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SEED DORMANCY IN CANCER BUSH (*SUTHERLANDIA FRUTESCENS*): A MAJOR HINDRANCE TO PRODUCTION

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Abstract

Cancer bush, Sutherlandia frutescens is a Southern African indigenous plant harvested for its medicinal properties against several human illnesses. One major challenge in the sustainable development and cultivation of medicinal plants is seed dormancy that prevents the seeds from germination even when exposed to favourable conditions or when sown in the field. In this study, the effect of various chemical (H_2SO), mechanical scarification, physical (hot-water, sodium chloride and cold-water soaking) and biological (Trichoderma harzianum) methods of breaking dormancy were tested. Among all other treatments, hot water was found to be moderately effective in breaking dormancy resulting in 48% seed germination, which is still below the minimum recommended standard germination percentage of 80%. However, the mechanical scarification was the most effective method, resulting in germination percentages of 100%. The other seed treatment methods resulted in less than 10% germination. In conclusion, cancer bush seeds exhibited physical dormancy and the mechanical scarification method is recommended for increased seed germination and germination speed of cancer bush, thus good for field establishment and uniform plant population production.

Key words*: cancer bush, germination, medicinal plants, seed dormancy, seed priming.*

INTRODUCTION

Cancer bush (*Sutherlandia frutescens* (L.) R. Br.) is one amongst well-known medicinal plants indigenous to Southern Africa (Fernandes et al., 2004) and it is broadly distributed in South Africa, Namibia and Botswana (Prinsloo & Street, 2012). In South Africa, it occurs in the drier parts of Eastern Cape, Northern Cape, KZN, and Mpumalanga Provinces, although it is more abundant in the Western and Northern Cape provinces (Aboyade et al., 2013). Cancer bush is so called because of its medicinal use originating from the Khoi-San and Cape Dutch people against internal cancer as reported by Prinsloo and Street (2012). It also has other potential medicinal uses such treating diabetes, HIV/AIDS symptoms, signs of anxiety, and wound healing (Mills et al., 2005; Van Wyk et al., 2008; Fu et al., 2009).

Cancer bush is one of the many medicinal plants recommended for conservation action by the South African government because of extinction threats, owing to overharvesting and habitat destruction of natural populations (Raimondo et al., 2009 cited in SANBI, 2010-2012). According to Raghu et al. (2018), merely 10% of medicinal plants are cultivated, indicating that harvesting of natural stocks for their benefit are more common. Bringing these species to cultivation (especially the identified endangered species) represent a viable alternative solution to unlimited supply and may also help in sustaining their availability for future generations (Xego, Kambizi & Nchu, 2016). However, there are challenges to cultivation and only a few studies have investigated that, opening a knowledge gap that is a limiting factor to successful commercialization (Nwafor, 2020). Farahani, Hajiberat and Hajiberat (2014), found seed dormancy to be one of the challenges that prevent successful development, mass cultivation and adoption of medicinal plants, as viable seeds remain in the soil for a very long time without germinating, even when exposed to favourable conditions or sown in the field. Seed dormancy acts as a plant establishment delaying mechanism, inhibiting germination leading to uneven crop stand in the field, making it difficult to plant simultaneously and properly maintain plant population (Yildiz, 2018).

Several dormancies that could potentially influence production have been identified by Baskin and Baskin (2000; 2004), and these include exogenous, endogenous and combinational dormancy. Germination-promoting stimuli such as scarification and others have been found to assist seeds in breaking their dormancy (Bentsink, Koornneef, & Hilhorst, 2002). Their use may help in improving seed germination and better the performance in cultivation of medicinal plants. The aim of the study was to use various dormancy breaking techniques to determine the mechanism of dormancy exhibited by the plant and their effect in improving germination of cancer bush.

MATERIALS AND METHODS

Description of study site and preparation of materials

The experiment was conducted at the University of Mpumalanga, (Mbombela campus), South Africa (25º27′06. 18″S 30º58′5.21″E) under laboratory conditions, using viable cancer bush seeds. The seeds were first sterilized in 1% NaOCl for 5min and subsequently rinsed five times with sterile distilled water before applying the different dormancy-breaking treatments (Farahani et al., 2014).

Experimental treatments and layout

Six experiments were conducted to determine the mechanism of dormancy in *Sutherlandia frutescens*: namely the mechanical scarification, acid scarification, hot and cold-water soaking, soaking with NaCl and with *Trichoderma harzianum.* The experiments were laid out in a completely randomized design (CRD) with three replications for each experiment and ten seeds per replication.

Mechanical scarification experiment

Seeds were scarified for 10s opposite the micropyle following the procedure described by Arowosegbe (2016).

Acid scarification experiment

Acid scarification was done under a laboratory fume hood following a procedure by Dada et al. (2019), using three different concentrations of H_2SO_4, 40, 60 and 100% for 2, 4, and 6 min. The seeds were then rinsed thoroughly using sterilized distilled water to terminate the chemical reactions.

Physical treatments

Hot water soaking

Hot water treatment was done by separately soaking seeds in hot water using an EcoBath at 60, 80 and 100°C for 2, 4 and 6 min (Dada et al., 2016). After the hot water bath, seeds were removed and allowed to cool down for 10 min.

Cold water soaking

Seeds were soaked separately in beakers containing distilled water at room temperature for 0, 24, 48, 72 and 96 h (Arowosegbe, 2016).

Soaking with sodium chloride (NaCl)

Seeds were separately soaked in four different levels of salt: 0, 2, 4 and 6 g L^{-1} for 24, 48, 72 and 96 h. All seeds from NaCl treatment were rinsed with double distilled sterile water to remove the salt prior to culturing.

Biological priming

Soaking with Trichoderma harzianum

Seeds were primed using *Trichodema*, 1x 10^7 cfu/g live cells (Dalil, 2014). Firstly, the fungi were grown on a potato dextrose agar (PDA) and incubated at 25 °C for 3 days. To prepare the inoculum, a few drops of water was added onto the plate while a hockey stick was used to detach spores from mycelia. The liquid containing the pores was then collected from the plate. Spore count was done using a haemocytometer and seeds were soaked in water with the *Trichoderma* spores for 3, 6, 12 and 24 h. After soaking, seeds were allowed to air dry under the laboratory laminar flow and then transferred into a growth chamber with standard conditions set at 25 °C.

After all the treatments were applied, seeds were then transferred into a 9 cm diameter sterilized Petri dish containing one-layer Whatman filter paper moistened with 5 ml distilled water daily (Tavili et al., 2010). Seeds were kept in a growth chamber with standard temperature set at 25 °C. Untreated seeds in each treatment were used as a control.

Data collection

Seed were considered to have germinated when the radicle had grown 2 mm beyond the seed

coat (Farahani et al., 2014). Germination was recorded in 24-hour intervals for 14 days or until no further germination occurred. Germination-related variables such as germination percentage (GP), mean germination time (MGT), mean daily germination (MDG), germination speed (GS), plumule length (PL) and radicle length (RL) were computed upon termination of the experiments using formulas below as described by Arowosegbe (2016).

The data collected was first tested for normality using Shapiro-Wilk test and then analysed using Statistix 10 software.

Treatment means were separated using LSD-test at 5% probability level. For the completely discrete data (obtained from mechanical scarification) Mann-Whitney U test (non-parametric test) was used to get the actual means rather than the transformed means.

GP % = [(G/N) *100]

Where G = Total number of germinated seeds

N = Total number of seeds in petri dish

GS = (10*n1) + (9*n2) + (8*n3) +… (1*n14)

Where n1, n2, n3...n14 are the number of germinated seeds of the 1st, 2nd and 3rd day and following days until the 14th day.

MGT = $\Sigma f.x/\Sigma f$

f = is the number of germinated seeds of day x

MDG = Total number of germinated seeds/ total number of days

RESULTS AND DISCUSSIONS

All measured variables were not normally distributed ($P \geq 0.05$) as presented by Shapiro-Wilk normality test, hence, were transformed accordingly.

Effect of treatments on germination variables of *Sutherlandia frutescens*

The cold water, *T. harzianum* and sodium chloride treatments had no significant effect on germination, except for the mechanical scarification, acid scarification and sulfuric acid.

Mechanical scarification

The treatments had significant effect ($P \leq 0.05$) on germination percentage, germination speed, mean germination time, mean daily germination, radicle and plumule length. Relative to the unscarified seeds, scarification treatment had significant higher germination percentage, contributing 100% (Table 1; Figure 1).

Figure 1. Cancer bush seeds

The untreated seeds had the lowest germination of 0%. The highest radicle and plumule length were measured when seeds were scarified with sandpaper compared to untreated seeds which recorded 0mm for both the plumule and radicle length.

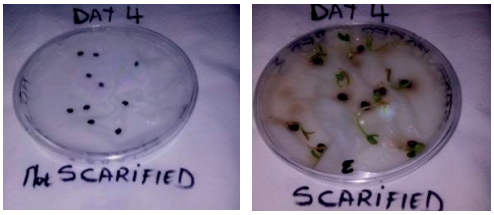

Figure 2. Cancer bush showing 100% germination within four days of culture

Table 1: Effect of mechanical scarification on germination variables of cancer bush (*Sutherlandia frutescens*)

Scarification	GP %	GS	MGT	MDG	RL (mm)	PL (mm)
Scarified seeds	100	23.86	44.58	1.25	13.93	18.73
Un-scarified seeds	0	0	0	0	0	0
U-stat	0	0	0	0	0	0
U-critical $_{0.05}$	0	0	0	0	316	316

Statistically different at $P \leq 0.05$ level according to Mann-Whitney U test (non-parametric test).

Sulfuric acid (H_2SO_4)

The results showed no significant interaction effect between concentration and duration of exposures. However, H_2SO_4 to some extent, was able to break seed dormancy even though it was not effective enough in improving seed germination variables. Seed exposure to 40% H_2SO_4 gave significantly higher GP, MGT, MDG, RL and PL and were significantly different from 60% and 100%, except for MGT at 100% (Table 2). Seed exposure to 60% recorded the lowest in all the variables and were not significantly different from 100%. The results show that the treatment with highest GP also had the highest MGT, MDG, radicle and plumule length and vice versa.

Hot water treatment

Exposing seeds for 4 minutes significantly recorded the highest germination percentage relative to 2 and 6 minutes (Table 3). At 6 minutes there were significantly lower GP, GS and MDG, although 6 minutes was not different from 2 minutes. The results show that the treatment with highest GP also had the highest GS and MDG, vice versa.

Exposing seeds to hot water at 80°C significantly improved GP, GS and MDG, relative to 60 and 100°C which had significantly less effect in all the measured variables (Table 4). Both 60°C and 100°C had lower germination effects and were both similar.

Although there were no significant interaction effects between temperature and time for GP, GS and MDG, a significant interaction effect was observed for MGT, RL and PL (Table 5). The 80°C treatment for 2 minutes had significantly higher MGT, RL and PL, relative to all the other treatments, 60°C for 2, 4 and 6 min, and 80°C for 4 and 6 min.

Table 2: Effect of H_2SO_4 on germination variables of cancer bush (*Sutherlandia frutescens*)

Conc[1] (%)	GP (%)	MGT (days)	MDG	RL (mm)	PL (mm)
40	0.35^{a}(13.3)	0.62^{a}(4.0)	0.07^{a}(0.2)	0.20^{a}(2.6)	0.23^{a}(4.5)
60	0.09^{b}(3.3)	0.19^{b}(1.4)	0.02^{b}(0.0)	0.03^{b}(0.3)	0.05^{b}(0.8)
100	0.16^{b}(5.6)	0.32^{ab}(1.9)	0.03^{b}(0.1)	0.06^{b}(0.9)	0.06^{b}(1.1)
F-value	5.61	4.01	4.71	6.66	6.92
P-value	0.0142**	0.0388	0.0247	0.0015**	0.0012**
$LSD_{0.05}$	0.1692	0.3290	0.0353	0.0940	0.1107

[X]Column means followed by same letter(s) are not significantly different at P ≥0.05 according to LSD All-pairwise comparisons. Values in brackets are untransformed means. **Highly significant (P ≤ 0.01), *Significant (P ≤ 0.05). [1]Conc = Concentration

Table 3: Effect of hot water treatment on germination traits of cancer bush (*Sutherlandia frutescens*)

Time (minutes)	GP (%)	GS	MDG
2	21.11^{b}(21.1)	0.38^{b}(1.8)	0.09^{b}(0.3)
4	34.44^{a}(34.4)	0.62^{a}(2.0)	0.15^{a}(0.4)
6	16.67^{b}(16.7)	0.31^{b}(1.4)	0.07^{b}(0.2)
F-value	5.80	7.98	7.23
P-value	0.0128**	0.0040**	0.0058**
$LSD_{0.05}$	11.519	0.1694	0.0457

[X]Column means followed by same letter(s) are not significantly different at P ≥ 0.05 according to LSD All-pairwise comparisons. Values in brackets are untransformed means; **Highly significant (P ≤ 0.01) *Significant (P ≤ 0.05).

Table 4: Effect of hot water treatment on germination traits of cancer bush (*Sutherlandia frutescens*)

[1]Temp (°C)	GP (%)	GS	MDG
60	12.22^{b}(12.2)	0.29^{b}(1.2)	0.06^{b}(0.2)
80	47.78^{a}(47.8)	0.76^{a}(3.5)	0.20^{a}(0.6)
100	12.22^{b}(12.2)	0.26^{b}(0.5)	0.06^{b}(0.2)
F-value	28.54	24.78	29.54
P-value	0.0000**	0.0000**	0.0000**
$LSD_{0.05}$	11.519	0.1694	0.0457

[X]Column means followed by same letter(s) are not significantly different at P ≥0.05 according to LSD All-pairwise comparisons. Values in brackets are untransformed means; **Highly significant (P ≤ 0.01), *Significant (P ≤ 0.05). [1]Temp = Temperature

Table 5: Effect of hot water treatment on germination traits of cancer bush (*Sutherlandia frutescens*)

Time (min)			
MGT (days)			RL (mm)
6	2	4	6
0.54^{bc} (3.8)	0.03^{e}(0.3)	0.38^{ab}(10.3)	0.13^{de} (2.2)
1.12^{a}(12.9)	0.84^{a}(14.4)	0.57^{bc} (7.1)	0.64^{ab} (10.2)
0.00^{d}(0.0)	0.14^{de} (1.33)	0.29^{de} (3.0)	0.03^{e}(0.3)
3.66			2.97
0.0267			0.0201
0.4453			0.2598

[X]Column means followed by same letter(s) are not significantly different at $P \geq 0.05$ according to LSD All-pairwise comparisons. Values in brackets are untransformed means; **Highly significant ($P \leq 0.01$), *Significant ($P \leq 0.05$).
[1]Temp = Temperature

Seed germination is the most important aspect of plant production and nutrition worldwide (Esan, Ayanbamiji & Abodunri, 2021). A wide understanding of the physiological processes in seeds is significant for crop stand establishment at the field. This study investigated the mechanism of dormancy and the effectiveness of different dormancy breaking treatments in cancer bush seeds which included cold water soaking, hot water, H_2SO_4 and sandpaper scarification, soaking with NaCl and *T. harzianum*. The results shown some variation in cancer bush response when treated with the different dormancy treatments.

Reports indicates that most seeds belonging to the family Fabaceae possess physical dormancy which prevents water and oxygen permeability thus delaying seed germination in such species (Ali et al., 2011). The results obtained from this study also confirm that cancer bush from the same family possess a physical dormancy which was broken by the different treatments, and as the results from untreated seeds was relatively low. This supports even more of the findings by Esan et al. (2021) who found that seeds of wild plant species including cancer bush are dormant relative to the cultivated plant species. Dormant seeds are alive but fail to germinate under conditions that are favourable for non-dormant seeds of the same species (Larson, 2002).

The results of this study revealed that mechanical scarification effectively improved seed germination relative to all other seed treatment methods. Other studies on leguminous seeds or seeds belonging to the Fabaceae family show that mechanical scarification is a very effective method for breaking dormancy of such species and improve the germination (Patane & Gresta, 2006). In species such as *Helianthemum* occurring in arid and semi-arid environments, the hand scarification of seeds was able to significantly improve germination (Pérez-García & Gonzalez-Benito, 2006). Patane and Gresta (2006); Travlos, Economou and Karamanos (2007) both reported that mechanical scarification of seeds is effective for breaking dormancy of leguminous seeds that are native in arid and semi-arid conditions and thus improving seed germination. The ability of the hand scarification method to improve germination is evidence that cancer bush seeds exhibit exogenous dormancy imposed by the hard seed coat.

The physical dormancy that many leguminous seeds exhibit can be broken or eliminated by exposing seeds to concentrated acids such as sulphuric acid (Nadjafi et al., 2006), and its use in breaking dormancy vary depending on the plant species (Uzun & Aydin, 2004). Studies that were conducted on other seeds of the same family, *Cassia occidentalis, C. obtusifolia, Indigofera astragalina, I. tinctoria, I. senegalensis, Tephrosia purpurea* and *Sesbania pachycarpa* (Sy, Grouzis & Danthu, 2001), *Parkia biglobosa* (J acq. Benth) (Aliero, 2003), *Astragalus hamosus* and *Medicago orbicularis* (Patane & Gresta, 2006), *Tylosema esculentum* (Buech) L. Schreib (Travlos, 2007), *Senna alata* (L.) Roxb. (Arowosegbe, 2016), *Senna alata* (Esan et al., 2021) revealed that immersion of seeds to concentrated H_2SO_4 resulted in improved final germination percentage of the dormant seed. The mechanism that led to improving the germination of the seed could have been the ability of the acid to break the hardened seed coat allowing water permeability and oxygen exchange (Ali et al., 2011). In this study, the

use of H_2SO_4 to some extent, was able to break seed dormancy giving the maximum germination of 13.3% which is still not effective enough in improving seed germination, yet it was significantly better than the untreated seeds. The results showed that increasing the concentration beyond 40 to 100% and the duration of exposure resulted very low final germination percentages to lower final germination percentages, lower than 13.3%. The factors that could have led to the decrease in germination percentage might be the loss of seed viability due to damaged embryo after seed immersion in H_2SO_4 (Pipinis et al., 2017). According to Pipinis et al. (2017), when the duration of acid scarification is prolonged, a significantly reduction in germination percentage will occur due to loss of seed viability. A response observed by Bhardwaj et al. (2016) is that any increase or decrease in the duration of *R. webbianum, C. carvi, S. lappa* and *B. persicum* seed immersion in the acid led to a significant reduction in germination because of damaging effects of acid on the embryo's vital parts.

Seed soaking in water at room temperature, irrespective of the duration of soaking, had no significant effect on the final germination percentage, however, when seeds were soaked in water at elevated temperatures it resulted in significantly higher final germination percentage. In this study it was observed that the treatment of 80°C, irrespective of time yielded the highest germination and anything beyond that resulted in subsequent reduction in final germination percentage. Same with the time at 4min, irrespective of the temperature improved germination. The results agree to the findings of Arowesebe (2016) who reported that increasing the water temperature improved germination. Aliero (2003) and Muhammad (2018) both reported hot water soaking act on the seed coat walls causing it to rapture and allow water to penetrate the tissues inside the seed causing physiological changes and improve germination. However, this was different when seeds of *A. muricata* were exposed to hot water baths which had no effect on germination percentage, irrespective of the duration of exposure (Dada et al., 2019). Pre-sowing treatments that produced the highest germination percentage include scarification, and hot water which also resulted in increased germination speed. All other priming methods including the cold-water soaking, NaCl, and *Trichoderma harzianum* resulted in a slightly higher final germination percentage compared to the control, however, they were not effective in improving the germination of cancer bush seeds.

CONCLUSIONS

Among all the various pre-sowing treatments used, hot water at 80°C, irrespective of the duration of exposure improved seed germination and were effective when compared to other seed treatment methods. However, mechanical scarification was able to completely break seed dormancy resulting in the best of all methods with a 100% germination. Medicinal plants are able to survive environmental conditions that are harsh or unfavourable, such as heat stress or mechanical damage which occurs in their natural habitat, and this is shown by the ability of the seed to germinate when exposed to elevated water temperatures or scarified with a sandpaper. Their ability to withstand such condition is of ecologically importance to them, such that it allow the seeds to accumulate into the soil increasing chances that some of the seeds will germinate producing new populations. However, this survival strategy is not effective when fast and constant seed germination is required, it is a limiting factor when the crop is to be cultivated. Based on the results it is suggested that cancer bush has physical dormancy caused by impervious seed coat. The two scarification treatments will therefore be good and recommended for good crop field establishment and obtaining uniform plant population. All other techniques are not effective practice as they resulted in seed germination of less than 20%.

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