Crop and Forest Biotechnology for the Future

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Ansvarig utgivare: Akademiens sekreterare och VD: Bruno Nilsson
Redaktör: Gunilla Agerlid

Crop and Forest Biotechnology for the Future

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Maria Larsson

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Introduction

Olof and Brita Stenström have established the Bertebos Foundation in order to promote research, development and education within the food industry. Through the auspices of KSLA, the Foundation has established a prestigious prize, the Bertebos prize. In 2001 this prize was awarded to Professor Donald Grierson, Nottingham University, UK, for his pioneering research on control of fruit ripening and quality. The Bertebos Foundation also holds a seminar in Falkenberg, close to Slöinge, where the Stenström family have their property, Berte Mill, "SIA Ice Cream" and a farm. In the seminar, the prize winner and other scientists from around the world gather as lecturers and participants. The 2001 conference was organised by the Bertebos Foundation in co-operation with the Royal Swedish Academy of Agriculture and Forestry (KSLA) and the Swedish Foundation for Strategic Research (SSF). This is the brief background to the current International Conference on Crop and Forest Biotechnology for the Future.

The conference focused on what biotechnology science can do for the long-term supply of agricultural and forestry products. Despite the absence of four speakers from the US and Australia (who were unable to come due to the events in New York on September 11) the programme was broad, thorough and inspiring. It is an intriguing task to document this conference properly and completely. The most important result of the scientific work, as Prof. Donald Grierson put it, is the increased understanding of how it all works, but of course it is also a challenge to be able to adapt this increased knowledge in practical horticulture, agriculture and forestry.

Olof Stenström, Donald Grierson och Brita Stenström. *Photo: Maria Larsson.*

Genetically modified organisms in food and agriculture: Where are we? Where are we going?

A transgenic plant has just one or a few genes altered and this generates a new variety with different properties. One gene might influence the aroma and other characteristics that seemed to be independent of the desired hereditary character. One fact that makes the transgenic plant different from a plant developed with traditional plant breeding is that the transgenic plant can contain genes from any living being, while the traditionally bred plant can receive hereditary characters only from plants from the same species.

It is easy to recognise the great potential as well as the complications with genetically modified organisms, GMOs. Louise Fresco, Asst. Director General of the FAO, claimed that the FAO needs to assess GMOs in terms of their impact on food security, poverty, biosafety and the sustainability of agriculture. Will GMOs increase the amount of food in the world and make more food accessible to the hungry? She wanted to discuss not the specifics of GMO technology, but the context in which they are developed and deployed and how public opinion and government policy on GMOs are formed.

In most countries there is no consensus on how biotechnology and GMOs in particular can focus on the key challenges of the food and agricultural sector. Scientists see genetic modification as a major new set of tools, the industry looks at GMOs as opportunities for corporate profit (but with public acceptance as a stumbling block) and governments often lack coherent policies in relation to GMOs.

The FAO has been conducting a worldwide inventory of agricultural biotechnology applications and products, with special reference to developing countries, to test the hypothesis that biotechnology applications in crop science – particularly GM crops – have not addressed the needs of developing countries.

The total area cultivated with GMO crops in the world is rapidly growing and currently stands at 44.2 million hectares. About 75% of the area planted with GM crops is in industrialised countries. Around 16% of the acreage of the four crops soybean, maize, cotton and canola is under GM varieties. Two traits – insect resistance (mainly based on Bt) and herbicide tolerance – dominate.

Only seven developing countries commercially cultivate GM crops. The dominant crops are soybean and cotton and the traits are herbicide tolerance and insect resistance. Only in China was one GM cotton locally developed and commercialised. Other countries obtained genetic constructs or varieties from industrialised countries.

However, to ensure that GM crops make an optimal contribution to world food security, food safety and sustainability, three great problems must be solved. Firstly, GMOs must be directed to the right research priorities such as drought and heat tolerance, improved nutrient uptake and rooting, biological nitrogen fixation, responses to carbon dioxide and tolerance to key abiotic stresses, such as salinity and drought. Secondly, agreed national and international instruments of governance must be developed. Thirdly, the access to research and new technologies for developing countries, poor producers and consumers must be facilitated.

Louise Fresco's speech formed a background to the scientific achievements that were presented during the seminar. Little of the research has been done to lighten the burdens of the poor, but many achievements can be utilised to develop a sustainable and secure food chain in poor countries.

Read Louise Fresco's full speech at http://www.fao.org/ag/magazine/GMOs/pdf

Ripening

Ripening and senescence go hand in hand but by delaying senescence in fruits and vegetables the sustainability is prolonged, which is an advantage to producers, trade and consumers.

Control of fruit ripening and quality

Tomato fruit development and ripening is influenced by the growth hormone ethylene (ethene). Twenty five years ago, Prof. Donald Grierson and his research team at Nottingham University and other scientists began to study genes that regulate ripening. One of these genes encoded a receptor for ethylene, which detects the presence of the hormone in fruit cells that switch on the ripening process. They later found that ethylene activates a cascade of genes that coordinate many biological changes and thereby affect fruit quality: colour, flavour, texture and even the rate of ripening in the tomato. Similar changes occur in many fruits.

Having identified some of the genes expressed during ripening, Prof. Grierson and his team wanted to find out whether they could control their expression with genetic modification techniques. They found that by using the antisense technique or sense gene silencing (co-suppression), messenger RNA (mRNA) and enzyme activity for polygalacturonase, phytoene synthase and ACC oxidase, and other enzymes could be reduced. This modified texture, changed succulence and colour, and demonstrated that senescence could actually be delayed if expression of the right genes was modified.

This is a very precise method and only one gene out of tens of thousands is inactivated. The effect is related to the specific antisense gene that is used and it is inherited. From tomatoes with the antisense gene, progeny tomatoes can be obtained that have a delayed ripening process or altered quality. This means that a new variety is created. Around 1990, Grierson found that if this was done with a sense gene, a similar knockout effect was obtained. The results of the two procedures are very similar, so there must be a common mechanism between sense gene activation and antisense gene activation.

The key point is that if ethylene production or the response to ethylene can be controlled, the rate at which produce progresses to overripeness and spoilage can be regulated. Between 20 and 50% of all fresh produce (including tomatoes) deteriorates before it reaches the consumer. Preventing this deterioration is an advantage to the producers, trade and consumers. The synthesis of ethylene requires a short biochemical pathway involving a compound found in all living cells called SAM (S-adenosyl methionine). To make ethylene the plant has to control two steps, first to convert SAM to ACC (1- amino-cyclo-propane-1 carboxylic acid); then to convert ACC to ethylene. Ethylene causes ripening and senescence in leaves, flowers and fruit and once the process is started, it is very difficult to stop. A low ethylene tomato, however, has a slower deterioration process and is therefore edible during a longer period.

A number of genes are needed to control different aspects of the ripening process. The expression by GM procedures affecting one gene can be inhibited without interfering with another and some interesting and potentially very useful biotechnological applications can be obtained.

To summarise, Prof. Grierson and his colleagues have found more than 25 ripening related genes and are beginning to understand the role of several of these genes in controlling processes like texture and succulence, pro-vitamin A contents, storage and post-harvest deterioration and ethylene.

Better keeping qualities

The methods used in Nottingham have been further developed and adapted to produce cantaloupe melons with better keeping qualities.

Cantaloupe Charentais melons are very tasty, sweet French melons with good flavour but with poor keeping qualities. They over-ripen quickly and are chilling sensitive. The cantaloupe Charentais melon therefore represented a good target fruit for improving storability through biotechnology.

Melons, bananas, pears and peaches and, of course, tomatoes belong to a climacteric type of fruit where ethylene plays a major role. They are characterised by a rise in respiration during ripening associated with a burst of ethylene production. In addition, ethylene can stimulate its own biosynthesis (autocatalysis). There is another type of fruit such as grapes and pineapples in which ethylene does not seem to play a major role but scientists have not yet identified the other factor that plays that major role, according to Dr. Jean-Claude Pech, INRA-ENSAT in Toulouse.

In order to increase the storage life of melons, he and his colleagues decided to try to slow down ripening by inhibiting ethylene production. They isolated the melon ACC oxidation gene (that Donald Grierson had discovered in tomato) and put it in antisense orientation. Ethylene was inhibited almost to 100% in the antisense melon in a way that gave a significant difference in storability between the antisense (AS) and the wild type (WT) melon. After 15 days of storage at 25°C, the WT was not edible any longer while the AS type had retained firmness and some other quality attributes. By comparing the composition of the two types of fruit, Dr Pech and his colleagues were able to show that sugar accumulation and the colour of the flesh were similar and therefore corresponded to ethylene independent processes. On the other hand, the colour of the rind and the formation of the peduncular abscission zone were ethylene-dependent processes. Softening of the flesh has two components, dependent and independent of ethylene. These data suggest that climacteric fruit ripening involves ethylene-independent events and therefore comprises some non-climacteric regulation.

By submitting AS fruit to ethylene, INRA-ENSAT scientists found that the various ripening pathways were sensitive to levels of ethylene (2.5 to 5 ppm) that are much lower than internal ethylene present in ripe fruit (80 to 100 ppm). Attached fruit had a higher rate of ethylene production than detached fruit but respiration was lower in attached fruit.

Wild type melons showed chilling injury after storage at low temperatures (2 weeks at 2°C). The injury was particularly visible upon rewarming. In contrast, the AS melon showed very little damage, demonstrating that ethylene was a stimulator of chilling injury.

Besides providing a tool for understanding the mechanisms of fruit ripening, these transgenic melons could give rise to new post-harvest handling methods:

- Harvesting all fruit at the same time in the field (possibly mechanically) when all of them have reached sufficient sensory qualities without risk of over-ripening.
- Storing at low temperature without chilling damage.
- Shipping to distant markets, treating with adapted levels of ethylene.
- Sorting for quality.
- Delivery to the consumer of fruit at an optimum sensory quality.

However, such a scheme needs to be validated at the practical level.

Fatty Acid Modification

Vegetable oils are important commercial products, of which about 90% are used for human consumption. Non-food applications include soap and detergents, paints, lubricants and fuel (bio-diesel). The use is dependent on the quality, which in turn depends on the fatty acid composition. Although several hundred fatty acids of different structures exist, it is likely that only about 50 enzymes/genes are involved in the synthesis of the most important fatty acids. The annual oilseed crops yield from 0.4–1.8 tons of oil per hectare, which is far exceeded by oil palm with up to 7 tons/ha/year. The annual global production/consumption of plant oils adds up to about 100 mio tons compared to about 20 mio tons of animal fats. All edible oils together would result in about 40 g oil/person/day given a global population of 6 billion people.

Desaturation of fatty acids

In industrialised countries, about three times as much oil, 120 g, is consumed per person per day despite the fact that for years medical and health authorities have been recommending a reduction in this consumption to about 60 g per person per day, which corresponds to about 30% of the recommended daily 2000 kcalories. In addition, the oils prevailing in present-day food items of the Western world are characterised by a predominance of saturated and at most diunsaturated linoleic acid. A healthy and balanced mixture of various fatty acids, particularly considering linoleic (ω-6) and linolenic acid (ω-3) in a ratio of about 5:1, is not provided. These two polyunsaturated fatty acids cannot be synthesized by man and, therefore, are essential fatty acids required for the biosynthesis of various effectors controlling human physiology on various occasions and at different points. The recommended daily 2 g of linolenic acid are contained in about 500 g of green vegetable fresh weight, whereas this ω-3-fatty acid is hardly found in any of the other food items appreciated by all of us for their convenience in storage and preparation.

From a biochemical point of view the daily consumed fatty acids should include about 10 g of linoleic and 2 g of linolenic acid and a significant reduction in all saturated fatty acids and a corresponding increase in oleic acid. Therefore, a reduction in cholesterol-containing animal (dairy) fats and the use of cholesterol-free plant oils whenever possible can be recommended. With respect to these criteria, in particular due to the presence of linoleic and linolenic acid in a ratio of about 3:1, rapeseed oil is superior to olive and sunflower oils, since both do not contain the essential linolenic acid. Since we all can hardly avoid the flooding of our daily diet by saturated and at most diunsaturated (ω-6, but not ω-3) linoleic acid, Prof. Ernst Heinz, Institut für allgemeine Botanik in Hamburg, recommends two servings of fat (not lean!) fish per week. This provides the C20- and C22-ω-3- and ω-6-long-chain polyunsaturated fatty acids that are the direct effector precursors made by the human body from dietary linoleic and linolenic acid.

In view of this situation it is an obvious idea to produce plant seed oils containing these very long-chain polyunsaturated fatty acids normally only found in fish ("oceanic" fatty acids). This goal may be realised by gene technology provided that the biochemistry of oil biosynthesis is known in detail. Also, all the required genes and the transformation technique to introduce these genes into annual oilseed crops must be at hand. Several groups world-wide have concentrated on this transgenic approach, because it would:

- Provide a healthy oil beneficial for human nutrition.
- Represent a consumer-orientated transgenic product.

- Guarantee a sustainable production.
- Protect the marine resources from overfishing.

At the end of the year 2002, most genes required for this task have been cloned and only one is still missing. The oilseeds of potential use for this project include rapeseed, soybean, linseed, sunflower and peanut, which all can be genetically modified. The most successful approach so far published has resulted in canola oil with 44% γ-linolenic acid, which is a good basis for the implementation of the final reactions leading to C20- and C22-polyunsaturated fatty acids.

Seed oils of higher plants contain three predominant fatty acids: oleic, linoleic and linolenic acid, which all have 18 carbon atoms and from one to three double bonds. These double bonds are introduced by desaturase enzymes, in which two large families exist in all plants: soluble enzymes for the first double bond and membrane-bound desaturases for the following double bonds. The gene-technologically most relevant enzymes belong to the latter group. In addition to the prevailing cis-desaturation, members of this group catalyse a wide variety of reactions such as trans-desaturation, hydroxylation, epoxidation, conjugation and decarbonylation. The acyl groups used as substrates are thioesters of coenzyme A or oxygen esters in glyco- and phospholipids. These seemingly unrelated reactions are due to the versatility of a di-iron complex believed to be bound in the active site of these enzymes. All the desaturases required for the above-mentioned project have been cloned from various organisms including algae, mosses and fungi. But apart from the desaturases, two elongases are required for converting polyunsaturated C18- to C20- and finally to C22-fatty acids. From these, the final elongase is still not available in cloned form, blocking the production of polyunsaturated C22-fatty acids.

Three milestones in oil biosynthesis

The fatty acids (FA) found in available vegetable oil qualities are:

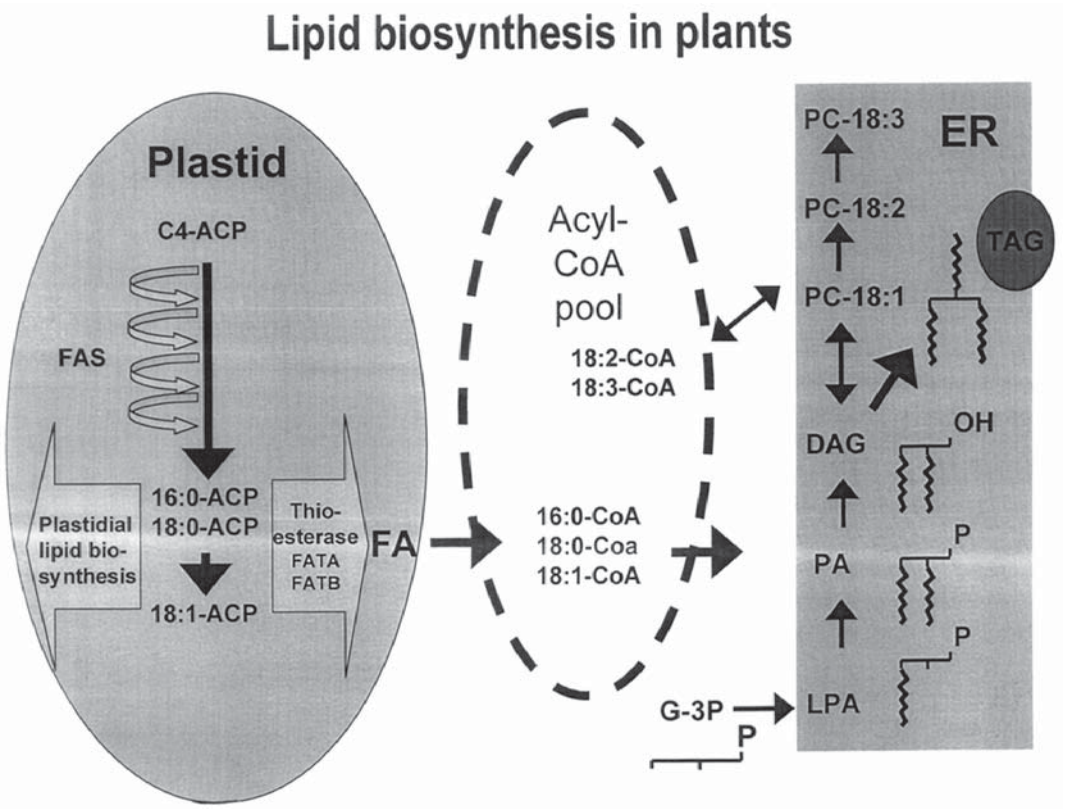
- The five common fatty acids: 16:0 (palmitic), 18:0 (stearic), 18:1 (oleic), 18:2 (linoleic) and 18:3 (linolenic), found in oilpalm, canola, sunflower, soya bean and linseed.
- Exotic fatty acids: 10:0 (capric), 12:0 (lauric) and 14:0 (myristic), found in palm kernel and coconut, 22:1 (euric acid) found in high erucic rape.

There are more than 200 exotic fatty acid structures identified in nature and many of these can be found in high levels, up to 90% of FA in natural vegetable oils. The chain lengths differ from C8 to C24 fatty acids. The desaturation can be at odd positions, trans, conjugated or triple. The substitutions can be hydroxy, epoxy, furanoid or keto. Exotic fatty acids can have branched, cyclic or fluoro modifications. They are used already today by the industry but many more have potentially interesting industrial applications. Most of the exotic fatty acids are naturally produced by plants that are not suitable for modern agriculture systems. However, biotechnology can in the future be used to design a desired oil quality in a desired oil crop. A desired fatty acid can be obtained with the usage of the big gene library in nature or by protein engineering.

The first milestone in the scientific challenge is to obtain a general understanding of lipid biosynthesis and metabolism in plants and especially in developing oil seeds. Oil seeds are highly specialised plant organs as regards their glycerolipid composition. Oil seeds contain 1% plastidial galactolipids, 1% plastidial phospholipids, 3% cytosolic phospholipids and 95% tricyglycerols.

The second milestone in the scientific challenge is to clone genes for the biosynthesis of exotic fatty acids. There is already a long list of cloned genes and it is rapidly growing.

Vegetable oils are already commercial products, but could with some biotechnology and protein engineering to a large extent exchange mineral oil. A general understanding of lipid biosynthesis and metabolism is an absolute condition. Illustration: Ulf Ståhl



The third milestone in the scientific challenge is to achieve high levels of exotic FA in vegetable oils. Dr. Ulf Ståhl, Swedish University of Agricultural Sciences in Uppsala, reported that so far there are two good examples of transgenic plants where the oil quality has been drastically altered. Both are transgenic rape, the first producing medium chain fatty acids and the second producing long chain wax esters. The levels obtained of hydroxy, epoxy, acetylenic and conjugated FAs in the oil of transgenic plants are considerably lower. It is possible to obtain a high content of exotic fatty acids as they occur in high proportions in natural seed oils such as castor beans and *Cuphea pulcherima*.

Starch

Starch is one of the most important bio polymers in nature. It is an energy source for animals and humans (food) and a source of raw material. It exists as small granules of different sizes (0.001–0.1 mm) and forms according to botanical origin. 55% of the trade with starch is within the food sector and 45% in the non food sector e.g. the paper, polymer and chemical industries.

Designing starch

Starch is the major form in which plants store their carbon – around 80% of the dry weight in potato tubers and the endosperm of cereals and up to 50% for peas and beans. Starch is the main source of carbohydrates in our diet but it also serves as a thickener, texturizer or stabilizer in both the food and non food industries.

Starch extracted from the crop (maize, wheat, potato, cassava etc.) undergoes post-extraction processing consisting of chemical, enzymatic or physical modifications. This generates starches with many different properties (different pastes and gels with varying characteristics) suitable for a wide range of different industrial uses.

But the post-extraction processing has some major disadvantages, it is energy consuming and expensive and can generate polluting waste. It would be better if we could design the right type of starch in the crop so that post-extraction modification would not be needed.

Starch consists entirely of glucose polymers. There are two types of glucose polymers in the granule: 20–30% long linear amylose and 70–80% highly branched amylopectin.

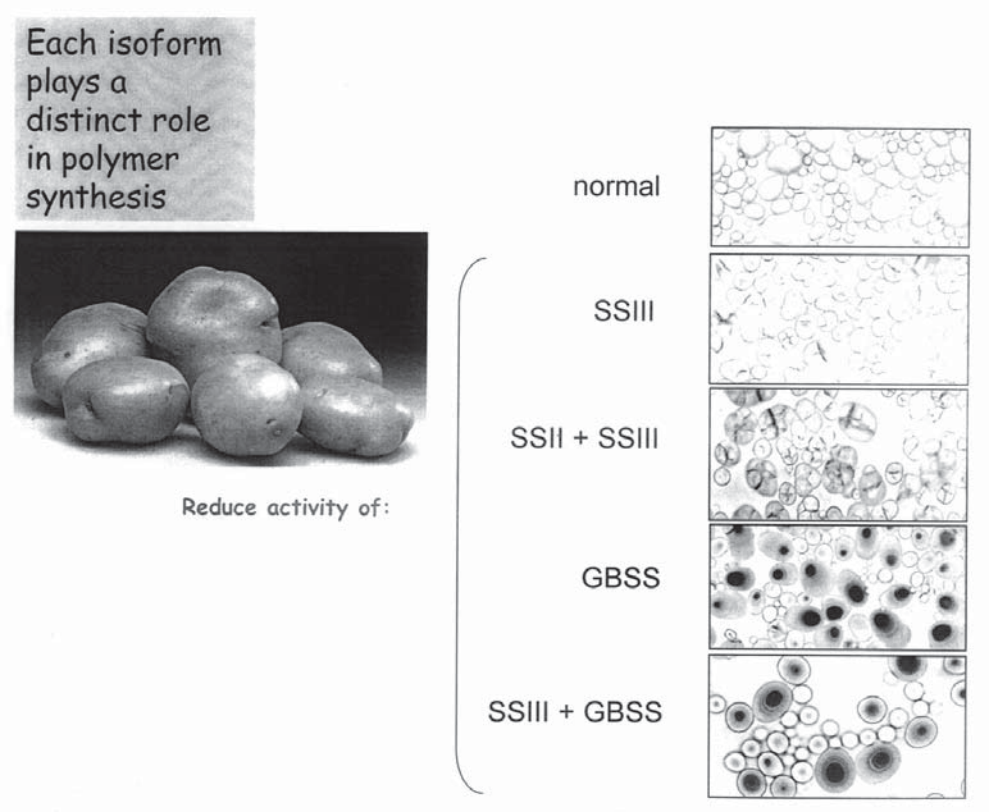
Only two enzymes are required to synthesize the polymers. They are starch synthase and starch-branching enzyme. The two enzymes exist as multiple, conserved isoforms: there are five different isoforms of starch synthase and two of starch-branching enzyme. Each isoform makes a different contribution to the synthesis of the glucose polymers, and without these different classes of isoforms the plant would not be able to make something so complex as a starch granule. For example, in a potato tuber three classes of starch synthase are found. SSII and SSIII are responsible for different aspects of the synthesis of amylopectin and GBSS is responsible for the synthesis of amylose. The function of each isoform cannot be replaced by other isoforms, and the contribution of each is dependent on which other isoforms are present.

Debranching enzyme (isoamylase) is also necessary for normal starch synthesis. When it is reduced or eliminated, the plant synthesises a highly-branched glucose polymer called phytoglycogen that is not normally present, and also synthesises many more starch granules than normal. The precise role of isoamylase is not known, but two possible explanations have been put forward.

1. The direct model proposes that isoamylase is directly necessary for the synthesis of amylopectin, because it removes some of the branches from a precursor called preamylopectin, allowing it to become organised to form the granule. If isoamylase is reduced or eliminated, less of the preamylopectin will become incorporated into starch granules. Instead it will become phytoglycogen.
2. The indirect model proposes that isoamylase is only indirectly involved in the starch synthesis. The starch synthase and starch-branching enzyme are allowed to be entirely responsible for the starch synthesis. The role of isoamylase is to degrade any soluble products of the action of these enzymes on malto-oligosaccharides present in the stroma surrounding the granule. If isoamylase is reduced or eliminated these malto-oligosaccharides are built up by starch synthase and starch-branching enzyme and become phytoglycogen.

Dr Alison Smith, John Innes Centre in Norwich, knows quite a lot about the enzymes that are necessary for starch synthesis but as yet has not successfully put them into a convincing pathway that tells us how starch is made. Nonetheless she and her colleagues are beginning to gain sufficient information for “designing” starch properties in a rational way.

Only two enzymes are required to synthesise the polymers, starch-branching enzyme (with two classes of isoform) and starch synthase (with five classes of isoform). Each isoform plays a distinct role in polymer synthesis. Illustration: Alison Smith



The starch synthesis in barley endosperm

Prof. Christer Jansson, Swedish University of Agricultural Sciences, Uppsala, is involved in a project on metabolic engineering of starch synthesis.

These scientists are working mainly on barley, but also on cassava and sorghum through a collaboration with East African countries. The long-term objective is to produce transgenic plants with modified starch for food production and for non food production.

The starch synthesis in barley endosperm in the seed is complicated. Regarding the regulatory aspects of the synthesis, a protein in nuclear extracts from leaves binds to an isoamine promotor. Since the gene is not expressed in leaves, it indicates that whatever binds is a repressor preventing the expression of this gene in non expressing tissue. The reason that the gene is expressed in the endosperm might be that the sucrose concentration is too high for the repressor to bind there.

The two genes SBIIA and SBIIB are to 60% similar but have some important differences. SBIIB contains a very large second intron. The second intron in SBIIA is much smaller. When the second intron in SBIIB was analysed it was found that it contained a sequence that could be involved in a regulation and was

similar to the bbox from patatine promotor (a storage protein in potato). This bbox confirms to specificity to the patatine gene. The project drew the conclusion that the gene was involved in regulation in barley. There is a protein or a protein complex binding to this element in the second intron. There is also, apparently, a protein binding somewhere outside. So again, a repressor binds to this element. The susiba 1-gene and susiba 2-gene have regulatory roles.

The hypothesis is that the susiba 2-gene (the longer of the two susiba genes that are cracked) is expressed only in endosperm. The encoding repressor binds to a sugar responsible element. In the endosperm the susiba 2-gene encoding transcription factor is active, while the transcription factor for susiba 1 is not active. The susiba 1-gene is instead active in the source of the leaves. When the susiba 1-gene is active it encodes a repressor which binds to the bbox-like element in the second intron.

Genomics, Proteomics and Metabolomics

Genomics is the analysis of genomes i.e. the sum of the genetic material in a cell, individual or species.

Proteomics is the systematic analysis of qualitative and quantitative changes of proteins expressed by a cell or tissue in response to a specific biological perturbation. Globally, it will increase understanding of biological mechanisms. It will also give an identification/screening of diagnostic markers.

Metabolomics is the analysis of the metabolism in the organism.

The sequencing of *Arabidopsis thaliana* was a great breakthrough that can be used to improve the genetic knowledge of other plants and to test different analysing instruments.

25 500 genes in the Arabidopsis genome

Arabidopsis thaliana has been the most widely studied plant the last ten years. It was the first plant to have its genome sequenced, in 2000, and by analysing epigenetics and chromosome structure it is possible to understand the role of methylation in regulating chromosome structure and gene expression.

The work with analysing every single gene will continue to 2005.

Arabidopsis has approximately 25 500 genes that are very closely packed in the genome. About 60% of the genes match ESTs (Expressed Sequence Tags) which is very good experimental evidence that most of the genes are functional and means that they express RNA.

Drosophila has round about 14 000 genes and the nematode *Caenorhabditis elegans* just under 20 000 genes (while the human genome has up to 40 000 genes). Arabidopsis, Drosophila and *C. elegans* make roughly about 11 000–14 000 different types of proteins. Yeast does not make more than about 5 000 proteins. Arabidopsis has a larger number of gene families than the other organisms mentioned. Around 1200 genes are present as tandem duplications.

There is evidence of duplication but not of triplication or greater repetitions. The process identifies duplicated regions of the genome. 60% are duplicated, which is the same proportion as is identified by the direct DNA comparison.

Once a sequence of genes is obtained, it can be compared with all other genes. A unique pattern is obtained. Arabidopsis devotes the largest proportion of its genes to metabolism, like any other organism, for example a bacteria. However, because it is multicellular it devotes a large amount of its genes to transcriptional control, cellular communication, signal transduction, control of cell division and cell growth etc.

30% of the genes are presently unclassifed. They are either false gene predictions, maybe 5–10%, or plant specific, which provides an interesting subject for future systematic analysis.

There are 29 gene families of 1700 known transcription factors in Arabidopsis and 16 of those families are unique to the plant. Prof. Michael Bevan, John Innes Centre in Norwich, finds the question of why plants should have evolved such a diverse range of DNA-bound proteins very interesting.

The genome sequence provides a complete record of all of the genes and of all the protein sequence because of the exquisite precision of nucleotide sequence. From that it is possible to study expression patterns of genes and to build up knowledge of co-regulated genes, the so-called regulon.

Brassica differs from Arabidopsis and Capsella

In the plant kingdom there exist a lot of differences in genome size. Whereas in other organisms there is quite a small range of genome size, in angiosperms there is really a tremendous variation in genome size in plants. Lilies have a much bigger genome but not so many more genes than Arabidopsis. Most likely it is repetitive elements spread between genes that make up the plant differences in genome size. Arabidopsis has 130 mbp/1C, rice has 450, tomato 950, maize 2 500 and wheat 16 000 mbp/1C.

Plants probably differ significantly in the number of repetitive elements. In spite of the big difference in genome size, the general structure of the chromosomes is often very similar. That means that the order of markers or genes along the chromosomes is often very similar, if not identical.

Comparative genetic mapping experiments reveal co-linearity of genomes. Tomato and potato genomes are almost identical while rice and maize genomes are very different. In maize there is some ancient duplication, so for every rice gene there are two genes in maize.

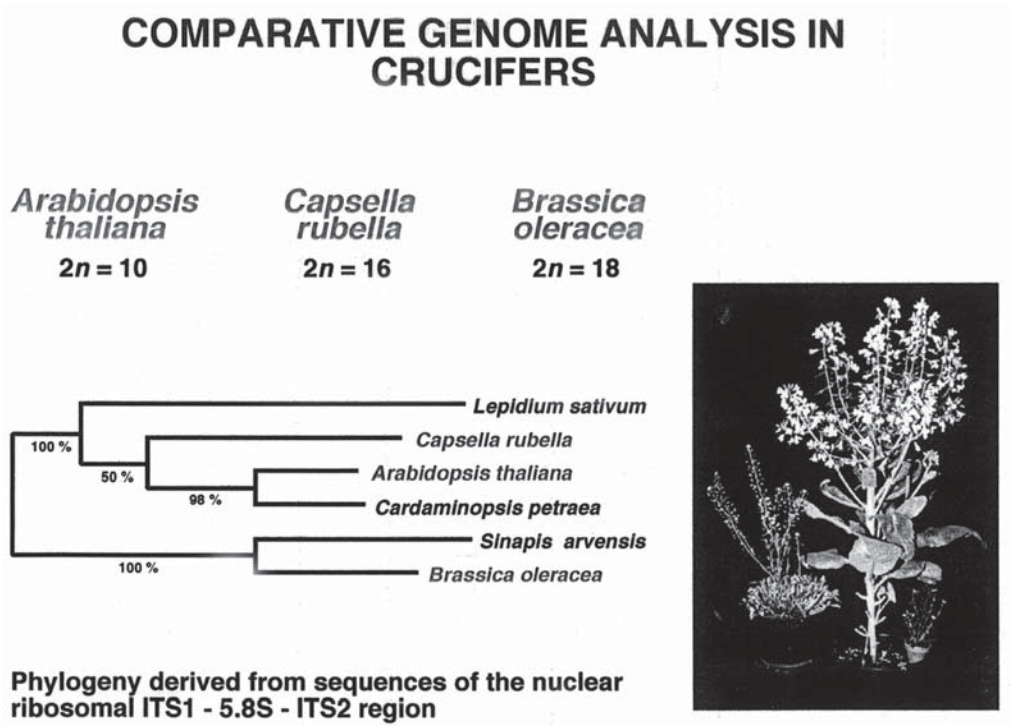
Dr. Renate Schmidt has worked in Max-Delbreuch-Laboratorium with comparative genome analysis in crucifers. She and her colleagues have chosen to compare *Arabidopsis thaliana* 2n=10, *Capsella rubella* 2n=16 and *Brassica oleracea* 2n=18. Arabidopsis and Capsella are much more closely related than either of them is to Brassica.

When maps of Arabidopsis and Capsella are compared, some growth chromosome changes would be expected. Despite the growth translocation events, the rest of the map looks largely co-linear. There are some small differences that could not have been seen if the Arabidopsis genome had not been sequenced, because all the markers in Capsella could actually be linked back to the Arabidopsis genome sequence.

The microsynteny analysis of *Arabidopsis thaliana* and *Capsella rubella* shows not only that most of the genes are similar but also that they are in the same order and the spacing between the genes is roughly the same, although not identical. The transposable elements are different in both species. A gene that is destroyed in Capsella seems to be functioning in Arabidopsis. Another difference between the genomes is that tandem duplications that are present in Capsella are the equivalent of a single gene in Arabidopsis. We know that a lot of tandem duplications occur in the Arabidopsis genome and the comparison between the Arabidopsis and Capsella shows that this is a general feature of plant genomes. Tandem duplication seem to be very important and very frequent.

More than 80% of the Capsella segments can be placed back on the Arabidopsis sequence. At least 50% of the repetitive DNA sequences go back to the low-copy nuclear DNA sequences. The sequences that match to the Arabidopsis sequences are not only

Arabidopsis and Capsella are much more closely related than either of them is to Brassica. All the markers in Capsella could be linked back to the Arabidopsis genome sequence. Illustration: Renate Schmidt



gene coding sequences but aligned sequences such as exon, intron and intergenic sequences.

When Arabidopsis and Brassica are compared, it is found that exon lengths and sequences are highly conserved, whereas intron and intergenic sequences are much more varied.

Two highly homologous partial copies of gene F are found on *Brassica leracea* Chromosome 3.

Dr Schmidt found large differences in transgene expression in independent transformants. The expression level itself, when it gets over a certain threshold, is responsible for the gene silencing.

Analyse 14 000 clones in a microarray

It is possible to identify in/dels between ecotypes of *Arabidopsis thaliana* using EST-microarrays.

With the microarray, all the genes can be viewed at the same time. Prof. Ellen Wisman from Michigan State University is studying gene expressions in different Arabidopsis ESTs to find out how many genes are regulated by daylight. With spotted microarrays, there is a glass slide which is the microscope slide and then inside this one can spot high density genome-DNA, EST-clones, CD-clones or any DNA source that is amplified by PCR. They are spotted in high density.

The AFGC array analyses about 14 000 clones: 9200 EST clones from all tissues, 2 000 EST clones from developing seeds and 3 000 GSTs (gene specific tags). The array controls are:

- Negative/spiking controls.
- Transgenes (BAR, BT, BASTA, Luciferase…).
- Positive controls.
- Dilutions of chromosomal DNA.

These are later needed to analyse the data.

Former arrays could analyse more than 11 000 clones and 99% of the re-sequenced clones in the old collection were correct. Ellen Wisman expects to have the same degree of resolution in the new collection.

The AFGC performs experiments for customers and 45% of these customer experiments are genotype comparisons of mutants, usually on Arabidopsis. The scientists take two biological repetitions, each with one technical repetition. That is a minimum. If small differences are expected, more repetitions are needed to provide higher confidence in the data; for example if comparisons between mutants and wild types are being made.

Gene expression studies have been used for gene discoveries. For the first time it is possible to have a global view of processes that were impossible before. This is a kind of experience where only the number of responding genes gives an answer.

Future perspective for global gene expression studies:

- Gene discovery.
- Global view (circadian rhythm, epigenic changes).
- Approach old problems (hybrid vigour).
- Diagnostic tool (transgenes).
- New hypothesis (looking for patterns over many experiments).
- Gene discovery in poorly characterized species.

Future perspectives: as Columbia ESTs were used, spots with higher intensities in other ecotypes are expected to result from an increase in gene copy number. In/dels can be mapped with QTLs to identify potential gene functions and interesting agronomic traits. Combining array expression in SMD may give clues to phenotypic differences between ecotypes. In/dels common between ecotypes might suggest a close relationship of groups of ecotypes. Phylogenetic relationships based on in/dels can be compared to more traditional methods.

Study the change in protein quantity

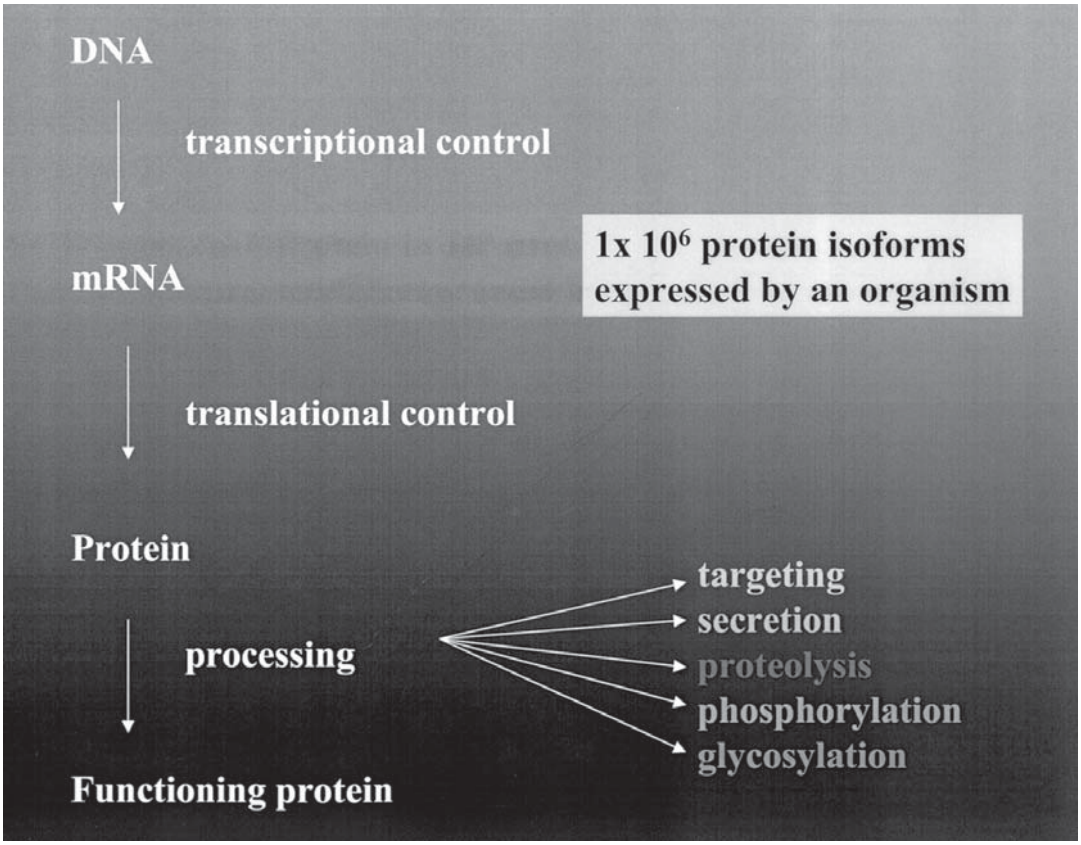
Descriptive proteomics analyse the proteome components under a set of defined circumstances. Quantitative proteomics analyse the relative amounts of proteins expressed under two or more sets of circumstances. To study the change in protein quantity scientists consider several factors such as tissue or cell type, development stage, infection, disease, mutation, environmental effects and pesticides and herbicides. In the quantitative analysis of the proteome, currently techniques are used that measure quantitative changes. They are 2-dimensional polyacrylamide gel electrophoresis (2D PAGE) and stable isotope labelling/multidimensional liquid chromatography.

The advantages of 2D PAGE are:

- 2D PAGE visualises many proteins at once (whole organisms, tissue types, sub-cellular fractions and multi protein complexes).
- It is a relatively quick technique
- The ease of detection (silver stain in the interval 1–100 ng, collodial coomassie at 50 ng–50 μg and SYPRO dyes in the interval 1 ng–100 μg.

The method also has some disadvantages: Only proportions of proteomes are visualised (membrane proteins, proteins with extremes

Proteomics is the systematic analysis of qualitative and quantitative changes of proteins expressed by a cell or tissue in response to a specific biological perturbation. Illustration: Kathryn Lilley



of pI and proteins with extremes of molecular weight (MW) are poorly represented), the resolution is co-migrated and there are low abundance species, (1 000–5 000 copies per cell), 90% of the protein is the product of 10% of the genome. Other disadvantages are poor gel to gel reproducibility and that only one sample is taken at a time.

The Difference Gel Electrophoresis (DiGE) advantages are that the method gives:

- Comparison of two samples simultaneously (it is the most sensitive method with broad dynamic range, it gives reproducibility – no gel to gel variation – and it has a multiplexing possibility).
- No staining (it requires fluorescent imager with integral spot cutter and it is compatible with mass spectrometry.
- Quantitation (image analysis performed by ImageMaster2D – output excel spreadsheet of Cy3/Cy5 ratios.

Dr. Kathryn S. Lilley and her group at the University of Cambridge were one of the first sites in the UK to work with the DiGE system. They are content with the method after analysing the data achieved with the DiGE system.

There are some points to be considered in plant proteomics: Phenols can cause protein cross-linking. The genetic background and

the standardised growth conditions must be consistent. The development stage must be matched. Contamination must be controlled. RUBISCO dominates 2D gel if photosynthetic material is used.

In the future there will be new techniques such as pre/sub fractionation techniques, isotope affinity tags, capillary electrophoretic techniques, multidimensional liquid chromatography and protein chips.

Metabolic profiling: A metabolic engineering perspective

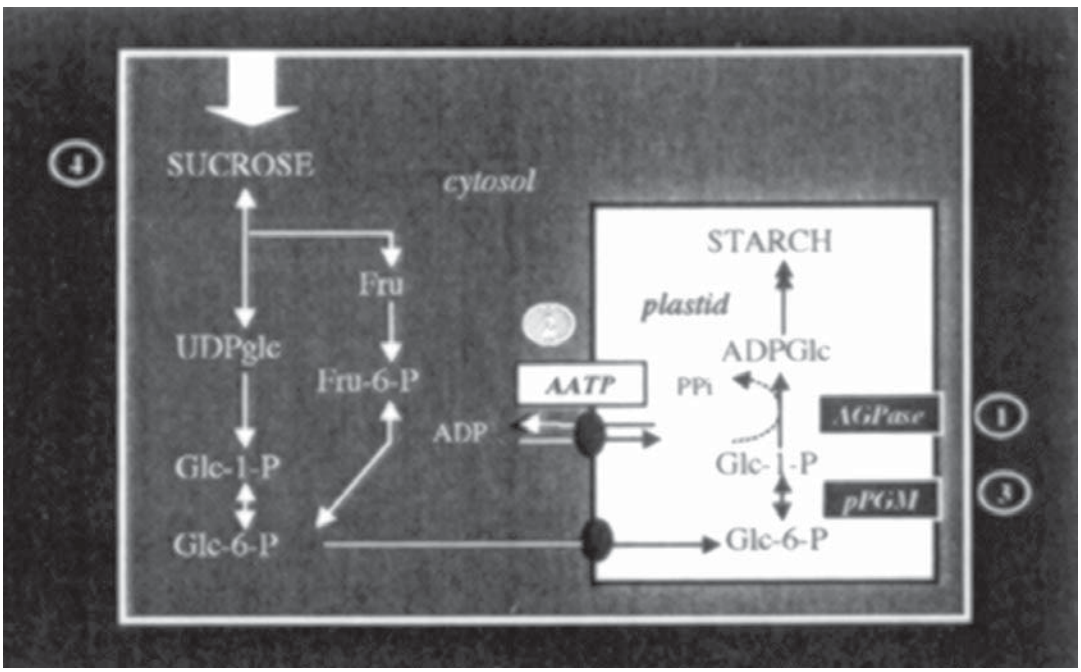
The approach for manipulation of starch and amino acid metabolism in the potato tuber is to move away from naive early approaches and towards predictable strategies. A framework of MCA is used for future strategies. Detailed characterisation based on metabolic profiling for understanding past "failures".

Dr. Alisdair Fernie, Max-Planck-Institute in Potsdam, describes four strategies:

1. Targeting AGPase: Overexpression of an unregulated bacterial AGPase resulted in increased starch yields. (Stark *et al*., 1991)

   However, in a separate experiment the increased flux to starch was coupled with an increased starch degradation (Sweetlove *et al*., 1996).
2. Targeting the plastid ATP:ADP *transporter*: Overexpression of an AtAATP has a control coefficient of 0.78 for starch synthesis.
3. Targeting the plastidial PGM. Alisdair Fernie's group generated transgenic plants in which the plastidial isoform of phosphoglucomutase was inhibited. These plants were characterised by severe reductions in starch contents, proving the involve-

The four strategies for manipulation of starch and amino acid metabolism in the potato tuber are 1) targeting AGPase, 2) targeting the plastidial ATP, 3) targeting the plastidial PGM and 4) targeting sucrose breakdown. Illustration: Alisdair Fernie



ment of pPGM in the starch synthesis pathway.

Antisense plants exhibiting inhibition in pPGM are a phenocopy of those exhibiting AGPase. pPGM has a relatively high control coefficient for starch synthesis. Therefore ectopic expressions of a non-plant PGM both individually and in combination with other proteins represent a novel strategy that may enhance starch yield.

4. Targeting sucrose breakdown. Primary characteristics of plants engineered seem to have more efficient sucrose metabolism.

The observed increase in amino acids was surprising given the use of a tissue-specific promoter. It had previously been thought that amino acids were synthesised in the leaves prior to transport to the tuber. This demonstrates that the tuber has the necessary machinery for the *de novo* biosynthesis of all amino acids and illustrates the importance of metabolic profiling in genomics approaches.

Metabolic profiling allows comprehensive phenotyping. When carried out on diverse systems this also allows the identification of phenocopies. The measurement of many different metabolites within a single extract allows evaluation of "dependency" relationships between metabolites.

Overall conclusions: A greater understanding of the control processes regulating metabolism is required before metabolism engineering becomes routine. Alisdair Fernie hopes to achieve this by assessing the steady-state concentrations of a wide range of metabolites and by the development of sensitive and accurate methods of determining cellular fluxes.

Future perspectives: Use of acquired knowledge for rational design of genetic manipulation strategies. To look deeper at the metabolic networks involved by a combinatorial approach using both radiolabelled and stable isotopes. To look at disparities in metabolism during different developmental stages.

Plant stress and signalling

Plants in all cultivation systems are more or less sensitive to stress which limits horticulture, agriculture and forestry. Stress can be frost, drought, heat, salinity and air pollution. All kinds of stress decrease the production. With more stress tolerant varieties, yields would be improved and new crops could be grown.

Hormonal regulation of oxidative cell death

To get a better understanding of stress, stress recognition and what kind of signalling the plant is using to resist the stress, Prof. Jaako Kangasjärvi, University of Turku, started by studying the effects of ozone (O_3). Ozone is a convenient tool for studying the role of oxygen radicals in signalling.

Ozone is an air pollutant and reacts with different components of the cell wall, forming reactive oxygen species there. It can never enter the cell.

Traditionally, people who have been studying ozone from an environmental point of view, as a pollutant, have believed that these radicals physically destroy the cells, like a wound response. But actually, there is plenty of evidence that reactive oxygen species are centrally involved in cellular signalling.

The classical example is the oxidative burst in plant-pathogen interaction, but it can also be found in the regulation of senescence and so on. Looking at recent papers, reactive oxygen species have been observed in many different stresses and also in some of the developmental aspects.

Arabidopsis columbia ecotype is tolerant but certain Arabidopsis mutants are sensitive to ozone, which leads to cell death. When

Prof. Kangasjärvi finds a mutant with one mutation in one gene that leads to acquired sensitivity he can later on identify that gene and say that this pathway is involved in the ozone lesion formation. The scientific group have named their mutants *rcd* (radically induced cell death). In the *rcd1* mutant, the cell death is spread from cell to cell in the leaf from the initiation sites. The cell death continues even after exposure to ozone in clean air, until almost the whole leaf is dead.

Rcd1 is a single co-dominant trait in chromosome one. It is hypersensitive to ozone and superoxide but not to hydrogen peroxide. *Rcd1* has been shown to increase initial cell death rates and prolong spreading cell deaths and superoxide is sufficient to trigger the lesion formation.

Instead of ozone damage, scientists are nowadays speaking more of the role of reactive oxygen species and how they interact with different signalling pathways, how these regulate expression of target genes and how they regulate cell death. Cell death in this case is an active genetically programmed process that requires metabolism, transcription and translation. Since there is a propagation of cell death, there must also be signals that go from the dying cells to the next ones – cell to cell communication.

Ethylene has been shown to be involved in several abiotic stresses, including oxidative stresses. Ethylene signalling is required in cell death and superoxide accumulation.

In the oxidative cell death cycle, salicylic acid enhances the toxicity, ethylene amplifies superoxide accumulation and jasmonates inhibit salicylic acid.

Conclusions: *Rcd1*, the radical hypersensitive mutant, exhibits a high ethylene peak which contributes to the spread of lesions. The ethylene insensitive *ein2* is highly tolerant to radicals because of the ethylene sensitivity and it has increased ethylene evolution because it has lost the feedback regulation of ethylene biosynthesis.

- ROS (reactive oxygene species) are important biological regulators of cell death during oxidative stress.
- ROS regulation of cell death is tightly coupled with hormonal regulation.
- ROS and hormones work really as an interconnective network where everything affects everything else and not as separate linear pathways. There is cross talk that affects the sensitivity of other pathways and so on, which is something that Prof. Kangasjärvi and his group are going to address in the future.
- Ozone is a convenient tool in probing the role of reactive oxygene species in the regulation of these things.

Defence responses to *Leptosphaeria maculans*

The fungal pathogen *Leptosphaeria maculans* (anamorph: *Phoma lingam*) causes blackleg or stem canker on several species within *Brassicaceae*. This plant pathogen is the major threat to crops like oilseed rape (*Brassica napus*) and turnip rape (*B. rapa*) and causes considerable crop losses in Canada, Australia and Central Europe.

The rapid evolution of new and more aggressive (virulent) isolates demands a more efficient use of resistance sources within the plant population. Resistance to *L. maculans* is mainly found in *Brassica* species containing the B-genome, i.e. *Brassica nigra, B. juncea* and *B. carinata*. Traditional breeding programmes have utilised this germplasm as a source of resistance, but transferring genes from interspecific hybrids demands much effort.

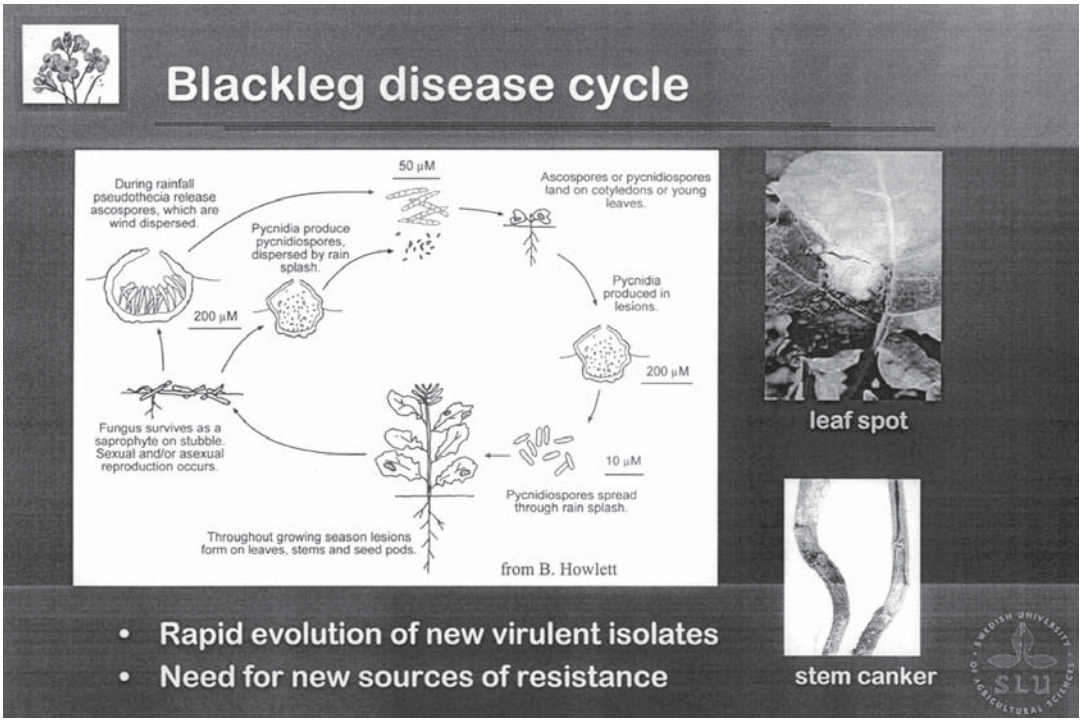
Dr. Christina Dixelius and her colleagues at the Swedish University of Agricultural Sciences in Uppsala have cloned the first gene, *Lm1*, from *B. nigra*, that confers resistance to *L. maculans*. Homologous sequences have also been found in *Arabidopsis thaliana*, rice and

partly in the *nin* gene from *Lotus japonicus*. The latter is interesting since some signal pathways in the symbiotic interactions are similar to those found in pathogenic systems. The Lm1 protein has two transmembrane regions and it is tempting to speculate that this unique part senses pathogenic attack, directly or as a part of a signal transduction pathway, while the part containing conserved motifs acts as a cytoplasmic effector domain.

Arabidopsis thaliana and *Brassica* species are closely related even if they phylogenetically belong to different genera within the *Brassicaceae* family. The similarity is further evident on nucleotide level where comparisons between *B. napus* genes and their *A. thaliana* orthologues show more than 85% identity even though the *Brassica* and *Arabidopsis* lineages diverged 12.2–19.2 million years ago. A large number of pathogen resistance genes are characterised to a more or lesser extent in *A. thaliana*. In addition to this, mutant studies have provided insight into *A. thaliana* basal defence responses against various pathogens. Interestingly, it has been demonstrated that the *A. thaliana* genome can be a source of resistance to *L. maculans*.

A number of *A. thaliana* mutants impaired in defined parts of various signalling pathways related to plant stress induced by other pathogens are known. Mutants such as *pad* (phytoalexin accumulation deficient), *npr* (non-expressor of *PR* genes), *eds* (enhances disease susceptibility), *ndr* (non-specific disease resistance), *lsd* (lesions simulating disease), and *cpr* (constitutive expresser of pathogenesis related (PR) proteins) have been evaluated in the *L. maculans* system. However, only *pad3* has given a susceptible phenotype. This finding is in contrast to e.g. the *Alternaria*

The fungal pathogen *Leptosphaeria maculans* causes blackleg or stem canker on several species within *Brassicaceae*. The disease is spread with the rain and the wind. Source: B. Howlett

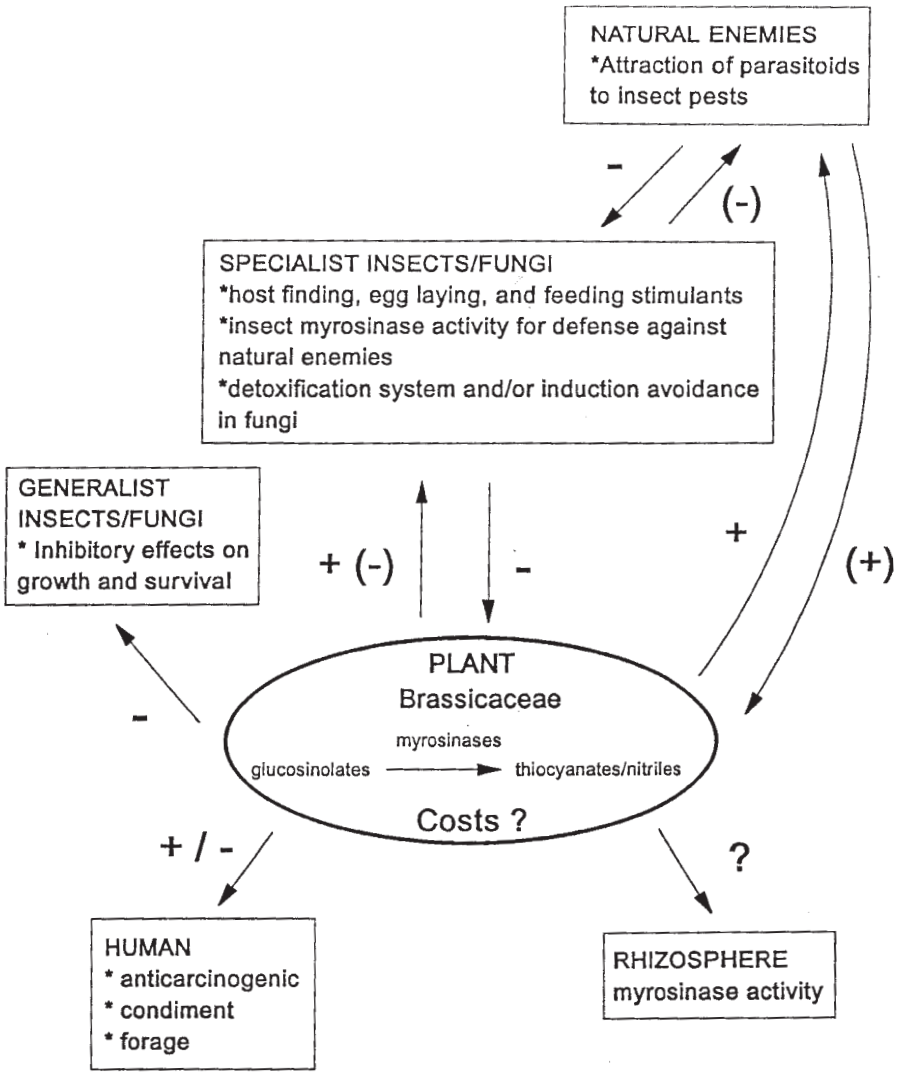


and *Perenospora* systems and suggests that there is another type of signalling in the *L. maculans* system. The *pad3* mutant is now being crossed with *lms1*, *lms2* and the *RacGap* mutant to reveal their reciprocal relationships. Such clarification is of high importance since *lms1* and *lms2* give a susceptible phenotype but show wild type level of camalexin.

Wound responses and insect defence

Dr. Johan Meijer, Swedish University of Agricultural Sciences in Uppsala, is studying biotic stress in plants and wants to understand the basic architecture of insect defence/ wound responses (constitutive – wound in-

The myrosinase-glucosinolate system serves as a defence in oilseed rape (Brassicaceae). Glucosinolates are secondary metabolites found in all Brassica plants. Illustration: Johan Meijer



ducible), the regulation of wound responses (signalling, cross-talk and gene activation) and the possibility to improve the insect pest resistance.

Model organisms are *Brassica napus* (oilseed rape), *Arabidopsis thaliana* and *Pichia pastoris* (for overexpression).

The myrosinase-glucosinolate system serves as a defence in oilseed rape. Glucosinolates are secondary metabolites found in all Brassica plants. These plants also house the enzyme myrosinase. Myrosinase degrades glucosinolates into toxic products e.g. thiocyanates/nitriles, which are harmful to pests. A number of other proteins are associated with myrosinase in seed extracts, such as myrosinase binding protein (MBP) which is a lectin and myrosinase associated protein (MyAP), and which houses a lipase motif. The role of complex formation is not clear.

Myrosinase cellular location: The distribution of myrosinase containing idioblasts is different in *Arabidopsis thaliana* and *Brassica napus*. In Arabidopsis, myrosinase is localised in idioblasts present in the phloem. In *Brassica napus,* myrosinase is localised in idioblastic myrosine cells in the ground tissue and in idioblasts in the phloem.

Myrosin "grains" form a continuous reticulum in *Brassica napus* myrosine cells as visualised by confocal laser scanning microscopy.

The third myrosinase gene (TGG3) in Arabidopsis is a pseudogene but is specifically expressed in stamen and petal.

Conclusions for oilseed rape seed analysis:

- SeedMBP ubiquitous expression except myrosin cells
- Myrosinase-MBP complexes do not exist *in vivo*
- SeedMBP necessary for complex formation
- Free MB myrosinase is soluble and active
- No effect of as seedMBP on pests tested.

Down-regulation of vegetative and wound-inducible myrosinase binding proteins in *Brassica napus* affect susceptibility differentially in different Brassica specialist herbivores.

Regulation of the plasma membrane H^+-ATPase by phosphorylation and 14-3-3

The plant plasma membrane H^+-ATPase is an abundant enzyme that builds up an electrochemical gradient across the plasma membrane by pumping protons out of the cell. This gradient provides the energy that drives secondary active transport of nutrients and solutes across the plasma membrane. Consequently, the H^+-ATPase is thought to play a major role in many cell processes and its activity is regulated by a number of physiological factors, including hormones, blue light and fungal toxins.

Prof. Marianne Sommarin and her colleagues at Lund University have earlier shown that treating intact leaves with the fungal toxin fusicoccin results in activation of the H^+-ATPase and that this activation is achieved by binding of 14-3-3 protein to the C terminus of the H^+-ATPase.

14-3-3 proteins constitute a family of eukaryotic proteins that are key regulators of a large number of processes ranging from mitosis to apoptosis. In plants, the most well studied examples are the plasma membrane H^+-ATPase and two enzymes of the primary metabolism, nitrate reductase and sucrose phosphate synthase. Nitrate reductase and sucrose phosphate synthase are both inhibited by 14-3-3 binding, in contrast to the activation seen with the plasma membrane H^+-ATPase. 14-3-3 proteins usually bind to phosphorylated motifs in their target proteins. However, there is no amino acid sequence in the C terminus of the H^+-ATPase with any obvious similarity to the established binding motifs for 14-3-3.

Following *in vivo* phosphorylation using radiolabelled $^{32}P_i$ and fusicoccin, Prof. Som-

marin and her group identified a phosphorylated amino acid in the C terminus of the H^+-ATPase, namely Thr-948. Thr-948 is the penultimate amino acid in the C terminus of most plasma membrane H^+-ATPase isoforms. They could show that phosphorylated Thr-948 is part of a binding site for 14-3-3 *in vitro*, a binding site that differs strongly from the established binding motifs. The physiological importance of this novel motif was shown by heterologous expression of a plant H^+-ATPase in yeast. A mutation in the motif (exchange of Thr-948 for alanine) abolished 14-3-3 binding and activation of the plant H^+-ATPase, and prohibited yeast growth, which in this yeast strain was dependent on H^+ pumping by the plant H^+-ATPase (the gene for the yeasts own H^+-ATPase was inactivated).

In all plants analysed so far, the plasma membrane H^+-ATPase and 14-3-3 proteins are encoded by multigene families. The genome of the model plant Arabidopsis harbours twelve H^+-ATPase genes and fifteen 14-3-3 genes, and most of them are expressed. An obvious question is whether the many isoforms in multicellular organisms reflect different functional roles, i.e. does isoform specificity towards target proteins exist? Is this achieved by differences in tissue/subcellular distribution and/or sequence differences in the interaction sites between the two components? The scientists are presently investigating the expression pattern and tissue/subcellular localization of the different isoforms in plants exposed to various forms of stress to address this issue. In addition, they are studying the interaction between the different 14-3-3 isoforms and phosphorylated peptides mimicking the C-terminal binding motifs of the different H^+-ATPase isoforms.

Forest genetics

According to the FAO report "The State of the World's Forests", there is a yearly decline of 12 million hectares due to an increasing consumption of wood and all kinds of wood products and due to an increased urbanisation. It is mainly in developing countries that forestry land is being converted to agricultural land. This decline is expected to continue. In the same time there is a growing environmental awareness of the need to protect the remaining forests. The simple conclusion is that there is a need to increase the yield and the quality of wood of tree species that can be grown in agro forestry systems. Forest genetics can be a valuable tool for this, especially considering the long rotation of forest trees and the natural slow improvement with traditional tree breeding.

Genetic engineering improve poplars

Breeding is the main approach to improvement of quality and yield. Genetic engineering can also be used. Disease resistance is the major selection criterion for breeding in poplar trees and an important factor determining yield.

When breeders started the resistance work against the major fungus

Melampsora larici – populina, they used interspecific crosses between the resistant American *Populus deltoides* and the native *Populus nigra*. The result was highly productive interspecific hybrids that were completely resistant to this pathogen. These clones have been spread over Europe.

Over the years, new varieties of the pathogen have occurred and broken down the resistance. As a consequence, there is currently not a single resistant clone that is commercially available.

Together with the Poplar Breeding Institute, Dr. Wout Borjean and his team at the University of Ghent have initiated a programme to understand the genetics of inheritance of the disease resistance in order to progress slowly to a marker resistant selection.

The first step was to create genetic maps of the three most important poplar species for breeding world-wide. The maps were used to dissect resistance to Melampsora. The scientists grew the poplars in greenhouses to make sure that they were not infected by other diseases. Approximately 50% are completely resistant while the other 50% are spread over all the other classes. This is an agreement with a model where the female parent, which is resistant, is heterozygous for a dominant gene which confers resistance and then segregates into a Mendelian ratio in the population.

Resistance to the three races completely co-segregates so there is a major locus.

Prof. Borjean was interested in cloning the resistance gene by positional cloning. He then had to saturate the locus with genetic markers. His team looked for primer combinations – "fingerprints" – that gave them markers that were present in the resistant parent, absent in the susceptible parent, will be present in the bulk of resistant individuals and will be absent in the bulk of susceptible individuals. Using 540 primer combinations they have identified 11 markers, and could, with the markers, identify 16 recombinants in their 502 progeny plants from two mapping pedigrees. The scientific team ended up with a locus of ten markers in coupling and one in reversion.

Interestingly enough, it looks as though this is a large resistance gene cluster.

The future plans for this project are to map these R-gen clusters and see whether they coincide with qtls. It is also important to integrate all the poplar maps world-wide.

Genetic engineering – in this case engineering lignin biosynthesis – is also a tool to improve wood quality for the pulp and paper industry. Roughly 50% of wood is made up of cellulose, 25% hemicellulose and 25% lignin. For making high quality paper this lignin fraction needs to be degraded by chemicals and needs to be extracted and separated from the cellulose. This is a very energy requiring and toxic process. The question is therefore whether it is possible to reduce the amount of lignin in trees or to change the composition of lignin in order to make it easier to extract.

A lignin molecule is a polymer with three units and with the creation of different types of interunit linkages. If the ratio of these units in lignin can be changed, this will also change the ratio of the linkages and as a consequence will interfere with the extractability of lignin.

The intentions of Prof. Borjean were to modify the composition of lignin. He cloned the different enzymes of this pathway, making antisense constructs and putting them in transgenic poplars. The first enzyme analysed was AS Comt. It did not change the amount of lignin but it drastically changed the composition. Prof. Borjean also found a novel unit incorporated into the transgenic plant.

The results so far indicate that it is possible to reduce the lignin in the tree as well as change the composition of the lignin and that less chemicals are needed to process the transgenic poplars.

Functional genomics of wood formation

A tree has round about 40 000 genes. Prof. Göran Sandberg, Swedish University of Agricultural Sciences in Umeå, and other Swedish scientists are working in a national EST-programme – The Swedish Tree Functional Genomics – on the first large scale sequence analysis of genes in a tree. They are especially interested in identifying all the genes that direct the formation and structure of the fibre.

The project concentrates on two different types of genes that are crucial to the practical implementation in forestry. One type affects formation of wood and stem growth. The other type of gene is important for stress tolerance. Wood formation and the ability to survive under harsh climates are unique characters for trees.

The initial stemcell can develop into different types of cells. Wood properties are determined in a developmental gradient, with the developmental decision being taken in the cambium.

In early spring, the number of xylem mother cells determines the amount of wood produced together with seasonal and other effects on the gradient. A bit later in the season, the cell has gone through the elongation zone and starts to form the secondary wall. When this cell enters the expansion zone, it has only the primary cell wall. In late season, the cell gets the secondary wall much earlier. There is also an effect of the climate on this gradient.

The dormant cambium provides a way for a conifer to survive for 700 years.

The point with the technology is that with the wide range of data and all pathways for amino acid synthesis etc, experiments can be initiated. The advantage is that it is possible to go in and look at all the pathways at the same time.

The most important thing for wood development is gene function. Functional genomics is just a way to get to gene function. The project has a database with real tissue specific expression of genes. If there is a gene that is expressed in the cell division zone, it might be a candidate for regulation of this process. This is a way to find key regulatory factors.

How can gene function be analysed? If there is high homology to an Arabidopsis orthologue, it can actually be done in Arabidopsis because secondary growth can be induced in Arabidopsis. If it cannot be done in Arabidopsis, it has to be done in poplars. Poplars are much closer to Arabidopsis than tomatoes or tobacco are to Arabidopsis. Poplar ESTs have a high similarity to Arabidopsis, but Arabidopsis has big families, which is a disadvantage if one wants to knock out genes and be sure they are compatible.

Basically, when going from the expression pattern in poplars it can be predicted that this has something to do with vascular formation and then it can easily be checked that the mutant is found in Arabidopsis.

If the process has to be carried out in poplar, there is a method to select good candidate genes and the project is now evaluating strategies to do this. The goal is to knock out at least 2 000 poplar genes at the Centre for Forest Biotechnology and Chemistry. The project will select interesting candidates, not knock out in random.

Metabolic profiling is another tool that is being developed.

Enzymes for making and breaking wood fibres

Enzymes can be used to change the structure, surface, composition and function of the fibres from the cell walls of plants/trees. By identifying, examining and modifying enzymes, scientists can make better and more exact changes.

Prof. Tuula Teeri, Royal Institute of Technology in Stockholm, is an expert on the enzymes that are involved in both the biosynthesis and biodegradation of wood fibres. The three major components of wood are cellulose, lignin and hemicellulose. Cellulose is the load-bearing structure that one usually wants to maintain in all kinds of application: papers, tables, houses etc. Chemically it is a very simple polymer, stacked together with other molecules in sheets and the sheets stack on each other forming crystals. These long fibrils pack further to long fibres, used by the pulp and paper industry.

The cell wall also consists of other components like hemicellulose and with a backbone

of one or different sugars which can be xylose, glucose etc. It is linked to different side groups which have the effect that these polymers usually are totally or partly soluble, amorphous or only very partially insoluble. The cell wall holes between cellulose and hemicellulose are filled in with lignin.

Sometimes it is desirable to get rid of or reduce the lignin when making paper. For example, residual lignin causes yellowing in paper. There are many mechanical and chemical processes involved in pulp and paper production but a possibility is that some of them could be displaced or facilitated with enzymatic processes.

In nature, there are many microorganisms, fungi and bacteria that, with the help of extracellular enzymes, break down and utilise plant material in a controllable way. Nature has probably developed thousands of enzymes to deal with the different aspects of wood degradation or biosynthesis. To achieve total hydrolysis, it is necessary to use mixtures of enzymes or even a whole organism, since no single enzyme is able to achieve complete degradation of the complex raw material.

In contrast, for pulp bleaching, paper making or the textile industry, one selects one or two enzymes that can remove selected fibre components but that do not harm the fibres further. The extracellular enzymes are specialised in different components in the cell wall and affect the composition as well as the structure and other characters.

With enzymes, it is possible to reduce the amount of lignin for bleaching or do some surface modification of the cell walls to perform better in paper making. Enzymes can make them react better with other agents.

In addition, enzymes could improve the reactivity of the main component, cellulose, because there is very little chemistry on the cellulose surface.

Prof. Teeri observes that efficient large scale production of enzymes is not yet possible, but she is sure that the pathways will be very different from now on and that there will find new enzymes to utilise.

The longer night length influences the phytochrome

Two major questions concern how the leaf adapts to its environment and what is the genetic basis of autumn senescence.

32% of ESTs (Expressed Sequence Tags) encode photosynthetic proteins and 37% encode chloroplast proteins in leaf protein synthesis. All plastid-encoded proteins are part of multi-protein complexes together with nuclear-encoded subunits for which synthesis can be estimated. The plastid protein synthesis in young Populus leaves corresponds to approximately 25% of nuclear protein synthesis. 50% of all leaf proteins are located in the chloroplast.

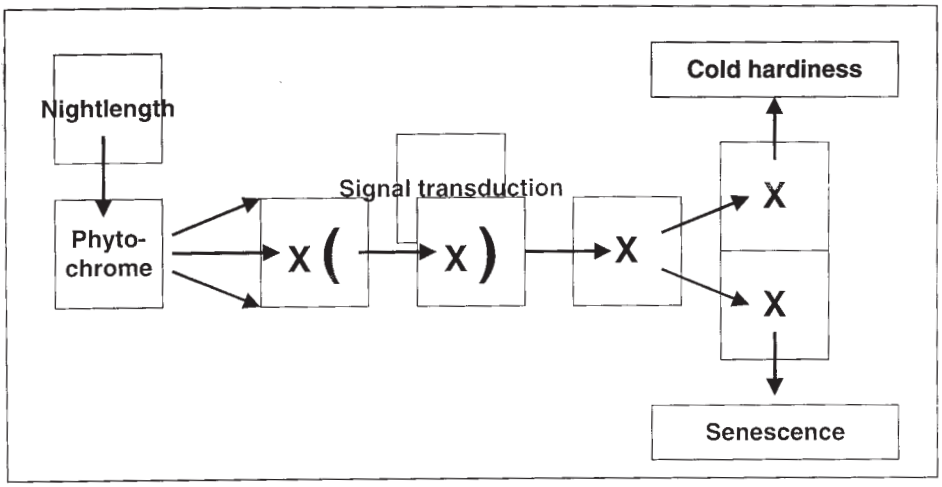
The previous day's weather is most important in determining PsbS mRNA levels, so there must be a signal that is stable overnight.

RNA accumulation precedes yellowing in autumn senescence. Different sets of genes are expressed and 2 700 different genes were found among 5 200 ESTs sequenced. Many of them have "unknown" functions. There are many proteases, stress-induced genes etc. During the senescence, there is a change from photosynthesis to respiration. RNA disappears two weeks after yellowing and the gene regulation patterns change. The genetic process is developmentally controlled.

The hypothesis for the genetic regulation of autumn senescence is that the longer night length influences the phytochrome. With signal transduction the tree obtains cold hardiness and is subject to senescence. The long-term goal is to analyse every step and learn to manipulate the senescence for different needs. Earlier senescence might increase the hardiness and later senescence might increase the yield.

The hypothesis for the genetic regulation of autumn senescence is that the longer night length influences the phytochrome. With signal transduction the tree obtains cold hardiness and is subject to senescence. Illustration: Stefan Jansson

Genetic regulation of autumn senescence Hyopthesis and long-term goals:



Earlier senescence > increased hardiness? Later senescence > increased yield?

Conclusions of Dr. Stefan Jansson, Umeå University:

- 45% of all proteins and 32% of nucleus encoded proteins synthesised in young Populus leaves have a role in photosynthesis.
- Plastid protein synthesis is approximately 25% of cytoplasmatic protein.
- The expression of PsbS is regulated by the weather.
- The weather of the previous day is most important.
- A new set of genes (for example many proteases and stress-related proteins) is expressed during senescence.
- Many "novel" genes are induced during senescence.

Plant development

A better understanding of plant development can give new tools to better growth in plants. Periods of growth and dormancy vary in trees. Can they be changed without disturbing the biological system? Members of the SHI gene family are involved in the control of cell expansion and perhaps also cell fate specification. They may regulate auxin responses. Auxin is one of the most important signalling molecules, which achieves the necessary balance between the root system and the rest of the plant.

A conifer perspective on reproductive organ development in plants

All the major angiosperm families were established 80–90 million years ago, which means that they are relatively young compared to other land plants. They are also closely related.

Most of the work on conifer trees has been focused on reproductive organs, mainly for practical reasons. Male and female reproductive organs are born on separate axes. The seed cone is surrounded by the remnants of the pollen cones. The angiosperm flower, on the other hand, is a truly unisexual organism.

Are there genetic similarities between the regulating mechanisms that control seed cone in conifers and the mechanisms that control the angiosperm flower (Arabidopsis)? Pollen cone is the male organ and seed cone the female.

The starting point of the work was data from Arabidopsis and other angiosperms. The basis for the ABC-model is two Arabidopsis mutants. In total, the Arabidopsis has a hundred MADS-box genes and 4 of these are part of A, B, and C functions. The genes present in Arabidopsis have evolved by a series of gene duplications indicated by branches in the "evolutionary tree" and followed by sequence divergence and functional divergenes. The increasing complexity of the gene family is perhaps related to the increasing complexity of the structure.

There are MADS-box genes that are active during the reproductive development in conifers as well. Prof. Peter Engström and his collegues at the Uppsala University isolated a few clones.

In angiosperms, a gene that is expressed in one type of organ has a role related to that specific organ. There are functions related to augiar development and augiar development is something different from genosperms and angiosperms.

The scientists found three genes that are phylogenetically related to AP3 and three pestilata genes that have the B-genes in the angiosperms. The DAL 11, 12 and 13 genes are all related to B but they have different functions.

Angiosperm genes can convert females to males.

B-genes and C-genes are present but no A-genes have been found. The B- and C-type system for regulating the reproduction development is an ancient feature common to genosperms and angiosperms.

By studying the reproductive development of Arabidopsis, the scientists have at least come up with a possible model for how reproductive development is controlled in its central aspects in conifers.

Regulation of cambial development

Dr. Rishikesh Bhalerao and his collegues at the Swedish University of Agricultural Sciences in Umeå are working with two model systems, Arabidopsis and poplars. The chosen process can be studied much better in poplars and the knowledge can be utilised in the further Arabidopsis research.

These scientists are studying the seasonal activity of the vascular cambium.

The tree shifts between active growth and dormancy. Some trees stop growing rather early in the north, which is because they need a longer preparation period for the winter. But different trees need various amounts of time for the preparation depending on how fast the tree gains cold hardiness. From a production point of view, a longer period of growth and consequently a short winter preparation period would be beneficial. The actual winter is hard to influence.

Dr Bhalerao wants to find the answers to three questions:

- What is the role of auxin transport in dormancy transition?

- What are the targets of dormancy inducing signals in the cell cycle machinery?
- How does auxin sensitivity regulate cell division?

Auxin transport has been shown to play a key role in cell division in the vascular cambium and in maintaining its identity as a merismatic tissue.

Poplars grow rather fast (compared with other trees), they have a limited genome size, some genetics work is possible on them and there exists a very excellent EST sequencing programme. Transcriptional profiling and genomics etc. are more reasons for using poplars.

The vascular cambium makes a transition from active to passive when the tree ceases to grow. The auxin transport is reduced as the tree stops growing, but even after the cell divisions have stopped there is considerable capacity in the cambium to transport auxin for a long time. That means there are two mechanisms operating. One which reduces the capacity first and the cells later. Those cells lose their capacity to transport auxin. The other mechanism operates when the poplars break. There is a tremendous increase in the transport capacity in the vascular cambium – prepared to handle all the auxin that comes down.

The auxin transport is reduced because the auxin handling genes are turned off.

Cell cycle machinery is very conserved from animals to plants and basically consists of four phases. Six enzymes (two in poplar) interact with a group of proteins, called cyclins. The cyclins are specific for the different phases and help the transitions. The dormant cambium is supposed to be "arrested" at one of the phases. The protein level is cut down during dormancy. This is the first level of regulation, which is post transitional. At the second level, the activity of protein goes down even when the level is constant. These activities are all driven by a pathway called cell division auxin transporters. There are very few genes in common between the active cambium and the dormant cambium. There are fewer expressed genes but they are expressed more often. Dr Bhalerao and his team cannot yet answer the question of how activity goes up and down when the switch from active to dormant cambium occurs.

In another project, the development of xylem is being studied. Xylem is arranged into distinct developmental zones. How does this kind of pattern get specified? There are several genes involved in different kinds of regulation. Chilling alters the sensitivity of the resting cambium to the applied 1AA gene. Dormancy is a complex but excellent system.

Members of the SHI gene family are involved in the control of cell expansion

Dr. Eva Sundberg, Uppsala University, is interested in genetic regulation of plant development and has focused on Arabidopsis and the SHI gene family, as it appears that they are involved in the regulation of development.

The SHI gene family consists of at least ten members and they interfere with GA- and auxin signalling when constitutively expressed. The SHI gene family encodes proteins that have a RING domain, which is characterised by a number of conserved cysteine and histidine residues. It has been suggested that RING domains are exclusively involved in protein-protein interactions. A large number of the RING finger proteins has been shown to be involved in ubiquitination for targeted degradation. In addition, some proteins including RING 1 itself appear to be involved in transcriptional repression via interaction with chromatin remodelling proteins.

The *SHI* gene was identified by activation tagging. The *shi* mutant, over-expressing *SHI* from a strong and constitutive promoter, resembles GA deficient dwarfs in several as-

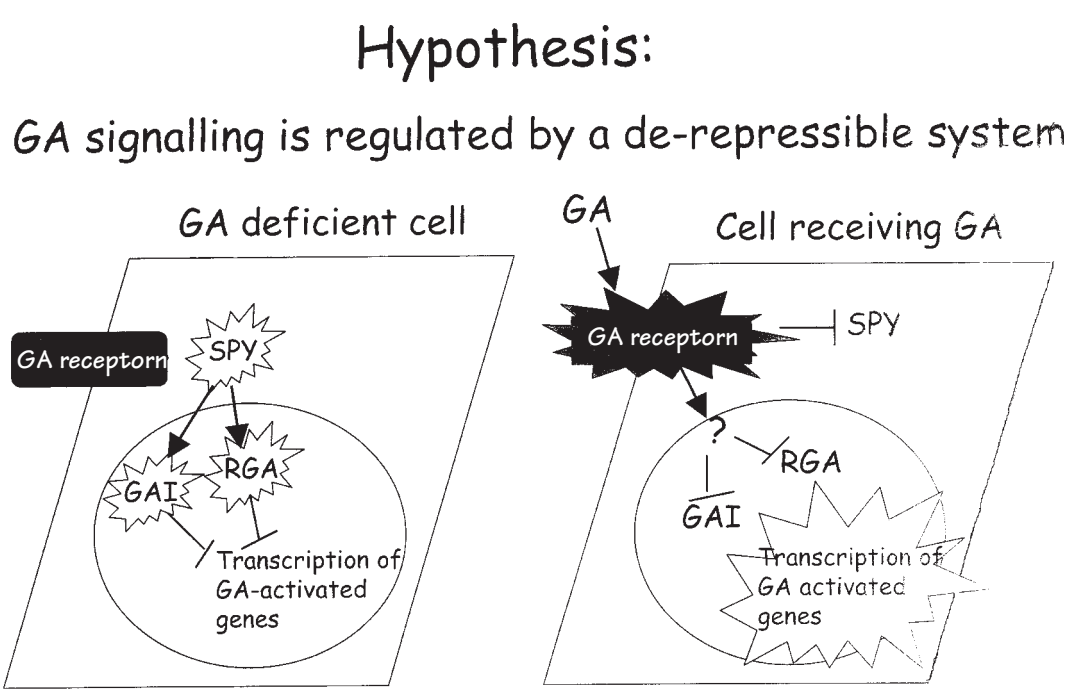
pects; reduced internode elongation, darker green rosette leaves and delayed flowering in short days. However, exogenous application of GA does not restore the dwarf phenotype of *shi*. A recent hypothesis predicts that GA signalling is regulated by a de-repressible system. Dr Sundberg is therefore testing whether *SHI* is acting as a repressor of GA responses. GA responses and signalling are well characterised in barley aleurones. At the time of germination, GA is transported from the embryo into the starchy endosperm of the barley grain. GA signalling in the aleurone tissue results in the activation of genes encoding hydrolytic enzymes. These hydrolytic enzymes are secreted into the starchy endosperm where the starch is degraded into solutes that will be used by the germinating embryo.

Dr. Sundberg could show that *SHI*, when expressed in barley aleurones, can repress GA induction of the genes encoding hydrolytic enzymes. This suggests that *SHI* at least when ectopically expressed can act as a repressor of GA induced gene expression.

Constitutive *SHI*-expression also appears to repress auxin responses, such as lateral root initiation. Dr. Sundberg has screened for insertion mutations in *SHI* and the other *SHI*-family members to get a better understanding of the gene function. Knock-out mutants of both *SHI* and another member of the gene family, *STY2*, show no phenotypic deviations from wild type suggesting that members of the family may have redundant functions. This is supported by the fact that constitutive expression of other SHI-family members confers similar changes as *35S::SHI*.

The only member of the gene family whose function cannot be compensated for by other family members is *STY1*. *Sty1* shows a subtle

A recent hypothesis predicts that GA signalling is regulated by a de-repressible system. *SHI*, when expressed in barley aleurones, can repress GA induction of the genes encoding hydrolytic enzymes. Illustration: Eva Sundberg



style and stigma phenotype, which is enhanced *in sty1 sty2* double mutants. The style cells are irregularly shaped and reduced in number in single and double mutants. In addition, a novel trait, leaf serration, appears in the *sty1 sty2* double mutant.

Conclusions: Members of the SHI gene family are involved in the control of cell expansion and perhaps also cell fate specification. They may regulate both gibberellin and auxin responses. They exhibit partly redundant functions.

The hidden half: Dissecting the genetic and hormonal control of root development

There must be a balance between the root system and the rest of the plant. The balance is achieved through root shoot signalling molecules. One of the most important signalling molecules is auxin, which is transported to the root where it regulates root architecture. Classically, for some seventy years or so, it has been imagined that auxin is transported to the root through polar auxin transport. Prof. Malcolm Bennett, Nottingham University, challenges that concept.

The group at Nottingham University has recently demonstrated that auxin produced in aerial tissues has a profound effect on root architecture. Following seedling germination, aerial derived auxin is transported to the root tip where it promotes lateral root initiation. Following seven days of growth, auxins produced in the very first leaf buds promote the emergence of lateral roots. Hence, the signals that go between the shoot and the root are of great importance to root architecture.

There are several different auxin carriers in plant cells. Auxin is transported into the cell through an influx carrier. Auxin then moves to the base of the cell where it exits through an efflux carrier. Mutations in both of these carriers have been identified in Arabidopsis. Mutations in two auxin influx carrier genes, AUX1 and LAX3, both affect lateral root development. The *aux1 lax3* double mutant exhibits an additive lateral root phenotype. The genetic additivity is likely to result from the non-overlapping expression patterns of AUX1 and LAX3. The function of the AUX1 protein is to provide the cells of the developing lateral root primordia with the ability to accumulate auxin and therefore promote cell division. In the case of LAX3, Prof. Bennett believes that its role is to facilitate the radial redistribution of auxin from a vascular source to the developing lateral root primordia. If both genes are knocked out it will be much more difficult for the root cells to obtain auxin and divide.

Transgenics: Not “if” but “why”

Let us now change perspectives and discuss the outlines and the results of the conference. In common opinion transgenics in agriculture – but not in medical use! – are controversial. GMOs are a fact of public preoccupation and opinion, which politicians must take into account. There is further discussion of this issue later in this report.

However, when scientists meet to report on and exchange scientific achievements there are very few objections against the technology itself. Genetically modified organisms (GMOs) are a fact of modern agriculture and are here to stay. The advantages are greater than the disadvantages.

The starting point for Louise Fresco, FAO, was that genetic modification has increased production in some crops but that the technology has so far addressed too few challenges, in few crops of relevance to production systems in many developing countries. Genetic modification is not a good in itself, but a tool integrated into a wider research agen-

da, where public and private science can balance each other. Scientists must not be blinded by the glamour of cutting-edge molecular science for its own sake. Louise Fresco is worried about the disconnection between the laboratory and the field and the decline of agronomy as an integrative science. She wants to see a scientific approach that in a globalised world serves local agriculture and local food security and helps keeping the rural areas liveable and attractive to young people in search of employment.

Where are we heading?

Gene technology can be utilised to increase the quality of the plant material. So far, the most widely used transgenics are resistant to pesticides rather than to pests. However, science seems – at least in the near future – to be able to meet the demand of consumers and producers in developing healthier, stronger and better producing plants. The New Scientist has just recently reported that biotech researchers in the US have knocked out a gene in soya that causes allergic reactions. They used a "gene silencing" technique called sense suppression, a technique that is also used by Donald Grierson and other scientists who participated in the seminar.

Louise Fresco's demands could have been challenging for the scientists present. Maybe they were – at least they agreed with her. However, none of the scientists at the seminar devoted themselves to the poor people in the third world – those for whom Louise Fresco had acted as a spokeswoman. Rather, the scientists devoted themselves to their science, albeit a science with many promising applications: non rotting tomatoes, healthier oils and crops that are resistant towards frost, drought and diseases.

Genetic engineering uses expensive techniques – how are we to share the money? How can we be most efficient? How can scientists, universities and research institutes retain their independence in scientific outline and control of the results? Many important questions – but beyond the aim of this seminar.

But what is a transgenic?

A transgenic plant is a plant with one gene altered – and all of a suddenit obtains completely new properties. One gene might influence the aroma and other characteristics that in the mother organism seemed to be independent of the desired hereditary character.

A genetically modified organism – GMO – is different from an organism developed with traditional animal or plant breeding, as the GMO can contain genes from any living being, while the traditionally bred animal/plant can receive hereditary characters only from individuals from the same species.

It is still unclear what consequences the origin of the gene might cause to the GMO.

Stable or unstable?

A critical point with GMOs is the stability. When a GM experiment is carried out, there can be a range of expression levels which vary from one plant to another. Some of those are unstable and unwanted for further use. However, according to Donald Grierson's experience of tomatoes, it is quite easy to identify, measure and select tens or maybe hundreds of lines that are stable. Some of his GM tomato lines are twelve years old.

Antisense technique

Antisense technique means that isolated genes are replaced one by one in a "backward" order. Donald Grierson first introduced the technique to study the ripening and

senescence process in tomatoes. However, the use is much wider than that. Silencing a gene allows one to study the reactions of the organism, to get a proper understanding of the biological function and role of the actual gene. It provides an exquisite and very precise view of a gene's function. The sense gene makes sense-RNA and the antisense gene makes antisense-RNA. Changing the structure of the sense gene affects the way in which silencing occurs.

Using anti-sense or knock-out techniques, beta-carotene genes in tomatoes and golden rice have been identified. Beta-carotene is a pro vitamin A and gives the tomato/rice a yellow colour.

What can be learnt from *Arabidopsis thaliana*

Arabidopsis thaliana is a tiny, plain plant – a weed to many people. Despite its humble nature, it has become famous because in the year 2000, it became the first plant to be totally genetically sequenced. By analysing epigenetics and chromosome structure it is possible to understand the role of methylation in regulating chromosome structure and gene expression. And now it seems that all scientists are working on Arabidopsis.

There are several reasons for working on *Arabidopsis thaliana*. Arabidopsis has a very short growth cycle – results can be achieved within five weeks – and the plant has naturally a lot of varieties, which makes it suitable to use for finding tools for expressing natural variation. For instance, there exists a Stockholm variety with a perennial behaviour, an adaptation to the short growth period to the latitude of the Stockholm area.

In addition, with very little experimentation plant genomes can be looked at in a very efficient way because Arabidopsis can be used as a template to learn more about other genomes. If there is high homology to an Arabidopsis orthologue, gene function in Arabidopsis can be analysed because it is possible to induce secondary growth in Arabidopsis.

Surprisingly enough, poplar trees are closer genetically to Arabidopsis than tomatoes or tobacco.

Food or non-food applications

Agriculture is in the first instance about food production. With a scarcity of food in vast areas of the world, the food problem and scientific work on food has a priority in agriculture and horticulture.

However, starvation and food shortages cannot be sustainably solved with distribution of food from the rich to the poor world. That fact opens up for non-food applications, at least in the rich world.

More and better food to feed the world

The biggest advantage with gene technology is that once all the difficulties with the technique have been solved, improvements will come much quicker than with conventional plant breeding.

Drought-stricken Southern African farmers would benefit from a drought resistant maize variety. A-vitaminised golden rice is already a fact.

Fruit and vegetables with a prolonged growing period (with later senescence) are suitable for farmers, trade and consumers. New unsaturated oils can reduce heart disease.

Replace mineral oils with vegetable oils

The annual consumption of mineral oil is 3200 million tonnes, of which 220 million tonnes are used in the chemical industry. Dr. Ulf Ståhl pointed out that after some more scientific work it would be possible to replace these finite, non renewable and chemically complex mineral oils, which require extensive chemical processing, with renewable vegetable oils with defined and desired qualities that do not require a lot of chemical processing.

Of course, that is a complicated task and it is a scientific challenge restricted by public opinion. The industrial capacity for industrial crops is uncertain, since the existing industry effectively forms a barrier against building a new industry. Mineral oil is a difficult competitor as it is relatively cheap. New oils might be toxic, which means that they could by no means be mixed with edible oils and that measures would have to be taken to protect the environment, birds and animal feeds.

Plastics are the most important potential of oilseed plants.

Energy in the field

Salix is a common energy crop in Sweden. It has been genetically improved and yields much more than the wild type. However, energy could also be produced in other forms. Enzymes could make methane production from manure more efficient, genetically modified grasses could yield more energy and vegetable oil could replace mineral oil.

Designing starch

Starch can be used both as a food and as a non food crop. “Designing” starch properties can be done in different ways.

- By modifying the amount of amylose it is possible to modify the properties in a radical way. A potato without amylose can for instance be used for textile fibres.
- Amylopectin can change the starch synthesis and the branching enzymes. The starch from an amylopectin free potato is hardly swollen at all.
- Little is known about controlling granule size, shape and number but they are believed to be important. When isoamylase is present, glucan polymers/precursors in the stroma are degraded by isoamylase. When isoamylase is absent, the glucan polymers/precursors build up in the stroma. Elaboration by synthases and branching enzymes are interesting possibilities of the technique. Accumulation of the starch granules and/or the phytoglycogen molecules are dependent on the synthetic enzymes available.

Forest engineering makes wonder

Trees have an extremely long growth rotation and therefore traditional breeding is a slow improvement process. Not surprisingly, scientists have turned to genetic engineering for solutions to quality problems, slow growth and different kinds of stress sensitivity.

Just as interesting are the attempts to reduce/replace chemicals in wood fibre processing with transgenics. The challenge is to design wood quality for different purposes, for instance produce a tree with less lignin to suit the pulp and paper industry. The new technique would save money and the environment. But while creating a new tree, it is important that laboratory studies, greenhouse experiments and field trials go hand in hand. So far, the few harvested trees with reduced lignin have proved to be safe and can withstand stormy weather, as well as other types of stress. The growth and yield are unaffected and it is important that they continue to be so.

Science, media and the consumers

Louise Fresco pointed out that as shareholders in the GMO debate, scientists must recognise that there is also a substantial public distrust of science. Maybe this distrust opens the way for non scientists and non scientific arguments in the debate. Donald Grierson has participated in many television debates and has been interviewed by the media many times. He was surprised and annoyed by the ignorance and sometimes even the lack of interest in finding out the truth about GM foods. Prof. Kristina Glimelius disagreed with this. Often in ongoing research, it is hard to find true facts and a number of facts must be presented – some for and some against.

However, scientists have an interest and a moral obligation in participating in the public debate. To reach the public, they have to explain their work in a simpler vocabulary than they use to address each other. Not everybody has that talent, but the talented scientists can get good publicity and outnumber ignorant media giants.

Consumers are concerned

Public perception is necessary for the acceptance of GM products.

Sometimes it is tempting to say that consumers are ignorant and overreact. There are many examples of strange headlines such as "GM food stunts your growth". But in fact there are also many sound and clever arguments against GM foods.

There are still unsolved problems in the technique and the applicability. And, probably more important, people want to know whether they are buying GM products. Such foods must be labelled so that if consumers do not approve of them, they have the choice of not buying them.

Monsanto reduced the British GM market to nil by marketing unlabelled GM soybeans, a practice that enraged the consumers. The market still has not recovered, nor has consumer confidence. There is a lesson to be learnt.

Legislation is needed

Many countries still lack a thorough legislation on GMOs. National and international agreements are important to set a standard but also to decide what is legal and what is illegal.

A functioning legislation is necessary for future applications.

Conclusions

What Science Can Do in Crop and Forest Biotechnology of the Future was the theme of an International Conference on Crop and Forest Biotechnology for the Future in Falkenberg. It was held on September 17–19, 2001 and organised by the Swedish Foundation for Strategic Research (SSF), The Royal Swedish Academy of Agriculture and Forestry (KSLA) and Bertebos Foundation.

That Genetically Modified Organisms (GMO) are a fact of modern agriculture and are here to stay was the starting point for Louise Fresco, Assistant Director General of the FAO. To ensure that GM crops make an optimal contribution to world food security, food safety and sustainability, three great problems must be solved, according to Louise Fresco. Firstly, GMOs must be directed to the right research priorities such as drought and heat tolerance, improved nutrient uptake and rooting, biological nitrogen fixation, responses to carbon dioxide and tolerance to key abiotic stresses, such as salinity and drought. Secondly, agreed national and inter-

national instruments of governance must be developed. Thirdly, the access to research and new technologies for developing countries, poor producers and consumers must be facilitated.

The speech of Louise Fresco formed a background to a wide range of scientific achievements in the genetics of horticultural and agricultural plants and forest trees.

Ripening and senescence: Ripening and senescence go hand in hand, but by delaying senescence in fruits and vegetables the sustainability is prolonged, which is an advantage to producers, trade and consumers. The Bertebos prize winner of 2001, Prof. Donald Grierson, showed from his own scientific work on tomatoes that this is possible with an antisense technique. This is a very precise method and only one gene out of tens of thousands is inactivated. The effect is related to the specific antisense gene that is used and it is inherited. From tomatoes with the antisense gene, one can produce progeny tomatoes that have a delayed ripening process or altered quality. The key point is that if ethylene production or the response to ethylene can be controlled, the rate at which produce progresses to overripeness and spoilage can be regulated. (Antisense technique means that the isolated genes are put back one by one in a "backward" order. By silencing a gene one can study the reactions of the organism, to get a proper understanding of the biological function and role of the actual gene.)

Fatty acid modification: From a biochemical point of view the daily consumption of fatty acids should include about 10 g of linoleic and 2 g of linolenic acid and a significant reduction in all saturated fatty acids and a corresponding increase in oleic acid. In view of this situation, it is an obvious idea to produce plant seed oils containing very long-chain polyunsaturated fatty acids normally only found in fish ("oceanic" fatty acids). This goal may be realised by gene technology, provided that the biochemistry of oil biosynthesis is known in detail. At the end of the year 2002, most genes required for this task have been cloned, and only one is still missing.

Biotechnology can in the future be used to design a desired oil quality in a desired oil crop. A desired fatty acid can be obtained with the usage of the vast gene library in nature or by protein engineering.

Starch: Starch is the major form in which plants store their carbon – around 80% of the dry weight in potato tubers and the endosperm of cereals and up to 50% for peas and beans. Starch is the main source of carbohydrates in our diet but it also serves as a thickener, texturizer or stabilizer in both food and non food industries.

Starch extracted from the crop (maize, wheat, potato, cassava etc) undergoes post-extraction processing consisting of chemical, enzymatic or physical modifications. This generates starches with many different properties (different pastes and gels with varying characteristics) suitable for a wide range of different industrial uses.

But post-extraction processing has some major disadvantages. It would be better if the right type of starch could be designed in the crop so that post-extraction modification would not be needed. Scientists are beginning to gain sufficient information for "designing" starch properties in a rational way.

Genomics, proteomics and metabolomics: *Arabidopsis thaliana* was the first plant to have its genome sequenced and by analysing epigenetics and chromosome structure, it is possible to understand the role of methylation in regulating chromosome structure and gene expression. Once a sequence of genes is obtained, this can be compared with all other genes. The genome sequence provides a complete record of all of the genes and of all the protein sequence because of the exquisite

precision of nucleotide sequence. From that it is possible to study expression patterns of genes and to build up knowledge of co-regulated genes, the so-called regulon.

In the plant kingdom there exist a lot of differences in genome size. In spite of the large difference in genome size, the general structure of the chromosomes is often very similar. That means that the order of markers or genes along the chromosomes is often very similar, if not identical.

Proteomics analyse the proteome components under a set of defined circumstances. To study the change in protein quantity, scientists consider several factors such as tissue or cell type, development stage, infection, disease, mutation, environmental effects and pesticides and herbicides.

A greater understanding of the control processes regulating metabolism is required before metabolic engineering becomes routine. This can be achieved by assessing the steady-state concentrations of a wide range of metabolites and by the development of sensitive and accurate methods of determining cellular fluxes.

Plant stress and signalling: Ozone is a convenient tool for studying the role of oxygen radicals in signalling (to get rid of the stress). ROS (reactive oxygen species) are important biological regulators of cell death during oxidative stress. ROS regulation of cell death is tightly coupled with hormonal regulation. ROS and hormones work as an interconnective network.

The fungal pathogen *Leptosphaeria maculans* causes blackleg or stem canker and is the major threat to crops like oilseed rape and turnip rape. It has been demonstrated that the *Arabidopsis thaliana* genome can be a source of resistance to *L. maculans*. The results of Arabidopsis work can be applied to Brassica.

The myrosinase-glucosinolate system serves as a defence in oilseed rape. Glucosinolates are secondary metabolites found in all Brassica plants. These plants also house the enzyme myrosinase, which degrades glucosinolates into toxic products, harmful to pests. Down-regulation of vegetative and wound-inducible myrosinase binding proteins in *Brassica napus* affects susceptibility differentially in different Brassica specialist herbivores.

The H^+-ATPase is thought to play a major role in many cell processes and its activity is regulated by a number of physiological factors, including hormones, blue light and fungal toxins. In all plants analysed so far, the plasma membrane H^+-ATPase and 14-3-3 proteins are encoded by multigene families.

Forest genetics: Genetics can be used to improve quality and yield in forest trees. A Swedish project concentrates on two different types of genes that are crucial to the practical implementation in forestry. One type affects formation of wood and stem growth. The other type is important for stress tolerance. Wood formation and ability to survive under harsh climates are unique characters for trees.

Lignin biosynthesis can be used to improve wood quality for the pulp industry. With enzymes it is possible to reduce the amount of lignin for bleaching or to do some surface modification of the cell walls to perform better in paper making. Enzymes can make cells react better with other agents. In addition, enzymes could improve the reactivity of the main component, cellulose.

Plant development: Periods of growth and dormancy vary in trees The vascular cambium makes a transition from active to passive when the tree stops growing. The auxin transport is reduced as the tree stops growing, but even after the cell divisions have stopped there is considerable capacity in the cambium to transport auxin for a long time. Auxin is one of the most important signalling mole-

cules, which achieves the necessary balance between the root system and the rest of the plant.

Members of the SHI gene family are involved in the control of cell expansion and perhaps also cell fate specification. They may regulate auxin responses and the study of gene control of these processes could be of great benefit to forestry.

Förteckning över tidigare utgivna nummer

År 2001; Årgång 140

Nr 1 Sälen – resurs eller problem
Nr 2 Skogliga konsekvensanalyser 1999
Nr 3 Framtida möjligheter till ökat utnyttjande av naturresurser
Nr 4 Verksamhetsberättelse 2000 Kungl. Skogs- och Lantbruksakademien
Nr 5 Landskapet: restprodukt eller medvetet skapat?
Nr 6 Ecologically Improved Agriculture – Strategy for Sustainability
Nr 7 Sverige i det europeiska samarbetet
Nr 8 Bekämpningsmedel i vatten – vad vet vi om förekomst och effekter?
Nr 9 Odlingssystem, växtnäring och markbördighet – 18 års resultat från en tidigare ej uppgödslad jord
Nr 10 Svenska fiskets framtid och samhällsnytta
Nr 11 Vem sätter värde på den svenska skogen? – Trämekanisk framsyn
Nr 12 Nutrition och folkhälsa i EU-perspektiv OCH Miljöpåverkan och framtidens matvanor OCH Framtidens mat i framtidens kök
Nr 13 Konkurrenskraftig virkesförsörjning– ett kraftprov för skogsteknologin!
Nr 14 Fritidsodlingens och stadsgrönskans samhällsnytta
Nr 15 Debatt – Hur kan marknad och miljö förenas? Exemplet spannmålsproduktion

År 2002; Årgång 141

Nr 1 Genteknik – en skymf mot Gud eller nya möjligheter för mänskligheten?
Nr 2 Genmodifierade grödor. Varför? Varför inte? – Genetically Modified Crops. Why? Why Not?
Nr 3 Skogsfrågor i ”Konventionen om biologisk mångfald”
Nr 4 Mindre kväveförluster i foderodling, foderomvandling och gödselhantering!
Nr 5 Bondens nya uppdrag OCH Shaping U.S. Agricultural Policy
Nr 6 Verksamhetsberättelse 2001
Nr 7 Sustainable forestry to protect water quality and aquatic biodiversity
Nr 8 Foder – en viktig länk i livsmedelskedjan!
Nr 9 Fortbildning för landsbygdsutvecklare
Nr 10 Hållbart jordbruk – kunskapssammanställning och försök till syntes
Nr 11 Närproducerad mat. Miljövänlig? Affärsmässig? Djurvänlig?
Nr 12 Nya kunskaper inom bioteknik och genetik för nya tillämpningar på husdjur SAMT Fria Varuströmmar – konflikt med djur- och folkhälsa? Vilka möjliga utvägar finns? SAMT Exempel på verksamhet inom Jordbruksverket
Nr 13 Bland skärgårdsgubbar och abborrar på Möja
Nr 14 EU och EMU – broms eller draghjälp för skogen?
Nr 15 Hur kan skogsbruk och kulturmiljövård förenas?
Nr 16 Vilket kött äter vi om 10 år? Rött, vitt, svenskt, importerat? Vi får det samhälle vi äter oss till!
Nr 17 Avsättning av skogsmark

År 2003; Årgång 142

Nr 1 Det sydsvenska landskapet, framtidsvisioner och framtidssatsningar SAMT Idéer för framtidens skogslandskap
Nr 2 Viltets positiva värden
Nr 3 Inför toppmötet i Johannesburg
Nr 4 Kapital för landsbygdsföretagare
Nr 5 Kompetensförsörjningen i svenskt jordbruk
Nr 6 Fiskets miljöeffekter – kan vi nå miljömålen?
Nr 7 Verksamhetsberättelse 2002
Nr 8 De glesa strukturerna i den globala ekonomin – kunskapsläge och forskningsbehov
Nr 9 Tro och vetande om husdjurens välfärd (Enbart publicerad på www.ksla.se)
Nr 10 Svenska satsningar på ökad träanvändning (Enbart publicerad på www.ksla.se)
Nr 11 Kapital för landsbygdsföretagare (Enbart publicerad på www.ksla.se)
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