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Review article

CONSERVATION OF BIODIVERSITY OF SOME IMPORTANT MEDICINAL PLANTS OF TROPICAL FORESTS OF INDIA THROUGH TISSUE CULTURE

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ABSTRACT:
Over the past three decades, the global environmental crisis has led to a belated acknowledgment that man is part of nature, a new paradigm challenging biological and ecological research, which has, in the past, tended to consider natural objects as totally independent of any social or political sphere (Larrere and Larrere 1997, Latour 1997). According to the Red List of threatened species 44 plant species are critically endangered, 113 endangered and 87 vulnerable (IUCN, 2000). Many medicinal plants are also in trouble from over harvesting and destruction of habitat. Population growth, urbanization and the unrestricted collection of medicinal plants from the wild is resulting in an over-exploitation of natural resources. Therefore, the management of traditional medicinal plant resources has become a matter of urgency. An ever increasing demand of uniform medicinal plants based medicines warrants their mass propagation through plant tissue culture strategy. Tissue culture technology is potent and has opened extensive areas of research for biodiversity conservation. Tissue culture protocols have been developed for a wide range of medicinal plants, which includes endangered, rare and threatened plant species. Some of these medicinal plants are *Oroxylum indicum, Asparagus racemosus, Costus speciosus* and *Chlorophytum borivilianum.*

KEYWORDS: Endangered, Medicinal Plants, Plant Tissue Culture, *Asparagus, Oroxylum, Costus*

INTRODUCTION

Biodiversity of plants collectively known as 'plant genetic resources' is a key component of any agricultural production system - indeed, of any ecosystem, without which natural evolutionary adjustment of the system to the changing environmental and biotic conditions would be impossible. India is a treasure chest of biodiversity which hosts a large variety of plants and has been identified as one of the eight important 'Vavilorian' centres of origin and crop diversity. Although its total land area is only 2.4% of the total geographical area of the world, the country accounts for 8% of the total global biodiversity with an estimated 49,000 species of plants of which 4,900 are endemic. In view of the tremendously growing world population, increasing anthropogenic activities, rapidly eroding natural ecosystem, etc the natural habitat for a great number of herbs and trees are dwindling. Many of them are facing extinction. To cope up with alarming situation, the recent exciting developments in biotechnology have come as a boon. One of them is the use of plant tissue culture technique. Most of the plant raised through seeds are highly heterozygous and show great variations in growth, habit and yield and may have to be discarded because of poor quality of products for their commercial release. Moreover many conditions, Besides, the callus derived plants exhibit huge genetic variation that could be exploited for developing superior clones/varieties particularly in vegetatively propagated plant species. In terms of the number of species individually targeted, the use of plants as medicines represents by far the biggest human use of the natural world. There is no reliable figure for the total number of medicinal plants on Earth, and numbers and percentages for countries and regions vary greatly (Schippmann *et al.,* 2002). Estimates for the numbers of species used medicinally include: 35,000-70,000 or 53,000 worldwide (Schippmann *et al.,* 2002); 10,000- 11,250 in China (He and Gu, 1997; Pei, 2002; Xiao and Yong, 1998); 7500 in India (Shiva, 1996); 2237 in Mexico (Toledo, 1995); and 2572 traditionally by North American Indians (Moerman, 1998). The United Nations Conference on Environment and Development (UNCED), held recently at Rio de Janeiro in 1992, Brazil helped to place the loss of biodiversity and its conservation on the global agenda. Conservation and use of genetic diversity for sustainable ecosystem or agro ecosystem should be continuous to meet food, clothing, shelter and health requirements of India's growing population.

PRESENT STATUS OF BIODIVERSITY OF IMPORTANT MEDICINAL PLANTS IN TROPICAL FORESTS OF INDIA

The ecosystems of southern peninsular India including the southern Western Ghats contain more than 6000 species of higher plants including an estimated 2000 endemic species. Of these, 2500 species representing over 1000 genera and

250 families have been used in Indian systems of medicine. Millions of rural mass use medicinal plants. In recent years the growing demand for herbal products has led to a quantum jump in volume of plant material traded within and outside the country. Very small proportions of the medicinal Plants are lichens, ferns, algae etc; the majority of the medicinal plants are higher plants. Though India has rich biodiversity and one among the twelve mega diversity centers, the growing demand is putting a heavy strain on the existing resources causing a number of species to be either threatened or endangered category. About 90% of medicinal plants used by industries are collected from the wild. While over 800 species are used in production by industry, less than 20 species of plants are under commercial cultivation. Over 70% of the plant collections involve destructive harvesting because of the use of parts like roots, bark, wood, stem and the whole plant in case of herbs. This poses a definite threat to the genetic stocks and to the diversity of medicinal plants. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security.

***Oroxylum indicum*:** *Oroxylum indicum* (Linn) Vent. (Sonpatha) belonging to the family Bignoniaceae. It is widely distributed in the deciduous and mixed miscellaneous forests of India. *O. indicum* is a small to medium sized deciduous tree with light grayish brown, soft, spongy bark and papery thin seeds with broad silvery wings. Most parts of plant are used in Ayurvedic medicine. The root bark is well-known tonic and astringent useful in diarrhea and dysentery. It is diaphoretic and used in rheumatism. Tender fruits are refreshing and stomachic and the seeds are purgative. The stem and root barks contain three flavor colouring matters, viz. Oroxylum-A, baicalin and chrysin. Young shoots and unripe fruits are eaten as vegetables. The tree is also frequently lopped for fodder. The *O. indicum* is propagated naturally by seeds, which germinate in the beginning of the rainy season. Seedlings require moderate shade in the early stages. However, the seed set is poor and seed viability is low. Problems related with its natural propagation and indiscriminate exploitation for medicinal purpose has pushed *O. indicum* to the list of endangered plant species of India.

Asparagus racemosus: *Asparagus racemosus* (Wild) locally known as "Satawar" belongs to the family Liliaceae. It is an undershrub climber with extensively branched woody stems, growing up to 2m in height. The succulent tuberous roots are 30-100 cm long and 1-2 cm thick in bunch attached at the stem base. The leaves are reduced to small scales or needle like spines called cladodes. The flowers are small, white, fragrant and in simple or branched racemes. This plant can found naturally in the tropical and sub-tropical forests throughout India. It is wildly used for multiple purposes in medicines, Roots, leaves and young shoots are of higher significance in Ayurveda medicines. In modern scientific tools many active compounds like several steroidal sponons, aglycones, alkaloids like *asparagin* anticancer agent. Leaves contain rutin, diosgenin and flavonoid glycoside. It is propagated by root suckers or seeds. For commercial cultivation, root suckers are preferred over seeds. Well developed root suckers are planted on the ridges. However, there are still major opportunities to produce and distribute high quality medicinal plant *A. racemosus*. The main advantage of tissue culture technology lies in the production of high quality planting material that can be multiplied round the season basis under disease-free conditions.

***Costus speciosus*:** *Costus speciosus* (Keukand) belonging to the family Costaceae is an important medicinal and ornamental plant, cultivated in India. *Costus speciosus* is propagated vegetative methods using rhizome pieces. However, the planting material shows a very low multiplication rate. The lack of material shows a very low multiplication rate. The lack of seed setting spoiled the breeding of this plant. Four different alkaloids responsible for pharmacological aspects and the presence of acetylcholine responses for anticholinesterase activity are also reported. Conventional propagation is beset with problem of poor seed viability, low percentage of germination and scanty delayed rooting of vegetative cuttings. Therefore there is a need for alternative propagation methods. Tissue culture techniques might be applied to generate large number for clonal propagates. Regeneration and reestablishment of the plant through *in-vitro* culture is one of the most effective biological techniques to maintain its diversity. Since the harvest of medicinal plants on a mass scale form their natural habitats is leading to a depletion of plant resources the conservation of these valuable genotypes is imperative.

***Chlorophytum borivilianum*:** *Chlorophytum borivilianum* (Safed musli) belonging to the family Liliaceae is endogenous medicinal plant to India and distributed in eastern part of India (Assam, Eastern Ghats, Eastern Himalayas, Bihar and Andhra Pradesh. Fasciculated roots of *Chlorophytum Borrivillianum* are used as tonic and constitute important ingredient of twenty ayuervedic and unani preparation. The production of high quality planting material propagated from vegetative parts has created global trading area, benefited growers, farmers, nursery owners and improved rural employment. However, there are still major opportunities to produce and distribute high quality medicinal plant *Chlorophytum borivilianum*. The main advantage of tissue culture technology lies in the production of high quality planting material that can be multiplied round the season basis under disease-free conditions. This species perpetuates both vegetatively by tubers and sexually through seeds. It has a sluggish perpetuation through seed on account of poor seed germination.

IN-VITRO CONSERVATION STRATEGIES USED FOR THE PROPAGATION

Micropropagation/Clonal propagation techniques using shoot tip and nodal segments are must for mass-scale multiplication and conservation of an endangered or threatened and medicinally important species within short

period and limited space. The plants produced from this method are true to type. Propagation through tissue culture provides solution for mass propagation of plants in general and threatened plants in particular. There is a need to conserve plants with medicinal values. Due to ever growing demand, the availability of medicinal plants to the pharmaceutical companies is not enough to manufacture herbal medicines. The powerful techniques of plant cell and tissue culture, recombinant DNA and bioprocessing technologies have offered mankind a great opportunity to exploit the medicinal plants under *in-vitro* conditions.

Micropropagation

In clonal propagation, plants are multiplied using shoot tips and seedling nodal sections as explants. For rapid *in-vitro* clonal propagation of plants, induced callus of species were cultured on modified MS medium and supplemented with various concentrations of auxin and cytokinin dividually for shoot and root proliferation. Shoot number increases logarithmically with each subculture to give greatly enhanced multiplication rates. As this method involves only organized meristems, hence it allows recovery of genetically stable and true to type progenies (Murashige, 1974; Hu and Wang, 1983).

Formation of Callus

Callus is a mass of unorganized cells resulting either as a consequence of wounding in plants or in tissue culture. Callus formation has been found in angiosperms, gymnosperms, pteridophytes and bryophytes. Callus is somewhat an abnormal tissue, which has the potentiality to produce normal roots and embryos, and in turn develops into plantlets. Although callus remains unorganized, as growth proceeds, some kinds of specialized cells may be formed and which can give rise to organs such as roots, shoots and embryos. For plant cells to develop into a callus it is essential that the nutrient medium contain plant hormones, i.e. an auxin, a cytokinin and gibberellins (Srivastava and Rajani 1999). Callus formation are the synthesis of starting compounds that are subsequently modified to yield the desired product. The starting material for the vegetative propagation of plants. Their reverting to tissue culture allows the conservation of virus or fungi free and resistant cell lineages.

Somatic Embryogenesis

Somatic embryogenesis is the process of formation of embryo like structure from somatic tissue. The somatic embryo may be produced either directly on the explant or indirectly from callus or cell suspension culture. For the first time, Haccius (1978) defined somatic embryogenesis as a non-sexual developmental process, which produces a bipolar embryo from somatic tissue. The first report of plantlet regeneration via in vitro somatic embryogenesis was in *Daucus carota* (Reinert, 1958; Steward et al., 1958). This pathway has offered a great potential for the production of plantlets and its biotechnological manipulation. In addition to the development of somatic embryos from sporophytic cells, embryos have been induced from generative cells such as in the classic work of Guha and Maheshwari (1964) with *Datura innoxia* microspores.

Tissue culture technique has been used successfully for *in-vitro* mass propagation of some medicinal plants (Table. 1), and its field survival trial (Table-2).

TABLE-1: *In-vitro* cultured important medicinal plants

Plant Species	Family	Explants	Nature of Response	Reference
Oroxylum indicum	Bignoniaceae	seedling nodal sections	Mass propagation	Mamta and Yogendra (2010)
Asparagus racemosus	Liliaceae	shoot tips / nodes	Shoot and Root Proliferation	Krishna and Sanu (2009)
Costus speciosus	Costaceae	young shoot	Shoot Proliferation	Chaturvedi and Mishra (1984)
Chlorophytum borivilianum	Liliaceae	shoot buds	Mass propagation	Archana et al (2008)

TABLE-2: Showing Percentage Survival on Field Trial

Plant Species	Family	No. of plants hardened	% of survival	Reference
Oroxylum indicum	Bignoniaceae	20	92%	Gokhale and Bansal(2009)
Asparagus racemosus	Liliaceae	20	94%	Krishna and Sanu (2009)
Costus speciosus	Costaceae	20	95%	Chaturvedi and Mishra (1984)
Chlorophytum borivilianum	Liliaceae	20	98%	Mathur and Kumar (2008)

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