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A review of conservation and sustainable use of medicinal plant with special reference of *Tecomella undulata* (Sm.) Seem.

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Abstract
Introduction- The north-western part of India is occupied by a desert ecosystem, the Thar Desert; having 682 plant species and 390 animal species. Many plant and animal species have become endangered due to zoogenic and anthropogenic activities, and *Tecomella undulata* is one of them. In Ayurveda classics it is known as Rohitaka and used mainly for *Yakritpleeha Roga* (Liver and spleenic disorders). *Tecomella undulata* is also used for treating syphilis, gonorrhea etc. and proved as Anti-HIV, antibacterial, antimicrobial, immune modulator, analgesic etc. Despite the great importance of *T. undulata* as economical, ethno botanical and medicinally important tree, attempts for its conservation, sustainable utilization and genetic improvement, are lacking.
Material & Methods- Different literatures, journals & internet media were used for collecting information regarding the topic.
Result- *In vitro* clonal propagation of *Tecomella undulata* has been reported by various workers through seedling. However, *in vitro* propagation through seedling has limitation. *In vitro* shoot cultures from nodal explants of mature trees were established and multiplied as reported. However, tissue culture protocol remains ineffective due to lack of reproducible rooting methods. Therefore, there is a need of improvement in shoot multiplication and long-term sub-culturing and better knowledge of root induction in this species.
Conclusion- *Tecomella undulata* is one among endangered plant species found in Thar Desert. For its conservation and sustainable use we have to adopt proper method of conservation. Propagation through seedlings takes more time so in-vitro shoot multiplication and sub- culturing methods can be applied.

Keywords: Thar Desert, *Tecomella* undulate, *Rohitaka*, Conservation, Sustainable use, Endangered, Clonal propagation.

Introduction
India is one of the world's top 12 mega-diversity countries with 10 biogeographic regions, and has over 40 sites which are known for their high endemism and genetic diversity [1]. It has more than one fourth (8000) of the world's known medicinal plant species (30,000). Medicinal plants are globally valuable sources of new drugs. Further more, up to 80 % of people in developing countries are totally dependent on herbal drugs for their primary healthcare, and over 25 % of prescribed medicines in developed countries are derived from wild plant species [2]. With the increasing demand for herbal drugs, natural health products, and secondary metabolites of medicinal plants, the use of medicinal plants is growing rapidly throughout the world.
Over 90% of the medicinal plants traded in India are harvested from the wild, most of them in an unsustainable manner. Due to an increasing demand for medicinal plants, a loss and fragmentation of natural habitats, close to 300 species of Indian medicinal plants have been so far assessed as under threat in the wild and around 1,000 species are estimated to be facing various degrees of threat [3]. Demand for medicinal plants is increasing, and this leads to unscrupulous collection from the wild causing threaten the existence of approximately 15,000 species worldwide due to a number of reasons such as overexploitation, destruction of natural habitats, and lack of regulations and standards for sustainable harvesting [4]. In India, forest land is losing its natural flora at an alarming rate – 1.5 m.ha. Every year and only 8% against a mandatory 33% of the geographical area (Shivarajan and Balachandran 1999) is left now. To

control the situation, various measures like commercial cultivation, habitat conservation, setting up of natural reserves, implementation of laws for restricting the export of plants (Rao *et al*. 2003), etc., have been taken up [5]. Providing high-quality planting material for sustainable use and thereby saving the genetic diversity of plants in the wild is important. For conservation and sustainable use of medicinal plants various sets of recommendations have been compiled including the establishment of systems for species inventorying and status monitoring, and the need for coordinated conservation practices based on both in situ and ex situ strategies. For medicinal plants with increasingly limited supplies, sustainable use of wild resources can be an effective conservation alternative [6].

Conservation Status of Tecomella Undulata- [17, 63, 67]

The north-western part of India is occupied by a desert ecosystem, the Thar Desert; having 682 plant species and 390 animal species. Many plant and animal species have become endangered due to zoogenic and anthropogenic activities, and *Tecomella undulata* is one of them [7]. *Tecomella undulata* (Sm.) Seem. (Family Bignoniaceae), a medicinally and economically important genus originating in India and Arabia [8]. In Ayurveda classics it is known as Rohitaka and used mainly for *Yakritpleeha Roga* (Liver and spleenic disorders) [9]. *Tecomella undulata* is also used for treating syphilis, gonorrhea etc. and proved as Anti-HIV, antibacterial, antimicrobial, immune modulator, analgesic etc [8, 9]. It is one of the most important deciduous, ornamental shrub or small tree of the dry regions. It is a multipurpose and vital species for arid-rural poor. It is the main source of timber amongst the tree species of desert regions of Rajasthan and highly valued for making engraved furniture, agricultural implements, carvings, turnery, toys etc. It is also used as source of firewood and charcoal also. So, it is heading towards extinction due to indiscriminate felling for timber and fuel by the local population, minimum conservation efforts, coupled with poor regeneration has severely depleted the natural population of this tree, with an associated loss of valuable germplasm [10, 11].

The species has been designated as ‘‘threatened’’ in Rajasthan, India (Pandey *et al*. 1983; Shetty and Singh 1987; Tripathi and Jaimini 2002). United Nations Environment Programme (UNEP), World Conservation Monitoring Centre (WCMC) Nairobi, Kenya, has included T. undulata into ‘‘Category 1— Indeterminate’’ to stop further exploitation of this species and the urgent need for conservation. Sharma (1986) suggested that it should be designated as a ‘reserved’ species to promote its conservation. Khan (1997) reported that *T. undulata* urgently requires conservation. Similarly, Hussain *et al*. (2010) compiled a ‘‘red data book’’ to determine the threatened status of native plants of Karachi and reported that out of 135 species, eight species including *Rohida* (*Tecomella undulata)* were threatened [8, 10]. So conservation of this plant species is a need of hour. The regeneration rates of this tree in natural surroundings is quite low, therefore alternative propagation methods would be beneficial in large scale multiplication, improvement and conservation of its elite clones. Monitoring genetic diversity can help in identifying the candidate populations for *in situ* and *ex situ* conservation, and thus lead to proper planning strategy for the conservation of this species. Biotechnology has opened a window for this.

Conservation Strategies of Medicinal Plants

In-situ conservation- It is the on-site conservation or the conservation of genetic resources in natural populations of plant or animal species, such as forest genetic resources in natural populations of tree species. It is the most common method of conservation. In situ conservation of whole communities allows us to protect indigenous plants and maintain natural communities, along with their intricate network of relationships. It strengthens the link between resource conservation and sustainable use. Natural reserves, National parks, wild nurseries are examples of in-situ conservation [1, 2, 6].

Ex-situ conservations- Ex-situ conservation is the preservation of components of biological diversity outside their natural habitats. This involves conservation of genetic resources, as well as wild and cultivated or species, and draws on a diverse body of techniques and facilities. it is an effective complement especially for overexploited and endangered medicinal plants with slow growth, low abundance, and high susceptibility to replanting diseases. Botanical gardens, seed banks etc are way of ex-situ conservation. The ex situ conservation programmes involve collection, preservation, multiplication and dissemination of economically important, endemic, rare and threatened species germplasms [1, 2, 6].

Cultivation of medicinal plants- Cultivation of medicinal plants is alternative way for conservation of natural resources and fulfills the demand of the market. Cultivation practices help to reduce dependency on natural resources. Although wild-harvested resources of medicinal plants are widely considered more efficacious than those that are cultivated, domestic cultivation is a widely used and generally accepted practice [2, 6].

Good agricultural and collection practices (GACP)- Under the overall context of quality assurance and control of herbal medicines, WHO developed the Guidelines on good agricultural and collection practices (GACP) for medicinal plants, providing general technical guidance on obtaining medicinal plant materials of good quality for the sustainable production of herbal products classified as medicines. These guidelines are also related to WHO's work on the protection of medicinal plants, aiming promotion of sustainable use and cultivation of medicinal plants [12]. In India GACP of medicinal plants is designed by National medicinal plant board in collaboration of WHO.

Conservation through Micro-propagation and Reintroduction - Reintroduction is the deliberate establishment of individuals of an extint/endangered species into an area and/or habitat where it has become extirpated, while Micro-propagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods [13]. The reintroduction of plants is becoming an increasingly utilized strategy in plant conservation and protected area management as proven successful in a variety of species [14].

Conservation through in Vitro and Cryopreservation- Cryo-preservation or cryo-conservation is a process where organelles, cells, tissues, extracellular matrix, organs or

any other biological constructs susceptible to damage caused by unregulated chemical kinetics are preserved by cooling to very low temperatures (typically −80 °C using solid carbon dioxide or −196 °C using liquid nitrogen) [15].

National Gene bank at Jawaharlal Nehru Tropical botanical garden and research institute, is one among the four to have an objective of conserving the medicinal and aromatic plants of Southern Peninsular India through biotechnological intervention, including in vitro and cryopreservation techniques [14].

Propagation and Cultivtion of Tecomela Undulata

It grows in well drained loamy to sandy loam soil and also thrives in stabilized sand dunes and even in rocky areas. Generally it is propagated through wind dispersal of winged seed naturally. Artificial propagation can be done with seeds or cuttings [9]. Collection of ripe fruits in the month of April is best suited for germination compared to unripe fruits, or ripe fruits collected in the month of June. Propagation is done in June to July by direct sowing fresh seeds and grows well if done under a nurse bush for protection. As per study freshly harvested seeds showed 82 % germination in wet paper lined petriplates at room temperature (35 ^{0}C) [16]. Planting of nursery-raised seedlings or rooted cuttings are preferable. Pre-treatment is not needed although soaking the seed in cold water for three to four hours has been effective in producing uniform germination. About 30 gm seeds are needed for 1000 plants. In seed beds, seeds are mixed with fine soil and fine sand is sprinkled over them. Germination is usually low (30-40%), starts in 2 weeks and is over in 4 weeks. The small germinant need to be kept clean weeded for rapid growth. Seedlings reach 30-45 cm tall in one year. Trees coppice strongly up to about 0.5 m and early pruning is often done to get a straight sapling. The major disadvantage of the species is that it is slow growing and this results in a slow return on breeding efforts [9].

Agro-Technique of Tecomella Undulata-[17]

Nursery Technique

Fresh seeds collected in month of April, are sown in the month of May on flat beds/raised nursery bed or polybags.

Plantation in field

After proper germination of seedlings, it should be transferred in well prepared field.

- **Land Preparation and Fertilizer Application:** Pits of 60x60x60 cm are to be prepared at an optimum spacing of 4 meter between row to row, 3 meter gap between plant to plant. Land is filled with FYM (farm yard manure) and NPK (Nitrogen, phosphorus and potassium) in the ratio of 750: 600:300 gm/plant is given as single basal dose.
- **Transplanting:** Plantation of seedlings is done at a spacing of 3x4 meter in the month of July-August.
- **Intercropping System:** Aromatic grasses / annual species of other medicinal plants can be cultivated as intercrop.
- **Inter culture and Maintenance Practices:** Proper maintenance of field is key factor for better yields so one to two harrowing is to be given in the middle space for keeping the field clean.
- **Irrigation Practices:** Irrigation may be given as per the season during winter at 30-40 days interval and summer at 20-30 days interval. Frequent irrigations is not necessary after establishment of the crop.
- **Weed Control:** Field should be weed free and for this regular weeding should be done.
- **Disease and Pest Control:** The plants need to be protected from termites and stem borer for which suitable insecticides are used.

Harvesting technique

- **Crop Maturity and Harvesting:** The crop matures after 3-4 years and should be harvested for its bark during summer (April-May) according to guidelines of good collection practices.
- **Post-harvest Management:** The bark is shade dried and stored in gunny bags in dry, ventilated places for marketing.

Micropropagation of *Tecomella undulata* (Sm.) Seem [14, 16, 18, 19].

Micro-propagation techniques are desirable in this species but commercially viable technique is still lacking. Thus *in vitro* propagation of *Tecomella undulata* using nodal segments of mature trees was refined. The *in vitro* shoot cultures can be established throughout the year but the most favourable months for bud break (75%) was January and February. Micro-propagation techniques are developed by standardizing various steps, such as source of explant, sterilization, shoot multiplication, rooting and hardening. Micro-propagation of *Tecomella undulata* can be summarize as following steps;

a. **Media Preparation-** Various culture media namely full and half strength Murashige and Skoog's (MS) B 5, Woody plant (WP), Broad-leaved Tree Medium (BTM), Heller's (HE) and White's can be used. Different auxins (IAA, IBA and naphthaleneacetic acid (NAA) and cytokinins (kinetin and BAP) at various concentrations and combinations are evaluated for rapid multiple shoot induction from the explants. Plant growth regulators are added according to the experimental requirement. The pH of media should be adjusted to 5.8

b. **Explant Collection and Sterilization** - Trees of Tecomella undulata 10-15 years old is selected and single node explants (2 cm) long is excised from mature trees as well as from new sprouts of the stem cuttings raised in mist chamber. After removing leaves, the explants are thoroughly washed with running tap water. The clean cuttings are washed thoroughly with distilled water containing 2-3 drops of Tween-80 and followed by treating with the solution of Bavistin and streptomycin for 20 minutes. After that, surface is sterilized with 5% NaOCl solution for 5 min followed by 3-4 washings in sterile distilled water. All the surface sterilization procedure is carried out in aseptic conditions in a laminar airflow cabinet. The explants are slightly trimmed at both ends to expose fresh tissue before planting them on Murashige and Skoog (MS) medium.

c. **Shoot induction and multiplication-** The surface-sterilized explants were inoculated on well prepared standard MS media for culture initiation. The explants producing shoots after bud break and few subcultures, healthy shoot cultures were maintained by repeated sub-culturing of the stock after 3-4 weeks on fresh MS + 1.0 mg/l BAP medium for their multiplication. These individual excised shoots were used for the rooting experiments.

d. **In vitro rooting**- Shoot segments of (3-5cm) in length is isolated from shoot multiplication cultures and used for in vitro rooting. To initiate rooting two step procedures are

adopted. In the first step the micro-shoots are given short duration treatment of autoclaved IBA and NAA (100 mg/l) solution and then transferred to the hormone free medium. Observations are recorded after regular interval of 10 days up to 40 to 60 days and root length and root number are recorded.

e. **Hardening and Field Trial of micro-propagated plants-** One and half month old rooted micro-shoots are removed from the culture tubes, washed thoroughly to remove the nutrient medium and transferred to the mixture of vermiculite and wetted with ½ MS liquid solution for in vitro hardening for 4-5 weeks. Then the plants are transferred to plastic cups containing vermiculite and placed in mist chamber. Acclimatization is carried out in mist camber (30 sec misting at 15 minutes intervals to maintain relative humidity between 85-90%).

After 4 weeks, acclimatized plantlets are transferred to pots containing normal garden soil and maintained in the greenhouse under normal day length conditions.

Discussion

Herbal medicines occupy a vital sector of health care system in India and also in rest of the world medicinal plants represent a major resource. With the increasing demand for herbal drugs, natural health products, and secondary metabolites of medicinal plants, a loss and fragmentation of natural habitats, overexploitation, and lack of regulations and standards for sustainable harvesting, medicinal plants have been so far assessed as under threat in the wild. So it is important to ensure their conservation for sustainable utilization. *Tecomella undulata* is a medicinally and economically important timber tree of the dry regions of India, Pakistan and Arabia [8], which is heading towards extinction. The natural propagation of this slow growing tree is through seeds is one of the method of propagation but it is not enough to fulfill the requirement. For its conservation and sustainable use, micro-propagation by tissue culture and other innovative methods will be an answer, while there are no suitable methods for its vegetative propagation and rapid multiplication. *In vitro* clonal propagation of *T. undulata* has been reported by various workers through seedling. However, *in vitro* propagation through seedling has limitation. *In vitro* shoot cultures from nodal explants of mature trees were established and multiplied as reported. However, tissue culture protocol remains ineffective due to lack of reproducible rooting methods. Therefore, there is a need of improvement in shoot multiplication and long-term sub-culturing and better knowledge of root induction in this species. A study concluded *in vitro* plant regeneration from vegetative parts is an easy and economical way to obtain a large number of consistently uniform and true-to- type plants within a short span of time. Shoot tips were excised from healthy young soft shoots of the mother plant and used as explant material for a study to establish the best conditions for their *in vitro* propagation. that shoot cultures from nodal explants initiated on MS basal medium in January-February months will give better results in propagation and apical part of the shoot subcultured on MS + 4.4 µM BA medium generated more rootable shoots. Rooting experiments done during January to March months with pretreatment of IBA (492.1 µM) and NAA (537 µM) solution for 15 minutes followed by transfer to ½ B5 basal medium will give best rooting results. It appears *in vitro* rooting is following also an annual pattern and optimal rooting was observed in February and March only.

Propagation through tissue culture offers a viable alternative to normal propagation because it can also be used as a complimentary strategy for conservation and utilization of genetic resources. Further, *in vitro* plant regeneration from vegetative parts is an easy and economical way to obtain a large number of consistently uniform and true-to- type plants within a short span of time. Shoot tips were excised from healthy young soft shoots of the mother plant and used as explant material for a study to establish the best conditions for their *in vitro* propagation [18]. As per study done, for plant regeneration from shoot apex explants of *Tecomella undulata* explants were isolated from 2-3 year old plants, cultured on a shoot induction media, and fortified with different concentrations of auxins and cytokinins (BAP or Kinetin). The results showed that greatest number of shoots was obtained on the medium fortified with BAP (1.0 mg l-1). The greatest root induction response (63.8%) was observed on MS half strength semisolid medium supplemented with 5.0 mg l-1 NAA. The regenerated plantlets were acclimatized and transferred to soil for normal growth under field conditions and 60% survived. Random amplified polymorphic DNA markers were used to analyze the genetic fidelity of these *in vitro* raised plants. Out of the forty three RAPD decamers screened; only ten primers resulted in two to twelve scorable bands. These ten RAPD primers generated 51 amplicons in total, ranging from 200-2, 500bp in size with an average of 5.1 bands per primer. The amplification products were monomorphic in micropropagated plants and similar to the mother plants, confirming the genetic fidelity of *in vitro* raised plantlets and corroborating the fact that *in vitro* multiplication through direct organogenesis is the safest method for multiplying true to type plants [16].

Conclusion

Conservation of natural resources of medicinal plant and its sustainable use is need of hour. For this proper strategies should be followed. In-situ conservation is the best option for conservation of natural resources but ex-situ conservation protocols are also good alternatives. The in vitro propagation protocols developed for medicinal plants are satisfactory in laboratory scale, but their use in commercial scale needs further field cultivation trials. The innovative methods of micro-propagation of *Tecomella undulata* will play a vital role for conservation of this threatened but economically important plant.

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