Achievements and Challenges in Micropropagation of Bamboo

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Abstract

There has been an increased demand for quality bamboo planting material in the wake of the recent interest in bamboo as a crop with diverse utilization potential. Traditional methods of seed and vegetative propagation has its limitations when the huge demand across the country has to be met since they are undependable and slow due to the peculiar flowering behavior of bamboo. There has been a renewed interest in the micropropagation of bamboo in recent years. Over the years, micropropagation of bamboo has matured from the techniques standardized using explants of seed and juvenile origin with limited practical applications to the feasibility of propagating selected superior mature clumps growing in the field. In spite of the long list of species that can be propagated through in vitro culture, there is scope of improving our knowledge in several areas. Problems of endogenous contamination, poor rooting of shoots, control over in vitro flowering etc still need attention of researchers. There is also a dearth of genetically superior and tested germplasm in most of the bamboo species. A description of the bamboo micropropagation technique is given along with a review of the progress made so far in bamboo around the world and the prospects for research in the area.

Introduction

Bamboo with its diversity of species, fabulous growth rates and versatility

of uses has come into limelight in recent years because of its potential as an industrial raw material. Large scale plantation programmes have been initiated in India under the aegis of the National Bamboo Mission. In the context of global climate change and the urgent need for mitigation measures, the potential of bamboo as a fast growing, multipurpose crop assumes great relevance. When interest in planting bamboo was awakened, it immediately became apparent that propagation on large scale was full of hurdles. The unavailability of seeds in sufficient quantity due to the unpredictable and long flowering periods and short viability meant that use of seedlings as propagules was often not feasible beyond a couple of years after flowering. The technique of macroproliferation (Adarsh Kumar, 1991) is a simple solution to the problem that can meet moderate scales of propagation. The traditional means of vegetative multiplication through simple techniques like rhizome transplantation and rooting of culm or branch cuttings (Banik, 1994), which is the mainstay of bamboo propagation in the absence of seeds, could be further improved by use of rooting hormones and mist propagators but yet does not meet the increased demand for planting material. *In vitro* techniques appeared to have great potential to overcome the constraints in propagation of bamboo on a large scale. Not only is micropropagation a prolific cloning method, it also has unique advantages like the potential for year around production of disease and pest free planting material.

Micropropagation technology

Micropropagation is the use of tissue culture techniques for rapid multiplication of plants from various plant parts. It requires facilities for maintenance of sterile conditions and control over the growth conditions such as temperature and light. Plant tissues are grown on a nutrient media of defined composition and the use of plant hormones gives control over the multiplication and regeneration of plantlets. The current technology of micropropagation in bamboo is based on two different pathways of plantlet regeneration. The first and more common method involves the use of axillary bud proliferation to produce multiple shoots from shoot tips and nodes of seedlings or adult bamboo shoots. In the presence of plant growth regulators especially cytokinins, high rates of shoot multiplication occur over several months during which the shoots are transferred to fresh media at regular intervals after separation into smaller pieces. When sufficient shoots have been produced rooting is carried out in culture usually on a media with auxins or simply by treating them as small cuttings and rooting them in a green house or mist chamber. The second method is somatic embryogenesis in which a mass of fast dividing cells called callus is first produced from meristematic tissues and formation of somatic embryo is induced followed by germination of the embryos to plantlets. Typically somatic embryogenesis has the potential for very high rates of multiplication and is amenable to mechanization using bioreactors.

Achievements in bamboo micropropagation

Research into tissue culture of bamboo has a history of more than two decades. Rapid progress was made, particularly in India, in developing micropropagation methods using seeds or seedlings (Mehta *et al*.,1982; Rao *et al.,* 1996, Gielis, 2001). Efforts have been ongoing to develop techniques to propagate bamboo from tissues taken from adult clumps and the list of species where success has been achieved is

growing (Godbole *et al*., 2002; Lin and Chang, 1998; Ramanayake and Yakandawala, 1997; Nadgir *et al*., 1984; Chaturvedi *et al*.,1993, Saxena and Bhojwani, 1993). From the mass propagation point of view, somatic embryogenesis has a great advantage in the potential for prodigious multiplication rates and amenability to automation. Most of the early reports were those in which seeds were used as explants. Somatic embryogenesis has also been induced in cultures of adult plant origin (Godbole *et al,*. 2002, Shali and Muralidharan, 2006, Muralidharan *et al.,* 2008). The phenomenon of *in vitro* flowering has been reported in about twenty species of bamboo (Gielis, 2002). These include flowering in cultures of seed origin as well as those of mature clumps but no progress has been made in understanding the factors responsible in its control or in putting the technique to any practical application.

In vitro culture of bamboo is at present a focus of research for a large number of groups around the world including India. The shortage of planting material of the important bamboo species for the plantation programmes being envisaged in the country points towards a high demand for micropropagated plants and a renewed interest in developing protocols, overcoming the constraints that prevent wider applicability of the technology for the bamboo species, preferred in plantation programmes.

Challenges

In vitro culture of bamboo from explants taken from adult plants, especially those growing in the field, is beset with problems like high levels of contamination, variability in sprouting of buds, insufficient multiplication rates and difficulty in rooting. The development of an efficient technology applicable for the important bamboo species is therefore still of significance.

Micropropagation of juvenile material like seeds is of limited value since bamboos usually produce copious quantities of seeds when they flower and with proper storage the viability could be retained for a few years. The utility of the technology is rather limited until better juvenile-mature correlation is established or molecular markers can be used to carry out selection for useful traits at the seedling stage. Currently therefore only a technology that permits micropropagation of mature clumps of proven superiority is of any practical significance.

A serious problem with establishment of cultures from plant material collected from the clumps growing in the field is that of high rates of contamination during the initial establishment stage. A major reason for the problem is the presence of endophytes, especially the fungi. Maintaining ramets of the selected stock plants under controlled conditions and prophylactic treatment with systemic antimicrobial compounds and growth regulators will facilitate the availability of explants with better chances of establishment *in vitro*. Other approaches like use of novel compounds like Lactic acid and use of low media pH has also been found to be successful (Saritha and Muralidharan, 2008)

Cloning of the selections from the field requires a suitable technique for large scale propagation. Currently the recalcitrance of shoot cultures derived from mature clumps to rooting is a serious hurdle in

several important species. It would be ideal to have an *ex vitro* rooting method where shoots could be rooted in the greenhouse concurrent with hardening.

Somatic embryogenesis offers great potential as a system suited for large scale production of plantlets. The induction and long-term maintenance of the embryogenically competent callus, the efficiency of plantlet regeneration and the risk of genetic variability are some of the issues that require attention of researchers for making large-scale cloning of bamboo feasible. Handling of somatic embryos in liquid media and improving the efficiency of conversion of embryos to plantlets are other issues where further work is required.

In the context of high cost of production through micropropagation, it is desirable that research should focus on improving efficiency at all stages of *in vitro* culture. A shift from heterotrophy to photoautotrophy can overcome some of these problems. Use of higher light intensity and carbon dioxide levels, improved gaseous exchange in culture containers and a reduction in the levels of sugar are some of the techniques that have been demonstrated in other species to be useful. The use of sunlight as the light source merits attention and initial results appear promising. The benefits of autotrophic micropropagation is realized in the improved hardening and survival rates of the regenerated plantlets (Kozai, 1991).

Significant reduction in energy and recurring costs can be achieved by adopting simple practices like use of stationary liquid cultures. The use of cheaper non-conventional culture containers, use of substitutes for media constituents also have potential in reducing the costs of bamboo micropropagation. Automation of the procedures involved in propagules production is yet another means of cost reduction. The potential for use of bioreactors for multiplication and development of propagule delivery systems particularly using somatic embryogenesis is high since bamboo is amenable to culture in liquid media.

One of the advantages offered by tissue culture is precocious induction of a rhizome *in vitro* (Shirgurkar *et al.,* 1996) which enables the plantlet to harden quickly and survive better after transfer to *ex vitro* conditions and to soil. The rhizome offers further potential for medium term to long term maintenance of germplasm or stock cultures. Further research into understanding the factors influencing rhizome induction in cultures is therefore important.

Crop improvement

To ensure productivity of plantations of bamboo under the wide range of climatic and edaphic conditions, it is essential that sufficient genetic variability is available to enable selection and genotype to site matching. Along with cloning of promising genotypes selected the natural habitat, efforts are required to generate variation and to screen for useful traits particularly for stress tolerance. There is much promise in the application of currently available *in vitro* techniques for generation of somaclones and screening against simulated salinity, drought and pollutants.

In spite of the fact that regeneration of plantlets from tissues is feasible in a large number of bamboo species, it is surprising that no reports of genetic transformation has been reported in bamboo whereas in

other grasses this has been very successful. It can be anticipated that transgenic bamboo will be a reality in the near future.

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"Unless we announce disasters, no one will listen."

-Sir John Houghton, First Chairman of IPCC