

Efficacy of *in vitro* tissue culture versus stem cuttings for propagation of *Commiphora wightii* in Rajasthan, India

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SUMMARY

To assist in the conservation of *Commiphora wightii* (an endangered medicinal tree), experiments were undertaken to develop an efficient, rapid and inexpensive method for large scale propagation. Two methods were trialed, propagation by stem cuttings and *in vitro* tissue culture. Propagation by the stem cutting method was found to be both more successful and produced plants of a suitable size for transplanting more rapidly than *in vitro* cultivation. Stem cutting propagation was also inexpensive and easier to perform, as compared to *in vitro* propagation. The cost to produce a plant of suitable size for transplanting was 3 Indian Rupees (INR) using the stem cutting method and 80 INR by the *in vitro* method.

BACKGROUND

Commiphora wightii (Arnott) Bhandari (Burseraceae) is a small tree (locally known in India as guggul) that grows in arid rocky tracts of the Aravali range of Rajasthan and Gujarat in northwest India, and adjacent Pakistan. The use of plants in the treatment of diseases occupies an important place in Ayurveda, the traditional system of medicine of India. The oleo-gum resin of *C. wightii* (gum-guggul) is mentioned in the classic Ayurvedic literature as an efficacious treatment for arthritis, obesity, bone fractures, inflammation, cardiovascular disease and lipid disorders. The plant has become endangered due to unsustainable gum harvest (with the often harmful methods used for resin tapping contributing to eventual tree death), combined with its slow growing nature, poor seed set and poor seed germination (Soni 2010). Guggul is considered endangered in India and is listed as 'Data Deficient' in the IUCN Red Data list (IUCN 2010) because of a lack of knowledge regarding its conservation status.

In this present study, efforts were made to develop an efficient, rapid and inexpensive method for large scale propagation of *C. wightii* plants, both for cultivation purposes to boost the income of subsistence farming communities, and for transplanting to bolster dwindling wild populations within India. I

attempted two methods of vegetative propagation, namely stem cuttings and *in vitro* tissue culture, during 2005-2006. A summary of the efficacy of *in vitro* tissue culture versus stem cutting propagation for the conservation of *C. wightii* is presented.

ACTION

***In vitro* propagation:** Nodal explants were collected during full flowering stage (April) of *C. wightii* from plants growing in the Grassfarm Nursery, Jaipur city (Rajasthan). Explants were surface sterilized with 0.1% mercuric chloride (w/v) for 6 min and then washed thrice using sterilized distilled water. Under aseptic conditions, the sterilized single nodal explants were cultured in 100-ml Erlenmeyer flasks (Borosil) on basal MS (Murashige & Skoog 1962) medium containing 3% (w/v) sucrose, supplemented with various combinations and concentrations of auxin and cytokinin for shoot differentiation. The pH of the media was adjusted to 5.8 prior to the addition of 0.8% agar and autoclaved at 121°C for 15 min. Cultures were then incubated at 26±2°C with a 16-h photoperiod at 3000 lux intensity by fluorescent tubes. Shoots regenerated from nodal explants were subjected to various concentrations (0.1-5.0 mg/L) and

combinations of auxins and cytokinins for root initiation.

Regenerated plantlets (5-6 weeks old) having well developed root-shoot system were transferred to plastic pots containing sterilized soil and soilrite (a mixture of horticulture grade expanded perlite, peat moss and exfoliated vermiculite in equal ratio) in the ratio of 1:3. The pots were covered with polythene bags to maintain humidity and kept in a growth chamber for 10 days under controlled conditions at $25\pm 2^\circ\text{C}$. The plantlets thus developed were then transferred to plastic bags (22.5 x 15 cm and 150 gauge thickness) containing soil and manure (ratio of 1:3) and grown on in a green-shade nursery (i.e. shaded with green plastic mesh affording 50% shade to prevent exposure to extreme temperature and sunlight). Plants were watered as required to maintain suitable soil moisture. Survival and growth were monitored daily.

Propagation through stem cuttings:

Approximately 400 stem cuttings (each 0.6-0.8 cm stem diameter and 12 cm long) were taken in April from mature *C. wightii* growing in the wild at Gulta Hills near Jaipur (Soni 2010). The basal portions of the freshly collected cuttings were dipped for 5 seconds in freshly prepared 1,500 ppm aqueous solution of Indole-3-butyric acid solution (Kumar *et al.* 2006). Cuttings were then planted (basal end of the stem 4 cm deep) in plastic bags (22.5 x 15 cm) containing soil and manure (ratio of 1:3) and maintained in the green-shade nursery.

CONSEQUENCES

In vitro propagation: Multiple shoot bud formation in nodal explants was noticed when two cytokinins (0.5 mg/L KIN and 3.0 mg/L BAP) along with IBA (0.5 mg/L) were added to the growth medium (Fig. 1a). The number of shoot bud induction per explant increased when IBA was replaced with IAA (Fig.1b). Other plant growth regulators singly or in combinations did not enhance any significant morphogenetic responses in nodal explants. MS basal medium supplemented with IAA (0.5 mg/L) and NAA (1.0 mg/L) was found most effective in root initiation in shoots regenerated from these nodal explants (Fig.1c). The 5-6 week-old acclimatized plantlets (238) transferred to soil and grown on in the nursery had around a 5% survival rate. A total of 22 plants suitable for transplanting were produced through *in vitro* method.

Propagation through stem cuttings:

Sprouting of stem cuttings was achieved within 20 days of planting (Fig. 2). The development of a root system was observed within 20 to 30 days of planting with 80% survival rate. Of the 400 or so stem cuttings taken, 319 plants were produced.

Transplanting: Plants developed through *in vitro* tissue culture (22) and stem cutting (319) were transplanted (approx. 30 cm tall) next year during the rainy season (August 2006) in natural habitat at a site near Gulta, Jaipur (Fig.3a, b). Survival was monitored with the help of local communities as an ongoing community-based conservation project (Soni 2010). As of June 2009, 80% of the transplanted plants are surviving.

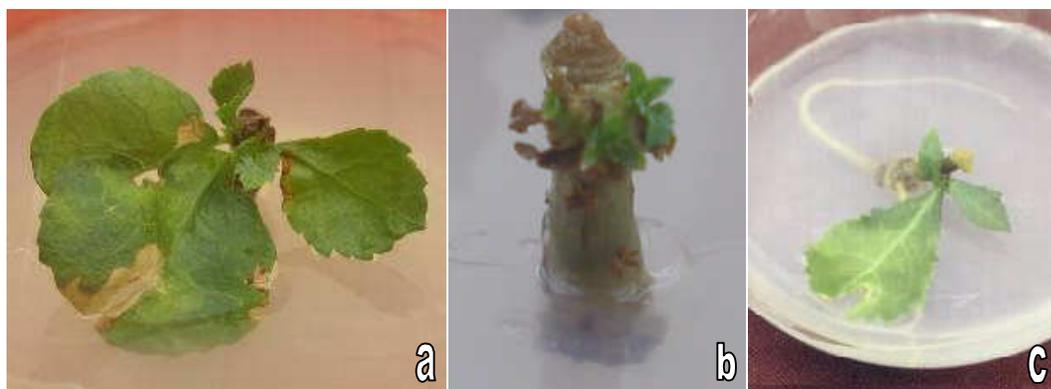


Figure 1. (a) *In vitro* multiple shoot induction from nodal explants of *Commiphora wightii* on MS medium containing KIN (0.5 mg/L), BAP (3.0 mg/L), IBA (0.5 mg/L) and (b) KIN (0.5 mg/L), BAP (3.0 mg/L), IAA (0.5 mg/L) and (c) IAA (0.5 mg/L) and NAA (1.0 mg/L).



Figure 2. Sprouting in guggul stem cuttings.



Figure 3. Transplanting guggul plants in the Aravali Hills.

Efficacy of stem cutting vs. *in vitro* propagation: In the present investigations it was found that the propagation of *C. wightii* through stem cuttings offers several advantages over *in vitro* tissue culture. The stem cutting method is inexpensive and does not require costly chemicals and equipment for propagation. It is easier to perform than *in vitro* tissue culture, and after out-planting into the nursery, tissue culture plantlets need higher levels of subsequent care (i.e. photoperiod and temperature maintenance, subculturing) than plants derived from stem cuttings. Plantlets developed by tissue culture were weak and fragile, those from stem cuttings far more robust. Another major drawback of *in vitro* propagation is that it is labour-intensive, further leading to a high price of micro-propagated plantlets. The cost to produce a plant of suitable size for transplanting was 3 Indian Rupees (INR) using the stem cutting method and 80 INR by the *in vitro* method.

Conclusions and discussion: The present findings clearly demonstrate that the stem cutting method trialed is suitable for rapid and large scale propagation of *C. wightii*. Since seed germination in *C. wightii*, even after application of different techniques to break dormancy is very poor (Soni *et al.* 2009), vegetative propagation through stem cuttings may be the only viable option to enhance natural populations. In addition, stem cuttings can continuously supply planting stock throughout the year for reforestation activities. The propagation of plants through stem cuttings will also help to support local communities by enabling farmers to cultivate *C. wightii* and thus earn an income from extraction and sale of gum-guggul. This, combined with ongoing education programs (Soni 2010) will hopefully serve to benefit nature conservation initiatives in the Aravali Hills region of Rajasthan.

ACKNOWLEDGEMENTS

The author is thankful to Prof. P.L.Swarnkar, Department of Botany, University of Rajasthan (Jaipur) for constant encouragement and support for the completion of the work.

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