Part 1
Linking Plants, Genes, and Biotechnology

Medicinal Plant Biotechnology. From Basic Research to Industrial Applications
Edited by Oliver Kayser and Wim J. Quax

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The Engineering of Medicinal Plants: Prospects and Limitations of Medicinal Plant Biotechnology

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1.1
Introduction

The use of medicinal plants is increasing worldwide. According to the World Health Organization (WHO), approximately 80% of the world's population currently uses herbal medicines directly as teas, decocts or extracts with easily accessible liquids such as water, milk, or alcohol [1]. Although modern synthetic drugs are mostly used in developed countries, the use of herbal drugs in the western world is well accepted, and a continuously high demand for plant material and extracted natural products can be observed. The top 10 ranked plants that have received greatest interest in the USA and Europe over the past 30 years, and account for over 50% of the over-the-counter (OTC) market, are listed in Table 1.1 [2]. It should be noted that these plants are only partly of interest for biotechnology and

Table 1.1 Top ten ranked medicinal plants medicinal herbs most commonly used in the United States and Europe.

Species	***Use***
Hypericum perforatum	Anxiety, depression, insomnia
Echinacea purpurea	Immune stimulation
Ginkgo biloba	Dementia, Alzheimer's disease, tinnitus
Sabal serrulata	Benign prostatic hyperplasia
Tanacetum parthenium	Migraine prophylaxis
Allium sativum	Lipid-lowering, antithrombotic, fibrinolytic, anti-hypertensive, anti-atherosclerotic
Zingiber officinalis	Antiemetic
Panax ginseng	Tonic, performance enhancer, "adaptogen", mood enhancer
Valeriana officinalis	Sedative, hypnotic, anxiolytic
Ephedra distachya	Asthma, rhinitis, common cold, weight loss; enhancer of athletic performance

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genetic modification, and today approaches of medicinal plant biotechnology focus more on distinct natural products and biosynthetic pathways. The main reason for this is that genetically modified plants as a source for extract preparations and for manufactured pharmaceuticals available in the pharmacy are not accepted by patients and consumers. Because of a gaining popularity for phytotherapy, and the wishful thought that products obtained from Nature are safe, patients do not consider genetically modified plants as a part of this philosophy. Hence, the approaches of medicinal plant biotechnology currently focus more on distinct natural products and biosynthetic pathways.

It is not only plants that are of great interest to the pharmaceutical industry, but also defined natural products. This situation is supported by the fact that some 25% of all drugs dispensed during the 1970s in the USA contained compounds obtained from higher plants [3]. Moreover, 11% of the 252 drugs considered to be basic and essential by the WHO are isolated and used directly from plant sources [4]. In addition, approximately 40% of pharmaceutical lead compounds for the synthetic drugs used today are derived from natural sources. It is due to this renewed interest in the use of plant sources in drug discovery that in this book we have included Chapter 6, which highlights the prospects and potential of plant-based natural products.

Today, only 10% of all medicinal plant species used are cultivated, with by far the larger majority being obtained from wild collections. Harvesting from the wild may, however, becomes problematic, as was seen in the case of *Podophyllum* and *Taxus* species, for *Piper methysticum*, for *Cimicifuga racemosa* and for *Arctostaphylos uva-ursa*, whereby both loss of genetic diversity and habitat destruction occurred. It is also not well known that conventional plant breeding methods are of major importance in medicinal plant biotechnology, due to improving agronomic and medicinal traits – a point which is discussed in Chapter 18. Thus, the domestic cultivation of medicinal plants is a well-accepted way in which to produce plant material. Moreover, such an approach also helps to overcome other problems inherent in herbal extracts, such as the standardization of extracts, variability of the plant material, minimization of toxic constituents and contaminations, increasing the content of the desired constituents, and breeding according to internationally accepted Good Agricultural Practise (GAP) guidelines. *Hypericum perforatum* and *Gingko biloba* are two examples of top-selling plants which have been cultivated for many years by European and American phytocompanies, and consequently are not greatly threatened by wild harvesting.

From this background the question arises as to whether there is a need for biotechnology and gene technology for medicinal plants. From its narrow definition, biotechnology does not focus on medicinal plants, and therefore it should be accepted in a broader sense. With regard to medicinal plants, biotechnology could be described as a method for enhancing the formation and accumulation of desirable natural products, with possible product modification in medicinal plants. Micropropagation, cell and hairy root culture as well as gene technology are all important techniques for plant propagation, but these are mostly used to improve the production and yield of desired natural products. Two well-described examples of this are artemisinin and paclitaxel, both of which are available in plants, albeit in only small quantities. As a consequence, not only are both natural products expensive, but *Taxus* species in the Himalaya region are also endangered due to unsustainable cutting and collection [5]. In an attempt to overcome these problems for both drugs, intensive research is being carried out worldwide, including combinatorial biosynthesis (see Chapter 13), or improved bioprocessing in bioreactors for both artemisinin (see Chapter 23) and paclitaxel (see Chapter 22).

The application of biotechnological techniques to medicinal plants has received considerable interest, especially when the final product is defined, purified, and natural. The manipulation of medicinal plants is well known and accepted both by scientists and consumers, if the pathways and product yield can be optimized to create precursors for semisyntheses (e.g., baccatin-III to paclitaxel), food components (e.g., vitamins), pesticide residence (e.g., *Atropa belladonna* [6,7]), and cellular storage conditions, as shown for *Mentha*×*piperita* with enhanced resistance against fungal attack and abiotic stress [8].

1.2
Genetic Transformation and Production of Transgenic Plants

The use of biotechnological tools in medicinal plant science is very limited as compared to other crops. However, in recent years the engineering of agronomic traits in medicinal plants led to interesting developments for genetic transformation, both *in vivo* and *in vitro*. Genetic transformation with bacterial vector systems has been widely used for several medicinal plants, including *Artemisia annua*, *Taxus* sp., *Papaver somniferum*, *Ginkgo biloba* (Chapter 21), and *Camptotheca acuminata* (Chapter 7), and species from the Solanaceae (Chapter 11), as reviewed in several chapters. Efficient gene vector systems are intensively discussed in Chapter 8, while the production of high-value phytochemicals is reviewed in Chapter 9.

The commercialization of many genetically engineered plants and plant products is currently being actively pursued by biotechnology and seed companies, and many genetically engineered plants are presently field-tested to determine their potential for commercialization. It remains an open question, however, as to which role transgenic medicinal plants will have in the future. Do we accept medicinal plants in the genomic era only as a source of chemicals, and should they acquire a new role as a genetic source or host for heterologous genes? An opportunity to discuss this question arises in Chapter 14, where the subject is plantibodies, while some answers – as well as further questions – are provided in Chapter 16.

1.3
Pathway Engineering and Combinatorial Biosynthesis

Increasing the production of pharmacologically attractive natural products represents on the main targets for the genetic manipulation of medicinal plants. An increasing number of natural products are being biosynthesized in low quantities, with known examples being artemisinin, paclitaxel, podophyllotoxin, and *Vinca*-alkaloids. The use of genetically modified plant cell cultures, such as hairy root cultures for Solanaceae (see Chapter 11) or for artemisinin (see Chapter 23), offers a rational approach to allow the overexpression of genes encoding biosynthetic enzymes, and to overcome the rate-limiting steps of the biosynthesis.

Combinatorial biosynthesis is a new tool in the generation of novel natural products, as well as for the production of rare and expensive natural products, and is explained and illustrated in detail in Chapter 13. The basic concept of combinatorial biosynthesis is to combine metabolic pathways in different organisms at the genetic level. Combinatorial biosynthesis is discussed for important classes of natural products, including alkaloids (vinblastine, vincristine), terpenoids (artemisinin, paclitaxel), and flavonoids. The main problem with combinatorial biosynthesis, however, is that most biosynthetic pathways are still poorly understood at the genetic level, with relatively few genes involved in regulation and biosynthesis in plants having been sequenced and functionally elucidated. Therefore, no complete biosynthetic pathway has been completely transferred to a heterologous host. Although today, for economic reasons, crops are at the focus of this new technology, in the future these new genomic approaches will be expanded, in both range and precision, to the area of medicinal plants and the development of secondary pathways of metabolism.

1.4 Bioprocessing

Although plant breeding and cultivation is widely accepted (for a review, see Chapter 18), the lack of consistency in the levels of bioactive chemicals in herbal medicines has been a crucial point, and variations in secondary metabolite production will certainly contribute to this problem. Plant tissue culture growth of medicinal plants can also be scaled-up using continuous culture systems such as "bioreactors", which would allow the automated, high-level isolation of medicinal secondary products. In this book, three chapters outline the possibilities and limitations of today's bioprocessing techniques with regard to the latest applications. These are made on a general basis in Chapters 7 and 15, and more specifically for the production of paclitaxel and artemisinin in Chapters 22 and 23, respectively. The biochemistry, enzymology and physiology of medicinal plant biotechnology, in addition to bioreactor design and the application of proteomics and genomics, represent other areas to be understood. For example, various points in a given metabolic pathway can be controlled simultaneously, either by overexpression and/or suppression of selected enzymes, or through the use of transcriptional regulators to control endogenous genes. Thus, multipoint metabolic engineering offers a new perspective for improving the production of plant-based chemotherapeutics, and this topic is discussed extensively in Chapter 7.

1.5 Plant Propagation

Biotechnological tools are important in order to select, multiply and conserve the critical genotypes of medicinal plants. In this book, plant tissue culture techniques are discussed in Chapters 8 and 12 as integrated approaches for the production of standardized quality phytomedicines, through the mass production of consistent plant material for physiological characterization, and the analysis of active ingredients. Micropropagation is the practice of rapidly multiplying stock plant material to produce large numbers of progeny plants by using modern plant issue culture methods. Protocols for the cloning of some medicinal plants such as *Catharanthus roseus* (Apocynaceae), *Chlorophytum borivilianum* (Liliaceae), *Datura metel* (Solanaceae), and *Bacopa monnieri* (Scrophulariaceae) have been developed and are discussed in part in Chapters 9, 11 and 19. The integrated approaches of plant culture systems will provide the basis for the future development of novel, safe, effective, and high-quality products for consumers.

1.6 Bioanalytics and Metabolomics

Despite recent advances in biotechnology, combinatorial biochemistry, and high-throughput screening, natural products from plants continue to serve as a major source of new chemical entities for pharmaceutical research. Although these new biotechnological approaches have allowed the creation of new and, in part, semi-natural products, the new innovative discipline of metabolomics will clearly open the door to explore thousands of plants and their constituents. What can be expected of metabolomics, and whether this new discipline will keep its promise, remains a matter of dispute, and is discussed in Chapter 2.

Recently developed analytical techniques are capable of identifying and structurally elucidating major constituents in a single plant, or assessing a single biological activity in *in-vitro* assays in parallel. Consequently, the potential of even relatively thoroughly investigated plants must be regarded as still largely unexplored. In order to overcome this problem and to accelerate the discovery process of mapping, the chemical diversity of plants is therefore of utmost importance, and this can only be achieved if new spectroscopic and genetic methods for the rapid dereplication of natural products from plant extracts is developed. Highlights of the latest LC-NMR techniques of spectroscopic analysis are discussed in Chapter 3, while genetically based techniques for DNA profiling of medicinal plants are reviewed in Chapter 5.

1.7 Future Prospects

The commercial viability of biotechnology and gene technology in medicinal plant research is strongly influenced by the common perception of both, the plant and biotechnology. As outlined above, genetically modified medicinal plants lose their "natural" status, and are considered – erroneously – by the public as unsafe and dangerous. The crop industry learned its lesson following the rejection in Europe of genetically modified crops that were introduced into the food chain. Clearly, companies producing herbal compounds would face the same problems in obtaining permission to conduct farm-scale trails, to document the safety of the final product, and to overcome the in-principle resistance of the consumer as a strong and perhaps immovable barrier [9].

Within an open scientific environment, the discovery and development of botanical therapeutics and medicinal plant biotechnology must be accepted, as its expansion is very unlikely to cease. It is difficult to predict the future for medicinal plants, but it is likely that herbal drugs, isolated natural products and recombinant low- and high-molecular weight products will hold at least the same significance. Plants, as a renewable source with low energy consumption that can offer complex biochemical syntheses, will be even more compatible in the future. Although the real potential of plants remains unexplored, we hope that with this book we can perhaps at least "scratch the surface" slightly to provide a better understanding of medicinal plant biotechnology, as well as its possibilities and limitations.

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