No. 34 Autumn 2001

European Tropical Forest Research Network

ETFRN

NEWS

BIOTECHNOLOGY

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Editorial

Dear readers,

In the popular media the word biotechnology is often used as a synonym for genetic modification, which is the theme of much hot debate. Biotechnology as a concept is much broader however; in fact, only a few of the articles in this issue even mention genetic modification as one of several biotechnological approaches.

Biotechnology is also often seen as 'hightech' and therefore not appropriate in the development context. The first article provides an overview of current biotechnological methods for tree propagation, reminding us that many of these techniques have existed for a long time, and they are practised by numerous tree growers, including poor farmers, all over the world.

The research presented in this issue focuses on the use of biotechnology for the identification and conservation of biological diversity, as well as for tree propagation. While both tree planting and conservation of biological diversity generally require an enabling environment in the sense of policies and economic and cultural situation, it is also important that the technologies to do so are available.

The research on genetic diversity of tree species, using isozyme and genomic techniques, has implications for conservation strategies, as well as for tree breeding. Several articles in this issue illustrate how there may be risks involved in the common assumption that samples of tree provenances (different geographic origins) reflect the true genetic variation within a tree species; in reality, most of the variation may be found within one single population, which must be represented by a large enough sample in order to capture the whole diversity within that species.

Hoping you will enjoy reading this issue; please remember that ETFRN CU always welcomes comments, and contributions for future issues.

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We are grateful to Olavi Luukkanen for editing this issue of the ETFRN News. Please note the themes and deadlines for the next issues on the back cover.

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Cover illustration:

Casuarina clones of improved genetic quality are vegetatively propagated using simple shoot cutting techniques in Thailand.

Photograph by Olavi Luukkanen.

ETFRN NEWS

ETFRN WEBSERVICE LAUNCHED

The ETFRN Coordination Unit website has been extensively revised and updated over the past year. We have tried to further improve access to the vast resources in the ETFRN web, and would like to hear what you think. We invite you to visit the **topics pages** on:

- *non-timber forest products,*
- *agroforestry,*
- *biodiversity,*
- *remote sensing and GIS, and*
- *people and forests*

http://www.etfrn.org/etfrn/topics/index.html

Newsletters

A new feature on the newsletter pages of the website is the possibility to download the newsletter as pdf file. At present, newsletters 32 and 33 are available. News 32 has been downloaded 329 times in total during August, September and October. News 33, which was made available online as pdf on October 4, has been downloaded 47 times in October.

The new webservice was formally launched at the occasion of the annual European Tropical Forest Advisers Group (ETFAG) meeting held in Wageningen last November 1 -2.

ETFRN WORKSHOPS

PARTICIPATORY MONITORING AND EVALUATION OF BIODIVERSITY

ETFRN web-based workshop and policy seminar convened by the Environmental Change Institute, University of Oxford
7-25 January 2002

All stakeholders who use, manage or conserve biodiversity assess it in some way. Local people have different objectives and ways of doing this, from policy makers and government departments responsible for commitments to the Convention on Biological Diversity. Improved understanding of each other's approaches to evaluating biodiversity, can have benefits for rural communities, governments and intermediary organisations.

Participatory monitoring and evaluation of biodiversity involves different stakeholders working *together* to assess biodiversity, which can help scientists to support local people in managing biodiversity, or local people to contribute to national biodiversity monitoring processes.

ETFRN and the Environmental Change Institute are convening a workshop to take stock of existing knowledge in this field, communicate findings to decision makers and provide recommendations for biodiversity monitoring and evaluation which benefits rural people and national level biodiversity managers.

The organisers would like to hear from researchers, NGOs, planners and policy-makers, as well as businesses and consultants, who have experience or questions to share through this workshop. Contributors will be recognised in the on-line proceedings, with the possibility of being included in a published outcome.

For further details please see:
http://www.etfrn.org/etfrn/workshop/biodiversity/index.html and read on below …

DEFINITIONS: although we recognise that there is a debate about 'what is biodiversity' we emphasise that perceptions of biodiversity depend on who you are, what you value and need from the environment. If we accept this, we can move on to look at practical issues – how do we better understand the different perspectives, and communicate them between stakeholders – and how useful is this communication to different stakeholders.

Some people are worried by the word '*participatory*'. We don't mean to imply any particular definition or methodology by using this word; we are more concerned to emphasise the need for 'inclusivity' i.e. recognising all the stakeholders and supporting their involvement in decision-making.

GEOGRAPHICAL FOCUS: The workshop is supported by the European Tropical Forest Research Network, but we believe the issues of communicating about biodiversity perception, addressing institutional gaps and providing policy support, are universal. So the focus is definitely not limited to the tropics. We are keen to hear from 'northern' initiatives and from people working with wildlife, to help us address our own 'tropical forestry' bias.

REGISTER NOW: By simply sending an email to express your interest, to ETFRN or Anna Lawrence at the address below. We would also be delighted to hear about your work or receive any tips about important reports we should be taking into account at this stage.

NEXT STEPS: We are preparing a 'state of the art' review and would like to know about projects / documents / websites / people we should be aware of at this stage.

The 'state of the art' review will identify key themes for the workshop (there is already a draft list of themes on the website), and in December we will contact you to seek people who are willing to write very short 'theme papers', or longer case studies, to post during the workshop. The workshop itself will run from 7 to 25 January 2002.

HOW DOES AN ELECTRONIC WORKSHOP WORK? There are different models, and we are aiming to minimise the amount of mail in your inboxes. The three weeks of the workshop will be divided into periods to address each theme. A short theme paper will go out to all participants at the beginning of each period, with links to case studies and background material on the website. Participants can then respond with comments and discussion, but messages will be posted on the website not in your inboxes. Each day the convenors will send out a synthesis and highlight key questions / recommendations emerging.

RESULTS OF THE WORKSHOP: The immediate result will be a report, posted on the website and acknowledging all contributions. We are exploring the possibility of a book which will however depend on the quality and novelty of the contributions. Perhaps most importantly, the internet workshop will define the recommendations and key discussion points for a one-day seminar for decision-makers, to be held on London or Oxford in February. We see this as an important opportunity for researchers and practitioners to communicate their experience to policy-makers and planners, in an accessible way. Limited funds are available to support the travel of key participants in this seminar.

We are excited to see the range of researchers, practitioners and decision-makers who have already expressed interest. We look forward to a productive discussion in January.

PLEASE REGISTER NOW TO FOLLOW DEVELOPMENTS AND PARTICIPATE IN THE WORKSHOP IN JANUARY 2002.

To register, simply send an email with your name and organisation, telling us of your interest in the workshop, to ETFRN or to:

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Tropenbos International Seminar:

"FOREST VALUATION AND INNOVATIVE FINANCING MECHANISMS FOR CONSERVATION AND SUSTAINABLE MANAGEMENT OF TROPICAL FORESTS"

March 20-21, 2002; The Hague, Netherlands
For many years the tropical forest ecosystem has been subject of debate at all levels of society, ranging from environmental NGOs, scientists and policy makers, to high level international Conventions and Agreements. Global warming, depletion of natural resources, violation of indigenous peoples' rights, biodiversity loss and soil degradation are major concerns affecting tropical forests. Voices have been raised to protect tropical forests because of their great value, but what exactly is this value of tropical forests? Tropical forests sustain a wealth of biodiversity, provide a wide range of ecosystems, services and products, and support livelihoods for millions of people. But if these biological, economic and social values are acknowledged why are forests continuously being depleted? To what extent have appropriate valuation and market mechanisms been developed and implemented? Could they help revert destructive practices? Tropenbos International (TBI) plans to organise a two-day seminar to discuss myths and reality of forest values, and to support the development and implementation of appropriate financing mechanisms for the conservation and sustainable use of tropical forests.

Day I: What is the value of tropical (rain) forests? Emotion and science
The keynote speaker will spark the discussion through a series of provocative statements on the perceived environmental or conservation values of tropical forests. He/she will touch upon discrepancies between statements/positions of environmental groups, private interest groups, politicians and scientific evidence. Five scientists will respond to the statements. Following each respondent an open debate will be launched involving various audience groups (policy makers, politicians, scientists, NGOs, and journalists).

Day II: Innovative Financing Mechanisms for Sustainable Forest Management and Conservation (ETFRN workshop)
The second day will explore the translation of these values into financial contributions for the conservation and sustainable use of forests. A discussion paper on innovative financing mechanisms, in particular market oriented mechanisms will be presented. The paper will describe the state of the art and identify policy recommendations and themes for further research. The draft discussion paper will be made available on Internet for comments one month prior to the seminar. A web-based discussion on key questions raised in the discussion paper will also be initiated, to facilitate feedback from those not able to participate in the seminar, and to help focus the discussion during the seminar. A summary of the Internet discussion will be presented at the seminar. Five cases of financing mechanisms in operation, development or in study will also be presented and discussed.

Innovative Financing Mechanisms is also the theme of the ETFRN News 2001-2002 winter edition for which Tropenbos is the guest editor. This newsletter will serve as a background document for the seminar.

Who should attend?
This seminar is designed to bring together academic scientists and researchers working in national and international organisations, NGOs, or the private sector; as well as decision makers (politicians, donors, managers); local/indigenous peoples; and journalists. A total of 250 participants are expected to attend. A limited number of travel grants will be available for participants from developing countries.

For Information please contact Tropenbos at tropenbos@tropenbos.agro.nl (Full address inside back cover) Or see:
http://www.tropenbos.nl

WHY BIOTECHNOLOGY FOR TROPICAL TREES? – A REVIEW OF APPLICATIONS IN TROPICAL FOREST MANAGEMENT

By Olavi Luukkanen

Forestry differs from agricultural cropping in one fundamental way: management interventions often concern natural or semi-natural ecosystems, and we are faced with the problem of how our work is related to the "natural" genetic processes.

Tropical forests are rich in biodiversity and have conservation needs related to specific patterns of reproductive biology in trees. Tree plantations and agroforestry systems, both often based on introduced tree species, have a central role in supplying the desired tropical forest products. These features make forest tree biotechnology applications especially attractive for the tropical zone and for the developing countries.

Plant biotechnology is often mainly associated with sophisticated DNA work related to genome mapping, with or without gene manipulation, or such rapidly advancing techniques as somatic embryogenesis, cryopreservation and artificial seeds. However, it is useful to remember that the history of forestry provides old examples on biotechnology applications in the form of vegetative propagation using stem cuttings, stratification and tree seed handling techniques, manipulation of flowering, and inoculation of trees with root symbionts. Recently, biotechnology has also made rapid advances in relation to wood quality and the underlying genetic, physiological, genetic and environmental factors (cf. Anonymous 2001).

Tree breeding and tree biotechnology are clearly linked. All traditional forms of biotechnology are still relevant, and in this review, the emphasis is on tropical tree biotechnology as related to the production of planting stock for forest regeneration. A more complete text by the author with full references is also available (see the comprehensive report on tropical tree biotechnology published by the International Foundation for Science (IFS, 1998).

Stem cuttings

Rooted stem cuttings are a traditional propagation method used globally mainly for broadleaved tree species but also in some conifers, such as *Cryptomeria japonica* and *Pinus radiata*. The largest clonal forestry programme of any forestry species or genus exists in eucalypts. Seedling and epicormic shoot ramets of eucalypts root easily, but mature shoots show an increasingly lower rooting percentage. Up to 100 % rooting can be achieved from basal epicormic shoot material, but problems may arise with species that do not form these shoots easily. Treatment of cut stump surfaces with auxins can induce basal shoots in otherwise recalcitrant eucalypt species. A continuous supply of ramets for stem cutting propagation is achieved from hedged seedlings or grafts; grafting followed by stem excision and epicormic shoot formation ensures juvenile material even when branches of mature trees are used as starting material; this procedure is applicable to most species used in stem cutting propagation. In lesser-known tropical trees, considerable improvement of conventional vegetative propagation has taken place in recent years.

Micropropagation in angiosperm trees

In tropical broadleaved tree species, the practical applications of micropropagation seem to concentrate on tissue culture, while propagation using somatic embryogenesis

seems to be more promising in (mostly temperate-zone) conifer propagation. Both groups of techniques require exact protocols for each species, but in several tropical forest tree species practical applications are already available. Forestry-related use of micropropagation for organogenesis has been successfully developed at least in the following genera: *Acacia, Bombax, Casuarina, Dalbergia, Eucalyptus, Ficus, Gmelina, Populus, Platanus, Salix, Shorea, Tectona* and *Terminalia*.

Most eucalypts can be vegetatively propagated using traditional stem cutting techniques, but *in vitro* micropropagation and rooting followed by transfer to soil substrate is gaining popularity because of the prospects of rapid genetic gain by using micropropagation for the production of stools for stem cutting production. Various plant parts have been used as explant material, but the best cultures are generally obtained from cotyledonal or other zygotic embryonal tissues.

Rattans are an example of insufficiently known species that are important in the tropical region but difficult to propagate and grow in plantations. Micropropagation has been suggested as a solution for commercial, agroforestry-type growing of *Calamus manan*, the most valuable rattan species in Southeast Asia. In bamboos, gregarious flowering and the limited capacity for branch cutting propagation are reasons for introducing micropropagation methods.

The very first woody species in which somatic embryos were produced in 1965 was the tropical sandalwood *(Santalum album)*. Somatic embryogenesis has now been recorded in more than one hundred angiosperm woody species alone. Tropical genera represented among these include *Albizzia, Azadirachta, Bambusa, Cassia, Citrus, Cocos, Dendrocalamus, Elaeis, Eucalyptus, Hevea, Mangifera, Phyllostachys* and *Sinocalamus*. The presence of fruit and estate crops and bamboos is conspicuous in this list; the most intensively studied genus is *Citrus*.

Right selection of the explant seems to be extremely important in somatic embryogenesis, and, commonly, immature embryos or nucellar tissue provide the best starting material. The fact that mature tissues have successfully been used for somatic embryogenesis in many tropical tree families opens up completely new prospects for gene conservation and silviculture based on indigenous tree species.

Field trials must also be used for proper verification of clone performance and especially for checks for somaclonal variation, early maturation and plagiotropism, which otherwise might cause problems. It is essential to include conventionally propagated material as control.

Micropropagation in conifers

Practical applications of micropropagation through organogenesis in conifers have become well established in *Pinus radiata* plantation management in New Zealand. The reason for not using rooted cuttings has been the fear of poor field performance due to ageing of the clones. This is particularly clear when ortets older than 4-6 years are used. No practical methods for somatic embryogenesis were available at the start of the programme in the late 1970s; therefore, the method chosen was micropropagation from embryonic cotyledons. The *in vitro* cycles needed include those for shoot formation, elongation and cutting (which can be repeated), callus formation, root induction with auxins, and finally transfer to non-sterile rooting media.

Since 1985, rapid progress has been achieved in micropropagation of conifers using somatic embryogenesis. Basically, there are three different methods for embryogenic culture initiation in conifers: (1) through continuation of natural cleavage polyembryony of explanted immature embryos; (2) through cell division in the epidermal or subepidermal layers of the hypocotyl, cotyledons or needles, and subsequent formation of callus which forms embryo suspensor masses (ESM); or (3) through cell division of small cells within the suspensor system of the explanted immature embryo.

In conifers as a whole, successful results on somatic embryogenesis have been reported in more than 30 species or hybrids. The few tropical species in this list include *Agathis australis* and *Pinus caribaea*. In pines, only immature zygotic embryos (often attached to the intact megagametophyte) can easily be used as starting material, which complicates the use of this technology.

For large-scale practical applications of somatic embryogenesis, automatic systems for culturing, delivering and preserving the embryos are being intensively studied and developed. However the production of mature embryos and especially coating them to produce manufactured (artificial, synthetic) seeds (complete with "manufactured megagametophyte") requires further development. Presently, full-scale commercialisation of this technology is prevented by inadequacies both in embryo quality and in the delivery systems. Cryogenic storage was developed early during the studies on somatic embryogenesis of conifers; it allows the preservation of propagation material over the extensive period needed in field testing of the clones.

Genomic techniques

From a forestry viewpoint the most important reason for the development of somatic embryogenesis technology may be its potential for gene transfer and subsequent multiplication of transgenic plants, but the genetic transformation methods used in agricultural plants are less easily applied to trees. As a result, the list of angiosperm species in which successful gene transfer has been reported is conspicuously void of trees.

In conifers, gene transfer to the level of regenerated plants was first reported only in four species: *Larix laricina, Picea glauca, P. mariana* and *Pinus radiata*. Much of the potential from gene transfer in conifers remains theoretical. Presently, the major limitation in conifers is the inability to regenerate plants from transformed single cells. It is interesting, howewer, that tropical pines *Pinus caribaea, P. oocarpa* and *P. patula* belong to the several conifer species in which protoplasts have been cultured and regenerated.

Once gene transfer has been achieved, genetic selection, using marker traits, such as antibiotic or herbicide resistance, must be carried out so as to separate the transformed cells. Thereafter, plant regeneration is achieved using either organogenesis or somatic embryogenesis. Thus the success of transformation in woody plants also depends of the availability of tissue culture protocols for the species in question. The final result in gene transfer also depends on gene expression as influenced by genetic interaction between DNA sequences and on the stability of the introduced trait. Unwanted escape of transgenes could be prevented by introducing sterility into transformed clones. The final check of gene transfer success is a field trial, but the

whole technology is affected by strict regulation and often polemic public discussion.

While genetic transformation (as well as somatic hybridisation) may be of limited practical use for a long time to come, biotechnology in the form of genetic markers already has a significant role in studying and utilizing the intraspecific and interspecific genetic variation in trees. Biochemical and molecular methods allow a precise study of the effects of forestry practices on biodiversity and thus provide tools for the application of criteria and indicators of sustainable forest management.

Virtually all trees can be genetically mapped, and the results have been applied, for instance, to host resistance screening and lignin biosynthesis modification in *Pinus taeda*. Fusiform rust resistance was found to be under oligogenic and, in some cases, dominant single gene control; since individuals homozygous for the resistance gene can be identified, all seedling progeny of such individuals would be resistant to the pathogen.

Conclusions

Micropropagation does not necessarily require expensive or sophisticated equipment. In fact, micropropagation easily competes with the maintenance of hedged seedlings for rooted cutting propagation -- stacks in a small laboratory can have as good a stock capacity as several hectares of hedges. Laboratory propagation allows year-round production of planting material and may reduce the labour input as compared to stem cutting production; micropropagation can also be combined with *in vitro* root symbiont inoculation. Micropropagation can and should be combined with the storage of genetic material, so as to allow proper field testing of new breeding material. Instead of cryopreservation, storage can be achieved in modified normal laboratory conditions. The two most important problems associated with micropropagation are maintenance of juvenile material and ensuring sufficient genetic diversity in plantations, using a sufficient number of clones.

Somatic embryogenesis is being rapidly developed for mass propagation of plantation trees. Its advantage is seen in the possibility to quickly produce millions of copies of superior individual trees. This method can be combined with a conventional breeding programme, to replace or complement propagation from stem cuttings or *in vitro* organogenesis. The available technology in embryogenesis is in most cases too expensive to compete with other methods, but the development of automated production systems may change this situation. Ideally, such systems would cover all steps from culture maintenance and embryo development to the conversion of embryos to autotrophic plants, whereby either direct-seeding of embryos or manufactured seeds could be used. Future research will concentrate on accomplishing all steps in a compact automated system, a bioreactor, and producing mature embryos in liquid medium.

The possibility for cryopreservation of clones in embryo cultures offers an added benefit to the application of somatic embryogenesis. Such storage can be used while field tests are carried out, and mass propagation would be done with the selected superior clones. There is a danger that the selection occurs in the embryogenesis process rather than the expected field performance. Further development of the embryogenesis technology for mature tissues would allow bypassing of cryopreservation during the testing of clones. Field testing is essential

in assessing the true value of new planting stock, and it is obvious that wood properties must also be considered in this work.

It must be admitted that the immediate benefits for forestry of sophisticated, high-input techniques such as genetic transformation or somatic hybridisation are not very clear. We should also perhaps be more aware of the danger involved in the perception of "modern" forestry as equal to high-input plantation management. In the tropics, where most forests still are (managed or unmanaged) natural forests, we should not promote intensive tree plantations or any high-input production techniques before studying the possibility to manage the original natural forest, perhaps after improving and rehabilitating it, for optimal production. Plantation performance (taking account of their socio-economic and environmental effects) should be compared with a range of alternative production systems, so as to provide tools for decision making on optimal land-uses.

The following statement, expressed in the introduction chapter of a comprehensive treatise on somatic embryogenesis in woody plants (Jain et al. 1995) tells an old truth about the role of forest management. It emphasises the fact that we have to actively *maintain* the genetic diversity, using such means as *in situ, ex situ* and *circa situ* gene conservation, and also to measure the actual genetic diversity in tree populations in natural and man-made forests when necessary for management decisions. On the other hand, we have to *select, multiply and use* the suitable genotypes in intensive culture. Technically, this management is fully comparable to growing such estate crops as coffee, oil palm or rubber:

"The challenge to forestry will be to maintain natural biological diversity in forest ecosystems in combination with intensive clonal culture of carefully diversified genotypes selected for the production of wood and other forest products" (Kriebel 1995).

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IN VITRO APPROACH FOR NATURAL AND INDUCED BIODIVERSITY CONSERVATION OF FOREST TREES

By S. Mohan Jain

The success of any crop improvement program primarily depends on the continuous availability of germplasm with desirable traits. Genetic resources conservation becomes an essential ingredient for breeding and continuous supply of raw material to the industry. Furthermore, large-scale industrialisation, environmental degradation, deforestation and ever-increasing human population growth are placing tremendous pressure on existing resources such as arable land, forests, and natural biodiversity. Deforestation has become a serious problem as a result of forest fires and the use of trees for firewood, timber and paper. In many developing countries, mismanagement and illegal destruction of trees are major serious problems added to deforestation (Jain, 1997). Industrially important trees are being destroyed much faster than the pace of reforestation, resulting in heavy economic looses and destruction of important biodiversity. In some cases, the paper industry is forced to import wood at a much higher price. Reforestation is a slow process often requiring superior planting material, and it is difficult to restore the vegetation of the primary forest. This would increase the demand of improved planting material and high-quality raw material for the industry. Moreover, the loss of natural habitats will narrow down the genetic diversity. Both adverse environmental conditions and industrial expansion may pose danger to the existing germplasm. Therefore, it is justified to induce genetic diversity, for instance, by mutagen treatment and to preserve it using tissue culture (Jain et al, 1998, 2001). Genetic markers are useful to evaluate the genetic diversity and the mechanisms that produce and maintain this diversity. The genetic diversity structure and distribution are important parameters to conserve biodiversity and also to follow the gene flow, especially in transgenic plants.

Tissue culture has facilitated large-scale plant multiplication in woody plants. This has come about with somatic embryogenesis and organogenesis or micropropagation (Jain et al, 1995, 1999, 2000; Ahuja, 1992). Somatic embryogenesis is very effective for clonal propagation, and it has made tremendous progress in some of the commercially important trees belonging to both angiosperms (*e.g.* bamboo*, Eucalyptus* spp*, Populus* spp*, Quercus* spp*,* sandalwood*, Tilia, Salix, Magnolia,* and *Betula*) and gymnosperms (e.g. *Picea, Pinus, Larix, Cycadales, Abies, Sequoia sempervirens,* and *Pseudotsuga menziesii).* Somatic embryogenesis is a process forming embryos from somatic cells without undergoing sexual cycles; it is similar to zygotic embryogenesis. Somatic embryogenic cultures can be multiplied in bioreactors for large-scale production and can readily be cryo-stored for a long period of time without losing plant regeneration capacity and genetic fidelity of regenerated plants. In Silvagen Inc., Canada, clone banks have been initiated for blister-rust-resistant western pine (7 families, 50 genotypes) and high-yielding coastal Douglas-fir (including 12 families and 220 genotypes). For three southern pine species, 400 genotypes and 27 families have been stored (Cyr, 1999). In USA, Weyerhauser Inc. also maintains cryo-storage facilities at several locations for long-term storage of somatic embryogenic cultures of elite forest tree germplasm.

The major disadvantages of somatic embryogenesis are a) high dependence on genotype, b) poor rate of somatic embryo production and c) gradual fluctuation and eventual decline in embryogenic culture potential; finally, since cultures are developed from seeds or seedlings, the material is of unproven genetic value.

Somaclonal variation can be prevented by cryo-storage of embryogenic cultures without initial subculture. Another approach for plant multiplication is by organogenesis without going through a callus phase, *i.e*. shoots can be directly initiated from *in vitro*-cultured explants such as mature embryos, shoot tips, and adventitious buds, or they can be excised from old trees, as found in Q*uercus robur*, *Eucalyptus*, *Betula*, poplars, etc. Micropropagated plants cannot be cryo-stored. However, they can be stored at low temperatures in the cold room. Usually, *in vitro* shoots or *in vitro*-rooted plantlets are stored at low light intensity. The major risk with this approach is contamination of the culture material, especially by viruses. This process becomes labour-intensive, since cultures require subcultures on fresh medium at regular intervals that depend on the plant species. Micropropagation is ideally suited for the developing countries because of low maintenance cost.

Molecular marker (AFLPs, microsatellites) and flow cytometer analysis are essential in order to study the genetic fidelity of micropropagated, cryostored, and cold-stored plants. Furthermore, molecular marker analysis would assist in studying the genetic population structure, gene flow, and transgene protection (Jain et al, 2001)

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GENETIC MODIFICATION IN FOREST TREES

S. Mohan Jain

Recent advances in plant biotechnology have enabled improvement of forest trees with varying success. This is mainly due to differences in longevity, heterozygous nature, life cycles, and the environment in which they grow (Ritter, 1999). Forest trees have long generation cycles, with vegetative phases ranging from one to several decades. Once trees have germinated in nature or transplanted to plantations, they remain anchored in one location where they are exposed to changing environments and other vagaries of nature. Some of these factors may influence their physiology and alter the complex morphogenetic processes. During the long life cycle of trees, many genetic and epigenetic changes are probably important to maintain the within-tree and

within-populations genetic diversity, and to assure a long-term survival of individuals and populations. However, it is not surprising that with an increasing world demand for pulp, paper and timber products, along with the growing awareness of the high ecological and social value of indigenous forests, more efforts have been directed using the molecular biology for forest tree improvement (Walter, 1999; Klopfenstein et al, 1997).

Now various techniques are available for gene transfer in forest trees. These include the bacteria *Agrobacterium tumefaciens* and *A. rhizogenes,* and the biolistic gun; somatic embryogenic cultures are commonly transformed; and clonal propagagtion of trees can be done with somatic embryogenesis and organogenesis (Jain, 1999; Jain et al, 1995, 1999, 2000; Klopfenstein et al, 1997). In most conifers and other forest trees, there is limited information on the genetic fidelity of somatic seedlings, which is vital for clonal propagation of elite trees (Jain and Ishii, 1998). Several forest tree species have been transformed such as conifers, poplars, sweet gum, eucalypts, elm, and European chestnut. There is a long list of angiosperm and gymnosperm forest trees that have been transformed with marker genes without plant regeneration (Jain and Minocha, 2000). Few tropical or subtropical forest trees have been genetically transformed; they include Japanese persimmon and acacias. The most common trait specific genes introduced in forest tree species are for herbicide resistance, marker genes, *rol*C lignin, cellulose, insect resistance (*Bt*, and trypsin proteinase inhibitor), Hepatitis B virus for vaccine, mercuric reductase (merA) for phytoremediation, disease resistance (against *Phytophthora cinnamomi* and *Ophiostoma novoulmi*), male sterility, and the glutamine biosynthase gene for efficient nitrogen utilization. World-wide up to the year 1999, a total of 68 field trials with transgenic trees have been approved, out of which 51 are in poplars alone. They include genes for lignin, cellulose, sterility, herbicides, insect resistance and marker genes (Walter, 1999). The long life cycle and extended vegetative phase of forest trees may hamper the monitoring of transgene expression. Transgenic effects may cause abnormality immediately or remain dormant for a long time or even might be lost during the long vegetative phase of the tree species, and therefore it is rather difficult to predict the behavior of transgenes in the future (Jain, 1999; Jain et al 2001).

The major challenges facing introduction of genetically modified forest trees include the stability of transgene expression or silencing effects, containment of transgenes by reproductive options, forest management for reforestation with transgenic trees, genetic engineering and biotechnology specifically for tropical forest trees, and desirable modification of lignin for future sustainable forestry. We will have to come up with technologies to guarantee correct expression of genes over the life span of the tree and for rapid assessment of a high number of genes and promoters using cost effective technology, starting with some model systems, such as genes involved in the reproductive development of *Arabidopsis* (Walter, 1999). In the case of lignin and cellulose formation, conifer tissue culture systems that provide cells developing lignin and cellulose-containing cell walls, can be used to test a wide range of constraints rapidly and economically. Tree genetic engineering has potential for contributing to a new green revolution, allowing us to grow trees with desired traits and produce high-quality end products. In the final analysis, however, public perception and acceptance of genetic engineering will determine the future of genetically modified trees in forestry. References (also see references in preceding contribution)

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AN INTEGRATED MOLECULAR POPULATION GENETIC APPROACH FOR FOREST TREE CONSERVATION AND MANAGEMENT IN THAILAND

By Suchitra Changtragoon

One of the fundamental requirements for conservation and use of forest tree genetic resources is understanding the biological dynamics of genetic variation within and between species. Considerable variation exists among tree species with respect to the extent of genetic diversity and the way such diversity is organised within and among populations. The extent and the pattern of this diversity are strongly dependent on the amount of genetic polymorphism, pattern of gene flow and mating system.

Molecular genetic marker screening is based on the survey of genetic diversity as revealed by variation at specific gene loci and provides information about the amount and distribution of genetic diversity within and among populations. Furthermore, analysis of gene marker data permits estimation of outcrossing rates and thus monitoring genetic changes caused by factors affecting reproductive biology of a species. Information gained from genetic marker screening is invaluable for identification of populations which are desirable for conserving and breeding purposes and improving forestry practices which inadvertently alter natural gene pools of domesticated species (Changtragoon and Szmidt, 1997).

Previous research on molecular population genetics of forest trees in Thailand. The genetic diversity and mating system in some economically important forest tree species in Thailand such as indigenous pines *(Pinus merkusii* and *P. kesiya*), neem (*Azadirachta indica* var. *siamensis, A. indica* and *A. excelsa*), *Dipterocarpus alatus*, teak (*Tectona grandis*), rattans (*Calamus spp.*), paper bark tree (*Melaleuca cajuputi)*, *Mitragyna brunonis*, *Pterocarpus macrocarpus*, *Cycad siamensis* have been evaluated by using

isoenzyme gene and DNA markers (cf. Changtragoon, 2001; Changtragoon and Finkeldey, 2000).

Among the populations examined, those genetically most diverse within and among populations of the species should merit a high priority for conservation. Since forest trees are long lived compared to annual or crop plants, high genetic diversity and outcrossing rate would guarantee higher possibility of their survival, viability, longevity, and disease and insect resistance for the present and forthcoming generations in a changing environment. Combination of marker-aided population genetic analysis and information about adaptive and quantitative traits as well as forest ecosystems would allow for the development of a comprehensive conservation programme for individual species in each forest type (Changtragoon, 2001).

Genetic diversity and gene conservation of teak in Thailand. Fifty-one RAPD (Random Amplified Polymorphic DNA) loci were identified and used to evaluate the genetic diversity in fifteen natural populations of teak in Thailand. Partitioning of genetic variation into within and among population components revealed that about 21 % of the total variation was attributable to differences among populations. The number of polymorphic loci in most of the investigated populations was very high with an average of 72.6%. The average expected heterozygosity was 0.310. Significant differences in allelic frequencies were found for most pairwise comparisons between populations (Changtragoon and Szmidt, 2000). The outcrossing rate ranged between 82-97%. These results suggest that natural populations of *T. grandis* in Thailand are highly differentiated genetically, implying that multiple sources of materials from at least one population of each province in the northern and central part of Thailand may be required for both *in situ* and *ex situ* gene conservation purposes (Changtragoon, 2001).

Ongoing research. At present, the investigation of genetic diversity and mating system of *Rhizophora apiculata*, *R. mucronata*, *Azadirachta spp.*, some *Dipterocarpus spp.* and bamboo species using molecular markers are on the way.

Acknowledgements. Some of the research mentioned in this paper was financially supported by the following organisations: Volkswagen Stiftung (VW), International Foundation for Science (IFS), International Plant Genetic Resources Institute (IPGRI), Center for International Forestry Research (CIFOR), Australian Centre for International Agricultural Research (ACIAR), Biodiversity Research and Training Program (BRT), Thailand; and Royal Forest Department, Thailand.

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ISOZYME VARIATION IN NATURAL POPULATIONS OF SAL (*SHOREA ROBUSTA*) IN THE TERAI REGION, NEPAL

By Jouni Suoheimo, Chunyang Li, and Olavi Luukkanen

Sal (*Shorea robusta*) is the most important commercial tree species in Nepal. The species belongs to the Dipterocarpaceae family and also forms extensive forests in the northern states of India. This species has been intensively studied for more than a century, but recent work has still revealed new aspects on its ecology and silvicultural management (Suoheimo, 1999). Until very recent date, only phenotypic traits of *S. robusta* populations have been studied, and little information exists on the intra-specific variation of *S. robusta* as revealed by biochemical (isozyme) or molecular (DNA) techniques.

Knowledge of the distribution of genetic variation within and between populations is essential for the conservation of plant genetic resources. The isozyme variation within and among populations has been extensively studied in many woody plants, including tropical species. There is also conclusive evidence showing significant correlation between isozyme variation and quantitative traits. Isozymes as genetic markers are thus of great importance in tropical tree breeding and conservation.

In our study, we investigated the genetic variation of *S. robusta* in three natural populations in the Terai region, Nepal, using 12 loci from 8 isozyme systems (Suoheimo et al. 1999). The mean number of alleles per locus was 2.16, and 58.3% of the loci were polymorphic (95% criterion for polymorphism). The mean observed and expected heterozygosities ranged from 0.105 to 0.129 with an average of 0.117, and from 0.130 to 0.158 with an average of 0.143, respectively. Only 4.7% of the total genetic diversity was due to differentiation among the populations, and the mean value of genetic distance was 0.018. The results indicated that the majority of the species' genetic variation was found within the studied populations and there was a high genetic similarity among these three natural populations of *S. robusta*. The sharing of one gene pool among the studied populations suggested a lack of barriers to gene flow.

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MOLECULAR GENETICS OF *FAIDHERBIA ALBIDA* (DEL.) A. CHEV.

By: Otto George Dangasuk

Faidherbia albida (Del.) A.Chev. (syn. *Acacia albida* Del.), a leguminous tree species belonging to the Mimosoideae subfamily, is an important agro-silvo-pastoral species in arid and semi arid lands throughout Africa. Its unique phenology of shedding leaves during the rainy season at the time of higher microbial activities in the soil improves the soil structure, stability and permeability, and provides a microclimate favorable for crops. Retaining the leaves in the dry season, it provides shade and green fodder rich in protein and carbohydrates for livestock. The resulting mulch reduces evaporation thus conserving the available soil moisture. *F. albida* has a remarkable capacity for recycling nutrients from underground to the surface due to its very deep root system. The tree also stabilizes sand dunes and prevents soil erosion. *F. albida* does not compete with inter-planted crops for soil nutrients as it enters a period of physiological rest during the crop growing season. It is also used for timber, medicines and food.

In Africa drought and human interference have endangered the genetic resources of this species, prompting its inclusion among the endangered plant species. It has not been domesticated, and it is little used outside its natural range. Plantation projects with this species in Chad and Niger have failed, possibly due to lack of basic knowledge on the amount of genetic diversity and breeding systems.

In our studies, an analysis of genetic variation based on morphological characteristics in twelve and sixteen African provenances of *Faidherbia albida* in Kenya, although it showed consistent genetic variation across Africa, could not separate the eastern provenances from the southern African ones, probably due to environmental influence and epistatic gene effects (Dangasuk et al. 1997; Dangasuk, 1999). This analysis was not effective in determination of genetic diversity or the phylogenetic relationship among the provenances.

Molecular and biochemical techniques provide a powerful set of tools for the study of plant population genetics. Isozyme analysis in *F. albida* shows significant deviation from Hardy-Weinberg equilibrium, deficiency in heterozygotes and less differentiation among provenances (Harris et al, 1997; Dangasuk and Gudu, 2000). This could be due to inbreeding; however, a high rate of inbreeding or selfing would have generated considerable genetic differentiation between provenances, which was not detected in the isozyme data. In addition, isozyme results do not allow detailed analysis of genetic structure within provenances of *F. albida* and hence firm conclusions on phylogenetic relationships among populations cannot be drawn (Dangasuk and Gudu, 2000). Reviews on the levels of variation detected in a range of plant species based on isozyme data reveal that tropical tree species maintain most of

their variation within populations (Hamrick, 1993). This suggests that the classical forestry approach, which considers provenance or geographic variation as an accurate predictor of the diversity spectrum within species, may be inappropriate.

For better understanding of population genetics of *F. albida*, for the purpose of germplasm conservation, there is need to use markers able to show more variations, which would allow the identification of individuals within populations that are genetically different. Restriction fragment length polymorphism (RFLP) is the commonly used DNA marker. RFLP requires large quantities of pure DNA; species-specific DNA probes, and generally uses short-lived radioisotopes in the detection system. Furthermore, RFLP analysis is laborious; making it impractical for many populations based studies. Polymerase chain reaction (PCR) is less technically demanding than RFLPs and requires only a small amount of DNA. In addition, PCR provides flexibility in detecting genetic variation as a variety of primers can be used which are design to reveal particular types of polymorphism (Rafalski and Tingey, 1993). PCR methods include microsatellites, endonuclease restriction of amplified products (ERAP), single–strand conformation polymorphism (SSCPs) and random amplified polymorphic DNA (RAPD).

Unlike other approaches, RAPD analysis requires no prior DNA sequence information and it relies on single short, random oligonucleotides for amplification of unspecified target DNAs. RAPDs therefore represent a PCR-based technology that is immediately applicable to organisms from diverse taxa and, because of the large number of primers available for analysis, potentially provides good overall genome coverage (Williams et al. 1990). Therefore RAPD method capable of detecting individual differences in populations will be used in a study of genetic diversity in sixteen four-year-old *F. albida* provenances representing the natural distribution range of this species in Africa. The DNA will be nuclear ribosomal DNA, which is well suited for evolutionary and phylogenetic study. The sixteen provenances are currently growing in a randomized complete block design (RCBD) with five replications in the semi arid Baringo District of Kenya.

The long-term objective of the research is the conservation of the genetic resources of *F. albida* in Africa. The specific objectives are: (1) determination of the phylogenetic relationship among the 16 provenances in order to establish the species center of origin; (2) determination of the extent of genetic diversity in *F. albida,* using the PCR method of RAPD; and (3) study of individual tree genetic variability using RAPD. Combination of morphological, isozyme and DNA data will then form the basis for conservation, domestication, breeding and improvement of this species in different regions of Africa.

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ASSESSING GENETIC VARIABILITY IN *LEUCAENA* HYBRIDS: ISOZYMES AS MARKERS

By Maria Teresa Schifino Wittmann and Liane Helena Cardoso de Freitas

Assessing the genetic variability is the first, essential step for plant breeding that indicates what can be done using morphological, physiological, cytological, agronomic and molecular characteristics. Isozymes are good markers, since most of them have simple inheritance, co-dominant expression, complete penetrance and no pleiotropic and epistatic interactions. In addition, they are selectively neutral. The isozyme technique is rather inexpensive as compared to DNA techniques. A research line is being developed by our group with hybrids between the legume trees *Leucaena leucocephala* and *L. diversifolia* ssp. *diversifolia*, aiming to select cold-tolerant plants adapted to the climatic conditions of Rio Grande do Sul (Southern Brazil), to be used as forage specially during the winter and also as an alternative protein bank throughout the year. In this case, isozymes, apart from estimating the population variability and possibly characterising the species, may also provide some markers related to characteristics of agronomic interest . Morphological and phenological variability showed to be expressive (Freitas et al., 1995). Among the three isoenzyme systems (esterase, superoxide dismutase, and malic enzyme) studied, two were polymorphic (EST and SOD), and at least for SOD, species-specific results were detected (Schifino-Wittmann et al.,1996). Twenty selected progenies of these plants are now being analysed. Morphological and physiological studies identified genotypes with good forage production and also cold-tolerance (as measured by leaf retention during winter; Simioni et al. in preparation). Besides EST, SOD and ME, MDH (malate dehydrogenase) was also examined in the selected progenies. Intrafamily variation was found for EST, SOD and MDH. In a further step of assessing the genetic variability, DNA techniques are planned to be used, in order to provide a thorough view of the existing variability at different levels of biological organisation.

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RAPD ANALYSIS OF THE GENUS *BAMBUSA "SENSU LATO"*

By Ye Sun, Nianhe Xia and Rushun Lin

Bamboo species of the genus *Bambusa* are distributed in the subtropical and tropical areas of Asia and the tropical areas of the American continent. There are about 70

species and four subgenera in southern and southwestern China. Subgenus *Bambusa* includes the armed species and subgenus *Leleba* accommodates the unarmed species of the genus. Subgenus *Lingnania* and subgenus *Neosinocalamus* were established by Chia and Fung when they revised the species of the genus *Bambusa* in China. At present, molecular markers provide a very powerful tool to study the biodiversity of bamboos. Random amplified polymorphic DNA (RAPD) is widely used in genetic diversity analysis because of its simplicity and low expense. In our research, we employed RAPD molecular markers to study the genetic relationships of species within *Bambusa "sensu lato".*

Method

Fifteen species were studied in our research, including members of the subgenera *Bambusa, Leleba, Lingnania, Dendrocalamopsis* and *Dendrocalamus*. Sixteen primers, selected from sixty primers, were used in amplification. The genetic distances were calculated between each two species by using Nei's genetic distance. The UPGMA (Unweighted Pair Group Method of Arithmetic Average) algorithm was made on the basis of distance matrix.

Results

The results were as follows:

- There were close relationships among *Bambusa oldhami* Munro, B. multiplex (Lour.) Raeuschel ex J.H.Schult., *B. tuldoides* Munro, *B. ventricosa* McClure, and *B. subaequalis* H.L.Fung et C.Y.Sia;
- *B. sinospinosa* McClure had a close relationship with *B. flexuosa* Munro.
- *B. contracta* Chia et H.L.Fung was closely allied to *B. emeiensis* Chia et H.L.Fung and *B. chungii* McClure.
- *B. membranaceus* (Munro) Stapleton & N.H.Xia was closely related to *Dendrocalamus latiflorus* Munro.
- *B. vulgaris* Schrader ex Wendland was highly divergent from other taxa

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GENETIC DIVERSITY IN NATURAL POPULATIONS OF *EUCALYPTUS MICROTHECA*

By Chunyang Li and Olavi Luukkanen

Eucalyptus microtheca F. Muell. is characteristically a species of open woodlands with a wide and patchy natural distribution in the arid and semi-arid zones of Australia. Different environmental conditions in its native habitats, such as seasonal change in water availability, have resulted in large intra-specific variation in leaf physiology and growth performance (Li, 1999). *E. microtheca* has great value as plantation species especially on marginal lands, since it tolerates heat, prolonged drought and calcareous and gypsum soils. In the Sudan, for instance, *E. microtheca* is the principal plantation tree species. However, there has been relatively little attempt to use biochemical and molecular

techniques to determine the genetic relationships among *E. microtheca* populations. In our studies, isozyme and random amplified polymorphic DNA (RAPD) markers were used to estimate genetic variation in 12 natural populations of *Eucalyptus microtheca* populations (Li, 1999).

Isozyme markers were analyzed at 13 loci in 9 enzyme systems. The mean number of alleles per locus was 2.25, and 62.2% of the loci were polymorphic (95% criterion for polymorphism). The mean observed and expected heterozygosities were 0.150 and 0.199, respectively. Most of the diversity was located within populations; only 10.36% of the total genetic diversity was due to differentiation among populations. Cluster analysis based on unbiased genetic distance and the UPGMA dendrogram revealed limited genetic distance among populations.

RAPD markers were analysed with 18 primers; the primers yielded a total of 102 polymorphic bands, i.e. an average of 6 polymorphic bands per primer. Molecular markers were used to calculate the similarity coefficients, which were then used for determining genetic distances between the populations. Based on genetic distances, a dendrogram was constructed. Gene diversity values for each population ranged between 0.176 and 0.232 with an average of 0.200. Total gene diversity for this species was 0.240, where 83.3% of the variation was found within populations and 16.7% between populations.

Therefore, according to these isozyme and RAPD analyses, in *E. microtheca* most of the genetic variation was found within each population and there was high genetic similarity among the natural populations. Levels of genetic diversity were similar to those observed in other *Eucalyptus* species which also have widespread distributions.

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ASSESSMENT OF LEVELS AND DYNAMICS OF INTRA-SPECIFIC GENETIC DIVERSITY OF LATIN AMERICAN TROPICAL TREES FOR CONSERVATION AND SUSTAINABLE MANAGEMENT

By Andrew Lowe, Eric Bandou, Peter Breyne, Henri Caron, Stephen Cavers, Nathalie Colpaert, Bernd Degen, Rogerio Gribel, Marvin Hernandez, Antoine Kremer, Patrick Labbe, Maristerra Lemes, Rogerio Margis, Marcia Margis-Pinheiro, Marc van Montagu, Carlos Navarro and Julia Wilson

Genetic variation is of paramount importance to the long-term survival of forest ecosystems, since its level and distribution will determine the forests' ability to adapt to changing environmental conditions. Tropical forests are under increasing threat, either directly through exploitation of their products, or indirectly through conversion to other land uses. However, very little is known about the effects of these disturbances on the genetic diversity of forest tree species. It is therefore essential to acquire a good understanding of the level and distribution of diversity in natural populations, before it is possible to predict or measure the impact of any ecosystem disturbance. Little work of this nature has so far been undertaken, and thus our ability to assess the scale of effect of human impacts such as logging, domestication, and fragmentation, is very limited.

The structure of genetic diversity at the population level is determined by factors such as the reproductive biology, and seed and pollen dispersal. The effect of human disturbance on levels of diversity therefore also depends, to some extent, on the disruption it causes to the mating system and gene flow of a target species. Selective logging, for example, may lead to an increase in inbreeding, which results in a reduction of genetic diversity, while forest fragmentation may disrupt pollinator behaviour, and thereby alter patterns of genetic diversity and gene flow.

Approach and methodology

The study we have completed has utilised recent developments in molecular genetics, which have produced many powerful, PCR-based techniques. Chloroplast (PCR-RFLP and sequencing) and nuclear markers (AFLPs and SSRs) were used, as appropriate, to assess levels of genetic diversity, seed flow and pollen flow, by considering the maternal and biparental inheritance of markers.

Activities

The project analysed the level and distribution of diversity in several tree species from different ecosystems in Latin America, and evaluated the effects of human influence upon diversity within these species. As the selected species represent a broad range of model characteristics related to human impacts and life history, this project should enable the identification of key factors likely to modify diversity levels in forest tree populations of importance throughout tropical forest ecosystems.

From the Atlantic coastal forest of Brazil, which has been reduced to about 2% of its original extent and is now internationally recognised as a priority for tropical moist forest conservation, attention focussed on two species, *Eugenia uniflora* (Myrtaceae) and *Anacardium occidentale* (Anacardiaceae). In addition, from the Brazilian Amazonian forest, of which about 15 % has been degraded, and which is under ever-increasing pressure, two other species were examined, *Swietenia macrophylla* (Meliaceae), the 'big leaf' mahogany, and *Ceiba pentandra*

(Bombacaceae).
From French Guyane, species were selected for their contrasting life history traits that are likely to affect levels of genetic diversity, and included the following: *Symphonia globulifera* (Clusiaceae), *Moronobea coccinea*, *Dicorynia guianensis*, *Sextonia rubra*, and *Cecropia sciadophylla* (Cecropiaceae). In addition, *Tabebuia heterophylla* (Bignoniacea) from the Caribbean Islands was investigated.

In Central America, attention focussed on *Cedrela odorata* (Meliaceae) and Costa Rican populations of *Swietenia macrophylla* (Meliaceae), *Vochysia ferruginea* (Vochysiaceae) and *Lonchocarpus costaricensis* (Leguminoseae), all timber or multipurpose trees of high economic and ecological value.

Conclusions
Molecular markers highlighted important differences in the partitioning of diversity within and among populations. In addition, the role of different breeding systems and colonising strategies in determining diversity was emphasised.

For example, studies indicated that chloroplast DNA variation was generally low, and often showed strong structuring related to population, geographic region and even ancient geological events. However, one species, *Symphonia globulifera*, showed very high variation and it is thought that cpDNA inheritance in this species may not be strictly maternal, as is usually the case.

In contrast to some other studies, it was difficult to relate the level and distribution of genetic diversity to pollinator or population density. In a study of *Symphonia globulifera*, it was emphasised that the territorial behaviour of pollinators and flowering patterns could be influential. While bird-pollinated *Symphonia* had low outcrossing rates, the bat-pollinated *Ceiba pentandra* showed long pollination distances (~18 km) and highlighted the influence of bat territoriality on genetic diversity.

Results for *Swietenia macrophylla* (mahogany), from both Central and South America, indicate that this commercially important and heavily logged species is very sensitive to disturbance, and may take generations (100s of years) to recover. Careful management is crucial to prevent populations descending into a downward spiral of genetic resource loss.
These results are only a small subset of those obtained within the project. Overall they highlight the value of molecular approaches for developing conservation and resource management strategies. Thus when challenged by different types of environmental change, species respond differently.

This project has been of enormous benefit to all the partners. The bringing together of high-level molecular expertise with the expert knowledge of distributions and history of populations of tropical tree species has been invaluable. Furthermore, the breadth of the project, which has tackled aspects of the molecular genetics of more than 14 different tropical tree species from a wide range of habitats and with a diversity of breeding systems, has been a tremendous strength. It is hoped that the quality of the collaboration will continue into a third phase of the project, which is about to start, and is be funded under the 5th Framework Programme (GENEO-TROPECO ICA4-2000-20056) of the EU, whom we gratefully acknowledge for their support and encouragement during all phases of this work.

Main Publications. For a complete list of publications, including theses and poster presentations, visit **http://www.nbu.ac.uk/inco/referenc.htm**

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GERMINATION SYNDROMES AS TOOLS FOR PLANT FUNCTIONAL TYPES

By Enrique Jurado

Traits associated with seed germination may be of great value for natural resource management if grouped into sensible functional types. For instance knowing the proportions of a given flora that germinate in particular seasons, as well as their germination speed, would help natural ecosystem management both for production purposes and large scale conservation plans associated to climate change. In this article I present some information for the Tamaulipan thornscrub (*matorral*) in northeastern Mexico from research mainly supported by IFS, CONACYT, and PAICYT.

Seed mass, dispersal syndromes and other plant attributes were investigated for 111 species native to northeastern Mexico. Tests were conducted to investigate whether seeds from Mexico conformed to patterns of seed size and dispersal syndrome spectra found for floras in arid environments from around the world. The distribution of seed mass in the Mexican flora (0.03 mg to 598 mg) was generally similar to that found elsewhere. All of the major seed dispersal syndromes previously found in arid environments were represented in Mexico, although vertebrate dispersal (33 species) was unusually common. There were 31 species with wind-

dispersed seeds, 4 ant-dispersed and 43 with no obvious dispersal morphology. Woody species were more likely to have large seeds and herbaceous species were more likely to have small seeds. Woody plants had proportionally more wind-dispersed and less animal-dispersed species than did herbaceous plants. We did not find the expected relationship between time of seed set of vertebrate-dispersed species and the presence of migratory birds or between time of seed set and optimum germination time.

In semiarid environments plants should be selected to germinate at times most suitable for seedling establishment. Germinability and the rate of germination and temperature preference for germination (12˚C or 28˚C constant) were analyzed in respect to growth form (grass, forb, woody), longevity and seed size. Short-lived species showed a preferential germination at either high or low temperature, whereas long-lived species tended to be season-indifferent. Germinability was >20% for 28 species, < 10% for 17 species, and 21 species did not germinate under any circumstances. Growth form or lifespan did not influence germinability. Woody species germinated rapidly. Germinability and germination rate were positively associated.

Seed germination was investigated for 47 herbaceous and woody species, representative of the Tamaulipan thornscrub flora of northeastern Mexico. More than half of the species had similar germination under spring and autumn conditions, 17 species showed a higher germination percentage under autumn rain conditions. No species had more seeds germinating under spring rain conditions. Germination season response was independent of plant habit, seed size and dispersal syndrome. Sixteen of the species germinated less under the moderate shade ofthornscrub canopy than under direct sunlight, and more than half of the species had similar germination under shade and direct sunlight. Germination in autumn, and away from the root competition of thornscrub, may reflect the importance of soil drought in mortality of young seedlings.

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TISSUE CULTURE PILOT PLANT FACILITY FOR UPSCALING AND TESTING TECHNO-COMMERCIAL FEASIBILITY OF FOREST TREE TISSUE CULTURE AT THE NATIONAL CHEMICAL LABORATORY, PUNE, INDIA

By R. S. Nadgauda

A tissue culture pilot plant facility with a

production capacity of 1 million plants per year with greenhouse was designed at the National Chemical Laboratory (NCL), Pune, with financial support from the Department of Biotechnology, New Delhi, and commissioned in 1992. This facility has a semiautomated, fully air-conditioned laboratory and a greenhouse, monitored by an advanced computer system. The forest tree species being multiplied in this unit, *E. tereticornis*, *E. camaldulensis* (eucalypts), *Dendrocalamus strictus* (bamboo) and *Tectona grandis* (teak) were selected on the basis of their economic importance.

The techniques for all these plant species reported earlier in the 1980's were refined considerably. The extensive research and development work carried out was focussed on the following points:

Development, refinement and upscaling of protocols by reduction in the number of stages, use of minimal media, rapid multiplication rate and improved survival rates;
Methods for successful transportation of plants from greenhouse to the field;
Field verificatory trials.

The main features of this project were identification of superior genotypes and cloning of these using tissue culture. Extensive research was carried out to develop the process for mass propagation of the identified genotypes. These were then planted in the field for evaluation. Over 1.5 million plants of all the above-mentioned species were planted all over India in different agroclimatic regions.These trials were undertaken in collaboration with forest departments, agricultural universities and the private sector or organisations.

The field trials were designed as progeny trials, provenance trials and clonal trials with the following objectives:

- To test the clonal homogeneity of the micropropagated plants;
- To assess the performance of micropropagated plants over seed-raised progeny;
- To identify suitable provenances for different clones so as to realise the full potential of individual genotypes.

The growth data collected from different sites and analysed revealed that the tissue culture plants exhibit high uniformity and higher biomass, leading to early rotation and indicating the possibility to use this technology to increase the production per unit area.

Advantages of tissue culture-raised plants over conventional plants. Field trials were conducted at more than 300 different locations in different states of India and Nepal covering an area of 1500 ha. The field performance of tissue culture-raised plantlets as assessed from different trials was as follows :

- Tissue culture-raised plants exhibit higher uniformity and higher biomass leading to early rotation.
- *Clonal trial on eucalypts*. In one of the field trials on eucalypts conducted in 1994 in Maharashtra State using different tissue culture-raised plants from clones of ERK-4, SR-5, R3 and R-2, plants of clone ERK-4 showed a growth of 9 m in height as compared to 7 m attained by other clones and 0.5 m by seed-raised progeny. This growth was obtained in spite of the fact that the soil pH was 8.0 as against normal pH 6.8 to 7.
- *Clonal trial on teak.* Among the teak clones numbered NC-21, NE, TD, DN, KLS, the clones NC-21 and NE proved to be best performers in Maharashtra and Gujarat. In one of the trials conducted at Gujarat the recorded

height for clone NE was 281 cm as against the average height of 200 – 215 cm in 10 months, recorded for the other clones. NC-21 was also found to be a promising clone in several agroclimatic zones in Maharashtra, Gujarat and Karnataka.

- The performance of tissue culture-raised plants of teak planted in 1994 at Madhya Pradesh indicated an average increase of 18% over the control for height and 21% for girth within 6 years for clone NC-21. In one of the trials planted in August 1997 using tissue culture-raised plants of clone NE in Chattisgarh, the height increase in tissue culture-raised plants was 41% and girth increase 62% over the control at the age of 3 yr.
- Wood density analysis revealed that the average wood density of clone NC-21 plants from a 9-year-old plantation was 547 kg/$m^{3,}$ which is comparable to 551 kg/m^3 found in a 27-year-old conventional plantation.

Demonstration plot on farmers' land. In order to disseminate the laboratory-based results, a demonstration plot was planted using tissue culture-raised eucalypt plants on farmers' fields with the help of the Cooperative Agroforestry Federation, Nashik, Maharashtra, India. This is an apex body of the Cooperative Tree Growers Society in Maharashtra, and it has been successful in developing a system for eucalypt marketing. In this programme the production is decentralised and marketing is centralised, whereas the price is linked with quality. This builds quality-consciousness, sets product evaluation standards, and gears the production in accordance with market needs.

These trials demonstrated higher growth rates and a shorter rotation leading to a higher cost-benefit ratio (1:3). The wood volume of tissue culture-raised plants was 45 m^3/ha higher and fetched 39% more value for wood, which resulted in a 42% increase in net profit.

Realizing the potential economic benefits of this technology, the Federation has encouraged farmers to undertake large scale plantations on their fields using micropropagated eucalypt plants of selected genotypes. This has created a market demand leading to the establishment of a commercial arrangement with EPC irrigation at Nasik having a production facility of 1 million plants per annum to cater to the needs of the farmers.

Molecular markers for testing clonal homogeneity. Genetic analyses of tissue culture-raised plants of *Eucalyptus tereticornis, E. camaldulensis* and *Tectona grandis* were carried out by Dr. S.N. Raina and his group at Delhi University, New Delhi, using cytochemical and molecular assays. Their results indicated that there is no difference in the total DNA content between progeny and mother clone. The genetic fidelity was further confirmed using RAPD techniques. The amplified genomic DNA fragments from *in vivo* and *in vitro* materials were monomorphic across the plants tested (Rani and Raina 1997).

Conclusions. These projects have contributed to building up of a strong knowledge base and resulted in the development of commercially viable indigenous technologies. The field verificatory trials and testing at molecular level have confirmed the feasibility of tissue culture propagules for clonal forestry. Commercial plantations have resulted in generation of confidence among foresters, farmers and industries on the benefits of micropropagation.

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THIRTY YEARS OF VEGETATIVE PROPAGATION RESEARCH ON TROPICAL TREES IN SCOTLAND

By Jan McP Dick

Research into vegetative propagation has been conducted at CEH Edinburgh (formally Institute of Terrestrial Ecology) since the 1970's. The early work was based in West Africa working with timber species (Bowen, Last, Leakey & Longman 1977 Forest Genetic resources information (FAO), no.6, 38-47). The aim was to increase timber production of commercial trees, and results showed the tremendous possibilities for selection of superior good-formed timber trees to be vegetatively propagated by leafy stem (soft wood) cuttings. During the 1980's the early emphasis on commercial timber species widened to consider other uses of vegetative propagation, including selection of superior trees producing non-timber forest products, biodiversity conservation-orientated issues of endangered species, and selection of individuals resistant to pests. Appropriate technology like the simple low-technology non-mist propagator was promoted.

Along with institute staff (including Alan Longman, Roger Leakey, Adrian Newton, Steve Hoad) an energetic group of tropical students and visiting scientists from Cameroon (Zac Tchoundjeu, Patrick Shiembo), Costa Rica, (Francisco Mesen), Kenya (Patrick Muthoka, Peter Odoul) Ghana (Daniel Ofori, Teresa Nketiah) Malaysia (Aminah Hamzah, Ernest Chai) and Tanzania (Francis Magingo) has conducted a series of experiments in Scotland and in their home countries investigating factors influencing the growing environment of stock plants (e.g. light quality and quality, nutrients, maturation), the effect of pre- and post-severance treatments (eg leaf area, stem length, auxin application) and propagation environment (eg light level, rooting media, mist/non-mist systems). Initial single-factor experiments led to more complex studies, and by utilizing multi-regression analysis it has been possible to determine some of the primary factors favouring the rooting of cuttings. An understanding of the primary importance of leaf area (~photosynthetic ability) and stem diameter (~stored starch) in the emergence of adventitious roots has led to the development of a mechanistic model. This provides a quantitative scheme for understanding how root development depends on properties of cuttings such as leaf area, internode length and initial carbohydrate content. The Vegetative Propagation Programme has created well over 100 papers many of which can be accessed via

http://www.nbu.ac.uk/ tropical/staff.htm

The Edinburgh station of CEH has been active in technology transfer and capacity building in tropical countries throughout the last thirty years and has worked with very many government and non-government organizations conducting in-country workshops and training MSc and PhD students. In 1993 Alan Longman wrote a simple guide answering the most frequently asked questions about leafy stem cuttings ("Rooting cuttings of tropical trees") which was also translated into Spanish. He followed this with the manuals "Growing good tropical trees for planting" and "Preparing to plant tropical trees". These manuals were produced by the Commonwealth Science Council, (Marlborough House, Pall Mall, London SW1Y 5 HX http://www.comsci.org). The

Forestry Research Programme (http://www.netcom.net.uk/~n/nri/frp1.htm) of the Department for International Development also funded the filming of a video showing the procedures necessary to propagate tree species by the technique of leafy stem cuttings ("Multiplying tropical trees: vegetative propagation and selection). The video has been translated into Spanish and Bahasah Malay.

The work of the group is still continuing primarily in a capacity building role. Recently the Darwin Initiative For The Survival of the Species (http://www.britishcouncil.org /science/science/pubs/briefsht/darwin/ darwin.htm) has approved two projects with elements of training in the techniques of vegetative propagation. The projects have quite different aims. In Vietnam the aim of the project is to help provide Vietnamese researchers and field staff with the knowledge and skills to enable them to undertake the sustainable management of the remaining montane forests. This will result in the utilisation of elements of the biodiversity by local communities and other stakeholders in a sustainable manner. The methods and techniques being exchanged include development of appropriate methods for the propagation and utilisation of threatened conifer taxa.

In Colombia the aim is to work together with Amerindian communities in Leticia and surrounding areas to exchange knowledge and techniques related to reforestation, rehabilitation and conservation of the Amazonian ecosystem. One aspect is the exchange and conservation of indigenous knowledge relating to propagation techniques and to contrast and compare their traditional techniques with our approach.

Conclusions

Working closely with our many overseas collaborators we have shown that there are now very few species, which cannot be manipulated to produce adventitious roots from juvenile material (i.e. seedlings or cuttings collected from coppiced adult trees). One of the next big researchable topics is to determine the factors influencing ontogenic ageing of trees which limits the rooting potential of cuttings collected from mature trees.

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GENETICS MARKERS (RAPDS AND rep-PCR) FOR ECOLOGICAL STUDIES OF THE NITROGEN-FIXING SYMBIOSIS *Frankia-Casuarina*

By María Valdés, Luis Vásquez and Néstor-Octavio Pérez

Introduction.

In developing countries the symbiosis between the fast-growing tree *Casuarina* and the nitrogen-fixing actinomycete *Frankia* has a great potential for agroforestry and forestry. *Casuarina* is noted for its rapid growth rate in marginal soils. The tree has been introduced in tropical and subtropical regions to reclaim disturbed sites, as windbreaks, for sand dune stabilization, and for production of high quality fuelwood.

This tree species is one of the most commonly propagated ones in the Mexican nurseries. An appropriately large-scale production of *Casuarina* implies the need of inoculation with an appropiate *Frankia*

strain, and to have information not only on its effectiveness, but also on its ecology (persistance and competitiveness in the soil). However this information is not commonly available, and plants devoid of nitrogen-fixing nodules are sent from the nurseries to the reforestation areas.

Microbial ecology has been dependent on the use of conventional microbiological techniques for a long time. Unfortunately these techniques do not allow identification or follow-up of the inoculated *Frankia* strains, because *Frankia* is a very slowly growing filamentous bacterium; its generation time can be up to 5 days. In order to avoid the isolation step from the nodules, we developed different genetic markers of the Mexican *Frankias*, so as to be able to perform ecological studies.

Methods.
Previous findings have shown that it not possible to discriminate between culturable *Frankia* strains nodulating *Casuarina* throughout RFLP techniques; these techniques utilized probes of the genes 16S and *nif*H, and PCR-RFLP of the intergenic spacer 16S-23S (Pérez et al 1999). We did utilize the resolutive technique RAPD, which is based on a random DNA amplification with a 10 bp primer (5'-CGG CCA CTG T-3') of arbitrary sequence. This technique allows fingerprinting of the whole genome, permitting to differentiate strains belonging to the same species (Williams et al . 1990).

We also utilized oligonucleotide primers of the short intergenic repeated sequences (5'CTA CGG CAA GGC GAC GCT GAC G-3'), repPCR, that contain highly conserved inverted elements for application to fingerprinting bacterial genomes; the primer was BOX A1R (Versalovic et al. 1994).

Results.
We obtained 143 bands after the RAPD reaction. The average number of bands per strain was 11 ± 2.9. The total of bands represented 39 common loci and 20 were specific loci. The reference strain *Frankia* BR showed five and the native strain Ce16 two specific loci. The constructed matrix, based on the data and analysed with the Jaccard, UPGMA and multidimensional scaling procedures (Rohlf 1994), showed low genomic diversity among the *Frankia* strains. However, the techniques provided specific genomic differences that can be used as molecular markers.

Resulting fingerprint patterns of the rep-PCR products when using the BOXA1R primer were not complex, and the average band number produced was 8.38 ± 1.59. Bands were also obtained that were unique to the fingerprint of some Mexican symbiotic strains of *Frankia* nodulating *Casuarina* (Pérez et al 1999). This technique enabled us to select the strains displaying different fingerprint patterns, e.g., Ce1, Ce17, Ce20, etc., to be used for ecological studies.

RAPD and rep-PCR procedures were shown to be effective and easy methods to differentiate *Frankia* strains infecting *Casuarina equisetifolia* that might be important for further ecological studies of this symbiosis.

Aknowledgements. We gratefully aknowledge grants from The Mexican Council for Science and Technology (CONACYT) and the Direction for Research and Graduate Studies (CGPI/IPN).

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MYCORRHIZAL AND RHIZOBIAL INOCULATIONS AS TOOLS FOR LEGUME TREE CONSERVATION AND MANAGEMENT IN THE ORINOCO FLOODPLAIN, VENEZUELA

By Zita de Andrade, Gladys Escalante, Saúl Flores and Rafael Herrera

Vesicular-arbuscular mycorrhizal (VAM) fungi and nitrogen-fixing microorganisms play an important role in nutrient cycling of natural ecosystems, especially under low nutrient availability. The seasonally flooded forest near the mouth of the Mapire River, a tributary of the lower Orinoco, is rich in legume tree species, the majority of which are nodulated with rhizobia and show infection with the VAM. *Acosmium nitens* (Vog.)Yakoul is the main species subject to anthropogenic pressure for its valuable timber which is used locally for construction purposes. In greenhouse studies, *A. nitens* was shown to be highly dependent on VAM fungi and rhizobia for its development. The aims of this work were: (i) to select the most effective VAM fungal species in combination with native rhizobia for the development of *A.nitens* during the first year of the seedling's life in a nursery, and (ii) to evaluate these under field conditions, so that the inoculated plants could be used later in reforestation of disturbed flooded areas in the Orinoco plains.

Seven treatments were carried out using sterilised forest soil in plastic bags: absolute non-mycorrhizal control, non-sterilised forest soil inoculated with *Glomus occultum, Scutellospora fulgida, Acaulospora laevis, Entropho-spora colombiana*, and a mixture of all the four mentioned fungi. All of these treatments were inoculated with native rhizobia and other rhizosphere microorganisms. At monthly intervals the height of each plant was measured. At 3, 6 and 12 months five replicates were harvested to determine biomass, nutrient content (N,P,K,Mg and Ca), nodular biomass and VAM fungal colonisation. The results showed that *A. nitens* is a broad host regarding its preference for VAM fungi. In fact, the best treatments in terms of growth and nutrient uptake were all those inoculated with VAM fungi species. The growth rate, nutrient uptake and biomass of the best treatments were all significantly higher than those of both controls, often by a factor of 5 or more. After one year in the nursery, 45 seedlings were inoculated and 36 seedlings used as uninoculated control plants. These were transplanted to the field where they were observed for three years. These plants performed in the field according to their development and not to their age; thus, mortality was low, growth was faster, and in general, the vigour was higher than in

naturally regenerated trees of a similar age. These experiments point towards the possibility of improving the natural regeneration of *Acosmium nitens*, saving the equivalent of five years in regeneration time and preventing the high mortality which had been occurring over the last few years.

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SYMBIOTIC INTERACTIONS BETWEEN WOODY LEGUMES AND ROOT NODULE BACTERIA (RHIZOBIA) UNDER FAVOURABLE AND ADVERSE CONDITIONS

by Leena A. Räsänen and Kristina Lindström

Introduction.
Nitrogen fixing, drought tolerant and multipurpose *Acacia* and *Prosopis* tree species are appropriate for reforestation of degraded areas in arid and semiarid regions of the tropcis and subtropics. Use of rhizobial inoculants may be crucial in order to exploit their nitrogen fixing capacity, because the soil might lack compatible rhizobia, or there may be too few of them. The infection of woody legumes by ineffective strains impaired in their symbiotic properties, or bacteria related to tumour forming agrobacteria can also be a problem in the field. These problems are also encountered in nurseries when local soils are used as growth media for leguminous tree seedlings. Thus, to ensure that seedlings develop effective, nitrogen fixing nodules at the nursery stage, it is important to inoculate them with well characterised and tested rhizobial inoculants.

The aim of our studies was to investigate the symbiotic properties (host specificity, infection mode and nodule type) of some putative inoculant strains inoculated on several *Acacia* and *Prosopis* species characteristic for semi-arid or arid regions. In addition, we investigated how the symbiosis will develop between *A. senegal* and *Sinorhizobium arboris* under heat and drought stress, and which factors might explain possible failures in the nodulation. We also studied possibilities to relieve detrimental effects of drought stress on plant growth by using exogenous osmoprotectants. In stress experiments, plants were inoculated with a *S. arboris* strain marked with the b-glucuronidase (GUS) encoding *gusA* gene. In this way, detection and comparison of infection processes, the early steps of the symbiosis, between stressed and nonstressed roots were easier and faster as compared to traditional staining methods.

Host specificity.
The five inoculant strains used were isolated from *Acacia* and *Prosopis* nodules in Sudan and Senegal, and they belonged to the fast-growing sinorhizobial species, namely *Sinorhizobium arboris*, *S. kostiense*, *S. saheli* and *S. terangae* bv. *acaciae*. All five strains induced nitrogen fixing nodules on all African *Acacia* species tested (*A. mellifera*, *A. nilotica*, *A. oerfota* (synonym *A. nubica*), *A. nilotica*, *A. senegal*, *A. seyal*, *A. sieberiana*, *A. tortilis* subsp. *raddiana*) and on Latin American *A. angustissima*, *P. chilensis* and *P. pallida*. The strains formed effective nodules also on *P. juliflora* and *P. chilensis*, which were introduced into Africa from Latin America, and on Afro-Asian *P. cineraria*. However, ineffective nodules were formed on African *P. africana* and *A. holosericeae* which has been introduced from Australia to Africa. The former case was surprising because growth sites of *P. africana* in Africa are rather similar to those

of the acacias or the introduced *Prosopis* spp. studied here. In general, Australian acacias seem to prefer slow-growing bradyrhizobia. According to recent molecular evidence, African and American acacias may be more closely related to each other than either are to the Australian acacias. This might explain why Australian acacias, like *A. holosericeae*, have different rhizobial preferences.

Infection mode and nodule type.
In general, all species had root hairs on their roots but particularly in *Acacia* spp. they were sparse. After inoculation, root hairs were deformed. Rhizobia could enter the root through root hairs and penetrate in a hair via a tunnel, formed by the plant. These so called infection threads were mostly formed on short hairs in *Acacia* spp. and on longer ones in *Prosopis* spp. Elongation and ramification of the nodules indicated that *Acacia* and *Prosopis* had indeterminate nodules, although a persistent apical meristem, characteristic feature of the indeterminate nodule, was not so clear in *Acacia* spp.

Infection and nodulation under stress.
Our plant experiments showed that at root temperatures of 42°C, *A. senegal* seedlings and *S. arboris* cells were able to survive and root hairs showed normal deformation capacity, but nodulation was inhibited. For some reason, rhizobial cells could not enter the hairs. However, the inhibition of nodulation was reversible, and nitrogen-fixing nodules developed on those parts of the roots which were formed after completation of the thermal stress.

In drought experiments, water deficit disturbed the infection process, inhibited nodulation and significantly decreased the numbers of culturable rhizobia in the *A. senegal* rhizosphre.
However, application of glycine betaine or trehalose to the drought-prone soils increased the numbers of culturable rhizobia to the same level as in well-watered jars. Exogenous osmoprotectants did not improve plant yield but helped *A. senegal* seedlings, which were incapable of synthesising glycine betaine, to survive under severe drought.

Heat induced peculiar changes in the culturability and cell morphology of sinorhizobia grown in batch cultures. Several complementary techniques (luciferase activity of *S. arboris* strain marked with the *luc* gene; plate counts; optical cell density; two fluorescent stains in order to microscopically identify metabolically active and dead cells) were applied. It appeared that although the culturability and cellular energy reserves decreased considerably during heat stress, a majority of the cell population maintained the basic enzymatic activity. In other words, under adverse conditions, rhizobial cells do not only die but may also enter into a state in which they are viable but non-culturable.

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For further information please contact the authors at:

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SOURCES OF FUNDING FOR ACTIVITIES IN THE FORESTRY SECTOR

Adrian Whiteman

As part of the international dialogue on forestry, countries have concluded that international organisations could play an important role by disseminating information about sources of funding for sustainable forest management (Report of the Intergovernmental Forum on Forests - fourth session, paragraphs 20 to 31). In response to this, FAO has started a small pilot project to improve public access to information about sources of funding for activities in the forestry sector. As a first step, a small database had been produced, describing some of the types of funding that are available. Currently, it only includes a very small number of funding organisations and it is limited to organisations that have placed information about funding on the internet. However, in addition to funding for "traditional" forestry projects, the database also includes information about funding for a range of other activities that could contribute to sustainable forest management (e.g. grants for overseas study and training, grants for feasibility studies). We estimate that this could grow to 200-300 sources(and probably twice this if national sources of funds were to be included).

The database has been loaded onto the FAO website for demonstration purposes,testing and evaluation and can be accessed at: http://www.fao.org/forestry/finance/sources Before taking this any further, FAO would welcome comments about how useful this information might be and suggestions for improvement. For example: should it include national as well as international sources of finance; is the classification system right (by country, by type of funding, by subject/activity, by target group etc.); are the descriptions (under "more information") too long or too short; should it include non-web-based information sources; should it be disseminated by other means as well (hard-copy, CD-Rom)? We would, of course, also welcome any suggestions for other sites to include in the database.

Please send any comments that you might have to catarina.moore@fao.org. Thanks.

CARBON CYCLING TERMINOLOGY IN INTERNATIONAL PROCESSES - CONTRIBUTION OF TERMINOLOGY SCIENCE FOR A BETTER MUTUAL UNDERSTANDING ON INTERNATIONAL LEVEL

IUFRO's terminology project SilvaVoc calls for co-operation in a forest terminology project on carbon cycling. The aim is to prepare a multilingual glossary on carbon cycling of approximately 300-400 terms in the official IUFRO languages: English, French, Spanish and German.

Special emphasis is placed on terms and definitions, which may cause confusion because of diverging national interpretation. An invitation letter calling for co-operation and input is presently circulated to International Organizations and Processes and forestry institutes. In order to assess the regional differences in definitions and meaning it is most important to identify partners and colleagues with different linguistic environments and mother tongues.
Please inform the SilvaVoc Coordinator Renate Prüller at prueller@forvie.ac.at

about your expertise and interest to contribute in this initiative.

PCTOC INVITES REVIEW ARTICLES ON FOREST TREE BIOTECHNOLOGY

By S. Mohan Jain

Plant biotechnology, including genetic modification, plant molecular and developmental biology and plant tissue culture, is making tremendous progress. The micropropagation industry produces worldwide hundreds of millions of high–quality, disease-free plants per year. Transgenic plants are in the field and genetic maps of several crops have been developed. Many useful mutant varieties have been obtained by induced mutations, and molecular markers are becoming an indispensable tool in the breeding of many crops. At the same time, public concerns have been raised about ethics, environment and health, and about patenting and commercialisation of biotechnology.

Large numbers of research papers are being published in numerous international and national journals. Often it is difficult to keep track of publications in the large pool of journals. Review articles are immensely helpful to researchers and students in updating their knowledge on a given subject, in integrating the present body of knowledge and in directing implicitly or explicitly future research. Therefore, *Plant Cell Tissue and Organ Culture* (PCTOC) will publish review papers under the name Reviews of Plant Biotechnology and Applied Genetics. Most will cover biological and agricultural aspects. However, the reviews will also deal with ethical, socioeconomic, and environmental dimensions of plant biotechnology, as well as with current debates on future lines of research and short commentaries on recent scientific events.

We aim to include one or two reviewsin each issue of PCTOC. The present, double issue contains only reviews. We call upon colleagues to contribute review articles to PCTOC.

For advice or any further information please contact the science editor of PCTOC responsible for reviews:

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International Atomic Energy Agency
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By Jelle Maas

The International Plant Genetic Resources Institute (IPGRI) is a Centre of the Consultative Group on International Agricultural Research (**CGIAR**) located in Maccarese, Italy. Its mandate is to advance the conservation and use of genetic diversity for the well-being of present and future generations. The IPGRI programme on Forest Genetic resources may be found at: **www.ipgri.org/programmes/grst/FGR/home.htm**. IPGRI publishes 'Backgrounders' short notes giving basic information on IPGRI and plant genetic resources, including a sheet on Genetically Modified Organisms (GMOs). **http://www.ipgri.org/institute/pawareness.htm**. More information on the institute and its activities are available at **http://www.ipgri.org/**

Danida Forest Seed Centre (DFSC) provides technical support to developing countries in the fields of procurement and handling of seed of tropical and subtropical tree species, basic tree improvement and conservation of forest gene resources. **http://www.dfsc.dk/**

The FAO Forest genetic resources programme **http://www.fao.org/forestry/FOR/FORM/FOGENRES/homepage/fogene-e.stm** provides access to the REFORGEN database **www.fao.org/forestry/foris/reforgen/index.jsp** on forest genetic resources; the bulletin Forest Genetic Resources **http://www.fao.org/forestry/FOR/FORM/FOGENRES/genresbu/genbul-e.stm** and other relevant publications.

Dendrome: **http://dendrome.ucdavis.edu/** is a collection of forest tree genome databases and other forest genetic information resources for the international forest genetics community. It includes the IUFRO World Directory of Forest Geneticists and Tree Breeders at: **http://dendrome.ucdavis.edu Colleague/index.html**. The Agricultural Research Service (ARS) Genome Database Resource (GDR) at Cornell University, New York hosts this collection of crop databases **http://ars-genome.cornell.edu/**

The CATIE (Latin American Centre for Research and Education on Tropical Agronomy and Natural Resources – based in Costa Rica) website has details (in Spanish) on the CATIE biotechnology laboratory, as well as on project activities including 'Development of micropropagation strategies to support improvement and conservation of forest species'. Please see: **http://www.catie.ac.cr/investigacion /investigacion.asp?pagina=proyectos** CATIE also provides a searchable database on seed resources for forest species: **http://www.catie.ac.cr/proyectos/prosefor/base/semillas.htm**

The projects 'Distribution of genetic diversity in tree species from the neotropics based on DNA fingerprinting assays: Implications for conservation, sustainable utilization and management', and the follow-up project: Assessment of levels and dynamics of intra-specific genetic diversity of tropical trees for conservation and sustainable management **http://www.nbu.ac.uk/inco/** are funded by the European Commission's DG Research International Cooperation Programme. The institutions collaborating on the projects are: the Centre for Ecology and Hydrology (CEH) in Scotland; CATIE; INRA stations in Bordeaux, Guadeloupe and Kourou; the Federal University of Rio de Janeiro and INPA in Brazil; and the Flemish Interuniversity Institute for Biotechnology. Please see **http://www.nbu.ac.uk/inco/partners.htm** for contact details.

CAMCORE **http://www2.ncsu.edu/camcore/** is the Central America and Mexico Coniferous Resources Cooperative. The website is located at the College of Forest Resources,

North Carolina State University, in Raleigh, NC. The objective of the cooperative is: "to conserve, test, and improve forest species in the tropics and subtropics for the benefit of humankind."

The Australian Tree Seed Centre (ATSC) is a central contact point for seed and information about the woody component of Australia's floral biodiversity. ATSC is part of CSIRO (Australian Commonwealth Scientific and Industrial Research Organisation) Forestry and Forest Products and has been Australia's national tree seed bank for over 30 years. **http://www.ffp.csiro.au/tigr/atscmain/index.htm**

The European Forest Genetic Resources Programme (EUFORGEN) is a collaborative programme among European countries aimed at ensuring the effective conservation and the sustainable use of forest genetic resources in Europe **http://www.ipgri.cgiar.org/networks/euforgen/euf_home.htm.** EUFORGEN operates through networks in which forest geneticists and other forestry specialists meet and work together to analyze needs, exchange experiences and develop conservation methods for selected species, including Mediterranean oaks, *Populus nigra,* European conifers, 'Noble hardwoods' and 'Social Broadleaves' (Temperate oaks and beech).

EBNIC is the European Biotechnology Node for Interaction with China, a virtual organisation aiming to assist European scientists and industries**. http://www.ebnic.org**

IPNI (International Plant Names Index) is a database of the names and associated basic bibliographical details of all seed plants. It is the product of a collaboration between Royal Botanic Gardens, Kew, Harvard University Herbaria and Australian National Herbarium **http://www.ipni.org/**

The internet based Information System on Genetic Resources (GENRES) is maintained by the Information Centre for Genetic Resources (IGR) at the German Centre for Documentation and Information in Agriculture (ZADI). GENRES connects the centralized meta, factual and evaluated data on genetic resources in Germany at the IGR with the decentralized data existing in institutions involved in GENRES. **http://www.genres.de/genreadk/genres_int/index-e.htm**

'Elaboration of recommendations for sustainable forestry to protect the genetic variation of forest tree and shrub species' is a project funded by the German Federal Ministry of Food, Agriculture and Forestry aimed at analysing the influence of forest management on the genetic variation of forest tree and shrub species. **http://www.rrz.uni-hamburg.de/OekoGenetik/Projects/P_Bernd.htm**

The Centre for Genetic Resources, the Netherlands CGN at: **http://www.plant.wageningen-ur.nl/about/Biodiversity/Cgn/** is part of Plant Research International at: **http://www.plant.wageningen-ur.nl/**. CGN executes the national plant genetic resources programme for food and agriculture of the Netherlands and holds collections of several agricultural and horticultural crops.

The Biodiversity and Genetic Resources (BIO) programme of CIFOR **http://www.cifor.cgiar.org/research/conservation/** is designed to facilitate improvements in the sustainable use of forest biological diversity at the landscape scale. This will result from an improved capability to assess biodiversity and new insights into relationships between human interventions, biodiversity and ecosystem processes.

INTERNATIONAL TROPICAL TIMBER ORGANIZATION (ITTO) FELLOWSHIP PROGRAMME (FREEZAILAH FELLOWSHIP FUND)

OBJECTIVE

To develop human resources and enhance professional expertise in member countries in tropical forestry, tropical timber industries and related disciplines, with a view to promoting sustainable management of tropical forests, efficient utilization and processing of tropical timber, and better economic information on the international trade in tropical timber.

ELIGIBLE ACTIVITIES

a. Participation in short term training courses, training internships at industries, research and educational institutions, study tours, lecture/demonstration tours and international/regional conferences;
b. Technical document preparation, publication and dissemination, such as manuals and monographs;
c. Small grants for post graduate studies.

AWARD

The maximum amount for a fellowship award is US$10,000. Budget Guidelines are provided in the application form. Please note the non-allowable costs, equipment purchase, personnel hiring and health insurance. For post-gradate studies, only a partial tuition fee or a small research grant can be provided.

ELIGIBILITY

1. Only nationals of ITTO Member countries are eligible to apply*/.
2. Awards are to be made to individuals not to institutions.
3. ITTO Fellowship awardees may not again apply for an ITTO Fellowship Award, within two years of receiving an award and submitting to the Secretariat his/her final report on the activity for which the award was made.

APPRAISAL

Applications are appraised by a Fellowship Selection Panel which meets during the Sessions of the International Tropical Timber Council (ITTC) in May and November each year. The Panel comprises six individuals, three from producer member countries and three from consumer member countries, and is chaired by the Vice-Chairman of the ITTC.

DEADLINES

Deadlines for receipt of the completed application are:

13 March 2002 for the 32nd Session of the ITTC (13 – 18 May 2002) and

4 September 2002 for the 33rd Session of the ITTC (4 - 9 November 2002).

Applicants are advised that fellowship activities should begin no sooner than July 2002 for the March deadline and December 2002 for the August deadline.

APPLICATION AND SUBMISSION

Application forms are available in English, French, and Spanish. Please write to the following address to request or submit the completed applications.

The Executive Director
International Tropical Timber Organization
Pacifico-Yokohama 5F, 1-1-1, Minato-Mirai,
Nishi-ku Yokohama 220-0012, JAPAN
Tel: (81-45) 223-1110

Fax: (81-45) 223-1111
E-mail: itto@itto.or.jp
Homepage: http://www.itto.or.jp
ITTO Member Countries as of 1 July 2001 are:

Producers:
Bolivia, Brazil, Cambodia, Cameroon, Central African Republic, Colombia, Congo
Côte d'Ivoire, Democratic Rep. of the Congo Ecuador, Fiji, Gabon, Ghana, Guyana Guatemala, Honduras, India, Indonesia, Liberia, Malaysia, Myanmar, Panama,Papua New Guinea, Peru, Philippines, Suriname, Thailand, Togo,Trinidad & Tobago,Vanuatu, Venezuela.

Consumers:
Australia,Austria,Belgium-Luxembourg, Canada, China, Denmark, Egypt, European Union, Finland, France, Germany, Greece, Ireland, Italy, Japan, Nepal, Netherlands, New Zealand, Norway, Portugal, Republic of Korea, Spain, Sweden, Switzerland, United Kingdom, United States of America.

SEARCH FOR PhD POSITION

Johannes Füüssel has a MSc. in Environmental Forestry (Bangor, UK), International Land-Management (Witzenhausen, FRG) and a post-grade in Ecology (Tüübingen, FRG). He has 12 years oversee- experience and momentary is focusing practical work with his consultancy ''Eco~Tierra'' on autochthonous vegetables and grains for food- security (mainly ''Breadnut-Tree'': *Brosimun alicastrum* and ''Spinach-Tree'': *Cnidoscolus urens & C. chayamansa*) in communities near Masaya, Nicaragua, in collaboration with ''Terre des hommes-Italia''. Both species have been basic food of the Central American Maya- culture.

Mr. Füüssel is looking for a PhD position to research this topic:

- Wild and Maya- cultivated populations of *B. alicastrum*: Yield, initial growth pattern, nitrogen fixation, nutritional value etc.
- Botanical revision of the six or more morphologically significantly different *C. urens* and *C. chayamanse*. Possible reclassification in species and/or variants and cultivars: Yield, growth pattern, propagation, nutritional value, nitrogen fixation etc.
- Socio-economic, historical and religious reasons for discontinue of cultivation and use of these crops in post- colonialist times.
- Potential as agroforestry and NTFP crops in modern agriculture and nutrition.

The practical fieldwork of this PhD would be the natural distribution and historical cultivation centre of these two species, Central America, but other countries may be considered for introduction by provenance- trials.

You may learn more about the work of Mr. Füüssel and revise his CV at www.eco-tierra.com or contact him directly at eco@ibw.com.ni

NEW ACTION PLAN FOR ITTO

YOKOHAMA, JAPAN, 3 November 2001: ITTO's course has been charted for the next five years under the new 'Yokohama Action Plan', announced today by the International Tropical Timber Council.

The Plan, which spans 2002-2006, was developed after wide consultation between Member governments, environmental non-governmental organizations, the timber trade and industry, and other international organizations. Its aim is to accelerate progress towards achieving exports of tropical timber and timber products from sustainably managed sources and it sets six major goals.

- Improving transparency of the international timber market;
- Promoting tropical timber from sustainably managed sources;
- Supporting activities to secure the tropical forest estate;
- Promoting the sustainable management of tropical forests;
- Promoting the increased and further processing of tropical timber from sustainable sources; and
- Improving industry's efficiency of processing and utilization of tropical timber form sustainable sources.

According to the Council Chair, Dr. Josefina Takahashi, the new Action Plan reflects the broad scope of the Organization.

"We have set a challenging agenda," she said. "But achieving sustainable tropical forest management and a sustainable timber trade requires nothing less".

According to Dr. Takahashi, member countries will need to increase their efforts if they are to make more rapid progress.

"Producer members will need to work harder to raise their capacity to manage their forests sustainably and to develop sustainable and profitable forest industries," she said. "Consumer members will need to increase their support for these efforts, both through direct assistance and through facilitating the trade of timber from sustainable sources. And the Organization itself will need to be highly energetic if it is to maintain its high standards in the face of what will undoubtedly be an increased workload".

For more information contact: Mr. Collins Ahadome, ITTO Secretariat; itto@itto.or.jp

THE NETWORK FOR NATURAL GUMS AND RESINS IN AFRICA (NGARA)

Natural gums and resins are among dryland resources in Sub-Saharan Africa that contribute to improved livelihoods of local communities in terms of food security, income generation and foreign exchange earnings. These resources also contribute to the amelioration of the environment. The increasing health consciousness among consumers internationally also favours their increased use.

The development of these resources and commodities is key to sustainable management and development of the drylands, which, due to harsh environmental conditions, have fewer options. However, irregularity of supply of these commodities accompanied by widely fluctuating prices and variable product quality has resulted in unfavorable long-term effects on the demand of these commodities. A coordinated strategy is therefore needed among producing countries and partners to take advantage of available opportunities and address the constraints.

Various initiatives have been undertaken since the mid nineties on how the plant gums and resins sector could be developed

to enhance food security, rural development and poverty alleviation in sub-Saharan Africa. One such initiative was the organisation of an inaugural workshop for producing countries and partners held in October 1997 in Nairobi, Kenya during which issues on the conservation, management and utilisation of plant gums, resins and essential oils were discussed and concrete recommendations made. A key recommendation of that workshop was the creation of a regional network to enable countries develop their own system of sustainable production, marketing and improvement of their products to international standards. A follow-up workshop was held in May, 2000 in Nairobi, Kenya where **the Network for Natural Gums and Resins in Africa (NGARA)** was established.

NGARA's mission is to assist in formulating a co-ordinated strategy for African producing countries and partners in the sustainable development of their natural gum and resin resources for improving rural livelihoods and environmental conservation. Its goal is to position African producer countries and partners as major global players in the production, processing and marketing of natural gums and resins.

The network's major objectives are:

- To promote exchange of information on production, marketing, processing and quality control among producer countries as well as with partners.
- To facilitate access to technological development and training.
- To support relevant research in the key areas of the sector.
- To promote the links between the primary producer, processor and end user.

Activities include creation of relevant databases, information dissemination and promotional activities; training and capacity building; and research and technology development.

NGARA was initially established with a membership of ten countries from sub-Saharan Africa which produce plant gums and resins (those who participated in the Nairobi workshop of May 2000). However, membership is by application from countries in sub-Saharan Africa which produce the commodities and organizations involved in the development of the resources and/or commodities.

The network has a steering committee comprising: representatives from three focal points (West and Central Africa, Eastern Africa and Southern Africa), experts on marketing and quality control and international observers (represented by FAO and the Association for the International Development of Natural Gums [AIDGUM]).

Each member country is represented by a national coordinator. The day-to-day activities are handled by a regional secretariat based at the Kenya Forestry Research Institute (KEFRI), Nairobi, Kenya. Currently, NGARA is working on a regional Project for member countries named "The Acacia operation " to be financed through FAO. The project's goal is to strengthen NGARA's structures for effective development of the gums and resins sub-sector among member countries in the framework of combating desertification. This represents just one of the initial activities.

For more information contact
The NGARA Secretariat,
P. O. Box 30241,
Nairobi, Kenya.
Tel: 254 154 32353,Fax: 254 154 32844
Email: kefri@wananchi.com or kefri@arcc.or.ke

PROTA FIRST INTERNATIONAL WORKSHOP

PREMIER ATELIER INTERNATIONAL
23-25 September 2002, Nairobi, Kenya

First Announcement
Organizer: PROTA Programme, Wageningen University, The Netherlands

PROTA will be the subject of an International Workshop to review the progress made, and to reach international consensus on the structure,organization, activities, and finances of the First Implementation Phase 2003-2007.

The workshop is a forum for scientists, policy-makers and donors, in order to highlight the importance of the Plant Resources of Tropical Africa through Commodity Group Reports, Country Reports and Plant Resources Reports; to review the progress made in the Phase 2000-2002 towards international cooperation, the documentation and information system, and the publication of the monographs; to make the recommendations for the Implementation Phase 2003-2012 on all aspects of the programme including organization, manpower, finances, publication policy and the databank.

PROGRAMME OUTLINE
Section 1: General
Invited papers on the many facets of the PROTA programme; its relation with new trends in agricultural, silvicultural and environmental policies and new developments in information technology

Section 2: Commodity Group Reports
Invited papers on general aspects of a number of Commodity Groups, like 'Cereals and Pulses', 'Vegetables','Timbers','Auxiliary plants' and 'Medicinal plants'.

Section 3: Country Reports
Invited papers on the plant resources of the various parts of Tropical Africa,, based on the documentation work of the PROTA Regional and Country offices.

Section 4: Plant Resources Reports
Contributed papers (Posters) on subgroups of plant resources, in particular treatment of the ecology, agronomy/management, uses and improvement of neglected and potentially important species.

Section 5: Phase 2003-2012
Working Groups and Plenary discussions on organization, financial aspects, publication policy and databank. Formulation of recommendations

Excursion
Half-day field-trip to interesting projects on plant resources in the surroundings of Nairobi

PREREGISTRATION
In view of the Workshop objectives, attendance will be limited to a maximum of 150 participants, to allow a close interaction between the participants from research and the public sector. Persons wishing to participate in the Workshop, please complete the Preregistration Form, and return it to the Secretariat PROTA FIRST INTERNATIONAL WORKSHOP.Some financial assistance may be available for delegates from institutions in Tropical Africa.

CONTRIBUTED PAPERS
Authors of contributed papers (Section 4) should enclose an abstract of 200 words or less with the Preregistration Form.
Authors of accepted papers will be invited to display the paper in a Poster Session and to submit a one-page summary for publication in the Workshop Proceedings

SECOND ANNOUNCEMENT
Details on venue, programme and logistics will be communicated in the Second Announcement, scheduled for March 2002

AIRMAIL TO: Secretariat,
Prota First International Workshop, c/o ICRAF,P.O. Box 30677,Nairobi,Kenya.
Email: prota@pros.dpw.wag-ur.nl
http://www.prota.org

FERN REPORT: BEHIND THE LOGO: AN ENVIRONMENTAL AND SOCIAL ASSESSMENT OF FOREST CERTIFICATION SCHEMES

FERN has produced a report based on case studies by WWF France, Taiga consulting, Taiga Rescue Network, Robin Wood, NRDC, FERN, Finnish Nature League and Greenpeace International. The report compares the four biggest forest certification schemes - the Forest Stewardship Council (FSC), the Pan-European Forest Certification (PEFC), the Canadian Standards Association's Sustainable Forest Management Standard (CSA) and the Sustainable Forestry Initiative (SFI), and analyses strengths and flaws of each scheme.

FERN aims to provide companies, governments and NGOs with the facts needed for informed decision-making.

FERN concludes that the Forest Stewardship Council is currently the only independent and credible certification scheme on the market, though it is also not perfect.

The report is available from FERN at
Fosseway Business Centre
1C, Stratford Road
Moreton-in-Marsh
UK - GL 56 9NQ
Tel: +44 1608 652 895
Fax: +44 1608 652 878
sara@gn.apc.org
www.fern.org

AVAILABILITY OF FOREST SEEDS ON THE INTERNET

In order to offer producers and consumers of forest seeds a useful and powerful

instrument, CATIE has established a database of forest seeds to facilitate commercialisation, exchange and consultation. The project was started in 1998 with the collaboration of the CATIE's Banco de Semillas Forestales and the Unidad de Ínformatica.

The database contains detailed information on each distributed forestry or agroforestry species and provides data of the companies involved in the distribution of the seeds. The database can be accessed in two ways:

- It is possible to derive information on the availability of seeds of a particular species, either using the common name or scientific name, or
- If one does not know which species, would be required, it is possible to execute "advanced searches" indicating the site conditions and the benefits that one hopes to derive from the species (timber, ornamental, etc.).

The database also provides access to other databases of forest seeds.

To date, information is available on more than 10 other databases and companies providing data on more than 100 species. The information is updated directly by the users on the Internet. All people interested, both consumer and producer of forest seeds, are invited to benefit from this service. You may visit the database through the following link:
www.catie.ac.cr/proyectos/prosefor /base/semillas.htm

If you are interested in incorporating the information of the seeds that your company distributes, please mail to arodrigu@catie.ac.cr ; wvasquez@catie.ac.cr or bsf@catie.ac.cr. A username and access code will be assigned to you.

RECENT ADVANCES IN BIOTECHNOLOGY FOR TREE CONSERVATION AND MANAGEMENT – IFS FLORIANÓPOLIS WORKSHOP PROCEEDINGS

Useful publications on tropical tree biotechnology

By Olavi Lukkannen

In September 1997, an international workshop on tropical tree biotechnology was organised by the International Foundation for Science (IFS) at Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brazil. Most of the 36 participants were current or former IFS grantees, especially from the Latin American countries. Dr Sinclair Mantell (Wye College, University of London, UK) and Dr Ana Maria Viana from UFSC were responsible for organising the scientific programme of this event. Presentations covered traditional approaches to forest inventory and tree population studies, as well as modern technologies to study genetic diversity in trees and practical approaches to conservation and propagation.

Selected titles from the proceedings are given in the following (see also articles in this newsletter by proceedings contributors Luukkanen; Jain; Schifino-Wittman and Cardoso de Freitas; Jurado; Valdés et al.; and de Andrade et al.).

Opportunities and challenges for the incorporation of genomic analysis in *Eucalyptus* breeding (by Dario Grattapaglia).

Genetic diversity and outcrossing rates in the Guanacaste tree *(Enterolobium cyclocarpum)* in the dry forests of Costa Rica (by Oscar J. Rocha and Jorge A. Lobo).

Allozyme variation in the Mexican closed-cone pines (by Carlos Ramirez-Herrera et al.).

Characterization of genetic variability of native plant species of the Atlantic rainforest: *Caesalpinia echinata* – a case study (by M.A. Cardoso et al.).

Use of RAPD markers in the study of genetic diversity of *Araucaria angustifolia* populations in Brazil (by Maria Cristina Mazza).

Random amplified polymorphic DNA (RAPDs) variation in hybrid swarms and pure populations of the genus *Prosopis* (Leguminosae) (by Beatriz Ofelia Saidman et al.).

Management of natural populations and maintenance of genetic diversity of *Euterpe edulis*, the heart-of-palm tree (by Maurício Sedres dos Reis et al.).

Use of genetic markers to conserve endangered species and to design protected areas for more widespread species (by Andrea C. Premoli).

Introduction of embryogenic cultures from mature-phase tropical and subtropical trees and control of somatic embryo maturation and germination (by Richard E. Litz et al.).

Somatic embryogenesis in *Ocotea catharinensis* (Lauraceae) (by Ana Maria Viana).

Development of cryopreservation technology applications in agroforestry and forestry (by Erica E. Benson).

Molecular diversity studies on

mycorrhizal associations in trees (by Francois Le Tacon et al.).

Networking of research in biotechnology and forest tree improvement in Central America and the Caribbean (by Markku Kanninen et al.).

Correct citation of the proceedings: International Foundation for Science (IFS) 1998, Recent Advances in Biotechnology for Tree Conservation and Management, Proceedings of an IFS Workshop (ed. Bruns,S., Mantell, S., Trägårdh, C. & Viana, A.M.). IFS, Stockholm. 334 p. ISBN: 91 5798 46 0.

A limited number of free copies is available from: IFS, Grev Turegatan 19, S-114 38 Stockholm, Sweden. Email info@ifs.se

GLOBAL BIODIVERSITY: EARTH'S LIVING RESOURCES IN THE 21ST CENTURY

B. Groombridge and M.D. Jenkins

The present decade has seen remarkable growth in concern at all levels for wildlife and the environment, and increased appreciation of the links between the state of the ecosystems and the state of humankind. Biodiversity has become a hot item. In 1992 in a joint effort UNEP and the World Conservation Monitoring Centre (WCMC) produced a substantial volume that introduced the many and varied themes covered by the unfamiliar term "biodiversity" in *Global biodiversity: status of the Earth living resources.* The book has since found wide application as an educational source and sourcebook of biodiversity data.

Global Biodiversity: Earth's living resources in the 21st century builds on nearly a decade of further research and analysis by WCMC and the conservation community worldwide. The purpose of the book is twofold: to outline some of the broad ecological relationships between humans and the rest of the biosphere, and to summarise information bearing on the health of the biosphere.

The first part of the book outlines some fundamental aspects of material cycles and energy flow in the biosphere. This is followed by discussion of the components of biological diversity, and an account of the expansion of this diversity through geological time and the pattern of its distribution over the surface of the earth. The second part deals with material relationships between humankind and biodiversity, covering salient features of human impact on the environment from the earliest times of human existence to the present, and the current use of elements of biodiversity. This part also summarises information on trends in condition to the main ecosystem types and the species integral to them.

In order to address biodiversity in a surveyable manner it is divided in marine biodiversity, terrestrial biodiversity and inland water biodiversity, each dealt with in separate chapters. The book contains a large amount of tables, graphs and maps to complement the text. Together, they provide a clear impression of what biodiversity is about, its importance to human kind and how it is influenced by man.

ISBN: 1 899628 15 0, 246 pages, UNEP-World Conservation Monitoring Centre. Orders: World Conservation Press, 219 Huntingdon Road, Cambridge, CB3 0DL, UK Tel: +44 1223 277314; Fax: +44 1223 277136 Email:info@wcmc.org.uk: http://www.wcmc.org.uk/

APPLIED ETHNOBOTANY PEOPLE, WILD PLANT USE & CONSERVATION

Anthony B. Cunningham

This manual focuses on practical steps to develop a better understanding of the values, vulnerability and resource management options for wild, non-cultivated plant resources. It is the third in a series produced by the People and Plants initiative, a joint programme of WWF, UNESCO and the Royal Botanical Gardens, Kew. Issue of the manual is the impact of harvesting of wild plants by people. It is intended as a practical guide to approaches and field methods for participatory work between resource users and field researchers. Emphasis is on how to identify the most urgent problems, needs and opportunities relating to wild plant use and resource management. Geographically,the focus is on Africa and attention is not only given to tropical forests, but to other vegetation types as well.

The book comprises 8 chapters, which are each well illustrated by figures, tables, boxes and photos, The first places conservation in the context of times and views. It reviews the interaction between vegetation , climate and human over time. The second chapter outlines methods such as mapping, transect walks with local people and satellite images to get a better understanding of people's preferences and the demand for particular plant species. In the third chapter an introduction to ethnobotanical surveys of markets is given, followed by a theoretical background to this type of studies. The next three chapters are of a quantitative nature dealing with the supply of plants, which are the focus of the demand described in the first two chapters. Chapter 4 presents methods to measure individual plants and how to assess the impact of harvesting, in particular the response of individual plants. In chapter 5 the same is done, but on the level of plant populations. It presents methods to measure the dynamics of plant populations and the impact of harvesting on them. Finally, Chapter 6 shows how patterns of harvesting relate to vegetation dynamics and disturbance, and what methods can be used to take this into account when developing conservation and resource management plans. It approaches the impact of man through harvesting on the level of landscapes, which enables to see the dynamic nature of natural processes and the close association between climate, soils, vegetation, land forms and human activity. In Chapter 7 methods are presented which may help to better understand tenure and boundaries, and resource user characteristics, like beliefs and rituals. Focus in this chapter is on social and cultural factors. Finally, Chapter 8 concludes the preceding chapters by suggesting fifteen basic steps to resource management.

ISBN: 1 85383 697 4, 300 p. Earthscan Publications Ltd. 120 Pentonville Road, London N1 9JN UK. Tel: 44 2072 780433 Fax: 44 2072 78114 Email:earthinfo@earthscan.co.uk http://www.earthscan.co.uk

A STATISTICAL MANUAL FOR FORESTRY RESEARCH

FORSPA-FAO Publications

K.Jayaraman (2000)

This manual covers some of the basic concepts involved in the theory and practice of statistics in forestry research. The manual was written on a specific request from FORSPA (The Forestry Research Support program for Asia and the Pacific), Bangkok to prepare a customised training

manual useful to researchers engaged in forestry research in Bhutan. It is intended to be a source of reference for researchers engaged in research on renewable natural resources especially forests, agricultural lands and livestock, in designing research investigations, collecting and analysing relevant data and also in interpreting the results.

The focus of the manual has been on introducing the researchers to some of the basic concepts and techniques in statistics, which have found wide application in research in forestry and allied fields. Basic statistical estimation and testing procedures dealt with in the first chapters, are followed by chapters that each deal with specific statistical techniques, such as methods of designing, analysing experiments and also some standard sampling techniques. Further, statistical methods involved in specific fields like tree breeding, wildlife biology, forest mensuration and ecology, many of which are unique to forestry research, are described. In order to serve as a handy reference manual description of only simple modes of design and analysis are given with illustrations and examples from the field which adequately support the text.

FORSPA Publication No. 25/2000, 240 p. Orders: S. Appanah, Forestry Research and Support Programme for Asia and the Pacific (FORSPA), FAO Regional Office for Asia and the Pacific, 39 Phra Atit Road, Bangkok 10200, Thailand. Tel.: (662) 2817844 ext. 136, Fax(662) 2804565. Email: Simmathiri.Appanah@fao.org

PROCEEDINGS OF THE SUB-REGIONAL SEMINAR "DIE-BACK OF SISSOO (*DALBERGIA SISSOO)*" Kathmandu, Nepal, 25-28 April 2000

S. Appanah, G. Allard & S.M. Amatya

In April 2000 a Seminar was held to address dieback of Sissoo, also called Shisham (*Dalbergia sissoo),* occurring both in natural stands as well as in plantations throughout its habitat. Sissoo is one of the most important timber species of the sub-Himalayan area, stretching from Bhutan in the east to as far as Afghanistan in the west. It is an important species for rural as well as industrial plantations. It is popular due to its fast growth and multiple use properties. The tree is therefore grown by government organisations, communities, individual farmers and private landowners. However, management of the stands appears to be inadequate or non-existent in most cases.

The proceedings of the seminar contain contributions form researchers from Bangladesh, India, Nepal and Pakistan, each dealing with the extent of the disease in their country, probable causes and possible solutions. The outcome of the seminar reveals that the cause of die-back may be multi-faceted and the primary causes are as yet to be confirmed. Nevertheless, symptoms seem to increase with increasing clay content of the soils and water logging, which impedes growth of the tree and increases stress, making it vulnerable to fungi and insect attacks. Recommendations of the seminar include the activities that should be undertaken by each country, individually as well as in a mechanism of co-operation with other countries involved. Furthermore, it is recommended to seek co-operation with international agencies/NGO's/Regional co-operatives to provide technical and financial support to the network.

FO: GCP/RAS/163/NET Field Document No.18, Forestry Research & Support Programme for Asia and the Pacific (FORSPA), 65 p. Orders: : S. Appanah,

Forestry Research and Support Programme for Asia and the Pacific (FORSPA), FAO Regional Office for Asia and the Pacific, 39 Phra Atit Road, Bangkok 10200, Thailand. Tel.: (662) 2817844 ext. 136, Fax(662) 2804565 Email: Simmathiri.Appanah @fao.org

DEVELOPMENT OF NATIONAL-LEVEL CRITERIA AND INDICATORS FOR THE SUSTAINABLE MANAGEMENT OF DRY FORESTS IN ASIA

Workshop Report & Background Papers

From November 30th to December 3rd 1999 the Workshop on National-level Criteria and Indicators for Sustainable Management of Dry Forests in Asia was held in Bhopal, India. The workshop was organised jointly by the Food and Agricultural Organisation of the United Nations (FAO), the United Nations Environment Programme (UNEP), the International Tropical Timber Organisation (ITTO), The United States Department of Agriculture Forest Service (USDA/FS) and the Indian Institute of Forest Management (IIFM). Workshop participants included representatives of forestry agencies from nine countries with dry forests in Asia: Bangladesh, Bhutan, China, India, Mongolia, Myanmar, Nepal, Sri Lanka and Thailand, and representatives of above mentioned organisations and WWF/India and the Regional Community Forestry Training Center for Asia and the Pacific (RECOFTC).

The workshop was organised in response to a recommendation by the 17th session of the Asia-Pacific Forestry Commission, held in 1998. This session revealed that some countries were making progress in the development and application of criteria and indicators for sustainable forest management, whereas others were remaining outside the established international processes. The workshop report describes the objectives and expected output and gives a brief overview of the inaugural session, the discussions, presentations and results. The annexes contain the list of participants, workshop programme, list of technical and discussion papers, recommendations for further action and definitions and basic principles of sustainable forest management in relation to criteria and indicators.

The presentations were compiled and published in the background papers. The papers help to highlight the special issues associated with managing the dry forests of Asia and to advance ideas for the development of criteria and indicators for the sustainable management of these forests, particularly in Bhutan, Mongolia, Nepal and China.

Workshop Report: ISBN 974-7946-04-1, FAO Publication: 2000/07, 20 p. Asia-Pacific Forestry Commission.
Background Papers: ISBN 974-7946-06-8, FAO Publication 2000/08, 97 p. Orders: Patrick B. Durst, Senior Forestry Officer, FAO Regional Office for Asia and the Pacific, 39 Phra Atit Road, Bangkok 10200, Thailand. Tel.: (66-2) 281 7844; Fax: (66-2) 280 0445; Email: Patrick.Durst@fao.org.

TROPENBOS PUBLICATIONS 2001

LA CHASSE ET LA FILIÈRE VIANDE DE BROUSSE DANS L'ESPACE DE TAÏ, CÔTE D'IVOIRE

Caspary, H.-U., Koné, I., Prouot, C. and Pauw, M. de (2001)

Game is an important food resource in Côte d'Ivoire, but hunting is forbidden. Hans Ulrich Caspary et al. argue that only regulated reopening of hunting will be able

to reduce poaching in protected areas. Sustainable wildlife management is urgently needed.
Tropenbos Côte d'Ivoire Series 2. Tropenbos International, Wageningen, the Netherlands. ISBN: 90-5113-048-1. Price: 20 Euro

RELATIONS FAUNE FLORE DANS LE PARC NATIONAL DE TAÏ: UNE ÉTUDE BIBLIOGRAPHIQUE
Chatelain C., Kadjo B., Koné, I. and Refisch J. (2001)
This study on the relationships between flora and fauna was carried out to provide the managers of Taï National Park in South-West Côte d'Ivoire with information that support the monitoring and evaluation of the flora and fauna in the area. Based on a bibliographic compilation the study is directed at the identification of those relationships of which the disappearing of one of its elements could endanger the conservation of a species. The study also identifies the gaps in knowledge that would be necessary to fill in for an improved management of the park.
Tropenbos Côte d'Ivoire Series 3. Tropenbos Côte d'Ivoire Series 2. Tropenbos International, Wageningen, the Netherlands.ISBN: 90-5113-049-X. Price: 20 Euro

FOREST FILLED WITH GAPS:EFFECTS OF GAP SIZE ON WATER AND NUTRIENT CYCLING IN TROPICAL RAIN FOREST. A STUDY IN GUYANA
Dam, O. van (2001)
Guyana's forests are selectively logged and aforest management is desired that is economically sustainable and ecologically responsible. Canopy gaps, created by selective logging, induce changes to microclimatic and edaphic conditions.
These changes influence the regenerationof the forest in general and of commercial tree species in particular. This study evaluated the influence of gap size on microclimatic conditions, water dynamic sand nutrient cycling. The study was carried out in the tropical rain forest of central Guyana in experimental gaps of 50 to 3200M2, and 13 years after felling. It generates insight into the potential impacts of logging gaps on abiotic and edaphic conditions affecting forest regeneration.
Tropenbos Guyana Series 10.Tropenbos-Guyana Programme, Georgetown,Guyana. ISBN: 90-5113-046-5. Price: 20 Euro

LES INSTITUTIONS COMMUNAUTAIRES DE GESTION DES PRODUITSFORESTIERS NON-LIGNEUX DANS LES VILLAGES PÉRIPHÉRIQUES DE LA RÉSERVE DE BIOSPÈRE DU DJA
Dkamela, G.P. (2001)
The role of community based institutions in non-timber forest products (NTFP) management was studies among Bulu, Badjoué and Baka people in or near the Dja Biosphere Reserve. Issues discussed include the importance of NTFPs for subsistence and trade, management of the resource of five NTFP species, the relating function of community-based institutions in a changing commercial and legal environment, and property rights.
Tropenbos Cameroon Documents 7.Tropenbos Cameroon, Kribi, Cameroon.ISSN:1566-2152. Price: 5 Euro

FOREST (AND) PRIMATES. CONSERVATION AND ECOLOGY OF THE ENDEMIC PRIMATES OF JAVA AND BORNEO
Nijman, V. (2001)
Biodiversity hot spots are said to be a cost effective route to species conservation. But local hotspots often do not coincide for different taxa, concludes Vincent Nijman.For the adequate protection of forest primates additional sites are needed.
Tropenbos Kalimantan Series 5. Tropenbos International,Wageningen, the Netherlands. ISBN: 90 5113-052-X. Price 20 Euro

***MACARANGA AND MALLOTUS* (EUPHIRBIACEAE) AS INDICATORS FOR DISTURBANCE IN THE LOWLAND DIPTEROCARP FORESTS OF EAST KALIMANTAN, INDONESIA**

Slik, J.F.W. (2001)

This publication explores the possibilities of indicator plant taxa to quantify forest disturbance in the lowland dipterocarp forests of East Kalimantan. The book provides a first step in the development of an easy-to-apply rapid assessment method for determining the level of forest disturbance.

Tropenbos Kalimantan Series 4. Tropenbos International,Wageningen, the Netherlands. ISBN: 90-5113-045-7. Price: 20 Euro

FOREST PRODUCTIVITY AND RESOURCE AVAILABILITY IN LOWLAND TROPICAL FORESTS IN GUYANA

Thomas, R.S. (2001)

This thesis is largely based on an assessment of tropical rainforest patterns in lowland tropical forest of Guyana. The thesis clearly demonstrates that spatial heterogenity in forest types in lowland tropical forests of Guyana can result in heterogenity of several factors, which may ultimately influence plant growth dynamics.

Tropenbos Guyana Series 7. Tropenbos Guyana, Georgetown, Guyana. ISBN: 976-8173-51-3. Price 12 Euro

SEMINAR PROCEEDINGS SUSTAINABLE MANAGEMENT OF AFRICAN RAINFORESTS HELD IN KRIBI, CAMEROON, NOVEMBER1999. PART I: WORKSHOPS.

Eds: Foahom, B.,Jonkers, W.,B.J., Nkwi, P.N., Schmidt, P.and Tchatat, M.

These proceedings provide an overview of recent trends in rain forest management in Cameroon and other West and Central African countries (Central African Republic, Côte d'Ivoire, Ghana, Equatorial Guinea), with a focus on management planning and societal aspects. Special attention is also given to the legal context, to ecological aspects and to lesser-known timber species.

Tropenbos International,Wageningen, The Netherlands. ISBN: 90-5113-047-3. Price: freeof charge

SEMINAR PROCEEDINGS SUSTAINABLE MANAGEMENT OF AFRICAN RAINFORESTS HELD IN KRIBI, CAMEROON, NOVEMBER 1999. PART II. SYMPOSIUM

Jonkers,W.B.J., Foahom, B. and Schmidt,P. (*Eds)* Proceedings of the Symposium Conducted November 1999, in Kribi, Cameroon

Tropenbos International, Wageningen,The Netherlands. ISBN: 90-5113-051-1 Price: free of charge

THE BALANCE BETWEEN BIODIVERSITY CONSERVATION AND SUSTAINABLE USE OF TROPICAL RAIN FORESTS. PROCEEDINGS OF A SEMINAR, 5-8 DECEMBER, BALIKPAPAN, INDONESIA.

Hillegers, P.J.M.and Iongh,H.H. de (Eds)

This publication presents the results of more than ten years of collaborative research of biodiversity conservation and sustainable forest management in Kalimantan.

Tropenbos International,Wageningen, The Netherlands. ISBN: 90-5113-050-3.
Price: free of charge

The European Tropical Forest Research Network is a network of European organisations involved in (sub)tropical forest research. It is presently supported by Directorate General for Research of the European Commission under the INCO-DEV Programme.

For further information on ETFRN, please contact your National Focal Point (see inside back cover) or the Coordination Unit (address below).

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ETFRN News is a publication of the European Tropical Forest Research Network. It is printed on 100% recycled paper and has a circulation of 3,600 copies.

Contributions to the ETFRN News are always welcome.
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New Instruments for Monitoring and Evaluation (tentative title)	**15 March 2002**