First International Workshop on Growing Plants for Increased Nutritional Value

University of Stavanger, Norway
May 12th- 14th, 2005

University of Stavanger

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14.30 - 15.15 ***Uwe Sonnewald,*** *Department of Biochemistry, University of Erlangen Nürnberg, Germany*. Genetic engineering of plants to reduce food allergens: Potentials and limitations.

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19.30 Dinner

Excursions on Saturday 14th of May.

Lysefjord and Flor og Fjære
We will leave the Rica Park Hotel at 2.30 p.m. and walk down to the harbour. The boat (”Helgøy express”) leaves from Tollbodkaien down town at 3 p.m.The boat will first take us to Lysefjord and Prekestolen (the pulpit). Arrival at Flor and Fjære is 5 p.m. where we will have a guided tour in the garden before having dinner. The boat will depart at 9.30 p.m. and the trip back to Stavanger is 20 min.

Wiig Gartneri
Information about the trip to Wiig Gartneri, a modern greenhouse facility producing heart-tomatoes, cucumbers, other vegetables, and flowers will be given on Thursday by Rune Slimestad.

Lectures

Search for new plant species for nutraceuticals, functional and medical food

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Epidemiological surveys performed during past 25 years, correlated diet as a factor in the etiology of five leading causes of death including ccoronary heart disease, certain types of cancer, stroke, non-insulin dependent diabetes mellitus, atherosclerosis. This correlation, however, frequently does not agree with the content of essential nutrients (protein, carbohydrates, fats and vitamins). Occurrence of other plant constituents correlate well with some kind of diseases. These constituents (phytochemicals, natural products) received common name "nutraceuticals" which *identify any substance considered a food, or part of food that provides medical or health benefits including the prevention and treatment of disease.* Food containing beneficial ingredients received common name "functional food" (pharmafoods, designer foods, vita foods, medical foods, dietary supplements), which *encompass potentially healthful products, including any food or food ingredients that may provide a health benefit beyond the traditional nutrients it contains.* Much attention has been paid to some of these chemicals to research their health promotional effect (carotenoids, catechins, phytoestrogens, lignans, flavones, flavonols, flavanols, procyanidyns, stilbenes, tocols, coumarins, diallyl disulphide and allicin, sulphoraphane and other isothiocyanates).

Huge number of papers has been published over last two decades to show structure related *in vitro* activities of these compounds, their absorption from gastric tract, availability, consumption rates and occurrence in plants. However, in spite of the fact that there is an agreement on beneficial effect of fruit, vegetable and grain consumption, the final conclusions of numeral scientific meetings and published papers indicate no straight evidence *in vivo* between phytochemicals and health benefits. Many clinical trials show no difference between placebo and phytochemical treatment, while others indicate beneficial effect. It seems we are still far away from the full understanding of their health promoting effect.

In vitro structure-activity relationship (SAR) studies performed recently, evidently show that quite limited number of natural compounds draws our attention. For example only in flavonoid family about 10 000 structures has been identified in plant material and only few of them (predominantly quercetin and kaemferol) were considered as valuable. Similar picture can be pointed in the other groups of phytochemicals. Additive or synergistic effects of phytochemicals from the same chemical class or even between classes still remain to be recognized and studied. For that there is a need to identify new plant species rich in desired phytochemicals to be used as supplements to our diet. In present lecture some examples of research on plant species that can be used in nutrition or as supplements will be presented. This will include flavonoids in buckwheat , saponins, stilbenes and yuccaols from *Yucca schidigera* and phytoestrogens and other phenolics from *Trifolium* species.

The Impact of Flavonoids on Human Health

Karl Egil Malterud, School of Pharmacy, Department of Pharmacognosy,The University of Oslo,Oslo, Norway

The flavonoids constitute a major class of secondary plant metabolites, and they are ubiquitous in higher plants. More than 7,000 flavonoids have been reported as natural products. Biological activity of flavonoids was first reported in the 1930s, and their properties in connection with protection against capillary permeability were studied by Szent-Györgyi and co-workers. These results were met with considerable interest. For some time, the flavonoids were regarded as vitamins and given the name Vitamin P. This is now regarded as obsolete; flavonoids are now usually regarded as non-essential, biologically active micronutrients.
In Norway, the pharmaceutical company Weider (now: Weifa) grew buckwheat commercially as a source of rutin, one of the first flavonoids to find clinical use. This practice is also long past.
After the first period of interest in the biological activity of flavonoids, a long period followed in which these activities were mostly forgotten. Towards the end of the 1970s, interest increased again, to a large extent because many flavonoids were found to be excellent antioxidants and radical scavengers, and also because they could modulate the activity of numerous important enzymes, e.g. those involved in arachidonic acid metabolism.
Today, many thousand articles deal with the bioactivity of flavonoids, and the number is increasing rapidly. Most of these articles are based on experiments in vitro (including our own work) or in animals, but several hundred clinical studies in humans have been published, as well.
Many publications deal with pharmacokinetics and metabolism of flavonoids in humans. Uptake of flavonoids after oral administration is usually moderate (but differs for different flavonoids). Flavonoids may be metabolized both by the GI tract microflora and by organs such as the liver. At present, the biological activities of flavonoids metabolites is known only to a limited extent.
Numerous reports on the antioxidant activity in the human body after ingestion of flavonoids-rich foods such as tea, chocolate, red wine, onions etc. have been published. Apparently, intake of these foods may lead to a higher antioxidant level in the body, although it may be difficult to ascribe this to one or a few substances.
Flavonoids may regulate enzyme activity. In this connection, enzymes of the arachidonic acid pathways, such as cyclooxygenase and the different lipoxygenases, have been intensively studied, and it may be possible that some of the putative biological effects of flavonoids may be ascribed to inhibition of these enzymes. Many other enzyme activities are modulated by flavonoids, but the relevance of this to human health is less well known.
Diseases such as cancer, cardiovascular disease and inflammatory diseases are among those where flavonoids have been suggested to play a role in amelioration or as inhibitors of the progression of disease. More research is needed on this.
Some important flavonoids or flavonoid-enriched preparations which have been subjected to clinical studies are:
-Isoflavones from soy, which are being extensively used against menopausal ailments in women, and also have been suggested to counteract bone loss and to have an advantageous effect on blood lipids.
-Flavonolignans from milk thistle, *Silybum marianum*, which has a long story of use against liver disease.
-Proanthocyanidins from hawthorn, *Crataegus* spp. are used in milder cases of congestive heart failure.
-Pycnogenol, a polyphenol mixture (mainly proanthocyanidins) from the bark of *Pinus maritima*, has been studied clinically in chronic venous insufficiency, retinopathy and some other diseases with promising results. A similar preparation from grape seeds is also used.
-Flavonoid preparations such as Daflon (diosmin + hesperidin) and troxerutin (a semi-synthetic rutin derivative) are employed against vascular disease.
For most of these, results from clinical studies have been contradictory; positive and negative results being reported in different studies. This is probably even more the case for flavonoid-rich health food preparations with purported health effects where it appears that the flavonoids may be involved in the biological activity of these preparations. In this area, documentation is often of variable quality, although some preparations, e.g. from *Ginkgo biloba* (against decreased blood circulation) and cranberry (against bacterial bladder infections) appear to have promising effects.

The utility of metabolic profiling in metabolic engineering

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Many attempts have been made to understand and manipulate plant metabolic pathways by the use of reverse genetic approaches. This is particularly true in the potato due to its amenability both for *Agrobacterium*-mediated gene transformation and for biochemical analyses. The generation of transgenic potato and tomato plants with modifications in carbon metabolism and partitioning have had mixed success when assessed from a biotechnological standpoint. In recent years we have used a combination of GC-MC based metabolic profiling techniques and contemporary statistical tools in order to allow the biochemical phenotyping of these transgenics. It was our hope that greater knowledge of both direct and pleiotropic effects of the introduced transgenes would lead to greater understanding of the interactions involved in plant metabolic networks. Along the same lines we have established a *Solanaceous* microarray that allows the assessment of around one thousand genes, primarily concerned with central metabolism. These tools allow us not only to characterise genetic diversity but also to replicate or *phenocopy* it by the application of diverse environmental conditions. By analogy to what has been achieved in microbial systems most recently we have generated several plants that were simultaneously modified in multiple metabolic pathways in an attempt to re-route carbon flux within the potato tuber system. The resultant transformants did indeed exhibit large metabolic shifts, however, not in the direction suggested. Recently we have extended these studies to transgenic and breeding populations of tomato, first results of which will be discussed also. These results allow several important conclusions to be made about heterotrophic carbon metabolism but they also suggest that yet further improvements as required with respect to the analytical tools used before our understanding allows routine metabolic engineering.

Methods to determine health-promoting effects of bioactive compounds

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Many epidemiological studies show negative correlations between the intake of vegetables and fruits and the incidence of several important diseases, including cancer and atherosclerosis. Many studies have attempted to define which bioactive compounds in vegetables are responsible for these protective effects, as a necessary prerequisite to attempt to grow plants for increased nutritional value. Most of these studies have focused on vitamins, essential minerals, antioxidants and fibres, components that are known to be essential, non-toxic or both. However, they have generally shown that simple supplementation by one or a few nutrients, antioxidants or fibres cannot reproduce the protective effect of their vegetable sources. There are in principle only two possible explanations for this discrepancy: Either the known compounds are only effective if they exist in a special matrix or combination, or vegetables contain other compounds with important health promoting effects, which are different from relief of malnutrition, reduction of oxidative stress or regulation of colon chemistry.

Several such non-nutrient beneficial compounds have been identified, which clearly exert their effects through other mechanisms than as antioxidants or prebiotics, such as glucosinolates (from Brassica), nitrate (from e.g. lettuce) and sulphoxides (from Allium). In contrast, it appears that most data cited to support the matrix/inter-action theory could just as well be explained by the actions of unknown bioactive compounds. A well-known example is that people with high plasma levels of food-derived beta-carotene are better protected against cancer than people consuming beta-carotene supplements. Either the beta-carotene must interact with a carrot matrix/component in order to be effective (carrots being the major dietary source of beta-carotene), or carrots contain one or more other cancer-preventing compound(s), with no need for any beta-carotene to obtain the effect.

If most of the beneficial effects of vegetables and fruits on health are due to not yet identified compounds, these putative beneficial compounds will have a set of specific properties that can be used as selection criteria to identify likely candidate compounds for further study. Basically, their bioactivity and uptake in the body must be large enough to affect human cells, and the effect in the relevant concentration range must be mainly positive.

Based on these principles, a step-wise screening procedure was defined and tested:

Step 1 is an initial literature based screening, according to three criteria: 1.1, presence of chemically reactive functional groups; 1.2, toxicity at high concentrations or other bioactivity; and 1.3, presence in healthy foods.

Step 2 is testing for minimum criteria defining health-promoting compounds, where additional (short term) experiments are often needed: 2.1, positive or biphasic ("hormesis") responses in bioassay; 2.2, human tissue concentrations corresponding to beneficial effects in bioassay; and 2.3, possibility to control content in food.

Step 3 is testing whether the effect in an intervention study (animal model or human study) can substitute for that of the corresponding food in the same concentration range.

Assessment of the relatively well-known bioactives in major vegetables showed several groups of compounds which fulfilled the criteria for step 1, including polyacetylenes, glycoalkaloids, sesquiterpene lactones and coumarins, as well as the already known glucosinolates and sulphoxides. Of these, the polyacetylene falcarinol was selected for experimental testing in step 2. It fulfilled all 3 criteria and progressed to step 3, where it showed anticancer effect in a rat colon cancer model, with a similar magnitude as a treatment with whole carrots. So falcarinol rather than beta-carotene is most likely the main anticancer compound in carrots, although much more research is needed before this knowledge can be translated into growing carrots for increased nutritional value.

Flavonoids do not fulfil criterion 2.2 as regards the free radical scavenging effect. While this does not rule out the possibility of other beneficial effects, it means that the antioxidant properties of flavonoids are not likely to be important for the nutritional value of plants. For other effects of flavonoids, which are exerted at physiologically relevant concentrations, the dose-response and structure-effect relations must be defined before it is possible to determine which plants have better nutritional value than others, or the plants tested directly.

The data needed for assessing the step 2 criteria for glycoalkaloids, sesquiterpene lactones and coumarins are not available from the literature. So additional research is needed to determine if increasing the nutritional value of the corresponding food plants means increasing or decreasing the contents of these compounds.

References:

Brandt et al. (2004), Trends Food Sci. Techno.15, 384-393.

Kobæk-Larsen,et al. (2005), Journal of Agricultural and Food Chemistry 53, 1823-1827.

The vegetarian salmon: Challenges for plant production and plant improvement

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One million tonnes of salmon are produced annually by intensive farming methods. This involves the use of 1.5 Mill tonnes compounded feed, which is shaped into pellets by extrusion processes. Traditionally, the main raw materials, which were used for making a salmon feed, were of marine origin. Fish meal and fish oil comprised 100% of dietary protein and oil.

The development to replace these traditional raw materials was fuelled by several pressures. Economically, plant raw materials in most cases lead to stabilization and long-term reduction of feed costs. On a global scale, proteins and oils produced by plants are seen as the more sustainable alternative, although fishing for fish meal and fish oil production is strictly regulated. Most recently, focus has been put on the presence of organic contaminants specifically in fish oils, which originate from polluted areas of the oceans.

Today, up to 50% of both protein and oil in salmon feeds can already be of plant origin. To reach this level of replacement, research had to answer many questions with regard to the correct balance and digestibility of nutrients (minerals, amino acids), the quality of the produced fish (fatty acid profile), and the negative effects of the so-called antinutritional factors (phytic acid, fiber, saponins, etc) in plant raw materials.

For the next generation salmon feed we expect an even more expanded use of plant raw materials. Not only is it intended to further replace fish meal and fish oil (maybe create a completely vegetarian diet), but also a more sophisticated use of other functional plant components is of interest. Areas of development are the use of immunomodulatory effects of plant compounds, natural antioxidants, and digestibility stimulants. The production of long-chain n-3 fatty acids and of the carotenoid pigment astaxanthin by plants would help to remove major bottlenecks for the expansion of salmon farming.

Transcriptional Regulation of Flavonoid Biosynthesis

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Flavonoid biosynthesis is the best understood secondary metabolic pathway in plants, both in terms of the biosynthetic enzymes involved and the regulatory proteins that control the activity of the pathway and the production of flavonoids. Several features have been determined as general in the mechanisms of control of flavonoid biosynthesis in higher plants. One is that anthocyanin biosynthesis is regulated by the combined activity of a MYB and a basic-Helix-loop-Helix (bHLH) protein. Another is that the steps (target genes) that these proteins regulate vary from one species to another; the entire committed pathway is regulated by the MYB/bHLH complex in maize, but only the genes encoding the late biosynthetic steps are regulated by the complex in dicotyledonous flowers. A third feature is that other branches of flavonoid and phenylpropanoid metabolism may be regulated by other MYB-related proteins, often operating without a bHLH protein partner.

We have focussed on the control of pigmentation intensity and patterning in flowers, using *Antirrhinum majus* as a model because of the large number of mutants affecting floral pigmentation available in this species. In *A.majus* flowers anthocyanin biosynthesis is regulated by three MYB related proteins (Rosea1, Rosea2 and Venosa) and two bHLH proteins (Mutabilis and Delila). Mutations in the genes encoding these proteins result in patterned flowers with altered distributions of anthocyanin. Differential activity of the regulatory proteins (especially Rosea1 and Venosa) underpins much of the natural variation in floral pigmentation within the genus *Antirrhinum*. The MYB proteins interact with the bHLH proteins to activate expression of the biosynthetic genes. However the different combinations of the proteins are not equivalent in terms of their ability to induce anthocyanin biosynthesis, some are stronger than others. Surprisingly, the different regulatory combinations also show distinct specificities in their abilities to activate different target genes. This is most likely a function of differential binding affinities of the regulatory complexes for the different target genes. Specificity in regulatory complex activity can account for the non-additive phenotypes resulting from particular double mutant combinations in *A.majus*. The differences in the activation of target genes by the MYB/bHLH complex in different plant species may, in part, be explained by differences in target gene recognition.

When the regulatory proteins from *A.majus* are over-expressed in plants they are able to induce not only the late biosynthetic target genes but also those like chalcone synthase operating earlier in the pathway. This result is unexpected because mutant analysis in *A.majus* has shown that only the late biosynthetic target genes are dependent for their expression on the activity of the MYB/bHLH complex. This paradox can be explained if the expression of the early biosynthetic genes can be fully rescued by other regulatory proteins when either the MYB or bHLH regulatory protein is non functional. Another MYB protein, encoded in Arabidopsis by *AtMYB12*, which regulates flavonol sythesis, may provide this complementing activity in flowers.

A third protein, with WD40 repeats, has been shown to be essential for the activity of the MYB/bHLH regulatory complex in Arabidopsis and Petunia flowers, and important in maize kernels. In *A.majus* the WD40 protein appears to play a relatively minor role in the activity of the regulatory complex.

Since the regulatory proteins controlling anthocyanin biosynthesis serve as very potent inducers of their target secondary metabolic pathway, they can be used to engineer flavonoid synthesis most effectively in plant tissues. Flavonoids are very significant dietary bioactives offering protection against cardiovascular disease, certain cancers and age-related degenerative diseases. Transcription factors can be used to engineer flavonoid biosynthesis in foods such as tomato fruits, which normally show very low flavonoid accumulation. Metabolic engineering using this strategy results in more than 150-fold increase in flavonoid accumulation and a three-fold increase in antioxidant capacity of the fruit. These methods can be used for crop/food improvement either using genetic engineering or through marker-assisted breeding. Increasing flavonoid biosynthesis can lead to health-promoting foods but can also result in crops that are more tolerant of biotic and abiotic stresses.

Arabidopsis seed as model for flavonoid metabolism

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Plants produce various secondary metabolites, including flavonoids, that influence their quality and nutritional value. The three major end-products of the flavonoid pathway are anthocyanins, flavonols and proanthocyanidin polymers (PAs; *syn.* condensed tannins). These polyphenolic compounds serve essential functions in plant (e.g. protection against diverse biotic and abiotic stresses, seed quality). Currently, there is a growing interest in the potential health benefits of flavonoids, and more especially PAs, as natural antioxidants*. In *Arabidopsis*, PAs accumulate specifically in the seed coat (or testa), giving the mature seed its brown colour after oxidation.

Genetic, molecular, and biochemical analyses allowed the identification of several loci, named *TRANSPARENT TESTA 1* (*TT1*) to *TT19, TTG1*, *TTG2, TDS1* to *TDS6* (*TANNIN DEFICIENT SEED*), *AHA10* and *BANYULS* (*BAN*). Most of the mutants and corresponding genes have been characterized, among which twelve can be placed in the flavonoid pathway. Recently, it has been shown that the Anthocyanidin Reductase (ANR), a core enzyme in PA biosynthesis that converts anthocyanidins to their corresponding 2,3-cis-flavan-3-ols, is encoded by *BAN*. We have characterized PA-accumulating cells demonstrating that both PA accumulation and the activity of the *BAN* promoter are restricted to the innermost cell layer of the integuments, also called endothelium (seed body and micropyle areas), and to the pigment strand (chalazal area). We demonstrated that a 86-bp DNA fragment of the *BAN* promoter functions as an enhancer specific for PA-accumulating cells, allowing to carry out a specific genetic ablation of these cells and to demonstrate the important role of PAs in seed quality (Debeaujon *et al.,* 2003).

Six regulatory loci required for PA biosynthesis have been previously described, namely *TT1*, *TT2*, *TT8*, *TT16*, *TTG1*, and *TTG2*. *TT1* encodes a zinc finger protein of the new WIP family (Sagasser *et al.,* 2002), *TTG1* a protein with WD40-repeats (Walker *et al.,* 1999), and *TTG2* a transcription factor of the WRKY family (Johnson *et al.,* 2002). *TT16* encodes the ARABIDOPSIS B-SISTER (ABS) MADS domain (Nesi *et al.,* 2002), *TT8* a bHLH (Nesi *et al.,* 2000) and *TT2* an R2R3 MYB domain proteins (Nesi *et al.,* 2001), respectively. Analyses of the *pBAN:GUS* expression in the different regulatory mutants have provided additional spatio-temporal information allowing to better understand the complex network of regulations involved in the differentiation of PA-accumulating cells, *BAN* activation, and finally tannin biosynthesis (Debeaujon *et al.,* 2003).

Interestingly, *TTG1, TT8,* and *TT2* control the expression of several genes, such as *DFR* and *BAN*, suggesting that the three proteins may interact to control PA metabolism. In addition, *TT2* expression was restricted to PA accumulating cells, consistently with *BAN* expression profiles (Nesi et *al.,* 2001, Debeaujon *et al.,* 2003). The interplay of TT2, TT8, and their closest MYB/bHLH relatives, with TTG1 and the *BAN* promoter has been investigated using a combination of genetic and molecular approaches, both in yeast and *in planta* (Baudry *et al.,* 2004). The results obtained using Glucocorticoid Receptor (GR) fusion proteins *in planta* strongly suggest that TT2, TT8, and TTG1 can directly activate *BAN* expression. Two- and three-hybrid experiments allowed to demonstrate that TT2, TT8, and TTG1 could form a ternary complex binding the promoter of *BAN*. TT2 is responsible for the specific recognition of the promoter, in co-operation with TT8. TTG1 regulates the activity of these proteins probably by regulating TT8 activity by an unknown mechanism (Baudry *et al.,* 2004).

Nevertheless, many gaps remain in the understanding of flavonoid metabolism, the nature and function of several *TT* loci, especially in relation to PA synthesis and modifications (e.g. polymerization, oxidation, or glycosylation), compartmentation and accumulation. For instance, we are currently investigating the functions of TT10 and TT15, for which we have identified candidate genes (Pourcel *et al.,* in preparation; Debeaujon, Nesi *et al.*, unpublished). To extend our knowledge of the flavonoid pathway and to characterise precisely the function of each protein under study, we have undertaken a comprehensive identification of all major classes of flavonoids during seed development, maturation and germination by means of LC-MS and acid-catalysed cleavage analyses (Routaboul *et al.,* in preparation). These metabolome analyses should also pave the way to study the natural variability of PA metabolism among various arabidopsis ecotypes.

* An EC funded project named "FLAVO", coordinated by our laboratory, is starting on this topic.

Nitrate as a key nutrient and signal compound influencing primary and secondary metabolites.

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Soil nitrate is the main nitrogen source for most higher plants and crops and is thus one of the limiting factors for both crop yield and quality. Besides its role as a nutrient, nitrate acts also as a signal regulating the expression of genes involved in nitrogen uptake and metabolism but also in photosynthesis, carbon and secondary metabolisms.

After uptake by the roots, nitrate is first reduced to nitrite by a cytosolic enzyme, nitrate reductase (NR). NR is submitted to a complex regulation. For instance, it has been shown that NR is phosphorylated in the dark on a conserved serine residue and that the phosphorylated enzyme is inactivated by subsequent binding of proteins belonging to the 14-3-3 family. The importance of this post-translational control on the overall regulation of the nitrate assimilation pathway has been investigated in Nicotiana plants overexpressing a deregulated NR which was mutated on the conserved phosphorylated serine residue. These plants showed, as expected, a high and constitutive NR activation state which demonstrates that this residue is indeed important for the NR inactivation in planta. Interestingly, these transgenic lines accumulated high levels of glutamine accompanied by vey low concentrations of nitrate. Furthermore, we have observed an increased rate of NO emission when NR expression was deregulated. Since NO is an important signalling molecule in plants, the question of the biological role of this NR-derived NO is of importance. Ectopic expression of NR has also been performed in potato and the resulting transgenic plants showed a dramatic reduction of nitrate accumulation in tubers and, in some cases, a higher biomass production.

Plant genes regulated by N supply have already been identified in different metabolic pathways and developmental processes. Yet the molecular mechanisms by which plants sense and respond to variations in N supply remain rather unclear. We have therefore used high density oligonucleotide arrays (Affymetrix ATH1 genome arrays containing more than 22 500 probe sets representing around 24 000 annotated genes) to perform extensive expression profiling on Arabidopsis plants grown in hydroponic conditions and subjected to N supply after starvation.

A limited number of genes were found to be regulated by ammonium supply whereas nitrate feeding influenced the expression of a much larger number of genes operating in different metabolic pathways. Moreover several putative regulatory genes were also identified among nitrate-responsive transcripts. We will present a characterization of these new N-responsive genes. The complete results will soon be available on our website : http://www-ijpb.versailles.inra.fr/nap/nap_accueil.htm.

Circadian and diurnal rhythms: Regulation of metabolite levels

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Circadian clocks produce an internal estimate of time that allows biological events to be synchronized with the external day/night cycle. The fact that clocks with similar properties and regulatory architecture are found in yeast, animals and plants, and that they have evolved at least four times, indicates that possessing circadian rhythms must confer a selective advantage. However, the nature of this advantage has proved elusive. We provide the first direct experimental evidence in eukaryotes, that the biological clock provides a competitive advantage in normal day/night cycles. Using a series of relatively simple, but surprisingly informative experiments involving *Arabidopsis thaliana*, we have shown that a circadian clock with a period matched to the period of light and dark cycles (i.e. the clock is resonant with the environment) increases growth via higher rates of photosynthesis. This is the first demonstration that the clock enhances a metabolic pathway. In wild-type, and long and short circadian period mutants, plants with a clock period matched to the environment contain more chlorophyll, fix more carbon, grow faster and survive better than plants with circadian periods differing from their environment.

To understand the molecular basis by which the clock regulates photosynthesis, we characterised the circadian regulation of transcript abundance in whole leaves using 70-mer oligonucleotide microarrays. Approximately 1800 circadian regulated transcripts were identified. The circadian clock regulates transcript abundance for 76% of genes coding for components of the light harvesting complexes (LHC), 55% coding for photosystem I and 50% coding for photosytem II. The true percent is likely to be higher, as not all genes are present on the arrays, The circadian regulation of photosynthetic genes far exceeds the 10 % average for the rest of the genome. It is noteworthy that the abundance of transcripts arising from nuclear genes coding for components of the photosystems and LHC's peak at 4 - 8 hours after dawn, and that of the genes coding for proteins involved in chlorophyll biosynthesis, only one (the H subunit of magnesium chelatase) is controlled by the clock.

Networks of negative feedback loops of gene expression underpin circadian clock function. Signal transduction networks provide the framework for synchronisation (entrainment) of the molecular clock with the external day-night cycle, and also communicate timing signals from the clock to clock-controlled aspects of physiology and metabolism. There are circadian and diurnal rhythms in the concentration of cytosolic free calcium ($[Ca^{2+}]_{cyt}$, but the function of these 24 h $[Ca^{2+}]_{cyt}$ oscillations remain unknown. We are investigating the position(s) of Ca^{2+}-based signalling events within the circadian signalling network. For example, circadian $[Ca^{2+}]_{cyt}$ oscillations might participate in clock entrainment, have a role in core oscillator function, or contribute to clock output signalling. We have investigated potential upstream regulators of circadian Ca^{2+} signals and are identifying candidate molecular and physiological targets for circadian $[Ca^{2+}]_{cyt}$ oscillations. We provide new data that the circadian regulation of signalling metabolites is central to clock function.

Phenolics, carotenoids and chlorophylls in organically grown broccoli (*Brassica oleracea* L. var. *italica*) and leek (*Allium porrum* L.) in Northern Sweden. Relation to latitude, mineral nutrition and growth.

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Broccoli and leek were grown in the greenhouse during spring 2003 and 2004 at Umeå (63°49'N, 20°17'E). They were sown in six different substrates certified for organic growing, and three different regimes for fertilizer additions were applied. The plants were transplanted into field at Offer (63°08'N, 17°43'E).

In 2003 the plants were analysed for 38 elements at the time of transplanting, and at peak harvest. At harvest they were also analysed for chlorophyll and carotenoids in the edible part. In 2004 the plants were analysed for 38 elements at the time of transplanting, and after 4–5 weeks in the field. At transplanting they were also analysed for phenolics and carotenoids. The mineral analyses were performed at LMI AB Helsingborg Sweden, and the biochemical analyses at PlantChem Sandnes Norway.

As different treatments did not achieve harvest stage at the same time in 2003, the broccoli heads were sampled at two occasions with one week between, and sent to the laboratory immediately. A reference sample was collected in a conventionally grown field near Stavanger (58°58'N, 05°44'E). The samples collected one week later (B) than the first one (A) contained much more chlorophyll and carotenoids, while the reference sample (C) had the lowest content of all three compounds (Fig. 1).

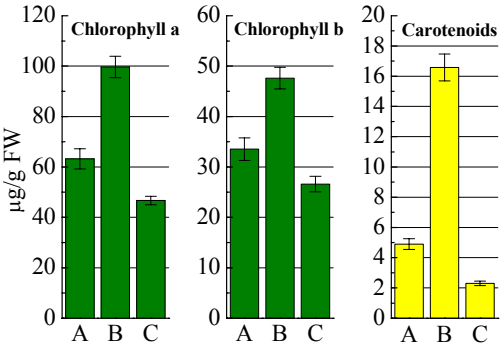

Fig. 1. Content of chlorophyll and carotenoids in 3 different samples of broccoli heads in 2003. A=sampled and analysed in week nr 33, B= sampled and analysed in week nr 34, C=Reference sample from a conventional field near Stavanger.

In 2004 the transplants of broccoli were analysed for 8 different phenolics (named X1–X8) of which two (X1 and X7) were positively correlated to plant weight (Fig. 2). All other showed a strong negative correlation to plant weight (Fig. 3). The same two compounds (X1 and X7) were positively correlated to marketable yield 2 months after transplanting into the field. The transplants of broccoli were also analysed for carotenoids which was positively correlated to plant weight and to marketable yield.

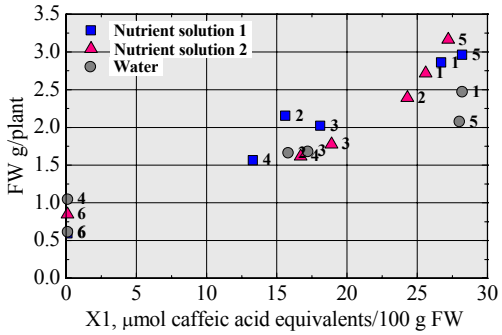

Fig. 2. Relationship between plant weight and X1 (invisible phenol, maybe a hydroxycinnamic acid-derivative, detected 280 nm) in transplants of broccoli in 2004. 1–6 refer to the different substrates.

The transplants of leek were analysed for 17 different phenolics (named Y1–Y17) of which one (Y3) showed some positive correlation to plant weight, while all the others were more or less negatively correlated to plant weight. Two of the compounds (Y3 and Y17) showed some positive correlation to marketable yield 3 months after transplanting into the field.

Iron, nitrogen, potassium and calcium in the plants showed a strong positive correlation to plant weight and yield in broccoli. In leek, nitrogen, sodium and magnesium in the plants showed a strong positive correlation to plant weight while phosphorus in the plants showed a strong positive correlation to yield.

In general, the different substrates had stronger influence on plant growth and content than the different fertilizer regimes applied to the transplants.

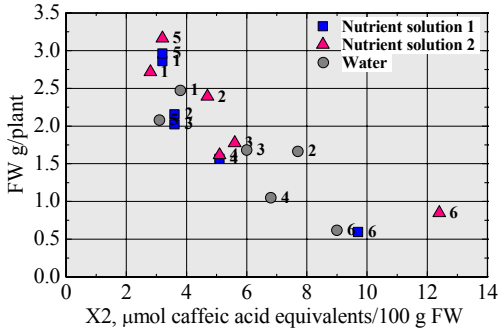

Fig. 3. Relationship between plant weight and X2 (unspecific flavonol, connected to both sugar and hydroxycinnamic acids, detected 320 nm) in transplants of broccoli in 2004. 1–6 refer to the different substrates.

Effects of light on production and quality of greenhouse vegetables grown at northern latitudes

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Plant production in northern countries is hampered by a short growing season. In Norway, waterpower provides relatively cheap and renewable energy that can be used for artificial lighting. Light is often the limiting factor for plant growth and productivity in greenhouses, and the use of artificial radiation became already early last century an important subject for investigation in Norway. Development of different lamp types gave rise to investigations of light quality on plant performance. Artificial irradiation was primarily confined to plant propagation. Large-scale irradiation of entire crops started in Norway in 1989 and has given rise to a marked increase in winter production of flowers and vegetables.

The Norwegian Crop Research Institute performs applied research in all chains of plant production. Research on greenhouse production is coordinated from Særheim Research Centre. Særheim is located in the southwest of Norway where 80% of tomatoes and 50% of the Norwegian cucumbers are produced. Research on the use of artificial light for cucumber production started here in 1990. After that, production systems for lettuce, herbs, strawberry, tomato and sweet pepper were developed. Growers that nowadays use our system for year-round production of cucumbers have increased their yield from 40 to 160 kg/m^2. In 2004, we were the first to reach an annual yield of 100 kg/m^2 in tomato.

In our applied research, consumer and wholesaler demands define the quality aspects of food to be produced. Consumers increasingly demand save and healthy food of high quality. At Særheim Research Centre, effects of environmental conditions (light, temperature, CO_2, air humidity, nutrients, growth media) and cultural practices (training, irrigation, harvesting) on size, colour, shelf life, taste and biochemical compounds are being quantified. It could be shown how light intensity and light quality effects the contents of chlorophyll, anthocyanins, ascorbic acid and nitrate in lettuce, shelf life and taste in herbs, taste and antioxidant activity in strawberry, titratable acidity and the contents of phenolics, ascorbic acid, lycopene and soluble solids in tomato. Results will be presented at the workshop. The favourable ratio between light and temperature in our region offers an opportunity to produce high quality products year-round.

Norway is known as a healthy country, with little problems with pests and diseases, with an availability of renewable energy, organic growth media and organic fertiliser and with a high water quality. This gives rise to a development of organic production. Research on organic production of greenhouse vegetables started at Særheim in 2003. By now, a rational growing system for organic production of tomato is developed and a system for cucumber production is on trial. Preliminary trials show differences in the content of phenolic compounds, ascorbic acids and antioxidant activity in fruits and plants grown on organic growth media when compared to rockwool. Results will be presented at the workshop.

Influence of salinity on phenolic compounds and mineral nutrient content in hydroponically cultivated broccoli plants

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Broccoli (*Brassica oleracea* L. var. *Italica*) is known to be a good source of phenolic compounds such as other bioactive compounds (glucosinolates, vitamin C). The presence of these compounds in vegetables is considered important in the prevention of some diseases, considering that a diet rich in broccoli can reduce the risk of a number of cancers. The contribution of phenolic compounds to health improvement is related to their antioxidant activity, as they provide bioactive mechanisms to decrease free radical. The aim of the present work was to use saline water for growing broccoli and to study the effects on water and nutrient uptake for obtaining broccoli containing high concentration of phenolic compounds. The behavior of the plant when exposed to increasing concentration of NaCl (0, 20, 40, 60, 80 and 100 mM NaCl) was examined. Phenolic compounds composition were determined in leaves, the ions composition was determined in roots a leaves and water uptake was determined as root hydraulic conductivity and stomatal conductance. Water uptake was reduced as the concentration of NaCl was increased. Phenolic compounds were increased with salinity probably due to the osmotic adjustment or as a response to the increase of free radicals. According to the nutrient concentration, an increase of NO_3^-, PO_4^{3-} y SO_4^{-2} were observed in roots and leaves with 40 y 60 mM NaCl treatments. The increased of sulfate concentration could be related to the glucosinolates synthesis. In conclusion, salinity did not cause any specific nutrient deficiency in broccoli and could active mechanisms of salinity tolerance that produced an increase in nutritional value.

Presentation of a Norwegian research programme (2002-2006) – "Bioactive phytochemicals (flavonoids) in fruit and vegetables: storage, processing and rapid sensor-based analytical methods"

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The population in northern Europe, and especially in Norway, has a very low consumption of fruit and vegetables. There is substantial evidence that a large intake is related to a reduced risk of cancer and cardiovascular diseases, probably because fruit and vegetables contain bioactive constituents in addition to nutrients. Very little is known about the behaviour of these phytochemicals during storage and processing of plant products, and how they can be rapidly measured for convenient quality control. The national research programme focuses on flavonoids being an important group of the bioactive compounds.

Programme objectives:

1. Investigate how industry relevant storage and processing techniques affect health-related bioactivities and the levels of flavonoids in selected fruits, berries and vegetables grown, stored and processed in Norway.
2. Identify individual or groups of flavonoids of relevance for antioxidant capacity, enzyme regulation and gene expression.
3. Develop rapid, preferably non-destructive methods for measurement of flavonoids in a selection of Norwegian fruits and vegetables and products thereof.
4. Investigate whether these methods in combination with other rapid methods of analysis can be used as a basis for storage and process quality control in the studied fruits and vegetables.

Some results from the programme:
Mice fed berries rich in antioxidants had increased activity in brain and muscle of genes that are involved in protection against oxidative stress. This suggests that antioxidants from the berries in fact are absorbed and transported to organs where they can regulate genes. In vitro antioxidant capacity (ORAC, FRAP, ARP) and content and type of flavonoids have been investigated in broccoli, kale, cabbage (white and red), apple, and high-bush blueberries during various storage and processing experiments. Irradiation of fruits and vegetables post-harvest with UV can increase flavonoid contents and vitamins (see posters). Rapid analytical methods based on chlorophyll fluorescence and light reflection have been tested, and they can measure flavonoid content non-invasively (see posters). After production and storage of jam and fruit juice the composition and level of flavonoids are very different depending upon the treatment conditions. There are therefore possibilities to improve the bioactivity of such products. Varieties of high-bush blueberries were found to have rather different contents of flavonoids and the level decreased during storage of fresh berries. On the contrary, total antioxidant capacity was stable during storage of jam, even if there were colour changes. A basic study has revealed that various methods for antioxidant capacity have different sensitivity for the different parts of the flavonoid molecules. The stable oxidation products can vary due to molecular structure and solvent type, and knowledge about them can shed light on antioxidant properties of flavonoids.

Participants:
Food Science Alliance with Matforsk (co-ordinator) and Norwegian University of Life Sciences, and University of Oslo, Dept. of Nutrition. Other participants are Uppsala University, Sweden and Kiel University, Germany.

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GENETIC ENGINEERING OF PLANTS TO REDUCE FOOD ALLERGENS: POTENTIALS AND LIMITATIONS

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Due to tremendous technological developments not foreseen a few years ago, plant biotechnology is reaching maturity. Plant genetic engineering has the potential to introduce new allergenic proteins into foods and at the same time might be able to remove or reduce established allergens. Removal of allergens requires the efficient and stable down-regulation of gene expression. This may be achieved by current dsRNAi technologies which allow down-regulation of any target gene in transgenic crop plants.
To explore different strategies to create transgenic plants with reduced allergenic potential we aimed at silencing the expression of lipid transfer proteins (LTP) and profilin in fruits of transgenic tomato plants and patatin in tubers of transgenic potato plants. In addition attempts were undertaken to reduce the content of allergenic glycan structures in tomato fruits and potato tubers by silencing plant N-acetyl glucosaminyltransferase I (GntI).
Lipid transfer proteins (LTPs) are small molecules of approximately 10 kD that demonstrate high stability. They have been identified as allergens in the Rosaceae subfamilies of the Prunoideae (peach, apricot, plum) and of the Pomoideae (apple). They belong to a family of structurally highly conserved cystein-rich proteins that are also present in non-Rosaceae vegetable foods including Solanaceae (tomato).
Profilins are recognised by IgE of about 20% of patients allergic to birch pollen and plant foods. They are ubiquitous intracellular proteins highly cross-reactive among plant species. Therefore, they were called panallergens and are made responsible for cross-sensitisation between plant pollen and food.
Peeling of raw potatoes may cause allergic symptoms, such as sneezing, wheezing, and contact urticaria, for adults. For children, potatoes as food may cause various allergic reactions. Recently, patatin, the major storage protein of potato tubers, has been recognized to be an important IgE-binding protein for children with a positive skin prick test response to raw potato. The glycoprotein is encoded by a large multi-gene family of at least 10 members per haploid genome. Interestingly one important natural rubber latex allergen, Hev b 7, is a patatin-like protein that shows cross-reactivity with its analogous protein in potato.
Approximately 30-50% of individuals who are allergic to natural rubber latex (NRL) show an associated hypersensitivity to some plant-derived foods, especially freshly consumed fruits.
Based on current biochemical and molecular knowledge we have designed transgenic potato and tomato plants accumulating reduced levels of the indicated target proteins in fruits and tubers, respectiviely. Beside a general discussion on GM strategies to reduce food allergens, first results obtained with transgenic potato and tomato plants will be discussed.

Biochemical characterisation of target steps in flavonoid pathway for improvement of metabolic engineering

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Metabolic engineering by introducing or suppressing specific genes is expensive and time consuming and often the result is not as that what was expected or assumed. Moreover, a specific flavonoid function is often determined by several factors (e.g. plant development, vacuolar pH, copigmentation, pathway performance). Therefore, a careful characterisation of the target plant and also protein is necessary to gain valuable information on the gene pool, the biosynthetic pathway, the substrate specificity of the concerned enzymes, and the availability of definite substrates. Simple chemical and biochemical approaches with well established methods, such as supplementation experiments with precursors or the application of specific enzyme inhibitor, can provide valuable information. Supplementing plants with flavonoid intermediates, which are not naturally present in the target plant, is a way to test whether the internal enzyme set can convert these intermediates to the desired flavonoid. The in vivo application of inhibitors might mirror the results of antisense or sense suppression strategies. Specific inhibitors are available for 2-oxoglutarate-dependent dioxygenases (FHT, FLS, ANS and FNS I) and for cytochrome P450 (F3'H, F3'5'H, FNS II and IFS). Both approaches may allow a prediction to be made on the outcome of a planned metabolic engineering experiment either by chemical analysis of alterations in the metabolomic profile, including the synthesis of novel compounds or, in the case of colour changes, even by visible inspection (Martens and Forkmann, 2001. Curr. Op. Biotech. 12, 155-160). Furthermore, the detailed enzymatic characterisation of key steps of the pathway is another important point for the success of metabolic engineering projects. With enzymatic methods the biosynthesis of flavonoids can be followed step by step starting from p-coumaric acid, or even at phenylalanin stage, up to the resulting anthocyanins and/or proanthocyanidins. All enzymes involved in the main pathway to form the different flavonoid classes and also some of the modifying enzymes, e.g. glycosyltransferases and methyltransferases, have been determined and characterized in plant crude extracts (Martens et al., 2003. Biochem Eng. J. 14, 227-235). Additionally, the structural genes coding for the respective proteins have been isolated from various plant sources and functionally expressed in bacteria, yeasts, insects or plant cells. The obtained recombinant proteins should be characterized regarding its biochemical properties. Such studies become more and more important since several proteins of the flavonoid pathway turn out as bi- or even multifunctional enzymes with broad substrate specificity (Martens et al., 2003. FEBS Lett. 544, 93-98; and references therein). This phenomenon can dramatically effect the result of metabolic engineering projects towards undesired metabolites. Finally, the biochemical characterisation of developed transgens should complete the results from metabolomics, proteomics and genomics.

Chloroplast genetic engineering for crop improvement and production of high value compounds

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There is a growing concern amongst scienstists, politicians, regulatory agencies and the general public regarding the widespread release of genetically modified (GM) food crops and this concern is mainly related to the risk of nuclear transgene spread by pollen from GM crops to other plant species. The use of GM food crops is increasing at a rapid rate and according to the International Service for the Acquisition of Agri-Biotech Applications (ISAAA) the world market for GM crops will rise to over 20 billion Euro by the year 2005/2006. In view of this the environmental concern surrounding the use of GM plants in agriculture can only be resolved by designing environmentally safe transgenic crop plants.

One way of resolving the above issue is to perform plastid genetic engineering by inserting transgenes into the plastid genome thereby generating transplastomic plants. The expression of transgenes in plastids has numerous advantages however, there are several bottlenecks with current transformation protocols which makes plastid tranformation a very time consuming and inefficient process. The main bottlenecks are: (i) To ensure that DNA-coated gold particles enter chloroplasts without extensive damage and (ii) To select and only regenerate cells containing transformed plastids into transplastomic plants. We have designed a new genetic system in *Arabidopsis*, based on temporal control of chloroplast size, that we believe will make DNA delivery into plastids a more efficient process. Furthermore, we are generating a new antibiotics-free transplastomic selection/regeneration system based on controlled temporal overexpression of the *isopentenyl transferase* (*IPT*) gene during initial selection followed by simultaneous removal of the *IPT* gene and transgene activation during subsequent regeneration of transplastomic plants using the Cre/lox system. Because genes inserted into the plastid genome are expressed to high levels we believe that transplastomic plants offer a great system for the expression and one-step purification of any protein. We have made a series of plastid transformation vectors based on the cytokinin selection system which will allow for rapid affinity purification of proteins expressed inside plastids. Examples will be given showing proteins that are currently being expressd in plastids for further biochemical and 3D structure studies.

Although *Arabidopsis* is a perfect model to optimise our system (*Arabidopsis* plastids have never been succesfully transformed) we are transfering our system to plants such as tomato and rice.

Posters

Chlorophyll fluorescence used for non-destructive assessment of broccoli epidermal flavonoids

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Fruit and vegetables have a limited postharvest shelf life. Fresh broccoli (*Brassica oleracea* L. var. *italica*) for food can be kept several weeks in cold storage without loosing its visual quality. However, little is known about the postharvest change of health-related quality (that is the levels of nutrients and secondary metabolites) and which storage conditions are optimal. Rapid and non-destructive methods to assess the actual quality are needed. Chlorophyll fluorescence has proven to be useful for measurement of the content of UV-absorbing epidermal flavonoids in leaves (Bilger et al. 1997). We have here tested the method on broccoli - a bulky vegetable – after various light treatments in order to induce different levels of flavonoids. Irradiation as a postharvest storage factor has been investigated in fresh fruit and vegetables to a very limited extent.

Fresh Norwegian broccoli heads were stored in the cold for 12 days under various combinations of visible light and UV irradiation (6h per day) by means of polymer films differing in light transmission characteristics. The contents of flavonoids as quercetin and kaempferol after acidic hydrolysis were measured by HPLC in methanol extracts. 'Oxygen Radical Absorbance Capacity' (ORAC) was also measured in the methanol extracts as an assay for total antioxidant capacity. Chlorophyll fluorescence (720-770 nm) was recorded under standardised conditions every 4 days by excitation at 382, 450, 530 and 685 nm.

Flavonoids and total antioxidant capacity had much higher levels in flower buds than in stalks of the inflorescence. Neither storage nor light treatment changed the total antioxidant capacity in flower buds. Flavonoid levels in flower buds tended to increase due to VIS + UV-A + UV-B treatment, but the change was not statistically significant.

By using the fluorescence signal excited at 685 nm as reference the non-destructive method for flavonoids worked well for the purpose. The content of flavonoids in flower buds correlated to absorption of excitation light at 382 nm ($r=0.40$, $p<0.05$), 470 nm ($r=0.69$, $p<0.001$) and at 530 nm ($r=0.39$, $p<0.05$). This is based on the large natural differences in flavonoid level between individual broccoli heads, these differences prevailing also after treatments. The highest correlation at 470 nm was, however, unexpected. Thus, conditions in flower buds of broccoli heads may be very different from the conditions in a green leaf.

Bilger, W., Veit, M., Schreiber, L., Schreiber, U. (1997). Measurement of leaf epidermal transmittance of UV radiation by chlorophyll fluorescence. *Physiologia Plantarum* 101, 754-763.

THE FLAVONOID CONTENT IN APPLES CAN BE INCREASED BY POST-HARVEST LIGHT TREATMENTS

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The flavonoids are secondary metabolites in plants and are assumed to have beneficial effects on human health when present in food. Even though apples do not have the highest content of flavonoids among plant foods, they are, because of a high intake, one of the most important sources of flavonoids in the European diet. "Aroma" is the most popular apple cultivar grown in Norway. Many of the flavonoids in the apple are induced by sun radiation. Beside the anthocyanins, which give "Aroma" the red colour, also colourless flavonoids such as flavones and flavonols are synthesised in response to the sun.

In this experiment the effects of post-harvest light treatments on the flavonoid content in apples have been studied. How does simulated sun radiation affect the flavonoid content in apples after being removed from the tree? Is it possible to optimise the flavonoid level in apples during storage? Are there any side effects? During the experiment the apples were treated with UV-B radiation with and without visual light. The flavonoid content was measured with HPLC and a rapid, non-destructive method based on chlorophyll fluorescence. Also the ORAC value and the content of chlorogenic acid, total phenols, ascorbic acid, soluble solids and titratable acids were measured. The results show that post-harvest treatment with UV-B and visual light can increase the flavonoid content in "Aroma" apples. The same light treatment also increased the levels of chlorogenic acid and ascorbic acid. The effects were strongest in suboptimal (green) apples harvested from the inner tree canopy. The light treatments had no influence on the content of soluble solids or titratable acid in any of the apples.

Nitrate reductase and biological clocks

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Nitrate reductase (NR) is the first enzyme in the assimilation pathway of nitrate into amino acids. Rhythms are observed in NR mRNA, protein and activity. The NR oscillations have been suggested to be self-sustained, and based on a positive feed-forward caused by light and nitrate, and a negative feedback caused by products of nitrogen assimilation (Lillo et al. 2001. These oscillations are still not thoroughly understood.

A transgenic *Nicotiana plumbaginifolia* line has been made in which the NR structural gene was mutated at a regulatory serine residue, and placed under the control of the constitutive S35 promoter. NR expression was confirmed to be deregulated in this transgenic (Ser) line. The endogenous NR gene had been inactivated by a transposon insertion, and the resulting truncated NR gene can therefore serve as a reporter gene for the NR promoter. Three lines were tested: WT (NR promoter-NR structural gene), C1 (S35 promoter-NR structural gene), and Ser (S35 promoter-mutated NR structural gene).

For WT and C1 plants, NR is rapidly activated when light is switched on. No increase in activity state was observed for the Ser line when light was turned on, and confirmed that the Ser 521 is indeed necessary for rapid activation/inactivation of NR. Although the NR gene is linked to the constitutive S35 promoter in C1 plants, diurnal variations in total NR activity (reflecting NR protein) is still observed. This points to light influence on NR expression on different levels. Diurnal variations of NR in Ser plants is strongly dampened, and these plants are therefore a suitable system for studying regulation of a reporter gene (truncated NR gene) linked to the NR promoter. Work with these plants should help in understanding the NR oscillating system and determine if expression of the reporter gene continue to oscillate independently of deregulation of nitrate assimilation.

References
Lea, US 2005 Deregulation of nitrate reductase; effects on physiology and gene expression. PhD thesis at the University of Stavanger, Norway. ISBN 82-7644-219-6
Lillo C, Meyer C, Ruoff P 2001. The Nitrate Reductase Circadian System. The Central Clock Dogma Contra Multiple Oscillatory Feedback Loops. Plant Physiology 125: 1554-1557.

Phenolics and other compounds with antioxidative effect in stone fruit – Preliminary results

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The consumers are becoming more aware of health related compounds in the diet. This has been related to compounds with antioxidative effects. Fruits, berries, nuts and vegetables are known for their high antioxidative capacity.

A study of the variation in the contents of phenolics, antocyanins and antioxidative effect in sweet cherry and plum cultivars has been performed. The fruit samples were picked in the experimental orchard at Planteforsk Ullensvang Research Centre. The samples were analysed for total phenolic content, content of anthocyanins and antioxidative effect (FRAP-method).

The total content of phenolics was more than seven times higher in the high phenolics cultivars than in the cultivars with low phenol content. The contents of phenolics were higher in cultivars with dark red juice than in cultivars with yellow juice. The total antioxidative capacity (FRAP) ranged from 436 µmol/100g in 'Sue' to 2,669 µmol/100g in 'Agila'.

In plums a similar variation was found between 9 tested cultivars. The total content of phenolics ranged from 27 mg/100g in 'Reine Claude Souffriau' to 54 mg/100g in 'Victoria'. The antioxidative capacity (measured by the FRAP-method) ranged from 655 µmol/100g in 'Opal' to 1,280 µmol/100g in 'Victoria'.

Highly significant correlations were found in sweet cherries between FRAP values and contents of anthocyanins and phenolics; $R(sq)=0.951$ ($p<0.001$) and $R(sq)=0.978$ ($p<0.001$) respectively. In plums the cultivar 'Victoria' had the lowest content of anthocyanins, and yet the highest antioxidative capacity of the tested cultivars.

Fructooligosaccharides and Phenolics in flesh and peel of spring harvested *Helianthus tuberosus*

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The old vegetable plant *Helianthus tuberosus* (Jerusalem artichoke) has been paid attention during the last years because of the high value as a functional food plant. The tuber of this plant is a rich source for fructooligosaccharides (mainly inulin). These are sweet tasting compounds that have shown decreasing effect on blood glucose and triglycerides. Inulin is not digestible by humans and has function as fibre in the digestion system. This group of compounds also increase absorption of calcium and synthesis of vitamin B. Interestingly; inulin could be utilized in the colon by bifidobacteria. This could favour healthy bacteria at the expense of other disadvantageous microorganisms that could not use inulin as substrate for growth and that cannot live under the lowered pH environment.

In our study genetic variants of *H. tuberosus* that originate from different parts of Norway are gown in a field experiment in sandy soil at the southern part of Norway. Analyses of fructooligosaccharides, phenolics and antioxidant capacity (DPPH) were performed in early April after exposure of tubers to frost during winter. At this time of harvest the levels of oligosaccharides are known to be at a minimum. One of the aims of our study was to search for genetic variants that stay high in fructooligosaccharide levels until spring harvest. For all the genetic variants tested approximately 50% of total carbohydrates was fructooligosaccharides and the rest was sugars (mainly sucrose and low levels of fructose). At this time of harvest 60-90 % of the sugars was sucrose. Kestose and Nystose was abundant fructooligosaccharides in all cultivars. The level of total fructooligosaccharides varied from 20 mg g^{-1} (FW) for the genetic variant 'Bergly' to 38 mg g^{-1} for 'Amerika'.

Analysis of the peel of tubers showed relatively high levels of total phenolics (51-128 mg GAE $100g^{-1}$ FW) and a high antioxidant capacity (97-296 mg ascorbic acid equivalents 100 g^{-1}). Level of total phenolics was correlated to antioxidant capacity (r=0,81). 'Moskva' had the highest level of total phenolics (128 mg GAE $100g^{-1}$ FW) followed by 'Kirkeøy', 'Solkroken' and 'Kapell' (70-79 mg GAE $100g^{-1}$ FW). The antioxidant capacity showed the same pattern. On the other hand, the flesh of tubers contained very low levels of phenolics and showed no antioxidant capacity.
The high phenolic content and antioxidant capacity of the peel may be a protection factor for the plant when exposed to pathogens and other stress factors. The different genetic variants tested varied with respect to this factor. As a functional food constituent for humans the content of fructooligosaccharides in flesh will play the most important role. Even with spring harvesting these compounds consist for 50% of the total carbohydrates with highest level for the variant 'Bergly'.

Identification and characterisation of male and female determining genes in *Populus tremula* L.

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Poplars belong to less than four percent of the plants on earth that show unisexual flowers. They are dioecious, that means normally one tree possesses only male or only female flowers. There are no morphological differences between the trees except for the inflorescences. Because there is evidence for genetically caused sex expression we want to find out how sex expression in poplar is steered and develop sex specific molecular markers.
By analysing the poplargenome with more than 200 primer-enzym-combinations (pec's) using the AFLP-technology we have identified nine DNA-fragments that segregate similar to the sex. We have cut three of them out of the AFLP-gel to clone and sequence them. One of these fragments bears high resemblence to EST-sequences from a cDNA-library of poplarflowers. The others show little affinity to known sequences.
We have developed a preliminary genetic map of *Populus tremula* in which we have detected 41 AFLP-markers on seven linkagegroups. We are localising microsatellite-markers in the map to adjust this map with already existing genetic maps of other poplar species.
The construction of a BAC-library is in process.

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ORGANIZATION

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