Metabolic engineering of plants – Molecular Pharming

Vinaya Roehrl (Oxford University)

Abstract

Plants can be metabolically engineered to produce industrially relevant compounds such as immunotherapeutics and biopharmaceuticals, also termed molecular pharming. To date, it has already been possible to produce vaccines, antibodies, glycohormones, enzymes, and metabolites using plants as bioproduction platforms. These compounds can exhibit challenging chemistries which would be difficult or sometimes impossible to reproduce synthetically. Therefore, plants provide a sustainable and cheaper alternative to chemical synthesis by decreasing energy and resource inputs as well as being biodegradable protein expression machines. Currently, research on plant biosystems still lags behind bacterial, yeast, and mammalian systems1. However, with the growing availability of advanced technologies, plants could become primary compound synthesis machines which would contribute to global health initiatives as well as supporting equal access to pharmaceuticals. Here, two examples of molecular pharming are described: the plant-based production of the Covid-19 vaccine CoVLP as well as the Ebola virus-neutralizing antibodies ZMapp.

The principles of plant metabolic engineering

The model species frequently used in plant engineering is the Australian tobacco plant *Nicotiana benthamiana* as its genome is well-characterized. The plant can also be easily infected with viruses, which is exploited to genetically engineer them. To introduce genes of interest into the plant leading to the production of the desired compounds, a process termed agroinfiltration is utilized. This involves the use of the bacterial species *Agrobacterium tumefaciens*, which usually causes crown gall disease in plants. It manipulates host cells through its T6SS (type VI secretion system), which allows it to integrate its DNA (T-DNA) into the host so that it can be transcribed to produce proteins favourable to the pathogen's survival and replication. This system can be manipulated by replacing the bacterial T-DNA with genes of interest. This technology provides incredible opportunities for fast, flexible, and inexpensive production of chemically complex compounds. Alternatives to the *Agrobacterium*-mediated engineering approach exist such as nanoparticle bombardment, which directly delivers DNA, RNA, or other proteins to target sites. This has been done in rice, whereby Cas9-gRNA ribonucleases were injected into plant zygotes. However, agroinfiltration is currently the most industrially scalable technology for plant metabolic engineering and has already been used successfully.

A plant-based Covid-19 vaccine: CoVLP

Medicago and GSK recently produced the world's first plant-based Covid-19 vaccine COVIFENZ (CoVLP). Using agroinfiltration technologies, they produced VLPs (virus-like particles) in living *N. benthamiana* plants. These VLPs are non-infectious SARS-CoV-2 virus S glycoproteins resembling the disease-causing virus and therefore elicit natural immune responses. Importantly, they are unable to cause disease in humans as they lack the core genetic material for replication. The full-length SARS-CoV-2 spike protein sequence was optimized for secretion in *N. benthamiana* and transformed into *A. tumefaciens* to be spray-inoculated onto the plants[2]. These VLPs spontaneously assembled and accumulated in the space between the plasma membrane and plant cell well. At first, VLP protein levels in the plants were low so that two additions were made to the system to allow for high levels of VLP accumulation. Firstly, hypertranslatable promoters derived from the cowpea mosaic virus were added to the sequence. Secondly, the tomato bushy stunt virus – derived protein p19 was added to the T-DNA sequence to overcome transgene silencing upon agroinfiltration. Co-expressing p19 with the glycoprotein sequence by mixing *Agrobacterium* cultures led to 50 times higher accumulation of VLPs in the tobacco plants. Within three weeks, the researchers were able to produce 10M vaccines. This result demonstrated a great success in plant metabolic engineering. The plant-based vaccine has also undergone clinical trials involving 2 doses of 3.75 micrograms of CoVLP adjuvanted with AS03 given 21 days apart. The companies announced the remarkable results stating an overall vaccine efficacy rate against all variants of SARS-Cov-2 of 71%, efficacy against the Delta variant of 75.3% and 88.6% against the Gamma variant[3]. This plant-based vaccine is therefore effective, as well as cheap to produce.

Anti-Ebola serum – ZMapp

The 2014 Ebola outbreak was another devastating disease causing over 11 thousand deaths. Using plants, researchers were able to produce virus-neutralizing antibodies ZMapp[4], which would serve as an Ebola treatment. A mix of three human-mouse chimaeric monoclonal IgG antibodies were engineered to be produced in *N. benthamiana* via agroinfiltration. These target the glycoprotein on the virus surface and block the entry of the virus into host cells. Trials of ZMapp on Rhesus monkeys (*Rhesus macaques*) showed that the serum was able to rescue all the monkeys that received the treatment. Advanced disease symptoms could be reversed leading to the full recovery of the treated monkeys. Clinical trials testing the efficacy of ZMapp in humans have unfortunately not been as successful[5], but the study still demonstrates the potential plants hold for future biopharmaceutical drug production. Especially the production of anticancer drugs could become vitally important.

Beyond pharmaceuticals

Even though molecular pharming only comprises the production of pharmaceuticals in plants, it is important to realize the wide-reaching potential plant compound production platforms hold. Not only medically relevant compounds but also plant-based industrial enzymes relevant to feed, biofuel, and papermaking industries will likely have a great impact[6]. A primary example of this is the successful production of spider silk in tobacco plants and potato tubers. Spider silk has remarkable mechanical characteristics as it has high tensile strength while also maintaining high elasticity. It could therefore be used as raw material to produce biodegradable fibers, foils, flexible and lightweight textiles as well as wound closure systems and scaffolds for tissue engineering. As spider silk cannot be obtained in high quantities from spiders (*Nephila clavipes*) researchers developed transgenic tobacco and potato plants which accumulated spider silk to high amounts without changing the growth or morphology of the plants[7]. Spider silk proteins were produced via *Agrobacterium*-mediated agroinfiltration to make up over 0.5% of the total soluble protein in the plants. The production costs also amounted to only 10-50% of production via bacterial fermenters.

Policy Recommendations

In conclusion, plants provide a promising tool for sustainably, efficiently, and inexpensively producing commercially important compounds. Their usage will be applicable to several fields of research from the production of biocatalysts, biopolymers, biofuels, to pharmaceutical compounds. These advantages will also greatly improve the distribution of compounds to developing countries, which will benefit from the cheap production technology. Molecular pharming therefore not only influences the SDG goal 3 of good health and wellbeing but also addresses goal 10 to reduce inequalities across the globe. Policies will therefore need to address the following challenges in the field:

1) *Promote research and provide funding to research in plant science:* We need to address our lacking understanding of complex plant metabolic pathways and continue to improve experimental approaches to achieve high yielding plant-based production of compounds[8]. This includes providing funding to research in fundamental plant science as well as metabolic engineering efforts.
2) *Promote GMO initiatives:* Governments should be encouraged to revise their GMO legislations to allow the widespread use of genetically modified plants.
3) *Encourage public acceptance of GMOs*: Even though there has been no indication that plant-based production systems are not safe to use, misinformation has led to the reluctancy to accept genetically modified plants among the general public. UN initiatives to educate people on the technologies and safety measures put in place could help reduce this stigma.

References

1. Roell, M.-S. & Zurbriggen, M.D., The impact of synthetic biology for future agriculture and nutrition. *Current Opinion in Biotechnology* 61, 102-109 (2020).
2. Ward, B.J., Gobeil, P., Séguin, A. et al. Phase 1 randomized trial of a plant-derived virus-like particle vaccine for COVID-19. Nat Med 27, 1071–1078 (2021).
3. Medicago. Medicago and GSK announce positive Phase 3 efficacy and safety results for adjuvanted plant-based COVID-19 vaccine candidate. (2021). Accessible at: https://medicago.com/en/press-release/medicago-and-gsk-announce-positive-phase-3-efficacy-and-safety-results-for-adjuvanted-plant-based-covid-19-vaccine-candidate/.
4. Qiu, X., Wong, G., Audet, J. et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature 514, 47–53 (2014).
5. PREVAIL II Writing Group et al. "A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection." The New England journal of medicine 375, 1448-1456 (2016).
6. Tschofen, M. et al., Plant Molecular Farming: Much More than Medicines. Annu Rev Anal Chem (Palo Alto Calif). 271-294 (2016).
7. Scheller, J., Gührs, KH., Grosse, F. *et al.* Production of spider silk proteins in tobacco and potato. *Nat Biotechnol* 19, 573–577 (2001).
8. Yang, X. et al., Plant Biosystems Design Research Roadmap 1.0. BioDesign Research 2020, 8051764 (2020)