Review TRENDS in Biotechnology Vol.xx No.xx Monthxxxx

Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology

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Consumption of herbal medicines is widespread and increasing. Harvesting from the wild, the main source of raw material, is causing loss of genetic diversity and habitat destruction. Domestic cultivation is a viable alternative and offers the opportunity to overcome the problems that are inherent in herbal extracts: misidentification, genetic and phenotypic variability, extract variability and instability, toxic components and contaminants. The use of controlled environments can overcome cultivation difficulties and could be a means to manipulate phenotypic variation in bioactive compounds and toxins. Conventional plant-breeding methods can improve both agronomic and medicinal traits, and molecular marker assisted selection will be used increasingly. There has been significant progress in the use of tissue culture and genetic transformation to alter pathways for the biosynthesis of target metabolites. Obstacles to bringing medicinal plants into successful commercial cultivation include the difficulty of predicting which extracts will remain marketable and the likely market preference for what is seen as naturally sourced extracts.

The growing pressures on wild medicinal plants

The World Health Organization has estimated that more than 80% of the world's population in developing countries depends primarily on herbal medicine for basic healthcare needs [1]. The use of herbal medicines in developed countries is also growing and 25% of the UK population takes herbal medicines regularly [1]. Approximately two-thirds of the 50 000 different medicinal plant species in use are collected from the wild [2] and, in Europe, only 10% of medicinal species used commercially are cultivated [1]. There is growing concern about diminishing populations, loss of genetic diversity, local extinctions and habitat degradation. Well-known species threatened by wild harvesting include *Arcostaphylos uva-ursa* (bearberry), *Piper methysticum* (kava), and *Glycyrrhiza glabra* (liquorice) [1]. Between 4000 and 10 000 medicinal species might now be endangered [2].

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The option of domestic cultivation

Although adequate protection of some species can be achieved through increased regulation and the introduction of sustainable wild harvesting methods, a more viable long-term alternative is to increase domestic cultivation of medicinal plants. Cultivation also opens up the possibility of using biotechnology to solve problems that are inherent in the production of herbal medicines. These include species misidentification, genetic and phenotypic variability, variability and instability of extracts, toxic components and contaminants. Cultivation offers the opportunity to optimize yield and achieve a uniform, high quality product. The prospective cultivator of medicinal plants must, however, make the difficult decision of which particular species to grow in what is a rapidly shifting, and fashion-prone, market [3]. The high-selling species with a large market share, such as *Ginkgo biloba* or *Hypericum perforatum* (St Johns Wort), are those most likely to be under cultivation already by the large European and American herbal companies and are those least threatened by wild harvesting. The future market for less well-known species is highly unpredictable and because many are perennials requiring several years to establish and become harvestable, investment in them could represent a considerable commercial risk.

Growth requirements of medicinal plants

Cultivation of some herbs has proved difficult because of low germination rates or specific ecological requirements [1]. There could simply be a lack of knowledge about the specific requirements for pollination, seed germination and growth. Low germination rates frequently result from fungal infection or mechanical damage to seeds and can be improved by seed treatments and by ensuring optimum storage conditions. Stratification, the artificial emulation of environmental conditions required for seed germination such as soaking or chilling, can sometimes provide the key to success. In *Panax quinquefolium* (American ginseng), the use of a controlled environment substantially shortens the stratification period required, increases germination rate and seed viability and enables seed germination at any time of the year [4]. Similarly, it might be necessary to optimize the conditions for pollinators or to conduct artificial pollination. The use of controlled environments,

 0167-7799/$ - see front matter doi:10.1016/j.tibtech.2005.02.002

including hydroponic systems, could be one way in which difficult-to-grow medicinal plants can be cultivated on a commercial scale.

Cultivation to control the content of active compounds

Controlled growth systems also make it feasible to contemplate manipulation of phenotypic variation in the concentration of medicinally important compounds present at harvest. The aim is to increase potency, reduce toxin levels and increase uniformity and predictability of extracts. The target compounds are almost invariably secondary metabolites, which, for the plant, frequently serve as adaptations to fluctuating temperature and light conditions (e.g. antioxidants), stress (e.g. proline), infection (e.g. flavanoids) or herbivory (e.g. alkaloids). For example, Caucasian-grown *Atropa belladonna* has an alkaloid content of 1.3%, compared to 0.3% in plants grown in Sweden [5]. Shade-grown *Mentha piperata* has a lower essential oil content (1.09% v 1.43%) and lower menthol content within the oil (57.5% v 61.8%) [5] compared with light-grown *Mentha piperata*. Cool-grown *Papaver somniferum* (poppy) contains more morphine but has a lower alkaloid content than warm-grown *P. somniferum* [5]. Secondary metabolite accumulation is similarly affected by water availability, exposure to soil microorganisms and variations in soil pH and nutrients [5].

Traditional breeding principles as applied to medicinal plants

By bringing herbs into cultivation, traditional and biotechnological plant-breeding techniques can be applied at the genetic level to improve yield and uniformity, and to modify potency or toxicity. Seed production and viability are target traits in which considerable success can be expected simply by selecting vigorous and fertile genotypes, a process that also establishes a population adapted to the growing conditions provided. Artificial selection is, however, time consuming and the process is accelerated if there are reliable methods to measure desired traits at an early stage in the reproductive cycle. For example, laser speckle technology uses quantitative and qualitative differences in speckle activity produced when seeds are illuminated by a laser to discriminate viable from non-viable seed [6]. Breeders can also aim for increased or stable concentrations of desirable biologically active compounds and reduced concentrations of intrinsic toxins. Extracts of *Ginkgo biloba*, for example, are standardized to contain 24–27% flavonoid glycosides and 6–7% terpene lactones, and the toxic component ginkgolic acid is kept below 5 ppm. These targets are usually achieved during the extraction process but deliberate selection of genotypes that yield extracts close to the desired chemical profiles, particularly if they could be identified successfully at an early stage, could simplify processing and reduce extraction costs.

Selection assisted by genetic markers is an extension of traditional crop breeding, which has been used extensively in food crop improvement. Again, it is a way to recognize desirable genotypes at an early stage to speed up the selection process. It relies on detecting specific DNA sequences that are either gene alleles directly concerned with the trait in question or that are closely linked to such genes. Identifying functional genes and useful marker sequences linked to them is a lengthy and expensive technical process but progress in this area of molecular biology has been facilitated and accelerated by the results of whole genome sequencing of model species, such as *Oryza sativa* (rice) and *Arabidopsis thaliana*. There is a high degree of similarity in the DNA sequences of functional genes between different plant species, therefore, DNA probes from one species can often be used to identify homologous sequences in another closely related species.

The availability of complete genome sequences for *Arabidopsis* and *O. sativa*, and the rapid increase in detailed genomics resources for several other model species, including those in the genera *Medicago*, *Lycopersicon* (tomato) and *Populus* (poplar), opens up opportunities for the improvement of medicinal species via comparative genetics. We can expect this technology to become useful even for less economically valuable species. The model legume *Medicago truncatula* [7] is closely related to *M. sativa*, recommended in herbals for a range of conditions. *Trifolium* species are also legumes that are sufficiently closely related to *M. truncatula* to benefit from read-across from the model into breeding programmes based on molecular markers and genetic maps. *T. pratense* (red clover) was traditionally bred for forage and in recent years has been revived as a sustainable source of on-farm protein for livestock systems [8]. The oestrogenic properties of this species have well-established agricultural value and are of increasing interest in the context of human health concerns about hormone replacement therapy [9]. The model *Populus* [10] is in the same plant family as *Salix*, the source of aspirin and, potentially, of other medicinally useful compounds.

To date, however, there have been relatively few reports of molecular marker-based approaches to medicinal plant improvement, and not even the most skeletal of genetic maps is available for any of the important species. However, in the case of *Cannabis*, a species receiving increasing attention for its potential medical uses, there have been reports since the 1940s of growers, both legitimate and illicit, successfully selecting for high or low THC and CBD. Molecular markers in *Cannabis*, including amplified fragment length polymorphisms and microsatellites, have been developed both for breeding the species as a fibre crop [11] and for forensic use [12]. Specific markers for codominant alleles thought to code for the two synthases responsible for cannabinoid biosynthesis have also been sequenced [13], specifically with a view to their use in breeding pharmaceutically useful lines. There have been several published accounts of the use of one particular type of molecular marker (RAPDs) for population analyses [14–16], but little work appears to have been conducted with marker types that would be usable for breeding. The high heritability and useful range of variation for artemisinin suggests that the development of molecular tags for the trait and their exploitation in a marker-assisted breeding programme are feasible [17]. Although the impact to date has been minimal, it is certain that the '-omics' revolution, as it spreads out from model species to those with more complex genomes (so-called

'muddle' species) will influence research and exploitation of medicinal species as it will plants in general.

Genetic transformation systems for medicinal plants

Direct manipulation of DNA sequences to alter gene expression in medicinal plants is an area that is ripe for expansion. Provided a trait can be related to one or a small number of genes, in principle, it is open to modification. Although the primary target for trait manipulation in medicinal plants is the content of active compounds, for development as crops, basic agronomic characters related to uniformity, stability, growth and development, and resistance to biotic and abiotic stresses, must also be improved [18–20]. The application of biotechnological approaches to medicinal plants does not start from a low baseline. There is already considerable interest in manipulating plant biosynthetic pathways to produce drug precursors, food components or pesticides; for example, through trichomes, specialized surface organs that manufacture, store and exude secondary metabolites [21]. In *Mentha spp* (mints), biosynthetic pathways have been engineered to modify essential oil production in the trichomes and to enhance the resistance of the plant to fungal infection and abiotic stresses [22]. There is a long history of experimental and commercial production of high-value phytochemicals by tissue culture, an *in vitro* system for growing plant organs, explants, tissues, cells or protoplasts. Genetic transformation of cultures using bacterial vectors to transfer genes into the cultured plant DNA has been widely employed to improve product output in such systems. For example, hairy root cultures, transformed by infecting cells with the bacterium *Agrobacterium rhizogenes*, often sustain stable and high productivity in hormone-free culture conditions [23]. An efficient transgene delivery system is thus already in place for several important medicinal plants, including *P. somniferum* [24], *Artemisia spp*. (wormwoods) [25], members of the family Solanaceae [26] and *Taraxacum platycarpum* [27]. Transformation systems based on *Agrobacterium tumefaciens* are well established for *Taxus* (yew) [28], *Echinacea* [29], *Scrophularia* (figwort) [30], *Digitalis* (foxglove) [31], *Thalictrum* (meadow rues) [32] and *Artemisia* [33]. Problems of regenerating whole plants from cultures have been overcome for many plants, but some important species remain recalcitrant, notably *G. biloba* [34].

As well as being essential elements of a system for rational engineering of the genetic makeup of medicinal plants, tissue culture and regeneration could make important contributions to other aspects of plant breeding [35]. Tissue culture often promotes genetic disturbances, which result in somaclonal variation, greatly extending the range of useful variation available to the breeder [35–37]. Tissue culture can also contribute to the conservation of valuable biodiversity [38,39] and somatic embryogenesis, the micropropagation of whole plants from cultured cells, has a potentially significant part to play in establishing breeding material from wild populations [40] and in mass-producing material for selection or engineering [41].

Pathway engineering in medicinal plants

Increasing the production of active phytochemical constituents is a well-established target for genetic manipulation but presents some severe challenges. In particular, the metabolic pathways by which active compounds are biosynthesized are mostly poorly understood, and relatively few genes for key enzymatic or regulatory steps have been isolated. Nevertheless, there are examples of pathway engineering leading to improvements of potential value in the breeding of medicinal plants (see reviews [42,43]). A recent article illustrating the challenges and opportunities of this approach [44] describes a ninefold enhancement in production of the sedative compound scopolamine in hairy root cultures of *Hyoscyamus niger* (black henbane), brought about by simultaneously overexpressing two genes encoding the rate-limiting upstream and downstream biosynthetic enzymes. Yun *et al.* [45] increased the production of scopolamine in *A. belladona,* from the naturally occurring chemical precursor hyoscyamine, by transformation with the enzyme hyoscyamine 6 β-hydroxylase from *Hyoscyamus*. Preliminary progress has been made towards engineering alkaloid production in *P. somniferum* [46]. A threefold enhancement in production of the putative anti-malarial, anti-cancer agent artemisinin has been reported in transgenic *Artemisia* plants overexpressing farnesyl diphosphate synthase, the enzyme immediately preceding the first committed biosynthetic step [25,47]. As an alternative to targeting an individual rate-limiting enzyme reaction, exploiting transcription factors that turn whole secondary pathways on or off shows great promise as a metabolic engineering strategy (Figure 1) [48]. New genomic approaches and efficient gene isolation methods applied to difficult secondary pathways in medicinal plant metabolism will undoubtedly expand the range and precision of manipulations via transgenesis, providing potentially superior material for the breeder.

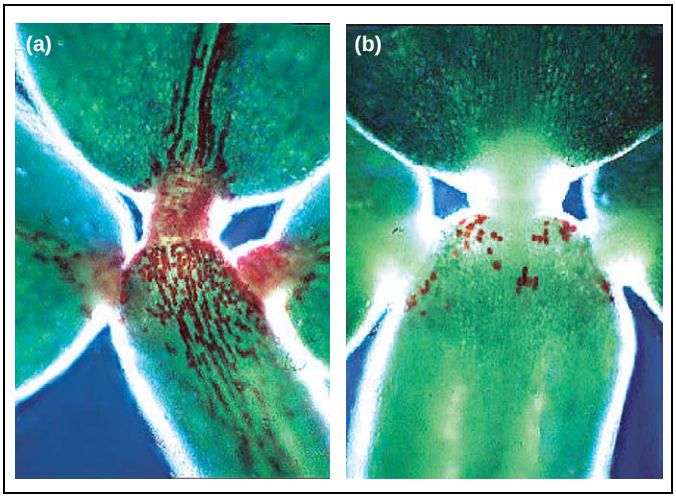

Figure 1. Manipulation of the levels and sites of expression of an entire pathway of secondary metabolism in *Lotus corniculatus* by transformation with a transcription factor. Introduction of *Sn* (a maize *myc*-class gene) results in enhanced phenolic metabolism, visible as accumulation of red anthocyanins in subepidermal cell layers of the leaf base and petiole. It also induces differentiation of cells that biosynthesize condensed tannins in lineages leading to spongy and pallisade mesophyll in the leaves. **(a)** Transgenic line. **(b)** Untransformed control. Reproduced with permission from Oxford University Press [48].

Engineering agronomic traits in medicinal plants

Just as resistance to herbicides, pests and diseases are the characters that have led the way in the introduction of transgenic crop species, so too have these characters been among the first targets for medicinal plant biotechnology. Transgenic *Atropa* plants resistant to the herbicides bialaphos and glufosinate have been described [49] and *Panax ginseng*, resistant to the herbicide Basta, has been generated by transformation with the enzyme phosphinothricin acetyl transferase [50]. Resistant biotypes can also be a useful germplasm source for breeding. Somatic hybridisation – the fusion of somatic cells from tissue culture – and atrazine selection have been used to regenerate herbicide-tolerant *Solanum nigrum* (black nightshade) [51]. Transgenesis will assist in breeding varieties resistant to fungal diseases. *Panax quinquefolium* (American ginseng) transformed with either a chitinase or a thaumatin-like antifungal gene have been regenerated successfully [52,53].

There have been relatively few reports of biotechnology applied to non-biotic stresses and other aspects of agronomic performance, although the approach is considered to have great potential [19]. Conventional breeding makes improvements through adjustments to growth and development [54] but transgenic intervention in such processes is limited by our understanding of their genetic basis. Nevertheless, studies have revealed promising new phenotypes. For example, morphological variation introduced to *Taraxacum platycarpum* regenerated from hairy root cultures was ascribed to the developmental effects of the *rol* genes introduced from *A. rhizogenes* [27]. When the bacterial gene *ipt*, which promotes the endogenous production of cytokinin growth hormones, is expressed in *Artemisia* there is a coordinated increase in hormone, chlorophyll and artemisinin levels [55].

Public perception of biotechnology: implications for medicinal plant cultivation

The commercial viability of bringing medicinal plants into domestic cultivation and the potential for increased use of modern biotechnologies are likely to be strongly influenced by the popular perceptions of both herbs and biotechnology. One of the main attractions of herbs as medicines is their 'natural' status and the associated, but erroneous, view that they must therefore be safe and intrinsically good for us. In stark contrast is the popular view of crops bred with the assistance of molecular biology and modern farming methods as highly 'unnatural'. This is particularly so for transgenic plants, and it is likely that organic growing methods will be received favourably by purchasers of cultivated medicinal plant extracts. It is worth mentioning that a field study of pollen flow from herbicide-resistant transgenic *P. somniferum* to non-transgenic varieties and weed relatives showed no gene transfer beyond 2.5 m and 20 cm, respectively [56]. Similar farm-scale trials would need to be performed on a species-by-species basis if the cultivation of transgenic herbs were to overcome the relatively minor and tractable initial obstacle of ecological risk; consumer on-principle resistance to plant biotechnology is an altogether more formidable, and possibly even immovable, barrier.

There is a danger that, if medicinal herbs were increasingly brought into domestic cultivation, then wild harvested plants would enjoy an increased cachet and commercial value, and non-sustainable harvesting methods would continue. This raises broad issues of intellectual property, the free exchange of germplasm and the rights of local farmers and governments, particularly where plants are of developing country provenance [57]. However, in scale, the biological consequences might overshadow even these difficult socioeconomic concerns: it is perhaps worth considering that any form of cultivation or wild harvesting of plants is bound to involve the application of selective forces, conscious or unconscious on the part of the grower or harvester. In the case of wild harvesting, continual selection of the largest wild-growing individuals or those with the traits considered desirable from a medicinal point of view, if it involves destruction of the whole plant or its reproductive organs, will inevitably lead to a degradation of the wild population. We cannot use medicinal plants on a large scale without modifying the characteristics of the plant populations available to us, be they wild or domesticated. If medicinal herbs are brought into cultivation, then we can at least attempt to do this in a controlled fashion, and at the same time attempt to conserve wild populations.

Future prospects

In regions such as Western Europe, the trend away from commodity-type agriculture towards niche production of high-value species for non-food markets offers major opportunities for the application of plant biotechnology. Medicinal herbs are taking their place alongside the likes of bioenergy crops, sources of renewable industrial feedstocks and bioremedials as potential beneficiaries of technological solutions originally devised for the food chain. The twin political issues of world energy security and management of landscapes to sustain rural communities and urban expectations are likely to keep the biotechnological option on the agenda. A particular challenge for medicinal plants is the degree to which synergistic effects (a major part of the herbalist rationale) can be not only conclusively demonstrated but also realistically defined for biotechnological intervention. Otherwise, increasing understanding of what the active components in herbs are and how they work will simply lead to their isolation as plant-derived drugs, and biotechnological interest in the plants and whole extracts from them will not be justifiable.

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