

GA₃-MEDIATED DORMANCY ALLEVIATION IN THE REPUTED AFRICAN POTATO, *HYPOXIS*
HEMEROCALLIDEA

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South Africa.*Email : shaiksh@ukzn.ac.za**Abstract****Background:** *Hypoxis hemerocallidea*, arguably the most well-known medicinal species in South Africa, has been the subject of intensive harvesting from the wild leading to recent conservation concerns. The seeds of this species do not propagate easily and can lie dormant for up to twelve months.**Materials and Methods:** In the *in vitro* germination experiments water, acid and chemical pre-sowing treatments were performed to determine the germination response of this species in both light and dark conditions. In the *ex vitro* experiment, intact seeds were sown and left to germinate in a potting soil mix under greenhouse conditions.**Results:** Highest levels of germination (36.7-60.0% in the light and 36.7-46.7% in the dark) were achieved by treating mechanically scarified seeds with GA₃ at various concentrations for 24 h. This was followed by scarified seeds soaked in water (26.7% in the light and 23.3% in the dark). Similar results (23.3 and 26.7%) were obtained in the 1% and 4% KNO₃ treatments, respectively, under light conditions only. The fastest time to germinate was two days and was obtained in the 1200 ppm GA₃ treatment in both light and dark conditions. *Ex vitro* germination of *H. hemerocallidea* seeds under greenhouse conditions was unsuccessful.**Conclusion:** *H. hemerocallidea* displays physical and non-deep physiological dormancy where pre-sowing treatments are required before the seeds will germinate.**Keywords:** medicinal plant; dormancy; GA₃; pre-sowing**Abbreviations:** FGP = final germination percentage; SSP = swollen seed percentage; HSP = hard seed percentage.**Introduction***Hypoxis hemerocallidea* Fisch., C.A. Mey. & Avé-Lall., the acclaimed African potato, is a member of the Hypoxidaceae family and is synonymous with *H. rooperi* T. Moore (Singh, 2007). This geophytic perennial thrives in open rocky grasslands and savannahs of most South African provinces (van Wyk et al., 2009). It is also found in dry regions of Lesotho, Botswana, Mozambique, Madagascar and Zimbabwe and is easily recognized by its bright yellow star-shaped flowers and strap-like leaves. The English name 'African potato' is somewhat incongruous since the tuberous rootstock is actually a corm with a dark brown or black colouring on the outside and bright yellow on the inside. The species is known by several vernacular names viz. *sterblom* (Afrikaans), *inkomfe* (isiZulu), *lotsane* (Sesotho) (Williams et al., 2008; van Wyk et al., 2009).*H. hemerocallidea* is a popular and widely traded plant on the African continent with a long history of medicinal use, and is arguably the most well-known medicinal species in South Africa. Folkloric and anecdotal uses of cormous and/or leaf infusions include the treatment of bladder disease, dizziness, insanity, burns, testicular cancer, prostrate hypertrophy and urinary system disorders (van Wyk et al., 2009). Scientific studies have demonstrated cormous extracts with antinociceptive, antiinflammatory and antidiabetic properties (Ncube et al., 2012b; Ojewole, 2006; Zibula and Ojewole, 2000), antioxidant properties (Laporta et al., 2007; Nair et al. 2007), antibacterial properties (Gaidamashvili and van Staden, 2002) and anticonvulsant activity (Ojewole, 2008). Recent reports document antibacterial, antifungal and antiinflammatory activity of leaf extracts (Ncube et al., 2012a,b). These pharmacological effects are purported to be the result of bioactive hypoxoside, phytosterols, lectin-like compounds and lipophilic compounds.It is evident that *H. hemerocallidea* corms are more widely used than the leaves in traditional medicine. Hundreds of thousands of corms are sold annually at various South African medicinal plant markets (Cunningham, 1988; Dold and Cocks, 2002; Williams et al., 2007) with corm size having little impact on sale. The corms are collected via unsustainable harvesting techniques by complete uprooting of plants to derive the medicinal plant organ, no matter the size. Katerere and Eloff (2008) reported that corms and leaves of *H. hemerocallidea* display clear chemical and bioactive differences so it is not possible to substitute corm use with leaves.Naidoo (1998) and Nomtshongwana (1995) reported that natural seedling establishment is not sufficient to sustain wild populations of *H. hemerocallidea* post collection. So the high demand on the plant poses a real and current threat to its survival, in fact, it has reached the 'declining' national status according to the Red List of South African Plants (Williams et al., 2008). Land transformation, urban sprawl and habitat loss are further compounding factors. Moreover, the seeds of this species do not propagate easily, are notoriously difficult to germinate and can lie dormant for up to twelve months (Hammerton and van Staden, 1988). Germination is known to be inconsistent with unpredictable seed viability (Hammerton et al., 1989) while vegetative propagation is rare. In dormant seeds pre-sowing treatments can be performed to enhance germination but this was last studied in *H. hemerocallidea* by Hammerton and van Staden (1988) twenty five years ago. The current study therefore, aims to improve on the seed germination of *H. hemerocallidea* by performing *in vitro* germination using further pre-sowing treatments.**Materials and methods****Seeds**One month old seeds of *H. hemerocallidea* were purchased in July 2011 from Silverhill Seeds and Books, South Africa. Seeds are black (containing phytomelan), hard and glossy in appearance. Seeds were stored in amber, air-tight bottles at room temperature (25°C) until experimental use.

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Ex vitro germination

In an *ex vitro* experiment, 90 intact seeds were sown in a potting soil mix in a seedling tray with one seed per insert. The tray was placed in a greenhouse for a germination period of 32 days and was watered daily by an automatic water sprayer.

In vitro germination

In the *in vitro* experiment, seeds were subjected to various pre-sowing treatments as outlined below:

Control: Intact seeds which were not subjected to any pre-sowing treatments were used.

Water soak: Intact seeds and mechanically scarified seeds were separately soaked in 50 ml of cold distilled water for 24 h at an ambient temperature of 25°C. Mechanical scarification was performed by rubbing intact seeds between two sheets of carborundum paper on the opposite side to the hilum to carefully remove the seed coat without damaging the embryo. In the hot water treatments, intact seeds were soaked separately in 50 ml of 80°C distilled water for 15, 30 and 60 min following which they were removed and left to cool for 10 min.

Acid scarification: Intact seeds were separately soaked in 50 ml of concentrated sulphuric acid (97% H₂SO₄) for 5, 15 and 30 min.

Chemical treatments: Mechanically scarified seeds were separately soaked in 50 ml of potassium nitrate (KNO₃) at 1, 2 and 4% for 24 h at an ambient temperature of 25°C. In addition, mechanically scarified seeds were separately soaked in 50 ml of gibberellic acid (GA₃) at 200, 500 and 1200 ppm for 24 h at an ambient temperature of 25°C.

Decontamination and seed culture

Following pre-sowing in all treatments (except the acid scarification) seeds were decontaminated by washing in 70% ethanol for 30 s followed by a 60 s soak in a commercial bleach solution (3.5% NaOCl) with Tween 20 as a surfactant. Thereafter, seeds were thoroughly washed in three 2 min rinses in autoclaved distilled water with intermittent hand agitation. Seeds in the acid scarification treatments were rinsed only in autoclaved distilled water as outlined above. Thereafter, seeds from each treatment were separately placed using a sterile forceps onto 1% agar in 9 cm Petri plates in a laminar flow hood. Parafilm was used to seal the edges of each plate before being placed in a growth room to record germination.

Experimental design

The experiments were performed in a completely randomized design with 15 treatments each containing 30 seeds which were replicated three times in the light and three times in the dark. Light was provided by fluorescent bulbs at 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a 16 h photoperiod. Dark conditions were emulated by completely covering the plates with heavy duty aluminium foil. All plates were kept for 32 days in a growth room at 25°C. Every 48 h germinated seeds were counted and recorded (germinated seeds in dark conditions were counted under a green lamp). Radicle protrusion from the seed coat was the criterion used to indicate germination (Auld et al., 1988). Parameters recorded were final germination percentage (FGP), swollen seed percentage (seeds that visibly absorbed water but failed to germinate) (SSP) and hard seed percentage (seeds that visibly failed to absorb water) (HSP).

Results and Discussion

Ex vitro germination of *H. hemerocallidea* seeds under greenhouse conditions did not occur (data not shown) after the equivalent *in vitro* germination period of 32 days. In the *in vitro* study, overall, FGP's in the light were better than in the dark and ranged from 6.7-60.0% and 3.3-46.7%, respectively (Table 1). In both light and dark conditions, the highest FGP's were achieved in the GA₃ treatments from 36.7-60.0% and 36.7-46.7%, respectively. This was followed by scarified seeds soaked in water with FGP's of 26.7% (light) and 23.3% (dark). Similar FGP's (23.3 and 26.7%) were obtained in the 1% and 4% KNO₃ treatments, respectively, under light conditions only. Hot water and 4% KNO₃ were less effective in promoting germination achieving between 3.3-13.3% FGP in both light and dark conditions. The control, intact seeds soaked in water and H₂SO₄ treatments were ineffective in initiating germination in either the light and or the dark. The fastest time to germinate was two days and was obtained in the 1200 ppm GA₃, 1% and 4% KNO₃ treatments in the light and only in the 1200 ppm GA₃ treatment in the dark (Figure 1). FGP's occurred between 20-28 days for the GA₃ treatments in the light and between 18-20 days in the dark.

Germination occurred in both light and dark conditions, therefore, *H. hemerocallidea* seeds are non-photoblastic. However, slightly better FGP's were achieved under light conditions. Soaking mechanically scarified seeds in water, in the plant growth regulator GA₃ and in the inorganic KNO₃ promoted germination whilst the control and soaking intact seeds in water had no effect on germination. This implies that dormancy is combinational and likely imposed by both the seed coat (physical) and the embryo (physiological) and can be classified as non-deep dormancy (Baskin and Baskin, 2001). These results are consistent with those reported previously by Hammerton and van Staden (1988) except that the latter reported 'deep' dormancy. Moreover, in the current investigation, FGP was improved to 60.0% in the light and 46.7% in the dark by increasing the GA₃ concentration to 1200 ppm at a temperature of 25°C as opposed to 36% (light) and 26% (dark) at a temperature of 30°C in the Hammerton and van. Staden (1988) study. In the latter study, GA₃ was exogenously applied for the entire duration of the germination period of 28 days but seems to have had little impact on the FGP and time (14-21 days) to reach this percentage. In addition, Hammerton and van Staden performed germination experiments on moist filter paper which became contaminated with endogenous seed fungi during the course of the investigation and could have hampered their results. Comparatively, in the current investigation, pre-soaking for 24 h in GA₃ at 25°C allowed optimal imbibition which promoted *in vitro* germination with no fungal contamination. GA₃ is an effective growth promoting agent when combined with light and suitable temperature and is widely used in breaking embryo-imposed dormancy of many seed types (Bewley and Black, 1994). This usually happens when seed storage reserves are mobilized and expansion of the embryo is stimulated. Germination of commercially important medicinal species (Nadjafi et al., 2006), fruit species (El-Dengawy, 2005) and cut flower species (de Mello et al., 2009) have been improved by pre-sowing in GA₃.

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Table 1: Percentages of final germination (means±standard deviation), swollen and hard seeds in *Hypoxis hemerocallidea* seeds as affected by various pre-sowing treatments in light and dark conditions after 32 days of incubation.

Treatment	Light			Dark		
	Final germination %	Swollen seed %	Hard seed %	Final germination %	Swollen seed %	Hard seed %
Control	0.0 ± 0.0	0.0	100.0	0.0 ± 0.0	0.0	100.0
Water 24 h intact	0.0 ± 0.0	0.0	100.0	0.0 ± 0.0	0.0	100.0
Water 24 h scarified	26.7 ± 6.8	53.3	20.0	23.3 ± 6.1	53.3	23.3
Hot water 15 min	6.7 ± 1.4	0.0	93.3	6.7 ± 4.0	0.0	100.0
Hot water 30 min	13.3 ± 4.0	0.0	86.7	3.3 ± 2.2	0.0	96.7
Hot water 60 min	6.7 ± 4.0	0.0	93.3	6.7 ± 2.9	0.0	93.3
KNO ₃ 1%	23.3 ± 9.0	43.3	33.3	6.7 ± 3.6	66.7	26.7
KNO ₃ 2 %	26.7 ± 7.1	50.0	23.3	0.0 ± 0.0	43.3	56.7
KNO ₃ 4 %	6.7 ± 5.8	33.3	60.0	3.3 ± 2.5	26.7	70.0
GA ₃ 250 ppm	36.7 ± 6.5	33.3	30.0	36.7 ± 6.7	50.0	13.3
GA ₃ 500 ppm	46.7 ± 8.9	30.0	23.3	36.7 ± 4.3	33.3	30.0
GA ₃ 1200 ppm	60.0 ± 7.1	23.3	16.7	46.7 ± 8.5	26.7	26.7
H ₂ SO ₄ 5 min	0.0 ± 0.0	0.0	100.0	0.0 ± 0.0	0.0	100.0
H ₂ SO ₄ 15 min	0.0 ± 0.0	0.0	100.0	0.0 ± 0.0	0.0	100.0
H ₂ SO ₄ 30 min	0.0 ± 0.0	0.0	100.0	0.0 ± 0.0	0.0	100.0

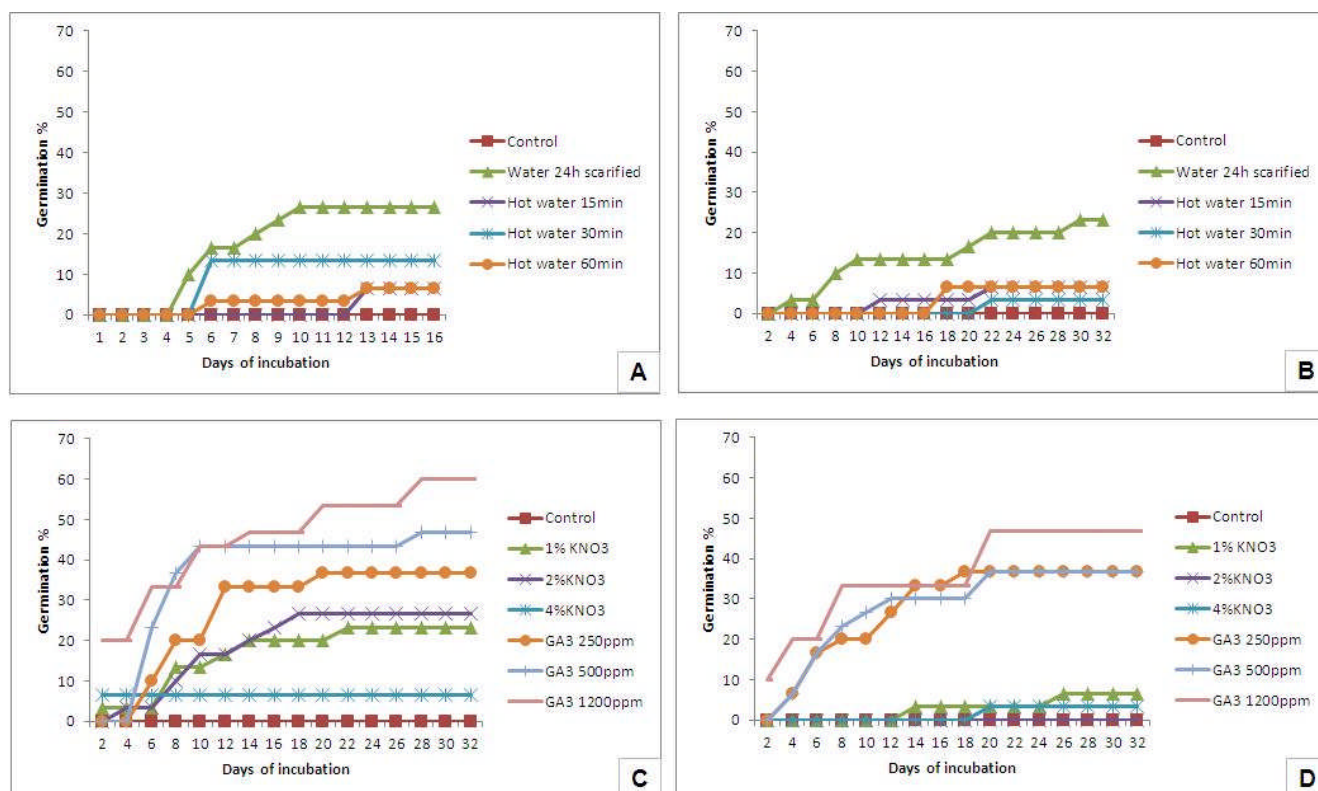


Figure 1: Time-course changes in *in vitro* germination percentage of *Hypoxis hemerocallidea* seeds as affected by water soak in the light (A) and dark (B), and chemical treatments in the light (C) and dark (D) over 32 days of incubation.

Nitrates, inorganic plant nutrients, which stimulate germination in the natural environment, had a positive effect on germination in this study but the FGP's were low and not as effective in promoting germination as GA₃. It is also possible that high seed content of nitrates inhibited germination. SSP for GA₃, KNO₃ and soaking scarified seeds in water (23.3-66.7%) reflected that scarification does enhance imbibition of the pre-sowing solutions but these embryos did not germinate either because they were immature or they required an extended period to emerge. HSP for these scarified treatments (16.7-70.0%) indicate that barrier tissues such as endosperm and the remaining testa may not have been weakened enough to promote penetration of the pre-sowing solutions and subsequent germination.

The seed coat of *H. hemerocallidea* seeds appears to carry an inhibition trait to germination. Intact seeds remained hard (100% HSP) in the control and water soak treatments for the germination test period of 32 days indicating that the seed coat may be impermeable to water. The testa may also restrict oxygen movement to the embryo (Baskin and Baskin, 2001) thus prohibiting germination. The endogenous abscissic acid to GA₃ ratio is unknown but if it is high, then soaking intact seeds for 24 h in water did not promote leaching since 100% of seeds remained hard in this treatment. The hard glossy testa protects the embryos from desiccation as a survival strategy. In its natural grassland and savannah environment, this

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characteristic is probably overcome by fire which is known to promote the germination of seeds (Hölscher, 2009). Heat shock treatments have been successful in increasing germination of other fire-adapted species (Zuloaga-Aguilar et al., 2011) by scarring the testa and increasing permeability to water. In the current investigation, heat shock (hot water) treatments in the current study did promote germination but the FGP's were low. Submersion in acid damaged the seed coat but the seeds did not swell (100% HSP). Perhaps the acid was injurious to the embryos and resulted in no germination which is consistent with that found by Wilsenach (1967); and Hammerton and van Staden (1988) who used HCl.

Combinational dormancy, unpredictable seed viability and poor seedling establishment has poor implications for restoration efforts of the declining *H. hemerocallidea*. The present investigation has provided a simple and cost effective method for the propagation and conservation of this species. It can be concluded that quick *in vitro* germination of *H. hemerocallidea* can be achieved in the light or dark by the application of 1200 ppm GA₃ to scarified seeds for 24 h at 25°C and that a maximum germination of 60.0% can be achieved in 28 days in the light. *H. hemerocallidea* has characteristic non-deep physiological dormancy where a dormancy-breaking treatment is required before the seeds will germinate.

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