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RESEARCH ARTICLE

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Germination and *ex-situ* conservation of *Terminalia arjuna* (Roxb.) Wight and Arn. seeds

Maitreyee Kundu and Shweta Tiwari

ABSTRACT : *Terminalia arjuna* is an important medicinal plant, the bark of which is useful for treatment of various diseases including fractures, ulcers and hypertension. This study involved: I) the effect of seed pretreatments for betterment of germination immediately after harvest and II) to evaluate the conditions for *ex-situ* conservation of arjuna seeds. Pretreatments tested to stimulate germination were: control, cold water, hot water, potassium nitrate solution, plant growth regulators and sulfuric acid. Germination response was highest by use IAA at the dose of 500 ppm, probably because of the hormonal nature of the dormancy in this species. Seeds were dried to 4.5 per cent, 8.2 per cent and 12.8 per cent moisture contents and stored at -20°C, 5°C, 15°C and 40°C for three years. Sampling for assessment of viability and moisture content was done at regular intervals. The seeds are tolerant upto 4.5 per cent moisture content, hence suggesting orthodox nature of the seed. The best results were obtained when the seeds were dried to 4.5 to 8.2 per cent moisture content prior to storage and stored in air-tight containers in 15 to -20°C. Use of these storage conditions may allow arjuna seeds to be stored for at least 3 years without a significant loss in viability and will help in conservation of this valuable species.

KEY WORDS : *Terminalia arjuna*, Pretreatment, Seed germination, Seed storage

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INTRODUCTION

Terminalia arjuna (family: Combretaceae) is a large evergreen trees with generally buttressed and fluted stem, spreading crown and drooping branches. It grows throughout the greater part of India in tropical moist and dry deciduous forests chiefly along rivers and streams; it is common in Central India like Bihar, Orissa, Madhya Pradesh, parts of Maharashtra and Tamil Nadu. The distribution is extended northward to sub-Himalayan tract, where it grows sporadically along the river banks. It is considered as an important medicinal plant, the bark of which is useful as an anti-ischemic and cardio-protective agent in hypertension and ischemic heart diseases especially in disturbed cardiac rhythm, angina or myocardial infarction. The bark powder possesses

MEMBERS OF RESEARCH FORUM

Address of the Correspondence : Maitreyee Kundu, Tropical Forest Research Institute, **Jabalpur (M.P.) India**

Email: spalliwest@yahoo.co.in

Address of the Coopted Authors : Shweta Tiwari, Tropical Forest Research Institute, **Jabalpur (M.P.) India**

diuretic, prostaglandin enhancing and coronary risk factor modulating properties (Halder *et al*., 2009). It apparently has a diuretic and a general tonic effect in cases of cirrhosis of the liver. The panicled spikes of white flowers of this species appear from April to July. Fruits ripen from February to May. Fruit is drupe, 2.5 cms long, ovate, thick with 5 rigid, longitudinal wing, 0.6 cm broad, the fruit is often notched near the top, marked with oblique upward curving striations.

It is predominantly propagated through seeds. But low germination capacity is the major constraint for efficient nursery management and plantation establishment. Again there is little information on seed storage behaviour of this species, which is an important aspect for *ex-situ* conservation of genetic diversity. Though it has been recorded that the seeds can be stored safely for one year (Luna, 1996), the viability of seeds at different storage conditions were not evaluated.

The objectives of the present study was to determine, the efficiency of different pretreatment methods to induce better germination and optimum storage condition for *ex-situ* conservation of genetic material (seeds).

EXPERIMENTAL METHODS

The experiments were carried out in the Silviculture laboratory and nursery of Tropical Forest Research Institute, Jabalpur. The soil used in the experiments was prepared by mixing soil, sand and farmyard manure in a ratio of 2:1:1. Fully mature seeds were collected from the Barha Experimental Station, Jabalpur, Madhya Pradesh from the randomly selected branches. After preliminary cleaning the seeds were stored at 15°C till they were used. Similar weighed seeds were selected for experiments to avoid non-treatment variation.

Pretreatment study:

The experiment used a Randomized Complete Block Design with treatments including control and three replications. The different pre-sowing treatments used in the treatments were:

Control (untreated seeds).

Cold water treatments:

Seeds were soaked in cold water for 24, 48 and 72 hours.

Boiling water treatment:

Seeds were poured in boiling water that was removed immediately from the heat source and allowed it to cool.

Hot water treatments:

Seeds were soaked in hot water (80°C), allowed it to cool and seeds were left in it for 17 hours.

Treatments with plant growth regulators:

Seeds were soaked in the following plant growth regulators: GA_3 250 ppm, GA_3 500 ppm, NAA 250 ppm, NAA 500 ppm, IAA 250 ppm, IAA 500 ppm for 17 hours.

Sulfuric acid treatment:

Seeds were immersed in concentrated sulfuric acid for 10 and 20 minutes, after which seeds were rinsed thoroughly in tap water six times and then soaked in cold water for 17 hours.

Potassium nitrate solution:

Seeds were soaked in KNO_3 (0.2%) solution for 17 hours and washed thoroughly before sowing.

After treatments 50 seeds for each treatment were sown in soil in three replications. The germination was recorded daily till the germination ceased. The seeds were considered germinated if at least 0.5 cm of the cotyledon and hypocotyls was protruded above the surface of the soil. Number of normal seedlings, abnormal seedlings, dead seeds, hard seeds and fresh seeds were counted (ISTA, 1985).

Storage experiments:

Freshly collected seeds were dried over regularly regenerated silica gel in a desiccator for adjustment to three moisture contents, 4.5, 8.2 and 12.8 per cent and then packed in thick sealed polythene bags for storage at four temperatures *viz.*, -20°C, 5°C, 15°C and 40°C. Moisture content was estimated with five samples of five seeds. These were cut into 2-3 pieces and dried at 103±2 ^{0}C for 17 hours in a forced-draft oven. Seeds were sampled at intervals upto three years for germination test as an indicator for viability. Before sowing, seeds were treated by soaking in IAA 500 ppm for 17 hours. Factorial in CRD was used as experimental design for this experiment. Analysis of variance was done according to ANOVA.

EXPERIMENTAL RESULTS AND ANALYSIS

The results showed that there were significant differences between the different pretreatment methods (Table 1). Highest germination percentage (62.5%) was achieved in the seeds treated with IAA 500 ppm. The second best treatments were IAA 250 ppm and GA_3 250 ppm that improved the germination percentage to 55.11 and 57.8 per cent, respectively over control (12.5%). However, GA_3 at the 500 ppm had an inhibitory effect on seed germination. The germination of seeds soaked for 24 and 48 h, those treated with hot water and potassium nitrate solution were not significantly different from the control. Sulphuric acid treatment for 10 minutes could not overcome the dormancy. Treatment with sulphuric acid for 20 minutes had a detrimental effect on seed and no seed germinated after this treatment.

The present work on storage trials supported the orthodox nature of the seed of this species. (Roberts, 1973) (Fig. 1, 2 and 3). The seeds are tolerant upto 4.5 per cent moisture content as the viability of seeds did not decline after drying the seeds upto and it was also observed the low moisture content and temperature can extend the viability of *Terminalia arjuna* seeds. Seeds of this species could maintain their initial viability for at least three years if stored at -20°C to 15°C with seed moisture content ranges from 4.5 per cent to 8.2 per cent. But seed viability gradually with increase in storage period at all moisture contents, if storage temperature

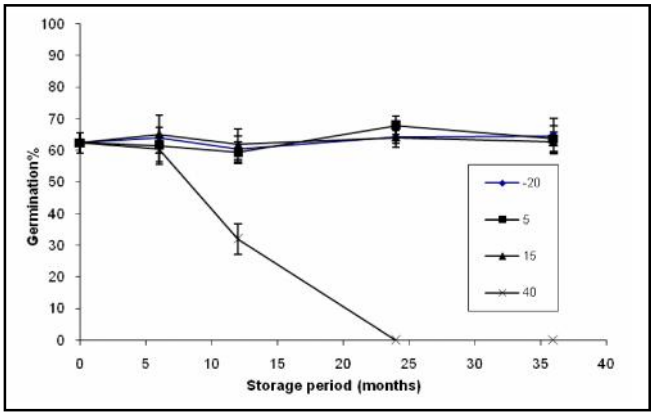

Fig. 1: Viability of seeds of *Terminalia arjuna* stored at different temperature with 4.5 per cent moisture content

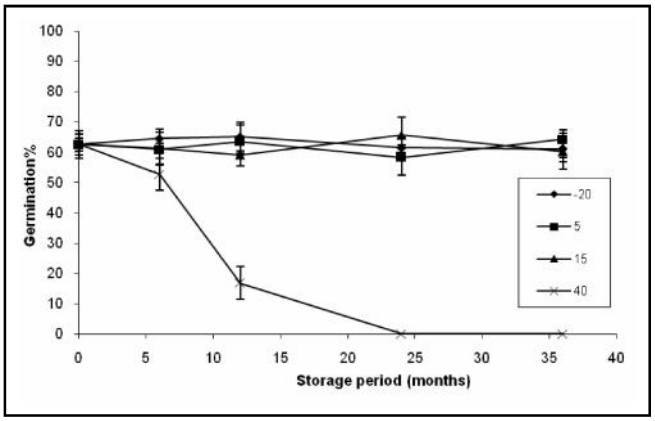

Fig. 2: Viability of seeds of *Terminalia arjuna* stored at different temperature with 8.2 per cent moisture content

Table 1: Effect of different pre-sowing treatments on germination percentage of *Terminalia arjuna* seeds

Treatments	Germination %
Control	12.5
24 hrs cold water	9.8
48 hrs cold water	9.6
72 hrs cold water	7.5
Hot water treatment	9.2
Boiling water treatment	20.2
GA_3 250 ppm	47.8
GA_3 500 ppm	6.3
NAA 250 ppm	6.8
NAA 500 ppm	6.2
IAA 250 ppm	45.1
IAA 500 ppm	62.5
Potassium nitrate solution (0.2%)	10.2
Sulphuric acid 10 minutes	9.3
Sulphuric acid 20 minutes	0
C. D. (P=0.05)	4.1

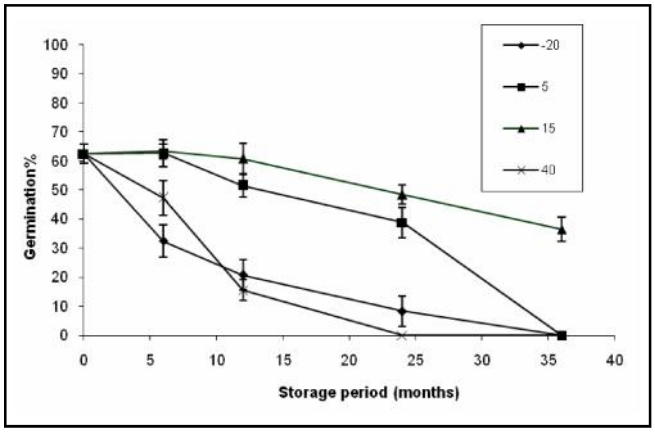

Fig. 3: Viability of seeds of *Terminalia arjuna* stored at different temperature with 12.8 per cent moisture content

was increased to 40°C. Deterioration was more pronounced in seeds with high moisture content (12.8%). It was also observed that the seeds stored with 12.8 per cent moisture content could not able to survive at -20°C due to freezing sensitivity of seed. No seed with 12.8 per cent moisture content became viable at 40°C after two years of storage, whereas some seeds at this moisture content remained alive, if stored at 15°C.

Successful seed germination constitutes the first essential step for successful establishment (Radosevich *et al*., 1997). After full maturation, the seeds may not germinate even under favourable condition. These can be termed as dormant seeds which can be stimulated to germinate using treatments that emulate natural conditions or satisfy certain physiological requirements. Stratification, leaching, scarification, light and plant growth regulators are effective dormancy releasing treatments (Bradbeer, 1988; Bonner *et al*., 1994 and Rahman *et al*., 2006).

Natural regeneration of *Terminalia arjuna* is poor. One healthy and mature tree produces hundreds of seeds, but only a few germinate in the natural habitat. Even in the nursery very few seeds germinate without any treatment. In this study, the fresh arjuna seeds recorded only 12.5 per cent germination indicates the presence of seed dormancy. The different pretreatment studies under this investigation indicated that seed of this species may not have seed coat dormancy as, boiling water or sulphuric acid treatment was found ineffective in increase of germination over control; though seed coat dormancy exists in other *Terminalia* spp. (Likoswe *et al*., 2008, Negi and Todaria, 1995). Slight improvement in germination was observed in seeds treated with boiling water, though earlier records opined that hot water treatment could be considered the most effective treatment of arjuna seeds (Luna, 1996). Seeds treated with IAA at the dose of 500 ppm achieved highest germination that indicates that there may be some growth inhibitory factors existing in the fresh seeds that impose endogenous dormancy of seeds shed in the unfavorable environmental conditions. Shivanna *et al*. (2007) also observed better germination in seeds treated with IAA 200 ppm. Auxins was also found to be critical in germination in seeds of *Thyrostachys siamensis* and *Dendrocalamus strictus* (Richa and Sharma, 1994). Several biochemical studies revealed that auxins modulate transcription and/or translation during protein synthesis. The mobilization of protein and lipid storage bodies upon specific enzymes which hydrolyze stored molecules and catalyse essential reaction in energy generating cycles (Noland and Murphy, 1984) result into the production of energy and substrates which in turn provide the structural components essential for the growth and emergence of the embryo.

Storage trials indicated that the fundamental factors for seed storage on quality of seed are the storage temperature and moisture content of seeds at which they are stored. Most of the tropical tree species lost their viability within a few months of storage at room temperature (Dent, 1948), where use of tin container or jute bag for storing seeds is a common practice. Arjuna seeds could maintain their viability and germination for more than three years, if stored at low temperature and low moisture content, whereas higher temperature and moisture content were found to be the main causes for seed deterioration.

Loss of viability of seeds depends upon the life span and storage condition (Dell'Aquilla, 1987). Most tropical seeds have low storage life at ambient conditions due to poor maintenance of seed moisture content and high temperature. During summer the temperature reaches 40°C in most of the parts of India and low moisture content of seed cannot be maintained by the common practice. Seeds dried to low moisture content should be stored in thick polythene bags or air-tight container with minimum air in it. It was observed that seeds stored in polythene bags showed superiority over tin container in both room and refrigerated condition due to change in moisture content (Chauhan and Nautiyal, 2007).

The best efficiency pre-sowing treatment for better

germination of *Terminalia arjuna* seeds is soaking in IAA 500 ppm for 17 hours. For *ex-situ* conservation of seeds the best suitable range of temperature and seed moisture content was -20°C to 15°C and 4.5-12.8 per cent, respectively.

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