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Original Research Article

Species-specific responses of “Critically Endangered” and “Least Concern” *Aloe* seed germination to environmental conditions in TanzaniaSiri A. Abihudi^{a, b}, Pavithravani B. Venkataramana^a, Hugo J. de Boer^{c, *},
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ABSTRACT

The majority of *Aloe* species are threatened by anthropogenic activities, trade, and the effects of climate change, but little is known on seed biology and appropriate conservation measures. Hence, understanding the germination behaviour of *Aloe* species will help in mitigating the negative effect of changing environmental conditions. “Critically Endangered” (*A. boscawenii* and *A. pembana*) and “Least Concern” (*A. lateritia*, *A. volkensii* and *A. secundiflora*) *Aloe* species’ seed germination was tested under various environmental parameters which are said to be crucial for *Aloe* species seed germination and included treatments of possible climate change scenarios. We varied temperature, light, scarification, KNO₃ addition and salinity and compared the response of the “Critically Endangered” versus the “Least Concern” *Aloe* species. “Least Concern” *Aloe* species were used as a control because they have adapted to a wide range of environmental conditions in contrast to “Critically Endangered” *Aloe* species, which often have a restricted range and specific environmental needs. All *Aloe* species germinated best at moderate temperatures (25 °C–30 °C) and low KNO₃ levels (0.01 mg/l). Dark conditions triggered higher germination percentages for all *Aloe* species except for *A. boscawenii*. Saline water suppressed the germination of all *Aloe* species compared to *Aloe* species grown in distilled water medium only. *Aloe* seeds grown in filter paper distilled water medium germinated better than *Aloe* seeds grown on a soil medium. The “Least concern” (IUCN Red List) *A. lateritia* germinated better than other species, followed by “Critically endangered” *A. pembana* and *A. secundiflora*. Generally, *Aloe* seed germination is nurtured by moderate temperatures and low concentrations of KNO₃. Hence, the effect of global warming will affect the survival of most *Aloe* species. The better germination performance under shade highlights the importance of parent plants, or at least a healthy canopy cover, in the *Aloe* species habitat. *Aloe* seeds showed species-specific responses to various environmental conditions (except for *A. pembana*), which reflects their Red List status.

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1. Introduction

Aloe species occur in the tropics, subtropics (Carter et al., 2011; Newton, 2004) and across a wide range of elevation and habitat types, including grassland, rocky areas, coastal sites and cliff faces worldwide (Arena et al., 2015; Carter et al., 2011; Carter, 1994; Newton, 2004). Biodiversity hotspots of the genus *Aloe* include the Arabic Peninsula, Madagascar, the African continent and the Indian Ocean islands (Grace, 2011; Grace et al., 2009; Newton, 2004). The genus is mostly known for its traditional and pharmaceutical uses for treating human and animal ailments related to the skin, urinary and respiratory tract, and nervous and female reproductive system (Bjorå et al., 2015; Grace et al., 2009; Wabuyele, 2006). Moreover, *Aloe marlothii* A.Berger nectar has been utilized by birds as food (Symes and Nicolson, 2008), many *Aloe* species have been planted as ornamental plants (Abihudi et al., 2019; Gadzirayi et al., 2005), and have been used in preventing soil erosion (Gadzirayi et al., 2005; King and Stanton, 2008).

Tanzania is a hotspot for biodiversity-rich ecosystems (Newmark, 2002; Tabor et al., 2010), in which the genus *Aloe* is represented by 46 different species, 25 of which are endemic to Tanzania (Bjorå et al., 2015; Carter, 1994; Newton, 2004). High biodiversity regions for *Aloe* species in Tanzania include the Katavi, Kilimanjaro, Rukwa and Tanga regions (Abihudi et al., 2019; Carter, 1994). There are about 500 species in the genus *Aloe* globally (Grace, 2011; Newton, 2004), and their seed biology and functional traits are yet poorly known. Many of the endemic *Aloe* species are also highly threatened by anthropogenic activities such as overexploitation for trade (CITES, 2003). Tanzania has signed up to the regulations of the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) (Cousins and Witkowski, 2012; Wabuyele et al., 2008) in the year 1979 (Majamba, 2000). Other threats include deliberately set fires, the extension of agricultural land and human settlements (Cousins and Witkowski, 2012; Pfab and Scholes, 2004). Furthermore, little is known about *Aloe* species cultivation and their recruitment success under various environmental pressures (Manvitha and Bidya, 2014). There is a need to test germination success of Tanzanian *Aloe* species experimentally. This will guarantee their conservation in the wild as well as enhance cultivation and trade activities. In the same way, the endangered *Paphiopedilum armeniacum* S.C.Chen & F.Y.Liu was successfully germinated and re-introduced at nature reserves for restoration purposes in China (Seaton et al., 2010).

Seeds are important for dispersal, persistence and the establishment of soil seed banks for the population to continue through different seasons and in hostile environments (Howe and Miriti, 2006; Howe and Smallwood, 2013). Rareness and endemism of most *Aloe* species are often associated with low seed establishment (Phama et al., 2014), poor seed quality (Arena et al., 2015; Cousins and Witkowski, 2012; Foden, 2002a; van Jaarsveld, 1989), retarded growth (Pfab and Scholes, 2004; Phama et al., 2014), low competitive ability (Imbert et al., 2012; Markham, 2014), lack of pollinator species (Botes et al., 2008; Cousins et al., 2013) and long seed dormancy (Cousins et al., 2014). The increase in temperature and shortage of rainfall as a result of climate change, modelled for four decades, has caused elevated mortality and population declines of *Aloe dichotoma* in the Namib Desert (Foden et al., 2007). These examples highlight that studying the seed ecology and germination behaviour of *Aloe* species will, therefore, help to determine favourable conditions for the survival and conservation of seeds that support maintenance and/or promotion of population growth. Literature shows that seed biology has been evaluated for only a few *Aloe* species, e.g., *Aloe greatheadii* var. *davyana* (Schönland) Glen & D.S.Hardy, *Aloe arborescens* Mill., *Aloe marlothii*, *Aloe ferox* Mill., and *Aloe plicatilis* (L.) Mill (Cousins et al., 2014; Smith et al., 2008; Symes, 2012). While for many endangered *Aloe* species, no experimental studies on seed germination have been conducted. Conservation of plant species through results of studies on their seed germination has been reported to be successful for various plant species, e.g., the restoration of "Critically Endangered" *Diospyros egrettarum* I.Richardson through mutualism relationship with frugivorous species, i.e., *Aldabrachelys gigantea* (Griffiths et al., 2011).

Leaves and fruits of some *Aloe* species are ingested and spread by wild animals and birds (Arena et al., 2013; Bani et al., 2016; Patil et al., 2017) while the majority of *Aloe* seeds are dispersed by wind (Cousins et al., 2014, 2013; Symes, 2012; Wilson et al., 2009). Feeding on fruits or seeds has been recognized to improve the viability of seeds compared to un-ingested seeds (Barnea et al., 2006; Kleyheeg et al., 2018). An improved germination percentage of scarified seeds, e.g., *Capsicum chacoense* Hunz. has been associated with frugivore species such as *Elaenia parvirostris* Pelzeln, 1868, which retain those seeds for some time in the gut (Fricke et al., 2013). While this is known for other species, scarification effect on *Aloe* species has not been studied. Moreover, the effect of climate change will have a negative effect on the dispersal of seeds as the result of reduced abundance of frugivores species (Mokany et al., 2014).

Being widely spread plant species, *Aloe* is associated with high adaptation to numerous environmental constraints (Brown, 1984). The "Least concern" *Aloe* species such as *Aloe lateritia* Engl., *Aloe volkensii* Engl. and *Aloe secundiflora* Engl. (according to the IUCN Red List) are widely distributed in Tanzania and Kenya (Carter, 1994). This suggests that they might be tolerant to a large variety of environmental factors. In contrast, some of the "Critically Endangered" *Aloe* species in Tanzania, such as *Aloe boscawenii* Christian and *Aloe pembana* L.E. Newton, are endemic and have a restricted distribution (according to the IUCN Red List) along the coast and on some islands of the Indian Ocean (Campbell-Barker, 2012; Carter, 1994; EAM and CFCPAP, 2009; Letsara et al., 2012). Coastal plants are associated with longer seed dormancy due to the high salinity from sea spray (Kahn and Durako, 2005; Willis and Chapman, 2006), low nutrients in soils (Willis and Chapman, 2006) and periodic rise in sea-level (Araujo and Pereira, 2007; Calvão et al., 2013), which can result in salt stress during seed germination and seedling growth of the *Aloe* species (Çavuşoğlu et al., 2016; Moghbeli, 2012). Fluctuating temperature can also affect the germination

of seeds and seedling establishment as has been tested in different studies (Kulkarni et al., 2006; Teketay, 1996). However, up to now, the varying environmental parameters have not been studied on any Tanzanian *Aloe* species.

Our study addresses the question whether the “Least Concern” *Aloe* species (*A. lateritia*, *A. volkensii* and *A. secundiflora*) (Carter, 1994) are linked to a more adaptive strategy against various environmental constraints as opposed to the “Critically Endangered” *Aloe* species (*A. boscawenii* and *A. pembana*), which have restricted distributions and are endemic to Tanzania (Carter, 1994; EAM and CFCPAP, 2009a, 2009b). We propose that *Aloe* seeds from “Least Concern” species will perform better across a wide range of environmental constraint treatments in comparison to “Critically Endangered” *Aloe* species, which are often endemic with a narrow home range. This is supported by Murray et al. (2002), who stated that the presence of ecological traits such as competitive ability and habitat tolerance is positively correlated with wide distribution of species. Laboratory experiments were used to test how the seeds respond to a range of temperature, nutrient conditions (potassium nitrate), mechanical scarification resulting from potential frugivores and granivores, saline water and how germination differs under controlled conditions compared to planting seeds directly into the soil.

2. Methodology

2.1. Study area and seed collection

A total of five Tanzanian *Aloe* species were used for this study. The *Aloe* seeds were collected from wild populations in Tanzania (Table 1). Since all collected *Aloe* species had been reported to flower at different times, usually immediately after the rainy season, based on local knowledge and our previous surveys (Abihudi et al., 2019), we stored seeds before the trials to allow the experiment to be conducted at the same time. Cousins et al. (2013) advocate the period of dry storage at room temperature to allow a higher germination percentage for *Aloe* seeds.

2.2. Experimental design

The seed germination experiments were conducted at the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratory in Arusha, Tanzania. The *Aloe* seeds were sorted by hand to obtain the intact seeds, in which the embryo was felt by the touch of the hand. This is a good indication that seeds were healthy and viable to be used for the germination experiment, according to Cousins et al. (2014). We evaluated the effects of light condition, scarification, temperature variation, growth medium and different concentrations of salt and potassium nitrate (KNO_3) on seed germination of the selected *Aloe* species (The details on different treatments are explained in Table 2). We washed the *Aloe* seeds (960 seeds per *Aloe* species) with 2% sodium hypochlorite for 15 min, then rinsed with distilled water medium three times for sterilization purposes (Arena et al., 2013). Each petri dish contained 20 seeds per *Aloe* species. Each treatment was replicated four times (Çavusoğlu et al., 2016), summing up to 80 seeds for each parameter. The *Aloe* seeds were placed in filter paper (except for the soil treatment) and moistened regularly with distilled water, KNO_3 treatment or saline water solution every two days. The experiments were conducted under normal daylight and night condition (except for dark treatment) and at a constant room temperature of 25 °C, which is the average temperature for Arusha when the experiment was conducted (except for moderate deviations). We recorded the germination of seeds daily, and we considered the seeds as germinated when the radicle length was equal or more than 2 mm. All experiments were terminated 21 days after planting, i.e., a broad margin after no further seeds germinated. None of the saline water treated seeds sprouted, and these were all washed three times and placed on new filter paper with distilled water for a subsequent 21 days to check if seeds germinated. Resulting seedlings for all treatments were planted in soil mixed with gravel to monitor their success and later were offered to the NM-AIST in Arusha, Tanzania.

2.3. Data analysis

The Final Germination Percentage (FGP = Final no. of seeds that germinated divided by the total number of seeds \times 100) (Kader, 2005) was calculated for all treatments. Data were tested for normality using Shapiro-Wilk & Kolmogorov-Smirnov test at a level of significance of $\alpha = 0.05$. We performed a two-factorial ANOVA to evaluate the main effect of each treatment (Temperature, light, scarification, potassium nitrate concentration, salinity, and soil medium) for the different *Aloe* species and their interaction effect on germination of *Aloe* species. *Aloe* species (20 seeds per petri dish) and treatments were assigned as

Table 1

Accession location, storage time (in months), and flowering months (according to Abihudi et al., 2019 and local knowledge) for the *Aloe* species that were germinated in this study.

IUCN Red List Status	Scientific name	District	Accession locations	Storage time	Flowering months
Critically Endangered	<i>A. pembana</i>	Pemba	5°21'S, 39°38'E	2	Nov – March
	<i>A. boscawenii</i>	Mkinga, Tanga	4°49'S, 39°6'E	5	
Least Concern	<i>A. lateritia</i>	Handeni, Tanga	5°25'S, 38°1'E	11	May – Aug
	<i>A. secundiflora</i>	Same, Kilimanjaro	4°15'S, 37°55'E	7	Nov – Feb
	<i>A. volkensii</i>	Serengeti, Mara	1°50'S, 34°40'E	14	

Table 2

Different treatments that were used in our experiment to mimic the environmental conditions and current or possible future climate change effects. n = 80 seeds per treatment, N = 960 per *Aloe* spp.

Environmental condition	Treatment	Settings	Representation
Constant temperature of seeds in treatments	25 °C	Low (control)	Average temperature of the regions where the species were found.
	30 °C	Moderate	Simulating the effect of global warming in Tanzania (Luhunga et al., 2018).
	35 °C	High	
Light treatment	Light	Alternating dark and light conditions (12 h) (Kulkarni et al., 2013).	Shade generated by older plants (Giddy, 1973).
	Dark	Light-proof box (Bairu et al., 2009; Kulkarni et al., 2013).	Seeds lying beneath the ground (Kazuhiko et al., 1997).
Scarification treatment	Scarified	Outer wing removed (except for <i>A. pembana</i> , Campbell-Barker, 2012), coat removed at distal end (Abrie and van Staden, 2001; Clemens et al., 1977) allowing water penetration into embryo (Copeland and McDonald, 1999; Orozco-Segovia et al., 2007).	Scratching the <i>Aloe</i> seed coats mimics the effect of granivores eating parts of the seed (Abihudi et al., 2019).
	Un-scarified	Control treatment	
Effect of KNO ₃	0.1 mg/l	Alkaline soil of rich organic matter and nutrients based on <i>Aloe</i> preference (Manvitha and Bidya, 2014).	Coastal soils contain excess salt, which decreases nutrient uptake through increasing osmosis (Kulkarni et al., 2013; Moghbeli, 2012).
	0.01 mg/l	Control treatment	
	0 mg/l		
Salt treatment	Salt plus water	Salt resembling seawater might reduce germination success (Jin et al., 2007; Moghbeli, 2012; Murillo-Amador et al., 2014)	Saline water representing coastal conditions to <i>Aloes</i> (EAM and CFCPAP, 2009a, 2009b) while distilled water stands for conditions for inland <i>Aloe</i> species
	Distilled water	Control condition	If sea spray gets washed off by rain
Soil treatment	Soil	Mixture of gravel and soil collected from the respective location at ratio 1/3 and 2/3, respectively	Gravel providing support for poorly established roots of <i>Aloe</i> seedlings (Hankey and Smith, 2006; Pascaline et al., 2010).
	Distilled water on filter paper	Control condition	

fixed factors while the response variable was the probability of Final Germination Percentage. Similarly, we compared Final Germination Percentage among *Aloe* species in all treatments by testing the differences using an ANOVA, followed by Tukey's post hoc tests (Hamasha and Hensen, 2009; Le Stradic et al., 2015). The significance level was set at $\alpha = 0.05$. OriginPro 2015 statistical package (Upadhyay and Mishra, 2015) was used for analysis. Student t-tests were used to compare differences in initiation of germination between seeds germinating in distilled water medium and seeds recovering after saline water treatment as well as to test the difference between seeds grown on soil medium and those on filter paper.

3. Results

There was a significant difference in the germination of *Aloe* seeds across the main effects of environmental factors and *Aloe* species (Table 3).

Further, species and temperature treatment interacted significantly, i.e., some *Aloe* species had higher germination rates at high, some at low temperatures (Table 3, Fig. 1). Most *Aloe* species germinated best at 30 °C, followed by 25 °C. *Aloe pembana* had high FGP in all the temperature treatments but highly preferred moderate and higher temperatures for germination. The FGP of *A. pembana* was 81%, which is more than twice as high as that for *A. lateritia* (39%), and almost five times higher than *A. secundiflora* (14%) at 30 °C. *Aloe lateritia* and *A. boscawenii* performed better at moderate temperatures of 25 °C and 30 °C, respectively. *Aloe secundiflora* generally had a low germination percentage but performed better at the higher and lower temperature regimes (with 20% and 29% of seeds, respectively) compared to the moderate temperature of 30 °C (14%). Unexpectedly, *A. volkensii* did not germinate at any temperature the seeds were exposed to. There was no significant difference in how *A. boscawenii*, *A. volkensii* and *A. lateritia* reacted at 35 °C. Although *A. pembana* germinated well in high temperature, it outperformed other *Aloe* species at low temperatures of 25 °C.

All *Aloe* seeds grown under constant-dark conditions for 24 h had a significantly higher FGP compared to the *Aloe* seeds grown under light conditions, except for *A. boscawenii*, which did not germinate at all (Fig. 1). In the dark treatments, the germination rate of 86% for both *A. secundiflora* and *A. lateritia* was about nearly twice as high as that of *A. pembana* (45%). There was no significant difference between *A. pembana*, *A. boscawenii* and *A. volkensii*, nor between *A. pembana* and *A. secundiflora*.

Table 3

Two-factorial ANOVA results to compare the main effect of different treatments, *Aloe* species, and their interaction effect on seedling emergence. N = 960 seeds per *Aloe* species, n = 80 seeds per treatment.

Fixed factors	Main effect		
	df	F	p
<i>Aloe</i> species	4	371.5	<0.0001
Temperature	2	58.5	<0.0001
Species x temperature	8	46.6	<0.0001
<i>Aloe</i> species	4	89.9	<0.0001
Light conditions	1	33.4	<0.0001
Species x light conditions	4	28.6	<0.0001
<i>Aloe</i> species	4	20.7	<0.0001
Scarification	1	15.4	<0.0001
Species x scarification	4	5.9	0.001
<i>Aloe</i> species	4	159.4	<0.0001
KNO ₃ concentration	2	34.3	<0.0001
Species x KNO ₃	8	18.5	<0.0001
<i>Aloe</i> species	4	50.2	<0.0001
Salinity	1	205.3	<0.0001
Species x salinity	4	5.6	0.002
<i>Aloe</i> species	4	22.4	<0.0001
Soil medium	1	318.7	<0.0001
Species x soil medium	4	11.7	<0.0001

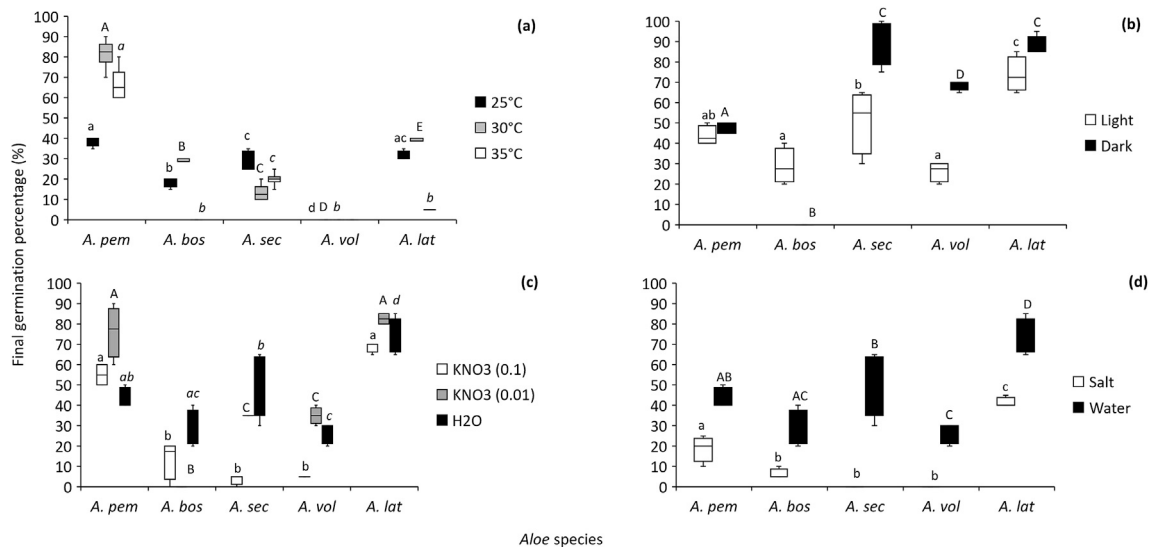


Fig. 1. Final Germination percentage of *Aloe* seeds grown at different (A) temperatures, (B) light condition, (C) potassium nitrate concentration and (D) salinity. Different letters (in capital, in small and in italics) indicate significant differences across germination percentage means of different species at specific treatment according to Turkey's post hoc tests ($p < 0.05$). Different colors indicate different treatment levels. The two *Critically Endangered* species are *A. pem* and *A. bos* while the species of *Least Concern* are *A. sec*, *A. vol* and *A. lat*. *A. pem* = *Aloe pembana*, *A. bos* = *Aloe boscawenii*, *A. sec* = *Aloe secundiflora*, *A. vol* = *Aloe volkensii*, and *A. lat* = *Aloe lateritia*.

There was a significant interaction between *Aloe* species and scarification treatment, with the majority of species performing better when not scarified. Un-scarified *Aloe* seeds had a slightly, but not significantly, higher germination percentage (44% for *A. pembana*, 16% for *A. boscawenii*, 20% for *A. volkensii* and 64% for *A. lateritia*) than scarified seeds (40%, 28%, 26% and 74%, respectively). Only *A. secundiflora* failed to germinate after scarification but germinated well (51%) when un-scarified. Further, the germination percentage of *Aloe* seeds differed significantly across KNO₃ concentrations (Fig. 1). High (0.1 mg/l) KNO₃ concentrations did not favour germination while low concentrations (0.01 mg/l) did for all *Aloe* species except for *A. boscawenii*. There was no significant difference between the control (water medium) and 0.01 mg/l KNO₃ (Fig. 1). Furthermore, there was no significant difference among the *Aloe* species that germinated well (*A. pembana* and *A. lateritia*) under high concentration of 0.1 mg/l KNO₃ and those that did not perform well (*A. boscawenii*, *A. secundiflora* and *A. lateritia*). The FGP of *A. pembana* (76%) was twice as high compared to *A. secundiflora* (35%) and *A. volkensii* (35%). For 0.01 mg/l KNO₃, *A. boscawenii*

differed significantly from other *Aloe* species while there was no significant difference between *A. pembana* and *A. lateritia* nor between *A. secundiflora* and *A. volkensii*.

Using saline water as a growth medium did not result in germination within the first three weeks. Nevertheless, when the saline water was added into distilled water medium, some seeds germinated except for *A. volkensii* and *A. secundiflora* (Fig. 1). The initiation of the germination process was faster (2.6 days) ($P = 0.003$, $t = 12.53$) for the seeds recovered from saline water compared to the seeds on distilled water medium only (5.6 days), with significant differences among *Aloe* species. *Aloe lateritia* seeds germinated most successfully (41%), followed by *A. pembana* (19%), and *A. boscawenii* (6%) after being recovered from saline water and remaining in distilled water for at least 21 days. Seeds grown only with distilled water medium outperformed those recovered from saline water for all *Aloe* species tested (Fig. 1).

There was a slight but not significant trend for most species to grow better without soil than on filter paper water medium, except for *A. volkensii* ($P = 0.001$, $t = 5.66$), which performed better with soil than in filter paper water medium. The FGP was 38% and 44% for *A. pembana*, 38% and 29% for *A. boscawenii*, 30% and 51% for *A. secundiflora*, 5% and 26% for *A. volkensii* and 60% and 74% for *A. lateritia* in the soil and filter paper water media treatment respectively. Tukey's post hoc tests indicates no significant difference among the "Critically Endangered" species (*A. pembana* and *A. boscawenii*) with the two "Least Concern" species (*A. secundiflora* and *A. lateritia*) although the latter had a significant difference across means.

4. Discussion

4.1. *Aloe* species and temperature

The optimum temperature for germination is 30 °C, followed by a lower temperature of 25 °C. *Aloe* species avoid cold conditions below 20 °C (Kulkarni et al., 2013; Liu et al., 2011) but some studies claimed that germination could also be low under high temperatures, i.e., from 30 °C upwards (Bairu et al., 2009; Kulkarni et al., 2013). In our study, most *Aloe* species germinated at moderate temperatures of 25 °C and 30 °C and germination success declined at a higher temperature (35 °C) as was supported by other researchers (Kulkarni et al., 2013). Hence, the rise in temperature might have a negative effect on the existence of many *Aloe* species that prefer moderate and low temperatures. Alternatively, a plant species can tolerate the rise in temperature if it is within its temperature range (Davis et al., 2005) or can shift towards an area within its temperature range as was observed for the tree-like *Aloe dichotoma* in the Namib desert (Foden, 2002; Foden et al., 2007). However, *Aloe* population declines and, consecutively, extinctions due to higher temperatures have also been observed (Foden et al., 2007). *Aloe pembana*, a species that tolerated both medium and high temperatures usually grew on rocky areas along the coast in our study. This exposes the species to heat during the day. On the other hand, *A. secundiflora* seeds have thrived both at high and low temperatures because this species tends to occur in both warm (Dodoma) and cold (Kilimanjaro) regions of Tanzania (Carter, 1994).

4.2. *Aloe* species and light

Further, we found that darkness stimulates germination (except for *A. boscawenii*) as opposed to light conditions. This might be due to the shade that parent plants cast on their fallen seeds as was seen for other plant species growing in tropical environments (Cousins and Witkowski, 2012; Kulkarni et al., 2006). In contrast, *A. boscawenii* has slender leaves facing up or hanging down on sea cliffs (direct observation), so growing in the shade did not result in an evolutionary advantage, and this was also seen in *A. arborescens* (Kulkarni et al., 2014). Our results are supported by a study on *A. ferox* (Bairu et al., 2009) and *A. greatheadii* var. *davyana* (Smith and Correia, 1992) that germinated well under dark conditions. Hence, diverse *Aloe* species will react differently when exposed to various light conditions.

4.3. *Aloe* species and scarification

Additionally, we found that *Aloe* seeds germinated well when un-scarified. The little positive effect of scarification we found on germination might indicate that most *Aloe* seeds are dispersed by wind and not by seed predators (Cousins et al., 2014; Symes, 2012; Wilson et al., 2009). Although animals and insects have been reported to be crucial in seed germination through scarification process, they have been reported to damage the *Aloe* species in our previous study (Abihudi et al., 2019) and by others (Bhaludra et al., 2013; Breebaart et al., 2002; Cousins et al., 2013; Wiseman, 2001). In contrast, wind-dispersed seeds will generally result in a higher survival rate of the seeds and *Aloe* species populations (Cousins et al., 2013). However, for the unwinged seeds, i.e., *A. pembana*, the dispersal and establishment of seedlings is likely close to the mature plants (Letsara et al., 2012; Stokes and Yeaton, 1995).

4.4. Growth medium for *Aloe* species

In our study, low KNO_3 concentrations (0.01 mg/l) favoured the germination of *Aloe* species as supported by other researchers (Kulkarni et al., 2013), indicating that *Aloe* seeds have adapted well to conditions of low soil fertility. Potassium nitrate has also been described to improve the concentration of flavonoids and phenolics compounds in *Aloe* species (Kulkarni et al., 2013) and to counteract the hostile effect of sodium chloride for the salt-tolerant plants (Akram et al., 2007;

Hasanuzzaman et al., 2018; Kaya et al., 2007). Therefore, the tolerance of coastal *Aloe* species such as *A. boscawenii* and *A. pembana* towards a high concentration of potassium nitrate (0.1 mg/l) can be explained by an adaptive mechanism for osmosis regulation in the cell membranes, which was also documented for other *Aloe* species (Cardarelli et al., 2013).

Our findings that germination percentage was increased after the seeds were recovered from saline water highlights that germination of coastal *Aloe* species occurs most likely after the rains. This happens when the salt is washed from the seeds, resulting in the recovery of the seed viability. As most of the coastal plants are associated with poor nutrients, wind and salt spray (Calvão et al., 2013), the saline conditions decrease the ability of *Aloe* to absorb important mineral elements (Cristiano et al., 2016). The negative effect of salt on germination for tested *Aloe* species has also been reported for *A. barbadensis* and *A. arborescens* (Cardarelli et al., 2013). The three *Aloe* species (*A. lateritia*, *A. pembana* and *A. boscawenii*) that germinated after being recovered from saline water indicate their salt tolerance. We expected the two coastal *Aloe* species (*A. pembana* and *A. boscawenii*) to germinate better under salt conditions but it was not expected that *A. lateritia* which is an inland species would germinate better under salt conditions. *Aloe lateritia* germination was twice as high as that for *A. pembana* and seven times higher than *A. boscawenii* under salinity conditions. .

Furthermore, the germination percentage and the initiation of sprouts were slightly higher on filter paper water medium on the soil as supported by other studies (Berthold, 2014). For cultivation of *Aloe* species in the future, we suggest germination of *Aloe* seeds on filter paper with distilled water medium and later a transfer of the seedlings into the soil medium to increase the number and pace of seeds germinating.

4.5. Species-specific responses

The *Aloe* species we tested in our study showed species-specific responses to different treatments, which reflected their current Red List status, except for *A. pembana*. Generally, *A. lateritia*, a species of “Least concern” (IUCN Red List) outperformed all other species in all experimental treatments, except at temperature conditions of 35 °C and 30 °C. This species was followed by the “Critically Endangered” *A. pembana* and the “Least Concern” *A. secundiflora*. Our results suggest that the rareness of *A. pembana* might not be related to germination as we had postulated before because it germinated better than the “Least Concern” *Aloe* species. Moreover, other factors, such as unwinged seeds can hinder its distribution to other areas (Campbell-Barker, 2012). Our results call for further studies on *A. pembana* distribution via herbivorous species on Pemba island, Tanzania.

The “Critically Endangered” species, *A. pembana*, exhibited a higher germination percentage in all treatments compared to *A. boscawenii*. The higher germination of *A. pembana* seeds suggests the greater adaptability of the species to various environmental conditions such as its ability to withstand different temperatures and KNO₃. In contrast, the low performance/germination of *A. boscawenii* across all treatments compared to *A. pembana* might be due to dormancy, as had also been reported for *A. verecunda* Pole-Evans (Craib, 2007). *Aloe pembana* has been documented but also observed in this study to produce side shoots on the upper section of its stem, thereby also promoting vegetative growth (Fig. 2; Cousins and Witkowski, 2012). This explains the better performance and persistence of *A. pembana* in the long run compared to *A. boscawenii*. Vegetative reproduction has been regarded as an outstanding approach in increasing the survival potential of a species (Alam and Ali, 2010). Nevertheless, *A. pembana* is still “Critically Endangered” as it is associated with a limited, restricted range (EAM and CFCPAP, 2009b). The *Aloe* species tested responded according to our predictions based on their IUCN Red List status (except for *A. pembana*), highlighting the importance of their protection in the future.

The species of “Least Concern”, *A. lateritia*, outperformed other species across all treatments in its germination success, followed by *A. secundiflora* and *A. volkensii*. This is supported by other researchers who reported the first two species being widely distributed across different geographical areas of various climatic conditions (Abihudi et al., 2019; Bjorå et al., 2015;



Fig. 2. *Aloe pembana* young vegetative shoot on the side of its upper stem at Pemba island, December 2018 (picture by Siri Abihudi).

Carter, 1994; Wabuyele et al., 2006). *Aloe volkensii* has a greater risk of becoming classified as a threatened species due to its narrow tolerance based on our tests on environmental parameters affecting germination.

5. Conclusion

Our study reveals that *Aloe* seeds germinate best under moderate temperature (25 °C–30 °C) and low potassium nitrate (0.01 mg/l). We found that an increase in temperature and potassium nitrate had a negative effect on most *Aloe* species seed germination. Saline water resulted in seed dormancy of all *Aloe* seeds tested, with viability being recovered when the *Aloe* seeds were washed with distilled water medium. Most *Aloe* seeds germinated well under dark conditions, highlighting the importance of parent plants or a healthy canopy cover in the germination habitat.

Our study on understanding the germination behaviour in response to the various environmental conditions can help in ex-situ conservation of *Aloe* species as the most suitable habitat, and growing conditions can be provided. This study is important for scenario-building models to predict future population trajectories under the current fluctuating environmental conditions. Our results further can support local conservation efforts and identify areas of threat to the current *Aloe* species populations. Currently, harvesting rates of *Aloe* species have been reported to be high (Grace, 2011) and in Tanzania, this harvesting is mainly conducted on *Aloe* populations in the wild (Abihudi et al., 2019). Our study provides information to local communities and various local stakeholders using the *Aloe* species on possible ex-situ conservation. This can be done through community farming of useful medicinal *Aloe* species by local people, which will, thus, reduce pressure onto the wild populations. For the *Aloe* species that had little FGP across all treatment i.e., *A. boscawenii*, other techniques such as propagation through cuttings and in vitro culture can be applied. Our study is further important for population development trajectories and can provide basic information for future species distribution models under a raising global temperature and more rainfall extremes in times of climate change.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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