

# MICROPROPAGATION OF TEMPERATE AND TROPICAL WOODY BAMBOOS - FROM BIOTECHNOLOGICAL DREAM TO COMMERCIAL REALITY

Johan Gielis and Jan Oprins

OPRINS PLANT N.V., Sint-Lenaartsesteenweg 91, B-2310 Rijkevorsel (Belgium)

Tel: 32 3 314 41 28 Fax: 32 3 314 78 98

E-mail: Johan.gielis@easynet.be

Website <http://www.oprins.be>

## ABSTRACT

World-wide interest in bamboo as a source of biomass in sustainable agriculture and agroforestry systems has increased rapidly in recent years. But while several classical propagation techniques are available, shortages of planting material and depletion of natural resources are both increasing rapidly. The potential of micropropagation for mass scale propagation has raised high hopes and a lot of research has been focused on the development of protocols for large and rapid scale propagation. But despite numerous publications few of the published protocols can be transformed into commercially viable propagation systems.

In our company research focus has been directed towards micropropagation of elite selected genotypes, using mature or juvenile selected genotypes of temperate and tropical bamboos. Currently we produce over 60 different temperate and tropical bamboos commercially, all from selected genotypes. Axillary branching is the preferred *in vitro* method for mass scale production of bamboos, and is highly efficient for large and even mass scale propagation, ensuring clonal fidelity and true-to-type propagation.

In the plant production system of the company special emphasis is placed on the optimisation of the process and of the added value of micropropagation. Added values are especially found in the selection procedures for elite plants and the use of micropropagated bamboos either as planting or propagation material in optimised quality bamboo production systems. Forward integration and product mix are also very important. The influence of micropropagation on bamboo availability and prices is discussed, both for ornamental bamboos and for tropical bamboos used in agroforestry. Other tissue culture techniques such as *in vitro* flowering of mature bamboos, cryopreservation and somatic embryogenesis are also under investigation in our laboratory.

## INTRODUCTION

In the subfamily Bambusoideae (Poaceae) we find both woody and herbaceous bamboos but only woody bamboos have economic potential. Historically woody bamboos have been used for different applications but rather recently interest from paper and wood industries has increased, both for tropical and temperate woody bamboos. Temperate bamboos of the Northern hemisphere are used for agroforestry, mainly in China, and as ornamental plants in Europe and the U.S., although some preliminary trials to use bamboo as source of biomass are ongoing in these regions (it was Thomas

Jefferson who said that the best contribution one could give to a culture, was to add a valuable crop to its agriculture).

Bamboo is often advocated as an ideal renewable resource for biomass, useful for wood and paper industry. Positive arguments thus also include ecological arguments; indeed in the future forests and agriculture, water conservation and carbon cycling will become very important criteria. However, the classical economic criteria (profit and added value) will remain very important. Moreover, if bamboo is to be used as source of biomass, it will have to compete with other plants, not to speak about competitions with industrial powers. This will certainly impose much pressure on bamboo, e.g. regarding selection of elite genotypes, silvicultural methods, new approaches for harvesting and the production of quality biomass. So, the time horizon of mass scale bamboo utilisation may be quite far beyond what the advocates of bamboo foresee (or hope for) at present.

One of the main problems with bamboo is that it has been regarded as a resource which is simply there to take, as has been done for thousands of years by people in rural economies. However, in industrial economies such practice leads to considerable overexploitation and rapid depletion of bamboo resources in the vicinity of the paper mills and factors. Up to the point that transportation costs have become too high for bamboo to be economical (indeed transportation of culms is a lot of air). Estimates regarding future use of bamboo all indicate that there will be an huge shortage for bamboo planting material in medium and long term (Subramanlam, 1994; Nadgauda, 1997). To cope with this forecasted shortage some large or mass scale bamboo planting has been planned/ carried out, such a Green Sarn in Thailand and the projected reforestation in India. Several pilot plantations or larger scale plantings are planned or carried out throughout the world. It is clear that bamboo propagation and silviculture are the pivotal upstream technologies in the whole process. If something fails there, it will have consequences down the line (in the worst case interest in bamboo may fade instead of increase).

The whole downstream process of bamboo production and transformation consists of many different steps. Each of these steps is important, and as in any successful industrial enterprise or chain of processes, optimisation in each step as well as integration of steps and feedback is important. Because, as a general rule, it will be the consumer who has to be satisfied. And the whole production scheme should be aiming at the market. At the production side propagation is crucial for mass scale utilisation of bamboo.

For bamboo different propagation techniques are available, such as seed propagation, clump division, rhizome and culm cuttings (Banik, 1994). But these methods suffer from serious drawbacks when one talks about large or mass scale propagation. Most of the classical techniques for clonal propagation are useful for the production on smaller scale (up to 10 000 plants per year), although some techniques such as macroproliferation (Adaresh Kumar, 1991) can go higher. Much will depend of course on the availability of mother plants. Large scale propagation (between 10 000 and 500 000 plants per year) requires highly efficient techniques. For mass scale propagation (> 500 000 plants per year) classical techniques are largely insufficient and inefficient, and tissue culture is the only viable method.

Indeed, the order of magnitude of the demand for bamboo planting materials indicates that micropropagation will inevitably be necessary for mass scale propagation (Subramanlam, 1994; Gielis, 1995). Classical techniques alone can never solve this problem. It is also important to point out that the

total world production of all tissue cultured plants in 1995, was estimated at 600 million (Debergh, pers.comm.), or less than the total projected needs for bamboo planting material.

## MICROPROPAGATION OF BAMBOO

**A historical note.** By now a large number of papers on micropropagation (the use of TC for propagation only) of bamboos have been published, original papers as well as reviews and some in which tissue culture is described in a more general aspect. Main centre of research has been Asia. The first paper on successful tissue culture is with Alexander and Rao (1968) who described embryoculture. In the eighties there was a considerable increase with propagation of seedlings in tissue culture (Nadgir et al., 1984), the induction of somatic embryogenesis in bamboo seeds of tropical species (Rao et al., 1985), clonal propagation of *Guadua angustifolia* (Manzur, 1988) and other species (Prutpongse and Gavinlertvatana, 1992), and the induction of organogenesis (caulogenesis) in mature bamboos (Huang et al., 1989).

Many researches have focussed on somatic embryogenesis of seedlings of tropical bamboos, eventually culminating in the INBAR publication "Propagation of Bamboo and Rattan through Tissue Culture" (1991). Major Research focussed also on the clonal propagation of elite genotypes, either juvenile and adult. The number of papers about this subject however is much less, and this is solely due to lack of success. Indeed, technically the propagation of adult plants via axillary branching is much more difficult than with seedlings of tropical bamboos. An inventory (BIC, 1994) shows that at a certain point at least 21 labs in South-east Asia were involved in bamboo tissue culture, mainly in India (reviews on tissue culture of bamboo can be found in INBAR, 1991; Saxena and Dhawan, 1994; Zamora, 1994; Nadgauda et al., 1997).

**A critical note.** Micropropagation via tissue culture attracted a lot of attention since it was believed that this method could solve most or at least many problems in propagation of bamboo. This biotechnological dream was strengthened by a large number of publications. However, the translation and transformation of these expectations into commercially viable propagation systems has been beset with a number of problems (which is in part normal in technological processes). Main problems were either technological, or related to marketing. The published research all lacked crucial parts. They were either not very efficient, or not applicable on other bamboos, or both.

The use of starting material (seeds or adult plants) and the choice of the propagation method are crucial. The two major advantages of using seedlings is that it is a new generation, and that the technology is easier. But the disadvantages are very considerable: (1) insufficient or no knowledge of genetic background, (2) restricted availability of seeds for most species and rapid loss of germination capacity, and (3) comparison of *in vitro* to *in vivo* performance has not been thoroughly evaluated. When using adult bamboos main problems are: (1) endogenous contamination, (2) hyperhydricity and instability of multiplication rates, and (3) many problems with rooting also in bamboos that root readily in nature. Rooting percentages for adult bamboos ranged from very low percentages to 73% for adult *Dendrocalamus longispatus* (Saxena and Bhojwani, 1993). Rooting percentages of 77% were obtained for adult *Dendrocalamus giganteus* in 3 or 4 weeks only (Ramanayake and Yakandawala, 1997). But, while 77% of success is good on 500 plants in a laboratory trial, it means a loss of 33 000 when you transplant 100 000 plants.

Initial trials for commercialisation were set up in some commercial companies in different parts of the world. An unknown number of commercial laboratories succeeded in propagating one or more genotypes of bamboo. Some of the originally produced bamboos were of bad quality, due to insufficient identification of the mother plants or due to the chase for short term profit.

In this way tissue culture gets bad press, while it is very important to show positive results, for market penetration; time to market is far away and feedback times are considerable (in fact 30 years or longer for flowering observation). It is not easy to convince foresters to invest, even with data which seem very convincing. But TC has to adapt to the market and not vice versa (or at least not in the first instance). Micropropagation is a tool, not an aim.

**A personal note.** Our view of these problems was that we had to develop a universal system for all bamboos that was highly efficient for the propagation of selected genotypes, at the same time ensuring genetic stability. In short tissue culture as *Best Available Technique*. This technology had also to be integrated in the plant production scheme in the company. With regard to commercialisation our strategy should combine the production of high quality plants with product mix and market diversification (both temperate and tropical species) and forward integration in the downstream process of bamboo propagation and transformation.

## SELECTION OF ELITE GENOTYPES

Because of the peculiar flowering habits in bamboo it has been almost impossible to breed for superior traits in woody bamboos. Bamboos are natural polyploids and a lot of variability occurs within seedling or natural populations (at infraspecific level in general), in tropical as well as in temperate bamboos (Banik, 1995). In seedling populations variability between genets occurs at the genetic and the phenotypic level. The various characteristics and properties are quite variable, and in general, overall characteristics are unfavourable for biomass production. Selection in existing populations to improve overall quality or yields is possible. Clonal forestry based on one or more elite selected genotypes, propagated via clonal propagation, allows a considerable "genetic" improvement (in this sense genetic refers to better yields, quality etc.).

Population enhancement of bamboo is relatively easy in first approach: selecting the plus clones (e.g. top 20 %) and using mass propagation for upscaling these plus clones will result in more homogeneous populations, with higher yields. The selection and subsequent vegetative propagation of superior clones allows to improve bamboo germplasm considerably, compared to natural populations. Simple selection procedures such as this can also be done for seedling populations. It is important to select clones in such a way that a broad genetic basis is maintained, since many tropical bamboo are natural monocarps (Gielis and Oprins, 1998).

Among the large number of species of woody bamboos, the economically most promising species can generally be used for a variety of purposes. Selection criteria depend on biological characters of the bamboo, either macro- and micromorphological and anatomical, directly related to ultimate transformation, or related to biomass production. Selection procedures should be refined for two main reasons. First is that the quality requirements imposed by industry (or some international standards) will get more severe, especially when bamboo is used as substitute for (and in direct competition with)

existing wood based biomass transformation. Secondly, refining the selection procedures, incorporating morphological, physico-chemical, anatomical characters, as well as molecular markers, will also allow to speed up selection through early assessment of seedlings considerably.

Molecular markers have been proposed as tools for identification of bamboos (fingerprinting), assessment of variation, and identification of characters or traits. We apply AFLP<sup>TM</sup>, which is considered to be one of the most sensitive and most reproducible methods among different laboratories. The advantage of AFLP patterns is that the same fingerprint can be used for identification, taxonomy, the study of natural variability and early assessment. It was shown that differences in DNA isolation procedures can seriously affect AFLP patterns (Gielis et al., 1997b). But DNA based markers are still in their infancy. In general they represent data not necessarily connected to phenotypic characters or loci in the strict sense, covering only a limited part of the genome. Therefore incongruences between morphological and molecular data can be expected and any result should be interpreted only with a maximum of precaution (Gielis et al., 1997a; Gielis et al., 1997b). But despite these difficulties molecular markers can be very helpful in the detection of useful characters and traits, or in the detection of elite genotypes using multivariate analysis, in careful combination with phenotypic markers.

In any case the selection of elite genotypes is at the basis of clonal forestry and at the same time the propagation of selected genotypes is one of the major advantages of micropropagation. In a very short period of time (e.g. 1 year) 100 000 or even 1 000 000 copies can be made from one single elite clone.

### **GENETIC STABILITY IN MICROPROPAGATION**

In tissue culture 3 basic methods exist for the propagation of plants (i.e. micropropagation): (1) *axillary branching*, which miniaturises the natural process of branching, (2) *organogenesis*, the neoformation of organs or shoots, and (3) *somatic embryogenesis*, the regeneration of embryos from somatic cells. In bamboo all these three methods have been investigated, but in bamboo both organogenesis and SE involve an intervening callusphase.

Tissue culture has been associated with molecular aberrations in general (including also chromosomal defects), and phenotypic aberrations may show up only some years later. To avoid such problems one should be very cautious using such techniques, especially when methodologies are used which are prone to higher chances of such genetic aberrations. Even when using axillary branching, the hifi technique in tissue culture, caution must be taken. The bulbous internodes of *Bambusa ventricosa* were lost after tissue culture (Huang and Huang, 1995) and variegated leaves appear very often during tissue culture in many species.

For large or mass scale production, efficiency of the propagation methods is important, but perhaps even more important is the genetic stability. The evaluation of clonal stability is very difficult and time consuming in bamboos and since feedback times are very long in bamboos to monitor the genetic stability, ensuring genetic stability is very important. The molecular tools to test for genetic stability are in general very rough approximations. Use of molecular markers may reveal some

differences but to detect a single gene mutation is very difficult and time consuming. Use of other tools such as flow cytometry to check ploidy level do not provide any information at the molecular level. So molecular tools to detect variation are not a watertight system. Therefore the best strategy seems to be the most prudent: use axillary branching as method for clonal multiplication.

From this perspective it is surprising that most of the efforts in bamboo have concentrated on the use of seeds and somatic embryogenesis for mass scale propagation (INBAR, 1991). For tropical bamboos like *Dendrocalamus giganteus*, *D. strictus* and *Bambusa bambos*, seeds are readily available in India. Certainly the ease of somatic embryogenesis in these species has attracted most attention, but somatic embryogenesis is a process with the higher chance for aberration at the molecular level. In fact, very few (if any) plants are micropropagated commercially via somatic embryogenesis, especially because of clonal fidelity (Cervelli and Senaratna, 1995). Only in bamboo, few people seem to care about this.

In most plants produced on large scale, axillary branching is used since this methodology has a lesser risk of aberration. All shoots originate from preformed buds, and no neoformation of organs is involved, also not in nodule cultures of pseudospikelets (Gielis, 1998). We have selected axillary branching as our method of choice for propagation of bamboo.

### **AXILLARY BRANCHING: A UNIVERSAL TECHNIQUE FOR BAMBOO**

From the beginning we had been confronted with the lack of good starting material. While for some tropical bamboos seeds were available for tissue culture studies, this was not so in temperate bamboos, where only adult material could be used. The development of an appropriate technology was thus technically far more difficult, especially if we wanted to develop a universal protocol. But once we had found how to do it, the technology worked with all bamboos. At present the remaining bottle necks for axillary branching, have been solved. Basic research has allowed to identify the endogenous factors involved in senescence and browning of explants, in hyperhydricity and variability of multiplication in general, and in inhibition of rooting of *in vitro* bamboos (Gielis, unpublished results).

In our laboratory commercially feasible micropropagation for large scale propagation is practised for species and varieties of *Arundinaria*, *Chimonobambusa*, *Fargesia*, *Phyllostachys*, *Pleioblastus*, *Sasa*, *Sasaella*, *Semiarundinaria*, *Shibataea* and *Yushania* (temperate bamboos) and *Bambusa*, *Dendrocalamus*, *Dinochloa*, *Oxythenanthera* and *Thyrsostachys* (tropical taxa). In fact with our technology it will probably be possible to propagate any bamboo (Gielis and Oprins, 1998). In total we propagate over 60 different species and cultivars of bamboo, with main emphasis on ornamental bamboos (*Phyllostachys*, *Fargesia*, *Semiarundinaria*....) and on tropical bamboos for reforestation and plantation (*Bambusa* and *Dendrocalamus* species).

In our greenhouse *ex situ* conservation of elite plants is combined with stage 0 conditions (Debergh and Maene, 1981), which allows to facilitate the initiation in tissue culture. In micropropagation practise, Stage 1, the initiation stage, involves all subcultures until a constant and stable multiplication rate is obtained. If prepared under proper Stage 0 conditions for about one month, the time for Stage I is reduced to less than 4 months for most tropical and temperate species, but for

some it can take up to 8 months. After this period already more than 1000 plants per species enter the production line (Stage II). Multiplication rates vary according to species from 3-10 every 3-4 weeks depending on the species (in practice multiplication is kept below 6). Within 6 or 7 months from initiation 100 000 plants can be produced, which corresponds to theoretical calculations (or rather earlier expectations) of TC technology. One skilled operator can easily transfer between 7-10 000 plants daily: indeed in handling clumps of 3-5 plantlets each, the transfer of clumps is much more efficient. New cultures are initiated at least every 2 years and stay in production for a maximum of about 20 cycles.

In our laboratory rooting is achieved either by a separate rooting medium or by administering liquid medium (Stage IIIb; Debergh and Maene, 1981). Thereafter plants are transplanted with 98-100% success under conditions of 100 % relative humidity. Under those optimal weaning conditions, rooted plantlets of e.g. most *Phyllostachys* and *Bambusa's* can be potted already 10-14 days after transplanting in the greenhouse. Rhizome development is equally fast. E.g. *Pleioblastus auricomus* rhizomes are visible already 8 weeks after transplanting.

In the classical scheme of tissue culture laboratories, the tissue culture phase terminates at Stage III, but in regard to marketing of plants one can also distinguish subsequent stages: (1) Stage IV, the transplantation stage with the end product a rooted plantlet in trays, (2) Stage V, the production of liners, either for production of saleable plants or for use as micromotherplant, and (3) Stage VI, the production of saleable plants. This distinction is important if the complete chain of production is integrated in a single company, since this determines the added values.

## COMMERCIAL MICROPROPAGATION OF WOODY BAMBOOS

A selected number of private laboratories have succeeded in mass propagating one to many bamboos, either from seedlings or from mature plants. Micropropagation is much more efficient when developed in private companies, when cost-benefit analyses have to be made and optimal strategies for a efficient implementation and integration of micropropagation into a complete plant production scheme, have to be developed and adapted when and if necessary.

While in most commercial plant production the tissue culture lab is a separate entity, forward integration is almost a necessity if a company wants to mass produce bamboos. Forward integration involves the integration of several steps of plant production in one company, sometimes also beyond the production part. This can have different goals: (1) to be able to monitor how the micropropagated plants perform downstream, (2) to get sufficient feedback to improve either selection or technology, (3) to increase the added value of the plant and/or (4) to improve market penetration by increased know-how of several steps in the downstream process.

Forward integration of bamboo tissue culture can indeed be rewarding. A straightforward comparison shows this: in Europe plants of *Phalaenopsis* selections and clones are sold at 0.25 \$ by a tissue culture lab. After hardening and growing, and through distributors, the ultimate retail price is between 10 and 20 US\$, so that the price of the tissue culture plant is between 1.25 and 2.5% of the retail price. In *Ficus benjamina* the situation is even worse. The TC lab sells clumps for about 0.25 US\$, but after hardening between 15 and 20 cuttings are taken from this plant, rooted and sold as liners to growers. Retail prices for certain sizes are around 10 US\$. So here, the price of the TC plant makes

up only 0.125-0.25% of the retail price. To generate sufficient turnover for the lab a large number of plants have to be produced.

In contrast, if the laboratory is integrated in the company, the situation is very different, especially when one operates in small niches in the market. For a *Hydrangea* plant from tissue culture, whole-sale prices after 6 months growth range from 2-4 US\$ depending on the species. For the company to have the same turnover as the big laboratories producing *Ficus* or orchids, only one tenth of the number of plants have to be produced; indeed the added value is gained on the saleable plant. At the same time this strategy can create and protect (stabilise) market niches. We try to apply the same strategy for bamboo. Besides increasing the added value for the plant producer/seller it also avoids a downward spiralling of bamboo prices in general, and tissue culture bamboos in particular.

In fact we aim at a product mix: tissue culture bamboos used for (1) production of saleable plants (end of Stage VI), sold only at this stage, (2) production of liners (end of Stage V), (3) hardened plantlets in trays (end of Stage IV). We aim at producing tissue culture plants which give a distinct added value to the company (new genotypes, high quantity and quality) and use those in the most profitable way.

With respect to return on investment, the higher added value will of course be for saleable plants, but faster return on investment is obtained for direct sale of tissue cultured plants. In terms of time to market the earlier sales can indeed be more profitable. On the other hand, this also is practical: logistically it is quite difficult to grow e.g. 2 million TC bamboos per year further to saleable plants. Most of the production of tropical bamboos is directly exported to several tropical countries for planting.

## **THE FUTURE IS HERE AND NOW**

The development of micropropagation systems for bamboos initially caused great excitement. But new technologies, or developments, often evolve in a typical way referred to as "double vision forecasting". A new technology can generate mass excitement and its short term impact is generally overestimated; because of the slow diffusion of the technology, time lags must be taken into account and short term expectations are not met. How interesting and promising the new technology may be, the initial excitement will fade and at the same time creates an under expectation of medium and long term results when the technology ripens. In bamboo tissue culture, problems of technological and of logistic nature have slowed down the development. Logistic factors involve the collection of elite germplasm, the incorporation of the novel technology in classical plant production and all aspects involved with market penetration.

We have presented the evolution of micropropagation of bamboos, starting from a biotechnological dream where tissue culture would provide the solution, to a down-to-earth practical and commercial reality. The problems to transform research papers into commercial technologies are numerous and include endogenous contamination, browning, lack of efficiency in rooting and transplanting, the choice of a specific propagation method, the choice of appropriate starting material, and so on. In this contribution we have outlined the approach we have adopted to develop a commercially feasible process. It involves three major components: (1) fundamental research on bamboo physiology and genetics, (2) the development of axillary branching into a universal technique

with a high clonal fidelity and very high efficiency, and (3) forward integration to optimise the added value of micropropagation.

In conclusion, it is now possible to micropropagate almost any selected bamboo at mass scale in a short time frame. The hypothetical numbers of propagation and short time frames proposed in earlier research papers, have been realised. The efficiency and time frames given in this paper are real figures and real productions, not on laboratory scale. Huge numbers of bamboo can be transported from lab to site of planting within 48 hours or less anywhere in the world. The quality of young plants derived from tissue culture is generally excellent. The plants are vigorous growers and in many ornamental bamboos the quality of plants of 1 year old derived from tissue culture is considerably better than of those propagated via conventional methods of propagation.

Micropropagated plants are also price competitive with other plants (if one includes quality and other long term factors), while for mass propagation of many bamboos, micropropagation is the only technique. As predicted some years ago (Gielis, 1995) micropropagation via axillary branching is a universal technique but above all, it is the *Best Available Technique* and will become the standard for mass scale propagation of bamboos. A comparison with *Ficus benjamina* seems appropriate: nobody in the tropics would even consider propagating a weedy fig tree via tissue culture. It has little value and can be propagated easily via cuttings. Yet, all *Ficus* plants produced in Europe today (about 50 000 000) derive from tissue culture; micropropagation has replaced propagation via cuttings completely.

Micropropagation of bamboo has also transformed into a medium technology at the same time the technology matured. So it can be applied on large and mass scale. But diffusion of technology will have to be based on normal market rules, such as maximising profits (profits are higher on ornamentals than for reforestation), protection of know-how (via patents or black-box), and technology transfer on a contract basis. Private companies are generally much more efficient, especially for production. Bamboo and its future are too important to leave it only to the scientists.

## REFERENCES

- Adarsh Kumar, 1991, "Mass production of field planting stock of *Dendrocalamus strictus* through macroproliferation- a technology". Indian Forester 117, 1046-4052.
- Alexander, M. P., Rao, T. C., 1968, "*In vitro* culture of bamboo embryo". Current Science 37, 415.
- Banik, R.L., 1994, "Review of conventional propagation research in bamboos and future strategy" . Constraints to the production of bamboo and rattan. INBAR Technical Report N°5 (Delhi), 115-142.
- Banik, R. L., 1995, "Selection criteria and population enhancement of priority bamboos". In: J.T. Williams, I.V. Ramanuja Rao, A.N. Rao (Eds.) Genetic enhancement of bamboo and rattan (New Delhi- INBAR Technical Report N°7), 99-110.
- Cervelli, R., Senaratna, T., 1995, "Economic aspects of somatic embryogenesis". In: Aitken-Christie J., Kozai T., Lila Smith M. (Eds) Automation and Environmental Control in Plant Tissue Culture (Kluwer Academic Publishers), 29-64.

- Debergh, P.C., Maene, L., 1981, "A scheme for commercial propagation of ornamental plants by tissue culture". *Scientia Horticulturae* 14, 335-345.
- Gielis, J., 1995, "Bamboo and biotechnology". *European Bamboo Society Journal*, May 6, 27-39.
- Gielis, J., Everaert, I., Goetghebeur, P., De Loose, M., 1997a, "Bamboo and molecular markers". *Bamboo, People and the Environment, Volume 2 Biodiversity and Genetic Conservation (INBAR Technical Report N°8)*, 45-67.
- Gielis, J., Ngoc, L.H.T., Angiolillo, A., Gerats, T., Van Gysel, A., Breyne, P., 1997b, "AFLP™ analyses in bamboos: Influence of DNA extraction procedures". *Mededelingen Faculteit Landbouw Universiteit Gent 64/2a*, 1471-1475.
- Gielis, J., Oprins, J., 1998, "Biotechnological approaches to germplasm improvement in woody bamboos". In: El Bassam, N., et al. (Eds) *Sustainable agriculture for food, industry and energy* (in the press).
- Gielis, J., 1998, "Micropropagation and in vitro flowering of temperate and tropical woody bamboos". (in the press).
- Huang, L.C., Huang, B.L., Chen, W.L., 1989, "Tissue culture investigations of bamboo IV-Organogenesis leading to adventitious shoots and plants excised from shoot apices". *Environmental and Experimental Botany* 29 (3), 307-315.
- Huang, L.C., Huang, B.L., 1995, "Loss of the species distinguishing trait among regenerated *Bambusa ventricosa* McClure plants". *Plant, Cell, Tissue and Organ Culture* 42, 109-111.
- Manzur, D., 1988, "Propagacion vegetativa de *Guadua angustifolia* Kunth". *Agronomia* 2(3), 14-19.
- Nadgauda, R.S., John, C.K., Joshi, M.S., Parasharami, V.A., Mascarenhas, A.F., 1997, "Application of *in vitro* techniques for bamboo improvement". In: Chapman, G. (Ed) *The Bamboos* (Academic Press), 163-177.
- Nadgir, A.L., Phadke, C.H., Gupta, P.K., Parsharam, V.A., Nair, S., Mascarenhas, A., 1984, "Rapid multiplication of bamboo by tissue culture". *Silvae Genet.* 33, 219-223.
- Prutpongse, P., Gavinlertvatana, P., 1992, "*In vitro* micropropagation of 54 species from 15 genera of bamboos". *HortScience* 27, 453-454.
- Rao, I.V. Ramanuja, Rao, I.U., Narang, V., 1985, "Somatic embryogenesis and regeneration of complete plantlets in the bamboo *Dendrocalamus strictus*". *Plant Cell Reports* 4, 191-194.
- INBAR, 1991, Rao, I.V. Ramanuja, Yusoff, A.M., Rao, A.N., Sastry, C.B. "Propagation of bamboo and rattan through tissue culture", INBAR (Delhi. IDRC, 1991), 60 pp.
- BIC, 1994, "Bamboo researchers and projects of south and south-east Asia. A directory". *Bamboo Information Centre India*, Trichur, India.
- Ramanayake, S.M.S.D., Yakandawala, K., 1997, "Micropropagation of the giant bamboo (*Dendrocalamus giganteus* Munro) from nodal explants of field grown culms". *Plant Science* 129, 213-223.
- Saxena, S., Bhojwani, S.S., 1993, "*In vitro* clonal multiplication of 4 year old plants of the bamboo *Dendrocalamus longispatus* Kurz". *In Vitro Cellular and Developmental Biology* 29P, 135-142.
- Saxena, S., Dhawan, V., 1994, "Micropropagation research in south Asia". *Constraints to production of bamboo and rattan. INBAR Technical Report 5 (Delhi)*, 101 - 113.
- Subramaniam, K.N., 1994; "Bamboos – Demand and supply of planting stock". *INBAR Newsletter N°5*, 24-25.
- Zamora, A.B., 1994, "Review of micropropagation research on bamboos". *Constraints to production of bamboo and rattan. IN BAR Technical Report 5 (Delhi)*, 45-100.