OIL CROP PLATFORMS FOR INDUSTRIAL USES

Outputs from the EPOBIO project

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EPOBIO: Realising the Economic Potential of Sustainable Resources
- Bioproducts from Non-food Crops

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EXECUTIVE SUMMARY

EPOBIO is an international project to realise the economic potential of plant-derived raw materials by designing new generations of bio-based products that will reach the marketplace 10-15 years from now.

EPOBIO is a "science-to-support-policy" project funded by the Framework 6 programme of the European Commission (EC). Partners from the European Union (EU) and United States (US), from academic research institutions and from industry, work together with an International Advisory Board of researchers, industrialists and policymakers. The aim is to ensure that a robust and holistic evidence-base is established to inform future national and international decision-making. This "EPOBIO process" considers new science-led projects and products within a wider context of their environmental impact, economics, regulatory framework, social acceptability and expectations of the public and policymakers. This holistic process underpins strategic recommendations that constitute the major outputs of EPOBIO.

Plants are already used widely to produce oil. Most of this oil crop cultivation globally is currently directed towards food and feed production. Increasingly, as the security and cost of supply of fossil reserves are becoming major issues, society is turning towards the need to use sustainable alternatives to the transport fuels of today. In this context, particularly in the EU, biodiesel is already a major biofuel produced from plant oil. In addition, oleochemicals have widespread use throughout many industrial sectors, as well as in healthcare and value-added applications. Thus, oil crops are already widely recognised for their utility and will undoubtedly continue to play a major role in the bio-based economy.

This report addresses the establishment of industrial crop platforms for oil. The report complements "Production of wax esters in crambe" prepared by EPOBIO in 2006 and available at www.EPOBIO.net.

Three crop platforms are considered. The first, rapeseed, is already a major crop globally; the second, oat, is explored as a potential new oil crop platform for Europe; the third, crambe, is relatively undeveloped compared to rape and oat but also holds significant potential for further development.

Rapeseed: The greatest strength of this crop for the production of industrial oil is the very considerable information-base and expertise available concerning its genetics, agronomy and molecular understanding of oil production. Current varieties however are relatively high input with respect to fertiliser and pesticide applications as well as requiring high levels of water. Given the negative environmental impact of these features, R&D should be directed towards improvement in these areas. There is also a major opportunity for increasing oil yield and the percentage of oleic acid in rapeseed oil. The existing science base is extensive and it is highly probable that these traits could readily be addressed. Rape improvement is likely to require a GM approach which may have social acceptability issues given the oil crop is used currently both as a food and a non-food feedstock.

Oat: Cultivation of oat is already widespread in many Member States of the EU but the grain is not established as an oil crop. Its major strength is that it is a low input crop, both with respect to fertiliser and pesticide, its weakness is its lack of cold tolerance. Whilst R&D could be designed to solve these issues it is likely that the technologies used will involve conventional breeding and tilling rather than GM due to high risk that oat can cross-fertilise with relatives that are known to be invasive weeds. There is considerable genetic diversity in oats which offers opportunities for breeding for increased oil content. Should strategic decisions be taken to develop oat as an industrial oil crop platform, the associated issues of using a traditional food and feed crop for non-food uses must be considered.

Crambe: The strength of the Crambe oil crop is that it is a dedicated non-food crop and can therefore potentially be developed into a highly efficient industrial crop platform for plant oils. Weaknesses include a narrow base of genetic diversity, comparatively low yield and low cold tolerance; there is also the possibility for potential gene flow to wild relatives but only in Member States of Southern Europe. However, on a timeline of 10/15 – 20 years, it is highly probable that GM solutions for these weaknesses will have been achieved. Crambe offers a good opportunity to develop designer transgenic lines each producing specialised fatty acids. There is a current issue in that the transformation systems for Crambe are not yet robust, but their optimisation is a component of developing Crambe into a large-scale commercial crop.

Strategic recommendations – science

Recommendations within this theme of industrial crop platforms must be viewed from the perspective of underpinning work that needs to be undertaken to ensure success in the market place in a 10/15 - 20 years time period. The global chemical industry would undoubtedly benefit from a sustainable source of basic carbon chain feedstocks and these could in principle be entirely provided by oleochemicals raising the significant possibility that oil crops could contribute to both the energy and non-energy sectors of the bioeconomy. It is likely that rapeseed oil will continue to contribute to the European industrial demand olecic acid, but R&D should be directed towards reducing the environmental impact of the crop as well as improving yield and proportion of the oil that is oleic acid to 90% or higher by composition. It is an issue for social acceptability whether the public will accept food and feed oil crops as industrial crop platforms. This issue is equally relevant for the development of oat as an oil crop. The high level of genetic diversity in the gene pool supports the possibility of breeding new oat varieties with higher oil yields by conventional breeding. It is a strategic decision as to whether the oat crop should be developed as an alternative to rapeseed for the production of oleic acid. For oat improvement, fast track technologies of breeding such as tilling should be encouraged in parallel to classical breeding. Given the potential risks of outcrossing to invasive weeds, GM improvement of oat is only to be used if no other technique is possible and then a marker-free selection strategy is advisable.

The advantage of Crambe is that it is not an existing food crop and there are no public perception issues involved in its development as an industrial oil crop platform. Crambe is an undeveloped crop currently requiring domestication and agronomic improvement as well as significant R&D to raise it to large-scale commercial cultivation. However, the possibility for producing a highly diverse range of designer oils in Crambe suggests the crop has considerable potential in the mid to long-term providing lack of social acceptability of GM industrial crops does not become a barrier.

The selection of a crop platform therefore requires careful consideration of the potential impact on existing markets, availability of production/processing infrastructure, challenges of identity preservation and appropriate risk assessment, as well as social acceptance of producing value-added non-food traits in food or non-food oil crops.

Strategic recommendations – policy

Our strategic recommendations on policy encompass six specific elements to ensure take up of the bio-based economy. These are:

- Policies must be coherent, integrated and coordinated.

Integration in Brussels and Member States is essential to develop a policy framework that will support the bioeconomy. As the bioeconomy represents a potentially huge strategic development consideration should be given to applying a ‘bioeconomy test’ to policies in development, in the same way that policies are assessed for their sustainable development impacts.

- Innovation in plant and industrial biotechnology should be supported.

Clear research objectives and a framework to achieve them are essential. An adequate level of targeted funding, selecting those novel and innovative processes and products likely to achieve success in the market place and deliver environmental benefit, should be an element of this.

- Policies should support development of the whole supply chain.

This will need to consider feedstock supply, processing and the production of bioproducts. There is a need to both stimulate the market side and build on the foundation of the Common Agricultural Policy which has moved from production subsidy to market-orientated developments. Financing along the supply chain needs to be considered as one aspect of feedstock supply.

- A communication strategy is essential.

The acute lack of awareness of the bioeconomy and the potential of biotechnology at all levels in society must be addressed by a strategic communications campaign designed to raise awareness and create an informed acceptance of bioproducts. This will need to explain the benefits of the processes and products delivered by the bioeconomy.

- Pilot projects have a role to play.

The establishment of proof of concept and testing under industrial conditions is a key step in moving research into product development. Scale-up during the research phase can develop and test industrial processes and also help to develop stronger co-operation between industrialists and academics.

- Measurable sustainability indicators should be developed.

The absence of validated techniques for the measurement of sustainability benefits needs to be addressed. This is important as these gains need be evidenced to enable all stakeholders to understand the rationale for the development of the bioeconomy.

In addition, Common Agricultural Policy support for the crops addressed in this report will primarily be delivered through the set aside mechanism. But the retention of set-aside is inconsistent with this market-led approach and the cultivation of non-food crops on set-aside land involves a significant degree of bureaucracy and cost. The future of set-aside should be reconsidered in the next round of CAP reform.

1 INTRODUCTION TO NON-FOOD OIL CROP PLATFORMS

Over the last century we have become dependent on fossil fuels not just as an energy source for transportation and heating but also for the provision of industrial feedstocks for a multitude of products that we use in every aspect of our daily lives. Fossil fuels are a finite resource and as this resource becomes limiting, oil prices will inevitably escalate to an even greater level than we currently experience. This will have a major negative impact on our economy and society.

The use of fossil fuels is adding a net input of carbon dioxide into the atmosphere. There are rising concerns among the public as well as scientists and decision makers, that this process leads to 'global warming', that is an increase in the average temperature of the Earth and the oceans. Global warming will have widespread influences on the environment, such as rising sea levels, changes in precipitation patterns, desertification, and so forth, which eventually will threaten our way of living.

Fossil fuels therefore need to be replaced with alternative, sustainable and renewable sources of energy and industrial feedstocks. The seed oils of plants are structurally similar to long chain hydrocarbons derived from petroleum, and thus represent excellent renewable resources for oleochemical production. Oils produced in oil seed plants include a wide range of fatty acids with five dominating fatty acids: palmitic, stearic, oleic, linoleic and linolenic acids, which are present in most food oils. In addition to these 'common' fatty acids, there are a great number of other fatty acids occurring in high amounts in seed oils from various wild plant species. These 'unusual fatty acids' include fatty acids with additional functional side groups such as epoxy and hydroxy groups, conjugated or acetylenic bonds, unusual mono-unsaturated fatty acids, and medium and very long chain fatty acids. Other unusual plant oils are ones made up of wax esters instead of triacylglycerols.

Both common as well as unusual plant oils are capable of satisfying demand from existing markets served by petrochemicals, and their use can also lead to the development of new market applications due to unique chemical functionalities present in unusual fatty acids. However, 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 industrial oils.

At an EPOBIO Workshop held in Wageningen in May 2006 the importance of robust crop platforms for the production of feedstock oils was highlighted. 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. A number of plant species were identified as potential candidates. After additional discussion and evaluation within EPOBIO, three crops, crambe, rapeseed and oats, were chosen to be evaluated in detail as non-food oil crop platforms.

Several criteria have been used in the selection process of the crop alternatives:

- 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 and out-crossing from the non-food crop are critical issues to ensure that industrial products do not enter the food chain.
- Fast track plant breeding such as ‘Tilling’ should be available to genetically improve the chosen plant species.
- It is recommended that various genetic tools are available, including transformation protocols, to further facilitate improvement of the agronomic traits through genetic engineering.

During the preceding discussions it was concluded that for the production of special plant oils designed to be used as feedstock for a broad range of industrial applications, specific non-food crop platforms are highly recommended. These designed plant oil qualities contain unusual fatty acids and should not mix with the food chain. The oil crop *Crambe abyssinica* (crambe) was identified as a candidate for such a non-food crop platform. Recognising the future development of a GM strategy for replacement, it was noted that crambe has good properties for GM technology that could be used in modifying its oil quality.

It was also acknowledged that a large part of the chemical industries in principle needs basic carbon chains as their feedstock. These can be provided by, for example, oleochemicals derived from high oleic acid oils produced by crop platforms such as rapeseed and oats. To meet the industries' demand of high volume - low price, the oleic acid content of plant oils needs to be at 90% or higher. This type of oil quality comprises only a minor problem if mixed up in the food chain since oleic acid is already a major component of vegetable oils.

In the present report the three crops, rapeseed, oat and crambe, are analysed for their potential to serve as non-food oil crop platforms producing industrial oils in 10/15 to 20 years from now.

2 THE POLICY CONTEXT FOR THE BIOECONOMY

2.1 Introduction

An opportunity exists to build a bioeconomy delivering sustainable economic growth with job creation and social cohesion as key outcomes. Creating such a bio-economy involves the substitution of fossil materials with renewable carbon. As a consequence of increasing the use of renewable resources for industrial feedstocks and for energy, the bioeconomy will bring benefits in a number of areas. Some examples of these benefits are:

- Reduced dependence on imported fossil oil.
- Reductions in greenhouse gas emissions.
- Building on the existing innovation base to support new developments.
- A bio-industry that is globally competitive.
- The development of processes that use biotechnology to reduce energy consumption.
- Job and wealth creation.
- The development of new, renewable materials.
- New markets for the agriculture and forestry sectors, including access to high-value markets.
- Underpinning a sustainable rural economy and infrastructure.
- Sustainable development along the supply chain from feedstocks to products and their end-of-life disposal.

In order to deliver these benefits it will be necessary to address a number of key challenges. Firstly, the potential of plant science to help deliver the bioeconomy is not well understood. This generally low level of awareness exists amongst politicians, policy makers, the general public and those likely to benefit directly, such as farmers and foresters. Secondly, large companies are reluctant to move away from production systems that are based on fossil oil. Feedstock costs will inevitably be a driver but there is also lack of experience of, and nervousness about, supply chains that originate in the agriculture and forestry sectors. There is also an absence of validated techniques for the measurement of sustainability benefits. Finally, building the bioeconomy requires the development of policies and a regulatory framework that recognise the linkages between a range of issues which include bio-resources, renewable feedstocks for energy and manufacturing, sustainable growth and employment, sustainable communities, climate change and other environmental issues and impacts.

2.2 Biorefineries and the bioeconomy

From a policy and regulatory perspective, the development of efficient and cost effective biorefineries is important for a number of reasons. Biorefineries are a key element in the bioeconomy, delivering renewable and sustainable products able to compete with existing fossil-derived products. Biorefineries already make a positive contribution to the delivery of international targets and governmental commitments for reductions in greenhouse gas emissions, whilst also addressing energy supply issues. Innovation directed to the development of new generations of more efficient biorefineries will deliver a major improvement in the level of the greenhouse gas emission reductions achieved.

Advances in plant science and biotechnology will underpin the future development of biorefineries that will support more diversified agriculture, forestry and industrial production systems that are more sustainable and deliver economic and societal advantages. Alternatives to food production will contribute to the redevelopment of rural areas.

The increasing concern about the environmental impact of the expansion of oil palm, soybean and sugar cane cultivation for biofuels feedstocks, leading to deforestation in Indonesia, Malaysia and Brazil can be addressed through the development of new generations of biorefineries. The future development of more efficient, second and third generation lignocellulosic biorefineries in Europe and the US affords the potential to track and evidence environmental impacts and benefits and increase the efficiency of production of biofuels. In parallel, it should be possible to reduce dependence on imported feedstocks and so help address environmental concerns about their production and use.

2.3 Fossil oil and biofuels

The production of biofuels in biorefineries and reducing dependence on fossil reserves is driven by a number of strategic imperatives including the price, finite nature and security of supply of fossil oil. Other factors include the detrimental environmental impact of fossil-derived fuels and mineral oils versus the renewable and sustainable nature of plant-derived alternatives.

There are also important regulatory drivers such as the indicative target in the EU of 5.75% biofuels by 2010, a target that has now been extended to 10% by 2020 'subject to biofuels becoming commercially available'. In the US, policy initiatives include the Energy Action Plan, mandating an increase in the use of bioethanol and biodiesel, and the Advanced Energy Initiative promoting the development of practical and competitive methods for the production of bioethanol from lignocellulose.

The expectation is that the biofuel industry will develop to significant size, consuming a significant proportion of biomass feedstocks. One concern is that the development of this industry depends on subsidising the product at the point of purchase. In addition, the environmental deliverables need to be compared to other potential options to, for example, reduce greenhouse gas emissions. This requires that a holistic view of the bioeconomy is taken, rather than feedstocks, products and markets being developed in isolation.

2.4 Common Agricultural Policy (CAP)

For the successful development of the bioeconomy it is essential that there is a robust agriculture sector that can provide a reliable source of feedstocks and deliver consistency of supply, price and quality. The 2003 reforms of the CAP, which were extended to the Mediterranean crop regimes (tobacco, cotton and olive oil) and to hops in 2004, broke the link between subsidy and production and brought a new focus on the market. Linked to actions designed to deliver sustainable farming strategies, these reforms provide a sound basis for farmers to take advantage of a new flexibility to innovate and seek out new markets. New income opportunities in farming are linked to the potential for diversification in agriculture and the new commercial markets of the bioeconomy will help farming, encourage sustainability and underpin the wider rural economy and its infrastructure.

Within the single farm payment scheme, land used for the cultivation of permanent crops (non-rotational crops that occupy the land for five years or longer and yield repeated harvests) is not eligible. Permanent crops include, for example, short rotation coppice and miscanthus. However, short rotation coppice and miscanthus can be grown on non set-aside land and the single payment received if the energy crop aid is also claimed. Any permanent crop or tree species used for a non-energy, non-food application would not be eligible for the EU single farm payment scheme unless grown on set-aside land. In this way, permanent crops grown as feedstocks for non-energy products are disadvantaged compared to those grown for energy products.

The retention of compulsory set-aside and the requirement to withdraw land from agricultural production, has maintained an opportunity and an incentive to produce feedstocks for biofuels and biorenewables. Under the set-aside rules, the production of crops for specified non-food uses is allowed subject to certain conditions, including a requirement for contracts and the payment of securities. Currently 8% of land must be set-aside. There is no guarantee that set-aside will be retained in any future review of the CAP and it could be said that it has no place in a market-focussed CAP. This inevitably builds uncertainty into the future production of bio-based feedstocks.

There is an energy crop aid of €45 per hectare for crops grown on non set-aside land. Originally this was paid for a maximum guaranteed area of 1.5m hectares of land, but has now been increased to 2m hectares to extend availability to the newer Member States of the EU. If this ceiling is breached the aid will be reduced pro rata.

Multiannual crops generally have considerably higher establishment costs than annual crops. Support for establishment costs is possible under EU rural development regulations. Regulations also allow Member States to grant national aid of up to 50 per cent of the costs of establishing multiannual crops.

A simplification exercise is currently underway for the CAP. This began in December 2006 with a proposal to establish a single Common Market Organisation (CMO) for all agricultural products, to replace the existing 21 CMOs. The aim is to provide a single set of harmonised rules in the classic areas of market policy, such as intervention, private storage, import tariff, quotas, export refunds, safeguard measures, promotion of agricultural products, state aid rules and communication and reporting of data. The substance of existing rules and mechanisms will not change. It is expected that the simplification will enter into force in 2008.

It is essential that policy frameworks are well coordinated. Agriculture and forestry have a critical role to play but the bioeconomy impacts on over fifteen policy areas in the EU and on the work of 10 of the EU's Councils. There is a crucial need to look holistically at the development of the bioeconomy, from feedstock production through to products and their end of life disposal. The full range of feedstock supplies needs to be considered, including material from agriculture, forestry and from the waste sector. There must also be an overview of the full range of industrial developments that are being promoted including biomaterials, biofuels and other forms of renewable energy from biomass.

2.5 Use of genetically modified plants

The implications for the use of a genetically modified plant, the impact of current GMO regulations in Europe and the associated substantial regulatory compliance costs have to be considered. Small and medium sized enterprises are unlikely to be able to bear the costs associated with these issues and so future exploitation is likely to be undertaken only by multinationals. Taken together, these constraints have the potential to limit development in Europe and lead to a continuing dependence on imported fossil oil and a continuing loss of competitive advantage to other countries and regions where the cultivation of genetically modified crops is not constrained.

The risks associated with the use of a genetically modified crop can be mitigated in a number of ways. The use of a crop which cannot be used for food or feed is important. This is considered essential from a regulatory perspective, given that the infrastructure in agriculture cannot ensure 'fail-safe' separation of different varieties/traits in the same crop species. However, the use of a non-food crop can have negative consequences since, for example, oil crops such as Crambe have not been optimised for mainstream agriculture and their oil yield needs to be improved.

Risks can also be mitigated by the choice of a crop for which inter-species crosses with the closest-related species give sterile offspring. A third means of risk mitigation is the adoption of the same identity preservation practices for the cultivation of non-food GM crops as those already in place for the cultivation of GM foodcrops.

2.6 Land use and availability

There are a number of studies, relating to the future development of the bioeconomy that address land use and land availability issues. But, to date, they have done so in isolation either looking at feedstocks for biofuels, feedstocks for other forms of energy such as heat or electricity, or feedstocks for non-energy bioproducts. Studies have not generally had regard to the totality of potential development. There will be an essential need to balance food security and supply with the production of raw materials for the bioeconomy as a whole. The development of the bioeconomy means that there are key issues and consequences:

- Increasing demand for productive land – land becomes scarce.
- Increasing questions about land use – security of supply.
- Increasing competition for land – range of different crops.
- Increasing use of marginal land.

Land use and availability issues will need to be addressed across the whole bioeconomy landscape in the near future.

2.7 Climate change

Climate change is regarded as one of the greatest environmental, social and economic threats facing the planet. There are international efforts to combat climate change and the two major treaties addressing this issue are the United Nations Framework Convention on Climate Change and its Kyoto Protocol. The Convention on Climate Change sets an overall framework for intergovernmental efforts to tackle the challenge posed by climate change. It recognises that the climate system is a shared resource whose stability can be affected by industrial and other emissions of carbon dioxide and by other greenhouse gases. The Kyoto Protocol to the Convention assigns mandatory targets for the reduction of greenhouse gas emissions in signatory nations.

Climate change presents an opportunity for the bioeconomy, through the use of plants and forest materials as feedstocks, to displace fossil alternatives and so reduce greenhouse gas emissions. Bio-renewables are a sustainable means of providing the essential products needed by society.

The potential of green plants to use solar energy and manufacture raw material feedstocks offers a way to help address these issues and to deliver the sustainable development needed to underpin future societal needs and demands. Crops provide a sustainable and clean technology with the potential for high capacity and the ability to produce feedstocks for energy or complex chemicals, yielding multiple products from a single crop. Agriculture, horticulture, forestry and aquaculture can provide products for all aspects of our lives including food, feed, medicines, chemicals and materials. Non-food applications of crops and the potential for renewable energy are also increasingly important.

Climate change does, on the other hand, also give rise to real concern in respect of the sustainable development of agriculture and forestry globally. Temperature changes, water availability and extreme weather conditions are among the issues that will impact on agriculture in the years to come. There will be an impact on crops in terms of types, locations and yields and a potential loss of production potential in some geographic regions. Crop patterns and management practices will need to adapt to new scenarios. This will raise serious challenges for bioeconomy feedstocks and for agricultural incomes.

2.8 Sustainable development

There is a growing realisation that our current model of development is unsustainable and that the increasing burden we are placing on the water, land and air resources and on the environmental systems of our planet cannot continue. Sustainable development is about meeting the needs of present generations without jeopardising the needs of future generations. It involves a better quality of life for everyone, now and for generations to come. It offers a vision of progress that integrates immediate and longer-term needs, local and global needs, and regards social, economic and environmental needs as inseparable and interdependent components of human progress.

The EU sustainable development strategy sets overall objectives, targets and concrete actions for seven key priority challenges for the coming period until 2010. Of the seven areas, five are relevant to EPOBIO and the bioeconomy. They are:

- Climate change and clean energy
- Sustainable transport
- Sustainable production and consumption
- Better management of natural resources
- Fighting global poverty

The use of crops for the production of bioproducts has the potential to help deliver these elements of the sustainable development agenda. In this as well as other policy areas, the absence of validated techniques for the measurement of sustainability benefits will need to be addressed so that the gains can be evidenced.

2.9 Developing countries

Developing countries have the potential to share in the expansion of the global bio-economy, and its commercial returns, through the production of feedstocks and their processing. This is an innovation that will be market-led and could develop an industrial base, trade and the underpinning agricultural production. The development of crop production and processing in developing countries has the potential to deliver wealth creation and access to trade.

The importance of the agriculture sector in developing countries means that the expansion of the agro-industrial sector would bring an opportunity to reduce poverty in a sustainable way. Biorefineries and the production of bioproducts in developing countries could readily deliver social and economic benefits through the production of biofuels and energy for local use, integrated with bioproducts for export.

These productive activities, based on market-led innovation, developing technology and innovation, would provide access to new and growing markets. Poverty reduction through the revitalisation of the agro-industrial sector would be a tangible outcome of the production of feedstocks and the development of bioproducts in developing countries.

2.10 Industrial competitiveness

Industrial competitiveness in the bioeconomy depends on a number of factors. There is a need for a policy framework that is integrated and coordinated. The range of feedstock and their applications means that the bioeconomy is relevant to a large number of policies from agriculture through to trade and waste management. Policy developments should be considered for their impact on the bioeconomy and its future expansion. Existing regulatory barriers to moving traditional industry to a more sustainable bio-based approach will need to be removed, for example in the approval of bio-based products that replace existing chemical alternatives.

Support for innovation is an essential underpinning for the development of the bioeconomy. Research funding, industrial engagement, the participation of small and medium sized enterprises and technology transfer are key elements.

Political and policy initiatives, such as future reforms of the Common Agricultural Policy, can set the framework within which the future production of feedstocks for the bioeconomy can take place.

The development of a competitive bioeconomy will deliver tangible outcomes in a wide number of sectors of industry. As well as access to markets and trade, outcomes will include job creation in agriculture, forestry, the transport sector and manufacturing. Support for rural communities and the rural infrastructure will be part of this.

2.11 Strategic conclusions and recommendations

There are key elements that need to be in place to set a policy and regulatory framework for the development of the bioeconomy:

1. Policies must be coherent, integrated and coordinated.

Integration in Brussels and Member States is essential to develop a policy framework that will support the bioeconomy. As the bioeconomy represents a potentially huge strategic development consideration should be given to applying a ‘bioeconomy test’ to policies in development, in the same way that policies are assessed for their sustainable development impacts.

2. Innovation in plant and industrial biotechnology should be supported.

Clear research objectives and a framework to achieve them are essential. An adequate level of targeted funding, selecting those novel and innovative processes and products likely to achieve success in the market place and deliver environmental benefit, should be an element of this.

3. Policies should support development of the whole supply chain.

This will need to consider feedstock supply, processing and the production of bioproducts. There is a need to both stimulate the market side and build on the foundation of the Common Agricultural Policy which has moved from production subsidy to market-orientated developments. Financing along the supply chain needs to be considered as one aspect of feedstock supply.

4. A communication strategy is essential.

The acute lack of awareness of the bioeconomy and the potential of biotechnology at all levels in society must be addressed by a strategic communications campaign designed to raise awareness and create an informed acceptance of bioproducts. This will need to explain the benefits of the processes and products delivered by the bioeconomy.

5. Pilot projects have a role to play.

The establishment of proof of concept and testing under industrial conditions is a key step in moving research into product development. Scale-up during the research phase can develop and test industrial processes and also help to develop stronger co-operation between industrialists and academics.

6. Measurable sustainability indicators should be developed.

The absence of validated techniques for the measurement of sustainability benefits needs to be addressed. This is important as these gains need be evidenced to enable all stakeholders to understand the rationale for the development of the bioeconomy.

2.12 Specific conclusions and recommendations

We make the assumption that the market should lead the expansion of the bioeconomy and biorefining. We consider that subsidies – which are inevitably unsustainable, distort the economics of the market place and can be removed at any time – should not be a feature of this sector.

In this context, the 2003 reforms of the Common Agricultural Policy provide a sound basis for the development of crop platforms to provide feedstocks for activities led by the market. The crops considered in this report – Crambe, rapeseed and oat – can be cultivated on both non set-aside and set-aside land and can access the single payment. The retention of set-aside as a mechanism to influence production is inconsistent with this market-led approach. The cultivation of non-food crops on set-aside land does also involve the producer in a significant degree of bureaucracy and cost. We recommend that the future of set-aside be reconsidered in the next round of CAP reform.

In the case of Crambe, the implications for the use of a genetically modified plant, the impact of current GMO regulations in Europe and the associated substantial regulatory compliance costs have to be considered. It is the case that small and medium sized enterprises are unlikely to be able to bear the costs associated with these issues and so future exploitation is likely to be undertaken only by multinationals. Consequently, there is potential to limit development in Europe and lead to a continuing dependence on imported fossil oil and a continuing loss of competitive advantage to other countries and regions where the cultivation of genetically modified crops is not constrained.

Crambe is a crop which cannot be used for food or feed. Risks are further mitigated given that Crambe is a crop for which inter-species crosses with the closest-related species give sterile offspring and so it is not anticipated that Crambe will be able to cross easily with its related species. Finally, it is possible to adopt of the same identity preservation practices for the cultivation of non-food GM crops as those already in place for the cultivation of GM foodcrops. Our research on social attitudes shows that the public are more accepting of genetically modified plants where they cannot be used for food or feed. We therefore consider their use a case for a review of the regulatory regime for GM crops where such crops cannot be used for food or feed.

3 RAPESEED (*BRASSICA NAPUS*)

3.1 Introduction

Rapeseed (*Brassica napus*) is one of the major oil seed crops in the world. Cultivars with various oil qualities are grown in large acreage in Europe, North America, China and Australia. There are currently three principal uses of rapeseed oil. The bulk use is for food oils, produced in the 'Canola' varieties. Oil with high erucic acid content is produced for industrial purposes by both traditional varieties and those specifically developed for the purpose. Thirdly, rapeseed oil is increasingly used for the production of biodiesel.

Cultivars of so-called 'Canola' qualities are those in which the content of erucic fatty acid and the levels of glucosinolates have been reduced to an absolute minimum. These cultivars are grown for the excellent qualities of their oils for food applications.

A different variety of rapeseed cultivars have been developed specifically for industrial oils and with a high content in their oil of the very long chain fatty acid (VLCFA) erucic fatty acid (22:1). This fatty acid is a valuable industrial feedstock and also accumulates in the seed oils of other plants, such as most *Brassicaceae* species (for example *Limnanthes alba, Eruca sativa* and *Crambe abyssinica)* but also in other plant families such as in *Tropaeolum majus* and *Simmondsia chinensis*, , (Ohlrogge et al. 1978; Muuse et al. 1992; Bao et al. 1998; Mietkiewska et al. 2004). Erucic acid is used for manufacturing industrial products such like surfactants, high temperatures lubricants, plasticizers and surface coatings (Friedt and Lühs, 1998; Wang et al. 2003c). Global demand is steadily increasing, rising from 18 million tonne in 1990 and up to a projected 35 million tonne in 2010. Similarly, the demand for behenic acid (22:0), a derivative of erucic acid, is estimated to rise to 46 million tonne by 2010 (Jadhav et al. 2005).

There is increasing interest in the production of biofuels from agricultural feedstocks and biodiesel is attracting considerable interest, particularly in Europe. Currently rapeseed oil is a major component produced from feedstocks grown in Europe (EBB, 2005).

Given that rapeseed is an extremely well developed oil crop world-wide, and given that technologies are now such that the chemical conversions of fatty acids are readily achievable, an opportunity exists to promote cultivation of rapeseed as an all-purpose oil crop platform producing oil that can subsequently be used for a variety of purposes. It is this potential that forms the focus of this section.

3.2 Rapeseed oil

Rapeseed is a good example of how market demands, for example of requirements for specifically nutritional qualities, influences the breeding of new varieties and demonstrates the developmental potential of an oil crop. Using the natural genetic variation in *B. napus* and among its close relatives in the *Brassica* genus, new varieties have been developed by conventional approaches such as intraspecific and interspecific crosses, as well as taking advantage of new variation introduced by induced mutagenesis. In addition, transgenic approaches have recently also provided important tools in order to alter the oil composition beyond what is possible through conventional breeding approaches.

Oil from traditional rapeseed (*B. napus*) or its related cultivars (*B. rapa* and *B. juncea*), has a typical composition of 5% palmitic (C16:0), 1% stearic (C18:0), 15% oleic (C18:1), 14% linoleic (C18:2), 9% linolenic (C18:3) and 45% erucic fatty acid (C22:1) (Table 1).

A number of reviews have covered the impact of breeding on rapeseed oil quality (such as for example, Burton et al. 2004; Scarth and Tang, 2006) and Table 1 below summarises the major oil quality changes brought about by breeding.

Table 1 A list of various oil qualities in *B. napus* and techniques used for accomplish the changes in oil composition.

Oil type/ seed variety	Origin/ method	12:0	14:0	16:0	18:0	18:1 n-9	18:2 n-6	18:3 n-3	20:1 n-9	22:1 n-9	Others
Original rapeseed[1.]	Traditional	-	-	5	1	15	14	9	7	45	4
'Canola' [2.] (Double-low)	Spontaneous mutant	-	-	4	2	60	21	10	1	1	1
Low-linolenic 'Canola'[3.]	Mutagenesis (EMS)	-	-	4	1	59	29	3	1	-	1
Low-linolenic 'Canola'[4.]	Mutagenesis (EMS)	-	-	4	2	66	24	2	1	-	1
High-oleic 'Canola'[2.]	Mutagenesis / transgenic	-	-	4	1	84	5	3	1	-	2
Laurate 'Canola'[2.]	Genetic engineering	37	4	3	1	33	12	7	-	-	3
High myristate/ palmitate[2.]	Genetic engineering	-	18	23	2	34	15	4	-	-	4
Very High-erucic acid rapeseed[5,6.]	Conventional	-	-	3	1	11	12	9	8	52-55	4

[1] (Ackman, 1990), [2] (Friedt and Lühs, 1999), [3] (Scarth et al., 1988), [4] (Scarth et al. 1995a), [5] (McVetty et al. 1998), [6] (Scarth et al. 1995b).

3.2.1 'Canola' (double-low)

Findings that nutritional intake of erucic acid could give rise to myocardial lipidosis and heart lesions in rat (Engfeldt and Brunius, 1975; Hung et al. 1977) and concerns that erucic acid also created health problems in humans (West et al. 2002) were driving forces in the development of low erucic acid rapeseed varieties (LEAR). Discovery of an accession of forage rapeseed 'Liho' that was low in erucic acid (Stefansson et al. 1961) led to an intensive work of backcrossing the LEAR profile into adapted cultivars. The first cultivars with low erucic acid were released in 1968 (Oro, *B. napus*) and 1971 (Span, *B. rapa*) (Stefansson and Downey, 1995). The impact of these new LEAR cultivars was immense and by 1974 more than 95% of the rapeseed that was growing in Canada was of a low erucic acid cultivar (Scarth and Tang, 2006). Continued breeding efforts led to the development of new cultivars with the additional benefit of low levels of glucosinolates. To distinguish these double-low rapeseed cultivars, i.e. low erucic acid and low glucosinolates, from traditional rapeseed cultivars, the term ‘Canola’ was introduced by the Canola Council for Canada. ‘Canola’ is a registered trademark and is defined as “An oil that must contain less than 2% erucic acid, and less than 30 micromoles of glucosinolates per gram of air-dried oil-free meal,” (Canola Council of Canada, 2007). As a consequence of the metabolic block in the synthesis of erucic acid, the double low ‘Canola’ cultivars have increased levels of oleic acid, as well as linoleic and linolenic acids.

3.2.2 Low linolenic acid varieties

A number of reports have demonstrated the nutritional importance of polyunsaturated FA (PUFA) in the diet. A regular intake of these nutrients is beneficial for cardiovascular and brain health (Lee and Lip, 2003) and inflammatory diseases (Simopoulos, 2002).

The double-low ‘Canola’ is rich in the essential PUFAs and has a ratio between linoleic and linolenic acid that is recognised to have nutrional benefits (De Lorgeril et al., 1994; Singh et al., 2002). However, the susceptibility of the oil to oxidation (Browse et al., 1988; Lauridsen et al., 1999) was known to be a problem (Tanhuanpää and Schulman, 2002). The conventional method of treating less stable oil with a hydrogenation process (removing double bonds) introduces trans fatty acids into the food chain (Demmelmair et al. 1996). Trans fatty acids are widely known for their negative effects on human health (Koletzko and Decsi, 1997). In addition, the hydrogenation process increases the cost of processing the oil (Ohlrogge, 1994; Kochhar, 2000). Thus, there was a need for developing ‘Canola’ cultivars with a lower level of linolenic acid to reduce the oxidation problem. In 1987, the first low linolenic ‘Canola’ cultivar ‘Stellar’ was released for commercial production (Scarth et al., 1988). This cultivar was developed using chemical mutagenesis treatment on a low erucic acid summer rape cultivar and cross/backcrossing that into a ‘Canola’-quality cultivar. A further reduction in levels of linolenic acid was achieved in a subsequent cultivar, ‘Apollo’ (Scarth et al. 1995a).

3.2.3 High oleic acid varieties

Brassica cultivars with medium- and high-oleic acid profiles attract great interest from both the nutritional and industrial sectors. Problems that arise from oxidation products formed in oils with high levels of linolenic and linoleic acid can be avoided if the unsaturated acids are reduced. The optimal oil composition for cooking performance, as well as shelf life and sensory properties of end products, were identified as 5-7% saturated acids, 67-75% oleic acid, 15-22% linoleic and about 3% linolenic acids (Scarth and McVetty, 1999). These mid-oleic ‘Canola’ cultivars are used for food applications that require high temperatures and in products that will have a long shelf-life. Oils with high oleic profiles (more than 75%) are greatly appreciated by the industry. Homogenous or near-homogenous feedstocks that require zero or minimum refining will of course reduce the total cost of the raw material (Katavic et al. 2000; Abbadi et al. 2004). Ideal oil for industrial feedstocks would be high oleic acid (above 90%), low linoleic and low linolenic acids. This level of oleic acid is therefore much higher than the content (about 61%) that is found in regular ‘Canola’ cultivars (Scarth and McVetty, 1999). Through mutagenesis treatment of either seeds or microspores, mutants with elevated levels of oleic acid have been achieved. Wong et al. (1991) found after selection specimens among *Brassica* genotypes with oleic acid above 85%. Auld et al. (1992) identified mutants with up to 88% oleic and less than 6% linolenic + linoleic acids. High oleic ‘Canola’ varieties have also been developed using genetic engineering: ‘Canola’ with oleic levels of more than 86% was developed using transgenic anti-sense technology (Debonte and Hitz, 1996). Silencing the delta 12 desaturase in *B. napus* by co-suppression led to an increase of oleic acid levels of up to 89% (Stoutjesdijk et al. 2000).

3.2.4 High erucic acid varieties

High erucic acid cultivars (HEAR) with low glucosinolates levels were developed through conventional breeding of a Swedish *B. napus* summer rape strain into an adapted high erucic rapeseed cultivar. Breeding focus was on high erucic acid, improved agronomic performance and low glucosinolates levels (Scarth et al. 1991, 1992). 'Mercury' with 54% erucic acid was released in 1992 and 'Castor' and 'MilleniUM01' were later cultivars with levels of up to 55% (Scarth et al. 1995b; McVetty et al. 1998, 1999). It has been estimated that a 10 % increase in the erucic fatty acid content in, for example, rapeseed would reduce the processing cost by half, since the main costs arise from the separation of the erucic from other fatty acids (Jadhav et al. 2005). Consequently, there is great interest to increase levels of erucic acid in optimised rapeseed cultivars and a number of strategies have been used. By re-synthesising *B. napus*, crossing the two ancestral diploids *B. rapa* and *B. oleracea*, the latter having *sn*-2 specificity for erucic acid (Taylor et al. 1995), levels of up to 60% were achieved (Lühs and Friedt, 1995). Somatic hybridisation has been attempted between *B. napus* and either *Crambe abyssinica* or *Lesquerella fendleri*, resulting in erucic fatty acid content in the progeny ranging from 51% up to 61% (Schröder-Pontoppidan et al. 1999; Wang et al. 2003c). However, theoretically, the highest level of erucic acid in rapeseed achievable by conventional breeding is 66% (Frentzen, 1993). This is because triacylglycerol (TAG) molecules in rapeseed oil only contain erucic acid at the *sn*-1 and *sn*-3 position since enzymes modifying fatty acids at the *sn*-2 position lack selectivity for erucoyl groups (Bernerth and Frentzen, 1990; Broun et al. 1999). To break the 66% barrier in rapeseed, genetic engineering has therefore increasingly been used as a tool for modifying enzymes in the pathway leading up to formation of trierucine (Puyaubert et al. 2005). Experimental approaches include enzymes targeting *sn*-2 position (*sn*-2 erucoyl specific LPAAT's), silencing of the oleic acid desaturase (*FAD2*) to increase substrate availability for the elongase producing very long chain fatty acids (*FAE1*) and expression of *FAE1* from species accumulating high levels of erucic acid (Lassner et al. 1995; Zou et al. 1997; Katavic et al., 2000; Katavic et al., 2001; Mietkiewska et al. 2004; Jadhav et al. 2005; Mietkiewska et al. 2006).

3.2.5 Medium chain acid varieties

Medium chain fatty acids such as caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0) and myristic (C14:0) acids are used as feedstocks for dietary and industrial applications. Through expressing a lauroyl–acyl carrier protein (ACP) thioesterase (MCTE) from the California bay (*Umbellularia californica*) plant in rapeseed, transgenic lines containing up to 56% lauric acid were produced (Voelker et al. 1996). A detailed study of the TAGs showed that the MCFA was found exclusively at the *sn*-1 and *sn*-3 positions. By further combining the MCTE and an acyltransferase with *sn*-2 specificity, the coconut 12:0-CoA preferring lysophosphatitic acid acyltransferase, levels of up to 67% were found (Knutzon et al. 1999). It was also found that in parallel with the accumulation of high levels of laurate in the TAGs there was a substantial amount of laurate that was incorporated into phospholipids (Wiberg et al., 1997). The genus *Cuphea* contain species that accumulate very high levels of MCFA such as *C. pulcherrima* (96% C8:0), *C. hookeriana* (75% C8 and C10) and *C. wrightii* (83% C10:0 and C12:0) (Dehesh, 2001). The introduction into rapeseed or 'Canola' varieties of specific medium-chain thioesterases from some of the Cuphea species resulted in the accumulation of up to 7, 29 and 63 mol% of 8:0, 10:0 and 12:0, respectively (Wiberg et al. 2000). However, the increased levels of MCFA in transgenic plants have been shown typically to stimulate an increased breakdown of the MCFA through β-oxidation (Ecclestone et al. 1996; Poirier et al. 1999). It is likely that β-oxidation is induced because of inefficiencies of the acylation enzymes in the transgenic plants to deal with medium chain fatty acids since the medium chain fatty acid content in the acyl-CoA pool is very high in these plants (Larson et al, 2002).

3.2.6 High stearic rape seed

Saturated oils such as stearate are used in a number of applications, for example, as emulsifying agents or as agents to achieve good consistency in cosmetic products, and also in the production of food products such as margarine and shortening (Pérez-Vich et al. 2004; Zarhloul et al. 2006). Stearate produced directly in plant oil will save the cost of hydrogenation as well as avoid the production of unwanted trans fatty acids (Pérez-Vich et al. 2004). 'Canola' cultivars normally only have 1 – 2.5% of stearate and several attempts by genetic engineering to raise this level have been reported. Expression of a 18:0 specific thioesterase (*FatA*) from Mangosteen (*Garcinia mangostana* L.), a plant accumulating up to 56% of stearate in the seed oil, increased the stearate levels in *B. napus* up to 23% (Hawkins and Kridl, 1998). By site-directed mutagenesis of the same enzyme, the level of stearate was increased to well above 30% in single seeds (Facciotti et al. 1999). Down-regulating the delta-9-desaturase by antisense suppression resulted in more than 32%, and up to 40%, of stearate in *B. rapa* and *B. napus,* respectively (Knutzon et al. 1992). A simultaneous overexpression of a thioesterase from soybean and down-regulation of delta-9-desaturase resulted in up to 45% of stearate in the seed oil of 'Canola' (Töpfer et al. 1995). It should be noted that germination problems have been reported in transgenic seeds with elevated stearate levels (Knutzon et al. 1992; Wang et al, 2001)

3.3 Genetics

The mustard family or *Brassicaceae* (*Cruciferae*) has a large size of about 338 genera and some 3700 species worldwide (Schranz et al. 2006). From an agricultural perspective, *Brassica* is the most important genus in the family, with 6 different species widely cultivated, of which rapeseed (*B. napus*) is the most significant. There are three diploids: *B. oleracea* (2n=18), *B. rapa* (2n=20) and *B. nigra* (2n=16), and three amphidiploids: *B. napus* (2n=38), *B. juncea* (2n=36) and *B. carinata* (2n=34) (Figure 1). Their genomic relationship is well understood with the three amphidiploids having evolved as a result from crosses between the corresponding diploids (Figure 1, Song et al. 1995; Berry and Spink, 2006; Leflon et al. 2006).

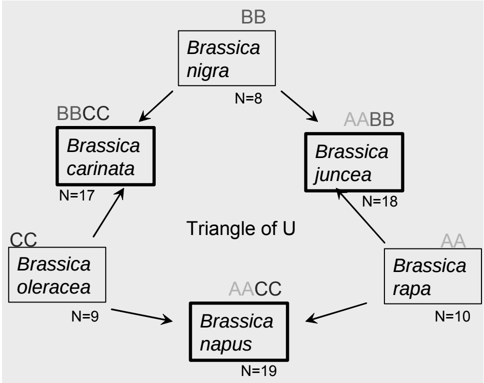

Figure 1 Genomic relationship between the three diploid and three amphidiploid species in the *Brassica* genus according to U, (1935).

B. napus (rape or rapeseed) is the most important of the *Brassica* oilseed species in cultivation. Its winter forms are most commonly grown in Europe and China, whilst in cooler climates such as Canada and areas of northern Europe, the spring forms are preferred. As the most cold-hardy oilseed cultivars are found in the *B. rapa* species, both winter and spring forms are also frequently cultivated in the cooler climate areas. Australia has large cultivation areas affected by water shortages and spring forms of *B. napus* have become the most frequently grown rapeseed. *B. juncea* is the preferred oilseed crop in regions with a short, hot to warm growing season and in which water supply is too unreliable for other oilseeds. These areas include southern Russia, northern and western China, and dryer parts of South Asia. *B. carinata* is grown in North Eastern Africa (the highlands of Ethiopia) and has recently been suggested as an oilseed crop for dryer areas of Canada and southern Europe and as a producer of oil for industrial purposes (Kimber and McGregor, 1995; Rakow and Getinet, 1998; Raymer 2002; Bouaid et al. 2005; Genet et al. 2005; Oram et al. 2005; Berry and Spink, 2006).

Major advances in understanding the genome of the *Brassica* genus has been made since the beginning of the 1990s. This coincides with the period of rapid progress in molecular marker technologies (Karp, 1998). Many important agronomic traits are inherited in a quantitative manner and are controlled by several loci termed quantitative trait loci (QTL). QTLs that cannot be resolved through Mendelian analysis are possible to track using different molecular markers. Through molecular marker analysis, linkage between the QTLs and the marker can be established, and the number and relative proportions of loci which determine complex traits can be elucidated. This information is compiled into linkage maps which provide important tools in the exploration across the *Brassicaceae* genomes as well in breeding programs to select for specific alleles. In the *Brassica* genus alone, more than 20 genome linkage maps have been constructed for *B. oleracea* (Slocum et al. 1990; Kianian and Quiros, 1992; Lan et al. 2000; Sebastian et al. 2000), *B. rapa* (Song et al. 1991; Chyi et al. 1992; Teutenico and Osborn 1994), *B. nigra* (Lagercrantz and Lydiate, 1996; Kim et al. 2006), *B. napus* (Landry et al. 1991; Uzunova et al. 1995; Lombard and Delourme, 2001; Qiu et al. 2006) and *B. juncea* (Cheung et al. 1997; Pradhan et al. 2003). The key findings according to Lysak and Lexer (2006) during the 1990s were:

- The widespread occurrence of duplications of genes and or chromosomal segments. For example, up to 50% of the studied loci in the species *B. rapa*, *B. nigra*, *B. napus* and *B. oleraceae* are duplicated, suggesting that in fact these species are ancient polyploids (Quiros, 1999).
- The presence of a high degree of genome plasticity among *Brassica sp*. including chromosome number variation, inversions, deletion and translocations (Marhold and Lihová, 2006). Such extensive genome rearrangements have been suggested to result from the polyploidisation process and that the considerable genetic diversity generated from such an event has contributed to the evolutionary success of the crucifers (Song et al. 1995).
- Comparing mapping studies between *Arabidopsis* and *Brassica sp*p. has revealed a high degree of colinearity (Parkin et al. 2005).

In the years leading up to the completion of the *Arabidopsis* genome sequence (The *Arabidopsis* Genome Initiative, 2000), and subsequently, a number of comparative mapping studies between *Arabidopsis* and other members of the *Brassicaceae* family have been reported (Cavell et al. 1998; Lagercrantz, 1998; Lan et al. 2000; Lukens et al. 2003; Boivin et al. 2004; Kuittinen et al. 2004; Koch and Kiefer, 2005). These studies have provided further insight in the similarities between *Arabidopsis* and *Brassica sp*p. genomes. Interestingly, genomes of diploid *Brassica sp*p. have as much as triplicated counterparts of the corresponding homologous segments of *Arabidopsis* (O´Neill and Bancroft, 2000; Rana et al. 2004) and in the amphidiploid *B. napus* up to six copies correspond to a particular *Arabidopsis* segment (Schmidt, 2002). Homologies of 80-90% are found between the exons of putative orthologous genes in *Arabidopsis* and *Brassica spp*. (Snowdon and Friedt, 2004), and therefore knowledge obtained from *Arabidopsis* is highly relevant for gene isolation and characterization in *Brassica* crops. A considerable amount of genetic information from research on *Arabidopsis* is available, such as the generation of expressed sequence tags (ESTs), and therefore can be directly applied to rapeseed (Lan et al. 2000).

Because of their great importance as producers of vegetable oil, fresh and preserved vegetables and condiments, the *Brassica* crops have been the focus of several genomic initiatives in recent years. The Multinational *Brassica* Genome Project (MBGP) is a major undertaking to accomplish the goal of having one of the cultivated *Brassica sp*p. fully sequenced (MBGP, 2007). The Steering Committee for the MBGP selected *B. rapa* subspecies *pekinensis* as the first species to be sequenced as it has the smallest genome (A-genome, ca. 550 Mb) among diploid *Brassicas* (Johnston et al. 2005). It has the lowest frequencies of repetitive sequences and communal BAC libraries, and in addition, mapping populations are available (Lim et al. 2006). The project organises a web portal (www.Brassica.info) which provide a comprehensive set of relevant resources, background information and links to databases with relevance to *Brassica* research.

The first step to be completed was the end sequencing of two BAC libraries from an in-bred line of *B. rapa* ssp. pekinensis (Hong et al. 2006). Data from all laboratories (200,553 end sequences) have now been submitted to GenBank. It is estimated that sequencing of chromosome 3 will be completed by the end of 2007 and chromosome 9 by the end of 2008. In addition, 22 cDNA libraries from different plant tissues have been sequenced and are about to be released by one of the MBGP partners, the 'Korea *Brassica* Genome Project' team of NIAB (Lim et al. 2006). The total number of expressed sequence tags (ESTs) from these libraries is 104 914 with an average length of 575 bp. Recently, the Korean partner established a 'Korea *Brassica* Genome Resource Bank' (KBGRB, http://www.Brassica-*rapa*.org/BGP/NC_brgp.jsp) for maintaining and distributing genetic and genomic resources of *B. rapa* ssp. pekinensis to the international community. Numerous additional data has been generated by different programs and deposited in the GenBank including 300 000 *B. oleracea* genomic shotgun sequences from TIGR (http://www.tigr.org/tdb/e2k1/bog1/), approximately 245,000 (seed-derived) *B. napus* ESTs from NRC (PBI) in Canada and 31 000 *Brassica* ESTs produced by the Genoplante Consortium. A tool to work with these data is the newly developed BASC bioinformatics system (Erwin et al. 2006) at http://bioinformatics.pbcbasc.latrobe.edu.au/basc/cgi-bin/index.cgi. The BASC system provides tools for the integrated mining and browsing of genetic, genomic and phenotypic data. It hosts information on *Brassica sp*ecies supporting the MBGP Project, and is based upon five distinct modules, ESTDB, Microarray, MarkerQTL, CMap and EnsEMBL. Typical results from the ESTDB module would include EST or consensus gene sequence, cDNA library information, sequence annotation, including UniRef, GenBank and significant GO categories and links to syntenic regions within *Arabidopsis*.

The BBSRC *Brassica* IGF programme (http://Brassica.bbsrc.ac.uk/IGF/) developed BAC-based physical contigs of the A and C genomes (*B. rapa* and *B. oleracea*). BAC clones were fingerprinted and contigs anchored to the *Arabidopsis* genome sequence through hybridisation to 1300 gene specific probes. All information has been made available in the public domain.

A public-domain *B. oleracea* EST programme was established under the BBSRC-supported UK-Canada interaction between Warwick-HRI and AAFC Saskatoon. Sequences so far obtained, representing approximately 16000 individual ESTs, have been deposited in GenBank. After submission to GenBank has been completed, the data will be incorporated into the *B. oleracea* mRNA alignments track (http://ensembl.warwick.ac.uk/info/about/index.html) to be viewed through The EnsEMBL software system tool (Hubbard et al. 2005).

GABI-GARS is a joint project with 9 partners focused on genome analysis in *Brassica napus*. It is run out of the GABI organisation that is supported by the German BMBF. The project is focused on identifying genes involved in the regulation of metabolic pathways and the development of the seed. This is accomplished through metabolic profiling using DNA chips for gene discovery and functional analysis, together with analysis of relevant biochemical and physiological parameters. In addition, synteny studies on *Arabidopsis* and *Brassica* have the aim of developing polymorphic markers for identifying genes for important traits in rapeseed. Other activities in the project are mapping and phenotypical characterization of QTL for important agronomical traits in rapeseed as well as development and evaluation of mapping populations and experimental lines in the greenhouse and the field. Data from the project is searchable from the database search tools at GABI primary database (GabiPD) (http://gabi.rzpd.de/index.shtml). Another source of information regarding *Brassica napus* is the *Brassica* Genome Gateway 2007 (http://Brassica.bbsrc.ac.uk/) organized by BBSRC at John Innes Centre. It provides links to projects involved in *Brassica* research and to several databases.

'The molecular biological mechanism of fatty acid synthesis and oil accumulation in seeds of Brassica napus' is a major ongoing (2006-2011) basic research program in China. It focuses on resolving three major key scientific problems:

1. The dynamic distribution patterns and molecular regulation pathway for the carbohydrate during the seed developmental process.

2. Regulatory mechanisms for fatty acid synthesis in some key steps and biological upper limit for the seed oil accumulation.
3. The mechanisms of interactions in fatty acid synthesis and oil accumulation between the related genes and the geographical environment such as temperature and photoperiod. The program is organised out of Huazhong Agricultural University, Wuhan (www.geboc.org).

3.4 Breeding

A consequence of the high significance of rapeseed as an oil crop world-wide is the extent of the literature available on breeding. The reader is referred to resources such as Labana et al. (1992), Nagata and Tabata (2003), Pua and Gong (2004), and the following summary highlights more recent approaches and data.

3.4.1 Conventional and marker assisted breeding

The quality of the rapeseed oil is determined by its fatty acid composition. A thorough understanding of the genetic control of the key coding genes involved in the desaturation of fatty acids is therefore important. However, the final oil composition is influenced by several factors such as multiple gene inheritance, maternal effects and environmental influence. There are often quite complex connections that the breeder has to handle. Identification of molecular markers that can be used in marker-assisted breeding (MAS) is therefore an efficient tool in breeding programmes (Javidfar et al. 2006). MAS provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers. Examples of their use in studies of *Brassica sp*p. are numerous, such as for cultivar identification (Hu and Quirós 1991; Mailer et al. 1994; Cassian and Echeverrigaray 2000; Lombard et al. 2000), relationships between genotypes (Hallden et al. 1994; Diers and Osborn, 1994; Diers et al. 1996; Riaz et al. 2001; Seyis et al. 2003; Plieske and Struss, 2001), determination of genetic relationships between different related species (Demeke et al. 1992; Ren et al. 1995), evaluation of seed purity in commercial hybrids (Crockett et al. 2000), and the genetic diversity and relationships among germplasm collections (Kresovich et al. 1992; Jain et al. 1994; Becker et al. 1995; Diers et al. 1996; Farnham 1996; Phippen et al. 1997; Zeven et al. 1998; Divaret et al. 1999; Cartea et al. 2005; Hasan et al. 2006).

When breeding for specific oil qualities, molecular markers enable an early selection of individuals equipped with the necessary genes. Desaturation of oleic acid (18:1) and linoleic acid (18:2) in rapeseed is catalysed by enzymes encoded by at least four different types of genes. These are respectively *DELTA 12 DESATURASE* (*e2*) and *DELTA 15 DESATURASE* (*e3*) located in the endoplasmatic reticulum, whilst the OMEGA 6 (*p2*) and *OMEGA 3* (*p3*) *DESATURASE* catalyse the same reactions in the plastids. Estimates based on Southern blot analysis indicate that *B. napus* has 4-6 gene copies of e2 and 6-8 copies per haploid genome of *e3*, *p2*, *p3* and *b5* (Scheffler et al. 1997). *B5* putatively encodes a fusion protein linking a cytochrome b5 segment with a desaturase-like domain.

Random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) as well as RFLP markers have been identified associated with genes controlling the level of linolenic fatty acid (Hu et al. 1995; Tanhuanpää et al. 1995; Jourdren et al. 1996; Thormann, 1996; Scheffler et al. 1997; Somers et al. 1998; Hu et al. 1999; Rajcan et al. 1999; Rajcan et al. 2002; Javidfar et al. 2006). Several of these studies showed a close association of the marker with the loci for the FAD3 gene as in Somers et al. (1998). To achieve a greater reliability, identified RAPD markers can be converted to sequenced characterised amplified regions (SCAR) markers as in Javidfar et al. (2006). SCAR markers have an advantage over RAPD markers in that they detect only single, genetically defined loci, whereas primers for the RAPD markers amplify multiple non-specific DNA fragments. Similarly, RAPD and AFLP markers associated with levels of linolenic and oleic acids have also been reported on (Tanhuanpää et al. 1996; Scheffler et al. 1997; Hu et al. 1999; Schierholt et al. 2000 Javidfar et al. 2006).

Markers such like RAPD and RFLP are low throughput markers and therefore not suitable for large scale screening through automation (Hu et al. 2006). Furthermore, these markers are not optimal for use in marker-assisted selection (MAS) because they are bi-allelic and therefore poorly polymorphic (Piquemal et al. 2005). Other types of PCR markers have therefore been developed. Microsatellites or simple sequence repeats (SSR) are randomly dispersed in eukaryotic genomes, are highly polymorphic, have a robust nature and are simple and inexpensive to use (Snowdon and Friedt, 2004). An increasing number of SSRs are becoming available in the public domain (SzewcMc-Fadden et al. 1996; Westman and Kresovich, 1999; Uzunova and Ecke, 1999; Plieske and Struss, 2001; Saal et al. 2001; Lowe et al. 2002; Suwabe et al. 2002; Tommasini et al. 2003; Lowe et al. 2004; Tonguc and Griffiths, 2004; Padmaja et al. 2005). Warwick HRI curates a web page that provides a summary of available information for all known *Brassica* microsatellites and enables information on the subject to be exchanged between researchers (http://www.Brassica.info/ssr/SSRinfo.htm). A tool for fine genetic mapping is single-nucleotide polymorphisms (SNPs) that constitute changes at single nucleotides. Recently two single nucleotide polymorphism (SNP) markers for detecting *fad2* and *fad3c* (C genome) mutations responsible for high oleic and low linolenic acid were identified (Hu et al. 2006).

Important properties such as the quality and the content of the seed oil are controlled by specific QTL. On genetic linkage maps such QTLs can be linked to the localisation of major genes and specific agronomic traits. Recently, several analyses of QTL controlling traits such like seed oil content and fatty acid composition have been reported (Burns et al. 2003; Delourme et al. 2006; Qiu et al. 2006; Zhao et al. 2006). Synthesis of erucic acid in rapeseed is controlled by two genes that also control the synthesis of eicosenoic acid (20:1). Two QTLs were identified that have a close association with the location of the two genes controlling erucic acid content (Ecke et al. 1995). Das et al. (2003) reported the cloning of one of the genes controlling erucic acid synthesis, *Fatty Acid Elongation 1* (*FAE1*), from two *Brassica* lines with low and high levels of erucic acid. Sequence analysis of the genes revealed specific differences in the amino acid sequence. The information from this study can be used to map the genomic region/s responsible for erucic acid synthesis in *Brassica sp*. Reproducible QTLs for seed oil content and erucic acid content have recently been identified (Qiu et al. 2006). QTLs controlling glucosinolate content have also been identified (Uzunova et al. 1995; Howell et al. 2003).

3.4.2 Genetic transformation

It was recognised at an early stage in modern approaches to rape breeding that tissue culture systems could be used to regenerate *Brassica* plants. Over time, shoots have been obtained from many different tissues such as stems, roots, leaves, hypocotyls, cotyledons and so forth, as reviewed in Poulsen (1996). Techniques for genetic engineering were therefore available at an early stage and could be applied to the different crops in this genus for breeding purposes. It is now some 20 years since the first successful transformations were accomplished by co-cultivating different *B. napus* explants with *Agrobacterium* (Fry et al. 1987; Pua et al. 1987; Charest et al. 1988). Since then there have been numerous reports on transformation systems using different tissues, *Agrobacterium* strains, *Brassica sp*ecies as well as different selection markers and transformation techniques. For a comprehensive list of gene transfers into *Brassica* prior to 1996 see Poulsen (1996). Although transformation using *Agrobacterium* has been and continues to be the dominating technique in *Brassica*, other methods have more or less successfully been carried out. These are reviewed in Earle et al. (1996), Earle and Knauf (1999), Poulsen (1996) and Sharma et al. (2005).

An important technique that takes advantage of the totipotency potential of plants is microspore embryogenesis. Embryos are produced from microspore (immature pollen) cultures and thus contain the gamete chromosome number. Plants derived through this method are therefore haploid. This technique has been used for transformation in *Brassica* (Dormann et al. 1998) but its main use currently is in *Brassica* breeding programmes to produce homozygous plant lines by chromosome doubling treatments of the haploids (Powell, 1990; Pauls, 1996; Shim et al. 2006; Zhang et al. 2006).

There is a growing list of reports on different traits for which *Brassicas* has been modified through *Agrobacterium*-mediated transformation protocols. These include modification of oil quality, introduction of herbicide or insect resistance, increase of abiotic stress tolerance, development of male-sterile lines and so forth (Cardoza and Stewart, 2004; Park et al. 2005; Tan et al. 2005; Wang et al. 2005a; Das et al. 2006; Sergeeva et al. 2006; Tan et al. 2006).

However, in parallel to the *Agrobacterium*-mediated transformation protocols presently in routine use, there is an ongoing effort to develop methods with higher transformation rates by modifying a range of different factors (Damgaard et al. 1997; Khan et al. 2003; Tang et al. 2003; Sparrow et al. 2004; Wang et al. 2005c; Jonoubi et al. 2005; Akasaka-Kennedy et al. 2005; Gribova et al. 2006). A much improved transformation protocol for 'Canola' (*Brassica napus* L.) was reported after optimizing two important parameters: preconditioning time and co-cultivation time (Cardoza and Stewart, 2003).

One of the most promising developments in transformation technology is in planta transformation for Brassica. This technique, which does not need the laborious work stage of tissue culture, is extensively used in Arabidopsis and is achieved by immersing intact inflorescences in *Agrobacterium* cultures which then targets the ovules for the transformation event (Ye et al. 1999; Pelletier and Bechtold, 2003). Species in which the ovary remains open for an extended developmental time may be more amenable to 'floral dip' transformation (Bowman, 2006). It is notable that the only *Brassicas* successfully transformed via *in planta* transformation to date are *B. napus* and *B. rapa*, and both have this feature (Qing et al. 2000; Wang et al. 2003a; Wang et al. 2005b).

3.5 Susceptibility to abiotic stress

Environmental stresses such as water, drought, heat or salt stress have adverse effects on plant growth and seed production. As these abiotic stresses are likely to increase in the near future, tolerance to such stresses is an important target for crop improvement (Shinozaki et al. 2003; Wang et al. 2003b; Mahajan and Tuteja, 2005; Yang et al. 2005). Given the significance of rape as an important oil crop globally, there have been substantial studies on the susceptibility of the crop to abiotic stresses and on the mechanisms for overcoming these stresses, including the development of new varieties. The literature is reviewed in, for example, Mendham and Salisbury, (1995), Rapacz and Markowski, (1999), Diepenbrock, (2000), Rife and Zeinali, (2003), Malhi et al. (2005), Berry and Spink (2006) and Rathke et al. (2006).

3.6 Susceptibility to biotic stress

Stem rot (*Sclerotinia spp*), black leg disease (*Leptospheria spp*), club root disease (*Plasmodiophora Brassicae*), *Alternaria* leaf and pod spot (*Alternaria spp*), downy mildew (*Peronospora parasitica*) and *Verticillium* wilt (*Verticillium dahliae*) are major diseases that are caused by fungal, bacterial and viral pathogens as well as pathogenic nematodes, and they all can affect the yield of *B. napus*. As above, there is extensive literature on these issues, the strategies in use and development needed to increase resistance to the different diseases. These are reviewed in Duke, (1983a), Rimmer and Buchwald, (1995), Tewari and Mithen, (2003), Sivasithamparam et al. (2005), Fitt et al. (2006), Hirai, (2006), Matthiessen and Kirkegaard, (2006), Rathke et al. (2006), and Rimmer et al. (2007).

3.7 Agronomy

Good agronomic practise and the choice of appropriate cultivar are essential to achieve the best crop results. There is extensive literature of agronomy on *B. napus* cultivated in the different geographical regions in which it is grown. Examples of reviews and books include Bearman, (1981), Kondra et al. (1983), Almond et al. (1984), Knott et al. (1995), Pouzet A, (1995), Walker and Booth, (2001), Jacobs et al. (2002), and Gunstone, (2004).

The climatic requirements of rapeseed (*B. napus*) make it a potential crop across most of Europe under present conditions. Field mustard (*B. rapa*), on the other hand, is much less widely distributed due to requirements for lower maximum temperatures and higher minimum rainfalls. By taking into consideration future climatic changes, Tuck et al. (2006) analysed potential changes in the cultivation of biomass crops in Europe. They predicted that rapeseed cultivation would remain very widespread throughout southern and central Europe (35-64° N), with only a small increase in potential cultivation areas (55-64° N to 65-71° N) by the 2080s. However, a decline in rape cultivation is predicted in southwest Europe (Spain) and *B. rapa* is predicted to disappear for southern Europe (35-44° N) and move further north (55-64° N).

3.8 Environmental impacts

3.8.1 Agrochemical inputs, nutrient and water requirements

As mentioned above, the cultivation of rapeseed is affected by numerous diseases and pests. To combat these problems, inputs can be excessive, for example in the UK (the season 2001/2002), rapeseed received applications on average of two fungicides, three herbicides, two insecticides and one molluscide (Garthwaite et al. 2002).

When conducting life cycle analysis (LCA) on the production of biodiesel (rape methyl ester), it was shown that the cultivation of rapeseed (at 140 kg N ha^{-1} input) is the dominating step when considering environmental load and energy consumption (Bernesson et al. 2004). Production intensities usually determine the consumption of mineral fertiliser (45% to 55% of the total energy input). The direct energy input due to diesel consumption is the second most important input factor (17%-46% of the total energy). The production of nitrogen fertiliser counts especially heavily on the energy balance and has been considered to be the most limiting factor for crop production. A high input of nitrogen is necessary to achieve a high yield of rapeseed (150-210 kg N for 3 t ha^{-1}) (Rathke et al. 2005). A spring rapeseed crop accumulates 50 to 60 kg nitrogen for every tonne of seed produced, whereas the equivalent for winter rapeseed is about 70 kg nitrogen (Pouzet, 1995). However the high N input results in high nitrous emissions. Assessments for the sustainability of biomass crops indicated that the emission of nitrogen oxide from bioenergy crops ranged from 0.7 to 4.4 kg ha^{-1} per year (Rapeseed; 2.6-3.0 kg ha^{-1} per year) (Hanegraaf, 1998). The succeeding crop in a rotation scheme and crop-and fertiliser management are therefore important factors to consider in relation to energy efficiency (reviewed in Rathke and Diepenbrock 2006). In this context it should be noted that perennial crops show lower emissions of nitrous oxide than annual energy crops.

Rapeseed has a high positive N balance (97 kg N ha^{-1}; Sieling and Kage 2006). In a rotation with other crops winter rapeseed can be a suitable crop to conserve nitrogen in its biomass throughout the winter, because of its large autumn N uptake of 40–60 kg N ha^{-1}. However, due to its early development, rapeseed only consumes a small proportion of the nitrogen mineralized in spring, especially when there is a slow increase in temperature. Due to the large amounts of easily mineralizable crop residues that return to the soil, rapeseed cultivation can result in a considerable increase in the leaching potential of the soil (Sieglin and Kage 2006). It is therefore advisable to maintain a careful nitrogen management to prevent leaching into the groundwater.

Calculation of energy balances can be a useful tool for optimizing inputs in agriculture (reviewed in Rathke and Diepenbrock 2006). An energy balance for winter rapeseed showed that for a high energy output (yield) and energy gain, high rates of N were required, and that the energy efficiency responds mainly to N management. The maximum output/input ratio (29.8) was obtained without N fertilisation whereas the most favourable N rate was 240 kg ha^{-1} in terms of energy gain (250 GJ ha^{-1}). 80 kg N ha^{-1} was needed for minimum energy intensity (Rathke and Diepenbrock 2006). Different N management strategies influence the productivity of winter rapeseed. Results analyzed by Rathke et al. (2005) showed that the seed yield, energy, CO_2 storage and crude protein content increased as N fertilisation rate increased, while the oil content of the seed declined. However, for 'Canola' such an inverse relationship was not observed (reviewed in Rathke et al. 2005). The optimum N supply depends on whether the goal is to produce high seed yield or high oil content. A high seed and a corresponding high oil yield were obtained following a pea crop and with high N-inputs (240 kg ha^{-1}) whereas the highest oil content was obtained without mineral fertilisation (Rathke et al. 2005).

As phosphorus and potassium are relatively immobile in the soil, the risk of polluting the ground or surface water by applying these nutrients is limited (Pouzet, 1995). The amount of phosphate in the plant is not very high and about 12 kg is removed per tonne of seed. In contrast, rapeseed needs large amounts of potassium as more then 200 kg K_2O is translocated to the plant, although only about 25 kg K_2O is removed from the ground per tonne of seed. The effects on yield are limited and are only detectable in case of deficiencies (Pouzet, 1995). Sulphates, like nitrates, are more prone to leaching into the groundwater and show interaction with yield and glucosinolate content. However, sulphur deficiencies are rare and may only develop after periods of heavy rainfall. Magnesium is required for chlorophyll production and for many enzymatic functions. Deficiencies may occur on specific soils. The magnesium to calcium ratio of the soil affects the magnesium absorption (Pouzet, 1995).

Water resources are becoming limited. Humanity presently uses an estimated 26% of total terrestrial evapotranspiration. Irrigation of agricultural land has dominated global water withdrawals, accounting for 65-70% of total water withdrawal and is expected to continue to do so (Berndes, 2002). Even if rapeseed is reported to tolerate an annual precipitation of 3 to 28 dm (Duke, 1983a), in order to produce well, it requires considerable amounts of water and responds with increases in yield to supplements of water. For example, around flowering time the evapotranspiration of *B. rapa* is as high as 8 mm and the crop is especially sensitive to drought at pod elongation stage as well as at the time of germination. Substantial areas of spring rapeseed and winter rapeseed have therefore been irrigated in western Canada and in the Mediterranean area, respectively. However, since water supplies became restricted, irrigation of rapeseed is not a normal practice nowadays (Pouzet, 1995).

3.8.2 CO_2 emission and carbon sequestration

The sequestration of carbon plays a critical role in regulating future climate change. This carbon sequestration on the other hand requires nitrogen availability for plant growth. As C and N become sequestered in long-lived plant biomass and soil organic matter, N may become limiting under elevated CO_2 levels. Average C and N pool sizes in plant and soil all significantly increase at elevated CO_2, ranging from 5% increase in shoot N to 32% increase in root C content. Also in litter the concentration of N and C are elevated and root to shoot ratios slightly increase. The net higher N accumulation under elevated CO_2 levels in plant and soil may help to balance carbon-sequestration in ecosystems (Luo et al. 2006).

Roots are an important sink for photoassimilates and carbon input into the soil. Pietola and Alakukku, (2005) showed that the root growth of spring rapeseed (*Brassica rapa* L.) was clearly faster compared to ryegrass, oats and barley. On the other hand, the number of roots of rapeseed also decreased more rapidly after the start of flowering, and their role in nutrient uptake probably diminished soon after. The average total root dry biomass at anthesis per m^3 (at a depth of 0-60 cm) was lower for rapeseed; 110 g compared to 260 g for oats, 160 g for barley 340 g for ryegrass. The fine roots of rapeseed produced the least biomass in the 0–60 cm soil profile layer. At anthesis or near the end of the vegetative growth biomass, the crops could be ranked on length density or surface area of roots (greatest to smallest); ryegrass>oats>barley>rapeseed in the 0-60 cm layer. The shoot to root ratios for barley, oats, rapeseed, and ryegrass were 7.1, 4.4, 4.2 and 2.5, respectively. In addition to having the lowest shoot to root ratios, ryegrass was considered to be the best soil C sink followed by oats (Pietola and Alakukku, 2005). Winter rapeseed concentrates energy in the seed due to the high oil content requiring high CO_2 fixation per unit of dry matter. The CO_2 fixation is closely related to both the biomass production per unit area and the energy concentration of the biomass (Greef et al.

1993). A model to predict canopy CO_2 and H_2O gas exchange rates of winter rapeseed is under development (Müller et al. 2005).

3.8.3 Gene flow and biosafety

When considering the use of GM rapeseed, mitigating the risk of gene flow to non-GM rapeseed or wild relatives is an important issue. The risk of gene flow depends on the type of transgene involved, the chance of out-crossing by pollination and the establishment of volunteer populations.

Early seed shedding is a serious problem in rape-seed and can result in high yield losses (up to 10 000 seeds m^{-2}). The seeds of rapeseed can persist dormant in the soil for up to 10 years. Seeds that are lost due to seed shedding before or during harvest therefore can remain in the soil for long periods and add to the soil seed bank of seeds that can germinate in subsequent growing seasons (volunteer plants). Volunteer rapeseed is very easy to control in cereals but it can be difficult to control in broad leaved crops and there form a weed problem. In case of GM-crops that are herbicide tolerant or in case of special oil qualities (such as high erucic rapeseed varieties) this may also result in an unwanted gene flow via volunteers resulting from pollen spread. Seeds of GM-rapeseed were detected in the soil two years after harvest (Roller et al. 2002). Volunteers that are not GM-herbicide resistant can generally be reduced effectively in subsequent crops if herbicide treatments are practised, but in a rapeseed crop the rapeseed volunteers can not be distinguished and form a problem (e.g. due to diseases, plant density, and seed quality). In Japan, a location at which no GM rapeseed crop is grown but imports are allowed, *B. napus* plants with ‘herbicide’ resistant transgenic seeds were found along the roadsides and nearby ports, suggesting that these feral plants were lost during transport from the harbour to the Japanese processing facilities. The transgenes were not found back in other locations or in the wild species (*B. rapa* and *B. juncea*) (Saji et al. 2005). Volunteer seeds in soil can be reduced in a number of ways (Gruber et al. 2004). Simulation models show that the proportion of contamination with volunteer plants within a crop of rapeseed will be relatively high, even though density of volunteers is low in other crops (Pekrun et al. 2005)

Breeding for reduced seed persistence or reduced seed shattering behaviour will reduce the risk. In *Arabidopsis*, pod shattering behaviour has been studied extensively and several candidate genes for the trait have been identified (reviewed in Liljegren et al. 2000, 2004; Ferràndiz et al. 2000). Recently, it was shown that ectopic expression of the *Arabidopsis* FRUITFULL gene in *B. juncea* is sufficient to produce pod shatter-resistant *Brassica* fruit (Østergaard et al. 2006). Also, based on complementation studies several GM approaches have been proposed to improve the pod shattering trait (Yanofsky and Kempin 2006).

B. napus can hybridize with several wild crucifers although the practical risks in the field are low with most members of the *Brassicaceae* except with *B. rapa* (Jorgensen and Andersen, 1994; Scheffler and Dale, 1994; Mikkelsen et al. 1996; Chèvre et al. 1997; Moyes et al. 2002; Tolstrup et al. 2003; Pekrun et al. 2005; Ford et al., 2006). Out-crossing rates of *B. napus* from 0.02% to 2.1% depending on the distance to the pollen source and plot size have been observed in various studies for GM rapeseed. In conventional rapeseed mean out-crossing rates from 20% to 40% have been found (Gruber et al. 2004; Pekrun et al. 2005). Rapeseed is 70% self-pollinating and 30% cross-pollinated. Even if wind and insects are absent, seed are still produced.

Concerns for problems arising after natural interspecific hybridization between transgenic *B. napus* and relatives like *B. rapa* and *B. juncea*, has led researchers to design approaches to minimise this risk. It has been suggested that there are safe sites for gene integration in *B. napus*, from where there is a low probability of gene transmission to backcross generations with *B. rapa* as the recurrent parent via homologous recombination (Mikkelsen et al. 1996). It was proposed that the risk of gene diffusion is small when transgenes are inserted into the C-genome of *B. napus* (Metz et al. 1997; Zhu et al. 2004a). In this context, Li et al. (2006) recently successfully integrated a transgenic trait into the C genome of *B. napus* and proposed this to be a useful approach to avoid migration of transgenes into other *Brassica sp*ecies since *B. napus* is easily hybridized with the *B. rapa* (A-genome) or *B. juncea* (AB-genome) (Metz et al. 1997; Jørgensen et al. 1998; Rieger et al. 1999).

3.8.4 Effects of climate changes

By the year 2080, due to increasing temperatures and drought, a drastic change can be expected that may reduce the choice of crops that can be grown in southern Europe. On the other hand the area of crops for northern Europe is extending more to the north and crops that currently are grown in the south are expected to move north. Currently, based on their climate requirements, rapeseed, crambe and oats can be grown across most of Europe. For all crops a decline is expected in Southern Europe, while they spread further north into latitude 65–71° N (Tuck et al. 2006). It should be noted that the predictions were based on climatic factors only and that a number of parameters such as soil type and profile, were not included in these predictions for the future.

3.9 Economics

3.9.1 Introduction

In assessing the economic potential of rapeseed as a feedstock platform for this sector of the bioeconomy, this report has looked at data for Italy, Germany, Poland, Sweden and the UK. Each of these countries represents a different climatic region of the EU with potential for the successful cultivation of different crops. It is not the intention of this analysis to propose monocultures in any of these regions but to draw out significant differences that currently exist and provide a basis on which future cropping plans can be considered. In addition, it will also be necessary, in the longer term, to consider the impact of climate change on agricultural production but little data currently exists from which conclusions can be drawn.

3.9.2 Results on rapeseed

Table 2 below sets out the value per tonne of yield and the value of the per hectare yield for rapeseed grown in the five countries chosen. From the figures shown the assumed per hectare yields of product can be derived. The data include a value for the straw produced as a by-product of the crop.

The value of the single farm payment has been shown in the table and net margins calculated to demonstrate the effect of including and excluding this subsidy. It should be noted that the value of the single farm payment has been allocated to the primary product and has not been split between grains/seed and straw. Costs have, in the main, been allocated to the primary product with the exception of land values and the costs directly associated with managing and handling the straw.

The variable costs shown in the table include:

- Cost of seed/planting material
- Agrochemical inputs – fertiliser, pesticides and herbicides
- Variable costs of straw baling, primarily identified in literature as baling string

Fixed costs include:

- Cost of machinery for cultivation, drilling and the application of agrochemicals
- Combining, including the management/handling of straw
- Labour costs
- Land costs
- For sugar beet only, the on-farm costs of collection, drying and storage.

Full details of the data used and assumptions made in the preparation of the economics information for this report are available on the EPOBIO website www.epobio.net.

Table 2 Revenues from rapeseed cultivation in Germany, Italy, Poland, Sweden and UK.

			Germany	Italy	Poland	Sweden	UK
Production	Grain	t ha^{-1}	3.3	1.5	2.4	2.4	3.2
	Straw	t ha^{-1}	4.1	1.1	2.6	2.6	3.9
Crop revenue	Main crop	(€ t^{-1} yield)	221.67	135.00	258.75	228.98	222.00
	Straw	(€ t^{-1} yield)	30.00	30.00	29.62	29.18	30.00
	Main crop	(€ ha^{-1})	731.50	202.50	621.01	549.55	710.40
	Straw	(€ ha^{-1})	123.30	31.50	76.42	75.29	118.20
	Total	(€ ha^{-1})	854.80	234.00	697.43	624.84	828.60
Single farm payment		(€ ha^{-1})	316.55	554.23	93.44	242.32	319.64
Total revenue (crop and single farm payment)	Main crop	(€ t^{-1} yield)	317.59	504.48	297.69	329.95	321.89
	Straw	(€ t^{-1} yield)	30.00	30.00	29.62	29.18	30.00
	Main crop	(€ ha^{-1})	1048.05	756.73	714.45	791.87	1030.04
	Straw	(€ ha^{-1})	123.30	31.50	76.42	75.29	118.20
	Total	(€ ha^{-1})	1171.35	788.23	790.87	867.16	1148.24
Summary of variable costs	Main crop	(€ t^{-1} yield)	88.04	241.51	54.16	79.29	134.68
	Straw	(€ t^{-1} yield)	1.89	1.75	4.02	17.75	1.50
	Main crop	(€ ha^{-1})	290.54	362.27	129.99	190.31	430.98
	Straw	(€ ha^{-1})	7.76	1.84	10.37	45.79	5.91
	Total	(€ ha^{-1})	298.30	364.11	140.36	236.09	436.89
Summary of fixed costs	Main crop	(€ t^{-1} yield)	151.19	360.15	80.41	170.28	135.62
	Straw	(€ t^{-1} yield)	41.05	227.68	20.13	49.43	43.10
	Main crop	(€ ha^{-1})	498.94	540.22	192.98	408.68	434.00
	Straw	(€ ha^{-1})	168.71	239.06	51.94	127.53	169.82
	Total	(€ ha^{-1})	667.65	779.28	244.93	536.21	603.81

Table 2 Revenues from rapeseed cultivation in Germany, Italy, Poland, Sweden and UK.

			Germany	Italy	Poland	Sweden	UK
Total costs	Main crop	(€ t^{-1} yield)	239.24	601.66	134.57	249.58	270.30
	Straw	(€ t^{-1} yield)	42.94	229.43	24.15	67.18	44.60
	Main crop	(€ ha^{-1})	789.48	902.49	322.97	598.98	864.97
	Straw	(€ ha^{-1})	176.47	240.90	62.31	173.32	175.73
	Total	(€ ha^{-1})	965.95	1143.39	385.28	772.30	1040.70
Net margins (without single farm payment)	Main crop	(€ t^{-1} yield)	-17.57	-466.66	124.18	-20.60	-48.30
	Straw	(€ t^{-1} yield)	-12.94	-199.43	5.47	-38.00	-14.60
	Main crop	(€ ha^{-1})	-57.98	-699.99	298.04	-49.43	-154.57
	Straw	(€ ha^{-1})	-53.17	-209.40	14.11	-98.03	-57.53
	Total	(€ ha^{-1})	-111.15	-909.39	312.14	-147.46	-212.10
Net margin (with single farm payment)	Main crop	(€ t^{-1} yield)	78.35	-97.18	163.12	80.37	51.58
	Straw	(€ t^{-1} yield)	-9.67	-199.43	5.47	-38.00	-14.60
	Main crop	(€ ha^{-1})	258.57	-145.76	391.48	192.89	165.06
	Straw	(€ ha^{-1})	-53.17	-209.40	14.11	-98.03	-57.53
	Total	(€ ha^{-1})	205.40	-355.17	405.58	94.86	107.53

Finally, it should be noted that the primary sources of the data used to derive this economic information are: Agro Business Consultants, (2006); Ask.com UK (2007); Ericsson et al, (2000); ERMES Agricoltura (2007); European Commission (2007); Food and Agriculture Organisation (2007); Nix, (2007); Stott, (2003) and Swedish University of Agricultural Sciences (2007).

3.9.2 Comments on the results for rapeseed

It should be noted that the yield data from FAOSTAT used for the calculation in Table 2 includes both spring and winter forms of rapeseed. As a result the yields presented are lower than can be expected from winter rapeseed alone. However, breeders are continuously improving winter forms of rapeseed (e.g. increasing their cold tolerance) and this new material can, to a large extent, replace acreage currently planted with spring rapeseed. Winter rapeseed means higher yield and consequently improved net margin for the farmer. For example, in the south and middle part of Sweden the average yield of winter rapeseed is 3.1 t ha^{-1}. Changing the yield in Table 2 from 2.4 to 3.1 t ha^{-1} improves the net margin from a loss of -147 to a gain of 26 € ha^{-1} (without single farm payment) and from 95 to 269 € ha^{-1} (with single farm payment).

3.10 SWOT analysis on rapeseed

Strength

- Established crop
- Extensive genetic data, MAS, QTL
- Biotechnology develops
- Valuable co-products
- Wide range of oil quality varieties developed

Weakness

- Relatively high input crop
- Potential high pest and disease pressure

- Both food and feed production
- High in water demand
- Outcrossing – Risk for gene flow

Opportunities

- High demand for biofuel
- Value of co-products could increase
- High fossil fuels prices

Threats

- Low fossil fuel prices
- Increased cultivation – increased pest pressure
- Public perception against GM technology

3.11 Research and development needs

As described in the preceding section, the greatest strength of rapeseed is the amount of information known about the crop, its genetics and its agronomy. In terms of further development as a crop platform for oil, R&D needs to be focussed on the development of new varieties requiring far less input both in terms of fertiliser application and pesticides. Rape grown currently across the EU has an increasingly negative environmental impact and this needs to be addressed by new R&D programmes.

Whilst an established oil crop, there remains room for improvement in terms of increasing oil yield and percentage of oleic acid in the oil. The R&D needs also to focus on these issues.

In summary, the R&D needed in order to further strengthen rapeseed as a provider of industrial feedstock oils is listed below:

- Breeding for increased oil yield
- Breeding for high oleic variety (> 90%)
- Breeding for pest resistance

3.12. Policy issues on rapeseed

The 2003 reforms of the Common Agricultural Policy, which were extended to the Mediterranean crop regimes (tobacco, cotton and olive oil) and to hops in 2004, broke the link between subsidy and production. This brought a new focus on the market, leaving farmers free to identify the best commercial opportunities for their production systems. The new single payment scheme includes the retention of compulsory set-aside, the requirement to withdraw land from agricultural production. This provides an opportunity and an incentive for the cultivation of crops for specified non-food uses. Rapeseed is a crop that is grown widely in Europe and which can be used for both food and non-food applications. It retains eligibility in the single payment scheme and can be grown both on set-aside and on other arable land. Production on set-aside is subject to conditions, including a requirement for contracts and payment of securities.

4 OAT (*AVENA SATIVA*)

4.1 Introduction

Historically oat was a major cereal crop in many regions of the world. Its principal use was for animal feed, particularly for the working horses that were used for agricultural practices prior to industrialization. The cultivation of oats has since reduced substantially and the crop has been replaced with others, in particular, by soybean, wheat, maize and barley (Frey, 2000).

Today, oat continues to be mainly used as animal feed. The greatest proportion of the crop remains on-farm for self-use with only a small proportion refined by feed industries. Thus, only a small part of the total oat production (5%) is used for human consumption. This part, however, is important since many different branches of the industrial food chain are involved in the use of oats. Most of this oat production is grown on contract and dealers in cereal seed as well as food producers are involved. Recently, the nutritional benefits of using oat as a food source have been recognised and this has led to an increasing trend in the use of oat as a food product (Truswell, 2002). In particular, there is a focus on the high content and unique functional properties of soluble fibers (beta-glucans) in oat (Lasztity, 1998; Blandino et al. 2003; Zekovic et al. 2005; Angelo et al. 2006). Other nutritional benefits including the relatively high levels of antioxidants, a desirable amino acid profile and high oil content are also well documented (Mälkki and Virtanen 2001; Peterson, 2001; Bratt et al. 2003; Liu et al. 2004; Drzikova et al. 2005; Angelo et al. 2006).

The high price for mineral oil and low market price for oat has created a recent increase in interest in the use of the oat crop for combustion as an energy source.

Significantly, in comparison to other cereals, oat accumulates substantial amounts of seed oil. Oat groats can contain up to 18% of oil (10% in breeding varieties) that contains relatively low levels of saturated fatty acids (FA), high levels of oleic FA and almost no linolenic FA. This feature raises the potential of oat as a highly interesting new opportunity for developing a new oil crop platform.

4.2 Oat oil

Oat groats are relatively rich in oil compared to other cereals and the content in groats varies substantially amongst different cultivars (Frey and Holland, 1999; Banas et al. 2000; Bryngelsson et al. 2002). Unlike other cereals, the major proportion (86-90%) of the groat oil in oat resides is in the endosperm tissue (Price and Parsons, 1979; Banas et al. 2007). It has been shown that groat oil content is strongly negatively correlated with starch content and positively correlated with protein content. In addition, microscopic studies have indicated that the oil content of the endosperm is higher in high-oil varieties (Peterson and Wood, 1997). These studies suggest that a proportion of reduced carbon is re-directed from starch into oil synthesis in high-oil oat varieties and that changes in metabolism occur within the endosperm cells. If this suggestion is further evidenced, identifying the regulatory enzymatic steps in the diversion of sugar into starch and oil, respectively in these cells, opens up the possibility to genetically engineer oats to further increase their oil content.

Most oat cultivars have some 5% of oil and 50% of starch in the grain (Welsh, 1995) with a grain yield of about 6 tonne per ha. If all of the sugar used for starch synthesis could be re-directed into oil in oat grains without any loss in total harvested energy, this would give an oil crop yielding about 1.7 tonne of oil per ha. Thus, it would be comparable to the oil yield of rape and with much less input and environmental impact in cultivation. Furthermore, oat grows with good yield at northern latitudes at which neither spring nor winter rape can be grown.

The dominating fatty acids in oat groat oil are 18:1 (40-50%), 18:2 (32-40%), 16:0 (13-16%) and 18:0 (1.6-2.7%) (Zhou et al. 1998; Holland et al. 2001a). The content of 18:1 and 18:2 varies substantially among species and cultivars, and there is a strong correlation between increased 18:1 and increased oil content (Schipper et al.

1991a; Holland et al. 2001a). Subsequently, the high content of 18:1 and its tendency of increasing with groat oil content constitute a good base for develop high oleic acid varieties of oat through approaches such as knocking out the *FAD2* gene coding for 18:1 desaturase.

4.3 Genetics

The genus *Avena* belongs to the tribe *Avena*eae of the family *Gramineae*. It incorporates a number of species and includes diploids, tetraploids as well as hexaploids (Table 1) (Baum and Fedak, 1985; Thomas, 1992; Leggett and Thomas, 1995; Loskutov, 2001). Four *Avena* genomes have been identified (A, B, C, and D) and a number of genome combinations are found in the nature (Li et al. 2000b). The A and C genomes are found in all the three ploidy groups, while the B and D genomes are only found in tetraploids and hexaploids respectively (Table 1). The A and D genome are highly similar to each other (Chen and Armstrong 1994; Jellen et al. 1994) while the C genome is more distantly related (Leggett and Markhand, 1995; Drossou et al. 2004). The B genome is also similar to the A genome (Leggett and Markhand, 1995) and it has been suggested that it arose from autotetraploidization of the diploid A genome (Katsiotis et al. 1997). The origin of the cultivated hexaploid *Avena sativa* with the three genomes A, C and D, is still unclear. It is highly probable that diploid members of the A and the C genome were involved in the process but there is no information on the progenitor for the D genome (Rajhathy and Thomas, 1974). The wild and cultivated hexaploid oats are interfertile and occasionally cross in nature (Hayasaki et al. 2000; Loskutov, 2001). It is therefore quite clear that the wild hexaploid oats are the direct progenitor to *A. sativa*, but when and where this occurred is unclear. Oat is not a cultivated crop in the region of its assumed origin i.e. south-western Asia, south-eastern Asia, northern Africa and coastal regions of the Mediterranean. It is believed that the domestication occurred in mid and northern Europe, where wild oats such as *A. fatua* and *A. sterilis* were spread as weeds together with emmer wheat (*Triticum dicoccum*) (Leggett and Thomas, 1995).

All *Avena* spp. are inbreeding annuals with the one exception, *A. macrostachya* that is an outbreeding perennial and considered to be an archaic member of the genus (Baum, 1974; Rodionov et al. 2005). Oats complete its life cycle from sowing/germination to harvest/maturity in 6-11 months. The distinction in length is mainly between winter and spring sown oat types (White, 1995).

The dominating cultivated species is *A. sativa* L. (2n=42) but species from each ploidy group have been or are still cultivated at a minor scale. Those are *A. strigosa* Schreb (2n=14), *A. abyssinica* Hochst. (2n=28) and *A. byzantina* C.K. (2n=42). For breeding purposes the wild *Avena* spp. represents valuable germplasm from which commercially important traits can be collected and used to improve cultivars (Zeller, 1998; Jauhar, 2006). Among the diploid species, resistance to powdery mildew are found in *A. pilosa*, *A. ventricosa*, *A. hirtula*, *A. prostrata*, *A. strigosa* (Hoppe and Kummer, 1991; Herrmann and Roderick, 1996; Loskutov, 2001) while *A. wiestii* is highly resistant to septoria leaf rust (*Septoria avenae*). *A. strigosa* is also highly resistant to stem rust (Steinberg, 2005). Among tetraploids, *A. barbata* confer resistance to powdery mildew (Aung et al. 1977; Thomas et al. 1980), stem and crown rust, and *A. macrostachya* is resistant to both stem and crown rust and *barley yellow dwarf virus* (*BYDV*) as well as to aphid injury (Weibull, 1988; Loskutov, 2001).

Even more important for breeding are traits among the hexaploid species since these can easily be crossed into *A. sativa*. An important species in this respect is *A. sterilis* that provides a number of key traits such as large grains, high protein content, a balanced amino acid composition, and high contents of oil and β-glucan (Loskutov, 2001). *A. fatua* also provides high content of oil and protein as well as resistance to stem and crown rusts, smuts, and *BYDV* tolerance. It is also highly recognised in oat-breeding program for its early ripeness, short culm and cold resistance (Frey, 1991).

Table 3 Species forming the *Avena* genus and their ploidy level and genomic configuration listed. Cultivated species are marked in bold.

(Adopted after Leggett and Thomas, 1995; Loskutov, 2001; Drossou et al, 2004).

No.	Species	Ploidy level	Genome
1	*A. clauda*	7	C_p
2	*A. eriantha*[a]	7	C_p
3	*A. ventricosa*	7	C_v
4	*A. bruhnsiana*	7	C_v
5	*A. prostrata*	7	A_p
6	*A. damascena*	7	A_d
7	*A. longiglumis*	7	A_l
8	*A. canariensis*	7	A_c
9	*A. brevis*	7	A_s
10	*A. hispanica*	7	A?
11	*A. lusitanica*	7	A_s
12	*A. nuda*	7	A?
13	*A. matritensis*	7	A_s
14	*A. wiestii*	7	A_s
15	*A. hirtula*	7	A_s
16	*A. atlantica*	7	A_s
17	***A. strigosa***	**7**	$\mathbf{A_s}$
18	*A. barbata*	14	AB
19	*A. vaviloviana*	14	AB
20	*A. agadiriana*	14	AB
21	***A. abyssinica***	**14**	**AB**
22	*A. maroccana*[b]	14	AC
23	*A. murphyi*	14	AC
24	*A. insularis*	14	CD?
25	*A. macrostachya*	14	AA
26	*A. fatua*	21	ACD
27	*A. occidentalis*	21	ACD
28	*A. sterilis*	21	ACD
29	*A. ludoviciana*	21	ACD
30	***A. sativa***	**21**	**ACD**
31	***A. byzantina***	**21**	**ACD**

[a] Previously known as *A. pilosa*, [b] Previously known as *A. magna*

4.4 Breeding

There is substantial literature on oat breeding and a selection includes (Stuthman, 1995; Walker et al. 1998; Frey and Holland, 1999; Cox et al. 2002; Palagyi, 2002; Zhu et al. 2004b; Nersting et al. 2006; Oats newsletter, 1998-2006). For the development of oat as an oil crop platform, particular issues are relevant and these are highlighted in the following section. Optimization of oat varieties as an oil crop is likely to include the use of GM technology. Therefore some emphasis in this section is placed on plant transformation, selectable markers and the availability of promoters.

4.4.1 Conventional and marker assisted breeding

The presence of groat-oil in oat is a quantitatively inheritated trait that is influenced of both additive and non-additive effects (Frey and Hammond, 1975; Thro et al. 1985; Brown et al. 1974). There is a strong genotypic variation for groat-oil content among adapted cultivars as well as wild relatives such as the progenitor species, *A. sterilis* (Brown and Craddock 1972; Frey and Hammond 1975; Martens et al. 1979). A survey of 4000 entries in the World Oat Collection showed groat oil concentration ranging from 3 to 11.6%, with a mean value of 7% (Brown and Craddock, 1972). These data revealed a wide diversity available for this trait among *Avena* spp. and the potential of improving oil yield in the cultivated forms by breeding. Thro et al. (1985) reported that alleles for higher oil content from *A. sterilis* and *A. sativa* are complementary. It has been shown that the procedure of recurrent selection is successful in raising various traits in self-pollinating crops (Carver and Bruns, 1993). Therefore, by adopting traditional gene recombination (sexual reproduction) and recurrent selection between the wild relative *A. sterilis* and cultivars of *A. sativa* increases in groat oil concentration could be achieved in relatively short time (Branson and Frey, 1989; Schipper and Frey, 1991b). Frey and Hammond (1975) had postulated that oat could be useful as an oil crop if oil content could be raised above 16%. In an attempt to further increase oil concentration, Frey and Holland, (1999) succeeded after nine cycles of recurrent selection to achieve genotypes with up to 18% of oil in their groats. It was concluded that the potential of further increases of the groat oil content through continued recurrent selection is not exhausted but that it is important to keep a focus on possible changes in grain qualities as well as agronomic performances in the population (Frey and Holland, 1999; Holland et al. 2001a). It should be noted that the increase in groat oil content was accompanied by an increase in the unsaturated to saturated fatty acid ratio mainly due to increases in stearate (18:0) and oleate (18:1), and decreases in linoleate (18:2) fatty acids (Schipper et al. 1991a).

In areas such as northern and central Europe oat suffers from competition with crops such as wheat and barley, due to its lower yields. However, the principal reason for this lack of competitiveness is that winter oat cultivars hardy enough to grow in those areas are not available. Winter cultivars of oat are grown in UK, Portugal and the Mediterranean region, and it has been estimated that winter cultivars developed for northern Europe would yield about 30% more than the present spring cultivars presently cultivated (Bräutigam et al. 2006). Developing winter varieties of oat has therefore been recognised as an important target in oat breeding and an accomplishment that would substantially extend the areas in which oat can be grown and compete well. Cold hardiness is a quantitative trait controlled by a number of genes (Thomashow, 1990) Traditional breeding programs have so far therefore been of limited success in improving the cold hardiness for any of the important crop species during the last decades. However, presently a program targeted on developing a Swedish winter oat is ongoing (Bräutigam et al. 2006). This has so far involved a thorough analysis of cold induced genes in oat, development of a unique collection of oat lines characterised by their relative winter survival and general vigour in the field, and development of molecular markers for cold hardiness. The research has so far resulted in 2800 identified transcripts, denoted the AsCIUniGene (*Avena sativa* cold induced UniGene) set (Bräutigam et al. 2005) and which is publicly available on a database that is denoted AGOD (http://www.agod.org). It was found that roughly 30% of the genes in the AsCIUniGene set showed similarities to cold related genes of other organisms.

4.4.1.1 Molecular markers

The development and use of molecular markers in the breeding of oat has been relatively slow compared with other cereals like barley, wheat and rice. However, a number of maps have been published for hexaploid oat as well as for diploid oat such as: *A. byzantina* C. Koch cv. Kanota and *A. sativa* L. cv. Ogle (O'Donoughue et al. 1995), 'Clintland64' x 'IL86-5698' (Jin et al. 2000), 'Ogle' × 'TAMO-301' (Portyanko et al. 2001), 'Kanota' × 'Marion' (Groh et al. 2001a), *A. atlantica* Baum et Fedak × *A. hirtula* Lag. (A ×H) (O'Donoughue et al. 1992) and *A. strigosa* Schreb. × *A. wiestii* Steud. (s × w) (Rayapati et al. 1994; Kremer et al. 2001). These maps have provided an important base for further genomic studies on identifying genes for important traits (O'Donoughue et al. 1995) and localization of quantitative trait loci (QTL) (Holland et al. 1997, 2002; Jin et al. 1998; Kianian et al. 1999; Groh et al. 2001b; De Koeyer et al. 2001, 2004; Bush and Wise 1996; Zhu and Kaeppler 2003; Zhu et al. 2003). For example, a QTL was found to have a major effect on the oil content in the groat. It was further shown that acetyl-CoA carboxylase (ACCase), an enzyme that catalyses the first committed step in de novo fatty acid synthesis, was linked to the same QTL (Kianian et al. 1999).

The major marker types that have been available and used for genomic mapping and marker-assisted breeding are RFLP and RAPD (reviewed in Wight et al. 2003). AFLP combines the simplicity of PCR analysis with the reliability of RFLP and has recently been used to identify useful markers in oats as well as analyzing relationship among *Avena* spp. and oat cultivars (Jin et al. 1998; Jin et al. 2000; Groh et al. 2001a; Pal et al. 2002; Fu et al. 2004; Fu et al. 2005). Paczos-Grzęda (2004) concluded that AFLPs would be the method of choice due to the higher efficiency and reproducibility. Recently, microsatellites or simple sequence repeats (SSR) have been produced for oat (Li et al. 2000a,b; Holland et al. 2001b; Pal et al. 2002; Jannink and Gardner 2005; Yu and Hermann 2006). A recent initiative to expand the number of microsatellites available is OATLINK (http://www.iger.bbsrc.ac.uk/OatLink/Overview.pdf) coordinated and managed by the Institute of Grassland and Environmental Research (IGER), Aberystwyth, Wales, UK. Its focus is on gathering the molecular tools (molecular markers and mapping for marker assisted selective breeding, MAS) that can be integrated into programs for developing new varieties for the milling, animal and organic sector. So far more than 250 polymorphic microsatellites have been identified and are now examined more closely. Another recent initiative that uses the new molecular technique Diversity Array Technology (DArT) (Jaccoud et al. 2001) is the DArT oat project with partners in US, Canada, Sweden and Norway. This initiative is aimed at developing a large set of common markers in oat and it is coordinated by N. Tinker at The Eastern Cereal and Oilseed Research Centre (ECORC), Ottawa, Canada. Presently up to 400 polymorphic markers have been developed.

4.4.2 Genetic transformation

When genetic transformation technology was starting to be used to modify traits in plants it was realized that monocots were quite recalcitrant to *in vitro* regeneration because only immature cells or tissue of a limited age range can be induced to regenerate efficiently (Repellin et al. 2001). It was also found that monocots did not respond to *Agrobacterium* mediated transformation (Chan et al. 1993). Promoters that worked well in dicots could not be used in monocots, selectable markers for monocots were lacking and there is a strong genotype specificity among monocots that influences the efficiency of the transformation. These obstacles led to a slow development of transformation systems for monocots so that transgenic cereals (Rhodes et al. 1988; Toriyama et al. 1988; Zhang and Wu, 1988) were only produced several years after the first transgenic tobacco had been reported (Barton et al. 1983).

However, most of these initial problems have now been overcome and transgenic lines of the major cereals are now routinely produced (Repellin et al. 2001). The first successful transformation of oats involved direct gene transfer via microprojectile bombardment (biolistics) using embryogenic callus derived from immature embryos (Somers et al. 1992). A number of successful attempts of biolistic gene transfers to callus derived from different oat tissues has been reported since then including the use of embryogenic callus derived from immature or mature embryos (Pawlowski and Somers, 1998; Torbert et al. 1998a,b; Kaeppler et al. 2000), leaf-base tissue (Gless et al. 1998) or highly regenerative cultures from seeds (Cho et al. 1999; Maqbool et al. 2004).

The varietal or cultivar specificity to transformation of embryogenic callus or suspension cultures and/or regeneration of transgenic shoots is a limiting factor in the application of transformation technologies in routine oat breeding for a broad range of commercial oat cultivars (Gana et al. 1995; Nuutila et al. 2002; Perret et al. 2003). Transgenic plant production has therefore been restricted to a small number of spring oat genotypes. However, a more recent report on the improvement and development of the use of embryogenic callus for the production of transgenic plants showed successful transformation of winter as well as naked oat commercial varieties (Perret et al. 2003). As an alternative to the use of embryogenic callus for transformation of oat, a system based on apical meristem multiplication has also been developed (Zhang et al. 1999; Cho et al. 2003; Maqbool et al. 2002). The advantage with such an approach is that the somaclonal variations that are caused by the prolonged tissue culture (Somers, 1999), is avoided. For an extended list of reported methods of oat transformation see Repellin et al. (2001) and Sticklen and Oraby (2005).

Most recently, a breakthrough in using *Agrobacterium*-mediated transformation with oat was reported (Bräutigam et al. 2006). In this protocol embryogenic callus derived from mesocotyles of dark germinated oat seeds, are co-cultivated with *Agrobacterium* and then placed on optimized root and shoot regeneration media. Regeneration is based on the use of mannose as the selectable agent and the mannose-6-phosphate isomerase gene (*manA*) as the selectable marker (Joersbo et al. 1998).

4.4.2.1 Selectable marker genes

The use of the bar gene for positive selection of transgenic shoots regenerated from callus has been very successful for oat (Somers et al. 1992; Kuai et al. 2001; Maqbool et al. 2002; Cho et al. 2003; Perret et al. 2003; Oraby et al. 2005). The bar gene encodes for phosphinothricin acetyl transferase (PAT), which confers plant resistance to herbicides containing phosphinothricin (PPT) and its derivatives (Thompson et al. 1987; De Block et al. 1987). However, there are concerns about using herbicide resistance as a selectable marker since cultivated oat easily cross-hybridise with its close relative's *A. fatua* and *A. sterilis*, both of which both are troublesome weeds in farming (Torbert et al. 1995). The transfer of an herbicide resistance trait to these weeds would eliminate the advantage of using the strategy of herbicide tolerant crops when it is based on the use of PPT-containing herbicides. A continued use of the bar gene in oat therefore ideally will have to be combined with techniques that include a marker-free selection strategy. Several alternatives exist such as the Cre/Lox system (Corneille et al. 2001), using a transiently cointegrated selection gene (Klaus et al. 2004) or combining an inducible site-specific recombinase for the precise elimination of undesired, introduced DNA sequences with a bifunctional selectable marker gene used for the initial positive selection of transgenic tissue and subsequent negative selection for fully marker-free plants (Schaart et al. 2004).

The hpt (hygromycin phosphotransferase) gene (Waldron et al. 1985) confers resistance to the antibiotic hygromycin. Hygromycin is a suitable selectable marker in for example rice (Chen et al. 1998) but has been used with varying success in oat. Kuai et al. (2001) for example could not recover stable transformants in oat from embryogenic callus. They suggested that this antibiotic is a possible inhibitor of somatic embryogenesis (Kuai et al. 2001). In contrast, hygromycin was successfully used as a selectable marker for obtaining transgenic shoots from seed-derived highly regenerative cultures (Cho et al. 1999). It was also successfully used as selectable marker for oat transformation of shoot meristem cultures (Cho et al. 2003).

The NPTII (neomycin phosphotransferase II) gene (Fraley et al. 1983) confers resistance to aminoglycoside antibiotics such as kanamycin, paromomycin and geneticin (G418). Kanamycin and G418 has been shown to be unsuitable as a selectable marker for transformed oat callus (Torbert et al. 1995) while at least G418 was successfully used to select for transgenic oat plants generated from *in vitro* shoot meristematic cultures derived from *in vitro* seedlings germinated from dry seeds (Zhang et al. 1999).

In contrast to the above methods, a number of alternative methods have been developed. They use non-antibiotic genes and are based on supplementing the transgenic cells with a metabolic advantage rather than killing the non-transgenic cells as in the selection system based on antibiotics and herbicides (Joersbo and Okkels, 1996). These methods are based on compounds such as mannose, xylose, and cytokinin glucuronides (Joersbo, 2001). By using the mannose-6-phosphate isomerise gene (*manA*) as the selectable marker and mannose as the selectable agent successful generation of transgenic plants has been accomplished for several major crops including wheat (Reed et al. 1999), maize (Negrotto et al. 2000, Wang et al. 2000a) and oat (Bräutigam et al. 2006).

Transgenes encoding luciferase, b-glucuronidase (GUS), and anthocyanins have been the most widely utilised as visual reporters in cereal transformation systems (McElroy and Brettell, 1994; Wilmink and Dons, 1993). However, recently the green fluorescent protein (GFP) was shown to work well as a visual selectable marker for transformation of oat (Kaeppler et al. 2000; Cho et al. 2003). The advantages with this system is that no substrates, as for example with GUS, are needed for the GFP expression and observations can be performed immediately on the living cell only by exposing it to light in the blue to ultraviolet range (Ormo et al. 1996). Furthermore, there are several modified GFP forms that have been successfully expressed in plants (Hraska et al. 2006) and it is therefore possible to simultaneous monitoring multiple transformation events in individual transformants (Davis and Vierstra, 1998; Heim and Tsien, 1996; Stauber et al. 1998).

4.4.2.2 Promoters

Seed-specific promoters are of vital importance for modifying seed storage products such as seed oil. In the literature there are only a few promoters reported with confirmed activity in the oat seed. The *act1* promoter consists of the 5' region of the rice actin 1 gene (*Actl*) (McElroy et al., 1990) and has been successfully used to drive selectable marker gene in transgenic oats (Gless et al. 1998; Cho et al. 2003; Perret et al. 2003). In barley, *Act1* is strongly expressed in callus, pollen, ovary, stigma, root, and immature embryo and endosperm tissues (Cho et al. 2002). Maize ubiquitin promoter (*ubi1*), including its first intron (*ubi1I*) (Christensen et al. 1992) is a useful promoter in cereals. It has been used to drive expression of the bar, GFP and the GUS genes in oat (Zhang et al. 1999; Cho et al. 1999; Kaeppler et al. 2000), and was shown to be active in anthers, ovary and stigma, in tissues related to shoot initiation, rooting, flowering and seed germination. Both the *act1* and the *ubi1* promoters confer constitutive expression in oat (Perret et al. 2003). The hordein promoter from barley shows endosperm specific expression in both barley and oat (Knudsen and Muller 1991; Cho et al. 1998; Cho et al. 2002).

The wheat high molecular weight (*HMW*) glutenin promoter (Colot et al. 1987) was shown to be highly and exclusively expressed in the endosperm and the aleurone layer of transgenic oat seeds (Perret et al. 2003). Expression was observed from day 12 after flowering and reached high levels within 3 days. Oat globulin promoter (*AsGlo1*) has been shown to be capable of driving strong endosperm-specific expression in barley and wheat (Vickers et al. 2006) but so far has not been used to drive transgenes in oat. The sugarcane bacilliform virus (*ScBV*) promoter from the sugarcane bacilliform virus (*ScBV*) is a badna virus belonging to the *Caulimoviridae* family as are the CaMV 35S (Bouhida et al. 1993). ScBV is highly expressed in the oat endosperm (Al-Saady et al. 2004). Finally, the CaMV35S promoter has been shown to have a relatively low activity in monocot cells compared with other plant promoters (Bruce et al. 1989; Christensen et al. 1992; McElroy and Brettel 1994; Perret et al. 2003), but still has been successfully used to drive bar, hpt and NPTII gene expression to generate transformed oats (Cho et al. 1999; Gless et al. 1998; Somers et al. 1992; Torbert et al. 1995).

4.5 Susceptibility to abiotic stress

Oat performs best in a moist and cool climate and can tolerate an annual temperature of 5°C to 26°C with a favourable temperature range of 13 to 19° C (Duke 1983b; Sorrells and Simmons, 1992). Compared to other cereals except rice, oat requires more moisture to accomplish high grain and straw yield (Coffmann and Frey 1961; Downes 1969). The requirement of oats for moisture explains why the crop finds optimal conditions in the moderate and humid climate of Europe and in regions of higher rainfall (Kirilov, 2004). Oats are also not as winter hardy as rye, wheat and barley and although winter oat cultivars have been developed they are still not hardy enough for cultivation in central and northern Europe (Bräutigam et al. 2005). A further constraint is osmotic stress inflicted on growing oats under drought or salinity stress, and which is a major cause of crop yield losses for oat globally (Murty et al. 1984; Verma and Yadava, 1986; Frey, 1998). For additional information on responses of oat to abiotic stresses the reader is referred to (Sorrells and Simmons, 1992; Forsberg and Reeves, 1995; Frey, 1998; Martin et al. 2001; Rizza et al. 2001; Tamm, 2003; Livingston et al. 2004).

4.6 Susceptibility to biotic stress

Crown and stem rust are two important diseases of oat. The fungus *Puccinia coronata* f. sp. *Avena*e Eriks. causing crown rust, is the most widespread and damaging disease of oats (Harder and Haber 1992). Stem rust is caused by *P. graminis* f. sp. *Avena*e Eriks. and Henn, and affects oats wherever the crop is grown (Harder 1994). Powdery mildew (*Blumeria graminis* D.C. (Speer) f. sp. *Avena*e Em. Marchal) is another important disease affecting oat especially in areas with elevated humidity such as maritime northwest Europe and along the Atlantic seaboard (Yu and Herrmann 2006).

For further information on biotic stresses on oats caused by fungal, bacteria, and nematodes the reader is referred to the following literature Clifford, (1995), PeltonenSainio et al. (1996), Thomas and Menzies, (1997), Murray et al. (1998), Bender et al. (1999), Cunfer, (2000), Sebesta et al. (2000), Taylor and Szot, (2000), Chongo et al. (2001), Yao et al. (2002), Newton et al. (2003), Mielniczuk et al. (2004), Strange and Scott (2005)

The oat cultivar 'Kaufmann' is a good example of how modern breeding can provide resistance to some of the pathogens that attack oat. 'Kaufmann' is resistant to more than 98% of the crown rust isolates [*Puccinia coronata* Corda var. *avenea* (W.P. Fraser & Ledingham)] found in western Canada. The cultivar is also resistant to a major proportion of all prevalent races of stem rust (caused by *P. graminis* Pers. f. sp. *avenae* Eriks & Henn.) and all races of loose smut (caused by *Ustilago avenae* (Pers.) Rostr.) and covered smut (caused by *U. kolleri* Wille). In addition, the 'Kaufmann' cultivar remains moderately susceptible to the PAV strain of *BYDV* (Kibite et al. 2003).

Several viruses are currently causing problems in oat cultivation. *BYDV* is one of the more important and cause problem for most cereals. It is caused by a group of related luteoviruses that as a group is called *BYDV* viruses and is transmitted by aphids (Miller and Rasochova 1997). The Oat mosaic virus (OMV) is classified as a Bymovirus and gives rise to stunted plant and yield reductions of 25–50% in tolerant cultivars (Monger et al. 2001; Clover et al. 2002). The Oat golden stripe virus (OGSV) is classified as a furovirus and like the oat mosaic it is also vectored by the slime mold *Polymyxa graminis* (Adams et al. 1988; Walker, 1998; Clover et al. 2002; Rush, 2003).

There are some insects that cause problems for oats such as: Stink bug (*Chlorochroa sayi*), Aster leaf-hopper (*Macrosteles fascifrons*), European corn borer (*Ostrinia nubilalis*), Armyworm (*Pseudauletes unipuncta*), Cereal leaf-miner (*Syringopais temperatella*) and Bluegrass billbug (*Sphenophorus parvulus*) (Duke, 1983b).

4.7 Agronomy

Oat is a well established crop that in comparison with many other crops grown world-wide has a good tolerance to pathogens and weeds. Oat is also a relatively low input crop in relation to nutrient requirement and is known to be an excellent rotation crop for other crops such as cereals and rape seed (Soriano et al. 2004; Bräutigam et al. 2006). For more information in these areas the reader is referred to standard literature on the subject such as Webster, (1986), Welch, (1995) and Green, (1999).

4.8 Environmental impacts

4.8.1 Agrochemical inputs, nutrient and water requirements

A major issue in oat cultivation is the impact of wild oats, especially *Avena* fatua. Yield losses of up to 70% have been observed in fields infested with wild oats (Willenborg et al. 2005a). Emergence of oat before wild oats is critical for oats to win competition against the wild oat intruders (Willenborg 2004; Wilderman 2004). Using selective herbicides in oat cultivation is difficult due to the inherent similarity of the wild and cultivated species. These issues are discussed in detail in Watkins, (1971), Ghersa and Holt, (1995), Willenborg et al. (2005b, 2005c) and Karlowsky et al. (2006). Typically in the UK (such as in the season 2001/2002), oats received an application of two fungicides, two herbicides, one growth regulator and one desiccant spray in the season (Garthwaite et al. 2002). Additional references can be found in Laverick, (1997), Swedrzynska and Sawicka, (2001), Browne et al. (2003), White et al. (2003), Gallandt et al. (2004), Henriksen and Elen, (2005), Long et al. (2006)

The application of nitrogen (N) fertiliser is dependent on a range of agronomic factors. Recommendations for N input for high yield ranges from 0 to 136 kg ha^{-1} (Rehm et al. 2001). Nitrogen use efficiency can be increased by rotation, limiting irrigation, forage-only production systems (no flowering), the use of cultivars with high nitrogen use efficiency, conservation tillage, use of NH_4-N (less prone to leaching or denitrification), timing of application (in-season or foliar, in irrigation water), precision (N-soil testing and N-balance). These issues are discussed in detailed in Raun and Johnson, (1999).

Among cereals, with the exception of rice, oat requires more moisture to achieve high grain and straw yield and therefore is the least efficient in producing dry matter per unit of water transpired (water use efficiency) (Coffmann and Frey, 1961; Downes, 1969). Common oat is reported to tolerate a maximum annual precipitation 12 to 18 dm y^{-1} and the minimum 2 to 4 dm y^{-1} (Duke 1983b, Tuck et al. 2006). Still, oat is more susceptible to water logging compared to other cereals (Brouwer and Flood, 1995).

4.8.2 CO_2 emission & carbon sequestration

The level of carbon sequestration and soil organic matter depends strongly on the cropping system applied. No-tillage coupled with annual cropping has been proposed to minimize or halt the loss of soil organic carbon content and maintain soil productivity. The effects of improved cultivation practices are only visible in the long term. Ecological processes are slow and the determination of the amount of carbon sequestration takes many years. Current cultivation practices which include tillage, lead to loss of soil organic carbon.

Removal of crop residues can lead to a decline in soil organic matter and nutrients and higher CO_2 emissions due to increased mineralization (Lal, 2005). Oats can produce hay, straw or grains and is also considered to be an excellent crop for weed suppression in slow-establishing legumes. In addition, it has been recommended as a good nutrient catch crop for excess N, P and K, a crop for erosion control, and as a good winter cover crop or low cost biomass/organic matter source (Welch, 1995; Duke, 1983b).

Several nitrogen-fixing bacteria, diazotrophs, have been detected in the rhizosphere of oat. Two genera were abundantly present: *Azospirillum* and *Herbaspirillum* (Soares et al. 2006). It has been reported that a combination of rye and oats was better than single species as cover crops in terms of mycorrhizal colonization, P uptake and yield of the subsequent sweet corn crop (Kabir and Koide, 2002). In sugar beet cultivation, experimental trials suggested that oats and other cereal cover crops have a positive effect on plant injuries caused by the feeding of the sugar beet root maggot, *Tetanops myopaeformis* (Dregseth RJ et al. 2003). Oats are also usefully cultivated as a companion crop for berseem clover. Adding the clover increased forage quality for livestock productivity (Ross et al. 2005). Kaspar et al. (2006) analysed oat as a cover crop in a rotation with corn and soybean in the US. No positive or negative effect was observed on the yield of the subsequent crop, nor on carbon levels in the soil and therefore in the soil and environment analysed, oats as a cover crop had no proven positive effect on carbon-balance (Kaspar et al. 2006).

Since roots are an important sink for photoassimilates and carbon input into soil, Pietola and Alakukku (2005) compared the root growth of oats (*Avena sativa* L), barley (*Hordeum vulgare*), spring rapeseed (*Brassica rapa* L) and annual rye grass (*Lolium multiflorum* Lam var. *italicum*) grown in a fine sand soil in Finland under Nordic long day conditions. They showed that the average total root dry biomass at anthesis per m^3 (0-60 cm) was lower for rapeseed, 110 g, compared to the other crops; 260 g for oats, 160 g for barley 340 g for rye grass. Ryegrass was considered to be the best soil carbon sink followed by oats (Pietola and Alakukku, 2005).

4.8.3 Gene flow & biosafety

Oat is mainly self-pollinating, but cross-pollination by wind can occur (Duke, 1983b). In two separate field experiments the degree of plant-to-plant outcrossing and pollen-mediated gene flow (PMGF) in wild oat were quantified (Murray et al., 2002). The purpose of the study was to determine the extent to which pollen movement could contribute to the spread of herbicide resistance in this species. It was concluded that that PMGF contributes to the evolution of resistance in wild oat populations. However, the contribution of pollen movement to resistance evolution and the spread of resistance in wild oat populations would be relatively small when compared with seed production and dispersal from wild oat plants that have become herbicide resistant. Outcrossing in *A. sativa* L. have been reported to range from 0 to 9.8%, but mostly it was less than 1% (Thurmann and Womak, 1961; Grindeland and Frohberg, 1966; Jensen, 1966; Schemske and Lande, 1985). Outcrossing in *A. sterilis* L. - *A. fatua* L. natural mixed populations, measured by the number of plants having an *A. sterilis* phenotype among plants derived from the *A. fatua* parent, was 4.8 and 1.2% in 2 consecutive years (Shorter et al., 1978). Wild oat species are present all over Europe and represents a weed problem in crop cultivation. It is advisable that the potential of outcrossing of traits is recognised when developing transgenic oats. Risk mitigation is ideally addressed through the restrictive choice of GM properities selected for the transgenic oats.

4.8.4 Effects of climate changes

By the year 2080, due to increasing temperatures and drought, a drastic change can be expected that may reduce the choice of crops that can be grown in southern Europe. On the other hand the area of crops for northern Europe is extending more to the north and crops that currently are grown in the south are expected to move north north. Currently, based on their climate requirements, rapeseed, crambe and oats can be grown across most of Europe. For all crops a decline is expected in southern Europe, while they spread further north into latitude 65–71^{o}N (Tuck et al. 2006). It should be noted that the predictions were based on climatic factors only and that a number of parameters such as soil type and profile, were not included in these predictions for the future.

4.9 Economics

4.9.1 Introduction

In assessing the economic potential of oat as a feedstock platform for this sector of the bioeconomy, this report has looked at data for Italy, Germany, Poland, Sweden and the UK. Each of these countries represents a different climatic region of the EU with potential for the successful cultivation of different crops. It is not the intention of this analysis to propose monocultures in any of these regions but to draw out significant differences that currently exist and provide a basis on which future cropping plans can be considered. In addition, it will also be necessary, in the longer term, to consider the impact of climate change on agricultural production but little data currently exists from which conclusions can be drawn.

4.9.2 Results on oat

Table 4 below sets out the value per tonne of yield and the value of the per hectare yield for oat grown in the five countries chosen. From the figures shown the assumed per hectare yields of product can be derived. The data include a value for the straw produced as a by-product of the crop.

The value of the single farm payment has been shown in the table and net margins calculated to demonstrate the effect of including and excluding this subsidy. It should be noted that the value of the single farm payment has been allocated to the primary product and has not been split between grains/seed and straw. Costs have, in the main, been allocated to the primary product with the exception of land values and the costs directly associated with managing and handling the straw.

Table 4 Revenues from oat cultivation in Germany, Italy, Poland, Sweden and UK

			Germany	Italy	Poland	Sweden	UK
Production	Grain	t ha^{-1}	4.7	2.2	2.5	4.0	6.0
	Straw	t ha^{-1}	3.5	1.7	1.9	3.0	4.5
Crop revenue	Main crop	(€ t^{-1} yield)	86.97	154.00	102.81	103.79	105.82
	Straw	(€ t^{-1} yield)	27.37	55.00	32.35	32.66	33.30
	Main crop	(€ ha^{-1})	408.76	338.80	257.02	415.16	634.92
	Straw	(€ ha^{-1})	96.47	90.75	60.66	97.98	149.85
	Total	(€ ha^{-1})	505.23	429.55	317.68	513.14	784.77
Single farm payment		(€ ha^{-1})	316.55	554.23	93.44	242.32	319.64
Total revenue (crop and single farm payment)	Main crop	(€ t^{-1} yield)	154.32	405.92	140.18	164.37	159.09
	Straw	(€ t^{-1} yield)	27.37	55.00	32.35	32.66	33.30
	Main crop	(€ ha^{-1})	725.31	893.03	350.46	657.48	954.56
	Straw	(€ ha^{-1})	96.47	90.75	60.66	97.98	149.85
	Total	(€ ha^{-1})	821.78	983.78	411.12	755.46	1104.41
Summary of variable costs	Main crop	(€ t^{-1} yield)	44.62	120.84	49.71	21.21	50.31
	Straw	(€ t^{-1} yield)	1.89	1.75	4.02	17.75	1.50
	Main crop	(€ ha^{-1})	209.71	265.85	124.29	84.84	301.85
	Straw	(€ ha^{-1})	6.65	2.89	7.53	53.24	6.75
	Total	(€ ha^{-1})	216.36	268.75	131.82	138.08	308.60

Table 4 Revenues from oat cultivation in Germany, Italy, Poland, Sweden and UK

			Germany	Italy	Poland	Sweden	UK
Summary of fixed costs	Main crop	(€ t^{-1} yield)	124.95	282.18	88.02	120.97	84.11
	Straw	(€ t^{-1} yield)	43.66	136.70	24.33	38.31	35.11
	Main crop	(€ ha^{-1})	587.27	620.79	220.04	483.88	504.67
	Straw	(€ ha^{-1})	153.91	225.56	45.62	114.93	157.98
	Total	(€ ha^{-1})	741.19	846.35	265.67	598.81	662.64
Total costs	Main crop	(€ t^{-1} yield)	169.57	403.02	137.73	142.18	134.42
	Straw	(€ t^{-1} yield)	45.55	138.46	28.35	56.06	36.61
	Main crop	(€ ha^{-1})	796.99	886.64	344.33	568.72	806.52
	Straw	(€ ha^{-1})	160.56	228.46	53.16	168.17	164.73
	Total	(€ ha^{-1})	957.55	1115.10	397.49	736.89	971.25
Net margins (without single farm payment)	Main crop	(€ t^{-1} yield)	-82.60	-249.02	-34.92	-38.39	-28.60
	Straw	(€ t^{-1} yield)	-18.18	-83.46	4.00	-23.40	-3.31
	Main crop	(€ ha^{-1})	-388.23	-547.84	-87.31	-153.56	-171.60
	Straw	(€ ha^{-1})	-64.09	-137.71	7.50	-70.19	-14.88
	Total	(€ ha^{-1})	-452.32	-685.55	-79.81	-223.75	-186.48
Net margin (with single farm payment)	Main crop	(€ t^{-1} yield)	-15.25	2.90	2.45	22.19	24.67
	Straw	(€ t^{-1} yield)	-18.18	-83.46	4.00	-23.40	-3.31
	Main crop	(€ ha^{-1})	-71.68	6.39	6.13	88.76	148.03
	Straw	(€ ha^{-1})	-64.09	-137.71	7.50	-70.19	-14.88
	Total	(€ ha^{-1})	-135.77	-131.32	13.63	18.57	133.16

The variable costs shown in the table include:

- Cost of seed/planting material
- Agrochemical inputs – fertiliser, pesticides and herbicides
- Variable costs of straw baling, primarily identified in literature as baling string

Fixed costs include:

- Cost of machinery for cultivation, drilling and the application of agrochemicals
- Combining, including the management/handling of straw
- Labour costs
- Land costs
- For sugar beet only, the on-farm costs of collection, drying and storage.

Full details of the data used and assumptions made in the preparation of the economics information for this report are available on the EPOBIO website www.epobio.net.

Finally, it should be noted that the primary sources of the data used to derive this economic information are: Agro Business Consultants, (2006); Ask.com UK (2007); Ericsson et al, (2000); ERMES Agricoltura (2007); European Commission (2007); Food and Agriculture Organisation (2007); Nix, (2007); Stott, (2003) and Swedish University of Agricultural Sciences (2007).

4.9.3 Comments on the results for oat

Oat is a well established crop manly used for feed production in Europe. However, on a revenue basis it only just makes a net margin in Poland, Sweden and UK and this is with the single farm payment added. The economical incentive to grow oat is therefore not very strong. Presently, it has an important role as a break crop in crop rotation. A decrease in oat production will result in crop rotations with a higher demand for fungicides.

Developing oat into an oil crop producing industrial oils feedstocks will provide it with an added value product. This in combination with improved cold tolerance and increased oil content should help in improving the net margin of oat.

4.10 SWOT analysis on Oat

Strengths

- Low input crop (fertiliser and pesticide)
- Important rotational crop
- High genetic diversity (oil content)
- Biotechnology developed
- Established crop
- Co-products (seed cake, straw)
- Extensive root-system

Weaknesses

- Lack of cold tolerance
- Invasive weeds as relatives
- Both food and feed production

Opportunities

- Breeding for high oil content
- Developing into a robust and high yielding oil crop

Threats

- Competition from oil crops such as rape
- Public perception against using oat for feedstock production

4.11 Research and development needs

Oat as grain crop is grown widely as a spring crop already throughout Europe where conditions are permissive since the cereal is not cold-tolerant. Oat is not established as an oil crop, but its many advantages in terms of underpinning science-base and agronomic knowledge as well as its low input requirement are all strongly suggestive of real potential. Thus, R&D needs to focus on breeding programmes to increase the oil content of the oat grain, which will necessarily involve understanding the metabolic pathways and flux control between starch and oil.

Additional R&D should address the need to develop winter oat varieties. This will further add to the yield increase of oat oil since winter crop varieties in general yields up to 30% more than spring varieties.

Given the major risk of oat is that it can cross fertilise with other oat species that are known as invasive weeds, alternative fast-track breeding programmes that do not involve GM should be encouraged. In particular, the use of TILLING for development of new varieties is to be highly recommended. However, GM technology will probably be required for the development of the high oleic acid cultivars. The uses of marker free technologies should be included.

In summary, the R&D needed in order to develop oat into a high yielding oil crop is listed below.

- Breeding (Conventional and Tilling) for increased oil content
- Breeding (GM) for high oleic and very high oil oat varieties
- Breeding (Conventional, TIlling and GM) for winter oat varieties
- Increased knowledge about metabolism (flux between oil and starch)
- Develop efficient seed and or endosperm specific promoters for oat

4.12 Policy issues on the oat crop

The 2003 reforms of the Common Agricultural Policy, which were extended to the Mediterranean crop regimes (tobacco, cotton and olive oil) and to hops in 2004, broke the link between subsidy and production. This brought a new focus on the market, leaving farmers free to identify the best commercial opportunities for their production systems. The new single payment scheme includes the retention of compulsory set-aside, the requirement to withdraw land from agricultural production. This provides an opportunity and an incentive for the cultivation of crops for specified non-food uses. Oats are grown widely in Europe, primarily for animal feed and also for human consumption but they can also be used in non-food applications. Oats retains eligibility in the single payment scheme and can be grown both on set-aside and on other arable land. Production on set-aside is subject to conditions, including a requirement for contracts and payment of securities.

5 CRAMBE (*CRAMBE ABYSSINICA*)

5.1 Introduction

The prospect of replacing the present petroleum-based feedstocks for the chemical industry with a renewable and biobased feedstock implies that a range of agricultural crops will be needed that are capable of supplying the necessary commodities. Oil crops bred with modern technologies to produce specialised oils with industrial qualities, have the potential to become a highly sustainable means to produce the future supplies that will be required. It will be essential to ensure industrial oils not intended for human consumption do not enter the food chain. Specific non-food oil crop platforms are one means to reduce this risk.

Crambe (*Crambe abyssinica*) is an interesting oil crop with good potential as a producer of these specialised oils for industrial use. As yet, crambe is still under development as an agricultural crop and is not widely grown. Due to its origin in the Mediterranean and highlands of east Africa, crambe is well adapted to the cool and wet climate conditions of large parts of Europe. Its history as a crop is somewhat diffuse but according to Mastebroek et al. (1994) cultivation probably started in the former USSR. There are reports from field experiments in Russia, Sweden and Poland before the Second World War (Grashchienkov 1959; Papathanasiou et al. 1966; White and Higgins, 1966; Zimmermann, 1962). After the war research efforts were extended to additional countries and some breeding activities occurred (Zimmermann and Ragaller 1961; Jablonski 1962; Hannich et al. 1970; Bengtsson and Olsson, 1981). In the 1960s testing of 11 strains introduced into the USA and Canada from Sweden and Russia did not show adequate diversity to achieve desired crop improvement (Papathanasiou et al. 1966). Breeding research by the Purdue University Agricultural Experimental Station (Maryland, USA) therefore started (Mastebroek et al. 1994) and resulted in the release of three cultivars ‘Prophet’, ‘Indy’ and ‘Meyer’ (Lessman, 1975). ‘Prophet’ and ‘Indy’ were obtained after mass selection in two accessions from Sweden and Ethiopia, respectively. ‘Meyer’ was selected from a cross between the two accessions (Lessman 1975).

Continued breeding efforts including introgression of wild populations into the 'Indy' cultivar lead to the release of the cultivars 'C-22', 'C-29', 'C-37', 'BelAnn' and 'BelEnzian' from USDA-ARS at Beltsville (Maryland, USA) (Campbell et al., 1986a,b). When Mastebroek et al. (1994) evaluated the released USA cultivars and old European accessions, they concluded that breeding activities for seed yield had been effective and resulted in 15% yield increase.

These early developments of crambe have continued in more recent years as the search for sustainable industrial oils has increased. The report highlights the benefits of crambe and the opportunities for its development as a new industrial oil crop platform.

5.2 Crambe oil

The seeds (including the pod) of crambe naturally contain some 37% of oil which consists of up to 57% of erucic acid. Due to the presence of this fatty acid, which has been implicated for creating health problem in humans (Eskin et al., 1996; Parke and Parke 1999; West et al. 2002), crambe oil is not suitable as food oil. Erucic acid is a very long fatty acid (22:1) that is a highly valuable industrial feedstock, used in applications such as the manufacturing of plastics and lubricants (Leonard, 1993; Lazzeri et al. 1997). The main use of crambe today is as a producer of this industrial feedstock (erucic acid) and the crop has been suggested as an excellent example of an oil crop producing oil for industrial uses (Fontana et al. 1998; Capelle and Tittonel, 1999; Wang et al. 2000b), that is to say a non-food oil crop platform. Furthermore, in a recent EPOBIO report, production of a highly valuable industrial feedstock i.e. wax esters for lubrication application, in genetically engineered crambe was exemplified (Carlsson et al. 2006).

5.3 Genetics

The annual oil crop crambe (*C. abyssinica* Hodhts. Ex T.E. Fries) is an allo-hexaploid (2n=6x=90) which belongs to the genus Crambe (*Brassicaceae*). The genus comprises of approximately 38 Old World species (Bramwell, 1969; Santos-Guerra, 1983, 1996; Khalilov, 1991a,b) including *C. abyssinica* as the only one cultivated for its oil that is rich in erucic acid (Lessman and Meier, 1972; Mulder and Mastebroek, 1996). However, all species in the section *Leptocrambe* has been shown to contain erucic acid at levels comparable to the level of *C. abyssinica* (Leppik and White, 1975). According to Francisco-Ortega et al. (1999) Crambe has a disjunct distribution among four major geographical regions: Macaronesian (12 species), Mediterranean (4 species), east African (3 species), and Eurosiberian-soutwest Asian (ca 20 species). Furthermore, it is subdivided into six sections: *Leptocrambe*, *Crambe*, *Orientcrambe*, *Dendrocrambe*, *Flavocrambe* and *Astrocrambe*, the first three including a greater part of the species. A phylogenetic analysis based on internal transcribed spacers (ITS) of the nuclear ribosomal repeat, place *C. abyssinica* in section *Leptocrambe* that also includes *C. hispanica*, *C. filiformis*, *C. glabrata*, *C. kralikii*, and *C. kilimandscharica*. Detailed information on the subdivision of Crambe species can be found in Francisco-Ortega et al. (1999). Together with *C. kilimandscharica*, *C. abyssinica* is endemic to east Africa (Francisco-Ortega et al. 1999), *C. glabrata*, *C. filiformis* and *C. kralikii* are found in the western Mediterranean basin (Prina, 2000), and *C. hispanica* is distributed in the Mediterranean region and Middle East (Warwick and Gugel, 2003). The three taxa *C. abyssinica* (n=45), *C. hispanica* (n=30) and *C. glabrata* (n=15) are morphologically similar and can be distinguished by chromosome number. Based on the morphological data *C. abyssinica* and *C. hispanica* has been suggested to be conspecific (Jonsell, 1976) and could be organised as subspecies or ecotypes under *C. hispanica* (Prina, 2000). A recent report based on morphological as well as molecular data sets including cpDNA restriction site and internal transcribed spacer regions (ITS) analyses, show that *C. abyssinica* taxonomically should be organised as a subdivision under *C. hispanica* and with *C. glabrata* as a distinct species (Warwick and Gugel, 2003). This is in comparison with earlier data where *C. hispanica* successfully could form hybrids with *C. abyssinica* but not with *C. glabrata* (Mulder and Mastebroek, 1996, Meier and Lessman, 1973a, 1973b). It has been reported that *C. abyssinica* lacks genetic variation for important agronomic traits (Papathanasiou et al. 1966; Appelqvist and Jönsson, 1970; Lessman and Meier

1972; Mastebroek et al. 1994). In comparison, *C. hispanica* has more variation for agronomic and seed quality traits (Warwick and Gugel, 2003).

5.4 Breeding

5.4.1 Conventional and marker assisted breeding

Following the historical development of crambe described in the introduction, breeding in Europe resumed in the beginning of the 1990s at Plant Research International (PRI), the Netherlands. The breeding target was aimed at improving seed and oil yield, enhancing the erucic acid content in the oil, improving the disease resistance and decreasing the glucosinolates content in the seed (Mastebroek et al. 1994; Mastebroek and Lange 1997). The breeding material was one late flowering old European land race and two early flowering newer American lines. The three crambe lines were intercrossed and, from F3 generation onwards, selection was performed for agronomical characteristics (Mastebroek and Lange 1997). After about five years the first new Crambe variety ‘Galactica’ (PRI selection 9103-16, *Abyssinica* x *Abyssinica*) was released with Dutch breeder’s rights for Europe (http://www.pri.wur.nl/UK/products/Varieties/Arable+Crops/Galactica/). This cultivar had a significant higher seed yield compared to previous cultivars such like the American ‘BelAnn’, ‘Prophet’, ‘Meyer’ and ‘Indy’. Oil content was higher than reference cultivars 35.8% compared to 33.3% and with an oil production of about 900 kg ha^{-1}. Erucic acid (C22:1) content in the oil was increased from 58.1% to 59%. An even higher improvement of the oil production seems only feasible when an autumn sowing or an early spring sowing is possible. A future breeding target for crambe therefore is increased cold resistance. Besides ‘Galactica’, another crambe variety ‘Nebula’ (PRI selection 9110-20), was released as a result of the 1990s breeding. In contrast to ‘Galactica’ this cultivar has a hairy appearance, is extremely early flowering and have small amounts of the fatty acid nervonic acid (C24:1) in its oil. The extreme earliness makes ‘Nebula’ suited for regions with a short growing season. Furthermore, early flowering and hairy crambe lines, like ‘Nebula’, performed well in hot and dry areas. In moderate climates, like Western Europe, hairiness combined with early flowering gives reduced yields. Additional cultivars with Dutch breeders rights are the early and non-hairy race ‘Charlotte’ (Innoseeds, (former CEBECO seeds bv) part of DLF Trifolium) and the hairy and early race ‘Carmen’ (Innoseeds). From the breeding material that produced ‘Galactica’ and ‘Nebula’ additional potential varieties were identified, such as those that are promising for regions with dry climates.

In 1995, the Instituto Sperimentale per le Colture Industriali in Bologna, Italy, released a new genotype, ‘Mario’, selected for the cultivation conditions of central-northern Italy. A three year field trial showed mean seed yields of 2.9 t ha^{-1} (Fontana et al. 1998). In a recent field trial over three years that included all the modern cultivars, ‘Mario’ produced seed yields of up to 4 t ha^{-1} (Fila et al. 2002). Yield values from 0.97 – 3.33 t ha^{-1} with an oil content of the pod of 23 - 38% were shown in field trials from Austria (Vollman and Ruckenbauer 1993). Additional objectives for improvement include the resistance to insects such as diamond back moth, resistance to lodging, pod dropping, and a short seed dormancy period.

As a result of the multi-location study (DiCra, 2003) carried out in Italy, France, UK, Netherlands, and Germany, useful information was received that confirmed the expectations that environment (=combination of in-Year weather conditions and Location) is the most prominent source of variation for seed yield and little genotypic differences existed among the set of crambe genotypes tested in the stability (of traits) over different environments. The variability of erucic acid content over cultivars and environments was lower compared to the variability for oil content (DiCra, 2003).

The protein content was lower in samples with high oil content. Glucosinolate content was highly variable between cultivation sites even for the same genotype. Results from work on genotypes with improved quality of the meal indicate that by breeding for low glucosinolate content a substantial improvement is possible. Mutation breeding efforts resulted in genotypes having a content of glucosinolate (epiprogoitrin) in their seeds, ranging from 16.8 to 55.0 micromole gram $seed^{-1}$. The average content of epiprogoitrin of 34.8 micromole gram $seed^{-1}$ is about half the content of the standards in the ‘BelAnn’ population and in the race ‘Galactica’. However, these lines still have to be tested for performance in the field. The protein content of the seed is about 20% of the whole pod and has a well balance amino acid profile very much like rapeseed (Liu et al. 1993). As *C. abyssinica* lacks genetic diversity for important agronomic traits (DiCra, 2003), several attempts have been reported on increasing its genetic variation. This has been done through inter-specific hybridisation and induced mutagenesis (Wang et al. 1999).

5.4.1.1 Molecular markers

There is no report presently available on work specifically targeted on developing molecular markers for traits in crambe. A few reports have used ITS, cpDNA, RAPD and similar techniques to analyse phylogenetic relationships between different members of the crambe genus (Francisco-Ortega et al. 1999; Warwick and Gugel. 2003). As crambe is part of the *Brassicaceae* there is a good potential in making use of the vast amount of genetic information gathered from research on *Arabidopsis* and on species from the *Brassicas* such as rapeseed.

5.4.2 Genetic transformation

A transformation protocol is critical in the process of providing Crambe with the necessary genes for novel industrial oil qualities. No public transformation protocol has so far been available for Crambe; however, recently a research laboratory in China established an *Agrobacterium*-based protocol for Crambe transformation (Banquan Huang, personal communication). However, this protocol will need to be developed further and optimised in order to transform other varieties and, this is an area where more research should be directed. In this respect it can be noted that like *Arabidopsis* and *B. napus*, Crambe is a member of the *Brassicaceae* family, where many members are routinely transformed.

A recent EPOBIO report suggested production of high levels of wax esters as a useful industrial feedstock from Crambe (Carlsson et al. 2006). However, such a production is depending on efficient and tissue-specific promoters. Seed-specific promoters such as napin or *FAE* (Stålberg et al. 1993; Rossak et al. 2001) have a good track record for expression in Arabidopsis and *B. napus* and are therefore considered to be a good choice for Crambe.

There are several advantages with choosing a strategy involving marker-free selection i.e. where the marker is removed when stable insertion of the transgene has been confirmed. There is public concern about the presence of selectable markers in the transgenic crops and these worries have especially been raised in respect of antibiotic selectable markers. Marker-free selection would therefore ease people's concern related to these antibiotic selectable markers and also herbicide resistance markers and their potential to be spread to other species like bacteria or weeds. However, EFSA (European Safety Authority) recommends that resistance genes towards antibiotics that are not in clinical use should be allowed in commercial GM plants (EFSA document ENV/04/27). Therefore EC recommendations for the use of selectable markers might change in the future.

The ability of plant biotechnologists to modify more complex metabolic pathways is accompanied by the transfer of multiple traits and genes to the plant. This calls for an increasing number of different selectable markers in order to distinguish the different traits from each other.

The above issues can be solved by using a marker-free selection strategy. Several alternatives exist such as the Cre/Lox system (Corneille et al. 2001), using a transiently cointegrated selection gene (Klaus et al. 2004) or combining an inducible site-specific recombinase for the precise elimination of undesired, introduced DNA sequences with a bifunctional selectable marker gene used for the initial positive selection of transgenic tissue and subsequent negative selection for fully marker-free plants (Schaart et al. 2004).

5.5 Susceptibility to abiotic stress

C. abyssinica originates from a warm-temperate area of Ethiopia. By adapting to a wide climate range including colder and more dry regions, crambe became native to the Mediterranean region but is at the same time also found across Asia, Western Europe and USA and grown as far south as Venezuela and as far north as Sweden and Leningrad. Crambe can tolerate mild frost from -4° C to -6° C (Duke 1983c, IENICA (2005). The base temperature for leaf expansion was determined to be 6.8° C for crambe (ref in Meijer and Mathijssen, 1996). Crambe can be grown as a spring crop (like spring rapeseed, 'Canola', *B. napus*) in Europe or as a winter-sown crop (like winter rapeseed, *B. napus*) in Mediterranean climates. Crambe seed is moderately tolerant to saline soils during germination over a range of soil temperatures of 10° C to 30° C. As soil temperatures decrease below 10° C in saline soils, crambe seed germination rate decreases. Established crambe plants are similar to wheat in saline soil tolerance (Endres and Schatz 1993; Francois and Kleiman, 1990).

5.6 Susceptibility to biotic stress

Crambe is susceptible to several diseases. Spores of many fungi including *Alternaria* are very common on crambe seeds at harvest (*Alternaria abyssinica* and *Alternaria brassicicola*). In North Dakota (USA) crambe is susceptible to *Sclerotinia* (white mould) and *Alternaria*. Excessive moisture conditions throughout the 1993 growing season in North Dakota resulted in significant yield loss due to these diseases. Also, blackleg and *pythium* root rot have been observed in this area. A four-year rotation cropping scheme may reduce the disease pressure (Endres and Schatz 1993). In Europe (The Netherlands) crambe may suffer from *Alternaria brassicicola* and to a less extend from *Alternaria brassicae*. Yield reductions of more the 50% have been observed. *Alternaria* is transmitted by the seed and the seed coat plays an important role. *Alternaria brassicicola* can propagate on *Brassica* *oleracea* and therefore cabbage should not precede crambe. The *Alternaria* spores produce toxins that can cause necrosis on the leaves (DiCra, 2003; Mastebroek et al. 1998). Infection with *Sclerotinia* spp. also has been observed. *Sclerotinia* infection occurs often halfway through the flowering period and can be treated with 1 litre Ronilan (vincholozoline) per ha. Early races are less susceptible to *Alternaria*. *Verticillium dahliae* which can multiply on crambe but thus far did not cause damage in the crop. In crop rotation experiments, higher levels of *Sclerotinia* on 'Canola' and crambe were observed when those crops followed a safflower crop. Crambe as a pre-crop showed a negative effect on sunflower regarding *Sclerotinia* disease (Krupinsky et al. 2006). Occasionally, infections with *Cladosporium spp*.; *Epicoccum spp*., *Stemphilium spp*., *Botrytis spp*; *Fusarium spp*. (Duke, 1983c) and *Plasmodiophora brassicae* (IENICA (2005) have been observed. Like beets and rapeseed, crambe is susceptible for the beet cyst nematode (*Heterodera schachtii*) the broad spectrum of host plants including members of the *Chenopodiaceae* and *Brassicaceae* families. At emergence the flea beetle (*Phyllotreta cruciferae*) can harm the young seedlings and at pre-flowering the pollen beetle (*Meligethes aeneus*) can eat the pollen. However, crambe has been found to be flea beetle resistant (Anderson et al. 1992). Also, especially in more stony soils, *Baris spp*. can eat the roots. Crambe does also seem to be less attractive to these insects compared to volunteer rape and weeds (Tittonel, 1998) Considerable damage can be caused by caterpillars (*Pieris brassicae* and *Pieris rapae*, NL: Koolwitje). Cabbage maggots cause damage in some areas and seed weevils, *Ceutorhynchus assimilis*, which transmit yellow mosaic virus, may infect crambe (Duke 1983c). Predation by birds is only a problem in small scale cultivations. Remarkably, birds seem to appreciate the non-hairy crambe types whereas the caterpillars like the hairy once. Granulate grains applied immediately after sowing can prevent caterpillar damage in small scale seed propagation plots. In the USA, grasshoppers have caused significant damage to crambe (typically in field margins). Crambe is most susceptible to grasshopper damage at the seedling stage. Grasshoppers tend to choose other crop foliage as crambe develops. Artificial infestation and defoliation experiments showed that neither method of defoliation caused yield reduction suggesting that crambe is a sink-limited plant (Kmec et al. 1998).

5.7 Agronomy

From an agronomic perspective crambe is competitive with spring rapeseed (‘Canola’), because both have the same growing period (depending on the location, from late March to late April until mid August to September). High erucic acid rapeseed (HEAR), the competitor of crambe on the oil market, is a winter rape which is sown in the last 10 days of August and the first 10 days of September and harvested the following July (IENICA, CSL report on Rapeseed and Turnip rape). One of the constraints to high productivity in crambe may be the inefficient use of radiation during seed formation. From emergence to mid-flowering the radiation use efficiency (RUE) was 2.2 g MJ^{-1} against only 0.9 g MJ^{-1} for subsequent developmental stages. The seed filling seemed to depend on actual photosynthesis by the pod tissue (Meijer et al.1999). As in rapeseed, green leaf area in crambe decreased rapidly from the beginning of pod formation. Thereafter the pod area index in crambe increased to 0.3-0.4 at most, which is low compared to rapeseed (1.5-2.0). This strongly suggests that the small area of the pods is a major constraint to dry matter and seed production in crambe (Meijer and Mathijssen 1996)

The medium light to heavy soils that are fertile and well drained are preferred by crambe but poor sandy soils are acceptable if nutrients are provided (50-75 kg N ha^{-1}). In North-West Europe all soil types are suitable for crambe cultivation although crambe prefers humid but well-drained soils with a pH of 6.0 to 7.5. A good humidity of the sowing bed is essential for germination (Endres and Schatz, 1993). Crambe does not grow well on stony and shallow soils. The soil must be deep, with a good moisture holding capacity but not prone to water logging. In loamy soils the seedling is often too weak to break the crust. Also, the structure of the soil is important and it must be ploughed or subsoiled in autumn so that the clods will be broken by frost. In spring compaction of the soil must be prevented (Dicra, 2003; Tittonel, 1998). Soils that have potential for crusting need to be managed carefully to prevent emergence problems. If a harrow or rotary hoe is used to break up soil crusting, crambe stands can be reduced if the seedling hypocotyl arch is immediately below the soil surface or the seedlings have emerged. Use of an empty press drill across a crusted soil has been successful in breaking the crust and minimizing crambe stand losses (Endres and Schatz, 1993; Francois and Kleiman, 1990).

Weed control is a critical management factor in crambe production (Endres and Schatz, 1993; DiCra, 2003). During the first three to four weeks after emergence the competition with weeds is critical and the use of relatively weed-free fields is recommended (Endres and Schatz, 1993). Seeding early will help establish a crambe stand that is competitive with weeds. A management practice is to allow weeds to emerge after the crambe seed bed preparation and kill them with non-selective herbicide (e.g. glyphosate, Treflan / trifluradin). Furthermore, drilling crambe in rows spaced at 12.5 cm to give 150-200 plants m^{-2} will provide early competition. If high weed populations should still emerge, reduced doses (25-50%) of appropriate herbicides suited for crambe can be applied (DiCra, 2003). If the weed is mechanically destroyed, a harrow or rotary hoe may be used for weed control three to seven days after crambe planting or after the crop has emerged. However, extreme caution must be exercised with these tillage tools as a high percentage of crambe seedlings may be damaged or destroyed (Endres and Schatz, 1993).

Oil seeds such as crambe can survive for more then ten years in the ground. Therefore, to avoid volunteer crambe plants in the next crop, the soil should be broken with a cultivator to allow spoiled seeds to germinate before winter to prevent growth in between the next crop. A part of the seed will germinate in spring. These volunteer crambe plants are easily managed in succeeding crops, especially in wheat, using tillage and/or herbicides (Endres and Schatz, 1993).

Rotation cropping of crambe with other crops is recommended to avoid a build-up of insects, diseases, and weeds. In crop rotation, crambe should not succeed itself or closely related crops such as ‘Canola’ or mustard. Instead, crambe can be grown after small grains, corn, grain legumes, onions or fallow ground. Crambe cultivation is most suited in a rotation with monocot crops (small grains, cereals, maize, and wheat) and fibre crops (hemp and flax). Crambe is also suitable as a companion crop for alfalfa or other biennial or perennial forage-type legume establishment. In potato cultivation a pre-crop of crambe is not preferred because the risk of contamination from *Verticillium* is high (Endres and Schatz, 1993). Competition for production occurs among crops belonging to the *Cruciferae* plant family such as beets, crambe and rapeseed. As was mentioned earlier, these crops are susceptible to the beet cyst nematode (*Heterodera schachtii*), and should therefore only be allowed once in a four year rotation scheme and therefore can only occupy 25% of the production area. Crambe production should reach economic values similar to that of sugar beet and rapeseed to constitute a competitive alternative in the crop rotation scheme. To establish annual crop rotation schemes ten crops (crambe, 'Canola', sunflower, safflower, dry bean, dry pea flax spring wheat and barley) were evaluated in crop matrixes for North America. The seed yield of several crops, including crambe, was influenced by the preceding crop. The lowest seed yields were of course obtained when a crop was grown on its own residue. The residues of crambe and 'Canola' showed a statistically significant negative effect on the yield of dry pea in one year and on dry beans in the other year of testing. 'Canola' and crambe are not associated with mycorrhiza and growing these crops may have a negative effect on the mycorrhizal fungi in the soil, affecting yields of preceding crops (Krupinsky et al. 2006). Therefore it is important to select a good crop rotation for either 'Canola' and/or crambe. In one out of two years, dry pea and sunflower had a positive effect whereas 'Canola' and safflower showed a negative effect as pre-crop on crambe yield. Sunflower and crambe appeared a good pre-crop for spring wheat (Krupinsky et al. 2006).

5.8 Environmental impacts

5.8.1 Agrochemical inputs, nutrient and water requirements

Annual rainfall in crambe growing areas is enough to fulfil the water requirements of the crop and additional irrigation is not practiced. The roots can reach depths of more then 15 cm rendering the plant tolerant to periods of drought. However, drought stress during flowering or seed set may cause loss of yield and reduction in oil content (IENICA 2005). Furthermore, crambe will not tolerate wet or waterlogged soils (Duke 1983c; IENICA 2005). In the US Great Plains, water-use efficiencies of 63 kg $(ha\ cm)^{-1}$ and 49 kg $(ha\ cm)^{-1}$ have been reported.

Table 5 Summary of agronomic and agrochemical data for crambe

Crop parameters	**Data**	**References**
Plant height	~116-183 cm (average 120)	DiCra, 2003
Root depth	15 cm	IENICA, 2005
Leaf area index (LAI)	2.0 – 3.3 m2 m-2 (maximum value)	Kmec et al. 1998
Total above ground biomass	400 – 600 g m-2	Kmec et al. 1998
Plant dry weight (max)	3.6-12.5 t ha^{-1}	DiCra, 2003
Root density	45 km m-3	Sharratt and Gesch, 2004
Seed Yield & oil yield	2353 kg ha^{-1} seed (range 546) 846 kg oil ha^{-1} (range 326)	DiCra, 2003
Water		
Water needs	spring rainfall	
WUE	63 kg ha^{-1} cm^{-1} (average 3 years)	Anderson et al. 2003
Fertilisers		
Max N in plant (without extra N fertilisation)	47-195 kg ha^{-1}	DiCra, 2003
N in soil after harvest (without extra N fertilisation)	24-87 kg ha^{-1}	DiCra, 2003
Fertiliser N-input	~75 kg ha^{-1} or adapted to soil reserves	DiCra, 2003
Sulphur (S)	~80 Units ha^{-1}	DiCra, 2003
Potassium (K)	~60 Units ha^{-1}	DiCra, 2003
Soil management		
Pesticides	Pyrethroid (flea beetle and pollen beetle. Deltamethrin (against altise) Carbendazim (*Sclerotinia*)	*Crambe abyssinica*. (1997)
Herbicides	Butisan (2 l ha^{-1}) but also Treflan (2 l ha^{-1}) and Ramrot (8 kg ha^{-1})	Laghetti et al. 1995.

The lower water-use efficiency number might be due to a non-uniform emergence of the seeds due to dryness at planting. Normally, in the same area crambe is tolerant to drought stresses (Anderson et al. 2003).

The fertility management of Crambe is comparable to that of small grains, summer wheat, mustard and 'Canola'. Normally the protocol for the fertilisation of summer barley is followed. The dry matter and the nitrogen content during the crop cycle were studied at three locations in France, Central Italy and Germany (DiCra, 2003). Accumulation of nitrogen started at stem elongation and continues to flowering time. When the plant was not in a good condition, the nitrogen remained in the leaves and did not migrate to the reproductive part. This indicates the importance of balanced Nitrogen fertilisation since higher fertilisation rates tend to result in a lower harvest index. This means that nitrogen supported the creation of dry mater but did not result in higher seed yields. The maximum nitrogen uptake ranged from about 115 to 46 kg N ha^{-1} without fertilisation, to 270 to 156 kg ha^{-1} with a fertilisation rate of 150 kg ha^{-1}. Besides added fertiliser, nitrogen from the soil is present (ranging from 32 to 62 kg ha^{-1}). The available nitrogen from soil and fertiliser was not always completely absorbed by the crambe plant, especially with the higher fertilisation rates. A high nitrogen fertilisation rate together with a high content of mineral nitrogen in the soil in spring increases the risk of pollution with nitrates because it may lead to a higher content of mineral nitrogen in the soil after harvesting. It has also been taken into account that considerable quantities of nitrogen were lost by the fall of the leaves and remain in the field in addition to the quantities after harvest, according to soil analysis. This amount increases with higher nitrogen fertilisation. This confirms the necessity of a well-adapted nitrogen fertilisation strategy to avoid pollution by nitrates. At all trial locations nitrogen fertilisation of 75 kg ha^{-1} resulted in a distinct yield increase compared with no nitrogen fertilisation while a fertilisation of 150 kg ha^{-1} did not significantly improve the yield compared to 75 kg ha^{-1}. From these experiments a nitrogen fertilisation of 75 kg ha^{-1} was recommended for crambe. However, this amount can vary depending on mineral nitrogen in the soil, soil type and the preceding crop. Fertilisation with sulphur is not necessary. The oil content slightly decreased and the raw protein content significantly increased with increased nitrogen fertilisation. The influence of nitrogen and sulphur fertilisation on the content of erucic acid was not very significant. (DiCra, 2003)

Pesticides: *Sclerotinia* infection occurs often halfway the flowering period and can be treated with 1 liter Ronilan (vincholozoline) per ha. Early races are less susceptible to *Alternaria*. *Alternaria* can be reduced by spraying with 1 liter Rovral (iprodion) per ha.

Herbicides: In the Netherlands no herbicides are allowed for crambe. Weed control should therefore be performed mechanically or by hand. Crambe is tolerant to frequently applied herbicides (Stougaard and Moomaw, 1991).

5.8.2 CO_2 emission & carbon sequestration

The level of carbon sequestration and soil organic matter depends strongly on the cropping system applied. No-tillage coupled with annual cropping has been proposed to minimize or halt the loss of soil organic carbon content and maintain soil productivity. The effects of improved cultivation practices are only visible on a long term. Ecological processes are slow and the determination of the amount of carbon sequestration takes many years. Current cultivation practices which include tillage lead to loss of soil organic carbon.

The soil water in spring is depending on remaining water in the soil profile retained by soil particles and crop residuals and in some areas additional melting-water. Crambe residual showed no extreme effects on the spring soil water content as compared to ‘Canola’ and several other crops. Also, other effects of crambe on the properties of the fertile soil were not observed under no-till management (Krupinsky et al. 2006).

Crambe stubble provides an acceptable cover for trapping snow, controlling erosion and establishing fall/autumn-seeded crops in a no-till production system. When planting fall-seeded crops, care must be taken to minimize stubble disturbance as crambe residue is brittle and easily destroyed (Endres and Schatz 1993).

5.8.3 Gene flow and biosafety

Airborne pollen of B. napus can travel at least 200m from the source crop (Flannery et al. 2004). However, as crambe has comparable flower morphology like *B. napus*, and this is related to pollination by insects, this is also the predominant way of pollination in crambe. It has been shown that bees can take the pollen from *Brassicas* over long distances (1.6 km-5 km). Theoretically there is potential for pollen to be transferred to distances of at least 10 km by mixing of bees foraging in different directions from the same hive (Flannery et al. 2004).

The introduction of novel crops or existing crops with new properties (e.g. introduced by genetic modification) may lead to the establishment of feral populations (Hancock et al. 1996). These populations may cause a threat to the ecosystem due to gene flow to wild relatives, the population becoming a persistent weed in arable fields or becoming dominant plants in wild vegetation. Feral populations often are initiated by spills of seeds from transport trucks or farm machinery. Locally these populations are often threatened by the large environmental fluctuations and usually do not persist longer than a few years

Outcrossing rates in *C. abyssinica* within the range of 4.8% to 10.7% have been determined in different types of plots between dominant and recessive genotypes (Vollmann and Ruckenbauer, 1991). Crosses between *C. abyssinica* and *C. hispanica* can result in hybrid lines although the offspring suffers from infertility because of the imbalance between the chromosome numbers of both species (DiCra, 2003). Analogous to this it is not expected that *C. abyssinica* can cross easily with the other related species and therefore the chance of a gene flow to wild relatives is low. Furthermore, *C. abyssinica* pollen is incompatible with *Brassica napus*, *Brassica campestris* and *Brassica juncea*. In *B. napus* and *B. campestris* pre-zygotic barriers occur to prevent the pollen tube growth and in the case of *B.*

juncea, embryo abortion prevented the formation of interspecific hybrids (Wang and Luo, 1998). Only by somatic hybridization and microspore cultures, were hybrids between *B. napus* and *C. abyssinica* obtained (Wang and Luo, 1998; Wang et al. 2003c), but such events will not happen in nature.

5.8.4 Effects of climate changes

By the year 2080, due to increasing temperatures and drought, a drastic change can be expected that may reduce the choice of crops that can be grown in southern Europe. On the other hand, the area of crops for northern Europe is extending more to the north and crops that currently are grown in the south are expected to move north. Currently, based on their climate requirements, rapeseed, crambe and oats can be grown across most of Europe. For all crops a decline is expected in southern Europe, while they spread further north into latitude 65–71^{o}N (Tuck et al. 2006). It should be noted that the predictions were based on climatic factors only and that a number of parameters such as soil type and profile, were not included in these predictions for the future.

5.9 Economics

5.9.1 Introduction

In assessing the economic potential of crambe as a feedstock platform for this sector of the bioeconomy, this report has looked at data for Italy, Germany, Poland, Sweden and the UK. Each of these countries represents a different climatic region of the EU with potential for the successful cultivation of different crops. It is not the intention of this analysis to propose monocultures in any of these regions but to draw out significant differences that currently exist and provide a basis on which future cropping plans can be considered. In addition, it will also be necessary, in the longer term, to consider the impact of climate change on agricultural production but little data currently exists from which conclusions can be drawn.

5.9.2 Results on crambe

As crambe is not yet an established crop, most of the yield data available are from field trials or limited cultivation. In DiCra (Diversification with Crambe: an industrial oil crop) an EC supported program on crambe (DiCra, 2003), it was calculated that if constant seed-yields of more than 1.8 t ha^{-1} can be reached on farm-scale cultivation, crambe could be a profitable crop (DiCra, 2003). In trial plots in different locations in Europe and over a range of cultivars, crambe yielded on average 2353 kg of seed ha^{-1} or 846 kg of oil, containing on average 57.8 % erucic acid. It was found that the yield and quality of crambe production depended more on the environment than on the crambe accession used, each location having a best performing accession (DiCra, 2003). Under optimal conditions high seed yields were obtained, for example in North-East Germany (2.72 to 3.19 t ha^{-1}), the Po valley (3.2 to 3.7 t ha^{-1} and up to1 t ha^{-1} oil), Southern England (on average 3.5 t ha^{-1}), France-Burgundy (up to 2.7 t ha^{-1}) showing the yield potential of crambe (*Crambe abyssinica*, 1997). Table 6 show yield data on crambe used in the economic analysis for Germany, Italy, Poland, Sweden and UK.

Table 6 Crambe yields in several areas in Europe for best performing cultivars (average over three years regarding the seed yield).

[a] (DiCra, 2003) [b] (*Crambe abyssinica*, 1997) [c] Estimated data.

	Average Yield (t ha-1 y-1)	**Highest seed yield (t ha-1 y-1)**	**Average oil yield (t ha-1 y-1)**	**Highest oil yield (t ha-1 y-1)**
Germany [a]	3.0	3.4	1.1	1.4
Italy [a]	3.7	4	1.3	1.5
Poland [c]	3.0	-	1.1	-
Sweden [c]	3.0	-	-	-
United Kingdom [b]	3.5	4	1.1	1.3

Table 7 Revenues from crambe cultivation in Germany, Italy, Poland, Sweden and UK.

			Germany	Italy	Poland	Sweden	UK
Production	Grain	t ha^{-1}	3.0	3.7	3.0	3.0	3.5
	Straw	t ha^{-1}	3.6	4.8	3.6	3.6	4.5
Crop revenue	Main crop	(€ t^{-1} yield)	266.00	162.00	310.50	274.78	266.40
	Straw	(€ t^{-1} yield)	30.00	30.00	29.62	29.18	30.00
	Main crop	(€ ha^{-1})	798.00	599.40	931.51	824.33	932.40
	Straw	(€ ha^{-1})	108.00	143.70	106.63	105.05	133.50
	Total	(€ ha^{-1})	906.00	743.10	1038.14	929.38	1065.90
Single farm payment		(€ ha^{-1})	316.55	554.23	93.44	242.32	319.64
Total revenue (crop and single farm payment)	Main crop	(€ t^{-1} yield)	371.52	311.79	341.65	355.55	357.72
	Straw	(€ t^{-1} yield)	30.00	30.00	29.62	29.18	30.00
	Main crop	(€ ha^{-1})	1114.55	1153.63	1024.95	1066.65	1252.04
	Straw	(€ ha^{-1})	108.00	143.70	106.63	105.05	133.50
	Total	(€ ha^{-1})	1222.55	1297.33	1131.58	1171.70	1385.54
Summary of variable costs	Main crop	(€ t^{-1} yield)	77.07	120.11	49.76	54.65	94.94
	Straw	(€ t^{-1} yield)	1.89	1.75	4.02	17.75	1.50
	Main crop	(€ ha^{-1})	231.21	444.39	149.28	163.94	332.31
	Straw	(€ ha^{-1})	6.79	8.40	14.47	63.89	6.68
	Total	(€ ha^{-1})	238.00	452.79	163.75	227.83	338.98

Table 7 Revenues from crambe cultivation in Germany, Italy, Poland, Sweden and UK.

			Germany	Italy	Poland	Sweden	UK
Summary of fixed costs	Main crop	(€ t^{-1} yield)	152.13	135.52	58.27	124.15	114.27
	Straw	(€ t^{-1} yield)	24.77	34.76	4.99	16.61	23.86
	Main crop	(€ ha^{-1})	456.39	501.42	174.81	372.45	399.96
	Straw	(€ ha^{-1})	89.16	166.51	17.97	59.80	106.18
	Total	(€ ha^{-1})	545.55	667.93	192.78	432.25	506.13
Total costs	Main crop	(€ t^{-1} yield)	229.20	255.62	108.03	178.80	209.22
	Straw	(€ t^{-1} yield)	26.65	36.52	9.01	34.36	25.36
	Main crop	(€ ha^{-1})	687.59	945.81	324.09	536.39	732.26
	Straw	(€ ha^{-1})	95.96	174.91	32.44	123.69	112.85
	Total	(€ ha^{-1})	783.55	1120.72	356.53	660.08	845.11
Net margins (without single farm payment)	Main crop	(€ t^{-1} yield)	36.80	-93.62	202.47	95.98	57.18
	Straw	(€ t^{-1} yield)	3.35	-6.52	20.61	-5.18	4.64
	Main crop	(€ ha^{-1})	110.41	-346.41	607.42	287.94	200.14
	Straw	(€ ha^{-1})	12.04	-31.21	74.19	-18.64	20.65
	Total	(€ ha^{-1})	122.45	-377.62	681.62	269.30	220.79
Net margin (with single farm payment)	Main crop	(€ t^{-1} yield)	142.32	56.17	233.62	176.75	148.51
	Straw	(€ t^{-1} yield)	1.20	-6.52	20.61	-5.18	4.64
	Main crop	(€ ha^{-1})	426.96	207.82	700.86	530.26	519.77
	Straw	(€ ha^{-1})	12.04	-31.21	74.19	-18.64	20.65
	Total	(€ ha^{-1})	439.00	176.61	775.05	511.62	540.42

Table 7, above, sets out the value per tonne of yield and the value of the per hectare yield for crambe grown in the five countries chosen. From the figures shown the assumed per hectare yields of product can be derived. The data include a value for the straw produced as a by-product of the crop.

The value of the single farm payment has been shown in the table and net margins calculated to demonstrate the effect of including and excluding this subsidy. It should be noted that the value of the single farm payment has been allocated to the primary product and has not been split between grains/seed and straw. Costs have, in the main, been allocated to the primary product with the exception of land values and the costs directly associated with managing and handling the straw. Since there is very limited data on management costs for crambe specifically, these data have been assumed the same as for spring rapeseed.

The variable costs shown in the table include:

- Cost of seed/planting material
- Agrochemical inputs – fertiliser, pesticides and herbicides
- Variable costs of straw baling, primarily identified in literature as baling string

Fixed costs include:

- Cost of machinery for cultivation, drilling and the application of agrochemicals
- Combining, including the management/handling of straw
- Labour costs
- Land costs
- For sugar beet only, the on-farm costs of collection, drying and storage.

Full details of the data used and assumptions made in the preparation of the economics information for this report are available on the EPOBIO website www.epobio.net.

Finally, it should be noted that the primary sources of the data used to derive this economic information are: Agro Business Consultants, (2006); Ask.com UK (2007); Ericsson et al, (2000); ERMES Agricoltura (2007); European Commission (2007); Food and Agriculture Organisation (2007); Nix, (2007); Stott, (2003) and Swedish University of Agricultural Sciences (2007).

5.9.3 Comments on the results for crambe

In regions where crambe can be grown as an autumn sown crop (like winter rapeseed) or in case early and more cold resistant cultivars should be available, high yields are expected (Tittonel, 1998; Wang et al. 2000b). Success of the crop greatly depends on a good emergence of the seeds. The best results have been obtained under favourable weather conditions with enough spring rainfall, optimal average daily temperatures (8-10^{o}C to14^{o}C) and a good weed management. Under these conditions a crop density of at least 150 plants m-2 should be reached. In Southern Italy, autumn sowing at the end of October is preferable (Tittonel, 1998). Crambe performed very well in the Po valley. Temperatures of more then 35° C during maturation did not harm the crop; enough rainfall during germination and good agricultural practice and crop-knowledge is more essential for a good yield.

The revenues, without the single farm payment, presented for crambe (Table 6) show a great variation from a loss of 377 € ha^{-1} in Italy to a profit of 681 € ha^{-1} in Poland. This difference is mainly due to the different levels of production cost in the five countries since yield data from field trials in Germany, Italy and UK does not show a huge difference (Table 5). The data in Table 6 points to that crambe preferable is a crop for the middle and northern part of Europe. This is, even after the addition of the single farm payment and taking into consideration the higher yields of crambe in Italy.

5.10 SWOT analysis on crambe

Strengths

- Strict non-food product
- Valuable industrial feedstock oil
- Knowledge-database from *Brassicaceae* available
- Outcross to other oil crop unlikely
- Co-products (seed cake)
- Existing processing infrastructure can be used

Weaknesses

- Narrow genetic diversity base
- Comparatively low yield
- High proportion hull (voluminous)
- Potential gene flow to wild relatives (Southern Europe)
- High glucosinolates
- Low cold tolerance
- Low disease resistance to *Alternaria*

Opportunities

- High fossil fuel price
- High yielding varieties
- Potential utilisation as a biocide
- Developing winter crambe

Threats

- Low fossil fuel price
- Competition from rapeseed
- Land use competition
- Public perception against using GM technology

5.11 Research and development needs

Crambe is not used for food and feed applications and can thus be considered as a non-food crop for development into an industrial platform for oils. R&D must focus on optimising crambe for use as a field crop. As yet, comparatively little development has taken place and programmes that address breeding for high yield are a priority.

Given the potential of crambe to produce a highly diverse range of specialised fatty acids using a GM strategy, transformation protocols for the crop must be optimised. Also, a much greater understanding of metabolic pathways and flux control will be necessary to realise the full potential of the platform for production of designer oils.

Low cold tolerance is a weakness of the germplasm currently available. R&D programmes therefore should focus on establishing new winter varieties of crambe.

In summary, the R&D needed in order to develop crambe into a non-food oil crop platform for production of special industrial oils is listed below:

- Breeding (Conventional and GM) for high seed yield
- Developing transformation protocol
- Breeding for increased *Alternaria* resistance
- Breeding (Conventional and GM) for winter varieties
- Metabolic engineering

5.12 Policy issues on Crambe

The 2003 reforms of the Common Agricultural Policy, which were extended to the Mediterranean crop regimes (tobacco, cotton and olive oil) and to hops in 2004, broke the link between subsidy and production. This brought a new focus on the market, leaving farmers free to identify the best commercial opportunities for their production systems. The new single payment scheme includes the retention of compulsory set-aside, the requirement to withdraw land from agricultural production. This provides an opportunity and an incentive for the cultivation of crops for specified non-food uses. Crambe is a crop used only for non-food applications and, in the EU, it retains eligibility in the single payment scheme and can be grown both on set-aside and on other arable land. Production on set-aside is subject to conditions, including a requirement for contracts and payment of securities.

6 EPOBIO RECOMMENDATIONS

The need for sustainable and reliable feedstock sources of plant oils is a prerequisite for replacing petroleum as the raw material choice in the chemical industry. The EPOBIO study has identified suitable crop platforms that can do well in producing the necessary feedstocks. Criteria that the crop platforms had to meet were:

- Production of industrial oil qualities must in many cases be avoided from mixing with food qualities by outcrossing or admixtures.
- Cultivation should be possible over large areas of Europe.
- Crops chosen should fit in existing crop rotations.
- The potential for modification using biotechnology should exist for the crop.

Suggested crop platforms should integrate with existing processing infrastructure to minimise the need for additional investments. The three crop platforms identified in the EPOBIO study are commodities for which cultivation are well known and handling of their seeds as well as oil processing can be done by existing infrastructure and therefore integrated into existing supply chains.

A significant part of the chemical industries feedstock requirements such as basic carbon chain, can be met by a supply of high oleic plant oils (90% and above). Such oils will decrease processing costs and provide the industry with the necessary carbon chains for its products while making use of existing manufacturing chains. As oleic acid is a constituent in food oil the high oleic oil crops constitute no problem if mixed with food oil. The EPOBIO study therefore recommends high yielding oil crops developed to produce high oleic acid oils as one type of non-food crop platform.

Over a large part of Europe rapeseed is the dominant crop producing plant oils for food applications as well as for the production of biofuels. Extensive breeding activities have resulted in high yielding cultivars with different oil qualities.

Substantial amount of data on the *Brassica* genomics have been accumulated over time and comprise an important resource for continuous improvement of the rapeseed crop.

To expand the use of rapeseed oil for feedstock use in chemical industries, the EPOBIO study suggest that rapeseed cultivars with high oleic fatty acid (90% and above) should be developed. This will require the use of genetic engineering.

The projected expansion of the total acreage of rapeseed cultivated in Europe will further increase the environmental load from this crop. This study therefore recommends substantial breeding activities to be directed towards increased disease and pest resistance and decreased demand for fertiliser.

Oat is a well known crop in Europe that is cultivated with low pesticide and fertiliser input. Significantly, it accumulates quite high amount of oil in its grain compared to other cereal crops. The EPOBIO study has identified this crop as having clear potential to be transformed into a high yielding oil crop by increasing its present oil content. This is accomplished by switching much of the carbon accumulating as starch into oil by modification of key enzymes in the starch and oil synthesis respectively. The preferred oat oil quality is high oleic acid and that will provide a means of meeting the increasing demand for oleochemicals as feedstock in the future biobased chemical industry. Conventional breeding and techniques such as “Tilling” as well as genetic engineering might be required to accomplish high oil oats producing high oleic oils.

This study recommends a program directed towards increasing oil content in oat. Inevitably this requires increased R&D to understand the fundamental switch between starch and oil accumulation. To further increase the yield of this crop, the EPOBIO study recommends support for a breeding program focused at developing winter varieties of oat.

To provide a means of producing specialised industrial oil qualities in oil crops the EPOBIO study recommends the use of oil crops designated for this production. Such oils can for example contain hydroxy, epoxy, conjugated, acetylenic or other unusual fatty acids, or primarily consist of wax esters. Although not necessarily toxic they are not intended for food use and must not enter the food chain. It is therefore important that the producing crop platform does not give rise to fertile offspring when crossed with dominating oil crops for food production. Its seeds must be easily recognised from other oil crops and it should be accessible for genetic engineering.

Crambe is identified by the EPOBIO study as a crop for production of specialised industrial oil qualities that must not be mixed with food quality oils. Crambe is already a non-food oil crop and it does not cross fertilise with *Brassicas* such as rapeseed. With regards to cultivation, crambe is a low input oil crop compared to many others and it can be grown over large part of Europe. Developing crambe cultivars producing special industrial oil qualities (designer oils) other than high erucic acid will require the use of genetic engineering.

The EPOBIO study recommends that crambe should be chosen as the crop for production of 'designer oils'. It further recommends substantial resources to be directed into improving the seed yield as well as cold tolerance in crambe.

It is important that the actions taken to provide a sustainable feedstock supply for the chemical industry will find a broad acceptance and support from the society. Results from several surveys and investigations performed among the public show a positive perception for a change to a biobased supply of feedstocks. Even the use of genetic engineering in order to achieve the goals finds substantial acceptance among wide groups of people in Europe if the purpose is confined to the development of non-food crops. The EPOBIO study suggests strategic communications campaigns designed to raise awareness and create an informed acceptance of bioproducts.

The importance of positive examples of the use of plant produced feedstock oils as replacement for fossil fuels is of vital importance. Developing these examples requires initial investments with risk of no major returns for an extended time when the market is developing. It is therefore advisable that the European Community initiates and supports projects along the whole supply chain of feedstock supply, processing and the production of bioproducts, ensuring the establishment of a bioeconomy market. The EPOBIO study therefore recommends directed support to pilot projects in the bioeconomy area, focused at establish a proof of concept and testing of the plant-based feedstock oils under industrial conditions.

To ensure a positive growth of the bioeconomy, EPOBIO recommends that strategic decisions and polices at all different levels in the European Community should be evaluated towards a ‘bioeconomy test’ in the same way they are now assessed for their sustainable development impacts.

The EPOBIO study recommends an open approach in communicating R&D actions taken to develop non-food crop platforms and when using techniques such as genetic engineering. This can be ensured by further strengthening the European Community resources supporting public research in plant biotechnology.

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