South African Journal of Botany xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Seed viability, germination and seedling emergence of the critically endangered stem succulent, *Adenium swazicum*, in South Africa

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ARTICLE INFO

Article history:
Received 8 August 2016
Received in revised form 14 December 2016
Accepted 8 January 2017
Available online xxxx

Edited by C Seal

Keywords:
Apocynaceae
Ex situ conservation
Germination rate
Regeneration ecology
Seedling establishment
Shading
Summer impala lily
Temperature
Tetrazolium staining

ABSTRACT

The regeneration ecology of many southern African threatened plant species is poorly understood. Temperature is considered the most important environmental factor governing seed germination. The germination temperature requirements of the critically endangered stem succulent, *Adenium swazicum*, were determined under controlled conditions, together with tetrazolium staining protocol tests to assess seed viability. The effects of soil medium, watering levels, depth of sowing and shading on germination/seedling emergence were also determined at the Lowveld National Botanical Garden (LNBG). Germination/seedling emergence was compared under nursery conditions within (Skukuza Nursery in Kruger National Park) and outside (LNBG) the natural distribution range of *A. swazicum*. The germination of viable seeds ranged 96.5–100% for a broad range of temperatures from 20 to 35 °C as well as for 30/20 °C (light/dark) alternating temperature. No germination was recorded at 5 °C and 10 °C, and relatively limited germination at 15 °C (42.9%) and 40 °C (15.4%). These results were modelled using a Generalized non-linear model, with a binomial error and a logit link function, best-fit models were second-order polynomials (*i.e.*, quadratic). Mean Germination Time ranged from 2 to 6 days among temperature treatments. Tetrazolium together with germination results for the 20 − 35 °C temperatures showed moderate to high seed viability across populations and between years within the same population. Seedlings readily emerged irrespective of soil media (3 types) or planting depth (surface, 5 and 10 mm) but higher emergence occurred under more frequent watering. Seedlings emerged equally well under sun or shade conditions, but only subsequently survived in the shade. Despite lower temperatures recorded during October/November 2010 at LNBG (outside range: 27.2 ± 0.9 °C) compared to Skukuza (within range: 32.6 ± 0.9 °C), final germination/seedling emergence was high at both locations (LNBG: 82%; Skukuza: 94%). Together with field observations, this study shows that (a) seed germination is rapid and with high percentages after being cued by warm to hot summer temperatures, a period when rainfall is also at its highest, and (b) that seedling recruitment depends on the availability of suitably shaded microsites. This is the first study on the regeneration ecology of an *Adenium* sp. and will aid both *in situ* and *ex situ* conservation of *A. swazicum*.

1. Introduction

South Africa has the world's richest temperate flora (Germishuizen et al., 2006), with more than 20,450 indigenous vascular plant species, but of these, 4809 (24%) are of conservation concern. Despite various conservation efforts, loss of biodiversity continues on regional and global scales due to increasing intensity of disturbances such as overexploitation of species, habitat destruction, climate change, invasion by alien species and plant diseases (Botha et al., 2004; Knowles and Witkowski, 2000; Mouillot et al., 2013; Royal Botanic Gardens (RBG) Kew, 2016).

Plant conservation biology has largely been based on decades of research into plant population dynamics and distributions and the factors that affect them (Van Dyke, 2003; Heard and Ancheta, 2011). However, a species long-term persistence often depends on reproduction, seed dispersal within the same community, expansion into new habitats and survival through times unfavourable for growth (Vasquez-Yanes and Orozco-Segovia, 1993; Weiersbye and Witkowski, 2002; Cousins et al., 2013). Variation in population size relates to underlying vital rates such as germination, seedling growth, reproduction and death, which in turn are influenced by multiple environmental factors including fire, herbivory and weather (Buckley et al., 2010). Two basic alternatives that limit plant recruitment include the availability of viable seed and the availability of suitable microsites at which seedling establishment is possible (Eriksson and Ehrlèn, 1992; Lamont et al., 1993). Areas under tree canopies in savannas have reduced soil temperature and higher nutrient levels compared to adjacent open spaces, which improves the survival and growth of seedlings (Kos and Poschlod, 2007). In the succulent thicket biome, nearly all the endemic succulent species

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http://dx.doi.org/10.1016/j.sajb.2017.01.011
0254-6299/

were recorded under *Euclea* shrubs (Moolman and Cowling, 1994). It has been argued that seedling establishment under or close to the mother plant could be indicative of localized seed dispersal and seed trapping (seed trapped in branches of mother plants or surrounding vegetation) rather than nurse effects *per se* on the seedlings (Haussmann et al., 2010; Soliveres et al., 2012).

Temperature is considered the most important environmental factor governing the maximum germination percentage and germination rate, and germination is usually only possible within well-defined temperature limits (Garcia-Huidobro et al., 1982; Hardegree, 2006; Mattana et al., 2010). The optimum germination temperature for a species is characterised by maximum germination in the shortest time, while no germination will occur beyond maximum and minimum temperatures (Probert, 2000; Shen et al., 2010). Once the temperature requirement for germination is reached, some species have a fast germination response to rain (Fenner and Thompson, 2005), with species such as *Colophospermum mopane, Acacia tortilis, A. karroo* and *Combretum apiculatum* having 90–100% seed germination within 4–5 days at 30 °C (Stevens et al., 2014). However, the availability of water is another major factor limiting germination, early seedling establishment and growth (Wilson and Witkowski, 1998; Carvajal et al., 2014). A germinated seed is highly vulnerable to lack of moisture for growth, fire, herbivores, burial under litter, being washed away by rain, and heat on bare soil, and hence up to 90% of released seed will not make it past the seedling stage (Leck, 2008). Sodic or brackish areas add another stress to seedlings due to high salt content of the soil (Medinski et al., 2010), as well as being hard when dry and lack structure (hence poor aeration) when wet. Such extreme conditions cause some species to become specialized by forming corms, bulbs or tubers under the soil surface to avoid drought or heat (Holmgren et al., 2006).

Adenium swazicum (Apocynaceae) is a critically endangered southern African savanna stem succulent species largely found on sodic soils. Although *Adenium* originally comprised six species, Plaizer (1980) in his revision of the genus reduced it to five, namely *A. obesum* Roem & Schult, *A. multiflorum* Klotzsch, *A. boehmianum* Schinz, *A. oleifolium* Stapf and *A. swazicum* Stapf. With the exception of *A. obesum*, all species are restricted to small areas in southern Africa. All *Adenium* species occur in savanna or open forests on sandy or rocky soils, which are often brackish (Plaizer, 1980). *Adenium swazicum* is approximately 0.2–0.7 m tall with a carrot-like tuber, which can be up to 1 m in diameter. The leaves are narrowly oblong, rounded and mucronate at the apex. The inflorescence is approximately 1.5–3.5 × 1–2.5 cm in size and tinged with red or pink, flowering occurs between October and April (Plaizer, 1980). The fruit are follicles 15–20 cm in length, with two follicles produced per flower. Follicles mature from late September. Seeds have a woody outer layer which protects the soft embryo; individual seeds weigh approximately 0.002 g and are 9–13 mm in length with hairy tufts on both ends (KvdW, unpublished data). No research has been conducted on its seed dormancy and germination, or requirements for seedling establishment, and this is the case for all *Adenium* spp. In order to better manage both *in situ* and *ex situ* conservation of *A. swazicum,* the aim was to study its seed germination, and seedling establishment requirements. The objectives were to determine and compare (a) seed viability between *A. swazicum* populations, (b) germination temperature requirements, (c) the importance of soil medium, depth of planting, moisture stress and shading on seedling emergence and establishment, and (d) to compare germination response and seedling establishment under nursery conditions within and outside the natural distribution range of *A. swazicum.*

2. Methods

2.1. Study area and species

Adenium swazicum occurs from the Kruger National Park (KNP) and Timbavati Private Nature Reserve in the north, extending to Komatipoort/Malelane and Swaziland in the south and Mozambique in the east. The area has strong summer (>80%) rainfall (October to April), receiving an average of 620 mm *per annum* (Mucina and Rutherford, 2006). The mean maximum temperature recorded in the Lowveld National Botanical Garden (LNBG) in Nelspruit, Mpumalanga (approximately 60 km west of the closest known population of *A. swazicum*) and KNP (Skukuza indigenous nursery) during the experimental period (12th of October 2010 until 10th of November 2010) was 27.2 ± 0.9 °C and 32.6 ± 0.9 °C, while the mean minimum temperature was 15.0 °C ± 0.4 °C and 17.7 ± 0.6 °C, respectively (KNP Scientific Services; LNBG weather records; TuTiempo weather; World Weather Online).

2.2. Seed collection and storage

Seeds were collected from three representative populations (Populations A–C; Table 1) in the lowveld of Mpumalanga (Komatipoort/Malelane), based on land use (protected areas and private land) and size of population (minimum of 50 plants/population). Given that *A. swazicum* is a critically endangered species, large numbers of seeds could not be collected. Details for all seed collections during the experimental period (October 2009 to October 2010 are provided in Table 2. All seeds were collected at the point of natural dispersal by securing nylon stockings over developing follicles, securing both ends lightly with a cable tie to avoid seed dispersal by wind. All seeds were stored under ambient conditions in a cool, dry storeroom in brown paper bags at the LNBG prior to the trials.

2.3. Seed viability

Standard tetrazolium staining protocol was used to assess seed viability (AOSA/SCST, 2010). The initial tetrazolium (TZ) tests were conducted using 25 seeds from each of the three seed batches in order to test the method for *A. swazicum* seeds (Table 2). Seeds were prepared for the TZ test by placing them in 50 mm diameter Petri dishes, which were suspended over distilled water inside an airtight container for 24 h at 20 °C. After 24 h, the seeds were transferred to 90 mm diameter Petri dishes that contained a 1% water-agar solution and stored at 20 °C for an additional three days. The seeds were then placed in plastic vials containing a 1% 2, 3, 5-triphenyltetrazolium chloride solution and each vial was wrapped in heavy-duty aluminium foil to prevent reaction to light. The vials were incubated at 30 °C in an 8/16 h light/dark cycle for two days. The seeds were then washed with distilled water and evaluated immediately. Seed viability was based on tissue characteristics as described in Patil and Dadlani (2009); red and uniform staining of the embryo was taken as viable. Seed batches were kept separate throughout the experiment.

2.4. Seed germination

A permit was obtained from Mpumalanga Tourism and Parks Agency (MTPA) to export the seeds to the Millennium Seed Bank of the Royal Botanic Gardens, Kew, at Wakehurst Place, England for the germination trial, in June 2010. Seeds were not treated or rinsed before germination experiments. Seeds were placed in 90 mm Petri dishes (5–10 seeds/dish) containing a 1% water-agar solution within nine different environmental control incubators set at a 8/16 h light/dark cycle at 5 °C constant temperature intervals between 5 °C and 40 °C as well as one alternating temperature of 30/20 °C, also at 8/16 h light/dark cycle (the 8 h light period coincided with 30 °C) The light/dark cycle was applied by means of fluorescent lamps providing a photosynthetic photon flux density of 80 μmol m^{-2} s^{-1}. Due to the limited number of seeds available, especially from population A, the more extreme constant temperatures of 5 °C, 10 °C, 15 °C and 40 °C only used seeds from population C (2009) (Table 3). Furthermore, the seed batches were run in sequence on the incubator (population C before A), and hence it was decided to prioritise

Please cite this article as: Van der Walt, K., Witkowski, E.T.F., Seed viability, germination and seedling emergence of the critically endangered stem succulent, *Adenium swazicum*, in South Africa, South African Journal of Botany (2017), http://dx.doi.org/10.1016/j.sajb.2017.01.011