Breaking dormancy in *Macroptilium* and *Clitoria* species for routine viability monitoring of genebank collections

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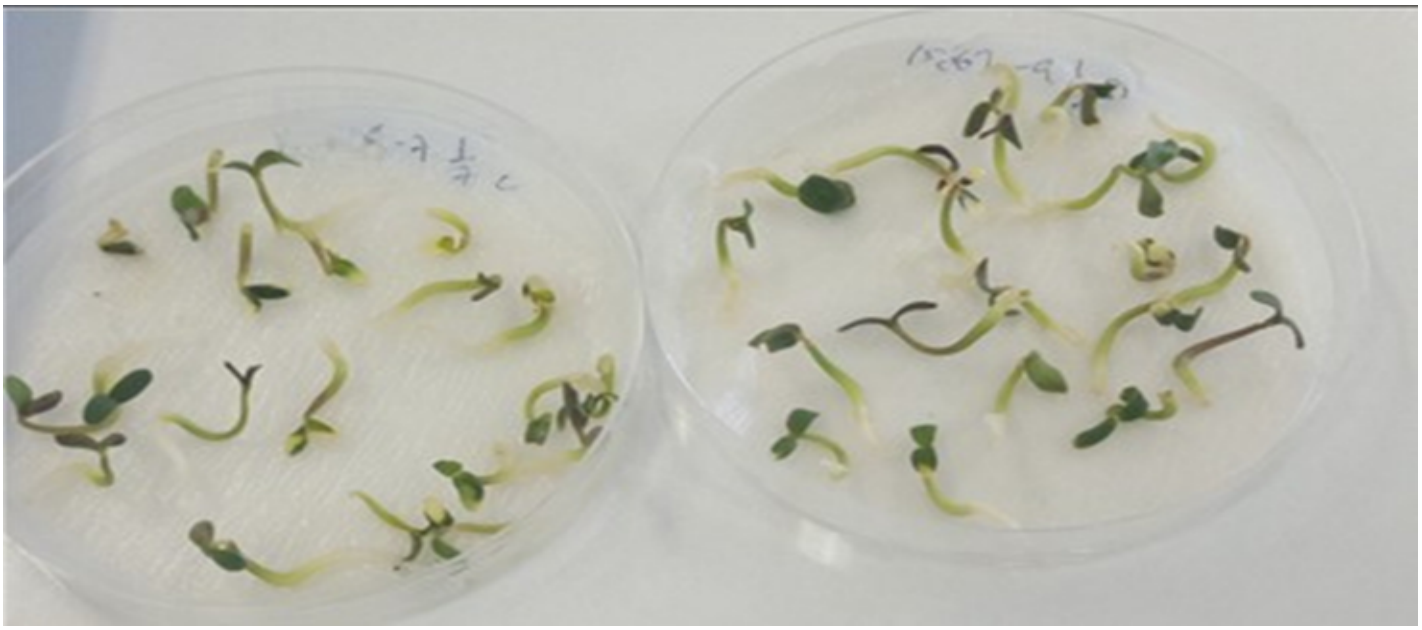

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Introduction

The International Livestock Research Institute (ILRI) genebank preserves a large collection of legumes, grasses and browses. The accessions are maintained and made available for use in agriculture and research to enhance food security. The collections conserved in the genebank need to be monitored to determine viability status and make the right decision before the viability falls below the threshold. However, most of the collections represent wild species and, unfortunately, there is limited information on their viability monitoring and dormancy status (Sartie et al. 2020). Monitoring the viability of such wild species is typically more challenging than other crop species, due to the higher prevalence of seed dormancy (FAO 2014).

Macroptilium and *Clitoria* are important perennial legume species that play a crucial role in sustainable agriculture. Both species serve as a valuable source of high protein feed for livestock. Their nutritional profile makes them an excellent feed option, contributing to improved animal growth and productivity. Additionally, these legumes can thrive in various environmental conditions, making them adaptable choices for farmers looking to enhance the quality of their pastures and provide superior nutrition to their livestock.

However, like many other forage species, they have a high seed coat dormancy problem (Rusdy 2016; Silva et al. 2020; Pincay-Ganchozo et al. 2021), challenging the growers and seed quality analysts. Both species exhibit physical dormancy, posing a major difficulty when assessing viability in genebank. The limited water absorption capacity of these species blocks the imbibition process, obstructing embryo growth and delaying germination, which results in poor field establishment. To enhance the percentage of normal seedlings a wide range of treatments and several experiments were done to break the physical dormancy in many forage species (Gresta et al. 2011; Baskin and Baskin 2014; Rusdy 2017; Olbana et al. 2023).

However, information on dormancy breaking methods for some forage species was scanty and there is a need to optimize the germination test protocol to monitor collections in the genebank. Also, most of the dormancy breaking methods were time consuming, labour intensive and difficult to work with when a large number of samples needed to be scarified. Therefore, the present study aimed to investigate the best scarification methods for dormancy breaking to enhance germination and to examine the possibility of release from dormancy during storage in *Macroptillium* and *Clitoria* species seeds stored under genebank conditions in medium-term storage (MTS).

Methodology

Experimental procedures

Ten accessions of *Macroptillium* and *Clitoria* were selected based on the quantity of seeds stored at medium-term storage from the genebank (Table 1). The experiment included four pretreatment methods. The pretreatment methods were 98% concentrated sulphuric acid treatment (15 and 20 minutes for *Macroptilium*, 5 and 10 minutes for *Clitoria*), hot water treatments at 80°C ±5 for 4 and 6 minutes, sandpaper scarification (Diamond GLASS Paper No. $1^{1/2}$) and dry heat treatments at 80°C (15 and 20 minutes). For both species, untreated seeds were used as a 'control' to compare against treated seeds.

The concentrated sulphuric acid treatment was carried out by soaking the seeds in the prepared solution for the allocated time and then washing the seeds repeatedly under cold running water and redrying. For hot water treatment, the samples were immersed in a water bath (Gallenkamp BKS-350-010 220/240 V) heated at 80 ±5°C for the given minutes using a muslin cloth and cooling at room temperature. Sand scarification was done using glass paper by rubbing the seed surface to soften the seed coat to facilitate water imbibition. The dry heat treatment was done in the oven at a given temperature for a given minute.

Table 1. List of accessions used in the experiment for dormancy breaking

Species	Acc. No.†	Lot No.	DOI
Macroptilium atropurpureum	111	6	10.18730/FQ18=
Macroptilium atropurpureum	396	7	10.18730/G4JWU
Macroptilium atropurpureum	9275	2	10.18730/G7S4F
Macroptilium bracteatum	7280	4	10.18730/G6B0K
Macroptilium bracteatum	11101	3	10.18730/FQ1B1
Macroptilium gracile	14558	7	10.18730/FSZGH
Macroptilium gracile	16693	4	10.18730/FVR6J
Macroptilium lathyroides	8	4	10.18730/G6V0D
Macroptilium lathyroides	6955	12	10.18730/G640H
Macroptilium lathyroides	9215	1	10.18730/G7QN5
Clitoria ternatea	7261	2	10.18730/G6AF2
Clitoria ternatea	9291	4	10.18730/G7SJX
Clitoria ternatea	9294	4	10.18730/G7SN*
Clitoria ternatea	9296	10	10.18730/G7SQ$
Clitoria ternatea	11000	5	10.18730/FPZ88
Clitoria ternatea	15435	1	10.18730/FTPQM
Clitoria ternatea	24656	5	10.18730/G3K9R
Clitoria ternatea	24657	4	10.18730/G3KAS
Clitoria ternatea	24678	3	10.18730/G3KZ9
Clitoria ternatea	24734	15	10.18730/G3NRX

† Refers to ILRI genebank accession number.

Germination test and seedling evaluation

Germination test was conducted using four replicates of 20 seeds each. Seeds were arranged on Whatman Grade 4 filter paper (TISCH Scientific) moistened with distilled water held in 90 mm diameter Petri dishes. The Petri dishes with seeds were incubated at a constant temperature of 25°C with 12 hours light/12 hours dark. The first germination count was made five days after sowing and the final count was made on the fifteenth day. At the final seedling evaluation, the hard seeds were dissected using a scalpel blade and the embryo was visually observed to check whether it was alive or not. The evaluation of seedlings as normal, abnormal, dead, hard and fresh ungerminated was done following ISTA guidelines (ISTA 2019).

Results

Dormancy breaking treatment had a significant effect on seed germination and seedling growth of *Macroptilium* species accessions. All pretreatments showed significant differences in all accessions tested. Dry heat treatment had a positive effect on the germination percentage of *Macroptilium* species compared to other treatments

(Figure 1). A higher percentage of dead seeds was observed for the seeds treated with concentrated H_2SO_4 and hot water.

Similarly, for *Clitoria* species, the dormancy breaking treatments showed significant differences in the percentage of normal seedlings germinated compared to untreated seeds (Figure 2). The sandpaper, hot water (4 minutes) and H_2SO_4 (5 minutes) treatments showed good germination and these treatments considered as best scarification methods for this species. The concentrated H_2SO_4 treatment for 15 minutes resulted in a moderate germination percentage and a low percentage of hard seeds; however, it also caused significant damage to the seeds. The control (untreated) seeds did not show good germination result and the seeds remained hard at the final evaluation in both species.

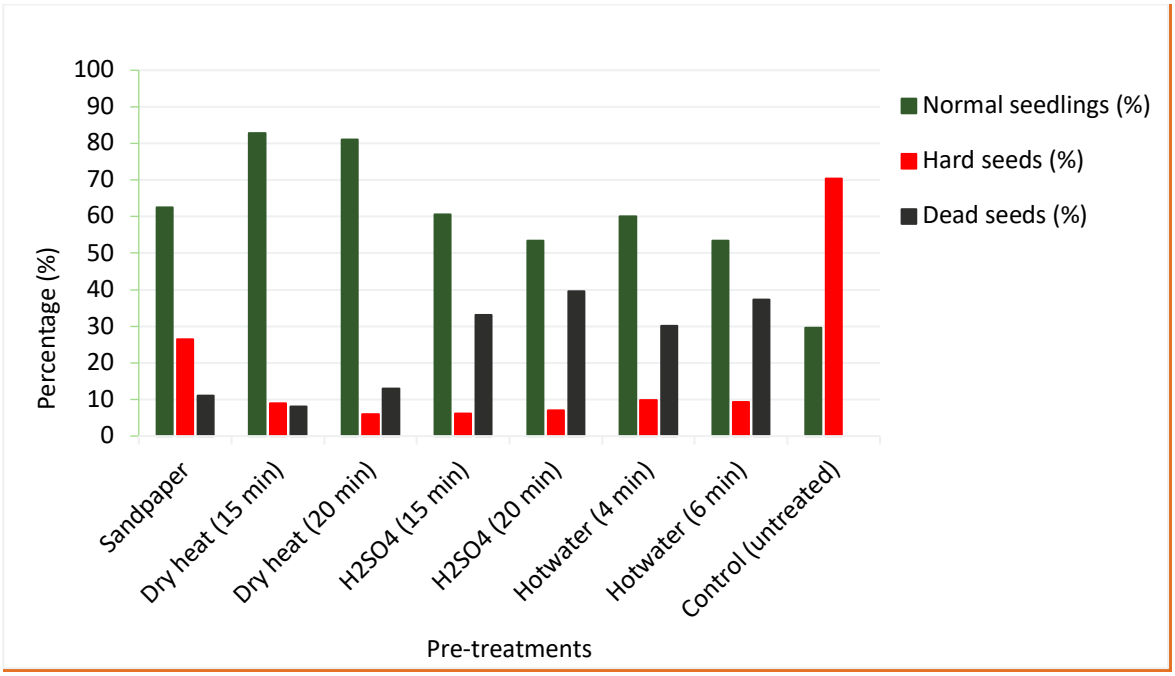

Figure 1. Effect of different pretreatment methods on the percentage of normal seedlings, hard seeds and dead seeds/abnormal seedlings in *Macroptilium* species.

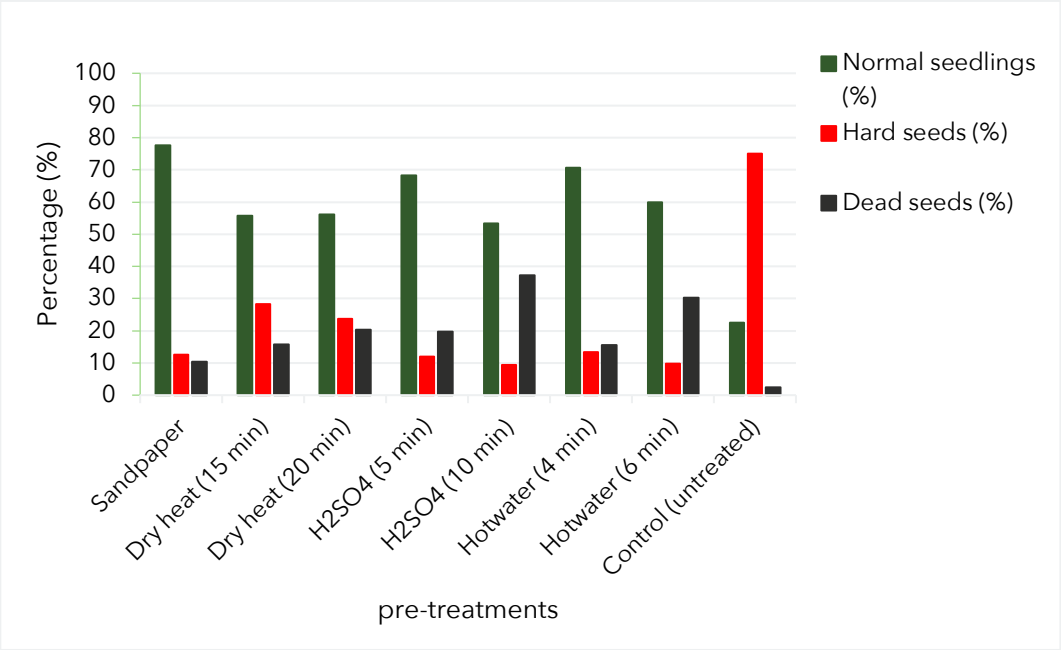

Figure 2. Effect of different pretreatment on the percentage of normal seedlings, hard seeds and dead seeds/abnormal seedlings in *Clitoria* species.

Discussion

Dormancy breaking treatments are important in enhancing seed germination and seedling development in crops. Different methods have been used to overcome seed coat dormancy in forage species. In this study, different pretreatment methods were explored for breaking dormancy in *Marcoptilium* and *Clitoria* spp and the result showed significant variations in germination percentage and subsequent seedling growth among the pretreatments. Among the treatments, dry heat treatment for 15 to 20 minutes at 80°C emerged as the most effective approach for increasing the germination percentage in *Macroptilium* species.

For Clitoria spp, the use of sandpaper to abrade the seed coat, immersing the seeds in hot water for four minutes and concentrated sulfuric acid (H2SO4) treatment for five minutes have contributed positively to germination percentage. Various scarification methods have also been found to significantly enhance germination percentage for *Clitoria* species (Nagar and Meena 2015; Shobharani and Sundareswaran 2018; Pincay-Ganchozo et al. 2021).

In general, the seeds of *Marcoptilium* and *Clitoria* spp exhibited physical dormancy that can be alleviated by different pretreatments and the result from this study provides invaluable insight for improving seed quality management of collections stored in the genebank.

References

Baskin, C.C. and Baskin, J.M. 2014. *Seeds: Ecology, biogeography and evolution of dormancy and germination. 2nd ed.* San Diego, California, USA: Academic Press.

FAO. 2014. *Genebank standards for plant genetic resources for food and agriculture.* Rome, Italy: Food and Agriculture Organization of the United Nations (FAO).

Gresta, F., Avola, G., Onofri, A., Anastasi, U. and Cristaudo, A. 2011. When does hard coat impose dormancy in legume seeds? Lotus and Scorpiurus case study. *Crop Science* 51: 1739–1747. https://doi.org/10.2135/cropsci2010.12.0700.

ISTA. 2019. *International rules for seed testing.* Bassersdorf, Switzerland: International Seed Testing Association (ISTA). https://doi.org/10.15258/istarules.2019.f.

Nagar, R.P. and Meena, S.S. 2015. Effect of physical and chemical scarification and ageing on hardseededness in *Clitoria ternatea*. *Range Management and Agroforestry* 36(1): 79–83.

Olbana, T., Muchugi, A., Woldemariam, Y., Hay, F.R., Ndiwa, N. et al. 2023. Overcoming dormancy in *Desmanthus virgatus* seeds for improved viability monitoring protocols of genebank accessions. *Seed Science and Technology* 51(3): 421–434. https://doi.org/10.15258/sst.2023.51.3.12.

Pincay-Ganchozo, R.A., Luna-Murillo, R.A., Cunuhay, K.A.E. and Suárez, H.O.E. 2021. Chemical and biological scarification in the emergence and growth of *Clitoria ternatea*. *CABI Digital Library* 48(3): 53–59.

Rusdy, M. 2016. Enhancement of seed germination and seedling growth of Siratro (*Macroptilium atropurpureum*). *International Journal of Science and Research* 5(10).

Rusdy, M. 2017. A review on hardseededness and breaking dormancy in tropical forage legumes. *Livestock Research for Rural Development* 29. http://www.lrrd.org/lrrd29/12/muhr29237.html.

Sartie, A.M., Woldemariam, Y., Hanson, J. and Ndiwa, N. 2020. Developing improved methods for cost-effective viability monitoring of *Aeschynomene abyssinica* and *A. americana*. *Seed Science and Technology* 48(2): 221–230. https://doi.org/10.15258/sst.2020.48.2.09.

Shobharani, M. and Sundareswaran, S. 2018. Effect of different dormancy breaking treatments on seed germination and seedling growth in Shankhapushpi (*Clitoria Ternatea* L.). *Journal of Pharmacognosy and Phytochemistry* 7(4): 1353–1355.

Silva, M., Yamashi, O., Rossi, A.A.B., de Carvalho, M.A.C., da Silva, I.V. et al. 2020. Germination of *Macroptilium lathyroides* seeds as a function of the presence of salts on the substrate. *Ibero-American Journal of Environmental Sciences* v. 11(2).

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