



## Conservation of some endangered and economically important medicinal plants of India – A review

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### ABSTRACT

India has a great wealth of medicinal plant biodiversity which is used by various tribal's and local people to cure different ailments. Unchecked commercialization, habitat loss and habitat degradation have placed many medicinal plant species at a risk of extinction. Therefore there is an immense need for their conservation. There are two basic methods of biodiversity conservation: *in situ* (on site) and *ex situ* (off site), both are complementary to each other. *In situ* methods allow conservation to occur at gradual rate with natural evolutionary processes while *ex situ* conservation involves conservation outside the native habitat. *Ex situ* conservation via tissue culture technology helps in achieving the objective at a faster rate. The present paper discusses the various *in vitro* protocols developed for some of the selected endangered and economically important medicinal plants of India such as *Tinospora cordifolia*, *Pterocarpus santalinus*, *Stevia rebaudiana* and *Tylophora indica*.

**Keywords:** Endangered, Ex situ, Tissue culture, Medicinal plants

### INTRODUCTION

India is rich in all the three levels of biodiversity such as species diversity, genetic diversity, and habitat diversity of medicinal plants.<sup>1</sup> The present demand for medicinal plants is approximately US \$14 billion per year estimated by WHO (World Health Organization). The demand for medicinal plants is likely to increase more than US \$5 trillion in 2050. The medicinal plant-related trade in India is estimated to be approximately US \$1 billion per year.<sup>2</sup> International Union for Conservation of Nature and Natural Resources (IUCN) has placed 560 species of India under the Red List of Threatened species, out of which 247 species are in the threatened category. IUCN recognises the following categories: extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, least concern, data deficient and not evaluated. Species with small populations that are not at present endangered or vulnerable but are at risk are called rare.<sup>3</sup> The genetic diversity of medicinal plants in the world is getting endangered at alarming rate

because of ruinous harvesting practices, over-harvesting, forest degradation, agricultural encroachment, urbanization etc.<sup>4</sup> Therefore, it is a matter of urgency to manage and conserve the traditional medicinal plant resources. Although species conservation is achieved most effectively through the management of wild populations and natural habitats (*in situ* conservation) but most of the medicinal plants either do not produce seeds or seeds are too small to germinate in soils and if the seeds are able to germinate then the raised plants are highly heterozygous. Therefore mass multiplication of uniform disease free planting material becomes a general problem. In order to overcome these barriers, *ex situ* techniques can be used to complement *in situ* methods and, in some instances, may be the only option for some species.<sup>5,6</sup> Tissue culture has greatly enhanced the scope and potential of mass propagation by exploiting the regenerative behaviour in a wide range of selected medicinal plants. Many important medicinal herbs throughout the world have been successfully propagated *in vitro*, either by organogenesis or by callus formation. The present study was therefore undertaken with a view to establishing efficient protocols for *in vitro* rapid propagation of the above endangered and economically important medicinal plants.

### MEDICINAL PLANTS AND CONSERVATION PROTOCOLS

***Tinospora cordifolia*** (willd.) Miers Ex Hook.F. & Thoms commonly known as Guduchi belonging to the family menispermaceae is a large, glabrous, succulent, perennial deciduous twiner with succulent stems and papery bark, climbing shrub. The aerial roots that arise from the stem are thread like. The leaf is heart shaped and smooth. The flowers are yellowish in colour emerges in bunch in rainy season.

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The fruits are pea like which are seen in winter in India. It is distributed throughout the tropical Indian subcontinent. It has been used in Ayurvedic preparation for the treatment of various ailments throughout the centuries. Ancient Hindu physicians prescribed it for gonorrhoea. The plant is used in Ayurvedic, "Rasayanas" to improve the immune system and the resistance against infections. The whole plant is used medicinally; however, the stem is approved for use in medicine as listed by the Ayurvedic Pharmacopoeia of India.<sup>7</sup> In folk and tribal medicine the whole plant, powdered root and stem bark, decoction of root and stem, juice of the root and paste or juice of leaves or stem are used to treat various ailments such as fever, jaundice, general debility, cough, asthma, leucorrhoea, skin diseases, bites of poisonous insects and venomous snakes and eye disorders.<sup>8</sup> It acts as a memory booster, develops intelligence, and promotes mental clarity. It is described as one of the Medhya Rasayana (mental rejuvenative) in the Charak Samhita (The oldest and most potent book of ayurvedic medicine). It is regarded as a liver protector.

*In vitro* micropropagation protocol for *T. cordifolia*.<sup>13</sup> The healthy plants of *Tinospora cordifolia* were collected and raised in pots containing soil and farmyard manure (1:1) in greenhouse condition. The explants were prepared from the nodal portion of the stems, internodes and shoot tip, which were first washed thoroughly in running tap water for about half an hour to remove soil and other superficial contamination, and then allowed to stay for 3–4 hrs in double distilled water to facilitate the phenolics and a characteristic gummy substance of polysaccharide leaching out of explants. The disinfected explants were surface sterilized under aseptic conditions in a laminar flow chamber. Later the explants (1cm each) with upper portion were washed with 1% sodium hypochlorite solution (v/v) for 5 min followed by thorough washing under running tap water for 15 min. Then single bud explants (1 cm each) with upper portion were washed with 1% Bavistin (w/v) for 5 min followed by 3 times washing with sterile distilled water. The explants were surface sterilized with 0.1% (w/v) mercuric chloride for 6 min. and later rinsed 4 or 5 times with sterile distilled water. Later the edges of the explants were trimmed with a sterile blade to eliminate possible residue of sterilant and the explants were then used for culturing. For, *in vitro* shoot initiation from various explants of *Tinospora cordifolia*, the surface sterilized explants such as nodes, internodes and shoot tips were cultured on full strength MS medium supplemented with various concentrations of Cytokinins BA (N<sup>6</sup> – benzyladenine) (0.44–13.31  $\mu$ M), KIN (Kinetin) (0.46–13.94  $\mu$ M) and 2ip (N<sup>6</sup> -2- isopentenyl adenine) (0.49–14.76  $\mu$ M) were tested individually for shoot induction. The cultures were incubated under a 16 hours photoperiod (50  $\mu$ m.m<sup>2</sup>/sec provided by cool-white daylight Sylvania fluorescent lamps) at 25 °C and 55% relative humidity in sterile environment condition. Different experiments were conducted to find out an optimum culture conditions for the maximum shoot initiation (budding) from the cultured explants. And use the different type of Phenolic exudation controlling substances such as polyvinyl pyrrolidone, activated charcoal, silver nitrate and ascorbic acid were used at the time of initiation. Shoot proliferation and elongations were cultured on MS medium supplemented

with various concentrations of Cytokinins BA (4.22–12.64  $\mu$ M), KIN (6.23–12.44  $\mu$ M) and 2ip (2.11–13.32  $\mu$ M) were tested individually and combine with Auxins like NAA ( $\alpha$ -naphthyl acetic acid) (4.22–12.88  $\mu$ M) and IAA (Indole -3-acetic acid) (1.32–11.86  $\mu$ M). After 20 days the culture was used to rooting, 1 to 2 cm long shoots were transferred to half strength of MS medium amended with 3% sucrose with Auxins IAA (1.76–17.13  $\mu$ M), IBA (Indole-3-butyric acid) (1.32–14.70  $\mu$ M) and NAA (1.34–16.11  $\mu$ M) tested individually. All the experiments were done in six cultures with ten replicated experiments. The *in vitro* rooted shoots were carefully removed from the culture vessel and they were gently washed with sterile distilled water to remove every trace of media. Thereafter plantlets were dipped in 0.05% Bavistin (systemic fungicide) for 10 seconds to minimize the microbial infection. Again a second wash was given with sterile distilled water. The treated plantlets were then transferred aseptically to small earthen pots containing mixture of vermiculite, sterilized red soil and farmyard manure at 1:1:1 ratio in growth chamber with controlled temperature, light and humidity to acclimatize with the outside environment. Half strength liquid medium was added periodically. The plantlets were covered with polythene bags to ensure a relative humidity of 70 to 80%. The acclimatized complete plantlets were then transferred to the field.

***Pterocarpus santalinus* L.** popularly known as Red Sanders belonging to family Fabaceae is an endemic species confined to Southern parts of Eastern Ghats of India especially in Andhra Pradesh. It is a small to medium sized deciduous tree having a dense, round crown reaching a height of 10 to 15 m with a girth of about 90 to 160 cm. The bark is typically dark brown in colour with rectangular plates and deeply fissured when matured. When blazed, it exudes a red colour gum with numerous pink streaks. The leaves are pinnately compound which are generally shed during January and March. With the onset of summer, new flush of leaves are produced along with large yellow coloured raceme flowers. Heartwood of Red Sanders has high demand in domestic as well as international market and the wavy grained wood is valued. Along with its extensive use in furniture, the red dye obtained from the wood is used as colouring agent for textile, medicine and food. The heartwood can accumulate various elements and rare earth elements like strontium cadmium, zinc, copper and uranium. The wood has different uses in traditional and folklore medicines and is used for the treatment of diabetes, prickly heat, skin diseases and for various other ailments. Yerukula and Irula tribes of Chittoor district in Andhra Pradesh use whole plant of *P. santalinus* for ulcer treatment.<sup>9</sup> Considering the wood demand, restricted distribution, slow regeneration, illegal harvest, trade and habitat destruction, the species has been categorized as endangered by IUCN.

*In vitro* propagation protocol of *Pterocarpus santalinus* L. (Red Sandalwood) through tissue culture.<sup>14</sup> Pods were picked directly from mature red sandalwood trees (over 25 years old) at the green to brownish stage. Seeds were surface sterilized using 10 % clorox for 20 min followed by 70 % ethanol for 2 min and cultured on Gamborg (B5) medium.<sup>18</sup> Six different explant types were used for the study. *In vitro* germinated seedlings were excised after 20 d to derive

mesocotyl segments, cotyledonary nodal segments and shoot tips. Mother plants were maintained under plant-house conditions to excise stem cuttings. Fresh and immature terminal shoot cuttings and subsequent nodal shoot segments (semi-hard wood, 3- 5 cm in length with dormant auxiliary buds) were collected during the early hours of the day at 2 wk intervals. *In vitro* germinated 20 day old seedlings were used as the control. As a rejuvenation procedure, decapitation and spraying of 10.0 ppm 6-benzyle amino purine (BAP) solution was done for the mother plants, at 2 wk intervals. Plants were maintained under plant-house conditions free from pests and diseases and watered when necessary. Overhead watering was avoided. A 100.0 ppm solution of thiophanate methyl 70 % (topsin) was sprayed 24 h before collecting the buds for culturing to prevent fungal infections. Plants were treated with 200.0 ppm Albert's solution (complete fertilizer mixture) at 2 wk intervals. Cultures were kept aseptically in a growth room ( $23 \pm 2$  °C temperature, 60 % RH) and cool photoperiod under 1220 lx of light intensity. All media contained 3.0 % sucrose and 0.01 % myo-inositol. The pH of the medium was adjusted to 5.8. Prepared media were autoclaved at 121 °C and 1.05 kg cm<sup>-2</sup> pressure for 20 min. Experiments were designed according to complete randomized design with 20 replicates in each treatment and all parametric data were analyzed using SAS statistical software. Mean separations were carried parametric data were analyzed using the Chi Square test.

***Stevia rebaudiana* Bertoni.** belonging to the family of Asteraceae is a perennial herb. This plant is a natural sweetener and famously known as "Sweet Weed", "Sweet Leaf", "Sweet Herbs" and "Honey Leaf", which is estimated to be 300 times sweeter than cane sugar. It naturally grows in low lying areas on poor sandy acidic soils adjacent to swamps, and so is adapted to and requires constantly wet feet or shallow water tables. It grows up to 1 m tall and has leaves 2-3 cm long. The leaves are the source of diterpene glycosides, stevioside and rebaudioside.<sup>10</sup> Stevioside is regarded as a valuable natural sweetening agent because of its relatively good taste and chemical stability. Stevioside is of special interest to diabetic persons with hyperglycemia and the diet conscious.<sup>11</sup> Natural sweeteners that can substitute for sucrose have caught great attention due to the growing incidence of obesity and diabetes.

The rapid micropropagation protocol developed.<sup>16</sup> Different parts of the plant were used as explants for the *in-vitro* regeneration of *Stevia rebaudiana* Bertoni. The seeds of the plant were obtained from J.N.K.V.V. Jabalpur, M.P. For *in-vitro* regeneration of *Stevia rebaudiana* Bertoni Murashige and Skoog's medium was used with various concentrations of plant growth regulators.<sup>15</sup> General aseptic techniques concerning in vitro culture of the explants were followed in the present experiment. After surface sterilization the explants were cut into very small pieces (about 1 cm) and inoculated onto shoot induction and proliferation media. When the shoot was fully growth (for 1 month) the shoots were taken out from the test tube a laminar flow hood and were cut (about) 1cm. Then the micro cuttings were sub cultured on root induction medium. All inoculations and aseptic manipulations were carried out in a laminar air flow cabinet. Before use the working surface of

the cabinet was cleaned by swabbing with 90% ethyl alcohol and UV light (for 20 minutes) to reduce the chances of contamination. The instruments like scalpels, forceps, needles etc were sterilized by an alcoholic dip followed by flaming inside the laminar air flow cabinet. Other requirements like Petri dishes, bottles, conical flasks, cotton, distilled water etc were sterilized by steam sterilization method. Before the onset of inoculations hands were washed thoroughly by soap and then swabbing with 70% ethyl alcohol. Cutting and transfer of the explants were carried out taking all possible care to ensure contaminations free inoculations. The rooted plantlets were first transferred to plastic pots containing garden soil, sand and peat moss (1:1:1), covered with transparent polythene bags and placed in acclimatization room at  $28 \pm 2$  °C with 70- 90% relative humidity, after five days temperature was increased from 28 to 32 °C. After two weeks, transparent bags were removed from pots for proper hardening. After four weeks, the plants were then shifted in greenhouse and in field under low light intensity. The data for various growth attributes were recorded such as % explants regeneration, multiple shoot formation, shoot length, % plantlets rooted, number of roots per plantlet, and survival of plants during acclimatization and in the field was recorded.

After the initiation of explant plantlet is a generated. In this experiment for the multiplication of plantlet, it is transferred to the multiplication MS medium supplemented with BAP (Cytokinin) at different concentrations viz. 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mg/l and 0.00 mg/l were used as control. The concentrations of BAP used found to be not significant differences for all parameters. Frequencies of bud break and bud elongation and mean number of regenerated shoots and shoot length were observed at 5mg/l BAP i.e. frequency of bud break is 82% and bud elongation is 88% while mean shoot number is (14.83) and mean shoot length is (4.51). While at concentration lower then 5.0 mg/l treatment resulted decline in all parameters. After bud break of explant plantlet is a generated. In this experiment for the multiplication of plantlet, it is transferred to the multiplication MS medium supplemented with KIN (Cytokinin) at different concentrations viz. 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mg/l and 0.00 mg/l were used as control. The concentrations of KIN used found to be not significant differences for all parameters. Frequencies of bud break and bud elongation and mean number of regenerated shoots and shoot length were observed at 5mg/l KIN. i.e. frequency of bud break is 72% and bud elongation is 88% while mean shoot number is (9.25) and mean shoot length is (5.51). While at concentration lower than 5.0 mg/l treatment resulted decline in all parameters. After multiplication of plants the plantlets clumps were dissected and plantlets were transferred in to rooting medium. The one way factorial design was carried to study the effect of IBA (auxins) and their interaction on frequency of Root number (FRN) and frequency of Root length (FRL) at different concentrations. IBA is a plant growth regulator play important role in the rooting of plants. No significant differences in rooting were observed by various concentration of IBA i.e. 0.5, 1.0, 1.5, 2.0, 2.5 mg/l. Frequencies of Root No. observed in 2.0 mg/l IBA (3.62) and frequencies of Root length observed in 2.0 mg/l IBA (3.71). While at concentration lower then 2.0mg/l



treatment resulted decline in all parameters. In this study experiments were conducted to standardize the explant source and culture media for multiple proliferation of shoot and result in mass propagation of homogenous elite plantlets of *S. rebaudiana*. The result of the experiment and other earlier research report clearly support the possibility of propagating *S. rebaudiana* by adopting *in vitro* techniques

***Tylophora indica* (Burm.F.) Merrill.** commonly known as “Antmool” or “Dama Bel” belonging to family Asclepiadaceae is an important endangered medicinal plant. It is a perennial branched climber having cylindrical, twinning stem with long fleshy roots. Leaves are ovate oblong to elliptic oblong and acute at tip. Flowers are bisexual, minute and grow in umbellate cymes. Corolla is greenish yellow outside and purplish within. It is found in the plains, forests, hilly slopes and outskirts of forests. It forms dense patches in moist and humid conditions and the plant shows stunted growth in the areas with lesser rainfall. It is indigenous to India native to the plains and hill forests of Eastern and Southern India. It is used as folk remedy for the treatment of number of diseases and ailments particularly bronchial asthma, bronchitis, allergies, rheumatism and dermatitis.<sup>12</sup> The roots and leaves of this plant contain pharmacologically active alkaloids tylophorine, tylophorinine and anticancerous tylophorinidine.

An efficient protocol for large scale multiplication of this endangered species developed.<sup>17</sup> In the present study, de novo adventitious shoot formation occurred on MS medium supplemented with different concentrations of BAP alone or in conjunction with adenine sulphate. However, the best results were observed on MS medium supplemented with BAP (22µM) and adenine sulphate (1.35 µM) where optimum growth of meristemoids occurred on the leaf segments. These meristemoids when cut into small groups and subcultured on fresh inductive medium multiplied further resulting in an exponential increase in number and eventually developed into green leafy shoots in nearly 85% of the cultures. Multiple shoot formation directly from leaf explants on MS supplemented with BAP (5mg/l) and adenine sulphate (0.5mg/l). The shoots thus formed were excised and transferred for rooting on half strength MS and MS medium supplemented with different concentrations of IAA, IBA and NAA. Among the various auxins used IBA proved to be the best. However, excellent root induction occurred on half strength MS medium alone. The rooted plants were first hardened under *in vitro* and green house conditions for 4 weeks before final transfer to the field. During the period of hardening, proliferation of roots and emergence of new leaves was observed and there was substantial improvement in the survival rate which was nearly 90%. Large numbers of uniform clones for commercial production were obtained.

## CONCLUSION

Medicinal plants occupy a vital sector of health care system in India and represent a major national resource. The above mentioned endangered medicinal plants are used by various tribals and local people to cure different ailments. Hence, there is an immense need for conservation of diversity of medicinal plant wealth for the present and fore

coming generations, by adapting the suitable strategy with most appropriate method of conservation. The development of efficient *in vitro* tissue culture protocols<sup>19</sup> would help to save the endangered status of the above medicinal plants.<sup>20,21</sup>

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