Bodede et al., Afr J Tradit Complement Altern Med. (2015) 12(5):70-73
http://dx.doi.org/10.4314/ajtcam.v12i5.10

GERMINATION RESPONSE OF *ZANTHOXYLUM CAPENSE* (SMALL KNOBWOOD) SEEDS TO DIFFERENT PRE-TREATMENT PROTOCOLS

Olusola Bodede[1], Shakira Shaik[2*], Roshila Moodley[1]

[1]School of Chemistry, University of KwaZulu-Natal, Westville Campus. Private Bag X54001, Durban, 4000, South Africa. [2]School of Life Sciences, University of KwaZulu-Natal, Westville Campus. Private Bag X54001, Durban, 4000, South Africa.
* Corresponding author **E-mail**: shaiksh@ukzn.ac.za

Abstract

Background: *Zanthoxylum capense* is an important medicinal species in South Africa. It is propagated by seeds which are dispersed by different animals but the seeds rarely germinate even under favourable germination conditions which could be the result of dormancy. In addition, the species is currently listed as threatened in the Red List of South African Plants; therefore, it is of importance to find ways of promoting its propagation.
Materials and Methods: The germination of *Z. capense* seeds in response to different scarification and stratification pre-sowing treatments was studied. Seed stratification included chilling at 4^{o}C. Seed scarification was performed mechanically, using hot and cold water, and using hydrochloric acid and sulphuric acid.
Results: The highest final germination percentage (FGP) (71.1%) was obtained from seeds soaked in hot water for 15 minutes and grown in dark conditions. The 30 days chilling treatment also gave a good response (57.8%) when grown under light or dark conditions. Other FGPs of note included seeds soaked with 500 ppm gibberellic acid (GA_3) (60%, light; 44.4%, dark) and 1000 ppm GA_3 (46.7%, light; 48.9%, dark) and soaking in H_2SO_4 for 5 minutes (42.2%, dark). Overall, the seeds sown under dark conditions produced better FGPs than those sown in light.
Conclusion: These results reveal that *Z. capense* seeds display combinational dormancy that imposed physically by the seed coat and that imposed physiologically by the embryo. These dormancy traits can be easily overcome by either chilling or soaking in hot water or GA_3.

Key words: Dormancy, scarification, germination, chilling, hot water treatment.

Introduction

The genus *Zanthoxylum* (Rutaceae) comprises approximately 200 species distributed globally. It includes the medicinally important *Zanthoxylum capense*, native to South Africa (Steyn et al., 1998). It is widely distributed across eastern and northern South Africa (van Wyk et al., 1997) and is also found in the flora of Mozambique and Zimbabwe (Luo et al., 2010). *Z. capense* is a small multi-branched tree about 4-10 m high with the bark armed with thorny prickles on younger branches which develop into cone-shaped knobs often scattered on mature trunks (Schmidt et al., 2002). The leaves are alternate and unevenly compound with 4-8 pairs of leaflets which have a strong smell of citrus when crushed (Schmidt et al., 2002). Ethno-botanically, the root or stem bark has been used to treat mouth ulcers and tooth ache (Steyn et al., 1998). Decoction of the leaves or root has found application in the treatment of fever, influenza, bronchitis, colds and infertility (Steyn et al., 1998; Steenkamp, 2003). Other uses include the treatment of syphilis, gastro-intestinal disorders, flatulent colic and epilepsy (van Wyk et al., 1997; Amabeoku and Kinyua, 2010). In addition to the wide array of anecdotal uses, recent scientific submissions have revealed the anticonvulsant (Amabeoku and Kinyua, 2010), anti-tubercular (Luo et al., 2013) and anticancer (Mansoor et al., 2013) potential of *Z. capense*.

Z. capense is propagated by seeds which are primarily dispersed by monkeys, birds and insects but they rarely germinate even when provided with favourable germination conditions (Netshiluvhi, 1996). This could be the result of dormancy, a phenomenon which describes the absence of germination of a viable and intact seed (Hilhorst, 1995). Many species use seed dormancy as a survival strategy (Li and Foley, 1997) but this could contribute to the declining numbers of many important plants. *Z. capense* is currently listed by the South African National Biodiversity Institute among the threatened species in the Red List of South African Plants (Raimondo et al., 2009). The survival of this species is at risk probably as a result of hard-seeded-ness and over-exploitation by medicinal herb collectors.

Seed dormancy could be imposed either by the seed coat (physical/exogenous) or the embryo (physiological/endogenous) (Bewley and Black, 1994). Over the years, extensive studies on dormancy breaking for the enhancement of seed propagation have brought about the application of diverse seed pre-treatments. Mechanical scarification, used to overcome impermeability of seed coats, can be carried out by rubbing the seed between two pieces of sandpaper (Faria et al., 2012). Cold stratification and chemical scarification have been reported to also stimulate germination (Baskin and Baskin, 2004). Sometimes, seeds with hydrophilic germination-inhibiting chemicals in their seed coats are subjected to leaching with water (Baskin and Baskin, 2004). Seed germination in several *Zanthoxylum* species has been reportedly poor and this is ascribed mainly to the dormancy imposed by the hard seed coat (Bonner, 1974, Sanon et al., 2005). *Z. capense* produces hard seeds but an extensive search of the literature did not produce any reports on whether the seeds are exogenously dormant. Therefore, the aim of this work was to establish the best pre-treatment protocol to overcome dormancy and enhance seed germination in *Z. capense*.

Materials and Methods

Seeds of *Z. capense* were procured from Silver Hill Seeds (Cape Town, South Africa) in October 2013. The seeds were preserved in air-tight containers under ambient laboratory temperature (18^{o}C) prior to the commencement of the experiment. The seed moisture content was 11.20%.

This was obtained by the difference in the fresh and dry weight as a percentage of the fresh weight of 100 seeds. Various pre-sowing treatments were used including scarification and stratification. Seeds were stratified using chilling by placing intact seeds on moist filter paper in sterile Petri plates at 4^oC for 7 and 30 days. Mechanical scarification was achieved by rubbing intact seeds over sandpaper for 8 to 10 seconds or long enough to slightly expose the embryo but not damage it. For water scarification, intact seeds were soaked separately in room temperature distilled water for 6 and 24 hours. Also, seeds were soaked separately in hot distilled water (80^oC) for 5 and 15 minutes. In the acid scarification treatments, intact seeds were separately soaked in 32% hydrochloric acid (HCl) for 10 and 30 minutes and in 98% sulphuric acid (H_2SO_4) for 1 and 5 minutes. In addition, mechanically scarified seeds were soaked separately for 24 hours in gibberellic acid (GA_3) (500 and 1000 ppm) and for 24 hours in potassium nitrate (KNO_3) (1% and 4%). Intact seeds that were not pre-treated were used as a control.

After pretreatments, all seeds were surface-sterilized by washing in sterile distilled water with a few drops of Tween 20 for 3 minutes and then rinsed in sterile distilled water for 20 minutes. This was followed by a 60 second rinse in 70% ethanol and then two sterile distilled water rinses. The seeds were then transferred into a 3.5% sodium hypochlorite solution for 10 minutes and finally rinsed three times in sterile distilled water before sowing onto 1% agar plates. There were 16 treatments with 6 replicates in each. Each replicate had 15 seeds. Three plates per treatment were incubated in a 16-hour photoperiod supplied by a fluorescent white light at 40 µmol m^{-2} s^{-1}, while the other three were kept in the dark (the plates were wrapped with foil). All the plates were incubated in a growth room at 23^oC for 30 days. Germinated seeds were counted every 3 days (seeds in the dark were counted under a green light). A seed was considered germinated following a visible emergence of the radicle. The final germination percentage was recorded after day 30 in culture. Final germination percentages were subjected to one-way analysis of variance (ANOVA) followed by Tukey's Post-Hoc test using the Statistical Package for the Social Sciences (SPSS version 21) to determine if there were significant ($P< 0.05$) differences among the treatment means.

Results and Discussion

The final germination percentage (FGP) across all treatments was in the range of 6.7 to 71.1% (Table 1). The FGP of the control in the light was higher (26.7%) than the dark (17.8%). However, out of the 16 treatments, 11 treatments had higher FGPs in the dark compared to the light. Fourteen treatments had higher FGPs compared to the control in the dark, while 10 treatments had higher FGPs than the control in the light. In addition, 12 treatments in the dark had higher FGPs than either the dark or light control. The highest FGP was achieved in the 15 minutes hot water soak in the dark (71.1%) which was significantly different from the control (17.8%). In the light, the highest FGP was achieved in 500 ppm GA_3 (60%), although this was not significantly different from the FGP for 30 days chilling (57.8%). The thirty day chilling was the only treatment that had equal FGP in both light and dark conditions (57.8%). The acid-treated seeds resulted in low germination both in the HCl 10 minutes (6.7%, light; 20%, dark) and 30 minutes (13.3%, light; 15.6%, dark) while those of H_2SO_4 were higher, particularly the H_2SO_4 5 minutes (28.9%, light; 42.2%, dark). There was no significant difference between the KNO_3 treated seeds and the control in both light and dark conditions. Both 1% and 4% KNO_3 had equal FGPs in the dark (33.3%). Seeds soaked for 6 hours in cold water had higher FGPs than the control in both light and dark, while seeds soaked in cold water for 24 hours had a higher FGP than the control in the dark only. Mechanically scarified seeds had higher FGPs (42.2, light; 55.6%, dark) than the control but this difference was only significant under dark conditions.

Table 1: Effect of different pre-treatments on the final germination percentage (FGP) of *Zanthoxylum capense* seeds after a 30 day culture period in the light and dark.

Treatment	**Average Final Germination Percentage**	
	Light	Dark
Control	26.7abcd	17.8b
Chilling 7 days	46.7acd	42.2abcd
Chilling 30 days	57.8d	57.8cd
Cold Water 6 h	35.6abcd	28.9abc
Cold water 24 h	20.0abc	31.1abc
GA_3 500 ppm	60.0d	44.4abcd
GA_3 1000 ppm	46.7acd	48.9abcd
HCl 10 min	6.7b	20.0be
HCl 30 min	13.3ab	15.5b
H_2SO_4 1 min	13.3ab	22.2abc
H_2SO_4 5 min	28.9abcd	42.2abcd
Hot Water 5 min	42.2acd	53.3cde
Hot Water 15 min	53.3cd	71.1d
KNO_3 1%	26.7abcd	33.3abc
KNO_3 4%	28.9abcd	33.3abc
Mechanical Scarification	42.2acd	55.6acd

Numbers with different letters (a–e) are significantly different by Tukey's Post Hoc Test at $p < 0.05$

The conditions that determine and regulate germination and survival of seedlings cannot be over emphasized as key factors that influence the expansion or extinction of populations (Rasmussen and Whigham, 1998). Seed dormancy, usually a survival strategy employed by plants, is one

of the important phenomena that can affect successful expansion of a species. Thus, pre-treatment of seeds prior to sowing has gained much attention in seed germination studies. It is evident from the findings of this work that the best pre-sowing treatment for seeds of *Z. capense* is soaking them in hot water to promote germination. Hot water pre-treatment promotes germination by influencing factors such as permeability of the seed coat to water and gases, and the release of germination inhibitors (Sharma et al., 2008). It is likely that the hot water softens the hard seed coat of *Z. capense* making it more conducive to water and gas uptake. Germination-inhibiting chemicals (like phenolic) may have been leached with the water. It is unknown whether *Z. capense* seeds are covered by an oil film as is the case with *Zanthoxylum gilletii* and some other species of Rutaceae (Okeyo et al., 2011). If so, decontaminating in NaOCl and pre-treating in hot water may have removed the oil film thereby promoting germination. Germination has been reported to be appreciably increased in other species by hot water treatment (Fariman et al., 2011; Gupta and Bandopadhyay, 2013; Irvani et al., 2012; Missanjo et al., 2013).

Cold stratification is known to promote seed germination by causing an increase and a decrease in the endogenous GA_3 and the abscisic acid (ABA) concentrations, respectively (Diaz and Martin, 1972). The latter has been reported to be involved in the control of dormancy whilst GA_3 promotes germination (Nicolás et al., 1996). It could be assumed that GA_3 production was enhanced by low temperature during cold stratification of *Z. capense* seeds in this study hence promoting its germination. Furthermore, the FGP of the chilling treatments were not significantly different to that of the GA_3 treatments. This finding agrees with the earlier study on seed germination in *Pistacia khinjuk* seeds in which it was suggested that chilling treatments could be replaced by GA_3 treatments to overcome dormancy (Baninasab and Rahemi, 2008). It is also likely that chilling promotes germination of *Z. capense* seed by activating the mobilization system of the embryo's reserve food (Davies and Slack, 1981). These results suggest non-deep physiological dormancy in *Z. capense* seeds. The positive response of *Z. capense* seeds to cold stratification in this study is consistent with a few *Zanthoxylum* species and some other genera (Bonner, 1974, Fariman et al., 2011, Gupta and Bandopadhyay, 2013, Irvani et al., 2012, Soltani, 2003).

In this study, mechanical scarification also promoted germination. The hard seed coat of *Z. capense* contributes largely to its germination barrier which mechanical scarification has appreciably overcome. The seed coat crack is assumed to ease the permeability of the pericarp to water and oxygen (Dewir et al., 2011) and also facilitate the emergence of the radicle (Bewley, 1997). These results are similar to those obtained from some other *Zanthoxylum* species (Etsè et al., 2011, Pérez, 2001). Mechanically scarified seeds of *Sabal palmetto* and *Thrinax morrisii* palms were also reported to have higher FGPs than their controls (Dewir et al., 2011).

Acids and other chemicals can break dormancy partially or completely, although their effects depend on the duration of the treatments and their respective concentrations. These include acids like H_2SO_4 and HCl; peroxides like H_2O_2 and $KMnO_4$; nitrogen compounds like KNO_3 and $NaNO_3$; and plant growth regulators like BA, GA_3, GA_7 and kinetin (Dewir et al., 2011, Yang et al., 2007). Nevertheless, seeds of some plant species either do not respond or demonstrate a poor response to them. Acid treatments did not significantly promote germination of *Z. capense* seeds in the current study. The higher FGP observed in H_2SO_4 5 minutes may be due to longer time exposure compared with the H_2SO_4 1 minute and the control which probably helped release chemical inhibitors. This shows that exposure time and concentration influences the effectiveness of acid scarification in breaking seed dormancy. Seed germination in many plant species is well known to be increased by pre-treating with GA_3 (Bewley and Black, 1994; Phillips et al., 2003, 2012). In this study, GA_3 also promoted germination possibly by increasing the embryonic physiological activities (Miransari and Smith, 2014).

` There are only a few reports on the conditions required for effective seed propagation in the genus. The present study presents a simple, economical approach to promote germination in *Z. capense* using either hot water or cold stratification treatments. It is concluded that *Z. capense* seeds display both exogenous and non-deep physiological dormancy which require pre-treatment to promote germination.

Acknowledgements

We are sincerely grateful to the University of KwaZulu-Natal for financial support.

References

1. Amabeoku, G. and Kinyua, C. (2010). Evaluation of the anticonvulsant activity of *Zanthoxylum capense* (Thunb.) Harv. (Rutaceae) in mice. Int. J. Pharmacol. 6: 844-854.
2. Baninasab, B. and Rahemi, M. (2008). The effect of scarification, cold stratification and gibberellic acid treatment on germination of Kholkhong seeds. J. Plant Sci. 3: 121-125.
3. Baskin, J.M. and Baskin, C.C. (2004). A classification system for seed dormancy. Seed Sci. Res. 14: 1-16.
4. Bewley, J.D. (1997). Seed germination and dormancy. The Plant Cell. 9: 1055.
5. Bewley, J.D. and Black, M. (1994). Seeds: physiology of development and germination. Plenum Press. New York.
6. Bonner, F.T. (1974). *Zanthoxylum* L. Seeds of woody plants of the United States. United States Department of Agriculture Handbook, pp 450.
7. Davies, H. and Slack, P. (1981). The control of food mobilization in seeds of dicotyledonous plants. New Phtyologist. 88: 41-51.
8. Dewir, Y.H., El-Sayed, E.M. and Naidoo, Y. (2011). Effect of some mechanical chemical treatments on seed germination of *Sabal palmetto* and *Thrinax morrisii* palms. Aust. J. Crop Sci. 5: 248-253.
9. Diaz, D.H. and Martin, G.C. (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. J. Am. Soc. Hort. Sci. 97: 651-654.
10. Etse, K.D., Aidam, A.V., De Souza, C., Creche, J. and Lanoue, A. (2011). *In vitro* propagation of *Zanthoxylum zanthoxyloides* Lam., an endangered African medicinal plant. Acta Bot. Gallica. 158: 47-55.
11. Faria, D., Lima, A. and Da Silva, N.C. (2012). Effect of scarification and nutrient mineral concentrations on the *in vitro* germination of *Senna macranthera* (Collad.) H.S. Irwin & Barneby seeds. Emir. J. Food Agricult. 24: 302-307.

12. Fariman, Z.K., Azizi, M. and Noori, S. (2011). Seed germination and dormancy breaking techniques for *Echinacea purpurea* L. J. Biol. Environ. Sci. 5: 7-10.
13. Gupta, S. and Bandopadhyay, A. (2013). Overcoming seed dormancy of *Acalypha indica* L. (Euphorbiaceae): an important medicinal plant. Indian J. Plant Sci. 2: 72-79.
14. Hilhorst, H.M. (1995). A critical update on seed dormancy I. Primary dormancy. Seed Sci. Res. 5: 61-73.
15. Irvani, N., Solouki, M., Omidi, M., Saidi, A. and Zare, A. (2012). Seed germination and dormancy breaking in *Dorema ammoniacum* D., an endangered medicinal plant. Trakia J. Sci. 10: 9-15.
16. Li, B. and Foley, M.E. (1997). Genetic and molecular control of seed dormancy. Trends Plant Sci. 2: 384-389.
17. Luo, X., Pinto, A., Duran, A., Mansoor, T., Mulhovo, S., Duarte, N. and Ferreira, M. (2010). Chemical constituents of *Zanthoxylum capense*. Planta Med. 76: 475.
18. Luo, X., Pires, D., Ainsa, J.A., Gracia, B., Duarte, N., Mulhovo, S., Anes, E. and Ferreira, M.U. (2013). *Zanthoxylum capense* constituents with antimycobacterial activity against *Mycobacterium tuberculosis in vitro* and *ex vivo* within human macrophages. J. Ethnopharmacol. 146: 417-422.
19. Mansoor, T.A., Borralho, P.M., Luo, X., Mulhovo, S., Rodrigues, C.M. and Ferreira, M.U. (2013). Apoptosis inducing activity of benzophenanthridine-type alkaloids and 2-arylbenzofuran neolignans in HCT116 colon carcinoma cells. Phytomedicine 20: 923-929.
20. Miransari, M. and Smith, D. (2014). Plant hormones and seed germination. Environ. Exp. Bot. 99: 110-121.
21. Missanjo, E., Maya, C., Kapira, D., Banda, H. and Kamanga-Thole, G. (2013). Effect of seed size and pretreatment methods on germination of *Albizia lebbeck*. ISRN Botany, 2013, Article ID 969026, doi:10.1155/2013/969026.
22. Netshiluvhi, T.R. (1996). Aspect of seed propagation of commonly utilized medicinal trees of KwaZulu-Natal. PhD Dissertation, University of Natal.
23. Nicolás, C., Nicolás, G. and Rodriguez, D. (1996). Antagonistic effects of abscisic acid and gibberellic acid on the breaking of dormancy of *Fagus sylvatica* seeds. Physiol. Plant. 96: 244-250.
24. Okeyo, M., Ochoudho, J., Muasya, R. and Omondi, W. (2011). Investigation on the germination of *Zanthoxylum gilletii* (African satin wood) seed. *Innovations as Key to the Green Revolution in Africa*, 683-691, Springer, Netherlands.
25. Perez, H.E. (2001). Germination of six Florida native endangered species. Thesis (M.S.), University of Florida.
26. Phillips, N., Drost, D. and Varga, W. (2003). Chemical treatments enhance seed germination *Perideridia gairdneri*. Acta Horticult. 618: 477-482.
27. Pipinis, E. et al., (2012). Effect of pretreatments on seed germination of *Prunus mahaleb* L. Not. Bot. Horti. Agrobot. Cluj-Napoca. 40: 183-189.
28. Raimondo, D., Staden, L.V., Foden, W., Victor, J., Helme, N., Turner, R., Kamundi, D. and Manyama, P. (2009). *Red List of South African Plants 2009*, South African National Biodiversity Institute.
29. Rasmussen, H.N. and Whigham, D.F. (1998). The underground phase: a special challenge in studies of terrestrial orchid populations. Bot. J. Linn. Soc. 126: 49-64.
30. Sanon, M.D., Gamene, C.S., Sacande, M., Neyer, O., Joker, D., Dulloo, M. and Thomsen, K. (2005). Desiccation and storage of *Kigelia Africana*, *Lophira lanceolata*, *Parinari curatellifolia* and *Zanthoxylum zanthoxyloides* seeds from Burkina Faso. *Comparative Storage Biology of Tropical Tree Seeds*, 16-23, International Plant Genetic Resources Institute (IPGRI), Rome.
31. Schmidt, E., Lotter, M. and Mccleland, W. (2002). Trees and shrubs of Mpumalanga and Kruger national park, Jacana Media, Johannesburg.
32. Sharma, S., Naithani, R., Varghese, B., Keshavkant, S. and Naithani, S.C. (2008). Effect of hot-water treatment on seed germination of some fast growing tropical tree species. J. Trop. Forest. 24: 49-53.
33. Soltani, A. (2003). Improvement of seed germination of *Fagus orientalis* Lipsky. Acta Universitatis Agriculturae Sueciae: Silvestria. 275: 1-15.
34. Steenkamp, V. (2003). Traditional herbal remedies used by South African women for gynaecological complaints. J. Ethnopharmacol. 86: 97-108.
35. Steyn, P., Van Den Heerver, J., Vosloo, H. and Ackerman, L. (1998). Biologically active substances from *Zanthoxylum capense* (Thunb.) Harv. S. Afr. J. Sci. 94: 391-393.
36. Van Wyk, B.E., Oudtshoorn, B.V. and Gericke, N. (1997). Medicinal plants of South Africa. Briza Publications, Pretoria.
37. Yang, Q., Ye, W. and Yin, X. (2007). Dormancy and germination of *Areca triandra* seeds. Sci. Horticult. 113: 107-111.