Session 8. INBAR

Table of Contents

Temperate bamboos in ornamental horticulture: differentiators and spillover effects into the 21st century.

Johan Gielis

Department of Bioengineering Sciences
University of Antwerp
Groenenborgerlaan
Wilrijk-Antwerpen, Belgium

Abstract

To understand the valorization chain of ornamental bamboos in ornamental horticulture, the historical foundations and specific differentiators that made mass production and marketing of ornamental bamboos possible are key. Based on a long tradition, these differentiators comprise the implementation of research results into propagation and production methods, together with modern (and very demanding) methods of marketing and branding with an increasing focus on clumping (non-invasive) bamboos. Such quality standards are much more demanding than for bamboo production in traditional forestry, but the same differentiators that drive ornamental bamboo, can lead to positive spillover effects, with applications of the developed technologies for mass propagation and genetic improvement for the 21st century in forestry and agroforestry with bamboo.

Outline of the paper

The production and trade of bamboo in ornamental horticulture has been increasing rapidly in the past decades. Sales have been rising year after year. The market for ornamental bamboos in Europe has increased by a factor of at least ten compared in the past 15 years, and an estimated 5 million bamboos are produced and sold today, not only as garden plant, but also for terraces and balconies and for public greening. More and more growers produce bamboo in larger numbers and of a quality that is much better than ever. Whereas bamboos were sold almost exclusively in garden centers earlier during limited periods of the year, top quality bamboos are now supplied year-round into markets and distribution channels that have been created by the ornamental industry for foliage and flowering plants in the past. This has required a complete rethinking of the supply and logistics chain of bamboo for a year-round production of top quality plants, in pace with the demanding developments in horticultural industry.

A quantification of the valorization chain of ornamental bamboos from real economic data is virtually impossible, neither in Western Europe nor in the USA. In trade of bamboo products, the plants are no separate category. Within horticulture, with cut flowers and vegetables as major products ornamental garden plants is only a minor part. And within this sector, bamboo and ornamental grasses constitute only a minor part of garden and orangery plant production. Partial market data are available but give no insight in the development of the sector. A qualitative approach, rather than a quantitative may be more valuable.

To understand the valorization chain of ornamental bamboos in ornamental horticulture, focus can be placed on the historical foundations and specific differentiators that made mass production and marketing of ornamental bamboos possible. Based on a long tradition, these differentiators comprise the implementation of research results into propagation and production methods, together with modern (and very demanding) methods of marketing and branding with an increasing focus on clumping (non-invasive) bamboos. Such quality standards are much more demanding than for bamboo production in traditional forestry, but the same differentiators that drive ornamental bamboo, can lead to positive spillover effects, with applications of the developed technologies for mass propagation and genetic improvement for the 21st century in forestry and agroforestry with bamboo. This analysis will draw for a large part on the research & development strategies and experiences of Oprins Plant of the past 25 years.

Ornamental horticulture in the West

The current trend, a wave of interest in bamboo as ornamental plant for gardens in Western Europe and to a lesser extent in the USA, is a revival rather than a new trend. Its foundations go a long way back. Horticulture and landscaping have been dominant factors of European culture mainly in the four past centuries. From the 17th century onwards there was a huge increase in collections from Asia and the New World, hotspots of botanical diversity.

Bamboos, as giant grasses, although already known in Europe from ancient times as Indian Reeds, attracted particular attention. The first known introduction of temperate bamboo into Europe (England) was the remarkable black bamboo *Phyllostachys nigra* around 1827 (timeline comparison: Belgium became an independent nation in 1830). It was reintroduced in France in 1846 , and since then collectors have introduced various temperate and tropical bamboos into Europe (Houzeau de Lehaie, 1906, 229-230) .

At the turn of the century, moving into the 20th century, there were many public gardens with bamboos all over Europe, from Scotland to the Black Sea, and many more private collectors, as can be read in "*The introduction, acclimation and culture of bamboos in Western Europe and esp. in Belgium*" (Houzeau de Lehaie, 1907, N°9&10, page 227). The most beautiful specimen were found in the botanical gardens of Italy (according to H.d.L.), and at that time the bamboo collection in the botanical garden of Edinburgh had over 40 species of bamboo, more than Kew. The Bambuseraie in Prafrance was one of the many private gardens. It had been established in 1855 by Eugène Mazel, and came into the hands of the Nègre family in 1902.

Actually history is always a good lesson for those who think that taxonomy, or science and technology started only yesterday. Bamboo has been used in technology long BCE in the Orient, but also in the West it was used in cutting edge innovations in technology. For example, in 1854 German Henricg Globel used a carbonized bamboo filament placed inside a glass bulb. He thus invented the first true light bulb. Edison had experimented with light bulbs from the 1870's onward and around 1880 he improved his light bulb until it could last for over 1200 hours using a bamboo-derived filament. In 1909 the Brazilian Santos-Dumont built an aeroplane (Demoiselle) made with linen and bamboo.

The 19th century was also a golden age for bamboo taxonomy, with scientific descriptions of many genera and species from all over the world. The genus *Bambusa* was described by Schreber in 1780 (herbaceous bamboo *Olyra* was described by Linneaus in 1759) but the majority of genera was described in the 19th century. Ohrnberger's *Bamboos of the World* (1999) also has a list of 'Generic Names in Chronological Order'. Names of botanists like Ruprecht, Trinius, Munro, Gamble, Jussieu, Bentham, Kunth, Franchet are still very well known. *Arundinaria* was described by Michaux in 1803, *Phyllostachys* by Siebold and Zuccarini in 1843 and *Fargesia* in 1893 by Franchet.

In the early twentieth century, Jean Houzeau de Lehaie (Mons, Belgium) published the first bamboo journal *Le Bambou, son Etude, sa Culture, son Emploi.* The opening paragraphs clearly describe the motivation (in French, English and German):

> "*Before creating the present periodical, we considered carefuly if it would not be better to ask from one of the influential horticultural papers, to insert our notices under a special title. But we feared, on one side that the paper should not reach the persons of different nations whom concern bamboo ; on the other side, that the necessarily little room granted to this one underfamily should nearly drown our notices among other articles.*
>
> *So we resolved to issue a periodical bulletin – probably monthly – totaly kept to the study of Bamboo on every way: scientific, horticultural and commercial and to the general questions thereto belonging. Our aim is the facility for botanists and lovers of Bamboo of communicating their studies and desiderata and exchanging their observations*".

Le Bambou, published from 1906-1908, is still a very rich source of information on the various collections and bamboo lovers in Europe and of the growth of bamboo in Belgium. It documents the intense communication between 'lovers of Bamboo'. In this period many books were already available: A *Monograph of the Bambusaceae* by Colonel Munro in 1866, *Les Bambous* by A. and C. Rivière in 1879, *Japanese Bamboos and their introduction into America* by David Fairchild in 1903, *The Bamboo Garden* by Freeman Mitford in 1896, and later *Les Bambusées* by Camus in 1913. Bamboo collectors, bamboo as ornamental for gardens, bamboo networks, journal and newsletters are not new at all.

At L'Ermitage, Houzeau de Lehaie cultivated well over 100 different types of bamboo and described various species of *Phyllostachys*. His efforts should be viewed in a general horticultural framework in Belgium. In 1968 Robert De Belder gave his impression on the development of horticulture in Belgium as follows (Adriaenssen, 2005):

> *"The nineteenth century was definitely the Golden Age for horticulturists in Belgium. Even more than in other European countries, many people were concerned with horticulture and were cultivating all sorts of exotic plants. At that time, an impressive number of species and varieties, usually tropical and subtropical ones, were introduced into the country. People kept*

> *these plants under glass and every large house in the country-side had its greenhouse: a hothouse, a temperate house and an orangery.*
>
> *We had in the nineteenth century several keen and famous botanists and horticulturists; Names like Louis Van Houtte, Ambroise Verschaffelt, Van Hulten, Morren, Bijls and Donckelaer are still well known in the horticultural world of today. An amazing number of periodicals were also published in Belgium during that century. They were publications of the highest standing, with good lithographic plates, comparable with the best ones in this country. Let us mention just a few:* Flore des Serres et des Jardins de l'Europe, L'Herbier de l'Amateur de Fleurs, Journal Horticole, La Belgique Horticole, Reveu de l'Horticulture Belge *and* Illustration Horticole. *Unfortunately, these lovely publications were too ambitious, and had to be discontinued. However they are still of the highest documentary interest for the study of horticulture".*

On his worldwide trip, David Fairchild (1947) was particularly charmed by Belgium:

> *"Belgium is full of horticultural interest, for the Belgians as a people are interested in plants. If they were not, one would not find everywhere all sorts of fruit trees espaliered against the walls of their houses. If you have ever tried to espalier a tree against a wall and every spring and every summer pruned back its hundreds of branches, or pinched out the buds which were not in the right place, you will be in a position to understand the Belgium patience in matters of plant culture. They seem to love to garden. They do not seem to be so restless as Americans, they love to stay at home and they build the romance of their lives right about them where they are, instead of trying to get it by gadding about over the surface of the earth….*
>
> *M. Charles Vuylsteke has spent his life in a comfortable but modest house just off the main street of Lochristi. Behind his dwelling are the orchids houses, where, for over fifty years he has spent most of his waking hours, for he began young with a passion for orchids. He cross-pollinated a species of* Odontoglossum *from the mountains of tropical America with* Cochlioda nutzliana*, an insignificant looking orchid from Peru…. The result was a beautiful new hybrid. From that time on he has been hybridizing orchids and sending the flowers to the great cities of Europe, where he charges his own price for them. He became one of the best-known men in the horticultural world, at least in that part which is interested in orchids. Never in my life have I seen such beautiful masses of orchids in bloom, and the handsomest of all were the Odontiodas, which had originated through the skill of this quiet, unostentatious man. When one thinks of the pleasure his orchids give, and of how they are worn at banquets and balls by the most beautiful women of society, is it surprising that M. Vuylsteke found a great satisfaction in having been the means of bringing such beauty into existence? He has the quiet lasting satisfaction of having created something, rather than the evanescent one of having merely seen something."*

Creating something new for a lasting satisfaction is deeply encoded in our genes. The twentieth century however was a dark period for Europe with World Wars I and II and the economic crisis of 1930, with a major impact on horticulture. De Belder:

> *"The Belgium amateur was mainly interested in greenhouse plants, unlike his British colleague, who collected all kinds of hardy plants in his garden. Perhaps as a result of this, practically nothing is left in Belgium of that glorious period. Devastation during two world wards, the economic crisis of 1930 and the evolution in taste and fashion are responsible for this situation.*
>
> *Most of the nurseries that provided the Belgian and foreign amateurs with plants were located at Ghent. This town is still an important nursery centre. However, the collections*

> *have disappeared and only commercially grown plants are produced:* Ficus elastica, Clivia, Sansevieria, Azalea indica *and tuberous begonia's".*

Robert De Belder's quote shows not only regional differences (and similar stories could be told for other countries as well), but points to 1) the devastations of large collections in the 20th century, 2) the revival of ornamental horticulture but with focus on a very limited number of commercial plants.
Things have changed however, and in the forty years following Robert De Belder's 1968 address to the Royal Horticultural Society (of which he was vice-president), we have witnessed an explosive growth of plant collections and awareness, and the applications of fundamental research for mass production and genetic improvement of ornamental plants.

Botanists, plant collectors and plantsmen

Only in a limited number of collections, mainly botanical gardens, collections of bamboo survived the world wars (Prafrance, Kew, ...) and only from the seventies onwards we witness an important increase in the interest in bamboo as an ornamental plant in Europe with the World Bamboo Congress at Prafrance in 1988 and the establishment of various national bamboo societies in the 1980's. Exchanges between plant collectors and botanical gardens (both public and private) intensified as well. Through connections between Belgium and Japan, the garden of Hemelrijk (private domain of Jelena and Robert De Belder) obtained about 69 different bamboo species, and Harutsugu Kashiwagi, the later director of the Fuji Bamboo Garden with over 450 species, came to Hemelrijk as a student (H. Van Trier, pers. comm.; Borin, 1995). To be sure, such exchanges were not only important for bamboo, but for other plant groups as well.
The commercial side for bamboo was initially driven by a limited number of larger players, with as distinguishing feature the entrepreneurial drive of Yves Crouzet, Wolfgang Eberts and Jan Oprins and others. However, it is also due to the enthusiasm of many collectors and gardeners that the current trend in bamboo sales finds it origin. Evolving from a limited number of species to the explosive growth of number of bamboo species into horticulture and horticultural trade has largely been the work of plant enthusiasts and collectors. It is here also that the 19th century spirit of collection and showcasing is found once more. One of the notions is that of a *plantsman*. David McClintock (Raphael, 1979) gave a simple definition:

> *"A plantsman is one who loves plants for their own sake and knows how to cherish them. This… concept… may include a botanist: it certainly includes a host of admirable amateurs who may not know what a chromosome looks like or what taxonomy means, but they know the growing plant, wild or cultivated, first-hand. To my mind they are the cream of those in the plant world, a fund of invaluable first-hand information."*

Building on traditions, Europe has some past and present notable plantsmen, like David McClintock and Jelena and Robert De Belder; the De Belder's biography (Adriaenssen, 2005) is a must read for any plant lover. In the bamboo world *plants(wo)men* like Houzeau de Lehaie, Gaston Nègre, David McClintock, Peter Addington, Max Riedelsheimer, Susanne Lucas, Jacques Van Dooren and Jos Van der Palen, are well known to those who witnessed the development of bamboo (and many names could be added, also from the USA). In many famous societies they play an important role. David McClintock was not only president of The Bamboo Society, the Heather Society and the Wild Flower Society, but also vicepresident of the International Dendrological Society, which was founded by Robert and Georges De Belder. Susanne Lucas served long as ABS's president, founded the World Bamboo Organisation and is a board member of the International Plant Propagators Society.
Their roles, in view of the valorization chain of bamboo, include collecting and disseminating genotypes, networking and evangelizing or including bamboos in important trade shows. One of Belgium's oldest organizations on ornamental plants organizes the Floralies of Ghent every five

years, a world-class event, where Jan Oprins now serves as its director. In 1906 Houzeau De Lehaie had convinced the organizers to include bamboo in the Floralies of 1908, but unfortunately spring was too early to have good foliage. The production methods have now allowed for excellent bamboo quality year round, so that in the most recent edition bamboos dominated the Floralies and obtained the second prize in "loveliest ornamental grasses".

The role of collectors and plantsmen is not that of a scientist or botanist and very often this has led to incongruences. For example in *The Bamboos*, published on the occasion of the international congress on bamboo by the Linnean Society in 1996, a valuable contribution of David McClintock was not deemed valid for publication (It was then published in Dutch translation in the *Newsletter of the Belgian Bamboo Society*; McClintock, 1996). But their role remains decisive and their observations and conclusions help decide which plants can be successful for our gardens. David Fairchild's (1947) continues:

> *I am sure there are scattered through Belgium many such plantsman as this orchid hybridizer (Charles Vuylsteke), but only one other was I fortunate enough to see, Houzeau de Lehaie who has gathered around his ancestral château at Mons a rare collection of bamboos. He has acquired these mainly by correspondence and through his acquaintance with the directors of botanical gardens all over Europe. He has set them out in the excellent soil of his garden wherever he had room for them, and through that knowledge one gets from growing plants one's self, he has become an authority on those species which are hardy in Europe. The bamboos are, of all plants, perhaps the most difficult to classify, for they bloom very infrequently, sometimes waiting forty years before doing so, yet the systematic classification of a species must be based on the characters in the flowers. If one were to try to classify the bamboo material found in any of the gardens of Europe, from the specimens of dried leaves, such as are generally all that one finds in the herbaria, one would make a sorry mess of one's classification. In fact, the bamboos are in a mess, scientifically speaking. Houzeau de Lehaie has done, perhaps, as much as anyone to clear this up, so far as the hardy species are concerned, by quietly studying the growing plants of his hobby and observing their relationships, and the world has had to turn to him for advise.*

It is very often the case that botanists have a lesser working knowledge of the plants proper they work with (or at least another kind of knowledge) than plantsmen, but that does not mean that there is no hope for botanists. They can develop "a feeling for the organism", title of a biography of Nobel Prize winning plant scientist Barbara McClintock, who unveiled the dynamic nature of the genome through her studies of genetics of corn (Fox Keller, 1987).

Plantsmen, botanists, growers, plant collectors.... have greatly contributed to a growing awareness about bamboo as ornamental plants. To such list taxonomists like Thomas Soderstrom, Lynn Clark and Chris Stapleton may be added, or bamboo scientists like Floyd Alonso McClure, Walter Liese, Jules Janssen. *The Bamboo Book – a comprehensive guide to this remarkable plant, its uses and its history* by David Farrelly became an instant classic. *The horticultural bamboo species of Japan* (Okamura and Tanaka, 1986*), Colored Illustrations of Bambusoideae in China* (Wen, 1993), *A Compendium of Chinese Bamboo* (Shilin et al., 1994) and *American Bamboos* (Judziewicz et al., 1999) became important references for bamboo enthusiasts and growers, often combined in one person.

Scientists, plantsmen, architects, designers and writers all contributed to the awareness of bamboo in the West. Europe had been building its own traditions with many books published in the last two decades, in various fields. Bamboo has become an important brand name and a household name, which has also been important in difficult times. When for example all *Fargesia murieliae* plants died in the 1990's due to mass flowering, this had little or no effect on the growing production and sales of bamboo.

Applications of research in ornamental horticulture

The development of novel propagation and production methods

Having built its own bamboo tradition and narratives, a second major differentiator in Europe was the reduction of ornamental plant production to just a few commercially interesting species as noted by Robert De Belder. This has perhaps been the major driving force for the rapid development of ornamental horticulture in The Netherlands and Belgium with the application of fundamental research in horticulture. The two major parts are methods for mass production (greenhouse technology and micropropagation) on the one hand, and for genetic improvement (breeding, molecular markers, plant tissue culture techniques and genetic transformation) on the other.

In my view it is important to address these issues since for the 21st century there can or will be important spillover effects: technologies developed and perfected in ornamental horticulture may be used for developing methods for both mass propagation and genetic improvement of bamboos in agroforestry and forestry for tropical and temperate regions.

In the past 50 years insights in plant physiology were translated into efficient greenhouse technologies (control of humidity, water, light, CO_2, temperature...), the application of plant growth regulators (auxins for rooting of cuttings, control of flowering and ripening by ethylene, cytokinins for increased branching in fruit trees, or the use of growth retardants to control plant shape and quality, are just a few examples), the use of efficient fertilization methods (inert soils, hydroponics, slow release fertilizers...) and methods for pest and disease control. These greenhouse technologies became a major driving factor for the increase in production and quality, the Green Revolution in horticulture. A good production planning and supply chain are, on the one hand, the result of automation (e.g. of climate control), but on the other also a prerequisite for automation in quality control, sorting and moving plants.

Nevertheless, the most distinguishing feature of ornamental horticulture has not been the production methods, but the methods for propagation of indoor and outdoor ornamental plants. The whole sector of foliage and flowering plants has been driven largely by the development of micropropagation methods (the use of plant tissue culture methods for plant propagation). It became possible to produce millions of plants from selected genotypes in well-controlled conditions, leading to high quality and low cost plants, and this completely transformed the valorization chain and the horticultural business and markets. Expensive orchids became cheap plants, available to anyone.

In the 1970's the plant tissue culture laboratories of P.C. Debergh in Ghent and of R.L.M Pierik in Wageningen became two of the world's leading schools for tissue culture research, and developed basic propagation protocols for *Ficus*, *Gerbera,* bulbous plants, ferns, Araceae, Maranthaceae, Bromeliaceae and many other plants. These protocols were adopted and adapted by several private laboratories and this rapidly led to the production of millions of plants. They also provided lab space for companies to develop their own protocols as in the case of bamboos. Their contacts and influence opened doors worldwide, and in the early nineties bamboo co-operations emerged with Thailand (Gavinlertvatana and Prutpongse, 1991) and with India (TATA Energy Research Institute, on somatic embryogenesis of *Dendrocalamus* species).

Today these vibrant centers of Ghent and Wageningen are still major hotspots of plant biotechnology with a combination of fundamental research and commercial spinoffs. In Ghent the first genetically transformed plants were produced (Van Montagu, 2011), which initiated the explosion of plant molecular biotechnology for the 21st century.

In 1990 the world production of micropropagated plants was roughly estimated to be 500 million units (Debergh, 1994) of which the majority were ornamental plants. In that period the production in Belgium was about 28 million (Debergh et al., 1990) on a total production of 212.5 million in Western Europe (Pierik, 1991). Two decades ago, Belgium was world leader in the micropropagation of ornamental trees, agricultural crops and herbs and second (after The Netherlands) for pot plants and garden plants, which illustrates the central role of the Low Countries in the development of ornamental horticulture. In the last two decades micropropagation of ornamentals has certainly doubled with many labs around the globe (actually orchids are the main product from tissue culture worldwide).

With a worldwide rise in number of plant tissue culture laboratories (In the 1980's Asia had around 150 commercial plant tissue culture operations and many more plant tissue culture labs in universities and research institutions; Gavintlertvatana and Prutpongse, 1991), companies in Western Europe had to specialize and automate. In 2010 the production in Belgium has more than doubled and 8 laboratories produce 65 million plants per year and five of these labs produce over 5 million plants each (Dhooghe et al., 2011). Over 50 million of these are pot plants and orchids, and over 10 million of the plants produced are garden trees and shrubs, perennial plants, including ornamental grasses and temperate bamboo. From Dhooghe et al. (2011):

> *When one looks more closely to the classification in plant families, the largest production of plants are species of the Bromeliaceae family (37.7%), followed by the Orchidaceae (17.4%) and Ericaceae (15.4%). The top ranking of the Bromeliaceae regarding production numbers is not unexpected since two world-leading breeding companies of Bromeliaceae are located in Flanders. Although the percentages of some other plant families are relatively minor, the impact on the world production might be high. For example, the majority of the world's micropropagated winter-hardy bamboo and some genera of the Maranthaceae originate from Flemish companies.*

Seven stages

Annually, over 1 million temperate bamboos are produced through micropropagation in Belgium, and as for most ornamentals the techniques used are axillary branching, which mimicks the natural process of division in bamboos. Axillary branching induces some rejuvenation (with a seedling-like vigorous growth) but it is the preferred method since the danger of genetic or epigenetic modifications are much smaller compared to organogenesis or somatic embryogenesis, where intermediate callus formation is needed. The latter methods are more useful for forestry, not for horticulture.

It is no exaggeration to say that every lab worldwide has at some point experimented with bamboos in tissue culture. In many countries various groups have developed efficient methods for mass production of tropical bamboos for forestry (for various references Fernandez and Gielis, 2003). In some cases commercial production of temperate bamboos was relatively successful, e.g. at Thai Orchids Labs (Prutpongse and Gavinlertvatana, 1991) and Piccoplant (Germany). Various other labs had one or more species of temperate bamboo, mainly species of *Sasa* or *Pleioblastus*. But all in all the success for temperate bamboos was quite limited. The lack of success is an indication that it is not easy to develop promising research results into successful production systems. Even today, in the scientific literature there are only a few papers with successful protocols. With successful is meant: starting from selected mature plants, with good and stable multiplication rates and transplanting success in the greenhouse between 95 and 100%. In additions, rooting in the greenhouse and re-growth after potting should be fast and reliable, independent of season or other influences. It is a fact that the bar in ornamental industry is considerably higher than in academic research, where still many papers are published with e.g. a low success rate for rooting. Such protocols are simply useless for commercial purposes in horticulture (Gielis and Oprins, 2002). Various recently published papers report findings that have been explored in private laboratories.

Apart from the fact that scientific research did not yield expected results, the integration of tissue culture into a complete ornamental production chain presents its own difficulties in bamboo.

Micropropagation has completely changed the markets and valorization chains in ornamental horticulture. Up till then the whole production chain was done *intra muros* (mother plants, cutting, liner and saleable plant production in one single company), but from the seventies, the production chain was cut up into pieces with specialized tissue culture laboratories, young plant growers (from tissue culture plants to liners) and growers for saleable plants. In the whole ornamental value chain this has led to mass production of plants at extremely low prices, a few cents or less in some cases. As a rule of thumb, the cost of hardened tissue culture plants is less than 10% of the final sales price (and decreasing).

There were some special issues to be dealt with when integrating bamboo micropropagation into any successful scheme for plant production for the ornamental market. Unlike foliage plants, with a long tradition, bamboos were found only in specialized trade. To open new markets, beyond garden centers, for mass sales of low cost bamboo plants of high quality required going against the trend of ornamental horticulture, namely to keep everything in house, from selection to production and marketing of saleable plants, not tissue culture plants.

Classically, micropropagation is limited to four stages (Debergh and Read, 1991) with Stage 0 the *ex situ* conservation of elite plants, Stage I, the initiation stage, involving all subcultures until a constant and stable multiplication rate is obtained. In Stage II about a 1000 plants per species enter the production line and multiplication rates vary according to species from 3-10 every 3-4 weeks depending on the species (in practice multiplication is kept below 6). Stage II is the preparation of tissue culture plants for transplanting into the greenhouse, for hardening and rooting.

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 micro-motherplant, and (3) Stage VI, the production of saleable plants. This distinction (seven stages) is important if the complete chain of production is integrated in a single company, since this determines the added values. In a mass scale production chain micropropagation, hardening, the development of liners, and the growth to saleable plant is then fully integrated.

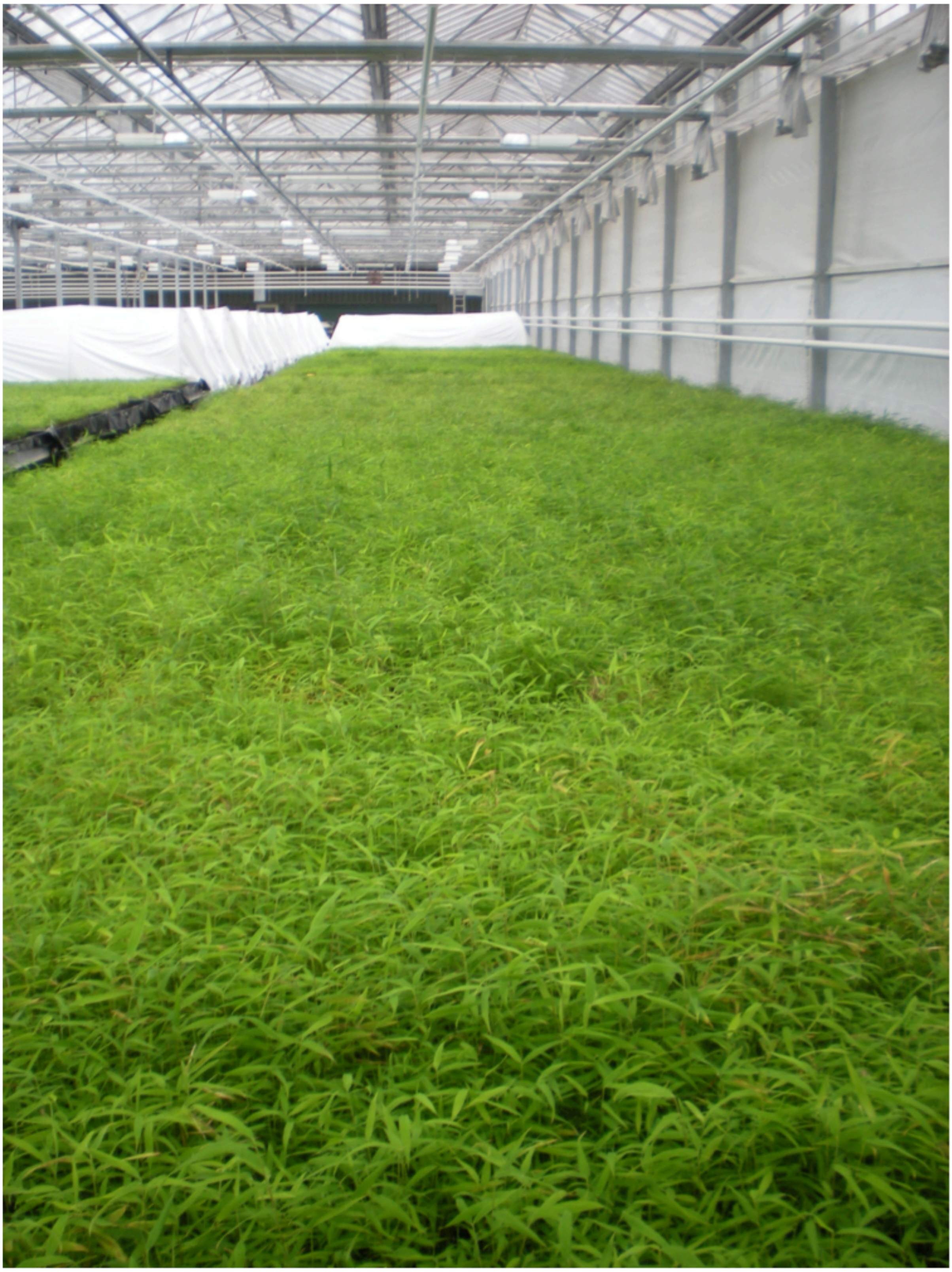

Figure 1 Hardening of micropropagated bamboos and the production of liners

As an additional advantage it has allowed to assess the quality up to the final stages of production. It is also important to study out the long-term effects: while it is true that the micropropagated plants are rejuvenated, with more and smaller culms per pot (which is a major advantage from a logistics and marketing point of view) and grow initially like seedlings with more culms. They follow the natural development from seedling to mature plants. Recent independent long-term observations in German gardens (Schütte, 2011) indicate that these plants are very vigorous, like seedlings:

> "*I have planted Lab-Fargesia's in various gardens and I have never observed any negative effect of their origin. On the contrary, these plants, starting from small initial plants, always surprise by their enormous vitality and vigorous growth.*"

These observations on BambooSelect^{TM} plants (see 4.4) showed that the adult plants grew true-to-type, without negative effects, also not of flowering. Although flowering has been a major concern in the trade, in 15 years of commercial bamboo production through tissue culture with a production of well over 10 million plants, we have never encountered flowering in *Fargesia*.

To assess the long term stability and genetic fidelity at the molecular level we have used various types of molecular markers: Random Amplified Polymorphic DNA (RAPD) markers (Gielis, 1995), Amplified Fragment Length Polymorphisms (AFLP) makers (Gielis et al., 2002), methylation sensitive AFLP (MSAP) markers to assess epigenetic effects (Gillis et al., 2007) and measurements of ploidy levels (Gillis et al., 2007). In none of the cases we could find any indication of changes in the DNA or epigenetic changes related with methylation (Smulders and de Klerk, 2011), using the most sensitive contemporary methods. Of course, this does not mean that there are no effects, but they will most likely be much more subtle than can be found with today's sensitive methods. Many of the cultivars in bamboo (e.g. color variations in *Phyllostachys*) are due to the effect of transposable elements, but this requires extensive research.

Rational Plant Tissue Culture

The final optimization and integration in a complete production scheme required yet another step. Tissue culture bamboos need to root faster and in a reliable way (i.e. 95% or more) and this high level needs to be achieved throughout the seasons and years. Although prices of tissue cultured *Fargesia*'s were already very competitive compared to foliage plants, lower production prices are always important for the price elasticity and the sales margin of the final products. Production of 50000 bamboos for one customer for one specific delivery on one specific day is no longer uncommon and this requires a very precise planning, from lab to greenhouse. This optimization was achieved through the study of the dynamics of phytohormones in tissue culture.

In general, micropropagation and tissue culture of plants is still much more art and skill than science. There is, however, a great need for a rational decision methodology in commercial plant production. Rational Plant Tissue Culture involves the efficient determination and dynamic analysis of auxins and cytokinins in plants and media. This allows us, to some extent, to look inside the plant and understand and predict its behavior and here we are at the forefront of developments in plant tissue culture in general.

To achieve this goal, we have developed Ultrahigh Performance Liquid Chromatography (UPLC) methods coupled with simplified methods for sample preparation and measurements, and mass spectrometry MS, and combined this into a high-throughput system for fast and accurate determinations of phytohormones in the service of the micropropagation industry. Due to the superior separation, in combination with robustness, UPLC-MS/MS is considered as the most accurate and reliable method up to now. This method can be used to quantify all plant growth hormones, not only cytokinins, and can be developed into high-throughput systems. These analytical methods allow for high-throughput measurements of cytokinins, auxins, gibberellins, abscisic acid and much more. Some of the results for cell suspension cultures are presented in Prinsen et al., 2012 (this congress).

From the perspective of commercial micropropagation this rational approach has developed into a decision-making method to improve the efficiency of the whole plant production process through monitoring of quality during the production cycles, allowing for considerable cost reductions. For example, using this approach we have been able to shorten the multiplication, rooting and hardening cycles, with an initial cost reduction of up to 25-28% for various species. A further cost reduction has been achieved since now Fargesia plants can be hardened in trays with 104 plants, compared to the trays of 54 plants used before. This increase in efficiency brings further automation and robotics within reach.

The liners (hardened and rooted plants) are more vigorous, and can develop into saleable plants for the ornamental market in less than four months. Since micropropagation is the first step in the whole production chain, everything downstream in the chain, from plant production to marketing and sales, benefits from this optimization. It allows an efficient integrated production planning needed for the demanding ornamental horticultural markets of today.

Figure 2: Growing saleable plants at Lacijns

The new insights in bamboo physiology have opened the door to drastically improve the quality of bamboo. For example, it has now become possible to produce bamboos, which can withstand longer periods without watering. When bamboos are sold in supermarkets, plant care or specialized personnel are not guaranteed and the risk of plant receiving little water in dry weather periods is considerable. In full sun bamboo leaves rapidly curl and become grayish in prolonged dry conditions, but our experiments indicated that with proper care, bamboos shipped from greenhouse to customer withstand 25 days without watering. After more than 3 weeks measurements of the photosynthetic capacity showed that the leaves were as healthy as control plants. Such strategy also opens the door, hopefully, for introducing bamboos as indoor plants. True bamboo for indoor use would be a major step, and some are in the product pipeline (Figure 4).

Figure 3: Results from experiments of 25 days without watering with control on the right

In any case, bamboo is a strong brandname: *Pogonatherum paniceum*, for which efficient methods of somatic embryogenesis were developed in the 1980's under the erroneous name of *Phyllostachys* viridis (Hassan & Debergh, 1987), became a market success under the name Baby Bamboo or House Bamboo, by Derooze Plants, one of Belgium's leading tissue culture labs. Another success story with a bamboo brand name is Lucky Bamboo, *Dracaena sanderiana* (also known as Belgian Evergreen).

Quality and Branding

In addition to propagation and production methods, also marketing and distribution have been completely changed compared to one decade ago. Two decades ago plants were offered sporadically at high prices, with limited availability. One decade ago, the production had increased dramatically, both because of the number of growers and the cultivated area per grower. Today, millions of bamboo plants find their way to the market through different distribution channels. Many of these channels are still classical (collectors, garden centers, landscapers), but sales have been increasingly successful through dedicated channels like supermarkets and wholesalers for garden center groups, or open market places like Groendirect where various cash & carry companies buy plants. The opening of these markets and channels is the direct result of the vision of mass propagation with micropropagation as the central technology.

There is still an increasing demand for high quality bamboos. The production of top quality bamboos should be organized in such as way that they are available in large numbers and at any time throughout the year. In the past, and as we speak in 2012, availability can highly fluctuate in years with harsh winters. Using greenhouse technology or production centers in warmer regions (Spain, Italy and Portugal for Europe) are necessary.

In these modern markets high quality means that all dried leaves have to be removed, green leaves should be of excellent quality (no spots, no dried leaf tips), and the pots should be extremely clean. Labels or pots used depend on the client. Integrating propagation, production and sales, requires an intensive monitoring system, in which each batch of bamboos produced in the lab is labeled with unique identification codes, which are used in the whole production and marketing system. This is necessary for any planning, but also allows feedback from sales to propagation.

To broaden the markets and develop new channels for bamboo sales (high volume – low prices) has also required another change, namely the naming of bamboo. From plants with (varying) latin names, today bamboos are marketed and sold under trendy brand names and a general brand BambooSelect™ (www.bambooselect.com and www.bambooselect.us). For this brand specific labels and a marketing campaign with brochures were developed. A decisive step towards a consumer market has been the development of trade names like "*New Umbrella" and "Green Hedge", "Red Panda", "Asian Wonder", "Great Wall*", *"Green Panda"* (also known as *"Rufa")*, *"Blue Panda*" and "*Green Screen"* for various *Fargesia* selections[1]; "*Black Jade"* and "*Green Perfume*" for *Phyllostachys nigra* and *atrovaginata* respectively, and *Sunshine* for *Bambusa* "Alphonse Karr".

Figure 4: BambooSelect™ *Sunshine"* for indoor use

From a market and marketing point of view, the ongoing debate about precise naming of bamboo is simply a nightmare. The many name changes in temperate bamboos ("To be or not to be *Fargesia, Sinarundinaria, Borinda, Thamnocalamus.....*") and the discussions among taxonomists can only create confusion and add nothing of value. For the horticultural markets clarity is necessary. As long as one knows the origin of the plant (e.g. *Great Wall* is a selected *nitida* type), with a specific DNA fingerprint (AFLP™ markers, stored in an open database), one can always trace back any plant. The origin of these selections is with plantsmen in Belgium, The Netherlands and Germany and rigorous selection at Oprins Plant Labs. Everyone familiar with the finesses of plant tissue culture understands that in vitro selection is crucial to successful products downstream.

[1] Selections of *F. murielieae* (NU & GH), *F. Jiuzhaighou* (RP), *F. scabrida* (AW), *F. nitida* (GW), *F.'Rufa'* (GP), *F. papyrifera* (BP) and *Fargesia robusta* (GP)

Figure 5: *"Green Hedge"* (left) and *"Blue Panda"*

This has also led to the strategy of growing from a pure plant producer to developing intellectual property rights, e.g. with BambooSelect^{TM} and its best clones (IlexSelect^{TM} for hollies is next in line). These include not only proprietary technology for propagation and production (Gielis et al., 1999), but also the commercial side with branding. This complex of technologies and strategies, based on fundamental research, will have various spillover effects in the next decades in agro-forestry and forestry with bamboo.

Into the 21st century: positive spillover effects

Valorization of "horticultural" technology to address major challenges

Botanist David Fairchild not only wrote about horticulture and his travels, but he was also the manager for the Department of Plant Introduction program (United States Department of Agriculture USDA). Fairchild (1947, p.57) writes about an impressive collection of non-native bamboos, growing at the USDA's plant introduction station in Savannah, Georgia:

> *"The vast possibilities of bamboo have interested me for years...The one hundred and twenty-five species of bamboo growing there represent what I presume is the largest collection of these useful grasses in the world today. The meager government support which it receives reflects, perhaps, the almost universal ignorance of the Western World with regard to the possibilities of these, the largest of all the grasses."*

Those who see the value of bamboo as ornamentals invariably are drawn to the potential for agriculture. The Savannah collection would become the source for interesting agricultural research into the possibilities of growing bamboo in temperate climates as a source of biomass. The results and techniques were encouraging, as were experiments elsewhere in temperate zones, but it was not until the 1990's that interest in bamboo as a source of biomass was awakened, both in the US, for example at the University of Washington (with a 1997 Pacific Northwest Agro-forestry Workshop in Port Angeles, Washington) and in Europe, with the Bamboo for Europe project.

Funding of the European Union for this *Bamboo for Europe* project allowed nine partners to work together on research on possibilities and restrictions on bamboo for agriculture and forestry in Europe, with the establishment of new plantations for research in Portugal, Spain and Belgium It was found that bamboo can be used as a source of biomass without major extra investments in the sectors of bio-energy and wood industry (boards and panels). Bamboo becomes then an agricultural plant, not forestry, with some specific advantages. Existing harvesting machinery in agriculture can be used directly for mechanical harvesting of bamboo (Gielis 2001; Temmerman et al., 2005; Potters et al., 2012; Schutte, 2011).

It is however, impossible to start any serious large-scale plantation scheme of temperate bamboos without mass propagation methods that have been developed and perfected in horticulture for temperate bamboos. It is impossible to turn the current bamboo resources into forestry liner production to supply enough cheap planting materials, without micropropagation.

A comparison with *Ficus benjamina* seems appropriate: nobody in the tropics in his or her right mind, would even consider propagating a strangler fig tree via tissue culture; it has little value and can be propagated most easily via cuttings. Yet, ALL *Ficus* plants produced in Europe today (approx. 100 million per year) are produced via tissue culture; in fact, micropropagation has replaced propagation via cuttings completely.

Whenever micropropagation methods can be developed successfully into an efficient production method, they will inevitably replace other methods of propagation, not only in ornamental horticulture (foliage plants, bulbs, orchids, ornamental trees and shrubs), but also in food sector (berries, nuts, rootstocks for fruit trees, palms, olives……) and the forestry sector. This is certainly not new (Debergh and Zimmerman, 1991), but an ever-growing process, although some methods intermediate between plant tissue culture and classical propagation have been developed, for example photoautotrophic (sugar free) micropropagation systems (Watanabe et al., 2000; Kozai et al., 2004), the use of micropropagated plants as mother plants for classical vegetative propagation techniques, including macroproliferation (Kumar, 2012).

The 21st century is the century of biotechnology, with plant tissue culture as one of the key ingredients. While developments in tissue culture and micropropagation have been quite impressive, the challenges are immense, of a different order of magnitude. For plantation and reforestation purposes hundreds of millions of bamboo transplants are needed annually in the future. This is only a few percent of the total need for transplants, and estimates range from 25-40 billion plants per year to be produced (Kozai, 2005):

> *"In order to solve these global issues in the 21st century, we are requested to develop a concept, a methodology and an industry to produce billions of plants every year, not only for food, feed and environmental conservation, but also for alternative raw materials to produced bio-energy, bio-degradable plastics and many other industrial products. By using plant-derived products, we can minimize the environmental pollution and the use of fossil fuels and atomic power. It has been predicted that in the forthcoming decades, demands for transplants will rise sharply in the pulp, paper, timber, energy, plantation, horticulture and furniture industries and in the desert rehabilitation for environment conservations. A large number of high quality transplants, woody and herbaceous horticultural plants, are also needed every year for people living in cities to improve their quality of life or green amenities. The same is true for medicinal plants."*

The production of genetically uniform and high quality transplant material through micropropagation is the start of a transformation that is ongoing in horticulture, agriculture and forestry. Protocols and methods have been developed worldwide for various genera and species of forestry bamboos and many have been tested in forestry, albeit on a relatively small scale (Yang and Hui, 2010). Somatic embryogenesis, esp. of tropical bamboos (Mehta et al. 1982; Rao et al., 1991) has proven an efficient, fast and cheap method for mass production of bamboos for forestry, including the potential for synthetic seeds (Gielis et al., 1999). In Europe it is far too costly to produce forest bamboos for the tropics and, consequently, production has to be done locally.

Time-keeping of media preparation and transfer times of these experiments, allowed for simulating the efficiency of mass production of *Bambusa balcooa* plants, comparing both

axillary branching and somatic embryogenesis. Economical evaluation of the methods allowed for comparing costs in Europe versus South Asia and showed a drastic cost reduction of SE compared to axillary branching (Gillis et al., 2007) and the necessity for local production (in this case at Bambu Nusa Verde, Indonesia; Peeters, 2011). The optimal plant production chain could still involve the production of embryogenic calli in Europe, and the regeneration of embryo's, multiplication and hardening of plants can be done locally. As they produce at low cost and can act in regional markets autonomously, this production chain allows for mass production of tropical bamboo plants at prices within the scope of forestry (Peeters, 2011).

One of the main challenges is to combine this knowledge and deploy the developed technologies in various parts of the world to establish sustainable plantations for biomass and wood of bamboo, adapting it to local situation and demands. Some of the building blocks have been developed, but to integrate these into highly efficient methods for mass propagation as in horticulture requires further steps. Toyoki Kozai (2005) writes:

> *"Micropropagation is one of the plant tissue culture technologies for producing a large number of genetically superior and pathogen-free transplant in a limited time and space. However, the widespread use of micropropagated plants is still restricted because of its high production costs, mostly attributed to its low growth rate and a significant loss of plants in vitro by microbial contamination, poor rooting, low percent survival at the ex vitro acclimatization stage and high labor costs*."

In contrast to in horticulture we have already tackled and solved most of these issues for ornamental bamboos, so our long-term experiences from horticulture, with extensions to agriculture (in temperate zones) and forestry (tropical zones), should certainly be useful and welcome in competitive markets of forestry where micropropagation "*is still an experimental or small scale production stage*" (Yang and Hui, 2010).

Research challenges for the future

There are still a lot of open challenges for micropropagation of bamboo. The control of somaclonal variation due to transposable elements is one important area for horticulture and somatic embryogenesis for temperate bamboos for example has hardly been successful. Clonal fidelity and long term follow up of the growth is of prime importance (Gillis et al., 2007; Negi and Saxena, 2010; Agnihotri et al., 2010). Even better for the future is that relations of certain molecular markers to development and relation to certain characteristics has been established (Rai et al., 2011; Bhattacharya et al., 2011; Rai et al., 2012).

Beside propagation and mass scale production, one of the main challenges for plant research and biotechnology is the genetic improvement of bamboo. Despite some initial trials in hybridization (Zhang Guangchu, 2002; for other references see Gielis, 1995), and the possibility to induce flowering into tissue culture, we are still far from any breeding program. So far, various research programs for the genetic transformation of bamboo have only yielded transient expression, or when a more stable integration is obtained in cell cultures, regeneration of plants is the bottleneck (Ojita et al., 2011; Sood et al., 2011; own unpublished results).

Since every disadvantage has also its advantage bamboo is probably one of the few cultivated plants that are still in their natural, wild state. Other crops have been domesticated for a long time or recently; extensive breeding programs have been developed and many crops can be genetically engineered (Van Montagu, 2011). Bamboo, on the contrary, is a completely green and natural plant, with a sufficiently large natural genetic variation. It will require concerted efforts of a research consortium to unravel this genetic variation, using molecular markers or high-throughput sequencing. The use of molecular markers alone will be insufficient. Part of the natural variation is, in my opinion, strongly related to transposable elements (compare color and culm shape variations in many forms and cultivars of bamboo) and thus difficult to study, but the use of state-of-the-art methods like AFLP™ based transposon display (De Keuckeleire et al., 2004) and sequencing (Diao et al., 2006; Zhou et al. 2011) will be of great help.

At present, however, our knowledge of the natural genetic variation of bamboo is extremely limited and fragmented. The use of molecular marker has yielded only a few clear-cut, practical results (Gielis and Oprins, 2009; Rai et al., 2012; BPG, 2012), yet they have become indispensible tools in research, with potentially practical results: the use of AFLP™ in Chinese clumping bamboos shows a geographical grouping, rather than supporting existing taxonomies and the AFLP™ fingerprint can be used to predict cold hardiness of genotypes (Gielis and Oprins, 2009). Despite the fact that we hardly know anything about the genetics of bamboo, it may become possible to close the gap with other plants with a targeted study of the genome of bamboo using state-of-the-art methods (high-throughput sequencing, bioinformatics, synteny (Gielis, 1995; Gui et al., 2010), transposable elements ,….).

High-throughput methods are available also for studies in bamboo physiology, for example in the dynamics of phytohormones (Prinsen et al., 2012), biogenic volatile emissions (Melnychenko et al., 2012) and potentially useful secondary metabolites (Van Hoyweghen et al., 2010). While studies have been performed on microbiomes in the rhizosphere, our understanding of the endophytes in bamboo and their ecology, which may play essential roles in plant physiology, has barely started (Moshynets et al., 2012). Bamboo tissue culture provides many opportunities as a basic method in research.

One major challenge however, is to bridge the gap from research to practice. There is a great need for, as Prof. Walter Liese calls it: Facts and Figures. Bamboo is a wonderful plant, but it has no magical powers for mitigating climate change (Düking et al., 2011), nor does bamboo yield 300 tons of wood per year in any sustainable way, as is often heard and read. A research community focused on 1) understanding and improving bamboo through physiology and molecular biology, combined with 2) a drive to generate real fact and figures is most needed.

There are many opportunities and challenges ahead to attain a better understanding of this wonderful natural resource, in which plant tissue culture is the key technology. Research on molecular and physiological aspects of bamboo flowering, where we have the possibility of induction of flowering *in vitro* (Nadgauda et al., 1991), should definitely be a number one target for an international research consortium, along with research on genetic transformation. Equally important will be the possibility for genetic transformation of bamboo, to further broaden its natural variability adapted to specific conditions. If we can begin to understand and control flowering, or be able to genetically transform bamboo, we hold the keys to making bamboo into one of the most valuable plants for mankind in the next centuries. Highly efficient mass propagation systems work, and markets are eagerly waiting.

Acknowledgements

The research at Oprins Plant has generously supported by IWT (Agency for Innovations by Science and Technology, Flanders) support. Special thanks to Bart Van Ransbeek (IWT); to Hilde Peeters, Susanne van den Akker, Koen Gillis, fellow members of the bamboo biotech research team at Oprins Plant 2000-2010, and to Guy Henderieckx, sales manager at Oprins Plant. The EU has supported applications in agriculture (Bamboo for Europe 1996-2000 and EFRO project IKEBANA 2010-2012) and networking actions with the Bamboo Thematic Network (2001-2004).

References

Adriaenssens D. (2005) Jelena and Robert De Belder. Laconti, Brussels.

Bamboo Phylogeny Group (2012) An updated tribal and subtribal classification of the bamboos (Poaceae: Bambusoideae). This congress.

Bhattacharya S., Ghosh J.S., Sahoo D.K., Dey N., Pal A. (2010) Screening of superior fiber-quality-traits among wild accessions of *Bambusa balcooa*: efficient and non-invasive evaluation of fiber developmental stages. Ann.For.Sci. 67: 611-620

Borin G. (1995) Bamboos in Belgium: a long but interrupted tradition. European Bamboo Society Journal. EBS Annual General Meeting, Meise, Belgium, May 6, 1995: 21-26.

Camus E.G. (1913) Les Bambusées. Monographie, Biologie, Culture, Principaux usages. Publisher Paul Lechevalier, Paris.

Chapman (Ed). (1997) The Bamboos. Linnean Society, London.

Colonel Munro (1866) A Monograph of the Bambusaceae, including descriptions of all species. Transactions of the Linnean Society Vol XXVI.

Clark L. (1995) Bamboo systematics today. European Bamboo Society Journal. EBS Annual General Meeting, Meise, Belgium, May 6, 15. 40-46.

Clark L. (1997) Bamboos – the centerpiece of the grass family. In: Chapman The Bamboos. Linnean Society Symposium Series N°19 Academic Press: 237-245.

De Keukeleire P., De Schepper S., Gielis J., Gerats T. (2004) A PCR-based assay to detect hAT-like transposon sequences in plants. Chromosome Research 12: 117-123.

Debergh P.C., Zimmerman R.H. (1991) Micropropagation: Technology and Application. Kluwer Academic Publishers.

Debergh P.C., Read P.E. (1991) Micropropagation. In: Debergh P.C, Zimmerman R.H. (1991) Micropropagation: Technology and Application. Kluwer Academic Publishers. 1-13.

Dhooghe E., Van Hylebroeck J., Geelen D. (2011) Ornamental plant tissue culture industry in Flanders (Belgium). Proc. 7th International symposium on in vitro culture and horticultural Breeding. Ghent, 18-22 September 2011.

Diao X., Freeling M., Lisch D. (2006) Horizontal transfer of a plant transposon. DOI: 10.1371/journal.pbio.0040005

Düking R., Gielis J., Liese W. (2011) Carbon flux and carbon stock in a bamboo stand and their relevance for Mitigating climate Change. Bamboo Science and Culture 24: 1-7.

Fairchild D. (1947) The World Grows Round My Door. Charles Scribner's Sons, New York

Fairchild D. (1903) Japanese Bamboos and their introduction into America. USDA Bureau of Plant Industry Bulletin 43.

Freeman-Mitford A.B. (1896) The Bamboo Garden. McMillan, London.

Farrelly D. (1984) The Book of Bamboo. A comprehensive guide to this remarkable plant, its uses, and its history. Sierra Club Books San Francisco.

Fernandez E., Gielis J. (Eds.) (2003) Compendium of bamboo research on Bamboo 1970-2003. EU Bamboo Thematic Network.

Fox Keller E. (1983) A feeling for the organism: life and work of Barbara McClintock. W.H. Freeman and Company. New York.

Gavinlertvatana P., Prutponse P. (1991) Commercial Micropropagation in Asia. In: Debergh P.C, Zimmerman R.H. (Eds.) Micropropagation: Technology and Application. Kluwer Academic Publishers.

Gielis J. (1995) Bamboo and Biotechnology. European Bamboo Society Journal. EBS Annual General Meeting, Meise, Belgium, May 6, 15. 27-39.

Gielis J., Woods S., Woods J., Oprins J. (1999) Micropropagation, synthetic seeds and germplasm storage of bamboos. US Patent letter 6677154, granted 2004.

Gielis J., Oprins J. (2002) Micropropagation of temperate and tropical woody bamboos: from biotechnological dream to commercial reality. Bamboo for Sustainable Development. INBAR Proceedings N°7. VSP Publishers: 333-344.

Gielis J., Peeters H., Gillis K, Oprins J., Debergh P.C. (2001) Tissue culture strategies for the genetic improvement of bamboo. Acta Horticulturae 552: 195-203.

Gielis J. (2001) Future possibilities for bamboo in European agriculture. Journal European Bamboo Society, Meise, Belgium, June 23-24, 2001: 24-32.

Gielis J., Oprins J. (2009) Identifying new *Fargesia* Introductions and Predicting their Cold Tolerance using AFLP markers. WBC VIII Proceedings, September 16-19, 2009 Bangkok, Vol 6: 56-67.

Gillis K., Gielis J., Peeter H., Dhooghe E., Oprins J. (2007) Somatic embryogenesis from mature *Bambusa balcooa* Roxburgh as basis for the mass production of elite forestry bamboos.

Godbole S., Sood A., Thakur R., Sharma M., Ahuja P.S. (2002) Somatic embryogenesis and its conversion into plantlets in a multipurpose bamboo, *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. Curr Sci 83: 885-889.

Gui Y.J., Zhou Y., Wang Y., Wang S., Wang S.Y., Hu Y., Bo S.P., Chen H., Zhou C.P., Ma N.X., Zhang T.Z., Fan L.J. (2010) Insights into the bamboo genome: syntenic relationships to rice and sorghum. J. Integr. Plant Biol. 52(11): 1008-15. doi: 10.1111/j.1744-7909.2010.00965.x.

Hassan E.H., Debergh P.C. (1987) Embryogenesis and plantlet development in the bamboo *Phyllostachys viridis* (Young) McClure. Plant, Cell, Tissue and Organ Culture 10(1): 73-77. Corrigendum 1988 PCTOC 12(1): 93.

Houzeau de Lehaie, J. (1906-1908) Le Bambou, son Etude, sa Culture, son Emploi. Mons, Belgium

Judziewicz E., Clark L.G., Londono X., Stern M.J. (1999) American Bamboos. Smithsonian Institute.

Komatsu Y.H., Batagin-Piotto K.D., Brondani G.E., Gonclaves A.N., Almeida M.D. (2011) In vitro morphogenic response of leaf sheath of *Phyllostachys bambusoides*. J Forest Res 22: 209-215

Kozai T. (2005) Introduction. In: Kozai T., Afreen F., Zobayed S.M.A. (2005) Photoautotrophic (sugar-free) micropropagation as a new micropropagation and transplant production system. Springer Verlag.

Kozai T., Afreen F., Zobayed S.M.A (2005) Photoautotropic (sugar-free) micropropagation as a new micropropagation and transplant production system. Springer Verlag.

Liese W. (1985) Bamboo: biology, silvics, properties and utilization. Schriftenreihe der GTZ, N° 180

Liese W. (2009) Bamboo as carbon sink: Facts or fiction? WBC VIII Proceedings, 16-19 September 2009,Bangkok. Vol 3: 71-86.

McClintock D. (1996) Bloei bij winterharde bamboos. Newsletter Belgian Bamboo Society 13: 5-11

McClure, F.A (1966) The bamboos – A fresh perspective. Harvard University Press., Cambridge, Mass. USA.

Melnychenko A.N., Rosenstiel T.N. Biogenic volatile organic compound emissions from bamboo: Exploring patterns of diversity across species.. In: Proceedings IXth World Bamboo Congress, Antwerp.

Mehta U, Rao IVR, Ram HYM (1982) Somatic embryogenesis in bamboo. Plant Tissue Culture. Proc. 5th Intl Cong Plant Tiss Cell Cult pp 109-110

Mehta R, Sharma V, Sood A, Sharma M, Sharma RK (2011) Induction of somatic embryogenesis and analysis of genetic fidelity of in vitro derived plantlets of Bambusa nutans Wall. using AFLP markers. Eur J Forest Res 130: 729-736

Moshynets E.V., Brunet J., Shpylova S.P., Bogaerts L., Kordium V.A., Potters G. (2012) Visualisation and identification of endophytic bacterial communities of *Phyllostachys* and *Fargesia.* In: Proceedings IXth World Bamboo Congress, Antwerp.

Nadgauda R.S., Parasharami V.A., Mascarenhas A.F. (1990) Precocious flowering and seeding behaviour in tissue-cultured bamboos. Nature 344, 335 - 336 (22 March 1990).

Nadha H.K., Kumar R., Sharma R.K., Anand M., Sood A. (2011) Evaluation of clonal fidelity of in vitro raised plants of *Guadua angustifolia* Kunth using DNA-based markers. J Med Plants Res 5: 5636-5641

Negi D., Saxena S. (2010) Ascertaining clonal fidelity of tissue culture raised plants of *Bambusa balcooa* Roxb. using Inter Simple Sequence Repeat (ISSR) markers. New Forests. Vol. 40: 1-8.

Negi D., Saxena S. (2011) In vitro propagation of *Bambusa nutans* Wall. ex Munro through axillary shoot proliferation. Plant Biotechnol Rep 5: 35␣43

Okamura H., Tanaka Y. (1986) The horticultural bamboo species of Japan, Kobe

Orhnberger D. (1999) The Bamboos of the World. Elsevier, The Netherlands

Ogita S, Kashiwagi H, Kato Y (2008) In vitro node culture of seedlings in bamboo plant, *Phyllostachys meyeri* McClure. Plant Biotechnol 25: 381␣385

Ojita S., Kikuchi N., Nomura T., Kato Y. (2011) A practical protocol for particle bombardment-mediated transformation of *Phyllostachys* bamboo suspension cells. Plant Biotechnology 28:43-50

Peeters M. (2012) Bamboo Tissue Culture to support plantation development. Proc. Of the International Bamboo Seminar: strategy and challenges of amboo and potential non-timber forest products, management and utilization. Bogor, Indonesia, 23-27 November 2011.

Pierik R.L.M. (1991) Commercial micropropagation in Western Europe and Israel. In: Debergh P.C, Zimmerman R.H. (Eds.) Micropropagation: Technology and Application. Kluwer Academic Publishers. 155-165.

Potters G., Schutte F., Van Goethem D., Denollin S., Samson R., Gielis J. (2012) Bamboo as a Crop in Western Europe – A SWOT Analysis. Acta Horticulturae (in the press).

Prinsen E., Van den Akker S., Bormans P., Peeters H., Gielis J. (2012) Cytokinin dynamics in cell suspension cultures of Bambusa balcooa Roxburgh using UPLC-ESI/MS/MS. Proc. of IXth World Bamboo Congress, Antwerp, April 10-13, 2012.

Prutpongse P., Gavinlertvatana P. (1992) In vitro micropropagation of 54 species from 15 genera of bamboo. Hort. Science 27:453-454.

Rai V., Ghosh J.S., Pal A., Dey N. (2011) Identification of genes involved in bamboo fiber development. Genedoi:10.1016/j. gene.2011.01.004

Rai V., Ghosh J.S., Dey N., Pal A. (2012) A genomic approach to identify genes expressed during fiber development in *Bambusa balcooa*. In Proc. IXth WBC Congress, Antwerp.

Rao R.I.V., Aziah Mohd. Y., Rao A.N., Sastry C.B. (1990) Propagation of bamboo and rattan through tissue culture. IDRC Bamboo and Rattan Research Network.

Raphael S. (1979) A Plantsman defined. The Plantsman Vol I Part I, (June 1979).

Rivière A., Rivière C. (1879) Les Bambous. Paris.

Schutte F. (2011) IKEBANA Brochure, University of Antwerp.

Schütte J. (2011) Fargesien aus Laborvermehrung. Bambus Journal (EBS-Germany), 2011, N°3: 17.

Shilin Z., Naixun M., Maoyi F. (1994) A compendium of Chinese bamboo. China Forestry Publishing House.

Smulders M.J.M., de Klerk G.J. (2011) Epigenetics in plant tissue culture. Plant Growth Regulation 63: 137-146.

Sood A., Ahuja P.S., Sharma M., Sharma O.P., Godbole S. (2002) In vitro protocols and field performance of elites of an important bamboo *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. Plant Cell Tiss Org Cult 71: 55-63

Sood P., Bhattacharya A., Sood A. (2011) Problems and possibilities of monocot transformation. Biologia Plantarum, Volume 55, Number 1, pp. 1-15(15)

Temmerman M., Van Belle J.F., Delcart J., Gielis J., Brias V. (2005) Bamboo as a source of bioenergy. Centre Wallon de Recherches Agronomiques, Gembloux, Belgium.

Ueda K. (1960) Studies on the physiology of bamboo with reference to practical application. Bulletin of the Kyoto University Forests, N°30.

Van Hoyweghen L., Karalic I., Van Calenbergh S., Deforce D., Heyerick A. (2010) Antioxidant Flavone Glycosides from the Leaves of *Fargesia robusta*. Journal of Natural Products 73(9): 1573-1577.

Van Montagu M. (2011) It is a long way to GM Agriculture. Annu. Rev. Plant Biology 62: 1-23.

Watanabe Y., Sawa Y., Nagaoka N., Kozai T. (2000) A new micropropagation system for *Pleioblastus pygmaeus* Nakai. In: Proceedings of the International Symposium by The Royal Project Foundation, Chiang Mai, Thailand, 2-4 August 2000, 94-101

Wen T.H. (1993) Colored Illustrations of Bambusoideae in China. Tapei, 175 illustrations.

Yang Y, Hui C. (2010) China's bamboo: Culture/resources/cultivation/utilization. INBAR Technical Report N°33.

Zhang Guangchu (2002) A Manual of bamboo hybridization. INBAR Technical Report N°21. VSP Publishers.

Zhou M.B., Liu X.M., Tang D.Q. (2011) Transposable elements in *Phyllostachys pubescens* (Poaceae) genome survey sequences and the full-length cDNA sequences, and their association with simple sequence repeats. Genet Mol Res 10: 3026-3037