Review

Biotechnology Intervention in Medicinal Plants of North East India-A Review

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Abstract: North East India is a rich phyto-diversity area positioned in the Indo-Burma and Himalayan Biodiversity hotspot. Around 1800 species of plants including several rare, endangered and endemic species have been recorded from this region with ethno-medico-botanical information. The use of plants for medicinal purposes with traditional know-how is a much prevalent practices throughout NE India. At this time, concern on various aspects of medicinal plants is gaining importance throughout the globe. Research focus is escalating on a wide spectrum of activities starting from *in vitro* propagation to metabolomics. Biotechnology tools and techniques are being used to select, multiply, evaluate and improve medicinal plants throughout the globe. Various techniques like micropropagation, cell suspension culture for secondary metabolite production, combinatorial biosynthesis, genetic transformation with desired gene, engineering of agronomic traits, metabolomics, profiling of germplasm using molecular markers are being used to achieve characterization and improvement of medicinal plants. During last two decades or so, biotechnology based R&D programmes are getting a momentum on utilization and development of medicinal plants in NE India. This paper attempted to highlight the success and lead in identification of diverse plant genetic resources of NE India using biochemical and molecular markers; high frequency *in vitro* regeneration of plants and plant parts for micropropagation and production of secondary metabolites; bioprospecting for novel phyto-chemicals, screening of high yielding elite clones and assessment and bioassay of medicinal properties.
Key words: Bioprospecting, Biotechnology, Endemic, Ethno-medicinal, Indigenous, North east India, Traditional.

Introduction

North East India is a rich phyto-diversity area positioned in the Indo-Burma and Himalayan Biodiversity hotspot. Around 1500 species of plants including several rare, endangered and endemic species have been recorded by a number of workers from this area which are being used in different traditional and modern medicinal uses (Handique, 2009). The use of plants for medicinal purposes with traditional know-how is a much prevalent practices throughout NE India. Around 50 plant species grown wild in NE India are exploited in large quantities for preparation of ayurvedic medicine and modern pharmaceuticals including healthcare products. In recent years concern on various aspects of medicinal plants is gaining momentum throughout the globe. Research focus is escalating on a wide spectrum of activities starting from *in vitro* propagation to metabolomics. Biotechnological tools are being used to select, multiply, evaluate and improve medicinal plants on a global scale. Biotechnological interventions are taking place in exploitation and improvement of medicinal plants that include micropropagation, cell suspension culture for secondary metabolite production, combinatorial biosynthesis, genetic transformation with desired gene, engineering of agronomic traits, bioanalytics, metabolomics and profiling of germplasm using molecular markers (Khan *et al.*, 2009) . However, such activities are still in infancy in NE India though

this region houses a large number of medicinal plants and a time tested tradition of herbal practices.

It is to be noted that a large amount of literature is available on ethno-medico-botanical exploration and enumeration of medicinal plants of NE India. But, only in recent years, around 1995, scientific investigation on medicinal plants of this rich biodiversity region using biotechnology tools and techniques has been taking place. Systematic efforts are seen to happen not only for botanical identification of important medicinal plants using molecular markers but to extract and characterize active principles, selection of elites for better production of important biomolecules, *in vitro* culture of plant and plant parts for production of phytochemical, and clinical trials of the medicinal plant products. Out of these activities *in vitro* regeneration of important medicinal plant species occupy a major share.

In vitro culture of plants and plant parts

Plant tissue culture has become an essential and powerful technique in plant biotechnology for improvement of crop varieties, hi-tech mass propagation and production of phytochemicals. Many newer aspects and techniques are being developed and added to the science of plant tissue culture. Micropropagation, a definite aspect of tissue culture, has been contributing in large-scale regeneration of various important medicinal plant species whose population is decreasing in nature due to various biotic and abiotic pressures. With refinement in the techniques, micropropagation has developed into a preferred method of cloning and bulking (Murashige, 1974). The second most important application of tissue culture is the establishment of pathogen free stock.

Efficient micropropagation protocols have been standardized for quite a good number of plant species that are either endemic or rare in NE India during last 25 years. In an early attempt, Rathore *et al.* (1991) were successful to regenerate *Nepenthes khasiana*, a rare insectivorous plants, in tissue culture using MS medium (Murashige and Skoog, 1968) containing adenine sulphate. Later Handique (2002) has developed an efficient *in vitro* mass propagation protocol for this species (*Nepenthes khasiana*) through high frequency multiple shoot regeneration. Tandon and Rathore (1992) standardized a protocol for regeneration of *Coptis teeta* via callus culture. Microshoots were developed from hypocotyls derived callus tissue on MS medium supplemented with 24-D and kinetin. Mao *et al.* (1995) developed a rapid *in vitro* clonal propagation system for *Clerodendron colebrookianum* Walp., which is used as anti-hypertension medicine in Assam. Handique and Bhattacharjee (1997) reported efficient shoot proliferation and plantlet development in *Citrus grandis* and *C. assamensis* using B5 medium (Gamborg *et al.*, 1968). Majumdar *et al.* (1998) reported *in vitro* growth and multiplication of shoots of *Albizia procera* Benth. Handique and Bora (1999) developed an efficient regeneration system for *Houttuynia cordata,* an important medicinal plant species extensively used in NE India. Sinha *et al.* (2000) accounted somatic embryogenesis and plantlet regeneration from leaf explants of *Sapindus mukorossi* Gaertn. They (Sinha *et al.*, 2000) also reported *in vitro* plant renegeration of *Albizia chinensis* (orb.) Merr.

Bhuyan *et al.* (2000) achieved multiple shoots from nodal explants and plantlet development of *Wedelia chinensis* on MS medium. Borthakur *et al.* (2000) developed a micropropagation protocol for *Eclipta alba* (L.) and *Eupatorium adenophorum* (L.) from nodal segments in MS Medium. During the same period, Handique and Bhattacharjee (2000) standardized regeneration protocol for wood apple (*Feronia elephantum* Correa) using shoot-tip explants in B5 medium. Chetia and Handique (2000) developed micropropagation protocol for *Plumbago indica*, a rare medicinal plant species through high frequency shoot multiplication using MS medium. Direct plantlet regeneration from male inflorescences of medicinal yam (*Dioscorea floribunda*) on medium supplemented with 13.94 mM kinetin was reported by Borthakur and Singh (2002). Das and Handique (2002) developed a protocol for *in vitro* regeneration of a rare medicinal plant *Polygonum microcephallum* through the nodal explants culture.

Efficient micropropagation protocols have been developed for a number of important medicinal plant species that are either indigenous or endemic to North East India. These species included *Phlogacanthus thyrsiflorus* (Baruah and Handique, 2002), *Phyllanthus fraternus* (Banu and Handique, 2003), *Wedellia chinensis* (Sultana and Handique, 2004), *Mentha longifolia* (Deka *et al*., 2005), Piper longum (Nesa and Handique, 2006), *Gomphostemma* species (Deka *et al*., 2006), *Lawsonia inermis* (Malakar and Handique, 2006), *Andrographis paniculata* (Purkayastha *et al*., 2008), *Tinospora cordifolia* (Handique and Choudhury 2009; Choudhury and Handique, 2013), *Valeriana jatamansi* (Das *et al*., 2013), *Stephania japonica* (Handique and Sharma, 2010), *Auilaria agalloca* Roxb (Debnath *et al*., 2013), *Acalypha Indica* (Saikia and Handique, 2014).

Kataky and Handique (2010, 2010a, 2011) has optimized *in vitro* regeneration protocol for *Andrographis paniculata*, by culturing shoot explants in ½ strength MS medium through high frequency multiple shoot induction. Singh and Chaturvedi (2010) reported clonal propagation of *Spilanthes acmella* Murr for production of scopoletin. They (Singh and Chaturvedi, 2012) also screened and quantified an antiseptic alkylamide, spilanthol from *in vitro* cell and tissue cultures of *Spilanthes acmella.* Srivastava and Chaturvedi (2010) described simultaneous determination and quantification of three pentacyclictriterpenoids - betulinic acid, oleanolic acid, and ursolic acid in cell cultures of *Lantana camara.* Ranyaphi *et al*. (2011) reported a protocol for *in vitro* organogenesis from leaf and internode explants of wintergreen plant (*Gaultheria fragrantissima*). Sandhyarani *et al*. (2011) developed an efficient *in vitro* clonal propagation protocol using elite triploid accessions of *Acorus calamus*. Dohling *et al*. (2012) reported *in vitro* mass propagation through multiple shoot induction from axillary bud cultures of *Dendrobium longicornu*, an orchid species with medicinal uses. Punyarani and Sharma (2012) developed micropropagation protocol and microrhizome induction in *Costus pictus*, an antidiabetic plant using nodal explants. Bora *et al*. (2013) optimized a protocol for micropropagation of *Capsicum chinense*, a highly pungent chili species found in Assam. Nongalleima *et al*. (2013) optimized surface sterilization protocol and induction of axillary shoots regeneration in *Zingiber zerumbet* (L.) Sm. Singh *et al*. (2013) made a comparative assessment of growth, yield potential and horticultural performance of conventional vs micropropagated plants of *Curcuma longa* var. Lakadong.

Deb *et al*. (2013, 2014) developed methods for *in vitro* regeneration of *Cinnamomum tamala* through callus mediated organogenesis from leaf and zygotic embryos explants as well direct organogenesis from cotyledonary segments. Singh *et al*. (2014) evaluated the horticultural performance of both conventional and micropropagated plants of *Zingiber officinale* from Manipur. Devi *et al*. (2014) optimized a high frequency plant regeneration system from transverse thin cell layer section of *in vitro* plant of 'Nadia' Ginger variety. Singh *et al*. (2014) developed a method for induction of *in vitro* microrhizomes using silver nitrate in *Zingiber officinale* var. Baishey and Nadia. Deb and Arenmongla (2014) reported a low cost protocol for micropropagation of *Malaxis acuminata*, a therapeutically important orchid species of Nagaland. Bhowmik *et al*. (2016) achieved direct shoot organogenesis from rhizomes of medicinal zingiber species - *Alpinia calcarata.*

Molecular characterization

Use of molecular markers for identification, characterization and assessment of species diversity are relatively few. However, a few investigators have carried out considerable work for characterization of species as well as to determine genetic fidelity of micropropagated plants using molecular markers. Pradhan *et al*. (2012) has optimized tissue culture of ginger varieties of Sikkim and determined the genetic fidelity of tissue cultured plants using RAPD markers. Singh *et al*. (2012) has developed a protocol for micropropagation of *Eclipta alba* through transverse thin cell layer culture and also assessed the clonal fidelity using RAPD analysis. Saikia and Handique (2014) reported uniform genetic fidelity of *In vitro* grown plants of Seabuckthorn (*Hippophae salicifolia*) using RAPD

markers. A detailed study was made on the diversity of genus Piper available in Manipur by Chongtham *et al*. (2015) using RAPD and ISSR markers. Sanabam *et al*. (2015) made an assessment of disease-free *in vitro* grown plants of khasi mandarin (*Citrus reticulata* Blanco) using DAS-ELISA and RT-PCR. They also optimized the micropropagation technique for disease-free plantlets through microshoot-tip grafting. Singh *et al*. (2013) evaluated the genetic stability in traditional ginger cultivated in Manipur using molecular and chemical markers.

In recent years, DNA barcode based molecular characterization of plants species is gaining popularity. Such activities are also taking place in NE India. Mahadani *et al*. (2013) had tested the efficiency of *matK* in species delineation like DNA barcoding in the members of the family Apocynaceae. They concluded that *matK* sequence information could help in correct species identification for medicinal plants of Apocynaceae. Medhi *et al*. (2014) reported genetic diversity in three *Zanthoxylum* species viz. *Zanthoxylum nitidum, Zanthoxylum oxyphyllum* and *Zanthoxylum rhesta* collected from the Upper Brahmaputra Valley Zone of Assam using 13 random amplified polymorphic DNA (RAPD) markers and 9 inter-simple sequence repeat (ISSR) markers. Pradhan *et al*. (2014) evaluated genetic diversity of Five elite cultivars namely, Bhaisay, Gorubthangey, Jorethangey, Charinangrey, Majhauley of *Zingiber officinale* Rosc and detected cultivar variations.

Investigation on phytochemical and medicinal properties

Medicinal plants are rich source of various medicinal compounds. Quite a good number of reports are available on phytochemical constitutions and medicinal properties of plants indigenous to NE India. Hazarika and Sarma (2006) studied the estrogenic effects of root extract of *Polygonum hydropiper* on induction of follicular recruitment and endometrial hyperplasis in female albino rates. Later, Goswami *et al*. (2008) investigated the TLC fraction of root extract of *Polygonum hydropiper* that induced vaginal epithelial cell maturation in adult overiectomized albino rate. Sharma *et al*. (2007) evaluated the antioxidant and cytotoxic properties of ginger (*Zingiber montanum*) and highlighted ginger as a promising radioprotective agent. Ramkete *et al*. (2007) evaluated DPPH radical-scavenging activity of leaf extracts of *Phlogacanthus tubiflorus.* Unni *et al*. (2009) has conducted a study on various biochemical parameters of some traditional medicinal plants of NE India viz., *Leucas aspera, Murraya koengigii, Oxali corniculata, Alternanthera sessilis, Pagostemon benghalensis, Hydrocotyl rotendifolia, Cyathula prostrata, Piper peepuloides, Potentilla mooniana*. It was observed that *L. lanata, M. koengigi, O. corniculata, A. sessilis, P. peepuloides* and *P. mooniana* shows antibacterial activity against *E. coli*. The nutritive values of these plants varied between 231.59 to 34.59 Cal/100 gm and the tannin content ranged from 3.8% and 0.62%. Das *et al*. (2011a, 2011b) made a comprehensive report on volatile constituents of *Valeriana Hardwicke* as well as terpenoid compositions and antioxidant activities of two Indian Valerian Oils from the Khasi Hills. Barooah and Konwar (2010) studied the antibacterial properties several medicinal plants of Assam traditionally used in the treatment of dysentery and diarrhea. Srivastava and Chaturvedi (2010) described simultaneous determination and quantification of three pentacyclictriterpenoids-betulinic acid, oleanolic acid, and ursolic acid-in cell cultures of *Lantana camara.* Srivastava *et al*. (2010) has reported the accumulation of betulinic acid, oleanolic acid and ursolic acid in *in vitro* cultures of *Lantana camara* L. and their cytotoxic activity against HeLa cell lines. Hui *et al*. (2010) evaluated *Chloranthus erectus*, a folk medicine of Arunachal Pradesh, for its entipyretic and antibacterial activity. Das and Handique (2011) evaluated antioxidant properties of methanol extract of *Phyllanthus fraternus.* Srivastava *et al.* (2011) has worked out the effect of culture conditions on synthesis of triterpenoids in suspension cultures of *Lantana camara*.

Singh and Chaturvedi (2012) also screened and quantified an antiseptic alkylamide, spilanthol from *in vitro* cell and tissue cultures of *Spilanthes acmella.* Srivastava and Chaturvedi (2010) described simultaneous determination and quantification of three pentacyclictriterpenoids-betulinic acid,

oleanolic acid, and ursolic acid-in cell cultures of *Lantana camara.* Thockchom and Sharma (2012) investigated free radical scavenging activity of several therapeutic plants of Manipur. They also reported protection of radiation-induced DNA damage by *Zingiber montanum*. Chowdhury and Handique evaluated phytochemical composition and antibacterial activity of *Garcinia lancifolia* (2012). Gogoi *et al*. (2012) evaluated antioxidant potential of three Garcinia species Namely, *G. pedunculata, G. xanthochymus and G. morella*. Moirangthem *et al*. (2012) evaluated antioxidant, antibacterial, cytotoxic and apoptotic activity of extracts of *Cephalotaxus griffithii,* a rare medicinal plants of NE India. Saikia and Handique (2013a, 2013b) has evaluated antioxidant and antibacterial activities of various plants parts of seabuck thorn (*Hippophae salicifolia*), a high altitude species rarely found in Sikkim and Arunachal Pradesh. Singh and Chaturvedi (2013) evaluated the antioxidant and anthelmintic properties of the extracts of dedifferentiated cultures of *Spilanthes acmella* Murr. Antibacterial properties of *Strobilanthes cusia,* a rare medicinal plant species found in Manipur was evaluated by Shahni and Handique (2013). A study was made on antioxidant activity of different parts of *Lysimachia laxa* and *Gymnocladus assamicus* using three different solvent extraction systems by Gupta *et al*. (2013). A method was optimized by Tsering *et al*. (2014) for determination of gallic acid from an important medicinal plant species - *Quercus griffithii* Acorn using HPTLC. Borah *et al*. (2014) investigated antioxidant activity of tender shoots of *Calamus leptospadix* Griff. They reported that the methanolic and ethanolic extracts of tender shoots possess the antioxidant activity in a dose-dependent manner. Rajan *et al*. (2014) estimated the trace element content in several selected medicinal plants traditionally used in the treatment of skin diseases in Mizoram.

Biosynthesis of nanoparticles from plants is considered as one of the most suitable method for synthesis of nanoparticles. Research activities in this area are also gaining an impetus in NE India. A method for synthesis of gold nanoparticles using ethanolic leaf extracts of *Centella asatica* was developed by Das *et al*. (2010). Babu *et al*. (2010) described the synthesis of gold nanoparticles using leaf extract of *Mentha arvensis,* a commonly used medicinal plant species. Babu *et al*. (2012) reported green synthesis of biocompatible gold nanoparticles mediated by piper-betle. Das *et al*. (2012) developed a rapid, microwave mediated method for synthesis of gold nanoparticles using *Calotropis procera* latex. Bhau *et al*. (2014) investigated the ability of *Nepenthes khasiana* to synthesis gold nanoparticles. They reported significant antimicrobial activity of the biosynthesized gold nanoparticles.

Das Gupta *et al*. (2014) made an assessment of diversity among and within the population of *Acmella paniculata* of Arunachal Pradesh using RAPD and ISSR markers. In a recent work, physico-chemical characteristics, proximate compositions and antioxidant properties were evaluated for five wild fruits species namely, *Garcinia pedunculata, Garcinia xanthochymus, Docynia indica, Rhus semialata* and *Averrhoa carambola* of Manipur (Bakleswshor *et al*., 2015). Rajendran and Chaturvedi (2017) has optimized the media constituents for enhanced production of medicinal N-alkylamide Deca-2 E,6 Z,8 E-trienoic acid isobutylamide from dedifferentiated *in vitro* cell lines of *Spilanthes paniculata*.

Conclusion

It can be concluded from the present review that considerable amount of scientific work had been accomplished by various workers on characterization, bioprospction, development and conservation of medicinal plants species of North East India using modern biotechnology tools and techniques. However, quantification of the results and products is required to assess the success of such research accomplishments. Moreover, this review is not exhaustive. There are more work and information are available on the subject which cannot be included due to several limitations of the present work. Despite that, it is expected that this review will be helpful for the beginners to choose tools and techniques as well as plant species for newer scientific exploration.

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