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REVIEW ARTICLE

In vitro propagation of pharmaceutical and endangered medicinal plants- a mini review

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ABSTRACT

Peoples and communities gain significantly from the use of medicinal herbs. The medicinal potential of these plants is related to the huge number of chemical compounds that have a specific physiological effect on the human body. Herbal drugs and phytonutrients, also known as nutraceuticals, are growing more popular around the world. The conventional strategy of propagation is the most popular and takes a very long time to multiply due to the low rate of fruit set, poor germination, and clonal homogeneity is not always preserved via seeds. Using optimal *in vitro* settings, elite plants may be rapidly multiplied to create pharmacognostic material delivering essential metabolites and also produce disease-free plants. This review summarises research on *in vitro* propagation methods established for medicinal plants, to use this technology to mass-produce medicinal plants that have been over-exploited.

Keywords: *In vitro* regeneration; micro propagation; callus; medicinal plants

INTRODUCTION

Traditional medications provide medicinal elements of knowledge that existed before the start-up of modern medicine across decades within diverse ethnicities. Up to 80 percent of the worldwide population in particular sections of African and Asian countries depend on conventional medicine for their basic healthcare needs. Around 70 percent of the countryside population in India relies on the ancient ayurvedic medicinal system. Many of the conventional users of the approach produce their formulations and deliver them to the patients through their recipes (Laha and Paul,

2019). Micropropagation technology can help to provide a great number of plants that are inexplicable by conventional means. The approach of *in vitro* regeneration or micropropagation relates to the formation of vast production of plants possessing unique traits within a short period (Espinosa-Leal et al., 2018). In addition, methods of plant tissue culture are already widely used as part of the engendering and conservation of therapeutic species, taking into consideration the ultimate objective to supplying the increasing demand of the pharmaceutical industry although protecting germplasm (Rout et al., 2000).

IN VITRO PROPAGATION

In vitro regeneration via tissue culture technique offers a viable technique for true-to-type rapid mass propagation and also conserving germplasm of rare, endangered, aromatic, and medicinal plants (Corral et al., 2011; Phulwaria et al., 2013; Rajasekharan and Sahijram, 2015; Saxena and Saxena, 2019; Shekhawat and Manokari, 2016). A necessity for the development of a genetic transformation protocol is the accessibility of a morphogenic culture system that is receptive to gene transfer techniques. Several investigations are known as regards *in vitro* propagation studies conducted in medicinal plants (Table. 1).

Table 1. *In vitro* propagated medicinal plants

Medicinal Plant	Explant	Reference
Aloe polyphylla	Shoot	Chukwujekwu et al. (2002)
Aloe vera (L.)	Shoot	Sivakumar et al. (2019)
Coleus forskohlii	Leaf	Sairam Reddy et al. (2001)
Henna (*Lawsonia inermis* syn. *Lawsonia alba*)	Aseptic seedlings	Rahiman and Taha (2011)
Cephalandra indica	Shoot buds	Kayalvizhi and Venkatachalam (2010)
Gymnema silvestre	Seeds	Komalavalli and Rao (2000)
Azadirachta indica	Seeds	Ashrafuzzaman et al. (2008)
Vetiveria zizanioids	Root	Widoretno et al. (2017)
Bael (*Aegle marmelos*)	Buds/Node	Pati et al. (2008)
Goldenseal (*Hydrastis canadensis*)	Leaf	He et al. (2007)
Wild yam (*Dioscorea villose*)	Node	Mahesh et al. (2010)
Rosy periwinkle (*Catharanthus rosse*)	Buds and Node	Amiri et al. (2019)
Zingiber cassumunar	Rhizomes	Chirangini and Sharma (2005)
Curcuma longa	Rhizomes	Naz et al. (2009)
North American ginseng (*Panax quinquefolius*)	Seeds	Uchendu et al. (2011)
lavender (*Lavandula coronopifolia*)	Shoot	Khateeb et al. (2017)
Withania somnifera	Shoot	Rani et al. (2016)
Valeriana wallichii	Node	Singh et al. (2015)
Coleus forskohlii	Node	Sivakumar et al. (2021)
Abutilon indicum L.	Leaf	Rout et al. (2009)
Cassia angustifolia	Leaf	Agrawal and Sardars (2006)
Ixora coccinea	Node	Sivakumar et al. (2020)

Regeneration via direct organogenesis

Pal and Roy (1991) found that leaf, node, and internode as explants removed from *C. speciosus* were able to grow many elongated shoots by 4.44 µg BAP supplemented MS media. They also noticed that elongated shoots were efficiently rooted on a half-strength MS medium. Subsequently, direct branch regeneration was obtained from the nodal explants of *C. speciosus* (Malabadi et al., 2005). They reported a high rate of numerous shoots from nodal explants on growth medium containing BAP (4.4 µM), kinetin (4.6 µM), and 3 percent sucrose. These plants matured appropriately, with no morphological variation between them and the mother plant. Thin rhizome section and triacontanol were utilised to get the greatest frequency of shoot regeneration in *C. speciosus*.

Shoot bud initiation was detected when rhizome was cultivated on 5.0 mg/l of TRIA supplemented B5 medium. Developed shoots grown on TRIA enriched B5 medium resulted in stronger roots. TRIA can be exploited as an efficient growth regulator in the *in*

vitro culture and conservation of *C. speciosus* (Malabadi et al., 2005).

A higher percentage of shoots (50.8%) from rhizome explants and multiple shoots (4.7) were seen using MS medium including BAP (2.5 mg/l) + kinetin (1.0 mg/l). A significant frequency of roots was reported in rhizome explant-derived shoots (50%) on half MS medium provided with IAA (1.5 mg/l). All the plantlets fetched to the field revealed similar morphological features to the mother plants (Bakrudeen and Arun, 2009).

Punyarani and Sharma (2010) evaluated nodes of *C. speciosus* (Koen.) having single axillary buds, cultivated on MS media supplemented with plant growth stimulants for generating plantlets. Nodal segments were grown on adenine sulphate (1-13 µM) or 40-70 gl^{-1} sucrose enriched MS medium with 5.0 µM BAP and 1.0 µM NAA to disturb the dormancy of axillary buds and also resulted in the formation of root and shoot at the same time. 5.0 µM BAP, 1.0 µM NAA, 50 gl^{-1} sucrose and 10.0 µM adenine sulphate (AdS) supplemented medium were proven most effective for breaking dormancy of axillary buds. The rooted shoots were put into the soil where they survived at a rate of 95 percent. The results revealed that axillary buds can be utilised for *C. speciosus* micro propagation.

Radha et al. (2011) reported successful regeneration of *Rubia cordifolia* utilising shoot tip and nodal explants. According to the data, nodal explants are a superior source of shoot development to shoot tip explants. The optimum shoots recorded were 5.9 and 5.2 per explants in MS media comprising 0.02 mg/l IAA + 1.0 mg/l BAP. The best root induction (98%) was achieved on 1.0 mg/l IBA and rooted plantlets were effectively moved to greenhouse conditions with 89% survival.

The nodal segments grown in NAA (0.25 mg/l) + BAP (2.0 mg/l) produced two shoots, whereas the internodal segments in NAA (0.5 mg/l) + BAP (2.0 mg/l) produced greater shoots per explant. Regenerated shoots rooted on half MS fortified with IAA (0.5 mg/l) exhibited the best root development from nodal segments. Rooted *in vitro* generated plantlets were moved to a greenhouse with an 80% survival rate was achieved. The regeneration methods used in this experiment provided as a foundation for conservating germplasm and future research of the active components of the herbal plants (Radha et al., 2015).

Khan et al. (2017) found that when rhizome was grown on MS medium blend with varied dosages of BA and kinetin, increased number of shoots was recorded in medium with BA 2.5 mg/l and kinetin 1 mg/l. A substantial rooting frequency was found in shoots from rhizome explants grown on half MS with IAA 1.5 mg/l. Maximum shoot development was reported in 0.6 µM NAA and 8.0 µM BAP rooted on 1-12 µM NAA and 3.0 µM BAP. The highest roots proliferation was achieved in 8.0 µM NAA and 3.0 µM BAP. A protocol for direct shoot regeneration and plantlet formation from nodal explants has been observed by (Sharma et al., 2019). Significant shoot multiplication frequency was detected on MS medium with 3.0 mg/l kinetin. Rooting of micro propagated shoots on 3.0 mg/l NAA supplied MS media. The *in vitro* generated plants were acclimatised in the greenhouse and eventually transplanted to the natural environment with 70% survival.

An optimal direct *in vitro* regeneration technique for *Saussurea costus* was accomplished by shoot tip explants taken from *in vitro* cultivated seedlings. MS medium reinforced with 1.14 µM TDZ and 2.68 µM NAA was observed to be most efficient for maximum shoot regeneration (73.33%), with the higher shoot numbers (11.4) and average shoot length (4.17 cm). Shoots grown on MS medium treated with 1.44 µM gibberellic acid, 2.32 µM kinetin and 4.44 µM BAP exhibited the greatest *in vitro* multiplication with an average shoot multiplication rate of 20.7 after 5-6 weeks (Sharma et al., 2019).

Malabadi et al. (2005) induced rapid shoot differentiation using thin slices of rhizome cultured on B5 medium without TDZ. Higher amounts of TDZ, especially 36.3 µM, 40.8 µM, and 45.4 µM, led explant browning which finally died. Shoot buds started to grow in the range of 11.3-7.3 µM TDZ and the explants retained greenness for 3 weeks on this medium and generated tiny chum structures in both the central and peripheral parts. The shoot buds had grown longer and formed two to three leaves after two weeks. Vani et al. (2016) suggested that BAP in combination with NAA demonstrated a synergistic impact, resulting in a larger number of shoots.

An efficient and elegant method for *in vitro* direct regeneration and multiplication of shoot was accomplished in *Alpinia calcarata*. Pre induction of rhizome sections in a media containing 8.8 µM BAP restored the buds from dormancy in 60% of the explants. An average shoot (6.2) was grown from the bud of rhizomatous explants on MS with 5.0 µM BAP, 10.0 µM kinetin, and 2.5 µM NAA (Bhowmik et al., 2016).

In vitro culture was described utilising sprouting branch buds of ginger rhizome. A dosage of 0.5 mg/l NAA and 1.0 mg/l BAP was reported to be excellent for shoot induction. Various plant growth hormones, photoperiod exposure levels, and sucrose concentrations were examined for micro rhizome induction (Musfir Mehaboob et al., 2019). TDZ (2.5 µM) in growth medium elicited more than 3 times of shoots (18.7) than BAP during direct plant regeneration (Zhang et al., 2011a).

Regeneration via indirect organogenesis

TDZ paired with BAP and 2, 4-D demonstrated a large influence on embryogenic callus initiation and multiplication during indirect plantlet culture. Explants grown on 2.5 µM TDZ, 2.0 µM BAP, and 1.2 µM 2,4-D provided MS media showed a callus generation rate of 91.1%. The organogenic shoots were shifted to MS media reinforced with 2.5 µM TDZ to generate many shoots (Zhang et al., 2011b). Callus were induced from the leaf, internode, node and rhizome explants on BAP (0.4 mg/l) + IBA (0.2 mg/l) + IAA (0.2 mg/l) + NAA (0.1 mg/l) + kinetin (0.2 mg/l) supplied MS media. These callus cultures failed to produce shoots on growth hormone combinations and concentrations in *C. igneus* (Radha et al., 2015).

Explants from two cultivars of ginger viz. Rio-de-Janeiro and Maran were grown in MS medium provided with varying amounts of 2,4-D and in paired with diverse BAP levels and these cultures were maintained under two different culture settings viz. light and dark. Callusing was noticed in all the explants except mature leaf in MS media reinforced with 2,4-D alone (1-2 mg/l) and is linked with BAP (0.5 - 3.0 mg/l). Shoot morphogenesis was achieved from one-month-old calli in BAP (3.0 mg/l) provided MS media (Resmi et al., 2007). On the medium containing NAA alone or in paired with BAP, only around 30% explant response was obtained with brown friable callus developing in 2 weeks.

It has been established that NAA might stimulate callus development on its own and that BAP had no interaction with NAA during callus formation. However, as the culture period progressed, the calli were unable to continue to proliferate and progressively changed into white roots, prohibiting shoot primordia to regenerate. Furthermore, swapping 2,4-D for NAA had no influence on the formation of calluses. Even though the 2,4-D and BAP combinations produced a few smooth calli in 62.3 percent of the explants, the formed callus was unable to multiply and finally wilted, demonstrating that callus growth was further restricted. As a result, either NAA and 2,4-D (both auxins) or BAP (cytokinin) would be adequate for *C. kwangsienesis* embryogenic callus survival whether treated individually or in a mixture (Zhang et al., 2011b).

Similarly, TDZ coupled with NAA, 2,4-D, BAP failed to generate callus. Surprisingly, explants performed effectively when TDZ was coupled with BAP and 2,4-D. Within 2 weeks, the explants grew and inflated at the cut area, and a soft viscous yellowish callus was raised from the cut surface (Zhang et al., 2011b).

Callus induction and sequential shoot organogenesis in *Costus* has been documented by (Roy and Pal, 1995). Callus induced from half cut seed explants grown on MS with 2, 4-D (2.0 mg/l) + BAP (0.1 mg/l) yielded 80% of brownish-white coloured callus. These callus cultures showed 55% of shoot regeneration response in MS medium with IAA (0.1 mg/l) + BAP (0.5 mg/l). Explants of *Pseudarthria viscida* cotyledonary nodes were used for callus production and subsequent shoot regeneration, as well as adventitious roots formation from the regenerated shoots (Cheruvathur and Thomas, 2011).

Ahmed et al. (2011) found callus initiation and the subsequent plants raised with alkaloids deposition in explants such as shoot tip and stem of *Phyla nodiflora*. When stem explants were cultivated on 1.5 mg/l NAA provided MS medium, they produced better callus biomass than shoot tip. 1.5 mg/l NAA and 1.0 mg/l BAP provided MS medium demonstrated a good shooting reaction from the callus. All of the *in vitro* generated shoots were efficiently rooted on a 1.0 mg/l IBA provided half MS medium. The plantlets were developed in the field with a 90% survival rate and displayed physical traits like the mother plant. Bakar et al. (2014) evaluated the impact of several medium on *in vitro* propagation of medicinal plant *Celosia argentea.* Multiplication of shoots was attained from a wide range of media tried, when nodal segments cultured in MS medium were invigorated with 0.5 mg/l NAA and 2.0 mg/l BAP.

Gymnema sylvestre is a perennial slow-growing woody medicinal herb. The major bioactive ingredient of this plant species, Gymnemic acid, is used to treat type II diabetes. Due to the intricacy and expense of propagating this plant, *in vitro* techniques were devised to induce callus and regenerate plantlets from numerous *G. sylverstre* explants. The best media for callus formation from leaf discs was discovered as an MS medium containing 5.0 mg/l 2,4-D. Even though the nodal segment raised on 1.0 mg/l BA supplied MS medium which had the highest shoot elongation (14.8), growth regulator-free MS also

exhibited a higher shoot elongation (14.2). When plantlets were acclimated in a medium including a 1:2 mixture of soil and sand, the best survival percentage (62.5) was achieved (Kaushalya and Senarath, 2014).

Effective callus initiation and plant regeneration technique has been regulated for an indigenous medicinal herb, *Elephanto pusscaber*. MS media given with 5.0 µM 2, 4-D, and 0.5 µM kinetin results in the optimum (89%) frequency of callus proliferation from seed explant. The data demonstrated that 6.0 µM BAP and 1.5 µM NAA supplied MS media had the greatest response of shoots per explant (56).

The most successful rooting of cultivated shoots was achieved on a half MS medium with 6.0 µM IBA. The clonal purity of regenerated plantlets was assessed by ISSR, and the banding patterns of callus-derived plants were having similar morphology to those of the mother plant, indicating that these plants were true-to-type (Abraham and Thomas, 2015).

Asteracantha longifolia is a therapeutic medicinal plant used to alleviate diabetes. The internodal explants cultured on 0.5 mg/l NAA and 0.25 mg/l BAP provided MS media improved the proportion of callus induction. The maximum percentage for shoot bud regeneration was reported in NAA 0.5 mg/l with BAP 2.0 mg/l. On the medium holding 0.5 mg/l of IBA, the largest percentage of shoot responsiveness for rooting and the considerable number of root developments per shoot were achieved. The survival rate (86.7%) of the *in vitro* grown plants was reported after 20 days of transplantation (Senthil Kumar and Nandi, 2015).

Leaves of *Cordia myxa* grown on 1.0 mg/l NAA and 1.5 mg/l BAP added MS medium efficiently induced callus with 94% frequency. The highest percentage acclimatization of plantlets reached 81% on a medium with one part of river sand to two parts of peat moos (Taha, 2016).

The tuberous root extract of Safed musli (*Chlorophytum borivilianum*) is used as an alternative to viagra, to cure diabetes, arthritis, chronic leucorrhoea, and delay menopause. Callus growth was better exhibited on MS media having 5 mg/l 2, 4-D. Differentiation of callus into shoots was noted on MS media with BAP or kinetin, whereas TDZ was unable to regrow any shoots. The rooted plantlets were effectively acclimatized and grown in soil resulting in 88.3% survivability (Nakasha et al., 2016).

Mun and Mun (2016) reported an efficient clonal mass growth of *Rheum coreanum* Nakai, a rare herb in Korea using rhizome *in vitro* culture. Induction of callus was efficiently obtained from rhizome explants on 2.4-D (0.2–0.3 mg/l) blended MS media. The MS medium containing BAP (2 mg/l) and NAA (0.2 mg/l) showed a larger number of shoots, roots, and plantlets.

An effective approach of shoot buds sprouting from nodal explants and indirect plant regeneration from *G. sylvestre* apical meristem-induced callus has been noticed by Isah (2019). Among the three growth hormones examined, BAP was found the most effective and 2.0 mg/l gave the efficient shoot development followed by TDZ and kinetin. The cultured micro shoots were forming roots on auxins supplied MS medium and rooted plantlets recorded an 87% survival rate.

Huang et al. (2020) reported a technique that is efficient and reproducible for *in vitro* regeneration by callus mediated organogenesis of *Neolamarkia cadamba* using hypocotyl and cotyledon. The most efficient media for bud initiation was revealed to be Douglas fir and sugar pine added medium with 22.7 µM TDZ and 0.27 µM NAA, with the largest bud-induction frequency and higher number of buds on hypocotyl and cotyledon. The regeneration plantlets adequately acclimatised in the greenhouse yielded above 95% survival rate.

CONCLUSION

In vitro culture of medicinal herbs has been noticed to be useful for large scale production of elite plants. It is achievable to develop disease-free healthy plants utilising tissue culture technologies for multiplication of superior and/or threatened genotypes of medicinal herbs, which might then be restored into their natural environment or grown on a vast scale for the pharmaceutical product. These are unique techniques of conserving medicinal plants in nature and lowering the probability of extinction. We endeavoured to highlight the notable and current research on plant tissue culture methodologies for *in vitro* bulk multiplication of medicinal plants.

CONFLICT OF INTEREST

The authors reported no possible conflicts of interest.

AUTHOR CONTRIBUTION

Jothi Kanmani Bharathi drafted the review and Muthu Arjuna Samy Prakash conceptualised and corrected the review.

REFERENCES

Abraham, J., & Thomas, T. D. (2015). Plant regeneration from organogenic callus and assessment of clonal fidelity in *Elephantopus scaber* Linn., an ethnomedicinal herb. *Physiology and Molecular Biology of Plants*, *21*(2), 269–277. https://doi.org/10.1007/s12298-015-0281-7

Agrawal, V., & Sardar, P. R. (2006). *In vitro* propagation of *Cassia angustifolia* through leaflet and cotyledon derived calli. *Biologia Plantarum*, *50*(1), 118–122. https://doi.org/10.1007/s10535-005-0084-8

Ahmed, A. B. A., Pallela, R., Rao, A. S., Rao, M. V., & Taha, R. M. (2011). Optimized conditions for callus induction, plant regeneration and alkaloids accumulation in stem and shoot tip explants of *Phyla nodiflora*. *Spanish Journal of Agricultural Research*, *4*, 1262–1270.

Amiri, S., Fotovat, R., Tarinejad, A. R., Panahi, B., & Mohammadi, S. A. (2019). *In vitro* regeneration of periwinkle (*Catharanthus roseus* L.) and fidelity analysis of regenerated plants with ISSR Markers. *Journal of Plant Physiology and Breeding*, *9*(1), 129–135.

Ashrafuzzaman, M., Haque, M. S., & Luna, L. N. (2008). *In vitro* clonal propagation of the neem tree (*Azadirachta indica* A. Juss.). *African Journal of Biotechnology*, *7*(4).

Bakar, D. A., Ahmed, B. A., & Taha, R. M. (2014). *In vitro* callus induction and plant regeneration of *Celosia argentea*-An important medicinal plant. *Braz. Arch. Biol. Technol.*, *57*(6), 860–866. https://dx.doi.org/10.1590/S1516-89132014026 11

Bakrudeen, A., & Arun, K. (2009). *In vitro* propagation of Monocot (*Costus pictus* D. Don) - an antidiabetic medicinal plant. *Journal of Agricultural Technology*, *5*, 361–369.

Bhowmik, S. S. Das, Basu, A., & Sahoo, L. (2016). Direct shoot organogenesis from rhizomes of medicinal zingiber *Alpinia calcarata* Rosc. and evaluation of genetic stability by RAPD and ISSR markers. *Journal of Crop Science and Biotechnology*, *19*(2), 157–165. https://doi.org/10.1007/s12892-015-0119-4

Cheruvathur, M. K., & Thomas, T. D. (2011). An efficient plant regeneration system through callus for *Pseudarthria viscida* (L.) Wright and Arn., a rare ethnomedicinal herb. *Physiology and Molecular Biology of Plants*, *17*(4), 395. https://doi.org/10.1007/s12298-011-0089-z

Chirangini, P., & Sharma, G. J. (2005). *In vitro* propagation and microrhizome induction in *Zingiber cassumunar* (Roxb.), an antioxidant-rich medicinal plant. *J. Food Agric. Environ*, *3*(1), 139–142.

Chukwujekwu, J. C., Fennell, C. W., & van Staden, J. (2002). Optimisation of the tissue culture protocol for the endangered *Aloe polyphylla*. *South African Journal of Botany*, *68*(4), 424–429. https://doi.org/https://doi.org/10.1016/S0254-6299(15)30368-9

Corral, P., Mallón, R., Rodríguez-Oubiña, J., & González, M. L. (2011). Multiple shoot induction and plant regeneration of the endangered species *Crepis novoana*. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *105*(2), 211–217. https://doi.org/10.1007/s11240-010-9854-x

Espinosa-Leal, C. A., Puente-Garza, C. A., & García-Lara, S. (2018). *In vitro* plant tissue culture: means for production of biological active compounds. *Planta*, *248*(1), 1–8. https://doi.org/10.1007/s00425-018-2910-1

He, S., Liu, C., & Saxena, P. K. (2007). Plant regeneration of an endangered medicinal plant *Hydrastis canadensis* L. *Scientia Horticulturae*, *113*(1), 82–86. https://doi.org/10.1016/j.scienta.2007.01.014

Huang, H., Wei, Y., Zhai, Y., Ouyang, K., Chen, X., & Bai, L. (2020). High frequency regeneration of plants via callus-mediated organogenesis from cotyledon and hypocotyl cultures in a multipurpose tropical tree (*Neolamarkia Cadamba*). *Scientific Reports*, *10*(1), 4558. https://doi.org/10.1038/s41598-020-61612-z

Isah, T. (2019). De novo *in vitro* shoot morphogenesis from shoot tip-induced callus cultures of *Gymnema sylvestre* (Retz.) R. Br. ex Sm. *Biological Research*, *52*.

Kaushalya, N. A. N., & Senarath, W. (2014). Callus induction and *in vitro* plantlet regeneration of *Gymnema sylvestre* R. Br. (Retz.) and the phytochemical screening of natural plants and callus cultures. *Plant Tissue Cult. & Biotech*, *23*(2), 210–210.

Kayalvizhi, T., & Venkatachalam, P. (2010). *In vitro* shoot multiplication from shoot tip explants of *Cephalandra indica* Naud: an important antidiabetic medicinal plant. *Plant Cell Biotechnology and Molecular Biology*, *11*(1/4), 37–42.

Khan, A., Sharma, V., & Shukla, R. K. (2017). Establishment of multiplication protocol for insulin plant (*Costus pictus*. D. Don) by using stem node as explants. *International Journal of Bio-Technology and Research*, *7*(1), 11–18.

Khateeb, W. Al, Kanaan, R., El-Elimat, T., Alu'datt, M., Lahham, J., & El-Oqlah, A. (2017). *In vitro* propagation, genetic stability, and secondary

metabolite analysis of wild lavender (*Lavandula coronopifolia* Poir.). *Horticulture, Environment, and Biotechnology*, *58*(4), 393–405. https://doi.org/10.1007/s13580-017-0342-7

Komalavalli, N., & Rao, M. V. (2000). *In vitro* micropropagation of *Gymnema sylvestre* – A multipurpose medicinal plant. *Plant Cell, Tissue and Organ Culture*, *61*(2), 97. https://doi.org/10.1023/A:1006421228598

Laha, S., & Paul, S. (2019). A therapeutic anti-diabetic herb with active phytoconstituents. *International Journal of Pharmaceutical Sciences and Research*, *10*(8), 3583–3591. https://doi.org/10.13040/IJPSR.0975-8232.10(8).3583-91

Mahesh, R., Muthuchelian, K., Maridass, M., & Raju, G. (2010). *In vitro* propagation of wild yam, *Dioscorea wightii* through nodal cultures. *Int J Biol Technol*, *1*(1), 111–113.

Malabadi, R., Mulgund, G., & Nataraja, K. (2005). Effect of triacontanol on the micropropagation of *Costus speciosus* (Koen.) SM. using rhizome thin sections. *In vitro Cellular and Developmental Biology - Plant*, *41*(2), 129–132. https://doi.org/10.1079/IVP2004586

Mun, S.-C., & Mun, G.-S. (2016). Development of an efficient callus proliferation system for *Rheum coreanum* Nakai, a rare medicinal plant growing in Democratic People's Republic of Korea. *Saudi Journal of Biological Sciences*, *23*(4), 488–494. https://doi.org/https://doi.org/10.1016/j.sjbs.2015.05.017

Musfir Mehaboob, V., Faizal, K., Thilip, C., Raja, P., Thiagu, G., Aslam, A., & Shajahan, A. (2019). Indirect somatic embryogenesis and agrobacterium-mediated transient transformation of ginger (*Zingiber officinale* Rosc.) using leaf sheath explants. *Journal of Horticultural Science and Biotechnology*, *94*(6), 753–760. https://doi.org/10.1080/ 14620316.2019.1624201

Nakasha, J. J., Sinniah, U. R., Kemat, N., & Mallappa, K. S. (2016). Induction, subculture cycle and regeneration of callus in safed musli (*Chlorophytum borivilianum*) using different types of phytohormones. *Pharmacognosy Magazine*, *12*(47), 460–464.

Naz, S., Ilyas, S., Javad, S., & Ali, A. (2009). *In vitro* clonal multiplication and acclimatization of different varieties of turmeric (*Curcuma Longa* L.). *Pakistan Journal of Botany*, *41*(6), 2807–2816.

Pal, A., & Roy, A. (1991). Embryo culture of *Costus speciosus* (Koen.) Sm. to regenerate variable diosgenin yielding clones. *Plant Cell Reports*, *10*(11), 565–568.

Pati, R., Chandra, R., Chauhan, U. K., Mishra, M., & Srivastava, N. (2008). *In vitro* clonal propagation of bael (*Aegle marmelos* Corr.) CV. CISH-B1 through enhanced axillary branching. *Physiology and Molecular Biology of Plants*, *14*(4), 337–346. https://doi.org/10.1007/s12298-008-0032-0

Phulwaria, M., Shekhawat, N. S., Rathore, J. S., & Singh, R. P. (2013). An efficient *in vitro* regeneration and ex vitro rooting of *Ceropegia bulbosa* Roxb.-a threatened and pharmaceutical important plant of Indian thar desert. *Industrial Crops and Products*, *42*, 25–29. https://doi.org/https://doi.org/10.1016/j.indcrop.2012.05.013

Punyarani, K., & Sharma, G. (2010). Micropropagation of *Costus speciosus* (Koen.) Sm. using nodal segment culture. *Notulae Scientia Biologicae*, *2*(1), 58–62. https://doi.org/10.15835/nsb213552

Radha, A., Balasubramanian, K., Shruti, B. S., & Nandhini, S. R. (2015). Studies on optimization of medium in induction and regeneration of callus and shoot from *Costus igneus* and its phytochemical profile. *Journal of Academia and Industrial Research (JAIR)*, *4*(2), 75.

Radha, R. K., Shereena, S. R., Divya, K., Krishnan, P. N., & Seeni, S. (2011). *In vitro* propagation of *Rubia cordifolia* Linn., a medicinal plant of the Western Ghats. *International Journal of Botany*, *7*(1), 90–96.

Rahiman, F. A., & Taha, R. M. (2011). Plant regeneration and induction of coloured callus from henna (*Lawsonia inermis* syn. *Lawsonia alba*). *Journal of Food Agriculture & Environment*, *9*(2), 397–399.

Rajasekharan, P. E., & Sahijram, L. (2015). *In vitro* conservation of plant germplasm. *Plant Biology and Biotechnology*, 417–443.

Rani, A., Kumar, M., & Kumar, S. (2016). Effect of phytohormones on shoot apex and leaf explants of *Withania somnifera* (Ashwagandha). *Journal of Applied and Natural Science*, *8*(1), 412–415.

Resmi, P., Shylaja, M. R., Nazeem, P. A., Valsala, P. A., & Nybe, E. V. (2005). Plantlet regeneration *in vitro* through indirect organogenesis in ginger (*Zingiber officinale* Rosc.). *Recent Trends in Horticultural Biotechnology, Vol. I and II. ICAE National Symposium on Biotechnological Interventions for Improvement of Horticultural Crops: Issues and Strategies, Vellanikkara, Kerala, India, 10-12 January, 2005*, 229–232.

Rout, G. R., Samantaray, S., & Das, P. (2000). *In vitro* manipulation and propagation of medicinal plants. *Biotechnology Advances*, *18*(2), 91–120.

https://doi.org/https://doi.org/10.1016/S0734-9750(99)00026-9

Rout, J. R., Mishra, M., Das, R., & Sahoo, S. L. (2009). *In vitro* micropropagation of *Abutilon indicum* L. through leaf explants. *Plant Tissue Culture and Biotechnology*, *19*(2), 177–184.

Roy, A., & Pal, A. (1995). Callus cultures from zygotic embryos of *Costus speciosus* and their morphogenetic responses. *Journal of Plant Biochemistry and Biotechnology*, *4*(1), 29–32. https://doi.org/10.1007/BF03262946

Sairam Reddy, P., Rodrigues, R., & Rajasekharan, R. (2001). Shoot organogenesis and mass propagation of *Coleus forskohlii* from leaf derived callus. *Plant Cell, Tissue and Organ Culture*, *66*(3), 183–188. https://doi.org/10.1023/A:1010697813852

Saxena, A., & Saxena, P. (2019). Synthetic Seeds: Relevance to endangered germplasm conservation *in vitro*. *Synthetic Seeds*, 21–60.

Senthil Kumar, M., & Nandi, S. C. (2015). High frequency plant regeneration with histological analysis of organogenic callus from internode explants of *Asteracantha longifolia* Nees. *Journal of Genetic Engineering and Biotechnology*, *13*(1), 31–37. https://doi.org/https://doi.org/10.1016/j.jgeb.2014.12.002

Sharma, S., Sharma, R., Sharma, P., Thakur, K., & Dutt, B. (2019). Direct shoot organogenesis from seedling derived shoot tip explants of endangered medicinal plant *Saussurea costus* (Falc.) Lipsch. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *89*(2), 755–764. https://doi.org/10.1007/s40011-018-0983-z

Shekhawat, M. S., & Manokari, M. (2016). *In vitro* regeneration frequency, micro-morphological studies and ex vitro rooting of *Hemidesmus indicus* (L.) R. Br.: a multi-potent endangered climber. *Indian Journal of Plant Physiology*, *21*(2), 151–160. https://doi.org/10.1007/s40502-016-0216-5

Singh, S., Purohit, V. K., Prasad, P., & Nautiyal, A. R. (2015). Micropropagation of *Valeriana wallichii* DC. (Indian Valerian) through nodes. *Indian Journal of Biotechnology*, *14*, 127–130.

Sivakumar, P., Ashokkumar, K., Rajalakshmi, K., Sangeetha, R., & Muthumalai, M. (2019). A review on in vitro propagation of miraculous physician *Aloe vera* (L.). *Research Journal of Biotechnology*, *14*(9), 126-130.

Sivakumar, P., Bavithra, V. S., Ashokkumar, K., Deepadharsini, R., Selvaraj, K. S. V., Gopal & M. R. (2021). Comprehensive review on phytochemistry and in vitro biotechnology of *Coleus forskohlii*. *Journal of Pharmacognosy and Phytochemistry, 10*(1)*,* 448-453.

Sivakumar, P., Pavithra, N., Tejashwini, B., Vineeta, M. E., Vijai Selvaraj, K. S., & Logeswaran, J. (2020). Effect of growth regulators in in vitro propagation of ixora spp.: an short review. *Journal of Current Opinion in Crop Science*, *1*(1), 31-34.

Taha, A. (2016). Callus induction and plant regeneration of *Cordia myxa* L. via tissue culture culture system. *International Journal of Pharma and Bio Sciences*, *4*, 18–29.

Uchendu, E. E., Paliyath, G., Brown, D. C. W., & Saxena, P. K. (2011). *In vitro* propagation of North American ginseng (*Panax quinquefolius* L.). *In vitro Cellular & Developmental Biology - Plant*, *47*(6), 710–718. https://doi.org/10.1007/s11627-011-9379-y

Vani, P., Kistamma, S., Reddy, K. S., Reddy, A. N., & Mohan, C. H. (2016). Antibacterial activity, antioxidant activity and micropropagation of *Gymnema sylvestre* R. Br. a valuable medicinal plant. *Journal of Pharmacognosy and Phytochemistry*, *5*(2), 207.

Widoretno, W., Fauziah, A., Indriyani, A., & Utomo, E. P. (2017). Clonal propagation of *Vetiveria zizanioides* L. through tissue culture technique. *Indonesian J. Essent. Oil*, *2*(1), 38–44.

Zhang, S., Liu, N., Sheng, A., Ma, G., & Wu, G. (2011a). Direct and callus-mediated regeneration of *Curcuma soloensis* Valeton (Zingiberaceae) and ex vitro performance of regenerated plants. *Scientia Horticulturae*, *130*(4), 899–905. https://doi.org/10.1016/j.scienta.2011.08.038

Zhang, S., Liu, N., Sheng, A., Ma, G., & Wu, G. (2011b). *In vitro* plant regeneration from organogenic callus of *Curcuma kwangsiensis* Lindl. (Zingiberaceae). *Plant Growth Regulation*, *64*(2), 141–145. https://doi.org/10.1007/s10725-010-9548-8