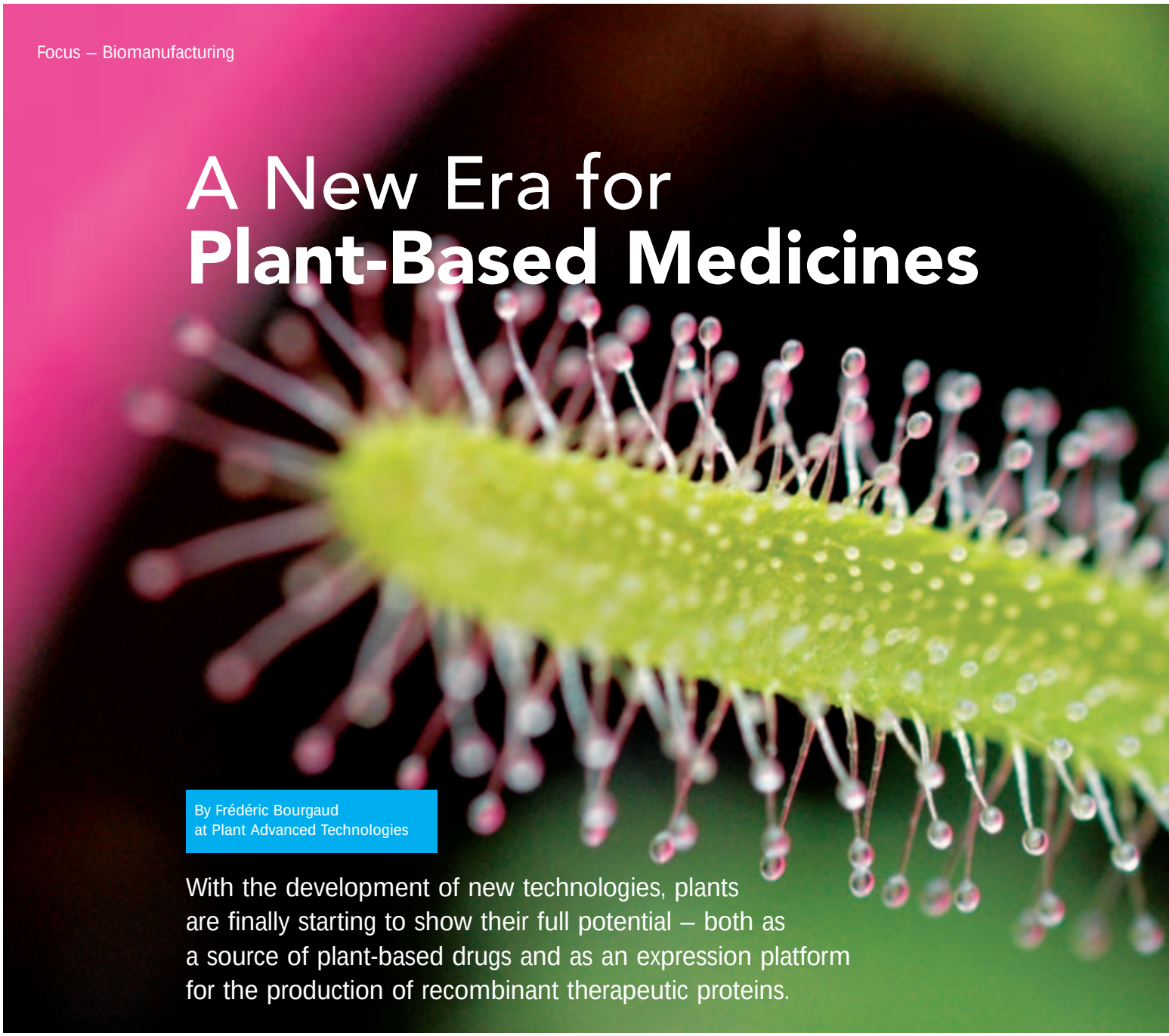
A New Era for **Plant-Based Medicines**



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With the development of new technologies, plants are finally starting to show their full potential – both as a source of plant-based drugs and as an expression platform for the production of recombinant therapeutic proteins.

Keywords

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Plant expression systems

Plants have historically served as the most significant source of new leads for pharmaceutical development. Today it is considered that about one-quarter of our modern medicines contain plant-derived molecules. Although not necessarily produced by plants as such, plant-derived compounds are often discovered in nature before being partially or totally synthesised through chemical processes; this was the case with the anticancer drug Taxotere® (docetaxel, Sanofi-aventis).

It is generally estimated that there are approximately 300,000 species of higher plants; out of these, only a few thousands are used as medicines. Thus, there are potentially many more important discoveries in the plant kingdom to be exploited for pharmaceutical application. In fact, there are literally millions of natural chemical structure types resulting from nature's combinational chemistry effort, supplying almost unimaginable chemical diversity – and in turn yielding stereochemically complex structures with diverse functional groups that are ideal for interacting specifically with biological target molecules. More importantly, nature has been 'doing' combinational chemistry for aeons – not just a decade or two – and has been selecting products from this natural library that have specific biological advantage.

Despite this obvious interest in natural plant products, a brief look at the drugs passing through the approval process over the last three decades clearly shows a decline in the development of plant-derived therapeutics. Concern over the availability of a chemical entity to put through development and that will meet market needs has been the single most limiting factor for the pharmaceutical industry's interest in natural products. Another limiting factor lies in the paradigm 'one illness:one molecule' that underpins modern drug development. This is clearly to the detriment of plant extracts that contain virtually thousands of active

Images: Plant Advanced Technologies

molecules, often displaying synergistic effects. On more than one occasion, plant extracts have shown remarkable potential, but a conventional approach based on fractionation/isolation of pure chemical entities has failed to reveal a 'single miracle molecule' because the synergistic association been broken.

A New Era

However, a new era is coming when these limitations will be overcome. First, as stated in the US FDA's guide to botanical drugs, identification of the active constituents is not a prerequisite to drug development. The critical point for any plant-based drug project lies clearly in the reproducibility of, first, the observed pharmacological/clinical effects, and second, the composition of the plant extracts that need to be rigorously controlled. Since the new millennium, the emergence of metabolomics has given a new tool-set to validate the quality/reproducibility of a given plant extract. Second, plants or plant parts can be cultivated under strictly controlled conditions, for example, bioreactors or confined greenhouses. Growing plants under controlled environmental parameters is a guarantee that a given plant accession/population will behave in a predictable manner between two different cultivation cycles, resulting in extracts of reproducible quality.

Finally, the new development of plant phenotyping platforms represents an opportunity to access the complex chemistry of plants and reveal new molecules from 'old' plants that have received insufficient attention in the past. Indeed, plant natural compounds are mostly secondary metabolites (phytopharmaceuticals) metabolites that protect a plant from biotic or abiotic stresses. Although their synthesis is usually highly inducible and triggered by stress factors, most phytochemical investigations are undertaken on plants collected in the wild with no special attention given to environmental conditions and physiological state. Hence, it is highly probable that the collected plant is not placed in the best conditions to express its pharmacological potential. Plant phenotyping platforms will help reveal cryptic molecules that are only produced upon elicitation.

Genetic engineering also offers the possibility to review plants' potential to produce interesting compounds. Metabolic engineering approaches can be used to enhance the production of a given compound naturally present in a plant species – that is, up-regulation of key genes encoding enzymes directly involved in the synthesis, or down-regulation of undesired biosynthetic pathways. Some recombinant DNA technologies can be used in a more straightforward 'synthetic biology' approach where a set of carefully chosen plant genes,

responsible for a given metabolic pathway, can be transported into another plant species (heterologous expression in plants) or even into microbial organism (heterologous expression in bacteria or yeast).

This approach is presently under development within the framework of the Smartcell Project (1); this is an EU-funded project that was started in 2009 and has been awarded EUR 6 million to develop tools to synthesise valuable pharmaceutical products using plant cells. The project is a consortium of 18 partners from the EU, Switzerland and the USA, including 14 leading research institutes and universities, as well as two small- and medium-sized enterprises and two major industrial enterprises. The project has a total budget of EUR 8.5 million and is scheduled to run for four years.

Production Platforms

Alternatively, plants can be used as 'fine chemicals factories' to produce recombinant therapeutic proteins. These biopharmaceuticals constitute the cutting edge of modern drug therapy as they represent half of the pharmaceuticals under development. Compared with other expression systems – such as mammalian cells, *E coli* or yeast cultures – plants show several advantages including cost-effectiveness, ease of scale up (surface extension), lack of contamination risk (viral/prion/endotoxins/oncogenicity) for humans, and a capacity to realise complex post-maturation processes (glycosylation, acylation, S-S bonds and so on).

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However, plant platforms for the production of recombinant proteins present several problems. The classic argument made against plant expression systems relates to their glycosylation patterns – that is, terminal glycan motives characterised by the presence of xylose and α1,3 fucose that confer a potential risk of immunogenicity to the recombinant protein. Yet, recent advances in the development of glucocerebrosidase, a protein developed by Protalix (Carmiel, Israel) for the symptomatic treatment of Gaucher's disease, have clearly shown that plant glycosylation in fact turned out to be an advantage with no reported secondary effects to patients under clinical investigation (2).

A more decisive drawback is the difficulty of purifying and isolating the recombinant protein from the rest of the plant tissue. It is classically admitted that this downstream process represents about 80 per cent of the overall cost of the purified protein. This has led several companies to invest in original plants or production systems with the objective of simplifying this critical step. Phytomedics (Jamesburg, NJ) has developed a technology based on guttation – a natural exudation process by which proteins are naturally excreted at the leaf surface, thereby facilitating their recovery (3). Alternatively, at Plant Advanced Technologies we are using the original carnivorous plants *Drosera* and *Nepenthes* to exploit their remarkable abilities to excrete large quantities of complex proteins (4).

Plant industrial platforms were started to be developed much later than mammalian cell technologies (hybridoma, CHO), but they are now entering into an age of maturity, as shown by ultimate market developments. To serve as an example, recombinant glucocerebrosidase is now being produced by Protalix from carrot cells with reactor culture (2); but most importantly this technology is being co-developed with Pfizer, a major pharmaceutical company that has the experience and funds to deal with the complex regulatory processes. Recombinant glucocerebrosidase is now entering the last step before an expected final approval by the US FDA and should represent the first plant recombinant protein dedicated to human health to reach the market. Another promising example is given by Medicago (Québec, Canada); this company has recently made a striking breakthrough by developing several production units (greenhouse facilities) around the world for the production of recombinant anti-viral human vaccines (5).

The Future

Finally, plants not only constitute an historical source of pharmaceutical compounds but also demonstrate many advantages for developing modern drugs. New recombinant DNA technologies together with adapted cultivation facilities enable exploitation of the extraordinary capacity of plants to produce rare natural compounds with remarkable pharmacological properties. Plants also constitute attractive biological systems that can complement other heterologous expression platforms for the production of recombinant therapeutic proteins. Plants will undoubtedly bring many pharmaceutical success stories in the very near future.

Further reading

1. Smartcell. http://www.smart-cell.org
2. Protalix. http://www.protalix.com
3. Phytomedics. www.phytomedics.com
4. Plant Advanced Technologies PAT SA. http://www.plantadvanced.com
5. Medicago. http://www.medicago.com