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Research Paper

Studies on habitat survey and seed germination of *Shorea tumbuggaia ROXB*. A globally threatened medicinal tree taxon of seshachalam biosphere reserve of India

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Abstract

Shorea tumbuggaia **Roxb. a globally threatened medicinal tree taxon is valued for its economic and pharmaceutical properties. Natural resurgence of this species is poor. The plant is propagated naturally through seeds. To improve the amplification of the species, seed germination studies were carried out. The habitat survey revealed that the more number of plants are available on Tirumala hills when compared to Talakona valley. The seed viability test indicated that is very high 92 percentage of seed viability in freshly produced seeds, in vivo seed germination was recorded upto 60% only in different soils.The growth of lengthy embryonal axis before shedding the seeds from plant is considered to be an adaptive strategy for seedlings to over come the hard rocky environment prior to establishment. The capacity of seed germination and establishment, growth and development of seedling was found to be vigorous in open conditions when compared with that of shaded seedlings. Whereas 100%** ***seed*** **germination was recorded in** ***in vitro*** **germinated seeds. In order to continue their existence, it requires powerful conservation procedure to achieve quick and reliable seed germination would be a valuable asset.**

Keywords Embryonal axis, plumule, radicle, seed germination, *Shorea tumbuggaia*

Introduction

The availability of medicinal plants is under serious threat in present days. Over 95% of medicinal plants used by Indian industry are collected from the wild. Threat assessment experiences as per latest IVCP guidelines, for southern and northern India have already listed about 200 species of medicinal plants that are rare, endangered and threatened. Government of India banned export of more than 50 species, believed to be threatened in the wild [1]. There are different factors contributing to the extinction that include habitat loss, introduction of new species, over exploitation indiscrete harvesting, environmental catastrophe, geographic or genetic loss of genetic variations and stimulation of deleterious mutations (genetic erosion), urbanization, shrinking forests, over testing, genetic drift and in breeding. Exploiding and bio prospection by trans natural pharmaceutical companies has further aggravated then due to which many important species of plants are becoming rare, endangered and threatened in nature [2]. Hence, it is imperative that feasible strategies to conceive the red listed medicinal and aromatic plants, assessment of genetic diversity in necessary to prevent further loss [3]. Conservation aims at preservation, maintenance, sustainable utilization, restoration and ultimately enhancement of quality of life [4].12 taxa are known from type collection only and 5 taxa are recollected from type locality only, 12 species are included under different IUCN threat

categories based on Red Data Book on Indian plants and Conservation Assessment and Management Planning (CAMP) 2001, Andhra Pradesh [1,5].

Shorea tumbuggaia species is an endemic and globally endangered semi-evergreen tree species, (Red list of threatened species IUCN-2006) restricted to the Southern Eastern Ghats up to 100 m, distributed in Seshachalam and Veligonda Hills in Cuddapah, Tirupati Hills in Chittoor District, Andhra Pradesh and North Arcot and Chingleput districts. *Shorea tumbuggaia* Roxb. is endemic to Middle Eastern Ghats of Andhra Pradesh and Tamilnadu [6], endangered globally according to the CAMP [1] and is critically endangered [7]. *Shorea tumbuggaia* Roxb. or *Vatica tumbuggaia*. Wt. & Arn. (Dipterocarpaceae). Commonly known as Green Dammar. It is large sporadic resinous tree attains the maximum height of about 20-30 m with maximum width of 150-190 cm. Bark is thick, rough dark brown longitudinally furrowed, within the furrows gum is exuded. Simple leaves with ultimate reticulate venation. Inflorescence is Axillary/ terminal panicles. Flowers white. Fruits with a woody pericarp with wings 2-3 times as long as capsule. Single seed with 4 unequal fleshy cotyledons. *S. tumbuggaia* trees bear adequate foliage during and after the rainy season. It has reduced foliage during late winter and early dry season. At the same time, gradual new leaf flushing occurs. The leaf transitional stage characterized by leaf fall and leaf formation is quite prominent during April-May. Flowering occurs during April to May and fruiting May to June. Each fruit produces only one seed against the actual number of six ovules. The sepals are acrescent 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 a thin inner membranous lining invaginated into the folds of cotyledons and split into two parts at the apex.

S. tumbuggaia is a tree taxon with economic and medicinal values. Stem is used in marine yards as a substitute for pitch. The tree trunk is in use as flag poles for temples [8]. The heart wood is similar to sal but much smoother and better for carpentry. The plant extracts used to cure ear-aches. Plant parts are to be used as an external stimulant. Leaf juice is used as ear drops for children [9].The bark having anti ulcer activity [10]. The stem is a source of resin, which is used as incense. The resin used to cure duodenal ulcers and Amoebic dysentery. The oleoresin which is exuded from the stem bark of *Shorea* can be used to cure hydrosis and alexiteric [11]. It is also used in indigenous medicine as an external stimulant and a substitute for arbutus[12]. The rise in human population with demand on land for farming, increased live stock, construction of road ways hydel power stations and allied works [13].

The modern agronomy, horticulture and forestry relies on seeds and seedlings to produce most of the world's food, fibre and medicine resources. The major advance in plants produced the marvelous diversity found in seeds and their accompanying fruit structures. The seed itself is the end product of process of growth and development within the parent plant. It is noted that about 10% of all plant species are endangered indicating widespread degradation of ecosystem and therefore the urgent need for strategic conservation action for the selected plant taxon. Hence the present study focused on suitable climatic conditions, soil characteristics, seed viability, seed germination, seedling vigour index, seedling establishment, growth and development. In order to increase the percentage of seed germination *In vitro* protocol would be very much helpful for mass cultivation practices of the *Shorea tumbuggaia*.

Materials and Methods

In vitro seed germination

a) Explant sterilization

Healthy seeds of *S. tumbuggaia* were collected in different places of Tirumala – Kadapa – Nallamalai hotspot, India during field visits and preserved for further studies. The seeds were washed thoroughly under running tap water for an hour and surface sterilized with 5% teepol solution for 10 min followed by running tap water until the foam was completely reduced. Then rinse in the distilled water for 5 min, these above preparation was done in normal lab conditions.Then the subsequent process was done in the laminar airflow under culture room conditions.The seeds were treated with 70% alcohol for 60 sec followed by sterile distilled water for 5 min at least two times and then wash with 0.1% $HgCl_2$ for 5 min, frequently swirrelled the conical flask to remove all microorganisms from all surfaces of the seeds followed by washing with sterile distilled water for 5 min for 3 times.

b) Phenolic treatment, inoculation and incubation

In order to reduce the phenolic exudation we were developed a protocol for successful seed germination i.e., washing seeds under running tap water for 2 hrs, stirring in 0.5 to 2.0% PVP solution and 1% citric acid solution. At each interval of the above treatment the seeds were washed with sterile distilled water for 5 min to remove the chemical residues. Finally seed coat was excised with sterilized scalpel. The seeds were inoculated on MS half strength and MS full strength basal media[14] at every regular intervals the instruments were dipped in alcohol followed by heating with spirit lamp. Later all the cultures were incubated in a culture room at 25 $\pm$ 2^0C with relative humidity of 50 to 60% under dark conditions.

***In vivo* seed germination**

a) Rhizospheric Soil analysis

Rhizospheric soil samples of *S. tumbuggaia* were collected from Tirumala and Talakona and air dried then sieved (2 mm). The pH of the soil samples was determined electrochemically by using pH meter. Organic carbon, alkalinity and moisture content [15], and total Nitrogen of soil [16], available phosphorus, exchangeable calcium and potassium [17] of the soils were estimated.

b) Seed Calorific value

Collected seeds were shade dried, powdered and estimated the energy content with the help of Oxygen bomb calorimeter. Platinum wire of 10 cm length was inserted in to each 1 g sample before compaction so that it could be tied up with the electrodes. The material was ignited electrically inside the oxygen filled steel bomb of the oxygen bomb calorimeter and the rise in the temperature was recorded by its thermometer.The energy content was calculated using the formula by Leith [18].

c) Seed viability test

The seed viability was assessed by performing tetrazolium (TZ) test.The seeds were separated from acrescent sepals, and incubated in 50ml of 1% (w/v) solution of 2, 4, 5-triphenyl tetrozolium chloride (TTC) prepared in 0.1 M Sorensen's buffer (pH–7.0) for 24 hours at 28^0C. After the incubation, the seed was longitudinally bisected and the embryo was observed.The seeds where in embryos turned reddish pink were scored as viable and the seeds that remained green were scored as non-viable according to Eplee and Norris[19].

d) Seed germination percentage, seedling growth and vigor index

Seeds were sowned in different soils like soil, sandy soil and sand and tested both open field and shade conditions. Then the percentage of the seed germination was calculated. The length of roots, shoots and leaves were measured with thread and scale.The seedling vigor index was calculated by the method of Singh[20] by multiplying the percentage of germination with root length.

Results and Discussion

***In vitro* seed germination**

Seed germination culture on half strength MS Basal media has registered the highest percentage at the seed coat excision condition within 7 days but in *Solanum trilobatum* takes 14 days at same media and same concentrations [21].Whereas MS basal full strength media showed 72% of seed germination. Half strength MS media was superior than full strength MS media[22].The survival percentage was recorded on full strength and half strength MS basal medium were not significantly different. The minimum number of days has been taken to seed germination (5 days) were recorded with half strength MS basal medium when compared with full strength MS basal medium. Lowest survival percentage (20.5%) was recorded on full strength MS media at the media fortified with 1% char coal and pre-soaking of seeds with 1% PVP even the increment of the concentrations also not encouraged in the seed germination. The superiority of reduced salt strength media in terms of survival may be attributes the fact that high salt strength did not suit the initial establishment of explants [23].

After 24 hrs of inoculation the phenolic substances were oozed out and media is getting brown it may be phenoloic compounds were secreted from seeds to media. The micro propagation efforts are largely failing due to the exudation of phenolics by the explants *in vitro* leading to the browning of the medium around them and arresting their *in vitro* growth[24]. Eventhough the plant having high phenolic content, *in vitro* seed germination was succeeded by different treatments like activated charcoal, 1% PVP added to the media, 1% Citric acid and seed coat excision as pre-inoculating treatment. Among

these, seed coat excision treatment given 100% (Table1 and Figure1 h-k) seed germination and best seedling growth and development on MS half strength media but MS full strength media was not encouraged. Browning intensity of the media is slightly reduced by adding 1 to 2 gm of activated charcoal to the media. Presoaking of seeds with 1% PVP also given good results in the browning of the media at 10-15 min.However, presoaking of 1% citric acid solution given better results towards the browning intensity at increasing the time from 10 to 15 min. Antioxidants could influence reduction of phenol accumulation but found a lag period at initial stages while might be due to relatively lower nutrient strength of the 3/4 MS compared to sole MS medium alone [25]. These results coincide with previous results of Dar et al. [26] in the regenerated on of some cherry through *in vitro* propagation.

***In vivo* seed germination**

Rhizospheric Soil analysis

A measure of soil pH buffering capacity is needed to understand rates of natural soil weathering, Talakona soil having 7.3 pH whereas Tirumala showed 6.9. This variation results in the variation of soil capacity to resist changes in soil pH due to soil reactions that either produce (or) consume H^+ [27]. Highest moisture holding capacity (12.5) has recorded in Talakona soils but Tirumala soil shows 10.5 only. Water holding capacity was greater in broad-leaved forests and poor in plains [28]. In our present investigation there is no variation in organic carbon (0.75%) between the two selected soils. The benefits of increasing soil organic carbon is not only improved soil structure and water nutrient relationships, but includes the ability to store carbon in the soil to reduce atmospheric CO_2. Similar results were reported by[20]. Several studies have been reported to compare the distribution of organic Nitrogen, Phosphorus, Potassium, Calcium Carbonate and Sulphur in different soils or among the soils under different management practices [29].There is no variation between the two soil samples in Nitrogen and Phosphorus content (400-500 kg/Hec) and 20 P_2O_5 Kg/Hec respectively. But potassium, calcium carbonate, sulphur contents have been varying from Tirumala soil to Talakona soil. i.e., 350 Kg/Hec to 145-340 Kg/Hec (P), 1 mg/lit to 2.5 mg/lit ($CaCO_3$), 10-15 ppm to 0-10 ppm respectively (Table 2). Tirumala hills are composed of granite complex soils and reddish brown loams [8].Talakona a sacred grove is a part of Tirumala – Cuddapah – Nallamalai micro hotspots of endemism and one of the sacred groves of Andhra Pradesh with rich plant resources, the grove comprises dry deciduous to moist deciduous types with a number of useful and under utilized plant taxa [30].

The TZ test revealed 92% viability in the seeds used.High seed calorific value (2441 Cal g^{-1}) is recorded when compared with flower buds (2138 Cal g^{-1}) Energy content for its calorific value was higher in *Rheum nobiles* than *R. emodi* [31].The seeds of *Shorea* has no dormancy and the embryo is chlorophyllous. It begins germination immediately after the fruit falls to the ground. The environment during seed development on the mother plant affected the levels and patterns of seed dormancy was observed in *Chenopodium quinoa*[32].Seed germination is cryptocotylar, semi hypogeal and rapid. The hypocotyl is red, long, cylindrical, takes different twists and eventually penetrates into the soil to produce the root system and the leaves.While the seed attains germination embryonal axis has pushed out and grows long is about 6-8 cm in length from this mid region of the embryonal axis plumule was arised and grows towards upper side as well as radicle was differentiated from the hypocotyls then lateral roots were arised and deepens in to the soil (Figure1 a-g).Growth of lengthy embryonal axis is considered to be an adaptive strategy for seedlings.

Seed germination and SVI

Seed germination in normal soil was scored just 10-30% with lowest seedling vigor index (65-195) in both open field and shade conditions. Maximum germination was observed in *Euryale ferox* during different months[33]. Whereas sandy soil proved good results towards the seed germination it was reached from 30 to 60% of seed germination with highest seedling vigor index (246-438) both in open field and shade conditions. But sand showed average seed germination (40-50%). With moderate seedling vigor index (300-325) both open field and shade conditions (Table 4).Germination percentage and vigour index can be improved by subjecting seeds.To presowing chilling, hydropriming for 8 days or chemical treatments observed in *Podophyllum hexan drum*[34]. Timing of seed dispersal and dormancy appear to control timing of seed germination and seedling recruitment of most of woody species [35]. Barmukh and Nikam [36] reported the effectiveness of wet heat, physical and acid scarification of seeds for inducing uniform and fast nursery germination in *Pterocarpus marsupium*. The seeds of *Aconitum heterophyllum* were germinated by overcoming dormancy with ethanol. Trivedi & Rachna Kumari [37] reported that the seeds of *Rauwolfia serpentina* showed seed coat dormancy scarified seeds showed highest germination.

Seedling growth

Open field conditions are the best suitable for the maximum root length ranges from 6.5 to 8.2 cm followed by shade conditions ranges from 5.2 to 7.3 cm in three different types of the selected soils (Table 3). Among these sandy soil proved maximum root length (8.2 cm) in the case of open field condition. But normal soil showed minimum root length (5.2 cm) from shade conditions. The largest shoot can be obtained from sandy soil under shade conditions, whereas shortest shoot was noted in the normal soil from open filed conditions.So, the shade conditions are most suitable for the maximum shoot length ranges from 24.0 to 27.3 cm in three different selected soils. Among these, sandy soil proved maximum shoot length (27.3 cm) especially from shade conditions. Soil temperature is an important edaphic factor that influences various processes such as evapotranspiration, soil aeration, growth and development of plants, biological activities etc. [38, 39]. The largest leaf can be obtained only at sand from open field, where as smallest leaf was recorded with normal soil in the case of shade conditions. Open field condition proved best suitability for maximum length and breadth which are ranges from 7.2 to 8.0 cm and 4.0 to 5.5 cm respectively (Table 3).The sand showed maximum leaf length (8.0 cm) and breadth (5.5 cm) was recorded especially in the case of open field conditions. But, shade conditions showed the maximum leaf size ranges from 5.0 to 5.8 cm length and 2.5 to 3.5 cm breadth. Among these sand showed the maximum leaf length 5.8 cm length and 3.5 cm breadth under shade conditions. 100% seed germination within short period has been obtained in *in vitro* conditions and the suitable environmental conditions were explained by *in vivo* seed germination. Moreover these finding will be useful to understand the physiology of seed germination and to develop strategies for cultivation of the *Shorea tumbuggaia* through seeds and to rehabilitate the degraded habitats and helps in conservation.

Table 1: *In vitro* Seed Germination on MS Basal media with phenolic treatment

S. No.	Treatment		Browning Intensity		% of germination		Survival % of seeds	
			F.S.M	H.S.M	F.S.M	H.S.M	F.S.M	H.S.M
1.	Adding activated char coal to the media	1 g/lit	High	Moderate	22	45	25.2	20.5
		2 g/lit	High	Moderate	23	46	30.5	21.5
2.	Pre-soaking of seeds with 1% PVP	10 min	Moderate	Moderate	46	64	35.6	34.2
		15 min	Moderate	Moderate	48	63	36.0	35.3
3.	Pre-soaking of seeds with 0.1% Citric acid solution	10 min	Moderate	Low	50	70.5	62.0	50.7
		15 min	Moderate	Low	52	71.0	65.0	53.2
4.	Seed coat excision		Moderate	Very low	60	100	70.0	72.0

F.S.M = Full strength medium, H.S.M = Half strength medium

Table 2: Rhizospheric Soil Analysis of the selected areas

S. No.	Soil characteristics	Tirumala	Talakona
1.	Moisture content	10.5	12.5
2.	pH	7.2	7.3
3.	Organic Carbon	0.75%	0.75%
4.	Nitrogen	400-500 Kg/Hec	400-500 Kg/Hec
5.	Phosphorus	20 P_2O_5 Kg/Hec	20 P_2O_5 Kg/Hec
6.	Potassium	350 Kg/Hec	145-350 Kg/Hec
7.	Calcium Carbonate	1 mg/lit	2.5 mg/lit
8.	Sulphur	10-15 ppm	0-10 ppm

Table 3: *In vivo* seed germination percentage and vigor index and measurements of the seedlings of two different group's *in vivo* seedling growth

S. No.	Sowing condition	Types of soil	%	Seedling vigor index	Root length (cm)	Shoot length (cm)	Leaf size (cm)	
							Length	Breadth
1.	Open field	Soil	10	65	6.5	10.5	7.2	4.0
		Sandy soil	30	246	8.2	17.6	8.0	4.2
		Sand	40	300	7.5	13.6	8.0	5.5
2.	Shade condition	Soil	30	156	52	24.0	5.0	2.5
		Sandy soil	60	438	7.3	27.3	5.0	2.7
		Sand	50	325	6.5	25.5	5.8	3.5

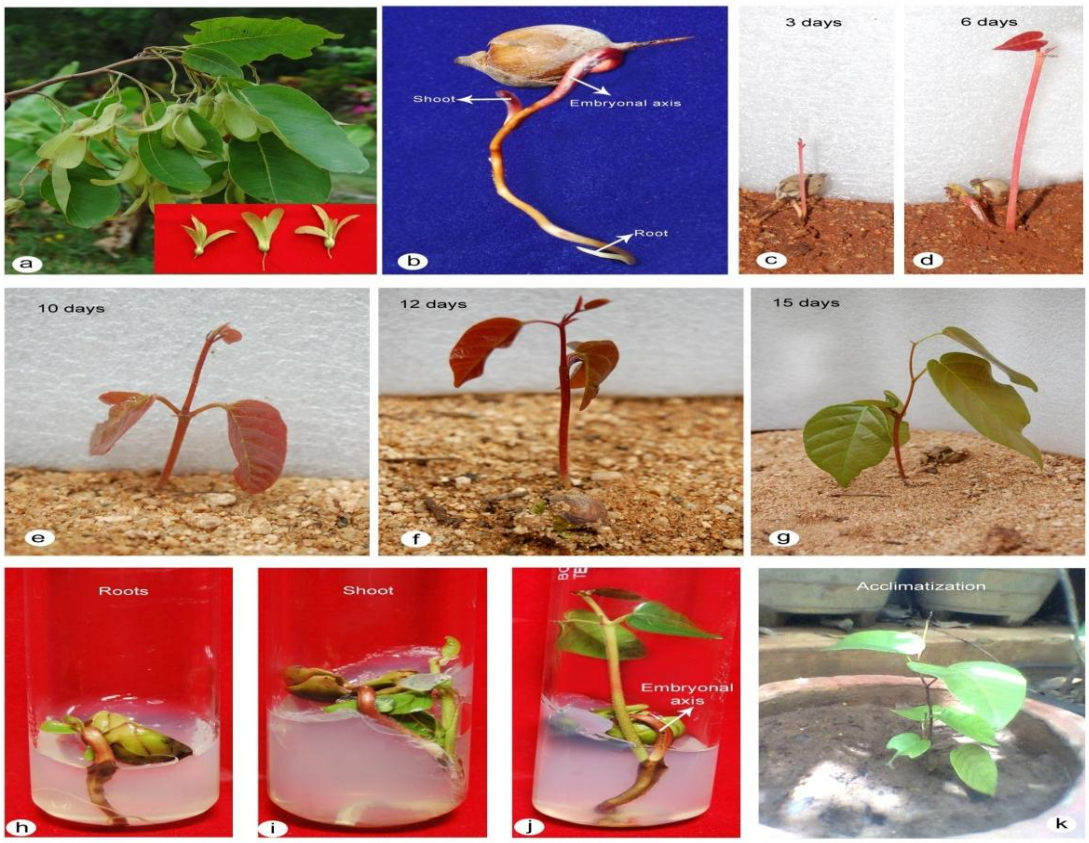

Figure 1 Seed germination of Shorea tumbuggaia
a) Twig with fruits, b-g) in vivo seed germination, h-k) in vitro seed germination

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