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INVITRO PROPAGATION OF SHOREA TUMBUGGAIA ROXB–AN ENDEMIC AND ENDANGERED SPECIES OF TIRUMALA HILLS.

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ABSTRACT

Shorea tumbuggaia is an endemic endangered medicinal important plant species of Dipterocarpaceae family. The present work is aimed to develop a protocol for micropropogation method for rapid and large scale propagation of the Shorea tumbuggaia by using seed. The percentage of germination is high but seedling production is very less of 0.01%. Now this tree facing high risk of extinction in wild due to ecological conditions. Invitro propagation is one of the best alternative method to conserve this plant to some extent. A high rate of success in percentage of germination was obtained by placing healthy seeds on MS medium supplemented with 2 mg/I BAP. The combination of (0.5mg/I) NAA or IAA is more effective in bringing quick germination. Shoot production was greatest in cotyledonary nodal explants on modified MS medium with BAP 5mg/l+ 0.5 mg NAA and the shoots were successfully rooted. There is an urgent need to protect this endemic plant by both *Invivo* and *Invitro* methods.

Abbreviations

Invitro propgatio -KN – Kinetin, BAP – N6 – Benzyl Aminopurine; IAA – Indole 3 – acetic acid; IBA – Indole butyric acid; NAA – 1 - Napthalene acetic acid.

INTRODUCTION

The genus *Shorea* (family Dipterocarpaceae) is native to Southeast Asia, from northern India to Malaysia, Indonesia and the Philippines. It is a tropical genus with 196 species of mainly rainforest trees, out of which 148 species are currently listed in the IUCNRed List; majority of them are listed as critically endangered. *Shorea tumbuggaia Roxb* is tree taxa, IUCN Red List of Threatened Species (Nayar MP & Sastry ARK. 1987; Reddy CS 2001), belonging to the family Dipterocarpaceae. It is endemic and medicinally (Ankanna S Savithramma N

2013; Ashton PS1980), very important plant of Seshachalum hills of India. So for there is no reports on the conservation status of this plant. This is the first report on germination studies and *Invivo invitro* studies of Shorea *tumbuggaia* from tirumala region of Eastern Ghats.

The *Shorea tumbuggaia seeds* produce only one seed against the actual number of six ovules. The fruit takes 4-6 weeks to mature; the sepals are accresent in that they are thickened and three of them expand into wings and are larger than the other two sepals. The fruit wall is free from calyx woody with thin inner membranous lining in vaginated into the fold of cotyledons and split into two parts at the apex. The main characteristic feature to identify these fruit is wings. The percentage of seed germination in these species is poor because.

The study reveals that non annual, massive flowering short flowering period partial flowering at tree level seed predation, short distance seed dispersal, absence of seed dormancy, low rate of seedling establishment and inability of seedlings to compete with other plants collectively contribute to the occurrence of the small population of *Shorea tumbuggaia* in a restricted area of the eastern ghats forests and interplay of all these factors might have led to the endangered status of the species. The fruit and nuts without its outer fleshy cover are variable and gave poor germination in *Shorea tumbuggaia.* The collection of seeds at the proper time enhances the germination rate. However, to achieve this aim, we should have well documented scientific information on in vitro practices for this species.

MATERIALS AND METHODS

The seeds of *Shorea tumbuggaia* were collected from fully natured fresh fruits obtained from mature trees in the months of June –August every year from different places in Tirumala forest. The seeds were carefully collected and immediately inoculated in the medium.

RESULT AND DISCUSSION

The seeds were obtained by drying the fruit in sunshade for a week. While one set of seeds was stored in a refrigerator at 13-14^{0}C, another set was kept in a wooden box in the laboratory at room temperature (20-30 C). Germination of seeds in both sets was studied at monthly intervals. For germination, 200 seeds were placed on different media. Effect of decoating on seed germination was studied in the laboratory using seeds with seed coat and without seed coat. Healthy and fully ripen seeds were selected. The problem of seed dormancy due to the presence of certain growth inhibitors has been overcome by treatment with some growth hormones like PVP or ascorbic acid. Seed germination was significantly

influenced by different treatments. The seed treatments for; healthy and collected seeds of *S. Tumbuggaia* were de-coated mechanically.

1. Then the decoated seeds were washed with tap water for 1 min.
2. Now, the seeds were treated with Bavistin (1%) [anti-fungal] plus 1 drop of tween-20 for 30 min and washed with tap water for 3 min
3. Seeds were washed with 0.1% of Labolene liquid detergent solution for 6 minutes followed by 3 washings with sterile distilled water.
4. Then the seeds were treated with Mercuric chloride (0.1%) for 10 min and followed by 3 washings with sterile distilled water under aseptic conditions.
5. The germination percentage was assessed after 4 weeks of inoculation.
6. Forty seeds were used for germination treatment and the experiment was repeated thrice.
7. Healthy seeds washed in water for 24 hours were treated with growth regulators such as GA3 (10, 20, 30, 40 mg/l), BAP (5, 10mg/l) + GA3 (20mg/l). Each of the samples was treated and cultured on different nutrient media like MS, B5 and WPM medium containing different concentrations of cytokinnin (BAP, KN) 1-5 mg/l alone or in combination with auxins (NAA, IAA) 0.5 mg/I.quick germination of seedlings was observed in on MS basal medium (1962) containing 3% agar and 0.8% of agar.

The *in vitro* regenerated plantlets were removed from the culture tubes, washed thoroughly to remove the nutrient medium and transplanted to small pots with 1:1 sterile garden soil and compost. For the initial period of transfer, potted plantlets were kept in culture room conditions and high humidity was maintained by covering the plantlets with polythene bags. Plants were subsequently transferred to large pots and gradually acclimatized to natural conditions.

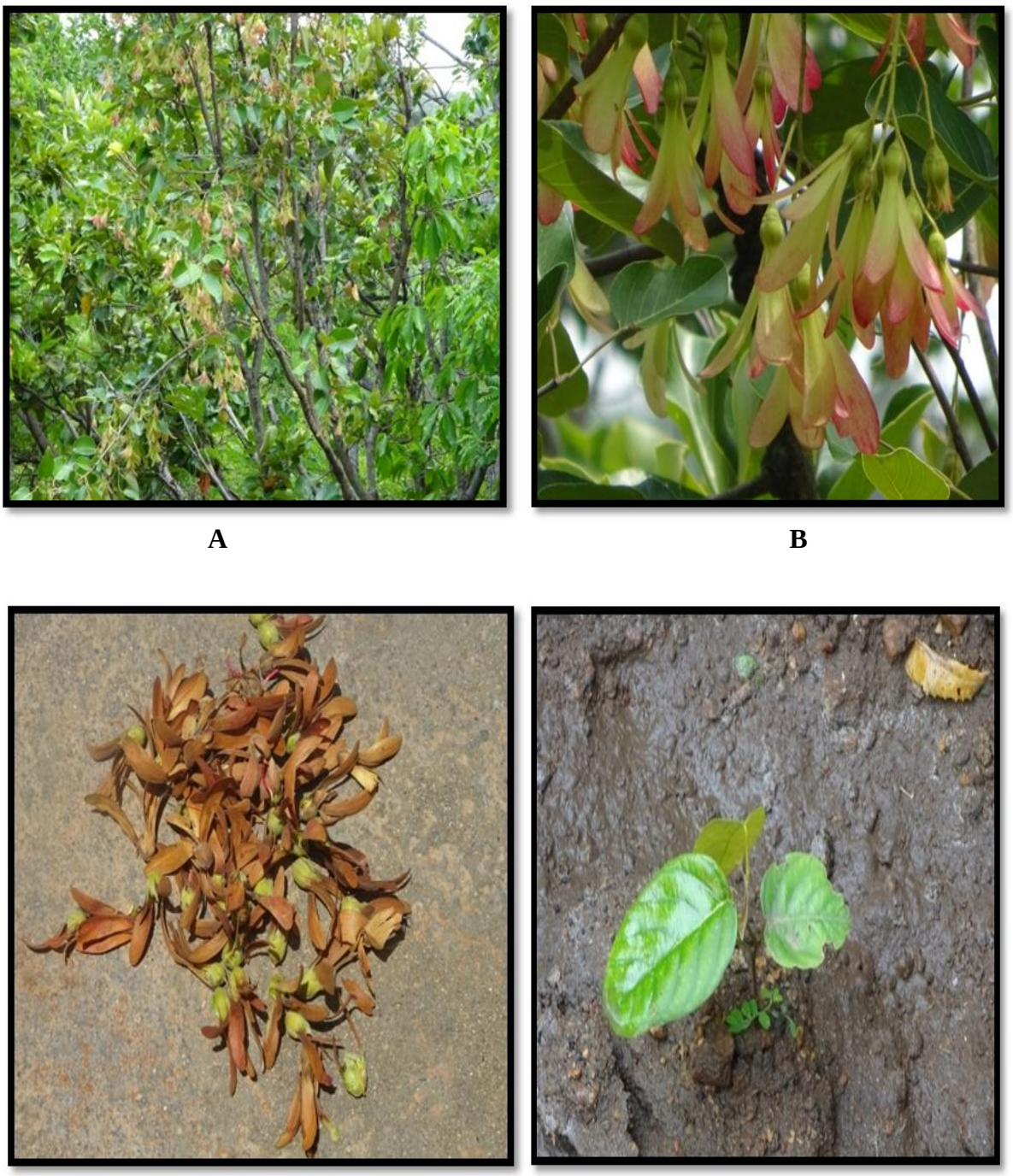
The present investigations were carried out to standardize nutrient media for the in vitro germination of *S. Tumbuggaia* and also to study the effect of growth regulators. Attempts were also made to devise methods for the maintenance of seed viability in different temperature and effect of seed size and weight and different treatments. From the data (table-1) the percentage of healthy seedlings, it is obvious that the ideal place for seed collection of *S. Tumbuggaia is* Tirumala. Seeds collected from Tirumala showed 80% germination. For natural germination, the germination percentage of 10-15%. Many reports say that the longevity of these seeds is variable and in majority of cases, low percentage of viability has been reported in these species. They are difficult to store for artificial regeneration and same

they are often described as recalicitrant. This is also reported by (Krishnapillay et al., 1998). The seed germination is cryptocotylar, semi-hypogeal and rapid. The hypocotyle is red, long cylindrical takes different twist and eventually penetrate into the soil to produce root system and leaves. Seeds die if moisture content is too low and temperature is too high. Therefore, the time of collection is important for propagation of these species. The seeds are immediately inoculated the seed germination is cryptocotylar, semi-hypogeal and rapid. The hypocotyls are red, long cylindrical takes different twist and eventually penetrate into the soil to produce root system and leaves. Seeds die if moisture content is too low and temperature is too high.

The germination of seeds was studied immediately after collection and 80% of germination was recorded within 48 hours in the fresh seeds of *S. Tumbuggaia.* Even though germination percentage is high due to the cryptocotylar semi hypogeal germination all the seeds are germinated within 2-3 days. To avoid the germination we kept the seeds in low temperature. In that also half of the seeds are germinated. When seeds are germinated the hypocotyle comes in the form of long red cylindrical structure. When the seeds kept outside the seeds become dry and moisture content become very low and germination percentage become very low(Table-2). The quality of the seedling is directly correlated to the size/weight of the seeds. Seeds with medium and heavier seeds weight performed significantly better than lighter seed weight class in terms of germination, survival and seedling height. The results confirm the earlier observation that seed germination and seedling development is highest in heavy seeds (Kandy 1975; Chauhan and Raina 1983) who reported that seed size of Leucanea leucocephala affected both germination and critical plant growth. Turnbull (1983) also indicated that the seed size affected the critical growth of Eucalyptus seedling. Goor and Barney (1976) also noted that the seed size influenced germination in *Eucalyptus citriodora.*

The percentage of germination is very hig in both room temperature and low temperature seeds. But when the days are increases. The room temperature seeds shows less percentage of germination due to the lose of moisture content and cryptogeal semi-hypogeal germination (figure 1). In low temperature seeds the percentage of germination also decreases due to to the seed born diseases and other microorganism on the seed. Finally the percentage of germination is totally decreased within 10-20 days (Table-1). These data shows a close relationship of moisture content with the viability and indicates that loss of moisture content

in room temperature stored seeds is primarily responsible for the loss of viability in *S. Tumbuggaia*.



A **B**

C **D**

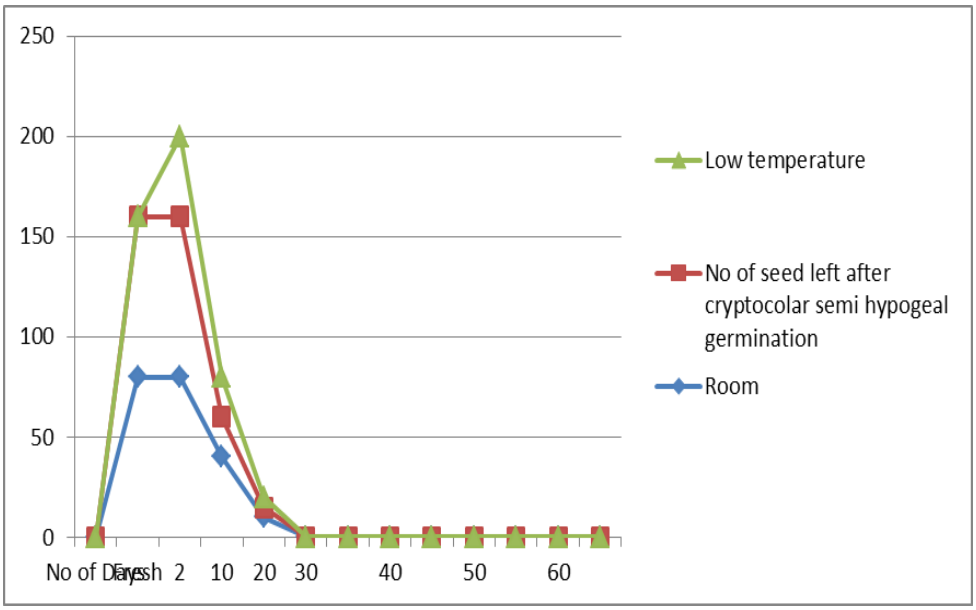
Figure: 1

A. Shorea tumbuggaia Roxb in tirumal forest

B. Shorea tumbuggaia Roxb in flowering stage

C. Shorea tumbuggaia Roxb seeds

D. Shorea tumbuggaia Roxb Invitro grown seed ling



Graph: Effect of Different Storage Temperatures on In Vitro Seed Gernimation of Shoreatumbuggaia.

Table 1: Effect of Different Sterilizing Agents on Aseptic Seed Germination of Shorea Tumbuggaia.

Treatment	Medium	Remarks
Washingwith0.1%HgC12 For 3 minutes	MS + Agar 0.8% + AC0.05%	Healthy seedlings germination is slow Contamination
Washingwith0.1%HgC12 For 5 minutes	MS + Agar 0.8% + AC0.05%	Healthy seedlings germination is slow Contamination
Washing with 0.1% $HgCI_2$ For 10minutes+ Bavistin (1%)	MS + Agar 0.8% + AC0.05%	Healthy seedlings germination is slow No Contamination
Washing with 0.3% $HgCI_2$ For 15 minutes	B_5 + Agar 0.8% + AC 0.05%	No contamination, Germination is slow
Before soaking washing in ascorbic acid Washing with 0.3% $HgCI_2$ For 15 minutes	MS + Agar 0.8% + AC 0.05%	Healthy seedlings germination is slow
Washing with 0.1% $HgCI_2$ For 10minutes+ Bavistin (1%)	MS + Agar 0.8% + 2 mg/I BAP+ 0.5 mg/I NAA	Healthy seedlings with quick germination
Washing with 0.1% $HgCI_2$ For 10minutes+ Bavistin (1%)	MS + Agar 0.9% + 2 mg/I BAP	Healthy seedlings with slow germination
Washing with 0.1% $HgCI_2$ For 10minutes+ Bavistin (1%)	MS + Agar 0.8% + 2 mg/I BAP+ 0.5 mg/I 1AA	Healthy seedlings with quick germination

In general for maintenance of seed viability in storage the lower the moisture content, the longer the period of their viability. But in recalcitrant seeds comparatively high level of moisture content is vital for the retention of viability (Roberties 1973 and Hartman and kester

1976), also reported that keeping seeds in a non-sterile moist warm medium for several weeks could soften the seed coat through micro organic action. The changes in the germinability of these seeds should be maintained at a high moisture content because they belong to the recalcitrant type of seeds. However in *Shorea tumbuggaia* if the seeds are maintained in low temperature, they will dry with in 2months or contatamination takes place due to the seed born diseases. However, among recalcitrant species also variation seems to occur in their ability to maintain the critical moisture content for long period while in others it is not. This is also reported in *Shorea robusta* where reductions in moisture content below a critical level cause death to its seeds (Nautiyal et al., 1985).

The main problem during germination of *Shorea tumbuggaia* is that of Exudation of Phenolics from The Seeds. To Over Come this Problem of Phenols Various experiments were conducted. Addition of PVP (50 and 100 mg/l) and activated charcoal (400 mg/l) suppressed the phenolic effect of released phenolic compounds and enhanced the seed germination. This is also reported in Teak (Gupta et al., 1980) and Guava (Amin and Jaiswal, 1988). Kumar et al. (1998) and Boulay (1979) also reported the inclusion of activated charcoal (400mg/l) for better germination. However, the best result in the. Present investigation was obtained when seeds were frequently subcultured 2-3 time on the same germinating medium after an interval of 5-7 days.

Before inoculation on different media, seed of *Shorea tumbuggaia* were treated with different concentration of growth regulators for early germination. A high percentage of germination was recorded in seed treatment with 24 hours incubation in 20 mg/lGA3. Before inoculation in the medium. Amoung different growth regulators represents tried BAP failed to record any superiority over the GA3 treatment. Application of GA3 (10 and 20 mg/l) showed quicker termination and developed plants in one week. But BAP (2 mg/l) + GA3 (20 mg/l) concentration the germination was slow and plants developed after one month only (figure-1).

Seeds were cultured on different media like WPM, B5, MS (Table -4). The present investigation has clearly demonstrated that MS medium is highly suitable for germination of seeds *Shorea tumbuggaia*. In *Shorea tumbuggaia* germination of seeds also takes place in B5 and WPM media. However the subsequent stages like first leaf, second leaf and fourth leaf were not supported on this medium. For all practical purpose and healthy growth of seedling MS media was found to be the best. Higher percentage of germination was obtained by

placing healthy seeds on MS medium supplemented with 2 mg/I BAP (Table). The combination of (0.5mg/I) NAA or IAA is more effective in bringing quick germination. Seeds were cultured on different media like WPM, B5, MS. The present investigation has clearly demonstrated that MS medium is highly suitable for germination of *Shorea tumbuggaia* seeds. In *Shorea tumbuggaia* germination of seeds also takes place in B5 and WPM media. However the subsequent stages like first leaf, second leaf and fourth leaf were not supported on this medium. For all practical purpose and healthy growth of seedling MS media was found to be the best. Higher percentage of germination was obtained by placing healthy seeds on MS medium supplemented with 2 mg/I BAP (Table-4). The combination of (0.5mg/I) NAA or IAA is more effective in bringing quick germination (Table) Kinetin at different concentrations did not improve the faster germination. The first leaf stage was however enhanced by 2 mg/I KN.

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