Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 559-563
Received: 18-05-2017
Accepted: 19-06-2017

Rajani Verma
Research Scholar Department of Plant Breeding and Genetics SKN Agriculture University, Jobner, Rajasthan, India

ML Jakhar
Professor and Head Department of Plant Breeding and Genetics SKN Agriculture University, Jobner, Rajasthan, India

Ravi Kumar
Research Scholar Department of Plant Breeding and Genetics SKN Agriculture University, Jobner, Rajasthan, India

Anamika Nath
Research scholar, Department of Plant Breeding and Genetics, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

Correspondence
Rajani Verma
Research Scholar Department of Plant Breeding and Genetics SKN Agriculture University, Jobner, Rajasthan, India

In vitro propagation of medicinal plant Guggul: A review

Rajani Verma, ML Jakhar, Ravi Kumar and Anamika Nath

Abstract
Commiphora wightii (Arnott) Bhandari is a medicinally important plant which is now considered a critically endangered species of the family Burseraceae. It is found in arid, rocky tracts of Rajasthan and Gujarat Maharashtra and Karnataka. In Rajasthan it is found in Jhunjhunu, Pali, Udaipur, Alwar (Sariska Tiger Reserve), Jaipur (Ramgarh, Jhalana area), Bhilwara and Rajsamand. It having a valuable active principle found in its oleo-gum-resin (guggulsterone E and Z), which are used in drugs preparation for lowering the cholesterol level in human body. Due to it's overexploitation, poor natural regeneration, this valuable plant is on the verge of extinction and thus a IUCN Red listed species. In the present study we focus on the development of an efficient micropropagation protocol from different explants of guggul.

Keywords: *Commiphora wightii*, endangered, IUCN, *in vitro*, meristem culture, virus free plants

Introduction
Commiphora wightii (Arnott) Bhandari is a medicinally important plant which is now considered a critically endangered species. The name *Commiphora* originates from the Greek words kommi (meaning 'gum') and phero (meaning 'to bear'). It belong's to family Burseraceae. It is a slow-growing, woody, medicinal plant having the chromosome number 2n = 26 (Sobti and Singh, 1961) [21]. The genus *Commiphora* is widely distributed in tropical regions of Africa, Madagascar, Asia, Australia and the Pacific islands (Good, 1974) [4]. Guggul is an important medicinal plant of herbal heritage of India. In Indian languages, it is known by various names like guggul in Hindi, gukkulu and maishakshi in Tamil, guggulu in Sanskrit and Indian bdellium in English. It is found in arid, rocky tracts of Rajasthan and Gujarat Maharashtra and Karnataka (Kumar and Shankar, 1982) [12]. In Rajasthan it is found in Jhunjhunu, Pali, Udaipur, Alwar (Sariska Tiger Reserve), Jaipur (Ramgarh, Jhalana area), Bhilwara and Rajsamand.
Guggul is critically considered endangered in India and listed as 'Data Deficient' in the IUCN Red Data list (IUCN, 2015) [25] because of a lack of knowledge regarding its conservation status. Over the past 84 years (three generation lengths) there has been a decline of more than 80% in the wild population as a result of habitat loss and degradation, coupled with unregulated harvesting and tapping of oleo-gum resin. This species is therefore assessed as critically endangered that mean a taxon is critically endangered when the best available evidence indicates that it meets any of the following criteria and it is therefore considered to be facing an extremely high risk of extinction in the wild:-

A. Reduction in population size based on any of the following:
1. An observed, estimated, inferred or suspected population size reduction of ≥ 90% over the last 10 years or three generations, whichever is the longer, where the causes of the reduction are clearly reversible and understood and ceased, based on (and specifying) any of the following:

a) Direct observation.
b) An index of abundance appropriate to the taxon.
c) A decline in area of occupancy, extent of occurrence and/or quality of habitat.
d) Actual or potential levels of exploitation.
e) The effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.

2. An observed, estimated, inferred or suspected population size reduction of ≥ 80% over the last 10 years or three generations, whichever is the longer, where the reduction or its causes may not have ceased or may not be understood or may not be reversible,

based on (and specifying) any of (a) to (e) under A1.

3. A population size reduction of ≥ 80%, projected or suspected to be met within the next 10 years or three generations, whichever is the longer (up to a maximum of 100 years), based on (and specifying) any of (b) to (e) under A1.
4. An observed, estimated, inferred, projected or suspected population size reduction of ≥ 80% over any 10 year or three generation period, whichever is longer (up to a maximum of 100 years in the future), where the time period must include both the past and the future, and where the reduction or its causes may not have ceased or may not be understood or may not be reversible, based on (and specifying) any of (a) to (e) under A1.

B. Quantitative analysis showing the probability of extinction in the wild is at least 50% within 10 years or three generations, whichever is the longer (up to a maximum of 100 years).

Over-exploitation, a narrow extent of occurrence, small area of occupancy, severe fragmentation of populations, very low regeneration and invasion of alien species mean that *C. wightii* is facing a high extinction risk (Reddy *et al.,* 2012 and Ved, *et al.,* 2015) [17, 25].

The plant has vast economic value and a wide array of medicinal uses in both ancient and modern therapeutics. The plant yields medicinally important natural gum resin. Guggul was first introduced to the scientific world by an Indian medical researcher, Satyavati, G. V. in 1966. Guggul-gum is known to be hypolipidemic, hypocholestrolemic and anti-obesity astringent and antiseptic, anti-arthritic, antimicrobial, anti-inflammatory, and anticancerous. It is also reported for the treatment of thrombosis and chronic bronchitis, nodulocystic acne, spongy gums, chronic tonsillitis and teeth carries. The gum is also used in perfumery, calicoprinting, fumigation, dyeing silk and cotton and as incense. Guggul has been also reported to cause side effects like gastrointestinal discomfort, possible thyroid problems and generalized skin rash. Guggul gum is a mixture of 61% resin, 29.3% gum, 6.1% water, 0.6% volatile oil and 3.2% foreign matter, (Jain and Nadgauda, 2013) [6].

The technique of plant tissue culture is used for growing isolated plant cells, tissues and organs under axenic conditions (*in vitro*) to regenerate and propagate entire plants. 'Tissue culture' is commonly used as a blanket term to describe all types of plant cultures, namely callus, cell, protoplast, anther, meristem, embryo and organ cultures. The different techniques of culturing plant tissues may offer certain advantages over traditional methods of propagation. Growing plants *in vitro* in a controlled environment, with in-depth knowledge of the culture conditions and the nature of the plant material, ensures effective clonal propagation of genetically superior genotypes of economically important plants.

The indiscriminate and faulty method of gum tapping has led to the destruction of the plants and coupled with inadequate replenishment; this species is now enlisted in the critically endangered plant (Tajuddin *et al.,* 1997) [23]. Guggul can be propagated by seeds and vegetatively through air layering and stem cuttings (Mertia and Nagrajan, 2000; Chandra *et al.,* 2001; Kumar *et al.,* 2002) [13, 2]. For propagation of stem cuttings earlier, one-meter long and 10 mm thick woody stem cuttings were reported suitable for raising of *Commiphora wightii* **(**Dalal and Patel, 1995) [3]. Traditionally propagating methods of guggul are not effective due to that *in vitro* micropropagation method is more desirable. In this review we are focusing on the micropropagation methods by which we can save this critically endangered plant by generating fast plant multiplication.

In vitro propagation

***In vitro* propagation/clonal propagation:** *In vitro* propagation is the production of whole plant from small section of plant such as a stem tip, node, meristem, embryo, or even a seed. The process of *in vitro* in guggul involves 5 distinct stages.

Stage 0: This stage consists of selection of elite mother plants which should be healthy (free from disease) and their preparation in such a way that they provide more responsive explants suitable for establishment in contamination free cultures.

Stage I: Explant: The selection of explant type is important for their response to tissue culture technique for callus initiation and regeneration. The quality of explants primarily determines the establishment of *in vitro* culture (John and Murray, 1981) [7]. *In vitro* growth of explants and degree of contamination may be influenced by seasonal condition at the time of explants collection. Barve and Mehta (1993) [1] investigated that the explants of *Commiphora wightii* were collected in the month of April-June gave a good response whereas, the contamination rate was higher for the culture during September-October.

Mishra and Kumar (2010) [14] developed reliable and reproducible protocols to get healthy and well formed plants from juvenile explants of the *C. wightii*. Fresh explants consisting of leaf, embryo and nodal segment of *C. wightti*, were selected for *in vitro* callus initiation. MS medium, supplemented with 2, 4-D and kinetin individually and in combinations were found to be effective for callus induction in *C. wightii*. For callus initiation MS medium was supplemented individually with 2, 4-D (1.0 – 5.0 mg/l) and Kn. (1.0 – 5.0 mg/l) and in combination of 2, 4-D (1.0 – 5.0 mg./l) with Kn. (0.1- 0.5 mg./l). Callus has been initiated in MS medium supplemented with 2, 4-D and Kn individually and in combinations. However good callus initiation and growth was observed with a combine effect of 2, 4-D and Kn. Maximum 5.78 g. fresh callus biomass was obtained in the treatment MS+ 2, 4-D (5 mg/l) + Kn (0.5 mg/l).

Surface Sterilants: Successful disinfection of explants is a pre-requisite for *in vitro* culture and often involves a standard set of treatments. Washing the plant material intensively in running tap water before the surface sterilization process. Mainly $HgCl_2$ & NaOCl used for surface sterilants.

Joshi and Mathur (2015) [8] found the fungicide Bavistin to be effective, at various time durations when used along with $HgCl_2$. However explants surface sterilized with NaOCl (1%) and $HgCl_2$ (0.1%) for different time intervals showed varying level of % of contamination. However sterilization of apical bud and nodal explant with 0.1% $HgCl_2$ for 4 minutes was more effective than NaOCl (1%). Maximum contamination free cultures were obtained when the explants were disinfected with 0.1% $HgCl_2$ for 4 minutes along with Bavistin.

Table 1: Effect of different sterilants on disinfection of nodal segments of *Commiphora wightii.*

Bavistin	0.1%$HgCl_2$	% of contamination
5 min.	2 min.	70
6 min.	4 min.	30
7 min.	5 min.	40

Media: Selected explants are surface sterilized and aseptically cultured on a suitable medium. Growth and morphogenesis of plant tissues *in vitro* are largely governed by the composition of the culture media. Although the basic requirements of cultured plant tissues are similar to those of whole plants, in practice, nutritional components promoting optimal growth of a tissue under laboratory conditions may vary with respect to the particular species. Media compositions are therefore formulated considering specific requirements of a particular culture system. The selection or definition of a culture medium is vital for the success of an *in vitro* process. The inorganic salt formulation can vary, but in most cases MS medium is the most commonly used culture medium in plant tissue culture (Smith, 1992) [20].

Parmar and Kant (2012) [16] reported an effective procedure of guggul (*Commiphora wightii*) micropropogation. Nodel segments were used as explants. Responsive treatments in terms of bud break response were tried on different media types MS, B5 and WPM media for suitability of the most responsive nutrient medium for improvement in terms of bud break response, length and numbers of shoots.

Stage II: Cytokinin: Effective explants from stage I, are sub-cultured on to a fresh medium. The time and concentration of auxins and cytokinins in multiplication medium is an important factor affecting the extent of multiplication. Cytokinins are purine derivatives that support cell division. The two main types of cytokinins used in tissue culture are benzyl amino purine (BAP) and kinetine. The cytokinin signaling pathway represents a potential target for manipulating *de novo* shoot organogenesis and *in vitro* plant regeneration.

Kant *et al*. (2010) reported an effective procedure of guggul micropropogation. Cotyledonary nodes were used as an explant and multiple microshoots were obtained on Murashige and Skoog, 1962 medium supplemented with 2.68 µM α-Naphthalene acetic acid (NAA) and 4.44 µM 6-Benzylamino purine (BAP) and on 2.68 µM NAA + 4.44 µM BAP with additives (glutamine 684.2 µM; thiamine 29.65 µM; activated charcoal 0.3%) and various other hormonal combinations. Elongation of microshoot was significantly observed on the 2.46 µM Indole-3-butyric acid (IBA) and 2.22 µM BAP supplemented MS medium.

Soni (2010) observed multiple shoot bud formation in nodal explants when two cytokinins (0.5 mg/l Kn and 3.0 mg/l BAP) along with IBA (0.5 mg/l) were added to the growth medium. The number of shoot bud induction per explant increased when IBA was replaced with IAA. Other plant growth regulators singly or in combinations did not enhance any significant morphogenetic responses in nodal explants.

Tejovathi *et al.* (2011) studied 40 single as well as combinations of NAA (1.0-4mg/l), IBA (0.1-4mg/l), BAP, Kn (1-4mg/l) and gibberellic acid- GA_3 (0.5-1.5mg/l) were tested, shows no shoot development on any cytokinin, auxin or GA_3 supplemented medium. Similarly, in the combination lower concentrations of BAP with IBA, also failed to induce shoot development from any of the explant. However, at higher concentrations of BAP (3.0mg/l) with IBA, shoot development was observed from both the explants. MS with BAP (3.0mg/l) + IBA (0.2mg/l) gave better response (20%) followed by 3mg/l BAP + 0.3mg/l IBA (13%).

Interactions of auxins and cytokinins

The ratio of auxins and cytokinins is important with respect to morphogenesis in the culture system. For embryogenesis, callus initiation and root initiation the requisite ratio of auxins to cytokinin is high, while the reverse leads to axillary and shoot proliferation.

Soni (2010) reported multiple shoot bud formation in guggul nodal explants when two cytokinins (0.5 mg/l Kn and 3.0 mg/l BAP) along with IBA (0.5 mg/l) were added to the growth medium. The number of shoot bud induction per explant increased when IBA was replaced with IAA. Other plant growth regulators singly or in combinations did not enhance any significant morphogenetic responses in nodal explants.

Joshi and Mathur (2015) [8] reported that MS medium containing BAP 3 mg/l and IAA 0.5 mg/l with additives (50 mg/l ascorbic acid, 25 mg/l citric acid and 25 mg/l arginine), activated charcoal 0.3%. 82% of cultures showed maximum bud break with 2.40±0.28 number of shoots with shoot length of 1.82±0.19 within 30 days. A low concentration of auxin along with a high concentration of cytokinin was most promising for the induction and multiplication of shoots in *Commiphora wightii.* The explants cultured on these media showed their first response by initial enlargement of the existing axillary buds followed by bud break.

Effect of photoperiod and light intensity

Photoperiodism is the physiological reaction of organisms to the length of day or night. Photoperiodism can also be defined as the developmental responses of plants to the relative lengths of the light and dark periods. Hence, it should be emphasized that photoperiodic effects relate directly to the timing of both the light and dark periods.

Singh *et. al.* (2011) [19] inoculated leaf discs on to the MS medium, fortified with different concentrations of 2, 4-D and IBA. The pH of the media was adjusted to 5.8 before autoclaving. All media were autoclaved at 1.06 kg cm/2 and 121ºC for 15 min. The cultures were incubated in growth room at temperature of 25 ± 2 °C and 16-h photoperiod. Leaf showed maximum callus formation on MS medium with IBA at the concentration of 1.5 mg/litre. Good amount of callus in creamish green healthy colour compact callus was obtained. But on 2, 4-D at 1.5 mg/litre concentration it was fragile and yellowish.

Stage III: Rooting: Proliferated shoots are transferred to a rooting medium. This phase is designed to induce the establishment of fully developed plantlets. Auxins are mainly concern with inducing cell division. In nature the hormones of this group are involved with such activities as elongation of stem, internodes, tropism, apical dominance, abscission and rooting. Different factors favoring root initiation have been tried. The auxin IBA was found the most effective of all auxin types. In contrast, IAA though being natural was least effective as it got degraded due to light. The availability of IBA induces primary / secondary roots where as NAA induces root hairs. In general rooting medium has low salt. It is the last period *in vitro* before transferring the plantlets to *ex vitro* conditions.

Singh *et al.* (2010) supplemented MS half strength medium with 2.0 mg/l IBA proved better with forty percent rooting after 22 days of implantation. Most of the roots were long and healthy. The micropropagated plantlets were hardened and acclimatized. They were successfully transferred in pots containing sterilized soil and sand mixture (1:1) with 60% survival rate under field conditions.

Micro-shoots of 2-3 cm length were initially given a 24 h treatment in liquid MS and White's medium supplemented

with 4.92 µM IBA and 5.71 µM IAA under dark condition, followed by transfer to semi-solid half-strength hormone-free MS and White's medium supplemented with 2% sucrose and 0.5% activated charcoal. High (86.7%) percent rooting was achieved after 4-5 weeks with 2.85 ± 0.5 multiple adventitious roots of 6.46 ± 0.4 cm length. (Kumar and Tarun, 2012).

For root induction regenerated individual shoots of 1-2 cm of *Commiphora wightii* were initially given a treatment in liquid MS media and White's medium fortified with 0.5 mg/L IBA under dark condition for 2 days, then they are transfer to light in semi-solid half-strength hormone-free MS media and White's medium supplemented with 2% sucrose and 1% activated charcoal. It was observed that continuous darkening during the rooting inductive phase, increases peroxidase activity which resulted in higher rooting rate. (Joshi and Mathur, 2015) [8].

Stage IV: Hardening: Hardening refers to the process of acclimating plants from indoor temperatures to the outdoors. The hardening of *in vitro* raised plantlets is essential for better survival and successful establishment. Direct transfer of tissue culture raised plant to field is not possible due to high rate of cosseted environment with a very high humidity, varied light and temperature condition and being protected from the attack of microbial and other agents.

Kant *et al.*, (2010) [9] developed well rooted plantlets and transferred them to glass jam jars filled quarter level with vermiculite and wetted with Hoagland's solution. After 4-5 weeks when plantlets showed new growth the plastic cap of the glass jar was unscrewed gradually over a period of 2-3 days to reduce relative humidity in the jar, then finally the caps were removed completely from the jars on the third day. The plantlets were then transferred to thermocol cups containing vermiculite wetted with Hoagland's solution at one-week interval. These plantlets were placed in mist chamber. After two weeks these were then transferred to soil: FYM 1:1 mixture in plastic plantation bags (poly-bags) of size 9x9x36 cm (2916 cm3). In mist chamber, 90 second misting at ten minutes interval was given to maintain Relative Humidity between 85 to 95%. The temperature of mist chamber was maintained between 28-30°C. After one month of transfer to poly-bags plantlets were transferred under green-50% agronet shade and after two week transferred to field, where they are growing well and have started to flower and set seeds. Growth data of the field grown plants is being collected

Advantages of *In vitro* Propagation in Guggul

1. The technique of *in vitro* propagation is an alternative approach to conventional methods of vegetative propagation, which has the enhanced rate of multiplication.
2. A million of shoot tips can be obtained from a small, microscopic piece of plant tissue within a short period of time and space.
3. The advantage in this type of propagation is that as shoot multiplication usually has a short cycle (2-6 weeks) and each cycle results in logarithmic increase in the number of shoots.
4. Stocks of germplasm can be maintained for many years using this method of propagation.
5. This method is more applicable where disease free propagules are wanted. This *in vitro* technique helps to raise pathogen free plant and to maintain them.
6. A major advantage of *in vitro* propagation happens to be the minimum growing space required in commercial nurseries. Thousands to millions of plantlets can be maintained within the culture vials.
7. This method is more helpful in case of slow growing plants where the seeds are produced after a long term and the seeds are the only propagule. This method can overcome the difficulty to obtain the propagules.
8. Through seed production genetically uniform progeny is not possible always. But the *in vitro* propagation method will help to maintain the genetic uniformity in the propagules.

References

1. Barve DM, Mehta AR. Clonal propagation of mature elite trees of Commiphora wightii. Plant Cell Tissue and Organ Culture. 1993; 35:237-244.
2. Chandra R, Kumar D, Jha BK. Rooting behavior of stem cuttings of *Commiphora wightii* (Indian Bdellium). J. Tropical Medicinal Plants. 2001; 2:269-274.
3. Dalal KC, Patel M A, Guggal. In: Chadha KL & Gupta R (eds) Advances in Horticulture, Medicinal and Aromatic Plants. New Delhi, Malhotra Publishing House. 1995; 11:491-501.
4. Good R. The geography of the flowering plants. London: Longman. 1974, 557.
5. IUCN The IUCN Red List of Threatened Species. Version 2015. 2. www.iucnredlist.org.
6. Jain N, Nadgauda SR. *Commiphora wightii* (Arnott) Bhandari—A Natural Source of Guggulsterone: Facing a High Risk of Extinction in Its Natural Habitat. American Journal of Plant Sciences. 2013; 4:(6A).
7. John A, Murray BW. Micropropagation of sitka, spruce (*Picea sitchensis*). In: Colloque international surla culture *in vitro* des essences forestries. AFoCEL, Fountainblean Publ. 1981, 65-70.
8. Joshi A, Mathur N, *In vitro* plant regeneration from nodal segments of *Commiphora wightii* an endangered medicinally important desert plant. International journal of advanced research. 2015; 3(10):729-737.
9. Kant T, Prajapati S, Parmar AK. Efficient micropropagation from cotyledonary node cultures of *Commiphora wightii* (Arn.) Bhandari, an endangered medicinally important desert plant. J Plant Dev. 2010; 17:37-48.
10. Kumar PA, Tarun K. Efficient micropropagation and evaluation of genetic fidelity of *in vitro* raised plants of *Commiphora wightii* arn. (bhandari) – a medicinally Important red-listed species of arid regions. J. Plant Develop. 2012; 19:29-40.
11. Kumar D, Jha BK, Chandra R. Response of auxins and planting time on the regeneration of stem cuttings of *Commiphora wightii* (Indian Bedellium). J. Tropical Medicinal Plants. 2002; 3:253-258.
12. Kumar S, Shankar V. Medicinal plants of the Indian deserts: *Comiphora wightii* (Arnott) Bhandari. Arid Environ. 1982; 5:1-11.
13. Mertia RS, Nagarajan M. Successful rooting in cuttings of *Commiphora wightii* (Arnott.) Bhand. Annals Arid Zone. 2000; 39:87-88.
14. Mishra SK, Kumar A. Biosynthesis of guggulsterone in the callus culture of *Commiphora wightii*. Arnott. bhandari (Burseraceae). Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2010; 1(3):35-41.
15. Murashige T. Plant propagation through tissue culture.

Annual Review of Plant Physiology. 1974; 25:135-165.
16. Parmar AK, Kant T. Efficient Micropropagation and Evaluation of Genetic Fidelity of *in vitro* Raised Plants of *Commiphora wightii* (Arn.) Bhandari—A Medicinally Important red Listed Species of Arid Regions. Journal of Plant Development. 2012; 19(1):29-40.
17. Reddy CS, Meena SL, Krishna PH, Charan PD, Sharma KC. Conservation Threat Assessment of *Commiphora wightii* (Arn.) Bhandari an Economically Important Species. Taiwania. 2012; 57(3):288-293.
18. Singh N, Garg A, Yadav K, Kumari S. Influence of growth regulators on the explants of *Commiphora mukul* (Hook. ex Stocks) Engl. under in vitro conditions. Researcher. 2010; 2(7).
19. Singh S, Tanwer BS, Khan M. *In vivo* and *in vitro* comparative study of primary metabolites of *Commiphora wightii* (arnott.) Bhandari. International Journal of Applied Biology and Pharmaceutical Technology. 2011*;* 2(1):162-166.
20. Smith R. Plant tissue culture: Techniques and experiments. Academic Press, San Diego. 1992, 171.
21. Sobti SN, Singh SD A. Chromosomal survey of medicinal plants Part 1. Proceed. Indian Acad Sci. 1961; 54b:138-144.
22. Soni V. Efficacy of *in vitro* tissue culture versus stem cuttings for propagation of *Commiphora wightii* in Rajasthan, India. Conservation Evidence 2010; 7: 91-93.
23. Tajuddin Agarwal SK, Tyagi BR, Ram M, Dwivedi S and Kumar S, Development of cultivar marusudha of guggul (*Commiphora wightii*). J. Med. Arom. Plant Science. 1997; 19:1043-1044.
24. Tejovathi G, Goswami H, Bhadauria R. *In vitro* propagation of endangered medicinal plant *Commiphora wightii*. Indian Journal of Science and Technology. 2011; 4:0974-6846.
25. Ved D, Saha D, Kumar RK, Haridasan K. *Commiphora wightii*. The IUCN Red List of Threatened Species. 2015: e.T31231A50131117.