Plants and human health in the twenty-first century

Article *in* Trends in Biotechnology · January 2003

DOI: 10.1016/S0167-7799(02)02080-2 · Source: PubMed

Plants and human health in the twenty-first century

Ilya Raskin, David M. Ribnicky, Slavko Komarnytsky, Nebojsa Ilic, Alexander Poulev, Nikolai Borisjuk, Anita Brinker, Diego A. Moreno, Christophe Ripoll, Nir Yakoby, Joseph M. O'Neal, Teresa Cornwell, Ira Pastor and Bertold Fridlender

The concept of growing crops for health rather than for food or fiber is slowly changing plant biotechnology and medicine. Rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics that include plant-derived pharmaceuticals, multicomponent botanical drugs, dietary supplements, functional foods and plant-produced recombinant proteins. Many of these products will soon complement conventional pharmaceuticals in the treatment, prevention and diagnosis of diseases, while at the same time adding value to agriculture. Such complementation can be accelerated by developing better tools for the efficient exploration of diverse and mutually interacting arrays of phytochemicals and for the manipulation of the plant's ability to synthesize natural products and complex proteins. This review discusses the history, future, scientific background and regulatory issues related to botanical therapeutics.

David M. Ribnicky
Slavko Komarnytsky
Alexander Poulev
Nikolai Borisjuk
Anita Brinker
Diego A. Moreno
Christophe Ripoll
Nir Yakoby
Teresa Cornwell
Ilya Raskin*
Biotech Center, Foran Hall, 59 Dudley Road, Cook College, Rutgers University, New Brunswick, N.J. 08901-8520, USA.
*e-mail: Raskin@aesop.rutgers.edu

Nebojsa Ilic
Joseph M. O'Neal
Ira Pastor
Bertold Fridlender
Phytomedics Inc., 65 Stults Road, Dayton, NJ 08810-1523, USA.

For centuries people have used plants for healing. Plant products – as parts of foods or botanical potions and powders – have been used with varying success to cure and prevent diseases throughout history. Written records about medicinal plants date back at least 5000 years to the Sumerians [1], and archeological records suggest even earlier use of medicinal plants. The strong historic bond between plants and human health began to unwind in 1897, when Friedrich Bayer and Co. introduced synthetic acetyl salicylic acid (aspirin) to the world. Aspirin is a safer synthetic analogue of salicylic acid, an active ingredient of willow bark, and was discovered independently by residents of both the New and Old worlds as a remedy for aches and fevers [2].

The twentieth century became a triumph for the synthetic-chemistry-dominated pharmaceutical industry, which replaced natural extracts with synthetic molecules that often had no connection to natural products. The spectacular rise of the pharmaceutical industry had a tremendous impact on disease treatment and prevention, saved countless lives and became one of the outstanding achievements of the twentieth century. However, the benefits of modern drugs are felt primarily in developed countries, and developing countries continue to rely on ethnobotanical remedies as their primary medicines, leaving almost 75% of the world population without access to the modern healthcare products taken for granted in the West. It is easy to overlook the fact that human medicines still contain phytochemicals – valued at US$22 608 million in 1997 and projected to reach a value of US$30 688.5 million in 2002 – with prescription products and over-the-counter (OTC) herbal remedies each comprising approximately 50% of the market [3].

The severed bond between plants and health was felt not only in the area of medicines. By providing a 'pill option', the pharmaceutical industry also diminished the historical connection between food and the treatment of disease. 'An apple a day keeps the doctor away' is the advice one usually gets from a mother, not from a professional health organization. Although plants are slowly making a comeback in several areas of human health (i.e. functional foods, dietary supplements and recombinant protein manufacturing), they are still losing importance in areas such as the traditional drug discovery process.

The plant therapeutic agents presented in this review are summarized and defined in Table 1. Although these definitions might not be ideal, they are helpful in structuring the discussion of the re-emerging connection between plants and human health. For the purposes of this review, the term 'plant' includes only seed plants (superdivision Spermatophyta), excluding the major contributions made by fungi and lower plants to human health and pharmaceutical discovery.

Single-ingredient drugs

Until recently, plants were an important source for the discovery of novel pharmacologically active compounds, with many blockbuster drugs being derived directly or indirectly from plants [4–6]. During the twentieth century, the emphasis gradually shifted from extracting medicinal compounds from plants to making these compounds or their analogues synthetically. Natural products were widely viewed as templates for structure optimization programs designed to make perfect new drugs, referred to by industry as new chemical entities (NCEs). Despite the current preoccupation with synthetic chemistry as a vehicle to discover and manufacture drugs, the contribution of plants to disease treatment and prevention is still enormous. In the 1970s, 25% of all drugs dispensed in the USA contained compounds derived from flowering plants [7], with an even greater proportion of phytochemicals used as drugs worldwide [4]; 16% of drugs dispensed in the USA were derived

http://tibtech.trends.com 0167-7799/02/$ – see front matter PII: S0167-7799(02)02080-2

Table 1. Categories of botanical therapeutics

Therapeutic	Description	Example	Availability
Drugs (NCEs)	Mostly single active ingredient pharmaceuticals originating from plants	Vinblastine, taxol or aspirin	Rx or OTC
Botanical drugs	Clinically validated and standardized phytochemical mixtures	None in the USA, several in clinical trials	Rx or OTC
Dietary supplements/ nutraceuticals	A plant component with health benefits	Garlic or *Echinacea* extract	OTC
Functional/medicinal foods	A food engineered or supplemented to provide health benefits	Healthy canola oil, golden rice or edible vaccine	OTC, Rx or Grocers
Recombinant proteins	Pharmaceutical protein expressed and isolated from plants	None commercialized, several in clinical trials	Rx

Abbreviations: NCE, non-chemical entity; OTC, over the counter; Rx, prescription drugs.

from microbial and animal sources [4]. Even at the dawn of the twenty-first century, 11% of the 252 drugs considered as basic and essential by the World Health Organization were exclusively of flowering plant origin [8]. The greatest recent impact of plant-derived drugs was probably felt in the antitumor area, where taxol, vinblastine, vincristine and camptothecin have dramatically improved the effectiveness of chemotherapy against some of the deadliest cancers.

The most important pharmaceuticals still derived from plants – directly or as precursors – are listed in Table 2. Galantamine and camptothecin are the most recent additions to the plant-derived NCE pharmaceutical pipeline, although both were isolated a long time ago and have spent many years in development. For reasons of brevity, semisynthetic compounds such as apomorphine (derived by chemical derivatization of morphine and recently

Table 2. Some of the most economically important pharmaceuticals or their precursors derived from plants, and the projected 2002 worldwide sales for the structural groups of plant-derived drugs (partially adopted from [3,23])

Name	Type	Source	Therapeutic use
Alkaloids: Projected 2002 sales of US$4 045 million			
Atropine[a], hyoscyamine, scopolamine	Tropane alkaloid	*Solanaceous* spp.	Anticholinergic
Camptothecin[a]	Indol alkaloid	*Camptotheca acuminata* Decne	Antineoplastic
Capsaicin	Phenylalkyl-amine alkaloid	*Capsicum* spp.	Topical analgesic
Codeine, morphine	Opium alkaloid	*Papaver somniferum* L.	Analgesic, antitussive
Cocaine	Cocaine alkaloid	*Erythroxylum coca* Lamarck	Local anaesthetic
Colchicine	Isoquinoline alkaloid	*Colchicum autumnale* L.	Antigout
Emetine	Isoquinoline alkaloid	*Cephaelis ipecacuanha* (Brot.)A. Rich.	Antiamoebic
Galanthamine	Isoquinoline alkaloid	*Leucojum aestivum* L.	Cholinesterase inhibitor
Nicotine	Pyrrolidine alkaloid	*Nicotiana* spp.	Smoking cessation therapy
Physostigmine	Indole alkaloid	*Physostigma venenosum* Balfor	Cholinergic
Pilocarpine	Imidazole alkaloid	*Pilocarpus jaborandi* Holmes	Cholinergic
Quinine	Quinoline alkaloid	*Cinchona* spp.	Antimalarial
Quinidine	Quinoline alkaloid	*Cinchona* spp.	Cardiac depressant
Reserpine	Indole alkaloid	*Rauwolfia serpentina* L.	Antihypertensive, psychotropic
Tubocurarine	Bisbenzyl isoquinoline alkaloid	*Chondodendron tomentosum* Ruiz, *Strychnos toxifera* Bentham	Skeletal muscle relaxant
Vinblastine, vincristine	Bis-indole alkaloid	*Catharanthus roseus* L.	Antineoplastic
Yohimbine	Indole alkaloid	*Apocynaceae, Rubiaceae* spp.	Aphrodisiac
Terpenes and steroids: projected 2002 sales of US$12 400 million			
Artemisinin	Sesquiterpene lactone	*Artemisia annua* L.	Antimalarial
Diosgenin[a], hecogenin[a], stigmasterol[a]	Steroids	*Dioscorea* spp.	Oral contraceptives and hormonal drugs
Taxol and other taxoids[a]	Diterpenes	*Taxus brevifolia* Nutt.	Antineoplastic
Glycosides: projected 2002 sales of US$9230 million			
Digoxin, digitoxin	Steroidal glycosides	*Digitalis* spp.	Cardiotonic
Sennosides A and B	Hydroxy-anthracene glycosides	*Cassia angustifolia* Vahl.	Laxative
Others and mixtures: projected 2002 sales of US$5014 million			
Ipecac	Mixture of ipecac alkaloids and other components	*Cephaelis ipecacuanha* (Brot.) A. Rich.	Emetic
Podophyllotoxin[a]	Lignan	*Podophyllum peltatum* L.	Antineoplastic

[a]Most often used as precursors in chemical synthesis of final products.

approved for the treatment of erectile dysfunction) and salicylic acid (present in plants but made synthetically) are not listed in the table.

The enthusiasm for using plant extracts for the discovery of novel pharmaceutical leads has declined in the past decade, with many pharmaceutical companies closing or downsizing their natural products groups. Throughout human history plants were unchallenged as sources of new drug discovery but the recent competition from combinatorial chemistry [9,10] and computational drug design [11] has put an end to the dominance of natural products in drug discovery. It is even possible to suggest that, after thousands of years of bioprospecting for plant-derived pharmaceuticals, people have now identified the most useful, relatively abundant molecules, and that finding new ones would require sophisticated approaches that have not yet emerged. However, many experts believe that the majority of plant-derived natural products – possibly valued at billions of dollars – remain undiscovered or unexplored for their pharmacological activity [12,13].

About 250 000 living plant species contain a much greater diversity of bioactive compounds than any chemical library made by humans. Few researchers doubt that plants and other sources of natural products are superior sources of molecular diversity and novel molecular chemotypes, particularly in the areas where good synthetic leads do not exist [14]; and the notion that evolution has been selecting and perfecting diverse bioactive molecules for much longer than any pharmaceutical company cannot be ignored. This became one of the arguments for the historic United Nations Convention on Biological Diversity, which opened for signature in 1992.

So should we abandon plants as sources of NCE-based drugs, embrace them again or redesign strategies for discovering novel molecules from plants? The last approach makes most sense because, despite the remarkable progress made by chemistry, pharmacology, molecular biology, genome research and high-throughput screening, the NCE pipelines of pharmaceutical companies are at historically low levels [15]. Not only is the pharmaceutical industry finding it difficult to replace old products with new and more effective alternatives, it is also having problems developing new products, although many new molecular targets have been discovered recently. A 40% increase in the R&D spending in pharmaceutical research from 1996 to 2001 did not overcome this problem [15].

So why not plants? The lack of reproducibility of activity for >40% of plant extracts [5] is one of the major obstacles in using plants in pharmaceutical discovery, despite the great diversity of compounds they synthesize. The activities detected in screens often do not repeat when plants are resampled and re-extracted. Moreover, the biochemical profiles of plants harvested at different times and locations vary greatly. In addition, the currently popular high-throughput drug discovery format favors single compounds over mixtures and is not compatible with complex plant extracts in which valuable bioactive molecules are often obscured by pigments and poly-phenols that interfere with screens. Equally important is the lack of efficient, rapid strategies to isolate and characterize NCEs, particularly those present in trace amounts, making phytochemical discovery a complex, laborious task incompatible with short lead discovery times. It often takes 6 months to isolate and structurally characterize a natural product from a plant extract. This is roughly equivalent to the lifetime of a high-throughput screen for a new target and is a prohibitively long time in an ever-accelerating lead discovery race.

Can something be done to make plants a more user-friendly discovery source? Unquestionably, the development of novel technologies that allow rapid isolation and characterization of putative lead molecules and new screening methods more compatible with complex mixtures will be imperative if plants are to return to the mainstream of drug discovery efforts. These technologies should consider and possibly use the fact that the biological activity of plant extracts often results from additive or synergistic effects of its components. Another strategy is to exploit the qualitative and quantitative variations in the content of bioactive phytochemicals, which are currently considered major detriments in phytochemical NCE discovery. Different stresses, locations, climates, microenvironments and physical and chemical stimuli, often called elicitors, qualitatively and quantitatively alter the content of bioactive secondary metabolites. Enzymatic pathways leading to the synthesis of these phytochemicals are highly inducible [16]. This is particularly true for phytochemicals that are well documented for their pharmacological activity, such as alkaloids [17], phenylpropanoids [18] and terpenoids [19,20] whose levels often increase by two to three orders of magnitude following stress or elicitation [21,22]. Thus, elicitation-induced, reproducible increases in bioactive molecules, which might otherwise be undetected in screens, should significantly improve reliability and efficiency of plant extracts in drug discovery while at the same time preserving wild species and their habitats. Standardization, optimization and full control of growing conditions should guarantee a cost-effective and quality-controlled production of many plant-derived compounds.

Botanical drugs

The US Federal Food and Drug Administration (FDA) recently published guidance for standardized multifunctional and multicomponent plant extracts, referred to as botanical drugs, thus making it possible to market these products under the New Drug Application (NDA) Approval Process [29]. In response to the public demand for trustworthy and

Table 3. Botanical drugs and companies currently involved in their clinical development in North America and the UK

Company	Areas of clinical testing
Ancile Pharmaceuticals. San Diego, CA	Sleep, anxiety disorders
CV Technologies. Edmonton, Canada	Respiratory infection
GW Pharmaceuticals. Salisbury, UK	Cannabis-based prescription medicines
Oxford Natural Products. Oxford, UK	Dysmenorrhoea, hepatitis-C symptoms, cognitive decline
PhytoCeutica. New Haven, CT	Cancer, neurovascular disease
Phytomedics. Dayton, NJ	Autoimmune diseases, cancer
Phytopharm. Godmanchester, UK	Appetite suppressant, inflammatory bowel disease, Alzheimer's disease, cancer

effective alternatives to NCE pharmaceuticals, the agency proposed abbreviated preclinical and clinical testing protocols for botanical drugs derived from plants with a safe history of human use. This has enabled US industrial and academic scientists to become involved in botanical drug R&D efforts. Botanical drugs are fully accepted and widely prescribed in China, Japan, India and other Asian and African countries. In addition, some countries in Europe, such as Germany, allow physicians prescribe botanical drugs. Currently, no botanical drugs are sold in the USA, but a few are under clinical development (Table 3).

So why do we need botanical drugs? The NCE paradigm of the twentieth century attempts to treat complex diseases with a 'single golden molecular bullet'. The first flaw in this paradigm appeared relatively recently when problems of resistance to antimicrobial and anticancer drugs became apparent. The multifactorial nature of many complex diseases, such as diabetes, heart disease, cancer and psychiatric disorders, is also an important consideration. Most of these diseases cannot be ascribed to a single genetic or environmental change but arise from a combination of genetic, environmental or behavioral factors [24].

Unlike the Western NCE paradigm, traditional medicinal systems of the East always believed that complex diseases are best treated with complex combinations of botanical and non-botanical remedies that should be further adjusted to the individual patient and to the specific stage of the disease. This approach, best articulated and developed in traditional Chinese and Ayurvedic medicinal systems, emphasizes the mutually potentiating effect of different components of complex medicinal mixtures. Ostensibly, plants have adapted a similar strategy in their biochemical warfare with pathogens, which are the main causes of plant disease and death. Relying on a single antibiotic to stop pathogens would probably be evolutionarily suicidal for plants because a resistance would develop. Although poorly studied, the ability of plants to produce families of structurally and functionally diverse antimicrobial compounds that act together to prevent the development of resistance has been documented. For example, *Berberis fremontii* Torrey produces both antimicrobial berberine alkaloids and inhibitors of a bacterial multidrug-resistant pump that strongly potentiate the activity of berberines [25]. Interaction between different molecular components can be also required for an optimal therapeutic effect of plant extracts. The root extract of a *Tripterygium wilfordii* Hook F has been used historically as a traditional Chinese medicine to treat rheumatoid arthritis, an observation recently supported by a Phase I/II double-blind, placebo-controlled trial in the USA [26,27]. Although the main active ingredient of this extract, triptolide, has been identified, it was shown to be toxic unless given as a part of the root extract, suggesting that other unidentified extract components increase its safety and, possibly, efficacy [28].

The future of botanical drugs in the USA depends on two factors: sustaining a favorable regulatory environment and developing technologies for the efficient discovery, development and manufacture of botanical drugs. At present, a majority of botanical drugs under development are derived from ethnobotanical sources and traditional medicinal uses. Common strategies involve upgrading well-known botanical dietary supplements, such as ginseng, to an ethical drug status through the NDA process. The difficulties in working with complex extracts and the relatively small size of companies developing botanical drugs do not encourage R&D spending on new botanical drugs.

In addition to the creative and innovative technologies needed for new botanical drug discovery, manufacturing botanical drugs presents a challenge not encountered by the modern pharmaceutical industry. To be FDA compliant, the process should involve 'seed-to-pill' and 'batch-to-batch' standardization of complex phytochemical mixtures, a challenge not encountered by chemical synthesis or single compound extraction processes. As stated above, environmental and genetic factors might dramatically affect the biochemical compositions of plant extracts. Therefore, production of botanical drugs will require genetically uniformed monocultures of source plants grown in fully standardized conditions to assure biochemical consistency and to optimize safety and efficacy in every crop. It is unlikely that field-grown plants can meet the quality standards for botanical drugs. Fully controlled greenhouse-based cultivation systems developed for high quality year-round vegetable

Table 4. Common botanical dietary supplements sold in the USA

							
1[a]	*Echinacea purpurea L. angustifolia*, DC. and *pallida* Nutt.	*Panax ginseng,* L.A. Mey (ginseng)	*Serenoa repens* (W. Bartam) Small (saw palmetto)	*Ginkgo biloba* L.	*Hypericum perforatum* L. (St John's wort)	*Valeriana officinalis* L. (valerian)	*Allium sativum* L. (garlic)
2	Shoots, roots	Roots	Fruit	Leaves	Shoots	Roots	Bulb, oil
3	Respiratory infections, immunostimulant	Fatigue and stress, high cholesterol, diabetes, gastro-intestinal disorders	Benign prostatic hyperplasia (BPH), inflamations, impotence	Dementia, cognitive decline, mental fatigue	Mild and moderate depression, epilepsy	Sleep improvements, anxiety, hypertension	Cancer, high cholesterol, diabetes, arteriosclerosis, hypertension, respiratory infections
4	Polysaccharides, alkylamides, chlorogenic acid, caffeic acid derivatives (echinacosides)	Ginsenosides, panaxans, sequiterpenes	Steroids (β-sitosterols), flavonoids (isoquercitin, kaempherol, rhoifolin)	Terpene trilactones (ginkgolides), flavonol glycosides	Hyperforin, adhyperforin, hypericin, pseudohypericin, flavonol glycosides	Valeric acids, valepotriates	Alliins, allicin, ajoens, oligosulfides
							
1	*Hydrastis canadensis* L. (goldenseal)	*Matricaria chamomilla* L. (German chamomile)	*Silybum marianum gaertn.* (milk thistle)	*Trigonella foenum-graecum* L. (fenugreek)	*Tanacetum - parthenium* Schultz-Bip. (feverfew)	*Ephedra sinica* Stapf. (Ephedra, Ma Huang)	*Cimicifuga racemosa* Nutt. (black cohosh)
2	Rhizome, roots	Flowering herb	Ripe seeds	Ripe seeds	Herb	Stems	Root
3	Diarrhea, respiratory and Gastrointestinal infections, Constipation	Intestinal disorders, wound healing, inflammations, anxiety	Liver disorders, lactation problems	Diabetes, loss of appetite, skin inflammation	Migraines, inflammation	Stimulant, obesity, asthma, congestion, fluid retention	Premenstrual symptoms, dysmenorrhea, menopausal symptoms
4	Hydrastine, berberine, canadine	Bisabolols, apigenins, luteolin, coumarins, proazulenes	Silymarins, flavonoids (apigenin, chrysoeriol, quercetin, taxifolin)	Mucilages, steroid saponins (trigofoenosides, dosgenin, foenugraecin), trigonelline	Sesquiterpene lactones (parthenolides, costunolide, reynosin, canin, artecanin)	Protoalkaloids (ephedrine, pseudo-ephedrine) cannins, saponins	Triterpene glycosides (cimifugaside, 27-deoxyactein, actein), isoflavones

[a]1,name; 2, parts used; 3, common indication/use; 4, putative active ingredients

production are much more suitable for the future production of botanical drugs.

Botanical dietary supplements

Botanical dietary supplements – also called botanical nutraceuticals or herbals (Table 4) – can be best defined as plant-derived materials with medical benefits aimed at disease prevention or treatment that go beyond satisfying basic nutritional requirements. This definition is somewhat vague because it reflects the current regulatory policy in the USA and is not a scientific definition. (Botanical drugs meet the same definition but are excluded from it because they go through different regulatory channels within the USA. FDA.) The use of botanical supplements in the USA increased dramatically after

passage of the Dietary Supplement and Health Education Act of 1994 (DSHEA). Under DSHEA, botanical supplements can be marketed with few regulatory impediments, providing that disease prevention, curing or detection claims are not made. Instead, so-called structure–function claims on products can relate to enhancing or maintaining normal physiological functions of human body. DSHEA does not require the manufacturers of supplements to verify that they are safe and effective. To block the sale of a dietary supplement, the FDA must go to court with the evidence and obtain a court injunction against the manufacturer. Botanical cosmetic supplements or cosmeceuticals (plant extracts with alleged beneficial properties, such as chamomile or aloe extracts) added to cosmetic and personal hygiene products have similarly relaxed regulatory supervision as a tradeoff for the absence of disease cure or prevention claims. Cosmeceuticals represent a relatively small segment of the total botanical supplement market.

Although the general public often considers botanical supplements natural and safe alternatives to conventional synthetic pharmaceuticals, there is relatively little scientific evidence behind this belief. In 1999 the global market for herbal supplements exceeded US$15 billion, with a US$7 billion market in Europe, US$2.4 billion in Japan, US$2.7 in the rest of Asia and US$3 billion in North America [30]. Most of the published studies suggest that the use of herbal medicine is widespread, with as many as three in ten Americans using botanical remedies in a given year. The demand for dietary supplements is driven by a variety of factors that include an aging population with substantial disposable income, a growing trend to self-medicate, mistrust in the conventional medical establishment, and the perception that natural is healthy and that plant products are safe. These trends suggest the growing demand for supplements and functional foods, providing that quality standards and efficacious new products are introduced. The growth of dietary supplements will be hastened by the dramatic advances in human genetics that have culminated in the recent sequencing of the human genome [31], which will soon allow the reliable assessment of the risk of life-threatening diseases long before their onset. The medical community is not fully prepared for this challenge because it uses monitoring as the main approach to high-risk groups. Botanical supplements and functional foods might hold the promise for a proactive preventive strategy that follows genetic identification of a medical risk.

Botanical supplements are frequently and rightly criticized for poorly proven efficacy and safety, lack of standardization and quality standards [32,33], and potential interactions with prescribed drugs [34]. Nevertheless, data from most of the randomized clinical trials performed with the top selling botanical supplements suggest that most are at least mildly effective against a specific indication [35,36]. Unlike ethical drugs, the active ingredients of very few botanical supplements have been fully characterized, despite significant efforts by many researchers. This difficulty in isolating active ingredients suggests that the therapeutic effects of many botanical supplements are the result of the combined effects of many compounds, which are often lost during standard activity guided fractionation, which separates extracts into their individual molecular components.

So what can be done to improve the efficacy, image and science behind botanical dietary supplements? Despite its size, the botanical food supplement industry is, to a large extent, marketing-driven, with almost no sustained R&D efforts directed towards creating credible product pipelines, quality control measures and discovery platforms. Instead, traditional medicinal plants are being repackaged, remixed and remarketed. Creating the environment that rewards better efficacy, quality and safety standards for dietary supplements is a major regulatory challenge that needs to be met to sustain the growth of the botanical supplement industry. Allowing more specific claims and some marketing exclusivity in exchange for more thorough preclinical and clinical data will be helpful, as will increasing governmental and private research funding of the botanical supplement R&D. Some of this funding could be directed through the recently created National Center for Complementary and Alternative Medicine (NCCAM) and used for studying the effects of complex phytochemical mixtures on human health and for developing innovative approaches to discovering and developing botanical food supplements.

Functional and medicinal foods

This is probably the best-known and best-reviewed area of botanical therapeutics because of its connection to the mainstream of plant biotechnology and molecular biology. As with other botanical therapeutics, the precise definition of functional foods is vague. This review considers only those crops engineered or selected to deliver certain health benefits above and beyond those normally present. Botanical functional foods produced by fortification, such as orange juice with calcium, or advertised for their innate health benefits, such as cereals with high fiber, will not be discussed here.

In the area of engineered functional foods, much attention was given to the development of golden rice, healthy plant oils from modified oil crops, edible vaccines and plants with increased levels of essential vitamins and nutrients. Golden rice was engineered with two plant genes from *Narcissus pseudonarcissus* and one bacterial gene from *Erwinia uredovora* to synthesize β-carotene, a precursor of vitamin A, at quantities sufficient to reduce vitamin-A deficiency, which affects more than 124 million children

worldwide [37,38]. Plant oils have many historical uses in food and industrial applications. The current health-related goals of plant oilseed engineering is to increase the content of healthy fatty acids and reduce unhealthy fatty acids in the four most important oilseed crops, which in descending order are, soybean, oil palm, rapeseed and sunflower [39]. For example, genetic engineering was successful in reducing levels of *trans*-unsaturated fatty acids and the ratio between omega-6 and omega-3 unsaturated fatty acids in some vegetable oils [40], thus reducing the risk of heart disease [41]. Proposed in the early 1990s, the idea of engineering tomatoes, bananas or potatoes to express a vaccine generated a lot of excitement as a simple way to distribute vaccines to developing countries [42,43]. Plant-produced oral vaccines were recently shown to be highly effective as boosters that have increased the immunity of mice to measles [44] and humans to hepatitis B [45]. Concerns about safety and correct dosing associated with direct consumption of vaccine-producing crops [46] might ultimately result in a more traditional approach, whereby plants are used to biomanufacture edible vaccines that are at least partially purified and delivered in a more conventional and properly dosed form such as a solution, powder or pill.

Other recent advances in functional plant foods include increasing vitamin E content in plants following initial demonstrations in *Arabidopsis* [47]; selecting high lycopene or vitamin C tomatoes [48] (although the link between dietary lycopene and cancer prevention is weak [49]); metabolic engineering of legumes [50] and tomatoes [51] for high content of bioflavonoids, known for their antioxidant, anticancer and estrogenic properties; and possible uses of thioredoxin to decrease allergenicity of foods [52].

Functional foods selected, bred and advertised for high content of therapeutically active molecules such as vitamins (A, C and E), isoflavones, antioxidants, folic acid and pigments are already making their cautious debut on supermarket shelves, leaving the determination of their true medical benefit to the consumer. However, none of the genetically engineered, plant-derived functional foods on the market today are advertised and labeled as such. In the USA, the Department of Agriculture and the FDA will most probably regulate genetically engineered functional foods, requiring thorough clinical validation, safety testing and strict quality control. A major constraint in engineering secondary metabolites in functional foods is the scarce information about their biosynthetic genes and pathways. Cross-species integration of proteomics and metabolomics with this genetic information will allow a better understanding and utilization of metabolic networks that enhance medicinal properties of foods. In parallel, better technologies for characterizing pharmacologically active compounds in foods have to be developed. Providing that scientific, regulatory and public acceptance issues are solved, the future of plant-based functional foods seems bright and, as a result, grocery and drugs stores might eventually look more alike. Functional foods with clear and direct health benefits for the consumer should lead to greater acceptance of crop genetic engineering, now almost exclusively, and controversially, used for crop protection.

Recombinant proteins

Recombinant proteins, such as antibodies, vaccines, regulatory proteins and enzymes, represent one of the most rapidly growing segments of the pharmaceutical industry. With dozens of proteins in clinical development today there is a substantial shortage of industrial capacity to manufacture future recombinant drugs [53]. During the past decade, plants have emerged as promising biopharming systems for commercial production of pharmaceutical proteins (Table 5). Tobacco was the first plant to express a recombinant antibody in 1988 [54], with further confirmation in 1989 [55]. Advantages offered by plants include low cost of cultivation and high biomass production, relatively fast 'gene to protein' time, low capital and operating costs, excellent scalability, eucaryotic post-translational modifications (i.e. glycosylation, folding and multimeric assembly), low risk of human pathogens and endotoxins and a relatively high protein yield. These advantages are potentiated by the ease of plant transformation through particle bombardment, electroporation, *Agrobacterium*-mediated transformation, or infection with modified viral vectors [56]. The relative advantages of the most common recombinant systems for pharmaceutical protein production are summarized in Figure 1.

Plants are generally considered to be low-cost, safe and relatively fast alternatives to many existing manufacturing systems, particularly when large quantities of multimeric recombinant proteins (i.e. antibodies) are required. Most major groups of human pharmaceutical proteins have been produced successfully in a diverse variety of crops and model systems (e.g. maize, rice, wheat, soybean, tomato, potato, mustard, oilseed rape, turnip, alfalfa, banana, tobacco and *Arabidopsis*) using stable nuclear and plastid transformations, as well as transient expression systems such as viruses (Fig. 1) [57,58].

Plants can successfully perform the post-translational modifications required by the majority of pharmaceutical proteins under development. Heterologous proteins requiring these modifications are usually retained in the endoplasmic reticulum (ER) using the C-terminal ER retention sequence KDEL or targeted into the protein secretion-modification pathway that delivers the recombinant proteins into the intercellular spaces (apoplast) via the ER and Golgi apparatus. Both strategies significantly enhance protein expression [59,60]. The highest yield of recombinant protein in plants is

Table 5. Examples of plant-based expression systems used for pharmaceutical protein production

System		Protein	Expression	Company[a]
Stable nuclear transformation systems				
Whole plant (cytosolic)		HbsAg, vaccine [65]	0.007% TSP	AltaGen Bioscience Inc. (potato)
		collagen [66]	1 mg/g DW	CropTech Corp. (tobacco)
				Medicago Inc. (alfalfa)
				Meristem Therapeutics (tobacco)
Cellular compartment	Vacuole	scFv, hepatitis B [67]	0.032% TSP	PlantGenix Inc. (not reported)
		sIgA/G [68]	not reported	
	ER	scFv, cutinase [69]	1% TSP	Novoplant GmbH (tobacco)
		scFv, T84.66 [70]	29 μg/g FW	
		scFv, ABA [71]	6.8% TSP	
	Apoplast	IgG1 [55]	1.3% TSP	Epicyte Pharmaceutical Inc. (tobacco)
		IgA/G [72]	500 μg/g FW	
		IgG1, Fab [73]	13% ISP	
Tissue-specificity	Seed	avidin [74]	6% TSP	ProdiGene Inc. (corn)
		hirudin [61]	1% FW	SemBioSys Genetics Inc. (canola)
				Applied Phytologics Inc. (rice)
				Epicyte Pharmaceutical Inc. (corn)
				IPT, Monsanto (corn)
				Meristem Therapeutics (rape)
	Tuber	scFv, oxalozone [75]	2% TSP	Meristem Therapeutics (potato)
	Root	IgM, RKN [76]	0.003% TSP	
	Fruit	RSV-F protein [77]	not reported	
Exudate		human SEAP [62]	20 μg/g DW/day	Phytomedics Inc. (tobacco roots)
		human SEAP [63]	2.8% TEP	Phytomedics Inc. (tobacco leaves)
				Biolex Inc. (duckweed)
Stable plastid transformation system				
Chloroplast		somatotropin [78]	7% TSP	
Transient transformation system				
Viral		α-trichosanthin [79]	2% TSP	Large Scale Biology Corp. (tobacco)

Abbreviations: DW, dry weight; ER, endoplasmic reticulum; FW, fresh weight; ISP, intercellular soluble protein; TEP, total exuded protein; TSP, total soluble protein.
[a]Companies sharing a row with a protein and reference are sources of this information.

achieved by chloroplast expression or possibly by transient viral expression. However, plastid expression suffers from the lack of glycosylation and correct folding of multimeric proteins, whereas viral expression is problematic for complex proteins and could introduce safety hazards. Seeds (corn) and tubers (potatoes) provide excellent long-term storage compartments for recombinant proteins before purification, but have lower protein yield.

Downstream protein purification is often as expensive as the biomanufacturing and should never be overlooked in the total 'cost of goods' equation. At least two approaches have been used successfully to lower the cost of downstream purification of plant-produced proteins: oleosin-fusion technology for heterologous proteins produced in oilseeds [61], and rhizo- and phyllo-secretion platforms based on continuous, non-destructive recovery of a target protein from plant exudates [62,63]. The latter also offers the advantage of continuous protein production that integrates the biosynthetic potential of a plant over its lifetime and might lead to higher protein yields than single harvest and extraction methods.

With no plant-produced protein drug on the market today, and only a few in clinical trials, one can only hypothesize that plants will provide substantial benefits and savings for protein manufacturing over the alternative systems. Nevertheless, the arguments favoring plants are appealing and a growing number of companies are trying to commercialize recombinant protein manufacturing in plants, with most concentrating on pharmacological applications (Fig. 1). It is likely that no single ideal system will ever emerge for the manufacturing of every recombinant protein, each system having distinct advantages and disadvantages [64]. Empirical analysis of several recombinant production systems might be required before the most efficient system is identified.

Conclusions

Plants are arguably poised for a comeback as sources of human health products. The hopes for this comeback are rooted in the unique and newly appreciated properties of phytochemicals *vis-à-vis* conventional NCE-based pharmaceuticals and are based on the: (1) enormous propensity of plants to synthesize mixtures of structurally diverse bioactive compounds with multiple and mutually potentiating therapeutic effects; (2) low-cost and highly scalable protein and secondary metabolite biomanufacturing capacity of plants; (3) diminishing return of the single NCE approach to drug discovery and disease treatment and prevention; (4) cost limitation on the

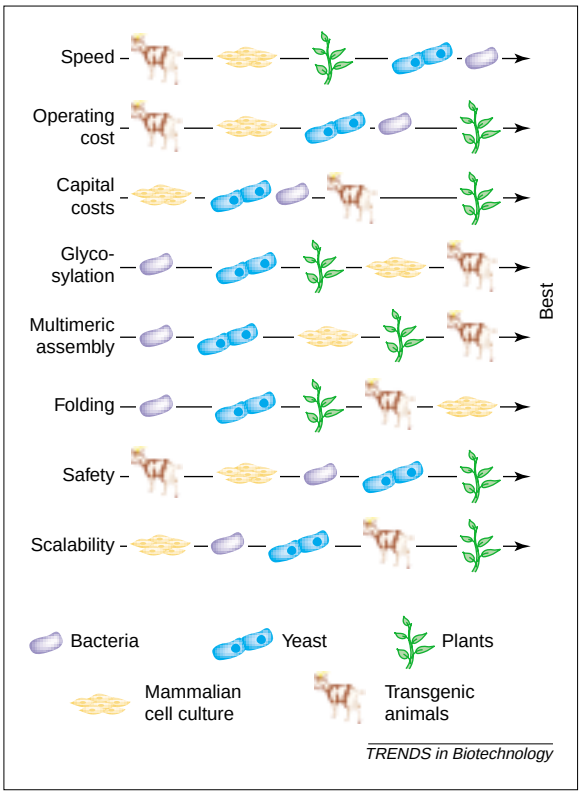

Fig. 1. Comparison of various commercial recombinant protein expression systems

chemical synthesis of complex bioactive molecules; and (5) perception that because of the history of human use and co-evolution of plants and humans, phytochemicals provide a safer and more holistic approach to disease treatment and prevention. Although the above properties have been known for a long time, the ability to better exploit the uniqueness of plant therapeutics was acquired only recently as a result of the dramatic advances in metabolic engineering, biochemical genomics, chemical separation, molecular characterization and pharmaceutical screening. A challenge for phytochemical-based botanical therapeutics is to integrate the ability to identify and genetically manipulate complex biosynthetic pathways in plants with better characterization of genetic targets for the prevention and treatment of complex diseases. Similarly, an important challenge is the development of discovery, validation and manufacturing technologies that are compatible with multifunctional phytochemical mixtures.

The discovery, development and manufacturing of botanical therapeutics, either isolated from plants or delivered as food constituents, is likely to be a major area of plant biotechnology expansion in the twenty-first century. It is likely that botanical drugs, dietary supplements and recombinant protein production will have a major role in this expansion. However, plants are unlikely to recapture their previously dominant role as sources for NCE discovery. It is harder to make predictions for the area of functional/medicinal foods. Consumer and regulatory acceptance of drugs delivered in foods will be a decisive factor in determining the future of these products. Whereas the increasingly holistic approach to treatment and prevention will continue to emphasize food, consumer food choices might still be determined by individual taste predilection, habits and cultural heritage rather than by health benefits. Thus, pills taken with 'not-so-healthy' meals might continue to be a preferred vehicle for delivering disease treatment and prevention.

The growth of botanical therapeutics might add more value to world agriculture than the more conventional application of plant biotechnology for yield enhancement. Plants are by far the most abundant and cost-effective renewable resource uniquely adapted to complex biochemical synthesis. The increasing cost of energy and chemical raw materials, combined with the environmental concerns associated with conventional pharmaceutical manufacturing, will make plants even more compatible in the future. Crops that will benefit the most include tobacco and corn as the major recombinant protein manufacturing crops and many minor crops and medicinal plants that will become sources of future botanical therapeutics. Farmers that adapt to growing crops for health, rather than calories, will profit from greater margins and higher values enjoyed by the health industry and, as a result, the planet could become greener and more healthy.

Acknowledgements
We thank Jim White, Barbara Halpern, Ellen Shlossberg and Rainer Becker for their help in preparing this manuscript. The Spanish Secretariat of Education and Universities provided funding for Diego A. Moreno.

References

1 Swerdlow, J. (2000) *Nature's Medicine. Plants That Heal*, National Geographic Society
2 Pierpoint, W.S. (1994) Salicylic acid and its derivatives in plants: Medicines, metabolites and messenger molecules. *Adv. Bot. Res.* 20, 163–235
3 Business Communications Company, Inc. (1998) *Plant-Derived Drugs: Products, Technologies and Applications*. Study RB-121
4 Farnsworth, N.R. (1988) Screening plants for new medicines. In *Biodiversity* (Wilson, E.O., ed.), pp. 83–97, National Academy Press
5 Cordell, G.A. (2000) Biodiversity and drug discovery. A symbiotic relationship. *Phytochemistry* 55, 463–480
6 Newman, D.J. *et al.* (2000) The influence of natural products upon drug discovery. *Nat. Prod. Rep.* 17, 215–234
7 Farnsworth, N.R. and Morris, R.W. (1976) Higher plants – the sleeping giant of drug development. *Am. J. Pharm. Educ.* 148, 46–52
8 Rates, S.M.K. (2001) Plants as sources of drugs. *Toxicon* 39, 603–613
9 Adang, A.E. and Hermkens, P.H. (2001) The contribution of combinatorial approaches to lead generation: An interim analysis. *Curr. Med. Chem.* 8, 985–998
10 Schreiber, S.L. (2000) Target-oriented and diversity-oriented organic synthesis in drug discovery. *Science* 287, 1964–1969
11 Clark, D.E. and Pickett, S.D. (2000) Computational methods for the prediction of drug-likeness. *Drug Discov. Today* 5, 49–58
12 Gentry, A.H. (1993) Tropical forest biodiversity and the potential for new medicinal plants. *ACS Symposium Series Washington, DC American Chemical Society* 534, 13–20
13 Mendelson, R. and Balick, M.J. (1995) The value of undiscovered pharmaceuticals in tropical forests. *Econ. Bot.* 49, 223–228
14 Shu, Y-Z. (1998) Recent natural products based drug development: A pharmaceutical industry perspective. *J. Nat. Prod.* 61, 1053–1071
15 Bolten, B.M. and De Gregorio, T. (2002) Trends in development cycle. *Nat. Rev.* 1, 335–336
16 Ebel, J. and Cosio, E.G. (1994) Elicitors of plant defense responses. *Int. Rev. Cytol.* 148, 1–36
17 Facchini, P.J. (2001) Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation, and metabolic engineering applications. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52, 29–66

18 Dixon, R.A. and Paiva, N.L. (1995) Stress-induced phenylpropanoid metabolism. *Plant Cell* 7, 1085–1097

19 Trapp, S. and Croteau, R. (2001) Defensive resin biosynthesis in conifers. *Annu. Rev. Plant Physiol. Plant Mol. Biol* 52, 689–724

20 Turlings, T.C.J. and Tumlinson, J.H. (1992) Systemic release of chemical signals by herbivore-injured corn. *Proc. Natl. Acad. Sci. U. S. A.* 89, 8399–8402

21 Darvill, A.G. and Albersheim, P. (1984) Phytoalexins and their elicitors – a defense against microbial infection in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 35, 243–275

22 Dixon, R.A. (1986) The phytoalexin response: Elicitation, signaling, and control of host gene expression. *Biol. Rev.* 61, 239–291

23 Balandrin, M.F. *et al.* (1993) Plant-derived natural products in drug discovery and development: An overview. *Acs Symposium Series Washington, D.C.: American Chemical Society* 534, 2–12

24 Kibertis, P. and Roberts, L. (2002) It's not just the genes. *Science* 296, 685

25 Hsiang, P. and Lewis, K. (2000) Synergy in medicinal plants: anitimicrobials potentiated by multidrug pump inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1433–1437

26 Tao, X. *et al.* (2001) A Phase I study of ethyl acetate extract of the Chinese antirheumatic herb *Tripterygium wilfordii* Hook F in rheumatoid arthritis. *J. Rheumatol.* 28, 2160–2167

27 Tao, X. *et al.* (2002) Benefit of an extract of *Tripterygium wilfordii* Hook F in patients with rheumatoid arthritis: A double-blind, placebo-controlled study. *Arthritis Rheum.* 46, 1735–1743

28 Su, D. *et al.* (1990) Comparative clinical study of rheumatoid arthritis treated by triptolide and an ethyl acetate extract of *Tripterygium wilfordii*. *Chin. J. Mod. Dev. Trad. Med.* 10, 144–146

29 U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (2000) *Guidance for Industry Botanical Drug Products*. Online: http://www.fda.gov/cder/guidance/1221dft.htm#P131_3293

30 Glaser, V. (1999) Billion-dollar market blossoms as botanicals take root. *Nat. Biotechnol.* 17, 17–18

31 Venter, J.C. *et al.* (2001) The sequence of human genome. *Science* 291, 1304–1351

32 Osowski, S. *et al.* (2000) Pharmaceutical comparability of different therapeutic Echinacea preparations. *Res. Compl. Nat. Classical Med.* 7, 294–300

33 Kressmann, S. *et al.* (2002) Pharmaceutical quality of different *Ginkgo biloba* brands. *J. Pharm. Pharmacol.* 54, 661–669

34 Izzo, A.A. and Ernst, E. (2001) Interactions between herbal medicines and prescribed drugs: A systematic review. *Drugs* 61, 2163–2175

35 Ernst, E. (2002) The risk-benefit profile of commonly used herbal therapies: Ginkgo, St. John's Wort, Ginseng, Echinacea, Saw Palmetto, and Kava. *Ann. Intern. Med.* 136, 42–53

36 Kroll, D.J. (2001) Concerns and needs for research in herbal supplement pharmacotherapy and safety. *J. Herbal Pharmacother.* 1, 3–23

37 Potrykus, I. (2001) Golden rice and beyond. *Plant Physiol.* 125, 1157–1161

38 Ye, X. *et al.* (2000) Engineering provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287, 303–305

39 Daniell, H. *et al.* (2001) Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Plant Sci.* 6, 219–226

40 Walmsley, A.M. and Arntzen, C.J. (2000) Plants for delivery of edible vaccines. *Curr. Opin. Biotechnol.* 11, 126–129

41 Bonetta, L. (2002) Edible vaccines: Not quite ready for prime time. *Nat. Med.* 8, 95

42 Thelen, J.J. and Ohlrogge, J.B. (2002) Metabolic engineering of fatty acid biosynthesis in plants. *Metab. Eng.* 4, 12–21

43 Liu, K. (2001) Modifying soybean oil through plant breeding and genetic engineering. In *Proceedings of the World Conference on Oilseed Processing Utilization, Cancun, Mexico, 12–17 November 2000*, pp. 84–89

44 Webster, D.E. *et al.* (2002) Successful boosting of a DNA measles immunization with an oral plant-derived measles virus vaccine. *J. Virol.* 76, 7910–7912

45 Kong, Q. *et al.* (2002) Oral immunization with hepatitis B surface antigen expressed in transgenic plants. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11539–11544

46 Hu, F.B. *et al.* (2001) Types of dietary fat and risk of coronary heart disease: A critical review. *J. Am. Coll. Nutr.* 20, 5–19

47 Shintani, D. and DellaPenna, D. (1998) Elevating the vitamin E content of plants through metabolic engineering. *Science* 282, 2098–2100

48 Frusciante, L. *et al.* (2000) Evaluation and use of plant biodiversity for food and pharmaceuticals. *Fitoterapia* 71(Suppl. 1), S66–S72

49 Barber, N.J. and Barber, J. (2002) Lycopene and prostate cancer. *Prostate Cancer Prostatic Dis.* 5, 6–12

50 Forkmann, G. and Martens, S. (2001) Metabolic engineering and applications of flavonoids. *Curr. Opin. Biotechnol.* 12, 155–160

51 Muir, S.R. *et al.* (2001) Overexpression of petunia chalcone isomerase in tomato results in fruits containing increased levels of flavonols. *Nat. Biotechnol.* 19, 470–474

52 Buchanan, B.B. *et al.* (1997) Thioredoxin-linked mitigation of allergic responses to wheat. *Proc. Natl. Acad. Sci. U. S. A.* 94, 5372–5377

53 Garber, K. (2001) Biotech industry faces new bottleneck. *Nat. Biotechnol.* 19, 184–185

54 During, K. (1988) Wundinduzierbare Expression und Sekretion von T4 Lysozym und monoklonalen Antikorpern in *Nicotiana tabacum*. Doctoral Dissertation, University of Koln, Germany

55 Hiatt, A. *et al.* (1989) Production of antibodies in transgenic plants. *Nature* 342, 76–78

56 Fischer, R. and Emans, N. (2000) Molecular pharming of pharmaceutical proteins. *Transgenic Res.* 9, 279–299

57 Giddings, G. *et al.* (2000) Transgenic plants as factories for biopharmaceuticals. *Nat. Biotechnol.* 18, 1151–1155

58 Daniell, H. (2001) Medical molecular pharming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Plant Biol.* 6, 219–226

59 Conrad, U. and Fiedler, U. (1998) Compartment-specific accumulation of recombinant immunoglobulins in plant cells: An essential tool for antibody production and immunomodulation of physiological functions and pathogen activity. *Plant Mol. Biol.* 38, 101–109

60 Firek, S. *et al.* (1993) Secretion of a functional single-chain Fv protein in transgenic tobacco plants and cell suspension cultures. *Plant Mol. Biol.* 23, 861–870

61 Parmenter, D.L. *et al.* (1995) Production of biologically active hirudin in plant seeds using oleosin partitioning. *Plant Mol. Biol.* 29, 1167–1180

62 Borisjuk, N.V. *et al.* (1999) Production of recombinant proteins in plant root exudates. *Nat. Biotechnol.* 17, 466–469

63 Komarnytsky, S. *et al.* (2000) Production of recombinant proteins in tobacco guttation fluid. *Plant Physiol.* 124, 927–933

64 Hodgson, J. (1993) Expression systems: A user's guide. Emphasis has shifted from the vector construct to the host organism. *Biotechnology* 11, 887–893

65 Mason, H.S. *et al.* (1992) Expression of hepatitis B surface antigen in transgenic plants. *Proc. Natl. Acad. Sci. U. S. A.* 89, 11745–11749

66 Ruggiero, F. *et al.* (2000) Triple helix assembly and processing of human collagen produced in transgenic tobacco plants. *FEBS Lett.* 469, 132–136

67 Ramirez, N. *et al.* (2002) Expression of a single-chain Fv antibody fragment specific for the hepatitis B surface antigen in transgenic tobacco plants. *Transgenic Res.* 11, 61–64

68 Frigerio, L. *et al.* (2000) Assembly, secretion, and vacuolar delivery of a hybrid immunoglobulin in plants. *Plant Physiol.* 123, 1483–1494

69 Schouten, A. *et al.* (1996) The C-terminal KDEL sequence increases the expression level of a single-chain antibody designed to be targeted to both the cytosol and the secretory pathway in transgenic tobacco. *Plant Mol. Biol.* 30, 781–793

70 Torres, E. *et al.* (1999) Rice cell culture as an alternative production system for functional diagnostic and therapeutic antibodies. *Transgenic Res.* 8, 441–449

71 Fiedler, U. *et al.* (1997) Optimization of scFv antibody production in transgenic plants. *Immunotechnology* 3, 205–216

72 Ma, J.K. *et al.* (1995) Generation and assembly of secretory antibodies in plants. *Science* 268, 716–719

73 De Wilde, C. *et al.* (1996) Intact antigen-binding MAK33 antibody and Fab fragment accumulate in intercellular spacers of *Arabidopsis thaliana*. *Plant Sci.* 114, 233–241

74 Hood, E.E. *et al.* (1997) Commercial production of avidin from transgenic maize: characterization of transformant, production, processing, extraction and purification. *Mol. Breed.* 3, 291–306

75 Artsaenko, O. *et al.* (1998) Potato tubers as a biofactory for recombinant antibodies. *Mol. Breed.* 4, 313–319

76 Baum, T.J. *et al.* (1996) Expression in tobacco of a functional monoclonal antibody specific to stylet secretions of the root-knot nematode. *Mol. Plant Microbe Interact.* 9, 382–387

77 Sandhu, J.S. *et al.* (2000) Oral immunization of mice with transgenic tomato fruit expressing respiratory syncytial virus-F protein induces a systemic immune response. *Transgenic Res.* 9, 127–135

78 Staub, J.M. *et al.* (2000) High-yield production of a human therapeutic protein in tobacco chloroplasts. *Nat. Biotechnol.* 18, 333–338

79 Kumagai, M.H. *et al.* (1993) Rapid, high-level expression of biologically active alpha-trichosanthin in transfected plants by an RNA viral vector. *Proc. Natl. Acad. U. S. A.* 90, 427–430