Temperature and light requirements for seed germination and seedling growth of two medicinal Hyacinthaceae species

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The effects of temperature and light on seed germination of two medicinal plant species Albuca pachychlamys and Drimia robusta were investigated. Temperatures of 25°C and alternating 25/20°C had a significant (P < 0.05) effect on seed germination of A. pachychlamys, resulting in 100% germination under 16:8h light/dark conditions. The highest percentage germination was recorded at a constant temperature of 20°C (87%) and alternating temperatures of 25/20°C (90%) for D. robusta. The seeds of A. pachychlamys exhibited significantly (P < 0.05) higher germination (100%) under constant dark conditions, compared to 87.5% germination under constant light. In D. robusta, the opposite effect was observed with 93% germination recorded under constant light and 80% in the dark. Different temperatures influenced the seedling survival and growth of both the species. Seedling survival of A. pachychlamys at 25°C was low (68%) but seedlings had significantly (P < 0.05) more roots as well as a higher total seedling and bulb mass compared to seedlings grown at other temperatures. More (82%) D. robusta seedlings survived at 25°C and their growth was significantly (P < 0.05) higher than at other temperatures examined.

Introduction

The sustainable use of traditional medicinal plants has become an important issue globally. In South Africa, where most of the rural people are dependent on indigenous medicinal plants for their primary health care needs, the demand for wild plants is increasing as the population grows. Many medicinal plants are being extracted from their natural habitats, threatening future supplies. It is believed that the demand for wild medicinal plants is already too large and meeting the demand by sustainable means would be extremely difficult (Van Staden 1999). It has been suggested that the only real solution would be to develop valuable medicinal plants as small-scale farming crops (Van Staden 1999). Although micropropagation protocols have been established for many indigenous medicinal species, these techniques are labour-intensive, making cultivation costly and therefore only feasible for high-value species (Ziv et al. 1995). Micropropagation is possible but seed propagation appears to be more promising and cost-effective for the mass production of seedlings (Zhou et al. 2003). Since most indigenous plant seeds have poor germination properties, it is difficult to commercialise medicinal plants for large-scale production (Okole and Odhav 2004). To improve the germination of indigenous medicinal plant seeds, a better understanding of the role of environmental factors, such as temperature and light sensitivity, is required.

Several indigenous members of the Hyacinthaceae are commonly used for medicinal purposes by traditional healers in southern Africa, including Albuca pachychlamys Bak. and Drimia robusta Bak. (Hutchings et al. 1996), which are sold at informal medicinal markets around South Africa (Cunningham 1993). Optimal conditions for seed germination and seedling growth of many of these species have not yet been determined.

Each species requires a range of temperatures for seed germination and seedling establishment (Bradbeer 1988). Since seedling development stages are sensitive to environmental changes (Shimono and Kudo 2003), seedlings may need to be established in the first growing season to survive in the following season (Maruta 1983, 1994). The objective of this study was, therefore, to determine the effect of temperature and light requirements on seed germination of A. pachychlamys and D. robusta, and secondly to examine the influence of temperature on seedling survival and growth.

Materials and Methods

Seeds of A. pachychlamys and D. robusta were collected between October and November of 2003 from the Botanical Garden, University of KwaZulu-Natal Pietermaritzburg, South Africa. The seeds were stored in brown paper bags at room temperature for a period of 30 days before being used for experimental trials. Seed mass was determined by weighing four replicates of 100 seeds. The moisture
content of seeds was measured by drying seeds at 110°C. The seeds were weighed repeatedly until a constant weight was reached. The moisture content was expressed as a percentage of the fresh weight.

In imbibition studies, the seeds were placed in 9cm disposable Petri dishes on two layers of filter paper (Whatman No. 1) moistened with 3.5ml distilled water and allowed to imbibe at room temperature (25 ± 0.5°C). At 2h intervals, for 12h, the seeds were blotted dry, weighed and returned to wet filter paper. The amount of water imbied by seeds is expressed as a percentage increase over the initial seed weight.

Seeds were surface-decontaminated by immersing them in 0.1% mercuric chloride for 2min and then rinsing in distilled water. Four replicates of 30 seeds each were germinated in disposable Petri dishes. The germination experiments were conducted under a 16:8h light/dark photoperiod with a photosynthetic photon flux density of 98 ± 5µmol m⁻² s⁻¹ provided by cool-white fluorescent lamps. Germination counts were made daily for 30 days. Germination was considered to have occurred when the radicle protruded 2mm. Mean germination time (MGT) was calculated by using the equation: MGT = \( \sum (n \times d) / N \) (Ellis and Roberts 1981). The optimum temperature for germination was calculated on the basis of constant temperature as: \( T_o = \sum \text{tp} / \sum \text{p} \) (Kochankov et al. 1998).

To determine the effects of different temperature regimes, the seeds were incubated at constant temperatures (10, 20, 25, 30, 35 and 40°C) and alternating temperatures (25/20°C and 30/15°C), under 16:8h light/dark. For continuous dark, the Petri dishes were placed in light-proof wooden boxes at 25 ± 0.5°C, and the seeds were inspected daily under green ‘safe light’ (0.3µmol m⁻² s⁻¹) conditions. In continuous light the seeds were exposed to a photosynthetic photon flux density of 98 ± 5µmol m⁻² s⁻¹.

To evaluate the effect of temperature on seedling growth, seven-day-old seedlings of A. pachychlamys and D. robusta were grown in growth chambers set at different temperatures (10, 15, 20, 25, 30 and 35°C) and with a 16:8h light/dark cycle and photosynthetic photon flux density of 101 ± 5µmol m⁻² s⁻¹. Seedlings were planted in pots (10cm) containing sterile quartz sand moistened with 100ml half-strength Hoagland’s solution (Hoagland and Snyder 1933). Each treatment consisted of 20 seedlings and the pots were arranged randomly in the growth chambers. The experiment was run for a period of 75 days, with 50ml half-strength Hoagland’s solution added weekly to each pot. At the end of the experiment the seedlings were harvested from the pots and the percentage survival, seedling and bulb mass, number of roots and leaf length recorded.

The germination data was arcsine-transformed before being statistically analysed using analysis of variance (ANOVA). All data were analysed using GenStat® Release 4.21 ( Rothamsted Experimental Station, United Kingdom). The least significant difference (LSD) at the 5% level was used to test differences between germination means and means of growth parameters of seedlings of different treatments.

Results

The weight of 100 seeds of A. pachychlamys and D. robusta was approximately 376mg and 110mg, with moisture contents of 55.3% and 50% respectively. The water uptake over 12h in both the species is shown in Figure 1.

Temperature had a pronounced influence on seed germination of A. pachychlamys, resulting in 100% germination at constant (25°C) and alternating temperatures (25/20°C) under 16:8h light/dark. Seeds did not germinate at 40°C (Figure 2). The calculated optimum temperature for seed germination was 22.9°C. All A. pachychlamys seeds germinated in the dark at 25°C, which differed significantly (P < 0.05) from the seeds grown under constant light conditions (Figure 3). Although more D. robusta seeds germinated at constant 20°C and an alternating 25/20°C, germination values were not significantly different from those at 25°C and 30°C. The calculated optimum temperature for seed germination was 23.8°C. None of the D. robusta seeds germinated at 40°C (Figure 2). Seeds of D. robusta exposed to constant light conditions at 25°C had a significantly (P < 0.05) higher percentage germination compared to the seeds germinated under constant dark conditions (Figure 3).

Different temperatures significantly affected survival and seedling growth of both species. In A. pachychlamys, the highest seedling survival rate was observed at temperatures of 10°C and 15°C (Table 1), while seedling and bulb mass was highest at 25°C. This differed significantly (P < 0.05) between temperature treatments (Figure 4). Roots and leaf number were also significantly higher at 25°C (P < 0.05) (Table 1). The survival rate of D. robusta seedlings at a temperature of 25°C was significantly (P < 0.05) greater compared to those grown at other temperatures. At low (10°C) and high (35°C) temperatures, only 36% and 32% of the seedlings survive, respectively (Table 1). The total seedling and bulb masses at 25°C were significantly (P < 0.05) greater than at other temperatures tested (Figure 4).

![Figure 1: Water uptake in Albuca pachychlamys and Drimia robusta seeds at 25 ± 0.5°C](image-url)
Similarly, root and leaf growth were also significantly (P < 0.05) greater at 25°C (Table 1).

Discussion

The water-uptake curve indicates that the seeds of both the species were non-dormant and that the covering structures did not restrict water uptake. Although seeds of a particular species may germinate over a wide range of temperatures, the time needed for maximum germination varies as temperatures fluctuate (Bradbeer 1988, Bewley and Black 1994). Temperature also influences the rate of seed germination in non-dormant seeds (Alvarado and Bradford 2002). In the present study, 40°C had a harmful effect on seed germination of both species. The best temperature for germination of *Albuca pachychlamys* seeds was 25°C and for *Drimia robusta* seeds 20°C, since the MGT was shorter than that calculated for other temperatures (Figure 2).

In *Echinacea purpurea* (L.), the highest percentage germination was also recorded for seeds with the shortest MGT (Kochankov et al. 1998). The fact that *Albuca pachychlamys* and *Drimia robusta* must be grown in warm temperatures (20–25°C) to achieve maximum germination can be correlated with the summer temperatures experienced in South Africa, since the yearly temperature cycle plays an important role in seed germination (Baskin and Baskin 1988).

Seed germination in many species is inhibited by continuous white light (Bewley and Black 1994). In the present study, continuous light resulted in a decrease in the germination response, while dark conditions promoted the germination of *Albuca pachychlamys* seeds. When seeds are on the soil surface, direct sunlight inhibits germination (Bewley and Black 1994). To achieve maximum germination, therefore, it seems that the seeds of *Albuca pachychlamys* must be sown at greater depths. In the case of *Drimia robusta*, however, seeds germinated readily under constant light, suggesting that the seeds could be sown near the soil surface. However, the light-requiring seeds are more susceptible to factors such as temperature and nitrate levels under field conditions (Bewley and Black 1994). Smoke extracts substitute for the light and dark germination requirements in many seeds (Van Staden et al. 2000). With the application of smoke extract, negatively photoblastic *Syncarpha vestita* (L.) B. Nord seeds germinate in the light (Brown and Van Staden 1997), while positively photoblastic seeds of lettuce (Drewes et al. 1995) and celery (Thomas and Van Staden 1995) germinate in the dark. With the application of smoke extract, light-requiring seeds could be sown deeper in the soil to avoid unfavourable soil surface factors like high temperatures and poor nitrate levels.
Although the survival of *A. pachychlamys* seedlings was low at 25°C, root number and leaf length were greater than those recorded for other temperature treatments (Figure 4 and Table 1). Thus to avoid mortality in *A. pachychlamys* seedlings, it may be best to grow them at low temperatures for a few days and then transfer them to higher temperatures, not exceeding 25°C. In *D. robusta*, the highest percentage seedling survival, as well as vegetative growth, was achieved at 25°C while low (10°C) and high (35°C) temperatures proved harmful.

Maximum germination can be achieved at optimum temperatures of 22.9°C and 23.8°C (calculated values) for *A. pachychlamys* and *D. robusta* seeds respectively, although seedlings of both the species should be grown at 25°C to ensure seedling vigour and health. Since these findings apply to germination requirements under controlled environmental conditions only, it is necessary to establish optimal conditions for germination and growth in field trials. However, under controlled conditions, large-scale seedling production can be achieved, with the healthy seedlings then being transplanted to the field.
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References


