Abstract

Thirty years after the production of the first generation of genetically modified plants we are now set to move into a new era of recombinant crop technology through the application of synthetic biology to engineer new and complex input and output traits. The use of synthetic biology technologies will represent more than incremental additions of transgenes, but rather the directed design of completely new metabolic pathways, physiological traits, and developmental control strategies. The need to enhance our ability to improve crops through new engineering capability is now increasingly pressing as we turn to plants not just for food, but as a source of renewable feedstocks for industry. These accelerating and diversifying demands for new output traits coincide with a need to reduce inputs and improve agricultural sustainability. Faced with such challenges, existing technologies will need to be supplemented with new and far-more-directed approaches to turn valuable resources more efficiently into usable agricultural products. While these objectives are challenging enough, the use of synthetic biology in crop improvement will face public acceptance issues as a legacy of genetically modified technologies in many countries. Here we review some of the potential benefits of adopting synthetic biology approaches in improving plant input and output traits for their use as industrial chemical feedstocks, as linked to the rapidly developing biorefining industry. Several promising technologies and biotechnological targets are identified along with some of the key regulatory and societal challenges in the safe and acceptable introduction of such technology.

Key words: Biofuels, biorefining, input traits, output traits, metabolic engineering, policy and regulatory frameworks.
concerns have reawakened an interest in renewable plant feedstocks.

The global scale of organic photosynthesis in plants is truly staggering, with 105 billion tonnes of carbon fixed naturally per annum, directly leading to the production of 210 billion tonnes of biomass (Kircher, 2012). Agriculture accounts for 7% of the total fixed carbon, with the majority directed into the synthesis of biopolymers, notably cellulose (40–55%), hemicelluloses (10–35%), and lignin (18–41%). In contrast, a smaller proportion of carbon flux is directed into non-fibrous natural products such as oils, proteins, sugars, and secondary metabolites, most of which are destined for the food and feed industries. These figures illustrate that, while plants are highly efficient in fixing carbon, the major sinks are chemically limited in terms of their diversity and accessibility, being in the form of insoluble polymers. To date, much plant biotechnological effort has been directed into making cell-wall components more accessible to downstream processing and hydrolysis to fermentable feedstocks (Vanholme et al., 2010). Perhaps a more fertile line of research would be to fundamentally re-engineer plant physiology and metabolism to divert just a small proportion of this fixed carbon into biopolymers with the rest directed into a more diverse range of metabolites which could be readily extracted and fed into existing chemical processing platforms.

To achieve the fundamental re-engineering of the primary metabolism of plants to render them better industrial feedstocks may ultimately be achieved using conventional approaches based on plant breeding or genetic modifications of existing crops. To this end, while such a goal is achievable, the route to its delivery will be long and torturous. Perhaps instead we should now be considering using novel approaches such as synthetic biology (SB) to construct new photosynthetic plant chasses (the SB term for the biological host which is the subject of re-engineering; Bubela et al., 2012) that are tailor made for the directed manufacture of specific chemicals and materials. While the latter proposition is clearly a major challenge, the development of useful traits in existing crops to meet future industrial needs is far from trivial. For example, to maintain the plant oil supply to the food sector and at the same time to replace 40% of fossil oils used by the chemical industry renewable oil production will have to triple in next 20 years, by a combination of both enhancing crop yield per hectare by 50% and by tripling oil content per plant (Carlsson et al., 2011). In contrast, crops that have been rationally re-engineered in form and metabolism using SB-based approaches to divert as much fixed carbon as possible directly into industrial oils could require a much smaller footprint in global agriculture and be less disruptive to food supply chains.

Biotechnological targets for plant synthetic biology: biorefining

A public dialogue around the development and applications of SB clearly identified areas where there was consensus that this was seen to be a useful emerging technology and where there was concern, or even hostility, regarding its development and adoption (TNS-BMRB, 2010). A clear message was the unease over using SB-based approaches to engineer or process foods. Bearing this in mind, it would seem far more sensible at this time to use the engineering principles of SB to improve plants for industrial applications in preference to altering crops which are to be used in the food chain. As a nascent technology, SB has a current focus on synthetic genomics, metabolic pathway engineering, minimal genome organism, protocols, and xenobiology. Typically these objectives are directed at engineering at the cellular or subcellular level, typically using microbes as the chasses. Applications to date have considered all major biotechnology sectors (Fig. 1). With respect to green biotechnology, effort has tended to focus on biofuels and biorefining, typically through improving microorganisms in their biotransformation of renewable feedstocks into a range of energy and chemical products (Connor and Atsumi, 2010).

The challenges of biorefining plants into chemicals using current technologies illustrate the need for new approaches, including SB (Jenkins et al., 2011). Thus, while significant progress has been made in chemical and biochemical engineering, the full potential of using plants as feedstocks for multiple chemical/material applications is fundamentally hampered by having to use crops which have been not been optimized for industrial applications. Major challenges include:

1. All major food crops have been selected for desirable nutritional properties, which has resulted in a reduction in the diversity of chemicals available for refining as compared with non-domesticated pro-genitor species.
2. Useful chemicals that can be accessed through biorefining are often in limited quantities, difficult to isolate, and contaminated with co-extractives which limit their efficient production.
3. Natural chemicals are also different from many of the synthetics commonly used as precursors and intermediates in industry. For example, natural chemicals have broader distributions of mass, greater diversity of ring systems, more chiral centres, more oxygen, and less nitrogen, sulphur, and halogen (Mitchell, 2011).
4. Agricultural systems use a very small range of crops: in addition to limiting the biochemical diversity available to refine from, this also causes potential issues of plants raised for non-food applications entering the food chain.

As a consequence of these challenges, at the moment biorefining uses a reductionist approach in which the biochemical complexity of plant metabolism is reduced through chemical conversion or fermentation to a spectrum of compounds similar to their petrochemical analogues (Edwards et al., 2011). While this rendering down has obvious advantages in allowing end products to directly feed into existing chemical industries, it is inherently inefficient in thermodynamic terms and does not play to the natural ability of the plant world in generating useful chemical scaffolds. Plants are sources of high-value natural products and produce a rich diversity of secondary metabolites comprising of more than 200 000.
structures with a complexity that matches and in some cases exceeds synthetic chemistry (Hartmann, 2007). Many plant secondary metabolites are too complex for chemical synthesis, with their value as pharmaceuticals, nutraceuticals, or fine chemicals inversely proportional to their abundance. This makes biomass fundamentally different from fossil feedstocks because plants, unlike oils, contain valuable and potentially labile entities within them which need to be isolated prior to degradative biorefining. As a result, plant SB applied to biorefining faces a 2-fold challenge: optimizing the technology to increase selectivity and efficiency, and improving yield and diversity of value-added products in industrial plant biomass.

To date, the greatest success in deriving value from plants using SB has been the engineering of microorganisms to express their natural product pathways to produce pharmaceuticals, fine and bulk chemicals, and fuels through the process of fermentation. One of the best-known examples of such engineering is the production (up to 100 mg l\(^{-1}\)) of the sesquiterpenoid artemisinic acid in yeast, with the derived compound artemisinin a major frontline drug used in the treatment of malaria. Genes taken from the medicinal plant \textit{Artemesia annua} (the natural source of artemisinin) and \textit{Escherichia coli} were used to reprogramme the host’s metabolism to efficiently provide terpene precursors (Ro et al., 2006). The approach developed to support large-scale artemisic acid biogenesis was then adapted to produce other members of the isoprenoid family, which represents some 50 000 different types of molecules with a wide diversity of potential applications, including uses as pharmaceuticals, flavour and fragrance compounds, and fuels. By switching a single enzyme in the artimisic acid biosynthesis pathway of the engineered yeast, the associated pathway was switched from producing a pharmaceutical intermediate to the biofuel precursor farnesene (Steen et al., 2010). Microbes have also been engineered using SB-based approaches for the consolidated bioprocessing of lignocellulosic biomass in a way that enzyme generation, biomass hydrolysis, and biofuel production are combined into a single organism. Such a consolidation strategy offers significant economic advantages over conventional biorefinery processes where biomass is first treated with hydrolytic enzymes, or chemicals, to release soluble fermentable sugars which can then be turned into biofuels through fermentation. Using such a consolidated approach, \textit{Escherichia coli} has been engineered to bioprocess lignocellulose plant biomass into the advanced biofuels pinene, butanol, and fatty acid ethyl ester (Bokinsky et al., 2011). While consolidated processing of lignin components in plant biomass is still at an early stage of research, current work on processing the carbohydrate component of the cell wall demonstrates the potential of synthetic biology in solving an important aspect of the lignocellulosic bioprocessing challenge. Such approaches...
in engineering consolidated pathways will no doubt benefit from the bottom-up factoring of specialist microbes based around minimal genomes to efficiently produce industrial products (Schirmer et al., 2010).

The advances in SB directed towards creating tailor-made microorganisms now point to similar approaches being applied for the improvement of plants as a renewable feedstock. Clearly, the challenges of engineering plants using the approaches adopted for manipulating microorganisms are considerably more complex and to date there are no equivalents of standardized BioBricks to rapidly engineer eukaryotic cells. There is, therefore, a need to invest in the underpinning technology to develop research tools, which will enable the industrial application of SB approaches in plants in the same way that this emerging technology has by now demonstrated its potential in yeasts and bacteria. However, there are already established efficient methods for the routine genetic transformation of the majority of agricultural crops, including methods for engineering complex pathways using ‘gene stacking’ approaches (Halpin, 2005). Global agriculture is also now well accustomed to the mass cultivation of transgenic plants with 170 million hectares currently dedicated to genetically modified crops (Marshall, 2013). With these objectives in mind, in 2008 the UK’s research councils funded the Synthetic Plant Products for Industry Network (SPPI-Net) to accelerate the development of plants for industrial biorefining applications. The network identified four themes which should be prioritized to advance the improvement of plants for biorefining, though synthetic biology: (1) constructing synthetic signalling pathways; (2) engineering metabolism through compartmentalization; (3) improving polysaccharide composition; and (4) diversifying secondary metabolism (Jenkins et al., 2011).

Since the early use of SB will almost certainly be a transition from conventional genetic modification, it is useful to review how transgenesis has been successfully used to improve plants over the last 20 years. To date, genetic modification has largely been used to engineer input traits into crops to drive greater yield efficiency and resilience in production. Notable examples include the development of Roundup Ready glyphosate-resistant soybean and maize and Bacillus thuringiensis-engineered cotton: advances that have revolutionized crop protection around the world. In contrast, the engineering of output traits such as Golden rice and long-life tomatoes have had a more difficult route to market and global acceptance. With respect to arable and horticultural production, it would seem unlikely that SB will result in any paradigm shift in consumer acceptance of engineered crops destined for the food chain. Rather, the initial potential for SB in plant biotechnological applications lies in engineering input and output traits in non-food crops and potentially even in generating wholly new plant varieties/species for specialized chemical and biomaterial production. Indeed, the generation of specialist ‘industrial’ plants which can be separated from crops destined for the food chain at the point of harvest and processing would seem to be a vital prerequisite to fully realize the potential of genetic and SB engineering of feedstocks for efficient biorefining and other industrial applications. Some of the promising targets for SB-based engineering of input and output traits are shown in Fig. 2, with some recent interesting developments and advances discussed in the following sections.

**Synthetic biology and input traits**

As the most fundamental plant input trait, photosynthesis itself has been identified as a target for SB re-engineering, based on the maximal theoretical conversion efficiencies of solar energy to biomass currently limited to 4.6 and 6% for C3 and C4 plants, respectively (Zhu et al., 2008). Many research programmes have attempted to re-engineer photosynthetic pathways in C3 plants, using components taken from crassulacean acid metabolism, or C4 pathways (e.g. phosphoenolpyruvate decarboxylase, pyruvate orthophosphate dikinase, phosphoenolpyruvate carboxykinase, NADP-dependent malic enzyme, NADP-dependent malate dehydrogenase), in an attempt to improve energy-conversion efficiency (Rosgaard et al., 2012). Alongside primary synthetic pathways, there has also been considerable interest in enhancing carbon fixation by re-engineering ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Approaches adopted include increasing the rates of catalysis of carboxylation as well as reduction of the oxygenation reaction catalysed by the enzyme, adjusting its activation state, suppression of the oxygenase reaction by increasing the localized concentration of CO2, and development of the natural regeneration phase of the Calvin cycle (Ducat and Silver, 2012). In another approach, the location of the genes encoding the subunits of RuBisCo (rbcL and RbcS) have been consolidated by applying a synthetic fusion strategy (Whitney et al., 2009). Overall, these studies have shown the difficulties in improving the carbon-fixation characteristics of RuBisCO in isolation. As an alternative approach, other carboxylating enzymes have been considered to drive photosynthesis. Using a computational approach to evaluate alternative routes to carbon fixation, 5000 carboxylating pathways identified from a Kyoto Encyclopedia of Genes and Genomes analysis were compared in terms of maximal fixation activity, NADPH and ATP consumption, and thermodynamic and topological capability. Intriguingly, a novel variant of the C4 pathway was identified, which was modelled as supporting a 3-fold-enhanced CO2-fixing capability for phosphoenolpyruvate carboxylase (Bar-Even et al., 2010). This interesting piece of work points to a new and powerful approach to re-engineer photosynthesis based on fundamental chemical principles applied through SB technology.

Continuing on the theme of engineering primary inputs to plant growth, nitrogen fixation is also now seen as a target for SB engineering, not in the least because of the cost and environmental footprint of using chemical fertilizers. Recently considerable progress has been made in understanding the signalling events underpinning the formation of N2-fixing nodules, so that this trait may ultimately be transferred to non-legume crops (Oldroyd and Dixon, 2014). In addition to the classic N-fixing *Rhizobium* species involved in nodulation, attention is also being directed to other N-fixing prokaryotes belonging to the *Azospirillum, Klebsiella,* and *Frankia* genera.
In terms of generating new tools for engineering N-fixing pathways, a ‘refactoring’ methodology has been developed in *Klebsiella oxytoca* allowing complete control over all of the functions encoded by the 20-gene cluster responsible for reducing atmospheric nitrogen into ammonia. Even though the synthetic cluster showed only 0.3% of the activity of the most closely related naturally evolved pathway, this research has demonstrated the potential of SB to re-engineer the complex biology that underpins the N-cycle (Temme et al., 2012).

In addition to nutrient input traits, SB has the potential to provide crop science with new traits to offset the increasing diversity of abiotic (environment), biotic (pathogens, herbivores), and xenobiotic (pollutants) stress which are now affecting agriculture. In particular, SB could provide a route to greater resilience in agriculture faced with an increasingly extreme and variable climate and associated pressures resulting from invasive pests and diseases. Two major SB strategies could be envisaged being deployed: one involving engineering the stress response systems themselves and the other reprogramming of associated signalling networks. As a logical extension, such synthetic signalling and defence networks would ultimately be used in combination to provide new defence mechanisms which may not have yet evolved in any single plant. Recent papers have suggested the potential utility of such approaches. For example, the transfer of the electron-shuttling flavoprotein flavodoxin from the cyanobacterium *Anabaena* into the plastids of tobacco to functionally replace the host’s ferredoxin carrier protein rendered the transgenic plants more stress tolerant (Zurbriggen et al., 2008). Similarly, an SB approach has been applied to use mitogen-activated protein-kinase signalling modules to reprogramme a range cellular and physiological responses (Šamajová et al., 2013). The ability to precisely engineer plant genomes with the aid of synthetic site-specific nucleases has recently been shown to be very successful in designing plants tolerant to biotic and abiotic stresses (Kathiria and Eudes, 2014). These site-specific ‘genome editing’ advanced techniques include transcription activator-like effector nucleases and zinc-finger nucleases. Applications include the selective engineering of resistance to *Xanthomonas oryzae* in rice (Li et al., 2012) and tolerance to imidazolinone and sulphonylurea herbicides in tobacco (Townsend et al., 2009). These tools are also proving useful in generating designer genomes for further metabolic engineering, as demonstrated by the introduction of regularly interspaced short palindromic repeats (CRISPR) in rice and wheat (Shan et al., 2013).

**Synthetic biology and output traits**

Output traits of interest to biorefining applications include the rational construction of crops that produce high yields...
of useful intermediates, which can then be readily used by existing chemical industries, requiring lower inputs of energy and materials in their processing and also with a usable set of byproducts to facilitate the move towards zero waste refining. The latter is an important point when introducing a disruptive technology in competition to the well-established and highly efficient oil-refining industry. As discussed earlier, these overall goals in producing ‘process-ready’ industrial crops effectively means diverting an increasing proportion of plant metabolism away from lignocellulose production into soluble, or readily extractable, primary and secondary metabolites. In terms of understanding the potential for diverting C into alternative sinks, a metabolic flux analysis had shown that, during steady plant growth, 80–85% of carbon is directed into sugar biosynthetic pathways (predominantly into cell-wall biosynthesis), along with smaller proportions diverted into the production of soluble polysaccharide and nucleic acids. Of the remaining total C-flux, 10 and 5% carbon are used in fatty acid and terpenoid biosynthetic pathways, respectively, mostly in the synthesis of essential membrane and photosynthetic components (Melis, 2013). The SB approach to be adopted should, therefore, be a push–pull strategy by which the sink represented by carbohydrate synthesis directed into the cell wall is reduced, thus enabling a new need for the released flux relating to industrial intermediate formation.

With respect to generating the ‘push’, to release more carbon into industrially useful primary metabolism would point to the directed re-engineering of the plant cell wall such that its essential structural and defensive roles are not compromised, while requiring less commitment in metabolic input. To date, attempts to remodel the cell wall have centred on improving the extractability and saccharification of cellulose from plants by conventional genetic modification of cell-wall composition. Approaches have concentrated on regulating monolignol biosynthetic genes, which ultimately control lignin content and composition and, in several instances, have led to deleterious phenotypes such as dwarfing, vascular collapse, or other abnormalities presumably caused by changes in cell-wall rigidity (Bonawitz and Chapple, 2013). To overcome this, Loqué and colleagues have recently reported using a systematic SB approach to decrease total lignin content in Arabidopsis cell walls, while maintaining the integrity of the vascular vessels (Yang et al., 2013). This was achieved by effectively reprogramming the metabolic network responsible for lignification through changing promoter-coding sequence associations and in doing so creating an artificial positive feedback loop (APFL) to enhance secondary cell-wall biosynthesis in very specific tissues. The first stage included a replacement of the promoter of the key gene, cinnamic acid 4-hydroxylase, with the vascular-vessel-specific promoter which is regulated by the transcription factor VND6. This lead to lignin biosynthesis relating to vessel formation being disconnected from the less-specific cell-fibre regulatory network. The second stage involved creating an APFL by using the promoter of the IRX8 gene, which encodes a glycosyltransferase essential for secondary wall synthesis, to express a new copy of the fibre transcription factor NST1. This two-stage modification resulted in enhanced polysaccharide deposition without excessive lignification. This directed approach showed promise as a means of further ‘pushing’ metabolic flux from the cell wall and into other potentially more useful pathways.

To create the metabolic ‘pull’ for industrial product formation, one emerging approach is to coordinately introduce a set of specific enzymes to interface directly with the Calvin cycle such that related metabolic intermediates, such as 3-phosphoglycerate, glyceraldehyde-3-phosphate, and fructose-1,6-bisphosphate, can be converted into industrial intermediates. An introduction of such ectopic branch points into novel sinks has the potential to increase the flux of output carbon from the cycle and enhance overall photosynthetic efficiency and, if designed effectively, could minimize the normal flux distribution of fixed carbon in cells (Ducat et al., 2012). Initially the concept has been proven using the cyanobacterium Synechococcus elongatus PCC 7942, engineered to produce the industrial intermediate 1,2-propanediol synthesis from CO₂ by building an NADPH-dependent pathway channeling the Calvin cycle intermediate dihydroxyacetone phosphate, with optimal yields of 150 mg l⁻¹ of the diol obtained (Li and Liao, 2013). It has also been proposed to link photosynthesis directly to introduce a synthetic pathway to butanol production (Lee, 2013) by direct coupling to the Calvin cycle to facilitate synthesis of this valuable biofuel and industrial intermediate (Fig. 3).

With respect to higher-value metabolic pathways, one of the more promising areas to apply SB in the immediate term lies in the engineering of secondary metabolism, with many of the required technologies already established in microorganisms. One of the more successful strategies adopted to date has consisted of producing a novel chemical scaffold formed through a core biosynthetic pathway and then allowing the new intermediate to be modified by the host cell to a series of new endproducts. Such an approach is compatible with all the major groups of plant natural products (polyketides, phenolics, alkaloids, terpenoids), with a notable example being the generation of novel biomedicinal tropane alkaloids based on the expression of a mutated scaffolding enzyme, strictosidine synthase (Runguphan and O’Connor, 2009). In terms of engineering other premium plant pathways, another interesting approach has been to use SB to directly link light-driven oxidoreductive chemistry to reactions catalysed by the haeme-containing cytochrome P450s (Jensen and Møller, 2010). The strategy adopted has involved the direct coupling of the electron transport of photosystem I (PSI) to P450-catalysed monooxygenation, in place of the reducing potential provided by NADPH via cytochrome P450 reductase. In plant cells, the membrane-bound P450s are typically localized in the endoplasmic reticulum in the cytoplasm, while PSI operates in the stromal lamellae of the chloroplasts. Through colocalization of the two pathways and the concerted re-engineering of electron transport carriers, the research group at University of Copenhagen is currently working on coupling PSI directly to a cytochrome P450 to develop a system in which oxidative catalysis is driven directly by the energy of solar light. They showed the possibility of combining biosynthetic pathways in vitro as well as in vivo, reassembling and
configuring new biosynthetic systems which do not exist in nature (Jensen et al., 2012).

In addition to using SB to engineer industrial products, another useful output trait which has been the focus of this technology relates to using plants in new biomonitoring applications to detect chemicals (e.g. pollutants, explosives) or pathogens as well as bioremediators. Plants have an innate ability to continuously sense and respond to the environment using signal transduction systems based on the recognition of small molecules. By introducing new sensor or reporter systems, these responsive signalling networks can be adapted using SB to provide new functionalities for sophisticated and low-cost applications in environmental detection. For example, it has been possible to computationally redesign periplasmic binding proteins which have a capacity to detect virtually any compound, including synthetic compounds such as explosives and pollutants, and then couple this recognition to histidine kinase-mediated signalling pathways that control the expression of reporter genes. For example, a synthetic degreening circuit that produced rapid chlorophyll loss upon presence of a specific input was constructed, which showed visible bleaching within 2 h after signal perception (Antunes et al., 2006). These first prototype detector plants have the potential to detect foreign compounds in soil as well as in air, allowing their potential use in a variety of remediation and security applications (Antunes et al., 2011).

Fig. 3. The engineering of plant metabolism to generate synthetic intermediates: proposed redirection of the Calvin cycle to interface with synthetic pathways leading to production of 1,2-propanediol (A; Li and Liao, 2013) and butanol (B; Lee, 2013).

Regulating the translation of synthetic biology innovations from laboratory to field

While the potential applications of ‘full-blown’ synthetic biology approaches in plant improvement are currently speculative, extreme genetic engineering and much of the technology required to deliver it already exist. Even though plant synthetic biology is still at the blue-sky stage, it is important to develop essential regulatory frameworks now if the technology is to be commercialized in the foreseeable future. On performing a SWOT (strengths-weaknesses-opportunities-threats) analysis of using synthetic biology to improve plants as renewable feedstocks (Table 1), it is clear that alongside technological and economic challenges, there are both societal and political obstacles to be overcome. In Europe, 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 from the conventional to the novel (i.e. synthetic) plant biomass platform need to be assessed. In particular, while an individual organism can be precisely engineered to exhibit new traits under conditions of containment, their behaviour in a more chaotic natural environment may be less predictable. As such, both the scientific and business communities have to take safety, security, ethics, and public perception into consideration and work together with governmental and non-governmental organizations to realize the potential and mitigate risks of any emerging technology. There is no doubt that biotechnology, either white or green, that uses SB-based approaches has to be tightly regulated. The question is whether there is the need for a related dedicated regulatory framework or, rather, whether the existing regulations can be adapted to cover SB-developed traits. There are three grounding principles of regulatory responses to new technologies (Bubela et al., 2012):

1. Proportionality: a proportional balance of risks to health and the environment against potential commercial benefits of research and novel technologies.
(3) Procedural justice: considering beneficiaries alongside those who will be adversely affected.

If we take the view that SB-derived organisms are a logical extension of genetically modified organisms, such novel microorganisms and plants with synthetic traits, are already controlled by a set of established regulations (Murphy and Yanacopulos, 2005). Even though the frameworks are significantly different in the EU and USA, robust methodologies for assessing the risks of genetically modified organisms are already in place, with many issues on biosafety, biosecurity, and ethics having been already addressed during the last decades. Instead of rewriting new policies and regulations, it has been suggested that similarities and differences between genetically modified and synthetic organisms need to be identified and more emphasis placed on addressing safety and security challenges unique to SB (Schmidt and de Lorenzo, 2012). If SB-derived organisms with synthetic traits can be contained in a controlled environment, the current regulation framework should be adequate. If, however, there is a possibility them entering the food chain, precautionary principals should be considered and methods of risk assessment have to be reviewed, adjusted and embedded into the existing regulations (Fig. 4). To date, the UK Health and Safety Executive had assessed current and future needs for the SB regulatory framework in Great Britain (Health and Safety Laboratory, 2012) and concluded that the current genetically modified organism framework adequately covers present and near future innovations in the field.

The other major consideration for synthetic biology applications is potential adverse public perception linked to ethical and intellectual property issues (i.e. fear of the fear of the public). As in the case with genetically modified organisms in the EU, public opinion could play a major role in hampering the development and commercialization of SB-technology. In the case of genetically modified organisms, this has led to product release and use being restricted in Europe, but it has not stopped the uptake of this technology in much of the rest of the world. In 2012, the Friends of the Earth released the first global declaration from an non-governmental organization on the development of SB technologies entitled ‘The Principles for the Oversight of Synthetic Biology’, where they proposed applying the precautionary principle to synthetic biology applications (i.e. a total ban on any attempt to change the human genome and a moratorium on a release or commercial use of synthetic organisms, cells, and genomes, until regulatory bodies have considered the risks). For their part, the UK’s research councils conducted a synthetic biology dialogue where scientists, policy makers, stakeholders, and the general public were encouraged to break down barriers of misunderstanding and develop a cohesive national strategy in the field of synthetic biology (UK Synthetic Biology Roadmap Coordination Group, 2012). The outcomes of the dialogue provided evidence that the use of SB was supported across the board for some applications (i.e. medical and energy), others, notably applications in the engineering of food, crops, and bioremediation, raised several concerns, pointing to legacy issues from earlier experience of the genetic modification debate.

**Future perspectives**

Alongside emerging technology challenges, plant synthetic biology has to deal with the genetic modification legacy, which could easily derail attempts to develop this powerful technology to improve crops and their utilization for food and non-food applications. It is therefore imperative to apply a cautious step-by-step approach in developing SB applications for plant engineering, starting from simple systems, learning from them, then iteratively further developing the technology based on evidence underpinned by sound knowledge, skills, and expertise. By drawing together white and green biotechnology, we

**Table 1. Plants as feedstocks for the post-petroleum economy: SWOT analysis**

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Weaknesses</th>
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<td>Usage of abundant sunlight, CO₂, and H₂O to produce a range of products (food, feed, materials, chemicals) and energy (fuels, power and/or heat) (i.e. novel feedstocks)</td>
<td>Limited plant yields</td>
</tr>
<tr>
<td>Sustainable, renewable resources for the post-petroleum economy</td>
<td>Biotic, abiotic, xenobiotic stress sensitive</td>
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<td>&quot;Public good&quot; concept</td>
<td>Low refinery conversions</td>
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<td>Economic and environmental advantages</td>
<td>Seasonal</td>
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<td>Variable contents and low density of biomass</td>
<td>Inadequate knowledge and evidence of long-term impacts of genetically modified plants</td>
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<tr>
<th>Opportunities</th>
<th>Threats</th>
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<td>New plant traits</td>
<td>Land availability: food vs. non-food</td>
</tr>
<tr>
<td>Integration into the existing industrial infrastructure</td>
<td>Regulatory frameworks (e.g. genetically modified)</td>
</tr>
<tr>
<td>Novel applications (e.g. bioremediation, biosensors)</td>
<td>Public perception</td>
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<td>Frontier research</td>
<td>Ethical issues</td>
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<td></td>
<td>Biodiversity</td>
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propose that biorefining offers an excellent learning ground for an initial assessment of synthetic biology applications.

In order to make plant biomass a fully viable alternative to petrochemical feedstocks, a long-term research and development programme aimed at improving their utilization for non-food uses needs to be initiated. Biorefining that utilizes conventional plant biomass and is developed in response to economic and environmental challenges, endorsed by public opinion, and supported through significant incentives provided by government offers a first step in the process (Fig. 5). It would then be envisaged that process optimization could be achieved by applying recent advances in plant and microbial biotechnology, using an incremental process which takes due account of environmental and socioeconomic concerns (e.g. crops-for-food vs. crops-for-fuel conflicts. To move from industries which are largely based on chemistry to those that adopt biotechnology to drive similarly efficient production processes will ultimately require the generation of microbial and plant systems which have been selectively re-engineered based on synthetic genomes. As such, it is most likely that first commercial applications of SB will be seen in the generation of synthetic microorganisms to significantly enhance selectivity and efficiency of biorefining.

In developing such designer biotransforming organisms, it will be imperative to initiate related, but independent, ‘public-good’ research into safe containment and biosecurity issues. If this is not done, a fledgling industry will put itself into an exposed position in the event of even minor breaches of containment. From these early studies on microbial bioconversion technologies, we will be able to gain knowledge and credibility in extending the technology to the greater prize of improving plants using SB approaches. Without a paradigm shift in plant productivity, it would be implausible for industry to switch increasingly to renewable feedstocks, while agriculture is still expected to feed a growing world population. Thus, any emerging biorefining industry will have to work within limits of plant productivity which are compatible with maintaining secure food supplies, while targeting output traits for improvements to increase the yield of valuable components and the efficiency with which these can be extracted from plants.

As this review has highlighted, while there are major challenges, the size of the prize is great, and with the increased global interest in agritechnological research, this review argues that basic research programmes in plant SB should now be initiated with a view to bringing the related technologies into translation over the next two decades, when food and energy/industry needs will require the large-scale introduction of new safe technologies.
Fig. 5. Applications of synthetic biology in biotechnology (this figure is available in colour at JXB online).

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References


Kircher M. 2012. The transition to a bio-economy: emerging from the oil age. Biofuels, Bioproducts and Biorefining 6, 369–375.


Li H, Liao JC. 2013. Engineering a cyanobacterium as the catalyst for the photosynthetic conversion of CO2 to 1,2-propanediol. Microbial Cell Factories 12, 2–9.


Whitney SM, Kane HJ, Houtz RL, Sharwood RE. 2009. Rubisco oligomers composed of linked small and large subunits assemble in tobacco plastids and have higher affinities for CO2 and O2. Plant Physiology 149, 1887–1895.

