Dormancy breaking of stored seeds of *Buchanania lanzan* Spreng. -An endangered medicinal plant of the Western Ghats

Ajith S., Krishna V.*, Sudhesh L. Shastri, Ravi kumar S.
Department of Biotechnology, Kuvempu University, Shankaraghatta, Shimoga, Karnataka, India 577 451
Address: Department of Biotechnology, Kuvempu University, Shankaraghatta, Shimoga, Karnataka, India 577 451

Abstract:

Buchanania lanzan Spreng. (*Anacardiaceae*) is an endangered medicinal tree of the Western Ghats of India, is at the verge of extinction due to the destruction of habitat, prolonged dormancy, uprooting of trees and over exploitation of seeds for its great medicinal value to cure diarrheal, diabetic and various skin diseases. An efficient method to standardize for breaking the dormancy of one year old stored seeds. Seeds are surface sterilized with 4% $HgCl_2$ for 10 min and seed coat is removed by gentle hammering. Scarification of seeds with different degree of hot and cold water and chemical treatment with various concentrations of H_2SO_4 and GA_3 was followed. Hundred Seeds each are sown in seed flats in greenhouse and germination was observed daily for a 30-day period. Treatment with 200ppm GA_3 and 4% H_2SO_4 are the efficient methods to break the dormancy of the seeds and resulted in 90% and 61% of germination respectively. In cold water treatment only 56% of seeds germinated, in control only 25% seeds germination in the untreated seeds and germination was not observed in the intact seeds with the impermeable seed coat. Results indicative of positive responses to treatments, while impermeable seed coats may be responsible for prolonged dormancy in intact control seeds.

Keywords: *Buchanania lanzan*, stored seeds, Dormancy breaking.

Introduction

Storage of seeds as *ex situ* germplasm is an essential step for the long-term conservation of plant genetic resources. Maintaining seed viability for a longer period is essential to preserve the genetic integrity of stored samples. *Buchanania lanzan* Spreng, commonly known as char, achar, and chironji, belongs to family *Anacardiaceae*. It was first described by Francis Hamilton in 1798. (Mahtab Zakira Siddiqui, 2014). It is endemic in the dry deciduous tropical forests of India and is an evergreen moderate-sized tree, with straight, cylindrical trunk, up to 10-15m height and tomatoes branches. Its bark is rough, dark grey or black, fissured into prominent squares, 1.25 to 1.75 cm thick, and is reddish inside. Flowering starts in the month of November and its leaves are coriaceous, broadly oblong with a rounded base. Fruit is a drupe containing a single seed, nuts are edible known as 'chironji', substituted for olive and almond oils, popularly known as wild almond.

In Western Ghats of Karnataka, India the plant grows on yellow sandy loam soil sand can be easily identified by its dark grey crocodile bark with the red blaze. Presently, this tree species is at the stage of extinction due to deforestation, a prolonged period of dormancy and over exploitation of seeds. Tribals of Karnataka state are using the seed oil as an edible oil (Mahtab Zakira Siddiqui, 2014). Seed coat extract is using to cure diarrhea and stem bark extract is using to cure diabetic wounds (Banerjee, s 2015) The major problem with *B. lanzan*is the availability of seeds only for a limited period (April) and there is a problem in the germination due to more prone to fungal and microbial invasion of the seeds. (Sharma et al. 1998). Another hindrance is the presence of a hard seed coat which leads to low germinating capability. Therefore, in order to ensure further supply of this commercially useful tree species, other breeding methods are required (Banerjee, et al.,2015) Dormant seeds does not germinate even under favourable conditions until it undergo series of changes favouring germination process (Kołodziejek J. 2015).Therefore the population

of this species is very scared and critically endangered (Rai, M. K. 2010).The Red Data Book published by International Union for Conservation of Nature and Natural Resources (IUCN) (Rai, P. K.2015) also recorded the threatening status of this species.

The breakage of these dormancy mechanisms is necessary for the completion of seed germination. Pre-treatment of dormant seeds by physical and chemical treatment to force germination could avoid the prolonged duration of seeds in germination. Many investigators were successful to break the dormancy of endemic and endangered species. (McDonnell.2012) Till present reporting, there is no publication available on the effect of long-term storage on seed germination in *B. lanzan.* The present study was undertaken with an aim to test the effect of physical and chemical treatments on breaking the dormancy of long-term storage seeds.

Materials and Method

Collection of the seeds

In the month of April 2017, freshly ripened seeds of *B. lanzana* were collected from a mixed population in Bhadra Wild Life Sanctuary reserve forest Shimoga district, Karnataka state, India. The seeds procured were pooled separately for each population and brought to the laboratory, cleaned thoroughly for impurities, and dried at room temperature for 15 days. Before further processing, all seeds for each population were mixed thoroughly to minimize effects of single source plant on germination and these seeds are coated with neem oil and air dried for 1 hour and stored in a plastic container for a future experiment in the lab condition.

Seed treatment

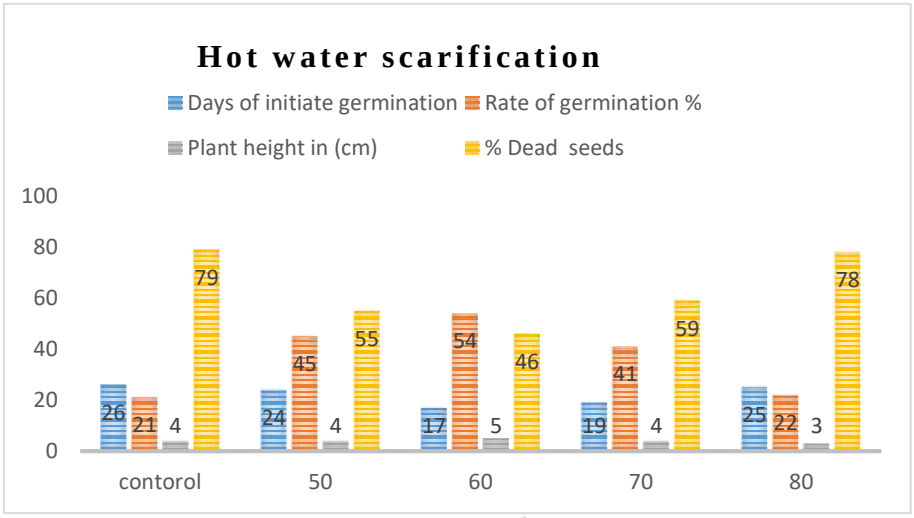
The germination potency of the one year old stored seeds was tested by employing physical and chemical parameters. For each germination test, seeds were at first disinfected with mercuric chloride solution (4% w/v) for 5min to reduce the incidence of fungal attack. For each experiment, 100 seeds were selected and seed coat was removed by mechanical scarification Treatment 1: scarification seeds were subjected to hot water treatment at different temperature 50°C, 60°C, 70°C, and 80°C for 12hour. Treatment 2: soaking of the seeds in cold distilled water at 10°C, 4°C, -1°C, and -5°C for 12hour. Treatment 3: scarification seeds were treated with different concentrations of gibberellic acid 100 mg, 200mg, 300 mg and 400 mg respectively, at room temperature for 12hours. Treatment 4: Soaking of the seeds in different concentrations of H_2SO_4 2%, 4%, 6% and 8% for 1hours.The experiment conducted in the greenhouse plastic trays containing 1:2 ratios (soil, sand) under the controlled condition the temperature maintained at 25 °C (±) 2 °C and relative humidity maintained at 95% under partial shade condition (50% shade). Observation data were recorded days to initiate germination, days are taken for germination, the rate of germination, plant vigor, plant height, and number of leaves, shoot length, of the plant were recorded daily up to 30days.

Statistical Analysis

The data were subjected to statistical analysis using ezAnova and Microsoft excel. The bars represent days of initiation, height of the plant (cm), percent of seed germinated and percent of dead seed, form three different individual experiments.

Results

The scarification data pertaining to the significant influence of treatments on germination efficacy and growth parameters such as days to initiate germination, the rate of germination, plant height (cm) and dead seeds of *B. lanzana*s influenced by physical and chemical factors are presented.



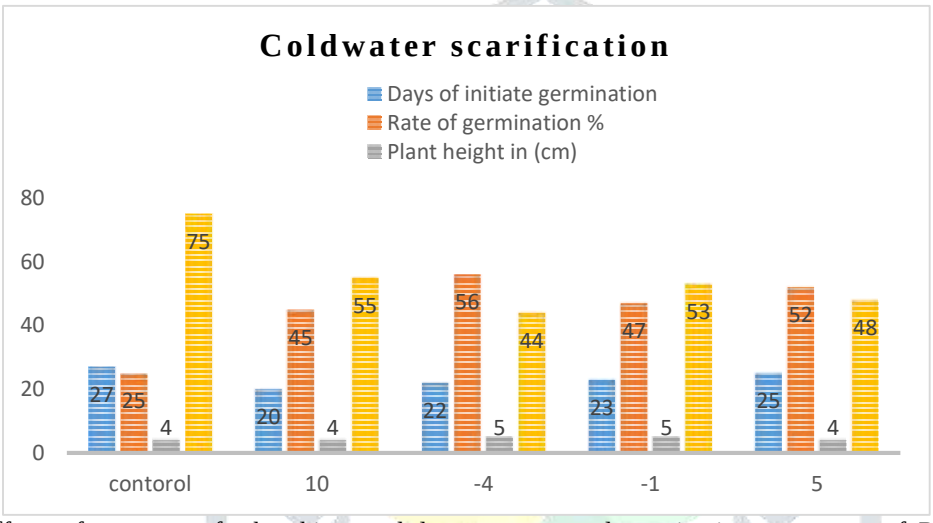


Fig. 1 & 2: Effects of treatments for breaking seed dormancy on seed germination percentage of *B.lanzan* (Hot & Cold Water Scarification)

In hot water treatment seeds are tested at 50˚C, 60˚C, 70˚C, and 80˚C respectively. Among them 54% of seeds germinated at 60˚C treatment shown in (**Fig 1**.). In cold watertreatment seeds are tested at10˚C,-4˚C, -1˚C and 5˚C, at -4˚C treatment 56% of seeds germinated as shown in (**Fig 2**.)

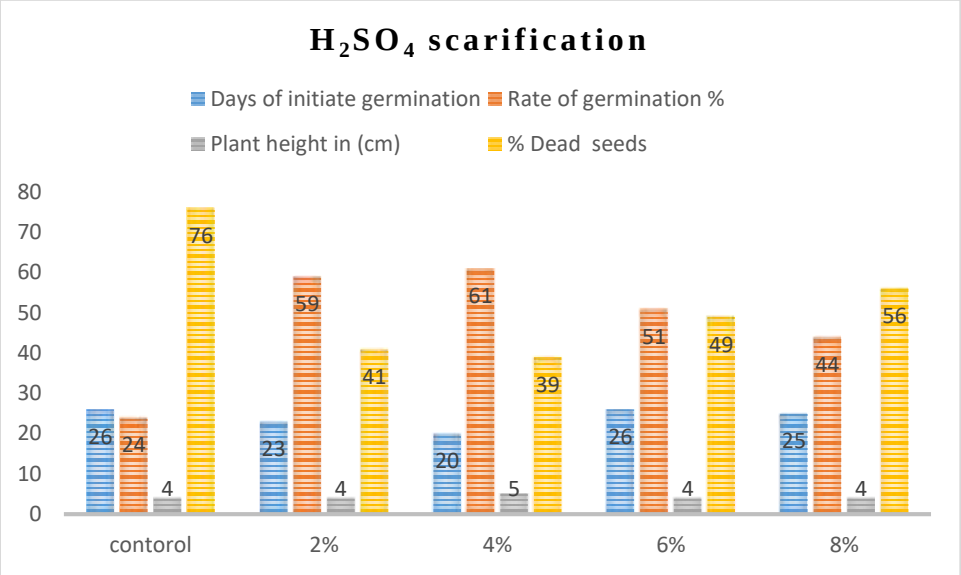


Fig. 3. Effects of treatments for breaking seed dormancy on seed germination percentage of *B. lanzan* (Sulphuric acid Scarification)

The effect of H_2SO_4 on breaking the dormancy of the seeds was tested at 2%, 3%, 4%, and 5%. For 1-hour Treatment of scarified seeds at the concentration 4% H_2SO_4 showed 61% of germination (**Fig. 3**) and at 30days incubated culture shoot length grew above 5cm as compared to control and least percentage of germination was noticed at 8% H_2SO_4.

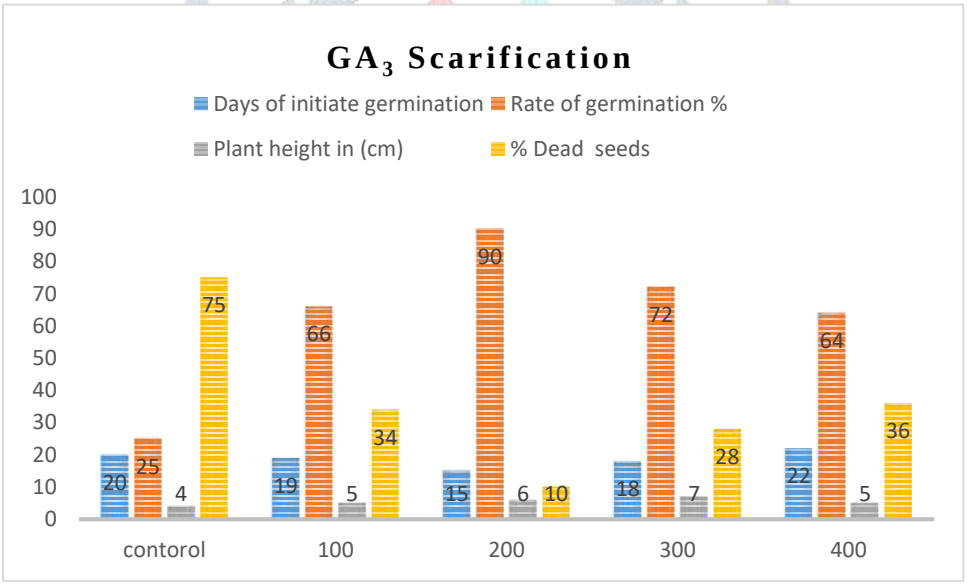


Fig. 4 Effects of treatments for breaking seed dormancy on seed germination percentage of *B. lanzan* (gibberellic acid Scarification)

The highest percentage (90%) of seed germination was noticed in GA_3 treatment at the concentration of 200 mg/l and the seeds were incubated for a period of 12hours as shown in **(Fig .4)**

Discussion

*B. lanzana*is an endangered tree species very sparsely distributed in the dry deciduous forests of the Western Ghats of Karnataka. Seeds exhibit poor germination even if exposed to favorable conditions owing to seed dormancy (Joshi, C. J.2017) Seed dormancy is a means to allow seeds to initiate germination when conditions are normally favorable for germination and survival of the seedlings. Dormancy can be regulated by the environment or by the seed itself. This may be due to morphological factors such as hard seed, thick test incorrect storage handling etc. and these factors ultimately lead to the reduced vegetative growth of the seedlings. Seeds of *B. lanzana*is very hard and mechanical scarification is required to open the seeds. In control condition percentage of germination was found to be 20% and the percentage of germination decreases in the stored seeds. In the present study dormancy of one year stored seeds of *B.lanzana* was broken by subjecting the mechanically scarified seeds to various physical and chemical treatments.

Hot water treatmentat 60˚C for 1 h induced germination potency in the scarified seeds. According to (Webster, R. E., 2016). Heat enhances seed germination and promotes cracking of the seed coat. Hot water treatment was successful in softening the hard seed coat and made permeable for water entry. Increase in temperature of water has resulted in quick breakdown of leginin content in outer seed coat and allowed water to entry through hilar region. (Smýkal P 2014). However, at 80˚C decreased seed germination compared with the control, this was due to the damage caused by high temperature for longer period on growing tip of embryo.

In many plant species, cold scarification is known to increase germination (Bewley& Black 1982). Seeds do not germinate immediately after being dispersed by the parent plant. They require a minimum period of cold scarification or dry storage at room temperature to acquire the capacity to germinate. Cold scarification is needed to initiate early and to reach maximum germination rates. (Cavieres, 2000). The scarification periods necessary for germination has also been found to increase with an elevation of seed source in other plant species growing along altitudinal gradients (Borghetti et al. 1989). Seed germination was higher in seeds from higher elevation stored for a longer time, suggesting that in addition to scarification, seeds also needed time to ripe to germinate (dry storage after-ripening). In *B. lanzana* also, cold scarification influenced the rate of seed germination at 60˚C. (Lohengrin and Arroyo 2000) also noticed that cold scarified *Juniperus procera* seeds for 4 years at −10°C and moistened in cold water induced higher germination percentage when compared with control seeds.

The acid scarification treatment of seeds also enhances the rate of germination there by increased the permeability of air and water through seed which favors the early germination. (Orozco-Segovia, A, 2007).In the present study, treatment of seeds with 4% concentrated H_2SO_4 for 1hrs resulted in 61% of germination. Similar results were also noticed by (Shukla and Solanki 2000), they recorded the early seed germination of mechanically damaged Chironji seed coat after treating with 5% H_2SO_4 for 24 hrs.

Gibberellic acid treatment increased 90% of seeds germination and growth parameter values as compared to control. The germination percentage increased clearly compared with the other treatments, the reason may relate to the removing the hard endocarp which did allow a large amount of GA_3 to enter the seed. GA_3 which promotes production alpha-amylase which translates starch into its simple sugar units which transfer to the embryo to be used as food. This may be due to the instigative action of GA_3 for germination of seeds. GA_3 induces the de-novo synthesis of photolytic enzymes like amylase and ribonuclease in turn hydrolyze starch in the endosperm, providing the essential sugars for the initiation of growth processes (Copeland and Mc-Donald, 1995). GA_3 treatment is also known to overrule the photo dormancy, thermo dormancy, dormancy imposed by incomplete embryo development, mechanical barriers and presence of germination inhibitors (Wareing, P. F. 1965). This may be due to a GA_3 role in cell division and cell enlargement and are largely controlled by the endogenous level of gibberellic acid which has been proved in a number of crops. The increased cell division and cell elongation reflected in increased plant height were observed in hybrid lilies (Qian Xu. et al.2016).Different concentrations of GA_3 caused significant difference over a percentage of germination in plants.

Conclusion

Storage condition highly affects the seed germination percentage in *B.lanzan* storage duration irrespective of storage condition. In the present study, physical and chemical scarification treatments are employed to break the dormancy of one year old seeds. It is concluded that germination of stored seed of *Buchanania lanzan* can be improved up to 90.% per cent using seed treatment *i.e.*, scarification of seeds with GA_3 200mg for 12hrs.

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