PRODUCTS FROM PLANTS – THE BIOREFINERY FUTURE

Outputs from the EPOBIO Workshop, Wageningen

22-24 May 2006

Contents

Foundation papers for each of the Flagships, providing academic underpinning for the Workshop discussions, can be found at www.epobio.net

PRODUCTS FROM PLANTS – THE BIOREFINERY FUTURE

1. Executive Summary

1.1 EPOBIO is an international project to realise the economic potential of plant-derived raw materials, funded through the European Union's 6th Framework Research Programme. EPOBIO will provide the science to support policy decisions of national and international funding agencies. Outputs will enable the design of new generations of plant-based bioproducts for the market 10-15 years from now. Through these activities EPOBIO establishes the evidence-base required for successful development of biorenewables and the supporting regulatory frameworks.

1.2 Agricultural feedstocks are the new sources of chemicals and energy as fossil reserves become increasingly expensive for manufacture. Photosynthesis, driven by solar energy, provides a sustainable means to make complex chemical products in large quantities. Global industry will benefit from bio-refining plant-derived raw materials for use in sectors as diverse as pharmaceuticals, chemicals and energy.

1.3 In the project, science, technology and supply chains are integrated to design new bio-based products. Uniquely, the EPOBIO process validates the decision to develop these products through analysing their environmental impacts, the economic case and the social attitudes of the public towards their development. This unique process ensures a thorough evidence-base to inform decision-making by funding agencies and underpins the future development of sustainable products of high utility to benefit society.

1.4 EPOBIO builds on work of the EC/US Taskforce on Biotechnology Research that established objective selection criteria for Flagship themes for plant-based bioproducts with significant potential to benefit end-users. At the 2006 Workshop, the international community of industrial and academic scientists with expert knowledge worked with EPOBIO to review independently and define further the priorities of the programme. This executive summary provides information on these priorities to inform uptake by the global community of stakeholders.

Plant Cell Walls - in relation to the biorefinery process

Improving the efficiency and reducing the cost of saccharification

1.5 Plant-based feedstocks, whether biomass crops, agricultural by-products or waste, mainly consist of cell walls, a complex composite and high energy resource. To reduce the costs, improve the energy balance and optimise the value of products from biorefining, the process of saccharification is all-important. New work is needed to: (1) design new bench-based micro-assays to determine the molecular characteristics of feedstocks (different species, different vegetable-based waste, natural genetic variation and introduced genetic variation), to link these to their digestibility and range of products formed under controlled chemo/enzymatic conditions; (2) design new strategies for gene discovery of novel hydrolases and their targeting to key components of the cell wall: approaches might include, for example, the use of metagenomics and proteomics for gene discovery and the use of microbial carbohydrate-binding modules to target enzyme delivery; (3) develop new microsystems to mimic miniature biorefineries to confirm the generic utility of laboratory-based method design.

A tailor-made platform to maximise cell wall utility in biorefineries

1.6 Current understanding of plant cell walls is limited, particularly the regulatory processes that determine the relative composition of polymers, whether protein, lipid, polysaccharide, or those based on phenylpropanoids. Regulation is both developmental and defence-/stress-related and the changes directly affect the efficiency of saccharification as well as the nature and quantity of value-added products that can be extracted from the wall in the biorefinery process. New work is needed to investigate carbon partitioning, the regulation of polymer synthesis and their assembly in the wall to underpin novel *in planta* strategies to decouple the synthesis of the different cell wall components and thereby design the raw material quality of the biorefinery feedstocks.

Plant Oils

Lubricants

1.7 The global market for lubricants, including engine oils, is immense and is currently dependent on fossil reserves. Plant oils offer a sustainable alternative, with wax ester replacements produced by agricultural crops. The oil crop, *Crambe,* has considerable potential to synthesise a diversity of wax esters, with their ratios in the oil tailored for different applications. New work is needed to optimise *Crambe* for this purpose, specifically to: (1) establish transformation methods, applicable to different sources of germplasm; (2) discover the genes, pathways and regulatory processes governing synthesis of defined wax ester species; (3) improve wax ester yield and agronomic performance of new, engineered varieties; (4) design improved processing technologies tailored to the use of oil products and the by-products from oil extraction.

Development of a generic non-food oil crop platform

1.8 Plant oils have the potential to fulfil market needs in a wide range of industrial sectors from health, bulk and speciality chemicals to energy and transport. The sustainable production of oil by agricultural crops offers major opportunities to reduce global economic dependence on mineral oil. There are key issues that must be addressed to realise this potential. New work is needed to investigate the regulatory processes controlling yield of native and novel oils to underpin *in planta* strategies for optimising a non-food oil crop platform that can be used as a generic field-based system to produce bespoke oils in quantities that make mineral oil replacements economically viable.

Biopolymers

Rubber

1.9 Rubber is a natural plant-based commodity used for high value and/or high volume applications for which there are often no alternative feedstocks. There is an emerging security of supply issue, given that demand will outstrip supply and existing supply is at risk from plant disease. New work is needed in two related areas: (1) greater understanding of the genes and metabolic processes involved in rubber production *in planta* to underpin the development of alternative rubber crops; (2) detailed analysis of the potential for new sources of rubber production, for example, in terms of the products formed, potential presence of allergens, agronomy, extraction process and environmental impacts.

A non-food crop platform for monomer and biopolymer production

1.10 The scale of current use of petrochemicals for monomer/polymer production by the global chemical industry is at threat from rising oil prices. Field crops offer the potential for high volume production of commodities. It will become increasingly necessary to underpin the chemical industry with agricultural feedstocks and sustainable replacements to those products currently made from petrochemicals. New work is needed to investigate the synthesis of those monomers and biopolymers that can be more effectively and cheaply produced in plants compared to microbial fermentation. Production of polymers or building blocks in plants will require the development of novel extraction and processing systems for cost-effective fractionation.

Next Steps

1.11 In each of the three Flagship themes, an immediate target has been prioritised together with the establishment of a major platform technology for generic development of plant-based bioproducts. It can be anticipated that information-sharing across the three platforms will ultimately lead to sharing of technologies to underpin ‘the integrated biorefinery’. The involvement of industry is key to ensure market focus and the means to ensure the new plant-based bioproducts are taken up by the consumer.

1.12 The advice from the international community emphasised the fundamental importance of multi- and interdisciplinary strategies to achieve the required outputs in the prioritised areas. This will necessitate teams incorporating a broad knowledge-base and skills-set within the biological sciences as well as the inclusion of complementary disciplines such as chemistry and engineering. Also, to achieve a successful systems approach and meet sustainability requirements, the involvement of life-cycle specialists, environmental scientists and socio-economists in project design and validation is strongly encouraged.

2. The EPOBIO project and its international relevance

2.1 Introduction to the EPOBIO initiative

2.1.1 EPOBIO is an international project funded through the European Union's 6th Framework Research Programme to realise the economic potential of plant-derived raw materials. Introducing the origins of the EPOBIO initiative, Dr Judy St John (Deputy Administrator Crop Production and Protection, Agricultural Research Service, United States Department of Agriculture) and Dr Laurent Bochereau (Directorate General for Research, European Commission) explained its background in the US-EC Taskforce on Biotechnology Research. This Taskforce has been an effective co-operative effort since it was established in 1990 as a forum for examining trends in biotechnology and exchanging ideas. It has strengthened research collaboration through workshops, short courses and joint publications in emerging fields.

2.1.2 A series of US-EC workshops identified and adopted three key Flagship themes – plant cell walls, plant oils and biopolymers – and a strategic vision paper on bio-based products was developed and endorsed. These actions provide the foundation that EPOBIO encompasses and is now building on. The EPOBIO objective is to design new generations of bio-based products derived from plant raw materials that will reach the market place 10-15 years from now.

2.1.3 Each of the three EPOBIO Flagship themes is being developed within a framework of its environmental impact, economics and regulatory frameworks, attitudes and expectations of policy-makers and the public, and a communication strategy. This holistic approach and analysis demand close co-operation between environmental scientists, agronomists, experts in legislation and regulations, socio-economists, policy-makers and the public to evaluate proposed products and projects and to ensure the products developed are beneficial to our society and for our planet. Placing scientific potential into this wider social context and appreciation of sustainability are unique features of EPOBIO.

2.1.4 In the US, 2006 has seen the launch of a number of new policy initiatives. The Energy Action Plan mandates an increase in the use of bioethanol and biodiesel and extends support for the production of electricity from biomass. The Advanced Energy Initiative promotes the development of practical and competitive methods for the production of cellulosic ethanol, part of the drive to reduce dependence on oil imports. A Department for Energy bio-based Task Force Initiative has been established with a programme of research and action supported by funding of $50 million from 2008 onwards.

2.1.5 In the EU, the Biomass Action Plan envisages a doubling of the use of bio-energy resources. The Strategy for Biofuels supports the development of these fuels in the EU and in developing countries, aiming to ensure a positive environmental impact and improved costs and competitiveness. Both of these actions have strong research components including relevant Technology Platforms and the biorefinery concept and the development of second-generation biofuels are being given high priority. Framework Programme 7 will see a 40% increase in research funding with a number of themes relevant to EPOBIO. The evolving European Knowledge-based Bio-economy will incorporate the sustainable management of biological resources, the production of a healthy and diverse food supply and the development of bio-based materials for health, industry and energy.

2.1.6 Focussing on the expected outcomes for the meeting, the need for on-going deep reviews of the three Flagship themes was recommended. It will be necessary to identify the key scientific issues and questions to underpin future proposals for consideration by relevant national and international funding agencies in either the EU or US. In particular, synergies for international networks and partnerships, such as clusters emerging from common research themes in different countries, should be investigated and recommended. A priority list of products and projects in each of the Flagship themes will underpin these future initiatives.

2.2 Keynote presentations addressing biorenewables

A Global Perspective: Dr James McLaren, StrathKirn Inc

2.2.1 Global technological development has led to improvements in transport, communications, housing, energy and consumables and contributed to a significant increase in living standards. However, it has to be questioned whether this current situation is sustainable. Global energy demand is expected to double over the next 30 years with China being a major driver and the USA and India increasing steadily in order to meet continuing economic growth.

2.2.2 Carbon dioxide emissions are expected to continue to rise, with potential impacts on global temperatures. Concerning energy sources, oil use is expected to increase, although economics will ensure it never runs out since alternative technologies will be needed and developed to provide competitive replacement energy sources. In the US, 7.5% of energy needs are currently met from renewable sources, primarily based on hydroelectricity and biomass from wood and corn grain.

2.2.3 Existing crops and approaches need to be improved to provide a major, sustainable bio-energy platform including biofuels, bio-power and bioproducts. New future approaches will need to include sources of raw materials designed for their specific end-use and the application of new processing methods. It must be recognised that it takes considerable R&D effort and a timescale of around 10-15 years to turn research into commercial products.

2.2.4 Biodiesel and bioethanol are expected to make modest contributions to total consumption of liquid fuels. The current commercial feedstock sources for ethanol are maize grain and sugarcane. There is potential for additional future feedstock sources including forestry, dedicated crops, crop residues and grains. New processing methods could see significant increases in the volumes of ethanol produced and also produce sugar streams for other products. The future can be characterised as tough challenges with high potential. However, success with critical technology applications will provide help for fuel security, rural economics and the potential delivery of environmental benefits.

2.2.5 The current success of maize ethanol expansion can be considered to be pioneering the biofuel market and creating potential for future lignocellulosic-based products. Future challenges are significant and include:

- A need for technical and market breakthroughs.
- Needs-based investment and policy inputs.
- Unified directions and actions.
- Petro-company support and involvement.

New technology has the potential to deliver 14-20 billion gallons of ethanol in the US from maize starch. This will also deliver a high volume glucose stream for bioproducts, and be available in the near future. Longer-term, maize stover residues could also be used with the potential to produce 150-200 gallons of ethanol per acre after 35% of the material is left to improve soil structure. If lignocellulosic processing technology breakthroughs are successful, maize stover would contribute an incremental 12-15 billion gallons of ethanol per year.

2.2.6 Fuels and bioproducts, based on the sugar platform which is expected to be generated from dedicated lignocellulose sources, still face significant challenges due to a lack of established agronomic cultural practices for dedicated crops. In addition, harvest, transport and storage methods need further development for the vast volumes of material that would have to be handled.

2.2.7 Biodiesel provides good technical performance and acts as a lubricity agent, and also delivers environmental benefits. The total EU diesel market is around 60 billon gallons per annum. In the US, soyoil has been highly successful in the food market and so achieves good returns which are not easily matched from fuel production. Improved yields of oil per unit area would help the economics of biodiesel production.

2.2.8 Technically it is possible to develop a biorefinery approach to produce energy in various forms and a huge range of industrial and consumer products. However, to achieve success there is a need for integrated research actions and cross-discipline debate so that issues are addressed together and sectors are not dealt with in isolation.

2.2.9 Biotechnology has the potential greatly to improve the production efficiency and the composition of crops and make feedstocks that better fit industrial needs. All crops are genetically altered but biotech-based transgenic crops show much higher improvement for specific gains. There are already 10 million farmers in 20 countries growing 85 million hectares of commercial biotech/GM crops, with significant benefits and no recorded problems. Crops are basically like effective solar panels that harness sunlight. Applying the modern tools of biotechnology to crops for enhanced production, improved pest resistance, and to channel the fixed carbon into more useful desired products would provide an excellent platform to drive the growth of future bio-products in the 21st century.

2.2.10 In developing strategies for biofuels and bio-based products the key stages to consider are the bio-feedstocks production (including bio-technology applications for primary products), harvesting/transport and storage, processing and the portfolio of materials/products produced. A key challenge for EPOBIO is to integrate along the path-to-market linking basic science, research, development and implementation, and processing while maintaining a realistic focus on economics, markets, and the needs of the consumer. If these tough challenges can be met then there will be an important role for bio-based products in our consumptive society.

A European industry perspective: Dr Bernward Garthoff, Bayer AG

2.2.11 Significant growth in companies active in renewable feedstocks has led to a worldwide stock/shares value of €55 billion. Currently, there is a large increase in the manufacture of equipment for biodiesel and bioethanol plants as around 60 such plants are set to be constructed in the EU by 2010. This will provide a commercial opportunity for agriculture and also for the construction and plant installation industry in Europe.

2.2.12 Current success in renewables is built on technological improvement, political support and high commodity prices. These factors have led to a significant increase in, for example, biodiesel production capacity in the EU, although the targets in the EU Biofuels Directive are unlikely to be met. Future progress is dependent on the close integration of crop science with agriculture and processing in order to ensure sustainable development.

2.2.13 Towards 2025 there will be significant increases in population, coupled with a changing age distribution and growing health and welfare demands. Demands for oil will increase as water availability falls and land use shifts towards providing feedstocks for the bio-economy. Rapid innovation, drawing on the science-base, is likely to lead to world regions competing to secure their long-term welfare through ‘in-house’ production of vital resources. The focus of agriculture is expected to change to serve these new and developing markets. At the same time it will be necessary to demonstrate benefit through ecological footprinting in order to secure support from stakeholders.

2.2.14 The demand for plant raw materials is not in doubt and the further emergence of the bio-based economy will provide the consumer with a broad range of plant-based speciality products which are expected to have a value in excess of €500 billion. This expected growth will be driven by innovation and technical breakthrough, increased demand, the development of new bio-based building blocks and by regulatory push, in a context where industry increasingly encourages the development of inexpensive, renewable feedstocks.

2.2.15 In Europe, plant biotechnology could easily improve the production of feedstocks for biodiesel but field trials are not permitted. Small field sizes in some EU countries, acceptance of biofuels and tax/subsidy issues can also be barriers. Environmental impacts and competition, between supermarkets and traditional fuel suppliers, are also issues that could present obstacles to development.

2.2.16 European industry welcomes and supports the EU strategy on life sciences and biotechnology but there are key challenges about providing the resources needed to meet society’s needs and, at the same time, increase competitiveness. Policies will need to deliver the confidence and support of citizens and Europe will need to respond to global challenges to pursue its interests internationally.

2.2.17 The knowledge-based bio-economy has huge potential, greater that that of the chemical and automotive industry combined. A common vision evolved through the development and implementation of the strategic research agenda has the potential to unite stakeholders and establish a value chain based on renewable feedstocks that can deliver a vast range of bio-products.

2.2.18 A coherent R&D programme within the 7th EU research framework programme (2006-2013), bringing together food, agricultural and biotechnology research to support the knowledge-based bio-economy, is essential to underpin progress. Joint interests mean that co-operation is essential with, for example, the EU and US, both having a broad knowledge base in this area, shared economic interests and a dependency on energy and fuel. Development in other parts of the world will increase competitiveness for energy and food. It will be important to seek joint solutions to future problems and the current situation calls for both an EU technology platform approach and also an inter-continental approach.

A US industry perspective: Dr John Pierce - DuPont

2.2.19 Experience in the US shows that taking bio-conversions to marketable products requires a high degree of multidisciplinarity alongside selecting the right targets, getting production platforms and costs correct and establishing strong partnerships. Whilst the bio-based economy can lead to a range of outputs and products, the development of Sorona®, an advanced polymer with superior properties and a range of uses, required 11-12 years of development and diligent work. Consequently, it is important not to underestimate the challenges that lie ahead.

2.2.20 DuPont is working in strategic partnerships which bring together unique competences. Complex processes make such partnerships an essential component of taking the bio-based economy through to the market.

2.2.21 Biofuels is one opportunity offering significant potential for development. These fuels can help address global warming issues and reduce dependence on imported fossil fuels. Biofuels are a sustainable alternative to traditional fuels and benefit the rural economy. Worldwide there is an opportunity totalling around 100 billion gallons with potential to use existing, emerging and advanced conversion technologies in production processes.

2.2.22 Starch and sugar, the existing raw material base for biofuels, make use of current infrastructure and technology. Future raw materials will need to produce high yields, meet environmental constraints and suit processing that requires little or no pre-treatment. Processing facilities will need to have low capital intensity and the flexibility to take a variety of feedstocks. Testing is being undertaken and will continue and corn stover is an example of a raw material available in large quantities in the US with the potential to produce both chemicals and biofuels in an integrated biorefinery.

2.2.23 The key essentials for cellulosic ethanol process development include a low energy input, inexpensive equipment, low cost and high yields. In respect of materials, high solids, fast conversion and high sugar yields are important. As well as the biofuel, it is also possible to produce syngas for use as energy to run the production process, helping the economics of production. An effectively integrated corn biorefinery will produce power, ethanol and also higher value products.

2.2.24 Keys to future development include improved conversion to sugar and new and cheaper enzymes. More tractable cellulosic biomass will help processing, alongside novel engineering approaches. Cheaper raw material prices and energy crops with improved cellulose yield are also important. All of this must be combined with the multidisciplinary approaches that can bring together the key competences needed.

2.2.25 In what will develop as a highly complex and competitive environment, good technical skills, integrated processes, cost control and strong partnerships will be essential to achieve success.

The European agriculture perspective: Marco Aurelia Pasti - Confagricoltura, Italy

2.2.26 European farmers are facing rapid changes, the origins of which can be seen in the 1992 reform of the Common Agricultural Policy. Set-aside was introduced, which gave a new impetus to the development of non-food crops. In Italy, small farm sizes and low returns for non-food crop feedstocks made development uneconomic and, initially, there was little progress. This was in contrast to other EU countries where the production of oilseeds for biodiesel progressed quickly.

2.2.27 The subsequent round of reform, known as Agenda 2000, saw limited change. However, the retention of set aside and the provision for the production of non-food crops on set-aside land continued to support the expansion of oilseed crops in particular.

2.2.28 In 2003, the Mid-Term Review of the Common Agricultural Policy brought about a big change in philosophy with the consumer placed at the centre of agricultural politics. Decoupling switched subsidies from an area-based approach to a system of direct payments to farmers that does not require them to produce crops, although certain environmental safeguards must be met. It was initially difficult to predict likely changes but, for example, durum wheat production has increased by 30% in Italy. Set-aside is no longer regarded as a tool to regulate production.

2.2.29 In 2004 new member states joined the EU and this had further impacts. Amongst the new Member States, Hungary and the Czech Republic are major cereal producers and this is leading to an imbalance in the Common Market Organisation (CMO) for cereals. It is likely that rising stocks will force down prices and the CMO will need to change. Alongside this, the phasing out of export subsidies will have an impact. Technological developments will also be a further factor for change with yields likely to increase in the new Member States as investment in their agriculture sectors increases.

2.2.30 Farmers must inevitably look for new alternatives. World Trade Organisation negotiations will lead to an opening up of markets and farmers need to access added-value opportunities by involvement in the biofuel and biochemical industries.

This could, for example, be through ownership of and participation in first-stage transformation of feedstocks.

2.2.31 To set farming issues in the wider context, good communication to the general public is a key essential. The presentation of genetic modification of crops has prevented development in the EU and it is very difficult to revisit such issues once opinions have been formed. The use of this and other new technologies depends on good communication, linked to informing and influencing public opinion.

Opportunities for the developing world – Professor Stanislav Miertus, ICS-UNIDO

2.2.32 Sustainable development is an important issue for both the developed and the developing world and it has become an everyday reality and not just a political debate. There is an urgent need to progress the use of renewable resources in the context that only 3.5% of the available resource was being utilised in 2002. A strategic approach to extend the use of this resource is urgently needed.

2.2.33 Technologies will need to be further developed in order to optimise processing and production. It is also important to take account of the need to underpin and demonstrate sustainability in those new technologies.

2.2.34 Many developing countries have a key driver because they lack fossil reserves but have an abundance of renewable feedstocks. This provides an opportunity to develop sustainable local industry through diversification and the use of waste materials. But it will be important to protect biodiversity, avoid over production and maintain a good balance between agriculture and industry.

2.2.35 There are inevitably a series of risks facing developing countries. These include addressing the wrong priorities by not balancing food production with renewables production and by worsening the technology gap through failing to develop national production capacity. In addition, there could be a lack of an integrated approach from crops to markets/products, and of an analysis of potential and the strategic planning needed to achieve that potential. Other important barriers include the cost of renewable products and their ability to compete with fossil alternatives. There may be limited capacity to support development because of a lack of experience and of potential investors. It is often the case that weak incentives and inadequate policy drivers hinder development.

2.2.36 Barriers can be overcome by, for example, promoting co-operative research programmes between developing and industrialised countries. Developing and demonstrating renewables programmes and building national capacity in developing countries are a key. In addition, appropriate subsidy programmes can be developed and industry can make voluntary global commitments in respect of developing countries.

2.2.37 ICS-UNIDO is well equipped to support such developments through its technical knowledge, linked to its worldwide focus on developing countries and countries in transition. Its awareness-building activities and the development of in-house expertise on decision support tools, priorities and strategies underpin these key activities. Examples are work on biodegradable plastics for the 2008 Olympic Games in Beijing, China and an extensive renewable and rural energy programme. Two renewable energy centres in China and India aim to disseminate information, facilitate technology diffusion, build regional/national capacities, create network and partnerships and promote renewable energy technologies through cooperation.

2.2.38 ICS-UNIDO is uniquely placed to link the EU 7th Framework Programme (FP7) and the outputs from EPOBIO with developing countries and countries in transition.

3. EPOBIO in the broader context

3.1 Vision, timescales and links to wider initiatives: Professor Dianna Bowles, EPOBIO Project Director

3.1.1 EPOBIO is funded under the 6th Framework Programme non-food policy research component. It is a Specific Support Action designed to assess how best to use plant-based resources so that sustainability can be achieved and the economic potential of the sector can be realised.

3.1.2 EPOBIO's origins can be found in the EC-US Taskforce on Biotechnology Research. The Albany Workshop of April 2004 addressed the prioritisation of scientific areas and technologies relevant to the design of future areas of collaboration - the Flagships. It also undertook to prepare a Strategic Vision paper to inform future research activities and to establish a Steering Committee to take the collaboration forward.

3.1.3 A subsequent Steering Committee meeting in Brussels in October 2004 defined the criteria for Flagship themes as:

- scientific challenge
  requires large-scale, complementary, multinational input
- user / consumer benefit
  societal benefit across the entire supply chain
- economic benefits and risk analysis
  the project as a continuum – research to proof of concept
- private sector involvement
  pre-competitive, demonstration of value.

3.1.4 The criteria provided the foundation for selection of the first three Flagship themes – Plant Cell Walls, Plant Oils and Biopolymers – at a meeting in Beltsville in March 2005 where the draft Strategic Vision paper was also endorsed. The proposal for EPOBIO for funding in FP6 was then submitted, envisaging worldwide participation and the incorporation of both plant- and microbial-based technologies.

3.1.5 Underpinning EPOBIO is the recognition that bio-based renewables are an essential way for the world to produce the energy and products needed for a sustainable future, using the sunlight to grow biomass that can act as feedstocks for biorefineries to produce chemical and energy products. Focussing across the supply chains from plants to products, EPOBIO aims to achieve a step change in sustainable innovation. EPOBIO brings together the science, technologies and supply chains needed to develop the high-utility products that will reach the market place in 10-15 years. EPOBIO has a unique focus in that it will address:

- entrenchment of standards based on petrochemicals
- consistency of quality/quantity: plant variability
- economic and environmental impacts
- existing supply chains that block new entrants
- regulatory frameworks
- perceptions of public and policy-makers

3.1.6 The three Flagship themes will identify products and projects that will be assessed in the contexts of sustainability, environmental impact, economics/regulatory issues, social attitudes/expectations and communication issues and strategies. Identified products and projects will be relevant both nationally and internationally, including developing countries, and will define the way forward to help secure future economic potential. Actions will link scientific potential with product development and the policy framework needed to facilitate uptake. International activities of EPOBIO beyond Europe include particularly the US and US expertise is provided through partnership in the EPOBIO Consortium from the USDA. EPOBIO also incorporates the BioMatNet database of information to make available the results of RTD projects supported by the European Commission in the area of biological materials from non-food crops.

3.1.7 The international Consortium of EPOBIO has academic and industrial representation, with a Director experienced in the science of plant-based renewables. Each Flagship theme is led by two Consortium partners, one based in Europe and a second based in the US. An appointment of a senior post-doctoral desk researcher in each Flagship theme to work alongside the partners ensures rapid progress in the analyses. The four support themes are led by Consortium partners based in Europe and, again, benefit from the involvement of a senior desk researcher who undertakes the analyses required. An additional Consortium partner undertakes the information dissemination and ensures full integration of EPOBIO findings with other databases such as BioMatNet. A full-time senior project co-ordinator with an extensive experience in government and EU policy manages the project and enables policy and regulatory issues to inform the development of the Flagship themes. There is an international Advisory Board and additional international experts are brought in to discussions such as through specialised desk research analyses and participation in EPOBIO workshops. This structure provides a framework for validation of research priorities by the international scientific and industrial community, such that EPOBIO recommendations can be useful for policy-makers and funding agencies.

3.1.8 Development of the supply chain for renewable resources involves the integration of ‘green’ biotechnology, optimising quantity and qualities of crop-based materials, with ‘white’ biotechnology, the use of enzymes and living cells in fermenters and reaction vessels to make products of high utility. EPOBIO consequently spans several of the Technology Platforms and addresses components in each Platform. Relevant Platforms for EPOBIO include: Plants for the Future, Sustainable Chemistry, the Forest-based Sector and Biofuels. EPOBIO draws on a range of expertise globally for the development of plant-based bio-products with a prime focus of science to support EU policy and inform national and international funders of validated themes for research funding support. EPOBIO’s activities are distinct from but complementary to the Technology Platforms and other European initiatives including the emerging networks of the European Research Area (ERA-Nets).

Plants for the Future Technology Platform: Professor Marc Zabeau

3.1.9 Technology Platforms (TPs) are one element of the vision for constructing the European Research Area. The aim is that for each technology area vital for European competitiveness, an advisory group, mainly made up from industry, will provide strategic guidance on future opportunities and the research needed to achieve them.

3.1.10 The Plants for the Future TP includes industry through the European Biotech Industry Association, academia through the European Plant Science Organisation and the farming sector through COPA GOGECA. The vision document launched in June 2004 and proposals for a strategic research agenda were published in July 2005. These present the long-term contributions of plant genomics and biotechnology to the transformation of the traditional agro-food and forestry economy into a knowledge-based bio-economy in Europe. Plants for the Future links the Technology Platforms in the agro-food sector with those in non-food.

3.1.11 The Plants for the Future vision document identified four main challenges:

- Developing sustainable agricultural production, while preserving the environment. Securing a healthy and safe food and feed supply. Developing new products for the bio-based industry.
- Ensuring Europe’s competitiveness and consumer choice.

3.1.12 The vision document was followed by stakeholder proposals for a Strategic Research Agenda (SRA) in which expanding the non-food, bio-product range concentrated on biochemicals (including pharmaceuticals), biomaterials and bioenergy, including biofuels. The importance of the environmental impact of agriculture was noted, as was the need to boost biodiversity.

3.1.13 Consultations on the SRA took place in 18 Member States and feedback will be incorporated in the final document, to be published in 2007. This will be a shared European vision with clear priorities for plant research. It will provide guidance for FP7, national research programmes, novel public/private research initiatives and international co-ordination of research.

3.1.14 Future plant research activities in Europe are currently being surveyed and results will also feed into FP7 and the European Research Area on Plant Genomics (ERA-PG). A summary report analysing the results will be published in July 2006 and will analyse the relevance of the research topics to realisation of the Strategic Research Agenda. It will recommend specific funding action under FP7 and the ERA-PG.

3.1.15 The survey will identify strengths and weaknesses in European science. Activities and proposals on non-food applications of plants in particular need to be stimulated. The engagement of the science community with the survey is essential for future progress.

Sustainable Chemistry – Industrial Biotechnology Technology Platform: Dr Colja Laane

3.1.16 Biotechnology is about using nature’s toolset to replace fossil feedstocks in industrial processes including energy, processing, product manufacture and environmental clean-up. Examples include biopolymers that deliver a reduction in greenhouse gas emissions of 55% but can be produced at equivalent cost to fossil alternatives. Antibiotics can be produced requiring 65% less energy and materials but at 50% lower cost. These examples illustrate the potential for biotechnology to deliver both environmental and economic benefit.

3.1.17 Agricultural products and by-products producing sugars can be used in the production of a range of chemicals from pharmaceuticals and food ingredients through to bulk materials such as ethanol and hydrogen for biofuels. Currently only 3-5% of chemicals are bio-based but this could reach 7-8% by 2010. Key growth drivers include the need for sustainable production, identifying alternatives to oil and gas and technical feasibility. The need for innovation, the delivery of cost savings and supportive legislative frameworks will also underpin and drive growth.

3.1.18 Global biotech performance in Europe showed a decrease in activity in 2003, in contrast to increases both in the US and, more strongly, in Asia. However, Europe is seeking to make rapid progress with FP7 including the knowledge-based bio-economy and an ERA-Net for industrial biotechnology. Key strengths in Europe include enzymes technology, with 70% of industrial enzymes being produced in Europe, and biospecialities.

3.1.19 EU Technology Platforms are a key component of future development. They bring together stakeholders and develop strategic visions that can be translated into road maps and implementation action plans. The preparation of research and development plans and policies can be complemented by demonstration activities. Technology Platforms can also help to stimulate public/private partnerships.

3.1.20 The vision document for Sustainable Chemistry – Industrial Biotechnology was developed in February 2005 with the Strategic Research Agenda following in November. The implementation action plan will be published in August 2006. Key research areas for white biotechnology are:

- Novel enzymes and microorganisms
- Microbial genomics and bioinformatics
- Metabolic engineering and modelling
- Biocatalyst function and optimisation
- Biocatalyst process design
- Innovative fermentation science and engineering
- Innovative downstream processing

3.1.21 Bioethanol is an example where processing needs to become more efficient and cheaper and a considerable amount of work is focussed to achieve this. Integrated, multi-purpose biorefineries could have a role in this.

3.1.22 EPOBIO and the SusChem Technology Platform link through the supply chain, from plant-based material to industrial processing. They therefore have strong complementarity and significant potential for collaboration.

Forest-based Sector Technology Platform: Mr Claes-Göran Beckeman

3.1.23 Wood is a strategic resource for Europe with forest cover expanding every year and has significant potential as a resource for the European bio-economy.

Products from wood are familiar to consumers and also represent a sustainable life cycle.

3.1.24 The Forest-based Sector Technology Platform has developed its vision and strategic objectives, the latter including:

- Innovative products for changing markets and consumer needs.
- Intelligent and efficient manufacturing processes, including energy efficiency.
- Availability and use of forest biomass for products and energy.
- Multifunctional demand on forest resources and their sustainable management.
- Placing the sector in a societal perspective.

The core idea for the wood biorefinery is an optimised utilisation of feedstocks for the production of chemicals. A key challenge will be to use scientific knowledge and expertise in commercial-scale production. New value-added chains will need to be identified including speciality chemicals and advanced materials and composites.

3.1.25 Future action by the Forest-based Technology Platform will include:

- The development of an Action Plan and a Communication Plan
- Securing participation of key stakeholders, specifically the industry
- Mobilising the National Support Groups
- Interacting with other Technology Platforms
- Promoting innovation, education and training in the Action Plan
- Securing transparency and involvement

3.1.26 Focussing on new products is seen as the key to future profitability. This is consistent with the EPOBIO approach to identify new products and projects in the Flagship theme areas.

3.2 Flagships in the wider sustainability agenda - the EPOBIO approach:

Professor Dianna Bowles, EPOBIO Project Director

3.2.1 EPOBIO brings together the science, technologies and support chains needed to develop high utility products from plant raw materials. Uniquely, it puts scientific potential into the wider social context. EPOBIO will, within each of the Flagship themes, identify a series of target products and projects that could be in the market place in 10-15 years. Some products and projects will have national relevance and some, international relevance requiring collaborative research and clustering of activities.

3.2.2 Each Flagship target product and project will be developed within a framework of its environmental impact, economics and regulatory frameworks, attitudes and expectations of policy-makers and the public, and a communication strategy. This will help ensure that science and technology are appropriately used to produce sustainable products of high utility to benefit society.

3.2.3 The EPOBIO Consortium partners and desk researchers together with the project co-ordinator and the international community of scientists from academia and industry will work together over 26 months. In this context, the breakout discussions at the Workshop are intended to identify and prioritise products and projects for each Flagship theme. Given the limited time available to EPOBIO it will be necessary to prioritise the analyses which will form the basis of desk researcher activities and interactions with international advisers and specialists.

3.2.4 Reports will be produced and published for each product and project analysed. These reports, validated by the international science and industrial communities, will contain funding recommendations to national and international policy organisations.

4. PLANT CELL WALLS FLAGSHIP

Breakout sessions co-chaired by Flagship leaders Markus Pauly and Sarah Hake, Report prepared by desk researcher Ralf Möller

4.1 Summary

4.1.1 The Workshop gathered together world-class experts in the field of plant cell walls and their use as feedstocks for biorefining. Each discussion topic in the breakout session was introduced by a leading expert. There was general agreement that detailed understanding of cell wall biosynthesis and architecture is crucial for the success of the biorefinery concept. For example, this understanding underpins the improvement of pretreatment technology, the cell wall degradation process, the *in planta* modification of cell walls for raw material quality and the identification of products available in the biomass. In-depth knowledge of the diverse components of the cell wall, their molecular structures and properties is urgently required. It is important to learn more about the connectivity of the polymer network *in muro*, for example the nature of covalent links between the different cell wall polymers. In particular, understanding must be gained of how cell wall synthesis is regulated, the genes involved and the cellular sensing mechanisms that determine cell wall composition. Similarly a better understanding of carbon partitioning in the plant is essential, together with a knowledge of how cells sense changes in the cell wall and react by regulating the relative proportion of different components.

4.1.2 To enable fast screening of cell wall phenotypes to determine the consequences of phenotype on biorefining processes/product extraction, new high-throughput cell wall assays must be developed. For example, since the degree of cellulose crystallisation is a major determining factor in the effectiveness of cell wall degradation, assays for this feature need to be designed. Similarly, small-scale assays specific for different wall products must be developed. Assays such as these will be instrumental in predicting the suitability of different cell wall phenotypes for biomass degradation/product manufacture in large-scale biorefineries. Assays are also required to investigate the value of specific cell wall modifications and their contributions to more efficient degradation/processing/extraction. In this context, cell wall material with different modifications can be derived from natural variation, mutant populations and transgenic plants. It is essential to analyse plant materials grown in greenhouses as well those from field trials to assess the impact of cell wall variation on environmental, mechanical and pathogen stresses to confirm the utility of beneficial variations for biorefining on overall crop performance.

4.1.3 New work on processing is required to optimise opportunities for extracting/fractionating pure wall components from biomass raw material – such as mono-, oligo- or polysaccharides or other cell wall constituents, that can then be more readily used for the efficient manufacture of products. An integral aspect of this is the design of assays to characterise fractionation outputs.

4.1.4 New approaches to discover novel hydrolases for use in cell wall degradation, such as those forming specific products such as mono-, oligo- or defined polysaccharides are required, together with new methods of targeting hydrolases to specific molecular sites, such as through the use of carbohydrate-binding modules.

Strategies for gene discovery will need to include the use of genomics, proteomics and metabolomics as well as novel approaches based on metagenomics.

4.1.5 The need to optimise hydrolase mixtures for digesting specific biomass feedstocks was recognised, together with the importance of developing micro-systems to mimic miniature biorefineries to confirm the utility of laboratory-based methods for scale-up.

4.1.6 Feedstocks for biorefineries were discussed within the context of regional and global availability, cheapness, sustainability and optimisation for processing. Dedicated biomass crops such as the perennial grasses, switchgrass and *Miscanthus*, and the woody species, willow and poplar, were discussed in addition to agricultural residues such as wheat straw and corn stover, and vegetable processing waste, for example fruit pomace.

4.1.7 It was concluded that plant cell walls will become increasingly high value feedstocks for biorefineries in the future. Whilst bioethanol production from biomass is the principal driver currently, plant cell walls represent a unique resource that can potentially be used to provide pure components capable of functionality in their own right as well as providing feedstocks for the manufacture of novel products. Thus, purpose-built biorefineries could in future be integrated into ‘a biorefinery village’ to manufacture a diversity of functional products derived from plant cell walls.

4.1.8 Short- to mid-term targets for research to realise this potential of cell walls were identified, together with the need to establish a bespoke platform to maximise cell wall utility in biorefineries. To achieve these goals and progress rapidly to functioning integrated biorefineries, the need for multi- and interdisciplinary research teams was highlighted. These teams should include biologists, geneticists, chemists, biochemists and processing engineers.

4.1.9 The first EPOBIO project for the Plant Cell Walls Flagship will aim at improving the efficiency and reducing the cost of the saccharification process. Sugars generated can both be used for fermentation to ethanol and converted to other chemicals for the production of a variety of novel products [1]. This high priority project will underpin the development of the subsequent work for the Plant Cell Walls Flagship which will go on to define the technical and non-technical issues necessary to establish a bespoke platform to maximise cell wall utility in biorefineries. To underpin the development of this platform, work will focus on the regulation of carbon partitioning in the plant and the synthesis and assembly of polymers in the wall. This research will inform novel *in planta* strategies to decouple the synthesis of the different cell wall components and thereby design the raw material quality of the biorefinery feedstocks.

4.1.10 The Plant Cell Wall breakout session involved many international experts and participants from industry and academia. All are recognised for their participation in the discussions.

Europe

Tony Arioli, Germany
Wout Boerjan, Belgium
Jim Coombs, UK
Matthias Dieter, Germany
Michel Ebskamp, The Netherlands
Wolter Elbersen, The Netherlands
Anne Mie Emons, The Netherlands
Ines Ezcurra, Sweden
Jens Freitag, Germany
Deborah Goffner, France
Claire Halpin, UK
Luc Harvengt, France
Katja Johansen, Denmark
Lise Jouanin, France
Antonio Levya, Spain
Benadetta Mattei, Italy
Simon McQueen-Mason, UK
Ralf Möller, Germany
Richard Murphy, UK
Patrick Navard, France
Michael Obersteiner, Austria
Markus Pauly, Germany
Henrik Scheller, Denmark
Peter Shewry, UK
Eva Stoger, Germany
Björn Sundberg, Sweden
Gail Taylor, UK
Simon Turner, UK
Pablo Vera, Spain
Waltraud Vorwerg, Germany

USA

Candace Haigler, NCSU
Sarah Hake, USDA-ARS, University Berkeley
Ron Hatfield, US DFRC, USDA-ARS
Arland Hotchkiss, USDA-ARS
Kenneth Keegstra, Michigan State University

4.2 Introduction

4.2.1 Plant cell walls are the most abundant renewable resource on earth, used as raw materials for the food and building industries, and playing an important role in carbon cycling in nature. About 70% of the carbon fixed by plants is allocated to their cell walls, and annually about 100×10^9 mt of cell walls are produced. Today, less than 1% of this abundant resource is used, whether in the form of wood, as timber for furniture making or house building, or in the form of fibres for the paper and textile industries. In the future, it is already anticipated that plant cell walls will also become key raw materials for biorefineries and a multitude of high value products will be produced from this cheap and abundant feedstock.

4.2.2 Although cell walls represent an abundant raw material, they are highly complex and have naturally evolved to resist breakdown from mechanical and microbial forces - precisely those processes needed for efficient and cost-effective biorefining. Unlocking the components of cell walls represents a massive scientific and technical challenge. The key issues that need to be considered for identifying scientific bottlenecks and the research needs required to unlock the economic potential of these biomaterials include: the chemistry of the cell wall polymers; the cell biology of the wall and post-genomic technologies (genomics, metagenomics, proteomics and metabolomics) to explore cell wall biosynthesis. In addition, enzyme biochemistry and associated technologies to optimise cell wall degradation will be necessary to underpin fractionation and bioprocessing of the raw materials. These issues have been reviewed in the Foundation Paper for the Plant Cell Walls Flagship [2].

4.2.3 The EPOBIO workshop in Wageningen brought together world-class experts from academia and industry in the US and Europe. The objective of the breakout sessions was to identify products/projects that could reach the market place 10 to 15 years from now. These products/projects would then be taken through the EPOBIO process [3] and analyzed for their technical, economic, environmental and social aspects to validate their choice for further work. The key issues listed above formed the main topics of the breakout sessions each of which was introduced by an expert in the field.

Discussion topics of the breakout sessions and names of the presenters.

Discussion topic	Presenter and discussion leader
Cellulose	Simon Turner (University of Manchester)
Hemicellulose	Kenneth Keegstra (Michigan State University)
Pectin	Henrik Scheller (Royal Veterinary and Agricultural University, Copenhagen)
Lignin	Lise Jouanin (INRA, Versailles)
Genomics	Sarah Hake (USDA/University of California)
Cell wall development, methods/imaging	Maureen McCann (Purdue University)
Grasses	John Vogel (USDA, Albany)
Forestry	Matthias Dieter (Federal Research Centre for Forestry and Forest Products, Hamburg)
Novel composites/biomimicry	Ines Ezcurra (KTH Biotechnology, Stockholm)
Enzymatic cell-wall degradation	Katja Johansen (Novozymes A/S)
Bioethanol production	Wolter Elbersen (Wageningen University)
Lignin-derived products	Ron Hatfield (USDA, Madison)
Final discussion	

4.3 Cell wall chemistry and genomics of cell wall biosynthesis

Cellulose

4.3.1 Simon Turner (University of Manchester, UK) introduced the topic of cellulose. Cellulose is one of the most abundant cell wall components and a major component of the lignocellulosic feedstock for biorefineries. Cellulose is currently used as a raw material for fibres, such as rayon, and other specialised chemicals. It was pointed out that energy production from ethanol made from lignocellulosics is predicted to be favourable in terms of greenhouse gas emissions compared to ethanol produced from corn starch [4]. Obvious goals to achieve more effective saccharification are to understand the organisation and inter-molecular interactions of cellulose in cell walls, increase its amount and, at the same time, make the polymer more accessible for breakdown into hexoses. A scientific bottleneck that was identified concerns the current lack of knowledge about the rate-limiting steps of cellulose biosynthesis and the relative importance of: (i) substrate availability; (ii) the level of cellulose synthase complexes in the plasmamembrane; (iii) the rate of crystallisation of cellulose chains to microfibrils within the wall; and (iv) the microfibril length. It is not known which, if any, of these is rate-limiting, nor whether cellulose synthesis can be altered without affecting the synthesis of other polymers in the wall, and indeed how these changes would affect the overall biomechanical properties of cell walls. An approach to make cellulose more accessible for breakdown processes could be the incorporation of molecular species between the cellulose microfibrils. However, again, it is not known how a manipulation such as this would affect cell wall properties and their impacts on plant development or responsiveness to stresses and disease.

4.3.2 During the following discussion, the formation of tension wood was suggested as a useful model system to learn more about cellulose synthesis. For example, tension wood formation could be used to identify regulators of cellulose biosynthesis since a cellulose-rich cell wall layer (gelatinous layer) is synthesised in fibres of this tissue. Tension wood formation can be induced experimentally and differential transcript and metabolite profiling can be readily carried out to investigate the regulation of cellulose biosynthesis. Trees with high proportions of tension wood grown in short-rotation plantations could potentially be used as feedstock for biorefineries. However, it was pointed out that the knowledge of the structure of cellulose in the gelatinous layer of tension wood fibers remains limited, and it is also not known whether this form of cellulose can be easily degraded in the biorefinery process.

4.3.3 The limited understanding of cellulose structure, for example the ratio between crystalline and amorphous cellulose, and of the structure of the amorphous regions, was identified as another scientific bottleneck. It was suggested that the crystallisation processes of synthetic polymers could aid understanding of the crystallisation process of cellulose, since our progress in understanding this process in the cell wall is hindered by the restricted accessibility. Similar problems beset the characterisation of cellulose structure, since it is highly probable that cellulose organisation will alter during extraction of cellulose from plant cell walls.

4.3.4 To optimise the biorefining process, an increased yield of released sugars from cellulose and other cell wall polysaccharides was recognised to be one of the major goals. The strategies adopted to tailor the cell wall would be informed by the range of products required from the borefinery. In this context the topic of feedstock was raised. The potential to breed crop-based or woody feedstock for increased biomass yield or selected cell wall properties was considered to be vast. The use of fruit and vegetable feedstocks that are not lignocellulosic, such as sugar beet pulp, citrus peel pulp, apple and grape pomace, as well as soybean residues, was also discussed. For example, it was estimated that about 1.6 Mio mt of sugar beet pulp annually are produced in the US, which would translate to about 200 Mio gallons of ethanol. Similar amounts of citrus peel and pulp are produced. This research must be underpinned by the design and development of new, reliable high throughput assays for cellulose content and cellulose crystallinity.

Hemicelluloses

4.3.5 The topic of hemicelluloses was introduced by Kenneth Keegstra (Michigan State University, USA). Hemicelluloses, such as xyloglucan, glucomannan or arabinoxylan, are complex polysaccharides that are diverse in sugar composition. Even the composition of a certain class of hemicellulose can vary depending on the plant species and the tissue-type analyzed. These data indicate clearly that the synthesis of hemicelluloses is highly regulated. A brief overview on the recent progress in understanding hemicellulose biosynthesis was given. For example, numerous genes encoding enzymes that form the backbone of glucomannan have been identified recently as well as those involved in the biosynthesis of mixed linkage glucans. However, neither the regulation of hemicellulose composition nor details of biosynthesis are understood. It may be possible that the proportions of different hemicelluloses in the wall could be controlled, and certain hemicelluloses in the secondary cell wall could be replaced by others – however, our limited understanding of these issues remains a major bottleneck. The bottleneck must be overcome if progress is to be made accurately to manipulate cell walls *in planta* and provide feedstock with tailored cell wall properties for ethanol production in biorefineries (refer also to a report from the US Department of Energy “From Biomass to Biofuels – a roadmap to the energy future” [5]).

4.3.6 During the following discussion it was emphasised that many other products besides ethanol could be produced from lignocellulosics and that biorefineries offer a major opportunity for multiple product streams. In this context, it was recognised that cell walls contain unique polymers with potentially novel functionalities for diverse applications if only they could be extracted, eg arabinoxylan containing high amounts of ferulic acid from the pericarp of corn bran. To obtain pure fractions of these oligo- and polysaccharides, new extraction and fractionation techniques must be developed. The short-term focus could remain increasing the yield of ethanol, through converting the entire polysaccharide fraction of the cell wall to individual sugars (saccharification). Ultimately, these biorefineries for ethanol production would provide a future platform for more diverse applications involving cell wall components. It was pointed out that the modern oil refineries also went through a similar developmental phase leading to those currently in operation producing a variety of products from crude oil.

4.3.7 The urgent need to develop reliable high-throughput assays to enable accurate measurements of the content of the hemicelluloses was emphasised.

Pectins

4.3.8 Pectins make up only a minor component of the lignocellulosic feedstock largely consisting of secondary walls, however, the polymers make up a high proportion of vegetable or fruit-based waste that consists principally of primary cell walls, explained Henrik Vibe Scheller (Royal Veterinary and Agricultural University, Denmark). Today, pectins are mainly extracted from plant tissues that have a high proportion of primary cell walls. Pectins are typically used as food ingredients, but a small proportion of these polymers is also used in non-food applications, such as coatings for pharmaceutical products and cosmetics. A European research programme (PECTICOAT) is underway to evaluate the utility of pectins for coatings of medical devices. The presentation stressed that an improved understanding of the structure/function relationships of pectin polymers is needed to design desirable molecular species for specific applications. Major scientific bottlenecks that were highlighted include: (1) the limited understanding of pectin biosynthesis and its regulation; (2) efficient processes for the extraction and fractionation of different classes of pectins; and (3) the limited understanding of pectin structure and availability of analytical methods for their rapid structural and functional characterisation. Understanding of the structure-function relationships of pectins will also contribute greatly to our understanding of how the localised composition of pectin in cell walls *in planta* affects the architecture of cellulose and hemicelluloses and their relative deposition.

4.3.9 During the following discussion, two major issues were raised. First, it became clear that a large amount of pectin-rich waste material, such as citrus peels or sugar beet pulp, is produced annually. Currently, this material is used as animal feed or has to be disposed of as waste. This material could potentially be a feedstock for ethanol production, however, compared to the abundance of lignocellulosic biomass its supply is limited. Nevertheless, it may be possible that these waste materials could be used to produce high value pectins for the pharmaceutical industry. Second, it was stressed that multiple uses of plant materials in biorefineries would be advantageous. Alfalfa was discussed as an example. Up to 15% dry weight of alfalfa consists of pectins, and these polymers could potentially be extracted from the biomass prior to converting the remainder to sugars. During the discussion of the concept of the ‘biorefinery village’, it was noted that ethanol biorefineries could become the central station of the village with streams of by-products redirected to satellite stations, each with optimised process technologies for their corresponding by-product streams. In the future, it was considered entirely feasible that additional applications for the by-product streams would be discovered and, in turn, those by-products would become the platform of future biorefineries.

Lignins

4.3.10 Lise Jouanin (INRA, France) introduced the topic of lignin. Lignin is a complex phenolic polymer and its composition and structure differs depending on the plant species, cell types and locations within the cell wall. The quality and quantity of lignin polymers are regulated during development and also during plant responses to distress and disease. It is well recognised that the process of lignification is highly regulated and little understood. Within agricultural feedstock, lignin is considered to be an undesirable polymer since its presence directly impacts on and limits the digestibility of the cell wall material. Lignin is also an undesirable polymer in the pulp and paper industry, since it must be removed from the fibres prior to their use. Currently, the bulk of the removed lignin is used for energy production by burning in paper mills. Lignification in plants has been studied widely using mutants and transgenic plants. These studies have indicated that a moderate reduction in lignin content is feasible without negatively affecting plant growth. Another avenue that has been explored to improve digestibility of lignin-containing walls is to modify the polymeric composition. For example, it has been shown that certain modifications can lead to a higher solubility of lignin in alkali. The following research needs were identified to overcome current scientific bottlenecks: (i) increased understanding of monolignol storage and transport; (ii) increased understanding of lignin polymerisation; (iii) increased understanding of the polymeric networks, their assembly and interactions in the secondary cell wall; (iv) production of plants with altered lignin composition and content to determine consequences on saccharification in cell wall processing.

4.3.11 During the following discussion it was emphasised that lignin is also an undesirable polymer in biorefinery feedstocks used for ethanol production, since some of its breakdown products act as inhibitors during subsequent microbial fermentation. The importance of understanding the secondary cell wall was again discussed since a knowledge of how the polymers are linked might underpin novel methods to induce a phase separation already existing within the cell wall, thereby leading to easier extraction and separation of lignin. Solubilised lignin from biorefineries could be burnt and used for energy production in a similar manner as its use in pulp and paper mills.

4.3.12 The plasticity of plants in responding to environmental conditions, particularly with respect to changes in cell wall composition and lignin deposition, makes it essential in any programme of work to compare and correlate the phenotypes of plants grown in greenhouses and field trials. For example, it is well established that lignification of maize culms differs depending on whether the plants are grown in the greenhouse or in the field. Similarly, the need for a better understanding of the feedback mechanisms linking cellulose and lignin biosynthesis was emphasised. Currently, it is simply not known how these feedback mechanisms are regulated, although transcript levels of genes involved in polysaccharide biosynthesis are known to be affected when the expression of genes encoding enzymes of the phenylpropanoid pathway is changed.

4.3.13 The vast potential for targeted breeding of biomass crops was stressed, both in relation to polysaccharide components of cell walls as well as phenolic components.

Genomics and Genetic Approaches to the Cell Wall

4.3.14 The topic of genomics and genomic approaches to the cell wall was introduced by Sarah Hake (USDA-ARS, University of California, USA). The main research need highlighted was the identification of genes that are involved in cell wall formation. How can these genes be identified, not only in model species, but also in crops? Strategies for exploring cell wall phenotypes and identifying the genes causally involved in producing those phenotypes include the screening of mutant populations by: (i) identifying visible phenotypes, for example *brown midrib* mutants; (ii) using digestibility assays to reveal biochemical/biophysical changes in the wall; (iii) through cell-wall digestion with known enzymes followed by oligomer profiling (OLIMP); and (iv) transcriptome analysis, eg coordinated expression analysis. In addition to the use of mutants and transgenic plants, advantage of natural genetic variation in plant populations, and for maize, the use of recombinant inbred populations, could also be used to link diversity in cell wall phenotype with the genes causally involved. Laser capture microscopy was suggested as a useful technique for isolating specific cell populations that could be analyzed. Through the use of bioinformatics, gene family members and their homologues in other plant species could be identified. The principal challenge will be to go from model species such as *Arabidopsis* or *Brachypodium* to other crop species and to identify the corresponding genes in those crops.

4.3.15 In the following discussion, potential energy crops and their genetic analysis were considered. A main theme throughout the discussion involved the urgent need to establish high throughput assays to determine the utility of different cell wall phenotypes for the production of ethanol and other potential products. It was emphasised that a wide range of relevant mutants and transgenic plants with altered wall phenotypes were already available but nothing was known of how these phenotypes would impact on ethanol production. Assay systems considered included, for example, digestibility assays in rumen, or the development of small-scale assays to mimic miniature biorefineries. It was recognised that a challenge for establishing reliable assay systems was going to be the correlation of laboratory-based assay results with the industrial manufacture of products in commercial biorefinery plants. Significantly metagenomic approaches could also be applied to identify enzymes that were highly effective in cell wall degradation, for example, the recent programme that involved sequencing of the genetic diversity of the microflora in the hind gut of termites.

4.4 Cell Wall Biology, Imaging and Methodology

Cell Wall Biology

4.4.1 Maureen McCann (Purdue University, USA) gave a presentation on cell wall biology in relation to biorefining. She emphasised that a better understanding of cell wall biology is essential to underpin the design of strategies to increase biomass production. Important research areas include those to improve understanding of: (i) carbon partitioning into cell walls; (ii) the coordination and regulation of cell wall synthesis; (iii) cell wall loosening and tightening; and (iv) the regulation of cell wall thickness. Biomass could be increased either through the production of larger plants, or through increasing the proportion of cell walls in plants.

4.4.2 A challenge for the biorefinery concept is the inherent heterogeneity of the feedstock in cell wall composition and architecture. The question was posed whether processing parameters should be optimised for specific cell wall types. At this stage of biorefinery development it was uncertain whether each industrial plant would process different types of feedstock simultaneously or sequentially. A challenge, in terms of engineering desired cell wall phenotypes in plants and tailoring cell walls to maximise their utility, might be the compensatory consequences of this engineering. For example, how will cell wall composition and architecture change, if the cellulose content of the wall is specifically increased? Also, how would these changes impact on functionality and parameters such as growth, development and defence?

4.4.3 Discussions addressed the cell biology parameters affecting the accessibility of cell walls in the feedstock to extractants or to hydrolytic enzymes. In this context different crop species would have different cell wall composition and architecture. The developmental state of the plant will influence cell wall composition, and this, in turn, will impact on the digestibility by hydrolases. Further, the anatomy of the plant tissues in the feedstock material will play an important role, for example the distribution of the lignified tissues, since these tissues will constitute a permeability barrier to extractants and enzymes. The proportion of primary and secondary cell walls in the materials, the degree of cell-cell adhesion and the structural water layer within the wall will also influence the potential for the feedstock to be hydrolyzed in a biorefinery, as will the nature of cross links between wall components. All cell wall cross links are going to impact on cell wall porosity, which will restrict the infiltration of hydrolytic enzymes. The current lack of understanding of how lignin and polysaccharides interact within the wall is an area requiring urgent research.

4.4.4 In the following discussion it was again confirmed that an important strategy would be empirically to test cell wall phenotypes for their suitability as a feedstock. Advantages could be taken of natural genetic variation, but also the wide range of cell wall mutants and transgenic plants with altered cell wall phenotypes that are available should be tested.

4.4.5 Another approach for more efficient degradation of cell walls could be to express hydrolytic enzymes in the plant. These enzymes could be placed under inducible control and predigest the cell walls prior to processing in the biorefinery.

Imaging and Methodology

4.4.6 This topic was also introduced by Maureen McCann. First, the methods already available were reviewed, such as the various spectroscopic, microscopic, chromatographic and enzymatic technologies. The usefulness of plant and cell culture model systems was highlighted. As an example for high throughput screening of cell wall phenotypes, FTIR spectroscopy was introduced. The urgent need for development of new methods was identified to enable the investigation of: (i) inter- and intra-chain interactions and polysaccharide conformations; (ii) side-chain distributions; (iii) the chemistry of cell wall polymers such as, for example, using 'glyco-arrays' that can be screened with antibodies.

4.4.7 Discussions focused on new assay systems and the design of miniature laboratory-based biorefineries to determine directly the utility of different cell wall phenotypes for eventual scale-up and product manufacture.

4.5 Biomass Crops and Cell Wall Biomimicry

Grasses

4.5.1 John Vogel (USDA-ARS, Albany, USA) introduced the topic of grasses and pointed out that corn starch is the most important feedstock for the US ethanol industry. However, the use of corn starch is well recognised to be unfavourable from an energy balance. Maize is an annual crop requiring the support of a relatively large input from fossil fuels, for example through the production of fertilizers. In the future it can be predicted that ethanol will also be produced from different types of feedstock, including lignocellulosic residues such as corn stover. Corn is likely to play a continuing role in biorefinery development since the associated infrastructures are already established. Nevertheless, an important research question in relation to the use of corn stover is the impact of its removal from agricultural land on future corn yield.

4.5.2 Dedicated perennial biomass crops such as switchgrass or *Miscanthus* will have a favourable energy balance compared to annual crop species such as maize. Other grasses such as Bermuda grass or giant reed could also play an important role in some geographical locations. Switchgrass has been chosen by the US Department of Energy as a promising biomass candidate for the US. Yields are as high as 12-18 mt ha^{-1}. *Miscanthus* is already being used in Europe and biomass yields range from 10-30 mt ha^{-1}. Both species are efficient perennial C4 grasses and need a low input after establishment. Harvesting is compatible with existing farm machinery. Another advantage is the comparatively easy re-conversion of the land for production of other crops. Grasses already represent a significant proportion of land use in the US and according to a report from the US Department of Energy [6] they are projected to be the major contributor to biomass production in the future.

4.5.3 Several research needs were highlighted: (i) advancement of our basic understanding of grass biology, especially the cell wall and how to optimise its composition, using model systems such as *Brachypodium distachyon*; (ii) accelerated domestication of dedicated energy crops through conventional breeding and biotechnology (vast potential since these are basically wild grasses); (iii) greater understanding of grass seed dormancy and early seedling growth; (iv) an understanding of mechanisms of yield loss due to residue removal from agricultural land.

4.5.4 The discussion confirmed that harvesting time of the grass feedstocks was important: it is common practice to harvest the dried biomass crops after senescence in winter. This practice ensures that nutrients are relocated to the perennial rootstock and therefore retained in the system to support grass growth in the following year.

4.5.5 Several questions were raised in relation to the use of *Brachypodium* as a grass model system and the research needs to be addressed including the need to characterise stem anatomy with focus on tissue types with secondary cell walls, and the need to explore in more detail the resistance of the model plant to pathogens.

Trees

4.5.6 Matthias Dieter (Federal Research Centre for Forestry and Forest Products, Germany) gave a presentation with the title “Woody biomass potential in Europe”. The raw wood potential for 28 European countries was analyzed and the results presented. Germany, France, Sweden and Poland have significant additional raw wood potentials. He expressed the view that the raw wood potential of all 28 countries will decline over the next 15 years from 170 Mio mt to 138 Mio mt. The decline is projected to be very significant in countries such as Germany and Romania. In addition, differences between the global round wood trade and the European fuel wood trade were highlighted. For example, round wood trade is very intensive globally, whereas fuel wood for Europe, due to the low price of the commodity, is only traded within the region.

4.5.7 In the following discussion, short rotation plantations were considered to have considerable potential to contribute to woody biomass in the future. It was also thought that forestry would become far more diverse and tree farms with species dedicated for specific uses could be established. The principal scientific bottlenecks to unlocking the full potential of woody biomass concern our limited knowledge of cell wall biosynthesis and wood development. Other constraints are the choice of the appropriate species, optimal rotation periods and the availability of land for forestry. Currently, forests represent a vast biomass resource globally, but wood is becoming an attractive material for energy use, and the future availability of wood may well decrease (refer also to “Where will the wood come from?” [7]) Whilst total forest area can be large this is often composed of relatively small stands that are individually owned. In this context many small forest owners might choose not to harvest their forests, because harvesting costs are sometimes not covered by the market prices and therefore the wood, although grown, is not managed nor available for use.

Novel Composites and Cell Wall Biomimicry

4.5.8 The topic of novel composites and cell wall biomimicry was presented by Ines Ezcurra (Royal Technical University (KTH), Sweden). The Strategic Research Centre for Biomimetic Fiber Engineering (BIOMIME) that brings together expertise from diverse areas such as wood biology, fibre biosynthesis, enzyme technology, polymer technology, surface technology, materials science and application research, was introduced. The aim of the Swedish research consortium is to learn how the structure and composition of plant cell walls affect its properties and how to use this knowledge to build new composites from fibres and polymers that are derived from cell walls. To gain a better understanding of secondary cell wall formation, differentially expressed genes can be identified during secondary cell wall biosynthesis in poplar and through proteomic approaches using poplar cell suspension cultures. Examples of modification of fibre surface properties were illustrated, such as using xyloglucan oligosaccharides modified by endotransglycosylases that are then used to coat fibre surfaces. It was suggested that hydrophobic packing material could also potentially be produced if fibres were coated with xyloglucan, decorated with hydrophobic groups. The strategic impacts of the BIOMIME project were described: (i) the development of novel, renewable raw materials; (ii) the renewal of the traditional fibre industry; (iii) the formation of a basis for new industries; (iv) to ensure a world leading position in fibre science and technology through combination of skills and expertise from different research areas.

4.5.9 In the following discussion, the multidisciplinary approach of BIOMIME was commended as an important means to take fibre science forward. The topic of cellulose nano-whiskers in biorefineries was raised since they have been shown to be very effective for reinforcing plastics. However, current bottlenecks include the lack of cheap methods to produce the nano-whiskers in sufficient quantities for industrial applications.

4.6 Cell Wall Degradation

Use of enzymes

4.6.1 Katja Johansen (Novozymes A/S, Denmark) presented the topic of enzymic cell wall degradation and introduced the cellulytic system of *Trichoderma reesei* as an example of high utility. The Novozymes work on degradation of pretreated corn stover was highlighted since their research in this area led to substantial decreases in cost and increased efficiency of ethanol production from the feedstock. Major research needs identified included the characterisation and identification of new hydrolases that would enable the utilisation of the unique carbohydrate chemistry of cell walls through their ability to release specific mono- and oligomer building bocks for further chemo/biotransformations.

4.6.2 In the following discussion the need to identify novel specific hydrolases was again strongly emphasised, and the potential of targeting hydrolases to specific locations in the wall composite through the use of microbial carbohydrate-binding modules was raised. However, it was pointed out that this discovery work should take place in parallel to research characterising the precise chemical structure of cell walls since both outputs are interdependent to achieve maximum utility of specific cell wall breakdown products.

4.6.3 The expression of cell wall degrading enzymes in plants was raised. This offers potential, but considerable research work is needed to take the strategy forward since it is not known whether the enzymes could survive current biomass pretreatments, nor how best to locate the hydrolases in the plant cells or cell walls.

Ethanol

4.6.4 An overview of ethanol production was given by Wolter Elbersen (Wageningen UR, The Netherlands). The demand for ethanol will increase in the future. Currently, ethanol is produced from feedstocks such as sugarcane, maize starch, wheat and barley starch, and to a limited extent from sugarbeet. These feedstocks are relatively expensive and require considerable agricultural land for cultivation. Attention is focusing on potential alternatives, particularly the lignocellulosic feedstocks such as softwoods, hardwoods and grasses as well as the agricultural by-products, straw and corn stover. Currently, most attention is focused on pretreatment of the biomass for ethanol production. Difficulties include the production of chemicals during the pretreatment that can inhibit downstream processes such as cell wall degradation and sugar fermentation. A number of useful research aims were identified including: (i) reduction of lignin content in cell walls; (ii) reduction of cellulose crystallinity; (iii) reduction of acetyl content of cell walls. Significantly, new markets for by-products must be found to contribute to increased cost-effectiveness of the overall biorefinery system.

4.6.5 In the following discussion it was pointed out that wheat straw could be a promising feedstock candidate for biorefineries in Europe, particularly if the ash content could be lowered. Other possible feedstocks could also be switchgrass, *Miscanthus* or wood pellets. In addition the need for empirical screening of cell wall mutants and transgenics of poplar, maize and *Arabidopsis* for ethanol production was again highlighted. Also, the issue of designing methods to ensure laboratory-based assays were relevant to large-scale production systems was again confirmed.

Products from Lignin

4.6.6 Ron Hatfield (USDA-ARS, USA) introduced the topic of lignin utilisation. First, the difference between lignified and unlignified cell walls was highlighted: lignified cell walls are much denser, have low porosity and are hydrophobic. Lignin is cross-linked to the carbohydrates in the wall and confers rigidity. There is great potential to alter the lignin structure and properties in plants leading to opportunities to design new plastics or other polymer-based products.

4.6.7 The following discussion focused on ferulic acid, its role in cross-linking lignin to other wall components and the role of these cross-links on lignin extractability, particularly from grass-based biomass.

4.7 Final discussion

4.7.1 The sessions concluded with the identification of short-, medium- and long-term research goals to underpin the development of new products/projects in the 10-15 year timescale. The process of saccharification was considered to be the highest priority for optimisation and the greatest bottleneck for realising the potential value of plant cell walls in biorefining. Any research on saccharification was recognised to need the involvement of multidisciplinary teams and the design and development of novel bio-assays linking cell wall phenotypes to their utility in biorefining and the nature of new degradation systems to release building blocks of high utility for further chemo/bioconversions. In parallel, it was recognised that mid- to long-term progress in this area would necessitate a co-ordinated programme of research leading to a much greater understanding of plant cell walls to be used as biorefinery feedstocks. This multidisciplinary programme needs to address both the cell biology and chemistry of cell walls as well as the regulation of synthesis and assembly of different cell wall polymers and the metabolic processes governing carbon partitioning in the different biomass crop species. This larger-scale research effort was considered essential to achieve the foundation for designing *in planta* strategies to engineer bespoke cell walls optimised for integrated biorefinery systems.

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5. PLANT OILS FLAGSHIP

Breakout sessions co-chaired by Flagship leaders Sten Stymne and John Dyer, report prepared by desk researcher Anders Carlsson

5.1 Summary

5.1. Our society is highly dependent on fossil fuels not just for its energy needs but importantly also for its supply of industrial feedstocks. It is also evident that this resource is limited and its prices inevitably will escalate to even greater levels than we currently experience. In order to avoid a major negative impact on our economy and society we will need to find reliable alternatives to petroleum. Plant oils are structurally similar to the hydrocarbon chains derived from petroleum and therefore oleochemical-based products represent a highly realistic alternative.

5.1.2 Plant oils are major agricultural commodities comprising 120 million metric tons total and worth about €40 billion, with 20% used for non-food applications that are worth about €8 billion. The two fatty acids, erucic and lauric acids, are the largest markets for specialty plant fatty acids, each market being worth more than €1 billion annually. Interestingly, these markets have been competing with petroleum alternatives for many years, even when petroleum was much less expensive than it is today. In light of the current upsurge in costs of crude oil, the relative costs for plant-derived oleochemicals are even more favorable compared to petroleum, which creates an immense opportunity for new products manufactured from plant oils to compete in the market. The further development of a global oleochemical market necessitating major increases in the production of plant seed oils offers significant new opportunities for agricultural businesses. This is particularly relevant for European agriculture following the reform of the Common Agricultural Policy and decoupling of subsidy from production.

5.1.3 The Workshop gathered a range of leading experts in the field of plant oils and products derived from these oils. During the Plant Oil Flagship breakout session, the focus of the discussion was to identify oleochemical-based products and related research and development necessary to underpin their future commercialisation. The session was organised in two parts, each containing different topics with invited experts leading the discussions. The first part focused on identifying examples of target products of industrial oils as well as the production systems necessary to manufacture them from plant oils and the traits that must be incorporated in those oils. In the second part the focus was on defining research and development areas and intellectual property issues necessary to achieve the products identified.

5.1.4 Several products were identified and were agreed to have high priority; in parallel, a number of target oils necessary to be developed for each of the products was also identified. The priorities identified are as follows:

Highest priority

Lubricants

- High 18:1
- C8-C10 fatty acid (FA)
- Wax esters
- Very long chain FA

BioDiesel

- High Carbon flux to FA
- High 18:1
- By-Products
- Yield

Polymers

- High 18:1
- Hydroxy, Epoxy, Conjugated FA

Non-food crop platform

Medium priority

Chemical feedstocks

- All selected FAs

Paints, solvents

- Conjugated FA

Lowest priority

Surfactants, Cosmetics

- High 18:1
- C8-C10 FA

Inks, dyes

- Conjugated FA

Further detail of these priorities and the associated research/development necessary is described later in the report.

5.1.5 A lubricant product and wax ester as the target oil was identified as the first priority for consideration. Lubricants comprise an immense global market. The market is currently dominated by petrochemical-derived products but, significantly, plant-derived oleochemicals also already capture a small proportion of this market. Wax esters represent a feedstock that can solve some of the existing problems associated with the use of pure vegetable oil as a lubricant. It has been demonstrated that wax esters can be produced in plants and although there are some issues associated with production (which require additional research to resolve), the experts agreed that it was a worthwhile and achievable objective. It was further agreed to develop this feedstock in *Crambe abyssinica*.

5.1.6 The need to establish a crop production platform(s) for novel oils was also highlighted.

5.1.7 The Plant Oil Flagship breakout session involved many international experts and participants from industry and academia. All are recognised for their participation in the discussions.

Europe

Amine Abbadi, Germany
Anders Carlsson, Sweden
Andy Pereira, The Netherlands
Corrado Fogher, Italy
Daniel Röme, Sweden
David Bennet, The Netherlands
Edward Worthington, The Netherlands
Elisabetta Biavati, Italy
Elma Salentijn, The Netherlands
Ernst Heinz, Germany
Fermin Azanza, France
Gareth Griffiths, UK
Grzegorz Kunikowski, Poland
Guy Barker, UK
Ian Graham, UK
Ivo Feussner, Germany
Judith Mitchell, UK
Kristina Georgieva, Bulgaria
Maarit Kivimäki, Finland
Manfred Kircher, Germany
Margrit Frentzen, Germany
Martin Kuiper, Belgium
Martin Miquel, France
Pablo Vera, Spain
Peter Dörmann, Germany
Peter Green, UK
Peter Griffee, Italy
Petra Sperling, Germany
Rene Lessire, France
Robert van Loo, The Netherlands
Sten Stymne, Sweden
Thomas Luck, Germany
Thomas Roscoe, France
Uwe Schneider, Germany

USA

Edgar Cahoon, USDA-ARS
Jan Jaworski, Danforth Center
John Browse, Washington State University
John Dyer, USDA-ARS
John Ohlrogge, Michigan State University
Kent Chapman, University of North Texas
Sevim Erhan, USDA-ARS

Canada

Jerome Konecsni, Canada
Xiao Qui, Canada

5.2 Introduction

5.2.1 Ian Graham (CNAP, University of York, UK) gave a general introduction to the breakout session. He stated that the aim of this workshop was to identify oleochemical-based products and related research and development necessary for their commercialisation. When choosing products it is important to think about the rationale for their development. It is imperative to know how well the identified products could compete with existing alternatives and what market sizes a new product can occupy. The market potential of specific products is therefore a central criterion, but also aspects such as governmental policies could be as important for the success or failure of a product. The biodiesel market is a good example of how policies have been vital in the creation of a market with a great demand of oleochemical raw materials.

5.2.2 Developing products to reach the market place 10-15 years from now inevitably includes removing barriers as well as taking on challenges of a technical nature. Products prioritised by the Breakout Session would be taken through the EPOBIO process and analysed for technical, economic, environmental and social aspects to further validate their choice for future research and development work.

5.3 End products, traits and oil targets

5.3.1 Jan Jaworski (Donald Danforth Plant Science Center, USA) introduced this section in which the aim was to produce an initial list of potentially significant products for the Plant Oil Flagship. In order to give a structure to the discussion, a list of end product areas was presented together with the type of traits and oil targets required. The importance of first prioritising the products based on pure economics and their market potential was emphasised.

End products	**Traits and oil targets**
Surfactants, Detergents, Soaps	Medium-chain fatty acids, etc.
Lubricants	Hydroxy fatty acids, wax esters, etc.
Solvents	Fatty acid methyl esters, etc.
Polymers	Hydroxy or any other reactive fatty acids
Paints, Coatings	Epoxy fatty acids, etc.
Inks, Dyes	Conjugated, epoxy-fatty acids, etc.
Cosmetics	Medium-chain fatty acids, wax esters, etc.
Chemical feedstocks	Ethylene, propylene, C8 and C10 mono- and di-carboxylic fatty acids, branched fatty acids, etc.

5.3.2 In the process of selecting products, it is important to consider the oil target(s) that will contribute to the product. For example, the nature of fatty acid or oil composition needed and level of oil purity required; the approaches necessary to achieve the desired fatty acid compositions and the associated feasibility and scientific/technical challenges; the nature of the optimal production process, such as biotechnology of field crops or the use of existing plant oils and post-harvest blending.

5.3.3 Surfactants, detergents and soaps - In the present production of surfactants, large volumes of organic solvents are used and this therefore is a nonenvironmentally friendly process. Plant oil-based surfactants have a much better environmental footprint and are therefore highly interesting.

5.3.4 Fatty acids important for these end products include, for example, 12:0 (abundant in coconut and oil palm kernel), 10:0 and 8:0 (either distilled from coconut or synthesised from mineral oil), phenolic compounds, and C9 dicarboxylic acids. Some additional examples of surfactants include plant-based sucrose fatty acid esters and alkyl polyglycosides. Fully acylated glucose with octanoic acid as a high temperature lubricant could have a major market, if it could be produced for 25-30 cents/lb. An example of a feedstock with a potential major market through avoiding the presence of organic solvents in the end product is virgin oil from coconut (VCO). The general idea of extracting the finished product directly from the seeds could be further exploited for other oils and products, provided the underpinning research and development is undertaken.

5.3.5 The potential of newly developed GM oil crops as alternative production systems for specialised oils that are already produced in existing crop species, such as coconut, was discussed. It was agreed that only in rare cases could this approach be justified, such as when there is a threat to the supply of an existing oil due to disease (for example, lethal yellowing disease and ganoderma basal stem rot in coconut and oil palm kernel). However, in each case the investment required to establish a new production system built around GM crops should be compared to the investment required to develop alternative crop protection strategies for the existing plants such as oil palm.

5.3.6 Lubricants present a major global market which is an excellent target for further development due to the significant growth potential for substitution of petrochemicals by plant oils. Lubricants are not required in high purity but are required in bulk amounts. A lubricant needs to be oxidatively stable over a wide temperature range and stable over a long lifetime. Boundary lubrication using vegetable oils is excellent. The thermal, pressure and oxidative stability problems encountered with vegetable oil-based hydraulic oils and other lubricants can be improved through the use of high oleic acid oils (and low linoleic and low linolenic acid), or blending with synthetic esters such as polyalcohols and diesters. Many synthetic ester oils are already made from vegetable oils. Chemical modifications by branching or polymerising the oil can also improve its performance. However, the market for vegetable oil-based lubricants is still limited due to the cost of production, rather than oil performance properties.

5.3.7 The discussion highlighted that wax esters will solve the problems associated with pure vegetable oils as lubricants, since they are oxidatively stable and more resistant to hydrolysis. The high melting point of waxes, however, may be a problem in certain applications, but addition of branched fatty acids to lower the melting point could be a solution. The feasibility to produce wax esters in transgenic plants has already been demonstrated[1] and although there are still some technical barriers, it is a system that is approachable through additional research. One potential constraint is the low germination of seeds derived from plants that transgenically produce wax esters, and this issue must be addressed for wide applicability of a crop-based production system.

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5.3.8 Other alternatives for lubricants are acylated sugars (sucrose and glucose esters with up to C12 and methyl branched fatty acids) produced in trichomes such as those of tobacco, and Meadowfoam oil (80% of 22:1 delta 5), which has good oxidative stability and very good performance. Using meadowfoam (*Limnanthes alba*) as a production system leads to a relatively high priced oil, but the same traits could be produced in greater abundance in GM crops to reduce the price. Erucic acid is used as a slipping agent for plastic products and is efficiently produced in high erucic acid rape and *Crambe abyssinica* seed (approximately 60% of seed oil composition). However, the production of oils containing higher than 60% erucic acid would substantially improve the overall economics.

5.3.9 Polymers - Polymer chemists require functionality on specific fatty acids at different positions along the carbon chain. Unusual fatty acids present a great opportunity in this field. Polymer chemists generally indicate that they need a relatively high purity (85-95%) of starting material in order to avoid purification of the desired fatty acid and thereby decrease the overall cost of the process. The major bottleneck in the production of industrially important fatty acids in transgenic plants has been the low yields of the desired fatty acids in seeds. Typically the novel fatty acids accumulate at much lower percentages in the transgenic plants than the level of natural fatty acids observed in the native seeds. Significantly, native seeds may produce oils containing very high amounts (60-90%) of the desired fatty acid, indicating that plants are clearly capable of producing these types of oils. A major collective research effort is required to achieve the production of high amounts of novel unusual fatty acids in transgenic plants. The types of molecules to consider are hydroxy, epoxy, conjugated fatty acids and double bonds at various sites that could readily act as feedstocks for polymer production systems. This area also presents opportunities to interface with white biotechnology since bacterial fermentation could, for example, through omega-oxidation of fatty acids, produce diacids that are ideal feedstocks for polyester production.

5.3.10 Since many of the industrially desirable fatty acids for use in polymer production are non-nutritional and might even be unhealthy to humans and animals, it was considered essential that the transgenic production system should involve use of a non-food oilseed crop.

5.3.11 Solvents - This is a field of considerable potential since the volatile organic solvents currently in use present major environmental concerns. Methyl esters from plants (e.g., methyl soyate from soybean oil) are excellent solvents and could readily replace petroleum-based products. Plant oil solvents have very low volatility and excellent solvation properties although they are slow in solubilising substances. There are a number of relatively small companies that are now marketing plant oil-derived solvents but, due to their size and lack of investment capability, their R&D into oil composition is restricted. This may present an opportunity in which increased research could rapidly improve products already on the market.

5.3.12 There is regulation in place that will increasingly force the paint industry to replace petroleum-based volatile organic solvents with alternatives. Replacements based on plant oils represent an interesting opportunity for reactive solvents, such as those with conjugated fatty acids as the drying agent. The methyl esters of soybean oil are also interesting but require increased reactivity. Epoxy fatty acids are also interesting in paint applications since they adhere to metals due to the polymerisation process during drying. Ideally, conjugated and epoxidised fatty acids could be used as drying agents to produce a variety of products, each tailored for specific end uses. In general, the higher the oxidising nature of the fatty acids, the better they are in providing a solid, protective film during the drying process.

5.3.13 Inks and Dyes - Soybean oil is already used in this market, in which the low oleic and high linoleic acid oils are preferred. For example, soyink has been highly successful in this field and is mainly used as a carrier for the ink such that drying takes place by partial absorption by the paper. Conjugated fatty acids or highly polyunsaturated oils such as those with more than 90% linolenic acid are the best drying oils for use on coated papers.

5.3.14 Cosmetics - Major oleochemical products in the cosmetics sector consist of different types of lotions. Ceramides, sphingolipids and triacylglycerols (TAGs) with medium chain fatty acids such as the 8:0 and 10:0 are the principal components. Increasingly, the cosmetic industry requires GLA (gamma linolenic fatty acid) in TAG and fatty acids are becoming recognised as important cosmeceuticals. For these applications, high value, low volume production systems are more appropriate.

5.3.15 Chemical feedstocks - This area is closely related to those described above and most of the fatty acids and oils that are relevant as chemical feedstocks are also relevant in other product areas, such as short and medium chained fatty acids, unusual as well as common fatty acids and very long chain fatty acids. As chemical feedstocks, the fatty acids serve as raw materials for further modification by green chemists to produce entirely new products. These chemical modifications, therefore, provide additional marketing opportunities for plant-derived oils. For example, as an alternative to using C8 and C10 produced by the plant, C18 fatty acids can be cracked with oxidative cleavage. Cleavage of C18:1 would render one molecule of 9:0 and one molecule of 9:0 dicarboxylic fatty acid. Other short fatty acids of different chain length could be obtained from longer fatty acids with double bonds at different positions, for example, C8 could be produced from calendic acid (8t10t12c-18:3) and C6 fatty acid and a three carbon unit from linoleic acid. Furthermore, the second product resulting from oxidative cleavage, *i.e*. the dicarboxylic acid, is a very interesting platform molecule in its own right, such as, for example, in polymer production.

5.3.16 Additional suggestions of fatty acids as chemical feedstocks included cyclopropane fatty acids, multi-epoxy fatty acids, lecithin and galactolipids. It was recommended that an “eco chemistry search” of exotic plant species to identify the types of fatty acids produced in nature might be useful using databases such as the SOFA (http://www.bagkf.de/sofa/).

5.3.17 Biodiesel - Biodiesel is already a major plant oil-based product on the global market. Ivo Feussner (Georg August University of Göttingen, Germany) presented an overview of biodiesel, followed by a discussion focused on yield and by-product issues.

5.3.18 Biodiesel is a mixture of methyl esters of any plant oil; through converting the oil into methyl esters the viscosity is lowered. Production of biodiesel in EU (EU 15) using rapeseed oil as the major source increased from 55,000 tonnes (1992) to almost 2 million tonnes (2004), with the leading countries as Germany (44% of production), France and Italy. The present prices (2005) for oilseed rape are €202 per tonne and €192 per tonne for food or non-food oilseed rape, respectively. In the EU, these are essentially the same oils having the same prices but the oil crop is cultivated for two different purposes. By 2010, the EU (EU 25) will have an estimated rape methyl ester (RME) consumption of 8.2 million tonnes. An additional 2.8 million tonnes will be required for food production. These RME/food volumes correspond to a cultivation area of 8.5 million ha. Today there are only 4.6 million ha in production. This highlights an urgent need to increase oil production that can be solved in a number of ways. One way is to increase the yield of rapeseed – this would necessitate a major new research effort. An alternative way is to increase rapeseed cultivation across Europe. A third way is to meet demand by importing low-cost plant oils from other countries.

5.3.19 The current oil and seed yield from rapeseed is 45% and 4.2 tonnes/ha, respectively. In comparison, oil and seed yield from soybean is 20% and 2.5–3.2 tonnes/ha. Changes in the carbon partitioning between oil and protein in the seed is suggested as one mechanism for increasing oil yield. Other factors influencing yield include farmers' practices, variations in disease pressure across Europe as well as optimising inputs such as fertilizer (nitrogen and sulphur) usage on the crop. It was considered important that a highly stable oil should be manufactured by the plant to minimise the amount of free fatty acid produced during the extraction process. Since free fatty acids cannot be converted into biodiesel by the alkaline production method normally used, their presence can reduce biodiesel yield by up to 5%. In the US, the desired increase in oil yield could be solved by simply replacing some of the acreage used for soybean with rapeseed which, in principle, could lead to a doubling of the yield. In Europe it was considered questionable that oil yield could be increased easily.

5.3.20 Glycerol as a by-product of RME production may not ultimately remain a significant problem since new markets will undoubtedly be found for its use. Protein is the other major by-product from the oilseed cake and this component has potential use in the bulk production of thermoplastics and adhesives. At current oil prices, replacement of these petroleum-derived products by vegetable oil by-products is economically unviable.

5.3.21 The discussions highlighted that the cost-competitiveness of biodiesel production requires improvement. Achieving this goal requires a integrated combination of approaches including the reduction of agricultural production costs, improvement of farming practices to increase overall yield, use of higher oil varieties to increase yields of oil per hectare, and discovery of value-added uses for low-value co-products. Research conducted within EPOBIO could readily focus on the issue of carbon partitioning, which would establish the knowledge base required to increase the amount of oil in seeds whilst in parallel reducing starch and protein content. Oil crop species yielding higher amounts of oil would be a specific deliverable from this research.

5.3.22 Alternative oil crops for local biodiesel production in developing countries were discussed such as, for example, *Jatropha*. Spring rapeseed and *Crambe* could also be considered as oil crops in geographical areas that are not suitable for cultivation of winter rapeseed. Soybean can be grown in southern Europe (4 tonnes/ha) and *Brassica carinata* (2 tonnes/ha) in drier European areas. Finally, starch-producing crops such as cereals could in theory be converted into oil crops to further increase geographical areas for cultivation and reduce costs for producing biodiesel.

5.4 Recommended product and target oil priorities

5.4.1 Jan Jaworski (Donald Danforth Plant Science Center, USA) opened the second day of the Breakout Session by presenting a re-prioritised list of the products and target oils compiled from the discussions, ranking highest priority as 5, lowest as 1. The list was used as a basis for more detailed analyses of potential targets, each evaluated by the participants. Significantly, several products were given similar rankings, such as lubricants, polymers and biodiesel, which were all ranked as very high priority.

Lubricants (5)
    Hi 18:1
    C8-C10 FA
    Wax esters
    Meadowfoam
Biodiesel (5)
    Hi Carbon flux into FA
    Hi 18:1
    By-Products
    Yield

Polymers (5)
    Hi 18:1
    Hydroxy FA

Chemical feedstocks (4)
    All modified FA
Paints (4)
    Conjugated FA

Surfactants, Cosmetics (3)
    Hi 18:1
    C8-C10 FA
Inks, dyes (3)
    Conjugated FA

5.5 Non-food oilseed platforms

5.5.1 The successful development of oleochemical-based products for global markets is critically dependent on the effectiveness and cost competitiveness of the strategies chosen for the production of the specialised industrial oils. In principle, this production could be achieved using specialty crops that naturally produce industrially important oils. An alternative production system involves general purpose, high-yielding crop platforms to produce the required specialty oils through genetic engineering techniques The advantages of specialty crops that naturally produce industrially important oils include better public acceptance due to the lack of GM technology and seed oils that often contain high concentrations of the desired fatty acid product. Disadvantages include typical poor agronomic performance (small seeds, low oil yields, etc.) and the need for considerable domestication. In contrast, the principal advantages with the use of non-food, general purpose crop platforms is that high yielding crop species can be chosen for the applications and only a limited number of genetic tools must be developed. A negative public attitude towards GM technology in Europe is, however, a current disadvantage.

5.5.2 Given the need for sustainable production and manufacture, as well as the relative stability in price and supply of vegetable oils, industrial use of oleochemicals is likely to increase. Therefore, development of production platforms for plant oils tailored for particular applications and cultivation in specific geographical regions should be considered. To underpin the future development of a GM strategy for replacement, the choice of the non-food oil crops to serve as platforms for industrial oil production is critical. Robert van Loo (Plant Research International, PRI, The Netherlands) led the discussion on this topic and provided a number of selection criteria for the choice of crops. Yield and agronomy are important parameters to avoid high production costs. Ease of processability of the crop is essential to avoid the need of developing new processing technologies and infrastructure. Cost competitiveness is highly influenced by the utility and value of by-products. Identity preservation, as well as out-crossing from the non-food crop, are critical issues to ensure that industrial products will not enter the food chain. There is considerable advantage if fast track plant breeding can be used to genetically improve the chosen plant species, as well as the availability of genetic tools, such as, for example, transformation protocols to further facilitate improvement of the agronomic traits.

5.5.3 Many specialty crops have already been investigated for their production capacity of non-food oils. These species include, for example, *Euphorbia lagascae* and *Vernonia galamensis* (epoxy fatty acid); *Lesquerella fendleri* (hydroxy fatty acid); *Lunaria* (very long chain fatty acid), *Cuphea* (medium chain fatty acid) and *Calendua officinalis* (conjugated fatty acid). However, in general, most of these plant species have significant constraints and require substantial plant breeding efforts to improve their agronomic properties. Whilst winter rapeseed yields 1.5 tonnes oil/ha, spring rape and *Crambe* yield 0.8 and 0.9 tonnes oil/ha respectively. *Crambe* is therefore already an alternative to spring rapeseed in terms of oil yield. Other oilseed species are yielding much less oil, such as *Lunaria*, *Euphorbia* and *Lesquerella* (0.4, 0.5 and 0.3 tonnes oil/ha respectively).

5.5.4 The participants agreed on the importance of developing a general purpose non-food GM oilseed crop as a platform to produce novel fatty acids for industrial applications. Use of a food crop for GM production of industrial fatty acids in Europe was considered not to be feasible due to potential problems of mixing of seeds into the food chain and general negative public attitudes towards GM technology. Thus, for the US, the crops considered as potential front runners in terms of cost for GM oilseed applications included *Brassica carinata*, tobacco, *Crambe*, cotton, soybean, flax and *Sinapis alba*. For European cultivation, non-food crops included *Crambe*, hemp and flax, although rapeseed, oat, wheat and sunflower were also considered, despite their uses in edible applications. Meal from crops with non-edible qualities would be directed for use in industrial processes, as well as in energy production. Detailed analyses of these potential crop platforms within the EPOBIO process should consider the usefulness of identifying different plant species for cultivation either in Europe or the US.

5.5.5 The choice of specialty oil crops for further development will be specific for different geographical locations. In this context, it was recommended that the development of a non-food oil crop platform is important outside the US and Europe, particularly in the developing countries. *Jatropha* was recommended for development and bulk production of biodiesel in Africa and Asia.

5.6 Metabolic constraints

5.6.1 The market price for oleochemical-based products is an important issue for their competitiveness. This in turn depends on the cost of production and processing the non-food oil. It has been estimated that, for example, a 10% increase in the erucic acid content of high erucic acid rapeseed would reduce the processing cost by half. Therefore, an obvious target is the development of high yielding crops that produce new specialty oils. To date, the yield of novel oils in transgenic oilseed crops has typically been low, indicating that a number of scientific bottlenecks must be overcome to remove the metabolic constraints and achieve a high yielding oil crop. John Ohlrogge (Michigan State University, USA) presented his views on this issue and led discussion of the following topics.

5.6.2 Low product yields - The low yield of many valuable unusual fatty acids produced in transgenic plants is a central problem. This is especially intriguing since many wild species produce the same specialty oils at very high levels (with unusual fatty acids accounting for up to 90% of seed oil fatty acid composition). It was pointed out that usually, in these transgenic strategies, a single gene encoding an enzyme for the synthesis on the novel unusual fatty acid has been expressed. Clearly, expression of a single gene is insufficient, since there is much lower accumulation of the desired fatty acid in the transgenic plants in comparison to the amount observed in the native plant species. Several speculative explanations have been put forward for this problem, such as, for example, protein levels from the expressed genes vary, expression of the target gene is too low, an optimised seed-specific expression strategy is required, reduced expression of endogenous genes, such as both plastid and cytosolic desaturases, may have occurred, or additional genes from the source plant may be required to enhance the production and accumulation of the desired fatty acid in transgenic seeds.

5.6.3 Recent experimental evidence has indicated that transgenic plants do not exclude the unusual fatty acids from their phospholipids, and this was raised in the discussion as a critical observation. In native plants such as castor bean, for example, an unusual fatty acid such as ricinoleic is present at approximately 90% in the TAGs of developing seeds, but is present at less than 5% in membrane phospholipids. Transgenic plants that are engineered to produce lauric acid have been found to have 63% lauric acid in the TAGs but also 38% in membrane phospholipids. A major conclusion from the discussion was the urgent need to improve understanding of how cells separate membrane lipid biosynthesis from storage lipid synthesis.

5.6.4 It was also suggested that accessory enzymes or the existence of a protein unit (“metabolon”) required for unusual fatty acid biosynthesis may be lacking in the transgenic plants. For example, accessory enzymes could include those that specifically transfer fatty acids from the phospholipids to the TAGs, or those that insert the fatty acids at site-specific positions on the TAGs. In this context, there was evidence to show that the production of high levels of petroselinic acid (cisΔ6-18:1) required at least 6 proteins specialised for petroselinic acid biosynthesis.

5.6.5 Another aspect of carbon flux is futile cycling. If an unusual fatty acid is produced in a plant cell and the plant lacks the accessory enzymes to incorporate that product into storage oils, then a cycle of beta oxidation may be induced to break down that fatty acid.

5.6.6 Whilst there are many examples of problems in producing unusual fatty acids in transgenic plants, the achievement of producing lauric acid in canola using a single gene and yielding more than 60% of laurate in the oil should be recognised.

5.6.7 If low yields of unusual fatty acids can be solved, additional metabolic constraints such as germination problems may remain. There is some evidence to suggest, for example, that high levels of stearate, as well as wax esters, in transgenic seeds leads to difficulties in the process of seed germination.

5.7 Lack of scientific knowledge

5.7.1 Several participants emphasised that in oil biosynthesis, and even for the very last step of assembling oil, the TAG biosynthesis, there is insufficient understanding of the enzymes involved. For many other steps, such as for example, handling the phosphatidyl choline (PC) the acyl-CoA synthetases, and the movement of acyl chains, a good understanding is lacking. Furthermore, it is not known how the unusual fatty acids move from the membrane PC, where they are made and how they are inserted into the TAG. It was also emphasised that a greater understanding of transcription factors and transcriptional regulation of oil synthesis is required to increase the oil yield in the field.

5.7.2 The 90% fatty acid target - There was general agreement that a proof of concept study to achieve 90% of a single specific fatty acid in the oil would be highly useful. High oleic, high erucic and high hydroxy were suggested as different examples in which the metabolic constraints would be different and the benefits of studying the constraints would also be applicable for different applications. It was also highlighted that it would be worthwhile to investigate a range of different plant species and compare those that store the oil in the endosperm with those that store the oil in the cotyledon, as well as those that accumulate the product in other tissues than the seed, such as tubers, leaves or the pericarp.

5.8 Molecular tools and knowledge base

5.8.1 Ed Cahoon (Donald Danforth Plant Science Center, USDA-ARS, USA) presented his views on the molecular tools and knowledge-base required to achieve the overall goal of high yielding, genetically enhanced oil crops. He described both the disadvantages and advantages of different approaches.

5.8.2 A direct approach to achieve the goal is to isolate genes encoding specialised protein products in the desired metabolic pathway and assay their effects and utility in model plant species. This approach can involve isolating genes for specialised fatty acid-modifying enzymes from seeds of species that make unusual fatty acids by EST and/or homology-based cloning strategies. The genes can also be identified through comparative genomics in which, for example, the castor bean genome, or that of any other plant genome that makes unusual fatty acids, is compared with the *Arabidopsis* genome. Genes encoding specialised enzymes and unique for the unusual fatty acid producing plants can be provisionally identified through bioinformatics and further analysed.

5.8.3 An open-minded approach would be the design of a genetic screen without any preconceived notions about genes involved in fatty acid metabolism. For example, if the goal is to increase the amount of hydroxy fatty acids in *Arabidopsis*, *Arabidopsis* lines that already have been engineered to produce hydroxy fatty acids could be retransformed with a cDNA library from developing castor bean seeds (that normally produce high amounts of hydroxy fatty acids). A high-throughput phenotypic screen will identify seeds that produce higher levels of ricinoleic acid and thereby provide a link to specific castor bean genes.

5.8.4 Another example of an open-minded approach is to start with a phenotype such as high stearate or conjugated fatty acids which leads to seeds with germination problems. Over-expression of a cDNA library with inducible promoters from a plant species that normally makes high levels of stearate or conjugated fatty acids yet enables those seeds to germinate, would enable the trait to be turned off or on. In this way, permissive/non-permissive conditions for a genetic screen can be used.

5.8.5 When interesting gene candidates have been cloned from either the direct or open minded approach, they need to be analysed in a model system. Whilst *Arabidopsis* is a well-known model, the species has some constraints for biochemical studies. *Brassica* seed tissues or somatic embryos of soybean have advantages and are good alternatives for these studies. It is also possible to use more high-throughput systems such as microorganisms, *Saccharomyces* or oleaginous yeasts (*e.g. Yarrowia lipolytica*) to analyse activities of specialised metabolic enzymes. However, it remains questionable whether microbial systems are appropriate models and whether they are capable of mimicking metabolic events in a developing seed.

5.8.6 To increase the yield of oil in plants, a much more comprehensive metabolic flux analysis of developing seeds is required to fully understand the basis for carbon partitioning. For example, a key issue that could form the basis for a collaborative project would be a metabolic flux analysis of acyl groups. Studying the metabolic flux of acyl groups in, for example, castor bean seeds, would probably lead to answers to many questions such as how to produce high levels of unusual fatty acids in transgenic plants. New advanced molecular tools are available to revisit plant species already studied in earlier years.

5.8.7 Whilst it was recognised that castor bean is an excellent model system for plants that produce industrial oils, the species has two major limitations that should be addressed. First, transformation methods are not available, and second, the presence of the ricin and allergenic proteins are problematic. Model oil seeds should be selected from plant species that produce unusual fatty acids in different ways, for example, by desaturases or desasturase-like enzymes located in the endoplasmic reticulum (e.g., castor, *Momordica charantia*) or by activities in the plastid (e.g., *Cuphea*).

5.8.8 Tools must also be developed to explore the level and activity of specific recombinant membrane-bound proteins in transgenic plants and relate these to optimised oil production without the requirement for extensive purification.

5.9 IP issues

5.9.1 Sten Stymne (Swedish University of Agricultural Science, Sweden) presented views on intellectual property issues. He considered that any existing IPR that can advance the field should be used since there is no reason to try and invent around the IP nor waste research time in doing so. In a research project involving industry there are hardly any fields in plant biotechnology that could be regarded as precompetitive. In European grants, funding with an industry partner typically includes a consortium agreement that states that the company has first access to any IP that comes out of the project. Consequently the company wants the universities involved in the project to also protect the IP.

5.9.2 Dr Stymne put forward the idea of an open source pool for common tasks relevant to many end products. His view was that with such an approach it should be possible in a research collaboration not to favour a single company and avoid locking the development into a single company. There are different models for this. One example could be that the IP for an enabling technology (e.g., a common crop platform) would be placed in a common pool. This IP would be freely accessible for everyone who is willing to agree to conditional use of that IP, for example, if someone improves on the technology they have to put the improved version back into the open source pool. If the technology is used to develop products, these could be protected from IP owned by the individual researcher or company.

5.9.3 It was raised that, for example, in the development of a non-food oilseed platform, the germplasm must be included in the open source pool. Without a variety, it would not be possible to develop a new crop regardless of the quality of the technologies used. It is therefore essential that institutes and companies with the interesting and relevant germplasm become involved in the initiative. The advantage for plant breeders in this context is a common platform from which the germplasm can be developed further by adding new traits that will enhance the value and acreage of the crop varieties.

5.10 Outcomes of the Plant Oil Flagship Breakout session

5.10.1 The focus of the session identified oleochemical based products and related research and development necessary for their commercialisation. During the closing section that was moderated by John Dyer (USDA-ARS, USA) and Sten Stymne (Swedish University of Agricultural Science, Sweden), the gathered experts agreed on a set of products listed according to a prioritisation scale (5 being the highest priority). For each product a number of critical oil targets were identified as necessary for achieving the products. In addition, for each of the oil targets within the highest prioritised products, oil seed production platforms were selected and the potential barriers for achieving the oil targets and research needed to overcome these barriers were identified. Finally, estimated time frames for delivering the products were defined.

Priority 5

Lubricants
- High 18:1
- C8-C10 fatty acid (FA)
- Wax esters
- Very long chain FA

BioDiesel
- High Carbon flux to FA
- High 18:1
- By-Products
- Yield

Polymers
- High 18:1
- Hydroxy, Epoxy, Conjugated FA

Non-food crop platform

Priority 4

Chemical feedstocks
- All selected FAs

Paints, solvents
- Conjugated FA

Priority 3

Surfactants, Cosmetics
- High 18:1
- C8-C10 FA

Inks, dyes
- Conjugated FA

Lubricants

Oil Target	Platform	Native species	Barriers	Actions	Time
High 18:1 (>90%)	Soybean, sunflower, canola, flax, *Brassica carinata* , cotton, *Crambe*		Residual PUFA	Gene silencing, GM	5 Yrs
C8-C10 fatty acid (FA)	Flax, *Crambe*	*Cuphea*	Channelling to TAG	Acyltransferases, acyl-CoA synth., acyl-ACP synthetases	10 Yrs
Wax esters	*Crambe*, *B. carinata*, flax, soybean		Germination	Genetic resources (synthesis, accumulation, mobilisation)	5-10 Yrs
Very long chain fatty acids	*Crambe*, *B. carinata*, flax, soybean, cotton	Meadowfoam, HEAR	Channeling to TAG, germination(?), membrane exclusion	Metabolic engineering, enzymes for membrane editing	10 Yrs

Biodiesel

Oil Target	Platform	Native species	Barriers	Actions	Time
Existing Oils	Rapeseed, soybean	*Jatropha*, palm	Cost; higher yield, reduced inputs, by-product value	Carbon partitioning	15 Yrs
		Oat, wheat, maize, *Lepidium*	Carbon flux, seed shattering	Carbon partitioning	15 Yrs
High 18:1 (>90%)	Soybean, sunflower, canola, flax, *B. carinata*, cotton, *Crambe*		Residual PUFA	Gene silencing, GM	5 Yrs

Polymers

Oil Target	Platform	Native species	Barriers	Actions	Time
High 18:1 (>90%)	Soybean, sunflower, canola, flax, *B. carinata*, cotton, *Crambe*		Residual PUFA	Gene silencing, GM	5 Yrs
Hydroxy FA	Soybean, *Crambe*, flax, *B. carinata*, cotton	Castor, *Lesquerella*	FA channelling, enzyme level, futile cycling	Native species characterisation (genes and enzymes)	10-15 Yrs
Epoxy FA	Soybean, *Crambe*, flax, *B. carinata*, cotton	*Vernonia, Euphorbia*	FA channelling, enzyme level, futile cycling	Native species characterisation (genes and enzymes)	10-15 Yrs
Conjugated FA	Soybean, *Crambe*, flax, *B. carinata*, cotton	*Calendula*, tung	FA channelling, enzyme level, futile cycling	Native species characterisation (genes and enzymes)	10-15 Yrs

5.10.2 In addition to the products identified, the central and urgent importance of developing a non-food oilseed crop platform was also recognised. In this context, a limited group of target crops should be identified and linked to their appropriateness for cultivation in different geographical regions. The following crops are those that the experts agreed should be evaluated further as potential oilseed crop platforms.

Non-food oilseed crop platform

Crop	Strength	Weakness	Time
Crambe	High yield, geographical distribution, transformation, similarity to *Arabidopsis*	Pod shatter	5-10 yrs
Cotton	High yield, processing infrastructure exists, geographical distribution, transformation, identity preserved, low value of seed oil and protein meal	Food crop in some areas High input crop, low drought tolerance	5 yrs
Flax	Processing infrastructure in place, transformation, IP	Food crop in some areas	5 yrs
Tobacco	Excellent transformation and genetic resources, good oil composition and yield	Lack of infrastructure for seed harvesting and processing, yield	10 yrs
Brassica carinata	Transformation, geographic distribution, processing infrastructure	Yield	5 yrs

6. BIOPOLYMERS FLAGSHIP

Breakout sessions co-chaired by Flagship leaders Yves Poirier and Bill Orts, report prepared by desk researcher Jan van Beilen

6.1 Summary

6.1.1 The EPOBIO Breakout Sessions on biopolymers discussed four classes of plant biopolymers, with the aim to clarify: 1) the prospects for the use of plant biopolymers as bioplastics or materials, on a scale that is relevant to European and US agriculture; 2) scientific and technological barriers currently preventing progress to market; and 3) the economic and societal factors that play significant roles in gaining access to global markets or increasing market share. Plant biopolymers, as all renewables, are becoming increasingly cost competitive as oil prices rise and the costs associated with greenhouse gas emissions lead to demands for more sustainable manufacture. As demand for renewables increases, plant products previously only used for food and some industrial applications are becoming considered as feedstocks for the fuel and chemical industries.

6.1.2 The four classes of biopolymers considered are starch, natural rubber, polyhydroxyalkanoates and proteins. These have very different properties and fields of application. However, in the context of their significant impact on agriculture, all of the classes can be considered as commodities that are traded on the basis of price and quality in a business-to-business market. The final usage of the polymers, such as for example packaging with a green image, and their production method, for example, transgenic or non-transgenic plants, determine to a large extent the attitude of business customers and society as a whole. Politics and regulatory frameworks have a considerable influence in setting standards, requiring life-cycle-assessments, compliance with sustainablility indicators and taxing undesirable by-products.

6.1.3 Starch is an abundant, cheap, versatile biopolymer produced worldwide from several different major crops. Only a small fraction of starch production is used in bioplastic applications since thermoplastic starch has a number of unfavourable properties. These problems are commonly tackled - but not completely solved - by extensive chemical modifications, blending, and thermal and physical treatments. The discussion on starch as a material did not identify clear novel targets. As a general goal, an improved knowledge-base and understanding of starch structure-properties relationships was recommended. The possibility of starch modifications *in planta* was also considered as a worthwhile objective. However, it was noted that starch has already been the focus of extensive EU and US research programmes in academia as well as in industry.

6.1.4 One of the most significant biological materials currently is natural rubber (NR). Chemically, it is a simple *cis*-1,4-polyisoprene, but many of its superior properties are determined by the ill-defined “impurities” present in *Hevea* latex. It is one of the few biopolymers lacking an affordable synthetic equivalent for many applications. At the same time, it is at great risk from a fungal pathogen (South American Leaf Blight - SALB), which has, to date, been excluded from the producing countries by a strict quarantine. An emerging issue with natural rubber is the growing number of people worldwide that are allergic to certain proteins present in *Hevea* rubber. The development of an alternative source of natural rubber was identified as a clear priority. Here, the primary focus should be on the breeding, agronomics and processing of guayule, currently the most promising alternative to *Hevea*. In Europe, guayule could be cultivated on marginal lands in Mediterranean countries. The development of a third crop, which could be Russian dandelion, Goldenrod or possibly transgenic sunflower or lettuce, was also recommended. Here, yield, agronomics, and the food-crop issue need to be taken into account. A clear requirement for a generic research programme aimed at studying the biochemistry and molecular genetics of rubber synthesis was identified. Knowledge from such a programme could be used to improve rubber quantity and quality in native or recombinant host plant species.

6.1.5 Many different polyhydroxyalkanoates (PHAs) can be distinguished with a wide range of monomer compositions and properties. If it is possible to produce these at a cost of €1-2 per kg, many potential applications become commercially attractive. According to some industry specialists, this price range is feasible with current, large-scale and fully integrated bioreactors and down-stream processing technology. Based on life-cycle-assessments, the production of one or two PHAs (potentially PHB and a PHA co-polymer) in plants could be attractive for large-scale/low value applications. Here, the identification of a suitable non-food host, reaching sufficient yield without compromising host fitness (by targeting to specific plant organs and metabolic engineering), and developing processing technology should have priority. Furthermore, a need for a generic research project on the carbon flux to and from acetyl-CoA, an important building block for both PHA and rubber, has been identified. Engineering PHAs as a co-product in a biomass crop has significant advantages in improving the cost effectiveness of biorefining for biofuels production.

6.1.6 “Protein polymers” brings together a very diverse collection of amino acid-based materials. Silk, collagen, adhesin, and numerous other fibrous proteins show great promise in that these materials have unique strength-to-weight, elastic, or adhesive properties, which justifies the extensive efforts that have been made to optimise the heterologous production of these proteins. Polyamino acids are non-ribosomally produced polymers that could replace chemically produced polyanionic and polycationic materials. A third even more heterogeneous group consists of the protein co-products from current agro-industry processes. Future large-scale biofuels production could lead to extensive amounts of proteins such as, for example, gluten, zein, soy and switchgrass protein, greatly exceeding that needed by the food and feed markets. This highly diverse group of protein biopolymers requires further analysis. The applications for fibrous proteins are not yet defined sufficiently well to consider the need for large-scale production in plants. As yet, no clear applications for protein co-products in biomass materials have been identified.

6.1.7 The principal discussions centred on the crop plant producing the functional biopolymers. However, additional areas for consideration are two fold. First, the plant can produce polymer feedstocks for use in industrial biotechnology and/or chemical synthesis. In polymerised form, monomer feedstocks are conveniently stored in the plant as osmotically neutral, and relatively easy to process biomaterials. In this context, as an example, cyanophycin was identified as a potential source of amino acids and derived chemicals. Second, the plant can produce monomers that are then polymerised into functional biomaterials post-harvest, such as again through industrial biotechnology and/or chemical synthesis. In these discussions it was recognised that a key issue is economics and the choice of targets in which synthesis in field crops provides a clear advantage over manufacture by microbial fermentation. These applications are at the interface of plant and industrial biotechnology since the plant production system is tightly linked to utility of feedstocks for fermentation.

6.1.8 The Biopolymers Flagship breakout session involved many international experts and participants from industry and academia. All are recognised for their participation in the discussions.

Europe

Kirsten Birkegaard Stær - Denmark
Simon Bright - UK
Peter Bruinenberg - The Netherlands
Udo Conrad - Germany
Michaelde Graaf - The Netherlands
Francesco Degli Innocenti - Italy
Steven Fish - UK
Corrado Fogher - Italy
Peter Griffee - Italy
Hinrich Harling - Germany
Barbara Hermann - The Netherlands
Kerry Kirwan - UK
Andries Koops – The Netherlands
Mats Levall - Sweden
Kerstin Lienemann - Belgiuim
Gordon McDougall - UK
Hans Mooibroek – The Netherlands
Marian Mours - Belgium
Richard Murphy - UK
Martin Patel – The Netherlands
Jens Pilling - Germany
J Dirk Schadot - Germany
Dietrich Scherzer - Germany
Peter Shewry - UK
Alison Smith - UK
Ewa Swiezewska - Poland
Pasi Vainikka - Finland
Peter van Dijk – The Netherlands
Jeroen van Soest – The Netherlands
Waltraud Vorwerg - Germany
Ralf Weberskirch – Germany

USA

Robert Anex - Iowa State University
Katrina Cornish - Yulex Corp.
David Kaplan - Tufts University
Bill Orts - USDA, ARS
Oliver Peoples - Metabolix

6.2 Techno-economic Feasibility

6.2.1 The Breakout Sessions on biopolymers started with an overview of a recent study entitled: Techno-economic feasibility of large-scale production of bio-based polymers in Europe (PRO-BIP), by Martin Patel of the Copernicus Institute, University of Utrecht, The Netherlands. In this overview and the subsequent discussions led by Robert Anex (Iowa State University, USA), several general issues were identified:

1. Many biopolymers can compete as replacements to their petrochemical equivalents based on price and performance. Natural rubber is unique as this polymer cannot be substituted by synthetic equivalents for many of its applications.
2. Competition between different uses (food, feed, fuels, chemicals and materials) of all renewables is strongly increasing. This leads to the expectation that the lower price limit will be set by the largest volume market, which is fuel.
3. Life-cycle-assessments indicate that plant biopolymers that are directly used as bioplastics or materials may offer considerable savings in energy use and greenhouse gas emissions over petrochemical equivalents.

4. Investment decisions could spoil the market for years to come (substantial petrochemical plastics production capacity of 50,000,000 tonnes/annum is being constructed in the Middle East and Asia).
5. The high investment cost to develop a new field crop for bio-based plastics in relation to the expected market volume is an important factor in view of the returns from low cost applications of bioplastics.
6. If transgenic plants are required, non-food plants should be used to achieve any prospect of acceptance in Europe. This factor is not relevant in the US.

6.2.2 The following sections focus on issues that are specific to each of the four classes of biopolymers.

6.3 Starch

6.3.1 Overviews on starch uses and potential modifications were presented by Francesco Degli Innocenti (Novamont, Italy), Waltraud Vorwerg (Fraunhofer Institute for Applied Polymer Research, Germany), and Peter Bruinenberg (AVEBE, The Netherlands). The subsequent discussion was moderated by Alison Smith (John Innes Centre, UK)

6.3.2 Starch is an abundant, cheap, versatile biopolymer produced worldwide from several different major crops. The fact that only a small fraction is used as a bioplastic can be attributed to the fact that thermoplastic starch has a number of unfavourable properties (Table 1). These problems are commonly tackled - but not completely solved - by extensive chemical modification, blending, and thermal and physical treatments. The discussion stated several times: “everything has been tried and patented”.

6.3.3 To make starch more suitable for bioplastics applications, it should be less crystalline, more hydrophobic, and stronger. A major aspect of the discussions focused on the possibilities to change starch *in planta*, to extend the range of currently available starch variants (for example, no amylose, high amylose, high phosphate, A- and B-type starches, different source plant starches), and their derivatives.

6.3.4 *In planta* modifications would typically involve transgenic plants. Here it was noted that 1) all starch plants are food plants, 2) the plant must be able to reutilise the modified starch (otherwise seeds or tubers will not germinate or sprout), and 3) the starch yield should not be compromised by the modifications.

6.3.5 During the discussions it was not possible to specify which modifications would be most useful to greatly improve starch for applications as bioplastic or material (which substitutions, at which degree of substitution). Moreover, developing methods to obtain these modifications without yield loss will take a considerable effort. Only limited suggestions were made and some have already been the subject of European funding:

1. Cationic starch (to replace a environmentally unfriendly process), has been the subject of several AIR and FAIR projects
2. High MW amylose (should have good properties for films and in food applications): this topic has been covered by the FAIR Bionanopack project

3. Low crystallinity starch, lowering the cost of hydrolysis (allowing cold starch hydrolysis, thus saving up to 30% of the energy costs), and facilitating introduction of modifications

6.3.6 A central point of the discussion was the necessity to better understand starch. However, this has been on the agenda for as long as starch is used in industry.

Table 1. Thermoplastic starch

Chemical composition	Amylose: linear -(1,4)-linked D-glucose polymer, MW 10^5-10^6 Amylopectin: -(1,4)-linked D-glucose polymer, -(1,6)-branches, MW 10^7-10^9	
Annual production	40'000 T/A, out of a total of 57'000'000 T/A starch	
Price	€ 0.20 to € 0.50 per kg, depending on source	
Main sources	Maize, potato, wheat, cassava, rice, pea, waxy and amylomaize, etc.	
Main general industrial uses	Diapers, cardboard, paper, fabrics, plastics, plaster, water treatment, detergent, oil drilling, filler for tyres, ……	
Main producers of TPS or starch foam	Novamont, BIOP, Biotec, Rodenburg Biopolymers, Green Light Products, National Starch and Chem., Earthshell	
Main use as bioplastic	Foams (for the loose fill foam market), mulch films, shopping bags, mouldable products (pots, cutlery, fast food packaging)	
Advantages	Cheap, widely available, many variant starches, many functional groups for derivatisation, grafting, and interaction with plasticisers	
Disadvantages	Mechanically weak, brittle, moisture sensitive, complex heterogeneous multiphase materials, sensitive to retrogradation, poor interaction with plasticisers and hydrophobic polymers, suitable only for short life applications (20% of the market), slow production rates in plastic film equipment	
Related materials	Glucans (polymers of D-glucose): glycogen, pullulan, cellulose, laminarin, dextran, lichenin, and other polysaccharides	
Important issues	1. The potential for starch bioplastics is several million T/A 2. Starch is a complex material (granule structure, amylose vs. amylopectin, crystallinity, chain-length) that is still not fully understood 3. Almost everything has been tried to improve properties 4. *In planta* modification of starch involves transgenic food plants 5. Starch yield should not be affected due to modifications 6. Composting vs. incineration vs. biogas production vs. re-use	
Recommendations & Timeline	1. Study starch structure-properties relationship 2. Feasibility study of *in planta* modifications of starch	15 years 5 years

6.4 Natural rubber

6.4.1 The prospects for alternative sources of natural rubber were introduced by Katrina Cornish (Yulex, USA) and Hans Mooibroek (Wageningen University, The Netherlands), with discussions being led by Bill Orts (USDA, USA).

6.4.2 One of the most successful biological materials is natural rubber (NR). Chemically, it is a simple *cis*-1,4-polyisoprene, but many of its superior properties are determined by the ill-defined "impurities" present in *Hevea* latex (Table 2). It is one of the few biopolymers lacking an affordable synthetic equivalent for many applications. At the same time, it is at great risk from a fungal pathogen (SALB), which has been kept out of the producing countries by a strict quarantine. The EU should consider the strategic nature of natural rubber and its security of supply with the possibility that current production in SE Asia 1) could be severely impacted by SALB, and 2) irrespective of the disease risk, supply is highly likely to fall short of demand in the near future.

6.4.3 It is clearly necessary to develop at least one good alternative natural rubber crop. Current efforts within the USDA target large-scale rubber production from the subtropical shrub guayule as well as other plants, such as sunflower. Since the American company Yulex (www.yulex.com) currently develops production of latex from guayule for high value/low volume hypoallergenic materials, development of guayule latex is not pre-competitive. However, large-scale production of guayule rubber for industrial applications is not yet planned, although the latex project provides an important stepping-stone to reach the required economy of scale. The present yield per ha is close to 1000 kg per year, which is actually in the same range as the productivity of *Hevea* plantations. Thus, the expected global shortfall of *Hevea* rubber by 2020 of 3,000,000 tonnes/annum could be obtained from 30,000 km^2 of guayule cultivation - an area similar to that under cultivation in Portugal (27’181 km^2).

6.4.4 It was noted that guayule has not yet been developed by a thorough breeding programme, and production processes for natural rubber from guayule have not been optimised. Research priorities for the EU could therefore include the identification of suitable regions (for example marginal lands in Mediterranean countries), agronomic studies, and the creation of a suitable germplasm by breeding for rubber yield, agronomic properties, and optimisation of post-harvest processing.

6.4.5 Several speakers raised the issue of continuing vulnerability to disruption of natural rubber supply chains even if there were two independent sources of production. The development of a third source of natural rubber would reduce the risk to security of supply. Russian dandelion may be such an alternative, but the latex is probably not hypoallergenic. Research carried out in the 1930’s and 1940’s indicate that although high quality rubber could be produced from Russian dandelion, the agronomics are unfavourable, and the yield is 10 fold lower than that from guayule or *Hevea*. Metabolic engineering of sunflower or lettuce as latex producing field crops are likely to be less appropriate for EU cultivation given they are food crops.

Table 2. Natural rubber

Chemical composition	*Cis*-1,4-polyisoprene and minor components (proteins, polysaccharides, minerals)	
Annual production	9’000’000 T/A	
Price	Up to € 1.80 per kg, depending on grade	
Main source	*Hevea brasiliensis* (rubber tree)	
Main exporting countries	Indonesia, Malaysia, Thailand, (Sri Lanka, India, China)	
Main uses	Tires, gloves, thread, condoms	
Advantages	High resilience, long fatigue life, very good tensile and tear properties, good creep and stress relaxation resistance, efficient heat dispersion, low-temperature flexibility, good balance of properties for demanding mechanical applications	
Disadvantages	Compared to some expensive synthetic rubbers: doesn’t age well, inferior resistance to sunlight, oxygen, ozone, solvents and oils, variable quality due to local production, re-use is difficult	
Alternative plant sources	Guayule (10’000 T/A in 1910), Russian dandelion, Goldenrod (R&D)	
Related natural materials	Gutta percha and Balata (poly-*trans*-isoprene) Chicle (mixture of *cis*- and *trans*)	
Synthetic alternatives	Synthetic rubber (total 10’400’000 T/A): styrene-butadiene copolymers (SBR: 2’400’000 T/A), acrylonitrile-butadiene copolymers, and others	
Important issues	1. *Hevea brasiliensis* is a genetically extremely narrow crop: South American Leaf Blight could destroy NR production in South-East Asia, as has happened in Brazil (now at only 1% of world production) 2. NR price strongly increases, a 25% shortfall is expected in 15 years 3. Increased competition for land-use by palm-oil plantations (for biodiesel and food applications) 4. NR production from *Hevea* cannot be mechanised, and work-force is getting more expensive 4. Synthetic rubber alternatives are non-renewable 5. Allergenic hypersensitivity to *Hevea* NR is increasing	
Recommendations & Timeline	1. Develop guayule as an alternative / additional source of NR: domestication, breeding, agronomics, processing	10 years
	2. Develop a third source of NR: Russian dandelion, goldenrod, others?	15 years
	3. Study resistance to SALB?	

6.5 Polyhydroxyalkanoates

6.5.1 Two industry representatives (Oliver Peoples of Metabolix, USA, and Dietrich Scherzer of BASF, Germany) presented their views on the future of polyhydroxyalkanoates, and the potential of producing these polymers in plants. The discussion was led by Simon Bright (Warwick HRI, UK).

6.5.2 Many different polyhydroxyalkanoates (PHAs) with a wide range of monomer compositions and properties can be distinguished (Table 3). If it is possible to produce these at a cost of €1-2 per kg, a diverse range of potential applications become commercially very attractive. According to some industry specialists, this price range is already feasible with current, large-scale and fully integrated bioreactors and down-stream processing technology.

6.5.3 Using industrial biotechnology, the varying monomer compositions can be obtained by using different bacterial hosts, feeding regimes, and co-feeding specific monomers. It is precisely this feature that appears to be difficult to replicate in plants, since at least two independent metabolic pathways (supplying the intermediates) would have to be quantitatively controlled during *in planta* production of the polymer. Taking into account the investment cost to create a transgenic plant and the time required to generate a commercial germplasm, it seems advisable to concentrate on the production of one or two standard PHA polymers in plants (PHB and perhaps mclPHA), leaving production of the wide range of other PHAs to fermentation schemes and industrial biotechnology.

6.5.4 Since production of PHAs in plants by definition involves the use of transgenic plants, from the EU perspective it is preferable to focus on a non-food crop, such as, for example, the switchgrass chosen by Metabolix (www.metabolix.com), energy-crops such as *Miscanthus*, or a non-food oil-crop such as *Crambe*. In the case of engineering biomass crops to produce PHAs the choice of crop should be based on a full analysis of potential, risks, and market prospects in collaboration with the Cell Wall and Plant Oil Flagships. As with all other bio-based products, life cycle assessment should guide investments: currently, PHAs produced by fermentation seem less favourable than some biodegradable polymers partially derived from petrochemicals. However, production of PHAs in field crops is predicted to have both a positive greenhouse gas and a positive energy balance.

Table 3. Polyhydroxyalkanoates

Chemical composition	Polyesters of 3-hydroxyalkanoates and related hydroxy acids 1. Poly-3-hydroxybutyrate (PHB), high crystallinity 2. PHB-co-valerate (PHBV), high crystallinity 3. PHB-co-hexanoate (PHBH or Nodax), moderate crystallinity 4. mclPHA, C6-C16 monomers, elastomers, low crystallinity	
Annual production	< 1’000 T/A (Metabolix, Biomer, Biomatera) Monsanto stopped production of PHBV in 1998 ADM & Metabolix announced construction of a 50’000 T/A plant in 2006	
Price	€ 1.50 per kg (expected), currently € 10-20 per kg (PRO-BIP)	
Main source	Bacterial fermentation using sugars and oils as starting material	
Main producing countries	USA, Brazil, Germany, Japan, China, Thailand, presently all at a very small scale	
Main (industrial) uses	1. Thermoplasts for bottles, packaging material, cutlery, cups, bags, mulching films 2. Latex for coatings and films 3. Blending with other biodegradable polymers 4. For mclPHAs: source of monomers, paints, pressure sensitive adhesives, biodegradable cheese coatings, and bio-degradable rubbers	
Advantages	1. Hydrophobic and moisture resistant compared to other biopolymers 2. Choice of feed strategy and host organism allows many different monomer compositions, resulting in a wide range of properties: for example PHBH (Nodax) is easier to process than PHBV due to lower melting T, lower crystallinity, and has greater toughness and ductility 3. High oxygen impermeability 4. Processing on conventional equipment for polyolefins possible	
Disadvantages	General: high production costs, hydrophobicity makes blending with cheap hydrophilic polymers such as starch and proteins difficult PHB: brittle, stiff, decomposes just above melting T, unfavorable aging PHBV: slightly lower melting T as PHB, long processing times mclPHA: weak, sticky, rubbery	
Related materials	Polylactate (PLA), polycaprolactone (PCL), other polyesters produced by condensation of diacids and diols, or hydroxy acids	
Important issues for production of PHAs in plants	1. Transgenic food plants will not be accepted in the EU 2. LCA and land-use favor plant GMO over bacterial fermentation 3. Deleterious effects on plant growth at high PHA levels 4. Lack of control over monomer composition 5. Processing 6. Stability in harvested material	
Recommendations & Timeline	1. Identify suitable non-food host plant in collaboration with Plant Oils and Cell Walls Flagships	1 year
	2. Develop production of one or two PHAs (PHB and mclPHA) by a) metabolic engineering to increase supply of acetyl-CoA and other suitable precursors, and conversion to PHA, and b) preventing deleterious effects by choosing the proper cell compartment	10-15 years

6.6 Fibrous proteins, polyamino acids, and protein co-products

6.6.1 David Kaplan (Tufts University, USA) and Udo Conrad (Leibnitz Institute of Plant Genetics and Crop Plant Research, Germany) gave their views on the potential of fibrous protein production, followed by a discussion moderated by Jan van Beilen (University of Lausanne, Switzerland).

6.6.2 Silk, collagen, adhesin, and numerous other fibrous proteins show great promise in that these materials have unique strength-to-weight, elastic, or adhesive properties (Table 4). Therefore, considerable effort has targeted the heterologous production of these proteins. In a few cases, plants have been tested as hosts. More often, microorganisms (but also cell cultures, and animals) were used as host. Problems such as clone instability because of repetitive sequences, inclusion bodies, and difficult processing have thus far prevented breakthroughs in plant-based production systems. It is also not yet possible to obtain materials (fibres, glues, elastic tissue) from recombinant material with the same quality as the original material (silk from silkworm, etc.). These difficulties, particularly the maintenance of quality, were highlighted as bottlenecks requiring solutions prior to any undertaking of large-scale research efforts involving transgenic plants.

6.6.3 From the EPOBIO perspective, the question should be asked if any of these materials has a (potential) market size that would lead to a significant role for agriculture and crop production of the biopolymers. Moreover, it should be noted that one of the most interesting aspects of the fibrous proteins is the ability to specify properties through the DNA template. This allows tailoring to specific applications and processing, but at the same time fragments this sector, clearly favouring production in more flexible organisms than green plants.

6.6.4 Polyamino acids are non-ribosomally produced polymers. One example that is of particular interest in the EPOBIO context is cyanophycin, a polymer composed of a polyaspartate backbone with arginines linked to the -carboxy-groups. For cyanobacteria, this polymer serves as a N- and C-storage compound. Manufactured on a large scale in plants, the product could serve as a source of asparate, arginine, and derived chemicals, whilst the polyaspartate backbone could find applications as a biopolymer. Other polyamino acids were not considered in the breakout sessions.

6.6.5 The amount of protein co-products from future large-scale biofuels production can be expected to greatly exceed the amount that can be absorbed by the food and feed markets. Before the advent of cheap petrochemical plastics (1930s and 1940s), attempts were made to convert such proteins (e.g. soy meal, gluten or zein) to fibres and bioplastics, using chemical cross-linking agents. However, the results were mixed. The inferior quality of protein fibres and bioplastics was pointed out by specialists at the Workshop. Nevertheless, the millions of tons of protein produced per year as by-products from biofuel biorefineries constitute an opportunity that deserves to be investigated and discussed in more detail.

Table 4. Fibrous proteins, polyamino acids, and protein co-products

FIBROUS PROTEINS		
Chemical composition	Proteins consisting of short peptide motif repeats: strong, tough, elastic, adhesive	
Annual production	In plants, cell cultures: R&D scale. Silk from *Bombyx mori*: 62'000 T/A	
Main source	Silk: domestic silkworm	
Applications	*Bombyx* Silk: fabrics Spider silk: fibres with high strength to weight ratio	
Advantages of production in plants	Large scale, potentially cheap and reliable source	
Disadvantages of production in plants	1. Synthetic sequences with tailor-made properties implies a large number of possible products, which are easier to handle in other production organisms 2. Scale may not be enough to justify production in plants	
Important issues	1. Early stage of development 2. Processing of recombinant fibrous proteins has not been established 3. New applications made possible using synthetic sequences or combinations of sequences (e. g. block co-polymers of silk and collagen)	
Recommendations	1. The feasibility of production and processing should first be demonstrated in other hosts (not EPOBIO)	Not applicable
POLYAMINO ACIDS		
Chemical composition	Polyamino acids: polylysine, polyaspartate, polyglutamate, etc.	
Annual production	Unknown	
Main source	Polyaspartate: chemical synthesis or cyanophycin Polyglutamate, polylysine: microbial fermentation	
Applications	Polylysine: antimicrobial in food Cyanophycin: source of polyasparate, amino acids and chemicals Polyaspartate: may replace polyacrylates Polyleucine, polyalanine: chemical catalyst	
Advantages of plant production	Presently, only cyanophycin is tested for production in plants Large scale, potentially cheap, and reliable source	
Important issues	1. Only cyanophycin potentially has a potentially large volume market 2. Early stage of development 3. Only N-rich biopolymer	
Recommendations & Timeline	1. Investigate potential of cyanophycin	1 year
PROTEIN CO-PRODUCTS		
Chemical composition	Proteins, often mixtures, also with polysaccharides, fillers, chemically cross-linked	
Annual production	1'000'000 T/A, current amount converted to bioplastics very small	
Price of raw material	Zein € 10-20 / kg, gluten € 1.50 / kg, soy protein $ 0.28 / kg General price of bulk protein € 1.50 to € 5.00 / kg	
Main source	Co-products of starch and vegetable oil production from soy, maize, wheat, potato, pea, rape, ……	
Main companies involved in protein bioplastics	DuPont Soy Polymers, Zein Protein Products, Showa Sangyo, Freeman Industries, Inc., Rohm and Haas	
Main uses	Fibres, films, food coatings, adhesives	
Advantages	Glossy, scuff-proof, good oxygen-barrier	

Disadvantages	Expensive, hygroscopic, low strength, properties difficult to control	
Important issues	1. Protein co-products will become available in large amounts (several million T/A) as by-product of biofuels production (maize, sugarcane, switchgrass, rape, etc.) 2. Current overproduction of gluten 3. Most biomass-to-fuel processes will yield complex mixtures of proteins and other compounds, very diverse in molecular structure, complicated chemistry 4. Specific proteins (for example zein) may be tailored by genetic engineering for applications as fibre or bioplastic 5. New environmentally friendly methods for cross-linking are required	
Recommendations & Timeline	1. Investigate future availability and potential alternative uses of protein co-products	1-2 years

6.7 Conclusion

6.7.1 Based on the presentation and the discussions during the Breakout Sessions, a list of immediate priorities was drawn up (Tables 1-4). In a closing session moderated by Yves Poirier (University of Lausanne, Switzerland), this list was discussed with the participants.

6.7.2 In the case of starch, the discussion did not identify clear target products. As a more general goal, an improved knowledge-base of starch structure-properties relationships was highlighted. The identification of possibly useful *in planta* starch modifications could be worthwhile. However, starch is already the focus of many EU and US research programs in academia as well as in industry.

6.7.3 The development of an alternative source of natural rubber was identified as a clear priority to safeguard the security of global supply chains. Here, the primary focus should be on the breeding, agronomics and processing of guayule, currently the most promising alternative to *Hevea*. In Europe, guayule could be cultivated on marginal lands in Mediterranean countries. The development of a third crop was also recommended. This third crop was not defined, but could be Russian dandelion, Goldenrod or possibly transgenic sunflower or lettuce. However, yield, agronomics, and the food-crop issue need to be considered in identifying the third crop.

6.7.4 Based on life-cycle-assessments, the production of one or two PHAs (specifically PHB and perhaps mclPHA) in transgenic plants could be a useful objective. Here, the two priorities are: the identification of a suitable non-food host, reaching sufficient yield without compromising host fitness (by targeting to specific plant organs and metabolic engineering) and, the development of appropriate post-harvest industrial processing technology. Further consideration of these issues should be undertaken in collaboration with the Plant Oils and Cell Walls Flagships.

6.7.5 Protein polymers form a group that requires further analysis. Fibrous proteins are in a stage of research and market development that is too early to support the investment needed for large-scale production in plants. Cyanophycin is a promising new biopolymer, potentially with multiple large-scale applications. No suggestions were offered for protein co-products from the production of biofuels from biomass crops, although these materials are likely to become available in vast quantities as biorefining applications increase.

7. Preliminary discussion on environmental, agronomic and economic analyses

7.1 The scope of the work to be undertaken in the support themes on environment/agronomy impacts and economic analysis was introduced to the specialist participants. The structure of life cycle assessments (LCAs) and the main micro- and macro-economic constraints for new products to enter established markets were set out. Each step of the life cycle cost calculation was proposed to include the estimation of fixed, variable and environmental costs. Analyses will include the feedstock production costs, transport costs in different scenarios, product production and disposal costs.

7.2 Issues relevant to the resource flow scheme of new crops were outlined. These include energy use in all stages of production, fertiliser use, crop protection, weed management, water use, land-use, the environmental impact of emissions and of disposal, and topsoil degradation. Biodiversity is another environmental, but complex, topic encompassing many interactions at different levels. There is a vast range of issues impacting on biodiversity including landscape, agronomical functions, ecological functions, soil quality, the soil microbial community, insect biodiversity, agro-biodiversity and bird predation on some pest-insect populations. Due to the complexity of these interactions and the limited timescales available to EPOBIO, it was proposed not to include assessment of the effect on biodiversity in the studies.

7.3 LCAs are already available that model the environmental impact of various crop/product combinations. These LCAs can serve as templates to estimate the environmental impact of newly identified bio-based products. However, it is unclear how to measure the impact of the different parameters that are relevant for the resource flow scheme. It was therefore proposed, as was the case in an LCA performed by Narayanaswamy et al. (2003) for wheat starch, to use a relative scoring of each stage in the flow scheme to assess the environmental impact of crop-product combinations to be defined in the EPOBIO project.

7.4 General and more specific questions will be applied to each Flagship project/product in order to determine the relevant state of the art in breeding and agronomy and environmental impacts. This work will be carried out in close collaboration with analysis of the economic potential of the defined crop/product combinations. The integration of these studies will ensure the Flagships reflect the need to achieve sustainability and offer environmental benefits in addition to those for the economy, industry and the consumer. This close linkage between the support themes for economics and environment/agronomy was seen as an essential way forward for the analyses.

7.5 It was noted that there is an important need to define clearly the scope of the economics work and agree the level of detail required for analysis. This is particularly relevant given the short timescales of EPOBIO.

7.6 A key component of these analyses is the manufacturing process since data are not always readily available, especially for new processes, and significant time may be needed to find and validate the quality of any data that are available. Gaps in the data sets could be closed by standard assumptions and adjusted standard charge rates of similar processes. Sensitivity analyses could be used to find a good approximation and assess the market entrance probabilities. Sensitivity analyses will, in any case, need to be applied to the future (10-15 years) scenarios.

7.7 On crop production, it was recommended that the ENFA model could be used to establish the projected production data for feedstocks, giving information about future feedstock prices and the area of agriculture and forestry needed.

7.8 Land-use and allocation are important factors and the reference land systems - arable or set aside land – will need to be decided. For all LCAs it is important to give a narrative on the issues that will be excluded and inventories should go into the public domain to ensure transparency. Such an approach gives potential for the process to be used in a target-setting sense in that economic and environmental targets can be set and scientists and industry will see the context in which they need to deliver new products.

7.9 Carbon dioxide level specification needs to be precise with tracking for all carbon and the CO_2 balance for the entire life cycle of any product. There is potential to use the EPIC model. Information on feral populations can be obtained from the Gressel Rockefeller workshop on feral underutilised crops

7.10 It was agreed that, in view of the timeframe, EPOBIO is not the platform to perform classical LCAs. The objective of EPOBIO is to apply data that are already available to the selected case studies, using agreed scenarios available on the agricultural performance of non-food crops. It will be important to show improvements of new products relative to current products and in relation to each region in which the crops are cultivated. It will be important to capture, for example, reduced pesticide use.

7.11 LCA data only model the realities under specific circumstances and a model may not always be wholly accurate. Other systems or scenarios may have a better environmental balance. LCA helps with the evaluation of the identified products and informs on actions, however, decisions are not based on LCAs alone and the results should reflect the essential points. A key is to keep the analyses as simple as possible and provide scenario options. This will need to include impacts analysed over timescales since future effects may be different.

7.12 The integration of the environmental and economic studies is seen as essential, bringing synergy to the analyses. Deciding and defining the scope of the work, in conjunction with a panel of experts, are vital. Publication of the work and transparency will be needed to underpin the results.

8. Preliminary discussion on social attitudes and communication

8.1 The analyses of social attitudes and communication are tightly linked to the products and projects that will be prioritised by the Flagship themes and further underpinned by the environmental and economic analyses, thus topics for these analyses will emerge subsequent to the workshop. As a consequence, discussion mainly centred on methodology with outputs from these analyses planned for presentation at the 2007 EPOBIO Workshop.

8.2 It was agreed that the two support themes should work very closely together and integrate the findings from the social attitudes analyses into the design and development of appropriate communication strategies.

8.3 Within the work plan for the social attitudes support theme a series of questionnaires was proposed, designed to assess the views of the general public towards the development of plant-based biorenewables and the applications of plant raw materials in industrial products. These questionnaires will be carried out in a number of European countries. The attitude of the public towards GM as well as biotechnology in general will be an important feature to explore since the public's views in Europe on GM applications may alter whether food or non-food uses are proposed. Based on discussions at the Workshop and prioritisation of the first topics for detailed scientific analyses by Flagship desk researchers, results from the first questionnaires can be expected by October 2006.

8.4 It was agreed that the first stage in development of the communication support theme would involve an analysis of how the European media report issues concerning biorenewables and non-food crops. The methodology underpinning this analysis will be first trialled for UK-based media. Results emerging from the wider European analyses, together with the results emerging from the socio-economic questionnaires will underpin the design of tailor-made communication strategies for the different EPOBIO products/projects.

9. Closing remarks

9.1 The closing session of the Workshop was chaired by Dr Antoinette Betschart, Associate Administrator of the US Department of Agriculture, Agricultural Research Service and Dr Christian Patermann, Programme Director for Biotechnology, Agriculture and Food Research in the European Commission. Participants were invited to discuss the priorities for future action identified by the three Flagship breakout sessions and the discussion also incorporated issues raised by the support themes, including particularly environmental issues, socio-economic analyses and the need for the development of an effective communications strategy addressing public opinion.

9.2 It was recognised that the bio-based economy/biorefinery concept impacts on all three Flagship themes and there are major opportunities for interactions between the Flagships, particularly in the development of non-food crop platforms that may produce a range of bioproducts for extraction and further processing within biorefineries. It was also recognised that it is essential to take full account of global developments and the potential for international collaboration, such as in clustering large-scale projects across many related initiatives.

9.3 EPOBIO impacts on every stage of the supply chains from the targets for plant breeding through to manufacturing industries and the increasing interests of the consumer in sustainability in all its aspects.

9.4 Plant-based biorenewables offer opportunities for both high volume and high value product development. Major issues include the identification of unique functionalities that can only be sourced in plant-based manufacture, as well as the need to ensure cost effectiveness of field-based production systems in comparison to microbial and industrial fermenter-based production. It is likely that the new bioproducts will emerge both as replacement products to those based on petrochemicals, as well as novel products.

9.5 Whilst scientific and technical bottlenecks may be overcome in the laboratory, it is essential that these advances can be scaled up, whether in the development of new crop plant varieties for widespread cultivation or in their utility for biorefining. In the latter case the ability to assess the economic and technical feasibility of laboratory-based solutions in pilot industrial plants will be necessary and this must be industry-led to confirm its relevance.

9.6 At the conclusion of the Workshop, the instability of world oil prices and their impact on global manufacturing was emphasised. Increased prices will necessarily impact on the choice of production systems for many products that society needs. This requires a constant reviewing and analysis of options and scenarios since the choice to take up plant-based biorenewables by global industry will change as feedstock prices alter. This is clearly illustrated in the many current global initiatives to produce biofuels. Significantly, the capital investment into infrastructures being established for biorefineries for biofuels provides an important basis for incorporating wider non-fuel applications. It was agreed that the EPOBIO process offers a unique means of validating the research required to develop sustainable options for manufacture of chemicals and energy.

10. List of Participants

EPOBIO Consortium

Dianna	Bowles	University of York
Anders	Carlsson	Swedish University of Agricultural Sciences
David	Clayton	University of York
Jim	Coombs	CPL Scientific Publishing Services Limited
Francesco	Degli Innocenti	Novamont S.p.A
John	Dyer	United States Department of Agriculture
Sarah	Hake	United States Department of Agriculture
Thomas	Heinzow	University of Hamburg
Eileen	Herrera	United States Department of Agriculture
Joanne	McDonnell	British Sugar
Ralph	Möller	Max-Planck Institute für Molekulare Pflanzenphysiologie
Bill	Orts	United States Department of Agriculture
Maria	Paschou	National Hellenic Research Foundation
Markus	Pauly	Max-Planck Institute für Molekulare Pflanzenphysiologie
Andy	Pereira	Plant Research International
Yves	Poirier	Université de Lausanne
Giorgos	Sakellaris	National Hellenic Research Foundation
Elma	Salentijn	Plant Research International
Uwe	Schneider	University of Hamburg
Philippe	Soucaille	Metabolic Explorer
Sten	Stymne	Swedish University of Agricultual Sciences
Marcel	Toonen	Plant Research International
Jan	van Beilen	Université de Lausanne
Louisa	Wright	University of York

EC & US Representatives

Antoinette	Betschart	United States Department of Agriculture
Laurent	Bochereau	European Commission
Christian	Patermann	European Commission
Judy	St John	United States Department of Agriculture
Piero	Venturi	European Commission

Workshop Participants

Amine	Abbadi	Norddeutsche Pflanzenzucht Hans-Georg Lembke KG
Klaus	Ammann	University of Bern
Robert	Anex	Iowa State University
Fermin	Azanza	Syngenta Seeds S.A.S.
Przemyslaw	Baraniecki	Institute of Natural Fibres
Guy	Barker	University of Warwick
Claes-Göran	Beckeman	SCA – The Forest-Based Sector Technology Platform
David	Bennett	European Federation of Biotechnology
Elisabetta	Biavati	S.F.I.R. - Società Fondiaria Industriale Romagnola S.P.A.
Wout	Boerjan	Flanders Interuniversity Institute for Biotechnology (VIB)
Simon	Bright	University of Warwick
John	Browse	Washington State University
Peter	Bruinenberg	AVABE Group
Camille	Burel	EuropaBio
Edgar	Cahoon	United States Department of Agriculture
Kent	Chapman	University of North Texas

Udo	Conrad	Institut für Pflanzengenetik und Kulturpflanzenforschung
Katrina	Cornish	Yulex Corporation
Aimery	de Dinechin	Pôle de Compétitivé Industries et Agroressources
Lina	Diamantopoulos	
Matthias	Dieter	Federal Research Centre for Forestry
Oene	Dolstra	Plant Research International
Peter	Dörmann	Max-Planck Institute für Molekulare Pflanzenphysiologie
Michel	Ebskamp	Genetwister Technologies
Gerrit	Eggink	Wageningen University and Research Centre
Wolter	Elberson	Wageningen University and Research Centre
AnneMie	Emons	Wageningen University and Research Centre
Sevim	Erhan	United States Department of Agriculture
Ines	Ezcurra	KTH Biotechnology
Alfonso	Ezquerro	Acciona
Ivo	Feussner	Georg August University
Corrado	Fogher	Plantechno Srl
Jens	Freitag	Max-Planck Institute für Molekulare Pflanzenphysiologie
Margrit	Frentzen	RWTH Aachen University
Bernward	Garthoff	Bayer Crop Science
Kristina	Georgieva	AgroBioInstitute
Deborah	Goffner	Université Paul-Sabatier, Toulouse III
Ian	Graham	University of York
Peter	Green	AlphaGalileo Foundation
Peter	Griffee	Food and Agriculture Organisation of the United Nations - now retired
Gareth	Griffiths	Aston University
Candace	Haigler	North Carolina State University
Claire	Halpin	University of Dundee
Hinrich	Harling	KWS SAAT AG
Luc	Harvengt	AFOCEL
Ron	Hatfield	United States Department of Agriculture
Ernst	Heinz	University of Hamburg
Barbara	Hermann	Utrecht University
Arland	Hotchkiss	United States Department of Agriculture
Jan	Jaworski	Donald Danforth Plant Science Center
Katja	Johansen	Novozymes Inc.
Lise	Jouanin	National Institute for Agricultural Research-INRA
David	Kaplan	Tufts University
Ken	Keegstra	Michigan State University
Tony	Kinney	DuPont
Manfred	Kircher	Degussa AG
Maarit	Kivimäki	Finnish Forest Research Institute
Gisbert	Kley	Germany
Jerome	Konecsni	Genome Prairie
Andries	Koops	Plant Research International
Rodian	Kopitsky	
Martin	Kuiper	Flanders Interuniversity Institute for Biotechnology (VIB)
Grzegorz	Kunikowski	EC Baltic Renew Energy Centre
Colja	Laane	DSM – Sustainable Chemistry Technology Platform
André	Laroche	Agriculture and Agri-Food Canada
David	Law	BP Chemicals
Rene	Lessire	CNRS Bordeaux
Mats	Levall	Syngenta Seeds AB
Steven	Lewis	York & County Press
Antonio	Leyva	Centro Nacional de Biotecnologia-CSIG
Kerstin	Lienemann	German Federation of Private Plant Breeders
Thomas	Luck	BASF

Benadetta	Mattei	University of Rome “La Sapienza”
Derek	Matthews	National Institute of Agricultural Botany
Maureen	McCann	Purdue University
Gordon	McDougall	Scottish Crop Research Institute
James	McLaren	StrathKirn Inc. USA
Simon	McQueen-Mason	University of York
Stanislav	Miertus	ICS-UNIDO
Martine	Miquel	National Institute for Agricultural Research-INRA
Judith	Mitchell	University of York
Hans	Mooibroek	Wageningen University and Research Centre
Nigel	Mortimer	North Energy Associates
Marian	Mours	Cefic
Richard	Murphy	Imperial College
Michael	Obersteiner	International Institute for Applied Systems Analysis
John	Ohlrogge	Michigan State University
Marco	Pasti	Confagricoltura
Martin	Patel	Utrecht University
Oliver	Peoples	Metabolix
John	Pierce	DuPont Central Research
Jens	Pilling	Bayer Crop Science
Reno	Pontarollo	Genome Prairie
Rongda	Qu	North Carolina State University
Christine	Raines	University of Essex
Guido	Reinhardt	Institute for Energy and Environmental Research Heidelberg
Daniel	Röme	Perstorp Specialty Chemicals AB
Thomas	Roscoe	Laboratoire Genome et Developpement des Plantes
J. Dirk	Schadot	BMBF
Henrik	Scheller	The Royal Veterinary and Agricultural University
Dietrich	Scherzer	BASF
Peter	Shewry	Rothamsted Research
Alison	Smith	John Innes Centre
Petra	Sperling	University of Hamburg
Henrik	Stålbrand	Lund University
Eva	Stoger	Institute of Molecular Biotechnology
Björn	Sundberg	Umeå Plant Science Center
Ewa	Swiezewska	Institute of Biochemistry and Biophysics
Gail	Taylor	School of Biological Sciences
Christian	Tobias	United States Department of Agriculture
Simon	Turner	University of Manchester
Pasi	Vainikka	VTT
Albert	van Dijk	CCL Research of Cehave
Peter	van Dijk	Keygene NV
Robert	van Loo	PRI
Jeroen	van Soest	CP Kelco BV
Pablo	Vera	Instituto de Biología Molecular y Celular de Plantas
Richard	Visser	Wageningen University and Research Centre
John	Vogel	United States Department of Agriculture
Waltraud	Vorwerg	Fraunhofer Institute for Applied Polymer Research
Neil	Westcott	Agriculture & Agri-Food Canada
Annita	Westenbroek	Kenniscentrum Papier en Karton
Peter	Winsley	Ministry of Agriculture & Forestry
Edward	Worthington	Axel Christiernsson
Marc	Zabeau	University of Ghent - Plants for the Future Technology Platform

Note: This list omits reference to delegates who indicated that their details should not be released.