



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

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Plant breeding gets a boost

Due to the development of new DNA sequencing techniques, the time needed to sequence the whole genome of an organism and the cost of sequencing is declining rapidly. In 2001, the year that the sequence of the human genome was published in Science, sequencing of one base took about US \$ 0.10. Nowadays, a Roche 454 machine sequences 400 million base pairs in ten hours for about US \$ 0.002 per base pair or less. This development enabled massive sequencing of many kinds of organisms and meant a substantial push for the tomato sequencing efforts in which EU-SOL participates. After the Tomato Next Generation Sequencing Initiative that was launched in October 2008, a first draft of more than 95% of the assembled tomato genome was released in December 2009. There is no doubt that the availability of this information will be very useful in future crop enhancement.

Decoding the genome is one thing, finding out what all this information means is another. The study of metabolic processes and gene regulation in plant cells is pivotal in EU-SOL. The study of the mechanisms underlying potato sprouting that is done at the Erlangen-Nuremberg University in Germany is a good example of research on mechanisms that are not yet well understood. The scientists involved think that their research could lead to a tool to predict potato sprouting, which is important both for planting and because of its negative effect on tuber quality.

Very recently, EU-SOL scientists from the Hebrew University of Jerusalem hit the news with the results of joint research with Cold Spring Harbor Laboratory in the US, published in Nature Genetics. They identified a single gene that causes 'hybrid vigor', a mechanism that pushes hybrid tomato plants to spectacularly increase yield. The yield-boosting power of this *florigen* gene works in different varieties of tomato, and crucially, across a range of environmental conditions. And perhaps equally important, the *florigen* gene also boosts the sugar content and sweetness of individual fruits.

Another part of EU-SOL focuses on the creation of libraries of mutagenised tomato plants that are later screened for the discovery of mutants. The technology used is called TILLING -an acronym for "Targeting Induced Local Lesions in Genomes". TILLING was also used by the Fraunhofer Institute for Molecular Biology and Applied Ecology to develop an amylase-free potato that only contains amylopectin, the type of starch that the thickening properties preferred for industrial uses.



Although the genetically modified eggplant, which also belongs to the Solanaceous family, with a Bt gene that makes the plant resistant to devastating insects has nothing to do with EU-SOL research, recent developments in the Indian approval process for commercial cultivation illustrate how societal issues can affect the introduction of new technologies. This is one of the reasons why EU-SOL puts considerable efforts in science communication, which includes activities to get societal feedback about the goals and methods used by EU-SOL. This time we asked one of the NGO's scientific advisors about her opinion on EU-SOL's objectives and methods. Notorious for its rejection of the introduction of genetically modified plants in the environment, the organisation is quite positive about the application of genomics based tools in plant breeding such as Marker Assisted Selection. This can be a big help to make breeding more efficient in developing climate-ready traits.

A tool to predict potato sprouting

EU-SOL's potato research is focusing on several quality aspects, such as flavour, texture and health properties. One aspect that affects the quality for consumers and processors as well as for growers is tuber sprouting. The mechanism underlying potato tuber sprouting is studied by Melanie Senning, Sophia Sonnewald and Uwe Sonnewald from the Erlangen-Nuremberg University in Germany. They explain how their work is progressing and how the results could be used.



The EU-SOL potato research group from the Erlangen-Nuremberg University. From left to right: Sophia Sonnewald, Uwe Sonnewald, Melanie Senning, Stephen Reid

Melanie Senning is a graduate student and Sophia Sonnewald is senior scientist leading the Molecular Physiology and Transcriptomics Research Group at the Chair of

Biochemistry of the Erlangen-Nuremberg University's Biology Department which is headed by Uwe Sonnewald. Their research groups study how the carbohydrate metabolism regulation in plants is influenced by environmental stress and plant pests and are interested in post-harvest physiology of vegetative and generative storage organs with the aim to contribute to the biotechnological improvement of crop plants.

How would you describe your field of research?

Plants use photosynthesis for tapping the energy from sunlight and converting carbon dioxide into carbohydrates (sugars). This process takes place in mature green leaves producing an excess of sugars which are distributed mainly as sucrose to other plant organs such as roots, flowers, fruits and seeds or tubers. There, sucrose serves as a source of energy to support growth or it is stored for later usage (e.g. during germination or sprouting). Thus, size and quality of tubers or seeds depend on the supply of sugars. We are specifically interested in how this mechanism of sugar metabolism and distribution is regulated, which genes play a role in this process and how those genes are activated or inactivated at different stages



during plant development. We use different model plants to study these mechanisms: Arabidopsis (mouseear cress), tobacco, maize, potato and tomato.

Why are you focusing on potato sprouting?

One of the stages in plant development under investigation is the regulation of dormancy and germination (sprouting) in potato tubers. After harvest, potato tubers enter a rest period which ends after several weeks or months with the appearance of a visible sprout. The length of this period varies between different cultivars and is controlled by environmental conditions such as the temperature during plant growth and tuber storage. The process of sprouting is accompanied by physiological changes like the conversion of starch into sugars and the remobilisation of proteins. The potatoes lose weight, become shrivelled and wrinkled, more difficult to peel, and also lose vitamins. They become even sweet if stored too cold. All these processes cause an unwanted loss of tuber quality.



Potato sprouts (Wikimedia)

Consumers and processors of chips, fries and other potato products want to avoid this. There are several solutions available. One of them is the use of chemical sprouting inhibitors (or essential oils for organic control of sprouting), but this should not be applied too early. Moreover, because of environmental concern, their application is problematic and could become restricted. Storage at temperatures lower than 4-5 °C keeps potatoes from sprouting but also has a negative impact on taste and processing quality because of reducing sugars that cause an undesired dark frying colour and bitter taste. In addition there is research going on to achieve sprout control by biotechnology approaches.

Sprout control is also important for farmers who need to plant their seed potatoes at the right time: Just as they start to sprout, but avoiding thick or long sprouts that might break.

So for both farmers and processors it would be useful to have a tool to predict when sprouting starts. Although this is not our primary intention, the knowledge we are developing may result in such a predictive tool which tells you when it's time to sell, to apply chemical treatment, to process, or to plant potato tubers.

So what is the mechanism you've discovered?

The time point of visible potato sprouting varies between different potato varieties and harvests and is not clearly predictable. Not much is known about the molecular mechanisms underlying the processes that induce sprouting. Recently, we have identified a candidate gene which is indicative for active tuber eyes.

Tuber sprouting occurs at tuber eyes containing a meristem. Meristems maintain a pool of undifferentiated cells, also called stem cells, and deliver cells for growth and development of new organs. Plant meristems can also be found at shoot and root tips. To start growing, the meristematic cells have to divide, proliferate and differentiate into specialised cells. To initiate cell division reactivation of DNA replication in the so-called cell cycle is needed. This process is controlled by the expression of several genes. In dormant tubers the cells of the meristem are arrested in a certain stage of the cell cycle and do not divide until reactivation of meristematic activity is initiated to induce sprouting and the formation of a new plant.

To find out which genes are involved in the onset of potato tuber sprouting we used the DNA microarray technique. Using this method, changes in the pattern of transcription of genes can be monitored, being the first step that leads to gene expression, which occurs by synthesis of a corresponding RNA copy of a DNA



sequence. So, the presence and the amount of RNA tells us about gene activity¹. We analysed the pattern of gene expression at different developmental stages, for example in dormant and sprouting tuber eyes, and compared them with those of other potato meristems or organs.

We discovered that the enzyme *deoxyuridine triphosphatase* (dUTPase) is very early up-regulated in the process of tuber sprouting. The dUTPase is essential for DNA replication and therefore a marker for active, dividing cells. We found a clear correlation between dUTPase gene activity and induction of tuber sprouting. For example, dUTPase gene expression increased strongly about one week before sprouting became visible. This would make dUTPase a good molecular marker to predict the transition from dormant to active potato tuber buds.

Could this be developed into a commercial tool?

We can imagine that it could be further developed into a suitable tool, for instance as a kind of stick that performs a quick PCR.

Senning, M., Sonnewald, U., Sonnewald, S. Deoxyuridine triphosphatase expression defines the transition from dormant to sprouting potato tuber buds. (Mol. Breed.) in press

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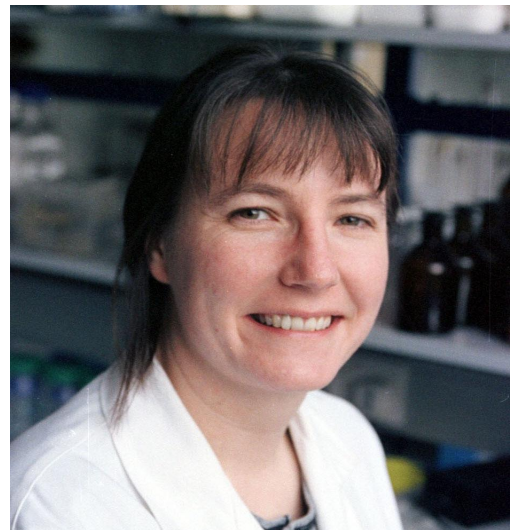
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Smart Breeding: Greenpeace's alternative to genetic engineering

Among plant biotechnologists Greenpeace is notorious for its rejection of genetically engineered crops. In August 2009 the environmental organisation published a report on Marker-Assisted Selection, which is advertised as 'a non-invasive biotechnology alternative to genetic engineering of plant varieties'. Against this background, we asked Janet Cotter, one of the organisation's scientific advisors, how she thinks about the goals of EU-SOL and the methods used.

Janet Cotter is one of the 6 scientific advisors of Greenpeace. She was awarded a degree in geology and geochemistry from Manchester University in 1987 and a PhD in soil science from Imperial College, University of London, in 1991. She has nine years of experience now in providing Greenpeace with advice and technical support on the environmental and food safety aspects of genetically engineered organisms and the interaction of forests with climatic change.



Why has Greenpeace become interested in MAS?

Greenpeace started to become interested in Marker-Assisted Selection (MAS) about one year ago. We decided to commission Benno Vogel to investigate literature about recent developments and actual applications. We knew about several examples where MAS was applied, such as breeding for drought

¹ Gene transcription is the first step to gene expression. It occurs by synthesis of an equivalent RNA copy of a sequence of DNA. So the presence of RNA tells us about gene activity. The cell's ribosome subsequently translates the RNA in proteins, the final gene products that play a role in the cell metabolism.



tolerance in maize, but we had not done any comprehensive assessment yet. It just did not attract the level of attention that genetic engineering got. We have always been stressing that Greenpeace is not against new technologies, which is something that people might think because of our campaigns on genetic engineering. Although genetic engineering is biotechnology, biotechnology is so much more than genetic engineering.

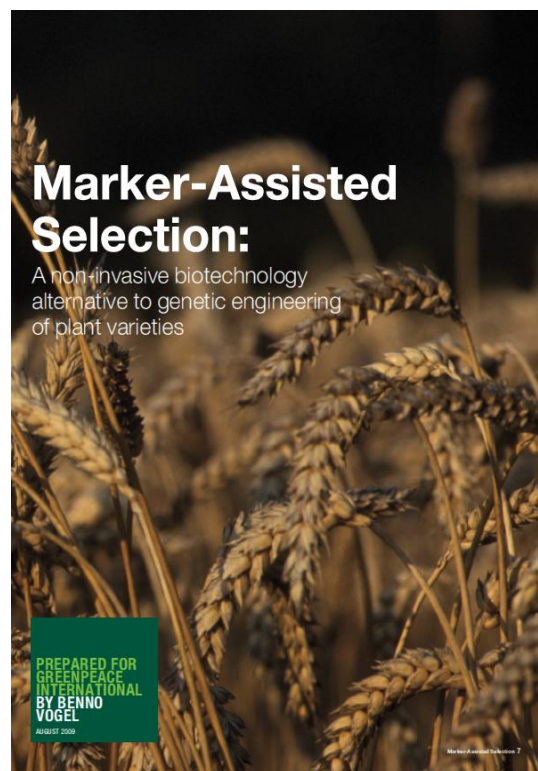
What makes MAS more acceptable than genetic engineering?

In our view, the random insertion of genetic material and the limited number of genes that new traits are based on makes genetic engineering a clumsy technique. We are aware that science moved on and that more approaches have been developed. Some of these approaches seem more sophisticated than genetic engineering, for instance because they explore the natural genetic diversity within a plant species and they take into account the complexity of the genome and the interactions between genome, environment and agricultural management. We think there are several other biotechnologies that we could consider an acceptable way forward, and using genomics knowledge in conventional breeding is one of them.

Because of our focus on environmental effects, we distinguish between experiments that are meant to gather knowledge about genetic and molecular processes in plants that are conducted in the lab or the greenhouse, the so-called 'contained use', and experiments that are done in the field, meant to introduce and test genetically modified plants in the environment. Even if the experiments in the lab or greenhouse experiments are designed to test the function of specific genes and require the use of genetically modified plants, we do not object. As long as they are securely contained.

Does that make MAS an ideal tool for plant breeding?

Although I am not sure, I can imagine that MAS can be a big help to make breeding more efficient in developing climate-ready traits, that is traits with higher tolerance for extreme climatic conditions: drought, flooding, cold, heat. Several MAS-developed bacterial blight resistant rice varieties are currently available for farmers and a rice variety that consumes 60% less water than traditional varieties has been developed with MAS. This technology may also help to develop plants that can be grown with less fertilizers, water, or pesticides, and promote biodiversity, thus supporting ecological farming. But we always warn for a 'technology fix' because technology is not the solution for everything. Some problems need other solutions. Take, for instance, the lack of a healthy and balanced diet. In our report we mention a sweet potato and maize, both bred by MAS to enhance levels of provitamin A. But a healthy diet does not necessarily require crops with improved nutritional value. Obesity, which is becoming epidemic in many countries, is a question of choice and education. In developing countries, a healthy diet is primarily a question of access to fresh and healthy foods.



Is your support to plant genomics research without any other restrictions?

A lot depends on how the results are being used. Free access to the data and technologies is important. Patent aspects could lead to some restrictions, e.g. a patent on a molecular marker may prevent other



breeders utilising this knowledge. The fact that EU-SOL is publicly funded means that in the end of the day, the data should be public.

What are your future expectations about MAS?

In the last five years we have seen an enormous progress and I think that further analyses of plant genomes will bring us a lot more. It really helps that the research is in the public domain. We'll be able to speed up the process of plant breeding for an increasing number of crops and traits. Of course, there is a limit to what MAS can bring: MAS is only one tool in the box, which will have to be combined with other strategies such as ecological farming.

Benno Vogel (2009), Smart Breeding – Marker-Assisted Selection: A non-invasive alternative to genetic engineering of plant varieties, Executive Summary and Report, Greenpeace International, Amsterdam, August 2009, <http://www.greenpeace.org/raw/content/international/press/reports/smart-breeding.pdf>

The tomato genome decoded

Tomato fruits occupy a central place in the global diet and human nutrition. They are widely appreciated by consumers throughout the world and they contain lots of components that greatly benefit human health. Tomatoes are a highly valuable crop being a multi billion € industry for both fresh market and processed food industries. Tomato is also a very useful system for studying fruit development and ripening, as well as plant genetics. Decoding the genetic blueprint of tomato, i.e. the order of the nucleotide bases –adenine, guanine, cytosine, and thymine- in the DNA (DNA-sequencing) will therefore be extremely useful in providing the information necessary for future crop improvement.



Tomato is a member of the Solanaceae, the nightshades. Other members of this medium-sized family of about 4,000 species, include eggplants, potatoes, petunias, peppers, and tobaccos. This family encompasses species with diverse appearances some are shrubs, others bushes and some even trees. They can survive in very challenging growing conditions including living in deserts and on mountains. Some Solanaceous species have been used in medicine. Despite the astonishing diversity of these plants, they share a remarkably similar genetic code raising the question, how do similar sets of genes and proteins generate such different plants? This is one of the major questions being answered by the International Solanaceae Genome Project (SOL), an initiative bringing together partners from across the world to create a coordinated network of knowledge about the Solanaceae.

EU-SOL and sequencing the tomato genome

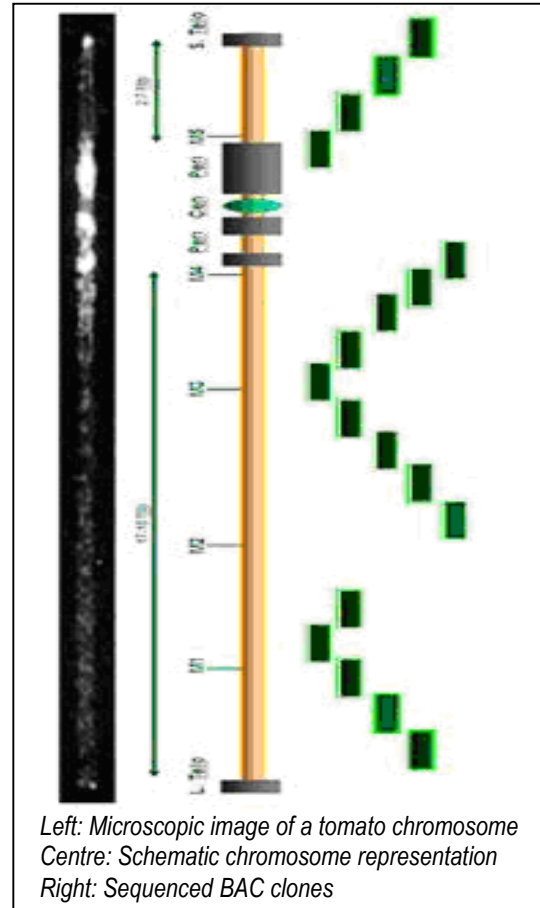
The worldwide SOL project was initiated in 2005 with research groups from 10 countries aiming to unravel the encoded information present in the tomato genome. Each country secured funding to sequence one or more of tomato's 12 chromosomes through their national funding agencies. The five participating European countries, Spain, Italy, France, United Kingdom and the Netherlands were also funded through their participation in the EU-SOL project. Part of the EU-SOL research programme supported the sequencing of



the tomato genome, both in producing sequence data and also in the development of computational methods to assemble, analyze and store the obtained DNA sequences.

Sequencing strategy : The first steps

The tomato selected to be sequenced was an old American processing-type cultivar named Heinz 1706. This particular tomato was selected because novel resources were already made for this cultivar and thus provided a rapid start to sequencing the tomato genome (DNA in the chromosomes). Genome sequencing is a great challenge as each chromosome is made up of a very long stretch of DNA (about 100 million base pairs). Therefore, initially chromosomes were split into manageable chunks of approximately 100,000 nucleotides or base pairs that could be grown in bacteria, called *Bacterial Artificial Chromosomes (BACs)*. These BACs were then split further and the building blocks read (sequenced) Overlapping chunks of sequence and overlapping BACs are then stitched together using dedicated computer software. This has been carried out for the 12 tomato chromosomes with long chains of overlapping BAC-sequences being produced, see figure. Due to cost constraints it was decided to focus the tomato sequencing project on gene rich regions ~ one quarter of the genome which is referred to as the euchromatin and which is known to contain approximately 90% of all the tomato genes. These gene sequences were considered as the most important targets to produce as the genes underlie all traits that make a tomato such a nutritious commodity.



After 3 years, approximately 50% of the DNA sequencing was completed. This represented very good progress from a diverse multinational effort but it became clear that further progress in certain regions would be difficult as BAC clones suitable for sequencing could not be generated for specific parts of the tomato genome and existing gaps in the euchromatin would remain.

Next Generation Sequencing

To overcome this the consortia led by EU countries adopted the use of novel DNA sequencing technologies that had emerged. These are collectively referred to as *Next Generation Sequencing (NGS)* technologies. Compared to the traditional sequencing technology, the NGS technologies allow more than a thousand-fold increase in sequencing speed and sequencing capacity. Moreover, the price per sequenced nucleotide has dropped from about \$ 5 per kb (1,000 bases) to about \$ 0.20 per kb using NGS technologies. This Tomato Next Generation Sequencing Initiative was launched in October 2008 and aimed to produce a so called “*whole genome shotgun sequence*” of the complete 950 Mb (950 million nucleotides/base pairs) tomato genome. This new sequencing initiative was carried out by the five participating countries from the EU-SOL project, Spain, Italy, France, UK and the Netherlands, in cooperation with research groups from India, Japan and from the United States. Next Generation Sequencing data was produced using two state of the art technologies (SOLiD and 454) and in addition, new computer software was developed to suit the needs of the tomato sequencing project. The latter was carried out in the framework of the Bioinformatics module of the EU-SOL research program.



The approach taken in the Tomato Next Generation Sequencing Initiative was highly successful. The first assembly of the newly produced DNA sequences, supplemented with only a small fraction of the already available BAC sequences, resulted in providing information of about 800 Mb of the entire tomato genome. The assembly process resulted in about 7,000 individual parts, the “scaffolds”, which are contiguous stretches of DNA ranging in size from 50 bases to approximately 10 million of bases. More than 95% of the assembled 800 Mb of tomato genome appears to be present on only 250 large scaffolds. A preliminary analysis of the tomato genome shows that approximately 34,000 genes are present. This first draft was released in December 2009.

Currently, the tomato sequence assembly is further improved and updated versions of the sequence are made available to the international scientific community at regular intervals. The latest version of the assembly (V1.03) can be downloaded from the following URLs:

<http://mips.helmholtz-muenchen.de/plant/tomato/index.jsp> and <http://solgenomics.net/>

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Single gene boosts tomato yield and sweetness

EU-SOL scientists at the Hebrew University in Israel and a Cold Spring Harbor Laboratory (CSHL) colleague have identified a gene that pushes hybrid tomato plants to spectacularly increase yield and the sweetness of individual fruits. The yield-boosting power of this gene, which controls when plants make flowers, works in different varieties of tomato, and crucially, across a range of environmental conditions.

“This discovery has potential to have a significant impact on both the billion-dollar tomato industry, as well as agricultural practices designed to get the most yield from other flowering crops,” says CSHL’s Zach Lippman, one of the three authors on the study, which appeared in the magazine *Nature Genetics* online on March 28th. The study is co-authored by Israeli scientists Uri Krieger and Professor Dani Zamir.

Hunting for genes that boost hybrid vigor

The team made the discovery while hunting for genes that boost hybrid vigor, a revolutionary breeding principle that spurred the production of blockbuster hybrid crops like corn and rice a century ago. Hybrid vigor, also known as *heterosis*, is the miraculous phenomenon by which intercrossing two varieties of plants produces more vigorous hybrid offspring with higher yields. First observed by Charles Darwin in 1876, heterosis was rediscovered by CSHL corn geneticist George Shull 30 years later, but how heterosis works has remained a mystery.

Shull’s studies suggested that harmful, vigor-killing gene mutations that accumulate naturally in every generation are exposed by inbreeding, but hidden by crossbreeding. “But there is still no consensus as to what causes heterosis,” says Lippman. “Another theory for heterosis, supported by our discovery, postulates that improved vigor stems from only a single gene – an effect called “*superdominance*” or “*overdominance*.”

To find overdominant genes, the team developed a novel approach by turning to a vast tomato “mutant library” – a collection of 5,000 plants, each of which has a single mutation in a single gene that causes defects in various aspects of tomato growth, such as fruit size, leaf shape, etc. Selecting a diverse set of mutant plants, most of which produced low yield, the team crossed each mutant with its normal counterpart and searched for hybrids with improved yield.



The florigen gene

Among several cases, the most dramatic example increased plant yield from 10Kg to 16.5Kg which was similar to AB2, the leading commercial variety in the region. This hybrid, the team found, produced greater yields because there was one normal copy and one mutated copy of a single gene that produces a protein called *florigen*. This protein, touted as the breakthrough discovery of the year in 2005 in *Science* magazine, instructs plants when to stop making leaves and start making flowers, which in turn produce fruit. In plants such as tomatoes, flowering (and therefore yield) is controlled by a delicate balance between the florigen protein, which promotes flowering, and another related protein, that delays flowering. "The fact that this modification affects a wide range of phenotypic traits is something amazing", Uri Krieger said. A mutation in only one copy of the *florigen* gene causes the hybrid to produce more flowers in less time (inflorescence number raised by 20% and flowers per inflorescence by 15%) – the key to improved yield. Also fruit weight increased fruit weight by 10%, sugar content by 15%.

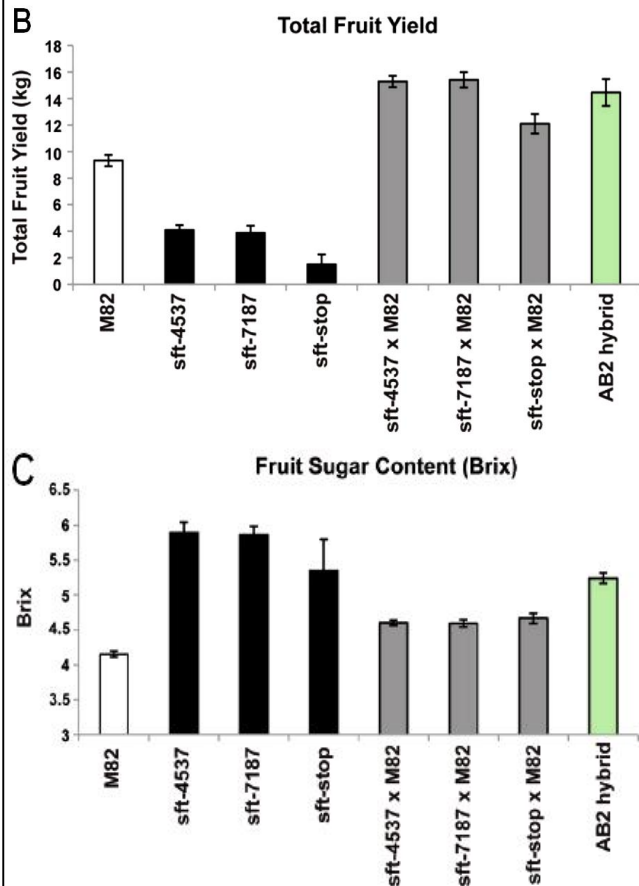
"It's the Goldilocks concept," explains Lippman. "What we find is that to maximize yield, you can't have too much or too little florigen. A mutation in one copy of the gene results in the exact dose of florigen required to cause heterosis." The scientists have observed the gene's heterosis effect in different varieties of tomatoes and in plants grown in different climate and soil conditions, both in Israel and locally in New York at CSHL and the Cornell Horticultural Experiment Station at Riverhead, NY.

In addition to superior yield, the hybrids also display another, perhaps equally important quality – taste. Tomato plants only produce a finite amount of sugar, which they distribute equally among their fruits. So higher yields usually result in each fruit having less sugar. But, remarkably, the *florigen* gene also boosted the sugar and sweetness of individual fruits.



M82 (control) **sft-4537 x M82** **sft-4537**

Representative plant and total fruit yield from a highyielding M82 inbred control plant (left), a low-yielding homozygous loss-of-function mutant allele of SFT (*sft-4537*, right) and a highly heterotic *sft-4537/+* heterozygote (middle).



Statistical comparison of mean values for total fruit yields (**B**) and sugar content (Brix value, **C**) between three independently derived *sft/sft* homozygous mutants, the inbred M82 control, the F1 *sft/+* hybrids of the *sft/sft* mutants with M82, and AB2, a leading commercial processing-tomato hybrid.

Courtesy: Uri Krieger



Further research in other crops

The researchers are already planning to explore if genes related to florigen in other crops can cause heterosis and improve yield. The concept that a mutation in only one copy of a single gene can improve yield has broad implications for plant breeding. "Mutant plants are usually thrown away because of the notion that mutations would have negative effects on growth," says Lippman. "Our results indicate that breeding with hybrid mutations could prove to be a powerful new way to increase yields, not only in tomato, but all crops."

"The flowering gene SINGLE FLOWER TRUSS drives heterosis for yield in tomato" is scheduled to appear in Nature Genetics online on March 28th. The full citation is: Uri Krieger, Zachary B. Lippman and Dani Zamir. The paper can be found at <http://www.nature.com/ng/journal/vaop/ncurrent/abs/ng.550.html>

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New plant breeding method could cut out generations of inbreeding

Plant biologists at University of California Davis developed a reliable method for producing plants that carry genetic material from only one of their parents. The technique could dramatically speed up the breeding of crop plants for desirable traits.

Plant cells usually contain at least one set of paired copies of each chromosome (diploid cells), one from the mother and one from the father. As a result, the offspring contains a mix of traits from the mother and the father. Therefore, plants usually have to be crossed with themselves (inbreeding) for several generations to achieve this and make a plant that will "breed true". This is a process that takes usually several years.

Creating haploid plants

But plant breeders want to produce plants that will pass the trait to all of their offspring. This requires plants that are homozygous, which means they carry the same trait on both chromosomes. When such plants are bred, they will pass the trait, such as pest resistance, fruit flavour or drought tolerance, to all of their offspring. This can be achieved with haploid plants with chromosomes from only one parent. Haploid plants are immediately, because they contain only one version of every gene. This produces true-breeding lines instantly, cutting out generations of inbreeding.

Existing techniques to make haploid plants are complicated, require expensive tissue culture and finicky growing conditions for different varieties, and only work with some crop species or varieties. A new method discovered by Simon Chan, assistant professor of plant biology at UC Davis, and postdoctoral scholar Ravi Maruthachalam should work in any plant and does not require tissue culture.

Chance observation

The discovery came out of a chance observation in the lab that could easily have been written off as an error. "We were doing completely 'blue skies' research, and we discovered something that is immediately useful," said Chan. Ravi and Chan were studying a protein called CENH3 in the laboratory plant *Arabidopsis thaliana* (mouse-ear cress). CENH3 belongs to a group of proteins called histones, which package DNA into chromosomes. Among the histones, CENH3 is found only in the centromere. The key role of the centromere in cell division makes CENH3 essential for life. Ravi had prepared a modified version of CENH3 by tagging it with a fluorescent protein, and was trying to breed the genetically modified plants with regular *Arabidopsis*. According to theory, the cross should have produced offspring containing one

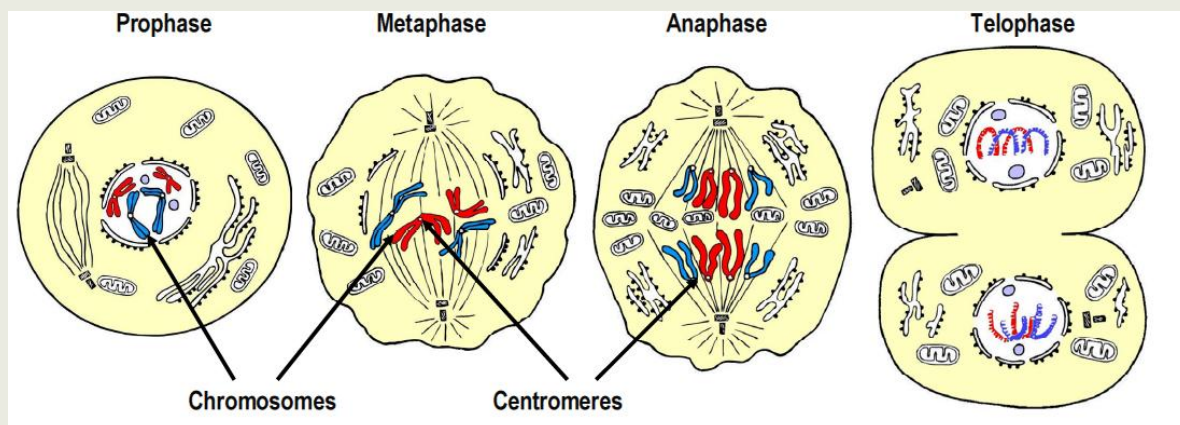


mutant gene (from the mother) and one normal gene (from the father). Instead, he got only plants with the normal gene. "At first we threw them away," Chan said. Then it happened again. Ravi looked at the plants again and realised that the offspring had only five chromosomes instead of 10, and all from the same parent. The plants appeared to have gone through a process called genome elimination. When plants from two different but related species are bred, chromosomes from one of the parents are sometimes eliminated. Genome elimination is already used to make haploid plants in a few species such as maize and barley. "But the new method should be much more widely applicable", Ravi said, "because unlike the process for maize and barley, its molecular basis is firmly understood. We should be able to create haploid-inducing lines in any crop plant".

Chromosomes, centromeres and histones

A **chromosome** is an organized structure of DNA and protein that is found in cells. It is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences. Eukaryotic cells (cells with nuclei) have large linear chromosomes that are packaged by proteins, the so-called **histones**, into a condensed structure called **chromatin**. The histones act as spools around which DNA winds, and play a role in gene regulation. Without histones, the unwound DNA in chromosomes would be very long.

Chromosomes may exist as either duplicated or unduplicated. Unduplicated chromosomes are single linear strands, whereas duplicated chromosomes contain two copies joined by a **centromere**. The centromere is involved in cell division. It's the part of the chromosome that controls how it is passed to the next generation. When cells divide, microscopic fibers spread from each end of the cell and attach at the centromeres, then pull the chromosomes apart into new cells (see graphic below).



The four phases of cell division (mitosis) and the role of centromeres therein. (Courtesy: Ymai, Wikimedia)

Once the haploid-inducing lines are created, the technique is easy to use and requires no tissue culture -- breeders could start with seeds. The method would also be useful for scientists trying to study genes in plants, by making it faster to breed genetically pure lines. After eliminating half the chromosomes, Chan and Ravi had to stimulate the plants to double their remaining chromosomes so that they would have the correct diploid number. Plants with the haploid number of chromosomes are sterile.

How species form in plants

The research also casts some interesting light on how species form in plants. CENH3 plays the same crucial role in cell division in all plants and animals. Usually, such important genes are highly conserved -- their DNA is very similar from yeast to whales. But instead, CENH3 is among the fastest-evolving sequences in the genome. "It may be that centromere differences create barriers to breeding between species," Chan said. Ravi and Chan plan to test this idea by crossing closely-related species.

Sources:

- Maruthachalam Ravi & Simon W. L. Chan, *Haploid plants produced by centromere-mediated*



genome elimination. In: *Nature* (464) pp. 615-619, 25 March 2010

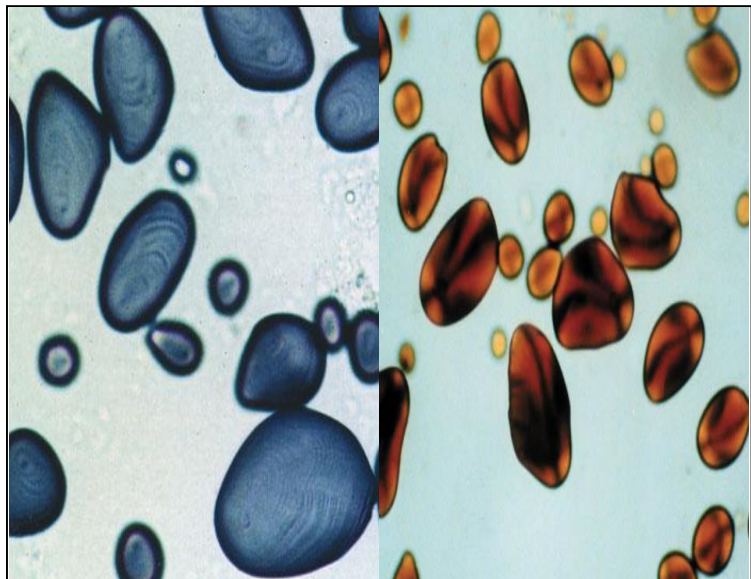
- UC Davis News & Information, *Chance observation leads to Plant Breeding Breakthrough*, March 24, 2010, http://www.news.ucdavis.edu/search/news_detail.lasso?id=9425

Precision breeding creates super potato

Researchers at the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) in Germany have applied TILLING to develop a potato that produces pure amylopectin, a starch used in the paper, textile and food industries.

Amylose-free potatoes

Starch forms the basis of numerous industrial applications, particularly in the paper, textile and plastics industries. In 2008 the EU used 21.6 million tonnes of raw material for starch production, more or less equally divided between wheat, maize and potato. Potatoes contain two types of starch: amylopectin and amylose. Both these components are of equal value in terms of human nutrition. In industrial processes they have different characteristics: usually, only the thickening properties of amylopectin are required, while the gelling amylose component is undesirable in many products and can interfere with certain processes. The chemical separation of these two components consumes energy and water. In order to reduce costs and environmental load the potato starch industry is seeking for amylose-free potatoes.



Starch grains under the microscope: the starch grains in the potato tubers turn blue in an iodine solution if they contain amylose as well as amylopectin. When amylose is no longer produced, because the gene for the starch synthase enzyme has been switched off, the starch grains turn red. Courtesy: MPIZ

TILLING: creation and genome analysis of mutants

Amylose-free potatoes have already been developed with the use of genetic engineering², which has disadvantages in terms of public acceptance and high costs for regulatory approval of genetically modified organisms in the European Union. The IME applied an alternative technology to produce amylose-free potatoes: TILLING. TILLING - an acronym for "Targeting Induced Local Lesions in Genomes" - combines a standard technique of mutagenesis with a chemical such as ethyl methanesulfonate (EMS) with a sensitive DNA screening technique that identifies single base mutations and alleles that are responsible for characteristics of interest (target genes). The IME produced a vast number of mutants with EMS and

² See for instance: Amflora – a potato for industrial applications, GMO Safety, <http://www.gmo-safety.eu/en/potato/starch/32.docu.html>



germinated the seeds in the laboratory. As soon as the first leaves appeared, the researchers took a leaf sample, broke apart the cellular structure, isolated the genome and analyzed it. This way they found out within a few weeks if a mutation had attained the desired traits. There are genes active in their genome responsible for the formation of amylopectin, whereas genes that trigger the formation of amylose are shut off. From this germ, they were able to generate the first generation of super potatoes. In the fall of 2009, 100 tonnes of the new super potato that exclusively produces amylopectin were harvested.

TILLING in EU-SOL

TILLING is also applied to create tomato mutants in the EU-SOL project. Metapontum Agrobios in Italy used EMS to create a collection of 5,500 M3 mutants from *Red Setter*, a processing tomato variety. In a similar way, researchers from the Vegetable Genomics Research Unit of INRA (URGV-INRA), France, produced M3 mutants from *Micro-Tom*, a cherry type tomato. Dani Zamir from the Cornell University produced M3 mutants from *M82*, another processing tomato variety. The mutant populations were phenotyped, DNA was extracted, and target genes were identified. This far, following requests from several EU-SOL partners, more than 30 genes have been TILLED. The TILLED genes and phenotyping data were then integrated in a database by the Vegetable Genome Research Unit of INRA in Evry, France. This database may be searched using a sequence, by keyword search or a phenotypic feature.

TILLING: an acceptable alternative to (safer than) genetic engineering?

Biotechnology critics like Greenpeace Germany claim that, from a technological point of view, there is a 'huge difference' between genetic engineering and TILLING. They present the TILLING method as an 'acceptable' breeding method that stays within the 'normal range' of plant characteristics. However, the editors of *GMO Safety*³ refer to plant experts who believe that concluding that TILLING leads to plants with a lower risk potential than genetic engineering is oversimplifying the situation. In a paper published in 2004, the National Research Council (USA) pointed out that, in principal, all plant-breeding methods can lead to unexpected negative effects. For instance, when potato plants were crossed in the 1960s and 1990s, two varieties were created, which displayed the same high, toxic content of alkaloids typically found in wild potatoes. Both potato varieties had to be withdrawn from the market as a result. Canadian regulators have gone one step further: all new plants with novel traits (PNTs), either as a result of mutation breeding or natural mutation or through genetic modification, have to go through a complex approval process.

The URG-INRA TILLING database is accessible at <http://urgv.evry.inra.fr/UTILLdb>. The Cornell University has its own database for M82 mutants, accessible at http://zamir.sgn.cornell.edu/cgi-bin/mutation_site/mutant04_search.pl. Metapontum Agrobios has a searchable database for Red Setter mutants at <http://www.agrobios.it/tilling/search.php>

Sources:

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- TILLING: The 'good' alternative to genetic engineering?, *GMO Safety*, <http://www.gmo-safety.eu/en/news/736.docu.html>
- Canadian Food Inspection Agency, *The Regulation of Plants with Novel Traits (PNTs) in Canada*, <http://www.inspection.gc.ca/english/plaveq/bio/pntchae.pdf>

³ A German Federal Ministry of Education and Research financed Internet portal that provides information about current and past biosafety research into genetically modified plants in Germany



Commotion about genetically modified eggplant in India

India will not cultivate genetically modified (GM) eggplant — also known as brinjal or aubergine — until the public and scientists are convinced it is safe, the country's environment minister Jairam Ramesh announced in February. India's Genetic Engineering Approval Committee (GEAC), under the country's environment ministry, cleared the GM eggplant for commercial cultivation last October but the final decision was referred to the government.

Ramesh decided to throw the issue open to public consultation after scientists and activists expressed concern about the decision, arguing that safety trials had not been conducted in a transparent manner. Ramesh also said he took on board decisions by ten state governments to ban cultivation even if approved. These states include Andhra Pradesh and Gujarat which were among the first to introduce Monsanto's GM cotton in 2002. The moratorium will last until independent scientific studies establish, to the satisfaction of both the public and professionals, the safety of the product from the point of view of its long-term impact on human health and environment.



Students of Punjab University express their protest against Bt Brinjal with their face painted with graffiti at a student centre in Chandigarh. Photo: Akhilesh Kumar

The eggplant *Solanum melongena* — which would have been the country's first GM vegetable⁴ — belongs to the Solaneceous family. The genetically modified plant was developed by Indian seed company Mahyco in collaboration with Monsanto. It contains a Bt gene that makes the plant resistant against lepidopteran insects like the Brinjal Fruit and Shoot Borer *Leucinodes orbonalis* and Fruit Borer *Helicoverpa armigera*. These insects are the greatest threat and can cause a major loss in the marketable yield.

Eggplant in Indian culture

The raging debate has probably risen from the popularity of this vegetable in the Indian diet and the cultural values attached to it. The crop is said to have originated in India and has been cultivated for over 4000 years. There are approximately 2,500 varieties of eggplant in India of various shapes extending from oval or egg-shaped to long or clubshaped; and colours ranging from white, yellow, green and purple to nearly black. The eggplant is among other nutrients an excellent source of minerals and vitamins and is rich in water soluble sugars and amide proteins. It is also an important ingredient in Ayurvedic medicine and is of special value in the treatment of diabetes and liver problems. There is several well-known eggplant dishes and folk songs that allude to the eggplant in different parts of the country.

⁴ Indian farmers have been growing GM cotton for several years now. Like the eggplant, the cotton contains a gene from the soil bacterium *Bacillus thuringiensis* (Bt) which makes the crop resistant to the bollworm pest. According to the latest report by the International Service for the Acquisition of Agri-biotech Applications (ISAAA), Indian farmers planted 8.4 million hectares with GM cotton in 2009, a 10.5% increase compared with the 2008 acreage.



Eggplant production in India

India is, after China, the second largest producer of eggplant in the world. The crop is cultivated across the country accounting for 9% of total vegetable production. The total area under eggplant cultivation in 2008 according to the UN Food and Agriculture Organization was 512,800 hectares (about 8% of the land under vegetable cultivation), with a total production of 8.45 million tonnes. The crop is typically grown in small plots or as inter crop. The major producing states in India are West Bengal, Orissa, Bihar, Gujarat, Maharashtra, Karnataka, Uttar Pradesh and Andhra Pradesh. Depending on the variety and the season, the average yield varies from 15 to 30 tonnes per hectare. Many of the hybrid varieties have shown a potential yield of upto 50 tonnes/ha. The eggplant is generally considered a high value crop yielding high net benefits for the farmer.

What is the controversy about?

Early 2010 public meetings on Bt-eggplant were organised at different places in the country, especially in the regions that are important for eggplant cultivation, such as Ahmedabad, Nagpur, Gujarat and Maharashtra. Almost 8,000 people from different sections of society participated in these seven public meetings.

The promoters say that Bt eggplant will be beneficial to small farmers because it is insect resistant, increases yields, is more cost-effective and will have minimal environmental impact. They argue that genetic improvement by conventional plant breeding has not been successful due to the lack of resistance to both pests in eggplant germplasm. Therefore, farmers usually rely on the use of pesticides. Because the larvae of shoot and fruit borer are often hidden in the fruit and do not easily come in contact with pesticides, the application of pesticides has to be critically timed by farmers in such a way as to kill the larvae before they bore into shoots and fruits. Small and marginal farmers use 25-80 sprays of pesticides in eggplant cultivation. About 60% of plant protection cost is for controlling fruit and shoot borer.

On the other hand, very much like in Europe, concerns about Bt eggplant relate to its possible adverse impact on human health and bio-safety, farmers' seed rights and livelihoods, consumer choice and biodiversity. Opponents refer to non-chemical alternatives for pest management, such as pheromone traps for mass trapping, sanitation of the field (timely removal and destruction of affected shoots & fruits), mechanical barriers, use of some local plant extracts for pest control etc., that result in similar yields as the Bt varieties. Opponents also refer to reporting of an increase in total pesticide usage in Bt-cotton over time due to increased secondary pest attacks or tolerance developed by the target pests.

Sources:

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