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Research Note

Influences of pre-sowing seed treatments on germination of the cancer bush (*Sutherlandia frutescens*), a reputed medicinal plant in arid environments

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Summary

Cancer bush (*Sutherlandia frutescens* L.) is a medicinally important perennial legume native to southern Africa. *S. frutescens* seeds exhibit dormancy like many other legumes. Experiments using physical, mechanical and chemical pre-sowing treatments were conducted to determine the germination response of this species. Among various treatments, soaking the intact seeds for 30 min in concentrated H_2SO_4 resulted in a high final germination percentage of 97.5% in day 14 of culture. However, seed dormancy was completely broken by mechanical scarification in which 100% germination was obtained in day 2 of culture. The results indicated that *S. frutescens* seeds possess exogenous dormancy due to the hard seed coat which is the main inhibitor of germination.

Experimental and discussion

Cancer bush (*Sutherlandia frutescens* L.), a member of the Fabaceae family, is a perennial shrub that grows in arid terrains of Southern Africa. The plant is a popular ornamental shrub due to its conspicuous scarlet flowers however; its medicinal benefits render it more widely utilized by traditional healers. Traditionally, the leaves and the aerial parts are used by boiling in water to yield an aqueous infusion used as an astringent tonic (Van Wyk *et al.*, 1997). It has been documented that *Sutherlandia* plays a significant role in the treatment of AIDS (Hartnett *et al.*, 2005), cancer (Stander *et al.,* 2007) and diabetes (Ojewole, 2004; Chadwick *et al*., 2007). It is also effective as an anti-inflammatory (Kundu *et al.*, 2005), antioxidant (Katere and Eloff, 2005) and anti-mutagenic agent (Reid *et al.*, 2006).

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Generally, legumes exhibit hardseededness resulting in dormancy (Tsiantis, 2005). Several studies have been conducted on legume germination using different seed coat pre-sowing treatments (Ibañez and Passera, 1997; Ortega Baes *et al.*, 2002; Patanè and Gresta, 2006; Travlos *et al.*, 2007). The mechanism of seed dormancy for *Sutherlandia* is unknown although it may have physical dormancy akin to other members of the Fabaceae family. Numerous attempts to germinate *S . frutescens* seeds in an array of soils in the East Coast region of KwaZulu-Natal have resulted in poor germination, not exceeding 10%. Moreover, there is a general lack of information on practices to overcome dormancy of *Sutherlandia* seeds. This study aims at evaluating the impact of various pre-sowing seed treatments on the germination response of *S. frutescens* and at developing an effective method of breaking seed dormancy. The present study is crucial for optimizing seed germination and growth of *Sutherlandia* since the increasing demand for fresh material as a medicinal herb could result in the attrition of natural populations to the point of extinction, and a resultant loss of biodiversity.

Seed materials and Pre-sowing seed treatments

S. frutescens seeds were obtained from the South African National Biodiversity Institute (SANBI), Kirstenbosch, South Africa in August 2007. The fresh weight of 100 seeds was 0.836 g and the moisture content was 5.5% which was estimated after drying the seeds at 100°C for 16h (Yang *et al.*, 2007). All seeds were surface sterilized in 70% ethanol for 30 s and then washed with sterile distilled water prior to an experimental procedure to prevent contamination. Seeds were subjected to different mechanical, physical and chemical treatments. Mechanical scarification was achieved by vigorously rubbing the seeds for 6 to 8 sec between two sheets of fine-grained sand paper to remove the testa without injuring the embryo (Pérez-García and González-Benito, 2006). Physical scarification was carried out by soaking intact seeds in distilled water for 24 h at ambient temperature (25°C) and in hot distilled water (80°C) for 15 and 30 min. After completion of the hot water treatments, seeds were removed from the water and left to cool for 10 min. Chemical scarification was accomplished using three different techniques. First, samples of intact seeds were soaked separately in concentrated sulphuric acid (97% H_2SO_4) for 5, 15 and 30 min. Second, samples of mechanically scarified seeds were soaked separately in potassium nitrate (KNO_3) at 0, 1, 2 and 4% for 24 h. Finally, samples of mechanically scarified seeds were soaked separately in aerated solutions of gibberellic acid (GA_3) at 0, 100, 250 and 500 ppm for 24 h. All seeds in the H_2SO_4, KNO_3 and GA_3 treatments were thoroughly washed in sterile distilled water before culturing. Intact seeds without pre-sowing treatments were considered as the control.

Experimental design and statistical analysis

All experiments were conducted in a completely randomized design. There were 15 treatments replicated 4 times, and each replication consisted of 10 seeds. Seeds were placed in sterile plastic Petri dishes (9 cm), containing 20 ml of 1% agar. All cultures were incubated for 20 days at 25°C and 16 h photoperiod provided by a fluorescent light at 40 µmol m^{-2} s^{-1}. Germinated seeds were counted every 48 h and then discarded. A seed was considered germinated when the tip of the radicle had grown free of the seed

coat (Auld *et al.*, 1988). The germination parameters including germination percentage (GP), germination rate index (GRI), corrected germination rate index (CGRI), time to 50% germination (GT_{50}), swollen seed percentage (seeds absorbed water but failed to germinate) and hard seed percentage (seeds failed to absorb water) were visually recorded (Esechie, 1994; Hsu *et al.*, 1985). Data were subjected to Duncan's Multiple Range test and Least Significant Difference test using SAS Program (Version 6.12, SAS Institute Inc., Cary, NC, USA).

Effect of water soak and H_2SO_4 treatments of the intact seeds on the time-course changes in germination percentage of S. frutescens

The effects of various pre-sowing seed treatments on the time-course changes in germination percentage of *Sutherlandia* are shown in figure 1. The germination percentage for the control did not exceed 10% whereas the water soak (24 h) treatment did not exceed 15% (figure 1A). This is in accordance with studies by Sy *et al.* (2001) in which germination capacities of several Sahelian legume species were similar to the control after soaking in water. In the hot water soaking treatments, germination percentage was improved with increasing exposure to hot water (figure 1B). Germination percentages in

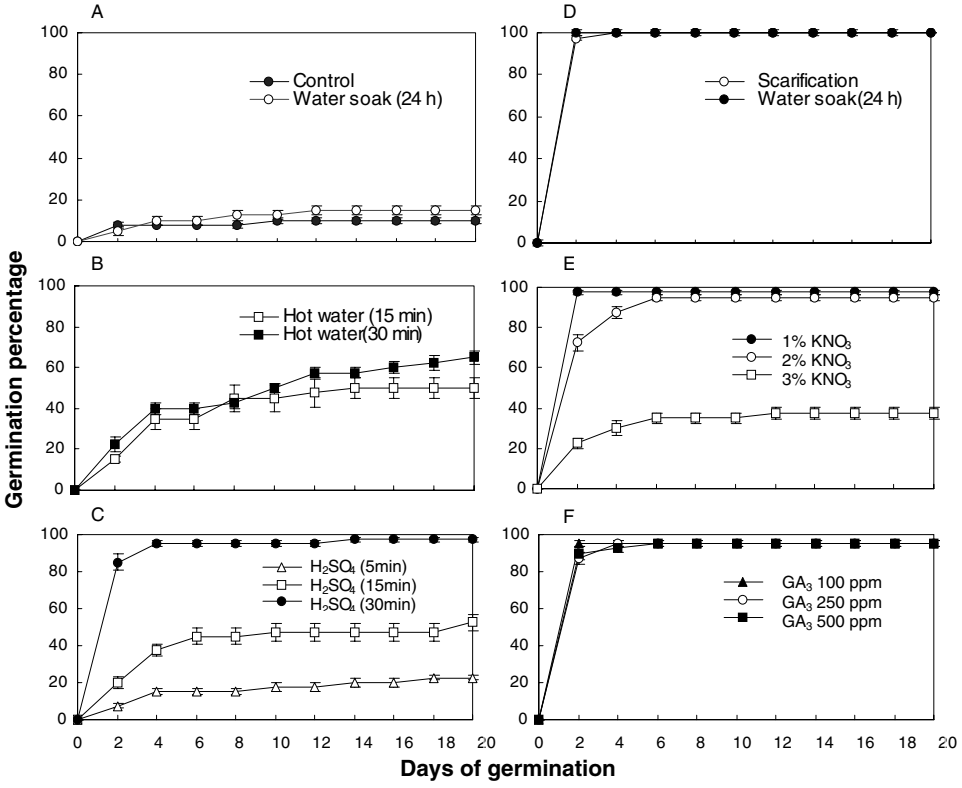

Figure 1. Time-course changes in germination percentage of *Sutherlandia frutescens* seeds as affected by different pre-sowing treatments {intact seeds (A,B,C), scarified seeds (D,E,F)} over 20 days of culture.

both 15 and 30 min hot water treatments were better than the control and reached 50 and 65% respectively. It has been reported that soaking in hot water improves the germination of seeds with hard seed coats (Patanè and Gresta, 2006). The improved germination percentages of *Sutherlandia* seeds using hot water are indicative of tolerance to high temperatures which is a characteristic of its natural habitat. In the H_2SO_4 treatments, germination percentage was improved with increasing exposure to the acid (figure 1C). Immersion in H_2SO_4 for 15 and 30 min resulted in germination percentages that were considerably higher than the control during the entire culture period. Similar results were obtained in studies carried out on other legumes (Patanè and Gresta, 2006; Travlos *et al.*, 2007) and in *Caper*, a plant that inhabits hot dry environments (Sozzi and Cheisa, 1995) similar to *Sutherlandia*. The most favorable germination pre-sowing treatment for intact seeds appears to be soaking in H_2SO_4 for 30 min since this treatment resulted in a high germination percentage (85%) on day 2 and reached its maximum germination (97.5%) on day 14.

Effect of mechanical scarification of the intact seeds and water soak, KNO_3 and GA_3 treatments of the scarified seeds on changes in germination percentage of S. frutescens

Both the mechanical scarification treatment and the water soak (24 h) treatment resulted in a maximum germination of 100% on day 2 and day 4, respectively (figure 1D). The mechanical scarification without soaking in water resulted in 100% germination in a minimum period of time indicating that seed dormancy was completely broken. Mechanical scarification has been shown to be advantageous for seed germination in other arid and semi-arid leguminous species with hard seed coats (Cavanagh, 1987; Patanè and Gresta, 2006; Travlos *et al.*, 2007) and also in non-leguminous species (Yang *et al.*, 2007). Among different KNO_3 treatments, the best germination percentages were achieved in 1% followed by 2% in which maximum germination of 97.5% and 95% was attained on day 2 and day 6, respectively (figure 1E). Higher concentration of KNO_3 at 4% considerably reduced the germination percentage to 37.5% by day 12 of culture. All GA_3 concentrations tested (100, 250, and 500 ppm) resulted in similar germination percentages (95%) at day 2, 4 and 6, respectively (figure 1F). It appears that seed germination of *Sutherlandia* is inhibited by the presence of the testa since removal of the testa resulted in high germination percentages regardless of KNO_3 or GA_3 treatments. Although nitrates and gibberellins have been widely used to overcome seed dormancy (Nadjafi *et al.*, 2006; Çirak *et al.*, 2007) its application in the present study was not required to break seed dormancy. This indicates that *Sutherlandia* seed dormancy is exogenous due to the impermeable hard seed coat.

Effect of pre-sowing treatments on FGP, GRI, CGRI, GT50, swollen and hard seed percentages of S. frutescens

The effects of various pre-sowing treatments on FGP, GRI, CGRI, GT50, swollen and hard seed percentages in *Sutherlandia* are presented in table 1.

In treatments using intact seeds, soaking for 30 min in either hot water or H_2SO_4 significantly increased the FGP from 10% (control) to 65% and 97.5%, respectively. This response to the chemical and physical scarification provides evidence that the seed coat of

Table 1. Effect of pre-sowing seed treatments on final germination percentage (FGP), germination rate index (GRI), corrected germination rate index (CGRI), time taken to reach 50% of final germination percentage (GT_{50}), swollen and hard seed percentages in *Sutherlandia frutescens* after 20 days in culture.

Treatments	FGP	GRI	CGRI	GT_{50}	Swollen seeds %	Hard seeds %
Intact seeds:						
Control	10.0 e*	11.0 c	73.2 c	3.5 bcd	0.0 b	90.0 a
Water soak (24 h)	15.0 e	13.5 c	104.2 abc	4.5 abcd	0.0 b	85.0 a
Hot water soak (15 min)	50.0 cd	46.7 b	89.0 bc	5.7 ab	5.0 b	45.0 b
Hot water soak (30 min)	65.0 b	51.2 b	78.7 c	6.6 a	7.5 b	27.5 c
H_2SO_4 (5 min)	22.5 e	18.2 c	81.0 c	6.8 a	0.0 b	77.5 a
H_2SO_4 (15 min)	52.5 bc	51.5 b	98.0 abc	5.6 abc	2.5 b	45.0 b
H_2SO_4 (30 min)	97.5 a	134.1 a	137.8 a	2.5 d	0.0 b	2.5 d
Mechanical scarification	100.0 a	145.2 a	145.2 a	2.1 d	0.0 b	0.0 d
Scarified seeds						
Water soak (24 h)	100.0 a	145.2 a	145.2 a	2.0 d	0.0 b	0.0 d
1% KNO_3	97.5 a	142.8 a	146.5 a	2.0 d	2.5b	0.0 b
2% KNO_3	95.0 a	126.0 a	132.5 ab	2.6 cd	5.0 b	0.0 d
4% KNO_3	37.5 d	43.8 b	116.3 abc	3.6 bcd	62.5 a	0.0 d
GA_3 100 ppm	95.0 a	139.1 a	146.5 a	2.0 d	5.0 b	0.0 d
GA_3 250 ppm	95.0 a	135.4 a	142.4 a	2.2 d	5.0 b	0.0 d
GA_3 500 ppm	95.0 a	136.0 a	143.0 a	2.2 d	5.0 b	0.0 d
LSD	7.04	9.45	21.71	1.35	3.81	5.46

* Mean separation within each column by Duncan's Multiple Range test at 5% level (n = 4).

Sutherlandia seeds is the main inhibitor of germination. Soaking in hot water and H_2SO_4 have been shown to enhance germination percentages of other species (Pérez-García and González-Benito, 2006; Travlos *et al.*, 2007). All other pre-sowing treatments resulted in slightly higher FGP than the control. Soaking in water at ambient temperature (25°C) did not significantly enhance the FGP, however, soaking in water at elevated temperatures (80°C) for 15 or 30 min resulted in significantly higher FGP than that of the control. These differences may be due to the inability of the water gaps to open (Baskin, 2003).

In treatments using scarified seeds, all treatments (except 4% KNO_3) had a significantly higher FGP than the control and ranged between 95 to 100%. FGP of seeds in the 4% KNO_3 treatment was much lower at 37.5%. Previous reports demonstrated that mechanical scarification is a very effective treatment in promoting the germination of leguminous seeds (Ibañez and Passera, 1997; Patanè and Gresta, 2006). In arid and semi-arid species such as *Helianthemum*, mechanical scarification significantly increased germination percentage (Pérez-García and González-Benito, 2006). The germination speed (GRI and

CGRI) and GT_{50} of the intact seeds were significant in the H_2SO_4 (30 min) treatment and seeds reached 50% of its final germination (GT_{50}) in a minimum time (2.5 days). Germination rate for seeds soaked in water (24 h) was slightly higher than the control. The germination rate of the scarified seeds with or without soaking in water was identical. The germination speed decreased with increasing KNO_3 concentration however, there were no significant differences in germination speed with increasing GA_3 concentration. GT_{50} was achieved in approximately 2 d in all treatments except that of 2% and 4% KNO_3. After 20 d of culture, the intact seeds that did not germinate either remained swollen (H_2SO_4 (15 min) and hot water 15 and 30 min) or remained hard for all other treatments. This could be explained by the resistance of the testa to the penetration of water, characteristic of many leguminous plants. Similar results were obtained at varying concentrations of H_2SO_4 in other legumes (Patanè and Gresta, 2006). In all scarified seed treatments with an FGP below 100%, seeds that did not germinate remained swollen with a significant percentage (62.5%) remaining swollen in 4% KNO_3.

The present investigation demonstrates that *Sutherlandia* seeds exhibit exogenous dormancy and is entirely imposed by the hard seed coat. The integument is able to withstand unfavorable conditions such as heat, teeth of dispersing agents and mechanical damage prevailing in the natural habitat. This avoidance of germination is ecologically advantageous to the plant in that seeds accumulate in the soil to increase the chance that some will germinate and create new populations to maintain the species. But this is limiting when quick and consistent seed germination is desirable for the successful establishment of economically important medicinal plant species. Our results also demonstrate that pre-sowing seed treatment with H_2SO_4 for 30 min is the most effective germination stimulant for intact seeds. However, mechanical scarification is required for optimal germination of seeds. The present study has established a successful methodology for overcoming seed dormancy and optimizing seed germination in *S. frutescens* in order to satisfy the demand for fresh material as a medicinal herb.

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