Plant Research Department annual report 2003

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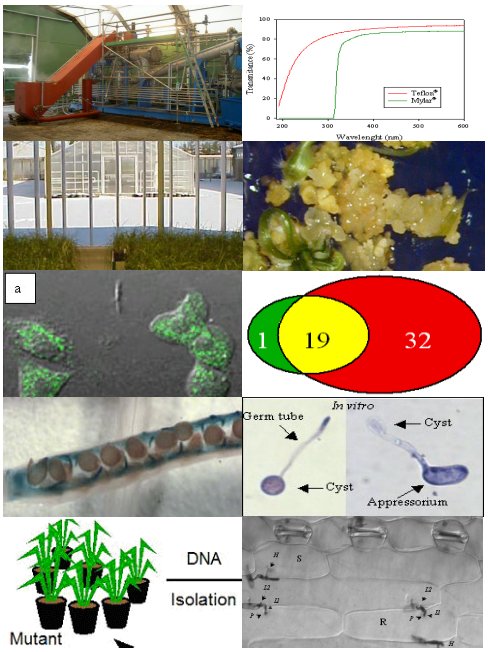
Plant Research Department



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Risø National Laboratory

Mission
Risø's mission is to promote an innovative and environmentally sustainable technological development within the areas of energy, industrial technology and bioproduction through research, education, innovation and advisory services.
Vision
Risø's research shall **extend the boundaries** for the understanding of nature's processes and interactions right down to the molecular nanoscale.
The results obtained shall **set new trends** for the development of sustainable technologies within the fields of energy, industrial technology and biotechnology.
The efforts made **shall benefit** Danish society and lead to the development of new large industries.
Risø's activities in 2003 are reported in the following publications: Risø Annual Report (available in Danish and English), Risø's Annual Performance Report (Danish) and the annual progress reports of the research departments (English). All publications and further information can be obtained from risoe.dk. Printed publications are available from the Information Service Department, tel.: +45 4677 4004, e-mail: risoe@risoe.dk, fax: +45 4677 4013.

Plant Research Department

Abstract
In 2003 the Plant Research Department (PRD) at Risø National Laboratory was involved in establishing the consortium Plant Biotech Denmark, which is unifying most of the Danish

Plant Biotechnology activities. Within the consortium, PRD has the unique opportunity to be the only life science department located in an environment that is largely dominated by physicists. PRD is challenged to optimally interface Plant Biology with the different fields of expertise that are established at Risø National Laboratory. These activities are mainly related to develop novel post-genomic tools to assign function to genes, which are widely applicable in the life sciences, such as non-invasive and non-destructive technologies to determine metabolite concentrations with high spatial and temporal resolution. The Plant Research Department applies these and state-of-the-art technologies to increase knowledge to develop crops with improved agronomic traits and to engineer high-value compound containing plants.

The department is divided into five research programs that are linked through their individual expertise delivered to the rest of the department. Three programs are engaged in improving the agronomic performance of plants. Genetic and molecular genetic tools are developed to enhance the nutrient efficiency of plants, to strengthen the withstanding of plants to fungal attack, or to adapt the flowering time to the optimal use of crops. One program is devoted to improve the market value of plant products. Plants with enhanced nutritional value or that contain novel renewable resources are designed to add value to the European Agro-Industries. A fifth program ultimately is studying the effects of the future climate on plant growth, and the performance of newly designed crops and their interaction with the environment. Finally, a Risø-wide multidisciplinary initiative has been started to establish systems that optimize the production of energy from biomass in order to promote sustainability in industrial societies.

Diverse activities in the area of Functional Genomics integrate the department within itself, within the research environment at Risø National Laboratory, and finally within the Plant Science environment in Denmark and Europe. Each program covers special expertises in the fields of genome, transcriptome, proteome, and metabolome analysis, which are delivered throughout the department and to other collaborators. It is unique to The Plant Research Department that these activities are supplemented with a broad expertise in environmental analysis, allowing the interpretation of large biological data sets in the context of factors affecting plant growth. In order to optimise the interpretation of these large data sets theoretical biophysicists have initiated the development of unique bioinformatic tools.

http://www.risoe.dk/prd/
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DLF-Risø Biotechnology Programme

Objectives
The biotechnology consortium between the Danish seed company DLF-TRIFOLIUM A/S and Risoe National Laboratory, Plant Research Department, is conducting a research programme focused on developing technologies for the control of flowering and improving quality traits in ryegrass, including abiotic stress tolerance, digestibility, and nutritional value.

Research fields

- Identification of key genes responsible for the switch from vegetative growth to flowering
- Gene activation systems allowing control of flowering and seed production
- Test of isolated genes in transgenic grasses
- Abiotic stress tolerance
- Digestibility
- Nutritional value (fructans)
- Establishment of a monocot model transformation system
- Transposon exon trapping lines in *Arabidopsis*

- Identification of novel specific promoters
- Identification of mutations affecting the response to vernalization

Selected report

- Monocot transformation platform
- *Arabidopsis* flowering time mutants under short day conditions

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Plant Environment Interactions Programme

Objectives

The aim of the programme is to study the structure, function, processes, and dynamics of agro- and semi-natural ecosystems, and the biological interactions between crops and wild plants. The goal is to predict the function of plant ecosystems in a changing environment and to assess the ecological risks of introducing genetically modified plant in the agricultural systems.

Research fields

- Soil-plant-atmosphere interactions in relation to air pollution, global change and other environmental aspects, and ecophysiology related to nutrient cycling and stress.
- Functioning of terrestrial ecosystems and impacts of global change.
- Plant fitness, competition, and environment
- Genetic resources for crops of the future.
- Ecosystem modelling. Developing and improving nutrient cycling models and ecophysiological models.
- Carbon sequestration in forest and grassland ecosystems.
- Emissions of non-CO_2 greenhouse gases and relationships with soil nutrient dynamics in organic and conventional grasslands and forest ecosystems.
- Gene flow between crops and wild relatives.
- Risk analysis of genetically modified crops.
- Development of sensors and sensor systems for site specific fertilisation. Improving the nitrogen utilisation by Precision farming.

Selected reports

- A new concept for evaluating effects of UV-B radiation on vegetation
- Are non-flowering grasses better competitors?

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Plant Nutrition Programme

Objectives

Our research aims at increasing the sustainability of plant production by enhancing the efficiency of plant nutrient uptake and thereby reduce fertiliser demands. Plant mutants, isotope labelling and advanced MS and NMR technologies are used to explore the role of mycorrhizal fungi and root hairs in phosphate and nitrogen uptake. Advanced MS is also used to investigate the proteomics of other plant-associated microorganisms.
Novel nanobiotechnology-based methods are being implemented to develop nanosensors and growth media for fungi. The nanosensors are porous polymer beads containing fluorophores and will be used for the spatio-temporal detection of specific metabolites in living cells. The growth media are topologically and biochemically tailored at the micro- and nanoscale and will be used to identify physio-chemical cues, which affect growth of eukaryote cells. The novel technologies have potential applications both in the plant and

health sciences and are being developed in close collaboration with the Polymer Department as part of the interdisciplinary Risø initiative *Nano- and Microscale Design of Interfaces*.

Research fields

- Pathways for phosphate uptake in responsive and non-responsive mycorrhizal plants
- Functional diversity of mycorrhizal fungi in relation to environmental perturbations (fungicide use and climate change)
- Selection of P efficient root hair mutants in mutagenised barley
- Development of polymer-embedded nanosensors for detection of metabolites in living cells
- Identification of physio-chemical cues to fungal growth by means of growth environments, which are topologically and biochemically tailored at the micro- and nanoscale
- The role of mycorrhizal fungi in phytostabilisation of radio-contaminated environments
- Proteomics as a tool to study the infection-involved proteins in *Phytophthora infestans*, a pathogen of potato
- Development of instrumental methods in plant biochemistry

Selected reports

- Polymer-embedded nanosensors for optical detection of metabolites in living cells
- The proteome of *Phytophthora infestans*

Contact:

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Plant Products Programme

(after 2004-01-01: Plant Quality Programme)

Objectives

The aim is to develop biochemical and genetic methods to improve the product quality and crop plant performance in an ever-changing climate and environment. Protein chemistry is used in studies of biosynthetic pathways, respiration and proteomics under abiotic stress condition. Rice serves as model species for the important northern European wheat and barley production. Rice is also important in the collaborative research carried out with developing countries to improve the bioavailability of iron and zinc for human consumption. The programme seeks to support a competitive and sustainable grain production through improved phosphate management and better cell walls and fibres for the benefit of human health and animal production. The training of students and young scientists at the University level is an integrated part of the research.

Research fields

- Plant mitochondria with emphasis on plant-specific processes
- Proteomics of rice mitochondria is focused on the identification of phospho-proteins in signal transduction and stress-related proteins
- Plant response to biotic and abiotic stress, production of reactive oxygen species - ROS - and oxidized proteins in mitochondria and other compartments. The cause of stress includes salinity, pathogens and ozone
- Plant fibres for new biodegradable materials and identification of high-value products
- Mutational breeding for improving the use of crop plants as a biomass resource
- Examination of starch biosynthesis with the aim of producing novel industrial products
- Phosphate uptake and grain phytate metabolism studied by induced mutation and in transgenic barley and rice.
- The biosynthesis of phytate in tagged *Arabidopsis* lines

- Biofortification of crop plants with micronutrients
- Cereal seed development, nutrient loading and unloading.
- Determination of metabolite concentrations as a tool to increase production through plant breeding
- Biosynthesis and function of antifungal compounds
- Crop plants: barley, rice, potato, flax
- Course on Plant Biotechnology with The University of Southern Denmark

Selected reports

- Identification of oxidised proteins in rice leaf mitochondria
- Identification of a potato gene involved in starch degredation in leaves

Contact

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Resistance Biology Programme

Objectives

The Programme is conducting research on different aspects of plant-pathogens interaction. The background for this is that diseases have serious impact on plant productivity, and therefore growers are forced to use agrochemicals to control the damaging pathogens. However, this is expensive and possibly harmful to the environment and to human health. The objective of the Programme is to provide alternative measures for pathogen control. These primarily include exploitation of the plants natural resistance mechanisms, based either on introduction of new resistance genes or on genetic engineering. Another aim is to identify genes responsible for tolerance towards abiotic stresses. In order to meet these objectives on longer terms, we do research on the fundamental processes controlling plant-pathogen interaction, and e.g. drought tolerance. In addition, we study disease development in response to different cropping systems, and thereby we can give recommendations for agricultural practices in order to alleviate disease problems.

Research fields

- Cellular and molecular mechanisms of resistance to the barley powdery mildew fungus in barley and *Arabidopsis thaliana*
- Characterization of barley genes involved in disease response to powdery mildew attack
- Characterization of barley powdery mildew stage-specific and avirulence genes
- Proteomics of the powdery mildew haustorium
- Mapping of barley and wheat genes for disease resistance and abiotic stress resistance, and development of marker-based selection systems
- Modelling dynamics of pathogen dispersal on wheat (yellow rust), and interspecific competition on barley (netblotch and scald)
- Plant health and induced resistance in relation to variety mixtures, intercropping and nutrient uptake
- Crop improvement by marker-assisted breeding for developing countries
- Genetic resources for crops of the future

Selected reports

- Transcript profiling in single epidemal cells attacked by the powdery mildew fungus
- Allele discovery by *eco*-TILLING

Contact

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BioMass Materials

Objectives

"Biomass materials" is a research group in the Department of Plant Research at Risø National Laboratory. The research concerns with the utilisation and conversion of biomass materials mainly in the form of plant fibre materials for production of energy products. Energy products include fuel products for the transport sector (e.g. bioethanol, biodiesel and in the long term hydrogen and methane), biomass for combustion and electricity production and low-density plant fibre materials to be use in energy production (wind turbines) or transport (vehicles, airplanes, trains).

Different pretreatment techniques such as wet oxidation and steam explosion, have been developed for the utilisation and characterisation of plant fibre biomass into these products. A number of ongoing projects including two EU-projects and several national projects are related to the utilisation and characterisation of natural biopolymers from plant fibres e.g. 1. Co-production of biofuels (ethanol and solid fuels), 2. Co-production of bioethanol and biogas, and 3. Strong biocomposites from hemp fibres.

Research fields

- Pretreatment of biomass materials by wet oxidation, steam explosion and hydrothermal treatment
- Chemical characterisation of the plant constituents: cellulose, hemicellulose and lignin in raw materials and treated products
- Separation and purification of these biopolymers
- Chemical derivatisation of biopolymers
- Fermentation by yeast and fungies
- Analyses of sugars and fermentation products
- Down stream processing

Selected report

- Wet oxidation used in co-production of biofuels

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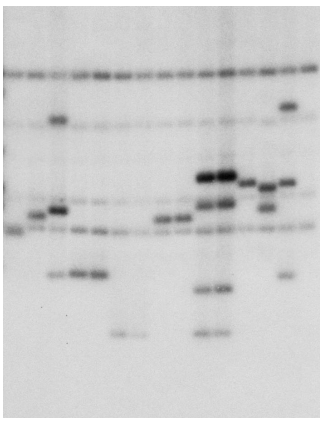
Monocot transformation platform

Ingo Lenk
DLF-Risø Biotechnology Programme

The Consortium is focusing on the establishment of a monocot transformation platform to serve as a novel model system allowing a fast and efficient testing of gene effects in grasses and cereals. The weedy grass species *Brachypodium distachyon* was chosen due to a number of unique features, making it a potential monocot counterpart to the widely used dicot model plant *Arabidosis thaliana*.
Brachypodium belongs to the Pooideae subfamily like many of the agronomical important temperate cereals. It possesses unique monocot model plant characteristics in having the smallest known genome size in grasses of down to 123 Mbp/1C (similar to *Arabidopsis*), a life cycle of less than four months and a physical size of down to 20 cm at maturity. *Brachypodium* is a self-fertile inbreeding annual with a chromosome base number of five. Di-, tetra- and hexaploid cytological genotypes have been collected mainly from South/East Europe.

Transgenic shoots of *Brachypodium distachyon* regenerating on selective medium



DNA blot analysis of transgenic *Brachypodium distachyon* lines reveals relatively simple integration patterns

Phenotypic appearance of *Brachypodium distachyon* belonging to the Pooideae subfamily

To establish *Brachypodium* as an efficient test bed for functional genomics in cereals and grasses, an efficient transformation system has been a focus area to the consortium.

Published protocols have demonstrated the readiness of *Brachypodium* in respect to ballistic transformation, however, with insufficient efficiency and reproducibility. Since year 2000, the Biotechnology Consortium has performed activities on the development of an improved *Brachypodium* transformation system based on both biolistic and *Agrobacterium* mediated transformation, different selection markers, and employment of genotypes with a difference in ploidy levels, vernalisation requirements and phenotype characteristics. Presently the transformation systems in both diploid and tetraploid accessions routinely are used in PRD projects focused on testing of gene function. Furthermore, the production of transposon tagged mutant populations has been initiated. Future aims are to prepare relevant cDNA and genomic libraries, to implement TILLING for *Brachypodium* and to develop a basal genetic map.

Arabidopsis flowering time mutants under short day conditions

Claus H. Andersen
DLF-Risø Biotechnology Programme

The major developmental transition in flowering plants is the switch from vegetative to reproductive development. The correct timing of this transition is crucial for reproductive success, and enables plants to optimize their use of available resources in the environment in which they grow. However, for many breeding programmes the ability to manipulate flower formation or flowering timing could be very beneficial. For some purposes it would be important to accelerate flowering or to increase the number of flowers. For example for many cereals it would be an advantage to shorten the time until flowering to achieve an extra growth period in the season. Also for some woody species, like citrus, a reduction of generation time would have multiple implications, as many woody species have a long juvenile phase that delays their reproductive development by up to 20 years. For other applications it would be useful to prevent flowering. For example, in order to genetic engineer native species, such as forest trees or forage crops, the spread of transgenes to wild populations has to be avoided.

In order to control flowering it is important to obtain knowledge on the genetic mechanisms regulating the floral transition. Genetic and molecular approaches in the model plant *Arabidopsis* have identified four major floral promotion pathways regulating the floral induction and flower development, namely the photoperiod, the vernalization, the autonomous, and the gibberellic acid (GA) pathway pathways (Simpson and Dean, 2002; Mouradov et al., 2002).

The functional analysis of *Arabidopsis* genes has largely been based on the phenotypic characterization of mutants selected by forward or reverse genetics. The screening for late-flowering mutants has been quite extensive and most screens have been performed under LD conditions. Therefore, the photoperiod pathway is well understood and the key regulators of this pathway have been characterized. However, only few early-flowering mutants under SD conditions have been identified. In contrast to most late-flowering mutants, early-flowering mutants are often very pleiotropic and often display a wide range of different phenotypes. This may be due to the fact that floral repressors are involved in several developmental programmes of which only one is the regulation of flowering. Therefore, the characterization and understanding of early-flowering mutants may prove very useful for the prediction of 'floral fine tuning' and constitute a basis to investigate flowering behaviour in changing environments.

Identifying *Arabidopsis* flowering time mutants

A screen for flowering time mutants has been performed on gene-trap transposon mutant lines generated under the EXOn Trapping Insertion Consortium (EXOTIC) (http://www.jic.bbsrc.ac.uk/science/cdb/exotic/) which was financed by The European Commission Fifth Framework Programme (2001-2003).

The EXOTIC project has used gene trap transposon tagging based on the Maize *Ac* and *Ds* transposable elements to generate approximately 30,000 insertions in the *Arabidopsis* genome disrupting approximately 5,000 genes (figure 1).

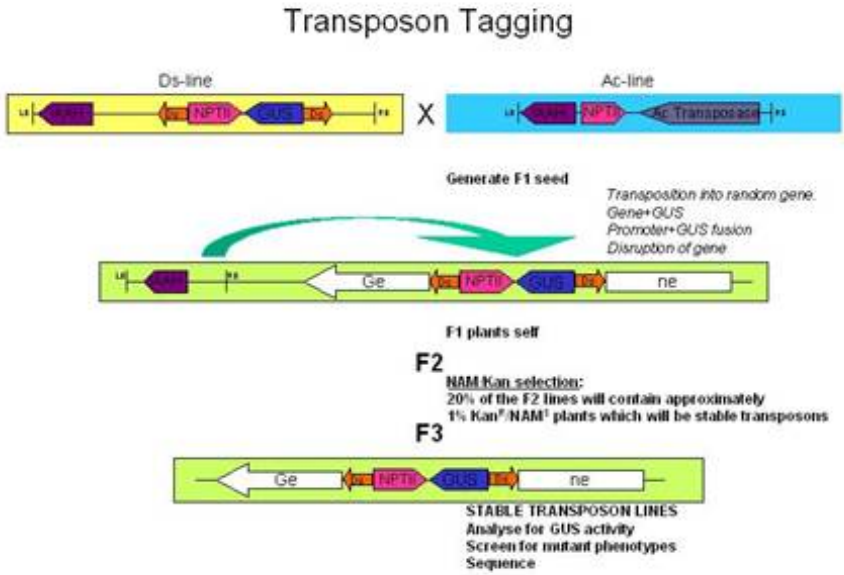


Figure 1. Illustrating the modified maize Ds transposon that contains a selectable kanamycin marker and a GUS gene with a three-frame splice acceptor. The transposon is mobilised by a T-DNA expressing Ac transposase, and transpositions to sites unlinked to the Ds launching pad are specifically selected using the iaaH counter-selection marker.

Insertions of the transposon in intron or exon sequences in the correct orientation lead to fusions of the GUS reporter gene in frame with the disrupted gene. In many cases the resulting GUS activity can be detected by sensitive histochemical staining, revealing the tissue-specific expression of genes (Figure 2).

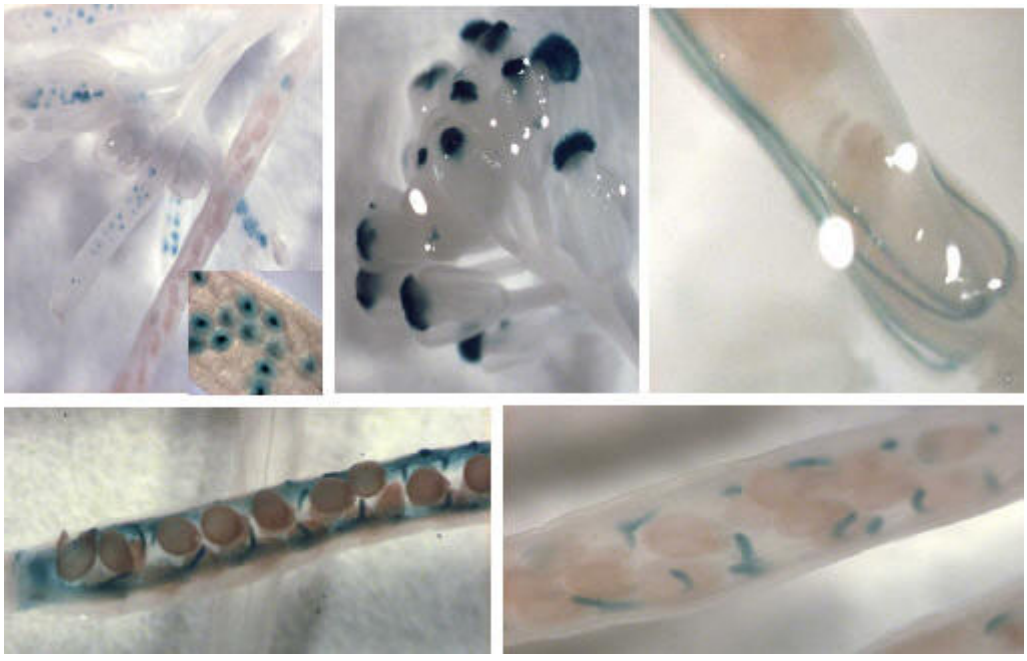


Figure 2. Illustrating different examples of tissue specific GUS-patterns

Ongoing work

Based on 8,000 EXOTIC gene-trap transposon lines, around 40 early-flowering and some late flowering *Arabidopsis* mutant candidates have been identified from a mutant screening under SD environment (figure 3).

Figure 3. Illustrating a screening for flowering time mutants under SD conditions

In order to further investigate and characterize the flowering mutants the initial strategy includes back-crossing with the *Ac* line to re-mobilize the *Ds* transposon to another locus or by complementing the *Ds* tagged gene with the corresponding genomic locus from wild-type. Further, a detailed expression pattern of the corresponding genes, and crossing of mutants to other well-characterized flowering mutants is performed. In addition, micro-array gene expression profiling will be performed on mutant and wild-type plants to determine the effect of the introduced mutations on other genes. And finally, ectopic expression of the corresponding genes will be performed to reveal additional roles for the genes that are normally obscured by genetic redundancy.

A new concept for evaluating effects of UV-B radiation on vegetation

Teis N. Mikkelsen[1], Helge Ro-Poulsen[2] & Kristian Albert[2]
1) Plant-Environment Interactions Programme. 2) University of Copenhagen

Risø and University of Copenhagen conducted from 2001 to 2003 a study of UV-B effects on the high arctic vegetation at Zackenberg, Northeast Greenland. Due to the degradation of the ozone layer, the ambient UV-B radiation will increase in the next 15-20 years. One way to study the effect was given in the Annual Report 2002 for Plant Research Department on page 12. Here, we used transparent filters with different abilities for UV-B absorption for reduction of the ambient UV-B in different treatments. To simulate increased UV-B another approach was developed: Shoots of *Salix arctica* growing on a south faced slope, were elevated from their horizontal position, by the use of aluminum frames, consequently the leaves were perpendicular to the sun around noon (figure 1). This increased the input of solar and UV-B radiation with approximately 70 %. Two types of transparent filters were placed 1 cm above the leaves.

Figure 1. A Salix arctica shoot fixed with nylon strings in an elevated position. A transparent Mylar® filter is placed above the shoot

One group of plants was covered with Teflon® foil (enhanced UV-B) and another group was covered with Mylar® foil (reduced UV-B). Plants covered with Teflon® received 93% of the Photosynthetic Active Radiation (PAR) and 84 % of the UV-B via the filter. Plants covered with Mylar® received 87% of PAR and 1 % of the UV-B via the filter. The transmittance of the filters measured in the laboratory is given in figure 2.

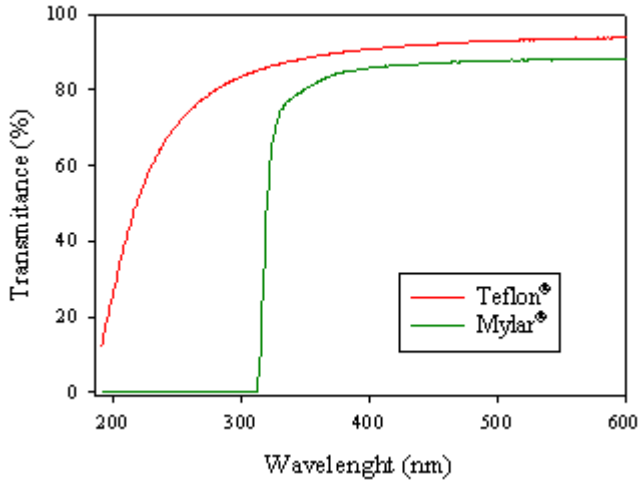


Fig. 2. Transmittance of Teflon® and Mylar® in PAR (400-700 nm) and UV-B (280-315 nm). Steen G. Hanson and Carsten Dam-Hansen from Optics and Plasma Research Department, Risø conducted the filter analysis

For plants exposed to increased levels of UV-B (*Teflon®*), measurements with a fluorometer (Handy PEA, Hansatech Instruments) showed significant lower values in the common index F_v/F_m and the new Performance Index (PI). The measurements were conducted throughout the growing season (July-August). The results indicate that enhanced UV-B will result in direct negative effect on the photosynthesis and thereby potentially on the whole high arctic ecosystem. A lower input from the primary producers (plants) will affect the herbivores and carnivores in the food chain.

However, PAR can be an artifact in this set-up, because the *Teflon*® treatment also receives enhanced PAR compared to the *Mylar*® treatment according to the measurements shown in figure 2. The concept by elevating plants and use filters can still be a valid method for investing UV-B effects if the difference in PAR transmission can be minimized in the future.

Are non-flowering grasses better competitors?

Thure P. Hauser
Plant Environment Interactions Programme

Plant breeders are in the process of developing genetically modified (GM) grasses that do not flower. The plants then don't have to spend their energy on flower stems but can instead use it for more leaf material with a better fodder or lawn quality. Another goal is that the inserted genes, the transgenes, then do not spread with pollen and seed to surrounding grass fields and natural populations. In an experiment at the Plant Research Department, we test whether non-flowering ryegrass instead has gained an increased potential to spread vegetatively, so they may outcompete normal ryegrass and the transgenes thereby may spread in the surroundings.

Pollen and seed often disperse from fields into surrounding weedy and wild populations, and so it does from fields cultivated with genetically modified (GM) crops. If the GM pollen flows into another field, cultivated with the same species, it may e.g. pollute organic products with higher frequencies of transgenes than wanted by consumer or allowed by regulation. If the pollen flows to related wild plants, they may inherit the transgene and the traits coded by the transgene. This is though in rare cases to create e.g. more aggressive weeds or wild plants with an increased tendency to increase and outcompete other plant species – with changes of the natural ecosystems as a consequence.

Plant breeders therefore try to reduce the spread of transgenes via pollen. Different methods have been proposed, e.g. to insert the transgene in parts of the cells, such as chloroplasts, that are not transmitted via pollen. Another possibility is to stop flowering altogether, so neither pollen nor seed is produced. This can of course only be used in species where we do not harvest seeds or fruits. Grasses are among such species. Plant breeding companies, and among them the Danish DLF-Trifolium A/S, are therefore working hard to develop genetically modified grass types that do not flower. In addition to blocking the spread of pollen and seed to the surrounding, they gain another advantage: flowering stems are a nuisance both for cows that like leaves much better, and for turf that is less attractive and more difficult to maintain with stiff flowering stems. Only for the

production of seeds for sowing is flowering needed, and a part of the development of the new grasses is to be able to switch the transgenes on and off.
Even though the majority of plants reproduce and disperse by pollen and seed, many species are also able to reproduce by runners, budding, and other vegetative means. Just think of potatoes and strawberries, which we never sow but plant out. In the same way, many grasses can spread by growth of an individual clone, by breaking into separate shoots or by forming runners. If the budded off parts sit on the hoof of a cow or the tire of a tractor, they may disperse and establish in new places. Of course not as fast and probably not so long as by pollen and wind. But vegetative reproduction is sometimes very efficient, and studies have found that old grass fields contain much fewer but larger clones than new ones.
When a grass clone lands in a new site, other grasses and plants probably grow there already with which the new one will have to compete. Genetically modified, non-flowering grasses may have an advantage here. They have been constructed to divert energy from reproduction into leaves, which may also increase their ability to initiate new shoots and perhaps runners. This may increase their competitive ability and potential to spread vegetatively.
In an experiment at Risø, we have therefore tested whether non-flowering clones of common ryegrass, *Lolium perenne*, are better competitors than flowering clones. At the start of the project, which is part of a larger EU research project Conflow we expected to test this with genetically modified types. However, this was not possible, and instead we have simulated non-flowering with normal ryegrass material. Our experiment has ended, but data have not been analysed yet.

The experiment and results

So how do we simulate non-flowering grasses, and especially, how do we get them to compete against other grass clones that *do* flower? We have done so by using plants from different geografical regions. Danish plants need a couple of months of winter temperatures before they flower, whereas plants further from the south do not have this requirement. In our experiment, we have used ryegrass from Denmark, Czechia, and Italy, from a cultivated variety and a wild population each place. We have let the Danish plants grow together with the Czech or Italian in pairs and kept them cold (5˚) for varying time periods, 0, 3, 6, or 10 weeks. To promote competition, we gave the plants winter conditions more often than once a year. This was possible because they grew in the advanced climate chambers at the Plant Research Department. Only vegetative reproduction took place in the experiments, as all seeds were harvested before maturity
As can be seen in Fig. 1, many of the Italian ryegrass plants indeed flowered without any cold, the Czech plants only after some cold, and the Danish only after 10 weeks cold. In some of our plant combinations, we have therefore succeeded in letting non-flowering clones compete against flowering ones. And we therefore expect that our data analysis will show that the non-flowering clones increased at the expense of the flowering clones

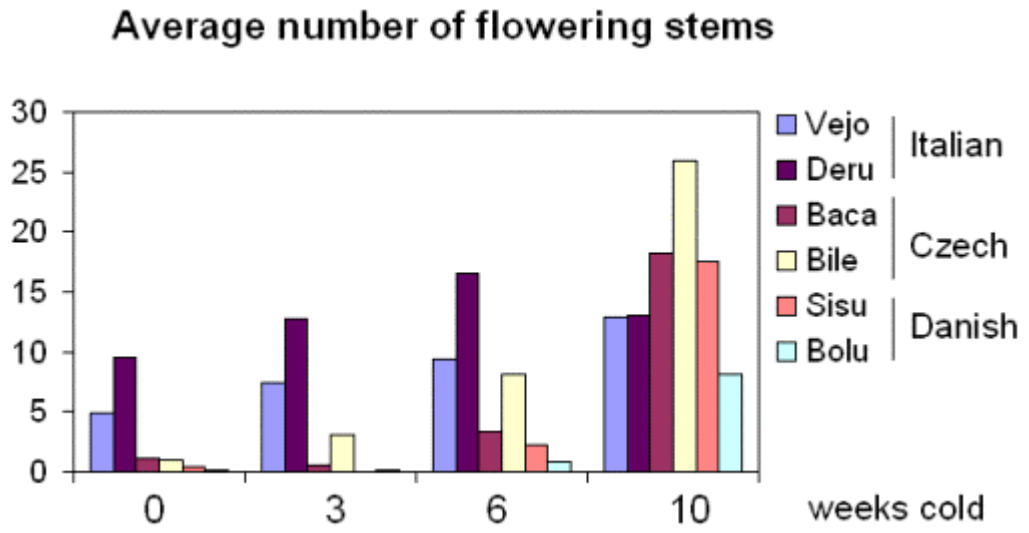


Figure 1. Number of flowering stems per plant for the last flowering period in the spring of 2003. Average of 80 plants per column

Limitations of the experiment
If our experiment shows that non-flowering grasses indeed have a better competitive ability, it still only indicates that there is an increased potential that such clones may spread at the expense of other clones and plant species. Other factors, which we did not include in our study, obviously affects plant growth and thus the competitive balance between species in a wild ecosystem. Even if non-flowering grasses in reality would turn out to be expanding competitors, this does not necessarily imply that they would harm agriculture or nature. Most plant species compete against each other most of the time, and there will always be some that are increasing and others that are decreasing. This doesn't mean that nature is deteriorating.
So when should we evaluate that a non-flowering clone, which has gone wild, creates so severe problems for agriculture or nature that we want to avoid using GM non-flowering grasses or try to limit their spread? In the worst cases, the genetically modified grasses could create worse weeds, or they could spread to new growth habitats in nature and disturb the composition of ecosystems. But even if new weeds should arise, it is probably not a problem for other than the farmers. And usually most of us don't care. Even if the composition of the ecosystems is changed, it is rather unclear how much it should change before we evaluate that it is seriously harmed and has a demand for help. Should we allow that a grass species changes its frequency from a given percentage to another?
Our experiment can not answer all these complicated questions. But it can give some of the biological information that we need in order to make qualified decisions

Polymer-embedded nanosensors for optical detection of metabolites in living cells

Anne Marie Scharff Poulsen and Hong Gu
Plant Nutrition Programme

One of the major limitations of physiological research in multicellular organisms is the lack of tools that permits measuring of metabolite levels in living cells with high spatial and temporal resolution. Classical methods for analysis of metabolite composition in organs, tissues, or cellular compartments often involve cell disruption and provide limited information. Current methods have low resolution, are prone to contamination by other cell

types or subcellular compartments, and neither measure metabolite changes in real-time nor account for variations in local concentration at the subcellular level.
Optical nanosensors for mammalian cells have recently been developed and provide a promising means for non-destructive metabolite imaging (Analytical Chemistry Research in the Kopelman Lab). These nanosensors consist of fluorescent reporter dyes embedded in a polymer matrix along with a reference dye, and the nanosensors have been used for ratiometric determination of a range of metabolites in mammalian cells (Fig. 1). This sensor type is superior over direct cell loading of fluorescent dyes by preventing cell toxicity of the reporter molecule as well as interference by non-specific binding to e.g. proteins and organelles. Such sensors have however not yet been used for *in vivo* imaging in plant cells.

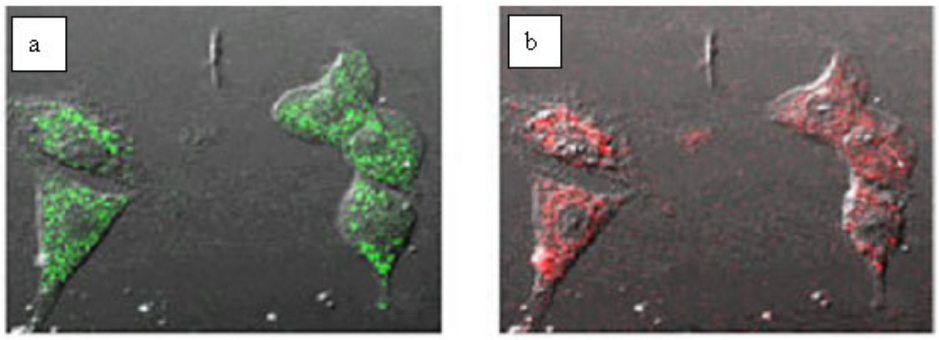


Fig. 1. Confocal images of rat C6 glioma cells loaded with fluorescent nanosensors containing a reference dye (a) and an oxygen sensitive dye (b) (Xu et al. 2001, Anal. Chem. 73, 4124-4133)

We have started out the development of nanosensors at Risø by synthesizing and characterizing polymer nanoparticles in collaboration with the Danish Polymer Centre. Our first strategy has been to use microemulsion polymerisation to synthesize crosslinked polyacrylamide particles with defined diameters in the range 20-30 nm. Size characterization is performed by size exclusion chromatography, dynamic light scattering and atomic force microscopy.

We have synthesised a prototype optical ratiometric pH nanosensor, which contains a pH sensitive fluorescent reporter dye as well as a pH insensitive reference dye. This has provided sensor particles that can be introduced into plant cells and used to optimise the delivery of particles into plant cells. Sensing of pH will enable the localisation of the nanosensors to either the cytosol or the vacuolar compartments. Protoplasts (plant cells without cell wall) are used as the first model system in order to facilitate the insertion of nanosensor particles. The nanosensors may be introduced into living cells by a range of methods including gene gun and microinjection technology. We have initiated optimisation of gene gun bombardment for the insertion of pH nanosensors into BY2 tobacco protoplasts. Confocal laser scanning microscopy will be used for imaging fluorescence responses in living cells.

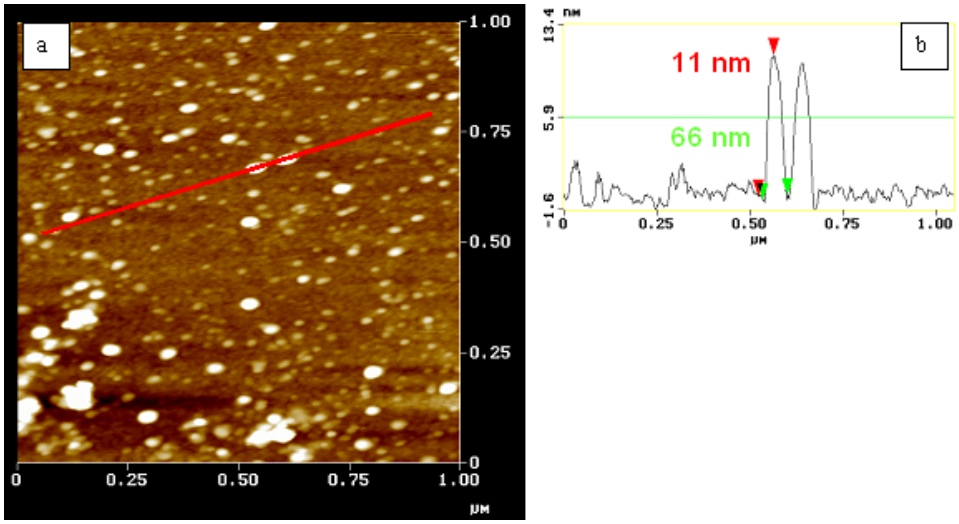


Fig. 2. Atomic force microscopy of crosslinked polyacrylamide nanoparticles produced by microemulsion polymerisation. The particles are depicted as a height image (a) and a linecut profile (b). Some deformation of the particles has occurred due to drying on a glass surface for microscopy

The future strategy for the development of optical nanosensors as a novel, versatile tool in the biological sciences includes the investigation of block copolymer micelles as a route to nanoparticle formation because the level of control of design of the particle matrix is increased. The exterior of the particle can be different from the interior as it is possible to covalently bond sensor moieties to the polymer backbone. It may also be necessary to include components that increase the mass density of the nanoparticles in order to be able to use gene gun insertion into plant cells with intact cell walls. The reporter molecules included in the nanosensors have so far been fluorescent dyes, but may also consist of protein based sensing systems. We collaborate with a group that have developed sensors based on bacterial periplasmic binding proteins fused to two variants of green fluorescent protein (GFP) (http://carnegiedpb.stanford.edu/research/research_frommer.php). Upon binding to their substrates, the binding proteins undergo a conformational change leading to a change in distance between the termini and thus to a change in Fluorescence Resonance Energy Transfer (FRET) between the two GFP variants.

The Proteome of *Phytophthora infestans*

Tine Ebstrup and Helge Egsgaard
Plant Nutrition Programme

The oomycete plant pathogen *Phytophthora infestans* is spread worldwide and causes the serious disease late blight in tomato and potato (Figure 1). A complete and durable resistance to *Phytophthora infestans* has never been found. At present the disease is mainly controlled by intensive use of pesticides. Besides pesticide resistance problems, this strategy has profound negative effect on the environment and is economically costly for farmers.

Figure 1: Field-grown potato plants infected with *P. infestans*

New strategies to combat *Phytophthora infestans* are therefore demanded. This requires more knowledge of the infection process at the biochemical and molecular level. *Phytophthora infestans* produce sporangia spores, which release zoospores that are able to swim in water on surfaces of plant tissues and in the soil. The zoospores encysts and form germ tubes, from where an appressorium (a hyphal swelling) is produced that enables the pathogen to penetrate the plant surface.

Our research focuses on identification of *P. infestans* proteins involved in these initial interactions between *P. infestans* and the host, potato. We use a proteomics strategy to achieve this goal. We have established and isolated the developmental stages of germinated cysts and appressoria on surfaces of petri dishes. Germinated cysts and appressoria formed *in vitro* resembles those formed *in planta* (Figure 2).

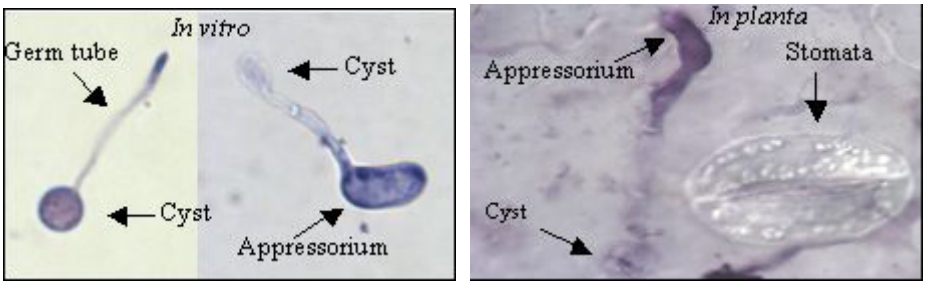


Figure 2: *In vitro* and *in planta* cyst germination

Proteins from different developmental stages of cysts were extracted and separated by two-dimensional (2D)-gel electrophoresis according to charge (by isoelectric focusing) and molecular weight (by SDS-PAGE) of the proteins respectively (Figure 3). The protein patterns are currently analysed for differences related to different stages of development.

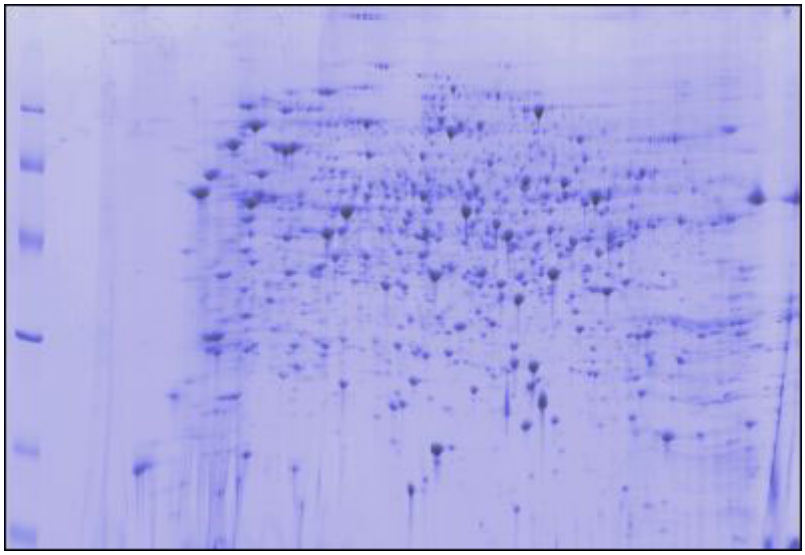
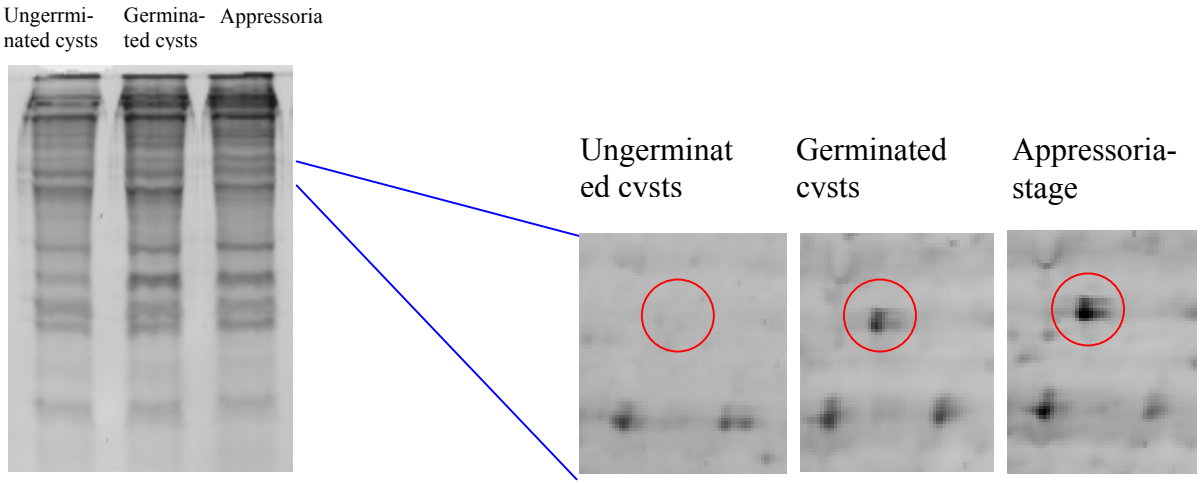


Figure 3: 2D-gelelctrophoresis of appressoria-stage proteins

In parallel, we search for differences in regulation, i.e. phosphorylation of the proteins. The binding of phosphate groups to proteins may strongly influence the activity/function of proteins. A fluorescent dye specific for phosphorylated proteins is used to quantify differences in the degree of phosphorylation of specific proteins. Separation of the proteins by one-dimensional SDS-PAGE showed a higher degree of phosphorylation of some proteins in the germinated cyst and in the appressoria-stage compared to the ungerminated cysts (Figure 4). Some of these phosphorylated proteins are currently being identified using a strategy combining 2D-gelelectrophoresis (Figure 5) and liquid chromatography/mass spectrometry.



Identification of oxidised proteins in rice leaf mitochondria

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Plant Quality Programme
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In plants biotic and abiotic stress phenomena are accompanied by an increased production of Reactive Oxygen Species (ROS) and this can lead to damage to proteins, lipids and DNA. In non-green plant cells and in green plant cells in darkness the electron transport chain in the mitochondria is probably the major ROS producer like it is in mammalian cells. There are three levels in the defence strategy against ROS damage - avoidance, detoxification and repair. Avoiding or minimising ROS production generally involves preventing the electron transport chain from becoming too reduced. Once formed, ROS can be detoxified by several enzymes or enzyme systems present in the mitochondrial matrix. Finally, if the first two strategies fail, ROS will accumulate and damage will occur that should somehow be repaired (Møller 2001).
The interaction between ROS and proteins is complex and can result in a large variety of modifications from oxidation of single side groups to chain breakage. The formation of carbonyl groups is considered to be an irreversible modification and as such be a valuable marker for oxidative stress. Carbonyl groups are one of the most studied protein modifications probably because a relatively simple detection method is available – conjugation with dinitrophenylhydrazine (DNP) and detection of the resulting DNP-tagged protein by a specific antibody.
We analysed the carbonylated proteins in the matrix fraction from green rice leaf mitochondria ("control matrix") and the effect of a mild in vitro metal-catalysed oxidation treatment ("oxidised matrix"). Carbonylated proteins were tagged with DNP, immunoprecipitated, and identified by two-dimensional liquid chromatography-tandem mass spectrometry followed by database searches.
Twenty oxidised (carbonylated) proteins were identified in the control matrix fraction. Of these, 10 are dehydrogenases or oxidases i.e. redox-active enzymes – four of the Krebs cycle or associated enzymes and six in the group "other redox enzymes". One of the oxidised protein, superoxide dismutase, is involved in ROS detoxification and two proteins, hsp60 and hsp70, are chaperones.
A further 32 oxidised proteins were detected in the oxidised matrix fraction (Fig. 1). Out of the 20 oxidised proteins in the control matrix, nineteen were also found in the oxidised matrix. The proteins oxidised by metal-catalysed oxidation reagent include five additional Krebs cycle or associated enzyme such that all the Krebs cycle enzymes present in the matrix were oxidised in the oxidised sample. The enzyme complex glycine decarboxylase appears to be particular susceptible to oxidation as all four component enzymes were found in the oxidised matrix.

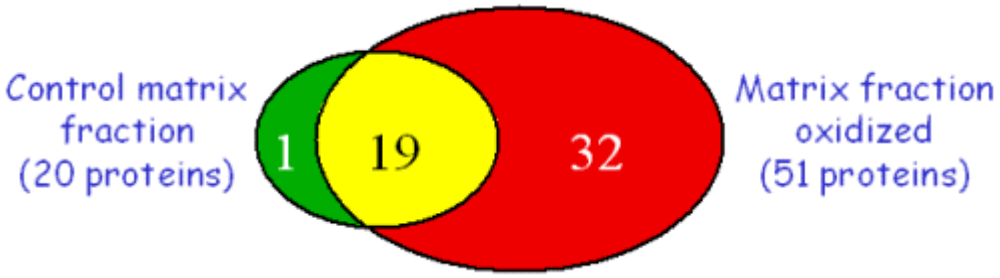

Figure 1

To check whether oxidation strikes “at random” we compared protein staining and carbonyl content on 2D-IEF/SDS-PAGE gels and blots, respectively, for each of our two matrix fractions (Fig. 2). The 2D protein pattern was almost unchanged by the oxidation of the matrix fraction (Fig. 2B) compared to the control matrix (Fig. 2A) indicating that little chain breakage had taken place. The amount of DNP-reactive proteins clearly increased in the oxidised fraction (Fig. 2D) compared to the control (Fig. 2C). Although most abundant proteins were labelled, there is little correlation between the amount of protein in a spot and the intensity of DNP-labelling.

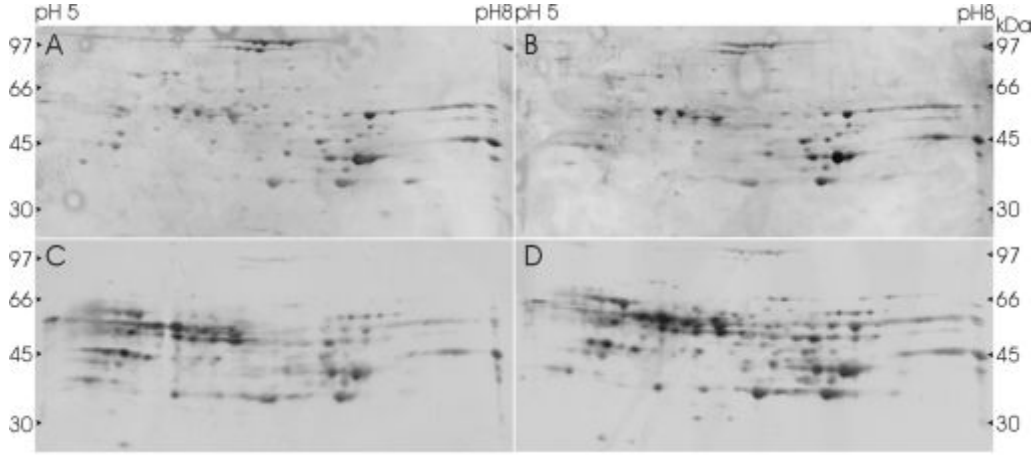

Figure 2

In summary this study shows that a group of 20 proteins is oxidised in the soluble matrix fraction from isolated plant mitochondria and that these proteins likely were oxidised *in vivo*. A further group of 32 oxidised proteins were identified when the matrix fraction was exposed to an oxidative treatment. These identified oxidised proteins are clearly more susceptible to oxidative modification than the other 2000-3000 proteins suggested to be present in this cellular compartment and are therefore potential sites of cellular stress response.

References

Møller, I.M. 2001. Plant mitochondria and oxidative stress. Electron transport, NADPH turnover and metabolism of reactive oxygen species. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52: 561-591.

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Identification of a potato gene involved in starch degradation in leaves

James R Lloyd
Plant Quality Programme

Starch is the main energy reserve produced by most plants. It is synthesised from sugars produced during photosynthesis, and then is degraded when the plant requires energy, such as during seed germination. The process of starch degradation is important for several reasons. For example, when beer is brewed, the alcohol produced comes from sugars released from starch. In addition, when potato tubers are stored at low temperatures they degrade starch and release sugars in a process known as cold-sweetening. When such cold-sweetened potato tubers are fried, the sugars react with amino acids and the fried potato product become discoloured.

The process of starch degradation is, however, poorly understood. One enzyme known to be involved in this process in potato is β-amylase. This enzyme degrades starch, and produces maltose as a product. To be further metabolised the maltose must be degraded to glucose, but the enzymes that do this have not been identified. To identify these, an *E. coli* expression system was established using a mutant that is unable to metabolise maltose. A potato cDNA library was transformed in, and colonies that stained red when grown on MacConkey media containing maltose were isolated. The red staining indicates ability to metabolise maltose. The DNA sequences of the cDNA's from these colonies were determined, and it was found that they coded for two different proteins, both of which were isoforms of disproportionating enzyme (D-enzyme, E.C. 2.4.1.25).

One of these (stDPE1) had been previously described in potato, but the other (stDPE2) was previously uncharacterised.Using this cDNA transgenic potato plants were manufactured which lack stDPE2. Firstly the rate of starch turnover in leaves of these plants was analyzed. Starch is manufactured during the day in leaves and is degraded at night to sugars, which are exported. It was found that there was a block on starch degradation in the leaves of the transgenic plants. This was demonstrated as leaves from the transgenic plants always contained significantly more starch than the control at the end of the night. This demonstrates that stDPE2 plays a role in starch degradation in leaves.

To see whether it is also important in cold-sweetening, tubers from these plants were stored at 4°C for eight weeks. No difference could be found in the amounts of sugars accumulating in the tubers of the transgenic plants in comparison with the control. These data show that starch degradation in leaves occurs using different enzymes than in tubers.

D-enzymes act by transferring an α1,4 bond from one sugar to another, however, the different sugar substrates can vary between different isoforms. To identify the substrates that stDPE2 uses the protein was purified from potato tubers. Using the purified protein it could be demonstrated that stDPE2 transfers glucose from maltose to glycogen, leading to the liberation of glucose.

Reference:

J.R. Lloyd, A. Blennow, K. Burhenne and J. Kossmann: Repression of a novel isoform of disproportionating enzyme (stDPE2) in potato leads to inhibition of starch degradation in leaves, but not tubers stored at low temperature. Plant Physiol. In press.

Cell specific gene transcript profiling of barley epidermal cells attacked by powdery mildew

Michael F. Lyngkjær, Torben Gjetting and Peter Hagedorn
Resistance Biology Programme

Activation of genes is important for plant responses to pathogen attack and knowledge of which genes, and when and where they are activated, is essential for our understanding of the processes leading to susceptibility and resistance. The gene transcripts accumulating in barley following powdery mildew (*Blumeria graminis* f.sp. *hordei*) attack are highly diverse. From literature we know that they encode pathogenesis-related proteins,

components of phenylpropanoid biosynthetic pathways, protein secretory mechanisms and components associated with oxidative metabolism. However, resistance and susceptibility is determined at the single-cell level. Even in generally susceptible barley leaves, attacked epidermal cells defend themselves against attempted fungal penetration by localised responses leading to papilla deposition and reinforcement of their cell wall (Fig. 1). This conveys a race non-specific form of resistance. However, this defence is not complete and a proportion of the penetration attempts succeed in infecting. The resulting mixture of infected and uninfected leaf cells makes it impossible to relate powdery mildew-induced gene transcript accumulation, in whole-leaves or even dissected epidermal tissues, to resistance or susceptibility.

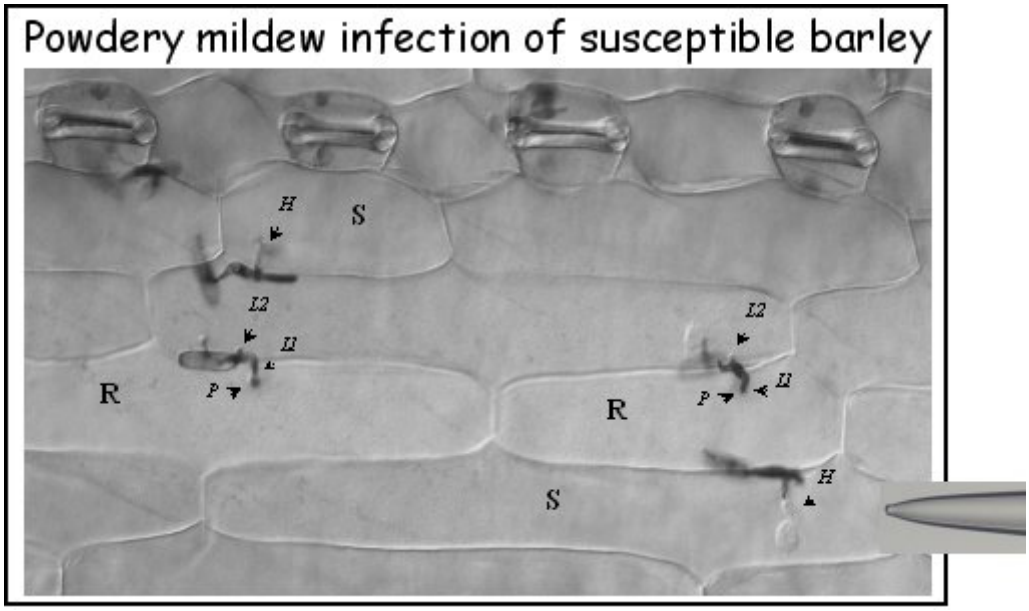

Figure 1. *Different outcomes of attempted penetration by* B. graminis *into leaf epidermal cells of a susceptible barley line, 18 h after inoculation. Two of the barley epidermal cells have resisted penetration (**R**) from the first* B. graminis *appressorial lobe (L1). This is recognised by the presence of a subtending papilla (P) and by the fact that a second B. graminis appressorial lobe (L2) has differentiated. Two cells have been penetrated successfully (**S**) and contain a rudimentary haustorium (H) beneath the first appressorial lobe. A glass micro-capillary needle was used to sample individual cells for gene transcript analysis.*

To solve this problem we have developed a procedure to examine gene expression in specific cells. In the present investigation, two situations were considered: 1) barley epidermal cells resisting fungal penetration, and 2) barley epidermal cells infected by the fungus (Fig. 1). Using glass micro-capillaries and micromanipulation we extracted samples from single barley epidermal cells after inoculation with powdery mildew fungus. The mRNA in the micro-extract was purified and processed into cDNA and amplified by PCR. The resulting cDNA pools were used as template in gene specific PCR of selected genes, as radioactively labelled samples in dot-blot/array hybridisation and for EST cloning. An expression analysis including genes previously reported to be induced upon mildew attack, showed that several genes, e.g. *PR1a* – encoding a pathogenesis related protein, and *GLP4* – encoding a germin-like protein, accumulated specifically in resistant cells, while *GRP94* – encoding a molecular chaperone, accumulated in infected cells. In collaboration with Dr. Patrick Schweizer, IPK-Gatersleben, Germany, a more detailed expression analysis was done on arrays spotted with 3,600 barley genes and showed very different profiles from resistant and infected cells (Fig. 2). 53 genes were expressed more than ten times higher than in non-inoculated cells and specifically induced in cells preventing fungal penetration, 26 genes were specifically and strongly induced in infected cells and 43 genes were strongly induced in both resistant and infected cells. Further characterisation of these genes and their possible involvement in resistance and susceptibility is in progress.

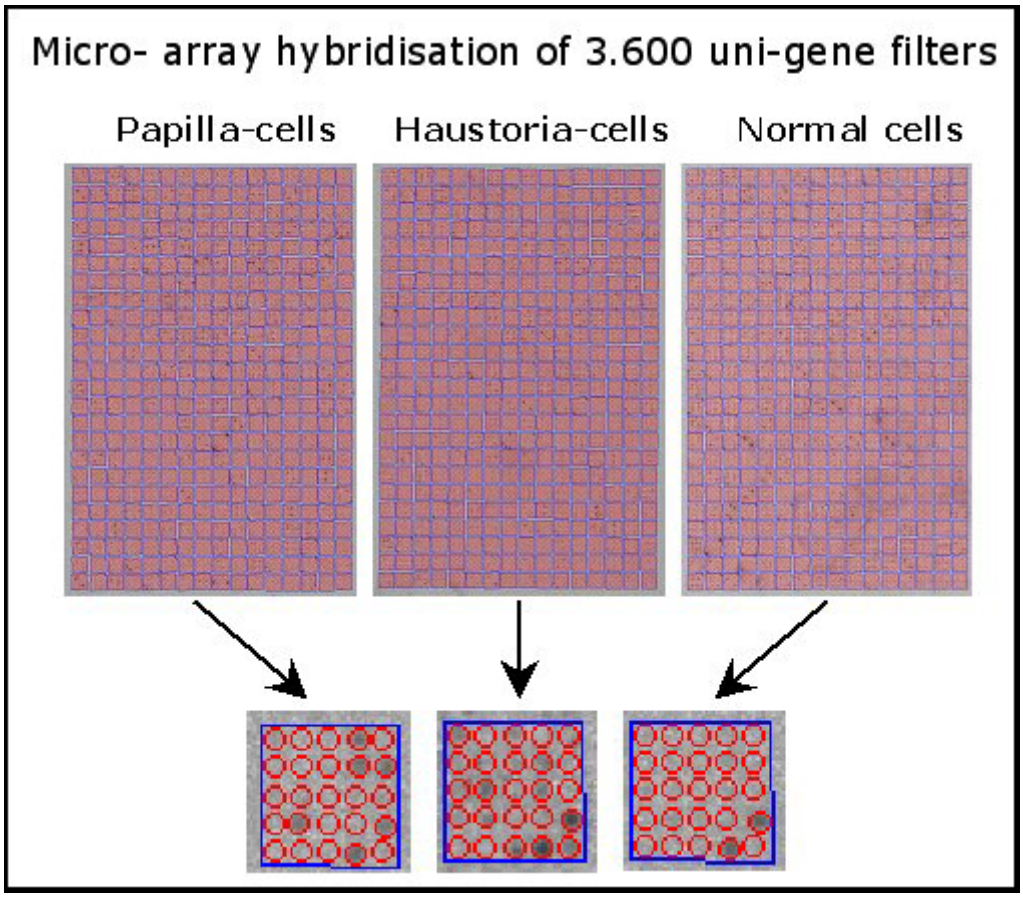

Figure 2. *Micro-array filters carrying 3,600 unique barley sequences hybridised with samples from individual barley epidermal cells that either resisted fungal penetration due to papilla formation, were successfully infected and contained a fungal haustorium, or were on non-inoculated leaves for control. The obtained profiles were very different between resistant and infected cells*.

Establishment of TILLING and application of Eco-TILLING

Gunter Backes, Nina Mejlhede Jensen, Zdenka Kyjovska and Ahmed Jahoor
Resistance Biology Programme

While classical (forward) genetics starts with a phenotype of interest and typically ends up with the isolation of the gene responsible for this phenotype, there is an increasing need for finding and analysing plants, where a specific gene of interest has been changed or destroyed by mutation. A growing amount of sequence data, without knowledge of the function of the genes represented in this information, demands that we take the sequence as starting point, in a reverse genetics approach, and search for phenotypic consequences of silencing or changing specific genes.
TILLING (Targeting Induced Local Lesions IN Genomes) is such a reverse genetics method. The technique is based on the creation of a mutation population and its analysis by the help of a DNA endonuclease, able to cleave at heteroduplexes. (Fig. 1).

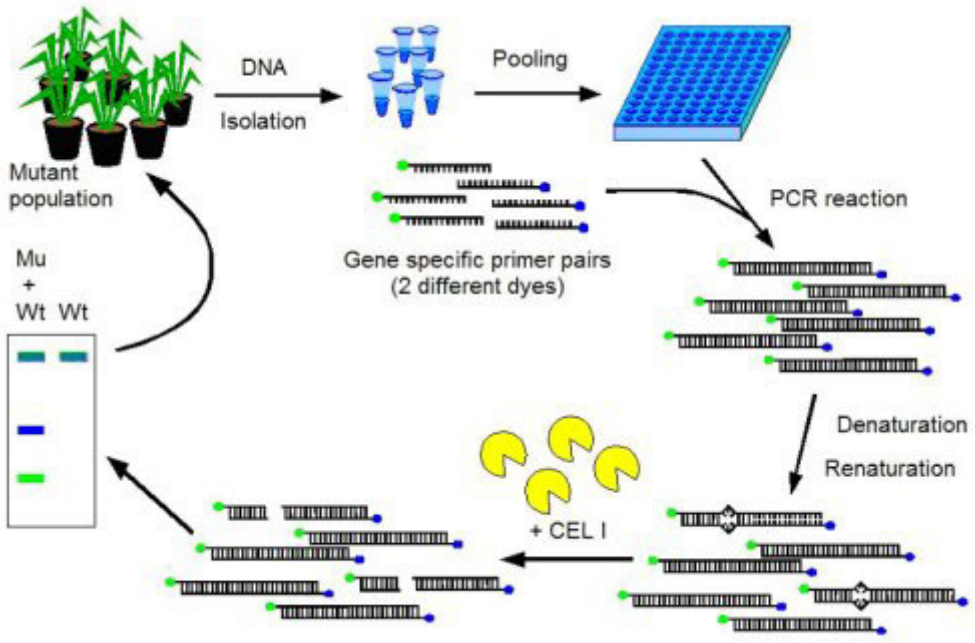

Fig.1: Principle of the TILLING procedure (Mu = mutant, Wt = wild type)
For this purpose, DNA from up to eight mutation lines is pooled and an amplification of a defined sequence of the gene of interest is performed on those pools. The two primers are carrying blue and green dyes. After the amplification, a denaturation and renaturation step leads to heteroduplex formation if there is any polymorphism in the DNA pool. The endonuclease CEL I cleaves at the heteroduplex. In the following gel electrophoresis, not only the initial PCR product labelled with the two dyes can be detected, but also one or more blue and green bands will result from CEL I cleavage.
At Risø, this important technique for the toolbox of molecular genetics was established, and Eco-TILLING was applied for the analysis of two powdery mildew resistance genes. In Eco-TILLING, the mixture of a standard line and several tester lines is used instead of a pool of mutation lines. For the highly polymorphic *Mla*-locus, 27 different marker patterns could be detected for 29 different alleles using a single primer pair. All newer resistance sources, derived from wild barley (*Hordeum vulgare* ssp. *spontaneum*) by our group, could be differentiated. The analysis of the *mlo*-locus (Fig. 2) revealed sequence differences within known alleles of this locus, and could correct an error in the allele description for a line with two different effective *mlo*-alleles in the pedigree.

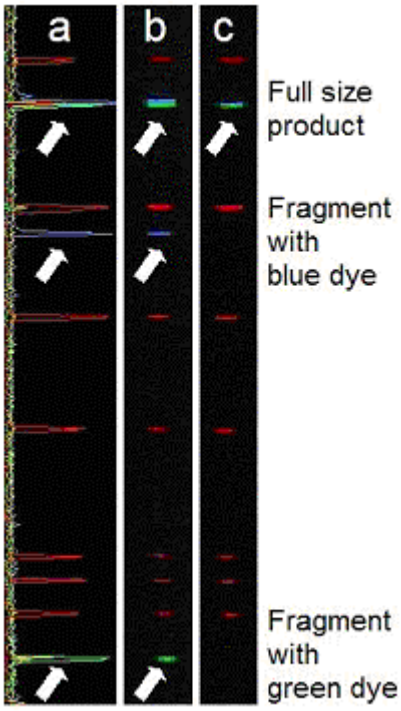

Fig 2:

Gel image of Eco-TILLING on the barley powdery mildew resistance gene *mlo* run on an ABI prism 377 sequencer and edited in GeneScan software.

Fig. 2a shows the intensities of the different colours, Fig. 2b and 2c show the gel pictures derived from those intensities. Fig. 2c shows the MLO-wild type (variety “Ingrid”) alone, Fig. 2a and 2b show a mixture of the wild type (“Ingrid”) and *mlo-3* (line “MC20”) with two additional bands, a blue and a green, adding up to the full product with both colours.

The red bands are from a size standard.

Eco-TILLING is not only an efficient tool for the characterisation of genotypes for specific sequences, but also represents an efficient MNP (multi-nucleotide polymorphism) marker type for the genetic mapping of known genes and for the use in association mapping. Besides, the rapid determination of sequence differences in many different genotypes, it also allows for the characterisation of sequence positions resulting in changes of function of the respective genes.

Acknowledgement
The CEL I enzyme was kindly provided by Dr. Kim Burhenne, Plant Quality Programme, Plant Research Department, Risø.

Wet oxidation used in co-production of biofuels

Anne Belinda Thomsen
BioMass Materials

In December 2002, a large EU-project was started for co-production of biofuels in collaboration with the Danish electricity company Elsam a/s. The overall objective is to develop cost and energy effective production systems for co-production of bioethanol and electricity based on integrated biomass utilisation.
During the first 12 months period of the project, two pilot plan reactors for pre-treatment of biomass by wet oxidation (Figure 1 and 2) were constructed and tested for their efficiency for pre-treatment of wheat straw that will be used for ethanol and electricity production. Pre-treatment of biomass by wet oxidation is performed for solubilising the hemicellulose fraction in straw as well as the alkaline salts (*e.g.* potassium chloride). Alkaline salts cause corrosion problems in conventional boilers during incineration of straw for electricity production. The solubilised hemicellulose is further converted by either enzymes or weak acid hydrolysis to monomeric sugar compounds for ethanol production. The cellulose fraction containing also lignin will be burned for electricity, however, some part of it may also be used for ethanol production if required. By-products from the pre-treatment and fermentation processes will be concentrated and used for animal feed.

Figure 1. Pilot plant reactor (100 kg/hour) for pre-treatment of biomass for ethanol production placed at Fynsværket in Odense

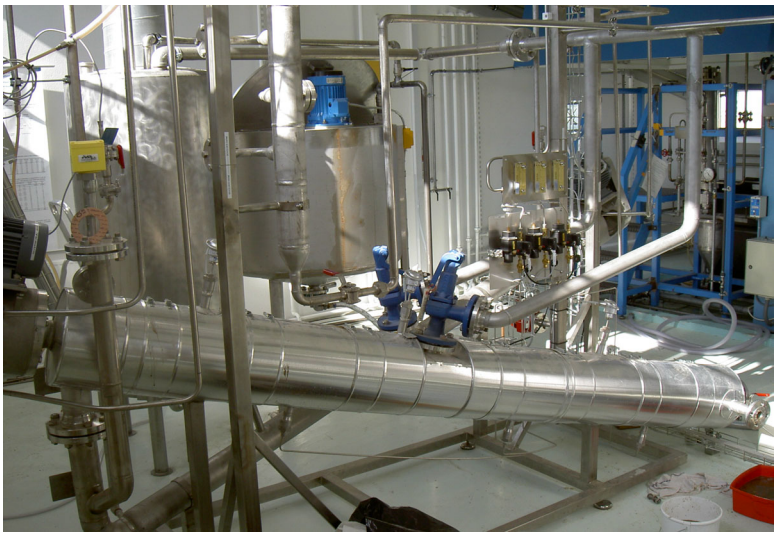

Figure 2. Risø's pilot plant reactor (1-10 kg/hour) for pre-treatment of biomass for ethanol production

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Jensen, Kim Poder (from 1/2 2003)
Korfitzen, Betina Hedegaard (1/2 2003)
Moestrup, Sofie Lund
Procopio, Beatriz Rocha
Vogeley, Corina Kehler

Summer-substitutes

Andersen, Mikael Rørdam
Azim Mohammed
Bustrup, Natacha
Hansen, Esben Halkjær
Hedberg, Lonnie
Hegazy, Nadia
Johansson, Inez
Kiær, Lars Pødenphant
Larsen, Annemette Møller
Larsen, Rasmus Kløcker
Lindell, Elsebeth
Lindhart, Ursula
Nielsen, Anja Haar

Visiting scientists

Abbas, Shahinaz, International Center for Agricultural Research (ICARDA), Aleppo Syria (6 months)
Bodea, Monica, University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Romania (1 months)
Chen, Bao-Dong, Chinese Academy of Science, Beising, China (3½ months)
Dayteg, Christophe, Sveriges Lantbruksuniversitet (SLU) Alnarp, Sweden (4 months)
Dupré de Boulois, Hervé, Université catholoque de Louvain, Louvain-la-Neuve, Belgien (5 months)
Fritz, Maendy, University of Giessen, Tyskland (6 months)
Gilson, Leena, INA P-G (Institut National Agronomique Paris Grignon) France (2 months)
Grigiene, Jurga, Kaunas University of Medicine, Lithuania (2 months)
Juszczuk, Izabela, Institute of Experimental Plant Biology, Warsaw University, Poland (5 months)
Krab, Klaas, Vrije Universiteit, De Boelelaan, Amsterdam, The Netherlands (7 months)
Kyjovska, Zdenka, Masaryk University, Brno, Czech (8 months)
Lababidi, Samer, International Center for Agricultural Research (ICARDA), Aleppo Syria (2 months)
Lissens, Geert,Laboratory for Microbial Ecology and Technology, Gent, Belgium (3 months)

Medina, Carlos Martín, University of Matanzas, Cuba (2 months)
Nejadghomi, Ali Asghar Nasrollah, University of Theran, Iran (6 months)
Nenninger, Anja, University of Wales, Aberystwyth, UK (8 months)
Orabi, Jihad, International Center for Agricultural Research (ICARDA), Aleppo Syria (12 months)
Pacurar, Daniel Ioan, University of Agricultural Cluj Napoca, Romania (4 months)
Penarel, Almudena Medina, Estación Experimental del Zaidín, Granada, Spain (5 months)
Prescha, Katrin, University of Potsdam, Germany (9 months)
Prieto, Eugenio Carrillo, University of Matanzas, Cuba (1 month)
Rysavka, Peter, MU Brno, Faculty of sciens, Brno, Czech (1 month)
Schulze, Silke,University of Potsdam, Tyskland (6 months)
Smith, Alice, University of Southampton, Hampshire, UK (3 months)
Trujillo, Carmen Gloria, University of Türingen, Germany (6 months)
Vítámuás, Pavel, Research Institute of crop production, Praha, CZ (3 months)
Wang, Linsong, Life Science College, Henan Normal University, Xinxiang, China (8 months)

Articles in international journals, books and reports

Aubinet, M.; Clement, R.; Elbers, J.A.; Foken, T.; Grelle, A.; Ibrom, A.; Moncrieff, J.; Pilegaard, K.; Rannik, Ü.; Rebmann, C., Methodology for data acquisition, storage and treatment. In: Fluxes of carbon, water and energy of European forests. Valentini, R. (ed.), (Springer Verlag, Berlin, 2003) (Ecological Studies, 163) p. 9-35

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Acronyms

2D	Two-dimensional
2D-IEF/SDS-PAGE	Two-dimensional isoelectric focussing/sodium dodecyl sulphate polyacrylamide gel electrophoresis
ABI	Applied Biosystems
Ac/Ds	Transposable elements, mobile pieces of the genetic material (DNA)
CDNA	Complementary DNA
CEL I	Endonuclease from celery cutting one of the two DNA-stands at mismatches
DNA	Deoxyribonucleic acid
DNP	Dinitrophenyl
E. coli	Escherischia coli
Eco RI	Restrictionendonuclease from *Escherichia coli*
EST	Expressed sequence tag
EXOTIC	EXOn Trapping Insertion Consortium
F.sp.	Forma specialis
FRET	Fluorescence Resonance Energy Transfer
F_v/F_m	Ratio of variable to maximal chlorophyll fluorescence
GFP	Green fluorescent protein
GLP4	Oxalate oxidase-like protein
GM	Genetically modified
GRP94	Endoplasmin
GUS	Beta-glucuronidase (Beta-D-glucuronoside glucuronosohydrolase)
hsp60	Heat shock protein 60 kDa
hsp70	Heat shock protein 70 kDa
IaaH	Indole Acetic Acid Hydrolase
LD	Long Day
Mbp/1C	Mega base pairs per DNA content of the unreplicated haploid set of chromosomes
Mla-locus	Gene locus on barley chromosome 1H conferring resistance against powdery mildew
Mlo-locus	Gene locus on barley chromosome 4H conferring resistance against powdery mildew
MNP	Multi-nucleotide polymorphism
MS	Mass spectrometry
Mu	Mutant
NMR	Nuclear magnetic resonance
P	Phosphate
PAR	Photosynthetic Active Radiation
PCR	Polymerase Chain Reaction
PR1a	Pathogenesis-related protein 1a
ROS	Reactive oxygen species
SD	Short Day
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
stDPE1	*Solanum tuberosum* disproportionating enzyme isoform 1
stDPE2	*Solanum tuberosum* disproportionating enzyme isoform 2
T-DNA	Transfer DNA
TILLING	Targeting Induced Local Lesions IN Genomes
UV-B	Ultra-violet radiation between 280-315 nm
Wt	Wild type

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