



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

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Exploring genetic diversity for breeding

"Plant genetic diversity, the result of a combination of farmers' selection over millennia, natural evolution and plant breeding is a foundation of agricultural development," said World Bank Vice President and CGIAR Chairman Ian Johnson when the International Treaty on Planet Genetic Food Resources for Food and Agriculture was approved by the U.N. Food and Agriculture Organization member states eight years ago. "Plant breeders all over the world rely on existing diversity to create new varieties of plants with higher yields and increased resistance to pests and diseases, while for many small farmers in developing countries, diversity is the basis of food security and income." EU-SOL's activities are meant to add knowledge to the possible uses of this existing diversity at the molecular level. The creation of the tomato core collection, which contains over 7,000 tomato varieties that have been collected from all over the world. In this issue, Roni Tadmor of the Hebrew University of Jerusalem explains what the value of this core collection is and how it is being used.

The application of this type of knowledge and resulting biomolecular tools in plant breeding is easily associated with genetic engineering of plants, which is a contested technology in Europe. In 2002 the board of the International Federation of Organic Agriculture Movements (IFOAM) adopted a position in which the organization calls for a ban of GMOs in agriculture. The European Regulation on organic production and labelling of organic products (834/2007) prohibits the use of genetically modified organisms and derived ingredients. Some of the principles that underlie the rejection of genetic engineering in organic agriculture apply to molecular approaches in life sciences in general, so it was not a surprise that experts from the organic sector gathered in a workshop in 2005 were skeptical about DNA markers. Nevertheless, the organizers of this workshop realized that rapid developments in DNA markers required reconsideration of the issue in another workshop in February 2009. In total 46 participants being scientists in the field of organic and conventional agriculture, breeders applying molecular markers or organic breeding as well as policy makers from the agricultural sector contributed to an open discussion on how breeding

programs for organic agriculture may benefit from molecular marker assisted selection (MAS). For this edition of the EU-SOL newsletter we interviewed one of the organisers, professor Edith Lammerts van Bueren. The balanced way in which she reflects on the opportunities and threats of MAS provides interesting feedback for the project.



The tomato core collection: Mapping genetic diversity for breeding

Last summer, over 30 EU-SOL scientists and breeders met for 6 days in Israel for a tomato phenotyping workshop in a field of local breeders near Akko. This field was planted with several thousands of tomato varieties, the so-called core collection. We asked Roni Tadmor of the Hebrew University of Jerusalem to explain what the value of this core collection is.

What exactly is the core collection?

The core collection contains about 7,000 tomato varieties that have been collected from all over the world. In 2006 Dani Zamir and Zach Lippman from the Hebrew University started collecting seeds from the gene banks in Western Europe and the United States and from the personal collections of EU-SOL scientists. We are still collecting seeds, especially from Asia. It concerns domesticated lines, so-called landraces, which have been bred and selected by farmers, adapted to the specific environmental conditions in the region and local farm management practices, along with several wild species. The number of varieties makes this a unique collection. Collections have been made for other crops such as potato, rice and wheat too, but not at this scale.



Roni Tadmor of the Hebrew University of Jerusalem, phenotyping in Akko

The value of the tomato Core collection is the amount of genetic and phenotypic diversity it represents. In general, the genetic diversity in the varieties used in commercial tomato breeding programmes is rather small and new traits of interest may be missing. It is possible to find interesting traits in wild tomato populations and cross them into the commercial lines, but that takes a lot of time because genetically wild tomatoes are very different from commercial tomatoes. The advantage of using landraces is that they also contain a lot of genetic diversity. Moreover, they have already been selected for cultivation and are therefore genetically closer related to the commercial varieties.

What are the traits of interest?

Well, last summer we took a closer look at some material selected from the ~4,000 accessions that were phenotyped¹ in 2008 plus a number of accessions that were not phenotyped last year. We took 400 accessions that were selected for colour, which can be red or a little more exotic, like purple, green, yellow, orange and more. 50 accessions were selected for the colour of the skin, ranging from solid red to semi transparent, or with a zebra pattern. The shape of the fruit is also an interesting trait, so we took about 100

¹ A phenotype is the observable characteristic or trait of an organism, such as the shape, size and colour of fruits and seeds, or the shape, number and position of the leaves. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and possible interactions between the two.



accessions with different shapes: round, oval, pear, fragmented. We also selected 100 accessions for differences in firmness, the size of the fruit, and plant architecture (determinate, semi determinate and indeterminate²). The content of soluble sugars (brix) is an interesting trait because it determines the sweetness of the fruit. Usually, sweet taste coincides with small size (little water), for instance in cherry tomatoes. One challenge for breeding is to find traits that combine a high sugar content with large size. A number of accessions were selected for differences in flower architecture mainly for scientific reasons.

What happened after the phenotyping?

After the phenotyping the tomatoes were yielded and the brix was measured. The yield was then taken to the lab in Rehovot where the seeds are cleaned. The seeds are available for further research by the EU-SOL partners.

The phenotypic is uploaded up to a database, part of which is freely available on the web. This database can be searched for several characteristics of the plants and the fruits. The idea is to combine this data with data that is generated in other parts of EU-SOL, such as data about metabolites and genetic marker analysis.

Until recently, genotyping was only done at a limited scale. Last year, Keygene started to analyse the whole core collection for 17 markers that were provided by the companies involved in the project. By now, we have approximately 2,500 accessions finished. Additionally, several EU-SOL participants will do some marker analysis. The ultimate goal is to create and maintain a limited collection that represents a wide range of genetic diversity, thus enabling the scientists to work with a relatively small set of germplasm and reducing the operational costs.

What is your role in the core collection?

My task is to continue to expand and maintain the core-collection. That means that prior to each season I select the material that we plant, according to the data collected so far. Once in the field, I coordinate the phenotyping process. Last year I arranged, together with Dani Zamir, a phenotyping workshop for EU-SOL partners. During the workshop we phenotyped about 2,000 accessions and prepared more than 1,000 fruit samples for future analysis in EU-SOL labs.

I also take care of the seed stocks of the core-collection, making sure they are catalogued correctly, and that there are enough seeds available from each accession, and manage the distribution of the seeds among EU-SOL partners.

² Determinate plants stop growing as soon as they have formed a certain number of trusses. Indeterminate plants can be kept alive and growing in controlled environments (the greenhouse) for a long time. Determinate plants are most suited for growing in the field. Usually, growers using indeterminate plants decide to replace the plants after several months because they tend to become more susceptible to disease and less productive as they grow older.



What will happen to the core collection when EU-SOL stops?

The creation of the Core collection is one of the major deliverables of the project. It is of great value for breeding and research, so it will certainly be continued. Several breeding companies have already indicated to be interested in taking over part of the Core collection. But most of the material of the collection belongs to the genebanks, and is therefore freely available.

See <https://www.eu-sol.wur.nl/dynamic/passport/aboutTheCC.php> for an impression of the core collection.

Plant genomics research and breeding for organic agriculture

In February 2009, a number of plant breeders, scientists and policy makers met in Wageningen to discuss the opportunities and threats of the use of molecular markers in plant breeding for organic agriculture. We asked Edith Lammerts van Bueren, one of the organizers of the workshop, head of organic plant breeding at the Louis Bolk Institute and [buitengewoon] professor in organic plant breeding at Wageningen University, whether and how organic agriculture could benefit from the knowledge generated in plant genomics research and Marker Assisted Selection.



In your reflection on a workshop on the use of molecular markers in organic plant breeding strategies in 2005 you wrote that the organic sector is skeptical about DNA markers. What is the reason for this skepticism?

Let me first stress that organic agriculture is characterized by a holistic approach, in which the plant production system is adapted to environmental conditions, rather than adapting the environmental conditions to the plant production system. This means that we have to deal with a wide variety of conditions, geographically defined, that can also fluctuate over time, for instance because of weather conditions. In this context, there is two major needs: **biological diversity** and **resilience**. **Biological diversity** allows to pick the variety that is best adapted to a specific range of environmental conditions. Molecular approaches require large investment, which may enforce further consolidation in plant breeding. This could result in a decrease of the number of available varieties, especially in the relatively small markets for vegetable seeds.

Resilience means: varieties with pretty constant performance under fluctuating environmental conditions. This usually requires a combination of traits. Not just drought tolerance or a specific pest resistance, but also morphological and physiological traits, such as waxy leaves or a positioning of the leaves that keeps tomato plants from staying wet after rainfall, the depth of rooting and adaptation to lower nitrogen availability. My impression is that molecular approaches tend to focus on single traits, which may be interesting for non-organic agriculture, but does not necessarily result in the type of resilience we are looking for in organic agriculture.



A second reason for being reluctant has to do with the fact that 'respect for life' is one of the key values in organic agriculture. In the case of plants this value has been translated in respect for the integrity of plants. Respecting integrity means that we prefer to support existing biological mechanisms, for instance by creating healthy soils, rather than changing biological processes in a radical way. You should not do something to the self-regulatory and reproduction capacity of a plant that this particular plant would never do without human interference.

We think that most problems in agriculture can be managed by focusing on the higher aggregation levels of the whole plant, cultivation in a specific environment, and the farm system rather than the interfering in the lower aggregation levels of molecular genetics and metabolic pathways in cells. Translated to breeding, this means that we prefer the use of higher aggregation level technologies that include the whole plant and its behaviour in different environments. We are wary about the use of breeding technologies at the cellular level, such as in vitro multiplication. In our view, technologies at the DNA level, such as protoplast fusion or genetic engineering tend to neglect the complexity of the whole plant, and are therefore not suited. We have to be aware of the fact that the complexity of life is (still) beyond our scientific knowledge. We still don't know the complex biological mechanisms in plants in relation to environment and management well enough to predict the effects of technologies that interfere on the molecular or cellular level on the whole plant, the crop and the farming system.

So far you have talked about invasive molecular tools, tools that interfere at the lower aggregation levels of DNA and cells. Molecular tools and plant genomics knowledge can also be applied for detection of specific traits at the whole genome level. Does organic agriculture reject those tools too?

No, we are prepared to consider the use of molecular tools as long as they do not interfere with the integrity of the plant, for instance when used as a diagnostic tool. At this stage, it is difficult for the scientists and practitioners involved in organic agriculture to value molecular technologies for plant breeding. There is a lack of knowledge resulting in prejudice. Marker Assisted Selection (MAS) is easily perceived as another reductionist approach, contrary to the holistic approach of organic agriculture, and regularly associated with genetic engineering. Many people fear for approaches that focus on a limited number of genes, without considering the whole plant and its interactions with the environment it grows in. The workshop on the role of MAS in breeding varieties for organic agriculture we organized demonstrated the importance of catching up with recent developments in knowledge and technology. I can say it was an eye-opener to some of the participants. Organic and molecular researchers have learned from each other during the workshop and can continue to learn from each other; they represent two different ways of looking and acting, and there was more awareness of the possibilities to combine approaches case by case.

What are specific breeding targets for organic agriculture and what could MAS contribute?

Like in any type of low-input agriculture, organic growers have less possibilities to influence environmental conditions. Therefore, we are looking for varieties that perform pretty constantly under varying conditions. We usually seek this in a combination of traits; not just in disease resistance but also in morphological and physical traits, such as waxy leaves, a positioning of the leaves that prevents the plant from staying wet for too long, the root structure and adaptation to lower nitrogen availability. In terms of taste we have to deal with consumers who expect better tasting organic tomatoes.

We are still in the stage of exploring and ... the opportunities and threats of MAS. In the workshop on MAS we focused on four cases: breeding for disease resistance in potato (*Phytophthora infestans*) and in wheat (general), and breeding for quality traits in tomato (flavour) and in wheat (baking quality). For each case both a breeder (researcher) working in the field of organic and of conventional agriculture was invited to give his/her view on a) the specific requirements for plant traits in the context of organic cultivation systems



and conventional low-input production systems and b) the state of the art and progress in relation to whether, when and how breeding programs for organic agriculture may benefit from MAS. It was concluded that MAS has the potential to contribute to the genetic diversity that is needed to build up resilience in agro-ecosystems. The technology makes access to new genetic resources more feasible by screening genetic resources with molecular markers and by making selection of newly introduced quality or resistance alleles from wild relatives more efficient when the targeted gene can more easily be followed and distinguished from undesired linkage drag. But it was also stressed by breeders that breeding research should also address the ongoing need for easy phenotypic selection methods. MAS is not the solution for every trait.

Suppose that organic agriculture would accept the application of MAS in plant breeding, would you be able to explain this to consumers?

Well, that really is a big challenge. Many people think that we can do with the varieties that stem from the days that artificial fertilizers and pesticides were not available, so we already have a hard time in explaining the need for breeding new varieties. I fear that few (organic) consumers will understand the difference between genetic engineering and the use of a diagnostic tool that operates on the DNA level. For the time being, I would focus communication efforts on organic producers, rather than trying to get a complicated message over to the public.

A full report of the February 2009 workshop can be downloaded from http://www.eucarpia.org/03publications/bioexploit_2009.pdf

Tomato draft genome assembly expected by the end of November 2009

KeyGene delivers sequence-based physical map of the tomato genome to CBSG and the International SOL Consortium. This map will be instrumental in achieving a “gold standard” sequence of the tomato genome that will be available to the scientific community soon.

Concluding a successful collaboration between CBSG (Centre for BioSystems Genomics) and the Crop Genome Center of KeyGene, KeyGene delivered a high quality sequence-based physical map of the tomato genome. The physical map was constructed using KeyGene proprietary Whole Genome Profiling (WGP) technology that is based on next generation sequencing of BAC clones³. Assembly of the BAC clones covered the complete 950 Mb tomato genome. Details on the tomato physical map will be presented during the 6th Solanaceae Genome Workshop on November 8-13, 2009 in New Delhi, India.

As part of the collaboration CBSG together with several other partners of the International Sequencing Consortium SOL produced a high-coverage shotgun sequence⁴ of the tomato genome using next-generation sequencing platforms: ultra fast sequencing machines that produce sequence data of short DNA strands between 25 and 500 basepairs. Roeland van Ham, coordinator of the international genome assembly effort and one of the scientists involved in EU-SOL says: ‘We are thrilled with the quality of the

³ A **bacterial artificial chromosome (BAC)** is a DNA construct, often used to sequence the genome of organisms in genome projects. A short piece of the organism's DNA is amplified as an insert in BACs, and then sequenced. Finally, the sequenced parts are rearranged, resulting in the genomic sequence of the organism.

⁴ **Shotgun sequencing** is named by analogy with the rapidly-expanding, quasi-random firing pattern of a shotgun. It is a method used for sequencing long DNA strands. The routine method of DNA sequencing can only be used for fairly short strands of 100 to 1,000 basepairs. Therefore, longer sequences must be subdivided into smaller fragments, and subsequently re-assembled to give the overall sequence. In shotgun sequencing, DNA is broken up randomly into numerous small segments, which are sequenced using the chain termination method to obtain *reads*. Multiple overlapping reads for the target DNA are obtained by performing several rounds of this fragmentation and sequencing. Computer programs then use the overlapping ends of different reads to assemble them into a continuous sequence.



WGP data. We expect that full integration of the WGP map will set a new quality standard in draft genome sequencing’.

‘The tomato genome assembly will support our molecular breeding programs directly’, says Marco van Schriek, Team Leader of KeyGene’s Solanaceae group. ‘A number of applications such as disease resistance and improved taste have already been developed using the insights we gained from the structure and sequence of the tomato genome’.

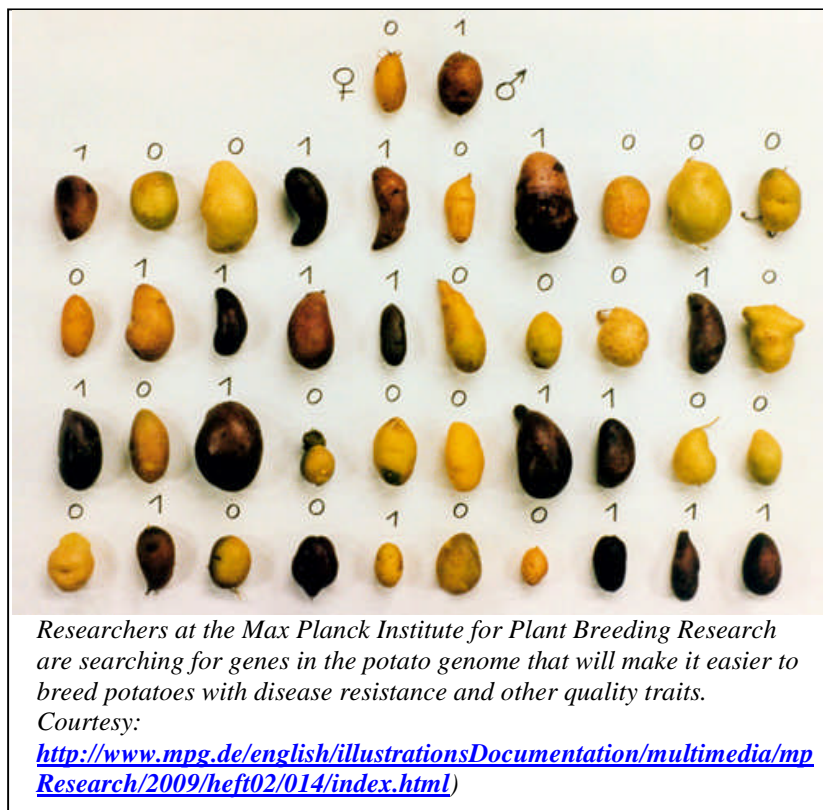
KeyGene’s WGP map will serve as a scaffold to anchor and order the sequence contigs - sets of readings that are related to one another by overlap of their sequences- produced by the consortium. René Klein Lankhorst, coordinator of the EU-SOL Project: ‘We are very pleased with the result of the physical map. The aim of the SOL Consortium is to deliver a “gold standard” sequence of the tomato genome to the scientific community and the new physical map will be instrumental in reaching this goal. We expect to present a tomato draft genome assembly expected by the end of November.’

First draft sequence of the potato genome released

The first draft sequence of the potato genome has been released in September by the Centro Internacional de la Papa (CIP). CIP scientists worked alongside scientists from 14 different countries as part of the Potato Genome Sequencing Consortium.

Access to the potato genome sequence gives scientists access to information about growth and replication of potato. This information can help improve potato yields, quality, nutritional value, and disease resistance. It also holds the promise of shortening the time it takes (currently 10-12 years) to breed new varieties of potatoes to respond more quickly to changing needs or conditions. The Consortium believes that releasing the draft sequence to the public allows for exponential gains in its development and utility, as more scientists and researchers access, use, and help refine the sequence.

The international Potato Genome Sequencing Consortium (PGSC) is a collaboration between 16 research groups; Argentina, Brazil, China, Chile, India, Ireland, The Netherlands, New Zealand, Peru, Poland, Russia, the United Kingdom and the United States, which started three years ago. Initially, the





approach of PGSC was to sequence a diploid⁵ potato line. The work was divided among the members based on the 12 chromosomes. During the last two years, the emergence of Next Generation Sequencing technologies allowed for the additional sequencing of a double monoploid¹ line, which originates from a diploid landrace.

Wageningen University and Research Centre in the Netherlands, who is the coordinator of the International Potato Genome Sequencing Consortium (PGSC), uses Whole Genome Profiling Technology to produce sequence-based physical maps. Such a DNA map is constructed in two steps. In the first step large DNA fragments are produced, clones of 120,000 base pair that can be fingerprinted by placing short, 30 base pair sequence tags spaced between 2,000 and 3,000 base pairs across these large DNA fragments. In the second step the large DNA fragments are ordered by overlapping regions with identical sequence profiles.

Using data generated by different sequence platforms and a new genome mathematical system developed by the Beijing Genomics Institute, the draft sequence covers 95 % of the genes in potato. Updates, including annotation of the genes⁶, identification of the transcriptome⁷ and analysis of genes relevant for potato production, will be made in the next five months.

Christian Baechem, involved in EU-SOL's research in potato tuber quality traits, says that although no part of the potato sequencing is being financed through EU-SOL, the PGSC is keen for the whole community (including EU-Sol partners) to benefit maximally from the potato genome sequence. Releasing the draft sequence to the public allows for exponential gains in its development and utility, as more scientists and researchers access, use, and help refine the sequence.

The first draft genome assembly is now available in the public domain at www.potatogenome.net

Other news

New computing tool could lead to better crops

A new computing tool that could help scientists predict how plants will react to different environmental conditions in order to create better crops, such as tastier and longer lasting tomatoes, is being developed by researchers from Imperial College London and Syngenta.

In order to develop new strains of crops that are more environmentally, scientists need to predict how the genes inside plants will react when they are subjected to different chemicals or environmental conditions. A new tool that is currently being tested, can analyse in a matter of minutes which genes are responsible for different processes inside a plant, and how different genes work together. It uses a type of computer programming that relies on 'machine learning', a set of sophisticated algorithms that allows a computer to

⁵ **Ploidy** is the number of complete sets of chromosomes in a biological cell. **Diploid** cells have two homologous copies of each chromosome (chromosomes that pair during cell division during the creation of fused cells), usually one from the mother and one from the father. **Haploid** cells contain only one copy of each chromosome. A **double monoploid** contains two similar chromosomes from one parent.

⁶ **Gene annotation** provides functional and other information, for example the location of each gene within a particular chromosome.

⁷ The **transcriptome** is the set of all messenger RNA (mRNA) molecules, or "transcripts," produced in one or a population of cells, in this case the total set of transcripts in potato. Unlike the genome, which is roughly fixed for a given cell line, the transcriptome can vary with external environmental conditions. Because it includes all *mRNA* transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time.



'learn' based on data that it is analysing. The researchers say the tool will recognise complex patterns in that data to find 'nuggets' of information about plant biology that might previously have taken months or even years to find. The 'machine learning' ability of the new tool means that researchers can develop an understanding of different plants even when they are lacking information about some aspects of their inner workings.

For the first project using the tool, scientists will look at how different genes affect the way a tomato's flesh hardens and tastes, and how the fruit's skin changes colour from green to red. The researchers hope that this will enable them to develop new tomato strains that are tastier, and that redden earlier and soften later so that they can be transported more easily to market. These qualities could be especially useful in developing countries, where factors such as poor transport can quickly spoil fruit and vegetables.

The tool will form part of a new 1.8 million Euro Syngenta University Centre at Imperial College London. All software developed by researchers at Imperial College is intended to be made publicly available over the next four years.

Source: *Imperial College London, 22 September 2009,*

http://www3.imperial.ac.uk/newsandeventspggrp/imperialcollege/newssummary/news_22-9-2009-11-57-55

Potatoes that fry up light even after cold storage

Genetic markers help selecting the right parents for breeding potatoes that can be stored at low temperatures without losing quality

When held at temperatures of 7 degrees Celcius or higher, potatoes won't produce dark fries that are unacceptable to consumers, but they're more likely to sprout, lose moisture and develop storage-related diseases. At the University of Idaho, postharvest physiologist Sanjay Gupta suspects we could have cold-stored potatoes and enjoy eating them, too. Potatoes that could be stored at or below 7 degrees Celcius and still fry up light wouldn't need as many sprout inhibition treatments. As living seed, they would respire less at colder temperatures, thereby retaining moisture and weight, and would be less prone to plant diseases.

Gupta has teamed with other scientists to select breeding lines for their resistance to cold-induced sweetening. While at the University of Minnesota, Gupta began developing two biochemical markers that reveal a potato's propensity to tolerate cold storage. One of the biochemical markers is a protein called UDP-Glucose pyrophosphorylase that controls the formation of sucrose from the potatoes' starches; the other, acid invertase, controls the formation of the reducing sugars glucose and fructose from sucrose. Together, they indicate not only how well a variety can be stored at lower temperatures but for how long. Gupta refined the markers at the University of Idaho. Along with other scientists, he then used the markers to screen about 300 experimental clones and commercial varieties from a dozen North American breeding programs. The markers predicted with about 90 percent accuracy a potato's response to storage temperatures.

By choosing the right parents, breeders could significantly accelerate the development of potatoes with the level of cold-sweetening resistance the market seeks, Gupta said.

Source: *University of Idaho, College of Agricultural and Life Sciences, October 23, 2009,*

<http://www.cals.uidaho.edu/newsDetail.asp?ID=1097>