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As part of the overall efforts of the Centre for Biodiversity and Biotechnology, St. Xavier's College affiliated to Manonmonium Sundaranar University to collect basic data on the distribution and threat status of RET plant species of the Western Ghats region in general and Agasthiamalai in particular, development of tools, techniques and strategies to achieve their practical conservation and possibly de-list them from the red list in due course, this present study was focused on Calophyllum austroindicum, an endemic tree species of Agasthiamalai whose conservation status has not been properly assessed. This species is included in IUCN list for RET Indian species, based on field observations on the distribution of the species in nature, critical studies on their current status was undertaken. The findings of the investigations are.

1. Areas of distribution of C. austroindicum were located through intensive survey and 50 m² quartets were laid in the designated areas of distribution. The occurrence of altogether 702 adult trees in 21 quartets ranging from 15 to 56 trees per quartet reveals that distribution of the species in the areas at best can be described as sparse. In other quartets no adult tree could be found. Rough estimates based on the populations studied suggest that in the Agasthiamalai as a whole, nearly 3000 trees may be occurring at present. Therefore, the target species (Calophyllum austroindicum) may be treated as vulnerable as per IUCN classification.
2. The distribution of the species was mostly on either sides of the river where microclimatic conditions like moist soil, high humidity (70-90% R.H) and moderate temperatures prevailed. Distribution may at times extended keep into the forests upto the tops of the hills upto 1500 m in altitude. As much the preferred altitudinal range of the species in the Agasthiamalai was between 750 m and 1500 m.

3. Unlike, *Hopea parviflora, H. utilis, Poeciloneuron indicum* and *Mesua ferrea*; the population of which were in abundance and dominant and were distributed at a stretch, all the populations of *C. austroindicum* were represented by well separated small number of individual trees. Its other associate planet species are *Gluta travancorica, Garcinia travancorica, Acampe congesta, Syzygium gambleanum, Rauwolfia serpentina, Calamus* species, etc.

4. *Calophyllum austroindicum* is not a strict riparian species as its distribution extends upto interior parts (upto 1 Km) away from the rivers and rivulets. However, such attributes as low frequency flowering, low frequency or absence of fruit formation, formation of small number of fruits and seeds, absence of seed shedding, poor germination of seeds and absence of seedling recruitment in the trees found in the interior parts of the forests suggest that continued survival of the species in nature depends on the riparian populations. Profusion of flowers, seed set and recruitment of seedlings are common only among trees found on either side of the rivers or rivulets. Large population located at Lower Kothayar
had more number of seedlings than the least number recorded in small populations of Oothu and Wooden Bridge.

5. *Calophyllum australindicum* is essentially an evergreen timber species found in areas inaccessible to the public and well protected by the Forest Department. The tree is seldom extracted for timber use and yet there is no evidence of its populations getting increased anywhere in the project area. However, only a few of the adult trees located at Kakkatchi, Nalumukku and Wooden Bridge may be related to anthropogenic pressure as these sites are occupied by tea estates and other related activities such as road construction, settlement for estate workers and grazing are not uncommon.

6. Thick or thin, within the same riparian population, the trees exposed to sun bore smaller and lighter fruits compared to shaded parts/trees which produced heavier and larger fruits. The size of the seeds also varied from tree-to-tree within the same riparian population which might be due to genetic characters. The single lofty tree found on the bank of the stream at Oothu well exposed to sunlight bore a number of small sized fruits.

7. Season wise productions of flosses and fruits showed considerable variations every year in the project area. Seeds were often collected in the month of May in Nalumukku and Wooden Bridge populations, whereas seeds could be collected
from Lower Kothayar population in February itself. However, during the subsequent two years seeds were not set in lower Kothayar population while seed production was not affected at Wooden Bridge. At Nalumukku, the well exposed population lost most of the blooms of the trees in the Tsunami winds of December 2005 and subsequent depression in the Bay of Bengal leading to poor fruit set.

8. Based on fruit and seed set characters the following generalizations could be made:

(a) Well exposed trees in the upper reaches of a population away from the river and also individual exposed tree on the bank of the rivulet set seeds fewer in number and smaller in size.

(b) Microclimatic conditions of the river banks minus exposure to sunlight favored healthy production of fruits in large numbers. However, shaded trees or shaded parts of the same tree produced heavier and larger number of fruits than the fully and partially exposed trees and tree parts.

(c) Flowering and of seed production characters were not strictly dependent on seasons. There was no guarantee that an adult tree or a population will produce flowers, set fruits the same way as in previous year. Though exact reasons could not be found out, factorial variability other than the season is presumed to determine the flowering and production characters of a tree / population.
(d) Due to unfavorable microclimatic/ environmental factors (sunlight, temperature, humidity, etc.) trees at the top of the hills seldom produced seeds in any part of the year.

9. Even in the riparian population, 70% of the seeds produced by the trees were damaged by the used squirrels on the trees and after fall on the ground by insect borers as evidenced from a number of embryo and endosperm free seeds collected on the ground otherwise rich. Part of the healthy seeds shed from the tree was lost in the flowing river down below or stagnant water in pits and puddles. There was no guarantee for the germinating seeds on the ground to develop into trees owing to stiff competition among various species that flowering on the bank of the river. This was evidenced from small trees or well grown saplings observed only very few in numbers in each populations. Therefore, the factors that affect the survival and performance of *Calophyllum austroindicum* (contributing to only 20% chances of survival) are natural, abiotic and biotic, the latter from squirrels and insect pests and not from human beings. Anthropogenic pressures are rare as the species is mostly distributed in populations or the sides of the river and in their extended distribution on the slopes of the hills.

10. Flushing, flowering, fruiting and seed shedding were season dependent though as indicated already, significant variations were observed in different populations. Studies on reproduction biology indicated that only 21-40% of the trees in a population had reproduction capability while those having less than 1.1m girth or
more than 3.6m girth did not have or lost the ability to reproduce. In order to ensure that at least 20% of the seeds germinate the tree has tuned its seed shedding to the arrival of the south west monsoon. However, the newly shed seeds take an year to germinate whatever be the favorable factors around are and of there, only 20% of them germinate under natural conditions, further limiting the chances of continued multiplication and spread.

11. The seeds of *Calophyllum austroindicum* were found to be recalcitrant as the seeds having less than 27% moisture content failed to germinate. During the 12 months long waiting period, the seeds under physiological dormancy which was easily broken with the application of 1000 ppm GA₃. In order to achieve high percentage germination (80%) of freshly collected seeds, a novel polybag method with GA₃ pre-treatment was developed. This is a significant contribution of the present study as against scarcity of available information on reproduction biology of endemic and endangered tree species of the tropical humid forests. High percentage of germination of seeds achieved so easily with the polybag methods offer an excellent opportunity to multiply the species *ex vitro* without loss of the seeds and use the seedlings so raised for re-introduction at 80% or more efficiency for revegetative purpose. The critical moisture content and viability of seeds was analysed and recorded.

12. Alternative approaches were also made to multiply and restock the species, through *in vitro* means. The successful demonstration of multiplication of the
species through *in vitro* seed and isolated embryo cultures is a useful indicator for the germplasm conservation of *Calophyllum austroindicum* with reasonable genetic variations in built into these cultures. In fact, seed and embryo cultures offer a system for *in vitro* conservation of the species without somaclonal variations that are common in typical tissue cultures. Besides, these cultures could be established without endophytic microbial contamination and phenolic exudates commonly encountered in tissue culture of this species.

13. Investigations on *in vitro* seedlings from mature embryos cultured on different nutritional formula devoid of plant growth regulators helped to develop a protocol for axenic seedlings. Studies on immature embryo cultures helped to develop a protocol involving initiation of cultures on $\frac{1}{2}$ MS medium containing a combination of BAP (2.0mg.l$^{-1}$), KIN (1.0mg.l$^{-1}$) and NAA (0.1mg.l$^{-1}$) to induce the formation of 11.1±1.8 shoot buds in 30 days followed by subculture in the same medium as such to produce upto 3-4 shoots which could be separated and rooted *in vitro*. The use of 1.5 g.l$^{-1}$ of PVP in the medium was found to be ideal to absorb phenolic oxidates and other inhibitors which facilitated complete regeneration of plants.

14. Difficulties were encountered in *in vitro* culture of nodal and shoot tip explants of nature trees. As the explants were loaded with endophytic bacteria and fungi, use of plant preservative mix (PPM) was indispensable to achieve upto 65% infection
free explants. Selection of young pale green shoots to dissect out the right type of explants and pretreatment with 1.5% PVP was essential to save at least 40-60% of the contamination free explants. Presence of BAP (2.0 mg.1⁻¹), KIN (1.0 mg.1⁻¹) and NAA (0.1 mg.1⁻¹) in a synergistic combination was again necessary to induce multiple shoot formation in nodal explants.

15. Nodal explants of axenic seedlings even in better than nodes of adult trees as only 3.0 shoot buds were obtained as against 2.7 in 6-8 weeks. However, the seedling explants cultures were relatively free from microbial contamination and excessive phenolic oxidates. Between nodes and shoot tips, the former proved to be better for multiple shoot initiation as apical dominance was more or less absent.

16. In all the tissue culture raised plants, \textit{in vitro} elongation of microshoot was induced on basal nutrient media and media fortified with GA₃, the later found to be better thereby conforming the inhibitory influence of cytokinins on shoot / internode elongation. Both \textit{in vivo} and \textit{in vitro} rooting obtained in the \textit{in vitro} raised shoots pointed to the use of the shoots for agroforestry appreciations by direct transfer to the hardening chamber while reducing the cost involved in root induction \textit{in vitro}. 

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17. The tissue culture raised plants were confirmed for their uniformity through RAPD assay. More than 80% of the plants micropropagated via embryo and seed culture got established while those from nodal culture showed roughly 60% establishment 12 months after reintroduction into the project area. These results have reinforced the utility of the *in vitro* methods developed for multiplication and restocking in natural habitats.

To conclude, *Calophyllum austroindicum* is probably the first endemic, valuable tropical tree species from the humid evergreen forests to be studied both in the field (for populations, flushing, fruit and seed set, seed shedding, seed generation and recruitment) and in the laboratory (for seed biology, seed germination, embryo and tissue culture) for evolving a suitable strategy for its effective conservation. The studies point out that the target species is least disturbed by human activities in the forests and most disturbed by its scanty distribution and performance due to varied abiotic (lack of microclimatic conditions, exposure to wind and sunlight, water flow during south west monsoon rain where seeds are shed, etc.) and biotic factors (squirrels and insect bests, poor seed formation, seed dormancy, irregular fruit set and non-adherence to seasons, variations between trees of the same population, competition between species on the same habitat, seed recalcitrancy) strategies. Experiments have been conducted to show that some of these hurdles can be overcome and the species established back in nature through reintroduction into selected pockets of the project area. Since nodal and shoot tip cultures are often plagued with endophytic contaminants, it would be desirable isolated embryo cultures which would retain certain amount of genetic diversity within the plants.
multiplied and reintroduced. Even if the species continue to decline in nature, it should now be possible to inference with appropriate tools and techniques of biotechnology not only to this species but also to enhance its population to de-list the species from the endangered category.