PLANT BIOTECHNOLOGY AND CONSERVATION OF BIODIVERSITY

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Abstract

Plant genetic resources are the major biological basis of the world food security. In all means they support the livelihoods of every life on planet earth. Biodiversity is the store house and acts as a cushion against potentially dangerous environmental changes and economic reforms. Such a buffer is facing threat due to manmade and ecological disasters. Hence, conservation of Biodiversity is considered fundamental and provided priority in all sectors of global development. Traditional means of germplasm storage and conservation of plant genetic resources has been immensely useful and are not without drawbacks. Thus utilizing the biotechnological approaches towards the improvement of in situ and ex situ conservation programmes are becoming vital. Integrating biotechnology in plant conservation programmes is a prerequisite to achieve success in sustainability and to complement the existing technologies. In this context, the conservation of plants through in vitro propagation and inducing slow growth in some of the economically important and threatened plant of Southern Western Ghats is presented. During the last ten years of intensive research programmes we are able to develop in vitro protocol for rapid regeneration and establishment of plants in the field conditions for about thirty different species of this region.

INTRODUCTION

India is a major centre of origin and diversity of crop and medicinal plants. It holds an extraordinary significance among the top gene-rich countries of the world relating to its abundantly rich land race diversity in agricultural and horticultural crops and their wild relatives. India possesses about 20,000 species of higher plants and one third of it being endemic and 500 species are categorized to have medicinal value (Mandal, 1999; Arora, 1988). The Southern Western Ghats is one of the major repositories of endemic and medicinal plants. It harbors around 4,000 species of higher plants of which 450 species belonging to 150 Natural orders are endangered. The red list category in this region is increasing and the valuable genetic resources are being lost at a rapid rate due to habitat destruction, environmental changes, natural calamities and more reasonably through over-exploitation. Tissue culture approaches have been vital in the reestablishment of endangered plant species. This paper highlights the integration of biotechnological programmes into conservation of biodiversity as well as the success achieved towards conservation of important plants through micropropagation.

ROLE OF BIOTECHNOLOGY

The tools of modern biotechnology are being increasingly applied for plant diversity characterization and undoubtedly they have a major role in assisting plant conservation programmes. However, their value is dependent upon ensuring that biotechnological methods are targeted effectively and utilized as complementary and enabling technologies. Most importantly, they must be applied in the appropriate context. Biotechnology is advancing so rapidly

that it may be sometimes difficult for potential 'conservation' users to assess the value and role of new techniques and procedures within their own specific area. It is important to recognize that the effective integration of biotechnology in conservation programmes requires multi- and interdisciplinary co-operation. Thus, present and future conservation teams should comprise personnel from a broad spectrum of disciplines.

There are four main areas of biotechnology, which can assist plant conservation programmes. 1) Molecular markers technology, 2) Molecular diagnostics, 3) Tissue culture *(in vitro* technologies) and 4) Cryopreservation. In addition, 'Information Technology' (IT) will have increasingly important role in facilitating conservation programmes and the interface between IT and biotechnology provides considerable potential for many aspects of plant genetic resource management.

MOLECULAR MARKERS AND DIAGNOSTICS

For economically important plant species, it is also essential to consider the relationship between conservation and utilization and to recognize that biotechnology can enable sustainability programmes (Benson, 1999). The elucidation of population structures and gene distribution patterns within ecosystems provides information, which can be used to support *in situ* conservation programmes. Contrasting examples of techniques include the assessment of restriction fragment length polymorphisms (RFLPs), which permit the detection of specific marker genes and polymerase chain reaction-based marker technologies (PCR) used in association with RAPD (randomly amplified polymorphic DNA) analysis.

TISSUE CULTURE PROGRAMMES

Tissue culture (or *in vitro)* technologies have had a major impact on the *ex situ* conservation of plant genetic resources and importantly, disease indexed *in vitro* - maintained germplasm provides an excellent means of mediating international germplasm exchange. Micropropagation, using somatic embryo and shoot culture techniques assists many crop plant improvement programmes and increasingly these methods are being used for the conservation of endangered plant species. Crop plants, which are vegetatively propagated present particular conservation problems, as their seeds are not available for banking. Whilst field genebanks provide important conservation options, germplasm maintained in this manner can be at risk from pathogen attack and climatic damage. For vegetatively propagated species, *in vitro* conservation using tissue culture methods is the only reliable, long-term means of preservation. Storage in the active growing state or under reduced (slow) growth provides cost effective, medium-term conservation options. Most major, international germplasm centers use *in vitro* conservation as their method of choice for vegetatively propagated crops.

CRYOPRESERVATION

Maintenance of plant germplasm in the active or slow growth state provides a medium-term storage option; however, the long-term conservation of *in vitro*-derived plant germplasm is increasingly achieved using conservation in liquid nitrogen. Cryoconservation is thus applied to plant germplasm, which cannot conserve using traditional seed banking techniques, and/or to vegetatively propagated germplasm. In 1980, Withers and Williams highlighted the

problems associated with difficult to store and seed-recalcitrant germplasm. It is encouraging to note that during the last decade or more, major advances have been made in the successful application of cryopreservation methods to once termed 'difficult' germplasm types (Benson, 1999). Particularly significant advances have been made in the cryopreservation of the recalcitrant seeds (and excised embryos) derived from tropical agroforestry and plantation crops and rain forest tree species.

Advances in biotechnology have only been equalled by the activity of the information science and technology sector. The ‘IT revolution’ is indeed rapidly changing the way and means in which conservation scientists perform their research and implement their conservation strategies. On a practical basis, IT does, and will continue to assist all aspects of documentation associated with genetic resource transfer and management, genome mapping, DNA data basing and gene bank inventories. However, in the future it will be important to enhance and consolidate the enabling role of IT in international training and technology transfer. Distance learning and electronic networking specifically designed for and targeted at plant conservation programmes will promote the expediency of concerted international conservation activities.

CONSERVATION BIOTECHNOLOGY AND THE SUSTAINABLE UTILIZATION OF PLANT GENETIC RESOURCES

Secondary metabolites are organic molecules synthesized and stored in plants in relatively low quantities. They may have no obvious role in growth and development of plant but have significant importance in self-defense. The

natural products have enormous potential as medicinal, nutraceutical and commercial value. These include terpenes-pigments, essential oils, steroids, rubber, phenolic compounds – coumarins, flavonoids, lignin and tannins, glycosides – saponins, cardiac glycosides, cyanogenic glycosides and alkaloids. All these compounds can alternatively be produced abundantly through cell culture without exploiting the natural resource. Plant cell suspension cultures involve growth of single or cell aggregates in liquid medium. Cultured cells are made to synthesize useful metabolites. Synthesis and accumulation processes are induced by altering media composition and feeding of precursors and elicitors. Induction of changes in the shikimic acid pathway plays central role in synthesis of useful pharmaceuticals.

Enhanced production by cell immobilization and biotransformation of new compounds are potential areas of this research. Alkaloids and terpenoids have extensively been produced *in vitro* conditions. Most of alkaloid compounds are pharmaceutical leads. Localization of specialized cells responsible for synthesis and accumulation of metabolites in plant tissues help extensively to choose the cell type for culture. Altering the genetic make up of the cells to go for enhanced production and quality drugs are the theme focus of this field. Hairy root cultures and bioreactor production of secondary metabolites have resulted in remarkable yields. Many prospective drugs including taxol, ajmalicine, atropine, codeine, dopamine, digitoxin and morphine are being obtained enormously from *in vitro* cell cultures and thereby conserving natural resource.

In 1995, the popular, UK-based, plant conservation journal, Plant Talk, produced a communication entitled ‘Yew

in the fight against cancer: sustainability or pillage?' The article refers, of course, to the use of taxus species for the production of the secondary metabolite taxol, which is used to produce a potent anti-cancer drug. Whilst synthesis of the secondary product has been reported, and indeed, the drug has been launched in the US, the article presents some interesting facts, such that it takes approximately ten Pacific Yew trees to yield enough bark for the 2g of taxol required to treat a single cancer patient. The link between plant conservation and sustainable utilization (as opposed to exploitation) is indeed of major importance.

Biotechnology can directly and indirectly enable conservation strategies, yet at the same time allow economically significant species to be both utilized and protected. This is a major issue for those global areas, rich in biodiversity and for which there is an urgent need for populations to realize the economic potential of their rich biological resources and yet at the same time preserve them for future generations.

CONCLUSION AND FUTURE PROSPECTS

Biotechnology is now integrated in all aspects of plant germplasm characterization, acquisition, conservation, exchange and genetic resource management. Future prospects are highly encouraging in terms of the development and application of new techniques and protocols within the context of germplasm conservation. The sustainable utilization of plant diversity can be greatly assisted by the application of direct and indirect biotechnological procedures. Future prospects and needs must target certain key areas including the development of appropriate structures for cryopreserved genebanks, the use of *in vitro* methods for

the safe transfer of disease free germplasm, and the application of genetic marker technologies for rationalizing germplasm procurement and genebanking, as *in vitro* and molecular approaches to plant conservation become more and more important to validate routine operational protocols within and between genebanks and repositories. This must be considered on an international basis and the provision of networking and training infrastructures, with the aid of IT, will assist by enabling cost-effective training, and collaborative communications.

Whilst considerable progress has been made in the application of biotechnology to plant conservation, there still remains the requirement to perform fundamental research. Seed recalcitrance, tissue culture recalcitrance, somaclonal variation and cryopreservation injury can be problematic for certain species. Similarly, whilst there has been considerable success in the use of molecular techniques, our current knowledge of the molecular biology of many groups of plants (eg. temperate woody perennial tropical rain forest trees) is still limited.

Unlike many biotechnological 'applications', conservation biotechnology programmes must be considered with a long-term perspective. Cryopreserved and *in vitro* genebanks, once created, must be maintained in perpetuity. Within an international context there is thus a need for individual governments and regional and global networks to have a commitment to provide sustainable long-term funding. To date, many advances in plant conservation biotechnology have had a short-term remit to solve a particular conservation problem or develop a certain procedure.

Micropropagation has been successfully achieved either through direct morphogenesis or indirect morphogenesis for about thirty species of angiosperms including some grasses for rapid multiplication and proper establishment in the field and wild (Table 1). The procedures developed can also be utilized for conservation by inducing medium term slow growth for all these plant species. A minimum of 15 plantlets were obtained form a single explant bypassing the callus stage and thus achieved clonal propagation. Plants like Wendlandia, Curculigo, Neem, Janakia, Decalepis, Bacopa could be maintained for years together by inducing slow growth. Slow growth was induced by altering the medium requisites and hormonal balance, particularly the levels of auxin and cytokinin. Certain plants such as Neem, Manisuris, Wendlandia, Bacopa, and Janakia could be maintained even without any hormonal supplements in the culture and maintenance media.

Visionary and sustainable funding policies, organized in concerted action by individual governments and appropriate international organizations will be essential to enable the next phase of conservation. Without such support it will not be possible to capitalize on the achievements to date and use them to implement long-term, and safe, plant diversity conservation programmes.

Table 1. Micropropagation of Rare and Economically Important Plants

S. No	SPECIES	FAMILY	STATUS/ USE	METHODS	REFERENCES
1.	***Aegle marmelos*** (L.) Corr.	Rutaceae	Threatened, Medicinal	Node, Embryo, seedling organs, Shoot tip	Parimala, 2004, Selvakumar, 2005.
2.	***Aloe vera*** L. Burm.f.	Liliaceae	Medicinal	Shoot tip and leaf	Vijayalakshmi, 2005.
3.	***Aristolochia ringens*** Vahl.	Aristolochiaceae	Exotic Ornamental	Node, Internodes	Thayammal, 2005.
4.	***Azadirachta indica*** L.	Meliaceae	Medicinal	Node, Embryo, seedling organs, Shoot tip	Ravichandran, 1996.
5.	***Bacopa monnieri*** L		Medicinal	Node, Internodes	Ebbi, Ravichandran, 2002.
6.	***Caesalpinia sappan*** L.	Caesalpiniaceae	Medicinal, Dye yielding	Node, Embryo, seedling organs, Shoot tip	Vinodhini, 2000, Lakshmichandra, 2004.
7.	***Chloris barbata*** Sw.	Poaceae	Fodder and Medicinal	Nodes, seeds	Ravichandran, 1994.
8.	***Curculigo orchioides*** Gaertn.	Hypoxidaceae	Rare Medicinal,	Seeds, Shoot tip	Thangavel and Ravichandran, 2002.
9.	***Cyperus pangorei*** Rottb.	Cyperaceae	Medicinal, Mat fibres	Inflorescence, Rhizome	Fathima Benazir, *et al.*, 2000.
10.	***Dactyloctenium aegyptium*** (L.) Willd.	Poaceae	Medicianal, Fodder	Seeds, Inflorescence	Ravichandran, 1994.
11.	***Datura metel*** L.	Solanaceae	Medicinal	Anther	Judi Cynthia, 2003.
12.	***Decalepis hamiltonii*** W. & A.	Periplocaceae	Rare, Endemic, Medicinal	Shoot tip, Node, leaf	Thangavel, 2002.

S. No	SPECIES	FAMILY	STATUS/ USE	METHODS	REFERENCES
13.	***Eryngium foetidum***	Umbelliferae	Medicinal and Aromatic	Leaf, Nodes, Shoot tip	Antonisamy, 1997. Ignacimuthu, *et al.*, 1999.
14.	***Emblica officinalis*** Gaertn.	Euphorbiaceae	Medicinal	Embryo, Cotyledon and Node	Rameshwari, 2004.
15.	***Gymnema sylvestre*** R. Br.	Asclepiadaceae	Rare, Medicinal	Leaf, Shoot tip, and Node	Ebbie & Ravichandran, 2002.
16.	***Hemarthria compressa*** (L. f.) R. Br.	Poaceae	Fodder	Node	Ravichandran, 1994.
17.	***Janakia aryalpathra*** (Joseph & Chandras) Venter	Periploccaceae	Endangered, Endemic and Medicinal	Leaf, Node, Shoot tip	Thangavel and Ravichandran, 2002, Agalyadevi, 2004.
18.	***Kingiodendron pinnatum*** (Roxb. ex DC.) Hams	Caesalpiniaceae	Vulnerable, Medicinal and timber, gum	Embryo, Cotyledon	Ganesan, 2002, Vinodha 2003. Vinu, 2003.
19.	***Manisuris myuros*** L.	Poaceae	Endemic, Rare	Inflorescence	Chandrasekar, 1999.
20.	***Musa paradisiaca*** L.	Musaceae	Fruit	Shoot tip	Roselin, 2004.
21.	***Nelumbo nucifera***	Nymphaeaceae	Medicinal and Ornamental	Embryo, cotyl	
22.	***Oryza sativa*** L.	Poaceae	Food grain	Caryopsis	
23.	***Pennisetum americanum***	Poaceae	Food grain and fodder	Inflorescence, Caryopsis	Beatrice Valdaris. 1999.
24.	***Pisonia alba***		Medicinal	Node, leaf	Sudarprakash, 2004.
25.	***Polygala javana*** DC.	Polygalaceae	Medicinal	ode, leaf	Lalitha *et al.,* 1995.

Biodiversity : Life to our mother earth

S. No	SPECIES	FAMILY	STATUS/ USE	METHODS	REFERENCES
26.	***Pterocarpus santalinus*** L. f.	Caesalpiniaceae	Rare, Timber, dye and Medicinal	Shoot tip	Rameshwari, 2004.
27.	***Saracca asoka*** (Roxb.) W.J. de Willd	Caesalpiniaceae	Rare, Medicinal	Embryo, shoot tip	Sudar Prakash, 2004.
28.	***Saccharum officinarum*** L.	Poaceae	Sugar, bagasse	Leaf roll	Manohari, 2003.
29.	***Spathoglotis plicata***	Orchidaceae	Ornamental	Seeds	Ravichandran 1997.
30.	***Solanum trilobatum*** L.	Solanaceae	Medicinal	Node, shoot tip	Manimekalai, *et al.,* 1995.
31.	***Sorghum bicolor*** (L.) Moench	Poaceae	Food grain and fodder	Caryopsis	Beatrice Valdaris. 1999.
32.	***Wendlandia angustifolia*** Wight ex Hook. F.	Rubiaceae	Rare presumed Extinct	Node, shoot tip	Chandrasekar & Ravichandran. 2001.

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