EU-SOL - High Quality Solanaceous Crops for Consumers, Processors and Producers by Exploration of Natural Biodiversity

Email newsletter no 2, May 2008

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The EU-SOL project is supported by the European Commission through the 6th framework programme. Contract number FOOD-CT-2006-016214

EU-SOL explained to the public is on the air!

One of the aims of EU-SOL is public outreach and education. At the Natural History Museum in London, Alex Garlick has developed a website that is meant to inform and educate the public about potato and tomato breeding and the use of the wider biodiversity of Solanacae therein. The website has been online since the beginning of March 2008.

Type www.eu-sol.net, and what you get is a website that is accessible to the public, and explains about the backgrounds, targets and methods of EU-SOL. So far, only the sections meant for the public at large and people interested in the science contain extensive content, but the other section –schools- will follow soon. Apart from news about recent scientific advances in jargon free language, the public section contains a range of sub-sections, such as the history of Solanaceous crops, explanations about DNA, plant breeding and biodiversity, recipes and a 'try this' section with easy-to-do-at-home experiments like the extraction of tomato DNA.

We asked Alex Garlick to explain how the website was developed and what it is about.

We got started in November 2008. We knew we wanted to write about the history of the tomato and potato (and other minor Solanaceous crops) and the fascinating journey they have made to our plates and we had other ideas as well but we felt first it was important that we collected the views of consortium members as to what they would like to see on the website. So at the annual meeting of EU-SOL we asked the participants what areas they would like us to communicate about. We asked for suggestions, and the reasons for doing so. The results made clear that it is important to explain where the plants that we eat originate form, so we should start with the history of tomato, potato, and other minor Solanaceous crops such as peppers and aubergine as well. The partners also indicated that there should be information about DNA and genetics: what it is, and how it works. This information would then be connected to the history of plants, showing how the plant breeding and the different techniques that came along the way changed the nature of plants. It was suggested that we show the gradual shift in those techniques, questioning the rather simple distinction between 'natural' and 'unnatural' methods as being used today. It was clear we should also provide information about the EU-SOL project itself: what it is, what it is doing, the different kinds of partners are involved, and the multi-nation character.

That sounds like a good first step, but it does not make a website yet, does it?

It was a good start indeed. The next step focused on developing a structure. Together with our partners from the Max Planck Institute in Köln we started looking at other websites and try to define the pros and cons of different approaches. We realised that we are dealing with different audiences that have different information needs: the adult educated public, including those young adults in formal education, may be looking for comprehensive background information, but the scientific information should also be accessible. The structure we developed allows the consortium to bring more sophisticated and complex issues to the public in a step-wise fashion.

People can already find a lot of content in the public section?

Yes, they can. As well as the history of the tomato and potato, information on DNA, plant breeding and tomato biodiversity we've put up quite a bit of fun-stuff, such as recipes that contain information about the characteristics (taste, bite) and preferred use of the type/variety of tomatoes or potatoes. The 'try this' sections explains how you can do simple experiments at home, such as the extraction of DNA from tomatoes, a blindfolded taste experiment with fruit flavoured sweets, and instructions how to listen to the electricity produced from a tomato. There is always a part that explains how this is related to particular qualities of the fruit, or to biodiversity and variety. Another key section is where we try to clarify the role of DNA and lead people from an appreciation of the traits EU-SOL is investigating such as taste to a basic understanding of how DNA could affect these traits. By linking between all the sections in the website we hope to stimulate people's interest in the hidden life of their food they may never have considered.

What are the plans for the school section?

The school section will contain lesson packs at a later stage. These lesson packs will be based on consultation with teachers in different EU countries. They are meant to introduce the principles of Mendelian genetics to secondary schools through hands-on practical experience using segregating populations. Probably starting from November 2008, through the EU-SOL bioinformatics platform, a dedicated website will be created where schools will be able to upload their data such that it will be possible to analyse the combined datasets, thus giving students practical experience with collaborative research.

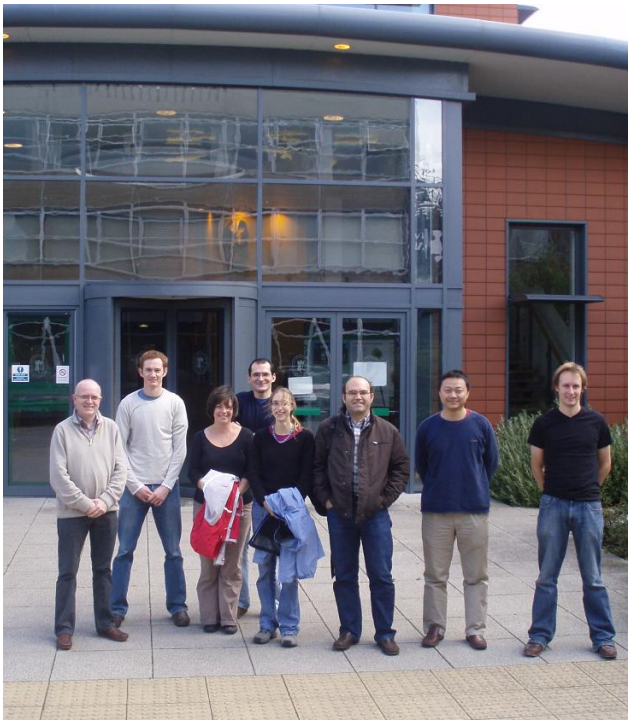
An effective website is never finished, so can we assume that there will be new content added in the near future?

Since 2008 is the International year of the potato (http://www.potato2008.org/en/index.html), we have recently introduced a Potato cam, which shows weekly pictures of a number of Charlotte potatoes, starting from chitting, following their growth until the harvest. At a later stage, the homepage will contain other topical items to attract people's attention. There will also be an interactive element with a discussion page. We will introduce links with other organisations and draw attention to specific events in the Natural History

Museum. In a couple of months, when there will be more content, we hope to start translations into a few more languages.

Fruit ripening is key to quality

Graham Seymour is Professor of Plant Biotechnology in the Plant Sciences Division at the University of Nottingham. In the EU-SOL project, Graham Seymour is responsible for tracking traits that are related to fruit ripening and fruit quality. He has been working for more than 20 years on the biochemistry and molecular biology of fruit ripening, and most of that has been focused on tomato.



Graham Seymour (left) and his research group at Nottingham University

What is your specific interest in tomato?

My research interests are developmental regulation of ripening in fleshy fruits and the genetic and molecular basis of fruit quality traits, for which tomato serves as a model system. Tomato makes an excellent model because there are so many biomolecular and genetic resources available, including many single gene ripening mutants and mutants of other types. There are excellent mapping populations and there is an emerging genome sequence.

What is the importance of fruit ripening as a trait?

The tomato industry is worth more than 10 billion dollars worldwide, and tomato is one of the most widely eaten fruit in terms of amount consumed. The ripening process gives the tomatoes the appropriate taste, colour, texture and smell. Consumer tests and surveys reveal that these are key quality attributes. The ripening process makes them also easy to digest, and produces good processed products as well. The raw material you start with can hugely affect the quality of the end product. This is very important, both in processing and in fresh market fruits. Ripening is key to quality. However, there is a kind of tricky balance between getting a good shelf life and other attributes useful to producers and say supermarkets and getting good fruit quality as well.

Quantitative Trait Loci (QTL) are stretches of DNA that are closely linked to the genes that underlie the continuous traits (traits that vary continuously - the trait could have any value within a range – such as height). They are often found on different chromosomes. Knowing the number of QTLs that explains variation in the phenotypic trait tells us about the genetic architecture of a trait. It may tell us that a specific trait is controlled by many genes of small effect, or by a few genes of large effect.
QTLs can be used to identify candidate genes underlying a trait. Once a region of DNA is identified as contributing to a phenotype, it can be sequenced. The DNA sequence of any genes in this region can then be compared to a database of DNA for genes whose function is already known.

How do you find appropriate traits?

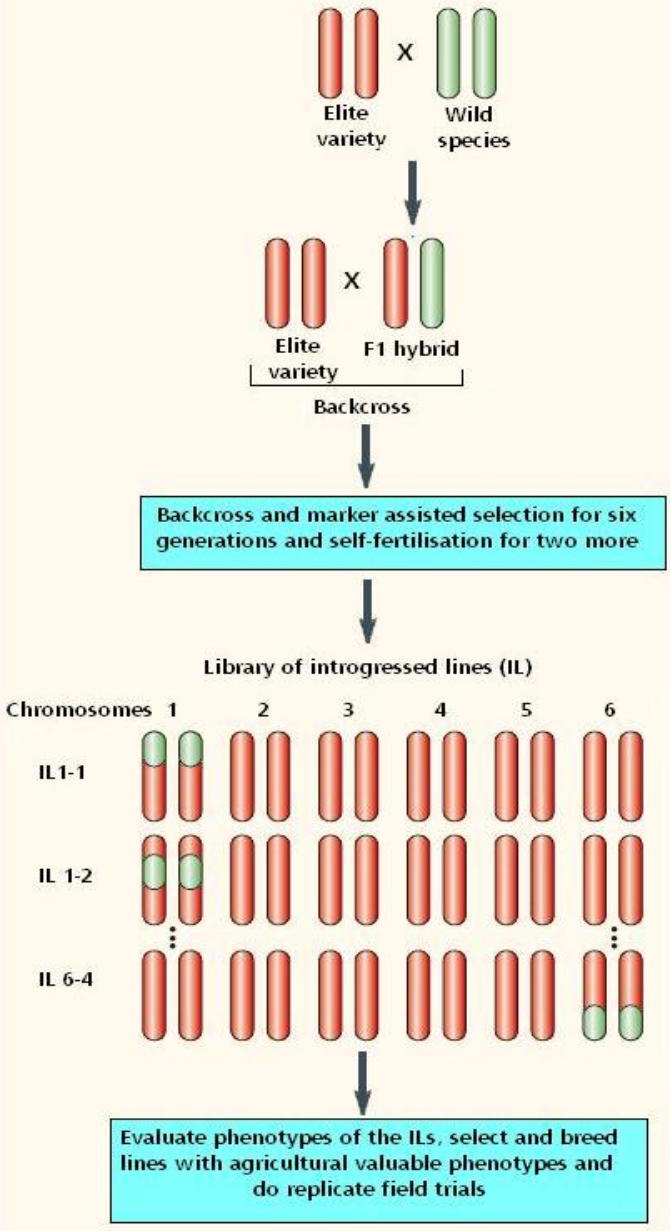
We are trying to harness some of the natural variation in genes that control fruit quality and my part in EU-SOL focuses on genes that affect texture. We want to look at how we might be able to use that natural variation to improve the keeping quality and the texture of the standard cultivating tomatoes. We use wild species to find those traits, to find the genes that control fruit texture, and then

understand what those genes are doing. Not only am I interested in the basic biology and molecular biology, I want to see this knowledge transferred into commercial practices as well.

Our approach can be best described in the following way.
We are working together with partners from INRA in France and from Argentina on resolving QTLs (see box **QTLs**) for fruit quality in tomato. In our case we start with already quite well characterised introgression line populations, these include some excellent resources created by Professor Dani Zamir from Israel, who is also a member of the EU-SOL project (see box **Introgression**). These lines include essentially small pieces of wild species DNA, which have been introduced by conventional breeding into cultivated tomato background. For instance, with the Zamir *Solanum pennellii* introgression collection, there are 76 lines, each of which has essentially a cultivated tomato background, but on one of the chromosomes on a particular place there is a fragment of wild species genome. You can grow these lines, in the field or in the greenhouse, and screen them for any trait of interest. So in our case fruit quality. You make a comparison between the control background, which is the cultivated tomato, and an introgression line background. Let us say in a specific introgression line you find a fruit that is much firmer, but they are as red as the control fruit background at the same stage of development.
In a second step, you can then take that line and break that introgression up by making further crosses. These crosses yield new combinations of the wild species and cultivated species DNA and by using DNA markers that indicate the presence of DNA from each species you can determine which part of the introgression carries the gene of interest. There is now a significant volume of published work using these lines where people have mapped and cloned genes underlying QTLs. Finally, once you have found a number of candidate genes that look of interest, you can examine their patterns of expression, and make transgenic plants to test their function. We also take some of the

Introgression is the movement of a gene (gene flow) from one species into the gene pool of another by backcrossing an interspecific hybrid with one of its parents.
Interspecific hybrids are bred by mating two species, normally from within the same genus, for instance between *Solanum pennellii* and *Solanum chmielewskii*. The offspring display traits and characteristics of both parents.
Introgression is a long-term process. It may take many hybrid generations before the backcrossing occurrs. It started with wheat around 20 years ago.

Scheme for generating and screening an exotic genetic library



(Source: Dani Zamir, Nature Genetics, December 2001)

QTL lines that have small introgressed fragments and begin to see whether we can relate changes in their biochemistry to alterations in fruit quality. This allows us to put the whole picture together.

Can the results of your work be applied in commercial breeding directly?
For breeders, once you have identified an introgression line, you can provide the breeders directly with those lines and they can introgress the fragments into their elite lines. This has already been commercially practiced for QTL linked to sugars. So the aim here is to both make the scientific discovery, to understand the biology, and to deliver the basic information through to practice.
So far, we have been focusing on tomatoes, but other vegetables would be targets too. It depends on whether you have the appropriate populations available.

What are your final expectations for EU-SOL?
These types of mapping experiments can take a long time, and we need the three or more years that we have in the project to identify the genes, and to deliver the appropriate material to the breeders. However with advances in our understanding of plant genetics and new molecular tools in the EU-SOL project, it will take a much shorter period of time than has been the case in the past. For me, EU-SOL does two things. One is the delivery of a few quite important discoveries and important deliverables to breeders. We have the advantage of so many industrial partners being involved in the project. Secondly, it has been able to bring our community in Europe together, and help people network and interact, which is something that never happened before to that extend. This will greatly benefit our Solanaceae community.

Hazera Genetics: a pre-breeder's role in EU-SOL

Hazera Genetics is one of the seed companies participating in EU-SOL. Since 1.5 years, Hazera Genetics is owned by Vilmorin & Company, which is part of the Limagrain Group. We asked Gil Shalev what Hazera's task in EU-SOL is about and why EU-SOL is important for his company.

By means of introduction, can you tell us what type of company Hazera Genetics is?

Hazera Genetics is an Israel based seed company with subsidiaries in Spain, Turkey, the USA, China and Brazil and activities in 60 countries. The company has about 400 employees; one-third of them are working in R&D. We spend 12% of our annual turnover in R&D (which is more than most seed companies/HdV). Hazera does the breeding, production and marketing of vegetable (tomato, pepper, cucumber, (water)melon, onion) and field crop (wheat, cotton sunflower, alfalfa) hybrid seeds. In the early 90s we introduced Daniela, a tomato variety that is famous for its long shelf life. More recently, we started focusing on so-called life style products. The competition is very hard, and we hope this will bring added value to the products. We have developed tomatoes and peppers with high concentrations of lycopene (tomatoes), vitamin E (peppers) and vitamin A and C (both tomatoes and peppers).

What is Hazera's specific task in EU-SOL?
EU-SOL is looking for interesting traits that can be produced with the use of the naturally occurring variation among the wild relatives of cultivated tomatoes. This involves the creation of a large number of so-called introgression lines (see box **Introgression**) that have to be characterised by phenotyping. Promising leads

will then be selected for high resolution DNA mapping. Hazera Genetics is involved in the phenotyping of the *Solanum chmielewskii* (see box **Solanum taxonomy**) population that was donated to the project by Keygene, and production of seeds for distribution. We work with 63 introgression lines, which we grow in greenhouses. We randomise the area and the collect quantitative data, because we are looking for QTLs (see box **QTLs**). With these data and the population with markers we can predict the effect of the QTLs on the phenotype. These lines have been created by crossing an elite variety with a wild species, followed by several generations of backcrossing. The result is a number of isogenic lines that are all the same, except for one piece of the wild species, the rest is background.
If you look at the population and you see a difference in phenotyping parameters (growth rate, plant architecture, fruit set, yield, fruit size and shape, colour, firmness, brix etcetera), you can assume that the difference between the lines is due to the difference between the small pieces of the wild species. This is a very powerful tool to recognise quantitative traits that involve several genes. Today you can find more than twenty introgression lines in tomato.

It sounds like an easy job….
The Keygene population is indeterminate background, which makes it very difficult to do the phenotyping. The indeterminate background causes the plant to keep growing up, and you can get more than thirteen clusters or inflorescences (as compared to three inflorescences in tomatoes with a determinate background). This means that you can not collect all the traits in one day . You have to phenotype it in three – four months and collect as many traits as you can get. Also the yield parameter is not simple because each inflorescence is ready to be picked two weeks after each other.

Indeterminate tomatoes are actually vines that continue growing in length throughout the growing season, also referred to as "vining" tomatoes. Indeterminate tomato varieties will continue to set and ripen fruit until killed off by frost. The majority of tomato varieties are indeterminate. Tomato growers seldom allow tomato plants to actually vine. Indeterminate tomato plants require substantial staking or caging to support what can become a large or heavy plant.
Most of the fresh market tomatoes is indeterminate background. Only the processing (ketchup) tomatoes is determinate background, and they are picked by machines.

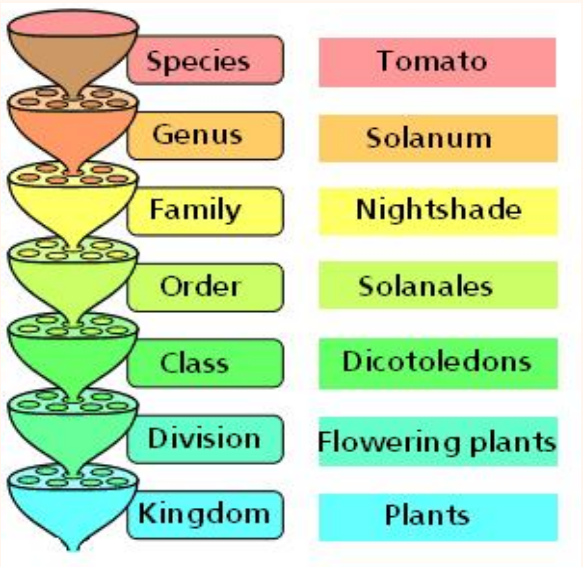
What makes EU-SOL interesting for your company?
Part of the project is dealing with new methods for measurement of fruit quality. Also a lot of the researchers in EU-SOL is dealing with introgression lines, for instance in the metabolic profiling. It makes it ease to find the gene area that influences the metabolic material that you are looking for. For me, it is interesting to pick the data: try it on the plants to see if it works or not, in a breeding context. Another part of EU-SOL that is about understanding the taste of consumers is interesting for us.

You told about the pre-breeding department of your company last year. This sounded like a new development.
There is an increasing gap between the academic world and the breeding companies. The academics are primarily interested in developing new knowledge and technologies. It is not new to phenotype introgression lines. It is a lot of work and in the end you'll have the same article as ten years ago. What counts for a breeding company is the amount of new varieties that you introduce in the market. If you work for a breeding company of the scale of Hazera, most of the work is dealing with varieties. Most of the time you have to travel around the world and you don't have time to read the articles and see what academic news is published. So you don't have the time to introduce new technology into breeding. In the past, the

breeding companies could count on the academics, but nowadays the gap between science and breeding has grown too wide. Because of that, the company decided to open a new department where I am working, which helps the breeders to introduce new technologies from the research institutes, form projects like EU-SOL, as to improve their own material. My product is a trait, not a variety. If a breeder comes to me and asks for specific characteristics, such as yield or sugar content, I supply him with QTLs with markers, with new genetic variation, with new landraces that I can allocate worldwide.
This is not a unique step: during the last year three of the five companies that are involved in Bioseeds opened a similar department.

How do you think the EU-SOL results will be applied in your company?

Solanum Taxonomy



Some examples of *Solanum* species are:

- *Solanum lycopersicum* is the cultivated tomato as we know it.
- *Solanum pimpinellifolium,* also known as the currant tomato is A sweet wild tomato from the coast of South America, useful for its drought tolerance and pest resistance.
- *Solanum cheesmaniae* is A rare wild tomato from the Galápagos Islands with unusual yellow or orange fruit.
- *Solanum peruvianum* is a small and tasty wild tomato from coastal deserts.
- *Solanum chilense* is wild species of tomato found in a variety of arid conditions in western South America.
- *Solanum pennellii* is an inedible wild tomato with sticky green fruit, very different to the commercial tomato.

More information on Solanum biodiversity is available at the EU-Sol public website: http://www.eu-sol.nl/public/biodiversity/tomatoes
More extensive information about Solanum biodiversity is available at the website of the Natural History Museum:
http://www.nhm.ac.uk/research-curation/projects/solanaceaesource/solanum/

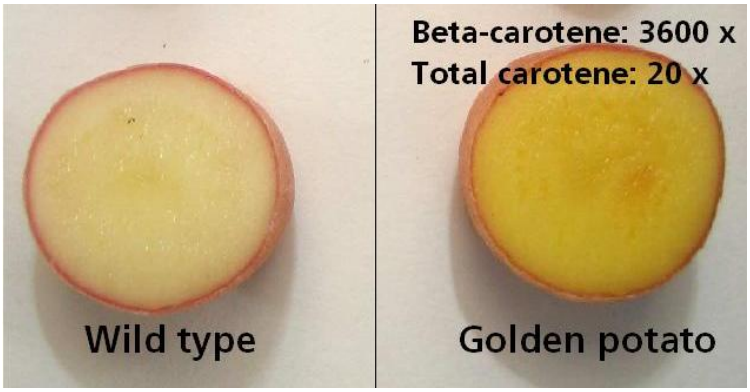
In the short term, I can pick the data and start looking into breeding. In the second stage, say the next 10-15 years, it depends on how much money and time I have to invest to make it a breeding tool. The more extreme the research is, the lower the chance that we will invest. Some of the research is dealing with genetic engineering. Today there is not a single vegetable in the market that is genetically modified. Maybe in the next 10-15 years there will be genetically modified varieties, but as a pre-breeder I am close to the breeder and I will not look for genetically modified varieties now.
The distance between academics and breeding is usually large. In this perspective, EU-SOL is very new to me. Even though the project is academic and pure science, it is very practic, so for a company like ours it is very useful.

Other news, events and reports

Scientists affiliated with EU-SOL create Golden potato

Scientists from Ente per le Nuove Tecnologie, l'Energia e l'Ambiente (ENEA) in Rome and the Universität Freiburg in Germany created biofortified potatoes with increased levels of provitamin A carotenoids. They

used genetic engineering to introduce a mini-pathway of bacterial origin, driving the synthesis of beta-carotene (Provitamin A). Three genes, encoding phytoene synthase (CrtB), phytoene desaturase (CrtI) and lycopene beta-cyclase (CrtY) from the bacterium *Erwinia*, were put under tuber-specific or constitutive promoter control. 86 independent transgenic lines, containing six different promoter/gene combinations, were produced and analyzed. Potatoes with 3600 times the original beta carotene level and 20 times the original total carotenoid levels were achieved. These potatoes have a deep yellow colour.



Assuming a beta carotene to vitamin A conversion rate of 6:1, 250g of this 'golden' potato would be enough to provide someone with 50% of their Recommended Daily Allowance of Vitamin A. Potato consumption is generally highest in developed countries and low in developing countries, where vitamin A deficiency prevails. Average daily potato consumption ranges between 316 kcal/person (420 grams) in Belarus and 0 kcal/person in several African countries (FAO Statistical Yearbook 2004). According to the FAO, world potato production has increased at an annual average rate of 4.5 percent over the last 10 years, and exceeded the growth in production of many other major food commodities in developing countries, particularly in Asia. While consumption of potato has declined in Europe, it has increased in the developing world, from less than 10 kg per capita in 1961-63 to almost 22 kg in 2003. Consumption of potato in developing countries is still less than a quarter of that in Europe, but all evidence suggests it will increase strongly in the future.

The full paper can be downloaded here: Metabolic Engineering of Potato Carotenoid Content through Tuber-Specific Overexpression of a Bacterial Mini-Pathway.

Events

- 5th Solanaceae Genome Conference (SOL2008), October 12 – 16 in Cologne, Germany. The conference is being organized by the Max-Planck-Institute for Plant Breeding Research (MPIZ).
- SOL Newsletter Nr. 18, March 2008, http://www.sgn.cornell.edu/documents/solanaceae-project/docs/SOL_newsletter_Mar_08.pdf
- Food4you is a food and health festival that will be organised in Wageningen, October 1-5, 2008. The festival will present all health knowledge available to scientists, other professionals in the food business (cooks), and the public. This year, the festival is focused on potato, genomics research, and sustainable resistance to *Phytophtora infestans* in potato. For more information, contact erik.toussaint@wur.nl
- International Year of the Potato Events: http://www.potato2008.org/en/events/index.html
- "Sharing the benefits of biotechnology with developing countries", Reconsidering Intellectual Property Policies in Public Research, international conference was held at Wageningen University and Research Centre on April 11th 200. Presentations can be downloaded at http://www.pri.wur.nl/UK/about/ipr