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

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP3: 246-251

Chandana BC
Assistant Professor (Contract), Department of Crop Improvement and Biotechnology, COH, Mudigere, Karnataka, India

Nagaveni HC
Assistant Professor (Contract), Department of Soil Science and Agricultural Chemistry, COH, Mudigere, Karnataka, India

Kumari
Assistant Professor (Contract), Department of Plant Pathology, COH, Mudigere, Karnataka, India

Lakshmana D
Profesor and Head, Department of Crop Improvement and Biotechnology, COH, Mudigere, Karnataka, India

Shashikala S Kolakar
Assistant Professor, Department Of Crop Improvement and Biotechnology, COH, Mudigere, Karnataka, India

Heena MS
Assistant Professor, KVK, Indi, Bijapur, Karnataka, India

Correspondence
Chandana BC
Assistant Professor (Contract), Department of Crop Improvement and Biotechnology, COH, Mudigere, Karnataka, India

National conference on "Conservation, Cultivation and Utilization of medicinal and Aromatic plants"
(College of Horticulture, Mudigere Karnataka, 2018)

Role of plant tissue culture in micropropagation, secondary metabolites production and conservation of some endangered medicinal crops

Chandana BC, Nagaveni HC, Kumari, Lakshmana D, Shashikala S Kolakar and Heena MS

Abstract
Tissue culture technology is potent and has opened extensive areas of research for micropropogation, secondary metabolite production and biodiversity conservation. Plant *in vitro* regeneration is a biotechnological tool that offers a tremendous potential solution for the propagation of endangered and superior genotypes of medicinal plants which could be released to their natural habitat or cultivated on a large scale for the pharmaceutical product of interest. Tissue culture protocols have been developed for a wide range of medicinal plants, which includes endangered, rare and threatened plant species. A review highlighting various *in vitro* protocols developed for some of the endangered medicinal species has been done to highlight the significance of plant tissue cuture in cases where regeneration through conventional methods is difficult to undertake and species are left with low population in the wild. Thus *in vitro* cell and tissue culture methodology is envisaged as a mean for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large scale re-vegetation and for enhancing the production of secondary metabolites.

Keywords: *In vitro* culture, micropropogation, secondary metabolites, *in vitro* conservation

Introduction
Medicinal plants have been the subjects of man's curiosity since time immemorial (Constable, 1990) [12]. Almost every civilization has a history of medicinal plant use (Ensminger *et al.*, 1983) [15]. Plants are the tremendous source of a wide range of secondary metabolites, which are used as base materials in pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides and food additives. Alkaloids, carbon compounds, hydrogen, nitrogen, glycosides, essential oils, fatty oils, resins, mucilage, tannins and gums are some of the important chemical substances found in plants. Most of these substances are potent bioactive compounds which can be used for therapeutic purpose or which act as the precursors for the synthesis of useful drugs (Sofowora, 1993) [42].

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Approximately 80% of the people in the developing countries rely on traditional medicine for their primary health care needs, and about 85% of traditional medicine involves the use of plant extracts (Vieira and Skorupa, 1993) [46]. India has 2.4% of world's area with 8% of global biodiversity and it is one of the 12th mega diversity hotspot countries of the world with a rich diversity of biotic resources. India is richly endowed with a wide variety of plants having medicinal value. About 2500 plant species belonging to more than 1000 genera are used by traditional healers and about 500 plant species are utilized by 159 different pharmaceutical companies**.**

With the ever increasing global inclination towards herbal medicine there is an obligatory demand for huge raw material of medical plants. The genetic biodiversity of traditional medicinal plants is under a continuous threat due to over exploitation environment, unfriendly harvesting, loss of growth habitat and unmonitored trade of medicinal plants. It is increasingly difficult to acquire plant-derived compounds with the rapid increase in population and extreme pressure on the available cultivable land. The harvest of medicinal plants on a mass scale from

their natural habitats, is leading to a depletion of plant resources. Therefore, there is an urgent need to adopt an alternate means of production and conservation, which could ensure large-scale and high quality plant materials as well as conserve them to fulfill the growing demand (Lange, 2004) [23].

Plant tissue culture with its rapidness and apparently limitless potential offers new and exciting opportunities to address the myriad problems in the field of medicinal plant cultivation, exploitation and conservation. Some of the important applications of biotechnology in medicinal plants are rapid clonal propagation to generate good quality planting material, exploiting somoclonal variation and genetic engineering technique for the crop improvement, *invitro* selection for resistance to biotic and abiotic stress, *in vitro* conservation and safe exchange of germplasm and secondary metabolite production.

Micropropagation

Conventional cultivation is affected by various environmental and climatic factors, but micropropagation insures a good regular supply of medicinal plants, using minimum space and time. Micropropagation is the process of vegetative growth and multiplication from plants tissues or seeds in aseptic and favourable conditions on artificial growth media. Tissue culture is based on concept of totipotency; the ability of plant cells and tissues to develop into whole new plant. Gottlieb Haberlandt, a German botanist is considered as the father of plant tissue culture, was the first to get the experimental evidence on totipotency by culturing plant cells on Knop's salt solution in 1898 (Krikorian and Berquam, 1969) [20]. Tissue culture success mainly depends on the genotype, age, types and position of explants in the mother plant. The advantages of *In vitro* micro propagation of medicinal plant are listed below:

- Rapid clonal propagation and multiplication.
- Controlled and altered environmental conditions aids to meet specific needs of the plant.
- Availability of planting material throughout the year irrespective of seasonal and regional variation
- Identification and production of clones with desired characteristics (somoclonal variantion).
- Production of secondary metabolites.
- Conservation of threatened plant species.
- Preservation of genetic material by cryopreservation.

Micropropagation of medicinal plants remained neglected till a miracle drug plant of India, *Rauvolfia serpentina* (L.) Benth plants were produced from its somatic callus tissue. Presently there are reports on organogenic differentiation in somatic callus tissue of medicinal plants, but there are only few reports on endangered medical plants, where complete plants have been regenerated *in vitro*.

Explant source

Explant is a material used as initial source of tissue culture. Tissue culture success mainly depends on the genotype, age, types and position of explants because ability to express totipotency differs among the plant cells. The most commonly used explants in medicinal plants are shoot tips, nodal segments, rhizome, seeds and root tips (Table 1).

Table 1: List of the some endangered and economically important medicinal plants of India

Plant species	Explants	Plant species	Explants
Aegle marmelos	Nodal segements and shoot tip	*Peganum harmala*	Seeds
Acorus calamus	Rhizome tip and sements	*Prosopis cineraria*	Seeds
Celastrus paniculatus	Seeds, nodal segments and shoot tip	*Glycyrrhiza glabra*	Nodal segements
Commiphora mukul	Leaf segements, apical and nodal segments	*Gymnema sylvestre*	Seeds
Bacopa monnieri	Leaf and nodal segments	*Holostemma ada-kodien*	Nodal segements
Ginkgo biloba	Apical and nodal segments	*Oroxylum indicum*	Nodal segements
Simmondsia chinensis	Nodal segements	*Picrorhiza kurroa*	Nodal segements
Spilanthes acmella	Nodal and internodal segments	*Saussurea lappa*	Shoot tip
Stevia rebaudiana	Apical and nodal segments	*Swertia chirata*	Shoot tip
Sapindus mukorossi	Leaf segements, apical and nodal segments	*Tinospora cordifolia*	Nodal segements

The highest frequency (73%) of shoot organogenesis was observed when rhizome was taken as the explant in case of *Acorus calamus* along with the treatment of IAA-BAP (Bhagat, 2011) [10]. First node (shoot tip) of *Commiphora wightii* performed better on BAP, IBA and GA3 supplemented medium (Tejoyathi *et al.*, 2011) [44]. The juvenile leaf explants of *Rauvolfia serpentine* responded efficiently in invitro regeneration (Singh *et al.* 2009) [41]. In *Thymus hyemalis* Lange maximum proliferation was observed for nodal explants (Nordine *et al.*, 2013) [29]. The Apical meristems was used as the explants in *Psoralea corylifolia* Linn (Pandey *et al.*, 2013) [31]. Successful initiation of tissue culture of *Saussurea esthonica* was achieved from seeds (Gailīte *et al.* 2010) [17]. Multiple shoots were obtained from the shoot apices on MS medium in *Curculigo orchioides* Gaertn. (Wala and Jasrai, 2003) [47]. Explants were prepared from stem in Tashnedari (*Scrophularia striata*) which gave better results (Lalabadi *et al.*, 2014) [22]. Trimmed shoot segments having two nodes of *Thymus bleicherianus* Pomel were used for *in vitro* culture establishment (Aicha and Abdelmalek, 2014) [1]. Maximum callus induction (100%) was achieved from internode explants of *Thymus persicus* cultured on MS medium (Bakhtiar *et al.* 2016) [7].

Tissue culture Media and growth regulators

Culture media contains vital nutrients and elements for *in vitro* growth of plant tissues. A wide range of media are available for plant tissue culture, but Murashige and Skoog (MS) medium is commonly used. Medium contains a carbon source, macro and micro nutrients, vitamins, growth regulators and other organic substances. Growth hormones regulate various physiological and morphological processes in plants and are also known as plant growth regulators (PGRs) or phytohormones. PGRs are synthesized by plants; therefore many plant species can grow successfully without external medium supplements. Hormones can also be added into cultures to improve plant growth and to enhance metabolite synthesis.

Multiple shoots were obtained from the meristem tip culture of *Curculigo orchioides* Gaertn. On MS medium supplemented with BA. The shoots were rooted either on half strength of MS basal medium or on the one supplemented

with NAA (Wala and Jasrai, 2003) [47]. Shoot regeneration in *Rauvolfia serpentina* was highest (75%) in BAP +IAA and usage of GA_3 provided better result for elongation of shoot. The frequency of root regeneration was 100% in MS Medium containing BAP (2.5) + IAA (0.5) + NAA (0.5) mg/l (Singh *et al.* 2009) [41]. The best results on *Saussurea esthonica* shoot proliferation were obtained using 6-benzilaminopurine and addition of 6-(γ,γ-dimethylallylamino) purine which promoted shoot elongation and root formation, but it was not effective on multiplication. The multiplication rate using kinetin was invariable without production of tissue swelling (Gailīte *et al.* 2010) [17].

The *Acorus calamus* explant cultured on M.S Media supplemented with (0.5-2mg/lit) IAA-BAP yielded 73% response, and 30% response in treatment with (0.5-1mg/lit) IAA-BAP, 18% response in any pytohormone (Bhagat, 2011) [10]. Highest Shoot regeneration (95%) results of *Psoralea corylifolia* were obtained on MS medium containing BAP with NAA and KN in. BAP was found to be best for shoot multiplication (Pandey *et al.* 2013) [31]. A high number of shoot was obtained on MS medium supplemented with 6-benzylaminopurine in *Thymus bleicherianus* Pomel (Aicha and Abdelmalek, 2014) [1].

Proliferation of *Rauvolfia serpentina* shoots was achieved when cultured on MS medium supplemented with thidiazuron (TDZ) (0.1_2.5 mmol/L) although with low regeneration response and few number of shoots per explant. Greater number of shoots was achieved from nodal explants pretreated with higher concentrations of TDZ (5_100 mmol/L) in liquid MS medium for different time periods (4, 8, 12 and 16 days) (Alatar AA, 2015) [2]. Maximum callus induction (100%) was achieved when *Thymus persicus* cultured on MS medium was fortified with NAA and KN. The highest frequency of shoot multiplication (96%) was observed with BAP and NAA. The maximum number of rootlets was induced on half-strength MS medium with IBA (Bakhtiar *et al.* 2016) [7].

Regeneration of endangered medicinal plants through somatic embryogenesis

Somatic embryogenesis is a process by which a non zygotic embryo is produced from plant tissue or cell, which can develop into a new plant (Zimmerman, J.L. 1993; Razdan, M.K. 2003) [48, 35]. Formation of somatic embryos occurs in two steps, first callus is cultured onto auxin rich medium (2,4-D commonly used) forming embryogenic clumps, these clumps are then transferred into medium without auxins resulting in the formation of mature embryos (Razdan, M.K. 2003) [35]. Somatic embryogenesis offers an alternative and efficient protocol for plant regeneration. This technique has also contributed for the genetic, morphological and physiological manipulation (Sharma *et al.*, 2010a) [36].

Somatic embryos were induced from hypocotyls segments of *Pimpinella tirupatiensis.* On MS + TDZ (1 mg/l) + BA (1 mg/l), somatic embryo formation was enhanced. Embryos isolated and germinated in the presence of MS + TDZ (1.0 mg/l) and GA_3 (1.0 mg/l) which showed normal flowering without any morphological variation on transplantation to soil (Prakash *et al.*, 2001) [32].

A first attempt on *in vitro* regeneration of *Psoralea corylifolia* L. *via* direct somatic embryogenesis was done by Faizal and his coworkers (Faisal M., *et al.*, 2008) [16]. The frequency of somatic embryogenesis was strongly influenced by the concentration of thidiazuron (TDZ) in the medium. The highest frequency of somatic embryogenesis was observed in MS medium containing 16 µM TDZ. The generated plantlets were successfully established in ex vitro condition with 90% survival.

The plant regeneration through indirect somatic embryogenesis was attempted from leaf-derived callus of *Calotropis procera* and the significant result was obtained. Somatic embryos at the highest frequency were induced in MS medium supplemented with Kinetin and 2,4- dichloro phenoxy acetic acid. High cytokinin concentration is required for the germination of these somatic emryos (Sundaram *et al.* 2011) [43]. Embryogenic callus were induced from *Kaempferia rotunda* Linn rhizomes on MS solid medium supplemented with 2.5mg/L 2,4-D and 0.5mg/L BAP. This embryonic callus was developed in to embryos on MS medium containing 0.25mg/L 2,4-D and 3.0mg/L BAP. Further plant regeneration was observed on MS medium supplemented with 5.0mg/L BAP (Mustafa anand, 2014) [28]. Plant regeneration via somatic embryogenesis was achieved from leaf and internode derived callus of *Wedelia calendulacea*. Transfer of embryogenic callus on a reduced concentration of 2,4-D facilitated somatic embryo development. Somatic embryos later germinated into plantlets upon transfer on MS medium containing 1.0 mg L^{-1} BAP plus 0.5 mg L^{-1} GA_3 (Sharmin, *et al.* 2014) [39].

A protocol for induction of direct somatic embryogenesis and subsequent plant regeneration for *Malaxis densiflora* has been developed. The best response was observed in seed derived protocorms cultured on half strength MS medium supplemented with 2,4-D at 3.39 µM and TDZ at 6.80 µM producing a maximum embryoids per explants. Complete plantlets were formed after 60 days of culture (Mahendran and Narmatha Bai, 2016) [24].

Secondary Metabolites in Plant cell and tissue cultures:

During metabolism plants produce enormous number of compounds as part of defense mechanism (Bennett and Wallsgrrove, 1994) [9]. These compounds do not play essential role like primary metabolites, hence they are called secondary metabolites. With the rapid increase in world population, extreme pressure on the available cultivable land, there is fast disappearance of natural habitats for medicinal plants together with environmental and geopolitical instabilities; it has become increasingly difficult to acquire plant-derived compounds (Mulabagal and Tsay, 2004) [26].

In vitro tissue culture offers an effective and potential alternative of production of bioactive compound because the amount of secondary metabolites produced in this technique can be even higher than in parent plants (Rao *et al.*, 2002) [34]. Amount of secondary metabolites was found significantly higher, in *in vitro* plantlets and callus compared to *in vivo* plantlets of *Swertia chirayita*. Higher heavy metal accumulation in *in vitro* as compared to *in vivo* plantlets correlated higher secondary metabolite production supporting that they play regulatory role in influencing the plant secondary metabolism (Kumar *et al.* 2014) [21].

An efficient and reproducible method for the induction of callus and hairy roots from explants of *Rauwolfia serpentina* is standardized to produce secondary metabolites *in vitro*. Hairy roots were induced from leaf explants and these leaf explants were infected with *Agrobacterium rhizogenes* to induce hairy roots for the production of secondary metabolites in large scale (Shetty *et al.* 2014) [40].

Plant cell cultures are an attractive alternative source to whole plant for the production of secondary metabolites. Plant cell cultures have the following advantages over conventional

agricultural production *viz.*, independent of geographical and environmental variations, defined production system with continuous supply of products, uniform quality, yield, rapidity of production and production of novel compounds. In recent years, various plant cell culture systems have been exploited for the enhancement of high value metabolites (Rao *et al.*, 2002) [34].

Homogenous cell suspension culture of *Celastrus paniculatus*, was established and multifold production of secondary metabolites like alkaloids and total phenols was obtained under the influence of monochromatic lights under *in vitro* condition (Billore *et al.*, 2016) [11]. The unorganized callus and cell suspension cultures contained fewer amounts of all phenolic compounds than redifferentiated shoots. The capacity of cell suspension culture to accumulate phenolics was enhanced after the application of salicylic acid and yeast extract thus provide a chance to improve yield (Owis *et al*., 2016) [30].

Jasmonic acid has been used to modulate the production of various secondary metabolites in plant tissue culture techniques. Jasmonic acid (JA) is most effective in eliciting total phenolic production of callus cell suspension cultures of *Celastrus paniculatus*. The total phenolics production was maximum when 250 µM, 250 µM and 50 µM of jasmonic acid was treated for 24, 48 and 72 hours respectively. Salicylic acid (SA) has also been used to enhance *in vitro* regeneration in several plant species. The total phenolics production was maximum when 100 µM, 250 µM and 50 µM of Salicylic acid was treated for 24, 48 and 72 hours respectively (Anusha *et al.* 2016) [4].

Among copper salts, copper sulphate and copper chloride were successfully used as abiotic elicitors in number of plant cell culture. The total phenolics production was maximum when 250 µM, 100 µM and 250 µM of CuSO4 was treated for 24, 48 and 72 hours respectively (Kasparova M. *et al.* 2008) [19].

In vitro conservation of endangered medicinal plants

Medicinal plants are under real jeopardy due to the uncontrolled collection and destruction of habitat, population growth, urbanization and the over harvesting of medicinal plants from the wild that ends with overexploitation of these natural resources (Al- Quran, 2011) [3]. More than 70% of the plant collections involve destructive harvesting which comprises a definite threat to these valuable genetic stocks. Moreover, the increase in demand for medicinal and aromatic plants is expected to continue threatening many species, taking in account 90% of medicinal plants that are used by industries are collected from the wild while 20 species of plants are under commercial cultivation (Shamra *et al.*, 2010b) [37]. Micropropogation, slow or reduced growth cultures and cryopreservation are some of commonly used *in vitro* techniques under *exsitu* conservation method.

Conservation through cryopreservation

Cryopreservation is long-term conservation method in liquid nitrogen (–196 °C) in which cell division and metabolic and biochemical processes are arrested. A large number of cultured materials can be stored in liquid nitrogen. Since whole plants can regenerate from frozen culture, cryopreservation provides an opportunity for conservation of endangered medicinal plants. For example, low temperature storage has been reported to be effective for cell cultures of medicinal and alkaloid producing plants such as *Rauvollfia serpentine, D. lanalta, A. belladonna, Hyoscyamus* spp (Bajaj YPS, 1988) [6].

Conservation through slow growth cutures

Slow growth is one of the major tissue culture techniques used for storage of *in vitro* grown plants by restrict growth of *in vitro* materials and to increase the intervals between subcultures by various means, i.e. reduced temperature in the presence of growth retardants, reduction in sucrose concentrations and/or addition of osmotically active additives (i.e. sorbitol and mannitol) under either low light intensity or complete darkness and reduction of the chemical compounds of the nutrient substance. The slow growth technique was applied to conserve germplasm of different plant species, i.e. *Garcinia indica* (Malik *et al.*, 2005) [25], *Glycyrrhiza glabra* L. (Uzundzhalieva *et al.*, 2014) [45] etc.

In vitro conservation of *Coleus forskohlli* Briq. by slow growth technique was achieved by employing osmotic regulators (sorbitol and mannitol). 3 M mannitol showed best performance for reduced or slow growth of the culture (Dube *et al.*, 2011) [14]. *In vitro* conservation using full strength MS salts with low concentrations of sucrose and sorbitol as a carbon source and osmotic agent, respectively, is a suitable slow growth medium for *in vitro* conservation of *Lavandula dentata* L and *Rhazya stricta* Decne (Attia *et al.* 2017) [5].

Synthetic seed (encapsulation) technology

The encapsulation technology can be considered as a promising approach that can be used for the exchange of plant materials between public and private plant tissue culture laboratories, and also to achieve germplasm conservation and the propagules that are derived from *in vitro* or by micropropagation applied directly in nurseries or in a field. The demand for artificial seed technology started after the discovery of somatic embryo production in various plant species *in vitro*. Artificial seeds (synseeds/ synthetic seeds), were firstly described by Murashige (Murashige, 1977) [27]. Artificial seeds are artificially encapsulated somatic embryos (usually) or other vegetative parts such as shoot buds, cell aggregates, auxiliary buds, or any other micropropagules which can be sown as a seed and converted into a plant under *in vitro* or in vivo conditions under disease and pest free environment. However, under the normal culture conditions, continuous growth of cultures is there induced by exogenous plant growth regulators and cultures are also subjected to frequent subcultures for long durations; may induce off types via somaclonal variations. Therefore, growth of *in vitro* cultures must be checked for storage of germplasm to overcome the risk of off type production and it is economic too in comparison to maintain fast growing cultures.

Synseeds of *Spilanthes mauritiana* produced by encapsulating axillary buds in calcium alginate gel exhibited a critical response to nutrient concentration for conservation into complete plant. Almost all the synseeds sprouted well and developed into plantlets when cultured on nutritional media after storage upto three weeks (Sharma *et al.*, 2009) [38].

Shoot tip and nodal segments of *Mimosa pudica* L. obtained from *in vitro* grown plants were encapsulated with sodium alginate solution followed by subsequent immersion in $CaCl_2$ solution which were later subjected to two storage temperature regimes at 4 ^{0}C and 0 ^{0}C and germination rate was examined which indicated that synthetic seed could be stored at 4 ^{0}C for 60 days without loss of viability (Banu *et al.*, 2014) [8].

In vitro rhizome production, encapsulation and cold storage of *Acorus calamus* were attempted for its propagation and 'true-

to-type' conservation. Synthetic seeds were produced from regenerated microtubers by encapsulation in calcium alginate beads. These synthetic seeds were stored in complete darkness at 10 ^{0}C temperature for different durations for mid-term conservation. After cold storage, synthetic seeds were re-cultured *in vitro*, 100% survival was recorded after the storage of 1, 3 or 6 months; and 80% survival was observed after the storage of 12 months (Quraishi, *et al.*, 2017) [33].

The single bead alginate-encapsulation for interim storing and conversion of *Tylophora indica* (Burm. Fil.) Merrill was done using nodal segments ensuing sphere shaped artificial seeds through 75 mmolL-1 $CaCl_2$-$2H_2O$ and Na-alginate with 93.3% conservation frequency. The conversion frequency after 30 days of storage at (15 $\pm$ 1) °C was recorded at 70% without further decline even following 45 days of storage, which evidently suggests that lower temperature (15 $\pm$ 1) °C is appropriate for storage and subsequent conversion of *T. indica* artificial seeds (Gantait, *et al.* 2017) [18].

Conclusion

Medicinal plants are the most important source of life saving drugs for the majority of the world's population. The biotechnological tools are important to select, multiply and conserve the critical genotypes of medicinal plants. In-vitro regeneration holds tremendous potential for the production of high-quality plant-based medicine. Thousands of plants are facing extinction risk due to extensive exploitation, habitat loss, deforestation and lack of conservation knowledge. Requirement of herbal products in the global market is ever increasing, and remarkably, the mass of plant material is still harvested. *In vitro* regeneration and conservation of endangered medicinal plants is pave the way for production of pharmacologically active substances. Tissue culture protocols have been developed for *in vitro* regeneration and conservation of several endangered medicinal plants but there are many other endangered and vulnerable species, which are over exploited in pharmaceutical industries and needs conservation.

References

1. Aicha N, Abdelmalek EM. Rapid *in vitro* regeneration and clonal multiplication of *thymus bleicherianus* pomel, a rare and threatened medicinal and aromatic plant in Morocco, *Med Aromat Plants*. 2014; 3(1):145-149.
2. Alatar AA. Thidiazuron induced efficient *in vitro* multiplication and ex vitro conservation of *Rauvolfia serpentine* - a potent antihypertensive drug producing plant, *Biotechnology & Biotechnological Equipment*. 2015; 29(3):489-497.
3. Al-Quran S. Conservation of medicinal plants in Ajlun woodland / Jordan. Journal of Medicinal Plants *Research*. 2011; 5(24):5857-5862.
4. Anusha TS, Joseph MV, Elyas KK. Callus Induction and Elicitation of Total Phenolics in Callus Cell Suspension Culture of *Celastrus paniculatus* – willd, an Endangered Medicinal Plant in India. Pharmacogn. J. 2016; 8(5):471-475.
5. Attia AO, Dessoky EDS, Al-Sodany YM, Ismail IA. Ex situ preservation for some endemic and rare medicinal plants in Taif, KSA. Biotechnology & Biotechnological Equipment. 2017; 31(5):912-920.
6. Bajaj YPS. Cryopreservation and the retention of biosynthetic potential in cell cultures of medicinal and alkaloid-producing plants. In: Bajaj YPS (ed). Biotechnology in Agriculture and Forestry, *Medicinal and Aromatic Plants I, Berlin: Springer- Verlag*, 1988; 4.
7. Bakhtiar Z, Mirjalili MH, Sonboli A. *In vitro* callus induction and micropropagation of *Thymus persicus* (Lamiaceae), an endangered medicinal plant, Crop Breeding and Applied Biotechnology. 2016; 16:48-54.
8. Banu LA, Rashid MHO, Miah MAB. Development of artificial seed and preservation in *mimosa pudica* l., an important medicinal plant in Bangladesh. J bio-sci. 2014; 22:89-99.
9. Bennett RN, Wallsgrove RM. Secondary metabolites in Plant Defence mechanisms. *New Phytologist.* 1994; 127:617-633.
10. Bhagat N. Conservation of endangered medicinal plant (*Acorus calamus*) through plant tissue culture. Journal of Pharmacognosy. 2011; 2(1):21-24.
11. Billore V, Khatediya L, Jain M. Sink - Source System of *In vitro* Suspension Culture of *Celastrus paniculatus* under Regulation of Monochromatic Lights, Plant Tissue Cult. & Biotech. 2016; 26(1):175-185.
12. Constable F. Medicinal plant biotechnology. Planta Med. 1990; 56:421-425.
13. Dornenburg H, Knorr D. Challenges and opportunities for metabolite production from plant cell and tissue cultures, Food Technology. 1997; 51:47-54.
14. Dube P, Gangopadhyay M, Dewanjee S, Ali MN. Establishment of a rapid multiplication protocol of *Coleus forskohlli* Briq. and invitro conservation by reduced growth. Indian journal of biotechnology. 2011; 10:228-231.
15. Ensminger AH, Ensminger ME, Konlande JE, Robson JRK. Food & Nutrition Encyclopedia. Pegus Press, Clovis, California, U.S.A. 1983; 2:1427-1441.
16. Faisal M, Shahzad A, Anis M. Somatic embryogenesis and plant regeneration from nodal explants in *Psoralea corylifolia* L. International Journal of plant developmental biology. 2008; 2(2):111-113.
17. Gailīte A, Kļaviņa D, Ievinsh G. *In vitro* propagation of an endangered plant *Saussurea esthonica*, Environmental and Experimental Biology. 2010; 8:43-48.
18. Gantait S, Vijayan J, Majee A. Artificial seed production of *Tylophora indica* for interim storing and swapping of germplasm. Horticultural Plant Journal. 2017; 3(1):41-46.
19. Kasparova M, Siatka T, Dusek J. Biotic elicitation of the *Trifolium pratense* L. suspension culture, *Ceska a Slovenska Farmacie*, 2008; 57(3):107-110.
20. Krikorian AD, Berquam DL. Plant cell and tissue cultures: The role of Haberlandt. *The Botanical Review*, 1969; 35:59-67.
21. Kumar V, Singh SK, Bandopadhyay R, Sharma MM, Chandra S. *In vitro* Organogenesis Secondary Metabolite Production and Heavy Metal Analysis in *Swertia chirayita*. Cent. Eur. J. Biol. 2014; 9(7):686-698.
22. Lalabadi MA, Jelodar NAB, Bagheri NA. Micro-propagation of Tashnedari Endangered Medicinal Plant *invitro* culture, Bull. Env. Pharmacol. Life Sci. 2014; 3:254-257.
23. Lange D. The German foreign trade in medicinal and aromatic plants during the 1990s (Newsletter of the IUCN species survival commission) Medicinal Plant Conservation. 2004; 9(10):38-46.
24. Mahendran G, Narmatha Bai V. Direct somatic embryogenesis of *Malaxis densiflora* (A. Rich.) Kuntze. J Genet Eng Biotechnol. 2016; 14:77-81.
25. Malik SK, Chaudhary R, Kalia RJ. Rapid *in vitro* multiplication and conservation of *Garcinia indica*: a

tropical medicinal tree species. *Sci Hortic*. 2005; 106:539-553.
26. Mulabagal V, Tsay HS. Plant cell cultures – An alternative and efficient source for the production of biologically important secondary metabolites. International Journal of Applied Science and Engineering. 2004; 2(1):29-48.
27. Murashige T. Plant cell and organ cultures as horticultural practices. In Proceedings of the Symposium on Tissue Culture for Horticultural Purposes, Ghent, Belgium, 1977.
28. Mustafaanand PH. *In-vitro* plant regeneration in *Kaempferia rotunda* Linn. Through somatic embryogenesis - a rare medicinal plant, Int. J Curr. Microbiol. App. Sci. 2014; 3(9):409-414.
29. Nordine A, Bousta D, Khanchoufi AE, Meskaoui AE. An efficient and rapid *in vitro* propagation system of *Thymus hyemalis* lange, a wild medicinal and aromatic plant of mediterranean region. Int. J Pharm. Biosci. Technol. 2013; 1(3):118-129.
30. Owis AI, Abdelwahab NS, Abul-Soad AA. Elicitation of Phenolics from the Micropropagated Endangered Medicinal Plant *Calligonum polygonoides* L. (Polygonoaceae). *Pharmacogn Mag*. 2016; 12(4):465-470.
31. Pandey P, Mehta R, Upadhyay R. *In-vitro* propagation of an endangered medicinal plant *psoralea corylifolia* Linn. Asian J Pharm Clin Res. 2013; 6(3):115-118.
32. Prakash E, Khan SV, Meru E, Rao KR. Somatic embryogenesis in *Pimpinella tirupatiensis* Bal. and Subr., an endangered medicinal plant of Tirumala hills, *Current science.* 2001; 81(9):1239-1242.
33. Quraishi A, Mehar S, Sahu D, Jadhav SK. *In vitro* Mid-Term Conservation of *Acorus calamus* L. *via* Cold Storage of Encapsulated Microrhizome, Braz. Arch. Biol. Technol. 2017; 60:e17160378.
34. Rao SR. Ramachandra, Ravishankar GA. Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnology Advances.* 2002; 20:101-153.
35. Razdan MK. Introduction to Plant Tissue Culture. 2nd Edition, Science Publishers, Inc., New Hampshire. 2003, 132.
36. Sharma MM, Ali DJ, Batra A. Plant regeneration through *in vitro* somatic embryogenesis in Ashwagandha (*Withania somnifera* L. Dunal), *Res*. 2010a; 2(3):1-6.
37. Sharma S, Rathi N, Kamal B, Pundir D, Kaur B, Arya S. Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology- a review. Agriculture and Biology Journal of North America. 2010b; 1(5):827-833.
38. Sharma S, Shahzad A, Jan N, Sahai A. *In vitro* studies on shoot regeneration through various explants and alginate-encapsulated nodal segments of *Spilanthes mauritiana* DC., an endangered medicinal herb. International journal of plant development biology. 2009; 3(1):56-61.
39. Sharmin SA, Alam MJ, Sheikh MMI, Sarker KK, Khalekuzzaman M, Haque MA, *et al*. Somatic Embryogenesis and Plant Regeneration in *Wedelia calendulacea* Less. An Endangered Medicinal Plant, Braz. Arch. Biol. Technol. 2014; 57(3):394-401.
40. Shetty MR, Harisha GA, Jayanth Y, Kumar HGA. Production of secondary metabolites from *invitro* cultures of *Rauwolfia serpentina (L.) Benth.*, International Journal of Scientific Research Engineering & Technology (IJSRET). 2014; 2(12):844-852.
41. Singh P, Singh A, Shukla AK, Singh L, Pande V, Nailwal TK. Somatic Embryogenesis And *In vitro* Regeneration Of An Endangered Medicinal Plant Sarpgandha (*Rauvolfia serpentina*. *L*), *Res.* 2009; 1(3):46-53.
42. Sofowora AE. Medicinal Plants and Traditional Medicines in Africa. 2nd edition. Spectrum Books, Ibadan, Nigeria, 1993, 289.
43. Sundaram S, Ahmad IZ, Dwivedi P. Study of different stages of somatic embryogenesis in a medicinal plant, Madar (*Calotropis procera*). Res. J Bot. 2011; 6(1):1-10.
44. Tejovathi G, Goswami H, Bhadauria R. *In vitro* propagation of endangered medicinal plant–*Commiphora wightii*. Indian J Sci. Technol. 2011; 4(11):1537-1541.
45. Uzundzhalieva K, Ruseva R, Kachakova S. Ex situ and *in vitro* conservation of *Glicyrrhiza glabra* L. Crop wild relative from Fabaceae. *Ecologia Balkanica*. 2014; 5:9-13.
46. Vieira RF, Skorupa LA. Brazilian medicinal plants gene bank. *Acta Hort*. 1993; 330:51-58.
47. Wala BB, Jasrai YT. Micropropagation of an Endangered Medicinal Plant: *Curculigo orchioides* Gaertn. *Plant Tissue Cult*. 2003; 13(1):13-19.
48. Zimmerman JL. Somatic embryogenesis- A Model for early development in higher plants. *Plant Cell*. 1993; 5:1411-1423.