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RESULTS PACK

Exploiting opportunities and addressing the challenges of biotech in the Agri-Food sector

EDITORIAL

EXPLOITING OPPORTUNITIES AND ADDRESSING THE CHALLENGES OF BIOTECH IN THE AGRI-FOOD SECTOR

Advances in biotechnologies promise to be one of the major leaps in human scientific understanding in the twenty-first century, offering new products and processes that may help to address some of the world's most pressing challenges. The Agri-Food sector is one area where biotechnologies could deliver on this promise. Innovative EU-funded research is aimed at ensuring that Europe can fully benefit from the opportunities created through Agri-Food biotech solutions, whilst maintaining high levels of environmental, food and consumer safety.

Biotech solutions for the Agri-Food sector encompass a range of modern techniques and tools, which can help with understanding the fundamental mechanisms of the basis of life and be used to provide substantial benefits to farmers, consumers and the environment. To this end, there is a need for an in-depth knowledge of the impacts of new technologies, addressing the needs and concerns of different stakeholders and society at large. This also requires transparent and accurate communication on scientific and technological advances and the broader engagement of different players to ensure that both opportunities and challenges are not overlooked.

Responsible policymaking

This is why the European Commission has been highly committed to stimulating an informed and open political debate amongst all interested stakeholders on how the EU can both benefit from modern biotechnologies and innovation in the Agri-Food sector, whilst maintaining high safety standards.

One of the ways the Commission facilitates this discussion is by ensuring impartial and thorough scientific advice to guarantee informed policymaking. In April 2017, the High Level Group of the Commission's Scientific Advice Mechanism (SAM) published an Explanatory Note on new techniques in the Agri-Food sector, covering applications in animals, plants, and microorganisms for food and feed production. The Note also outlined the agricultural application of new techniques in the fields of synthetic biology and gene drive.

Alongside informed policymaking, the EU has also supported, through the FP7 and Horizon 2020 framework programmes, ambitious research projects that are at the forefront of exciting biotech developments and new discoveries within the Agri-Food sector.

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On track for enhanced photosynthesis in certain crops

Not all plants are made equal. Whilst they all use an enzyme called Rubisco to capture carbon, some can only count on the inefficient C_3 photosynthesis pathway to do so, resulting in the loss of already-fixed carbon in a process called photorespiration. The 3TO4 project aimed to enhance photosynthesis in these plants, drawing inspiration from the more efficient C_4 photosynthesis.


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Plants use photosynthesis to convert carbon dioxide and water into carbohydrates using energy from light. Central to this carbon fixation process is an enzyme called Rubisco, which first evolved 3.5 billion years ago in photosynthetic bacteria.

Many crop plants, including wheat, barley, rice, soybean and potatoes, use Rubisco in an inefficient pathway – known as C_3 photosynthesis – to fix carbon. More recently evolved grasses like maize have altered their leaf structure and biochemistry to concentrate CO_2 around Rubisco in the more efficient C_4 photosynthesis pathway. In general, plants with C_4 photosynthesis account for about 50 % of known grasses, 3 % of flowering plant species, and 40 % of the world's grain harvest. But what if these percentages could be raised by means of green biotech?

'If photorespiration, a process that works against photosynthesis, could be reduced in C_3 crops, or if C_3 crops could be converted to use C_4 photosynthesis, large economic and environmental benefits would be ensured because of the reduced inputs per unit yield associated with the C_4 pathway,' says Richard Leegood, coordinator of 3TO4 and Professor of Plant Biochemistry at the University of Sheffield.

C_4 photosynthesis results in improved rates of carbon fixation, improved nitrogen use and improved water use, but bringing it to C_3 plants is far from easy. 'Efficient C_4 photosynthesis is associated with alterations to leaf development, cell biology and biochemistry,' Prof. Leegood explains. 'Transferring these traits into C_3 crops is a long-term undertaking, but even partial long-term success would already have significant economic and environmental benefits.'

3TO4 has been laying the groundwork by trying to uncover the fundamental aspects of C_4 biology. The team's ultimate aim is to use the C_4 mechanism to reduce the extent of photorespiration.

'The work proposed largely proceeded according to plan,' says Prof. Leegood. 'However, although rape lines with a

Efficient C_4 photosynthesis is associated with alterations to leaf development, cell biology and biochemistry.

photorespiratory by-pass were generated, the plants transformed with the by-pass did not show a sufficiently strong phenotype to warrant the extensive programme of work originally proposed.'

To overcome this problem, the team refocused their work on C_3-C_4 intermediate plants such as *Moricandia arvensis*, which is closely related to rape and features a natural photorespiratory by-pass.

Another of the project's main ambitions was to contribute to the C_4 Rice Project funded by the Bill & Melinda Gates Foundation. 'C_4 rice is destined to increase food production in its major markets in South-East Asia and Africa, but once the development of a C_4 crop (or any crop with reduced photorespiration) has been achieved, it should be relatively straightforward to apply to technology to other crops, including C_3 crops in Europe, such as wheat,' says Prof. Leegood.

Although the project has now been completed, work is continuing in partners' labs in areas such as C_4 leaf development and anatomy, the photorespiratory by-pass, post-translational regulation of C_4 proteins, function of transcription factors and regulation of gene expression. If all goes according to plan, Prof. Leegood believes C_4 crops could become a reality in 15 to 20 years.

Project	**3TO4: Converting C_3 to C_4 photosynthesis for sustainable agriculture**
Coordinated by	UNIVERSITY OF SHEFFIED
Funded under	FP7-KBBE
Project website	http://www.3to4.org/

Strengthening legume crops

The EU-funded ABSTRESS project has used genetic engineering to create new legume varieties that can better withstand drought and disease.

Along with climate change, food security – our ability to produce enough safe and nutritious food to feed Europe's population – is a major concern, making improvements in the crop varieties we breed more important than ever. For the last 5 years an EU-funded consortium, ABSTRESS has been investigating how state of the art genetic technologies can be used to introduce sustainable commercial crop varieties, with direct resistance to adverse environmental or biological conditions.

The 13 partner consortium, from 7 EU countries, studied legumes because, 'uniquely amongst crops, they work with microorganisms to take nitrogen from the atmosphere into the plant roots and act as a natural fertiliser,' says project coordinator Adrian Charlton from Fera Science in the UK. However, European legumes such as beans and peas can be quite susceptible to diseases, such as *Fusarium oxysporum* – a group of plant pathogens that live in the soil and attack plants through their roots, causing the plants to wilt. 'It results in unpredictable crop yields and is made worse by the sort of drought conditions that might become more common in Europe due to climate change,' adds Charlton.

The ABSTRESS project applied comparative genomic approaches to study the networks of genes in pea plants that control the plants ability to grow in drought conditions or resist the effects of pathogens such as *Fusarium*. They also studied *Medicago truncatula*, a small low-growing clover-like legume native to the Mediterranean region. 'It's a fast growing plant that is commonly used as a model for legume crops as it has a small genome that is well understood and allows us to understand the molecular mechanisms involved in how the plant responds to drought and disease,' explains Charlton.

The genetic data generated was used to identify control 'hub' genes in the crop and then to further identify similar genes in other crops such as tomato plants. From this information new seeds were created with mutated versions of the important genes.

As well as genetic analysis, plant material grown under stress was analysed with molecular imaging technologies such as x-ray and infra red imaging, to look inside the plants for early signs of stress. This, says Charlton, allowed the team to understand why some plants did better than others. 'We were looking at pre-symptomatic drought and *Fusarium* stress so that we could work out which mechanisms the most successful plants use to protect themselves,' he explains.

In addition the ABSTRESS team wanted to understand the role of soil microorganisms in plant health. 'We showed that changing the genetics of the microorganisms that live in the root nodules of legumes can also potentially help to improve resistance to drought in particular,' concludes Charlton.

The consortium was ultimately able to produce pea mutants with promising genetic characteristics. 'The novel collection of new seed varieties we have created will be put into commercial breeding programmes to assess its usefulness as a resource for breeding drought and *Fusarium* resistant plants,' says Charlton. 'This will be a benchmark for applying the latest technologies to breed a new generation of crops more able to cope with the challenges of climate change.'

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Project	**ABSTRESS: Improving the resistance of legume crops to combined abiotic and biotic stress**
Coordinated by	FERA SCIENCE LIMITED
Funded under	FP7-KBBE
Project website	https://secure.fera.defra.gov.uk/abstress/

The impact of environmental stress on plant seed quality

The fact that environmental stress can affect animals and humans before their birth, and how they do so, is relatively well-established. Yet somehow, the same cannot be said of plant seeds. An EU-project set out to complete the puzzle.

'Despite seeds' pivotal importance for agricultural productivity and ecosystem preservation, there is still much we don't know about the role played by the environment on seed development and storage, and most importantly on how it affects quality,' said Prof. Ilse Kranner, Head of the University of Innsbruck's Institute of Botany, back in 2014.

Specialised in plant stress response, in particular in extreme environmental conditions, Prof. Kranner was then presented with a one-of-a-kind opportunity in the ECOSEED project: a consortium bringing together 11 partners with well-established track records in seed biology and converging sciences from Austria, the UK, Germany, France and Spain – with the sole purpose of exploring seed fate under environmental stress.

The first step consisted in providing a definition for 'seed quality': 'Seed quality was defined as "the sum of all seed traits that are acquired from the time of seed development on the mother plant to seed germination",' Prof. Kranner explains. 'The quality traits we investigated include seed viability, vigour, dormancy, longevity and ageing, as well as other markers important for seed traders such as the "1 000 seed weight" and the seed number per plant.'

Four crop plants were identified as excellent candidates for the research: Arabidopsis, or thale cress, because of the high amount of scientific data already available; Brassica oleracea for its representativeness of vegetable crops; barley because of its importance for worldwide agriculture and sunflower for its status of second most important oil crop (after rapeseed) in the EU. The plants were grown under stressful conditions that are expected to increase due to climate change, namely elevated temperature and drought.

The consortium considered all stages of these seeds' life cycle: while still on the mother plant, during storage and during germination. 'We applied state-of-the-art scientific techniques to identify important molecular regulatory pathways and key cellular switches that mediate seed environmental sensing and

We applied state-of-the-art scientific techniques to identify important molecular regulatory pathways and key cellular switches that mediate seed environmental sensing and signalling, along with their importance for downstream seed quality and seedling establishment.

signalling, along with their importance for downstream seed quality and seedling establishment,' Prof. Kranner explains.

Once this was done, the team was able to identify genes and technologies contributing to the prediction of seed quality. This knowledge was then translated to wild species, and was fed into a model for seed quality trait dependency on environmental factors.

'The markers of seed quality could help with the regular monitoring of the quality of seeds in storage, also providing a better basis for the use of genetic material of wild plants, to the benefit of agriculture and conservation,' Prof. Kranner enthuses. 'Seed quality is an important determinant for the potential of prolonged dry storage, a trait that is not only important for plant conservation in seed banks but that also underpins the international agricultural seed trade worth an estimated $42 billion.'

Besides these markers, ECOSEED also provided guidelines for improved seed handling through ISTA (the International Seed Testing Association), including recommendations on the seed trade and conservation for seed harvest and storage to improve breeding, crop management and conservation activities.

'We hope that our results will benefit plant conservation and agriculture alike. From a more scientific standpoint, we believe that our results made a step-change in our understanding of seed performance in a changing environment.'

Meanwhile, the consortium is considering the possibility of further EU-funded projects such as an Innovative Training Network (ITN) through Marie Skłodowska-Curie actions. Bilateral project applications are expected between various ECOSEED partners, if and when calls for funding are announced by their respective national research councils.

Project	ECOSEED: Impacts of Environmental Conditions on Seed Quality
Coordinated by	UNIVERSITAET INNSBRUCK
Funded under	FP7-KBBE
Project website	https://www.uibk.ac.at/botany/ecoseed/home/

Finding fertile ground for dairy cows

Created to examine decreasing fertility in dairy cattle, the EU-funded FECUND project has collected detailed genetic data that is already helping to reverse the decline.

Since the 1990s there has been a decline in cow fertility which has in turn shortened the productive life of cows. 'It's now often less than 3 calvings per cow,' says Filippo Biscarini, from the Institute of Agricultural Biology and Biotechnology of the National Council for Research (CNR-IBBA) in Milan/Lodi, Italy. Biscarini is coordinator of the recently completed four-year EU-funded FECUND project that has tried to explain why this has happened and how dairy cattle breeding and farming can reverse the decline.

The decline in cow fertility, particularly in Holstein cattle, has led pregnancy rates after insemination to drop from 80 % 20 years ago to less than 40 % today. Poor fertility is now one of the main reasons for early culling. The FECUND consortium, with 13 partners from seven EU countries, sought to study this phenomenon in dairy cows by looking at the metabolic and genetic factors associated with high and low fertility potential.

To understand what is going on, we looked at two models, one based on metabolics – or energy use and input, and the second based on genetics.

'Our starting hypotheses was that today's high-producing cows divert most of their energy towards milk production, whilst neglecting other important physiological activities like reproduction, which has led to lower conception rates and intervals of more than 14 months between calvings,' explains Biscarini.

As well as a cow's metabolic state, pre-determined genetic factors also play a role in its fertility. 'To understand what is going on, we looked at two models, one based on metabolics – or energy use and input, and the second based on genetics,' says Biscarini. FECUND focused on the early phases of reproduction from egg development to foetal implantation. They sampled from the animals reproductive organs, taken at different stages in the reproductive cycle. The consortium generated a large set of data on the animals' genetic make-up and where and when specific genes were switched on and off, to fully understand how genetics could be influencing fertility.

The biological samples came from commercial dairy cattle in different countries, from cattle with high and low estimated breeding values (the industry measure of their genetic potential for fertility), and from cows under the energy stress of early lactation as well as dry cows and younger animals before pregnancy.

FECUND's results have provided a better understanding of how lactation stress and genetics affect egg and embryo quality, for instance, how components of the fluid inside the follicles where eggs form influence the egg quality and its ability to develop.

FECUND has used genetic data mined from their studies to find novel mutations related to fertility, and to predict carriers of desirable or undesired mutations, like the TUBD1 mutation, recently discovered, which is associated with embryo mortality and, consequently, reduced fertility.

'The project has contributed with the generation of large datasets available to the community, a new toolbox and a better understanding of the problem,' concludes Biscarini. This project and other work in the area is already paying dividends, he adds. 'The concerted efforts from the scientific and breeding communities have been effective, and the declining trend in cow fertility has been stopped and in some cases reverted.'

Project	**FECUND: Optimisation of early reproductive success in dairy cattle through the definition of new traits and improved reproductive biotechnology**
Coordinated by	FONDAZIONE PARCO TECNOLOGICO PADANO
Funded under	FP7-KBBE
Project website	http://www.fecund-project.eu/

Innovative pollination research promises an agri-boost

Plants need animals and insects for pollination and attract them through a range of chemicals that create colour, scent and nectar in their flowers. Recent Marie Skłodowska-Curie Fellow, Dr Monica Borghi has studied the genetic basis of these traits.

Looking, smelling and tasting good is important for plants. It's what allows them to become pollinated by insects or small animals, transferring pollen from flower to flower. But what are the biological processes within plants that create these signals for pollinators? Marie Skłodowska-Curie Fellow, Dr Monica Borghi, then at Wageningen University in the Netherlands, undertook a study looking at the development of these traits. 'Plants lure animal pollinators with a range of products produced in flowers, such as scented and coloured molecules to attract pollinators as well as nectar as food. We wanted to better understand how the production of these features in flowers contributes to successful pollination of crops,' explains Borghi.

We have generated a large amount of data on gene expression and metabolite composition during flower development and now hope to generate and validate new hypotheses about how plants are able to regulate the production of scent, colour and nectar.

Pollination contributes to more than $200 billion of agricultural revenue globally and to higher yields and better quality fruits and vegetables. The process of pollination allows plants to be fertilised and while crops such as wheat, maize, and rice self-pollinate, many agricultural crops, including strawberries, apples, pears, onions, melons and coffee, need the assistance of animals. So understanding the cues that attract and sustain pollinating animals is important and could lead to new bio-based procedures being developed to increase yields and quality.

Borghi, working in the plant biology lab of Harro Bouwmeester was looking into the Arabidopsis plant to study the chemical compounds, or metabolites that plants produce and release

from flowers. This includes nectar and pollen, which are made of sugars and proteins and other biological molecules that produce scent ad colour. 'Arabidopsis is a plant of the cabbage family. In this family pollination is important for seed production. This includes rapeseed and also in many vegetable brassica's such as cabbages,' says Borghi. The genome of Arabidopsis was sequenced in 2001 and a large database of information on the plants' genes already exists that make it useful to identify the genes that regulate plants production of fragrance, colour and nectar.

In the two year project, completed in June, Borghi extracted the chemicals responsible for colour and scent and the sugars and proteins present in nectar, from frozen flowers. These were analysed via a variety of chromatography and spectrometry techniques. She then tried to correlate this data with genetic information. 'We are trying to work out which genes control the production of pollinator signals in flowers,' Borghi explains.

To assess if a particular attribute made a flower more or less attractive to animals for pollination, Borghi experimented with hoverflies. She did this using 'knock-out Arabidopsis' – plants breed with disrupted genes to stop the production of some of the metabolites that produce colour, scent and nectar in flowers. 'For these experiments we allowed the flies to choose between the wild-type plant and each plant with a disrupted gene and recorded their preferences. Our preliminary results show that the flies were able to discriminate and quickly learn to visit the plants that offer the most abundant rewards, for example more nectar,' says Borghi.

'We have generated a large amount of data on gene expression and metabolite composition during flower development and now hope to generate and validate new hypotheses about how plants are able to regulate the production of scent, colour and nectar,' says Borghi. The project is currently being continued at the Max Planck Institute for Molecular Plant Physiology in Potsdam-Golm, Germany.

Project	**MEPOL: The role of plant primary and secondary metabolism in pollination**
Coordinated by	WAGENINGEN UNIVERSITY
Funded under	Horizon 2020-MSCA-IF

Creating a better hemp plant

EU researchers are developing new varieties of hemp capable of feeding an innovative hemp bio-refinery and serving as a sustainable alternative to cotton.

Hemp is a sustainable, high-yielding crop that is particularly well adapted for growing in European climates. Not only does it have the potential to bring numerous environmental, agronomical and economic benefits, it can also be used to produce a range of biomaterials that would contribute to the growth of Europe's overall bioeconomy – everything from fibres to seeds and even cosmetics and pharmaceutical products.

With all these advantages, it would seem a no brainer to promote the agricultural production of hemp. Yet surprisingly, in Europe hemp remains a minor crop and the hemp industry struggles to expand its acreage.

'Although hemp was once a key industrial crop for fibre, hemp production disappeared over the last century in favour of cotton and synthetic fibres,' explains Stefano Amaducci, project leader for the EU-funded MULTIHEMP project.

To change this, the project uses a cutting-edge genomic approach to achieve rapid, targeted improvements in hemp productivity and the quality of raw material. At the same time, it is working to advance the scientific understanding of gene-to-trait relationships in the crop. 'By combining this genetic work with innovations in agronomy, harvesting and processing methods, we have generated sustainable products from improved hemp varieties,' adds Amaducci.

A renewed interest

As Europe continues to look for new ways to mitigate the challenges of a changing climate, the need for more sustainable plant-based biomaterials has taken centre stage. For example, although most of our clothes and many other products are made from cotton, the cotton plant itself is an environmental disaster. As cotton fell from grace, a new solution was needed and, according to MULTIHEMP researchers, that solution is hemp.

'There is a renewed interest in hemp because it requires less water and chemicals to grow, and it provides a superior quality of fibre and oil than what can be derived from other plants,' says Amaducci. 'What we are doing is essentially taking an already good crop and turning it into a really great crop.'

There is a renewed interest in hemp because it requires less water and chemicals to grow, and it provides a superior quality of fibre and oil than what can be derived from other plants.

Hemp-based bio-refinery

The project's focus is on a natural, bio-refinery hemp plant that can combine the production of superior food (high quality proteins and oil) with a wide array of sustainable bio-materials. New hemp varieties are being developed to feed an innovative hemp bio-refinery where the value of each part of the plant is exploited.

At harvesting, the stem and seeds are separated and the threshing residue is collected to recover valuable secondary metabolites. The stem is then separated, with the short fibre used for producing an innovative flock insulation system and the long fibre for high value bio-composites. 'We're even analysing the dust produced during the decortication of the stems for possible industrial applications,' says Amaducci.

Big benefits

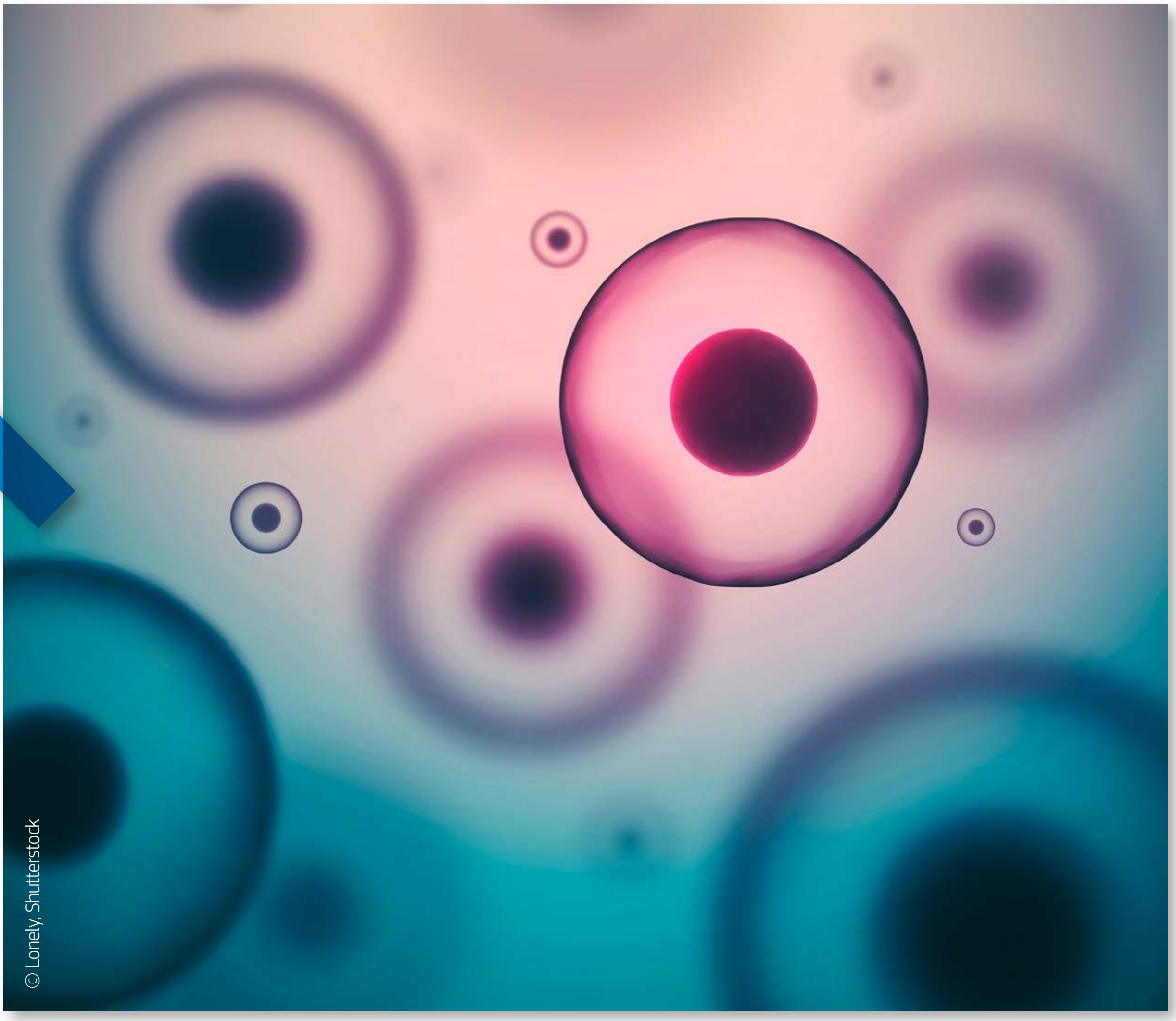
MULTIHEMP's natural bio-refinery hemp plant looks set to produce numerous benefits – both economic and environmental. For example, the project is supporting the release of new varieties suitable for multipurpose applications of the plant. Furthermore, the knowledge generated during agronomic and physiological research will be useful in fine tuning the cultivation techniques and choosing the most appropriate variety for a specific environment and end use.

Project	**MULTIHEMP: Multipurpose hemp for industrial bioproducts and biomass**
Coordinated by	UNIVERSITA CATTOLICA DEL SACRO CUORE
Funded under	FP7-KBBE
Project website	http://multihemp.eu/project/

Fostering a dialogue on synthetic biology

New areas of science, such as synthetic biology often cause public concern and require responsible governance. SYNENERGENE has engaged citizens across Europe in thinking about the challenges and opportunities synthetic biology poses.

Synthetic biology represents the latest phase in the development of biotechnology. By manipulating the genetic material, scientists now have the ability to design, manufacture and modify organisms that can perform useful tasks such as producing more durable crops or food flavourings. But is society ready for this unprecedented level of control over biology? Do we understand its possibilities and risks and how best it should be regulated? The EU-funded SYNENERGENE project was set up four years ago to explore these issues.



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While some conflicts, for example around the use of synthetic biology for food products and in green biotechnology in general, were hard to handle, we at least managed to bring the opponents together.

'From enthusiasts and promoters to harsh critics of synthetic biology, a great diversity of people and groups engaged and contributed to SYNENERGENE,' says project coordinator Christopher Coenen, a researcher in the impacts of technology at Karlsruhe Institute of Technology in Germany.

Synthetic biology, for instance, allows micro-organisms to be built almost like Lego, with different genetic bricks combined together. 'These organisms are becoming increasingly more estranged from those we may find in nature which poses new risks. Moreover, our notions of what we mean by "natural" may itself change as synthetic biology allows us to put "life" and "nature" on the drawing board like never before,' says Coenen. He suggests the public should also be fully informed about the field's potential to contribute to address current grand societal challenges such as sustainability, food security and climate change.

The SYNENERGENE consortium consisted of 10 European universities, plus 14 other partners. These including companies, think-tanks, a network of science centres and museums, an art society, science journalists, a public theatre and activist civil society organisations. There was involvement from 14 European countries as well as Brazil, Canada and the US.

The project organised more than 140 single events, many of which were open to the public. They developed a wide range of learning tools, information materials and options of engagement for the public. 'We used well-established means of public communication, engagement and dialogue, but also innovative means, in particular at the interfaces of art and science,' explains Coenen.

The project aimed to engage a wide variety of perspectives, with a special focus on young people. In order to reach a very broad spectrum of people, they co-organised or supported additional forums, with a wide range of organisations and at large public events. 'We also targeted activities to specific groups such as the new DIYbio/biohacker communities,' says Coenen. This group includes largely young enthusiasts who conduct biological experiments and other scientific activities and build tools for biological research outside of academic settings.

A major outcome of the project was to create spaces for reflection and debate which included a diversity of voices. 'While some conflicts, for example around the use of synthetic biology for food products and in green biotechnology in general, were hard to handle, we at least managed to bring the opponents together,' suggests Coenen. 'The project also helped create relationships of trust across a great diversity of stakeholders and fostered mutual learning.' With a great diversity of event types and its content-rich website, the project also reached thousands of citizens in Europe and beyond.

Project	**SYNENERGENE: Synthetic biology – Engaging with New and Emerging Science and Technology in Responsible Governance of the Science and Society Relationship**
Coordinated by	KARLSRUHER INSTITUT FUER TECHNOLOGIE
Funded under	FP7-SIS
Project website	https://www.synenergene.eu/

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Melinda KURZNE OPOCZKY

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