Print : ISSN 0970-7662
Online : ISSN 2455-7129

Journal of Tree Sciences
online available at www.ists.in

Volume 36 No. 1 June, 2017

Seed dormancy and Storage Behavior of *Strychnos potatorum* – A Fast Depleting Tree resource

Rekha R. Warrier*, R. Anandalakshmi, V. Sivakumar, B. Gurudev Singh

Institute of Forest Genetics and Tree Breeding, Indian Council of Forestry Research and Education, P.B No. 1061, Coimbatore – 641002, India
**Email address: rekha@icfre.org, rekhawarrier@gmail.com*

DOI: 10.5958/2455-7129.2017.00007.3

Key words:

Strychnos potatorum, morphophysiological dormancy, desiccation, GA_3

ABSTRACT

As a first step to develop an *ex-situ* conservation strategy, the seed storage behavior of the species was studied. The initial moisture content of mature seeds was 37.14 per cent with a germination of 56 per cent. Freshly matured seeds had physiological dormancy which could be overcome by use of GA_3 when germination was enhanced to 81 per cent. Seeds did not tolerate desiccation and lost viability. Storage of fresh seeds in wet vermiculite helped retain seed viability for only ten days beyond which viability declined rapidly. Considering seed size, shape, weight, moisture content and inability to withstand desiccation the storage behaviour of *S. potatorum* seed is predicted as recalcitrant with simple non-deep morphophysiological dormancy.

INTRODUCTION

Ex situ conservation of plant germplasm through seed storage under conditions which maximize their post-harvest life spans is the most convenient method adopted. Orthodox seeds which are desiccation-tolerant can be stored in dry state in equilibrium at low relative humidity (15 %) and sub zero temperatures (FAO 2013). Recalcitrant seeds on the other hand are desiccation sensitive and have short life spans under dry state conditions. A database by the Royal Botanic Gardens Kew (2008) suggests that if it is not possible to carry out the necessary experiments to determine seed storage behaviour, though it may be predicted according to taxonomy, origin, and other seed traits. However, some plant families or genera have representatives showing all categories of seed storage behavior. Therefore, seed storage behaviour continues to be determined experimentally for diverse species as this knowledge is likely to improve predictive models of storage behaviour and phylogeny-/ecology-based understanding of the occurrence of each category of seed storage behavior (Jayasuriya et al. 2013).

Strychnos potatorum Linn (family: Loganiaceae) is a moderate sized tree found in southern and central parts of India, Sri Lanka, and Burma (Yadav et al. 2014). In traditional system of medicine the seeds are used for the treatment of various ailments like jaundice, bronchitis, diabetes, conjunctivitis, chronic diarrhoea, dysentery *etc.* They are also used to clear muddy water by their coagulant action (Sanmuga and Venkatramanan 2013). As a result, there is a growing demand for fruits/seeds of this species by the Indian pharmaceutical industries (Tewari 2000). A recent estimate puts the demand for fruits as 100 MT per annum (Anonymous 2008).

The species occurs naturally in the wild and no large scale cultivation has been adopted despite heavy demand for the seeds. This reduces the availability of propagules in the wild for the establishment of new seedlings. With the natural resources getting depleted due to procurement of raw material from the wild, the resources get exhausted. As propagation by seed is the most often used and cheapest method. Knowledge of seed handling is a prerequisite for growing this species successfully. Research has been carried out to establish the medicinal value of the species (Sanmuga and Venkatramanan 2013, Yadav et al. 2014); however, information to support conservation aspects is scanty. Therefore, it is important to investigate the seed germination and storage behaviour characteristics for effective conservation of the germplasm of *S. potatorum.* In the present study, the main aim was to identify seed dormancy type and conditions for dormancy breakage, dessication tolerance and storage behaviour of *S. potatorum.*

MATERIAL AND METHODS

Seed collection and processing

Mature fruits were collected during the peak fruiting season (December to February) from Chettipatti (10°22' N; 78°81' E), Tamil Nadu, India. The fruits were de-pulped manually. The seeds were separated by macerating the fruits by hand. The extracted seeds were washed and shade dried under ambient conditions (30 ± 1 °C) for half an hour before conducting the experiments. The processed seeds were tested for moisture content on fresh weight basis by the oven dry method following the standard rules (International Seed Testing Association 2003). Five grams of seed in duplicates were weighed before and after drying at 103 °C for 16h, and the moisture content was calculated as the percentage of water in the seeds on a fresh weight basis. Seed samples were drawn to measure length and breadth using Image Analyzer (Leica 500). 100 seed weight was taken in quadruplicates as per ISTA (2003) rules.

Pre-sowing treatments

To examine whether *S. potatorum* seeds possess dormancy, presowing treatments were applied. The presence of physical dormancy was investigated by soaking seeds in cold and hot water overnight. To investigate whether dormancy in S. potatorum seeds is physiological, seeds were soaked in 1000 ppm gibberellic acid (GA_3) for 24 h and tested for seed germination.

Desiccation trials

The effect of moisture content on the germination of *S. potatorum* seeds was investigated by reducing the initial moisture content (34.71%) to different target moisture levels 16, 8 and 5 % by sun drying. The corresponding target weight for each target moisture content was determined using the following equation (Danida Forest Seed Centre 1999):

$$\text{Seed weight at target moisture content (\%)} = \frac{\text{100 seed-Initial moisture content (\%)}}{\text{100 seed-Target moisture content (\%)}} \text{ X Weight of seed}$$

The water loss in desiccated seeds was monitored by weighing the seeds at regular intervals. Once seed samples attained the target moisture content, they were subjected to germination tests without further pretreatment.

Storage with wet vermiculite

The seeds of *S. potatorum* with an initial moisture content of 37.41% were mixed with equal weight of wet vermiculite (10 %) and stored at 25-30°C for 3 and 6 days. The seeds were then stored at ambient (25–30°C) in sealed polybags (400 gauge) after dusting the vermiculite and tested for germination characters in intervals of 10, 20 and 30 days in sand bed following GA_3 pretreatment.

Germination assay

Germination tests were conducted at ambient temperature and with a photoperiod of 10 h light and 14 h dark. For each experiment, four replicates of 50 seeds were sown. Watering was done everyday throughout the experiment period.

The number of days for first seedling emergence was recorded. The final germination count was taken 120 days after initiation of germination. Germination parameters were derived using standard formula (Willan 1985).

Statistical analysis

The germination data collected from different experiments were subjected to arcsine transformation wherever required and statistical analysis was carried out. The means were tested at 5 per cent level of significance.

RESULTS AND DISCUSSION

Being a deciduous species, leaf fall was noticed from the first week of May. Leaf bud break was observed in July which followed leaf fall. The development of leaves and flowers were simultaneous. Fruiting was observed during first week of December. Fruit fall was observed from January. Mature fruits of *S. potatorum* are black, globose shaped while immature fruits are green in colour. Immature fruits were smaller in terms of fruit length and width (14.13 and 13.15 mm respectively) with a seed weight of 34.52 g. The seeds were soft and pliable, the length and width were 10.23 and 4.86 mm, respectively and MC was 47.0 %. Mature fruits were 15.44 mm long and 14.21 mm broad with a seed weight of 72.22 g. The seeds were hard and compressed and length and width were 1.48 and 7.95 mm, respectively with an MC of 37.14 %. The initial germination percentage of mature and immature seeds of *Strychnos potatorum* was 56.0 and 31.0% respectively. Germination was epigeal and first seedling emergence occurred after 44 days (Photo 1).

Dormancy Studies

Untreated seeds of *S. potatorum* showed 56.0 per cent germination. The seeds soaked in 1000 ppm GA_3 for 24 hours and sown on sand beds showed enhancement in germination. There was 40.8% increase in the germination compared to control. In morphologically dormant seeds, germination occurs without any special dormancy-breaking treatment within 30 days (Baskin and Baskin 1998, 2004). Seeds with morphological dormancy and having a physiological component of dormancy require specific treatments to break physiological dormancy. They are classified as morphophysiological dormant seeds (Baskin and Baskin 2004). Fresh seeds of *S. potatorum* showed an initiation of germination beyond 30 days of sowing. This prolonged duration suggests morphological dormancy. The seeds soaked overnight in hot and cold water did not differ significantly in germination per cent. When subjected to GA treatments, the germination per cent increased indicating physiological dormancy coupled with morphological dormancy. Germination energy (Table 1) and germination values were also high for seeds soaked for 24 hours in 1000 ppm concentration of GA_3 irrespective of the germination medium (Fig. 1).

Table 1. Effect of pretreatments on germination in *Strychnos potatorum*

Pretreatments	Germination Percent	Mean Time of Germination	Germination Energy
Control (untreated)	56.00[b]	50.4[b]	14.25[b]
Cold water (24 hours)	57.00[b]	51.8[b]	13.40[c]
Hot Water (24 hours)	59.00[b]	53.5[b]	14.03[b]
GA3 1000 ppm (24 hours) in sand bed	81.00[a]	65.8[a]	28.20[a]
(n=100); F value= 43.95		P value = 0.00026 (0.01%)	

Earlier records on germination studies in nursery sown seeds of *S. potatorum* show a germination percentage of about 14 and plant percent around 10 (FRI 1985) following cold water soaking for 12 hours. Peak value and final DGS (Daily Germination Speed) were also higher for the GA treated seeds. The results reveal that *S. potatorum* produces morpho-physiologically dormant seeds.

Desiccation studies

The fresh seeds of *S. potatorum* were estimated to have 37.41 % moisture content with 56.0 % germination. The seeds desiccated to 16.0 and 8.0% showed decrease in germination percentage. Lowering moisture content to less than 8 per cent resulted in complete loss of viability (Fig. 2). This suggests recalcitrant behaviour of the species. Recalcitrant seeds remain sensitive to dehydration both during development and after they are shed from the parent plant (Berjak and Pammenter 2008). Similar observations were made by the group in *Calophyllum inophyllum* (Singh et al. 2008) and *Myristica dactyloides* (Sivakumar et al. 2006a).

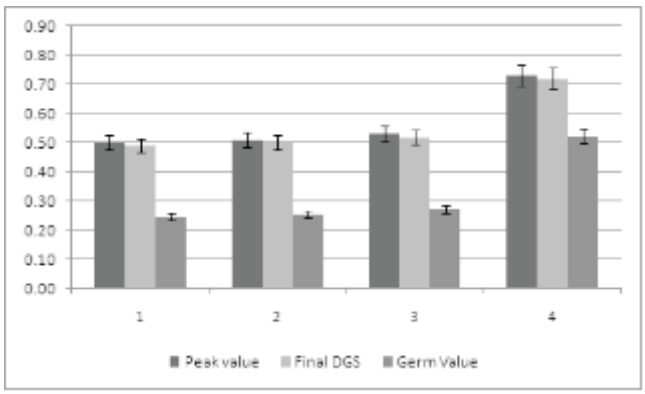

Fig 1. Effect of pretreatments on germination parameters in *Strychnos potatorum* (n=100; F value- 22.48; P value – 0.00003) (1-Control (untreated); 2- Cold water (24 hours); 3- Hot Water (24 hours); 4- GA_3 1000 ppm (24 hours) in sand bed)

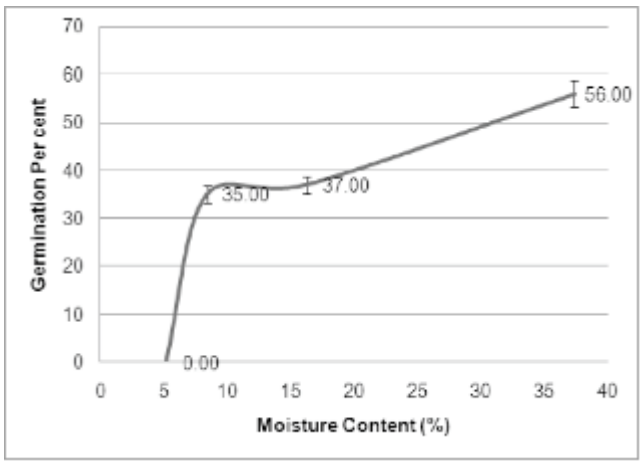

Fig 2. Relationship between germination percentages and moisture contents for *Strychnos potatorum* seeds. Error bars represent the S.E.

Storage studies

Seedling emergence occurred within 35 days. The initial viability was maintained for ten days. When the seeds were stratified for 3 and 6 days, and then stored, viability was prolonged for 30 days with 15 per cent germination. This suggests that stratification helped retain the moisture content within the seeds. There was no significant difference between germination per cent after 3 and 6 days of storage (Fig 3).

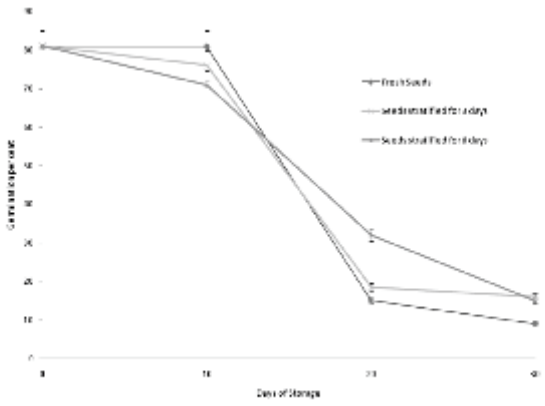

Fig 3. Relationship between germination percentages, storage conditions and storage period for *Strychnos potatorum* seeds. All seeds were incubated at 25/20°C with a 12 hours photoperiod. Error bars represent the S.E. (F statistic - 0.008)

Photo 1. Fruits, Seeds and Seedling of *Strychnos potatorum*

Physiological dormancy can be classified into three levels: non-deep, intermediate and deep (Baskin and Baskin 2004). In the present study, since $GA_3$and warm stratification could break seed dormancy of *S. potatorum.* It could be concluded that the physiological dormancy exhibited by *S. potatorum* is non-deep.

Storage behaviour of few loganiaceae species has been studied. The present group reported that *S. nux-vomica* seeds possess physiological dormancy that can be broken effectively by after-ripening (Sivakumar et al. 2006b). Studies conducted on *Strychnos cocculoides* seeds revealed that desiccation from 24 to 10.4% moisture content was found to improve the germination from 75 to 91% (Danida Forest Seed Centre 2000). However, germination was highly reduced following extended drying (3% moisture content). When moisture levels are considerably reduced, several metabolic processes are disturbed, the most prominent among them is loss of membrane integrity (Vertucci and Farrant 1995). However, Matasyoh (2000) and Silva et al. (2012) reported orthodox behavior in *Strychnos spinosa* and *Strychnos pseudoquina.* Roeder at al. (2013) reported that *Strychnos amazonica, S. glabra* did not germinate after desiccation and seeds lost viability. This indicates that the family loganiaceae has representatives showing different categories of seed storage behavior. Therefore, seed storage behaviour needs to be experimentally determined for the different species within the genera Strychnos for knowledge to improve predictive models of storage behavior.

CONCLUSIONS

From this study, it is clear that *S. potatorum* seeds have a non-deep simple morphophysiological dormancy, which could be overcome by GA3 and warm stratification. The fresh recalcitrant seeds of *S. potatorum* could maintain viability for almost a month under warm stratification. This information could be used and applied for conservation of germplasm of *Strychnos potatorum.*

Acknowledgements

The authors gratefully acknowledge the financial support provided by National Medicinal Plants Board to carry out this study (TN - 49/GO/2003).

REFERENCES

Anonymous 2008 Agro-techniques of selected medicinal plants, National Medicinal Plants Board Department of AYUSH, Ministry of Health and Family Welfare Government of India, Vol 1, p. 183.

Baskin CC and Baskin JM 1998 Seeds: *Ecology, Biogeography,* and *Evolution of Dormancy and Germination.* Academic Press, San Diego.

Baskin JM and Baskin CC 2004 A classification system for seed dormancy. Seed Science Research, 14: 1-16

Berjak P and Pammenter NW 2008 From Avicennia to Zizania: Seed Recalcitrance in

Perspective. Annals of Botany, 101(2): 213–228. doi:10.1093/ aob/ mcm168

Danida Forest Seed Centre 1999 Desiccation and Storage Protocol. Newsletter No. 5: 23–40. Humlebaek, Denmark

FAO 2013 Genebank Standards for Plant Genetic Resources for Food and Agriculture. Rome: FAO.

FRI 1985 Troup's Silviculture of Indian Trees. vol. 6. FRI & Colleges, Dehra Dun. pp. 172-173.

International Seed Testing Association 2003 International Rules for Seed Testing. International Seed Testing Association (ISTA), Bassersdorf, CH-Switzerland.

Jayasuriya KMGG, Wijetunga ASTB, Baskin JM and Baskin CC 2013 Seed dormancy and storage behavior in tropical Fabaceae: a study of 100 species from Sri Lanka. Seed Sci. Res., 23: 257-269.

Matasyoh LG 2000 Variation in fruit, seed and germination characteristics of provenances of Strychnos spinosa Lam. *Subsp. Lokua in Malwi. Zomba Malwi: University of Malwi.* 159p.

Roeder M, Ferraz ID and Holscher D 2013 Seed and Germination Characteristics of 20 Amazonian Liana Species. Plants, 2:1-15.

Sanmuga PE and Venkatraman S 2013 Pharmacognostical and Phytochemical Studies of *Strychnos potatorum Linn Seeds*. Pharmacognosy Journal, 2(8):190-197.

Silva VF, Sales JF, Silva FG, Campos RC, Branquinho AC and Silva VA 2012 Cryopreservation of quina seeds *(Strychnos pseudoquina A. St. Hil).* Int. Res. J. Biotechnol., 3(4): 55-60.

Singh G, B, Warrier RR, Anandalakshmi R and V Sivakumar 2008 Desiccation and Temperature Tolerance of Recalcitrant Seeds – Madhuca longifolia (Roxb.), Calophyllum inophyllum Linn. and Artocarpus heterophyllus Lam. In: ENVIS Forestry Bulletin: Forest Seed Science and Technology Special, 8(1): 78-85.

Sivakumar V, Anandalakshmi R, Warrier RR, Tigabu M, Oden PC, Vijayachandran SN, Geetha S and Singh BG 2006b Effects of presowing treatments, desiccation and storage conditions on germination of *Strychnos nux-vomica* seeds, a valuable medicinal plant. New Forests, 32(2): 121-131

Sivakumar V, Warrier RR, Anandalakshmi R, Tigabu M, Oden, PC, Vijayachandran SN, Geetha S and Singh BG 2006 Germination requirements and storage behavior of *Myristica dactyloides* Gaertn. *Seeds.* Seed Science and Technology, 34(3): 729-733.

Tewari DN 2000 Report of the Task Force on Conservation & Sustainable use of Medicinal Plants. Government of India Planning Commission March-2000, India.

Vertucci CW and Farrant JM 1995 Acquisition and loss of desiccation tolerance. In: Kigel J. and Galili G. (eds), Seed Development and Germination. Marcel Dekker, Inc., New York/Basel/ Hong Kong, pp. 237–271.

Willan RL 1985 A guide to forest seed handling. FAO For. Pap. 20/2. Rome: Food and Agriculture Organization of the United Nations. 379p

Yadav KN, Kadam PV, Patel JA and Patil MJ 2014 *Strychnos potatorum:* Phytochemical and pharmacological review. Phcog. Rev., 8:61-6