Plants in progress

PROFESSORS MARGIT LAIMER AND FATEMEH MAGHULY

As the demand for sustainable energy sources grows, **Professors Margit Laimer** and **Fatemeh Maghuly** explain why they are hoping a little known perennial plant will, with a little help, provide the answer

Can you offer a brief outline of your research and the context from which it emerged?

The primary objective of our research is to develop a renewable energy source from non-domesticated, non-edible plants, especially for countries without oil reserves or access to alternative energy options.

We are concentrating on *Jatropha curcas*, which seems to be the ideal plant for biodiesel production. *J. curcas* is a perennial, non-edible plant with a lifespan of over 30 years that thrives on poor soils, which are unsuitable for food production. The species itself even allows the 'greening' (the planting on sites traditionally deemed unusable for farming) of degraded areas. In addition, different tissues of *Jatropha*, including seeds, have long been used as raw material for lamp oil, soap production, paints, lubricating oils and medical applications.

Why has this plant not been used more successfully to date?

The reasons why *J. curcas* is still not successfully cultivated on a large scale can be attributed to its non-domesticated state. Although the fuel properties of its oil correspond to the standards of biodiesel, the plant lacks genetically uniform varieties. The health status of current planting material is uncertain, since to date pathogen detection tests are largley missing.

Plantations are currently planted with seeds from wild plants, where fruiting is fairly irregular, and inefficient management further hinders the ability to reliably predict yield. The species itself is susceptible to many pests and diseases, requiring particular attention prior to large-scale planting.

Most of the *Jatropha* accessions are toxic, which renders the seedcake unsuitable for use as animal feed. This deprives the producers of valuable byproducts, currently known in soybean for example.

In light of the issues you have outlined, what methodology are you employing?

The project is the first of its kind to employ a systems biology approach to deciphering important metabolic pathways and enzymes, such as the oil and toxin synthesis during seed development for *J. curcas*.

We have addressed the issue with a series of technologies:

- Establishing *in vivo* and *in vitro* germbanks of 1,300 accessions from 14 countries worldwide
- Successfully ear-marking germplasm with molecular markers in order to define the best specimen, the best breeding partners etc.
- Conducting genomic, proteomic and metabolomic analyses on the identical samples to address important questions from various perspectives and speed up knowledge gain, together with international partner institutions
- Application of chemical and physical mutagenesis to create novel germplasm
- Identification of potential hazardous pathogens and developed rapid virus detection tools for mass screening of planting material

The Plant Biotechnology Unit (PBU) has been working collaboratively with Bioplant R&D. What have been the outcomes so far?

In 2010, in collaboration with Bioplant R&D, our group succeeded in deciphering the transcriptome of *Jatropha* seeds for the first time worldwide. Transcriptome analyses were performed in successive stages of developing seeds. 19,000 contigs were obtained, of which approximately 6,000 genes could be annotated, basically a function could be attributed; to them in one of 145 known metabolic pathways.

We designed oligo microarray chips from in-house transcriptome analyses,

which allow very clear differentiations, for example between toxic and non-toxic plants, as well as of gene expression patterns of different developmental stages of seeds.

If successful, what are the likely outcomes of this work?

Data collected from *J. curcas* will also boost research on other Euphorbiaceae and will open new perspectives:

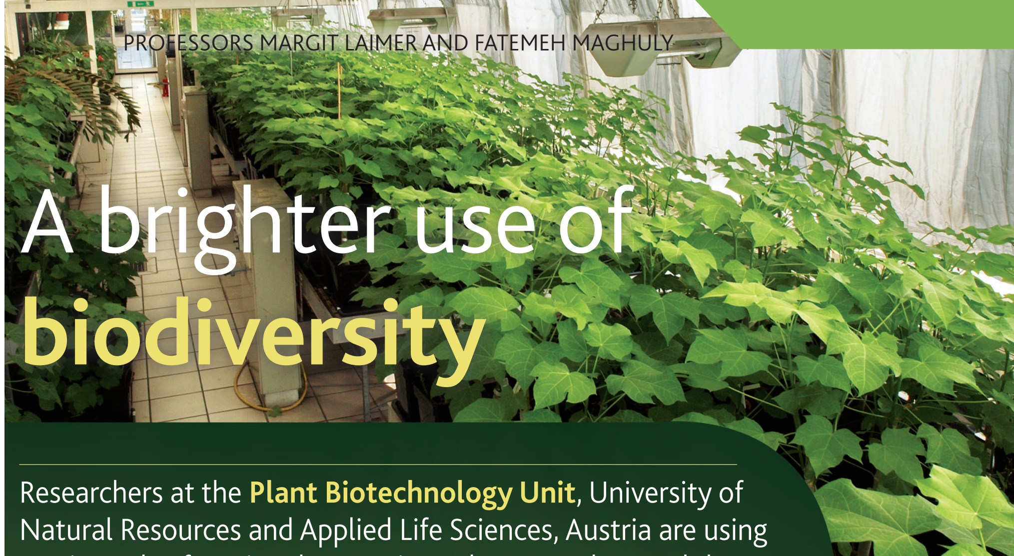
- Providing a better understanding of the basis of phenotypic diversity in Euphorbiaceae
- Uncovering the mechanisms of gene regulation, signalling, disease resistance and defence, and vegetative and generative development in renewable energy crops
- Reducing the time required for the analyses of large sample numbers for the presence of pathogens
- Providing marker-based approaches for genetic diversity assessment and germplasm characterisation to improve breeding strategies

All this will hopefully contribute significantly to the agronomic improvement of other non-domesticated species.

Ultimately, who will this research benefit?

In developing regions like many Sub-Saharan African countries, the limited access to energy sources implies that trees are cut for firewood as only energy resources, which results in rapid degradation, erosion and desertification. Therefore to achieve energy security and stimulate rural economic development, a plant like *Jatropha* can be used as an alternative energy and also improve the livelihood conditions, land restoration, reforestation, rural development and mitigate desertification.

Even for industrialised countries one of the promising ways to shift from the substitution of fossil fuels, whose combustion results in large net CO_2 emissions, to alternative energy sources like the perennial tree *Jatropha*, whose combustion releases gases sequestered through cultivation and which are therefore considered greenhouse gas neutral.



A brighter use of biodiversity

Researchers at the **Plant Biotechnology Unit**, University of Natural Resources and Applied Life Sciences, Austria are using cutting-edge functional genomics to better understand the *Jatropha* plant with the aim of creating novel cultivars for a better future

THE CREATION OF cheap and sustainable biofuel to replace fossil fuels is a pressing goal for researchers worldwide. Currently, biofuels provide almost 3 per cent of the world's energy, but with the unpredictability of the oil market and peak oil no longer a concept but a reality, this figure will need to grow significantly in coming years to meet demand in a more sustainable manner. With continuing population growth placing pressure on food production, many concerns have been raised concerning the use of food crops as biofuel. The possibility of developing a renewable energy source from non-edible resources is therefore a high priority.

Scientists at the Plant Biotechnology Unit (PBU) of the University of Natural Resources and Applied Life Sciences in Austria are foreseeing that *Jatropha curcas* – a perennial plant originating from Central and South America – could provide the answer. The plant has long been known to produce high-quality biodiesel from its oil. Moreover, it can survive in the most demanding of environments, but its cultivation and harvest – on the scale demanded by energy markets – has proved problematic with the crop returning sporadic and unpredictable yields, leading many biofuel farmers to dismiss it.

The PBU Researchers in Vienna, led by Professors Margit Laimer and Fatemeh Maghuly, are using techniques specially adapted for the in-depth study of *J. curcas*. The aim is to better understand its genetic makeup in the hope of producing novel cultivars that are sustainable, economically viable and manageable. If successful, the application of their findings has the potential to improve millions of lives.

BREAKING IT DOWN

Any breeder needs to know the breeding populations to anticipate a good pedigree. Today we do not look for morphological indicators, but we go for the information the -omics technologies tell about a potential crossing partner.

By systematically deciphering the genetic code of the plant, researchers at the PBU are working on a strategy to produce *J. curcas* cultivars for a wide variety of applications, and with a number of special features to improve its usage, as Maghuly explains: "The understanding of the biology of *J. curcas* and precise plant breeding represent the real key to succes".

There are important challenges to be overcome by PBU researchers. Despite the many potential benefits of *J. curcas*, the plant is susceptible to many pests and diseases; toxic, which renders the seedcake unsuitable for use as animal feed; and in addition, fruiting is fairly irregular which increases the cost of harvesting.

The first stage of the research involved the collection of different specimen of *J. curcas*, from a range of tropical and subtropical regions. So far the PBU collection maintained *in vivo* and *in vitro* contains over 1,300 accessions from 14 different countries on three continents.

DISCOVERY OF VARIATION

The team is using newly developed techniques to understand, at a genetic level, how the species differ and how they can be used in the creation of new, stronger and more durable *J. curcas* cultivars. Molecular markers are developed, allowing PBU researchers to characterise the genetic diversity through Targeting Induced Local Lesions IN Genomes (TILLING) and EcoTILLING in a wide range of natural germplasm of this species and induced mutants.

TILLING is a reverse genetic technology to identify polymorphisms in DNA samples. A variation of this technique (EcoTILLING) represents a means to determine the extent of natural variation in selected genes.

These processes, developed and augmented specifically by the collaborators on this project, enable the speedy detection of Single Nucleotide Polymorphisms (SNPs) amongst samples. SNPs are the simplest form of DNA variation amongst individual sub-species of plants. By understanding these minor differences, and analysing them, researchers can specify their impact on gene function. In practice, the process allows researchers to identify natural mutations in individuals as part of the wider analysis of population genetics.

> The process of functional genomics allows researchers to decipher the important metabolic pathways and essential enzymes of *Jatropha curcas*

By registering and understanding the minute genetic differences amongst sub-species, researchers are laying the foundations to the creation of different cultivars of *J. curcas* that are resistant to known pathogens, producing both a reliable yield and a high-quality of biofuel for specific regions across the world.

Climatic conditions, differing levels of rainfall, soil conditions and even neighbouring plants (sharing the same pathogens) in a region can affect the creation of a new cultivars of *J. curcas* capable of thriving year after year in areas where pests and disease may otherwise have rendered the plant unusable.

FUNCTION OVER FORM

The analysis of natural genetic variation and the interest in distinguishing adaptive variation from neutral variation is becoming a key issue in functional genomics. The approach of functional genomics – the dynamic study of genes in a variety of live-action scenarios – allows scientists (for the first time in a study such as this) to decipher the important metabolic pathways and enzymes of *J. curcas*. This is key to understanding potential and building upon it.

It is a challenge that PBU are tackling through strong collaborative efforts, including work with Bioplant R&D: "In our area of research, national and international cooperation is of vital importance," Laimer highlights. Worldwide connections have allowed not only the collection of a huge sample base, but scientific investigation and analysis has taken place in partnership with universities and organisations throughout the world, strengthening the research and providing much needed specialist skills.

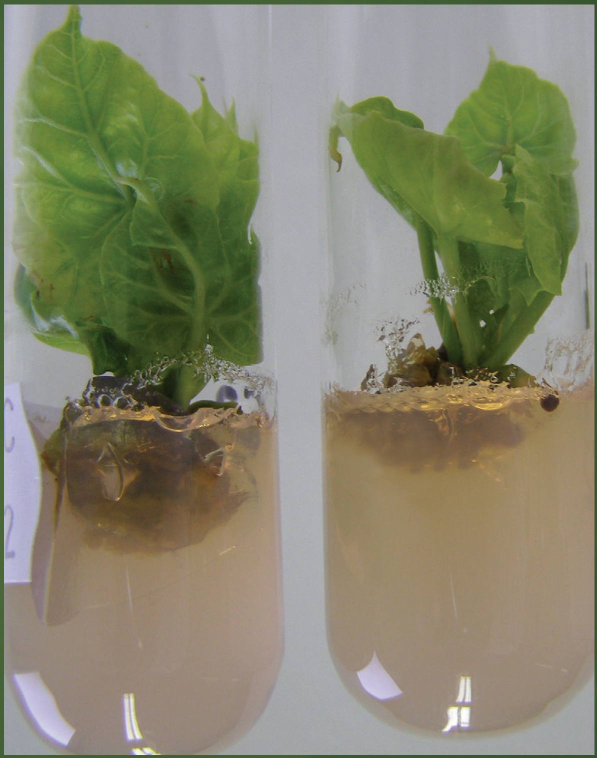
Health status also plays an important role. PBU researchers have already been able to identify the presence of the Begomoviruses for the first time in *Jatropha*. It will therefore be crucial to monitor novel planting material for the absence of pathogens that decrease vigour and yield of crop plants.

THE ENERGY OF REFORESTATION

At the heart of the research is a vision beyond creating a genetically robust plant. The focus is on the creation of a sustainable energy source that will significantly impact the wellbeing of some of the poorest areas on Earth.

By creating a viable biofuel source that thrives in such tough conditions, the benefits would be felt by rural communities through greater employment and resulting infrastructure improvements, offering a robust and long-lived crop that produces a reliable yield, and a regular income.

The group's activities contribute to the objectives of four of the international community's Millennium Development Goals, including the eradication of extreme poverty, ecological sustainability and building a Global Partnership for Development. In addition, and perhaps more profoundly, this project will have a positive impact on the quality of life and strengthen the role of women as they will carry out majority of the care of crops and harvesting of seeds.



GERMPLASM COLLECTION *IN VITRO* OF *JATROPHA CURCAS*

INTELLIGENCE

SNPS DISCOVERY FOR TARGETED GENOTYPING IN *JATROPHA CURCAS*

OBJECTIVES

The strategy is to develop *Jatropha* cultivars for a wide variety of applications and with a number of special features, allowing a future economic use of the plant in different ecosystems.

PARTNERS

Bioplant R&D, Austria • **International Atomic Energy Agency**, Austria • **Austrian Institute of Technology**, Austria • **Egerton University**, Kenya • **Ethiopian Institute of Agricultural Research**, Ethiopia • **Gadjah Mada University**, Indonesia • **Universidad Nacional Autónoma de México**, Mexico • **Universidad de Leon**, Nicaragua

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PROFESSOR FATEMEH MAGHULY was born in Shiraz, Iran, and received a PhD in Food and Biotechnology from BOKU and Austrian Research Centre Seibersdorf in 2003. She is Deputy Head of the Plant Biotechnology Unit, Institute of Applied Microbiology, Department of Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria. Her main research interests address genetic diversity and plant functional genomics.

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