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

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Abstract

Shorea tumbuggaia is an endemic endangered medicinal important plant species of Dipterocarpaceae family. In general, seed is the most common form of propagation for afforestation in which breeding stock is usually retained. The fruits are winged and mature with 20-30 days. The seeds lack Dormancy, some seeds germinate before falling from the tree and some seed germinate as soon as they fallen from trees. 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:

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

Introduction:

Shorea tumbuggaia Roxb. Belonging to the family Dipterocarpaceae, It is endemic to India found widely in Seshachalam and Veligonda hills in Cuddapah and Tirupati hills of Chittoor district of Andhra Pradesh to North Arcot and Chingleput, districts of Tamil Nadu. It is globally threatened medicinal tree tax is valued for its timber and pharmaceutical properties. Now this tree is facing high risk of extinction in wild due to ecological conditions specially Forest fires and other anthropogenic pressures seem to have affected the vegetation considerably. There is an urgent need to protect this endangered and endemic plant in Seshachalum area (Figure-1). The reason for endangered status of the plant in tirumal region is phenology status of the flower that is continued up to the seed formation. Seed is the natural vehicle for gene movement and storage. It is the usual form in which germplasm is collected. In general, seed is the most common form of propagation for afforestation and is the form in which breeding stock is usually retained. There are, however, considerable problems remaining in the use of seed. Some of these are discussed below for dipterocarp species in relation to the underlying seed physiology processes.

Invitro plant regeneration among the members of Dipterocarpaceae has been quite difficult. The explant source has been one of these important factors that greatly affected the frequently of plant regeneration. The results presented below provider a complete and rapid clonal propagation system of *Shorea tumbuggaia Roxb* r. To our knowledge this is first report on *Shorea*. Unfortunately there is no such report available for clonal propagation and seed germination studies of *Shorea,* in this paper we presented rapid multiplication of this medicinally important plant through high frequency axillary shoot proliferation from seeds and cotyledonary node explants followed.

Material and Methods:

Seeds were collected from the Tirumala hills of Sechachallum hills. Wings and seed coat from Seeds were mechanically separated from and were surface sterilized for 10min in tween 80, then dipped in 70% alcohol for 3.5 min and subsequently in the 0.5% H_2O_2 for 2-3 min 3-4 times in the sterile distilled water. Seedlings were micropropagated on MS medium in the 2 mg/l BAP. Cotyledonary node explants were aseptically excised from 15-30 days old seedling. The explants were transferred to the MS medium with various concentration and combinations of auxins and cytokinin and sucrose 3% is as the carbon source. The PH of the medium was adjusted to 5-6 by adding 0.8% agar before sterilization was observed within 15 days and callus was subculture one in every fifteen days well developed shoots were excised and transferred to rooting media for rooting. All the experiment was repeated at least thrice.

Result and discussion:

Even though massive flowers were present in that there is no anthers found in the flowers. From flowering stage on words more bees and Wild Red big Red Aunts are fond on the trees. Out of this only 40% of the plants pollinated by pollination and seed set happens. The fruits are winged and mature with 20-30 days. The seeds lack Dormanancy, some seeds germinate before falling from the tree and some seed germinate as soon as they fallen from trees. The poor seed set is due to the high temperature. The healthy fruiting occurs in ever 1-3 years, but not in every year. This is common in *Shorea* species. The germination is very fast as soon as the seed is fallen Hypocotyl comes out in the form of long ,cylindrical red color structure and penetrate into the litter and produce root, only 1or2 plants are produced with root, shoot and leaf. The percentage of germination is high but seed ling production is very less of 0.01%. Even though rich nutrient soil is present under the tree the growth of the seed is suppressed due to the seed borne diseases, some unknown beetles and due Ecological conditions like high temperature suppress the growth of the plant. Because fruit set, seed fall and germination takes between May-July. The seeds are produced irregularly and sparsely in some species and fruit production varies in quantity and quality from year to year. Mass fruiting appears to favor seed predators.

In *Shorea tumbuggaia* each fruit produces 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 invaginated into the fold of cotyledons and split into two parts at the apex. The fruit are winged, mature quickly within a month and each fruit invariably produces a single seed with a large chlorophyllous embryo (figure-1B and 1C). As the seeds lack dormancy and germinate soon as they fall from the tree, the large chlorophyllous embryo may aid in better survival in unpredictable habitats with irregular supply of light nutrients and water during germination period. The seed germination is cryptocotylar, semi-hypogeal and rapid (Figure-1D). The hypocotyl 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. They are difficult to store for artificial regeneration and same they are often described as recalcitrant. This is also reported by (Krishnapillay et al., 1998). A considerable amount of empirical work on the storage of forest tree seed has been carried out. A more physiological research approach is relatively new. Many tree species have seed that is desiccation-sensitive (recalcitrant), so that moisture physiology is especially important for this group. However, in a recent review article on water in relation to seed storage, the section on desiccation- sensitive seeds comprised only 4% of the article (Roberts and Ellis 1989). More attention is, however, now being given to recalcitrant seeds (Berjak and Pammenter 1996). The winged character of the sepal allows the single seeded fruits to gyrate towards the ground and hence the seed dispersal is anemichorous. The distance of seed dispersal by wing is up to 10m only due to the semi-closed nature of the canopy cover the forest. The

dispersal of winged fruit takes place much more efficiently by wind if the forest is of open seasonal dry deciduous type. The seeds fallen on the ground have no possibility for further dispersal by sweeping action of the wind due to the litter accumulation and grass growth in the study area. The ripe dry winged fruit fell to the ground and dispersed with in the 10m area of the tree due to the wind action. The flora of the forest in the surroundings of *Shorea tumbuggaia* was found with the mixture of rocks and soil. The forest flora is rich in nutrients was found to be good for the seedlings to establish there but soon the seedlings were suppressed by competition of other plants. But in *S. alba* seed dissemination by wind is up to the 2km. (Ashton PS 1982). Fruit infestation rate was 70%. In the healthy fruit seedlings establishment rate was 48%, but it was only 14% compared to the total fruits produced. The fruit can store at least for one month. Because the seed are germinated with in falling. If we store the seed res hypocotyl will come and all the seed were germinate with in the week. The germinated seed cannot store. After one month the hypocotyl will dry and become black. In *Shorea tumbuggaia* other cause for damage is fruit were found to be infested with an unidentified Bruchid beetle. It is found at an early storage of fruit development had pierced the pericarp and deposited a single egg. When the egg hatched, the young larva burrowed into the developing seed to use it as food source. The pierced part from the pericarp into the seed formed a hole throughout and larva used this hole for exit. Fruit fall occur when the larva was still in the growing stage. The larva left the seed and fruit through the hole after completion of its development and purpated in the soil. The pupal stage was observed 6 weeks, but there was no emergence of the adult. This long period was considered as the dormant stage of the pupa for the emergency of the adult when conditions were favorable in the forest floor (Solomon Raju AJ, 2009).

Different insect's species attack the seeds of *Shore s*pecies during their development. Insect pest attacks at the pre or post dispersal stage of the seed in the pre dispersal stage. The pest attacks the fruit on the tree before dispersal while in the post dispersal stage the pest attacks fruits on the ground (Toy RJ 1988). Kattu and Chakrabarti reported that many Buchid species send a dormant stage as pupa in the soil and this holds true in case of Bruichid pest in *Shorea tumbuggaia*. They also reported that in India, the seed weevil *Sitophilus rugicollis*, attacks seeds of *s. robusta*, surviesas a dormant adult in the forest floor and emerges with the first monsoon rain, which coincident with the commencement of seed fall (Khattua AK and Chakrabarti S 1990). This is also reported in some Dipterocarp seeds, seeds are produced irregularly and sparsely in some species, and fruit production varies in quantity and quality from year to year. Mass fruiting appears to favor seed predators, but it can also be a strategy to escape complete seed destruction (Janzen 1974). Seed predation can be very high, and the crop can be completely wiped out. Curran and Leighton (1991) reported that the 1986 crop was entirely destroyed (100,000 seeds/ha). The major losses are caused by insect pests. Natawiria *et al.,* (1986) observed weevils (Curculionidae) damaged 40-90% of the seeds of *Shorea pauciflora, S. ovalis, S. Iaevis, S. Smithiana and* Dipterocarpus *cornutus.* Daljeet-Singh (1974) reported that weevils were responsible for more than 80% of the total seed damage in all case studies except *Shorea*

macrophylla, in which the most important pests were the Colytidae. Kobayashi (1974) observed that 80% of the mature seed crop *of Hopea nervosa* was damaged by squirrels. Parrots (*Psittacula* sp.) have been observed feeding on dipterocarp seeds (Natawiria *et al.*, 1986).

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.

For *Invitro* cultures of seed of *Shorea tumbuggaia* were treated with different concentration of growth regulators on different media for early germination. A high percentage of germination was recorded in seed treatment with 24 hours incubation in 20 mg/l GA_3. Before inoculation in the medium, different growth regulators tried but BAP failed to record any superiority over the GA3 treatment. Soaking of seeds in of GA_3 (10 and 20 mg/l) before inoculation showed quicker germination and developed of plants in one week. But BAP (2 mg/l) + GA3 (20 mg/l) medium the germination was slow and plants were developed after one month only.

Seeds were cultured on different media like WPM, B5, and MS. 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, and 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 (Table8).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. The number of shoot buds generally decreased as seedlings were aged. The youngest seedlings of 15-30 days produced more shoot buds than 30-60 days.

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Figure :1

Shorea tumbuggaia Roxb. In flowering in tirumala forest

A **B**

C **D**

A. *Shorea tumbuggaia Roxb* in tirumal forest
B. *Shorea tumbuggaia Roxb* in flowering stage
C. *Shorea tumbuggaia Roxb* seeds
D. *Shorea tumbuggaia Roxb* infected seed