Biotechnology in Agriculture Series, No.19

Biotechnology and Plant Genetic Resources

Conservation and Use

Edited by

J.A. Callow,

B.V. Ford-Lloyd

and

H.J. Newbury

CAB INTERNATIONAL

BIOTECHNOLOGY AND PLANT GENETIC RESOURCES

Conservation and Use

Biotechnology and Plant Genetic Resources

Conservation and Use

Edited by

J.A. Callow,
B.V. Ford-Lloyd
and
H.J. Newbury
School of Biological Sciences
University of Birmingham
UK

CAB INTERNATIONAL

CAB International
Wallingford
Oxon OX10 8DE
UK

Tel: +44 (0)1491 832111
Fax: +44 (0)1491 833508
E-mail: cabi@cabi.org

CAB International
198 Madison Avenue
New York, NY 10016-4341
USA

Tel: +1 212 726 6490
Fax: +1 212 686 7993
E-mail: cabi-nao@cabi.org

A catalogue record for this book is available from the British Library, London, UK
A catalogue record for this book is available from the library of Congress, Washington DC, USA

ISBN 0 85199 142 4

Typeset in 10/12pt Palatino by Columns Design Ltd., Reading
Printed and bound in the UK by Biddles Ltd, Guildford and King's Lynn

Contents

11 Biodiversity for Bioindustries

G. Tamayo, W.F. Nader and A. Sittenfeld

Instituto Nacional de Biodiversidad (INBio), Apartado Postal 22-3100, Santo Domingo, Heredia, Costa Rica

11.1. Biodiversity and its Benefits

Biodiversity refers to the variety and variability of living material and ecological complexes in a given area and comprises species, genetic and ecosystem diversity. Biodiversity is not only the basis of life on earth, but also provides the goods and services essential to support every type of human endeavour. Accordingly, biodiversity enables societies to adapt to different needs and situations (US National Research Council, 1992).

Biodiversity generates economic value in different ways. Populations are interconnected, for instance where predators and disease organisms control populations of their prey, or when pollinators and seed dispensers promote the growth of plant populations. Thus agriculture directly benefits from a functioning ecosystem, allowing the extensive use of agrochemicals to be avoided. Biodiversity also generates economic value from extractable products obtained from individual species (Wilson, 1992). For centuries biodiversity has provided fuels, medicines, materials for shelter, food and energy. The use of compounds, genes and species is essential to meet industry needs. Furthermore, ecosystems contribute to climate regulation, maintenance of hydrological cycles and nitrification of soils. In addition, recreation, science and education also figure among the vast array of social, ethical, spiritual, cultural and economic goods and services provided by biodiversity that are recognized as fundamental for human livelihoods and aspirations.

Bioprospecting links biodiversity and industry. Previously, this activity generated benefits almost exclusively for industry, leaving biodiversity

 Biotechnology and Plant Genetic Resources (eds J.A. Callow, B.V. Ford-Lloyd and H.J. Newbury)

conservation and source countries to generate benefits and returns elsewhere. The rapid loss of biological diversity, with the extinction of 30 to 300 species per day (Japan Economic Newswire, 1995), has initiated a new attitude towards the exploration of natural resources. Costa Rica's Instituto Nacional de Biodiversidad (the National Biodiversity Institute, INBio) has pioneered a new concept of bioprospecting that integrates product discovery with financial and intellectual returns to 'nature'. INBio's Biodiversity Prospecting Department links the understanding and non-damaging exploration of biodiversity to conservation activities and economic development of the countries where bioresources were first obtained (Sittenfeld and Villers, 1994). The exploration and conservation of the world's biotic resources require an approach involving bioindustries, research centres and developing countries, all collaborating towards a common goal, each participant benefiting from the relationship. Presently, a natural resource conservation strategy based on the three overlapping steps – saving, knowing and using biodiversity – is paving the way towards implementing joint activities for the benefit of industry, biodiversity conservation and source countries (*Global Biodiversity Strategy*, 1992; Janzen and INBio, 1992).

Gene technology opens a new dimension for bioprospecting. However, because in its current stage of development it represents more of a threat than a benefit to the primarily agricultural societies of the developing world, new strategies must be implemented to combine benefits to the biotech industry with biodiversity conservation and the development of biodiversity-rich nations.

11.2. Industry and Biodiversity

11.2.1. Development of drugs and pesticides

Up until the present, the primary beneficiaries of biodiversity have been the pharmaceutical and agricultural industries. Sales of drugs based on natural products from plants were estimated at $US43 billion in 1985 worldwide, accounting for approximately 40% of the total drug market (Principe, 1989). Earnings from a single new successful pharmaceutical on the market can be in the range of a billion dollars. The value of yet undiscovered pharmaceuticals in tropical forests is estimated at $US3–4 billion for a private pharmaceutical company, and as much as $US47 billion to society as a whole (Mendelsohn and Balick, 1995).

On the other hand, sales of pesticides in the US by 18 major suppliers were estimated at $US6.5 billion in 1992 (Frost & Sullivan Inc., 1992). Although humans have traditionally used plant products like rotenone or nicotine as agricultural insecticides, most industrial pesticides on the

market have not been derived from natural compounds (Wink, 1993). However, the toxicity and ecological hazards created by most of these chemicals have initiated intensive research for more specific and biodegradable pesticides, including new biological pest control agents based on natural compounds and microorganisms. The first results of these screening efforts are already on the market. For example, avermectins are macrolides derived from *Streptomyces avermitilis* and possessing insecticidal properties (Babu, 1988). Dehydration of avermectins yields the even more efficient ivermectins, which generate approximately $US1 billion in annual sales, and are useful not only for the treatment of infestations of parasitic worms and insects in livestock, but also in humans. Pyrethroids, on the other hand, were derived by chemical synthesis from pyrethrins as lead structures as natural insecticides found in chrysanthemum flowers (Wink, 1993). Pyrethrins are more stable and active than their natural precursors (by a factor of 1000), but also produce more side-effects in mammals. These side-effects have led Germany to consider banning pyrethrins from the market (Wink, 1993). Still undergoing development is a nematocide, the pyrrolidine alkaloid 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine from *Lonchocarpus* species, developed through a collaboration between the British Technology Group, the Royal Botanical Garden at Kew and INBio (Janzen *et al.*, 1990; Birch *et al.*, 1993).

Many of the technical aspects of screening and developmental processes involved in creating new drugs or pesticides are related to more general issues like biodiversity, technology transfer and biodiversity conservation, and will be described in more detail below.

Sources for new drugs or pesticides

Three major sources for the screening of new compounds, suitable for drug or pesticide development, are available: (i) molecule libraries created by combinatorial chemistry; (ii) fermentation broths of microorganisms; and (iii) plant and animal extracts. Modern automated methods have created high-throughput screening, allowing thousands of substances to be tested for their biological activity rapidly and inexpensively. Therefore, access to these three sources can be regarded as a prerequisite rather than a privilege. The goal is the discovery of a 'leading structure' that will guide the development of new drugs, pesticides or fine chemicals.

Although combinatorial chemistry plays an increasingly important role, half of the ten best-selling drugs are derived from secondary metabolites originally isolated from microorganisms or plants (O'Neill and Lewis, 1993). Obviously, organic chemistry has not yet caught up with the capacity of nature to create new structures with a complex molecular diversity (for review see Ecker and Crooke, 1995).

Screening for natural compounds has traditionally concentrated on plants and microorganisms, identifying a huge variety of pharmacologically

active alkaloids, terpenoids, aromatics and glycosides. Fungi and bacteria can easily be isolated from soil samples and other sources. Once in a strain collection, microorganisms and their products are readily accessible. Plants, on the other hand, are more difficult to collect, but offer a higher molecular complexity and diversity. Marine organisms yield new structures with high molecular diversity and important biological activities (König *et al*., 1994). For example, briostatin and didemnin B are compounds found in molluscs and shown to have strong antitumour activity; they have reached preclinical and clinical trials, respectively. Shark and tunicate alkaloids are also currently undergoing intensive investigations (Moore *et al.*, 1993; Research Foundation of the State University of New York, 1994). In contrast, insects, spiders and other invertebrates have mainly been examined for their potential to produce bioactive peptides and proteins rather than small molecules (see below). The same applies to vertebrates like frogs, snakes and bats. Certain alkaloids obtained from the skin of frogs (see below), and insect hormones and pheromones useful for pest control are the exceptions. Nevertheless, drug research is also heading in this direction. In collaboration with Merck & Co. and Bristol-Myers Squibb (within an International Cooperative Biodiversity Group together with Cornell University), INBio is screening insects for small bioactive compounds (Sittenfeld and Lovejoy, 1995).

However, years of research may fail if the initial collection and documentation of biological material are not done properly. Problems may arise if further material from the same species or subspecies, from the same environment or even from the same location, is not available for later investigation. Calanolides, for example, were isolated from *Calophyllum lanigerum* var. *austrocoriaceum* (Kashman *et al*., 1992). These compounds inhibit HIV *in vitro*. Although fully characterized (Cardellina *et al*., 1993a) material from the same tree is now unavailable because the tree was subsequently cut down. There is a lesson to be learned from this event as other specimens from the same area did not yield even trace amounts of the desired compounds.

The collection strategy

Natural compounds can be accessed through ethnobiological information, evaluation of chemotaxonomic relationships, random sampling or biorational observations.

Approximately 3000 million people use traditional medicines (Balick, 1994). Many of today's multinational giants in the pharmaceutical industry made their first millions with products derived from ethnobotany. Prior to Bayer's aspirin, American and Eurasian peoples treated fevers, inflammation and pain with salicin-containing plants like willows and poplars. The worldwide market for phytomedicines derived from ethnobotany is estimated at $US12.4 billion, headed by products derived from ginseng, ginkgo, garlic, horse chestnut and echinacea (Grünwald, 1995). Today

ethnobotanical information is readily accessible through Internet's databases, e.g. NAPRALERT or AGIS. But most companies avoid the search for new compounds on the basis of ethnobiological information. An evaluation of 'hits' during the Natural Products Drug Discovery Program at the National Cancer Institute revealed no appreciable differences between samples collected at random and those screened on the basis of ethnobotanical leads (Cragg *et al.*, 1994). These results were obtained after dereplication, which eliminates a substantial number of substances. Interpretation of illness descriptions from the 'native pharmacologists' is also problematic. However, ethnobotanical information can be extremely useful when applied to diseases that *can* be translated into the language of Western medicine, e.g. diabetes, skin infections, wounds, etc. Aspects of intellectual property rights in relation to this approach are discussed in more detail in a recent study of the Rural Advancement Foundation International (1994).

Chemotaxonomy is based on the assumption that related species from the same genus or family produce the same type of secondary metabolites. For example, most members of the *Asteraceae* produce sesquiterpenelactones. An example is artemisinin, isolated from *Artemisia annua* on the basis of ethnobotanical information. Likewise, members of the *Rubiaceae* may produce alkaloids (quina alkaloids, for example), and species of the genus *Taxus* may contain taxanes, etc.

Most large pharmaceutical companies, not limited by their screening capacity, collect biological material randomly. They may show a preference for certain plant families, but in general only the taxonomic identification and exact documentation of the collection site of the sample are required.

The biorational approach requires the systematic study of interactions between organisms within the ecosystem. The leaf-cutter ant, *Atta cephalotes*, for example, avoids feeding the symbiotic fungus found in its nest with leaves from *Hymenaea courbaril* (Harborne, 1989). The tree contains a terpenoid (caryophillene epoxide) that inhibits the growth of the fungus. Observations like this may provide decisive information leading to the discovery of antifungal or formicide compounds. Evaluation of larvae or adult insect feeding preferences may lead to the discovery of new insecticides.

Technological aspects

The amount of material that can be taken from a particular environment without causing damage is a primary concern for biodiversity conservation. Bioassays can be performed on 10–100 mg of a compound mixture, the usual yield from 10 g of dry plant material. For confirmatory assays and further fractionation and isolation, amounts in the range 100–1000 g must be collected. Depending on a given compound's yield, preclinical trials to evaluate toxic side-effects and effectiveness in animals may require

large amounts (tonnes) of dried plant material if the compound is too complex to be synthesized. There is no doubt that the survival of certain species has been threatened in the past by drug researchers. The exploitation of pilocarpine, for example, threatened the survival of *Pilocarpus pignatifolios*, *P. microfilla* and *P. jaburandi* species in South America (Balick, 1994). In another case, clinical trials with taxol have affected the survival of *Taxus brevifolia* in its natural habitat.

When considering the possibility of countering this danger through sustainable breeding or planting, one must keep in mind that cultivation of a desired species may lead to a loss of the desired compound. The isolation and identification of epibatidine from *Epipedobates tricolor*, a frog used by indigenous people to poison arrowheads, has been described (Sapnde *et al*., 1992). Epibatidine is a remarkably simple, but highly efficient alkaloid that is 200 to 500 times more potent than morphine in analgesic assay systems. However, investigation of the alkaloid required the skins of 750 frogs collected from the wild. Attempts to breed these frogs in an artificial environment led to a loss of epibatidine in the skin of the second generation of frogs. Therefore, the compound is probably produced by complex interactions with other organisms in the environment.

Extraction of plant material is a philosophy in its own right. An efficient protocol for organic extraction was developed at the National Cancer Institute (McCloud *et al*., 1988). The decision whether to use raw extracts, prepurified fractions or even randomly isolated pure compounds for bioassay depends on a drug company's philosophy. Depending on the bioassay, natural compounds like polysaccharides, tannins, saponins or fatty acid esters must be removed prior to testing. This is of particular importance for bioassays involving cell cultures (Cardellina *et al*., 1993b).

In recent years, drug bioassays have become increasingly specific, rapid, reproducible and sensitive. At the same time, they have become less susceptible to matrix and other effects that tend to cause false positive or negative reactions. Even during the 1970s, companies were screening with receptors isolated from cell cultures, a technique that yielded various top-selling drugs. Today, gene technology allows the cloning and expression of receptors, enzymes and other proteins important for signal transduction, metabolic conversions, or cell or viral structures. Once produced on a large scale, they can be immobilized on ELISA (enzyme-linked immunosorbent assay) plates and integrated into a chromogenic assay to measure ligand–receptor interactions. The end result is the introduction of a huge variety of new assays, which has increased the industry's screening activities and the overall demand for new compounds. Nevertheless, the development of drugs against diseases not investigated down to the molecular level still requires complex cell culture assays or even screening in animal models. This is the case for most types of anticancer drug.

Drug development, from the collection and taxonomic classification of

biological material to compound extraction and fractionation, bioassays, structure elucidation and final clinical testing, is usually not performed by a single drug company, but by various research partners, working under contract. Therefore, the transfer of related technology to developing-world source countries is a possible, and even logical, approach for the technological development of these regions. Collection, taxonomic classification and extraction are not trivial tasks, but require a high standard of documentation and reliable and reproducible work that can easily be conducted in Southern nations with the aid of technology transfer. Drug companies would benefit from carrying out further bioassaying and chemical structure elucidation directly in the source countries, not only because of developmental issues and cheaper labour costs, but also because it would ensure these companies gained more direct access to important markets.

11.2.2. Prospecting for genes

Genetic engineering opens a totally new dimension for bioprospecting. The search for new genes and their applications is the primary objective of the biotech industry. Today's biotech products, already on the market, are based on genes from humans, domesticated animals and cultivated plants. Examples are human cytokines and growth factors like interferons, colony-stimulating factors, erythropoietin and bovine somatotropin, and also Calgene's Sav-R-Flavr tomato. The market value of erythropoietin alone amounts to several billion dollars per year worldwide. Hence the tremendous appetite of this new industry for novel genes; indeed, the hunt for them in tropical rainforests is already on. In contrast to random screening in natural compound research (see above), gene technology allows a more straightforward approach, as illustrated by the following examples.

The pharmaceutical biotech industry and biodiversity

Biodiversity and protein engineering. Minor changes to the amino acid sequences of pharmaceutical proteins and peptides (biologics) and enzymes may lead to new or improved activities. Computer simulation in combination with site-directed mutagenesis are the basis for a new technology, called protein engineering, which creates its own 'molecular diversity'. Nevertheless, the first successful examples of amino acid sequence improvement are the result of screening genes from the wild. Calcitonin is a peptide hormone that inhibits the release of calcium ions and phosphate from the bones, and has therapeutic uses for osteoporosis (MacIntyre *et al.*, 1987). The investigation of related hormones from animals revealed that the calcitonin from salmon is more active and has a longer half-life within the human body than the human peptide structure

(Epand *et al*., 1986). Today, chemically synthesized 'salcatonin' is on the market under tradenames including Calsynar and Miacalcic.

Industrial enzymes, used to catalyse chemical processes, can be improved to increase their heat stability, activity and specificity. Naturally thermophilic bacteria have become a useful source of industrial enzymes. Hydantoinase, for example, catalyses the conversion of chemically synthesized hydantoins to precursors of D-amino acids. D-Amino acids, like D-hydroxyphenyglycine and D-phenylglycine, are needed to derive amoxicillin and ampicillin from penicillin, just as D-serine is a precursor for pesticide production. The first enzymes to be used for this conversion on an industrial scale were isolated from common soil bacteria. The characteristics of these enzymes limited the maximal temperature for the catalysis to 40°C (Kanegafuchi Co., 1978; Yamada *et al*., 1978), conditions which do not permit hydantoins to dissolve well in water. Researchers at BASF screened thermophilic bacteria from Yellowstone geysers and found two new hydantoinases with much improved heat stability and specificity characteristics (BASF AG, 1987). Through recombinant DNA technology, these enzymes are now produced in *Escherichia coli* and already on the market. The catalytic process can be performed more efficiently and more competitively at temperatures reaching 75°C.

Protein engineering based on computer simulation is doubtless a very powerful tool. However, the screening of natural products for improved principles still has a higher success rate, proving again that the computer cannot yet rival Mother Nature.

Animal defence and attack mechanisms as a source for biologics. For several decades now spider, snake, frog and bee venoms and squid and leech salivas have been investigated for pharmaceutically active peptides and proteins. Leeches (e.g. *Hirudo medicinalis*) have been used in traditional medicine to treat thrombosis since ancient times. The active principle from their saliva, the protein hirudin is now an ingredient of numerous ointments and gels and thus used against varicosis and haemorrhoids. Hirudin was one of the first proteins isolated from wild biodiversity. Recombinant hirudin has now been produced in *Escherichia coli* (Fortkamp *et al*., 1986). Other leech species are currently under investigation to discover new hirudin variants with improved therapeutic applications (Sacheri *et al*., 1993). Promotion of blood clotting during wound healing can be achieved using proteins from snake venom (e.g. from the Egyptian sand viper) that induce platelet aggregation (Baheer *et al.*, 1995).

The mammalian enzyme tissue plasminogen activator (tPA) dissolves thrombotic blood clots. Recombinant human tPA, developed and patented by Genentech, has been approved as a therapeutic agent against heart attack in the USA and Europe. Researchers at Schering AG found four similar proteins in the saliva of *Desmodus rotundus*, the common vampire bat,

that are more efficient and safer for therapeutic application than their human counterparts (Schleuning *et al*., 1992). These products are presently undergoing preclinical studies.

Eledoisin is a hendecapeptide isolated from the salivary gland of certain squids (*Eledone* spp.). Its physiological action resembles that of other tachykinins. The peptide stimulates extravascular smooth muscles; it is a potent vasodilator and hypotensive agent (Pisano, 1968), and has potential therapeutic use to counter dry-eye syndrome.

Through combining common biological knowledge with simple observation and commonsense, a new biotech. company with millions of dollars in venture capital may be formed. In the following case, common knowledge of frogs and the Gram-negative bacteria found in wet environments was sufficient to launch a successful search for antibiotics. Although frogs live in ponds infested with Gram-negative bacteria, they rarely become infected by these pathogens. Based on their research, the biotech company Magainin Sciences Inc. is named after a peptide (magainin) that is highly effective against Gram-negative bacteria and occurs naturally in the skin of frogs. Effective antibiotics against this type of bacteria are rare and as a result this peptide is currently undergoing clinical trials (Jacob and Zasloff, 1994). This same way of thinking led to the discovery of the steroid squalamine, which has antibiotic properties and occurs in the stomach, liver and other organs of the shark (Moore *et al.*, 1993).

These few examples indicate that there is a whole new world to be found in wild biodiversity, accessible by gene technology, and merely awaiting exploration by the pharmaceutical biotech industry.

The agricultural biotech industry and biodiversity

Recombinant genes found in wild biodiversity may be even more important for agriculture than for the pharmaceutical industry. Classical breeding has quite successfully used genes from wild ancestors of cultivated plants to promote pest resistance and develop new and improved crop variations. Gene technology now enables humans to integrate revolutionary new properties into cultured plants through interspecific gene transfer. As with recombinant pharmaceuticals, research and development does not require random screening, but is rather a product-orientated engineering approach.

Exploiting natural defence and attack mechanisms for pest control. Plants protect themselves against pathogens through various enzymes, enzyme inhibitors and lectins. For example, the basic chitinases from rice and the acidic β-1,3-glucanase from alfalfa are directed against the cell walls of fungi. Transfer of the genes encoding these compounds into tobacco has yielded resistance against these pests (Zhu and Lamb, 1991; Maher *et al.*, 1994; Zhu *et al*., 1994). The α-amylase inhibitor in the common bean makes

the starch of the seed indigestible for insects, and this property can be transferred into the garden pea (*Pisum sativum*) (Shade *et al.*, 1994).

The transfer into plants of genes from viruses, bacteria and animals is becoming a standard procedure in crop protection. Numerous successful field trials with recombinant crops expressing genes for the δ-endotoxins of *Bacillus thuringiensis* prompted intensive screening of bacterial species with insecticidal proteins (e.g. Koziel *et al.*, 1993). Researchers at Monsanto found a cholesterol oxidase in a streptomycete which lyses the midgut epithelium of pest insects; expression of the gene in transgenic plants could promote insect resistance (Corbin *et al.*, 1994).

Spider, scorpion and mite venoms contain neurotoxic peptides which specifically kill insects. The expression of their respective genes in plants also leads to resistance against insect pests (FMC Corporation, 1993).

Resistance against bacterial infections can be achieved by the production of peptides with antibiotic activities, such as cecropin B produced by wounded silk moths (Florack *et al.*, 1995). The production of viral coat or nonstructural proteins in plants protects the plant not only against infection from that same virus, but also against related virus types (Murray *et al.*, 1993). Even mammalian enzymes can be useful. For instance, 2'-5'-oligoadenylate synthetase from rats, when produced in potatoes, protects the plant against virus X under field conditions (Truve *et al.*, 1993).

Finally, resistance against herbicides can also be achieved through heterologous gene expression. A detoxification pathway for 2,4-dichlorophenoxyacetic acid, an agonist of indoleacetic acid, can be created in plants through the expression of a monooxygenase gene from the bacterium *Alcaligenes eutrophus* (Lyon *et al.*, 1989).

Although only few of these examples deal with the transfer of genes from wild biodiversity, there is no doubt that tropical environments, especially tropical rainforests, engender a multitude of defence and attack mechanisms among their inhabitants. This might lead us to suspect that these survival mechanisms must be highly sophisticated, and may represent a rewarding resource for the genetic engineer. It can be expected that the investigation of novel defence mechanisms will increase dramatically in the future as pest resistance develops to counter the first generation of recombinant plant variations. However, DNA sequences coding for defence proteins and peptides can be patented, and this may give cause for socioeconomic problems in the source countries, many of which are developing countries. This issue will be discussed below.

Engineering of metabolic pathways. Expression of recombinant genes in cultivated plants is under intensive investigation to improve oils, proteins, starch and other polymers for the food industry. A comprehensive overview of the state of the art is given by Beck and Ulrich (1993). However, it is not solely the food industry that stands to benefit: paper,

packaging and chemical industries will also be greatly affected. For example, Zeneca's method (Zeneca Ltd., 1995) to suppress cinnamyl alcohol dehydrogenase through antisense technology in trees facilitates the removal of lignin from cellulose, and will therefore have an impact on the aforementioned industries. In most cases, new plant variations are engineered by interspecific transfer of genes, coding for enzymes, which alter metabolic pathways. Examples based on genes from the wild biodiversity are still rare, but already quite impressive. For the production of soaps, chocolate and candies, medium-chain fatty acids found in coconut and palm kernel oil have great economic importance. In 1992, the US alone imported 600 000 tons of these oils, which contain up to 50% of trilaurin, a medium-chain dodecanoic unsaturated fatty acid. Recently, the USDA approved a high-laurate canola oil developed by the US ag-biotech company Calgene (PR Newswire, 1994). Calgene researchers investigated the synthesis of lauric acid in certain plant families and found a thioesterase which prematurely hydrolyses the growing acyl thioester of the fatty acid with an acyl-carrier protein in the wild Californian bay (*Umbellularia californica*). This 12:0-acyl-carrier protein thioesterase from the bay's developing oilseeds was expressed in transgenic *Arabidopsis thaliana* and *Brassica napus* ssp. *napus*, with the result that laurate and stearate became the most abundant types of fatty acid found in the oil of these plants (Voelker *et al.*, 1992). Three of Calgene's patents cover the purified enzyme, the recombinant nucleic acid construct with the gene for the enzyme, and a method to produce laurate in recombinant *Brassica* seeds (Calgene Inc., 1994a,b,c). The socioeconomic consequences of these patents will be discussed below.

Long-chain wax esters are required for a variety of industrial applications including pharmaceuticals, cosmetics, detergents, plastics and lubricants. Such products, especially long-chain wax esters, have previously been available from endangered species such as the sperm whale, or more recently, from the desert shrub, jojoba (*Simmondsia chinensis* or *S. californica*). Waxes are fatty alcohol and fatty acid esters, and their synthesis requires a fatty acid reductase as well as a synthase. The jojoba genes for the wax synthase (fatty acyl-CoA:fatty alcohol acyltransferase) and the fatty acyl reductase have been cloned and patented by Calgene (Calgene Inc., 1995a,b), and as a result, wax may be produced in rape seed in the future.

New biodegradable plastics produced from the bacterial storage compound polyhydroxybutyrate have been developed and are already on the market. However, production of the compound through fermentation is not cost efficient. In recent development, the transfer of the entire anabolic pathway, consisting of three enzymes from the bacterium *Alcaligenes eutrophus*, into *Arabidopsis thalania* (Nawrath *et al.*, 1994) may transform the farm field into a chemical factory for plastics in the near future. Examples for pathway engineering do not yet involve genes from the tropical rainforest, but a thorough and product-orientated survey of oils, fats, waxes and

other polymers (formerly not of commercial interest because of low productivity or abundance) from tropical plants, animals and microorganisms may lead to new compounds for industrial applications. These new compounds would have the advantage of being both biodegradable and available for farm production through genetic engineering.

Gene technology will also lead to the more efficient production of natural compounds presently used for pharmaceuticals and pesticides. The chrysanthemyl diphosphate synthase gene from *Chrysanthemum cinerariaefolium* was patented by Agridyne Technologies Inc. (1995) and promises to open new paths for producing insecticidal pyrethrins, pyrethroids and their derivatives with greater efficiency and higher purity levels in cultivated plants.

Metabolic engineering of medicinal plants has been performed successfully with *Atropa belladonna* to produce the alkaloid scopolamine. The naturally occurring alkaloid in this plant is hyoscyamine, known for its anticholinergic activity, and an active ingredient in eye drops, antidotes and spasmolytics (Yun *et al.*, 1992). Scopolamine, found throughout the *Solanaceae*, is an epoxy-derivative of hyoscyamine but with a broader therapeutic spectrum, including, e.g., antiemetics and hypnotics. Expression of the hyoscyamine 6-β-hydroxylase gene from *Hyoscyamus niger* in *Atropa* led to an almost exclusive accumulation of scopolamine in the plant's leaf and stem. Flux through a pathway to a plant secondary product can be elevated by genetic engineering. For example, over-expression of the yeast ornithine decarboxylase gene in transgenic roots of *Nicotiana rustica* led to enhanced nicotine accumulation (Hamill *et al.*, 1990). The same effect can be obtained to produce sterols in plants by over-expressing 3-hydroxy-3-methylglutaryl CoA reductase, which catalyses the production of the sterol building block mevalonate (Amoco Corporation, 1994).

11.3. Modern Bioprospecting: Linking Industry, Biodiversity Conservation and Developing Country Technology Acquisition

The rapid loss of biological diversity – indicated by the extinction of an estimated 30 to 300 species per day (Japan Economic Newswire, 1995) – together with the potential opportunities and threats of gene technology, led to the United Nations Convention on Biological Diversity (UNEP, 1992). In light of the vast potential of biotic materials and the need to ensure their survival, in addition to measures taken to improve biodiversity conservation activities, it is imperative that industries move from the passive role of simple users to the more active one of reinvesting part of their revenues into conservation efforts. Companies should be aware that

they are among the first to lose as a consequence of species extinction, and indeed such an awareness is growing.

The principle of this modern approach to bioprospecting may be simple, but the link between biodiversity conservation and its sustainable use requires a careful design and strategic planning. The goals are to maximize those uses which generate information, and to reinvest part of the benefits obtained from bioproducts into acquiring knowledge and improving biological resource management. As a consequence, wildland biodiversity can be developed as part of a country's national economy at the same time as its preservation into perpetuity is guaranteed. The bioindustries are thereby encouraged to initiate relationships with partners in biodiversity-rich countries. Following the guidelines of the Biodiversity Convention such partnership can facilitate sustainable and nondamaging biological and genetic resource use for research and development, while taking care to share economic and intellectual benefits with the owners of the biological resources.

The process of collecting bioresources, extracting and testing constituents (either chemicals or genes) for biological activity, and the further development of a product is long and expensive (Reid *et al*., 1993). Based on the research and development costs of 93 randomly selected new chemical entities during the period 1970 to 1982, the development of a single drug to the point of market approval was estimated at $US114 million for the USA (DiMasi *et al*., 1991). Although rewards might be high, the chances of failure are equally so: of 10 000 different products tested, only one will make it to the market (Farnsworth, 1994).

However, the real challenge for this new generation of bioprospectors is to find a way to capture part of the financial revenues for the source country's biodiversity conservation efforts and economic development. As an example of this innovative approach to prospecting activities, INBio is negotiating agreements with scientific research centres, universities and private enterprise that are mutually beneficial to all parties (Sittenfeld and Lovejoy, 1994). These pioneering agreements provide significant returns to Costa Rica while simultaneously assigning economic value to natural resources, and providing a new source of income to support the maintenance and development of the country's Conservation Areas (Sittenfeld and Lovejoy, 1995).

11.3.1. Bioprospecting frameworks

Modern biodiversity prospecting requires the creation of appropriate frameworks and the cooperation and involvement of governments, intermediary institutions, private enterprise, academia, and local communities and entities. This activity also requires the involvement of lawyers,

lawmakers, scientists, managers and economists from developing and developed countries (Sittenfeld and Lovejoy, 1995).

The fundamental point of departure for a biodiversity prospecting framework is *macro-policy*, the set of governmental and international regulations, laws and economic incentives that determine land use patterns, access to and control of biological resources, intellectual property rights regimes, technology promotion, and industrial development. Macro-policies are formed on the international, national and social levels. On the international level, agreements, conventions and other mechanisms establish the relationships and protocols for sharing biological resources between countries. Documents considered important in providing the guidelines and regulations for biological resource use include: the Biodiversity Convention, the Trade Related Intellectual Property Rights (TRIPs) of the General Agreement on Tariffs and Trade (GATT), the Draft Declaration on Indigenous Rights of the United Nations Working Group on Indigenous Populations, and also subregional agreements, such as the North American Free Trade Agreement (NAFTA), the Amazonian Treaty, and the Pacto Andino.

Nevertheless, conventions, agreements and organizations still leave open the major responsibilities of designing adequate legislation and regulations regarding land ownership, land tenure rights, the creation of protected areas, the use of biological resources, nationally recognized intellectual property rights, the definition of public-domain resources, and the creation of market incentives or deterrents for private enterprise and research investments to each individual country. Such legislation and regulations promote stability and manoeuvrability of in-country partners, characteristics considered attractive to private industry and academic research counterparts.

Deterrents, such as national policy vacuums or legislation drafted outside the framework of the Biodiversity Convention, still exist in many countries and continue to create obstacles to establishing collaborations with academic and industrial research partners. In general, changes in laws and policies governing the ownership of and access to genetic resources are needed as well as changes in the way bio-business has evolved to date. The importance of favourable national policies, regulations and laws becomes obvious when considering international intellectual property rights. Drug research within the source country itself is an important step towards national economic development, but will only be attractive to the industrial partner if results can be patented. It is for this reason more than any other that international patent laws should be recognized by national law.

At the same time, there is concern within the industrial sector that countries, spurred by the Biodiversity Convention, may promulgate new laws restricting access to biological and genetic resources, and reducing

renewed enthusiasm for natural products (Putterman, 1994). Yet the recognition by the Convention of sovereign rights of nations over their genetic resources is intended to encourage world trade in genetic resources, since it commits countries to facilitate access, based on mutually agreed terms (Putterman, 1994). National governments should implement rules, regulations and policies that take advantage of Articles 15 and 16 of the Convention. These Articles encourage source-country participation in researching their own biological resources, transferring technologies to utilize these resources and reaping a fair share of the benefits from their commercial exploitation (Sittenfeld and Lovejoy, 1995).

Finally, on the social level, heavy investment in education and other social services has created a scientific environment of qualified institutions, researchers and educated personnel in Costa Rica. Such an environment is a prerequisite for research collaborations with private enterprise and is essential for integrating biodiversity into economic development (Sittenfeld and Lovejoy, 1995).

11.3.2. Inventories, business development and technology access

Supported by a favourable international and national macro-policy, three basic elements guide the rational and productive use of biological resources in prospecting agreements: (i) biodiversity inventories and information handling; (ii) business development; and (iii) technology access.

Inventories and information management

As pointed out in Section 11.2.1, screening for drugs and pesticides will only be successful through the development and management of biological, ecological, taxonomic, and related systematic information on living species and systems. Even with these data, further information is required for the more systematic screening approach used in gene technology. For example, biochemical data must be evaluated for, e.g., the occurrence of certain biopolymers, metabolic pathways, enzymes and defence or attack mechanisms. Biodiversity inventories create catalogues of available resources and their location. They prevent damage to ecosystems, areas, species and populations by indicating what resources are available, and where they can be collected without damaging the environment (Raven and Wilson, 1992). Simultaneously, the source-country collaborator becomes a more attractive, knowledgeable and reliable business partner because the inventory-generated information reduces the uncertainties of collecting further material should this prove necessary.

Business development

Building upon inventory-generated knowledge, business development defines markets, market needs, major players, and national capacities in

science and technology as well as institutional strategies and goals. Important requirements include knowledge of one's assets and drawbacks, market surveys and evaluation of conservation needs. The key to business development is interacting with international industry in order to approach the market in a realistic and practical way. Because bioprospecting should promote source-country economic development, business development must encourage the sustainable use of biodiversity by local entrepreneurs. However, this is a considerable challenge in developing countries where industry normally cannot take the financial risk of applying innovative technologies, let alone those that are sustainable and non-damaging to the environment.

Technology transfer

One of the major issues discussed in the Biodiversity Convention refers to technology transfer, allowing source countries to convert raw biological materials into products of greater value in exchange for access to their biodiversity (Putterman, 1994). This issue is of tremendous importance, particularly in a decade of patentable genes, and will be discussed in more detail below. In the near future, genes isolated from tropical biodiversity may provide the farmers in developed countries with advantages over the farmers of the source countries. These advantages stem from the bioindustries' historical development and their physical proximity to the developed agricultural economies of the North. This may lead biotechnological research and development to concentrate solely on improving the properties of crop and livestock in the North (for discussion, see Shand, 1993). Technology transfer may enable source countries to keep pace with the developed countries, and avoid being left out of important agricultural developments (Lesser and Krattiger, 1993). This scenario is realistic because gene technology, in contrast to natural compound chemistry, does not particularly rely on expensive investments in laboratory equipment, and would therefore be easier to implement.

11.3.3. Contract negotiations

In general, contract negotiation is divided into three basic sets of issues: scientific issues, business issues and legal issues. To negotiate, an organization must have a good sense of its own fundamental needs and those of its potential collaborator. The typical source-country needs are: the generation of income to support protected areas and conservation management activities through direct contributions as well as royalties; the transfer of processing technologies and a guaranteed future profit-sharing if commercial products are forthcoming. Sampling must be done under best ecological

practice without damaging the ecosystem. For bilateral contracts with industrial partners, exclusivity and time limitations are further requirements.

In summary, modern bioprospecting requires that the source country:

1. Creates an infrastructure guaranteeing a reliable supply of natural products (including correct taxonomic identification, quality control, full support from government and adherence to national or local regulations on access to resources).
2. Acquires technology that adds value to natural products wherever possible (from extracts to partially purified or pure compounds or gene sequences).
3. Takes advantage of local capabilities using all types of organisms as biological resources attractive to industry (from plants and microbial resources through marine or freshwater life forms to arthropods).
4. Develops a reputation as a reliable business partner over the time.
5. Reinvests part of the revenues in improving biodiversity management and conservation.

In exchange for access to biological resources, the industrial partner must agree to:

1. The fair and equitable sharing of benefits, both in intellectual and monetary terms.
2. The implementation of collection and production methods with minimum effects on biodiversity.
3. The use of equitable bioprospecting practices for further research on tropical diseases and problems specifically associated with developing countries.

11.4. How to Face the New Challenge of Gene Technology

With few exceptions classical bioprospecting for drugs has not proved economically beneficial for developing nations, but nor has it directly damaged these economies. In contrast, bioprospecting for genes may soon pose a real threat to the economic survival of these biodiversity-rich countries. The farmers of the North are currently suffering under low prices and overproduction of certain traditional crops and livestock. As a result they are looking for new markets and products.

Modern biotechnology promises to aid Northern farmers in this endeavour, eliminating or displacing traditional export commodities from developing countries and transferring production or substitutes from the farm fields of the South to those of the North. Quite possibly the transfer may even skip the Northern farms and jump straight into bioreactors. In Africa alone, US$ 10 billion in exports are vulnerable to industry-induced

changes in raw material prices and requirements (Shand, 1993). Most developments in plant biotechnology have been achieved with crops cultivated mainly in industrialized countries. This denies the farmers of developing countries the chance to benefit from the new agricultural opportunities of gene technology.

As mentioned above, Calgene's high-laurate canola oil may displace coconut and palm kernel oil, posing a threat to the economic survival of millions of farm families in the South. In the Golfito region of Costa Rica the government has started a programme to grow oil palms on banana fields deserted by the US fruit multinationals. All these efforts, which are partially financed with Northern developmental aid, may vanish into thin air as a result of Calgene's new rape seed. Thaumatin, a sweet-tasting basic protein from the tropical plant *Thaumatococcus*, has been traditionally used in West Africa as a sweetener. With the collection of *Thaumatococcus* fruits for the British food industry, the population in this region earned a large part of its income. However, the thaumatin gene has now been cloned and the sweetening protein can be produced by large-scale fermentation of brewer's yeast at low cost (Lee *et al*., 1988). The same applies to natural compounds like indigo, which can now be produced by the fermentation of *Escherichia coli* engineered with genes from a toluol-degrading subspecies of the soil bacterium *Pseudomonas putida* (Ensley *et al.*, 1983). Products like vanilla, pyrethrum and rubber may follow this same path.

Along the same lines, the extension of patent laws to the developing nations through the GATT could mean that the biotech industry obtains a monopoly on genetically engineered livestock and crops, which farmers in developing countries must cultivate under constraint in order to remain competitive. For example, US-Patent No. 5 159 135 of Agracetus (a subsidiary of W.R. Grace & Co.) covers all genetically engineered cotton. This patent is a warning of potential future problems, and has already caused an outcry in developing countries like India (Kidd and Dvorak, 1994). If the biotech industry continues developing without adequate controls, the consumers and farmers of developing countries may even end up paying royalties on biotech products that were originally developed from their very own resources and knowledge.

As a step in the right direction, the US Patent and Trademark Office reversed its decision to grant the Agracetus patent at the end of 1994, but primarily as a result of pressure from the US biotech industry which argued that patents like this will inhibit research and development (AGWEEK, 1994). Such issues bring important questions to light: How can developing nations be motivated to conserve their biodiversity under these threatening circumstances? Are the regulations and tools of modern bioprospecting, as described above, sufficient to face this challenge?

The Biodiversity Convention attempts to address this new threat by requiring that access to biodiversity's genetic potential be combined with

the biotechnology transfer to the South in order for those countries to develop their own methods of sustainable biodiversity utilization. Nevertheless, the Convention suffers from three major drawbacks.

1. The treaty was not ratified by the USA, the leading country in biotechnological research, development and application.
2. It specifically excludes (under US pressure) *ex situ* genebank material collected before the enactment of the treaty (Shand, 1993). As a result, huge stocks of germplasm collected by the North, mostly in tropical and subtropical countries, are not restricted by the Convention. The recent transfer by the Consultative Group on International Agricultural Research of its 12 genebanks to the auspices of the UN must be the first step in keeping the access of developing countries open to their own resources (Madeley, 1994).
3. It still remains nearly impossible to control the illegal transfer of genetic material into the North. Genes can be cloned from minute amounts of DNA or RNA and isolated from biological material that easily fits into an airmail envelope. Genes do not have tags designating their country of origin, and once they are cloned, they are no longer controlled by their source country. This is quite different from the isolation of natural compounds from plants, where larger amounts of plant material must be collected and, for the process of isolation and structure elucidation, must be re-collected. In this last case, controlling the flow of biological material is possible simply because industry will eventually require sample resupply at a given point, and for this the industry needs reliable partners in countries of origin.

These issues must be approached in an active and more aggressive manner than traditionally used. During collaborations with traditional pharmaceutical and biotech companies like Merck & Co., Bristol-Myers Squibb and the British Technology Group, INBio used such an approach, proving to industry that fair partnerships are mandatory and conducive to success. More importantly, INBio demonstrated that reliable applied research is possible in a developing country, and that technology transfer to acquire necessary know-how and equipment works to the advantage of the industrial partner as well.

The same applies to the biotech industry. In this case, the transfer of gene technology is not critical, in contrast to natural compound chemistry, because it does not require million-dollar investments in laboratory infrastructure. Rather, the industry relies on the know-how already existing in many source countries. Gene technology also represents a promising development opportunity for countries that do not have large research budgets at their disposal. Moreover, gene technology is a very straightforward approach, relying on natural history observations (e.g. whether certain plants show natural resistance to pathogens), and not involving

the automated random screening of thousands of samples. Biodiversity inventories, which are already in place in countries like Costa Rica, are a reasonable and advantageous prerequisite for successful 'gene prospecting'.

Costa Rica's aggressive strategy to foster collaborations with the international industry and academic institutions in drug research, gene technology and agriculture actively seeks to develop and patent natural compounds, proteins and genes in Costa Rica based on a foundation of national research. Collaborations of this nature will help launch Costa Rica onto a scientific and technological plane that offers services and goods that are both competitive and compatible with those of industrial nations.

Simultaneously, INBio works within this strategy to increase knowledge about Costa Rican biodiversity in general, access revenues for further conservation efforts, and to assign biodiversity a higher value than it has had in the past. There is little doubt that such activities will encourage society's willingness to preserve biodiversity for future generations, by making it worthwhile for the Costa Rican population to maintain tropical forests and other ecosystems on their own.

Acknowledgements

We thank Mrs Annie Lovejoy for critical reading, comments and major improvements of the manuscript. This work is supported in part by a grant (to Dr Nader) of the Centre for International Migration and Development, Frankfurt, Germany, and by grant No. 5 U01 TW / CA00312 from the National Institute of Health (Fogarty International Center).

References

Agridyne Technologies Inc. (1995) Chrysanthemyl diphosphate synthase, corresponding genes and use in pyrethrin synthesis. US Patent No. 5 443 978.

AGWEEK (1994) Plant patent dispute reaches standoff. December 12.

Amoco Corporation (1994) Method and composition for increasing sterol accumulation in higher plants. US Patent No. 5 365 017.

Babu, J.R. (1988) Avermectins: biological and pesticidal activities. In: Cutler, H.G. (ed.) *Biological Active Natural Products: Potential Use in Agriculture.* American Chemical Society, Washington DC, pp. 91–108.

Baheer, A.R., el-Asmar, M.F. and Soslau, G. (1995) Characterization of a potent platelet aggregation inducer from *Cerastes cerastes* (Egyptian sand viper) venom. *Biochimica et Biophysica Acta* 1250, 97–109.

Balick, M. (1994) Ethnobotany, drug development and biodiversity conservation – exploring the linkages. In: Chadwick, P.J. and Marsh, J. (eds) *Ethnobotany and*

the Search for New Drugs. Ciba Foundation Symposium 185. John Wiley, Chichester, pp. 4–18; discussion pp. 19–24.

BASF AG (1987) Verfahren zur Herstellung von mesophilen Mikroorganismen, die eine bei höherer Temperatur aktive D-Hydantoinase enthalten. German Patent No. 3535987.

Beck, C.I. and Ulrich, T.H. (1993) Biotechnology in the food industry. *Bio/Technology* 11, 895–902.

Birch, A.N.E., Robertson, W.M., Geoghegan, I.E., McGavin, W.J., Alphey, T.J.W., Phillips, M.S., Fellows, L.E., Watson, A.A., Simmonds, M.S.J. and Porter, E.A. (1993) DMDP – A plant-derived sugar analogue with systematic activity against plant parasitic nematodes. *Nematologica* 39, 517–530.

Calgene Inc. (1994a) Plant thioesterase having preferential hydrolase activity toward C12. US Patent No. 5 304 481.

Calgene Inc. (1994b) Plant medium-chain-preferring acyl-ACP thioesterases and related methods. US Patent No. 5 298 421.

Calgene Inc. (1994c) Plant thioesterases. US Patent No. 5 344 771.

Calgene Inc. (1995a) Jojoba wax biosynthesis gene. US Patent No. 5 445 947.

Calgene Inc. (1995b) Fatty acyl reductase. US Patent No. 5 411 879.

Cardellina, J.H., Gustafson, K.R., Beutler, J.A., McKee, T.C., Hallock, Y.F., Fuller, R.W. and Boyd, M. (1993a) National Cancer Institute intramural research on human immunodeficiency virus inhibitory and antitumor plant natural products. In: Kinghorn, D.A. and Balandrin, M.F. (eds) *Human Medicinal Agents from Plants.* American Chemical Society, Washington DC, pp. 218–227.

Cardellina, J.H., Munro, M.H.G., Fuller, R.W., Manfredi, K.P., McKee, T.C., Tischler, M., Bokesch, H.R., Gustafson, K.R., Beutler, J.A., and Boyd, M. (1993b) A chemical screening strategy for the dereplication and prioritization of HIV-inhibitory aqueous natural products extracts. *Journal of Natural Products* 56, 1123–1129.

Corbin, D.R., Greenplate, J.T., Wong, E.Y. and Purcell, J.P. (1994) Cloning of an insecticidal cholesterol oxidase gene and its expression in bacteria and in plant protoplasts. *Applied and Environmental Microbiology* 60, 4239–4244.

Cragg, G., Boyd, M.R., Cardellina, J.H., Newman, D.J., Snader, K.M. and McCloud, T.G. (1994) Ethnobotany and drug discovery: the experience of the US National Cancer Institute. In: Chadwick, D.J. and Marsh, J. (eds) *Ethnobotany and the Search for New Drugs. Ciba Foundation Symposium 185*. John Wiley, Chichester, pp. 178–190; discussion pp. 191–196.

DiMasi, J.A., Hansen, R.W., Grabowski, H.G. and Lasagna, L. (1991) Cost of innovation in the pharmaceutical industry. *Journal of Health Economics* 10, 107–142.

Ecker, D.J. and Crooke, S.T. (1995) Combinatorial drug discovery: which method will produce the greatest value. *Bio/Technology* 13, 351–361.

Ensley, B.D., Ratzkin, B.J., Osslund, T.D., Simon, M.J., Wackett, L.P. and Gibson, D.T. (1983) Expression of naphthalene oxidation genes in *Escherichia coli* results in the biosynthesis of indigo. *Science* 222, 167–169.

Epand, R.M., Epand, R.F., Orlowski, R.C., Seyler, J.K. and Colescott, R.L. (1986) Conformational flexibility and biological activity of salmon calcitonin. *Biochemistry* 25, 1964–1968.

Farnsworth, N.R. (1994) Ethnopharmacology and drug development. In: Chadwick, D.J. and Marsh, J. (eds) *Ethnology and the Search for New Drugs. Ciba Foundation Symposium 185*. John Wiley, Chichester, pp. 42–59.

Florack, D., Allefs, S., Bollen, R., Bosch, D., Visser, B. and Stiekema, W. (1995) Expression of giant silkmoth cecropin B genes in tobacco. *Transgenic Research* 4, 132–134.

FMC Corporation (1993) Insecticidally effective peptides. US Patent No. 5 441 934.

Fortkamp, E., Rieger, M., Heisterberg-Moutses, G., Schweitzer, S. and Sommer, R. (1986) Cloning and expression in *Escherichia coli* of a synthetic DNA for hirudin, the blood coagulation inhibitor in the leech. *DNA* 5, 511–517.

Frost & Sullivan Inc. (1992) *The U.S. Market for Chemical Pesticides.* Frost & Sullivan Inc., New York, p. 8.

Global Biodiversity Strategy: Guidelines for Action to Save, Study, and Use Earth's Biotic Wealth Sustainably and Equitably (1992) WRI / IUCN / UNEP, USA.

Grünwald, J. (1995) The European phytomedicines market: figures, trends, analyses. *HerbalGram* (Austin, Texas) 34, 60–65.

Hamill, J.D., Robins, R.J., Parr, A.J., Evans, D.M., Furze, J.M. and Rhodes, M.J. (1990) Over-expressing a yeast ornithine decarboxylase gene in transgenic roots of *Nicotiana rustica* leads to enhanced nicotine accumulation. *Plant Molecular Biology* 15, 27–38.

Harborne, J. (1989) *Introduction to Ecological Biochemistry*, 4th edn. Academic Press, London, UK, pp. 171–172.

Jacob, L. and Zasloff, M. (1994) Potential therapeutic applications of magainins and other antimicrobial agents of animal origin. In: *Ciba Foundation Symposium 186.* John Wiley, Chichester, pp. 197–216; discussion pp. 216–223.

Janzen, D. and INBio (1992) A north–south perspective on science in the management, use and development of biodiversity. In: Sandlund, O.T., Hindart, K. and Brown, A.H.D. (eds) *Conservation of Biodiversity for Sustainable Development.* Scandinavian University Press, Oslo, pp. 27–54.

Janzen, D.H., Fellows, L.E. and Waterman, P.G. (1990) What protects *Lonchocarpus* (Leguminosae) seeds in a Costa Rican dry forest? *Biotropica* 22(3), 272–285.

Japan Economic Newswire (1995) Biodiversity report proposes government action. March 29.

Kanegafuchi Company (1978) Verfahren zur Herstellung von D-N-Carbamoyl-a-Aminosäuren. German Patent No. 2757980.

Kashman, Y., Gustafson, K.R., Fuller, R.W., Cardellina, J.H., McMahon, J.B., Currens, M.J., Buckneit, R.W., Hughes, S.H., Cragg, G.M. and Boyd, M.R. (1992) The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*. *Journal of Medicinal Chemistry* 35, 2735–2743.

Kidd, G. and Dvorak, J. (1994) Agracetus' cotton patent draws opposition. *Bio/Technology* 12, 659.

König, G., Wright, A.D., Sticher, O., Angerhofer, C.K. and Pezzuto, J.M. (1994) Biological activities of selected marine natural products. *Planta Medica* 60, 532–537.

Koziel, M.G., Beland, G.L., Bowman, C., Carozzi, N.B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Lauitis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M.R., Merlin, E., Rhodes, R., Warren, G.W., Wright, M. and Evola, S.V. (1993) Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Bio/Technology* 11, 194–200.

Lee, J.H., Weickmann, J.L., Koduri, R.K., Gosh-Dastidar, P., Saito, K., Blair, L.C., Date, T., Lai, J.S., Hollenberg, S.M. and Kendall, R.L. (1988) Expression of synthetic thaumatin genes in yeast. *Biochemistry* 27, 5101–5107.

Lesser, W. and Krattiger, A.F. (1993) *Facilitating South–North and South–South Technology Flow Processes for "Genetic Technology"*. Working paper R7W. International Academy of the Environment – Biodiversity / Biotechnology Programme, Geneva.

Lyon, B.R., Llewellyn, D.J., Huppatz, J.L., Dennis, E.S. and Peacock, W.J. (1989) Expression of a bacterial gene in transgenic tobacco plants confers resistance to the herbicide 2,4-dichlorophenoxyacetic acid. *Plant Molecular Biology* 13, 533–540.

McCloud, T.G., Nemec, J., Mushik, G., Sheffield, H.G., Quesenberry, P., Suffness, M., Cragg, G. and Thompson, J. (1988) Extraction of bioactive molecules from plants. In: *Proceedings of the International Congress on Natural Products Research, July 17–21, Park City, Utah.*

Maclntyre, I., Alevizaki, M., Bevis, P.J. and Zaidi, M. (1987) Calcitonin and the peptides from the calcitonin gene. *Clinical Orthopedics* 43, 45–55.

Madeley, J. (1994) International plant genebanks come under UN auspices. *Financial Times* October 26.

Maher, E.A., Lamb, C.J. and Dixon, R.A. (1994) Stress response in alfalfa (*Medicago sativa* L.). XVII. Identification of multiple hydrolyases and molecular characterization of an acidic glucanase. *Physiological and Molecular Plant Pathology* 43, 329–342.

Mendelsohn, R. and Balick, M.J. (1995) The value of undiscovered pharmaceuticals in tropical forests. *Economic Botany* 49, 223–228.

Moore, K.S., Wehrli, S., Roder, H., Rogers, M., Forrest, J.N., McCrimmon, D. and Zasloff, M. (1993) Squalamine: an aminosterol antibiotic from the shark. *Proceedings of the National Academy of Sciences of the USA* 90, 1354–1358.

Murray, L.E., Elliott, L.G., Capitant, S.A., West, J.A., Hanson, K.K., Scarafia, L., Johnston, S., DeLuca-Flaherty, C., Nichols, S., Cunanan, D., Dietrich, P.S., Mettler, I.J., Dewald, S., Warnick, D.A., Rhodes, C., Sinibaldi, R.M. and Brunke, K. (1993) Transgenic corn plants expressing MDMV strain B coat protein are resistant to mixed infections of maize dwarf mosaic virus and maize chlorotic mottle virus. *Bio/Technology* 11, 1559–1564.

Nawrath, C., Poirier, Y. and Somerville, C. (1994) Targeting of the polyhydroxybutyrate biosynthetic pathway to the plastids of *Arabidopsis thaliana* results in high levels of polymer accumulation. *Proceedings of the National Academy of Sciences of the USA* 91, 12760–12764.

O'Neill, M.J. and Lewis, J.A. (1993) The renaissance of plant research in the pharmaceutical industry. In: Kinghorn, D.A. and Balandrin, M.F. (eds) *Human Medicinal Agents from Plants.* American Chemical Society, Washington DC, pp. 48–55.

Pisano, J.J. (1968) Vasoactive peptides in venomes. *Federation Proceedings* 27, 58–62.

Principe, P.P. (1989) The economic significance of plants and their constituents as drugs. In: Wagner, H., Hikino, H. and Farnsworth, N.R. (eds) *Economic and Medicinal Plant Research*, vol. 3. Academic Press, London, pp.1–17.

PR Newswire (1994) Calgene receives USDA approval to produce laurate canola. November 1.

Putterman, D. (1994) Trade and the biodiversity convention. *Nature* 371, 553–554.

Raven, P. and Wilson, E.O. (1992) A fifty-year plan for biodiversity surveys. *Science* 258, 1099–1100.

Reid, W.V., Laird, S., Meyer, C A, Gámez, R., Sittenfeld, A., Janzen, D.H., Gollin, M.A. and Juma, C. (1993) *Biodiversity Prospecting: Using Genetic Resources for Sustainable Development*. World Resources Institute, Washington DC, 341 pp.

Research Foundation of the State University of New York (1994) Biological applications of alkaloids derived from the tunicate *Eudistoma* sp. US Patent No. 5 278 168.

Rural Advancement Foundation International (1994) *Conserving Indigenous Knowledge: Integrating Two Systems of Innovation*. United Nations Development Programme, New York.

Sacheri, F., Nitti, G., Valsasina, B., Orsini, G., Visco, C., Ferrera, M., Sawyer, R.T. and Sarmientos, P. (1993) Novel hirudin variants from the leech *Hirudinaria manillensis*. Amino acid sequence, cDNA cloning and genomic organization. *European Journal of Biochemistry* 214, 295–304.

Sapnde, T.F., Garraffo, H.M., Edwards, M.W., Yeh, H.J.C., Pannell, L. and Daly, J.W. (1992) Epibatidine: A novel (chloropyridyl)azabicycloheptane with potent analgesic activity from an Ecuadoran poison frog. *Journal of the American Chemical Society* 114, 3475–3478.

Schleuning, W.D., Alagon, A., Boidol, W., Bringmann, P., Petri, T., Kratzschmar, J., Haendler, B., Langer, G., Baldus, B. and Witt, W. (1992) Plasminogen activators from the saliva of *Desmodus rotundus* (common vampire bat): unique fibrin specificity. *Annals of the New York Academy of Sciences* 667, 395–403.

Shade, R.E., Schroeder, H.E., Pueyo, J.J., Tabe, L.M., Murdock, L.L., Higgins, T.J.V. and Chrispeels, M.J. (1994) Transgenic pea seeds expressing the alpha-amylase inhibitor of the common bean are resistant to bruchid beetles. *Bio/Technology* 12, 793–796.

Shand, H. (1993) Agbio and third world development. *Bio/Technology* 11, S13.

Sittenfeld, A. and Lovejoy, A. (1994) Biodiversity prospecting. *Our Planet (UNEP: Magazine for Sustainable Development)* 6(4), 20–21.

Sittenfeld, A. and Lovejoy, A. (1995) Biodiversity prospecting frameworks: The INBio experience in Costa Rica. In: McNeely, J. and Guruswamy, L. (eds) *Their Seed Preserve: Strategies for Protecting Global Biodiversity*. Duke University Press.

Sittenfeld, A. and Villers, R. (1994) Costa Rica's INBio: Collaborative biodiversity research agreements with the pharmaceutical industry. Case Study 2. In: Meffe, G.K., Carroll, C.R. and contributors. *Principles of Conservation Biology*. Sinauer Associates, Sunderland, MA, 500–504.

Truve, E., Aaspollu, A., Horikanen, J., Puska, R., Mehto, M., Hassi, A., Teeri, T.H., Kelve, M., Seppänen, P. and Saarma, M. (1993) Transgenic potato plants expressing mammalian 2'-5' oligoadenylate synthetase are protected from potato virus X infection under field conditions. *Bio/Technology* 11, 1048–1052.

UNEP (1992) *Convention on Biological Diversity*. United Nations Environmental Programme, Nairobi, Kenya.

US National Research Council (Panel of the Board on Science and Technology for International Development) (1992) *Conserving Biodiversity: A Research Agenda for Development Agencies*. National Academic Press, Washington DC.

Voelker, T.A., Worrell, A.C., Anderson, L., Bleibaum, J., Fan, C., Hawkins, D.J.,

Radke, S.E. and Davies, H.M. (1992) Fatty acid biosynthesis redirected to medium chains in transgenic oil seed plants. *Science* 257, 72–74.

Wilson, E.O. (1992) Resolution. In: Wilson, E.O. (ed.) *The Diversity of Life*. Harvard University Press, Cambridge, MA, pp. 311–342.

Wink, M. (1993) Production and application of phytochemicals from an agricultural perspective. In: van Beek, T.A. and Breteler, H. (eds) *Proceedings of the Phytochemical Society of Europe: Phytochemistry and Agriculture*. Clarendon Press, Oxford.

Yamada, H., Takahashi, S., Kii, Y. and Kumagai, H. (1978) Distribution of hydantoin hydrolyzing activity in microorganisms. *Journal of Fermentation Technology* 56, 484–491.

Yun, D.J., Hashimoto, T. and Yamada, Y. (1992) Metabolic engineering of medicinal plants: transgenic *Atropa belladonna* with an improved alkaloid composition. *Proceedings of the National Academy of Sciences of the USA* 89, 11799–11803.

Zeneca Ltd. (1995) Modification of lignin synthesis in plants. US Patent No. 5 451 514.

Zhu, Q. and Lamb, C.J. (1991) Isolation and characterization of a rice gene encoding basic chitinase. *Molecular and General Genetics* 226, 289–296.

Zhu, Q., Maher, E.A., Masoud, S., Dixon, R.A. and Lamb, C.J. (1994) Enhanced protection against fungal attack by constitutive co-expression of chitinase and glucanase genes in transgenic tobacco. *Bio/Technology* 12, 807–812.

Index