Chapter 2

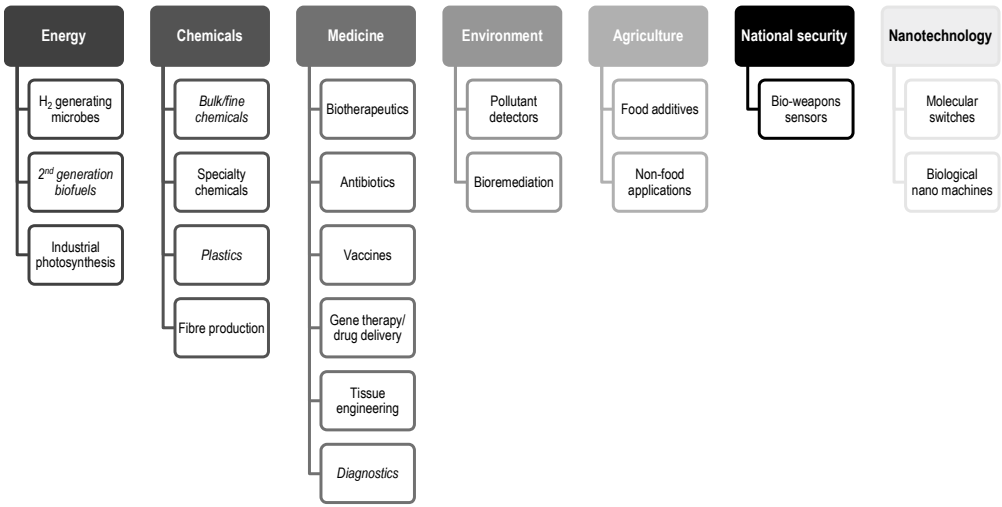
The applications and potential benefits of synthetic biology

Synthetic biology can be regarded as a platform technology that cuts across several key market sectors, such as energy, chemicals, medicine, environment and agriculture. Its formative years have been spent in developing the basic tools for applications in biofuels and other bio-based products, where the earliest products have been seen. It holds out very high expectations and potential for applications to human and animal health, with the potential for greatest benefits in the developing and poor nations. With a growing global population and threats to water and soil quality, agricultural applications are envisaged that could have far-reaching consequences for productivity and efficiency, but in many parts of the world such agricultural applications are controversial.

Introduction

One of the reasons synthetic biology attracts such a high level of interest is that it can be seen as a platform technology that cuts across business sectors. Figure 2.1 identifies some of these sectors and some specific applications of synthetic biology in each. The earliest interest concerned energy applications: several start-ups have been formed in the United States for these applications. Applications in medicine and health care are much more diverse, but it takes a great deal of time to bring the products to the market. The chemicals sector also has a large body of research on the application of synthetic biology to the production of bio-based plastics, for example (e.g. Jung and Lee, 2011).

Figure 2.1. Applications of synthetic biology across sectors



Note: Italics denote the earliest industrial applications.

Source: Adapted from Collins (2012), "Win-win investments: synthetic biology for growth and innovation", paper presented at the Science and Technology Options Assessment (STOA) workshop "Synthetic biology – enabling sustainable solutions for food, feed, bio-fuel and health: New potentials for the European bio-economy", European Parliament, Brussels, 6 June 2012, www.europarl.europa.eu/stoa/cms/home/events/workshops/synthetic_biology.

Industrial biotechnology and synthetic biology

As a large-scale commercial activity, industrial biotechnology foundered after the failure of single cell protein (Bud, 1993). It has since rebounded strongly in the form of liquid biofuels, ushering in a new wave of growth following the success of bioethanol in Brazil (Goldemberg, 2008). Between 2005 and 2010, fuel ethanol production worldwide more than doubled (FO Licht, 2010a), and biodiesel production more than quadrupled (FO Licht, 2010b).

Industrial biotechnology has matured rapidly and has produced a large number of bio-based chemicals and bioplastics (OECD, 2011a). Bio-based production can also partially replace petrochemical production in order to mitigate climate change. As biomass is the feedstock for industrial biotechnology, significant savings in greenhouse gas (GHG) emissions are possible compared to production from oil (OECD, 2011b).

For the vast majority of its applications, industrial biotechnology faces a difficulty: the best biocatalyst for a particular conversion or synthesis rarely occurs in the best organism for industrial exploitation. Production microorganisms have to be engineered, both to maximise yield (e.g. prevention of the loss of plasmids during fermentation) and to tolerate the artificial, and sometimes extreme, conditions of the fermentation process (Murakami et al., 2008). This is an area in which synthetic biology holds great promise.

Synthetic biology has already made significant contributions to industrial biotechnology and is poised to make more. Recent market research (Bergin, 2009) predicted that the world market for synthetic biology products could expand to USD 2.4 billion by 2013, largely in the chemicals and energy sectors. Between 2008 and 2013 this would mean a compound annual growth rate of 59.8%. Although the focus of the synthetic biology biofuel community has been on the production of diesel, jet fuel, automotive fuels and other industrial oils, the biofuels campaign will enable the development of generic synthetic biology technologies and platforms, including the creation of a technical metabolic engineering knowledge base, the training of a cohort of practitioners skilled in the discovery of practical solutions to important problems in metabolic engineering and their dissemination as general principles.

Some examples of metabolic engineering/synthetic biology for the production of industrial materials are given below and indicate the extent to which synthetic biology already contributes to industrial biotechnology. BIO, the Biotechnology Industry Organization, maintains a resource centre on its website and publishes overviews of member companies' development of commercial applications of synthetic biology, such as OPX Biotechnolo-

gies (bioacrylic), Goodyear/Danisco-Genencor (rubber for tires), Modular Genetics (converting agricultural waste into surfactants), and DSM (synthetic antibiotics and vitamins). Other companies investing in synthetic biology include Codexis (enzymes and catalysts), DuPont (polymers), and BP (butanol). Dozens of biofuel or related start-ups have emerged since 2005, including LS9, Solazyme, Gevo, Synthetic Genomics and Joule Unlimited (Dress et al., 2011).

Bio-isoprene

Isoprene is an important commodity chemical with a range of applications. Before the efforts of the Goodyear Tyre and Rubber Company and Genencor, there was no obvious biological route to this compound. There is an increasing need for isoprene and a simultaneous environmental imperative to reduce GHGs, but neither natural rubber nor synthetic rubber compounds can be sourced in sufficient quantities to meet anticipated future demands. Before the recent economic recession, more than 70 million motor vehicles were sold every year around the world, bringing the total number on the road to over 800 million recently. By 2030, this figure could reach 1.3 billion, increasing the demand for rubber in parallel.

The development of bio-isoprene represents a major achievement for industrial biotechnology and synthetic biology because it has the potential to enable production of isoprene from renewable raw materials and represents a key bio-based intermediate that can be converted to a drop-in transport fuel additive (using chemical catalysis) to C_{10} and C_{15} bio-based hydrocarbon fuels for performance gasoline, jet fuel and biodiesel markets. Current state-of-the-art technology has resulted in production, recovery, polymerisation and manufacture of tyres with the isoprene component produced via fermentation.[1]

1,3-propanediol (1,3-PDO)

The appealing properties of 1,3-propanediol for many synthetic reactions, such as polycondensation, and for uses in solvents, adhesives, resins, detergents and cosmetics (Zeng and Sabra, 2011) make it a classic platform chemical. It has long been known that it is produced by microorganisms but none of these would be treated seriously as an industrial biocatalyst.

Nakamura and Whited (2003) described the strategy and progress of an effort by DuPont and Genencor International, Inc. to design and build a single organism catalyst for the direct conversion of D-glucose to 1,3-PDO as a textbook example of metabolic engineering. The strain is based on an *E. coli* K12 strain, which is eligible for favourable regulatory status in the United States, and is also in Risk Group 1 (the lowest risk under NIH guidelines).[2]

In contrast to processes that use naturally available organisms, the DuPont/ Genencor process is aerobic and inherently more efficient. By introducing a four-step pathway consisting of genes from PDO-synthesising bacterial species, together with targeted changes to the host central metabolism, researchers were able to achieve PDO production with high rate and titre. This led to a commercial process.

It is worth mentioning the need to shorten the innovation cycle in bio-based production, as its lengthy duration often dissuades potential investors, especially venture capitalists. It took DuPont and Genencor approximately 15 years and 575 person years to develop and produce 1,3-PDO (Hodgman and Jewett, 2012). One of the great hopes for the integration of software and wetware development in synthetic biology is to shorten the innovation cycle for making new bio-based products drastically.

Marine biotechnology: a potentially disruptive technology

Production of algal biofuels has the potential to be disruptive owing to the very high potential yields (Table 2.1), as oil crops cannot significantly replace petroleum-derived liquid fuels in the foreseeable future.

Table 2.1. Comparison of some sources of biodiesel

Crop	Oil yield ($l\ ha^{-1}$)	Land area needed [a] (M ha)	% of existing US cropping area
Corn	172	1 540	846
Soybean	446	594	326
Canola	1 190	223	122
Jatropha	1 892	140	77
Coconut	2 689	99	54
Oil palm	5 950	45	24
Microalgae[b]	136 900	2	1.1
Microalgae[c]	58 700	4.5	2.5

Notes:

a. For meeting 50% of all transport fuels needs in the United States.

b. 70 % oil (by weight) in biomass.

c. 30 % oil (by weight) in biomass.

Source: Tan, T., J. Yu and F. Shang (2011), "2.58 – Biorefinery Engineering", in *Comprehensive Biotechnology* (2nd edition), Vol. 2, pp. 815-828.

The magnitude of the difference in oil yield from microalgae and the relatively low land area requirements have meant that algal biofuels technology is being intensely researched. Many of the major oil companies are investing heavily. But technical hurdles, particularly in production and harvesting, mean that algal biofuels will be among the last of the biofuels to be commercialised.

As an example of the potential of synthetic biology, Joule Unlimited Inc. of the United States is working on a direct algal process that combines an engineered cyanobacterial organism supplemented with a product pathway and secretion system to produce and secrete an alkane diesel product continuously. The process is closed and uses industrial waste CO_2 at concentrations 50-100 times higher than in the atmosphere (Robertson et al., 2011). If successful, this technology has the potential to change the dynamics of biofuel production as it does not require the extraction of fuels from large amounts of biomass. The company now has a commercial arm, Joule Fuels.[3]

How much of the oil barrel can be replaced?

Many governments are sceptical about the potential of bio-based production to have a real impact on energy security and reduction of GHG emissions. To do so, industrial biotechnology products cannot be limited to a few specialty and platform chemicals. However, Jay Keasling, a leader in the field, has stated[4] that he believes that "through synthetic biology all petroleum-based products can be produced from sugar-based microbes resulting in cleaner processes and slowing global warming". There is mounting evidence, especially from efforts in metabolic engineering and synthetic biology, that even completely unnatural compounds can be manufactured using microbial cells.

For example, 1,4-butanediol is non-natural and highly reduced and very difficult to biosynthesise from carbohydrates. However, its biosynthesis as a combination of software design and metabolic engineering (Yim et al., 2011) has shown that, at laboratory scale, such improbable syntheses can be achieved. On 18 October 2013 it was announced that a joint venture (Mater-Bi) between Novamont and Genomatica will start commercial production of butanediol in 2014 in Italy.

The short-chain olefins are the building block chemicals for making many other petrochemicals and polymers, and thus are at the heart of the petrochemicals industry. Global Bioenergies[5] plans to make short-chain olefins through microbial fermentation rather than from fossil resources. Late in 2013, Choi and Lee (2013) reported on metabolic engineering of *E. coli* to produce short chain alkanes. This opens up the possibility of bio-based petrol as well as short-chain chemicals derived from fatty acid.

In 2013 the scope of bio-based production of fuels and chemicals has significantly increased. Such developments, if commercially viable, may open the door to greater replacement of the oil barrel. Demonstrating this to governments may reduce scepticism and improve the prospects for a supportive policy environment.

Environmental applications and biosensors

The European Environment Agency estimates that, in Europe, potentially polluting activities have occurred at about three million sites, of which more than 8% (nearly 250 000) are highly contaminated and require remediation. The total number of contaminated sites requiring remediation may increase by more than 50% by 2025 (European Environment Agency, 2007). In fact, the scale of the problem has not yet been properly identified. Although it is seldom acknowledged in discussions of agricultural genetic resources, soils are the critical life-support surface on which all terrestrial biodiversity depends. Meanwhile, the world's soil is being lost 13-80 times faster than it is being formed. It takes some 500 years to form 25 mm of soil under agricultural conditions, and about 1 000 years to form the same amount in forest habitats.[6] In the face of soil destruction, more crops will have to be grown more efficiently, and methods will have to be sought to halt or limit soil destruction. Bioremediation can be applied to contaminated soil to bring it back into productive use.

Bioremediation is used for site clean-up in approximately 10% of applications (Roelofsen et al., 2011). This is a surprisingly low figure, given that it may improve soil quality and appears more sustainable than other remedial technologies (e.g. treatment of contaminated soil by incineration offers greater certainty but completely destroys the soil). This is principally because of a still widespread perception that bioremediation is less reliable than other means, difficult to predict in terms of the rate and extent of remediation (in particular whether specified endpoints will be reached), and requires more extensive, intrusive and expensive site assessment. The result is a lack of confidence among stakeholders, especially land developers and regulators.

Laboratory research can address these problems in broadly two ways. The utility of bioremediation in the field, and confidence in its use, could be enhanced by research directed at understanding and improving predictability. A plethora of "-omics" technologies, biosensors and community profiling techniques could act as enabling technologies (so-called "ecogenomics") to achieve these ends. Ecogenomics approaches might be used to characterise contaminated sites and monitor the bioremediation process (Stenuit et al., 2008), especially for sites with many recalcitrant pollutants. Eventually,

these ecogenomics techniques could be combined with software tools in order to translate knowledge about biodegradation into the ability to predict the power of bioremediation. This will take time, since the technology will have to be proved and then approved by the regulators of contaminated land, who are comfortable with the certainties afforded by chemical analysis and characterisation. It would free bioremediation contractors from uncertainties about the applicability of bioremediation and would in turn improve the confidence of other stakeholders.

Synthetic biology has a role in environmental sensing. Microbial resistance to heavy metals and hydrocarbon biodegradation is often encoded on genes and operons. These genes can be combined with a convenient reporter function to determine the concentrations of metals or hydrocarbons in soil and water. Whole cell biosensors that detect arsenic have been developed. Arsenic in groundwater used for potable water is a serious health problem in some parts of the world. The current recommended World Health Organization (WHO) limit for drinking water is 10 parts per billion (ppb) arsenic. Bangladesh and some other countries maintain an earlier limit of 50 ppb, but many groundwater wells in Bangladesh exceed this by a large margin. Chronic consumption of water with high arsenic concentrations leads to arsenicosis, which results in the skin lesions and various cancers that affect 0.8% of the population in Bangladesh (Bryce and Philp, 2005). French et al. (2011) have shown that an arsenic detection system linked to a simple pH change using synthetic biology techniques gives robust and reliable responses to arsenic concentrations as low as 2.5 ppb.

Medical applications

According to Donald Johnston, former Secretary General of the OECD, good health for all is a vital pillar of sustainability, and it is for OECD countries to shoulder much of the responsibility for delivering it (OECD, 2003). Synthetic biology holds the promise of solutions to a range of medical conditions, from microbial infections to cancer therapies (Xiang et al., 2006), from diabetes (Ye et al., 2011) to artificial insemination (Kemmer et al., 2011). Perhaps the most progress to date has been made in drug discovery and synthesis, but many fronts in biomedical research are being investigated using synthetic biology approaches.

Drug discovery

Many of the scourges that were thought to have been defeated during the golden age of antibiotics have come back, more lethal than ever owing to acquired drug resistance. With globalisation, these and other infectious agents can spread rapidly across the world, bringing new challenges.

Some infections are now resistant to all current anti-bacterials, and bacteria are becoming resistant to antibiotics faster than effective replacements are developed (Dwyer, 2009). While some drug candidates currently in preclinical development have generated optimism, there is nevertheless an urgent need for new agents to combat these resistant organisms. There is no evidence that this need will be met in the foreseeable future (Boucher et al., 2009). In the European Union alone, some 25 000 deaths a year are due to multi-drug resistant bacteria (European Centre for Disease Prevention and Control and the European Medicines Agency, 2009).

Combinatorial chemistry has failed to deliver the anticipated wealth of new drug candidates (Weissman, 2004). Natural products, derived from the secondary metabolism of bacteria, fungi and plants, have long been a reliable source of new therapeutic leads. However, large collections of pure natural products are rare because they are hard to build through classical fermentation methods, and in recent years this source has fallen into disfavour. The convergence of next-generation sequencing and synthetic biology opens the door to the creation of large, reliable libraries of pure natural products for drug discovery (Mitchell, 2011). Lee et al. (2009) cite over 30 drugs and drug precursors being produced by metabolically engineering microorganisms; they include a range of antibiotics, anti-cancers, anti-oxidants, anti-parasitics, anti-tumours, anti-virals, hormones, cholesterol-controlling drugs, human gamma-interferon, human interleukin-3 and IgG antibodies.

The marine environment is seen as a particularly important source of future drugs. The wealth of the marine pharmaceuticals pipeline is evidenced by at least three compounds in Phase III trials, seven compounds in Phase II trials, three compounds in Phase I trials. Numerous marine natural products representing potential clinical candidates are also being investigated (Mayer et al., 2010). Moreover, the impact of genomics and proteomics on the biotechnological exploitation of marine organisms has hardly been felt. Given the overall importance of the marine environment, it is inevitable that a large number of marine organisms (and microorganisms) will be brought into genome programmes (Borresen et al., 2010).

Artemisinin is an often-cited example of the potential of synthetic biology for drug design and development. Artemisinin is a botanical anti-malarial isolated from *Artemesia annua*, a wormwood related to *Artemesia absinthium*. Like other natural products, artemisinin is biosynthesised in multiple, sequential steps by a suite of functionally related enzymes, which in bacteria are coded on an operon. By transferring plant genes for the artemisinin pathway into a fermentable chassis organism and forcing production of the artemisinin precursors, the cost of artemisinin was cut in half, opening access to artemisinin combination therapy for low-income malaria victims in developing countries (Hale et al., 2007). It should be noted, however, that

programming microbes for expression of artemisinin is still laborious; it has taken 150 person years of work (Kwok, 2010).

Another example is taxol, a diterpenoid derived from the Pacific yew tree (*Taxus brevifolia* Nutt.) with a high chemotherapeutic value in lung, ovarian and breast cancer (Chang and Keasling, 2006). Taxol precursors are currently produced from plant cell culture and transformed into taxol by chemical synthesis. This is a costly process, given the low yields from plant cell culture. Synthetic biology offers a cheaper, more efficient route to production by assembling complete biosynthesis pathways in *E. coli* and *Saccharomyces cerevisiae* (Weber and Fussenegger, 2009).

Disease prevention

Synthetic biology principles are providing new opportunities for the design of attenuated pathogens for use as vaccines. Wimmer and Paul (2011) described the first synthesis of a virus (poliovirus) in 2002 accomplished outside living cells. They commented on the reaction of lay people and scientists to the work, which shaped the response to *de novo* syntheses of other viruses. In pioneering a safe live vaccine Coleman et al. (2008) synthesised *de novo* large DNA molecules for the rational design of live attenuated poliovirus vaccine candidates. They postulated that this strategy could be used to attenuate many kinds of viruses.

Similarly, the synthetic attenuated virus engineering approach was applied to influenza virus strain A/PR/8/34 for the rational design of live attenuated influenza virus vaccine candidates. Mueller et al. (2010) state that the approach can be applied rapidly to any emerging influenza virus in its entirety, an advantage that is especially relevant for seasonal epidemics and pandemic threats, such as H5N1 or the 2009 H1N1 influenza. During the latter pandemic, vaccines for the virus became available in large quantities only after human infections peaked. To accelerate vaccine availability for future pandemics, a synthetic approach that rapidly generates vaccine viruses from sequence data has been developed (Dormitzer et al., 2013).

The mosquito-borne viral disease dengue fever, including dengue haemorrhagic fever and dengue shock syndrome, is an increasing public health problem, with an estimated 50–100 million new infections each year. Suppression of insect vectors using transgenic insects containing a synthetic gene network could provide pest control by disseminating a conditional flightless female phenotype (a female-specific indirect flight muscle promoter) among natural insect populations (Fu et al., 2010). In future this strategy may control the transmission of malaria parasites and could eventually control the spread of untreatable diseases (Weber and Fussenegger, 2012).

Cancer therapies

With a global mortality rate of 12%, malignant tumours are among the most severe of human pathologies. Surgery remains a common cancer treatment, and when radiation and chemotherapy work, off-target effects on patients can result in considerable damage to healthy tissue. New therapies that exclusively target diseased tissue while leaving normal tissue intact would make landmark changes in cancer treatment. This has been the goal of some synthetic biologists.

Naturally occurring bacteria that self-propel towards tumours have been engineered to invade and proliferate selectively in tumour tissues, produce cytotoxic compounds to kill tumour cells, and contain reporter proteins for non-invasive follow-up on tumour regression (Forbes, 2010). Forbes proposed that synthetic biology techniques can be used to solve many of the key challenges associated with bacterial therapies, such as toxicity, stability and efficiency, and can be used to tune their beneficial features, allowing the engineering of "perfect" cancer therapies. Synthetic virus particles have also been designed that exclusively package therapeutic proteins and can be released in a dose-dependent manner. This approach has been shown to eliminate tumour cells both *in vitro* and *in vivo* (Link et al., 2006).

Pharmacogenomics and personalised medicine

The inability to predict the pharmacology and toxicology of drug candidates in preclinical studies has led to a decline in the number of new drugs that make it to market and to the rise in cost associated with drug development (Gresham and McLeod, 2009). Generally speaking, the challenge is to find the balance between patient benefit, economic value and clinical merit for biomarker-based diagnostics (Jakka and Rossbach, 2013). Today, a majority of drugs in the developmental pipeline have associated biomarker programmes, and the number is likely to increase.

In oncology, genome-based diagnostics are rapidly evolving as many pharmaceutical companies focus on the development of targeted therapies and consider the benefits of a diagnostic test that pairs with a specific treatment. Such tests are showing potential in reducing the costs of clinical trials tremendously (around 60% of clinical trial costs in some cases). A recent report estimates over USD 130 million in savings per approved compound for pharmaceutical companies. Diagnostic tests are likely to be the first synthetic biology health-care products on the market.

Recent synthetic biology health-related projects

Grand Challenges Explorations, an initiative funded by the Bill and Melinda Gates Foundation, supports "creative projects that show great promise to improve the health of people in the developing world". The grants (see Annex A) awarded in May 2012 were on the topic "Apply Synthetic Biology to Global Health Challenges". They exemplify the range of applications of synthetic biology to medical challenges.

There is much work to do before synthetic biology-based health-care solutions find clinical application. Progress on strategies for classical biomedical applications has advanced substantially and may ultimately lead to shorter drug discovery and development timelines, increased precision of drug delivery, and the production of new and more affordable medicines as the human population expands towards nine billion.

In the near term there will be an increasing need to move towards mammalian systems. Most constructs so far have been made in microbes, but moving towards clinical practice will require more complex, clinically applicable circuits, the identification of new mammalian modules and components, and synthesis and characterisation of diverse component libraries (Ruder et al., 2011). The clinical use of these devices and therapeutic scenarios will face the same legal, ethical, regulatory and governance issues as any gene- and cell-based therapy (Weber and Fussenegger, 2012).

Agricultural applications

Bioeconomy strategies envisage the expansion of agriculture both to feed the world's population and to provide the raw materials for bio-based industries, including biomass for fuels. One of the primary drivers of bio-based production is rural regeneration. This expansion will however mean an increasing use of land to produce crops not intended for food or feed and will have to take place against a backdrop of rapid destruction of soil, a trend that urgently needs to be reversed.

Discussions of agriculture and synthetic biology revolve around increasing efficiency to feed more people and accommodate other demands on agriculture. Over the past decades, agricultural efficiencies have increased, and global agriculture has been characterised by policy-induced production surpluses in industrialised countries and stagnating growth in developing countries (OECD/FAO, 2013).

Synthetic biology can play a role, for example in producing crops with higher yields per acre through increased resistance to disease to reduce crop losses. It is important to clarify that there are no synthetic biology applica-

tions in agriculture at present. However, an increasing number of applications of genetic engineering have resulted in safe genetically modified (GM) products in modern agriculture. Table 2.2 shows the top 18 countries (by acreage) of GM crop production in 2012. An excellent searchable approval database on genetically modified organisms (GMOs) is available.[7]

By 2008 GM crops were grown on almost 300 million acres in 25 countries, of which 15 were developing countries (James, 2009). Acceptance and planting of GM crops has continued to increase, but bottlenecks continue to exist in Europe. At present the poorer countries of the world would benefit most from synthetic biology or GM technology. For example, 40 grams of GM Golden Rice a day (modified for the production of vitamin A) are sufficient to prevent the severe health consequences of vitamin A deficiency in rice-dependent poor populations (Potrykus, 2013).

Table 2.2. Land used for GM crops, countries growing 50 000 hectares or more

Country	Million hectares	Crops
Americas		
United States	69.5	Maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash
Canada	11.6	Canola, maize, soybean, sugar beet
Mexico	0.2	Cotton, soybean
Brazil	36.6	Soybean, maize, cotton
Argentina	23.9	Soybean, maize, cotton
Paraguay	3.4	Soybean, maize, cotton
Uruguay	1.4	Soybean, maize
Bolivia	1.0	Soybean
Chile	<0.1	Maize, soybean, cotton
Colombia	<0.05	Cotton
Honduras	<0.05	Maize
Cuba	<0.05	Maize
Costa Rica	<0.05	Cotton, soybean
Europe		
Spain	0.1	Maize
Portugal	<0.05	Maize
Czech Rep.	<0.05	Maize
Romania	<0.05	Maize
Slovak Rep.	<0.05	Maize

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Table 2.2. Land used for GM crops, countries growing 50 000 hectares or more *(continued)*

Country	Million hectares	Crops
Africa		
South Africa	2.9	Maize, soybean, cotton
Burkina Faso	0.3	Cotton
Sudan	<0.05	Cotton
Egypt	<0.05	Maize
Asia		
India	10.8	Cotton
China	4.0	Cotton, papaya, poplar, tomato, sweet pepper
Pakistan	2.8	Cotton
Philippines	0.8	Maize
Myanmar	0.3	Cotton
Australia	0.7	Cotton, canola

Source: International Service for the Acquisition of Agri-Biotech Applications (2012), www.isaaa.org.

So far there is little literature on synthetic biology applications in agriculture. Some obvious areas of interest for agriculture are: reduced water use (crops that use less water); more efficient nitrogen use (less fertiliser); greater disease resistance; more "efficient" plants (increased yield, less production of CO_2). Other, less strategic, applications could include: better quality products (flavour, aroma, colour, anti-oxidant content, altered oil content, improved fibre quality); and improved processing characteristics (high solids tomatoes, high cellulose cotton).

Resistance to drought and other abiotic stresses

Water is the primary limiting factor in global agriculture, yet water availability and quality for crops diminish as cities grow and as irrigation and land-clearing salinise soil and underlying water tables. Humans are expected to appropriate from 70% to 90% of all accessible freshwater by 2025. As agriculture accounts for almost 70% of all human use of water (Sophocleous, 2004), measures to conserve water in agricultural use are of the utmost importance.

Water deficit, salt and other abiotic stresses are exacerbated by global warming and climate change (Fedoroff et al., 2010). Yields of the most important food, feed and fibre crops decline precipitously at temperatures much above 30°C (Schlenker and Roberts, 2009), and water shortages amplify the problem. The 1988 drought in the mid-western United States re-

sulted in a 30% reduction in US corn production and cost about USD 39 billion (Mishra and Cherkauer, 2010). The United States has also just experienced its most widespread drought in more than half a century (Reardon and Hodson, 2013).

A looming gap between water supply and demand calls for major advances in adapting crops to drought and salt stresses through more efficient use of water and increased tolerance to saline soil. Increasing evidence suggests that plants' adaptation to shortage of water and other abiotic stresses is under genetic control and epigenetic regulation, so that the rational design approach of synthetic biology may lend itself to crop modification.

Excess water can also be a problem. Rice is a crop well adapted to wet, monsoon climates and allows farmers to produce food in flooded landscapes. Of the lowland rain-fed rice farms worldwide, over 22 million hectares, representing 18% of the global supply of rice, are vulnerable to flash flooding. Most rice varieties can tolerate only a few days of submergence and die after about a week. Success in fine mapping of SUBMERGENCE 1 (SUB1), a robust quantitative trait locus from the submergence-tolerant FR13A landrace, has enabled marker-assisted breeding of high-yielding rice capable of enduring transient complete submergence (Bailey-Serres et al., 2010).

Reducing fertiliser and pesticide use

Nitrogenous compounds in fertilisers are major contributors to waterway eutrophication and GHG emissions, and the Haber-Bosch process for making fertilisers is very energy-intensive. When the price of Brent crude oil rose from around USD 50 per barrel to about USD 110 by January 2013, the prices for ammonia in western Europe and the mid-western corn belt in the United States roughly tripled.[8] An important goal of synthetic biology research could therefore be more efficient uptake and use of nitrogen in crops.

Although there is plenty of nitrogen in the atmosphere, atmospheric nitrogen is not in a form plants can use. Atmospheric nitrogen must be "fixed" or converted into compounds that make the nitrogen available to plants. Synthetic biologists at Washington University have taken the first proof-of-principle steps towards inserting the genes needed to fix nitrogen (otherwise found only in bacteria and the bacteria-like Archaea) into the cells of crop plants.[9] This opens up the possibility of creating plants that make their own fertilisers. This could revolutionise agriculture and would significantly decouple agriculture from the oil industry.

The first few GM crops that have been widely grown, including insect-resistant and herbicide-tolerant corn, cotton, canola and soybeans, are reported to have increased agricultural productivity and farmers' incomes

(Federoff et al., 2010). They have also had environmental and health benefits, such as decreased use of pesticides and herbicides and increased use of no-till farming (Brookes and Barfoot, 2010). No-till farming of GM crops reduced GHG emissions in 2008 by the equivalent of removing 6.9 million cars from the roads.

Resistance to disease

Sugar cane is a good example of a crop much in demand for different uses, especially its increasing non-food use for biofuels production. Sugar cane is attacked by over 1 500 insect species and over 80 diseases from bacteria, fungi and viruses. *Telchin licus* Drury (the giant cane borer) was recorded for the first time in 2008 in the São Paulo region, the main sugar-growing region of Brazil (Goebel and Sallam, 2011). The larva causes severe damage to sugar cane and significantly reduces biomass and sugar yields, thereby lowering both sugar and ethanol production. The struggle with plant disease is constant and is more difficult for large areas of monoculture. This is an area in which early success with synthetic biology could enhance its reputation and perhaps diffuse some of the political angst associated with genetic modification (Philp et al., 2013).

Furthermore, climate change and global warming are likely to result in changes in the microbial and insect disease patterns in crops (Gregory et al., 2009). Synthetic biology may be able to develop understanding of disease mechanisms and resistance to disease faster than is possible through GM technology. This would improve responsiveness to changing patterns of plant disease under stresses of global warming.

Molecular farming

The use of transgenic plants as bioreactors is relatively new in the biosciences but is gaining some momentum. It involves the genetic modification of the host plant through the insertion and expression of new genes. It can be argued that this approach is in the grey area between genetic modification and synthetic biology but, with the passage of time, projects will arise that appear to be closer to synthetic biology. Products currently being researched for production in plant bioreactors include bioactive peptides, vaccine antigens, antibodies, diagnostic proteins, nutritional supplements, enzymes and biodegradable plastics (Sharma and Sharma, 2009). The other links to the bioeconomy are the potential GHG emissions savings and creation of rural jobs.

For example, Somleva et al. (2008) demonstrated that polyhydroxybutyrate, a biodegradable plastic, can be produced at less cost from switchgrass. This non-food crop has proven amenable to the complex meta-

bolic engineering necessary to produce high-value biomaterials with lignocellulose-derived biofuels as a co-product.

Astaxanthin is a carotenoid found in microalgae, yeast, salmon, trout, krill, shrimp, crayfish, crustaceans and the feathers of some birds. It provides the red colour of salmon meat and the red colour of cooked shellfish. It is employed widely as a component of the feed used by fisheries and poultry farms (Aflalo et al., 2007), but it adds significantly to costs, as synthetic astaxanthin costs some USD 2 000 a kilogramme (Guerin et al., 2003). Non-synthetic sources are limited and extremely expensive.

Recently, Huang et al. (2013) described the engineering of tomato for high-yield production of astaxanthin by expressing a specific pair of algal genes that were identified as the best combination for astaxanthin production from β-carotene. Compared to the microalga *Haematococcus pluvialis,* which needs a well-controlled environment (e.g. growth in an enclosed photobioreactor) for pure culture, tomato is a food crop cultivated cost-efficiently worldwide with very high yields. Therefore, astaxanthin production in tomatoes might be an effective commercial production route for the natural compound.

Conclusion

This chapter should demonstrate why synthetic biology has created the excitement that it has. It has potential applications in a broad range of economic sectors. Moreover, it can be used to address some of the grand challenges facing society: climate change mitigation, energy security, applications in agriculture to address water, soil and food security, improving the health of the world's poor and of ageing populations, and environmental protection. The earliest products of synthetic biology, bio-based chemicals, are now arriving in the market place. The large scale associated with transport fuels is a problem still being addressed. The first synthetic biology food ingredient is due to be released in 2014. Medical applications are clearly going to be in the next generation of synthetic biology achievements. Meanwhile, fuel and chemical applications are also producing the required platform tools and technologies.

Notes

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