Biotechnological interventions to save a medicinally important, threatened species of the Indian desert – *Commiphora wightii* (Guggal)

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Introduction

The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs (Bannerman, *et.al.,* 1983). Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor. Medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of India.

In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. Though India has a rich biodiversity, the growing demand is putting a heavy strain on the existing resources. While the demand for medicinal plants is growing, some of them are increasingly being threatened in their natural habitat.

About 90% of medicinal plants used by the industries are collected from the wild. While more than 800 species are used in production by industry, less than 20 species of plants are under commercial cultivation. Many plants are completely destroyed by the collectors because of the use of parts like roots, bark, wood, stem and the whole plant in case of herbs. This will pose a definite threat to the genetic stocks and to the diversity of medicinal plants if not used sustainably in the very near future.

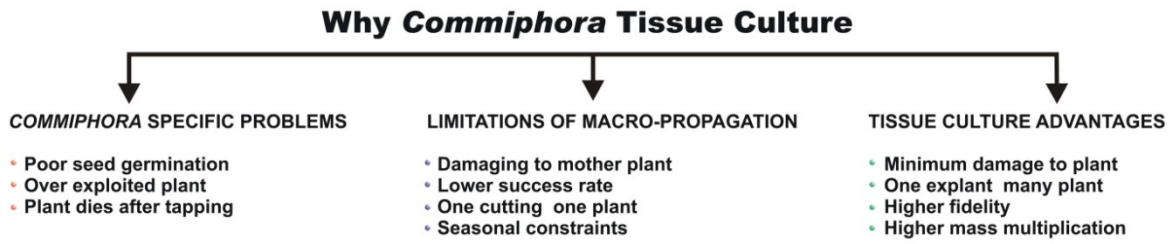
Commiphora wightii popular by its vernacular name GUGGUL is one of most important medicinal plant of our traditional system of medicine and also having a nice status in modern research and drug system. It is the source of valuable oleo-gum-resin known as GUGGUL gum. GUGGUL gum have many medicinal and non medicinal properties.

Once a prominent species of the arid tracts of Rajasthan and Gujarat states (northwest India), *Commiphora wightii* is now on the verge of extinction over much of its Indian range and is listed as endangered (IUCN, 2010). The predominant reasons for its fast diminishing populations are over-exploitation (tapping of woody shoots for its oleo-gum-resin), poor natural germination rate and slow growth rate. This resin has tremendous value as cholesterol reducing agent and hence a favorite of Ayurvedic medicine industry. This has resulted in widespread indiscriminate tapping for the resin. The magnitude of the conservation problem facing C. wightii through this exploitation is greatly exacerbated by the fact that a plant after being tapped through deep cutting, usually dies within two to six months of

a single tapping episode (Bhatt *et al.,* 1989; Paliwal, 2010). It is not yet clear as to why plants die after tapping. Various theories exist, but none are scientifically proven. Several researchers are currently working to ascertain the reason for this, and only if this becomes known can strategies of sustainable gum tapping perhaps be implemented.

Tissue culture holds the promise to save Guggal and why

It is imperative that urgent measures be taken up for production of planting stock to enable species restoration efforts through transplanting initiatives. The state forest departments (SFDs) of Rajasthan and Gujarat are not able to produce enough plants through propagation by seeds and stem cuttings to meet their own demands for plantation activities. In this situation, tissue culture technique can supplement such demands due to the high potential of mass multiplication at faster rate, though at slightly higher costs. These costs may be considerably reduced if protocols are further refined to make them more efficient and reliable (Kant *et al*., 2010a). The benefits of tissue culture based mass multiplication verses propagation via cuttings are highlighted in chart given below (adopted from Kant *et al*., 2010a).



Highlights of research on Guggal related biotechnology research activities at AFRI, Jodhpur

At Arid Forest Research Institute, Jodhpur we have been working on biotechnological aspects, primarily the research towards developing an efficient and a scalable protocol for mass multiplication of this amazing medicinal plant that is now on the verge of extinction.

The various research projects on *in vitro* studies (and various other aspects of Guggal) that have been completed or ongoing presently are presented in the following table:

Project title	***Funding agency***	***Period***
Micropropagation of an important medicinal plant of the arid and semi-arid regions –*Commiphora.*	ICFRE, Dehradun	2002-2007
Source variation, extraction and cultivation practices for Commiphora wightii (Arn. Bhandari).	NMPB, New Delhi	1006-2009
Network Research Project on Guggul	NMPB, New Delhi	2009-2013
Assessment of guggul germplasm for studying population density, diversity, female-male plant's ratio for in situ and ex situ conservation in Rajasthan	SFD, Rajasthan	2010-2013

Tissue culture of Guggal

Two micropropagation protocols (one somatic embryogenesis based and the other cotyledonary node based) have been developed at the AFRI, Jodhpur. Plants obtained from these micrcroprogation techniques have been out-planted in an experimental field site to evaluate their performance and to establish the suitability of this system for *ex situ* conservation efforts for the species. In the longer-term, it is hoped that the plants produced through these protocols can be used in *C. wightii* species recovery programmes.

Cotyledonary node based micropropagation protocol: For the development of the micropropagation protocol from cotyledonary nodes, mature fruits (40 fruits per treatment) were collected from the identified mother plants. These fruits were subjected to flotation technique to segregate the 'floaters' from 'sinkers' (approximately 50 % each). Only sinkers were used in our experiments as floaters were usually found to be empty fruits, without viable seeds. These selected fruits were de-pulped to remove the exocarp and mesocarp. They were then surface sterilized by washing in running tap water for 2 min (to remove dirt) and shaken in 100 ml RO water (Millipore RiOS5) with two drops of Tween-80® (polysorbate 80; Ranbaxy Fine Chemicals Limited) for 10 min, rinsing three times with autoclaved RO water. The cleaned fruits were then treated for 10 min with a solution of Bavestein and Streptomycin and were finally treated with NaOCl solution (providing 5% available chlorine) for 5 min and rinsed with autoclaved RO water thrice. The endocarp was then broken open carefully with a sharp sterile scalpel and seeds were scooped out and inoculated on germination medium. The seeds were germinated and cotyledonary nodes were used as explants. The cotyledonary nodes were inoculated on MS medium with various hormonal combinations to achieve bud break and subsequent steps leading to development of complete plantlets (Fig 1) (Kant *et al.,* 2010b).

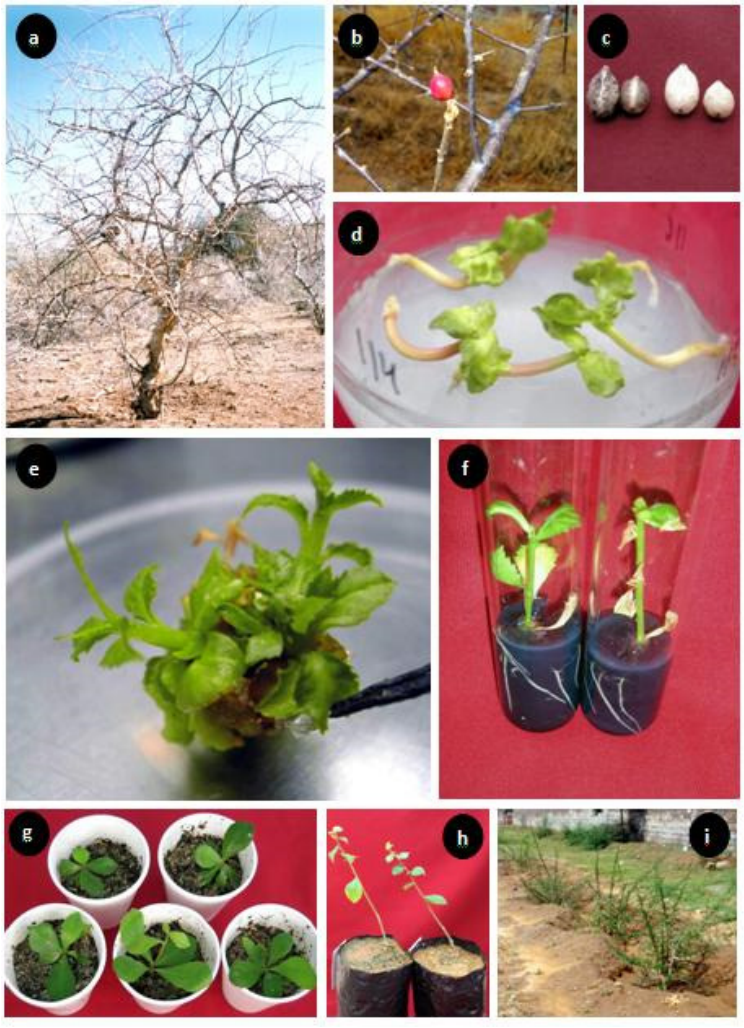


Fig 1: Cotyledonary node derived micropropagation in Commiphora wightii. A, a mature tree in May; B, mature fruit; C, seeds with black and white endocarp; D, Germination after BAP pretreatment; E, micro-shoot multiplication; F, in vitro rooting; G, ex vitro hardening; H, hardened plantlets ready for field trial; I, plants growing under field conditions (after 1 yr. of transplantation)

Somatic embryogenesis micropropagation protocol: For the development of somatic embryogenesis based protocol, immature fruits (rather than mature ones) were collected from the mature selected mother plants; somatic embryogenesis was found to be higher through use of immature fruits compared to mature fruits where somatic embryogenesis percentage was very poor. The sinkers were selected and surface sterilized as described above and the seeds extracted and inoculated on various types of media [B5 (Gamborg, 1968) and MS (Murashige and Skoog, 1962)] with and without hormones (2,4-D, IBA and BAP) to derive somatic embryo based plantlets. All experimental treatments consisted of 25 explants, each replicated three times (Fig 2) (Kant et al 2010a).

Fig 2: *In vitro* mass multiplication of Commiphora wightii through somatic embryogenesis. A: Embryogenic callus; B: Somatic embryo (SE) multiplication; C: SE maturation ; D: SE germination; E: Hardening of plantlets; F: Hardened plants ready for field plantation.

Acclimatization of in vitro raised plants: Acclimatization was carried out in mist chamber (90 sec misting at 10 min intervals to maintain a relative humidity between 85 to 95%). The temperature of the mist chamber was maintained between 28-30°C. Somatic embryo derived complete plants and rooted micro-shoots derived from cotyledonary nodes were transferred to vermiculite and wetted with Hoagland's solution (Hoagland, 1950) for primary hardening for 4-5 weeks and then finally transferred to plastic cups containing vermiculite, placed in mist chamber. Plantlets were transferred to a soil: farm yard manure (FYM) mix (ratio 1:1) in polythene plantation bags (9 x 9 x 36 cm). After one month of transfer to polythene bags, plantlets were transferred under green-50% agronet shade for complete acclimatization and finally transplanted to the experimental field site in April 2007 and July 2010

Cost considerations and concluding remarks: Tissue culture protocols (through cotyledonary node cultures and somatic embryogenesis) for in vitro mass multiplication of C. wightii are effective and provide a viable alternative to propagation via stem cuttings and seeds, for quality plant production. The

plants so obtained can directly assist in various conservation efforts, including restoration of wild populations. Through a tissue culture based system, continuous supply of quality planting stock can be ensured at a reasonable cost. At a per plant cost (calculated using Tomar *et al*., 2007) ranging from INR 19 to 27 for somatic embryogenesis and cotyledonary node based pathways respectively are economical. Long term survival and production of fertile tissue cultured plants demonstrates that these are performing very well under natural field conditions and hold a promising future.

Future plans: DNA fingerprinting techniques will be used to assess the extent of variability (or the lack of it) amongst the regenerated plants that have been out planted in the field conditions. This will help in deciding the final application of the tissue culture protocols.

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