination was comparable across the four treatments for 4-month stored seed (78.0–90.4%) (Fig. 2a). Within the four-month stored seed lot, seeds kept at 4 °C had the lowest GV due to a slow germination rate (Table 2, Fig. 2a). Those kept at ambient and 25 °C had similar GVs and shared the same PV, while the −80 °C seeds had the highest GV, since they had the highest germination rate and percentage germination of all the 4-month stored seeds (Table 2, Fig. 2a).

The GV and PV of the 9-month stored seeds at ambient and 25 °C were substantially higher than those at 4 °C and −80 °C owing to a greater germination rate and percentage (Table 2, Fig. 2b). The highest GV and PV overall were for 9-month stored seeds under ambient conditions (97 and 19.6 respectively) (Table 2). Percentage germination of 4-month stored seeds at −80 °C was the highest overall (90.4%) — in sharp contrast to seeds stored at −80 °C for nine months. This treatment shared the lowest overall percentage germination (39%) with 9-month stored seeds at 4 °C (Fig. 2).

Despite the low percentage germination of 9-month stored seeds kept at 4 °C and −80 °C, 82% and 87% of the ungerminated seeds respectively, were still viable as demonstrated by tetrazolium salt staining (Fig. 3). Overall, there was no difference in post-storage total viability (as shown by germination and tetrazolium tests combined) of 4-month and 9-month stored seeds ($\chi^2_3 = 0.507$, $p = 0.917$), which ranged from 89% to 98% across the treatments (Fig. 3).

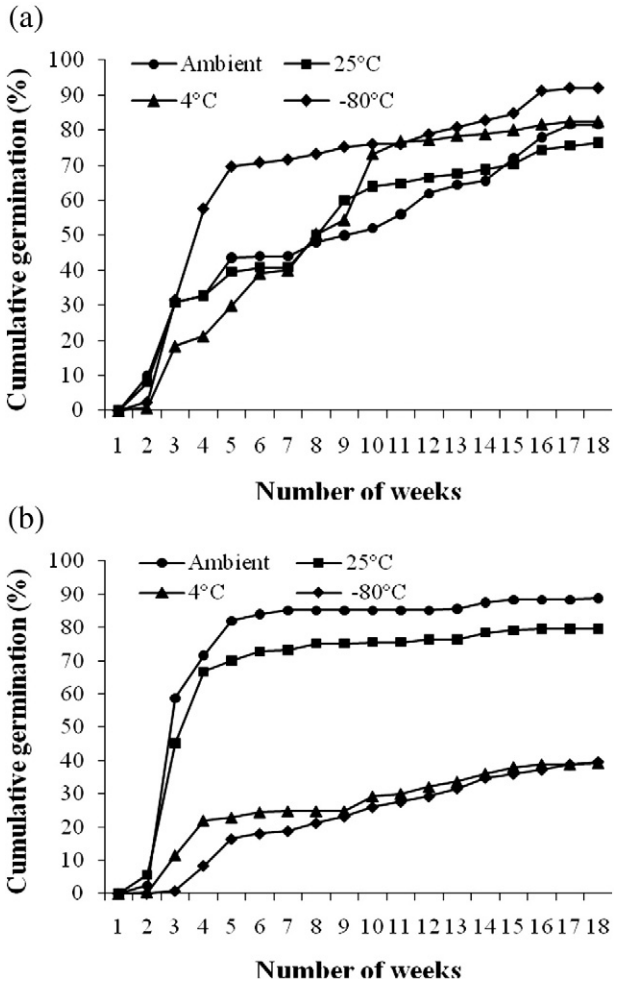

Fig. 2. Cumulative germination of *Kumara plicatilis* seeds stored at four different temperatures for (a) four months and (b) nine months.

4. Discussion

The most important factors that influence extended seed longevity during *ex situ* storage are seed moisture content and storage temperature (Hartmann et al., 1997). The low water content of *K. plicatilis* seeds (6.91%) indicates that they are orthodox seeds, and can therefore probably be stored for long periods at sub-zero temperatures (Bonner, 1990). The initial germinability of the seed lot as determined by Cousins et al. (2013) indicated that an after-ripening period is required before *K. plicatilis* seeds will germinate, since after six weeks of

Table 1
Water content of *Kumara plicatilis* seeds stored at four different temperatures for four and nine months.

Treatment	Mean ± S.E. water content (%)	
	Four months	Nine months
−80 °C	6.09 ± 0.73	6.18 ± 0.50
4 °C	7.17 ± 0.31	5.92 ± 0.40
25 °C	5.36 ± 0.74	4.63 ± 0.60[a]
Ambient conditions	6.44 ± 0.20	5.77 ± 0.52

[a] Significantly lower than initial water content of 6.91 ± 0.18%.

Table 2
Peak value (PV), germination value (GV) (*sensu* Czabator, 1962), mean weeks to germination (MWTG) (mean ± S.E.) and percentage germination (PG) for *Kumara plicatilis* seeds stored under four different temperature treatments for four and nine months (n = 250 seeds/treatment).

Treatment	Four months			Nine months		
	PV	GV	MWTG	PV	GV	MWTG
Ambient	10.3	46.5	7.8 ± 0.4	19.6	96.7	7.8 ± 0.4
25 °C	10.3	43.6	6.8 ± 0.3	16.7	73.9	7.1 ± 0.3
4 °C	7.3	33.5	7.3 ± 0.2	5.5	12.0	7.4 ± 0.3
−80 °C	14.4	73.6	5.9 ± 0.3	3.3	7.2	5.9 ± 0.3

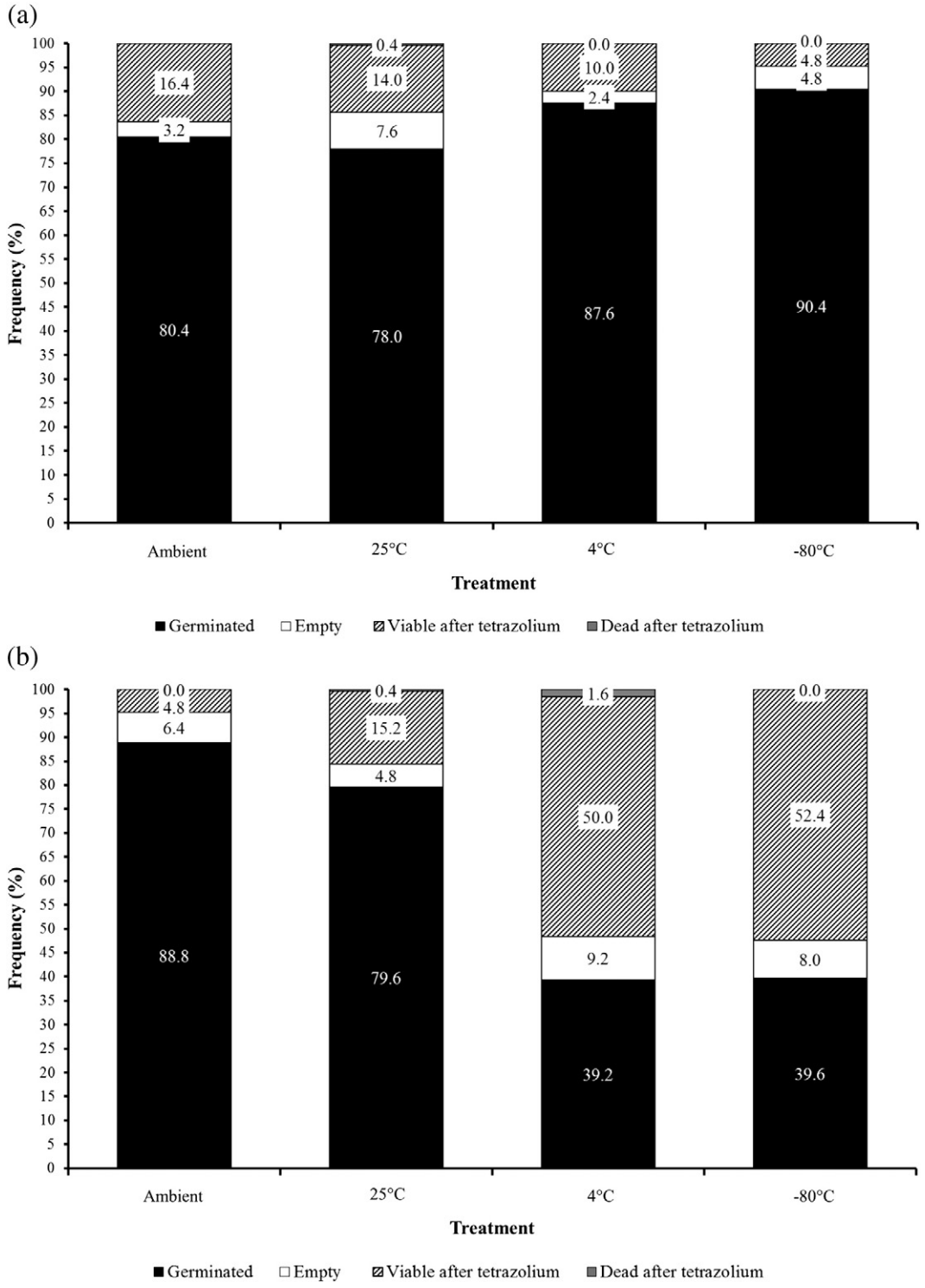

Fig. 3. Fate of *Kumara plicatilis* seeds after an 18-week germination trial following storage for (a) four months and (b) nine months using four different temperature treatments.

germination trials, only 28% of 3-month-old seeds stored under ambient conditions germinated, while ~80% of the remaining ungerminated seeds were still viable. By seven months of storage under ambient conditions (the initial three months, plus the four months reported here) 81.6% of seeds germinated. This after-ripening period (between three and seven months) likely corresponds with the months of summer drought that seeds in the wild must endure directly post-dispersal (December–April). The end of the after-ripening period therefore coincides with the onset of the winter rains, when conditions for germination and establishment are more favourable.

Short-term storage of *K. plicatilis* seeds at all four temperature treatments appeared to have very little impact on seed vigour and viability, as germination rate and total germination were high for all four, and almost all the ungerminated seeds were viable. A striking result was that seeds stored at the coldest temperature (−80 °C) for four months showed the highest germination rate and percentage overall, but those stored at the same temperature for a further five months displayed the exact opposite trend. Storage under cold conditions (both 4 °C and −80 °C) for nine months had similar effects on germination rate and percentage, and appeared to induce dormancy in the majority of seeds

(~60%). It is most likely that the *K. plicatilis* seeds entered physiological dormancy, whereby an inhibiting mechanism of the embryos prevented radicle emergence (Baskin and Baskin, 1998). While it is widely known that chilling can be used to break dormancy, particularly among species adapted for spring germination, there is considerable evidence that low temperatures can also result in the induction of dormancy (Probert, 2000). Furthermore, within a single seed population, genetic differences in the depth of dormancy between individual seeds can result in differential effects of chilling (Probert, 2000). The germination of a small proportion of *K. plicatilis* seeds after extended cold storage may have been a result of such genetic differences. Extended cold storage of *A. plicatilis* seeds may have acted as an environmental stressor, and triggered a response to a reduction in the probability of the arrival of conditions favourable to germination and establishment. Since reduced temperature invariably lengthens the storage life of seeds (Hartmann et al., 1997), by entering dormancy, the aloe seeds would extend their longevity, and thereby improve their chances of survival after release from cold storage.

Kulkarni et al. (2013) showed that treatments of 10-month-old *Aloe arborescens* seeds with smoke water (1:500 v/v) and smoke-isolated KAR1 (10^{-8} and 10^{-9} M) significantly increased percentage germination in comparison with the untreated control seeds when incubated under a 16 h photoperiod at 25 °C. Bairu et al. (2009) obtained similar results for *Aloe ferox* using smoke-water-treated seeds germinated under the same conditions. Although the effect of smoke water treatment on the germination of *K. plicatilis* seeds was not tested, the high percentage germination (76–92%) in its absence suggests that smoke is unlikely to act as a germination cue in the species. This hypothesis ties in with a total absence of recruitment at a population 22 months post-fire reported by Cousins et al. (2013), suggesting that mass post-fire recruitment (which is common in many other fynbos plant species) as a general rule in *K. plicatilis* is unlikely. A more probable recruitment strategy involves the germination and establishment of small numbers of seedlings over time in the absence of major disturbances as suggested by Cousins et al. (2014).

The high percentage germination achieved using seed stored under ambient conditions (80%) shows that *K. plicatilis* seeds germinate readily without any special treatment such as cold stratification, varying light conditions, pH modification and smoke water treatment. However, the higher germination rate and percentage after storage at −80 °C suggests that chilling enhances germination in the species. Further studies on long-term storage, germination cues and dormancy breaking mechanisms in *K. plicatilis* seeds, and those of other taxa in the Alooideae, would be beneficial for developing long-term seed storage protocols for the family Alooideae for *ex situ* conservation.

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References

Arena, G., Symes, C.T., Witkowski, E.T.F., 2013. The birds and the seeds: opportunistic avian nectarivores enhance reproduction in an endemic montane aloe. Plant Ecology 214, 35–47.
Bairu, M.W., Kulkarni, M.G., Street, R.A., Mulaudzi, R.B., Van Staden, J., 2009. Studies on seed germination, seedling growth, and in vitro shoot induction of *Aloe ferox* Mill., a commercially important species. HortScience 44, 751–756.
Baskin, C.C., Baskin, J.M., 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, California.
Bonner, F.T., 1990. Storage of seeds: potential and limitations for germplasm conservation. Forest Ecology and Management 35, 35–43.
Cousins, S.R., Witkowski, E.T.F., 2012. African aloe ecology: a review. Journal of Arid Environments 85, 1–17.
Cousins, S.R., Witkowski, E.T.F., Pfab, M.F., Riddles, R.E., Mycock, D.J., 2013. Reproductive ecology of *Aloe plicatilis*, a fynbos tree aloe endemic to the Cape Winelands, South Africa. South African Journal of Botany 87, 52–65.
Cousins, S.R., Witkowski, E.T.F., Pfab, M.F., 2014. Elucidating patterns in the population size structure and density of *Aloe plicatilis*, a tree aloe endemic to the Cape fynbos. South African Journal of Botany 90, 20–36.
Czabator, F.J., 1962. Germination value: an index combining speed and completeness of pine seed germination. Forest Science 8, 386–396.
Daru, B.H., Manning, J.C., Boatwright, J.S., Maurin, O., Maclean, N., Schaefer, H., Kuzmina, M., Van der Bank, M., 2013. Molecular and morphological analysis of subfamily *Alooideae* (*Asphodelaceae*) and the inclusion of *Chortolirion* in *Aloe*. Taxon 62, 62–76.
Fenner, M., Thompson, K., 2006. The Ecology of Seeds. Cambridge University Press, Cambridge.
Finch-Savage, W.E., Leubner-Metzger, G., 2006. Seed dormancy and the control of Germination. New Phytologist 171, 501–523.
Grace, O.M., Klopper, R.R., Smith, G.F., Crouch, N.R., Figueiredo, E., Rønsted, N., Van Wyk, A.E., 2013. A revised generic classification for *Aloe* (Xanthorrhoeaceae subfam. Asphodeloideae). Phytotaxa 76, 7–14.
Hartmann, H.T., Kester, D.E., Davies, F.T., Geneve, R.L., 1997. Plant Propagation: Principles and Practices. Prentice-Hall Inc., New Jersey.
Klopper, R.R., Retief, E., Smith, G.F., 2009. The genus *Aloe* L. (Asphodelaceae: Alooideae) in the Free State province of South Africa. Haseltonia 15, 41–52.
Kulkarni, M.G., Amoo, S.O., Kandari, L.S., Van Staden, J., 2013. Seed germination and phytochemical evaluation in seedlings of *Aloe arborescens* Mill. Plant Biosystems 148, 460–466.
Mukonyi, K.W., Oeba, V.O., Chiteva, R., Lelon, J., Gathara, M.W., 2011. Germination and growth performance of *Aloe turkanensis* and *Aloe secundiflora* under different substrates. Journal of Agricultural Research 6, 2132–2138.
Probert, R.J., 2000. The role of temperature in the regulation of seed dormancy and germination, In: Fenner, M. (Ed.), Seeds: The Ecology of Regeneration in Plant Communities, 2nd editionCABI Publishing, New York, pp. 261–292.
Rowley, G.D., 2013. Generic concepts in the *Alooideae* Part 3 — the phylogenetic story. Alsterworthia International 13, 24–26.
Savonen, E.-M., 1999. An improvement to the topographic tetrazolium testing of Scots pine (*Pinus sylvestris* L.) seeds. Seed Science and Technology 26, 49–57.
Smith, G.F., Van Wyk, B., 2009. Aloes in Southern Africa. Struik Nature, Cape Town.
Symes, C.T., 2012. Seed dispersal and seed banks in *Aloe marlothii* (Asphodelaceae). South African Journal of Botany 78, 276–280.
Tang, S., TeKrony, D.M., Egli, D.B., Cornelius, P.L., 1999. Survival characteristics of corn seed during storage: II. Rate of seed deterioration. Crop Science 39, 1400–1406.
Van Wyk, B.-E., Smith, G., 2008. Guide to the Aloes of South Africa. Briza Publications, Pretoria.
Vertucci, C.W., Roos, E.E., 1990. Theoretical basis of protocols for seed storage. Plant Physiology 94, 1019–1023.