Plants: biofactories for a sustainable future?

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Depletion of oil reserves and the associated effects on climate change have prompted a re-examination of the use of plant biomass as a sustainable source of organic carbon for the large-scale production of chemicals and materials. While initial emphasis has been placed on biofuel production from edible plant sugars, the drive to reduce the competition between crop usage for food and non-food applications has prompted massive research efforts to access the less digestible saccharides in cell walls (lignocellulosics). This in turn has prompted an examination of the use of other plant-derived metabolites for the production of chemicals spanning the high-value speciality sectors through to platform intermediates required for bulk production. The associated science of biorefining, whereby all plant biomass can be used efficiently to derive such chemicals, is now rapidly developing around the world. However, it is clear that the heterogeneity and distribution of organic carbon between valuable products and waste streams are suboptimal. As an alternative, we now propose the use of synthetic biology approaches to ‘re-construct’ plant feedstocks for optimal processing of biomass for non-food applications. Promising themes identified include re-engineering polysaccharides, deriving artificial organelles, and the reprogramming of plant signalling and secondary metabolism.

Keywords: biorefining; lignocellulosics; SPPI-Net; synthetic biology

1. Biorefining chemicals from plants

The global economy is currently dependent on fossil feedstocks to generate electricity and heat for industrial and domestic markets and to produce liquid fuels for the transport sector. These fossil reserves drive our global economy but are finite in nature, with the major proven reserves of oil and gas being located in politically unstable, or environmentally sensitive, environments [1]. Also, the declining quality of these reserves requires energy-intensive technologies for their use in conventional petrochemical refineries [1]. In addition to their use in energy generation, heating and fuels, petrochemicals also have a high-value use as feedstocks for the chemical and pharmaceutical industries, being used as raw materials for the manufacture of a wide range of consumer goods

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including plastics, healthcare and drug products, agrochemicals and fertilizers [2]. There is a growing realization that our current industrial manufacturing practices, which are dependent upon fossil oil and gas, will be unsustainable in the coming century [1,2]. Energy production and the growth of the transport sector in developed and emerging economies are placing a burden on our environment, particularly through greenhouse gas (GHG) emissions and their contribution to climate change. GHG emissions are twice their pre-industrial revolution level and, with increasing industrial and economic growth in the BRIC (Brazil, Russia, India and China) countries and other emerging economies, it will be challenging to reduce society’s impact on the environment with a rapidly expanding transport sector and increasing demands for consumer products [1].

There are therefore significant drivers to move all industrial sectors currently dependent on petrochemicals towards a more sustainable manufacturing platform that uses renewable feedstocks from agriculture, forestry and aquatic resources to reduce society’s dependence on fossil-derived energy and raw materials for industrial production [2]. Initially, emphasis has been placed on the development of liquid biofuels from plants. The associated technology whereby an increasingly diverse range of crop products are used for the fermentation of ethanol and to a lesser extent butanol has advanced rapidly from the pioneering use of sugar cane in Brazil to the large-scale adoption of maize and wheat as feedstocks [1,3]. In the course of this rush for first-generation biofuels, a tension has rapidly evolved regarding the ethics of using effectively edible crop products for industrial versus food use [4]. It is clear that such biofuel production is a transitional technology and that instead we must use non-food biomass for fuel production if this technology is to be sustainable in the longer term. Currently, work on developing plants for biofuel applications is concentrating on biomass production for combustion/chemical processing, the use of algae for oil production and the digestion of saccharides in cell walls for fermentation into simple alcohols [3,5]. Concentrating on the last case, the challenges of using cell walls, or lignocellulosics, as starting materials for extracting useful chemicals from plants have been an enormous driver for thinking how else we could use biomass for chemical production. For example, the primary products derived from the plant cell wall which are currently used in biofuel fermentation are derived from its D-glucose (cellulosic) component. It means that, even if we were to achieve efficient digestion of the cellulose for fermentation, we would be left with large quantities of non-fermentable by-products composed of pentose sugars in the hemicellulose fraction, as well as polyphenolics (lignin) and cross-linked proteins [3].

The adoption of lignocellulose as a primary source of biofuel alcohols has renewed interest in the use of plants as feedstocks for multiple non-food applications in the chemicals and materials industry and has established the concept of biorefining [2,6]. In biorefining, we can consider plant biomass as a direct replacement for petrochemicals, from which we can derive all the chemicals needed to sustain a modern industrialized society [2,6]. Interestingly, this concept is far from new: William Hale’s book *The farm chemurgic* extolled a similar use of plants as chemical feedstocks in 1934 [7]. Currently, two lines of research are being developed to use refined plants; namely, thermochemical and biochemical processing technologies [8]. In the thermochemical approach, the dried biomass is largely considered as a relatively uniform hydrocarbon feedstock.
Table 1. Biomass-derived platform chemicals with potential for biorefining applications.

<table>
<thead>
<tr>
<th>number of carbon atoms</th>
<th>BREW report [10]: 21 identified white biotechnology chemicals for production from renewable resources</th>
<th>US Department of Energy report [2]: top 12 building block chemicals that can be produced from sugars via biological or chemical conversions</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>hydrogen</td>
<td>hydrogen</td>
</tr>
<tr>
<td>2</td>
<td>acetic acid; ethanol</td>
<td>acetic acid; ethanol</td>
</tr>
<tr>
<td>3</td>
<td>acrylamide; acrylic acid; lactic acid; 3-hydroxypropionic acid; 1,3-propanediol</td>
<td>glycerol; 3-hydroxypropionic acid</td>
</tr>
<tr>
<td>4</td>
<td>aspartic acid; fumaric acid; 1,4-dicarboxylic acids (succinic, fumaric and malic acid); 3-hydroxybutyrolactone</td>
<td>aspartic acid; 3-hydroxybutyrolactone</td>
</tr>
<tr>
<td>5</td>
<td>arabinol/xylitol; glutamic acid; itaconic acid; levulinic acid</td>
<td>arabinol/xylitol; glutamic acid; itaconic acid; levulinic acid</td>
</tr>
<tr>
<td>6</td>
<td>adipic acid; caprolactam; citric acid; lysine; sorbitol; 5-hydroxymethylfurfural</td>
<td>glutaric acid; sorbitol; 2,5 furan dicarboxylic acid</td>
</tr>
<tr>
<td>complex</td>
<td>polyhydroxyalkonates; natural fats and oils derivatives</td>
<td>polyhydroxyalkonates; natural fats and oils derivatives</td>
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To be treated through either gasification or pyrolysis to produce gases or liquids, respectively, which through further catalytic or biocatalytic processes can be converted to useful platform chemicals [8]. This approach has the advantage of using well-established technologies, though the plant required is both expensive to commission and operate. In the biological process, the plant biomass is digested using a combination of simple chemical processes and enzymes to release low molecular weight monomers, which can then be assimilated into useful chemicals using microbial fermentation [3]. Using either process from a bulk chemical manufacturing standpoint, the platform chemicals required from biomass for subsequent conversion to traditional industrial chemicals via biotransformation or chemical catalysis have been documented in a range of reports and are summarized in table 1 [2,9,10].

Whether using thermochemical or biological processing of the biomass, efforts on biorefining appear focused on reductionist approaches in which the biochemical complexity of plants is reduced to converting the thousands of primary and secondary metabolites to the simple spectrum of compounds shown in table 1. While this approach has obvious advantages with respect to interfacing biorefining to the existing chemical industry, there needs to be a recognition...
that plants, whether terrestrial or aquatic, are also able to produce a variety of important chemical compounds and secondary metabolites with wide-ranging functionalities in their own right, with applications in market sectors ranging from niche pharmaceutical, nutraceutical and cosmeceutical sectors to bulk chemicals, e.g. speciality starches [9]. It would therefore follow that biorefining differs from the refining of petrochemicals in one very important aspect; namely that plants, unlike oil, contain valuable and potentially labile entities within them which need to be isolated from the biomass prior to mainstream degradative processing. Figure 1 provides such a schematic of the current thinking behind the biorefinery concept and follows the process flow from biomass input through to value-added products via biochemical or thermochemical transformation. The development of integrated biorefineries, which incorporate several technologies to generate multiple product through a combination of chemical and biological processes, is now seen to be essential in underpinning this new technology and hastening the move to a competitive bioeconomy [6,8].

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2. Towards integrated biorefining and feedstock improvement

Drawing parallels with refining fossil oils, in order to be competitive and sustainable, biorefineries will have to be highly efficient, produce minimal waste streams, and allow the fractionation of raw materials and recovery of multiple products which can match those currently available from petrochemical feedstocks. In terms of bulk supply, these feedstocks will be primarily derived from terrestrial and marine plants, as these are responsible for photosynthetic primary productivity and carbon fixation in these environments. While these ambitions are theoretically possible, there is a need to introduce innovative processes and products to biorefining and feedstock production, respectively, if we are to realize these ambitions in the coming two decades at a time when mankind is approaching the ‘perfect storm’ of a crisis in global availability of food, energy and water [11]. While this change in paradigm is challenging, history tells us that it is perfectly possible. When mineral oil and natural gas were exploited at the turn of the twentieth century for transportation and energy production, so the science and technology around oil refining rapidly accelerated, with new chemical manufacturing industries emerging. Similar maturation of biorefining can be envisaged in the coming century as the bioeconomy evolves alongside our current dependence on petrochemicals.

The major plant feedstocks currently being exploited for non-food applications are maize in the USA, sugar cane in Brazil and India, cereals and oilseed rape in Europe and North America, and palm oil in southeast Asia. Interestingly, despite the many other crops potentially available for development, it seems likely that this small number of plants will continue to dominate both the food and non-food sectors for the foreseeable future, mainly as a consequence of the associated investment in their agronomy and breeding. Table 2 provides a summary of the major crop species being cultivated in the world today and their global production in 2009. These crops have predominantly been selected and improved by commercial breeders and biotechnology companies to increase yield, protect crops against major agronomic pests, and to improve traits of benefit for food and feed applications. Optimizing yield will remain the primary target for commercial breeders to maximize agricultural productivity per hectare. However, the emerging biorefining industry will have new target traits for crop improvement programmes to increase both the yield of valuable components (e.g. for the chemical industry) and the ease with which these can be extracted from crops. It will be important to establish links between the supply and the demand ends of a biorefining value chain to ensure that communication of desirable feedstock traits by end-users, such as chemical manufacturers, is passed down to seed breeders and biotechnology companies so that new industrial targets can be identified and selected for in future crop improvement programmes [8]. Examples from the sugar beet manufacturing industry have identified how this type of communication has helped commercial breeders to increase the yield of the high-value natural product betaine in high-yielding sucrose varieties of beet. Considering biorefinery process development, there are already encouraging signs of integrative thinking in the industrial sector. For example, here in the UK, the world’s largest beet sugar factory in Wissington, Norfolk, aims to transform all inputs into sustainable products, including the large-scale production of biofuels, the recovery of valuable products such as betaine as a by-product of sucrose extraction, and an imaginative...

<table>
<thead>
<tr>
<th>crop</th>
<th>harvested area (ha)</th>
<th>yield (Hg ha(^{-1}))</th>
<th>crop production (tonnes)</th>
<th>oil/seed production (tonnes)</th>
</tr>
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<tbody>
<tr>
<td>oil palm</td>
<td>14731 230</td>
<td>140 740</td>
<td>207 327 608</td>
<td>413 340 178(^a)</td>
</tr>
<tr>
<td>oilseed rape</td>
<td>31 023 788</td>
<td>19 865</td>
<td>61 630 798</td>
<td>21 222 886(^b)</td>
</tr>
<tr>
<td>sugar cane</td>
<td>23 727 751</td>
<td>709 118</td>
<td>1 682 577 768</td>
<td>27 927 887</td>
</tr>
<tr>
<td>maize</td>
<td>159 531 007</td>
<td>51 219</td>
<td>817 110 509</td>
<td>5 856 396</td>
</tr>
<tr>
<td>wheat</td>
<td>225 437 694</td>
<td>30 248</td>
<td>681 915 838</td>
<td>32 077 505</td>
</tr>
<tr>
<td>rice</td>
<td>161 420 743</td>
<td>42 044</td>
<td>678 688 289</td>
<td>17 526 569</td>
</tr>
</tbody>
</table>

\(^a\)Figure shown is for tonnes of palm oil production.
\(^b\)Figure shown is for tonnes of rapeseed oil production.

... and efficient use of waste heat (http://www.britishsugar.co.uk/Files/Factory PDFs/About-Wissington-Factory-pdf.aspx). With respect to downstream processing of plant feedstocks, enormous efforts are currently being expended in the more efficient digestion of plant biomass. This includes the sourcing of exotic microbes and enzymes that are able to degrade biomass at high efficiencies while being compatible with chemical processing and the development of fermentation routes which are tolerant of the many inhibitory sugars and secondary metabolites released during cell wall degradation [3]. In addition, the repertoire of endproducts that are being produced through fermentation is also being extended beyond the intermediates already identified as starting points for chemical synthesis (table 1). For example, in one process, genetically modified yeast has been engineered to produce bioplastics directly from extracts derived from plant biorefining [12]. As the processing technologies of biorefining are further developed, we will continue to see further extensions to the range of products and an increasing sophistication as to their optimization through metabolic modelling and systems-based approaches [13].

While the progress being made in biorefining technologies is encouraging, the full potential of using plants as feedstocks for multiple industries is fundamentally hampered by harnessing plants in processes and applications for which they have never been optimized. As a result, the precursors of the useful chemicals that we can access through biorefining are often in limiting quantities, difficult to isolate and contaminated with co-extractives which limit their efficient production. An excellent example is seen in the celluloses present in the plant cell wall needed for bioethanol production. These can be readily digested into glucose by the existing cellulolytic degrading enzymes. However, the efficiency in the enzymic degradation of cellulose fibrils in the cell wall is limited by the co-polymerization of occluding hemicelluloses and lignin, with the partial degradation products of these secondary wall products also inhibiting the efficient fermentation of the released hexoses into ethanol [3]. The example of optimizing lignocellulose for biorefining identifies many of the difficulties in using plant feedstocks in new applications. Thus, until recently it was more likely that, as part of breeding programmes aimed at improving yield and stress tolerance in arable crops, modifications to the cell walls would have been directed at traits such as improved...
mechanical strength to reduce lodging and resistance to attack and degradation by pathogenic fungi. As these traits are more likely to involve an increasing degree of cross-linking of cell wall polymers, such improvements would actively work against their digestibility in biorefining applications [3].

While the tradition of genetic improvement of crops for food applications may work against the biorefining agenda in many cases, the technology required to improve plants for non-food applications is already established. For example, varieties of oilseed rape have been bred for high erucic acid production for industrial use [14], while natural variation in the content of the galanthamine in daffodils has been used as the basis for commercial production of this valuable medicinal alkaloid [15]. As an alternative route to crop improvement for non-food applications, genetic modification of multiple new genes into existing crops such as soybean is already well established, with a series of trait improvements planned over the next 10 years [16]. The problem with these approaches is that they all tend to focus on improving the availability and yield of single products and take limited account of how to process the remaining biomass; a key consideration in the development of biorefining feedstocks. As such, instead of these incremental improvements, we need to consider new approaches if we are to accelerate the use of plants for sustainable chemical production and food production in the short time-window we have available to us to meet the ‘perfect storm’ of 2030 [11].

3. Synthetic biology: a new technology for biorefining?

Synthetic biology is an emerging science which can be defined as a new area of research that combines science and engineering in order to design and build (‘synthesize’) novel biological functions and systems [17]. This ability to design biological organisms to serve specific functions clearly has a number of attractions for generating feedstocks for industrial manufacturing processes and for improving microbes for the biotransformation of renewable raw materials into a range of energy and chemical products [18]. In the case of engineering plant feedstocks, the need is particularly urgent, since, as identified in table 2, we are expecting to derive an increasing variety of chemical products from what is essentially a very restricted group of crops. Therefore, we could rationally extend the biochemical diversity present within the germplasm of our major crop species through synthetic biology approaches, such that they can produce a range of bespoke compounds for industrial biorefining. Such systematic re-engineering would involve the introduction of specific metabolic pathways in crops, probably derived from a combination of plants and microbes, which would result in the production of platform chemicals, or precursors, that could also be readily converted into value-added speciality chemicals. While such metabolic ‘re-engineering’ of primary and secondary metabolism would initially seem daunting, exciting processes such as the production of bioplastics in plants using microbial pathways have already been demonstrated [19]. These attempts to produce industrial chemicals in plants have also shown unexpected pitfalls, which will need to be addressed in applying synthetic biology in plants. For example, while bioplastics based on polyhydroxybutyrate chemistry can be produced in plants, this results in autotoxicity and a reduction in crop yield [19].
To date, the greatest success in synthetic biology has been recorded in the re-engineering of micro-organisms, notably to produce novel products or biosensor-signalling pathways [20]. The iGEM competition (http://ung.igem.org/), in which teams of university researchers construct new biological circuits and machines in model organisms such as *Escherichia coli*, has been formative in the development of this emerging science and has driven the concept of the BioBrick—a DNA sequence encoding a component of a metabolic or signalling pathway which can be efficiently assembled with other BioBricks to generate a new operon [21]. Such thinking has led to the assembly of collections of BioBricks as standardized components which can be readily assembled to generate new biological machines. Indeed, the concept of standardizing the toolkits used in synthetic biology based on long observed engineering principles has been central to the emergence of the science [20,21].

In terms of industrial applications, synthetic biology has focused on engineering micro-organisms for pharmaceutical and bioenergy applications. Research being developed by Jay Keasling *et al.* at the Joint BioEnergy Institute at Berkeley has led to the introduction of genes from the Chinese medicinal plant *Artemisia annua* into the yeast *Saccharomyces cerevisiae*, resulting in the high-level production (up to 100 mg l\(^{-1}\)) of the antimalarial drug precursor artemisinic acid [22]. Artemisinin, a drug that shows high efficacy in killing the multidrug-resistant malarial parasite *Plasmodium* spp., can be synthesized from this precursor using established and simple chemistry to form artemisinin as well as a series of related therapeutic derivatives. Importantly, the synthesized artemisinic acid is transported out and retained on the outside of the engineered yeast, providing a way to purify it from other intracellular chemicals via an inexpensive purification process to obtain the desired product [22]. The artemisinic acid-producing yeast were engineered by constructing a 12-gene hybrid biosynthetic pathway using genes from *A. annua*, *S. cerevisiae* and *E. coli*, which has enabled the simple and renewable feedstock glucose to be efficiently converted into terpenoid pathways, which do not normally operate as major routes of metabolism in this host, and be converted by two specific plant enzymes into artemisinic acid. The lessons learned in this project as to the reprogramming of the existing metabolism to efficiently provide precursors for an ectopic pathway have provided a framework for the development of renewable biofuels and high-value chemicals through synthetic biology approaches [23]. Thus, artemisinin is a member of the isoprenoid family of natural products, which includes some 50,000 different types of molecules with applications as pharmaceuticals, nutraceuticals, flavour and fragrance compounds, and fuels. Switching a single enzyme in the artemisinic acid-producing yeast can change the production from artemisinic acid to farnesene, which on hydrogenation produces the highly combustible fuel farnesane, with properties similar to those of diesel [22]. Commercial development of farnesene and fatty acid derivatives as fuel and platform chemicals produced using synthetic biology approaches is being taken forward by the Berkeley spin-out company Amyris [23].

Researchers at the J. Craig Venter Institute (JCVI) are also working towards the development of synthetic organisms that are able to produce various kinds of biological products and renewable fuels. A synthetic version of the bacteriophage PhiX 174 was generated in 2003, and in 2008 researchers at the JCVI successfully synthesized a small bacterial genome. The first synthetic
microbe capable of self-replicating was reported earlier in 2010 by Gibson et al. at the JCVI [24]; the method of construction of this organism is worth describing in some detail, as it illustrates the power of modern molecular biology. The work involved synthesizing a 1.08 million basepair chromosome derived from a modified Mycoplasma mycoides genome, using a yeast assembly system [24]. This synthetic genome was constructed using specific cassettes of DNA that were 1080 bp long. The cassettes were designed so that each end would overlap the neighbouring DNA cassette by 80 bp. The first step in the process to generating the synthetic genome involved taking the 1080 bp cassettes 10 at a time to build 10,000 bp segments, with 110 of these assembled. The second step in the construction of the synthetic genome took the 10,000 bp segments 10 at a time to produce eleven 100,000 bp (100 kb) segments. In the final step, the eleven 100 kb segments were assembled into the complete synthetic genome in yeast cells and grown as a yeast artificial chromosome. The complete synthetic M. mycoides genome was then isolated from yeast cells and transplanted into recipient cells of Mycoplasma capricolum that had the genes for its restriction enzymes removed. The synthetic M. mycoides genome was transcribed into messenger RNA, which was then translated into new proteins, with the host genome either destroyed by M. mycoides restriction enzymes or lost during cell replication. After 2 days, viable M. mycoides cells containing only synthetic DNA were visible on Petri dishes [24]. The approach developed in this study, whereby artificial genomes can be constructed to carry out specific metabolic functions, is a significant step forward and opens new opportunities for the application of synthetic biology to design micro-organisms that can efficiently produce industrial products such as biofuels, vaccines and pharmaceuticals [23,24]. In addition, such customized organisms could be deployed in bioremediating recalcitrant pollutants, or in desalinization applications to provide clean water for food and agriculture [24].

With respect to the links with biorefining, the pioneering work in synthetic biology at Amyris and JCVI demonstrates how renewable simple feedstocks, which could be derived from plants (e.g. glucose), can be converted into a series of high-value products of direct application to the chemical and pharmaceutical industries. Indeed, the ability to use engineered microbes in fermentation applications to produce an ever wider range of endproducts will be a major feature of the biorefinery of the future. In addition, the fact that this work has been sponsored by the private rather than public sectors also points to the increasing role of industry in driving the associated innovation.

4. Future perspectives: applications of synthetic biology in next-generation biorefining

The advances in synthetic biology directed towards industrial chemical production in microbes now points to similar approaches being applied for the improvement of plants for biorefining applications. Clearly, the challenges of engineering plants using the approaches adopted for microbes are considerably more complex. However, we have already established efficient methods for the routine genetic transformation of the majority of our major crops and, using these technologies, have made major advances in our understanding and manipulation
of plant primary and secondary metabolism. Clearly, a good starting point for synthetic biology in plants would be the manipulation of single-celled algae [25]. There is already considerable interest in using algae as a source of oils for biofuel applications, and the recent discovery of the associated pathways leading to alkane production [25] is a particularly exciting development for synthetic biologists in terms of producing feedstocks with similar performance characteristics to petrochemical products. While industrial chemical production in algae offers many possibilities in the future, the current focus for biorefining is naturally focused on terrestrial biomass and crops. As identified in the drivers for moving towards integrated biorefining, the ideal plant feedstock should be amenable to efficient processing towards the manufacture of a range of products (both bulk and speciality chemicals) and produce minimal waste streams. Depending on their chemical composition, such plants could be specialist ‘non-food’ industrial crops, which are segregated from the food and feedchain. For example, biomass plants such as *Miscanthus*, willow and poplar currently grown for energy production would represent such a platform for producing products which should not enter the food supply chain. Perhaps, more realistically, in view of the continuing pressure on our agricultural supply chain, the ideal plant engineered through synthetic biology would render edible food products in its grain or tubers, while vegetative tissues (straw, stems) would be separately harvested and converted into chemicals through biorefining.

With these objectives in mind, as part of a national competition in 2008 sponsored by the UK’s research councils, it was proposed to establish a network in synthetic biology to use this rapidly developing technology to accelerate the development of plants for industrial biorefining applications. The network, termed ‘Synthetic Plant Products for Industry’ or SPPI-Net, was convened in 2008 and since then has organized a series of workshops attended by industrialists and academics to develop interdisciplinary science themes and projects (www.sppi-net.org).

Recognizing the complexity of the task, the SPPI-Net steering group, aided through a formative initial workshop, developed four themes to advance the improvement of plants for biorefining though synthetic biology. These related to constructing synthetic signalling pathways, engineering metabolism through compartmentalization, improving polysaccharide composition and diversifying secondary metabolism (figure 2).

We have proposed using synthetic biology approaches to engineer synthetic signalling pathways *in planta* (figure 2). This approach could use a composite of signalling components from plants, animals and microbes to selectively drive the expression of specific metabolic or developmental pathways. For example, a scenario whereby one could control the type of endproduct being laid down during grain filling (oil versus starch) based on market forces and food security issues would be a very attractive trait to engineer into several of our major crops (table 2). Again, while this concept is in its infancy, we already know that plants are highly specific in their responses to a chemically diverse range of compounds, including synthetic compounds not found in nature [26].

The theme of engineering plant metabolism by controlling compartmentalization is a very interesting approach to obtain major changes in plant products essentially through altering development pathways. For example, the recent work on artemisinin production in *A. annua* has shown the power of increasing the
Figure 2. Themes to advance the improvement of plants for biorefining applications. (a) Synthetic signalling—methods which could be adopted under this theme area include: generation of synthetic chemical switches that do not exist in nature and can respond to unique xenobiotics or artificial transcription factors with altered binding characteristics. Potential applications could include the switching between assimilation of lipids and carbohydrates for biofuels production. Using operon-like BioBrick approaches to engineer signalling and metabolism networks could also be taken forward in tractable organisms such as algae. (b) Synthetic organelle—metabolism in the plant is highly compartmentalized, making it an attractive target for new synthetic pathway engineering. Two approaches could be envisaged, as follows. Top-down—potential for engineering self-replicating organelles such as chloroplasts and mitochondria. Production of organelles with a minimal genome would provide a platform to engineer ‘biofactories’ synthesizing desired industry products. Bottom-up—opportunity to make new synthetic ‘proto-organelles’ with raw materials and DNA that is not derived from living cells. (c) Cell wall polymers—production of plant cell wall polymers for commercial applications has potential in the chemical and material industries. Current applications have focused on the deconstruction of plant cell wall material for biofuel production. However, there are future synthetic biology approaches with potential for the production of natural and synthetic polymers with novel and well-defined performance characteristics. (d) Harnessing chemical diversity—the engineering of the artemisinin pathway in yeast has illustrated the potential for synthetic biology approaches in plant secondary metabolism. The modular construction of secondary metabolic pathways is well established in prokaryotes, but less well developed in plants that provide approximately 25% of all registered pharmaceuticals. There is potential to take forward the advances that have been made in microbial and fungal engineering towards future plant-based production systems. (Online version in colour.)
production of this pharmaceutical through using a directed breeding programme to enhance the abundance of the organs (trichomes) responsible for synthesizing and accumulating this compound on the leaf surface [27]. Through a similar line of thinking at the subcellular level, virtually all primary and secondary plant metabolisms are organized within specific compartments; therefore, engineering their relative types and abundance will have major effects on the types and diversity of endproducts. Following on from this approach, we have also considered the possibility of generating synthetic organelles which have been designed for the efficient production of one type of industrial feedstock. Essentially two approaches could be envisaged. First, a top-down strategy in which existing organelles are stripped down of all non-essential components, which could divert metabolism away from the desired endproducts. This approach would be best applied to organelles that contain at least some of their own DNA for replication, facilitating their direct engineering, with the plastid being an obvious target. Interestingly, the plastid has already been successfully engineered to massively overproduce proteins by taking advantage of its microbe-like characteristics in transformation and expression [28]. Another attraction of using the plastid is that this organelle can support the operation of bacterial operons, allowing the coordinated activation of microbial biosynthetic pathways [28]. In the other approach, a bottom-up assembly process would be envisaged, in which the organelle effectively auto-assembles itself. Again, while the development of metabolically functional minimal vesicles is currently a scientific curiosity driven by studies examining potential routes to the origin of life, the commercially useful overproduction of proteins in plants using auto-assembling protein bodies has recently been reported [29].

With respect to improving polysaccharide composition, plants are consummate producers of sugar polymers, yet most of our current attention has been turned towards their deconstruction to produce fermentable intermediates for biofuel production. Current studies on engineering cell wall composition have already shown that it is possible to greatly enhance the extractability and saccharification of cellulose from plants by conventional genetic modification approaches, such as reducing xylan content or altering the availability of lignin intermediates [3]. Such deconstruction technologies will be useful in the future in helping recover other high-value polymers produced through synthetic biology from the cell wall; for example, biomedical polysaccharides derived from animals or industrial matrices from microbes. Another area of promise for synthetic biology approaches is the engineering of speciality starches, a group of compounds which have already found wide usage in non-food applications.

The use of synthetic biology to diversify plant metabolism, notably of secondary metabolites, is one of the more accessible themes with many of the required technologies already established in microbes. Basically, the approach adopted is to produce a novel chemical scaffold formed through a core biosynthetic pathway and then allow the intermediates produced to be modified by the host cell to a series of new endproducts. This strategy maps onto the process of metabolic diversification in secondary metabolism in the natural world and is compatible with all the major groups of natural products (polyketides, phenolics, alkaloids, terpenoids). A notable recent example has been the generation of novel biomedicinal tropane alkaloids based on the expression of a mutated scaffolding enzyme, strictosidin synthase [30].
While much of the potential application of synthetic biology approaches in plant improvement is currently speculative, one could argue that most of the technology required is already well developed. We already know how to efficiently transform many of our major crops with multiple transgenes to produce novel products. However, unlike the work undertaken in microbes, we do not yet have the equivalent of standardized BioBricks to rapidly develop a truly synthetic biology approach to crop improvement. There is therefore a need to invest in the underpinning technology to develop research tools, which will enable the industrial application of synthetic biology approaches in plants in the same way that this emerging technology has already demonstrated its potential in yeast and bacteria. It is also clear that we will need to carefully consider the life cycle benefits of adopting plant-based production methods for chemicals, as well as ethical questions concerning the diversion of crop resources away from food production, and food safety issues. In Europe, in particular, several non-governmental organizations have already identified synthetic biology as a threat to food security and sustainability, and these concerns will need to be carefully considered and the risks versus benefits of moving to plant-based chemical production platforms assessed. Interestingly, set against this backdrop, as we have recently observed in stem cell science, the major drivers for advances in synthetic biology and its application may actually come from the private rather than the public sectors.

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