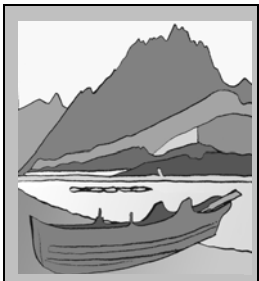

European Plant Science Organisation

4th EPSO Conference

"Plants for Life"

Toulon (Côte d'Azur), France

22 – 26 June 2008

www.epsoweb.org/catalog/conf2008.htm

SPONSORS

The 4th EPSO conference is sponsored by:

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FESPB - The Federation of European Societies of Plant Biology

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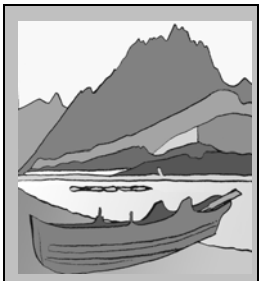

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Editorial

The **European Plant Science Organisation, EPSO**, has established itself as a platform for dialogue for all that have an interest in plant sciences. This includes its members, academia, and related stakeholders such as industry, consumer representatives and the general public.
EPSO aims to:

- Increase impact and visibility of the European plant science community
- Articulate the vision of the European plant science community for the future and advise on decisions of funding agencies at the European and national level on long term strategies to support plant science
- Communicate with academia, industry and the general public to ensure independent dissemination of plant science information
- Contribute to tighten the link between plant science and the development of agriculture, horticulture, forestry and ecology.

Members of EPSO are 168 academic organisations from 26 countries, such as institutes and universities, and over 1000 personal members. They interact with the EPSO observers from industry and other related organisations. EPSO has links to specialised organisations in the area of plant and life sciences in Europe, and plant science organisations worldwide.

The **EPSO Conferences**, the European Plant Sciences Forum, play a vital role in contributing to the development of plant sciences in Europe to ensure that they remain

- On the forefront of plant sciences worldwide
- Beneficial to humankind and our environment
- Valuable to society at large
- Committed to ethics.

We are delighted to welcome you to the **4th EPSO Conference** to actively take part in the debates that will have a significant impact on the most crucial aspects of plant science and its contribution to our society.
This conference brings together world leading plant scientists from 34 countries from Europe, Australia, Hong Kong, Japan, Malaysia, New Zealand, USA and Vietnam to discuss cutting edge science and organise networks in four thematic areas:

- Understanding, preserving and using plant diversity
- Preserving our future by reducing the inputs in agriculture
- Improving plant product quantity and quality
- New products

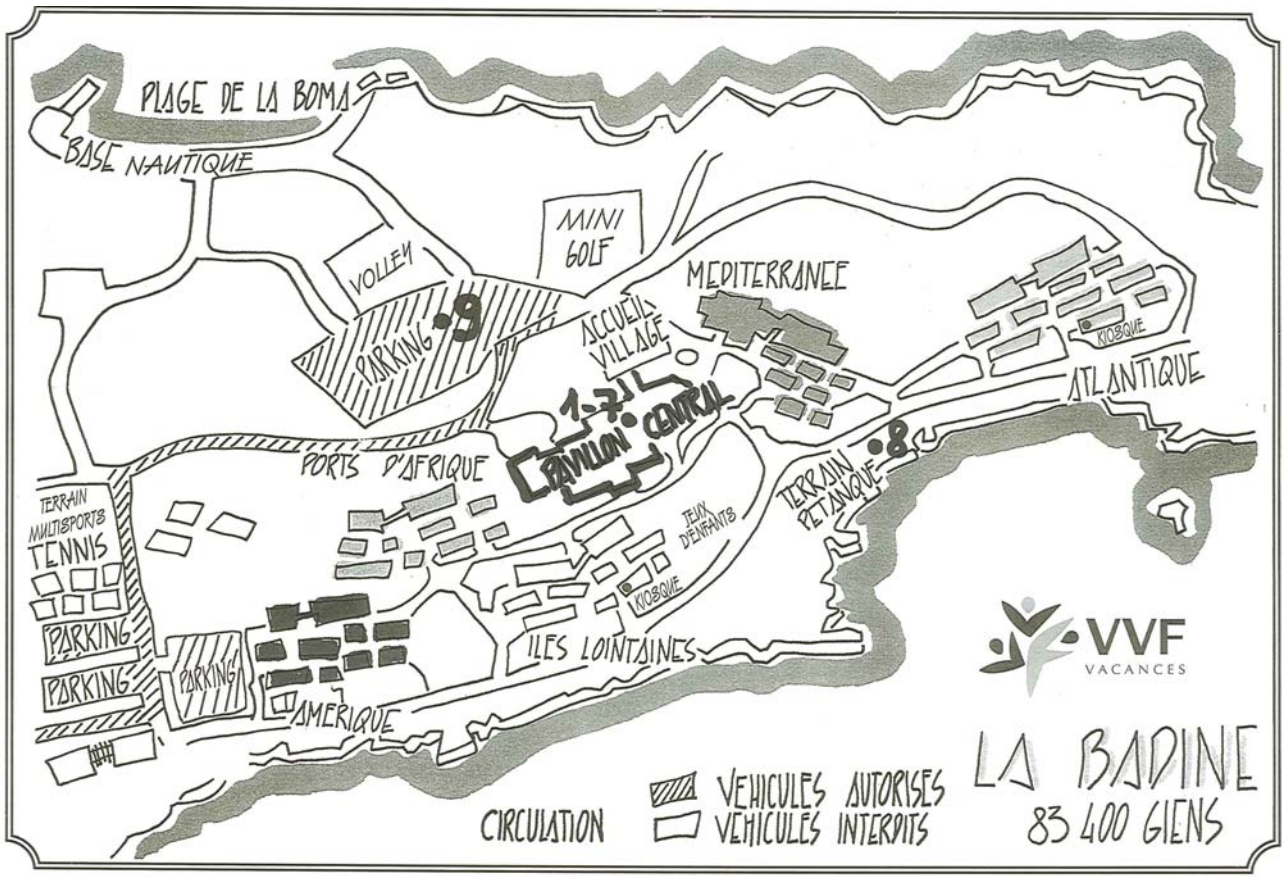
Representatives from academia, industry and politics discuss socially relevant topics, such as

- Plant science in Europe – science policy
- The challenges for tomorrow's agriculture

We wish you an enjoyable conference in the inspiring surroundings of the Giens peninsula. At the heart of a 34 hectares pine-wood forest, encircled by small coves, this is one of the most beautiful sites on the Côte d'Azur.
We would like to thank our French colleagues, the organising committee and the conference secretariat for their enthusiasm in preparing this conference.

Karin Metzlaff, EPSO, Brussels **Hélène Lucas, INRA Versailles, France**

Conference information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VOLLEY
MINI GOLF
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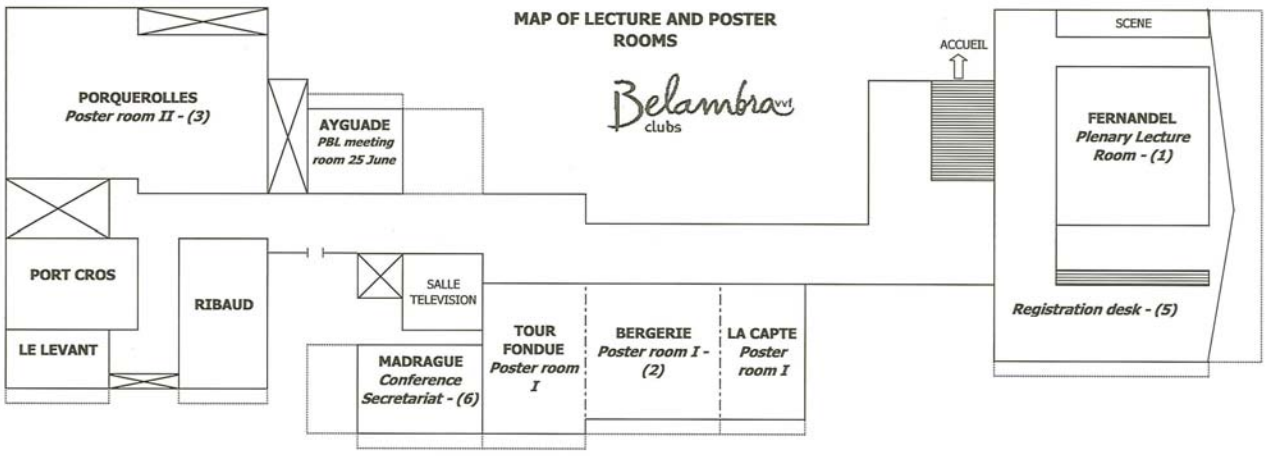

MAP OF LECTURE AND POSTER ROOMS
Belambra clubs
PORQUEROLLES
Poster room II - (3)
AYGUADE
PBL meeting room 25 June
ACCUEIL
SCENE
FERNANDEL
Plenary Lecture Room - (1)
PORT CROS
LE LEVANT
RIBAUD
SALLE TELEVISION
MADRAGUE
Conference Secretariat - (6)
TOUR FONDUE
Poster room I
BERGERIE
Poster room I - (2)
LA CAPTE
Poster room I
Registration desk - (5)

Venue

Club Belambra vvf La Badine
406 avenue de l'Esterel
Presqu'île de Giens
83400 Hyères
Tel: +33 (0)4 94 58 36 60
Fax: + 33 (0)4 94 58 96 38
E-mail: giens.groupesseminaire@belambra-vvf.fr
Web: www.belambra-vvf.fr

Belambra VVF club "La Badine" is a residential village, located in sumptuous Mediterranean surroundings at the end of Giens peninsula, facing the Gold islands. At the heart of a 34 hectares pine-wood forest, encircled by small coves, this is one of the most beautiful sites on the Côte d'Azur, only 15 minutes away from the Toulon-Hyères airport.

Tranfers to and from airports, stations, or any other place can be arranged for you. We strongly advise you to book your transfer to and from the conference venue before the start of the conference. **Pre-booked transfers always get priority to other requests.** You can complete the travel reply form at www.epsoweb.org/catalog/conf2008/Travel_to_VVF_Giens.pdf and send it back to Katrien Molders, EPSO Conference secretariat, Tel/Fax: +32 2 213 62 63/69, Katrien.Molders@epsomail.org. All information on traveling to and from the conference venue can be found at the same link.
Should this not be possible, contact us during the conference at the registration desk in front of the plenary lecture room (salle Fernandel), we will try to arrange a transfer on the spot (without guarantee).

Conference facilities:

1. Lecture room: room "Fernandel" — pavillon central, level -1
2. **Poster room I**: rooms "La capte + Bergerie + Tour Fondue" — pavillon central, level -1
3. Poster room II: room "Porquerolles" — pavillon central, level -1
4. Restaurant: next to the VVF reception desk — pavillon central, level 0

5. Registration desk: in front of the plenary lecture room (Fernandel) — pavillon central, level -1
For registration, social programmes, excursions, transfers and shuttle service.

6. Conference secretariat: room "Madrague" — pavillon central, level -1
Please deliver your presentation files here at least 2 hours before the onset of your session.
We assist with any management issues and other questions you might have.
Four computers with internet connection are available here for participants. For wireless internet access see page 13.
Opening hours: from 8:00 to 20:00.

7. VVF reception desk: — pavillon central, level 0
Check in and check out, wireless internet connection, taxi (should the conference vans not be available), send faxes, print documents etc.

8. Welcome reception at the beach
9. Excursion start

In your participant bag you will find a detailed map of the venue indicating the exact location of these conference facilities.

Registration

The registration desk will be located in front of the lecture room (Fernandel) at the -1 level of the "pavillon central". Registration starts on Sunday 22 June 2008 at 13:00.

Opening hours of the registration desk:

Sunday 22 June:	13:00 – 19:30	Monday 23 June:	08:00 – 19:30
Tuesday 24 June:	08.00 – 11:00 and 17:00 – 19:30		
Wednesday 25 June:	08:00 – 19:30	Thursday 26 June:	08:00 – 14:00

Posters

The poster rooms are:

Poster room I:	La capte + Bergerie + Tour Fondue	pavillon central, level -1
Poster room II:	Porquerolles	pavillon central, level -1

The size of the poster boards is 100 cm (width) x 200 cm (length). The poster boards are made of wooden panels in which it is forbidden to make holes. Therefore, posters can only be **attached with tape or blue tack**.You are **not allowed to use pins** for attaching your poster.

The poster boards have the same numbering as the posters in the abstract book. In addition, the poster author and numbers list will be displayed in the poster room to guide you to the poster board reserved for you.

Posters can be installed on the boards from 13:00 on Sunday 22 June 2008 and should be set up latest by 10.30 the next morning. Posters can be viewed from Sunday on throughout the conference and should be removed at the end of the meeting by Thursday noon.

Poster sessions will be held on:

- Monday evening 23 June from 20.30 to 22.30. Posters with <u>even numbers</u> should be attended from 20.30 to 21.30, those with <u>uneven numbers</u> from 21.30 to 22.30. Drinks will be served in the poster room.
- Tuesday evening 24 June from 20.30 to 22.30. Posters with <u>uneven numbers</u> should be attended from 20.30 to 21.30, those with <u>even numbers</u> from 21.30 to 22.30. Drinks will be served in the poster room.

A jury will select the best three posters for a poster price awarded at the conference dinner.

Speakers

The presentations will be shown on Windows Vista-PCs / Ms Office 2007. The presentations should be Microsoft PowerPoint either .ppt, .pptx, .pps or .ppsx, alternatively you can bring presentations converted to Adobe pdf. Movies can be shown on Apple QuickTime. The files can be brought as CD-ROM, DVD-ROM or USB-stick. For all further plug-ins, software and for all other technical questions contact Markus Fauth at <u>m.fauth@cellbiology.uni-frankfurt.de</u>.
If you wish Markus Fauth to test-run your presentation on the conference computer before the conference, you can send it before Wednesday 18 June to Markus by email.

Recommendations for Mac-users:
- As the presentations will be shown on a PC environment, we recommend bringing a pdf-presentation as back-up. This should eliminate most compatibility problems.
- When editing a picture file (e.g. jpeg), please edit it on the original file outside PowerPoint and re-import it to PowerPoint instead of editing the picture directly in PowerPoint.
- If you have movies or clips, please bring them also as separate files so they can be imbed freshly if a problem occurs.
A good way to circumvent problems is to test the presentation on windows PC in your institute before going to France.

Speakers are requested to load and test their presentation on the conference PC at least 2 hours before the onset of their session. This can be done on Sunday between 14:00-15:00 and 16:30-16:45 and all other days between 8:00–8:15; 10:30-10:45 (not on Tuesday); 13:00-13:15 (not on Tuesday); 14:45-15:00 (not on Tuesday) and 17:00-17:15 directly at the projection desk in the lecture room (responsible person is Markus Fauth).

Time slots available for presentation and discussion are stated in the programme following your name. We trust you respect the time slots, to have sufficient time for discussion, in the interest of all conference participants.

Internet access

Wireless internet will be available in the VVF reception area (pavillon central, ground level) and is charged only for the time you access the wireless internet. Therefore we recommend the conference participants to bring a laptop set up with wireless internet connection facilities and to buy access for 24 hours (15€) or alternatively 1 hour (5€) or 30 minutes (3€). When the time you bought is used up, you can buy a new ticket.
In addition, 4 computers with internet connection and 4 internet cables for laptops will be available for conference participants at the conference secretariat between 8:00 and 20:00h.
It will not be possible to connect to the internet from your room.

Coffee breaks, lunch and dinner

Lunch and dinner will be offered in the restaurant, level 0 of the pavillon central. Please, bring the correct meal ticket for every meal.
On the excursion day Tuesday 24, all participants can pick up a lunch bag at the VVF reception desk (before leaving for the excursion). Please bring the correct ticket to the VVF reception desk.

Coffee breaks (see programme) will be held on the outside terrace of the bar/restaurant at level 0 of the pavillon central.

Welcome reception

The welcome reception will be held at the beach on Sunday evening 22 June 2008 at 19:30 and will be followed by a dinner at the restaurant.

Conference dinner

Wednesday 25 June 2008 at 20:00 at the Restaurant and Terrace.
We would like to invite all registered participants, registered accompanying persons and invited speakers to attend the conference dinner, which is included in the registration fee. Please, bring the correct meal ticket.

Excursions

Tuesday 24 June 2008 from 11:00 to 17:00.

You can choose between four different excursions. Participating in an excursion is optional and included in the registration fee. However you can only participate if you inscribed yourself for one of the excursions by sending the excursion reply form to the EPSO conference secretariat before the start of the conference. You can download the excursion information and reply form at www.epsoweb.org/catalog/conf2008/4CF_Excursions_Web.pdf.

All excursions will start at 11.00h at the parking close to the VVF reception building and will finish at the same place approximately at 17.00h. Do not forget to pick up your lunch bag at the VVF reception desk before leaving. Please, bring the appropriate meal ticket.

Option 1: Porquerolles Island, part of the Golden Islands (walk and boat)
20 minutes walk from the conference venue to "La Tour Fondue", from here by boat to the Porquerolles Island. Visit of the Island: tour of the village, walk through the oliver grove towards the 14th century Fort Ste Agathe with its underwater archaeological exhibition. Return to the village and visit the Saint Anne's church (1850), patron saint of the Islands.
You can also choose to discover the Island on your own, please check when the boat will return.

Option 2: Toulon (city, port and boat)

Toulon, hosting the main French naval base, was built in one of the most beautiful Mediterranean bays. We visit this beautiful bay by boat, and then there is free time to visit the port or the old city district.

Option 3: Vignes et Terroir (Vine and land)
Visit of an olive oil mill "Le Moulin du Partegal" in La Farlède (http://www.moulindupartegal.com/): olive oil, tapenade and anchoiade production. Visit the old and the new mill and discover how the products are made. Outside visit of the waterwheel and the oliver grove. Tasting of oils, tapenade and anchoiade.
Afterwards visit of a winery "chateau et cave: Côte de Provence", followed by a wine tasting.

Option 4: Hiking (choice between 3 different hikes)
Hike 1: Discover the rich fauna and flora of the "La Presqu'Ile de Giens" with an official guide from the National Forest Office (ONF).
Hike 2: Nature walk with an official guide of the National Forest Office (ONF) in the "Massif des Maures".
Hike 3: Bird watching: Guided discovery of numerous bird species and their habitat at the saltpans of La Presqu'Ile de Giens, with a guide from the Bird Protection League.

Accommodation

Accommodation has to be booked (and paid) before the start of the conference via the EPSO Conference Secretariat. Contact Katrien Molders at Katrien.Molders@epsomail.org or Tel/Fax: +32 2 213 62 63.

Transport

Two minibuses (and if needed larger buses) will be available at the conference venue to take you to and from airports, stations and other places for a charge of 0.3€ per kilometer per person. The transfer fee has to be paid in cash to the driver or at the registration desk before departure.

The conference venue is located near Toulon at:
- approx. 20 km from the Toulon airport (charge for a single way transfer is 5€)
- approx. 80 km from the Marseille airport (charge for a single way transfer is 25€)
- approx 150 km from the Nice airport (charge for a single way transfer is 45€)

Please, book your transfer to and from the conference venue before the start of the conference. **Pre-booked transfers always get priority to other requests.** You can complete the travel reply form at www.epsoweb.org/catalog/conf2008/Travel_to_VVF_Giens.pdf and send it back to Katrien Molders, EPSO Conference secretariat, Tel/Fax: +32 2 213 62 63/69, Katrien.Molders@epsomail.org. All information on traveling to and from the conference venue can be found at the same link.

For a transfer to a city tour or similar, we will include this as far as possible. If we do not have vacant cars, please contact the VVF reception desk to book a taxi for you.

Currency

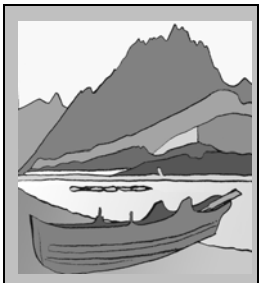
The currency in France is EURO.

Coins: 2 and 1 Euro; 50, 20, 10, 5, 2 and 1 Eurocent
Bank notes: 50, 20, 10 and 5. Bank notes of 100, 200 and 500 EURO are often refused, due to forgeries circulating
The exchange rate of 1€ is approximately: 1,5 USD; 158 JPY; 1,64 AUD; 2 NZD; 0,8 GBP.

Conference secretariat

Mrs. Katrien Molders	Office phone: +32 (2) 213 62 63	on working days before 20.06 and after 27.06 from 9:00 – 17:00
	Mobile phone: +32 (0)473 88 27 29	every day from 20.06 to 27.06
Miss Jacqueline Breitlid	Mobile phone: +32 (0)473 68 20 65	every day from 20.06 to 27.06
EPSO office	+32 (0)2 213 62 60	on working days from 9:00 – 17:00

Conference programme



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Sunday	**22 June 2008**	
From 13:00	Registration	
15:00 – 16:30	**Opening and Keynote** **Chair: Karin Metzlaff**, Brussels, BE **& Hélène Lucas,** Versailles, FR	**Speakers:** **Karin Metzlaff** and **Hélène Lucas** (10 min) *Executive Director of EPSO* *Head of Genetics and Plant Breeding Division, INRA and Local Conference Organiser* Welcome from EPSO **André Le Bivic**, FR (10 min) *Deputy Scientific Director, Department of Life Sciences, CNRS* **François Houllier**, FR (10 min) *Scientific Director for Plant and Plant Products, INRA* **Richard B. Flavell**, USA (60 min) **S 001** *Chief Scientific Officer of Ceres, Inc. - The Energy Crop Company* What do we need to improve crops faster and cheaper?
16:30 – 17:00	Break	**Speakers:**
17:00 – 19:00	**Plant Science in Europe – Science Policy** **Chair: Wilhelm Gruissem**, Zürich, CH **& Karin Metzlaff**, Brussels, BE	**Timothy Hall**, EU (25 + 5 min) **S 002** *Acting Director of Directorate Food, Agriculture, Fisheries and Biotechnology, DG Research* The knowledge-based bio-economy from a "plant" perspective **Babis Savakis**, GR (25 + 5 min) **S 003** *Senior Advisor to the President of the ERC* The European Research Council: A benchmark for frontier research funding in Europe **Wilhelm Gruissem**, CH (25 + 5 min) **S 004** *President of EPSO* Plant science in Europe – Breaking new ground **Mike Gale**, UK (25 + 5 min) **S 005** *Member of the CGIAR Science Council* Plant science, the basis for farm, food, non food and energy
19:30 – 22:30	Welcome Reception	
Monday	**23 June 2008**	
8:30 – 10:30	**Understanding, preserving and using plant diversity I: Genome structure and evolution** **Chair: Catherine Feuillet**, Clermont-Ferrand , FR	**Speakers:** **Catherine Feuillet**, Clermont-Ferrand , FR (30 + 5 min) A glimpse into the impossible: physical mapping of the giant hexaploid wheat genome using a chromosome based approach **S 006** **Graham Moore**, Norwich, UK (25 + 5 min) **S 007** It's not size but coordination that matters **Joachim Messing**, Piscataway, USA (25 + 5 min) **S 008** Evolution of grasses by comparative genomics

		Anne-Françoise Adam-Blondon, Evry, FR (15 + 5 min) The grapevine genome sequence suggest hexaploïdization in major angiosperm phyla **S 009**
10:30	Coffee Break	
11:00 – 13:00	**Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation** **Chair:** Stephen Hopper, Kew, UK	**Speakers:** **Stephen Hopper**, Kew, UK (30 + 5 min) **S 010** Rethinking agriculture and urban green space management: plant adaptation, domestication and conservation **Simon Hiscock**, Bristol, UK (25 + 5min) **S 011** Hybrid speciation in flowering plants **Thomas Städler**, Zürich, CH (25 + 5min) **S 012** Assessing demographic history in a population-genetic framework: A multilocus case study in wild tomatoes **Benjamin Kilian**, Gatersleben, DE (15 + 5 min) **S 013** A dispersed-specific model of plant domestication
13:00 – 15.00	Lunch	
13:45 – 14:45	**SEB Careers Workshop**	**Making the most of your research position**
15:00 – 17:00	**Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades** **Chair: Ulrich Schurr**, Jülich, DE	**Speakers:** **Ulrich Schurr**, Jülich, DE (5 min) Introduction **Franco Miglietta**, Firenze, IT (25 + 5 min) **S 014** From ecosystems to genes: understanding the diversity of plant response to elevated CO_2 **Andrew D. Friend**, Cambridge, UK (25 + 5 min) **S 015** Impacts of global environmental changes on the distribution of plant production to 2100 **Bruce Osborne**, Dublin, IE (25 + 5 min) **S 016** Using comparative assessments of net ecosystem exchange and carbon sequestration to identify mitigation options for managed ecosystems **Shravani Basu**, Nottingham, UK (15 + 5 min) **S 017** Promoting indigenous crops as a tool for tackling climate change and food insecurity in semi-arid Africa
17:00	Coffee Break	
17:30 – 19:30	**Science and Society: The challenges for tomorrow's agriculture** **Chair: Robert Watson**, London, UK	**Speakers:** **Robert Watson**, London, UK (20 min) **S 018** Is multifunctionality the future of agriculture or simply a trade issue? **Tim Lang**, London, UK (15 min) **S 019** A food system which ticks all the policy boxes: Can it be done? What would it look like? Is anyone pushing for it? **Matin Qaim**, Göttingen, DE (15 min) **S 020** Economic consequences of Golden Rice **Joachim Schiemann**, Braunschweig, DE (10 min) **S 021** Regulation and risk assessment of transgenic plants at European level **Discussion** (60 min)
19:30 – 20:30	Dinner	

20:30 – 22:30	**Chaired Poster Session I with drinks**	20:30 – 21:30 Even poster numbers will be attended 21:30 – 22:30 Uneven poster numbers will be attended
Tuesday	**24 June 2008**	
8:30 – 10:30	**Improving plant product quantity and quality I: Developmental biology** **Chair: Ottoline Leyser**, York, UK	**Speakers:** **Ottoline Leyser**, York, UK (25 + 5 min) **S 022** Regulation of shoot branching **Enrico Coen**, Norwich, UK (25 + 5 min) **S 023** Modelling genes, growth and form in plants **Björn Sundberg**, Umeå, SE (25 + 5 min) **S 024** Wood development – what do plant hormones do? **Raffaele Dello Ioio**, Rome, IT (15 + 5 min) **S 025** Cytokinins control root meristem activities and root growth by antagonizing auxin action
10:30	Coffee Break	
11:00 – 17:00	Excursion	
17:30 – 19:30	**Preserving our future by reducing the inputs in agriculture I: Reducing fertilisers** **Chair: Mark Stitt**, Golm, DE	**Speakers:** **Mark Stitt**, Golm, DE (30 + 5 min) **S 026** Genomics analysis of responses to nutrients **Javier Paz-Ares**, Madrid, ES (25 + 5 min) **S 027** Phosphate starvation signalling in plants **Nicolaus von Wirén**, Hohenheim, DE (25 + 5 min) **S 028** Nitrogen uptake and signaling networks **Heike Schneider, Jülich, DE** (15 + 5 min) **S 029** A new approach for imaging nutrient distributions in plant tissue using time of flight secondary ion mass spectrometry and scanning electron microscopy
19:30 – 20:30	Dinner	
20:30 – 22:30	**Chaired Poster Session II with drinks**	20:30 – 21:30 Uneven poster numbers will be attended 21:30 – 22:30 Even poster numbers will be attended
Wednesday	**25 June 2008**	
8:30 – 10:30	**Preserving our future by reducing the inputs in agriculture II: Reducing pesticides** **Chair: Jonathan Jones**, Norwich, UK	**Speakers:** **Jonathan Jones**, Norwich, UK (30 + 5 min) **S 030** Monitoring and manipulating information flow at the host/pathogen interface **Sophien Kamoun**, Norwich, UK (25 + 5 min) **S 031** Filamentous pathogen effectors **Frank Takken**, Amsterdam, NL (25 + 5 min) **S 032** Resistance proteins: scouts of the plant innate immune system **Montserrat Solé**, Barcelona, ES (15 + 5 min) **S 033** A family of bacterial effectors promote disease by interfering with plant MAP-kinases
10:30	Coffee Break	
11:00 – 13:00	**Preserving our future by reducing the inputs in agriculture III: Reducing water input** **Chair: Peter Langridge**, Glen Osmond, AUS	**Speakers:** **Peter Langridge**, Glen Osmond, AUS (30 + 5 min) **S 034** Genetic and genomic approaches to deal with subsoil constraints to yield **Jian-Kang Zhu**, Riverside, USA (25 + 5 min) **S 035** Small RNAs and epigenetic regulation in abiotic stress resistance

		François Tardieu, Montpellier, FR (25 + 5 min) **S 036** An integrated approach of tolerance to water defecit involving precise phenotyping and modelling **Laszlo Szabados**, Szeged, HU (15 + 5 min) **S 037** Controlled cDNA overexpression system to isolate novel stress genes in *Arabidopsis*.
13:00 – 15:00	Lunch	
13:45 – 14:45	**SEB Careers Workshop**	**Identifying and selling your skills**
15:00 – 17:00	**Improving plant product quantity and quality II: Improving yield** **Chair: Lothar Willmitzer**, Golm, DE	**Speakers:** EMBO **Lothar Willmitzer**, Golm, DE (30 + 5 min) **S 038** Metabolic composition and biomass **Ian Bancroft**, Norwich, UK (25 + 5 min) **S 039** The identification of molecular markers for yield components **Wim Van Camp**, Gent, BE (25 + 5 min) **S 040** Yield increase by transgenic approaches **Teresa Penfield**, York, UK (15 + 5 min) **S 041** Increasing artemisinin yield in *Artemisia annua L.*
17:00	Coffee Break	
17:30 – 19:30	**Improving plant product quantity and quality III: Food and feed** **Chair: Kaisa Poutanen**, Espoo, FI	**Speakers:** **Kaisa Poutanen**, Espoo, FI (30 + 5 min) **S 042** How to optimally exploit grains for food? **Roberto Ranieri**, Parma, IT (25 + 5 min) **S 043** Food product innovation taking advantage of plant selection **Søren K. Rasmussen**, Frederiksberg, DK (25 + 5 min) Presentation of the white paper of the EPSO workshop on "The European Feed Value Chain" held in Copenhagen from 26 to 27 June 2007. **S 044** **Wessel van Leeuwen**, Wageningen, NL (15 + 5 min) An *Arabidopsis* genetical genomics approach to improve phytonutrient quality in *Brassica* vegetable crops **S 045**
20:00	Conference Dinner	Prices for the three best posters will be awarded
Thursday	**26 June 2008**	
8:30 – 10:30	**New Products I: Plant based biofuels: how to improve them?** **Chair: Michael Bevan**, Norwich, UK	**Speakers:** **Michael Bevan**, Norwich, UK (30 + 5 min) **S 046** *Brachypodium distachyon* genomics for bioenergy research **Jay D. Keasling**, Berkeley, USA (25 + 5 min) **S 047** Engineering microbial metabolism for production of advanced biofuels **Birgitte K. Ahring**, Lyngby, DK (25 + 5 min) **S 048** Second generation bioethanol production from lignocellulosic material **Hélène Zub**, Peronne, FR (15 + 5 min) **S 049** Effect of early plant development and genotypic variation in frost tolerance for 3 species of *Miscanthus*
10:30	Coffee Break	

11:00 – 13:00	**New Products II: Biomaterials, biopharmaceuticals and other new products** **Chair: Yuri Gleba**, Halle, DE	**Speakers:** **Yuri Gleba**, Halle, DE (25 + 5 min) **S 050** New materials from new plants **Inge Broer**, Rostock, DE (25 + 5 min) **S 051** Biomaterials, synthesis of the biopolymer cyanophicin in tobacco and potato **Dirk Bosch**, Wageningen, NL (25 + 5 min) **S 052** Controlling of quality of biopharmaceuticals in plants **Melanie Oey**, Potsdam, DE (15 + 5 min) **S 053** High efficient synthesis in chloroplasts of a protein antibiotic active against human pathogenic bacteria
13:00 – 13:30	**Closing**	**Karin Metzlaff** and **Hélène Lucas** *Executive Director of EPSO* *Head of Genetics and Plant Breeding Division, INRA and Local Conference Organiser*
13:30	Departure	

We would like to thank our committees and secretariat members for organising this conference:

Members of the organising committee: Wilhelm Gruissem, Jacek Hennig, Dirk Inzé, Jonathan Jones, Hélène Lucas (local coordinator), Karin Metzlaff (EPSO coordinator), Kirsi-Marja Oksman-Caldentey, Pere Puigdomenech, Ulrich Schurr, Chiara Tonelli, Erkki Truve.

Members of the local committee: Hélène Barbier-Brygoo (CNRS), Hélène Lucas (INRA), Jean-Christophe Glaszmann (CIRAD).

Conference secretariat: Katrien Molders, AnnaKarin Hedin and Agnès Hubert

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European Plant Science Organisation

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European Plant Science Organisation

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Speaker abstracts

What do we need to improve crops faster and cheaper? S 001

Keynote

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Societies continue to depend on improved plants to cope with the many critical problems associated with sustaining necessary food, energy and fibre supplies as well as managing the environment. Indeed now, more than for a very long time, there is a momentum for including agriculture and production of plants much more centrally in strategies for sustaining the planet and all its inhabitants, human and non human. This creates opportunities and responsibilities for the plant science communities. There have been major successes in plant breeding programmes but the speed of progress looks depressingly slow from the point of view of today's needs and fast-moving science base. What do we need to do to improve crops faster and cheaper? We need much greater commitments to establishing the necessary infrastructure, public and private, for modern plant breeding that integrates the spectacular innovations of the past three decades into applied projects. Only when this occurs can the promise that plants and agriculture offer for sustaining the planet be fully realized. Without these commitments the gains from progress in plant science cannot be realized adequately by societies. What are the technical needs to make a plant breeding program go faster? The diversity in germplasm i.e the natural resources available for each crop, needs to be characterized much more extensively, so that all the forms of allelic and other variation are known and the links between the allelic variation and traits are known. We need to deploy the new DNA sequencing technologies as rapidly as possible to characterize genomes and their variants. The genetic basis of the major traits needs to be known so that breeders can target trait improvements rationally, using informed markers and sentinels and/or surrogate assays for speed and ease. We need to exploit knowledge across species, whether it is based on conservation of biochemical processes or genetic synteny. We need to be able to add genes and/or silence genes readily. All these and additional ones will be illustrated with an emphasis on improving high biomass crops that can serve as sources of energy for tomorrow's world.

S 002

The knowledge based bio-economy from a "plant" perspective

Session: Plant Science in Europe – Science Policy

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Funding for R&D has evolved from the first to the current 7th Framework Programme in terms of budget, the priorities for collaborative research, and to a certain extent modalities of funding. While for example, the agricultural research agendas in FP5 and FP6 were driven by wider concerns about food quality, the strategic orientation in FP7 takes a broader view to address equally food quality, sustainability and productivity issues as well as promoting research into new uses of plants and plant products.

The 7th Framework Programme (FP7) is ambitious in scope and amount, providing through its various programmes support to collaborative research (COOPERATION), infrastructures (CAPACITY) , mobility and training (PEOPLE), as well as to bottom-up frontier research (IDEAS). Under the FP7 COOPERATION programme 1.9 billion EUR have been allocated to Theme 2 “Food, Agriculture and Fisheries, and Biotechnology" with the aim of enhancing the development of the "Knowledge-based Bio-economy" (KBBE).

The KBBE concept acknowledges the increasing importance of the "bio-sector" not only as a source of primary food production but also for the generation of renewable energy, high-quality materials and for industrial biotech applications based in particular on advances in life sciences and biotechnology. Accordingly, activities under Theme 2 are structured around the three headings: (1) Sustainable production and management of biological resources from land, forest and aquatic environments; (2) Food, health and well being; (3) Life sciences and biotechnologies for sustainable non-food products and processes.

A strong and dynamic KBBE is considered as crucial to enhance sustainable economic growth and employment, and to find solutions to major global challenges such as climate change and its necessary mitigation, reducing the environmental footprints of agricultural production, finding alternatives to fossil fuel, food security and feeding an increasing world population, health issues and globalisation. The implementation of the KBBE is further promoted at European level through "policy" instruments, such as the European Life Sciences and Biotechnology Strategy and a LEAD-market initiative for bio-based products.

In order to further maximise the output of increased but still limited EU research funding, it is vital that a more coherent approach is applied across Europe in terms of pooling research efforts, biological infrastructures and resources, where necessary also in collaboration with countries outside the EU. Equally important, synergies between national and EU research activities need to be further exploited. The European Technology platforms such as "Plants for the future" also have an important role to play in achieving greater coherence across Europe by focussing efforts on areas of high priority to industry and by bringing together different stakeholders.

The European research council: a benchmark for frontier research funding in Europe

S 003

Session: Plant Science in Europe – Science Policy

The ERC is a nascent institution focused on delivering the Ideas Programme as an excellence initiative to reinforce frontier research in the European Research Area within the EC 7th Framework Programme. It supports with substantial grants individual researchers of any nationality and in all fields of science and scholarship, provided that they will work in a European Member State or Associated Country. Excellence of the proposal and the proposer are the only criteria for selection of applications. Two funding streams are implemented, the ERC Starting Grants for academically young investigators and the ERC Advanced Grants for accomplished investigators who are at the forefront of their fields. It is expected that in the next years ERC will become a world-leading funding agency for support of frontier "bottom-up" research. The lecture will summarize the origins of ERC, its structure, the outcomes of the first Starting Grants competition and the challenges that lie ahead.

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S 004

Plant science in Europe – Breaking new ground

Session: Plant Science in Europe - Science Policy

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Climate change, population growth, food crisis, energy shortage, loss of biodiversity…the media are abuzz with disconcerting news, and challenges that societies are facing seem to accelerate at an ever-increasing pace. Many of the challenges are linked to plants and their performance in natural and agricultural systems. How can we tackle these challenges to support sustainable growth and economic wellbeing, while at the same time reducing the environmental impact of agricultural production, meeting societal request for sustainability, and preserving healthy natural ecosystems and biodiversity? Common to all challenges is the need to increase public awareness of the critical importance of plant research to advance breeding, sustainable agriculture, horticulture and forestry, as well as our understanding of plant function in ecosystems. During the last few years we have witnessed major breakthroughs in understanding the molecular function of plants, the development of technologies to improve breeding and crop production, and the interaction of plants with their environment. Since EPSO was founded in 2000, the organization has become a strong advocate for plant research in Europe and an important voice to articulate the needs of plant scientists at national and European levels. Many tasks remain, and the EPSO vision of a strong European plant research and technology platform for sustainable future growth must become firmly embedded in public and political decision-making. Only then will rising public awareness and innovative plant science break new ground for Europe to lead the world in building a knowledge-based economy that can support sustainable development.

Plant science, the basis for farm, food, non food and energy

S 005

Session: Plant Science in Europe - Science Policy

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The increasing productivity of agriculture per ha, per man hour, per kg of external input and per animal has created ample opportunity for development of land use change that may lead to a substantial reduction in the environmental side effects of agriculture, ample opportunity to restore fragile ecosystems and expand European nature and forests. (Ground for choices, 1994).
The possibilities for a productive agriculture and sophisticated European food system that may fulfill the changing needs of European consumers and producers are impressive. Productivity may increase per ha with at least 2-5 times, per kg of input through precision agriculture with at least 40% and pesticide use may be reduced with at least 70%, GHG emission may decrease with 80%. All this sounds as utopia, but is possible when the best ecological and best technical means are applied on the agriculturally speaking, best lands. Utopia is possible but may change in dystopia when big scale biofuel production is stimulated through regulation, tax measures or subsidies. The GHG reduction is, due to changed land use, very limited or negative and the land, water and nutrient use very substantial. The way to sustainable development in Europe is in agriculture and food and feed security possible but will be jeopardized when energy security through biofuel or biomass is promoted.

S 006

A glimpse into the impossible: physical mapping of the giant hexaploid wheat genome using a chromosome based approach

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

Etienne Paux *
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Together with rice and maize, wheat provides more than 60% of the calories and proteins for our daily life and their improvement for food and non food uses is critically important if we are to meet human needs in the next decades. Genome sequencing is a widely accepted mechanism to understand the molecular basis of phenotypic variation, accelerate gene cloning and marker assisted selection, as well as improve the exploitation of genetic diversity for efficient crop improvement. While rice and maize improvement is profiting already from information derived from their genome sequences, wheat is lagging behind without a genome sequence project underway. Bread wheat is grown on over 95% of the wheat growing area and has been chosen by the International Wheat Genome Sequencing Consortium (IWGSC, www.wheatgenome.org) as a target for genome sequencing. However, with a genome size 40 times that of rice, it represents a challenge for molecular studies. Physical maps anchored to genetic maps are the substrate for genome sequencing and they provide efficient tools for marker development, map based cloning, QTL mapping, as well as for structural, functional, and comparative genomics studies. Currently, whole genome physical mapping is hampered by the size (16,000 Mb), allohexaploid nature, and high repetitive DNA content (~80%) of the wheat genome. Using laser flow cytometry and aneuploid lines, individual chromosomes or chromosome arms can be sorted at high speed thereby providing an alternative approach for a chromosome-based dissection of the wheat genome. In the framework of the IWGSC, we have developed a physical map of chromosome 3B, the largest wheat chromosome (1 Gb, 2.5 times the rice genome) and established the proof of concept for physical mapping of the 21 bread wheat chromosome through a chromosome based approach. The 3B physical map consists of 1,036 contigs with an average size of 783 kb that cover 811 Mb *i.e.* 82% of the chromosome. To date, the physical map is anchored to cytogenetic and genetic maps with 1,397 markers thereby providing a framework for efficient map based cloning and marker development through BAC end and contig sequencing. Application of the 3B physical map for studies of recombination, LD, genome composition, organisation, function, and evolution will be presented.

It's not size but coordination that matters

S 007

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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Despite possessing multiple sets of chromosomes, hexaploid wheat and tetraploid wheat behave as diploids at meiosis. Correct pairing of homologous chromosomes is controlled by the *Ph1* locus which stabilises their polyploid genomes. By exploiting comparative genomics and deletion mapping we have defined the *Ph1* locus to a cluster of *Cdk-like* (CDKL2) genes containing a segment of heterochromatin. This dominant locus arose by gene amplification and insertion during wheat's polyploidisation. The 5B locus suppresses the expression of the corresponding loci on the homoeologous chromosomes 5A and 5D. The *Cdk-like* genes show homology to *Cdk2* in mammals. Cdk2 affects replication, chromatin remodelling and the recombinational machinery. It's disruption causes non-homologous synapsis at meiosis in mammals. Our working hypothesis is that CDKL2 is functional similar to Cdk2 and this explains the *Ph1* phenotypes observed. *Ph1* affects replication, controls the remodelling of heterochromatin and the recombinational machinery, all important for stabilising the genome.

References:
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S 008

Evolution of grasses by comparative genomics

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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The genomes of rice and sorghum have been sequenced and assembled into chromosomal molecules. The maize genome is being sequenced with more than half of its genes already anchored as sequence tags to its chromosomes. Conserved gene order between all three genomes (synteny), provides us with important insights in 50 million years of grass evolution. Progenitors of rice, sorghum, and maize split about 50 million years ago (mya), while progenitors of sorghum and maize only 11.9 mya. Despite the uneven distances between these genomes, rice and sorghum exhibit a greater degree of gene collinearity than sorghum and maize. It appears that the accelerated change of maize chromosomal organization was due to allotetraploidization. Allotetraploidization is a whole genome duplication event of two diverged although closely related species. Therefore, maize chromosomal regions match rice and sorghum regions at a ratio of 2:1, where the two maize homoeologous regions diverged further than between rice and sorghum. Collinearity or the lack of it permits us to reconstruct ancient chromosomal breakages and fusions, the shedding of centromeres, and the formation of new centromeres. It also allows us to distinguish between gene insertions and deletions. Besides gene duplications, transposable elements have played a major role in the diploidization of the maize genome and its size. While chromosome expansion occurred in sorghum and maize recently, they resulted in different chromosomal organizations of the two genomes.

The grapevine genome sequence suggest hexaploïdization in major angiosperm phyla

S 009

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

The grapevine *Vitis vinifera L.* is in economic terms the principal fruit crop in the world. Its haploid genome is estimated to be about 500 Mb, organized in 19 chromosomes. The grapevine is the fourth plant whose genome sequence has been made public after *A. thaliana*, rice and poplar. Here we present a public consortium project that completed a 12X Whole Genome Shotgun sequence of a quasi-homozygous genotype, PN40024,which was derived from repeated selfings of Pinot Noir. All data were generated by paired-end sequencing plasmid, fosmid and BAC libraries of different insert sizes, using Sanger technology. Using a 8.4X coverage an intermediary assembly of 498 Mb was obtained, composed of 3830 scaffolds. Half of the assembly is represented by scaffolds longer than 1.9 Mb and a large majority of these are anchored on linkage groups. Different approaches revealed that approximately 41% of the grape genome is of repetitive/transposable elements (TE) origin. The proteome was determined by an annotation strategy reconciling proteins, cDNA alignments and *ab initio* predictions that led to an estimate of 30434 protein coding genes. Several large expansions of gene families with roles in aromatic features are observed. The grape genome was shaped by two ancient whole genome duplications, that were not followed by extensive rearrangements, thus enabling the discovery of ancestral traits and features of the genetic organization of flowering plants.

The French-Italian Public Consortium for the Sequencing of the Grapevine Nuclear Genome[1]
Anne-Françoise Adam-Blondon[2]

[1] http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/
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S 010

Rethinking agriculture and urban green space management: plant adaptation, domestication and conservation

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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Climate change and the need to deal with carbon emissions through ongoing deforestation require fundamental rethinking of major global land uses in both regional and urban lands. Given that most of the world's arable land is already sequestered for agriculture, people are attempting to move into marginal lands to continue to feed the world. This strategy has delivered marginal temporary gains but long-term damage to the biosphere. An alternative approach is to get better use out of the most productive lands through broadening the plant diversity used, particularly under rapidly changing climate. Here, we highlight how RBG Kew has experimented with introducing plant biodiversity into agricultural and urban lands, and worked with people in South Africa to domesticate new medicinal crops. While there are potent economic, political and cultural challenges to rethinking the model and implementing new approaches, we must, as a matter of urgency, foster significant research investment in this arena if we are not to further erode the global life support systems provided by plant diversity.

Hybrid speciation in flowering plants

S 011

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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Interspecific hybridization is a major force in plant evolution. Most hybrid speciation events are associated with an increase in the chromosome number (allopolyploidy), but hybrid speciation without a change in chromosome number (homoploid hybrid speciation) is also possible, although its occurrence appears to be less frequent. Notable examples of recent hybrid speciation have been described in the Poaceae (e.g. *Spartina*), Asteraceae (e.g. *Senecio*), and Rosaceae (e.g. *Sorbus*), which provide excellent model systems for studying speciation 'in action'.

In the genus *Senecio* there are recent examples of both homoploid and allopolyploid speciation. *S. squalidus* (Oxford ragwort) is a recent allopatric homoploid hybrid species that originated from material derived from a hybrid zone between *S. aethnensis* and *S. chrysanthemifolius* on Mt. Etna, Sicily. During its colonization of the UK in the last 150 years, *S. squalidus* has hybridized with native *S. vulgaris* (tetraploid) to create two new allopolyploid hybrid species *S. cambrensis* (allohexaploid), and *S. eboracensis* (allotetraploid). We are studying genetic changes to genome and transcriptome associated with the origin of *S. squalidus* and *S. cambrensis* using wild and resynthesized plant material.

In the genus *Sorbus* hybridization, polyploidy, and apomixis have combined to generate new reproductively isolated taxa in sympatry within the Avon Gorge in Bristol. This evolutionary 'hot-spot' is one of the richest areas of *Sorbus* diversity in the world, containing at least six endemic *Sorbus*, including the Red Data Book species *S. bristoliensis* and *S. wilmottiana*. We are using molecular markers to determine the genetic relationships between the nineteen *Sorbus* taxa in the Avon Gorge. Our preliminary data indicates that *Sorbus* speciation is ongoing and that conservation strategies for *Sorbus* in the Avon Gorge should aim to preserve this evolutionary process rather than individual rare taxa.

S 012

Assessing demographic history in a population-genetic framework: A multilocus case study in wild tomatoes

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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One of the principal aims of molecular population genetics is to infer aspects of evolutionary history from currently observed patterns of DNA sequence polymorphism and divergence. To this end, approaches based on coalescent theory have become the standard inferential tools of population geneticists. One of my goals in this presentation is to provide an intuitive understanding of certain properties of gene genealogies, as reflected in levels and patterns of Single Nucleotide Polymorphisms (SNPs). In particular, I will focus on two demographic factors that are likely to be of general relevance for (but by no means limited to) plants, namely population-size changes and population subdivision.

A robust understanding of these (past and/or present) demographic features has largely remained elusive in empirical studies, with the partial exception of certain economically important crop species where genome-wide impacts of domestication bottlenecks have been characterized using population-genetic approaches. Background information about demographic history, population subdivision, and effective population size is important in trying to interpret patterns of sequence diversity at loci screened for putative adaptive functions ('candidate' loci). Accruing evidence for the adaptive nature of a subset of molecular variation, or of natural selection from current patterns of sequence diversity at particular loci or genomic regions, can in principle be achieved by comparisons with SNP data from neutrally evolving 'reference' loci.

Our study system comprises several species of wild tomatoes (Solanum section Lycopersicon), which are native to western South America and close relatives of the cultivated tomato. We have generated DNA sequence data from multiple nuclear loci and multiple population samples per species. In particular, data from S. peruvianum and S. chilense (four population samples each) will be used to illustrate population-genetic inferences with respect to species-wide nucleotide diversity and the extent of population subdivision. Moreover, these empirical data were instrumental in understanding the importance of sampling schemes for observable properties of the underlying sample genealogies, in particular the frequency spectrum of polymorphic sites (which is widely used to assess deviations from neutral expectations and/or population-size changes).

While most of the tomato population samples would seem to be compatible with demographic equilibrium when analyzed separately, locus-specific sets of pooled (combined) sequences exhibit mutation-frequency spectra skewed towards low-frequency mutations. Coalescent simulations implementing both population subdivision and population-size changes suggest that such patterns are expected under demographic (or range) expansions. Importantly, every sampling scheme that includes more than one allele (sequence) per local population is biased against detecting the full extent of species-wide demographic changes. Because these effects of sampling scheme disappear only under very high rates of gene flow (connectivity) between populations, virtually every species should be regarded as deviating from the population-genetic 'ideal' of being panmictic.

A dispersed-specific model of plant domestication

S 013

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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Over the last decade, a consensus has been reached on the existence of a core area of plant domestication in the Fertile Crescent, located in south-eastern Turkey, where the closest wild relatives of einkorn, emmer, barley, rye, chickpea, and lentil still grow today. Similar wild populations were necessarily the starting material at the origin of agriculture in the Fertile Crescent. Detailed archaeological reports describe how the pre-domestication cultivation of wild cereals lasted even for centuries in the region, and how it was followed by domesticate phenotypes. The genetic and cultural mechanisms underlying the origin of those phenotypes are the issue. If geographically distinct domestication events each entailed random sampling from local genotypes, domesticate lines should trace to different localities across the range of the wild progenitor.
This is not observed for einkorn wheat (*Triticum monococcum* L) that we used as a model species: Einkorn was among the first crops domesticated by humans in the Fertile Crescent 11,000 years ago. During the last 5,000 years it was replaced by tetraploid and hexaploid wheats and largely forgotten by modern breeders. Einkorn germplasm is thus devoid of breeding bottlenecks and has therefore preserved in unfiltered form the full spectrum of genetic variation that was present during its domestication. We investigated haplotype variation at 18 loci across 321 wild and 92 domesticate *T. monococcum* lines (> 12 Mb sequenced). Our broad sample of wild lines reveals that wild einkorn underwent a process of natural genetic differentiation, most likely an incipient speciation, prior to domestication. That natural differentiation brought forth three distinct wild einkorn races. Only one of those natural races, β, was exploited by humans for domestication. We present also evidence that einkorn underwent no reduction of genetic diversity during domestication and propose a new model of plant domestication that we designate as dispersed-specific model.
In essence, our model supports the adoption of a wild population specific for the core area: this wild population colonized cultivated fields and gradually became domesticated while conserving the original genetic variation. This hypothesis accounts for our molecular data and accommodates the results of archaeological excavations.

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S 014

From ecosystems to genes: understanding the diversity of plant response to elevated CO2

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

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Although global biomass resources are vast and underutilized, over the coming decades, in the face of a growing population and a changing climate, there is likely to be increased pressure on plant resources for food, fuel and other plant products as we move from an oil-based to a bio-based economy. All of this will put pressure on global agricultural productivity. Plant biologists, agronomists, and breeders should therefore consider the future of crop production in a changing climate; bearing in mind a multiple objective that is to maintain acceptable production levels while minimizing degradation of soil and water resources, preventing environmental impacts such as ground water pollution and greenhouse gas emissions.
Increasing atmospheric CO_2 stimulates photosynthesis in C3 crops and CO_2 is likely to rise by a further 150 ppm or more in the next 20-25 years. In theory this could increase light-saturated C3 photosynthesis and yield by approximately 40%, but past and current experiments in elevated CO_2 in FACE (Free Air CO2 Enrichment) have revealed a plethora of responses to this treatment, depending on plant species, age and exposure duration and this could eventually limit potential gains in crop biomass and seed yield. The ability of crop plants to benefit from rising CO_2 will depend on adequate genetic variation being present to enable adaptation to these new conditions and selection of appropriate traits – maximizing the benefits of CO_2 whilst offsetting the negative impacts of water and temperature stress.
We propose that new and novel experimental work investigating the CO_2-responses of major food crops under representative field conditions, worldwide, is now justified, with a proper methodological approach. There is a need to understand the molecular genetic basis of complex traits that are key to the productivity of crops using a new genomics toolbox that is now available that includes high throughput sequencing, identification of QTL, transcriptomic approaches for important genes and SNPs discovery in those genes. Coupled with a new generation of experimental facilities allowing large scale experimentation under elevated CO_2 and under realistic field conditions.
This paper will outline the most critical requirements in terms of methodology, by reviewing molecular methods that will be required and describing a range of opportunities that are currently available to establish large scale collaborative facilities to implement a proper scientific policy.

Impacts of global environmental changes on the distribution of plant production to 2100

S 015

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

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Quantifying, explaining, and predicting the temporal and spatial dynamics of photosynthesis from regional to global scales is important for understanding the potential impacts of rising atmospheric CO2 and changing climate on future plant production and the global carbon cycle. A new high-resolution modelling approach is described, tested, and applied. Photosynthesis is modelled using a detailed mechanistic algorithm, within a full treatment of the surface energy and hydrological balance. Canopy processes are parameterised at an intermediate level of detail. Photosynthetic capacity is either prescribed from remote sensing and plant-type specific properties, or simulated prognostically. A range of sensitivity tests are used to highlight key processes and key areas for future research. Simulations of the distribution of plant production to the end of this century are performed using a range of global climate model predictions, and the implications for future food production, forestry, and ecosystem services are discussed.

S 016

Using comparative assessments of net ecosystem exchange and carbon sequestration to identify mitigation options for managed ecosystems

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

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One of the challenges for agriculture in the future is to reduce carbon and other greenhouse gas emissions, whilst still maintaining crop productivity. To identify potential management options that might lead to a reduction in C emissions that don't compromise crop productivity we have made a comparison of three different land uses, cropland, grassland and forest-that are located within ~30km of each other, using measurements of net ecosystem exchange (NEE) based on eddy flux technology. Annual carbon sequestration was highest in the forest ecosystem (7.69-9.44tC ha^{-1}) and lowest in the cropland (1.83-2.69tC ha^{-1}). This was principally a consequence of the shorter duration of vegetation cover in the cropland, as peak values for NEE or gross primary productivity were as high as or higher than the other two ecosystems. Forest thinning increased NEE, possibly due to the exposure of a larger photosynthetic surface to the incoming radiation; the impact of this may also depend on the proportion of diffuse to direct radiation. Increases in the diffuse component would also likely lead to enhanced productivity in the grassland and cropland ecosystems. The introduction of a cover crop enhanced NEE in the cropland whilst reduced tillage had only a small impact on NEE. The effect of the cover crop (mustard) on NEE was found to depend on the number of freezing-nights, with significant and persistent depressions in carbon uptake that lasted for ~7 days. Leaf level measurements indicated that these reductions in carbon uptake were due to impaired photochemistry. These results indicate that simple management practices could significantly enhance carbon uptake and sequestration in different agro-ecosystems. In cropland increased carbon sequestration may be achieved by the introduction of cover crops with enhanced carbon sequestering capacity and improved resilience to freezing temperatures. This requirement may, however, be modified by the projected increases in winter temperatures associated with climate change.

Promoting indigenous crops as a tool for tackling climate change and food insecurity in semi-arid Africa

S 017

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

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The semi-arid region of sub-Saharan Africa is characterized by extreme climatic conditions that vary hugely between years. The origins of some of the most resilient crop species such as sorghum, pearl millet and cowpea can be tracked back to this region. Globalization is pushing the existence of these indigenous crops to the verge of extinction in a competition for research and resources with less resilient, but popular introductions like maize and phaseolus bean, which yield well under favourable conditions. Further, the intensive breeding of a few major crops has widened the gap between the cosmopolitan and traditional crops.

Bambara groundnut (*Vigna subterranea* L. Verdc.) is one such indigenous legume grown primarily for subsistence in many parts of Africa. It has the potential to contribute to food security in extremely drought-prone regions, especially in much of the semi-arid tropics where rainfall is often insufficient to support the cultivation of other leguminous crops. It is the third most important legume in Africa after cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogaea*). In January 2006, an EU-funded project, BAMLINK, was launched to assess and link nutritional, ecophysiological and molecular characteristics of bambara groundnut landraces in order to promote it as a major food crop for semi-arid Africa and India. This has led to the construction of a microsatellite enriched library and evaluation of the DArT marker technology aimed towards developing markers for assessing genetic diversity in over 200 bambara groundnut accessions. The first genetic linkage map based on AFLP markers in a wide cross of bambara groundnut identified many important QTLs responsible for domestication and agronomic traits. Massively Parallel Signature Sequencing technology was exploited for gaining a comprehensive insight into metabolic processes under water deficit, for identifying drought induced genes and for assessing differences between genotypes adapted to contrasting environments in bambara groundnut. Physiological traits like photoperiodic responses, heat stress, Δ13C discrimination and WUE measured in landraces produced noteworthy results. Detailed nutritional and functional evaluation of bambara groundnut consolidated some scattered information available until now. All these information pulled together led to the successful development of the first fully functional model for predicting growth rate and productivity of bambara groundnut landraces under heat, cold and drought stress conditions.

We will present research progress and demonstrate that by adopting a holistic approach towards developing one indigenous crop how underutilised, but locally adapted species with obvious evolutionary advantages, have the potential to contribute towards food security in an ever changing global climate, especially in some of the poorest and most hostile regions of the world.

S 018

Is multifunctionality the future of agriculture or simply a trade issue?

Session: Science and Society: The challenges for tomorrow's agriculture

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The recent food price increases are a major cause for concern around the world. In developing countries in particular, they are undermining attempts to reduce hunger and pushing some of the world's poorest people into abject poverty. The underlying causes are complex and include factors such as increased demand from rapidly growing economies, poor harvests due to an increasingly variable climate, the use of food crops for biofuels, higher energy prices, export bans on agricultural products from a number of significant exporters and speculation on the commodity futures market. But are these price increases a momentary blip - the result of an unfortunate series of events, or are they a harbinger of the future? And if they are more than a blip, what else do we need to know if we are to provide sustainable and nutritious food for the world?

Meeting the goal of affordable nutritious food for all in an environmentally sustainable manner is achievable, but it cannot be achieved by current agricultural 'business as usual'. Instead, if a large part of the world isn't to go hungry in the 21st Century, we need nothing short of a new 'agricultural revolution', with a more rational use of scarce land and water resources, an equitable trade regime, as well as widespread recognition and action on climate change. We also need to recognise that in this changing world we need new tools, which means increased investments in agricultural knowledge, science and technology.

Agriculture can no longer be thought of as production alone, but the inescapable interconnectedness of agriculture's different economic, social and environmental roles and functions must be explicitly recognized. **Multi-functionality is the future of agriculture – it is not simply a trade issue.**

Thankfully, many of the technologies and practices we need to meet the challenge of sustainable agriculture already exist

Meeting the goal of affordable nutritious food for all, in an environmentally sustainable manner is achievable. The future is not pre-ordained, but is in our collective hands. While we can build upon our successes, we must also recognise that an extrapolation of business-as-usual will not suffice. Instead, we need to be bold enough to rethink agriculture. Most importantly, if we are to help today's and tomorrow's poor and disadvantaged, we need to acknowledge that the time to act is now.

A food system which ticks all the policy boxes: Can it be done? What would it look like? Is anyone pushing for it?

S 019

Session: Science and Society: The challenges for tomorrow's agriculture

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For many years, evidence has been growing that the global food system is under considerable stress. Policy-makers have not until recently recognized this evidence for many reasons, ranging from lack of champions, difficulty in accommodating the enormity of the challenges and 'lock-in' to existing policy perspectives and institutions. This paper sets out the new fundamentals which a food policy fit for the 21st century will have to accept and be built upon. The paper proposes that the new food policy will have to address many previously discrete policy 'boxes'. It asks whether existing institutions are appropriate for the task and proposes that reform of both policy and institutional architecture is required. Scientists will need to be better organized and engaged with civil society if the pace and scale of reform that most of us think necessary has a chance to map let alone deliver the change in policy directions needed.

S 020

Economic consequences of Golden Rice

Session: Science and Society: The challenges for tomorrow's agriculture

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Golden Rice (GR), which has been genetically modified to produce beta-carotene in the endosperm of grain, has been proposed to control vitamin A deficiency (VAD), especially among the poor in developing countries. However, the usefulness of GR is questioned by some, and the technology has become one of the centerpieces in the public controversy over genetically modified crops. Because GR is still at the stage of research and development, its actual effectiveness remains unknown. We have developed a methodology for ex ante evaluation, taking into account health and nutrition details, as well as socioeconomic and policy factors. The methodology has been used for empirical analyses in India. Building on a disability-adjusted life year (DALY) framework, we show that VAD is a serious public health problem in India, causing a sizeable disease burden, especially in terms if increased child mortality. Using a nationally representative data set of household food consumption, we have simulated the nutrition and health effects of GR under different assumptions. With public support, if GR were to be consumed widely, the disease burden of VAD could be reduced by 60%, while under more pessimistic assumptions the reduction would be around 10%. When valued in dollar terms, these positive health effects also translate into large economic benefits. Regardless of the underlying assumptions, GR is likely to be more cost-effective than alternative vitamin A interventions, such as food supplementation or fortification. Therefore, it should be considered seriously as a complementary intervention to fight VAD in rice-eating populations.

Regulation and risk assessment of transgenic plants at European level

S 021

Session: Science and Society: The challenges for tomorrow's agriculture

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The following topics will be included in the talk: (i) GMO Regulation in the EU, (ii) European Food Safety Authority (EFSA), (iii) Environmental Risk Assessment, (iv) Future Developments and (v) Biosafety Research.
The European Food Safety Authority (EFSA) is the keystone of EU risk assessment regarding food and feed safety. In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides independent scientific advice and clear communication on existing and emerging risks. The EFSA Panel on genetically modified organisms provides independent scientific advice on the safety of (i) GMOs such as plants, animals and micro-organisms, on the basis of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and (ii) genetically modified food and feed, on the basis of Regulation (EC) No 1829/2003 on genetically modified food and feed. The GMO Panel carries out risk assessments in order to produce scientific opinions and advice for risk managers. Its risk assessment work is based on reviewing scientific information and data in order to evaluate the safety of a given GMO. This helps to provide a sound foundation for European policies and legislation and supports risk managers in taking effective and timely decisions.

S 022

Regulation of shoot branching

Session: Improving plant product quantity and quality I: Developmental biology

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Shoot system architecture is an important agronomic trait, with for example a profound influence on light capture, shoot biomass and fruit number. The degree of shoot branching is under both genetic and environmental control. Integration of these inputs is achieved through the action of a network of interacting plant hormones. As a result of this hormonal network, branching can be modulated throughout the life of the plant depending in the environmental conditions. For example, damage to the primary shoot apex can be detected and mitigated by the activation of axillary shoot apical meristems. Thus shoot branching control is also an important factor in achieving yield stability.
We are investigating the network of interacting hormones that regulate branching, and the mechanisms by which they act and interact with one another and the environment. Our progress in understanding the operation of this network will be presented.

Modelling genes, growth and form in plants

S 023

Session: Improving plant product quantity and quality I: Developmental biology

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Much progress has been made recently in our understanding of how genes control patterns of cell types or regional identities with in an organism during its development. However, the link between this process of patterning and growth or morphogenesis is much less well understood. Bridging this gap requires a quantitative understanding of how genes modify growth of multicellular tissues in 3D space at multiple scales. We have been addressing this problem using a combination of genetic, morphological, computational and imaging approaches in collaboration with Andrew Bangham (University of East Anglia) and Przemyslaw Prusinkiewicz (Calgary). The results provide new insights into how genes interact with patterns of growth at various scales to modify shape. The talk will illustrate how integrating biological and computational methods may lead to a quantitative mechanistic framework for development.

S 024

Wood development – what do plant hormones do?

Session: Improving plant product quantity and quality I: Developmental biology

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Wood is a sustainable raw material that will become increasingly used in both traditional and novel processes to supply energy and materials. Commercial use of wood is based on only a few selected conifer and angiosperm species that provide raw materials with various properties that are suitabile for different uses. Wood and fiber properties are also highly variable within a stem, reflecting the plasticity of wood development. The large within and between species variation in wood properties highlights the immense potential for targeted breeding towards improved feedstock and performance in service. This can best be achieved by a basic understanding of wood development and the use of molecular tools in breeding programs. Plant hormones have long been known to be potent modifiers of growth and development when applied to wood forming tissues, and are generally thought of as mediating internal and environmentally induced growth patterns and growth responses. Auxin, gibberellin and ethylene have all been observed to affect basic events in wood development, i.e. cell division, cell expansion and secondary wall formation. They are therefore putative key players in signalling pathways of commercial traits such as biomass production and fiber chemistry. However, despite the increasing knowledge of plant hormone signal transduction pathways, and hormone cross talk, emerging mainly from research on *Arabidopsis* the very basic understanding of the regulating role of endogenous hormones in wood development is still rudimentary. Tree size and the physically broad developmental progression of wood relative to other tissue types offers advantage as an experimental system. It is possibile to sample wood from specific developmental stages during their formation. This technique has, for example, been used to visualize the distribution of endogenous hormones with physicochemical tools. We took advantage of this technique to establish that the auxin gradient across wood forming tissues peaks in concentration in the cambial meristem, suggesting its pivatol role in cambial growth. GAs also stimulate cambial cell division when applied to wood forming tissues. Endogenous GAs, however, show a strict compartmentalization to expanding cells with an absence in the cambial meristem. This indicates that GAs are not involved in cambial growth, but rather have a function in wood fiber expansion. Moreover, physiological and molecular experiments demonstrate cross talk between IAA and GA in regulating each others homeostasis and also in inducing target genes. Ethylene is a gas and therefore not likely to be restricted to specific tissue compartments. It is also generally thought of a mediator of environmental stress signals and not required for cell division and cell expansion. By imposing ethylene insensitivity, we could investigate its endogenous affects. We created ethylene insensitive trees by ectopic expression of the dominant negative ethylene receptor At*etr1-1*. This approach allowed us to demonstrate a role for endogenous ethylene in mediating increased cell divisions in the cambial meristem as a response to gravity when the tree forms tension wood.

Cytokinins control root meristem activities and root growth by antagonizing auxin action

S 025

Session: Improving plant product quantity and quality I: Developmental biology

Plant postembryonic development and growth arise from localized regions called meristems. Within the meristems, a subset of stem cells self renew and produce daughter cells that differentiate, giving rise to all plant organs and structures. Cell differentiation is initiated at the meristem transition zone (TZ), the boundaries between dividing and expanding cells of the different cells files. We recently demonstrated that cytokinins control cell differentiation at the root TZ while auxin induces cell division in meristem: thus, the antagonistic and coordinate action of these two hormones is responsible of the establishment of a balance between cell division and cell differentiation necessary to maintain meristem activities and root growth. Here we show that cytokinins position the TZ and regulate the cell differentiation by regulating auxin perception and homestasis.

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S 026

Genomics analysis of responses to nutrients

Session: Preserving our future by reducing the inputs in agriculture I: Reducing fertilisers

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Nutrients act as an essential resource for plant growth, and also modulate metabolism and development. To decrease fertiliser use, we need to create plants that can acquire nutrients more efficiently form the soil, and that can use these nutrients more effectively to promote growth and yield. This will require an understanding of the signalling pathways that plants use to adjust their metabolism, growth, allocation and development to changes in the nutrient supply. While some of these responses like changes in cellular growth may be generic, other aspects like the adjustment of transport activity and metabolism can be expected to very from nutrient to nutrient. This talk will focus on nitrogen, phosphate and sulphate, which are the three nutrients that enter metabolism.

I will first present results that document the response of global transcript profiles to long tern carbon, nitrogen, phosphate and sulphate starvation, and the rapid responses after resupply of the limiting resource. In order to approach pre-transcriptional responses to nutrients, we are using quantitative (phospho)proteomics to identify proteins that are rapidly phosphorylated or dephosphorylated after resupply of nutrients to starved material. These results will be used to characterise network responses and to highlight commonalties and differences between the responses to these different nutrients. They also allow the identification of candidate genes that could be involved in regulator responses to nitrate.

I will then discuss the insights gained by functional analysis of selected signalling pathways that mediate plant adaptation to changes in the nutritional status. Examples will include (i) the elucidation of a novel systemic signalling pathway involving miR399 and PHO2 that regulates phosphate allocation between the shoot and the root, (ii) the identification f a small family of transcriptional regulators that allow nitrate to regulate sectors of secondary metabolism and (iii) the analysis of a signalling pathway that allows early flowering in low nitrate and phosphate.

In a complementary approach, we are using Arabidopsis natural diversity to characterise responses and identify QTLs and eventually genes that promote biomass accumulation in limiting nutrient conditions. To do this we have established a growth system in which decreased nutrient supply leads to a steady and sustained inhibition of growth, and have used this to compare growth rates and metabolites across a set of 94 Arabidopsis accessions. This approach is revealing which metabolic characteristics correlate with growth in low nitrogen conditions, and will allow the application of association mapping to identify genes that contribute to nitrogen use efficiency.

Phosphate starvation signalling in plants

S 027

Session: Preserving our future by reducing the inputs in agriculture I: Reducing fertilisers

Phosphorous is an essential nutrient for all organisms. Plants absorb P from preferentially as phosphate, (pi) a quite immobile ion in soils. As a consequence, Pi availability is a major constraint to plant productivity in many soils. On the other hand, plants, as well as other organisms directly taking Pi from the media, have evolved adaptive responses that allow their growth under Pi limiting regimens. In this communication, we review our work on the regulatory system of the Pi starvation rescue system in plants. In particular we will report on the role of PHOSPHATE STARVATION RESPONSE REGULATOR1, as a master transcription factor that largely controls transcriptional activation and repression responses to phosphate starvation in Arabidopsis. Thus, transcriptome analysis showed that impairing PHR1(-like) function reduces not only most of the transcriptional activation responses but also repression responses to Pi starvation. Induced genes showed enrichment in P1BS (PHR1-binding sites) in their promoters while repressed genes did not, indicating direct and indirect action of PHR1-(like) TFs. Induced genes containing P1BS are shown to be direct targets of PHR1, and are on average more highly responsive to Pi starvation. Moreover, we demonstrate that a minimal promoter containing multimerised PHR1-(like) binding sequences (P1BS) recapitulates Pi starvation specific responsiveness. Likewise, mutation of P1BS in the promoters of Pi starvation responsive genes impairs responsiveness to this but not to other stresses. Additionally, we will report on the identification of a novel riboregulatory mechanism of miRNA activity operating in the control of Pi starvation. This mechanism is based on the existente of highly Pi starvation responsive non-coding RNAs, the IPS1 family, which sequester miR399, a highly specifically Pi responsive miRNA. miR399 sequestration depends on the capacity of IPS1(-like) RNAs to be recognised by miR399 but remain resistant to miR399 guided degradation, thus defining an inhibitory mechanism based on target mimicry mechanism. Altogether, these results highlight the important regulatory novelties in Pi starvation signalling, whose potential biotechnological applications will be discussed.

Regla Bustos
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S 028

Nitrogen uptake and signalling networks

Session: Preserving our future by reducing the inputs in agriculture I: Reducing fertilisers

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Agricultural plant production strongly depends on the application of mineral nitrogen fertilizers, which are mostly supplied in the form of urea, ammonium or nitrate. Even though transport systems for these nitrogen forms have been extensively characterized at the molecular level in model and crop plants, an upregulation of their transport activities has remained unsuccessful to improve nitrogen uptake efficiency.
Nitrate has been shown to act as a signal for metabolism and plant development in physiological studies. However, so far no use is made of the signalling effect of different N forms in cereal plant production.
To investigate the effect of different N forms on shoot development, we performed nutrient solution experiments with spring barley and observed that tillering decreased with an increasing amount of nitrogen being supplied in the form of urea. The influence of different N forms on tillering was neither associated with nutrient disorders nor ammonium or urea toxicity. Instead, we observed that cytokinin translocation rates in the xylem increased under nitrate nutrition, while they were low under ammonium or urea supply.
To reproduce this N form-dependent effect in field trials, winter wheat plants were fertilised with stabilised N forms in the starter dressing. In fact, supply of nitrate stimulated tillering, while ammonium und especially urea led to decreased tiller numbers per plant. This change in plant architecture also had an effect on grain yield. Our study indicates that the use of different N forms for N fertilization to cereal crops can serve as a means to manipulate plant architecture and help in guiding individual yield components along seasonal variations.

A new approach for imaging nutrient distributions in plant tissue using time of flight secondary ion mass spectrometry and scanning electron microscopy

S 029

Session: Preserving our future by reducing the inputs in agriculture I: Reducing fertilisers

A new approach to trace the transport routes of water and nutrients in plants at the level of cells and tissues, and to measure their elemental distributions, was developed. With this technical approach we aimed at gaining insight into the dynamics and structure–function relations of transport processes in order to better understand the general principles underlying high water and nutrient use efficiency. Stem samples from *Phaseolus vulgaris* were used as a test system. Shock-freezing and cryo-preparation were combined with cryo-time-of-flight secondary ion mass spectrometry (cryo-ToF-SIMS) for element and isotope specific imaging. Cryo scanning electron microscopy (cryo-SEM) was integrated into the cryogenic workflow to assess the quality of structural preservation. We evaluated the capability of these techniques to monitor transport pathways and processes in xylem and associated tissues, using element and stable isotope tracers added to the transpiration stream. Cryo-ToF-SIMS imaging yielded detailed mappings of water, potassium, calcium, magnesium and sodium. Lateral resolutions ranged from 10 µm in survey mappings at high mass resolution to ca. 1 µm in high-lateral resolution imaging of reduced areas at lower mass resolution. The selected element and stable isotope tracers were imaged with high sensitivity in xylem vessels and surrounding tissues. Cryo-SEM confirmed that tissue structures had been preserved at particularly high quality allowing recognition of sub-cellular details. Overlays of cryo-ToF-SIMS images onto corresponding SEM images allowed detailed correlation of nutrient images with sub-cellular structures. The technique proved to be suitable for elucidation of the fate of taken-up water and nutrients and to open up new possibilities to evaluate plants with regard to their water and nutrient use efficiency. Studies of altered transport properties and their effects on growth and performance of plants will be possible in a very detailed manner, including plant phenotyping after genetic modification of water and/or solute transport.

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S 030

Monitoring and manipulating information flow at the host/pathogen interface

Session: Preserving our future by reducing the inputs in agriculture II: Reducing pesticides

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Plant pathogens use small molecules and also proteins to render their hosts susceptible. Several pathogens either make plant hormones, or perturb host hormone signalling networks by other means. In addition, many bacteria and other pathogens use a specialized secretion system to deliver proteins into host cells that interfere with host defence. We have taken advantage of the bacterial T3SS secretion system to investigate effectors from filamentous pathogens such as oomycetes. We are part of an ERA-PG project involving Jim Beynon, Jane Parker and Guido van den Ackerveken, in which we use this method to investigate the effector complement of the downy mildew pathogen *Hyaloperonospora parasitica (Hpa).* I will report recent data on Hpa effector functions and on the use of the Solexa/Illumina sequencing instrument to advance *Hpa* and other oomycete genomics and transcriptomics.

Filamentous pathogen effectors

S 031

Session: Preserving our future by reducing the inputs in agriculture II: Reducing pesticides

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Eukaryotic plant pathogens, such as fungi and oomycetes, secrete an arsenal of effector proteins to modulate plant innate immunity and enable parasitic infection. Deciphering the biochemical activities of effectors to understand how pathogens successfully colonize and reproduce on their host plants became a driving paradigm in the field of fungal and oomycete pathology. This presentation will focus on the oomycete *Phytophthora infestans*, the Irish potato famine organism that causes late blight of potato and tomato and is arguably the most destructive pathogen of solanaceous crops. Tremendous progress has been made recently in understanding the biology of *P. infestans* effectors. Two classes of effectors target distinct sites in the host plant: apoplastic effectors are secreted into the plant extracellular space, while cytoplasmic effectors are translocated inside the plant cell, where they target different subcellular compartments. Of particular interest are the RXLR and Crinkler effectors that are characterized by conserved motifs following the signal peptide. The RXLR domain is functionally interchangeable with a malaria host targeting domain and appears to function in delivery into host cells. The recent completion of the genome sequence of *P. infestans* enabled genome-wide cataloguing of the effector secretome. Using computational analyses, we identified several hundred candidate RXLR effector genes. These were frequently organized in clusters of paralogous genes, many of which exhibit hallmarks of positive selection probably as a result of a coevolutionary arms race with host factors. Predictably, effector genes are typically expressed and often up-regulated during infection. We also utilized the discovered RXLR and Crinkler effectors in high-throughput *in planta* expression assays to screen for alteration of plant defense response and gain an insight into their function. Understanding the perturbations caused by effectors is helping us to unravel mechanisms of pathogenicity as well as further illuminate mechanisms of plant defense and innate immunity.

S 032

Resistance proteins: scouts of the plant innate immune system

Session: Preserving our future by reducing the inputs in agriculture II: Reducing pesticides

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With the growing concern for the environment and a consumers wish to reduce, and eventually eliminate, residue levels in foods there is a growing demand for production systems that do not rely on the use of pesticides. One way to reduce the need for pesticides is to exploit natural disease resistance sources present in the plant germplasm. Breeders have successfully utilized these natural resistances over the last century and introgressed many, so called, resistance (*R*) genes into their elite lines, thereby making a substantial contribution to the green revolution.
To understand the molecular mechanisms underlying *R* gene function, in the last decade research efforts have focussed on the cloning of these genes. By now, >50 *R* genes have been cloned from both model and crop plants including woody species such as apple and poplar. The identified *R* genes can be divided into two groups. One contains genes encoding transmembrane proteins with an extracellular receptor-like domain while members of the second group encodes intracellular proteins. The intracellular receptors perceive the presence, or actions, of pathogen-derived proteins that enter the host cell, whereas the extracellular receptors monitor the presence of such proteins in the apoplastic space.
The majority of intracellular resistance proteins are multi-domain proteins containing a central nucleotide binding (NB) domain fused to a leucine rich repeat (LRR) domain. This dual NB-LRR core is often linked to variable N and C –terminal domains. In our group we are interested in how NB-LRR proteins trigger plant disease resistance. As model we focus on the interactions of tomato (*Solanum esculentum*) with the fungus *Fusarium oxysporum* and the root-knot nematode *Meloidogyne incognita* .Disease resistance towards *F. oxysporum* strains producing avirulence factor 2 (Avr2) is mediated by the R protein I-2, whereas resistance to *M. incognita* requires the R protein Mi-1.
In this seminar I will present a structure-function analysis of the NB domain of I-2 and Mi-1. Furthermore, I will report on our analysis of loss-of-function and autoactivation mutations in the NB domain of Mi-1 and the effects these confer on intramolecular interactions in this protein. These data culminate in a testable working model on how R proteins function as molecular switches controlling disease resistance.
The second part of the presentation focusses on proteins secreted by *Fusarium* in the xylem vessels when colonising a susceptible tomato plant. Among the identified proteins three were shown to be R protein recognition determinants: Avr1, 2 and 3 matching I-1, I-2 and I-3. Besides disclosing the presence of the pathogen to a resistant plant, these proteins were found to be important for virulence as they enhance colonization of plants lacking the corresponding *I* gene. Surprisingly, the virulence function of Avr1 turned out to be suppression of *I-2* and *I-3* function. This cross-talk provides an insight in the ongoing warfare between host and pathogen and the defence and counter-defence strategies employed.

A family of bacterial effectors promote disease by interfering with plant MAP-kinases

S 033

Session: Preserving our future by reducing the inputs in agriculture II: Reducing pesticides

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Proteins which are secreted via the Type III Secretion System (T3SS) by bacteria, named effectors, are a key element in terms of pathogenesis. The *awr* gene family from the pathogen *Ralstonia solanacearum* is a group of 5 effectors that are translocated to the plant cell in order to manipulate the host. Deciphering the targets of the AWR effectors will let us know more about their mechanism of action.
A Yeast Two Hybrid screening was performed using one of the *awr* as a bait to identify interacting proteins from an *Arabidopsis* root cDNA library. Amongst all cDNA clones found to interact, one encoding the Mitogen-Associated Protein (MAP) kinase ATMPK6 was of particular interest. In the MAP-kinase signalling pathway, ATMPK6 plays an extremely important role, integrating several stimuli including oxidative stress and defense to pathogen infection. One of the outcomes of such pathway is to produce a Hypersensitive Reaction (HR), which allows the plant to limit the spreading of the pathogen by a rapid programmed cell death. Interference of this process by the AWR bacterial effector could facilitate bacterial spread and render the plant more susceptible to the pathogen.
In order to assess the AWR functions *in planta,* gain-of-function approaches with transient expression in *Nicotiana benthamiana* leaves have been performed. The localization of AWR and ATMPK6 in the plant cell, overexpression phenotypes and the biochemical validation of their interaction will be presented. Finally, a possible mode of action of the AWR proteins in disease will be discussed.

S 034

Genetic and genomic approaches to deal with subsoil constraints to yield

Session: Preserving our future by reducing the inputs in agriculture III: Reducing water input

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Abiotic stresses such as extreme temperature, low water availability, high light intensity, high salt, and mineral deficiencies or toxicities can severely reduce crop plant productivity. In many cases, several types of abiotic stress challenge crop plants simultaneously. High temperatures, high irradiance, scarcity of water and nutrient deficiencies are commonly encountered under growing conditions but are frequently not amenable to management through traditional farm practices. Higher plants have evolved multiple, interconnected strategies that enable them to survive unpredictable environmental fluctuations. However, these strategies are not always well developed in the cereal cultivars grown by grain producers and typically they focus on plant survival at the expense of yield.

This presentation will focus on wheat and barley where the genetic control of traits determining yield in water limited and low yielding environments are generally expected to be of low heritability, polygenic and many of the key loci will show epistatic rather than additive effects. Current breeding and mapping techniques make it very difficult to detect and select for these types of loci. Know confounding factors, such as maturity, height, resistance or tolerance to soil diseases, and tolerance to related stresses such as boron, acidity, salinity and nutrient deficiencies must be taken into account. In many cases the genetic control of tolerance to these factors is known so that they could be fixed in both breeding and mapping populations.

In comparison to model organisms, wheat and barley have the advantages of extensive monitoring and archiving of genotypes and associated phenotypic data and the availability of unique populations adapted to specific environments and end-uses that have resulted from a long history of selective breeding. These advantages are becoming increasing significant as analytic tools improve. However, application of markers and genomics research in wheat and barley still faces a number of serious issues. In particular, many of the key traits influencing yield are poorly understood at the physiological level, hard to reliably phenotype and the genetic control is frequently poorly understood. However, whole genome approaches and systemic analysis of the molecular basis of stress tolerance responses are starting to reveal key pathways and process involved in maintaining yield in difficult environments.

Small RNAs and epigenetic regulation in abiotic stress resistance

S 035

Session: Preserving our future by reducing the inputs in agriculture III: Reducing water input

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The research in my lab is focused on the molecular mechanisms of salt, drought and cold stress signaling and resistance. Recently, we began to study the roles of microRNAs and small interfering RNAs in abiotic stress response pathways, the mechanisms of active DNA demethylation and small RNA-directed DNA methylation, and the contribution of these epigenetic mechanisms to stress resistance. Recent results concerning abiotic stress-regulation of small RNAs and DNA methylation in Arabidopsis will be presented.

S 036

An integrated approach of tolerance to water deficit involving precise phenotyping and modelling

Session: Preserving our future by reducing the inputs in agriculture III: Reducing water input

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Unlike the tolerance to other abiotic stresses, drought tolerance does not consist in identifying resistance mechanisms but in a changed optimisation strategy. Because photosynthesis and transpiration have essentially the same determinisms, namely leaf area and stomatal control, drought tolerance is an optimisation of "risk limitation vs potential production", and "carbon vs water". For an agricultural purpose, we look for less conservative strategies in several processes such as leaf growth or kernel abortion, which are adaptive processes which limit the risk of total seed loss under water deficit. The mechanisms driving the reduction in expansive growth with water deficit are multiple (e.g. changes in cell division rate, in cell wall mechanical properties, in turgor, and/or in their signalling pathways). Bottom-up approach from genes to phenotype cannot be envisaged for predicting phenotypes in these conditions. We have developed an approach in which the phenotype was first dissected in fluctuating conditions by using a model ; parameters of the models were then subjected to a genetic analysis. Time courses of transpiration and of leaf elongation rate were analysed in the platform *Phenodyn* which deals with 400 plants simultaneously over a large range of environmental conditions in the greenhouse and growth chamber. Leaf growth rate and transpiration were followed together with soil water status and micrometeorological conditions. Sensitivities to soil water deficit and to evaporative demand were determined for all genotypes of mapping populations, by the use of response curves whose parameters are valid in several experiments and experimental conditions. They can therefore be considered as stable characteristics of each genotype. We then identified QTLs of these sensitivities which were partly common to three mapping populations. These QTLs were confirmed in an analysis of near isogenic lines, and were partly common with QTLs of silk development under water deficit. The mechanisms driven by genetic responses, in term of cell wall properties, turgor maintenance, root hydraulic conductivity and ABA signalling, have been analysed and will be discussed. The combined genetic - ecophysiological model predicts the time courses of leaf growth under any climatic scenario in genotypes known by their alleles only. It has also been inserted in a whole-plant model which simulates total leaf area and biomass accumulation as a function of environmental conditions and of alleles of the considered genotypes. This opens the way to the use of "virtual genotypes" in breeding programmes, for the evaluation of the appropriate alleles for each climatic scenario.

Controlled cDNA overexpression system to isolate novel stress genes in *Arabidopsis*

S 037

Session: Preserving our future by reducing the inputs in agriculture III: Reducing water input

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Adaptation to extreme environmental conditions in higher plants requires coordinate changes in metabolism, cell growth, division and differentiation, which depend on a large set of genes controlling complex regulatory mechanisms. Responses to abiotic stresses are controlled by a complex web of ABA dependent and independent signalling pathways. Genetic approaches are best suited for the identification of regulatory genes and the majority of genes controlling responses to high salinity, drought and cold were discovered using forward genetic screens of mutagenized Arabidopsis populations.

To perform genetic screens for identification of novel Arabidopsis loci involved in the control of abiotic stress responses, a cDNA expression library was created in a Gateway version of estradiol-inducible XVE binary vector (Controlled cDNA Overexpression System, COS). The COS system was tested in three genetic screens by selecting for ABA insensitivity, salt tolerance and activation of a stress-responsive alcohol dehydrogenase-luciferase (*ADH1-LUC*) reporter gene. More than thirty cDNAs conferring dominant, estradiol-dependent stress tolerance phenotype, were identified by PCR amplification and sequence analysis. Several cDNAs were recloned into the XVE vector and transformed recurrently into Arabidopsis, to confirm that the observed conditional phenotypes were due to their estradiol-dependent expression. Characterization of a cDNA conferring insensitivity to ABA in germination assays has identified the coding region of heat-shock protein HSP17.6A suggesting its implication in ABA signal transduction. Screening for enhanced salt tolerance in germination and seedling growth assays revealed that estradiol-controlled overexpression of a 2-alkenal reductase (2AER) cDNA confers considerable level of salt insensitivity. Screening for transcriptional activation of stress- and ABA-inducible *ADH1-LUC* reporter gene has identified the ERF/AP2-type transcription factor RAP2.12, which sustained high level ADH1-LUC bioluminescence, enhanced *ADH1* transcription rate and increased ADH enzyme activity in the presence of estradiol. These data illustrate that application of the COS cDNA expression library provides an efficient strategy for genetic identification and characterization of novel regulators of abiotic stress responses.

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S 038

Metabolic composition and biomass

Session: Improving plant product quantity and quality II: Improving yield

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Yield is probably the most complex trait displayed by a plant. It is the final and integrative descriptor of plant growth during its entire life cycle. Yield is thus influenced by and dependent on numerous genetics and environmental parameters.
We are interested in the identification and analysis of processes influencing biomass. Using *A. thaliana* as the model organisms we on the one hand follow a genetic approach aiming at the parallel identification of biomass and metabolic QTL´s using segregating *Arabidopsis thaliana* ecotypes and derived RIL´s and NILs.
On the other hand we are interested in investigating the potential use of metabolite profiles as a predictor for biomass.
Both approaches show a very strong association between mQTL´s respectively metabolic composition and biomass.
As stated above biomass is influenced by numerous genetics and environmental parameters. Temperature and light are two obvious environmental parameters which constantly change over even short times. We thus set out for a systems approach following metabolic and gene expression changes as a result of changing light and/or temperature. Results of this ongoing analysis will be presented.

The identification of molecular markers for yield components

S 039

Session: Improving plant product quantity and quality II: Improving yield

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Understanding the control of yield in crops is perhaps the most complex problem in plant biology and, as it underpins the world's staple food supply, the most important. Plant breeders have made spectacular advances in improving yield, using largely empirical approaches. In contrast, our mushrooming knowledge of the molecular bases of biological processes in plants has had relatively little identifiable impact.
We have taken two approaches to better connect our knowledge of plant biology with pathways that can lead to the outcome of improved crop yield. The first is to dissect the genetics of components of yield, in order to identify the most relevant for targeted genetic improvement. The second is to identify markers that are relevant for these components. These markers will then be available for both the identification of beneficial alleles in collections of natural or induced genetic variation, and to underpin marker-assisted breeding strategies. Examples will be presented, from ongoing work in Arabidopsis, oilseed rape and maize, illustrating some of the key messages emerging from these activities.

S 040

Yield increase by transgenic approaches

Session: Improving plant product quantity and quality II: Improving yield

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Considering that yield improvement has been the focus of breeding programs for several decades, it is remarkable how little is known about the plant genes that determine yield. With the advent of genomics, classical genetic approaches have made substantial progress in identifying plant genes that control processes such as disease resistance or flowering. Yet, for a complex multifactorial trait like yield, which additionally shows a strong genotype to environment interaction, genetics has generally not been able to reach the resolution power that is required to dissect QTLs to the level of single genes.

Reverse genetics approaches have been more successful, particularly for the identification of genes that control processes underlying yield - such as photosynthesis, carbon partitioning, flower development and seed production. However, only a limited number of these genes have been demonstrated to effectively improve overall crop yield. One of the major factors that hampers progress in this area is the need for new tools and technologies to measure yield reliably in the setting of a plant molecular biologist, which is typically a small plant population in a greenhouse environment.

CropDesign has developed a high-throughput reverse genetics platform, named TraitMill, to evaluate the effects of single genes on yield. This platform uses rice as a model crop and has a capacity for testing 500-1000 different gene constructs per year in a controlled environment. The yield evaluation set-up makes use of robots for automated plant transport, digital imaging tools for plant evaluation and proprietary image analysis software for data production and statistical analysis of the results. Phenotypic parameters include the most relevant yield components for cereals, such as total seed yield, seed number, seed filling and seed size, number of panicles, flowering time, growth rate, vegetative and root biomass. Greenhouse conditions are adaptable, so that the same parameters can be measured for plants grown under water- or nutrient-deficiency stress.

Over the last years, CropDesign has in this way identified a range of genes that improve one or several of these yield components. Single genes contributing more than 20% yield increase in greenhouse conditions have been found. Several of these effects have been validated in small-scale field trials, with similar increases in yield, showing that many of the identified genes operate quite independently of the environment. Our results demonstrate the potential of single gene approaches to modify complex quantitative traits such as yield. Moreover, these results provide breeders with new tools and alleles for yield improvement programs that can be integrated in crop plants either by genetic engineering or by conventional breeding.

Increasing artemisinin yield in *Artemisia annua* L.

S 041

Session: Improving plant product quantity and quality II: Improving yield

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Artemisia annua L. (Asteraceae) is currently the only source of the antimalarial drug artemisinin. The low yield of artemisinin (<1% of plant dry weight) and increasing demand, for use in Artemisinin Combination Therapies (ACTs), has resulted in an expensive drug with an unstable supply chain. Improving artemisinin yield from *A. annua* would reduce costs and increase availability, making it more accessible to the 300- 500 million individuals who contract malaria, worldwide, every year.

While *A. annua* is currently the only source of artemisinin, little work has been done to improve yield. Artemisinin yield could be improved in 3 ways:

1. by increasing flux into the artemisinin biosynthetic pathway, for example, by reducing carbon flow to competing compounds,
2. by increasing the number of glandular trichomes on the leaves, which are the site of artemisinin synthesis,
3. by increasing the amount of leaf biomass per plant, the tissue from which artemisinin is extracted.

The CNAP Artemisia Research Project is undertaking a combination of strategies aimed at creating high yield varieties of *A. annua* which will be suitable for commercial cultivation. Strategies include developing genetically diverse populations of *A. annua* from which high yielding individuals are being identified (using both reverse and forward genetic approaches), fast-track breeding technologies and a gene discovery programme, which is identifying genes with the potential to impact artemisinin yield. This work is being supported by the collection of biochemical and morphological data which will inform upon the most important factors influencing artemisinin biosynthesis and yield in *A. annua*.

S 042

How to optimally exploit grains for food?

Session: Improving plant product quantity and quality III: Food and feed

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Intake of both cereal dietary fibre and whole grain is increasingly shown to protect against rapidly expanding chronic diseases such as cardiovascular disease and type 2 diabetes. The mechanisms are as yet not known, but the protection is suggested to be due to the concerted action of dietary fibre and various bioactive compounds such as lignans, phenolic acids, alkylresorcinols, phytosterols, folates, tocopherols and tocotrienols, other vitamins and minerals. Being concentrated in the outer layers of the grain, these compounds are often removed in current milling processes, optimised to deliver products made of refined grains.
Foods should be made available containing more grain fibre and outer layers of the grains, addressing also the consumer expectations of palatability and convenience. This demands for new ingredients high in grain phytochemicals and showing good technological properties. The natural diversity in grains offers a good basis for tailored fractionation and bioprocessing.The grain chain from plant breeding and crop selection should take into account the nutritional quality criteria set by the end-use. This is the approach in the EU integrated project HEALTHGRAIN running in 2005-2010.

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Food product innovation taking advantage of plant selection

S 043

Session: Improving plant product quantity and quality III: Food and feed

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To compete in the global market food companies need to continuously propose new products on the shelves. Modern Distribution and Hard Discount have recently reinforced this need since they are competing with branded companies just on cost and using often branded end product-like to attract people. Therefore, innovation is one of the few tools in the hand of the branded companies to compete and hopefully to expand on the market.
The main areas where branded companies are active to deliver innovative end products are: technology/processing, service/packaging, function/usages and raw materials, which represent really a powerful tool of differentiation.
Barilla's approach on a strategic raw material is to know in depth the whole production chain and to understand the critical points to study and to develop research projects. Often, the answer is breeding. Conventional and molecular assisted breeding. Adopting breeding on durum wheat (*Triticum turgidum* var. *durum* Desf.) and on processing tomatoes (*Solanum lycopersicum* L.), Barilla has been able to differentiate some of its products in terms of cost, texture, nutrition and appearance.
Barilla is the pasta worldwide leader and durum wheat semolina is the only raw material used to obtain the end product. Barilla is vertically integrated too because it has durum wheat mills. Tailor made durum wheat varieties (i.e. "Svevo") obtained together with breeding companies (i.e. Produttori Sementi Bologna) are cultivated under cultivation contracts and allow to have top desired quality (texture, mouth feel, consistency) at a better price (cost) than if sourced from elsewhere. "Svevo" was obtained by conventional breeding, but what made the difference were the input and the analytical support given by Barilla to the breeding company during the selection cycle. Barilla followed this approach also in the case of "Aureo" (a new durum variety awaiting registration), but the requests could not be met using only conventional breeding, therefore, a study of a mapping population was undertaken obtaining two results: "Aureo" (a Recombinant Inbred Line meeting Barilla's requirements) and a set of QTLs controlling quality traits and of linked molecular markers, which are being exploited in new cycles of Marker Assisted Selection.
In the sauces business, Barilla uses also a tomato variety (i.e. "Scarpariello") exclusively cultivated in a dedicated production chain and selected for outstanding and taste which withstands during the thermal applications. It allows obtaining innovative and distinctive sauces that have been "branded" with the variety name too. In the future, through breeding, it could be possible to differentiate even more among sauces in terms of taste and why not, colour and nutritional traits, too.
The raw material relevance on final product innovation is so distinctive that "Svevo" and "Scarpariello" stories become the core of the TV advertising campaigns of both pasta and sauces categories.
Modern plant breeding may help food branded companies in the daily fight in the market because the new tools offered by genomics allow a very efficient selection of the traits required for some end product innovations.

S 044

Presentation of the white paper of the EPSO workshop on "The European Feed Value Chain" held in Copenhagen from 26 to 27 June 2007

Session: Improving plant product quantity and quality III: Food and feed

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The global demand for meat, dairy products and eggs is increasing rapidly as relative incomes are rising. The demand is shifting from plant based diets towards livestock derived products. At the same time, in Europe, the environmental concerns related to the large and intense animal production and its environmental impact require new and improved production technologies. Finally, the EU is facing increasing competition on the world markets and rationalizations and cost reductions are essential to defend world market positions. The environmental impact of the large and intense animal husbandry is of increasing importance on national and European policies and regulations. In consequence, strict limitations are put on the use, and loss, of nitrate and phosphorous both directly from the livestock and indirectly from the feed crop production. The effects of global warming on feed and livestock production require increased attention. Agriculture is a major emitter of greenhouse gasses in the form of CO2 as well as methane from ruminants. Besides, climate change will in itself with predictions of raised temperatures, changed patterns in rain fall with dry summers and wet winters, and with more extreme weather conditions have a major effect on feed production. Major challenges to plant breeding will be securing crop yield and stability in situations with unfavorable or even harmful growth conditions. Likewise, extreme rainfall situations increase the likelihood of loss of nutrients from arable land to the aqueous environment. Taken together, the whole value chain from plant breeding, feed crop production, feed formulation and to meat, dairy products and eggs is in a difficult position facing increasing international competition from low cost countries, national and EU restrictions and regulations on environmental impact and animal welfare, and potential changes in the climate.
Significant genetic improvement of the plants is required in order to maintaining high product quality. These traits includes high quality macro and micro nutrients content, factors affecting digestibility, palatability, gut health in relation to feed, mycotoxin and xenobiotic contamination in the feed chain. Water use, nutrient efficiency, pesticide use, greenhouse gas emissions, landscape, energy efficiency of agriculture has be solved as well.
Plant research contribution to solutions thus involves many facet's. Most important is the cereals followed by and forage crops – grasses and grain legumes for which high and stable yields should be maintained. Sustaining crop diversity in European agricultural systems seems an attractive option.

An *Arabidopsis* genetical genomics approach to improve phytonutrient quality in *Brassica* vegetable crops

S 045

Session: Improving plant product quantity and quality III: Food and feed

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Brassica vegetables contain a wide variety of secondary metabolites that contribute in both positive and negative ways to their nutritional qualities. The influence of these compounds on nutritional quality has stimulated interest in breeding new *Brassica* vegetable varieties with improved nutritional profiles. We use *Arabidopsis thaliana* as a model species to study the phytonutrient biosynthesis pathways, to identify new regulatory genes or unknown biosynthesis genes.
Twelve Arabidopsis accessions for which well-genotyped RIL populations are available, were analysed using LC-UV/Vis, LC-QTOF MS and ^{1}H-NMR analysis to identify phytonutrients. Based on the metabolic differences of the parent accessions, we chose a genetically characterised segregating Arabidopsis recombinant inbred line (RIL) population (Landsberg *erecta* x Kashmir). This population was grown hydroponically for four weeks under short days until the rosette-stage. Pooled leaf material of six plants was used for metabolic profiling using both targeted and untargeted approaches. This metabolite survey focussed on the identification and quantification of phytonutrients such as glucosinolates, phenolic compounds (phenyl-propanoids and flavonoids), folate and isoprenoids (carotenoids and tocopherols). In addition to the metabolome analysis, the same leaf samples were used for gene expression analysis using a distant-pair micro-array design. Using both the metabolite and transcriptome data as trait data we subsequently performed a QTL analysis. The results of this analysis, focussing on glucosinolate biosynthesis as well as isoprenoid biosynthesis, will be discussed. All metabolome, transcriptome and QTL information will be combined to predict metabolic networks. In this way, new regulators or biosynthesis genes can found. These genes, identified in Arabidopsis, will then be used to identify the corresponding *Brassica rapa* orthologues and develop molecular markers for breeding purposes.

This work is financially supported by the IOP Genomics project IGE05010.

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S 046

Brachypodium distachyon genomics for bioenergy research

Session: New Products I: Plant based biofuels: how to improve them?

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The small annual grass *Brachypodium distachyon* is a close relative of wheat and other members of the Pooideae subfamily. It's ~300 Mb genome is very small and contains genes that are highly similar to wheat genes and occur in a closely similar chromosomal order. It has a rapid generation time, small stature and undemanding growth requirements that enables 500 plant/m^2 to be grown in simple conditions. It is self fertile, sets abundant seeds and exhibits natural variation in many important features such as flowering time, vernalisation responses, polyploidy and seed size. These features have led to major interest in developing genomics and functional genomes resources in Brachypodium to support research in cereal and grass crops for food and fuel production. The DOE Joint Genome Laboratory is conducting whole genome shotgun sequencing and transcriptome sequencing projects that will be completed by June 2008. A check point assembly of the 4x sequence has already been distributed to users through dedicated databases (www.brachypodium.org and www.modelcrop.org). Two physical maps of BACs support the sequence assemblies, and genetic maps are currently being produced. By the end of 2008 a thoroughly annotated and well- assembled genome sequence will be available.

The availability of high quality annotated genome sequence has raised considerable interest in Brachypodium both as a comparative genomics resource for "bridging" into the largest more complex genomes of closely–related wheat, barley and forage grasses, but also as an experimental system for exploring the biology of environmental adaptation, growth control and disease resistance in temperate grasses. Biological studies in *Brachypodium* can also form a useful "bridge" between the extensive biological research conducted in Arabidopsis and strategic research goals in wheat, barley, forage and bioenergy grass crops, especially projects focussed on grass-specific traits. Consequently there has been an increased demand from the research community for resources to conduct biological research in *Brachypodium*.

In my lecture I will describe recent progress in the *Brachypodium* genome project and illustrate how comparative genomics can aid genomics research in wheat and barley.

Engineering microbial metabolism for production of advanced biofuels

S 047

Session: New Products I: Plant based biofuels: how to improve them?

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Today, carbon-rich fossil fuels, primarily oil, coal and natural gas, provide 85% of the energy consumed in the United States. As world demand increases, oil reserves may become rapidly depleted. Fossil fuel use increases CO_2 emissions and raises the risk of global warming. The high energy content of liquid hydrocarbon fuels makes them the preferred energy source for all modes of transportation. In the US alone, transportation consumes around 13.8 million barrels of oil per day and generates over 0.5 gigatons of carbon per year. This release of greenhouse gases has spurred research into alternative, non-fossil energy sources. Among the options (nuclear, concentrated solar thermal, geothermal, hydroelectric, wind, solar and biomass), only biomass has the potential to provide a high-energy-content transportation fuel. Biomass is a renewable resource that can be converted into carbon-neutral transportation fuels.

Currently, biofuels such as ethanol are produced largely from grains, but there is a large, untapped resource (estimated at more than a billion tons per year) of plant biomass that could be utilized as a renewable, domestic source of liquid fuels. Well-established processes convert the starch content of the grain into sugars that can be fermented to ethanol. The energy efficiency of starch-based biofuels is however not optimal, while plant cell walls (lignocellulose) represents a huge untapped source of energy. Plant-derived biomass contains cellulose, which is more difficult to convert to sugars, hemicellulose, which contains a diversity of carbohydrates that have to be efficiently degraded by microorganisms to fuels, and lignin, which is recalcitrant to degradation and prevents cost-effective fermentation. The development of cost-effective and energy-efficient processes to transform lignocellulosic biomass into fuels is hampered by significant roadblocks, including the lack of specifically developed energy crops, the difficulty in separating biomass components, low activity of enzymes used to deconstruct biomass, and the inhibitory effect of fuels and processing byproducts on organisms responsible for producing fuels from biomass monomers.

We are engineering the metabolism of platform hosts (*Escherichia coli* and *Saccharomyces cerevisiae*) for production of advanced biofuels. Unlike ethanol, these biofuels will have the full fuel value of petroleum-based biofuels, will be transportable using existing infrastructure, and can be used in existing automobiles and airplanes. These biofuels will be produced from natural biosynthetic pathways that exist in plants and a variety of microorganisms. Large-scale production of these fuels will reduce our dependence on petroleum and reduce the amount of carbon dioxide released into the atmosphere, while allowing us to take advantage of our current transportation infrastructure.

S 048

Second generation bioethanol production from lignocellulosic material

Session: New Products I: Plant based biofuels: how to improve them?

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Residues from agriculture and forestry are suitable raw materials for production of biofuels. In contrast to the use of corn and grain this biofuels will be sustainable and possess a high degree of CO2 reduction when used as transport fuel. In the presentation I will describe a special biorefinery concept: "The Maxifuels concept" whereby the biomass raw materials will end as different energy products in the form of biofuels, a solid fuel, hydrogen and methane. By optimizing the outcome of the raw material it is possible to ensure high energy efficiency and a high CO2 reduction of the produced fuels. The concept has been tested for two years in pilot phase and the results show promises for a future production scheme of second generation biofuels for a price lower than gasoline today. Results from pilot testing will be presented.
The Maxifuels concept is now being commercialized through the spin-off company BioGasol. BioGasol is currently building a demonstration project on the island of Bornholm and has just initiated the work on a DOE funded demonstration project in the state of Oregon, USA together with the US partner Pacific Ethanol.

Ref.
BirgitteK. Ahring & Niels Langvad. Sustainable low cost production of lignocellulosic bioethanol- "The carbon slaughterhouse". 2008. International Sugar Journal. Pp. 184-191.

Effect of early plant development and genotypic variation in frost tolerance for 3 species of *Miscanthus*

S 049

Session: New Products I: Plant based biofuels: how to improve them?

In view of its high yield potential under low input demands, the perennial rhizomatous C_4 grass *Miscanthus* seems a good candidate for biomass production in Europe as a potential source of agro-energy. However, it can be susceptible to frost, in particular during the establishment of the crop. To evaluate the genotype variability for frost tolerance according to plant development at early stages, three species of miscanthus (*M. x giganteus*, *M. sinensis*, and *M. sacchariflorus*) were studied. They were tested at three development stages (3, 5 and 7 visible leaves) under controlled conditions mimicing those found in Northern France during spring time, with temperatures up to -8°C repeated for two successive days. Plants produced by rhizomes were cold acclimated during 8 days at 12°C before frost exposure, whereas control plants were not. Tolerance was scored with respect to damage to the plants, and was noted from 0 (low) to 3 (high). The first results for *M. x giganteus* showed a correlation of 0.62 between frost tolerance and leave stages; 77% of "3 leaves-plants" were able to stand the frost exposure, against only 33% for "5 leaves-plants", and 4% for "7 leaves-plants". New experiments are on the way to validate these results and to determine the response of the other miscanthus species at the same stages of crop establishment and under the same temperature regimes.

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S 050

New materials from new plants

Session: New Products II: Biomaterials, biopharmaceuticals and other new products

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Plant biotechnology as a commercial process is a reality. During 1996-2007, the global GM crop area has grown for 12 consecutive years and has reached 81 million hectares. Such numbers undoubtedly reflect benefits enjoyed by the various participants in the business, including 10 or so million farmers. However, all GM crops grown at present were modified to facilitate crop production, thus, they do not benefit the consumers. Promises to create engineered plant hosts-producers of novel materials, medicines and improved foods made by plant biotechnologists did not materialize so far. It is safe to predict that all this and more will be 'delivered' during the 21st Century, but the timing will depend on our ability to develop both the sound science leading to new products as well as the new engineering processes that satisfy the requirements of an exploiter (technical efficiency, compliance with business requirements, compatibility with existing or predicted infrastructure), a government regulator (regulatory compliance, safety, sustainability), and an end user. The products most likely to reach the market in near future are high-value proteins such as biopharmaceuticals. Several injectable biopharmaceuticals including plant-made 'biosimilar' glucocerebrosidase, interferon alpha, insulin have reached clinical trials, many more are nearing that stage. Several materials such as industrial enzymes and immunoadsorbents are in advanced testing phases and at least one has reached the market. The purpose of the presentation is to review the rapid progress in this exciting area of plant biotechnology.

Biomaterials, synthesis of the biopolymer cyanophycin in tobacco and potato

S 051

Session: New Products II: Biomaterials, biopharmaceuticals and other new products

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The production of biodegradable polymers that substitute petrochemical compounds in commercial products, in transgenic plants is an important challenge for plant biotechnology. The polymer Polyaspartate is used to substitute polycarboxylates. It can be isolated from the bacterial storage protein cyanophycin, composed of L-Aspartat and L-Arginin. Cyanophycin is produced via non-ribosomal protein biosynthesis by a cyanophycin synthetase. Potato tubers are particularly suitable for the production of biopolymers since they allow a cost effective manufacture as a by product of starch. To produce cyanophycin in plants, three different Cyanophycin Synthetase genes (Berg et al. 2000) were expressed constitutively in tobacco and potato plants. Only one of the three synthetases produced cyanophycin in plants with up to 0.1 % polymer in dry weight (dw). Granula containing cyanophycin were detected by electron microscopy in different transgenic lines in leaves and for potato also in tubers. Unfortunately the transgenic tobacco and potato lines exhibited different stress symptoms like reduced growth, variegated leaves and early flower induction due to the production of the polymer (Neumann et al. 2005). In order to increase polymer synthesis, the functional cyanophycin synthetase gene was fused to different transit peptide sequences for import into chloroplasts. In transgenic tobacco and potato lines cyanophycin content increased up to 3 % in dw. Additionally, these plants did not exhibit any phenotypic damage but a slightly thicker cell wall (Hühns et al 2008). The reduction of polymer synthesis to potato tubers results in very small tubers with a polymer content up to 2 % in dw. Up to now, the highest cyanophycin content was observed by a tuber specific production in plastids without phenotypical changes.

S 052

Controlling the quality of biopharmaceuticals in plants

Session: New Products II: Biomaterials, biopharmaceuticals and other new products

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Plants are considered promising factories for the production of recombinant therapeutic proteins. PhaseIII clinical trials with plant produced proteins are currently in progress. Their full potential is however limited by the fact that plants and mammals differ with respect to the formation of complex N-glycans on glycoproteins. In a previous study, we showed that expression of a human β(1,4)-galactosyltransferase (GalT) in tobacco resulted in the introduction of terminal galactose residues but that these N-glycans remain unaltered with respect to the presence of β(1,2)-xylose residues and α(1,3)-fucose residue linked to the glycan core. The latter epitopes are normally not found in mammals and are potentially immunogenic.
We designed an innovative strategy to prevent the incorporation of these potentially immunogenic epitopes. This strategy is based on the observation that the identity of N-glycans is not only determined by the mere presence of enzymes involved in their biosynthesis, but also by the order in which these enzymes act on the N-glycan substrates. Ordering is largely determined by the sequential positioning of the enzymes along the secretory pathway. This notion offers the possibility to steer the N-linked glycosylation by controlling the localization of the enzymes in the secretory pathway. The so-called CTS anchors of type II membrane bound glycosyltransferases play a central role in their sub-Golgi distribution. As an example of this type of pathway engineering we exchanged the CTS region of human GalT by that of a plant xylosyltransferase (XylT). Expression of the hybrid galactosyltransferase in tobacco resulted not only in galactosylation of N-glycans but simultaneously in a dramatic decrease of xylose and fucose epitopes on plant glycoproteins as well as on N-glycans on a recombinant antibody. A radioallergosorbent inhibition assay with proteins purified from leaves of the transgenic tobacco plants using sera from allergic patients suggested a significant reduction of potential immunogenicity.
A further characteristic of N-glycan biosynthesis is that often enzymatic reactions do not go to completion. This may result in complex mixtures of glycoforms of even a single protein. This is undesirable when product homogeneity and consistency is important. By interfering in an early step of N-glycan biosynthesis in the ER, we were able to by-pass several subsequent enzymatic reactions thus preventing the accumulation of high mannose intermediates and resulting in a more homogeneous N-glycan profile. These data show that knowledge on biochemistry as well as cell biology of N-glycan biosynthesis in plants facilitates the control over N-linked glycosylation enabling the production of therapeutic quality antibodies in plants.

High efficient synthesis in chloroplasts of a protein antibiotic active against human pathogenic bacteria

S 053

Session: New Products II: Biomaterials, biopharmaceuticals and other new products

There is a pressing need to develop new and inexpensive antibiotics to keep pace with emerging bacterial resistances. Here we report extreme overexpression of a proteinaceous antibiotic against pathogenic streptococci from the plant's plastid (chloroplast) genome. The antibiotic, a phage lytic protein, accumulated to enormously high levels (>70% of the plant's total soluble protein), proved to be extremely stable and efficiently killed the target bacteria within minutes. These unrivaled expression levels, together with the chloroplast's insensitivity to enzymes degrading bacterial cell walls and the eliminated need to remove bacterial endotoxins by costly purification procedures establish an effective production platform for next-generation antibiotics.

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Session: Preserving our future by reducing the inputs in agriculture
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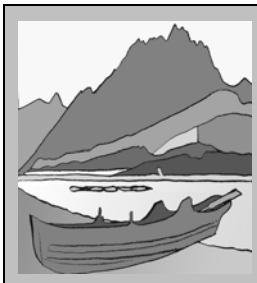

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Poster abstracts

ProfiCrops – our commitment to crop production in Switzerland

P 001

Session: Plant Science in Europe – Science Policy

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The three Agroscope research stations, Changins-Wädenswil ACW, Liebefeld-Posieux ALP and Reckenholz-Tänikon ART, are jointly carrying out three multidisciplinary research programs during the period 2008-2011 promoting interdisciplinary cooperation between Agroscope and external partners: ProfiCrops (a future for crop production), NutriScope (healthy nutrition) and AgriMontana (production systems in mountainous areas). Focused synergies and communication efforts are intended to create added value for Agroscope, its co-workers and the stakeholders. Switzerland's agriculture has assumed a pioneering role for years which deserves to be adequately represented in the media.

ProfiCrops is aiming at acquiring, providing, assessing and transferring knowledge in order to safeguard the future for Swiss crop production in a largely liberalized market, and to enhance consumers' trust in local products. These goals are supposed to be achieved by:

• innovations within the production chain on the breeding, cultivating, grafting and disposing level, e.g. with food and non-food crops as well as niche products, novel products of high additional value, new technologies (precision farming), waste management and resources efficiency;
• enhancing the awareness of customers and the non-agricultural part of society as to the importance of Swiss crop production with the aim of promoting local products and the appreciation for multifunctional services;
• creating the basics for optimized economic structures (on operational and inter-operational levels) and labour planning;
• analyzing and demonstrating economic and technical requirements for crop production, and issuing corresponding recommendations as to optimizing current potentials;
• hands-on examinations to determine the prerequisites for selecting suitable crop species and cultivation sites in relation to the corresponding requirements and foreseeable developments.

ProfiCrops takes a positive stance in that we are committed to agricultural activities and crop production in Switzerland; to achieve that goal we need professional, interdisciplinary and networked research projects. Agroscope acts as a hub and constitutes the critical mass for such research work. By launching horizontal programs like ProfiCrops Agroscope provides «interfaces» for network partners – a truly innovative approach of applied research in Switzerland.

More than 130 research projects have been announced to contribute to the mentioned goals of ProfiCrops. In parallel mega projects strongly involving stakeholders and providing a transdisciplinary approach are being developed for the main crop types. The contribution of the crop variety towards a future-oriented high quality Swiss agriculture or strategies to drastically reduce production costs in arable crops are being investigated with the intense involvement of the stakeholders and research partners. In fruit crops a higher awareness and the strategic positioning of our fire blight research competence centre in Switzerland and internationally are aimed. In viticulture, as a last example, development of disease resistant cultivars and its significance will be promoted at the stakeholders and consumers levels.

More information is available under www.proficrops.ch

P 002

System-thinking essence in decision-making procedures. A conceptual approach integrating weed population dynamics and possible economical outputs

Session: Plant Science in Europe – Science Policy

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The agri-food sector is facing global challenges that cannot be met without support of an integrated decision-making system. In all areas of science, especially in agriculture nowadays, integration across disciplines is an important source of ideas, leading to new avenues for theoretical and empirical investigations, an approach that can be especially useful in the management of sustainable food production, complex requirements on quality assurance, reliability and flexibility in the provision of food, sustainability in people's trust, control on environmental effects, market and trade organization and so many others. By developing common conceptual perspectives for different management problems it is possible to detect previously unobserved patterns, to understand the processes shaping these patterns more clearly and to use them as a broad basis for decision-making between professionals of various disciplines. In this paper we present a conceptual model by applying a system dynamics approach through an appropriate software tool (STELLA®) particularly designed for this kind of modelling. More specifically, using basic building blocks, a hierarchical flow chart is constructed, which incorporates biological procedures with biological weed control managerial decisions and possible economic outputs. A simple weed population model is developed taking into account seedling emergence, seedling population and recruitments that contribute to the final weed population size. Possible loses due to intrinsic or extrinsic causes are considered as well. The application of a biological agent for the control of weeds extends the level of population loses resulting in reduced inputs (e.g. conventional weed control methods). This in turn increases crop's output and consequently the final price and income. Investments and capital rate can be further improved not only because increased income but also of hiring rate. Our purpose is to highlight the capabilities of a system thinking essence hence facilitating the communication and eliminating most of information gap between various disciplines needed for decision-making procedures.

Investigating meiotic recombination in rice - examining *OsDMC1A* and *OsDMC1B* through phenotypic, genotypic, cytological and complementation analysis

P 003

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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Meiotic recombination is a fundamental aspect of sexual reproduction plant evolution and produces the novel allele combinations which are the basis for breeding selection in crop plants. Understanding the basis of homologous recombination could also lead to gene targeting methodologies in crop plants which could revolutionise plant science and crop genetic modification.

OsDMC1 is the rice homologue of the yeast *DMC1* gene that plays a role in combination with the *RAD51* gene, binding ssDNA to form a nucleoprotein filament in the homology search and strand invasion at early meiosis. *OsDMC1* is duplicated in rice (*OsDMC1A* and *OsDMC1B*). We have investigated the functions of these orthologues by using retrotransposon insertion mutagenesis lines (TOS17) and through cloning and over-expression of these genes in *Arabidopsis thaliana*, both wild-type and *at*dmc1 mutants.

Two lines, NF6843 (TOS17; intron 5 insertion) and NF8016 (TOS 17; exon 10 insertion) of *OsDMC1A* and one line, NE1040 (TOS17; exon 12 insertion) of *OsDMC1B* were studied over four generations by a number of approaches. The single mutants of *osdmc1a* and *osdmc1b* showed retardation of the root at seedling stage but grew normally during vegetative stages and during panicle development. The pollen viability in both single mutants was reduced (51.3% and 54.3% in *osdmc1a* and *osdmc1b*, respectively). These produce seed set of 16.2% and 23.1%, respectively, compared with wild-type segregants which typically showed >85% seed set. These single mutants differ from the *osdmc1* double mutant generated using RNAi which exhibits almost completely sterile and produced less than 5% seed set (Deng and Wang, 2007). Further cytological observations of male meiocytes revealed the single mutant of *osdmc1a* and *osdmc1b* led to defects in bivalent formation at Prophase I and subsequent unequal chromosome segregation and irregular spore generation producing 18.6% triads and 7.9% polyads (n=125). However, the single mutants still produced 73.5% normal tetrads. *OsDMC1A* and *OsDMC1B* expression were analyzed by semi-quantitative RT-PCR. *OsDMC1A* was highly expressed in leaf and flower at R2-R6 stages but showed low expression in root. On the other hand, *OsDMC1B* was expressed in root, leaf, and flower at R2-R6 stages but not at high levels. These results differ from Ding *et al.* (2001) in that *OsDMC1* was expressed at low levels in root and undetectable levels in leaf. However, the single mutants, *OsDMC1A* and *OsDMC1B* were not expressed in every tissue. These results suggest that TOS17 insertion in the exon causes complete disruption of the *OsDMC1A* and *OsDMC1B* translation. In this study, our data demonstrate that two copies of *OsDMC1* are essential for normal rice meiosis and play an important role in homologous pairing. However, the single mutant of *osdmc1a* and *osdmc1b* decrease the efficiency of chromosome pairing, without abolishing it.

In on-going work, the *OsDMC1A* and *OsDMC1B* have been over-expressed in wild-type *Arabidopsis thaliana* and are currently being tested for their ability to complement the *atdmc1* T-DNA knock-out line. Lines over-expressing *Os*DMC1A and *Os*DMC1B will be crossed to a tester line for genetic recombination developed by Greg Copenhaver, to test for effects on genetic distances in pollen in a *quartet* background.

P 004

Forward & Reverse genetic approaches for elucidating the role of Meiotic Recombination gene homoeologues in hexaploid wheat

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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Meiotic Recombination is a fundamental process occurring in all sexually reproducing organisms. Meiotic Recombination (MR) fulfils a critical biological function ensuring correct chromosome disjunction and is responsible for generating new combinations of gene alleles. An understanding of the genes involved in MR has potential applications, such as allowing manipulation of the level of genetic recombination in plant breeding programs and facilitating introgression from wild relatives. Many of the genes involved in MR are well conserved among eukaryotes. Meiotic recombination in eukaryotic cells mainly requires two orthologues of *E. Coli* RecA proteins RAD51 and DMC1. But the role of these genes and gene homoeologues in MR in hexaploid wheat (*Triticum aestivum* L.) is not well known. Here we describe forward and reverse genetic approaches used for elucidating the role of *TaRAD51 & TaDMC1* genes and their genome-specific homoeologues in MR. In diploid species, deletion of either DMC1 or RAD51 orthologues usually leads to sterility. Wheat, as a polyploidy, offers a unique opportunity to examine the effects of the deletion of specific homoeologues, while maintaining a level of fertility. We wish to use this property to examine events in wheat meiosis further.

We have isolated the full length coding sequences for *TaRAD51 & TaDMC1* homoeologues using consensus primers based on *OsRAD51 & OsDMC1* cDNA sequences. Genome-specific primer sets were developed for *TaRAD51 & TaDMC1* based on intronic sequence differences between the three genomes and their specificity confirmed through Nulli-tetrasomic analysis. A Gamma radiation mutant population of a spring wheat (Paragon) was used for reverse genetics purposes. Initial screening of 200 Paragon deletion lines with *RAD51* genome-specific primers identified 1 deletion line for *RAD51* A genome, 3 deletion lines for *RAD51* B genome and 1 deletion line for *RAD51* D genome and screening with *DMC*1 genome specific primers identified 1 deletion line for *DMC1* A genome and 1 deletion line for *DMC1* D genome. No deletion lines have so far been detected for the *DMC1* B genome. Screening for the rest of 300 paragon mutant lines is in progress.

Phenotypic and Cytogenetic characterization of paragon deletion lines of *DMC1 & RAD51* homoeologues in the field will be carried out this year. Also *TaRAD51 & TaDMC1* genome specific primer sets based on exonic sequences are being developed for screening both mutant and wild type Paragon through qPCR, to evaluate the level of expression of different homoeologues and explore whether there are compensatory changes in gene expression in the Paragon genome-specific deletion lines.

High levels of coding sequence conservation and the identification of deletions for 5 of the 6 gene orthologues/homoeologues argues for extensive redundancy of function in these genes in wheat. The current results will be discussed in relation to published results from other crop species.

Identification and analysis of SNPs on a large scale using high-throughput sequencing in maize

P 005

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

New sequencing technologies produce now up to 1 billion bases per run. The main difficulty is that with the produced short reads ranging between 25 and 40 bases direct de novo sequencing is difficult. We have used reference sequences generated for genic fragments for the identification of SNPs in pools and individual lines in maize. In experiments, we have amplified fragments with an average length of 560 bases from 4000 maize genes and sequenced these fragments using the Illumina/Solexa platform. Based on the reference sequences, it was possible to assign many of the reads back to the individual reference sequences. Methods were developed which permit the identification of SNPs in individual lines and for allele frequency estimations in pools of lines. The results were validated using available Sanger sequencing data. The results demonstrate that it is possible to simultaneously analyze a large proportion of the genes for the presence of SNPs and in the long term establish a genotyping by sequencing procedure for maize.

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P 006

Towards the characterization of a distant cis-acting enhancer element associated with flowering time in maize

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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Flowering time is a fundamental trait for the adaptation of maize to different latitudes and altitudes. The Vegetative to generative transition1 (*Vgt1*) QTL controls most of the variability for flowering time in a population derived from the cross of two nearly isogenic lines (Salvi et al., 2002, Plant Mol Biol 48:601-613). Through positional cloning and association mapping carried out on a set of ca. 100 inbred lines representative of the cultivated temperate germplasm, the QTL has been resolved to an approximately 2-kb non-coding region positioned 70 kb upstream of an Ap2-like transcription factor (*ZmRap2*.7) that has been shown to control flowering-time (Salvi et al., 2007, PNAS 104:11376-11381). *Vgt1* functions as a cis-acting, long-distance enhancer as suggested by the correlation of the effects of the *Vgt1* alleles with the transcription levels of *ZmRap2*.7. One of the hypotheses that we are currently testing is that *Vgt1* might function by modifying *ZmRap2*.7 chromatin through an epigenetic mechanism. To investigate this possibility, the methylation state of both *Vgt1* and *ZmRap2* will be monitored. Additionally, by comparing the maize-rice genomes within the *Vgt1* region, we identified conserved non-coding sequences (CNSs) despite an evolutionary distance of about 50 million years between the two species. Interestingly, the two parental lines have an indel polymorphism caused by a 144-MITE element within one CNS. These results support the notion that modifications in distant cis-acting regulatory regions are a crucial component for the regulation of quantitative traits of pivotal importance for the evolution and breeding of maize.

How to deal with landrace-based crops? Estimating 'genetic breadth' for crop improvement -an example from an indigenous African legume

P 007

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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The genus *Vigna* (Family: Leguminosae, subfamily Papilionoideae) comprises of around 80 species originating from different regions of Africa, America and Asia. It includes several agriculturally important species like *V. unguiculata* (Cowpea), *V. subterranea* (bambara groundnut), *V. radiata* (mungbean), V. mungo (blackgram), *V. aconitifolia* (mothbean) and *V. umbellata* (rice bean) among others. The most important African grain legume species are *V. unguiculata* (Cowpea) and *V. subterranea* (bambara groundnut).

Bambara groundnut has a diploid genome (2n=22) with an estimated C-value of 0.90 pg. It is a self-pollinating, herbaceous annual plant. It consists of two botanical forms: var. *spontanea*, comprising the wild forms, encountered in a limited area from Nigeria to Sudan; and var. *subterranea* comprising the cultivated forms found in many parts of the tropics particularly sub-Saharan Africa.

The crop has the ability to tolerate a wide range of agroecological conditions and it is popular among resource-poor farmers. It is also valued for its drought tolerance and resistance to pests and diseases. The crop therefore has the potential to play a crucial role in alleviating poverty and hunger, and thereby enhancing food security in sub-Saharan Africa.

Despite bambara groundnut being one of the most important African legume crop, it has no established varieties. Marginal and subsistence farmers grow locally adapted landraces, which are genetically diverse populations selected under low-input agriculture. The aim of this research is to exploit the potential of microsatellite markers in understanding bambara groundnut genetics and breeding. Developing a rapid method to estimate the 'genetic breadth' of a landrace is important for breeding and for physiological assessment. We are developing a rapid microsatellite method based on bulked individual plant samples of landraces to estimate this parameter for modelling and for G x E estimates. Our results to date are presented.

P 008

Insights into a giant genome: physical mapping of chromosomes 3S in wheat and barley

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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Sequencing of crop genomes is the ultimate goal of current plant genomic projects. However the sequencing of a giant and repetitive genome such as wheat with its 1.7 Gb is like facing Goliath with a sling. The construction of a physical map is a preliminary step and gives information of the genome structure. To date, the wheat genome has been studied by analysing single or small clusters of BACs covering a few hundred kb or through genetic analysis at the whole genome scale. Neither of these two strategies has allowed us to build a picture of the physical structure along a chromosome; the first strategy focused on specific loci while the second was too broad to be accurate. We now have huge variation in the physical/genetic relationship ranging from 100kb/cM to 10Mb/cM. By focusing on chromosomes 3 and 7, we investigated the relationship between genetic and physical location of genes in the polyploid genome of wheat. In association with the European Project FP7 TriticeaeGenome, we will generate fine maps of wheat and barley chromosomes 3 and 7.

We made a first physical map of chromosome 3S by using the BAC library of *Aegilops tauschii*, the ancestor of wheat D genome. The target region is the telomeric 20 cM of the short arm of Group 3 chromosomes, delineated by an X-ray induced deletion mutation called *ph2a* and estimated to be around 80 Mb in size. We anchored about 90 EST on the contigs by using information from the physical map of 3BS, the wheat deletion bin map, the barley genetic map and sequences of the *Brachypodium* genome. The BAC libraries of barley H genome and of the wheat 3DS chromosome will also be screened. The contig assembly will allow us to compare the organization of the genes along the chromosome 3S in the H, B and D genomes.

To tie the physical map to a high resolution genetic map of wheat, we developed three large populations, consisting of 300 doubled haploid lines plus 3,000 single seed decent lines (F5) which will give a resolution of less than 0.01 cM. We also investigated a new method of SNP detection based on sequencing and successfully identified SNP in the anchored EST and in BAC-end sequences of chromosome 3S.

Characterization of WRKY transcription factors in barley (*Hordeum vulgare*)

P 009

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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WRKY proteins constitute a family of zinc-finger transcription factors that are characterized by a conserved ~60 amino acids spanning DNA-binding domain, the WRKY-domain. Based on structural features, WRKY proteins can be divided into three major groups and subgroups. Phylogenetic analysis revealed a monophyletic origin from basal eukaryotes and enormous radiation events in higher plants, which might account for their enrolment in adaptation to biotic and abiotic stresses. Yet, there have been 72 and 81 WRKY genes identified for the model plant species *Arabidopsis thaliana* and *Oryza sativa*, respectively. For the cereal crop barley, three WRKY proteins have been described so far. Hence, we have to assume that the majority of barley WRKY proteins remained uncharacterized until now. Using the publicly available sequence information, we identified a minimum number of 45 barley WRKY (HvWRKY) proteins.
Comparative phylogenetic analysis of HvWRKYs and WRKY proteins from Arabidopsis and rice identified clusters of orthologous and paralogous WRKY proteins for all three major groups. Strict clusters of only rice and barley WRKY proteins indicate a monocot-specific radiation for some of the subgroups. We used publicly available microarray datasets to monitor gene expression for the 20 barley WRKY genes. Based on this data we conclude HvWRKYs being involved in both, plant development and response to biotic stresses. To gain further insights in the function of barley WRKY genes, we are currently analyzing the expression of a subset of genes in caryopses on sub-organ level.

P 010

Application of cytoplasmic and nuclear DNA based marker systems for elucidation of phylogenetic relationship of Musa acuminata and M. balbisiana

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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Musa (Musaceae) is one of the most important staple crops widely cultivated in tropics and subtropics. The present day edible bananas originate mostly from the diploid species (2n= 22) Musa acuminata and M. balbisiana. The diploid or polyploid cultivated banana varieties mostly are sterile intra- or inter-specific hybrids of these two species and have been fixed through hundreds of years of human selection. Therefore, knowledge on the putative fertile ancestors would be beneficial for breeding programs. In the present work cytoplasmic (chloroplast and mitochondria) as well as nuclear genome based (rDNA) marker systems were applied for the identification of putative ancestor gene-pools of banana in a model study based on a mini core collection of 52 genotypes including ten M. acuminata and eight M. balbisiana wild types along with ten AAA, ten AAB, eight ABB triploid hybrids. The collection contained three AA, two AB diploid and a single tetraploid cultivar as well.
Both cytoplasmic and nuclear marker systems revealed nearly identical grouping of the wild type M. acuminata subspecies analysed. The data revealed three main groups formed by ssp. burmannica, burmannicoides, siamea and ssp. banksii, errans and ssp. zebrina. However the affiliation of ssp. malaccensis and ssp. microcarpa is still ambiguous. Based on these results the identification of putative ancestor gene pools contributing to the formation of the hybrid cultivars was attempted with special focus on the Cavendish sorts.

Excess heterozygosity and scarce genetic differentiation in the populations of *Phoenix dactylifera* L.: Human impact or ecological determinants

P 011

Session: Understanding, preserving and using plant diversity I: Genome structure and evolution

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Although extensive research has been conducted on the characterization of thousands of date palm (*Phoenix dactylifera* L.) cultivars worldwide, the population genetics of date palms has never been studied. In this study, we collected 200 individuals from 19 populations from different geographic locations in Sudan. The collection sites grouped according to the type of dates (date palm fruits) that dominates in the area. Ten microsatellite markers were used to investigate the genetic diversity within and among populations, and the correlation between the genetic and geographic distances. The tested microsatellite markers showed a high level of polymorphism. A total of 261 alleles were detected at the ten loci. The overall mean value of fixation indeces equalled -0.163, which shows the present of excess heterozygosity. However, the chi-square tests conducted for every locus in each population indicated no significant deviation from the Hardy-Weinberg equilibrium. The AMOVA analysis indicated that about 95% of the total genetic variation existed within populations, while significant differentiation within the type groups could be detected. Although significant isolation by distance ($r^2 = 0.552$, $p < 0.022$) was detected by a Mantel test, it seems that the spatial effect has become complicated as a result from the exchange and introduction of different kinds of plant material by date palm growers and traders as well as seed dispersal. This complexity was clearly apparent in the weak clustering relationships among most of the tested populations.

P 012

The Arabidopsis ROF1 (FKBP 62) is essential for acquired thermotolerance by affecting the level of small heat stress proteins

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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The heat- stress response is the adaptation of organisms to heat, regulated by heat- stress transcription factors (Hsfs). Plants adapt to variation in temperatures by a mechanism named acquired thermotolerance by which exposure to non lethal temperature provides the ability to cope with higher temperatures that will follow. An essential component of acquired thermotolerance is the induction and synthesis of chaperones and heat stress proteins. The regime following the initial exposure to mild heat stress plays a major role in discovering factors involved in acquired thermotolerance. By prolongation of the recovery times after original exposure treatment, new phenotypes were discovered for the *rof1* knockout and *rof1* over expressor plants. *rof1* knockout plants collapsed after exposure to 45C if a delay of 24h occurred after the initial exposure to 37C, whereas the transgenic plants over expressing ROF1 were highly resistant to exposure to 45C. These observations were followed by finding a decrease in the expression level of the sHsps ,Hsp17.6-CII, 18.1-CI, 25.3-P and Hsa32. The level of these Hsps is also very low in the *HsfA2* knockout mutants. HsfA2 is a major transcription factor shown to participate in their transcription .HsfA2 interacts with HSP90.1 in the plant nucleus as demonstrated by the BiFC method. HSP90 is a major player in the heat stress response and has many cellular partners. HSP90.1 was shown to interact with the chaperone ROF1 in the cytoplasm and heat stress causes translocation of the complex to the nucleus. Similarly, addition of HsfA2 to the ROF1-HSP90.1 induces translocation of the ROF1-HSP90.1 to the nucleus and complex appears in the nuclei in addition to the cytosplasm. We propose a model which integrates the ROF1 in the long term acquired thermotolerance. Under normal growth conditions the ROF1-HSP90.1 complex is present in the cytoplasm. After heat stress HsfA2, HSP90.1 and ROF1 are induced and a complex ROF1-HSP90-HsfA2 is formed .The complex appears in the nucleus apparently carried by HsfA2. Members of this complex or the whole complex regulate the transcription and/or stability of sHsps which are essential for direct coping with high temperatures .The sHsps disappear in the cells after 24 hours and in the absence of ROF1 or HsfA2 they are not detected. We propose that the absence of the sHsps is the casual factor of collapse of plants exposed to 45C revealed in the phenotypes of the ROF1 and HsfA2 knockout mutants.

The genomics of germplasm exploitation

P 013

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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Consumers of whole foods, such as fruit, demand consistent high quality and the development of new varieties with enhanced health, convenience, novel taste, and reduced impact on the environment. The domestication of temperate fruit crops such as the apple and kiwifruit, a focus at HortResearch, exploits both existing cultivars and the extensive germplasm collections of related species and novel accessions.

Our genomics research is focused on the key producer and consumer traits. We achieve this by defining the biology of our key fruit traits and developing an understanding of the processes in model plants. Our translation genomics research then transfers this molecular information to our target crops. To do this we have developed extensive fruit EST sequence database and are in the process, through collaboration, of developing Whole Genome Sequence for these crops.

In our work on fruit colour, we have described both the metabolic and regulatory genes involved in anthocyanin accumulation. In addition we have analysed novel germplasm of apple with red-flesh and shown that this is due to the ectopic expression of a MYB regulatory gene. A simple rearrangement in the promoter DNA is sufficient to account for this desirable phenotype and we will discuss the molecular mechanism responsible for this altered phenotype and how this information is being used to develop novel red-fleshed cultivars that retain the flavour, texture and long-term storage of cultivated apples.

This, along with our work on carotenoids, chlorophyll, flavonols and vitamin C, provide compelling evidence that genomic research on temperate fruit can accelerate the development of novel cultivars with improved quality and consumer appeal.

P 014

Twenty two years since Chernobyl disaster: What seed proteome can tell us?

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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The explosion of one of the four reactors of Chernobyl nuclear power plant (CNPP) on 26 of April 1986 caused the worst environmental nuclear disaster in the history. A total amount of about 12.5 EBq ($12.5*10^{18}$ Bq) radioactivity was released not only to the close surroundings of the power plant but also to large parts of Europe. In the present time, the Chernobyl contaminated area represents a unique area for radioecological and radiobiological research difficult to perform elsewhere. Despite the fact that since 1986 radiation levels in the affected environment have declined several hundred folds, dangerous long-living isotopes such as ^{137}Cs and ^{90}Sr remains as main contaminants. Now, 22 years after the accident, the question how plants in contaminated Chernobyl were able to adapt is still open, and needs to be fully answered. Plants are stationary and thus must adapt to extreme conditions in order to survive. The main objective of our research is to characterize quantitative differences on protein levels between soybeans (*Glycine max*) grown in contaminated (~5 km from CNPP) and control (~100 km from CNPP) experimental fields in order to elucidate molecular mechanisms plants used for adaptation. To acquire complex proteome information about expressed proteins in the seeds grown in Chernobyl condition, the total protein was quantitatively analyzed using two-dimensional gel electrophoresis (2-DE) using wide (pH3-10) and narrow 2-DE (pH4-7) IPG strips. In total 84 2-DE spots were found to be differentially expressed between contaminated and control seeds. These spots were excised from the 2-DE gels and analyzed by liquid chromatography tandem mass spectrometry for the protein identification.
The project has received the funding from FP7 of the European Union (MIRG-CT-2007-200165). This abstract reflects only the author's views and the Community is not liable for any use that might be made of information contained herein.

Life in the margins – a multidisciplinary approach to understanding the mechanisms that allow Restharrow to colonise saline beaches

P 015

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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The term 'Restharrow' was originally used to describe *Ononis* species, as they arrested the progress of the harrow before mechanisation of ploughing. Restharrow is a common weed throughout Europe, colonising calcareous clay soils and wastelands. Some subspecies *O. repens subsp. Maritima* and *O. reclinata* have developed the remarkable ability to colonise sand dunes, shingle beaches and cliff tops. A low growth habit and a long tap root system are thought to allow the plants to survive in exposed habitats with high salinity and variable water availability. Restharrows contribute towards habitat sustainability, aiding the formation of sand dunes and inhibiting erosion along riverbanks, cliff tops and shingle beaches. Restharrow (not a hyper-accumulator), tolerates heavy metals, frequently being found on contaminated waste lands. Onocerin is a secondary metabolite which contributes up to 0.5% (dry weight) in Restharrow roots. The ecological function of Onocerin is poorly understood, with suggestions that it has waterproofing properties, potentially inhibiting the flow of sodium chloride ions into root cells, or preventing desiccation in arid environments. The occurrence of Onocerin across such a diverse range of plant groups, raises questions regarding the evolutionary history and function. Onocerin has been found in groups of angiosperms, pteridophytes and club mosses which have an association with water. That Onocerin has arisen a number of times in distantly related taxa argues for a relatively simple mutation from non-producing antecedents. With the increasing evidence of climate change and the expected increase in world population in the coming decades, adaptive mechanisms which permit crop survival and growth on marginal or saline soils are an important area of future research.

A multidisciplinary approach is being used to investigate the biosynthesis and ecological function of Onocerin. Genome mining in model crops, comparative genetics and molecular genetics, have been used to follow the expression of phytosterol precursors; squalene synthase, squalene epoxidase and β-amyrin synthase. Metabolomic (GC/MS) and biochemical techniques such as cell free systems, have been used to follow metabolite accumulation in Restharrow and *A. thaliana* throughout development and under environmental treatments. By combining expression data (qRT-PCR) and comparing directly to metabolic profiles we will be able understand how plants respond to change in terms of phytosterol biosynthesis. The developed systems have potential to be applied to other secondary metabolites, of a wide range of candidate species under aboitic or biotic stresses and may reveal a wide-spread, but as yet uninvestigated, novel route to plant survival in the margins.

P 016

Antirrhinum genes differentially regulate Tam3 transposition

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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Suppression of the activities of transposons is normally essential for host genomes to prevent high frequencies of mutations. To maintain the integrity of their genomes, hosts may evolve various defense systems to counteract the activities of their transposons. Here, we describe the complex mechanisms underlying the regulation of *Tam3* in *Antirrhinum*.
In *Antirrhinum*, several unique regulations of the transposon, *Tam3*, have been described. *Tam3* activity in *Antirrhinum* is strictly controlled by the growing temperature of plants (low-temperature-dependent transposition: LTDT), by chromosomal position of *Tam3* copy and by two specific repressor genes *Stabiliser* (*St*) and *New Stabiliser* (*NSt*). We compared the effects of the *St* and *NSt* loci on *Tam3* transposition. In cotyledons and hypocotyls, *Tam3* was active even at high growing temperatures indicating that LTDT does not operate when these organs are developing. This developmental regulation of *Tam3* activity was differentially influenced by the *St* and *NSt* loci: *St* permits *Tam3* transposition in cotyledons and hypocotyls, whereas *NSt* suppressed it in these organs. We examined the effects of these host genes on *Tam3* activity at the molecular level. We found that neither of these genes inhibit the transcription of the *Tam3 transposase* gene nor its translation, and that the *Tam3* transposase has the potential to catalyze transposition in the *St* and *NSt* lines. The differences between the effects of *St* and *NSt* imply that they regulate *Tam3* activity independently. Our molecular data represent that their influence on *Tam3* transposition seems to be non-epigenetic. *Tam3* activity is regulated by a complex interaction of cues from the environment, development, host genes and chromosomal position. This leads us to suppose that various regulatory systems specific for *Tam3* still remain to be discovered in addition to the generally adopted epigenetic regulation of transposons.

Vernalization and Photoperiod Responses among High-Latitude/Altitude Accessions of *A. thaliana* from Norway

P 017

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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Flowering time is a crucial trait in plants adaptation and a complex network of pathways regulating flowering has been identified in *Arabidopsis thaliana*. Vernalization (prolonged period of cold) promotes flowering by repressing expression of the FLC gene. Variation in the FLC gene, which prevent flowering before onset of favourable spring conditions, contributes to differences in vernalization response. Flowering is also regulated by the photoperiodic pathway with CONSTANS and GIGANTEA genes involved in promoting flowering in response to long day.

We have collected a number of *A. thaliana* populations from high-latitude and high-altitude locations in Norway. These arctic/sub-arctic environments have unique combinations of photoperiod, light quality and temperature found nowhere else where *A. thaliana* naturally occurs. We present the results of phenotypic screening of flowering time variations as affected by vernalization and photoperiod, and its association with climatic variables and sequence variation in functional genes.

Flowering time after 5 different vernalization treatments (0, 3, 6, 9, 12 weeks of vernalization) was scored in 27-36 populations using 5-12 lines per population. Variation in flowering time among populations and among lines within populations was high. Regression of flowering time against temperature, precipitation, altitude and latitude revealed significant clinal variations. Sequence analyses of the flowering pathway genes *PHYC*, *FLC*, *CRY1*, and *CRY2* were performed in 15-25 of the populations. No sequence variation was detected in *CRY1/2* and SNPs detected in *PHYC* did not show any geographic/phenotypic pattern. However, sequence analyses in the *FLC* gene in 25 populations revealed two main clusters among Norwegian populations. Northern populations cluster together and flower significantly later than Southern ones.

Phenotypic screening of flowering time responses to 5 photoperiods (8, 16, 19, 21 and 24 h of light) was performed in 10 populations using 3 lines per population and 5 individuals per line. The screening revealed diverse responses to photoperiod among Norwegian populations, and photoperiod response is not correlated with latitude but rather with climatic factors such as winter temperature and precipitation. Real-time RT PCR of CRY2, PHYA, GI, FKF1, TOC1, CO, and FT was performed in 5 populations that had been subjected to 8, 16, and 24 h photoperiod. The results revealed significant variation in expression of CRY2, TOC1, and CO in response to 16 and 24 h among these populations. Variation in expression of CRY2 and TOC1 is correlated with phenotypic response to photoperiod, which suggests that adaptation of Norwegian populations to their local areas may be partly mediated by photoreceptor and circadian clock pathways.

P 018

Chilling and freezing responses of *Eucalyptus globulus* L. clones differing in drought resistance

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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The effect of chilling and freezing was evaluated in acclimated and unacclimated plants of two *Eucalyptus globulus* L. clones (ST51 and CN5) that differ in their sensitivity to drought (ST51 is more drought sensitive). We studied changes in carbohydrates and pigments content, plant water status and osmotic potential, antioxidant enzymes and membrane injury. At six months old 30 cuttings per clone were growing in a chamber with controlled conditions (24/16° C, day/night). One group of plants were subjected to a gradual temperature decreases from 24/16° C to 10/6° C (day/night), which took 14 days. After acclimation plants were exposed to further temperature decline and measurements were done at Day 1, 5 and 9 under 10/6° C, 10/2° C and 10/-2° C (day/night), respectively. Another group of plants was examined after transference 24 h before from the control to the low temperatures without acclimation at the same days (direct chilling / freezing). The differences in the responses to low temperatures in *E. globulus* L. clones were due to different alterations in carbon metabolism, including a faster and higher capacity for osmotic regulation as compared to ST51 clone. Results support our hypothesis regarding higher cold tolerance of the drought-resistant CN5 clone for partial or incomplete levels of acclimation despite the fact that there was no difference in membrane injury between CN5 and ST51 acclimated plants.

How do plants adapt to highly weathered tropical soils? Emerging clues from a case study with *Brachiaria* grass species

P 019

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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Highly weathered tropical soils used as grasslands are characterized by a low available P concentration and often by a high P sorption capacity. *Brachiaria* grasses are the most widely planted forages in tropical grasslands. What is an adapted *Brachiaria* species to low-P soils? In addition to high yield, quantitative plant traits such as fine root development combined with high root exudation rates of organic acids and acid phosphatases might hold the clue. Evolutionary ecologists agree that phenotypic plasticity is as important as real genetic adaptation. However, morphological plasticity represents a high carbon/energy-cost solution and may not be sustainable in slower growing species that are adapted to less productive natural environments. Thus, it is essential to develop a strong cost-benefit understanding of key plant traits involved in plant adaptation.

B. decumbens has better field persistence than *B. ruziziensis* in soils of tropical America. We investigated morphological and physiological traits that might underlie the differential adaptation in the two species and asked two main questions: the role of root morphology, inclusive of a mycorrhizal contribution in nutrient foraging, and the function of organic acids and acid phosphatases in P acquisition. Ecophysiological approaches were applied to understand plant growth. The study involved greenhouse experiments in sand culture as well as hydroponic experiments in growth chambers. The morphological trait profile differed between species and the physiological basis for understanding differences in biomass production and allocation involved interactions between P, C and N. Increased root exudation was associated with decreasing plant P concentrations. The consideration of a mycorrhizal contribution revealed a strong effect of mycorrhizal strain specificity on root growth and P uptake. Species differed with regard to the degree of change in various traits during acclimation to low P availability.

Our results agree with the general notion that although certain traits are conserved in a wide variety of species from different environments, they are by no means identical in all plants. More specifically, our results shed some light on the poor understanding of tradeoffs between plant traits that are important for P acquisition.

P 020

The genetic diversity of wild species *Prunus tenella* and *Prunus webbii* in Serbia and Montenegro, assessed by the polymorphism of S-locus

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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Prunus tenella and *Prunus webbii* are endangered wild species, potentially useful in plant breeding. Both species are resistant to economically important patogen *Taphrina deformans* and also tolerant to draught conditions. Molecular methods allow designing a sampling strategy that provides good representation of the genetic diversity of plants studied. Those species are native to Balkan peninsula, but little is known about the extent of variability in their populations. The genetic diversity of these plants was screened using highly polymorphic *S* locus which exerts one of the highest levels of allelic polymorphism known for any gene.

The S-locus controls self-incompatibility phenomenon in plants which prevents fertile hermaphrodite plants producing zygotes after self-pollination. The *S*-locus is considered to contain two complementary genetically linked parts, encoding a stylar-specific product and a pollen-specific product. Stylar *S*-proteins were identified as glycoproteins with ribonuclease activity (*S*-RNases). We studied self-(in)compatibility in 18 accessions of *Prunus tenella* and 10 accessions of *Prunus webbii* by characterising stylar-expressed *RNase* alleles using IEF, PCR and DNA sequencing,

Nine *P. tenella S-RNase* alleles (S_1–S_9) were cloned; their sequence analysis showed very high Ka/Ks ratios and revealed that *S*-RNase alleles, unlike those of *P. dulcis*, show positive selection in all regions except the conserved regions and that between C2 and RHV. Remarkably, one of the alleles, S_8-RNase was found to be identical to that of S_1-RNase allele from *P. avium*, a species which does not interbreed with *P. tenella* and, except for just one amino acid, to S_{11} of *P. dulcis*.

BLAST analysis of the six sequences of *P. webbii* confirmed those as a new *S-RNase* alleles. Also, one of sequenced alleles, named S_9, was found to code for an amino acid sequence identical to that for *P. dulcis* S_{14}-RNase, except single conservative amino acid replacement in the signal peptide region, while another, named S_3 , was showed to differ only by three residues from *P. salicina S*e-RNase. Allele named S_7 was found to be inactive by stylar protein isoelectric focusing followed by RNase specific staining, but the reason for the inactivity was not at the coding sequence level. Furthermore, in five out of ten analyzed accessions we detected the presence of one active basic RNase (marked as PW_1) that did not amplify with *S*-RNase specific DNA primers. Two of them were amplified with primers designed from the PA1 RNase nucleotide sequence (basic «non-*S* RNase» of *P. avium*). Obtained PW1 sequence showed high homology (80%) with the PA1 allele.

The evolutive implications of the obtained data should be discussed.

Temperature-dependent intracellular localization of *Tam3* transposase in *Antirrhinum*

P 021

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

In the adaptation to the environment where the plants grow, the temperature is one of the most important factors. In *Antirrhinum, Tam3* transposition is activated at low temperatures around 15°C, while it is strictly inhibited at high temperatures above 25°C. Activation of *Tam3* that is rapid and reversible response to temperature change occurs during the lifetime of a single plant. Such low-temperature-dependent transposition (LTDT) of *Tam3* is a typical example of a response to environmental stimuli. On the other hand, transposable elements perturb the order of the host genomes, and have been immobilized for restriction of their activities to maintain the genome integrity. Previous reports have shown that LTDT of *Tam3* is unlinked with the known regulatory mechanisms for transposon activity. Here, we reveal that LTDT is brought about by different subcellular localizations of the *Tam3* transposase (TPase) between the low (15°C) and high (25°C) temperatures. The low temperature can locate the TPase in nuclei, while the high temperature cannot locate the TPase in nuclei. At high temperature, absence of the TPase in nuclei is caused not by nuclear export, but by inhibition of nuclear import. Such arrest of the nuclear import did not occur in tobacco BY-2 and onion cells, thus subcellular localizations of *Tam3* TPase is considered a unique mechanism to *Antirrhinum*. Our results suggest that the LTDT of *Tam3* is regulated by host factor(s) in *Antirrhinum*.

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P 022

Allelic diversity of candidate genes for drought tolerance in a rice reference collection

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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Considering climatic changes, improving crops for drought tolerance is one of the major challenges of plant breeders for the coming years. During domestication and selection events of major food crops in different eco-geographical environments, humankind has generated some selection pressure acting on key adaptive genes that could be still useful for crop improvement. Generation Challenge Program has funded in 2006 - 2008 a commissioned project called “allelic diversity of orthologous candidate genes” (ADOC) aiming to assess the allelic diversity of candidate genes or gene families for drought tolerance, in reference collections of about 300 accessions of seven mandate crops of the CGIARs (rice, barley, sorghum, bean, chickpea, potato and cassava). Here we report results obtained on rice for several genes families, including ASR (ABA-stress ripening), ERECTA and SuSy (sucrose synthase) genes, involved at different steps of drought stress response. A detailed analysis of nucleotide polymorphism from aligned DNA sequences, representing total or partial coding and non coding regions of the different genes studied, reveal different diversity patterns within gene families. Several genes present evidence of selection acting on specific subgroups of this germplasm collection. Implications in term of plant adaptation, domestication and use in plant improvement for drought tolerance will be discussed.

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Phosphoinositides regulate a plant K^+ -efflux channel in tobacco cultured cells

P 023

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

Membrane inositol phospholipids (PIs) affect various ion channels in animal cells and a few plant K channels expressed in frog oocytes. To find out whether PIs affect depolarization-activated Kout channels in plant cells in situ, we monitored outward K^+ currents in protoplasts from cultured tobacco (Nicotiana tabacum) cells with genetically modified PI levels, using patch clamp in whole-cell configuration. Currents identification was based on their reversal potential and block by Cs^+ and Ba^{2+}. "Low-PIs", i.e., protoplasts with low levels of PIs, expressing constitutively the human type I InsP(inositol polyphosphate) 5-phosphatase, including $InsP_3$ (inositol 1,4,5-trisphosphate) and $PtdInsP_2$ (phosphatidylinositol 4,5-bisphosphate), had higher I_{KSS} (net steady-state K^+ currents) than all controls, and "High-PIs", i.e., protoplasts with 50- and 100-fold higher levels, respectively, of InsP3 and PtdInsP2 (mimicking a constantly PI-stimulated plant cell), expressing constitutively the human phosphatidylinositol phosphate 5-kinase, had lower I_{KSS} than controls. ABA, known to activate phospholipase C (PLC), activated Kout channel in the High-PIs, and inhibited the Kout channel in the Low-PIs. U73122, a specific PLC inhibitor, inhibited the Kout channel in all cell lines, and reversed the promoting effect of ABA in the High-PIs. These results support the involvement of PIs in the regulation of the Kout channel. Boltzmann analysis of conductance-voltage relationship revealed its shift in a depolarizing direction in the High-PIs, consistent with increased negative charge density on the inner side of the plasma membrane, likely due to increased PtdInsP2 levels at the inner membrane leaflet. Additionally, the maximum membrane conductance was much lower in the High PIs relative to controls and in the Low PIs it was much higher than in controls, reflecting, perhaps, PIs-dependent differences in the number of channels and/or in unitary conductance.

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P 024

Characterization of *CER1* gene activity for cuticular wax biosynthesis in *Arabidopsis thaliana*

Session: Understanding, preserving and using plant diversity II: Plant adaptation, domestication and conservation

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The plant aerial organs are covered by a hydrophobic layer composed of very long chain aliphatic components which are assembled in cristals called epicuticular waxes. This layer is involved in the resistance to drought and UV, in male sterility and in the interactions between plants and pathogens. The wax components derive from very long chain fatty acids (VLCFAs): aldehydes, alkanes, primary and secondary alcohols, ketones and esters. In the plant model *Arabidopsis thaliana*, the major components are the alkanes and their biosynthesis mainly depends on one gene : *CER1* (eceriferum 1). Indeed, the mutation of cer1 induces an 80% decrease in the alkane content and an increase in the aldehyde amount (precursors of alkanes in the biosynthetic pathway). *CER1* might encode for a protein involved in the epicuticular wax biosynthesis : an aldehyde decarbonylase.
We have analysed the relative transcript abundance of the genes involved in the wax biosynthesis by Quantitative-PCR, and the quantity and the quality of the wax components by GC-MS, with or without stress (hydric stress, osmotic stress, hormonal stress). Among several candidate genes, *CER1* was the most regulated gene. This is why we decided to focus our researchs on this gene and to study its promoter activity. Transgenic plants transformed with *CER1* promoter fused to a reporter gene were constructed in the laboratory. They allowed us to localise the *CER1* promoter activity in the epidermal cells and in the young aerial tissues. Moreover, the hydric stress increases the promoter activity.
At the same time, we have constructed transgenic plants in which *CER1* is overexpressed or inactivated under the control of an inducible or a constitutive promoter. In the leaves, the overexpression of *CER1* induces an increase in the total epicuticular wax amount more than seven-fold compared to the wild type plant. In low humidity conditions, the leaves are more round and the flowering is earlier in the transgenic plants than in the control plants. The roots are not affected.
The fact that the modifications of epicuticular wax quantity and quality depend on the amount of *CER1* transcripts and abiotic stress proves that *CER1* encodes for an aldehyde decarbonylase and that waxes are involved in abiotic stress resistance, and especially, in hydric stress resistance.

Can crop adaptation mitigate the effect of climate on food security in the Sahel?

P 025

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

Several trends indicate an increase in average temperatures on a global scale. Among the potential important consequences of climatic change are those on food security. Sahelian countries have experienced a significant climatic shift to drier climates in the last four decades. However, it is yet unclear if cultivated plants rapidly adapt to such climatic shifts. One of the major cereal crops in Sahelian countries is pearl millet. Pearl millet contributes heavily to food security in the entire Sahelian region and covers more than 65% of the cultivated land in Niger. In this study, we analyzed samples collected in the same villages in 1976 and 2003 across the entire cultivated area of Niger. Comparisons of phenological and morphological evolution in a common garden experiment of 600 traditional varieties were performed over three field seasons. We observed a statistical significant shift in adaptive traits: compared to 1976 samples, samples collected in 2003 displayed a shorter life-cycle, and a reduction in plant and spike size. In the context of a changing climate, shorter life cycle may mitigate the effect of climatic change by allowing flowering and seed production in drier environment than 30 years ago. However, this adaptation is very modest and might not to be effective enough to cope with a rapidly changing climate.

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P 026

Dynamics of phytohormones during the response of tobacco plants to drought and/or heat stress

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

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Plants have evolved considerable capacity to cope with adverse environmental conditions, including water deficit and elevated temperatures. Stress responses include substantial modulation of plant growth and development, which is mediated, at least partially, by phytohormones. However, the function of plant hormones, apart of the key stress hormone, abscisic acid (ABA), is still far from being understood.
We found gradual decrease in the levels of bioactive cytokinins (CKs) in tobacco leaves during the drought progression. As upper leaves retained CK content more efficiently than the other ones, gradient of bioactive CKs in favour of upper leaves was formed. Unequal CK distribution affected sink-source relationships in shoots, facilitating protection preferentially of upper leaves. Under severe drought, CK gradient was maintained by the enhancement of CK degradation with cytokinin oxidase/dehydrogenase in lower leaves. Application of heat stress at the end of drought period led to further decrease of CK levels in leaves. In roots, accumulation of bioactive CKs during drought took place. The effect of heat stress on bioactive CK levels was dependent on the temperature, stress duration as well as on physiological state of plants, ranging from mild increase (accompanied by the overall decrease of CK degradation) to strong decrease (associated with elevation of cytokinin oxidase/dehydrogenase activity, especially in upper leaves and roots). Both stresses, which are unfavourable for plant growth, were associated with the decrease of auxin levels in upper and middle leaves and their increase in lower leaves and roots. The extent of auxin accumulation was proportional to the stress strength. Accumulation of both auxin and CK in drought treated roots seemed to be involved in the stimulation of primary root growth, which resulted in change of root morphology. ABA levels increased highly significantly at drought, correlating well with the water deficit. Application of heat stress at the end of drought period led to mild decrease of ABA content. Heat stress had a mild negative effect on ABA levels. The results indicate that abiotic stresses impose apart fast changes in ABA levels, which affect both regulation of stomata conductance and stimulation of plant defence mechanisms, also significant changes in the pool, and especially the distribution, of CKs and auxin, i.e. phytohormones controlling plant growth and development.

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The Xspecies approach to genomics and transcriptomics – a new way to work in minor and underutilised crops for the future

P 027

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

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Affymetrix expression arrays are currently available for 15 plant species (http://www.affymetrix.com/products/arrays/index.affx; 21-04-08) with additional ones becoming available in 2008. All of these (with the exception of the *Arabidopsis thaliana* ATH1, AG and *Medicago* arrays) are for major crop species. For genomic analysis, the *Arabidopsis* tiling 1.0R array is the most comprehensive resource available and the first SNP chip for this genome is expected in Summer 2008. Such resources are very powerful, but require extensive sequence and genome information to design.

The predicted scenarios for climate change in the next fifty years suggest increasingly erratic weather patterns and severe pressure on natural resources, particularly water. This is without taking into account levels of predicted population growth. Diversification of agriculture in the developing world and the development of new crops to reduce reliance on the 10 or so major crops which are responsible for the majority of food production at the moment, are critical steps for future food security. For many minor or underutilised crop species a lack of sequence-based resources are likely to limit our understanding of the genetic and physiological processes underlying important traits. Even with high throughput approaches, such as 454 Pyrosequencing and Solexa, such resources may never be developed for some crops.

The Xspecies approach (http://affymetrix.arabidopsis.info/xspecies/) uses existing Affymetrix expression arrays to work in species for which no alternative array exists. The basic approach is to carry out a genomic hybridisation of the species of interest against an existing array. A software mask is generated that contains those features which appear to show genuine hybridisation to the gDNA. A 'custom array' is thereby generated that can be used to analyse RNA samples from the newly enabled species.

To test the potential of this approach in minor and underutilised crop species, we have examined applications in bambara groundnut (*Vigna subterranea* L. Verdc.), an indigenous African legume crop with good drought tolerance. Genomic hybridisation profiles and a derived comparative expression data analysis are presented, together with an evaluation of the potential for bulked segregant mapping of simple traits. To finish, we present an assessment as to whether this approach can bridge the gap in available resources for such crop species.

P 028

Barriers to the conservation of recalcitrant-seeded plant species: requirement for an enhanced understanding of cryopreservation protocols

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

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Seeds are part of our landscape genetic memory, and through storage in seed banks, of our genetic insurance against climate change. However, an estimated 10%, and in some habitats such as tropical forests, up to 48% of seed-bearing plants produce desiccation sensitive 'recalcitrant' seeds, while the remaining species produce desiccation tolerant 'orthodox' seeds. Storage in seed banks is a key strategy for the conservation of plants with orthodox seeds, but involves drying and is therefore not suitable for recalcitrant seeds. For some recalcitrant seeds, cryopreservation protocols have been developed to conserve their germplasm, involving isolation and drying of embryonic axes, and subsequent storage in liquid nitrogen. However, there are still major challenges regarding efficiency and applicability of cryopreservation protocols to a wider range of taxa with recalcitrant seeds.

The present work aimed at understanding the stresses that accompany the first steps in cryopreservation protocols, wounding and desiccation, both of which are likely to lead to the formation of reactive oxygen species (ROS). Excision of embryonic axes from Sweet chestnut (*Castanea sativa*) seeds was accompanied by an immediate burst of superoxide ($O_2^{\bullet-}$) production on the cut surface. Cell wall fractionation in combination with gel electrophoresis revealed that peroxidases bound to the cell wall by strong electrostatic forces are involved in extracellular $O_2^{\bullet-}$ production. Isolated axes subjected to variable levels of desiccation stress showed a decrease in viability and vigour and increased electrolyte leakage, indicative of impaired membrane integrity. Mild desiccation enhanced extracellular $O_2^{\bullet-}$ production by the embryonic axes. Exogenous application of H_2O_2, the dismutation product of $O_2^{\bullet-}$, significantly improved the viability of mildly desiccated seeds. Overall, $O_2^{\bullet-}$ production showed a typical pleiotropic pattern in response to increasing desiccation, reflecting both the adaptive and the detrimental stages of the responses of organisms to stress.

In conclusion, our results indicate a complex interaction between excision and subsequent drying. ROS production, although often viewed as deleterious, appears to be an essential part of the response of isolated embryonic axes to wounding, and is modulated by desiccation. In mildly desiccated seeds apoplastic $O_2^{\bullet-}$ and H_2O_2 may ameliorate the effects of desiccation stress, suggesting that extracellularly produced ROS play an important role in the stress response of recalcitrant seeds. Fundamental roles of ROS in stress response are discussed with a view of manipulating ROS production as a key strategy for the optimization of cryopreservation techniques.

Ecogenomics of extreme submergence tolerance in Arabidopsis wild relatives

P 029

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

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Flooding is a widespread natural catastrophe with an often devastating impact on survival of wild and cultivated plants. Engineering crop tolerance for waterlogging or whole-plant submergence, either through marker assisted breeding programmes or genetic transformation, is therefore of major agronomical importance, especially given the expected increase in flooding frequency as a consequence of global climate change. Efforts in monocot crops like rice and barley have already resulted in high yielding varieties with strongly enhanced tolerance for the detrimental effects of excess water. The dicot model organism *Arabidopsis thaliana* displays natural variation in submergence tolerance within the range of several days which is currently being explored for QTL discovery. Moreover, we have recently demonstrated extreme submergence tolerance lasting over three months in Arabidopsis wild relatives. Interestingly, two contrasting *Rorippa* (yellow cress) species were found to strongly differ in their survival time underwater, associated with higher growth rates of the intolerant *R. amphibia* compared to the tolerant *R. sylvestris*. Furthermore, whole-genome transcript profiling using Affymetrix ATH1 GeneChips© of roots after 24 hours of submergence revealed striking differences in the expression of genes involved in anaerobic metabolism, as well as various other functional categories. Genomic DNA of both *Rorippa* species was hybridized on the Arabidopsis microarrays beforehand to generate a so-called probe mask which proved to greatly enhance the number of genes found to be significantly regulated in our cross-species microarray dataset. Quantitative RT-PCR confirmed the differential transcriptional regulation of key enzymes in anaerobic metabolism between the two species. We postulate that the extreme submergence tolerance of *R. sylvestris* as compared to *R. amphibia* (and almost all crop species) is based on restriction of growth underwater and repression of genes associated with the utilization of carbohydrate reserves through anaerobic respiration. The cloning of *Rorippa* genes associated with submergence tolerance provides a novel source of natural diversity with the potential to ultimately improve resistance to excess water in other plant species.

P 030

Cotton response to low temperatures: Isolation, characterization and expression analysis of membrane modifying enzymes from *Gossypium hirsutum*

Session: Understanding, preserving and using plant diversity III: Climate change and challenges for the next decades

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Lipid modifying enzymes play a key role in the development of cold stress tolerance in plants. However, little is known about the role of the endogenous enzymes in cold sensitive species such as cotton. In order to study the response of *G. hirsutum* to cold stress, expression analysis of genes known to participate in cold sensing and adaptation to low temperatures through second messenger supply and acyl chain modifications was performed. The genomic and cDNA sequences of PLD*alpha1* isoforms were isolated and characterized from *G. hirsutum* and used in an expression study along with previously isolated desaturase isoforms (FAD2). An induction in PLD*alpha1* was observed as it has been previously described in *A. thaliana*. The induction of microsomal *delta12* fatty acid desaturases at an mRNA level under cold stress is shown for the first time in plants (Kargiotidou et al., 2008). Quantitative PCR showed that though both *delta 12 omega 6* fatty acid desaturase genes FAD2-3 and FAD2-4 were induced under cold stress, FAD2-4 induction was significantly higher than FAD2-3. The induction of both isoforms was light regulated, in contrast a third isoform FAD2-2 was not affected either by cold or light. Expression analysis patterns were correlated with the observed increase in both total and microsomal fatty acid unsaturation levels suggesting the direct role of the FAD2 genes in membrane adaptation to cold stress. Stress tolerance and light regulatory elements were identified in the predicted promoters of *Gh*PLD*alpha1*, *At*PLD*alpha1* as well as of *Gh*FAD2.

Plant production in Switzerland - Perspectives 2050

P 031

Session: Science and Society: The challenges for tomorrow's agriculture

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Economic globalisation, the increase in the world's population and changes to its demographic structure, climatic changes together with the increasing scarcity of natural resources are going to have a profound effect on the framework for plant production worldwide. For example, does plant production still have a future in Switzerland? What are the possible development scenarios? What would the consequences be of the disappearance of plant production? These are questions and controversial issues that are often raised. Answers must be found that will enable plant production to adapt to the changes that are taking place and to minimize the risks. Following a workshop organized early 2006 by the Swiss Society of Agronomy (SSA) called «Towards the abandonment of large scale cultivations in Switzerland? Thoughts and Perspectives», the committee observed that, to this day, very little research work has been carried out on this subject and that the interests and concerns of parties associated with plant production are real. A working group called «Plant Production Perspectives 2050» was created with the main objective of identifying and characterizing the changes and challenges to be faced during the next 50 years. Such a vision would allow, in the medium term, the formulation of objectives for the production, research and development, public relations and management of agricultural policy. This action should also allow our reflections to be integrated into similar initiatives developed within the European Union, such as those supported by the SCAR or EPSO (action "plant for the future").
From its inception, the working group wanted to develop such a vision in cooperation with representatives of various bodies from the plant production sector, with the SSA fulfilling the role of a platform for the management of the project and the integration of the various points of view. Two sub-working groups were created at the end of 2006 bringing together Swiss experts from all areas concerned. The first one called «Basic Conditions» discussed questions concerning the evolution of the frame-work conditions of Swiss agriculture until 2050. Its considerations included climatic concerns, demands of society, resources and socio-economic conditions. The second sub-working group called «Systems» defined four scenarios for the future of Swiss agriculture. The group's willingness to develop scenarios that could be considered extreme such as «Agrotourism», «Regional Intensification», «Sustainable Hightech Agribusiness» and «Bioland Switzerland» aimed to stimulate discussion and get away from a traditional narrow way of thinking concerning vegetal production. During a next phase, these four systems of production have been compared to the framework conditions forecasted. A synthesis of this work resulted in a long-term vision for vegetal production for our country. The report Plant Production Perspectives 2050 as well as a information flyer are available online under the website of the Swiss society of agronomy (www.sgpw.scnatweb.ch). The conclusions of the study were, that the production of sufficient foods of high quality is only possible based on scientific and technological progress in plant sciences and production. In addition, conservation of fertile agricultural land and public commodities such as recreational landscapes, secure supply of drinking water and conservation of biodiversity are a necessity. The SSA highlights the requirements for research and development for enabling a plant production of high quality and quantity in the future.Formulated improvement and research needs have now to be prioritized and better defined with the involvement of stakeholders. This coming preparation phase aims the formulation of a research program at a national level.

P 032

Sensing low-phosphate at the root tip

Session: Preserving our future by reducing the inputs in agriculture: Reducing fertilisers

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With the rise of environmental concerns and the ineluctable reduction of natural resources, new crops will be needed to face new agrobiological conditions. The yields of future crops must satisfy human needs, but with reduced supplies of fertilizers. Reducing mineral inputs is though to reduce plant growth by reducing metabolic activity, however this might be an oversimplified view. By using the Arabidopsis natural variation we have identified a major QTL (*LPR1 = Low Phosphate Response1*), and its paralogue *LPR2*, two genes that reduce the primary root growth when seedlings are on a phosphate-deficient medium. The molecular origin of the *LPR1* QTL is explained by the differential allelic expression of *LPR1* in the root tip (mainly the root cap) (*Nature Genetics*, 2007). Interestingly, physical contact of the primary root tip with low-Pi medium is necessary and sufficient to arrest root growth. These results provide strong evidences for the involvement of the root cap in sensing and/or responding to nutrient deficiency. *LPR1* and *LPR2* encode multicopper oxidases (MCO), highlighting the essential role of MCO for plant development. Our results suggest that when the root encounters a mineral-deficient zone, a signaling pathway restraining growth is triggered in the root tip.

Hormonal control of nitrate influx and nitrogen allocation in wheat plants

P 033

Session: Preserving our future by reducing the inputs in agriculture: Reducing fertilisers

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Many data indicate that cytokinins are involved in signaling nitrogen availability to shoots. An analysis of the sequence of events following restoration of the nitrate supply to nitrogen-depleted maize roots revealed an early accumulation of cytokinins in roots and increased flux of cytokinins *via* xylem flow (reviewed in Sakakibara *et al*., Trends in Plant Sci. 11:440-448, 2006). We have demonstrated the opposite effect (i.e. an enhancement of nitrate influx and nitrogen accumulation) in wheat plants in response to an increase of cytokinin content due to up-regulation of cytokinin biosynthesis or to application of cytokinin. Plants grown hydroponically absorbed most of their nitrate (over 60%) during the phase of vegetative growth and its uptake decreased sharply after anthesis. Following anthesis most of the nitrogen required for grain development was mobilized from other parts of the plant, especially the leaves. Measurements of ^{15}N accumulation in various wheat organs from a pulse of $^{15}NO_3^-$ supplied through the nutrient solution showed that some 60% to 90% of the nitrogen located in mature grains was absorbed prior to anthesis. When leaf senescence was delayed by the enhancement of cytokinin accumulation in leaves due to the expression of the *ipt* gene under the control of a senescence-induced SAG12 promoter, nitrate influx increased significantly and the period of active nitrate uptake doubled up to 30 days after anthesis. The delayed leaf senescence also deferred remobilization of the absorbed nitrogen from the slowly senescing leaves thus reducing its availability for the grains during the early stage of their development. The retention and accumulation of nitrogen in leaves did not occur when cytokinin was applied to the whole plant so there was a significant increase in grain yield. Interestingly, the promotive effects of both endogenously synthesized and applied cytokinin described above were found only when the plants were grown under conditions where nitrogen supply was limited. This suggests that cytokinins can enhance the ability of plants to cope with nitrogen deficiency and prevent the yield losses. This effect was confirmed in field experiments where application of cytokinin increased the grain yield by up to 10% depending on the availability of nitrogen and weather conditions.

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P 034

Reduced nitrogen forms in top dressings increase grain protein concentrations via changes in cytokinin levels

Session: Preserving our future by reducing the inputs in agriculture: Reducing fertilisers

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Urea, ammonium and nitrate are the most important nitrogen forms employed in agricultural plant production. Although nitrate has been shown to act as a signal for metabolism and plant development in physiological studies, so far no use is made of the signalling effect of different N forms in cereal plant production.
To investigate the effect of different N forms on cytokinin concentrations in leaves, we performed nutrient solution experiments with spring barley. We observed that cytokinin concentrations and protein remobilisation in flag leafs were strongly influenced by the N form supplied to the nutrient solution after flag leaf appearance.
To reproduce this N form-dependent effect in field trials, winter wheat plants were fertilised with stabilised N forms in the late top dressing. Supply of nitrate increased the cytokinin concentration in the flag leaf, while ammonium und especially urea led to decreased cytokinin concentrations. Lower cytokinin concentrations in flag leaves coincided with protein enrichment in the grains. This indicates a more efficient re-translocation of nitrogen from the flag leaf after ammonium or urea fertilisation.
This study points to the possibility that the use of different N forms for N fertilization to cereal crops can serve as a means to manipulate N remobilisation and N use efficiency of lately applied N fertilisers.

Modeling systems response to sulfur deficiency stress

P 035

Session: Preserving our future by reducing the inputs in agriculture: Reducing fertilisers

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Cysteine and methionine essential for human and animal nutrition are sulfur-containing amino acids synthesised in plants. That's why understanding of how inorganic sulfur is uptaken by plants and built into the organic molecules in the process of sulfur assimilation is important on the way to sustainable agriculture. As complex biological systems, plants subsist as integrated molecular, organelle, cell, tissue and organ entities being in permanent synergistic coordination. The process of sulfur uptake and assimilation is an integral part of this dense network of influences, its reconstruction may help in manipulating the bio-production of organic sulfur-containing compounds and reducing fertilisers. New high throughput technologies allow the systems view on the coordination of complex processes in living organisms. Among them, transcriptomics and metabolomics studies were applied to Arabidopsis plants subjected to sulfur deficiency stress. From the integrated analysis of the obtained data the mosaic picture of distinct sulfur stress response events and processes is starting to be assembled into the whole systems network of sulfur assimilation. In the time trajectory of sulfur stress response, two systems' states can be distinguished. The first state of short-term responses is characterized by the development of enhanced lateral roots exploring the space in search for the lacking nutrient. When this physiological reaction can not be accomplished by bringing the system back to the initial state of sulfur sufficiency, a new program is toggled aiming at saving the organismal resources for vital seed production. We describe an approach for representing and reasoning about these two system states, as well as the state transitions between them, using the concept of action languages.
To build the model of Arabidopsis plants responding to hypo-sulfur stress, we compiled the available data on the behaviour of the particular system elements and on their mutual coherence. This data was translated into a formal causal model by formalising states in terms of individual fluents and known knowledge of changes between these states in terms of actions (as causally directed connections between fluents and actions). Fluents were represented by genes, metabolites, or more complex phenotypical traits and actions corresponded to particular cellular processes. In such a way, a systems' state is described in a query by a combination of fluent/action states, and examined for the following constraints:

1) Analysis of an initial state and a modelled time of a query fluent to hold
2) Combinatorial manner and synergism in functioning of biosystem constituents
3) Essentiality of causal hierarchies for systems functioning
4) Redundant side branches of informational flows through analysis of action essentiality
5) Fluent essentiality through comparative simulation of alternative models.

The approach has proved to be useful for reconstructing and reasoning about the regulation of nutrient uptake and assimilation by plants. It showed also promise for the in silico probing of putative effects of the mutations on the stability and flexibility of a biological system.

P 036

Repression of anthocyan synthesis by three nitrate-induced transcription factors acting upstream of *PAP1*

Session: Preserving our future by reducing the inputs in agriculture: Reducing fertilisers

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Signals derived from nitrate per se have a broad impact on gene expression, resulting in the reprogramming of nitrogen and carbon metabolism to induce efficient nitrate uptake and assimilation. Root growth and architecture, induction of flowering, senescence and anthocyan production are also adjusted by signals derived from internal and external nitrate. To obtain insights in the regulatory infrastructure underlying these changes, transcription factors (TFs) strongly regulated by nitrate were identified using Affymetrix genechips and high-throughput real time RT-PCR. Out of these, three related genes were selected for functional analysis.
Constitutive over-expression (OX) of each of the three TF genes led to a "stay green" phenotype with strongly reduced anthocyan accumulation in nitrogen-deprived conditions both in young seedlings grown in axenic culture as well as in adult plants grown on soil. Additional visual phenotypes of the OX plants include accelerated development, a higher germination rate and increased length of the main root in axenic culture as well as early flowering with more lateral branches when grown on soil under low nitrogen conditions.
Real time RT-PCR and Affymetrix genechip analysis reveal strongly reduced expression of regulatory genes (*PAP1*, *PAP2*) and several key enzymes of anthocyan biosynthesis (e.g. *DFR*, *ANS*, *AGT*, *GST*) in all OX lines grown in N-deprived conditions, thus confirming the reduced anthocyan synthesis phenotype at the molecular level. A strongly decreased amount of cyanidin glucosides in OX plants confirm the reduced anthocyan synthesis at the metabolite level.
Other conditions, like phosphate deprivation, high light, high sugar and cold, that normally induce anthocyan synthesis, were unable to override the effect of the over-expression.
Expression and metabolite data also show that these TF influence the uptake and assimilation of nitrate in the plant.

CLE peptide signalling during nodulation on *Medicago truncatula*

P 037

Session: Preserving our future by reducing the inputs in agriculture: Reducing fertilisers

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Legumes form symbiotic interactions with soil borne bacteria called rhizobia. Those interactions are characterized by the development of new root organs, the nodules in which bacteria colonize the inner cells and fix nitrogen which is used by the plants. In return the microsymbiont receives carbon sources and a protective niche.
The classical hormones ethylene, cytokinin and auxin are involved in the initiation and coordination of the nodulation process. We suspect that a new class of hormones, the peptide hormones, and more specifically the CLE peptides also have an important function. Until now, CLE peptides were only assigned a role in shoot, flower and root meristem maintenance, in vascular development and in nematode feeding cell formation.
By sequence analysis of the *Medicago truncatula* genome and *Mt*EST libraries, we identified 25 *Mt*CLE peptide genes. qPCR analysis revealed that 3 of them, *Mt*CLE4, 12 and 13 are upregulated from early stages of nodulation on.
Promoter-GUS analysis of the three *Mt*CLE peptide genes indicated that each of them is activated in the nodule meristem. Moreover, *Mtcle13* is also expressed very early in the nodule primordia. RNAi analysis of *Mtcle13* by use of *Agrobacterium rhizogenes* transformation, resulted in a retarded and diminished nodulation. No major RNAi phenotypes could be observed for the other two CLE peptide genes. This could be the result of redundancy, as this has often been observed for CLE peptides. In parallel to the RNAi, the ectopic expression of the three CLE peptide genes was performed. A Nod^{-} phenotype was generated when overexpressing *Mtcle12* and *Mtcle13*. In addition, systemic effects were observed on the non transformed parts of the composite plants.
The results gained until now, suggest a role for at least one of the nodule specific *Mt*CLE peptide genes in the dedifferentiation of cortical cells during the early stages of nodulation.

P 038

Potassium and nitrogen: where do they meet?

Session: Preserving our future by reducing the inputs in agriculture: Reducing fertilisers

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Regulation of nutrient levels involves perception and signalling mechanisms to regulate nutrient uptake, assimilation and storage in plant cells. At the whole plant level, mineral nutrient levels are adjusted to best meet specific developmental, environmental and organ requirements through regulation of assimilation, storage and re-allocation in/between different tissues. The complexity of nutrient homeostasis is further increased by the fact that deficiency in one nutrient can have serious effects on the uptake and usage of other nutrients, for example, interactions between N and K are well known to farmers. Despite intensive research into the molecular processes underlying perception and integration of mineral nutrients we still lack basic understanding of how the adaptive responses to individual nutrients and the signalling pathways involved interact with each other. Microarray studies from our lab revealed the down-regulation of several NRT2 nitrate transporters in response to K deficiency, which was quickly reversed after K re-supply. This down-regulation of NRT2 transcripts can explain reduced nitrate uptake capacity observed in K-starved crops but raises questions about the physiological implications of such a response and the nature of the signal. To answer these questions we carried out a comprehensive comparative analysis of primary metabolites and enzyme activities in control, K-deficient and K re-supplied plants. The results from these studies provide first hints as to which enzymes might be primary targets of K-deficiency, and which metabolites might act as metabolic signals for K-deficiency. Since several of these targets and signals are known to regulate K-and nitrate-transporters our data provide exciting new evidence for feedback regulation between metabolism and ion transport. A detailed view of carbon and nitrogen metabolism during K-deficiency and re-supply will be presented at the meeting.

Role of the Arabidopsis MYB transcription factor A*tMYB30* in the control of disease resistance and hypersensitive cell death

P 039

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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The Hypersensitive Response (HR), characterized by a rapid and localized cell death at the inoculation site, is one of the most efficient resistance reactions to pathogen attack in plants. A better understanding of the molecular mechanisms leading to the HR will enable us to reduce pesticide use in agriculture.
We previously found *AtMYB30* as specifically, rapidly and transiently expressed during incompatible interactions between *Arabidopsis* and bacterial pathogens. We also demonstrated that AtMYB30 is a positive regulator of the hypersensitive cell death. Transcriptome analysis, together with recent molecular, genetic and biochemical studies, show that putative AtMYB30 target genes are involved in the lipid biosynthesis pathway leading to the production of very long chain fatty acids (VLCFAs), suggesting a role of this pathway in the control of the HR and plant defence responses.
New strategies aiming at (i) studying the subcellular localization of AtMYB30, (ii) characterizing posttranslational modifications within the protein, and (iii) identifying proteins that may interact and work together with AtMYB30 in the initiation of the HR will be presented. These studies indicate that ubiquitination of AtMYB30 may modulate AtMYB30 activity and its control of the plant HR and defence responses.

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P 040

Gene expression profiling to identify new bioactive compounds acting on plant defence

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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The ability of plants to defend themselves against pathogens depends on the perception of signalling molecules, called elicitors, released during infection. Since elicitors induce plant defence, they might be considered as alternative tools for disease control in agronomic crops. Industrial use of elicitors needs the identification of abundant sources of these molecules and characterization of their biological activity. Recently, we used the model legume *Medicago truncatula* to show that a crude extract of the green algae *Ulva* spp. was a able to elicit defence reactions and to induce protection against a fungal pathogen (Cluzet et al., 2004, Plant Cell Environ. 27 : 917). Here we report on the identification of a new polysaccharide purified from this *Ulva* spp extract and the use of global gene expression profiling to characterize biological activity of this compound.
High and low molecular weight molecules contained in the extract were sized-fractionated. Analyses of the fractions revealed that biological activity was present only in the fraction of high molecular weight. Physical and chemical analyses of this fraction showed that it contained most exclusively a high molecular weight sulfated polysaccharide named ulvan, whose main constituent is a disaccharide unit, □-D-glucuronosyluronic acid (1→ 4) L-rhamnose 3 sulfate. This purified polysaccharide was sprayed on *M. truncatula* leaves and gene profiling analyses were performed using oligo microarrays allowing the monitoring of more than 16,000 genes. Responses to ulvan were compared to those induced upon methyl jasmonate and acibenzolar-S-methyl (a salicylic acid analog) treatments. These analyses revealed that ulvan treatment induced distinct functional gene classes including defense-related genes and genes involved in nitrate and sulphate metabolisms. Interestingly, ulvan gene expression signature showed significant similarity to methyl jasmonate. Altogether, the results suggest that plant responses to ulvans are mediated by a methyl jasmonate dependent signalling pathway and highlight the use of functional genomics to develop new bioactive compounds for plant protection.

Co-inoculation with a beneficial endophytic fungus as a promising strategy to reduce clubroot disease symptoms

P 041

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

The control of the clubroot disease, one of the most damaging within the familiy of Brassicaceae, is difficult due to the obligate life style of the pathogen *Plasmodiophora brassicae*. Consequently, it is of high interest to understand the underlying mechanisms of pathogenesis. In addition, the search for alternative methods to control this devastating plant diesease is desirable. In this study we have therefore investigated the ability of an endophytic fungus of the genus *Acremonium* to influence clubroot formation in the model plant *Arabidopsis thaliana*, which is a good host to the clubroot pathogen *P. brassicae*. When host plants were infected with *P. brassicae* alone, they formed the typical root galls accompanied by stunted growth of the aereal parts of the plant. After co-inoculation with *Acremonium* sp. we found smaller root galls and the phenotype of the shoots was comparable to that of uninfected plants. In addition, the smaller root galls were accompanied by fewer pathogenic structures in the galls and especially a reduction in resting spore formation was found. This led us to the hypothesis that development of *P. brassicae* was delayed. Using quantitative RT-PCR to monitor the expression of a small number of *P. brassicae* genes the delay in development was confirmed. The fungus *Acremonium* had colonized the root as well as shoot tissue of the host plant as shown by Real Time RT-PCR. Furthermore, we identified a time window in which the endophyte had to be administered in green house experiments. These results are promising to be further developed in the context of a complex disease management to reduce clubroot symptoms. Future studies will aim at the elucidation of the mechanism by which *Acremonium* sp. can delay the development of the clubroot pathogen.

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P 042

Activity-based protein profiling to study plant-pathogen interactions

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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Activity-based protein profiling (ABPP) is a powerful technology to display the activities of classes of enzymes in plants during their infection by pathogens. The technology is based on biotinylated inhibitors that covalently react with the active site residues of enzymes in an activity-dependent manner. The biotinylation is irreversible, which facilitates further analysis by mass spectrometry and protein blots. The activity of papain-like cysteine proteases can be monitored using DCG-04, a biotinylated version of E-64, a mechanism-based inhibitor of papain-like cysteine proteases (PLCPs). We used this technology to show that tomato plants create a proteolytic environment in the apoplast during defense. Of these PLCPs, transcription of only PIP1 and RCR3 is induced by treatment with benzothiadiazole (BTH), which triggers the salicylic acid-regulated defence pathway. Sequencing PLCP alleles of tomato relatives revealed that only PIP1 and RCR3 are under strong diversifying selection, resulting in variant residues around the substrate binding groove. The doubled number of variant residues in RCR3 suggests that RCR3 is under additional adaptive selection, probably to prevent autoimmune responses. AVR2 selectively inhibits only PIP1 and RCR3, and one of the naturally occurring variant residues in RCR3 affects AVR2 inhibition. The higher accumulation of PIP1 protein levels when compared to RCR3 indicates that PIP1 might be the real virulence target of AVR2 and that RCR3 acts as a decoy for AVR2 perception in plants carrying the Cf-2 resistance gene. Besides Avr2, we have evidence that other tomato PLCPs are targeted by secreted inhibitors form Phytophthora and Pseudomonas. Current work in the Plant Chemetics lab is focused on understanding the role of these enzymes, and further expanding ABPP to monitor activities of other enzyme classes in living tissues during infection.

Detection of candidate genes for useful traits in potato using different molecular tools

P 043

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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Molecular markers are useful to construct linkage maps and to localize monogenic and polygenic traits, allowing the efficient introgression and selection of individuals with specific characteristics already in breeding material. They can accelerate breeding programs to obtain superior cultivars for sustainable agriculture and better adapted to present and future environmental threats. In potato large amounts of genomic resources are being established within the frame of international projects including a potato genome sequencing project.

Traditionally, markers used for linkage mapping and QTL analyses in potato were neutral markers and identify generally only genomic DNA. Linkage distances to QTLs and varying allelic configurations restrict the application of these markers to specific genetic backgrounds. Therefore, it is necessary to detect directly the genes which influence a trait of interest and to analyze and compare the effects of their different alleles. Such marker types can be applied directly in marker-assisted selection, independent of the genetic background and are useful to establish functional maps.

Different molecular tools have been applied to detect resistance or response genes to various potato pathogens and quality genes. These include transcriptome mapping based on the cDNA-AFLP technique combined with co-location analyses between QTLs and TDFs, the use of differential cDNA-AFLP and microarray analyses.

The cDNA-AFLP technique targets partial cDNAs and allows to monitor differential gene expression genome wide using appropriate mRNA populations. Moreover, allelic differences of constitutively expressed genes can generate segregating polymorphisms for linkage mapping. In this way a transcriptome map containing around 700 cDNA markers was constructed. The map was anchored to the bins of a high-density reference map of potato. Subsequently over 200 published QTLs and genes were projected onto this map. cDNA markers which are co-located with published QTLs for pathogen resistance represent potential candidate genes controlling the trait. Such bands were cloned, sequenced and homology searches were performed. Several interesting homologies were detected which have a relevant biological meaning.

Differential cDNA-AFLP was applied to detect resistance or response genes for nematode (*Globodera pallida)* and *Phytophthora infestans* infections and for water stress in a set of *Solanum* wild species. In all three case studies several differentially expressed transcripts showed significant homologies with known resistance genes or stress proteins. The global response was much higher in resistant accessions and concentrated in the infected organs.

Microarray analyses were applied to monitor differential expression of cDNAs in three different *Solanum* accessions after inoculation with *G. pallida* and in five accessions after infection with *P. infestans*. Several cDNAs with homologies to known resistance genes were detected in all cases. Comparative analyses revealed a variable structure of the response depending on the particular genotype.

The results of the different studies were integrated by mapping the detected cDNAs onto the reference map. In part, they were found to be co-located with relevant published QTLs.

P 044

Revealing role of environmental factors of accessibility of pesticides with special regard to the soil characteristics

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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Bioavailability of pesticides is tailored by several crucial factors including both biotic and abiotic feature. Among abiotic factors soils' characteristics play the most relevant role, as the extent of interactions between pesticides and soils may vary on a large scale depending on the soil-type. In order to be able to characterize these adsorption processes it is of high interest to model accessibility of widely used pesticides, as well as to provide extensive comparison of different methods. Our work signifies major innovations in terms of applying distinctive extraction model systems and involving pesticides have not been studied so far for accessibility.

Impacts of environmental conditions, including major soil types and parameters were also assessed in our studies. Applying 5 diverse extraction methods provided solid basis for proper comparison and selection of the best method, as well as led to authentic estimation of pesticide residues. The accessibility and the extent were investigated for 3 different types of soil (sandy, brown forest and alluvial soil) and 5 pesticides (simazin, chloropyrifos, acetochlor, diuron and) at different pH values, in cases of diverse organic matter contents. The effect of microbiological activity has also been studied to estimate the contribution of the microbiological systems to pesticide bioavailability. Marked differences were observed between extractable amounts of pesticides from sterilized and non sterilized soil samples.

Major goal was to make a comprehensive comparison between different experimental methods to model accessibility of pesticides. Six chemically much different pesticide (simazine, acetochlor, chlorpyrifos, diuron, pirimicarb) were applied to three soil types (brown forest soil, sandy and alluvial soils). The extracted amounts were determined by GC/MS and HPLC/MS technique.

The five distinctive types of applied extracting solvents displayed different effectivity for mobilizing pesticides from soil. The extracted pesticide amounts were in inverse proportion to the increase of the organic content in cases of all the three soil types. In general it might be stated that natural-like extraction methods provided significantly efficient and excellent models for estimation of bioavailabilty of pesticides. Pesticides were adsorbed in soils having distinctive pH values to different extent in accordance with their chemical characteristics. Pesticides were not equally accumulated in different segments of the plants exhibiting major role of pesticides' chemical feature. Differences in accumulated amounts in terms of the examined soil types were observed, while the extent of plant uptake of pesticides has exhibited no correlation with the parameters of the examined soils.

Volatile chemical cues involved in plant-insect interactions

P 045

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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Plant semiochemicals play an important role in the ecological interactions between plants and insects. Plant parasitic insects such as aphids and whiteflies use plant-emitted "odours" to find their host plants. Here it is investigated which plant volatiles are involved in repellence by tomato species of the pest insect *Bemisia tabaci* (sweet potato whitefly), a well-known vector of many devastating plant viruses.
Free choice bioassays of *Bemisia tabaci* released among cultivated tomato and wild relatives revealed a clear preference for the cultivated tomato and a differential and reduced preference for several wild tomato accessions. Also, it was shown that host-choice behaviour was no different between two genetically different whitefly populations. It was demonstrated that cultivated tomato (*Solanum lycopersicum* cv Moneymaker) could be made less attractive by supplying it with the repellent volatile chemicals of the headspace of *Solanum pennelli* or *Solanum habrochaites*, indicating that repellence is based on volatile chemicals.
The headspace volatile 'fingerprints' of repellent and cultivated tomatoes were determined using GC-MS techniques. This dataset in combination with the preference behaviour of the whiteflies resulted in identification of several semiochemicals with possible involvement in either attracting or repelling *Bemisia tabaci*.
To demonstrate causal effects, free-choice bioassays were repeated with susceptible tomato Moneymaker plants supplemented with pure components of repellent headspace on rubber septa. Thus, it was possible to identify two single terpenoids that act as repellents of whitefly in wild tomato accessions. Using high-throughput sequencing of trichome cDNA libraries, the corresponding terpene synthase genes are being identified and cloned.

P 046

Increased resistance against smut and bunt diseases by specific anti-fungal virus genes in genetically engineered wheat

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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A viral gene (KP4) encoding for an anti-fungal protein in genetically modified spring wheat varieties (*Triticum aestivum*) resulted in a 30% reduction in symptoms of *Tilletia caries* (stinking smut). In a dose-response greenhouse based experiment using isolated fungal strains, in which the infection pressure was varied *via* the spore concentration, the transgene behaved as a quantitative resistance gene and shifted the S-shaped dose-response curve towards greater resistance. A field test confirmed a 10% increase in fungal resistance against *T. caries* under high infection pressure. To the best of our knowledge, this is the first report of improved resistance in wheat to fungus achieved using genetic engineering techniques. The same genetically modified wheat lines also showed up to 60% increase in resistance to *Ustilago tritici* (loose smut) in greenhouse experiments. The transgene was shown to be highly specific for fungi of the order *Ustilaginales*. Toxicity tests of the transgene using cultures of eukaryotes, including hamster and human cells, showed no significant side effects with respect to bio-safety. Endogenous pathogen-related genes were also activated upon fungal infection in the presence of the *kp4* transgene as shown by micro-array analysis and confirmed by real-time PCR. Flavonoid content, as an example of a metabolic profile of putative environmental interaction, showed greater difference between different conventional varieties than between KP4-GM wheat and wild type plants.

Towards transgenic lines of *Picea abies* (L.) KARST. showing toxicity to bark beatle species

P 047

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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The aim of our research is the utilization of *Bacillus thuringiensis* var. *tenebrionis* delta-endotoxin to production of the transgenic spruce lines toxic towards bark beatle (*Scolytidae*). Somatic embryogenesis potentially provides a highly regenerative source of explants for genetic transformation. A plant's sensitivity to antibiotics is species specific showing either inhibition or promotion of explant growth and regeneration.
At the beginning of our experiments we determined the sensitivity of 9 lines of *Picea abies* embryogenic calli to antibiotics. Two groups of antibiotics were tested: (1) antibiotics commonly used to eliminate *Agrobacterium* from tissue culture (augmentin, carbenicillin, cefotaxime, ticarcillin and timentin), and (2) antibiotics for the selection of transformed tissue (kanamycin, paromomycin and hygromycin).The effect of antibiotics was evaluated after 3 weeks of culturing on the modified Litvay's medium.
The effect of antibiotics on growth inhibition was very cultivar specific. Among antibiotics of the first group ticarcillin reduced the growth of embryogenic calli in all 9 *P. abies* lines. Carbenicillin reduced the growth in 7 lines and timentin in 5 lines tested, but in comparison to carbenicillin, timentin was less toxic. The lowest growth reduction was observed in the case of augmentin. The influence of cefotaxim is not completed yet.
In regard to second group of antibiotics the most of embryogenic tissue lines proved better tolerance to paromomycin than to kanamycin or hygromycin. The growth of embryogenic calli was strongly inhibited at 50 – 150 mg/l paromomycin, 25 – 100 mg/l kanamycin and 10 – 20 mg/l hygromycin after 3 weeks in culture.

This work was supported by NAZV QH7 1290 and AV0Z50510513.

P 048

Different viral RNA silencing suppressors have different effects on virus infection in two *Nicotiana* species

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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We have constructed transgenic *N. benthamiana* and *N. tabacum* plants, expressing the following RNA silencing suppressors: P1 of *Rice yellow mottle virus*, P19 of *Tomato bushy stunt virus*, P25 of *Potato virus X*, HcPro of *Potato virus Y*, AC2 of *African cassava mosaic virus* and 2b of *Cucumber mosaic virus* (strain Kin). Expression of several suppressors caused changes in the phenotype of plants, whereas the reaction of two *Nicotiana* species to the expression of suppressors was different in most cases.
In this work we have analyzed how the different suppressors affected the accumulation, systemic spread, symptom severity and possible recovery of *Tobacco ringspot virus* (TRSV) infection. Potato *calico* strain of TRSV, a nepovirus that induces very clear initial ringspot symptoms, with obvious later recovery in *N. benthamiana*, was used to challenge the plants.
In *N. benthamiana*, this virus produces initial infection with severe systemic symptoms, but the infection is strongly reduced within a few weeks as the plants recover from the infection. In this host, the P25 and HcPro silencing suppressors effectively prevented the recovery, allowing continuous accumulation of viral RNA as well as virus-specific siRNAs in the systemically infected leaves.
Susceptibility of *N. tabacum* to the *calico* strain of TRSV was temperature sensitive. In cool temperatures, up to 25 ^{o}C, the plants became systemically infected, but at higher temperatures, the non-transformed *N. tabacum* plants were resistant to the infection. In these preventive conditions, all silencing suppressor transgenes (except P25, which was expressed at very low level in the used transgenic line) allowed the establishment of both the local and systemic infections. The systemic infections was much weaker in the P19 expressing plants, suggesting that these plants still had active defence against the systemic accumulation of the virus. The virus level remained stable through the course of the infections in the HcPro and AC2 expressing plants and in some cases, in the 2b and P1 expressing plants.

Multifunctional viral genome-linked protein of *Potato virus A* is an intrinsically unstructured phosphoprotein

P 049

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

Genome-linked protein VPg of *Potato virus A* (PVA; genus *Potyvirus*) has essential functions in all critical steps of PVA infection, i.e. replication, and movement. It can be an avirulence determinant in certain resistant hosts in which the potyvirus fails to achieve systemic infection Structural features of the recombinant PVA VPg were investigated with the aim to understand the structure-function relationships. Circular dichroism data revealed a distinct near-UV spectrum indicating that the environment around its aromatic residues is structured but rather flexible, and a far-UV spectrum that contained features typical for intrinsically disordered proteins. Acrylamide fluorescence quenching and 1-anilino-8-naphthalene sulfonate binding experiments together with an NMR analysis further verified that PVA VPg behaves as a partially folded species having a loose tertiary structure. Regions predicted to be disordered in PVA VPg were cut the fastest by trypsin. Regions predicted to be structured and to contain the most conserved amino acids among potyvirus VPgs were trypsin-resistant. The properties of intrinsically unstructured proteins are often regulated by phosphorylation. In order to be able to analyse post-translational modifications of VPg taking place during PVA infection, an affinity-tag based purification system was developed by inserting a sequence encoding for 6xHis- and hemagglutinin (HA)-tags to the 3' end of the VPg coding sequence within the infectious cDNA clone of PVA. The engineered virus was infectious and the HisHA-tag encoding sequence remained stable in the PVA genome through the infection process. Purification under denaturing conditions resulted in a protein sample that contained multiple VPg and NIa (VPg and proteinase fusion) forms carrying post-translational modifications that altered their isoelectric points. Non-modified tagged VPg (pI 8) was a minor product in the total leaf protein sample, but when the replication-associated membranes were used as a starting material its relative amount increased. Phosphatase treatment verified that some of the PVA VPg isoforms were modified by multiple phosphorylation events. Further experimentation is required to understand the molecular mechanisms of functional regulation achieved via the flexibility of VPg structure and its phosphorylation.

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P 050

The *dotcom* mutant series: ORMV-MP transgenic *Arabidopsis* mutants impaired in their ability to complement the movement of MP-defective *oilseed rape mosaic tobamovirus* (ORMV)

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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Intercellular movement of macromolecules has become a central issue in many aspects of plant molecular physiology. Numerous processes have been described that involve the movement of protein or nucleic acids at short and long distances within the plant body. These include important developmental transitions, systemic spread of gene silencing, and viral movement among others. Intracellular dynamic macromolecular distribution, symplasmic macromolecular movement through plasmodesmata, and long distance vascular movement reside in the mechanistic bases of this intercellular exchange of biological information. However, information about genes and gene products mediating these processes is scarce and fragmented, largely due to the lack of good genetic approaches to identify and interrogate the plant genes involved.

We have developed a genetic approach specifically designed for the identification of Arabidopsis genes involved in cell-to-cell movement of a tobamovirus, used as a representative of macromolecular movement. These mutants DOn´t Transgenically Complement ORMV Movement (*dotcom* mutants, *dcm*). In this communication we describe the experimental system developed, and the preliminary characterization of some mutants.

Tomato genotypes specifically modulate the interactions with beneficial fungi of the genus *Trichoderma*

P 051

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

One of the most promising opportunities for reducing the use of chemically synthesised pesticides in agriculture is to booster the use of biopesticides based on beneficial micro-organisms. Fungi of the genus *Trichoderma* have been widely studied for their ability to effectively protect plants from pathogen infection. Besides, they also exert beneficial effects on plant growth and development, thus representing a low-input alternative to synthetic fertilizers. Several formulations of *Trichoderma*-based biopesticides and biofertilizers are already commercially available. However, the complex mechanisms underlying their beneficial activities are not fully understood yet, especially as far as the ability of *Trichoderma* to induce plant natural defences, which adds to direct competition and mycoparasitism in protecting plants against pathogens.

We have recently demonstrated that the molecular and physiological plant responses induced by *Trichoderma* species are plant genotype-specific. In order to further investigate this aspect, we have studied the effects of either of two *Trichoderma* species (*T. harzianum* T22 and *T. atroviride* P1) on several cultivated and wild tomato genotypes (*Solanum lycopersicum* and *S. habrochaites*) in terms of promotion of seed germination and plant development, protection against pathogens and transcriptional modifications of pathogen responsive genes. Plant genotype-dependent changes were recorded for all tested morpho-physiological parameters in response to *Trichoderma* treatment, indicating for the first time that the interaction between tomato and these beneficial micro-organisms is specific.

Identification of key differences between tomato genotypes in their ability to respond to *Trichoderma* treatment will pave the way to the understanding of the genetic determinants involved in this specific interaction. This will ultimately help in the selection of the most favourable *Trichoderma*/plant genotype combinations and thus favour the diffusion of Trichoderma-based biopesticides and biofertilizers for sustainable agriculture.

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P 052

Yielding ability and competitiveness of wheat cultivars against weeds

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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One of the most important, although least investigated, issues in integrated weed management strategies is to grow more competitive crops. Crop's competitiveness manifests itself either as an ability to tolerate stresses imposed by weed populations, or as a capacity to suppress these populations. Choosing a competitive cultivar, as part of an integrated weed management scheme, might reduce herbicide inputs considerably. This work examines the potential for crop's competitiveness enhancement, without interfering with traits that confer high yield. Data were obtained from two experiments in split-plot field layout, having as main plots wheat cultivars x crop density combinations and as sub-plots the presence or absence of the naturally occurring weed flora. Cultivars were selected based on their different growth attributes: two traditional tall varieties Maris Huntsman (first experiment) and Maris Widgeon (second experiment) and five modern semi-dwarf varieties Fresco, Riband, Flame, Buster and Hussar, the latter replaced by Rialto in the second experiment. Sowing densities ranged from 50 to 380 plants/m^2. Cultivars were classified according to their tolerance, as estimated by comparing yield losses in the presence of weeds relative to weed free conditions Although grain yield losses did not differ significantly among the cultivars in either experiment, there was a clear trend indicating that Fresco, Rialto and Flame suffered greatest when facing weed competition, whereas under weed free conditions all cultivars performed similarly. On the contrary, Buster suffered less grain yield loss in the presence of weeds. Similar results were observed for Riband and M. Huntsman. Furthermore, the lack of correspondence between weed dry matter in a crop: weed mixture and grain yield of the same cultivar in weed free condition suggests that competitive ability does not necessarily coincide with the high yielding properties of a wheat cultivar. For example, Fresco showed the least weed dry matter reduction compared to other cultivars, producing at the same time an appreciable yield under weed free conditions. These observations indicate that, when increased competitive ability is a desirable attribute, cultivar selection should also be based their productiveness under weed stress conditions.

Durable leaf rust resistance in durum wheat is controlled by a major QTL in the distal region of chromosome arm 7BL

P 053

Session: Preserving our future by reducing the inputs in agriculture: Reducing pesticides

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The genetic basis of leaf rust (*Puccinia triticina* Eriks.) resistance carried by the durum wheat cultivars Creso and its derivative Colosseo was studied using a recombinant inbred population of 176 lines (RILs) from the cross Colosseo x Lloyd, a set of 62 advanced lines from multiple crosses and a collection of 164 Mediterranean durum wheat accessions. The genetic materials were tested under field conditions and artificial rust inoculation. The RIL population was tested in 2006. The two accession panels were evaluated in 2006 and 2007. The percentage of infected leaf area was evaluated through the disease developmental cycle and the area under disease progress curve (AUDPC) was obtained for each field trial. A major QTL (*QLr.ubo-7B.2*) for leaf rust resistance was identified on the distal region of chr. 7BL with the favourable allele inherited from Colosseo. The QTL showed R^2 equal to 72.9% and LOD peak of 44.5 for AUDPC. The presence of this major QTL was validated by a linkage disequilibrium-based test using the two accession panels. The association results confirmed that the QTL is most probably located on the small support interval flanked by SSR markers *Xbarc340.2* and *Xgwm344.2*, with the corresponding AUDPC R^2 values ranging from ca. 10 to ca. 35% across the two panels and years. The SSR-based long-range haplotype homogeneous to cv. Creso is widespread in the cultivated durums adapted to the Mediterranean region and is particularly frequent among the elite accessions bred in Italy and at the ICARDA durum germplasm program. *QLr.ubo-7B.2* maps in a gene-dense region (7BL10-0.78-1.00) known to carry several genes/QTLs in wheat and barley for resistance to rusts and other major cereal fungal diseases. Colinearity has been reported between the distal portion of rice 6L and the distal ends of wheat group 7 chromosomes. Therefore, genes on rice 6L lying in the region between *Xbarc340.2* and *Xgwm344.2* were used in BLASTn searches to identify wheat ESTs mapped on 7BL10-0.78-1.00, thus corresponding to those rice genes. The wheat ESTs identifying single-copy genes in the rice chromosome 6 genomic sequence in reciprocal BLAST searches were selected to develop PCR markers in order to help the fine mapping of *QLr.ubo-7B.2*. Rice annotations were exploited to identify exon/intron boundaries, so PCR primers could be designed from exons to amplify predicted wheat genomic fragments spanning intronic regions, assumed to have the highest number of sequence polymorphisms. Chinese Spring wheat nulli-tetrasomic (CS-NT) chromosome substitution lines were used to design 7B-specific primers,. Of 19 primer pairs, 7 detected polymorphisms between cv. Langdon and the *dicoccoides* accession Israel A, while two identified monomorphic fragments that will be used as probes in RFLP analysis.

P 054

A predictive model for drought tolerance selection using leaf anatomical characteristics and physiological parameters in *Ziziphus mauritiana* Lam

Session: Preserving our future by reducing the inputs in agriculture: Reducing water input

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The aim of this study was to determine if selected leaf anatomical characteristics are associated with drought tolerance in *Ziziphus mauritiana* Lam. Six *Z. mauritiana* cultivars, Seb, Gola, Umran, Keitly, Q-29 and B-5/4, grown under field conditions in Israel's Negev desert were studied. Anatomical characteristics were investigated using light microscopy and histological techniques while drought tolerance was investigated by monitoring plant response (leaf transpiration, diffusive resistance, and chlorophyll fluorescence (Fv/Fm)) to water stress in two field experiments. Greater epidermis-, mesophyll-widths and xylem diameters and densities were associated with increased drought tolerance. Proportional differences between initial and final physiological parameters, before and after drought were significantly greater in Keitly, Umran, Gola and B-5/4 as compared to Seb and Q-29, indicating that the former cultivars are more sensitive to drought conditions. A predictive model for the relation between anatomical indicators and drought tolerance indicators based on physiological responses was proposed. Significant positive linear relations were determined with regression coefficients: r= 1.5, 1.4 and correlation coefficients: R^2=0.75, 0.88 for data collected at Sede Boqer and Beer Sheva respectively. The data presented may provide the basis for developing improved selection tools for drought tolerance in *Z. mauritiana* breeding programs.

ABA affects root hydraulic conductance and leaf growth via aquaporin content

P 055

Session: Preserving our future by reducing the inputs in agriculture: Reducing water input

The respective effects of ABA and drought on leaf growth and root hydraulic conductivity (Lp_r) are controversial. While it is accepted that ABA increases the expression of plasma membrane aquaporin (*PIP*) genes, it is not clear if it has a long-lasting effect on Lp_r and to what extent ABA-related changes in leaf growth rate are due to differences in Lp_r. We addressed these questions with a series of transformant maize lines deregulated in the expression of the *VP14* gene encoding NCED, a key enzyme of ABA synthesis. One sense (S) and three antisense (AS) lines with contrasting ABA biosynthesis capacities were analysed in moderate water deficit. As expected, increased ABA synthesis caused stomatal closure and increased leaf water potential. The protein contents of 3 PIP aquaporins were strongly increased in roots and leaves in S plants, and decreased in AS plants. This resulted in large differences in Lp_r measured on excised root systems, with 4-fold values in S compared with AS plants, and an intermediate Lp_r in WT. The hydraulic conductance of transpiring whole plants was also largely affected. The recoveries of leaf elongation rate and leaf water potential after rewatering were quicker in S and slower in AS plants than in WT. A model of water transfer accounted for these changes and suggested a important role for both root and leaf hydraulic conductances in the recovery rates. Overall, these results suggest that ABA has long-lasting effects on both plant hydraulic and stomatal conductances, which contribute to maintain a favourable plant water status.

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P 056

Over-expression of the HyPRP *At*CWLP forms a cell wall-plasma membrane-cytosol continuum that improves drought tolerance of transgenic *Arabidopsis* and potato plants

Session: Preserving our future by reducing the inputs in agriculture: Reducing water input

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HyPRPs (Hybrid Proline-Rich Proteins) are a group of more than 500 plant proteins which contain a large N-terminal hydrophilic proline rich domain (PRD) followed by a hydrophobic 8CM domain predicted to be localized to the plasma-membrane.
The *Arabidopsis thaliana* hybrid proline-rich Cell Wall Linker Protein (*At*CWLP), has been chosen as a model protein for estimating the role of proline rich membrane proteins in stress tolerance and the need for available free proline for their accumulation. Bioinformatical analysis and C-terminal GFP translational fusion confirmed the localization of CWLP in the plamsa-membrane with a cytoplasmic protruding C-terminal end and an external proline rich N-terminal domain.
Dehydration experiments showed that CWLP over-expressing (CWLP-OE) *Arabidopsis* and Potato (*Solanum tuberosum*) plants are more tolerant to water shortage than WT plants. Moreover, higher levels of CWLP were assembled in the PM of *Arabidopsis* cells when proline accumulation was induced during stress imposition.
Plasmolysis experiments with detached leaves followed by Confocal LSM observations revealed that while leaf cells of the CWLP-OE plants could maintain normal cytoskeleton structure and did not show protoplast shrinkage (plasmolysis) during exposure to 30% glycerol or 0.6 M sorbitol, the WT cells revealed protoplast contraction and destruction of microtubule structure. However, plants that overexpressed the CWLP lacking the cytoplasmic-protruding domain or the cell wall anchoring proline-rich-part were sensitive to plasmolysis, suggesting a role of those domains in delaying plasmolysis.
Taken together our results suggest that CWLP molecules form a cell wall-plasma membrane-cytosol continuum required for tolerance to cellular water loss and highlight the novel role of HyPRPs in plant stress withstanding.

Plant growth control by water deficit: which process(es) to lead the game?

P 057

Session: Preserving our future by reducing the inputs in agriculture: Reducing water input

The nature of plant growth limitation by environmental stresses such as water deficit is a central question for physiologists and breeders because this knowledge could help to target key processes for breeding programs and help designing plants able to maintain growth under stressful conditions. In order to grow, plant cells must loosen their walls, absorb water, reduce and process enough C and minerals to match the plant demand. Therefore, plant cell growth can be limited by cell wall rheological properties, cell or tissue hydraulics or by metabolism. In addition, cell division can be an important process to consider as cell number, together with cell size, contributes to the whole organ size. Over the past few years, our group has questioned the importance of these limitations using combinations of ecophysiological tools, spatio-temporal growth analysis and modelling in ranges of genotypes (including natural variants and mutants). Among the outcomes of these studies, I will show that (i) hydraulic limitation plays a great role on organ growth on the short term, (ii) distinct members of the cell wall loosening expansins family are downstream, unspecific targets of a range of converging developmental, genetic, and environmental cues (iii) metabolism and growth are tightly connected, possibly through a remote control of leaf expansion by starch metabolism and (iv) leaf cell size is more a consequence of growth control at higher levels of organization than vice-versa.

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Muller et al. (2007) Association of specific expansins with longitudinal and lateral expansion in maize leaves is maintained under environmental, genetic and developmental sources of variation Plant Physiol. 143(1):278-290

Aguirrezábal et al. (2006) Plasticity to soil water deficit in Arabidopsis thaliana: dissection of leaf development into underlying growth dynamic and cellular variables reveals invisible phenotypes. Plant Cell Environ. 29(12):2216-2227

Voisin et al., (2006) Are ABA ethylene or their interaction involved in the response of leaf growth to soil water deficit? An analysis using naturally occurring variation or genetic transformation of ABA production in maize Plant Cell Environ. 29(9):1829-1840

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P 058

Differential expression of sugar transporters in *Arabidopsis thaliana* during water stress

Session: Preserving our future by reducing the inputs in agriculture: Reducing water input

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When plants are subjected to water shortage, source/sink relations between organs are altered and the maintenance of some organs is favoured over that of others, leading to a successful adaptation to drought
The maintenance of sink organs rely primarily on the import of sugars (mostly sucrose) by the phloem. As a first step towards understanding the changes in sugar fluxes during water stress, we are studying the expression of selected genes coding for sugar transporters (sucrose, hexose and polyol transporters) in *Arabidopsis*. For this purpose specific macroarrays were produced and hybridized with RNA from different organs (shoot, root, floral stem) under normal and stress conditions. Particular attention was devoted to the way stress was applied and 2 different protocols were compared : plants were either cultivated on soil or hydroponically. Experiments will also be extended to different ecotypes and selected mutant lines of *Arabidopsis*. The most responsive genes will be selected for further analysis.
In a second step, the results obtained will be compared with physiological data (plant size, biomass, sugar content…) to analyse the role of sugar transporters in drought adaptation.

Growth-dependent expression of aquaporin genes in developing barley (*Hordeum Vulgare)*

P 059

Session: Preserving our future by reducing the inputs in agriculture: Reducing water input

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Water and mineral uptake is essential for the physiology of the plant cell, especially during growth. However it is not known along which pathways water moves within growing tissues. The membrane intrinsic proteins (MIP) or aquaporins are likely to play a key role, allowing the passage of water or small-molecular weight solutes through membranes. Previously, microarray expression studies have been carried out on the developing barley leaf, and several aquaporins contigs showed a differential expression between growing and non-growing and between transpiring and non-transpiring leaf regions. The aim of the project is to characterize further some of these candidate aquaporins in terms of functionality (water channel function), organ-and tissue-site of expression and regulation. Data from expression analyses and test of functionality (through expression in *Xenopus laevis* oocytes) will be shown.

P 060

OPEN STOMATA 3, an ABC transporter implicated in ABA signalling, drought and light response

Session: Preserving our future by reducing the inputs in agriculture: Reducing water input

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Mutants sensitive to progressive water deficit are characterized by excessive transpiration due to the failure of stomatal closure and can therefore be detected as cold plants by remote infrared imaging (Merlot *et al*., 2002).
Among the signalling mutants, three are collectively named *open stomata* (*ost*). The corresponding *OST1* and *OST2* genes encode an ABA-activated kinase and a P-type H^+-ATPase, respectively (Mustilli *et al*., 2002; Merlot *et al*., 2007).
The current work deals with *OST3* which encodes an ATP-binding cassette (ABC) transporter. There are over 120 members of the ABC protein in the superfamily of *Arabidopsis thaliana*. Most of them are membrane-bound proteins that transport a diverse range of substances across the phospholipid bilayer.
Characterisation of the mutant phenotype confirmed that *ost3* transpires excessively. We have also shown that the *ost3* mutations reduce seed dormancy but seed sensitivity to exogenous ABA seems unaffected. The guard cells of *ost3* are impaired in responses to ABA and light, but are normal with respect to low level of CO_2 which stimulates stomatal opening.
OST3 is expressed mainly in leaves, particularly in guard cells, but it is low in root tissues. Transgenic expression of the OST3 protein fused to GFP in the *ost3* mutant can rescue the phenotype and moreover, the fusion protein seems targeted exclusively to the plasma membrane suggesting that it has a role in intercellular transport required for ABA signal perception.
Using the yeast two-hybrid system, we found that OST3 interacts with the OST2 P-type H^+-ATPase and the OST1 protein kinase. The last observation is also consistent with the fact that OST3 can be phosphorylated by OST1 *in vitro*. Therefore we suggest that the trio of proteins identified by our genetic screen may function in the same signalling complex in mediating stomatal response.

Establishing a system for monitoring aquaporin expression under drought in strawberry (*Fragaria* spp)

P 061

Session: Preserving our future by reducing the inputs in agriculture: Reducing water input

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The strawberry genus, *Fragaria*, contains over 20 species that are present throughout the temperate regions of the world, of which the cultivated strawberry is a highly economically important soft fruit species. Increased competition between sectors for dwindling water resources means that in future only limited irrigation will be available for commercial production of strawberry. Hence it is increasingly important to exploit any tolerance of water deficits that exists within the genus. Aquaporins are transmembrane proteins of the Major Intrinsic Protein (MIP) family that control the transport of water molecules across cell membranes and there is strong evidence that aquaporins play key roles in plant water relations. Aquaporin isoforms expressed in roots are of particular interest for studying water uptake. Six partial root cDNA sequences and four genomic DNA sequences of putative *Fragaria* aquaporins of the plasmamembrane intrinsic protein (PIP) subfamily have been obtained using primers designed from heterologous sequences retrieved from the EMBL database. A system was developed that provides normal plant growth and root development similar to that in soil, and which allows for root sampling without washing the root tissue. Deficit irrigation (to replace approx 66% of evapotranspiration) was applied to *F. vesca* plants from flowering through to fruit production. Stomatal conductance, whole plant transpiration, and leaf water potential were reduced in the plants under deficit irrigation compared to control plants throughout the treatment, and leaf development was also limited by deficit irrigation. Plants were harvested at intervals after imposing the drought treatment and expression analysis was conducted on the root tissue to determine the influence of water deficit on expression of selected putative aquaporins. Patterns of expression are discussed.

P 062

Cloning and functional analyses of hop transcription factors (TFs) to develop TF biotechnology of *H. lupulus*

Session: Improving plant product quantity and quality
Developmental biology

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Hop (*Humulus lupulus* L.) is plant cultivated for commercial use in brewing industry and known in traditional medicine since medieval times. Glandular trichomes (lupulin glands) developing in hop female inflorescences (cones) contain a specific part of hop metabolome including α and β bitter acids valuable for the brewing process and other compounds like prenylated chalcones (e.g. xanthohumol) that are of particular recent interest in view of their medicinal and especially anticancerogenic properties (e.g. Stevens, J.F. and Page, J.E. -Phytochemistry 65:1317, 2004). The composition and levels of valuable secondary metabolites in hop are strongly developmentally regulated. In our previous work we described oligofamily of genes encoding for so-called "true" hop chalcone synthases CHS_H1 (EC 2.3.1.74) (Matoušek, J. e.a. -Plant Sci. 162:1007, 2002; Matoušek, J. e.a. - J. Agric. Food Chem. 54:7606, 2006) that are the key enzymes in hop, showing strong expression in maturating hop cones and having capability of catalyzing both, chalcones as precursors of xanthohumol, and bitter acids. In order to solve the principal question how *chs*_H1 genes are regulated, we analyzed *chs*_H1 promoter elements and cloned the first hop transcription factors (TFs). According to *chs*_H1 sequence, promoter binding motifs include Myb-like boxes, H-box P- and G-boxes. Initially, we cloned the first R2R3Myb factors from cDNA library prepared from glandular tissue-enriched hop cones and showed their specific expression in hop and pleiotropic action on metabolome and plant morphogenesis in heterologous transgenotes (Matoušek, J. e.a. - J. Agric. Food Chem. 53:4793, 2005; Matoušek, J. e.a - J. Agric. Food Chem. 55:7767, 2007). Recently we isolated first authentic bZIP transcription factor *Hl*bZIP1 (clone 2327) from the hop library. This TF has predicted molecular mass of 34.2 kDa and pI 8.51. A combinatorial action of *Hl*bZIP1 and other cloned hop TFs was analyzed in *P. hybrida* and *N. benthamiana* model systems. TF *Hl*bZIP1 was found to be strong activator of *chs*_H1 promoter fused to GUS reference gene in infiltrated *N. benthamiana* leaves and modulator of *Hl*Myb1 and *Hl*Myb3 TFs from hop. Simultaneously, both hop R2R3 Myb TFs were proven to be involved in *in vivo* interaction with *chs*_H1 promoter in these systems. Together with PAP1 TF from *A. thaliana* that was shown previously (Matoušek, J. e.a. - J. Agric. Food Chem. 54: 7606, 2006) to stimulate *chs*_H1, cloned hop TFs will form the basis of TF biotechnology to modulate and modify hop metabolome.

This work is supported by GAČR 521/08/0740 and NAZV QH81052.

Leaf and root growth dynamics: How can plants reach their full growth potential in a dynamically fluctuating environment?

P 063

Session: Improving plant product quantity and quality
Developmental biology

Plant growth occurs in an ever-changing environment. Prominent changes are the daily rhythms of atmospheric temperature and light intensity, to which leaves are exposed. Leaves of dicot plants cope with these rhythms by using the endogenous clock to adjust growth to predominant environmental fluctuations. In some species, such as Arabidopsis, the leaf growth rhythm shows a maximum at dawn, while in other species such as poplar, maximal growth happens at dusk. Both types of growth patterns ensure that maximal growth occurs, when water loss of the growing tissue due to transpiration is low and carbon availability is high. In leaves of monocot plants and roots, where the growing tissue is not subject to water loss via transpiration, growth is synchronized with the environment in a different way. There, growth is almost directly correlated with temperature of the growing tissue, leading to maximal growth at noon for monocot leaves and to an often constant diel growth pattern in roots. Leaves and roots live in completely different habitats, but are parts of the same organism. Thus, sudden alterations of environmental parameters in the root or the leaf habitat can affect growth dynamics of both organs strongly and unexpectedly. Elucidation of the mechanisms, how different plants manage to reach their full growth potential and optimal resource use efficiencies in a fluctuating environment, will hence require joint analysis of gene x environment and root x leaf interactions.

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P 064

Impact of long and small non-protein coding RNAs in root developmental plasticity

Session: Improving plant product quantity and quality
Developmental biology

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Long non-protein coding RNAs (npcRNA) represent an emerging class of riboregulators, which act either directly in this long form or are processed to shorter miRNA and siRNA. Plant and animals use small RNAs (microRNAs and siRNAs) as guide for post-transcriptional and epigenetic regulation. In plants, miRNAs and trans-acting siRNA (tasiRNA) result from different biogenesis pathways but both interact with target transcripts to direct their cleavage. Genome-wide bioinformatic analysis of full-length cDNA databases identified 76 *Arabidopsis* npcRNAs. Thirteen npcRNAs were antisense to protein-coding mRNAs, suggesting cis-regulatory roles. Numerous 24-nt siRNA matched to five different npcRNAs, suggesting that these npcRNAs are precursors of this type of siRNA. Abiotic stress altered the accumulation of 22 npcRNAs among the 76, a fraction significantly higher than that observed for the RNA binding protein-coding fraction of the transcriptome. One npcRNA expressed in root tissues corresponded to TAS3a, a tasiRNA precursor target of miR390. Using reporter constructs for TAS3a and miR390 loci, as well as analysis of the accumulation of their derived RNAs, we have characterized the expression pattern of the TAS3 pathway and its interaction with ARF3 targets during root development. Overexpression of another npcRNA, antisense to a coding transcript, identified a regulator of root growth during salt stress. Hence, long npcRNAs and small RNAs, sensitive components of the transcriptome, may control expression patterns of regulatory genes and contribute to modify root development and architecture in the soil environment.

Unravelling transcriptional regulatory networks that control seed maturation in Arabidopsis

P 065

Session: Improving plant product quantity and quality
Developmental biology

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In Arabidopsis seed, the accumulation of both, storage compounds (i.e. oil and proteins) or secondary metabolites (e.g. flavonoids) are tightly regulated at the transcriptional level providing interesting models for the analysis of regulatory networks.
In seed, flavonoid biosynthesis (e.g. flavonols or proanthocyanidins/PA) is controlled through the specific expression of several structural genes. For instance, the *BANYULS* (*BAN*) is specifically expressed in PA-accumulating cells of the seed coat. We have previously shown that TT2 (MYB), TT8 (bHLH), and TTG1 (WDR) form a ternary complex that directly controls the expression of some of these structural genes, including *BAN*. TT16 (a MADS box protein) has been shown to be essential for the correct differentiation of PA-accumulating endothelial cells, at least through the regulation of *TT2* expression. Interestingly, TT2-TT8-TTG1 also regulates the expression of *TT8* in a self-activated feedback loop. In addition, TT8 and TTG1 can interact with other regulators including a small MYB protein (MYBL2) modulating the activity of this complex. Taken together, the relationships between these different regulators provide an interesting model of transcriptional fine-tuned regulation.
Although the main metabolic pathways necessary for the accumulation of oil, starch, or protein during seed maturation are well characterized, the overall regulation and partitioning between these pathways remain unclear. *LEAFY COTYLEDON* genes, namely *LEC1*, *LEC2*, and *FUSCA3* (*FUS3*) encode key transcriptional regulators of seed maturation, together with ABSCISIC ACID INSENSITIVE 3 (ABI3). Interestingly, LEC2, FUS3, and ABI3 are structurally related proteins sharing a “B3” DNA-binding domain. These proteins display some partially redundant functions involving other factors such as LEC1, PICKLE, or ABI5. During the recent years, genetic and molecular studies have shed new light on the structure and robustness of this regulatory network. Interestingly, we have recently shown that *WRINKLED1* (*WRI1*, encoding a transcription factor of the AP2 family) is a target of LEC2 and is necessary for the regulatory action of LEC2 towards fatty acid metabolism.

P 066

New barley species-specific genes are required for pollen and tapetal development

Session: Improving plant product quantity and quality
Developmental biology

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Traits mediating sexual reproduction often undergo rapid evolution and are encoded by a unique set of genes also expressed during flower and seed development in plants. We described the new *jekyll* gene in barley possessing unique primary sequence but showing similar genomic organization and protein secondary structure with glycine-rich proteins, known to be involved in sexual reproduction in Arabidopsis. Earlier we studied the function of the JEKYLL during barley seed development and established its pivotal role in the nutrient supply to filial endosperm by cell death in maternal tissues (Radchuk et al., Plant Cell; 2006). Here we show that *jekyll* is also expressed in the developing gynoecium and anthers, which plays an important role in sexual plant reproduction. *In situ* hybridization and GFP expression under the *jekyll* promoter revealed the *jekyll* localization only in those tapetal cells of anthers which surround the developing pollen grains. Transgenic plants with RNAi-mediated down regulation of *jekyll* gene expression showed altered pollen formation. We supposed that the *jekyll* gene is critical for viable pollen formation being involved in nutrient supply to the developing pollen, similarly to its already established function in the developing seeds. Moreover, the transgenic plants were characterized by insufficient release of pollen from the anthers. The role of *jekyll* in tapetum degeneration and in establishment of the outer coat wall (an exine) of a pollen grain will be discussed.
Plants, pollinated with transgenic pollen, produced reduced seed set indicating involvement of *jekyll* gene in the pollination and subsequently contributing to the fertilization event. It can be due to the following reasons: insufficient accumulation of storage compounds in the transgenic pollen further leading to the pure pollen germination and growth, ineffective pollen release from the anthers, or due to insufficient pollen/stigma recognition because of the affected structure of exine. All these results underlie the crucial role of the *jekyll* gene in sexual plant reproduction. Using macro-array analysis of transgenic anthers and nucellar projection tissue from developing grains of barley we have identified regulators connected with altered nutrient supply and cell death.
Searching the EST collections, we further found two genes in barley and two genes in wheat sharing similarity with JEKYLL. All these genes are expressed exclusively during plant reproduction cycle indicating their involvement in the process.
Identification of genes that control pollen development and species-specific pollen recognition has agricultural applications. The ability to control gene flow between species would facilitate the creation of new hybrids and the containment of genetically modified plants.

Regulation of *AP1* transcription by the floral integrators LFY and FT

P 067

Session: Improving plant product quantity and quality
Developmental biology

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The transition between the vegetative phase to the reproductive phase in *Arabidopsis* is controlled by factors which integrate different environmental signals and have therefore been called floral pathway integrators: LEAFY, FT and SOC1 (Simpson and Dean, 2002). Two of these integrators, FT and LFY have been shown to be able to activate the transcription of the floral marker gene *APETALA1* (*AP1*), whose expression establishes the floral fate in a non-reversible manner in the newly formed meristems. The biochemical basis for this activation is not known. Our aim is to understand how LFY regulates the activation of AP1 and how the different environmental cues are integrated at the level of its promoter. To reach our goal we have combined biochemical (emsa and florescence anisotropy) with genetic approaches (promoter-GUS fusions).
The work presented provides insights in the biochemical interaction of LFY with different regulatory elements present in *AP1* regulatory regions (Bush *et al.,* 1999; Parcy *et al*., 1998). Several potential LFY binding sites are present in *AP1* promoter. Our studies in vitro and in plants show that LFY activates *AP1* mainly through a single high affinity site. Finally, the possible synergy between LFY and FT is analyzed as both transcription factors are capable of activating *AP1* transcription independently. This study of the integration of different environmental signals at the level of the *AP1* promoter provides more details into the tightly regulated and fine-tuned network of interactions controlling phase change in *Arabidopsis*.

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P 068

Dissection of oligogalaturonide-mediated signalling: role in defence and development

Session: Improving plant product quantity and quality
Developmental biology

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The structure and integrity of pectin in the cell wall is critical for both defence and development. Upon tissue injury or pathogen infection, homogalacturonan (HGA), the main component of pectin, is broken down into lower size fragments (oligogalacturonides: OGs). OGs activate the plant innate immune response, acting as endogenous elicitors and alerting the cells of a breach in tissue integrity. OGs are therefore signals derived from an altered-self (Host-Associated Molecular Patterns or HAMPs) and microarray analysis show that they induce responses largely overlapping the responses activated by pathogen-associated molecular patterns (PAMPs). The formation of OGs may be favoured by the interaction of fungal endo-polygalacturonases with plant cell wall leucine-rich-repeat proteins (PGIPs: PolyGalacturonase-Inhibiting Proteins). Despite their simple primary structure, OGs have a wide range of effects including the regulation of plant growth and development. Their activity as regulators of growth and development is related to their antagonism with the action of auxin. In Arabidopsis, OGs increase resistance to the necrotrophic fungus *Botrytis cinerea* independently of jasmonate-, salicylic acid- and ethylene-mediated signalling. OG-induced resistance to fungal infection is suppressed by exogenous auxin. By using both biochemical and genetic methods we are dissecting the OG perception/transduction pathway to elucidate the molecular basis of the OG/auxin antagonism and its significance in defence and development.

Increased sensitivity and decreased cost using DeepSAGE – sequence tag based transcriptomics

P 069

Session: Improving plant product quantity and quality
Developmental biology

DeepSAGE transcriptomics using DNA sequencing-by-synthesis of sequence tags provides high sensitivity and cost-effective gene expression profiling. We have developed protocols for greatly simplified sample preparation with multiplexing capacity for both 454 and Solexa (Illumina). Using the Solexa version we have prepared and sequenced tags from 2.5 µg of total RNA from 27 different tissue samples (in triplicates) from Lotus japonicus roots in symbiosis with Rhizobium in only two sequence runs. The resulting dataset contains more than 60 mio high quality sequence tags and is equivalent to the sensitivity of approximately 400 DNA microarray experiments. Preliminary analysis of the dataset implies that transcriptome analysis using tag-based sequencing platforms might be able to provide complete transcriptomics with the ability to detect even the lowest abundance transcripts.

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P 070

Cytokinins-dependent molecular mechanisms necessary for the stem cell niche mainteinance of Arabidopsis thaliana root meristem

Session: Improving plant product quantity and quality
Developmental biology

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In plants postembryonic development occurs from localized regions called meristems. In the Arabidopsis root meristem, stem cells for all root tissue types surround a small group of organizing cells known as the quiescent centre (QC). Together they form a stem cell niche (STN) whose position and maintenance depend on the combinatorial action of the *PLETHORA* (*PLT1, 2*), *SCARECROW* (*SCR*), and *SHORTROOT* (*SHR*) genes. Root meristem size and activity depend on the coordinate action of cell division in the STN and in the meristem and cell differentiation at the meristem transition zone (TZ). We recently demonstrated that cytokinins are crucial signalling molecules determining meristem activity by controlling cell differentiation at the (TZ). Here we provide preliminary data on the molecular mechanisms trough which the cytokinin mediated cell differentiation input is coordinated with genes necessary for the STN maintenance and position.

The effect of culture media (*in vitro*) on the acclimatization of micropropagated pineapple (*Ananas comosus*)

P 071

Session: Improving plant product quantity and quality
Developmental biology

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It is acknowledged that one of the most difficult and important stages of micropropagation for any plant is the acclimatization phase, i.e. the transfer from the *in vitro* environment to the glasshouse. This stressful transition can only be overcome by the plant through developing a normal physiology and functional roots. It is important especially in commercial micropropagation systems that all of the plants come through this transition as material losses can be costly. Some plant species can be more difficult than others to wean. It has been reported that pineapple microplants can be difficult to establish, with poor survival rates in some cases, a prolonged stage in the nursery (of up to 5 months) with sometimes high mortality rates and very slow root and shoot development This experiment examines the effect of a range of auxin concentrations on acclimatization and plant development during weaning.

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P 072

Identification of defense-related genes in sorghum responding to the challenge by *Colletotrichum sublineolum*

Session: Improving plant product quantity and quality
Developmental biology

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Sorghum is one of the five top cereal crops in the world. It is an annual grass that is extremely drought tolerant, making it an excellent choice for arid and dry areas. *Colletotriclum sublineolum*, one of the more important genera of plant pathogenic fungi, causes anthracnose in sorghum, affecting leaves, stems, peduncles, panicles and grains, either separately or all together. Sorghum anthracnose, one of the most important sorghum diseases, limits grain production in most growing regions around the world.
Suppression subtractive hybridization was used to identify sorghum genes induced in defense response. Two cDNA libraries enriched for transcripts differentially expressed in *C. sublineolum* -infected and uninfected sorghum (cultivar DK18, which is resistant to *C. sublineolum*) were generated. After differentially screening by membrane-based hybridization and subsequent confirmation by reverse northern blot analysis, selected clones were sequenced and analyzed. Seventy-five unique cDNA clones were obtained and assigned into fourteen different groups according to the putative functions of their homologous genes in the database. Most of these clones were not previously classified as being induced in response to pathogens. Further analysis and characterisation will be discussed.

Increased nitrite reductase activity in tobacco reveals a stay-green phenotype

P 073

Session: Improving plant product quantity and quality
Developmental biology

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The common form of nitrogen taken up by plants is nitrate and ammonium. Nitrate is reduced in the cytosol to nitrite by the enzyme nitrate reductase (NR). Nitrite itself is reduced to ammonium in the chloroplasts by the enzyme nitrite reductase (NiR) or in the plastids of non-photosynthetic organs.
Residual nitrate/nitrite in harvested leaf material can lead to the formation of undesirable compounds such as N-nitrosamines. The major nitrosating agent is nitrite (Yamasaki *et al.,* 2000; Morikawa *et al.,* 2004). By modifying the nitrogen pathway it should be possible to influence the build up of nitrite in the cell by controlling the activity of NiR.
In this study an *Arabidopsis thaliana NiR* (AtNiR) was isolated and used to transform tobacco plants under the control of a constitutive promoter (CERV – Carnation Etched Ring Virus (Hull *et al*., 1986)). The aim was to over express NiR in an attempt to alter the level of residual nitrite in the leaf. The expression of the introduced AtNiR protein was analysed by western blot. A stay-green phenotype was observed in this primary AtNiR population. Further investigation of the T_1 homozygous population demonstrated an increased NiR and NR activity, lower nitrite levels as well as a stay-green phenotype. This reveals the importance of NiR in primary nitrogen assimilation and how modification of this key enzyme affects both the nitrogen and carbon metabolism of tobacco plants.

P 074

Function characterization of an anther-specific DFR gene in *Arabidopsis thaliana*

Session: Improving plant product quantity and quality
Developmental biology

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Previously it was estimated that approximately 3500 genes were expressed specifically within the Arabidopsis anther. Characterization of anther sterile mutants in molecular level not only provides insights into the regulation of male gametogenesis, but also offers potential applications to plant breeders. An Arabidopsis T-DNA mutant with an insertion within a gene encoding a homologue of dihydroflavonol reductase (DFR) was recently identified in our laboratory. Homozygous mutant plants are entirely devoid of mature pollen. Normal silique elongation fails to proceed and no seeds are produced. . Reciprocal crossings demonstrated that the mutant pistils were fertile. Reverse Transcriptase PCR (RT-PCR) revealed a significantly high level of the gene transcripts in flowers. Furthermore, transgenic Arabidopsis plants expressing a promoter::GUS construct demonstrated that the gene is anther-specific and is associated with tapetum development.

Root enhancement by root-specific reduction of the cytokinin status

P 075

Session: Improving plant product quantity and quality
Developmental biology

The root system is an important plant organ, which anchors the plant in the soil, takes up water and nutrients and may be transformed into a storage organ. Cytokinin is a negative regulator of root growth. Here we show that transgenic Arabidopsis and tobacco plants expressing a cytokinin-degrading *CKX* gene under control of a root-specific promoter show an enhanced root system and lack the detrimental effects of cytokinin-deficiency on shoot growth. Elongation of the primary root, root branching and biomass formation was increased up to 80%. Thus it was shown that (i) targeted interference with the cytokinin status may produce localized effects and (ii) that a single dominant gene can be used to regulate a complex trait, root growth. The content of several micro- and macro-nutrients was increased consistently and significantly in leaves of *Arabidopsis* plants with an enlarged root system, which shows the potential usefulness of the approach for biofortification of plants as well as phytoremediation.

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P 076

Highly complex, random-primed domain libraries for yeast two-hybrid analysis of *A. thaliana* interactome

Session: Improving plant product quantity and quality
Developmental biology

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Yeast two-hybrid (Y2H) protein interaction screening has proven to be a method of choice for the analysis of the model plant *Arabidopsis thaliana* interactome, mostly thanks to pairwise testing[1] or screening of oligo dT-primed cDNA libraries[2, 3]. However, interaction map completeness has been limited by the use of full-length proteins and C-terminal polypeptide fragments which result in significant false negative rates.

To circumvent these limitations, we have used a domain-based strategy to construct two highly complex, random-primed cDNA libraries. The first library has been prepared from one-week-old seedlings which grew in vitro at 24°C with 16 hours of light per day. The second library was obtained by combining opened and unopened flowers. The complexity of each library is greater than 10 million independent fragments in yeast, with an average fragment size of 800 bp.

To ensure exhaustive and reproducible Y2H results, these libraries are screened to saturation using an optimized cell-to-cell mating procedure. This allows the testing of 97 million interactions per screen on average, corresponding to a 10-fold coverage of the library. As a consequence, multiple independent fragments are isolated for each interacting partner, enabling the immediate delineation of a minimal interacting domain and the computation of a confidence score[4].

These two *A. thaliana* libraries have been integrated into our high-throughput yeast two-hybrid platform and are available for screening on a fee-for-service basis. Results from representative screens performed on both libraries will be presented at the meeting.

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Chloroplast-to-nucleus retrograde signalling contributes to photoperiodic development in *Arabidopsis*

P 077

Session: Improving plant product quantity and quality
Developmental biology

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The biogenesis and function of chloroplast is largely controlled by nuclear-encoded chloroplast-targeted regulatory and functional proteins, the number of which ranges from 2100 in *Arabidopsis thaliana* to 4800 in rice. It has also become clear that retrograde signals from plastid to nucleus contribute to the regulation of nuclear gene expression. This signalling involves multiple factors including tetrapyrrole biosynthetic pathway (producing chlorophyll) and redox state of chloroplast electron transfer components. The retrograde signalling is crucial for the acclimation to environmental changes, especially under stress conditions. Light periodicity (day length) affects multiple developmental phases of plants, including timing of flowering and seasonal cycle of active growth and dormancy in perennial plants. In this paper, we demonstrate that chloroplast biogenesis is interconnected with photoperiodic development of *Arabidopsis*. Dysfunction of chloroplast biogenesis, caused e.g. by mutation in chloroplast regulatory component, impaired the perception of light periodicity, especially under short day conditions.

Thioredoxins are small regulatory proteins, which catalyze disulphide-dithiol interchange in their target proteins thus being crucial for the regulatory redox networks in cellular compartments. Thioredoxin reductases mediate the internal and external signals to thioredoxins. Mutation in the nuclear *NTRC* gene encoding chloroplast NADPH-thioredoxin reductase (NTRC) severely reduced the growth of *Arabidopsis thaliana*. Besides retarded growth, T-DNA insertion *ntrc* line showed distinct developmental and metabolic defects when grown under short-day conditions: small cell size, reduced number of chloroplasts, delayed flowering and senescence, low chlorophyll and anthocyanin content, and low carbon assimilation rate. The mutant phenotype was less severe in plants grown under long-day conditions. Transcript profiling of *ntrc* plants revealed a pattern of differentially-expressed genes coupled to the *ntrc* phenotype. Chlorophyll biosynthesis-related genes differentially expressed in *ntrc* included the key regulatory genes in this biosynthetic pathway, *HEMA1* and *GUN5*. The latter gene has also been identified as a component of retrograde signalling pathways from chloroplast to the nucleus. Furthermore, *ntrc* plants showed defects in the perception of blue light, presumably due to a distinct repression of *CRYPTOCHROME2* (*CRY2*), which encodes the blue-light receptor in *Arabidopsis*. CRY2 controls plant circadian clock that regulates the photoperiodic development in plants. Our results indicate that chloroplast retrograde signals are crucial to correct function of light perception systems in plants. Thus apart from being a source of energy, functional chloroplasts are important factors controlling plant development and responses to environmental changes.

P 078

Analysis of barley (*Hordeum vulgare*) grain development using an LC-based approach

Session: Improving plant product quantity and quality
Developmental biology

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As a consequence of increasing importance in the brewing industry, for human and animal nutrition, and plant reproduction, intensive research on cereal seed proteins has been in the focus of plant breeders. Many factors affect embryo development and accumulation of storage compounds, being the final stage of growth in cereals, and thus determine the final grain weight and quality [1]. Therefore, understanding of the physiological, metabolic, and biochemical aspects of the development of cereal caryopses is of big interest. Recently, intensive work has been performed on monitoring transport and accumulation of nutrients in plant embryos mainly based on metabolite and gene expression analysis [2, 3]. But, despite numerous reports on proteome analyses of mature and germinating barley seeds and seedlings, kinetic analyses of developing barley grains are still rare.

We aim at qualitative and quantitative protein profiling to monitor changes in protein composition during seed development using barley as model system. In our presentation we will focus on LC-based label-free techniques for comparative protein analysis. Barley seeds of various developmental stages (5, 7, 10, 12, and 16 days after flowering) were analysed. Therefore, whole crude extracts were digested and tryptic peptides directly analysed using a nanoLC system combined with ESI-Q-TOF MS/MS (Waters). Data acquisition was performed by a data independent strategy, called MS/MS^E. For data processing and protein profiling Expression Software (Waters) was utilised processing the intensities of molecular ions for quantification and the fragment and molecular ions for identification. Quantification between any two samples can be performed at either the peptide or protein level [4], in which quantification at the protein level involves mapping of detected peptides to proteins in the database. Besides, quantification at the peptide level allows also groups of unidentified peptides.

For an elucidation of statistically significant and objective kinetic patterns and biomarker identification multivariate statistics was applied. Prior to this, some data pre-processing and initial visualization was performed to ensure the quality of the data and the appropriateness of the subsequently applied clustering algorithm. A number of computational intelligence based clustering algorithms, such as Self-Organizing Maps (SOM) and Neural Gas (NG), that have proven to be highly suitable in similar context, were applied for the clustering task.

The obtained results indicate the validity of our approach for the elucidation and visualisation of changes in protein patterns during developmental processes. In fact, using LC-based approaches is especially advantageous when investigating samples with high degrees of complexity and huge dynamic range. The future task will be to transfer the developed analytical methods from the scale of the whole organ down to the level of an individual cell to monitor spatiotemporal patterns in dissected seed tissues.

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Identification of *A. thaliana* mutants affected in the iron-dependent expression of the *AtFer1* ferritin gene

P 079

Session: Improving plant product quantity and quality
Developmental biology

In plants, iron homeostasis needs to be tightly regulated to avoid deleterious effects of iron deficiency or excess. Ferritins play an essential role in these processes by sequestering iron in a bioavailable and non toxic form. Ferritin mRNAs are strongly accumulated in response to iron excess leading to protein synthesis and iron storage. To identify molecular events involved in this signalling pathway, we developed a Luciferase (LUC) reporter-gene based strategy to screen mutants affected in the regulation of the iron-responsive ferritin gene *AtFer1*. After EMS mutagenesis of *Arabidopsis* seeds carrying a p*AtFer1*::LUC construct, a screen for mutants showing a high LUC activity in iron sufficient condition was done by bioluminescence imaging. We identified 5 *dif* (Deregulated In Ferritin) mutants in which *AtFer1* expression was strongly up-regulated and we performed further studies on the *dif3* mutant. The *dif3* mutant displays chlorotic symptoms reverted by iron excess. Positional cloning allows us to identify the *dif3* mutation in the *TIC* (Time For Coffee) gene, a nuclear factor involved in circadian-clock regulation. Preliminary results indicate that the circadian clock do not directly regulate *AtFer1* expression, suggesting a novel role for *TIC*. Results regarding the physiological and molecular characterization of *dif3* will be presented.

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P 080

The use of pea (*Pisum sativum* L.) as a model plant

Session: Improving plant product quantity and quality
Developmental biology

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Pea (*Pisum sativum* L.) is an important plant for the study of plant productivity and also for plant growth correlations. The changes linked to the release of axillary buds from apical dominance were studied on pea seedlings cv. Vladan. Previously we have proved that polar auxin transport in the inhibited axillary buds is not established and soon after decapitation export of auxin from axillary buds was traced by the use of labeled [^{14}C]-IAA and the establishment of polar auxin transport was visualized by immunolocalization of PIN1 protein. Now we show that also in the stem below and above the axillary bud there are significant changes in *PsPIN1* and *PsAUX1* gene expression due to canalization of the auxin exported from the outgrowing bud. In the stem above the bud the expression of both genes drops to zero in six hours after decapitation due to the absence of an auxin source. In the stem below the bud a slower decrease of gene expression and after six hours an increase due to the auxin exported from the outgrowing bud could be observed.

This work was supported by grants of the Ministry of Education CR – 1M06030.

Functional characterization of B-type MADS box transcription factors in *Gerbera hybrida*

P 081

Session: Improving plant product quantity and quality
Developmental biology

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MADS box transcription factors are main components in the ABCDE-model of flower development that describes how organ identities are determined. The ABCDE-model is based on analysis of mutants from *Arabidopsis* and *Antirrhinum*. However, studies conducted in diverse plant species have shown interesting diversification of this model. For example, most core eudicot species have three B-function genes belonging to PI-, euAP3- and TM6-lineages while both *Arabidopsis* and *Antirrhinum* have lost their *TM6*-type gene. In contrast to the classical B-function genes that define petal and stamen identity, the function of *TM6*-type genes in Solanaceae-species has specialized in determining stamen but not petal identity.
Gerbera hybrida is a member of the large sunflower family (Asteraceae), which is characterized by composite inflorescences consisting of morphologically different types of flowers. We have studied the function of the three *Gerbera* B-type MADS-box genes: the *PI*-type gene *GGLO1*, the eu*AP3*-type *GDEF2* and the *TM6*-type *GDEF1*. Expression analysis and transgenic phenotypes show that *GGLO1* and *GDEF2* mediate the classical B-function. However, in addition to the expected interaction of the GDEF2 and GGLO1 proteins, GDEF1 strongly interacts with GGLO1 in yeast. The pattern of *GDEF1* expression deviates from the expression of conventional B-type genes, suggesting a more specialized function. Comparison of phenotypes of the transgenic *Gerbera* lines with reduced expression of *GDEF1* and *GDEF2* also suggests functional diversification.

P 082

Pattern formation of the *Arabidopsis* root

Session: Improving plant product quantity and quality
Developmental biology

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Plant vasculature is of great importance for plant growth and development as it connects all parts of the plant and allows the transport of water, nutrients, and signalling molecules. Currently, our knowledge regarding how the development of plant vasculature is regulated is relatively limited. The influence of various hormones have been emphasized by several studies, but few regulatory factors have been identified, and only one, *APL,* has been shown to determine phloem identity (Bonke et al., 2003, *Nature* 426:181-186). APL is a transcription factor necessary, but not sufficient for phloem differentiation, which implies that it represents an intermediate hierarchical level in the transcriptional network determining phloem development. We aim to characterize this network in detail and, furthermore, to identify the targets of APL using a functional genomics approach.
In order to to identify novel mutants defective in the phloem development, we have performed a genetic screen using EMS-mutagenized plants expressing the AtSUC2::GFP phloem marker (Imlau A et al., 1999, *Plant Cell* 11:309-322). This resulted in the identification of a set of novel mutants with patterning defects specific to the stele which were named *distorted root vascular pattern1-7* (*dva1-7*). These mutants all have short primary root, lack AtSUC2::GFP expression at the root tip and are accompanied by delayed and distorted phloem development. Interestingly, in dva1 and dva2 mutants xylem develop ectopically in the pericycle layer adjacent to xylem axis. Expression of APL and cytokinin signalling inhibitor, AHP6 (Mähönen et al. 2006, *Science* 311:94-98) is reduced. This suggests that *dva1* and *dva2* act upstream of the phloem identity determining gene APL and they may interact with cytokinin signalling pathway.
Dva1 and *dva2* are both necessary for normal development of vascular tissues in the root. Mapping and characterization of these genes and their putative roles in vascular development will be discussed.

Study of the genetic and physiological control of juvenility in plants

P 083

Session: Improving plant product quantity and quality
Developmental biology

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The juvenile phase (JP) of vegetative growth can be defined as the early period of development during which the plants are incompetent to initiate reproductive development, and they are effectively insensitive to photoperiod. It is during the adult phase of vegetative growth that the shoot apical meristem acquires the competence to respond to floral inducers required for the transition to reproductive phase. The juvenile to adult transition within the vegetative phase is associated with several physiological and biochemical markers whilst very little is known about the molecular mechanisms involved in this process. Significant advances in our understanding of the genetic control of developmental transitions derive from studying the vegetative to reproductive phase change in *Arabidopsis*. During this transition, FLOWERING LOCUS T (FT) protein, an output of the photoperiod pathway, acts at the apex in concert with the *FLOWERING LOCUS D* transcription factor, resulting in floral initiation.

Here we exploit *Antirrhinum* and *Arabidopsis* as model systems to understand the genetic and environmental factors that regulate the floral incompetence during JP. We approached this by hypothesizing that plants are florally incompetent during their JP due to inactivity of the photoperiodic floral induction pathway, FT protein is not translocated to the apex or that the apex is incapable of responding to FT.

A physiological assay has been developed in *Antirrhinum* that allows the length of the JP to be measured. Irradiance has been found as a key modifier of the length of JP; reduced light levels prolonged juvenility. The effect of irradiance on carbohydrate accumulation and its effect on the juvenile to adult transition within the vegetative phase were studied in *Antirrhinum.* HPLC analysis indicates a correlation between limiting photosynthetic assimilates and transition within the vegetative phase. Furthermore, experimental data suggest that a carbohydrate threshold level may be required before plants undergo a transition from a juvenile to an adult phase of plant development. Studying the effect of CO_2 on the length of the JP further confirms the linkage of the length of JP and assimilation availability.

Using the physiological assay to determine the length of juvenility in *Arabidop*sis, differences in JP length in Col-0, Ws-4 and Ler have been revealed. Col-0 was found to have the shortest JP length. Moreover, by using this assay with defined mutants, it was possible to identify genes involved in regulation of the vegetative phase transition in *Arabidopsis.*

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P 084

Mechanics of morphogenesis at the shoot apical meristem of Arabidopsis thaliana: an interdisciplinary view

Session: Improving plant product quantity and quality
Developmental biology

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During the development of multi-cellular organisms the regulators of growth and patterning must somehow interfere with physical processes to generate specific shapes. How this is achieved, i.e. how molecules assemble into complex systems with a particular form is not known in any organism. Here, we address this central issue in developmental biology using the shoot apical meristem (SAM) in the higher plant Arabidopsis. The shoot apical meristem is a population of stem cells which continuously generates aerial organs and to do so undergoes complex shape changes. Using a combination of physical, mathematical and biological approaches we provide evidence for a model where molecular networks would impact on two separable processes.
First a microtubule control of cell wall anisotropy which resists to and feeds back on local stress patterns. Second, an auxin dependent control of the growth rate which define the patterning events. Here we have investigated to which extent both actors are coupled or not, by 1) analyzing the polarity of auxin efflux carrier PIN1 and the orientation of the microtubules in the SAM, 2) analyzing the behavior of PIN1 in the absence of microtubule, 3) analyzing the dynamics of the microtubules when auxin transport is inhibited.
Using mechanical models we show that this hypothesis is sufficient to explain all morphogenetic processes observed at the shoot meristem.

Comparing non-cell-autonomomy of miRNAs and tasiRNAs in *Arabidopsis thaliana*

P 085

Session: Improving plant product quantity and quality
Developmental biology

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RNA interference (RNAi) or post-transcription gene silencing in plants (PTGS) is a mechanism which complexes of proteins and small RNA molecules (sRNA) act to downregulate gene expression in a sequence specific manner. Shortly after the discovery of the RNAi mechanism, it was already clear that the silencing signal caused by small interfering RNAs (siRNA, sRNAs originated from long dsRNA, usually related to virus replication) could spread from its production site to neighboring cells and even throughout the whole plant. The existence of a systemic silencing mechanism suggested that siRNAs themselves could act as messenger molecules. In contrast to siRNAs, the cell-to-cell spreading of other classes of sRNAs, such as microRNAs (miRNAs) and tasiRNAs, has been controversial, with some publications supporting miRNA movement and others arguing against it. We have used artificial miRNAs and tasiRNAs to address the questions of whether miRNAs and tasiRNAs move, and, if so, what determines their trafficking. Our analyses suggest that miRNAs behave in a way similar to siRNAs, spreading 10-15 cells out of their production site, while tasiRNA can travel much farther. However, genetic data suggest that miRNAs and tasiRNAs do not require the same factors necessary for the siRNA movement, suggesting the existence of an alternative trafficking pathway.

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P 086

Disturbance of *Arabidopsis thaliana* development by a potyviral infection maps to the P3/p6k1 viral genomic region

Session: Improving plant product quantity and quality
Developmental biology

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Infections of plants by viruses induce plant disease and associated symptoms result in economic losses in crops. The study of viral infections has led to the discovery of RNA silencing as a plant defence mechanism against plant pathogens and of viral suppressors of gene silencing as the viral mechanism to counter such plant defence. In addition, it has led to the unravelling of the role of small RNAs (sRNAs) in plant development. Developmental symptoms associated with plant disease have been attributed in some systems to the effects of the viral suppressors of gene silencing on the normal performance of the plant sRNA machinery.
In the model system *Arabidopsis thaliana - Turnip mosaic virus*, a potyvirus two different strains of which induce very different disturbances of the plant development, we have identified the viral determinant of developmental symptoms in the P3/p6k1 region, different from the described viral suppressor of gene silencing (HC-Pro). This result emphasises the role of the different viral proteins in disease induction, opens the way to deepen our knowledge of the potyviral proteins in the viral cycle and also to better understand plant growth regulation. Results will be presented and discussed.

Manipulation of *Arabidopsis* orthologue for characterisation of embryogenesis-related genes from the oil palm

P 087

Session: Improving plant product quantity and quality
Developmental biology

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EgPK1 and *EgHOX1* are amongst a number of genes from oil palm that have been shown to be up-regulated during somatic embryogenesis. To dissect the role of these genes *in planta,* analysis of the expression of orthologues of these genes in the model plant *Arabidopsis* is being carried out. *EgPK1* which has sequence similarity with *ATPK3* (putative serine threonine protein kinase) of *Arabidopsis* which has unknown function. This gene is closely related to animal protein S6 kinase. There are only a few members of the S6 kinase subfamily found in plants and these include *ATPK1*, *ATPK2, ATPK6 and ATPK19*. These genes appear to have a role in the response of a plant to its immediate environment. To examine the *ATPK3* expression in *Arabidopsis*, RT-PCR was performed on RNA extracted from stems, leaves, flowers, siliques and seeds. *ATPK3* mRNA is expressed in all tissues tested with slightly different levels of expression and these results were consistent with *in silico* data. Promoter:GUS analysis of *ATPK3* and *EgPK1* is being carried out to determine spatial and temporal expression of these genes. We have explored the role of the *ATPK3* gene by examining the phenotypic characteristics of a T-DNA knockout line of *ATPK3*. Our results indicate that this gene plays a key role in *Arabidopsis* development. Further analysis will be performed to dissect the function of this gene and determine whether the oil palm orthologue gene is able to rescue the *ATPK3* knockout mutant phenotype.

P 088

AtCYP38 ensures early biogenesis, correct assembly and sustenance of photosystem II

Session: Improving plant product quantity and quality
Developmental biology

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AtCYP38 is a thylakoid lumen protein comprising the immunophilin domain and the phosphatase inhibitor module. Here we show the association of AtCYP38 withphotosystem (PS)II monomer complex and address its functional role using AtCYP38 deficient mutants. The dynamic greening process of etiolated leaves as well as the early development of seedlings in light under short photoperiod failed in the absence of AtCYP38, due to problems in biogenesis of PSII complexes. Detailed biophysical and biochemical analysis of mature AtCYP38 deficient plants from favourable growth conditions (long photoperiod) revealed (i) intrinsic malfunction of PSII, which (ii) occurred on the donor side of PSII and (iii) was dependent on growth light intensity.
AtCYP38 mutant plants also showed decreased accumulation of PSII, which was shown not to originate from impaired D1 synthesis or assembly of PSII monomers, dimers and supercomplexes as such but rather from the incorrect fine-tuning of the oxygen evolving side of PSII. This, in turn, rendered PSII centers extremely susceptible to photoinhibition. AtCYP38-deficiency also drastically decreased the in vivo phosphorylation of PSII core proteins, probably related to the absence of AtCYP38 phosphatase inhibitor domain. It is proposed that during PSII photoinhibition-repair cycle the AtCYP38 protein first assists the dephosphorylation of PSII core proteins, thus enhancing the degradation of damaged D1 protein, and then guides the proper folding of D1 (and CP43) into PSII thereby making the correct assembly of the water-splitting Mn4-Ca cluster feasible even upon high turnover of PSII.

Role of a GDSL lipase-like protein as sinapine esterase in Brassicaceae

P 089

Session: Improving plant product quantity and quality
Developmental biology

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Members of the Brassicaceae accumulate sinapate esters with sinapoylcholine (sinapine) and sinapoylmalate as major compounds. Sinapine is a characteristic antinutritive seed component found mainly in the embryo of the seed and sinapoylmalate in the cotyledons of the seedling. During early stages of seed germination sinapine is hydrolyzed to sinapate and choline by an esterase activity (SCE). The enzyme has been described biochemically, but the protein structure and the corresponding gene have not been characterized. Based on enzyme purification from germinating seeds of oilseed rape (*Brassica napus*), peptide sequences of SCE were generated and used to clone a full-length cDNA. Heterologous expression of this cDNA in *Nicotiana benthamiana* conferred SCE activity to the leaf protein extract. Sequence analysis of the purified oilseed rape SCE reveals homology of the protein with a newly described group of GDSL lipases of Arabidopsis giving rise to the hypothesis that SCE has been recruited from lipolytic enzymes of primary metabolism in the course of evolution. Further biochemical experiments indicate that the SCE has broad substrate specificity towards choline esters including phosphatidylcholine. Also of interest is the reduction of the sinapat ester content due to the overexpression of the *SCE*. First results show a strong decrease of sinapine in transgenic plants. Future work includes promoter analyses, studies on gene expression and protein localization as well as evaluation of the evolution of this lipase-like enzyme family.

P 090

Development of a technique for non-invasive monitoring of intracellular phosphate changes in plant cells

Session: Improving plant product quantity and quality
Developmental biology

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Inorganic phosphate (Pi) represents as well a major essential macronutrient for all living organisms and a contributor to contamination of lakes and soils. A better understanding of phosphate absorption and transport within the plant is therefore needed.
In plants, Pi is taken up at the root periphery plant and transported to the shoot of an actively transpiring via the dead cells of the xylem. Transport of Pi through plant membranes is not only controlled by specific transporter proteins, but also by the activity of the proton pump energizing them. This control has a high significance for root hairs, endodermis and xylem parenchyma which are key interfaces for uptake, long distance transport and partitioning of nutrients.
Recently a nanosensor has been designed, a genetically encoded fluorescent indicator protein from *Synechococcus*, which can report the cytosolic phosphate levels in real time. The method is based upon the physical process fluorescence resonance energy transfer (FRET) between two reporter proteins CFP and YFP. Self-reporting cells will become an important tool to monitor metabolite fluxes in a plant non-invasively, since they will uncover how plant cells are adjusting their cytosolic metabolite concentrations to a set value. Generally, metabolite homeostasis is maintained by transport processes at plasma membrane and tonoplast.
The Pi-nanosensor was incorporated into plant cells at key interfaces using protein transduction domains as well as different transient transformation methods. The nanosensor was localised to the cytoplasm in tobacco, Arabidopsis and onion root epidermis including root hairs, as well as to leaf epidermis in tobacco. Preliminary results further suggest that the sensor may be incorporated into xylem parenchyma via Agrobacterium-mediated transient expression.

100 years after its discovery, cloning of the tomato gene *Potato Leaf* unravels a common mechanism in the regulation of leaf, shoot and inflorescence architecture

P 091

Session: Improving plant product quantity and quality
Developmental biology

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Shoot and inflorescence branching are the main determinants of plant architecture and are of agronomic importance for many crop species. In tomato, the MYB transcription factor *Blind* is an important regulator of shoot branching and inflorescence development. Database searches and molecular cloning revealed three close homologs of *Blind*: *Blind-like1*, *-2* and *-3* (*Bli1*, *Bli2* and *Bli3*). Reverse genetics approaches demonstrated that these four genes regulate leaf, shoot and inflorescence architecture in an overlapping fashion. Loss of function of these genes leads to reduced shoot branching, inflorescences with lower flower numbers, elevated vegetativeness and simpler leaves.

Bli2 acts as a key regulator of leaf complexity, lobing and serration. The phenotype of *bli2* tilling lines resembled that of the *potato leaf* mutant, first described in 1908. Sequencing of *Bli2* in six *potato leaf* accessions proved that *Bli2* and *Potato Leaf* are the same gene. The second gene analysed, *Bli3*, seems to play a less important role, but interestingly *Bli3*-RNAi plants show defects in all the three aspects of development. *Blind* and *Bli1* affect shoot branching and inflorescence development, *Blind* playing the most important role.

Taken together the phenotype of *Bli3*-RNAi plants and the fact that *Potato Leaf* and *Blind* show 92% sequence identity in the MYB domain, we suggest that regulation of shoot, inflorescence and leaf branching involve a common mechanism. Deeper insights into this mechanism are the aim of future research and may be instrumental for breeding purposes.

P 092

Pleiotropic effects to (1,3;1,4)-β-D-glucan biosynthesis during endosperm development in barley mutants

Session: Improving plant product quantity and quality
Developmental biology

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Starch and (1,3;1,4)-β-D-glucan (BG) are the most abundant carbohydrates in barley endosperm. The biosynthesis of these carbohydrates is closely linked in cereals, as the substrates for starch and BG biosynthesis, ADP-glucose and UDP-glucose, respectively are both derived from the same pathway and can easily be interconverted. An example of this is the observation that the barley *lys5* mutant is found to compensate for starch deficiency by incorporating elevated levels of BG compared to the parental variety (Patron *et. al*. 2004). A detailed characterization of the *lys5* mutant revealed that the starch deficiency was caused by a point mutation in the *Nst1* gene encoding a plastidial ADP-glucose transporter, and that the mutant had increased levels of cytosolic ADP- and UDP-glucose. Thus, the compensatory biosynthesis of BG in the *lys5* mutant is most likely due to a regulation at the substrate level. However novel work by L. Munck indicate that the perturbation of carbon metabolism in the *lys5* mutant appears to cause pleiotropic effects in not only starch and BG biosynthesis but also fatty acid and vitamin E biosynthesis (Munck *et. al.* 2007).
The purpose of this work is to carry out a transcriptional profiling of the putative pleiotropic effects of the *lys5* mutation, including genes involved in BG biosynthesis, substrate interconversion processes and sugar transport. This may contribute to the unraveling of the complex carbohydrate metabolic network in cereals and to an improved understanding of the limiting factors for biosynthesis of cell wall polysaccharides.

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The wheat GCN2 signalling pathway: Does this kinase play an important role in the protein content of wheat?

P 093

Session: Improving plant product quantity and quality
Improving yield

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When yeast and mammalian cells are starved of amino acids, general protein synthesis is down-regulated whilst genes involved in the amino acid biosynthetic pathway are up-regulated; this helps the cell maintain homeostasis and survive. This paradox is controlled by a protein kinase that phosphorylates the eukaryotic translation initiation factor eIF2α; the kinase is General Control Non-derepessible-2 (GCN2).

This activation pathway has been well characterised in all eukaryotic kingdoms except plants. Recently, however, GCN2 was cloned from Arabidopsis, suggesting that plants also have a co-ordinated response to amino acid starvation induced by GCN2. The potential link between amino acid signalling and nitrogen-use efficiency make the study of this regulatory protein kinase of particular importance in crops.

The aims of this research are to elucidate this stress response signalling pathway in wheat by identifying, cloning and characterising GCN2 as well as the upstream and downstream effectors.

Latest research from mammalian systems suggests that GCN2 may also play a wider role in virus defence and UV light stress. So far, we have cloned GCN2 and raised specific antibodies. Data will be presented on the effects of nutrient deficiency, UV light and other abiotic stresses on wheat GCN2 as well as eIF2α. Using RNAi we have manipulated GCN2 activity in wheat seeds and the whole plants and the effects on the GCN2 signalling pathway in these plants will be discussed.

P 094

Gene expression in the disease response of potato to PVY infection

Session: Improving plant product quantity and quality
Improving yield

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In nature plants encounter many factors, which have an influence to their growth and development and consequently to the plant product quantity and quality; and which are important from the agronomical, environmental and the social aspects. Among agronomical extremely important biotic factors are also viruses. The plant's responses to viral infection and disease development are different and much less explored in comparison to the bacterial or fungal infection. There are no chemical means for virus control available (such as fungicides for the control of fungi), and therefore the knowledge of plant – virus interactions is even more important as it provides basis for development of new molecular diagnostic tests, faster progress of agronomic expertise and alternative ways of virus spread control.

Plant responses to plant pathogens are complex, involving a range of signaling pathways, and show a broad spectrum of physiological and histological changes. Depending on the pathogen type, plants can exhibit resistance or sensitivity. It has become increasingly apparent that the speed and extent of the plant response determines the outcome of the plant-pathogen interaction. Hosts react to virus infection in complex ways defined by the demands of the virus, host defenses, host stress factors, cellular responses and local and remote tissue responses. Studying single components of the response in isolation can lead to limited conclusions or results, which fail to take into account the complex interactions between the different pathways of the response. Omics technologies are a major step forward in understanding plant - pathogen interactions as they offer a more holistic view of the processes involved. Expression microarrays are currently the most established technique for studying the trancriptome.

Potato virus Y (PVY) is of extreme economic importance as it is responsible for yearly losses in production of crops from family Solanaceae in Europe, and thus the subjects of investigation in many research groups all over the world. The tuber necrotic strain of Potato virus Y (PVY^{NTN}) causes potato tuber necrotic ringspot disease in sensitive potato cultivars. In our studies, gene expression in the disease response of the susceptible, tolerant and resistant potato (*Solanum tuberosum* L.) cultivars to PVY infection was investigated at different times after infection, using omics approaches, among them subtractive hybridization, cDNA microarrays and real-time PCR. The expression of several genes in several metabolic pathways during the infection process, including those involved in photosynthesis, sugar and starch metabolism, cell wall processes and secondary metabolism, suggests their important role in the potato – PVY interaction.

Increasing wheat yields through increasing grain number

P 095

Session: Improving plant product quantity and quality
Improving yield

Wheat (*Triticum aestivum* L.) is the staple food for nearly 35% of the world's population. Wheat yield potential has been estimated at around 21 ton ha^{-1} but the average of observed wheat yield in the world is less than 3 ton ha^{-1}. It has been estimated that the global demand of wheat by the year 2020 will be around 1050 million tonnes. The human population by the same year is estimated to reach 6.4 billion, increasing annually at a rate of 1.06%. With limited prospects for an increase in available arable land area, the challenge for wheat breeders is to increase wheat yield per unit of land in order to satisfy the growing demand.

From the cross of a 'large-ear' spring wheat (Line14) developed at CIMMYT, Mexico and the UK winter wheat Rialto (a high photosynthetic efficiency wheat), 138 double haploid lines were developed. The Line14 parent expresses a longer rachis with 2-3 more spikelets than conventional CIMMYT spring wheats. A total of 69 lines were identified as photoperiod insensitive types and grown in Ciudad Obregon, Sonora, Mexico in 2004-5 and 2005-6. The full 138 DH lines were grown in the UK both at Cambridge and Sutton Bonington in 2005-2006 and 2006-7, as Ear Rows and at commercial planting density (300 seed/m^2; Sutton Bonington, 2006-7). Detailed physiological analysis for up to twenty-three traits related to ear fertility and yield components were measured in three different environments. Using 411 DArT and 80 microsatellite markers a linkage map for the L14 x Rialto DH population was developed and Quantitative Trait Loci (QTL) analysis was carried out.

Initial results from this analysis will be presented and the potential for the 'large-ear' phenotype to increase wheat yields will be discussed.

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P 096

A novel role of pectic arabinan: involvement in resistance against the fungal pathogen *Botrytis cinerea*

Session: Improving plant product quantity and quality
Improving yield

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The cell wall is one of the most important structural components of plants. The wall defines cell shapes, provides strength to withstand the turgor pressure, influences cell development, and serves as the last physical barrier against invading pathogens. Pectins constitute ca. 30% of the cell wall polysaccharides and fill space between the load-bearing cellulose-hemicellulose network. Only a few examples are known about the defense roles of the pectin polymers. Previously studies have identified that oligogalacturonides released after digestion of homogalacturonan by pathogen-derived endopolygalacturonase elicits a defence response in the host, thereby functioning as an endogenous signal for the host defence activation.

We have recently identified a pectin mutant of *Arabidopsis thaliana*, *arabinan deficient 1 (arad1*), defective in the pectic arabinan biosynthesis. Detailed cell wall composition analyses identified that *arad1* has 70% less arabinose in the pectic rhamonogalacturonan I fraction. The mutant did not show a visible growth phenotype distinct from the wild type, indicating that arabinan is not essential for plant growth. However, *arad1* mutants showed increased susceptibility to the necrotic fungal pathogen *Botrytis cinerea*, while they appear to show the wild-type level of susceptibility to the bacterial pathogen *Pseudomonas syringae*. These results indicate that arabinan plays a role in interaction with *Botrytis* by alleviating the disease development. Molecular mechanism that account for the arabinan function in disease alleviation is currently under investigation. We have identified arabinan-degrading activities in the supernatant of the *Botrytis* culture both by AZCL-conjugated arabinan plant assay and LC-MS analysis. Our current working hypothesis is i) that oligoarabinosides released by the fungal arabinan-degrading enzymes act as an elicitor that activates the host defence response and thereby diminishes the disease development, or ii) that arabinan plays a structural role in reducing the rate of fungal penetration and/or diffusion of virulence factors within the host wall.

The current study provides the first evidence that the pectic arabinan plays roles in plant defence. Detailed characterization of the underlying molecular mechanisms will be presented.

Genetic dissection of a QTL for grain size in wheat

P 097

Session: Improving plant product quantity and quality
Improving yield

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The yield of wheat is determined by the factors spike number per plant, grain number per spike and grain weight. Grain size also constitutes an important component of the domestication syndrome of crop plants. Since these traits are usually inherited in a quantitative fashion the use of the usual mapping populations, such as recombinant inbreds or doubled haploids only leads to the detection of QTLs, however, does not allow to trace the single genes. Therefore the concept of advanced backcross breeding proposed by Tanksley and Nelson (1996) and the subsequent development of nearly isogenic lines (NILs) was applied to detect and further dissect a QTL for grain weight into a single Mendelian gene.

The previously described QTL for grain weight *QTgw.ipk-7D* associated with microsatellite marker *Xgwm1002-7D* was originally detected in a BC_2F_3 advanced backcross population of the German winter wheat variety 'Prinz' and the synthetic wheat line W-7984 (lab designation: M6) (Huang et al, 2003). We developed nearly-isogenic lines (NILs) carrying introgressions of M6 in the genetic background of 'Prinz' with varying size on chromosome 7D. The BC_4F_3 NILs had a 10% increased 1000-grain weight compared to the control group and the recurrent parent 'Prinz' and 84.7% of the phenotypic variance could be explained by the segregation of marker *Xgwm1002-7D*. The trait increased grain weight was strongly correlated with increased grain length and increased plant height, while the trait grain number per ear was stable between the NILs and the control group. It was possible to delimit the QTL *QTgw.ipk-7D* to the interval *Xgwm295-Xgwm1002* which is located in the most telomeric bin 7DS4-0.61-1.00 in the physical map of wheat chromosome arm 7DS. We propose the presence of a gene modulating grain weight with the preliminary designation *gw1* which has a recessive or intermediate mode of inheritance for the phenotype large grain. Furthermore, our data suggest the presence of a novel plant height reducing locus *Rht* on chromosome arm 7DS of 'Prinz'. The two phenotypes large grain and increased plant height may reflect the pleiotropic action of one gene or may be caused by two linked genes. Currently we are in the process of developing a large number of homozygous recombinant lines for a further fine mapping of *QTgw.ipk-7D.*

In general, our data support the concept of using nearly isogenic introgression lines for validating and dissecting QTLs into single Mendelian genes and open the gateway for map-based cloning of a grain-weight QTL in wheat.

P 098

Regeneration and genetic transformation of Russian sugar beet cultivars and production of herbicide-resistant plants

Session: Improving plant product quantity and quality
Improving yield

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Sugar beet (*Beta Vulgaris* L.) is traditional and basic domestic source for sugar production in Russian Federation. On average 25-30% its yield are lost because of weeds. Over the half of the sugar beet cultivation costs are incurred as a result of weeds control. Genetic engineering methods allow to product sugar beet plants with improved agricultural characteristics, for example, herbicide resistance.

The aim of our research was to study regeneration and transformation competence of Russian sugar beet cultivars and to produce transgenic lines expressing the *bar* gene, which determines resistance to herbicides based on the phosphinothricin.

In vitro regeneration techniques have been optimized for seven lines and varieties of sugar beet (*Beta vulgaris* L.) of Russia selection. The frequency of shoot regeneration from somatic cells and tissues varies from 10 to 97% depending on the explant type, the culture-medium composition, and the genotype. The *Agrobacterium*-mediate transformation parameters were optimized (the explants pre-cultivation time, the time of co-cultivation with *Agrobacterium* in liquid and solid media). Also selection system of the transgenic cells on phosphinothricin (ppt) (the ppt concentration and the time of selection) was optimized. Thus, it be came possible to avoid the formation of chimerical shoots among the initial transformants. Transgenic plants of the five varieties were obtained via an *Agrobacterium tumefaciens* transformation system, using the optimized regeneration and transformation techniques. Stable integration of the *bar* gene into the genome was confirmed by Southern blot analysis. Transgenic plants showed high resistance to Basta herbicide under the following conditions: *in vitro* (400 mg/l ppt), greenhouse (9 l/ha) and field conditions (3 l/ha). Now we research *bar* gene expression in sugar beet plants generations.

Genetic dissection of seasonal vs recurrent flowering for better management of the production of fruits in the cultivated strawberry

P 099

Session: Improving plant product quantity and quality
Improving yield

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In France, among the most important agricultural productions, strawberry (*Fragaria*) is important for rural development and for maintaining an activity in rural regions. Today, this species is subjected to evolutions due to the global warming and due to modification of agronomical techniques (e.g. development of soiless culture). The new challenge for this crop is to control flowering in order to better manage fruit production. In this species, two different modes of flowering exist. These modes affect the flowering duration and therefore the period of fruit production. Flowering can occur only once a year in spring (seasonal-flowering genotypes) or can occur all along the growing period of the plant (recurrent flowering genotypes). Between these two extremes, all intermediate modes of flowering can exist. Our research aims to better characterize the molecular and genetic determinism of flowering. The applied objective of this research is to develop novel strawberry cultivars with extended production for better competitiveness and easier management of farmer work. This work is conducted in collaboration with private companies in order to give benefit to consumers as to strawberry industry.

Mapping of quantitative trait loci (QTL) controlling the flowering duration of cultivated strawberry (*Fragaria* x *ananassa* Duch., 2*n*=8*x*=56) can be used to provide a better understanding of its genetic control and to develop marker assisted selection for breeders. For this purpose, a segregating population of 213 individuals of a cross between 'Capitola' and CF1116, two genotypes with contrasting flowering modes, was used for genetic mapping. In order to evaluate the seasonal *vs* recurrent flowering, the number of inflorescences was measured at the end of July for seven years. In addition, the number of runners was evaluated at the same period but only for three years. For the number of inflorescences, a total of two significant QTLs was detected by composite interval mapping, both located on the female map. One of these QTL was detected each of the seven years of observation. Since its percentage of phenotypic variance explained was high to very high according to the year (from 20% to 88%), it can be considered as major QTL. Considering the polyploidy of the cultivated strawberry, these two QTLs were localized on linkage groups belonging to different homoeology groups. For the number of runners, one significant QTL was detected two of the three years of observations. This QTL colocalized with the major QTL linked to the flowering mode (seasonal *vs* recurrent flowering) on the female map and its percentage of phenotypic variance explained ranged from 18% to 50% according to the year.

The identification of QTLs linked to the mode of flowering is a first step to understand genetic and molecular control of everbearing strawberry in order to better manage strawberry production.

P 100

Characterization of genes improving cotton fiber quality from allotetraploid (*Gossypium hirsutum*) cultivated cotton and its diploid progenitors

Session: Improving plant product quantity and quality
Improving yield

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In cotton, the most important industrially cultivated crop for its fibers, fibre initial cells undergo a rapid cellular re-programming around anthesis to form the long cellulose fibres. On the day of anthesis the cotton fibre initial cells balloon out from the ovule surface and so are clearly distinguished from adjacent epidermal pavement cells. Microarray experiments indicated that in these cells are predominantly expressed gene families including proteins involved in cell wall biosynthesis, lipid metabolism, and cuticle biosynthesis, indicating the essential role of these cellular components during rapid elongation. To elucidate the role of genes involved in cotton fiber development we isolated and characterized genomic clones encoding cotton xyloglucan endotransglycosylase/hydrolases (XTH) and Profilin (PRF) isoforms from cultivated cotton (*G. hirsutum*) and its diploid progenitors (*G. arboreum* and *G.raimondii*). Furthermore we analyzed the expression patterns in different *G. hirsutum* varieties, differing significantly in fiber percentage, as well as in the allotetraploid species *G. barbadense* that has significantly higher fiber length in comparison to *G. hirsutum*. Quantitative real time PCR and High Resolution Melting experiments indicated that in *G. hirsutum* cultivars, in cotton fibers during early stages of fiber elongation different expression patterns exist among the XTH and PRF homologs from G. arboreum and G. raimondii. We also isolated the promoters of XTH and PRF and we performed *in silico* analysis to identify putative regulatory elements. DNA blotting analysis indicated that at least two copies of XTH and PRF are present in *G. hirsutum* whereas the diploid progenitor species *G. arboreum* and *G. raimondii* has only a single copy.
These results suggest that the XTH and PRF genes are positive regulators of both cotton fiber elongation and density and suggest that overexpression of these genes in cotton species with low fiber yield would probably result in improvements of cotton fiber characteristics.

Characterisation of bioavailability of distinctive pesticides by applying model-plants and optimised extraction method

P 101

Session: Improving plant product quantity and quality
Food and feed

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The bioavailability of pesticides represents a considerable factor in respect of both environmental protection and food safety as pesticides are among the most frequently applied agrochemicals. Estimation of bioavailability is indispensable for appropriate food safety and risk assessment of plants. Up to now comparative study on bioavailability of distinctive pesticides from several soil types in cases of more than one model plants has not been performed yet.

The objective of this study is to model the plant uptake of pesticides in wheat *(Triticum aestivum)* and corn *(Zea mays)* samples, in order to acquire information regarding ″biologically incorporated″ amounts of examined pesticides. Bioavailability of 5 pesticides (pirimicarb, diuron, simazine, Acetochlor, chlorpyrifos) has been investigated in cases of three soil types (alluvial, brown forest and sandy soil). Comparison of efficiency of different extraction models was also implemented, as 4 methods have been applied to gain deeper insight into the biological relevance of pesticide application. 2 extracting solvents (humic acid, $CaCl_2$ solutions) were found to be the most appropriate procedures for further studies.

One hundred pregerminated wheat, and in parallel 25 corn seedlings were potted in pesticide-treated soils (100ppm, 50ppm, 20ppm). After 21 days plants were harvested and soil samples collected. Pesticide residues from plant and soil were determined by GC-MS technique.

The examined soils adsorbed pesticides to significantly different extent, the highest amounts of pesticides were determined in case of brown forest soil. The bioavailable and accumulated amounts of pesticide were observed in the largest quantities in case of soils treated by 100ppm of pesticides, and the detected amounts changed in parallel with the decrease of the initial concentrations of pesticides.

It was realised that pesticides were accumulated not equally in different organs of the plants. Depending on the soil type, aerial parts of wheat sample's treated with 100ppm simazine contained 3.43-4.85 µg/g pesticide at the end of the cultivation period, while in the roots approximately 1.5 µg/g of simazine could be detected. In the 21-day-long period 0.05-0.11 µg/g acetochlor amounts were detected in wheat root, while no traces of pesticide were observed in the aerial parts of wheat. Chlorpyrifos may not penetrate into any segments of test plants. Considerable amounts of pirimicarb (47-55µg/g) were detected in soils with significant differencesin case of the different soil types.

Soil samples sowed by maize contained less pirimicarb (18µg), in case of wheat (49µg). The aerial part of maize contained higher amounts of pirimicarb (36µg/mg) than the wheat samples (14µg/mg) (100ppm). Pirimicarb could be detected in roots of maize, while in case of wheat the regained amounts were under the detection limit. Neither wheat nor maize segments contained detectable amounts of pesticide in case of 20ppm concentration. Wheat and maize samples incorporate diuron mostly into roots.

P 102

Identification of Arabidopsis mutants with an altered response to zinc deficiency

Session: Improving plant product quantity and quality
Food and feed

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Zinc is an essential micronutrient in all organisms, being the co-factor or structural element of many enzymes and other proteins. A tightly regulated network of metal transport, chelation, trafficking and sequestration exists to insure a constant supply of zinc: the zinc-homeostasis network. Despite the emerging knowledge on the nature of zinc uptake and translocation transporters in plants, it is not known how these genes respond to changes in the internal and/or external zinc status. Thus the regulation of the zinc-homeostasis network remains unraveled.
A better understanding of the zinc-homeostasis network will be important for the future application of phytoremediation of metal-polluted soils and will also have important implications for human health, through improved nutritional quality of plants, and for ensuring stable crop production on marginal soils.
In this work we describe a mutant screening approach developed with the aim of identifying Arabidopsis mutants that have an altered response to zinc deficiency and identifying the genes in the signalling pathway leading to the zinc deficiency response.
ZIP4 is an Arabidopsis zinc deficiency responsive metal transporter gene that shows strong induction and high expression upon zinc deficient conditions (Grotz et al., 1998; van de Mortel et al., 2006). A transgenic Arabidopsis line, stably transformed with a pro*ZIP4*::GUS construct and showing a stable GUS-Zn-deficiency induced expression, was mutagenised by gamma-irradiation and the M2 progeny was screened for mutants with an altered GUS-expression using a non-lethal GUS assay (Martin et al., 2006).
With the described mutant screening and the developed mutagenised population it was possible to identify positive mutants. Here we show four positive mutants identified, their phenotypes upon zinc deficiency/sufficiency supply and their endogenous *ZIP4* gene expression. The most interesting mutants will be genetically mapped and used for positional cloning of the gene and further characterization.

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Molecular dissection of sensory traits in the potato tuber

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Session: Improving plant product quantity and quality
Food and feed

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Tubers from *Solanum tuberosum* group Phureja cultivars score consistently higher than *S. tuberosum* group Tuberosum cultivars in professional sensory evaluation panels. A recently developed 44,000-element potato microarray was used to identify tuber gene expression profiles that correspond to differences in tuber flavour and texture. Gene expression was compared in two *Solanum tuberosum* group Phureja cultivars and two *S. tuberosum* group Tuberosum cultivars; 309 genes were significantly and consistently up-regulated in Phureja whereas 555 genes were down-regulated. Almost half of the genes in these lists can be identified from their annotation and amongst these are candidates that may underpin the Phureja/Tuberosum trait differences. For example, a clear difference in the cooked tuber volatile profile is the higher level of a sesquiterpene compound in Phureja compared with Tuberosum. A sesquiterpene synthase gene was identified as being more highly expressed in Phureja tubers and its corresponding full-length cDNA was demonstrated to encode the appropriate sesquiterpene synthase. Other potential "flavour genes", identified from their differential expression profiles, include those encoding branched-chain amino acid aminotransferase and a ribonuclease suggesting a mechanism for 5'-ribonucleotide formation in potato tubers on cooking. Major differences in the expression levels of genes involved in cell wall biosynthesis (and potentially texture) were also identified including genes encoding pectin methylesterase, pectin acetylesterase and xyloglucan endotransglycosylase.

In addition to volatile compounds, tastants associated with the potato matrix have been put forward as key determinants of potato flavour. Such compounds include the tastants giving rise to the umami taste sensation. Phytochemical analysis was used to assess the levels of the major umami compounds in boiled potato tubers, in cultivars previously assessed for sensory quality. The free levels of the major umami amino acids, glutamate and aspartate and the umami 5'-ribonucleotides, GMP and AMP, were measured in potato samples during the cooking process. The levels of both glutamate and 5' nucleotides were significantly higher in mature tubers of two *Solanum phureja* cultivars compared with two *Solanum tuberosum* cultivars. Calculation of the equivalent umami concentration for five cultivars showed there were strong positive correlations with flavour attributes and acceptability scores from a trained evaluation panel suggesting that umami is an important component of potato flavour.

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High sugar ryegrasses for livestock systems - Gene expression profiling of cultivar, tissue and temperature dependent fructan accumulation

Session: Improving plant product quantity and quality
Food and feed

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There has been mounting interest over the production and environmental benefits from using perennial ryegrass cultivars bred to have higher water soluble carbohydrate content (high sugar grasses - HSGs). HSGs offer opportunities to mitigate greenhouse gas emissions (nitrous oxides) from grazed pastures and to improve meat and milk production in livestock.
The major reserve carbohydrates in cool-season grasses are fructans, which accumulate predominantly in pseudostems. HSGs have been bred in the UK by IGER and these cultivars are targeted to accumulate high levels of fructans in the blades, the major grazed component of pasture grasses. However, previous experiments with these HSGs have revealed critical constraints to the expression of the trait, possibly showing a strong gene x environment interaction. Achieving a more consistent, and greater than current, expression of the high sugar trait requires a better understanding of the molecular regulatory mechanisms of fructan biosynthesis, accumulation and relocation.
Fructans in ryegrass are synthesised by several fructosyltransferases (FTs). First, 1-SST (sucrose: sucrose 1-FT) initiates *de novo* synthesis of the trisaccharide 1-kestose. 1-FFT (fructan: fructan 1-FT) transfers fructose from 1-kestose or fructans with a higher degree of polymerisation to a variety of pre-existing fructans or sucrose resulting in fructans with (□2-1) linkages only. The biosynthesis of the neoseries fructans, comprising the majority of fructans in ryegrass, requires 6G-FFT (fructan: fructan 6G-FT) activity leading to (□2-1) or (□2-6) linked fructose units, respectively.
In the present study, we tested eight *L. perenne* breeding lines for fructan accumulation under three different temperature regimes in controlled environment chambers. Three of these lines showed significantly higher levels of fructans in blades at warm temperatures and were selected together with one control grass (Fennema) for detailed gene expression analysis. Eight genotypes of each line were clonally propagated, grown at three different temperature regimes (20°C/ 20°C, 20°C/ 10°C, 10°C/ 10°C) and separated into pseudostems and blades. Quantitative RT-PCR was used to analyse the expression of 1-SST, a putative 1-FFT, two 6G-FFT isoforms, and a fructan exohydrolase (1-FEH).
Fructans were increased 2.5 to 6.6-fold in both blades and pseudostems at 10°C/ 10°C compared to 20°C/ 20°C, depending on the ryegrass line. All five genes were also highly expressed at 10°C/ 10°C compared to higher temperatures, but only in pseudostems. In contrast, expression of 1-FFT, 6G-FFT, and 1-FEH was lowest at this temperature in the blades.
Fructan levels were 3 to 5-fold higher in pseudostems compared to blades and all five genes analysed were significantly more expressed in this tissue. There was a significant line x tissue interaction, showing that only the putative 1-FFT and one of the 6G-FFT isoforms were highly expressed in blades of the line with the highest levels of fructans. Interestingly, the second 6G-FFT isoform was not expressed in this line, but showed highest expression in the control grass Fennema. This clearly indicates that fructan related gene expression is differentially regulated in ryegrass lines differing in their capacity to accumulate high levels of fructans in the blades. These findings also show that transcriptional activation of structural fructan genes is likely to play a major role in fructan accumulation and the identification of transcriptional regulators might offer novel opportunities for the manipulation of fructan biosynthesis in pasture grasses.

Improved carbon supply results in higher protein content and increased yield of winter wheat grains

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Session: Improving plant product quantity and quality
Food and feed

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Generally, cereal grain yield increases by improved storage of the low-energy compound starch at the expense of the high-energy compound protein. Consequences of decreased grain protein content are both, reduced baking and feeding quality. The reduced feeding quality requires expensive protein-rich additionally supply. In cooperation with a regional breeding company, we decided to use transgenic approaches to meet the challenge of negative correlation between yield and grain protein content in winter wheat.
Based on scientific knowledge about seed-specific activities of transport proteins, we pursued a strategy to improve the sucrose transport into developing seeds to achieve positive effects on grain filling. To reduce the possibility of endogenous suppression, a sucrose transporter from another cereal crop species, *Hordeum vulgare,* was integrated into the genome of a selected elite wheat cultivar.
At the moment, seven transgenic winter wheat lines exist harbouring at different integration loci one copy of the transgene in the homozygous state (HOSUT lines). All lines showed significantly increased grain protein content, but no reduction of the thousand grain weight under green house conditions. Based on a newly developed transformation technology, five of the lines are free of any marker gene. Line HOSUT 10 was grown under different environmental conditions (green house, semi-conditioned green house with growing of the plants in natural soil, field conditions). The line shows significantly increased protein yield per plant (up to 137%) under all growing conditions. The higher protein yield results from both, increased grain protein content and increased yield.
HOSUT 10 was crossbreed into seven selected elite cultivars to test the stable occurrence of transgene-mediated characteristics in different genetic backgrounds. 104 individual transgenic lines carrying the HOSUT gene resulted from crossing. They were tested under field conditions. For 72% of the field-grown progenies, significantly increased grain protein content was measured. The grains contain up to 18.9 % grain protein in comparison to 15,6 % and 15.2 % measured for the parental lines. Currently, a second field trial is running to confirm these previously obtained results and, in addition, to estimate yield-related parameters.

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ERGI (European Rosaceae Genomics Initiative)

Session: Improving plant product quantity and quality
Food and feed

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The plant family Rosaceae contains many of the most important food crops and ornamentals grown in Europe, such as almonds, apples, apricots, black-blueberries, cherries, nectarines, peaches, pears, plums, raspberries, strawberries, roses and various other ornamentals. In 2006, the commercial import/export value of sales of various types of foods produced from these crops in the EC countries exceeded 10 billion euros with a total production of 23.5 million tons on 2.3 million ha (www.fao.org). Rosaceous crops are, overall, valuable targets for the development of functional foods as they are rich in health-related compounds including vitamins, fibers, carotenoids, and beneficial polyphenols. Rosaceous ornamentals have a strong aesthetic value and as such are important for the well-being of humankind. The Rosaceae also includes some timber species and medicinal or nutraceutical plants. Thus, a collective research strategy based on genomics of this family, has the possibility to solve many key issues critical for sustainable and profitable production of rosaceous crops in Europe. Key issues for European Rosaceae industry are quality, biotic stress resistance and abiotic stress tolerance, labour inputs and diversification of production systems for profitable production. Efficient and sustainable production of safe, high-quality and health-promoting food is recognized as a priority by the EU. To these ends, the mission of ERGI (European Rosaceae Genomics Initiative) is to contribute to the improvement of the quality of life and well-being of Europeans by promoting the development of novel and improved fruit and ornamental products derived from rosaceous crops. This will be achieved through the development of genomics-based tools and resources that will generate new knowledge and will lead to targeted, marker-assisted breeding of better performing cultivars, insertion of desirable natural genes from wild germplasm by means of cisgenesis, and more environmental-friendly agricultural and commercialization practices that will enhance and preserve the quality of fruits and flowers that reach the marketplace. The membership of ERGI is composed of European scientists from public and private research institutes that are involved in research projects focussing on rosaceous genomics, genetics, proteomics and breeding. Despite the fact that national and international collaboration among research groups involved in Rosaceae genomics projects have already been initiated, a coordinated action at the EU level is needed along with substantial EU and national funding to face key issues critical for the sustainability and profitability of the European Rosaceae-based industries and to meet consumer needs over the coming years.

Valorisation of waste foliage in industrial chicory

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Session: Improving plant product quantity and quality
Food and feed

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Different varieties of chicory (*Cichorium intybus* L.) are cultivated for their leaves (salads, witloof endive, forage) or roots (industrial chicory). The roots of industrial chicory are processed to obtain products used in pharmaceutical, food and feed industry: e.g. inuline, flour for bakery and a coffee-like drink named "chicorée". This means that, in contrast to salad chicories, the foliage of industrial chicories are not harvested, and their waste cause environmental and phytohygienic problems. Studies have demonstrated that chicory byproducts could form a natural source of antioxidants, known for reducing the risk of cancer and vascular diseases. Breeding of chicory as a functional food, and as a source of natural antioxidants that could replace synthetic ones, requires an understanding of the genetic control of the metabolism of these molecules. To identify genes implicated in the production of antioxidants in chicory, especially phenolic compounds, we will apply a combination of QTL (Quantitative Trait Loci) analysis and candidate gene approach. This requires a high through-put method to extract and identify the molecules of interest adapted to genetic analysis.

The method we developed simplifies sampling and extraction, and reduces the variability induced by the manipulator. A progeny of 192 genotypes, already used for the construction of a molecular genetic map for chicory, was analysed by a chemical test for antiradical activity (DPPH) and by HPLC. In contrast to the parents of this progeny, significant differences were found between the 192 plants for the concentration of chlorogenic and chicoric acid identified by HPLC, and for the antiradical activity (AR). In addition a good correlation was found between the AR and the concentration of chicoric acid (R^2= 0.85), suggesting that chicoric acid is at least in part responsible for the AR found in our extracts. Other compounds found by HPLC analysis but not identified yet, will be determined by mass spectrometry.

For 192 genotypes, three to five clones were obtained by cutting and the analysis of these plants will reveal if differences in the production of phenolic compounds is under genetic control. This information, associated with the molecular markers of the genetic map, might identify QTL involved in the biosynthesis of antioxidant molecules.

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Harvesting maturiy and ripening temperature to degreen harumanis mango

Session: Improving plant product quantity and quality
Food and feed

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A study was conducted to determine the effect of harvesting maturity and ripening temperature to degreen Harumanis mango (*Mangifera indica* cv Harumanis). The fruits were harvested at 11, 12 and 13 weeks after flower anthesis. The mangoes were divided into four lots with each lot containing six fruits of mangoes. The fruits were packed in 35.5 cm x 29 cm x 15 cm of fibre board cartons and induced for ripening using 50 mL/L of ethylene gas. The fruits were then kept in the chamber of 15, 20, 25 and 30°C temperatures with 75% relative humidity for 24 h. After 24 h, the fruits were removed from the chamber and allowed to ripen at 25°C of 75% relative humidity. The fruits were analyzed for its quality characteristics at day 0, 1, 3 and 5. The quality characteristics were determined for peel and pulp colour, flesh firmness, soluble solids concentration (SSC), titratable acidity (TA), pH, vitamin C, water loss and chlorophyll content. The experimental design was a randomized complete block design with factorial arrangement. The experiment was repeated three times. All the data was subjected to analysis of variance while the mean separation was separated by least significant different. From the study conducted, the chromaticity (C*) and hue (h°) of peel, pulp lightness (L*), flesh firmness, TA and SSC were not affected by the different harvesting maturity. However, the L* values of peel, C* and h° values of pulp, pH, vitamin C content, water loss and chlorophyll content were affected by the different harvesting maturity. The ripening temperatures have a significant effect on the pulp colour (L*, C* and h°), flesh firmness, pH and water loss but not affecting the peel colour (L*, C* and h°), TA, SSC, vitamin C and chlorophyll content. The ripening days shown a significant effect on L* values of peel, pulp colour (L*, C* and h°), flesh firmness, SSC, pH, vitamin C content, water loss and chlorophyll content but did not have a significant effects on C* and h^o values of peel and yet TA. There was no significant interaction effects between harvesting maturity and ripening temperatures on peel colour (L*, C* and h°), pulp colour (L*, C* and h°), flesh firmness, TA, SCC, pH, vitamin C content, water loss and chlorophyll content. The interaction between the harvesting maturity and ripening days have significant effect on the L* and C* values of peel colour, flesh firmness, TA, water loss and chlorophyll content of the fruits but not the h^o values of peel colour, pulp colour (L*, C* and h°), SSC, pH and vitamin C content. The interactions between ripening temperatures and ripening days have significant effects on h° values of pulp colour, flesh firmness, pH and water loss. However, there were no significant effects on peel colour (L*, C* and h°), pulp lightness and chromaticity, TA, SSC, vitamin C and chlorophyll content. The interaction between harvesting maturity, ripening temperatures and ripening days were not significant on peel colour (L*, C* and h°), pulp colour (L*, C* and h°), flesh firmness, TA, SSC, pH, vitamin C content, water loss and chlorophyll content. The result indicated that different harvesting maturity and ripening temperatures failed to degreen Harumanis mango. The peel colour of Harumanis mango remains green even at the end of the ripening day 5.

Iron, zink and selenium content of lentil (*Lens culinaris* Medik.) lines in winter and spring crop

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Session: Improving plant product quantity and quality
Food and feed

In Haymana, Turkey (Altitude 1050 m) 64 green lentil lines were planted as spring and winter crop in 2006/07 season. Lines and cropping seasons were evaluated for iron, zinc and selenium content. Lentil lines for different cropping seasons showed high variability for Iron, zinc and selenium. In a winter crop, mean Fe, Zn and Se content of lines were 28.67 mg/kg, 8.26 mg/kg and 0.38 mg/kg while in a spring crop, micronutrient contents were 91.47 mg/kg, 9.71 mg/kg and 0.36 mg/kg respectively. Lentil lines in spring crop had higher micronutrient content (Fe, Zn and Se) than winter crop. In conclusion, one can say that spring crop in lentil has higher quality than winter crop for micronutrient concentration.

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Towards map-based cloning: fine mapping of the *D* gene involved in peach fruit acidity

Session: Improving plant product quantity and quality
Food and feed

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Peach (*Prunus persica* (L.) Batsch) is the second most important fruit tree crop in Europe after apple. It is a diploid species (2n=16) with a short juvenile period (2-3 years) and a small genome (262 Mb) about twice the size of *Arabidopsis thaliana*. Therefore, peach is considered as a model for *Rosaceae*. The sugar/acid ratio is an essential component of the organoleptic quality for fruits in the *Rosaceae* family. The *D* gene, controlling the low-acid trait in peach, is dominant and segregates as a mendelian character. A peach F_2 progeny, obtained from a cross between Ferjalou Jalousia® and Fantasia, segregating for several mendelian traits, was analyzed for fruit quality traits and used for the construction of a genetic linkage map. The *D* gene was mapped on linkage group 5 and co-localized with QTLs with major effects involved in the control of pH, titratable acidity, organic acid contents and with QTLs with low effect for sugar contents. To understand the molecular and physiological bases of the *D* gene, a positional cloning strategy is in progress. Using a BSA-AFLP method, 11 AFLP markers were located within 10 cM containing the *D* gene, with 2 markers co-localizing with this gene. Three SSR markers and six AFLP markers transformed into SCARs were used to identify recombinants among 1510 F_2 additional individuals. The fine genetic map of the region around the *D* gene was realized after genotyping and phenotyping of these individuals that allowed the precision of gene position. In parallel, a new BAC library was realized for the isolation of the *D* gene using F_1 hybrid DNA (obtained from the JxF cross). Screening of the BAC library is in progress using flanking markers in order to construct two physical maps for *D* and *d* alleles. Two clones containing the gene (one for each allele) will be identified and the two sequences will be compared in order to identify the *D* gene. The same strategy can be used for other traits segregating in this progeny and the results could be transferred to other Rosaceae.

Spatio-temporal leaf growth of *Arabidopsis thaliana* and characterisation of diel growth dynamics of starch metabolism mutants

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Session: Improving plant product quantity and quality
Food and feed

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Modifications of plants to improve yield and food/product quality often involve changes in metabolism. The link between metabolism and growth thus needs to be well characterised to obtain plants with high yield and also high productivity for metabolites of interest. We investigate the molecular control underlying diel growth dynamics to unravel the link between plant growth and its metabolism.
A digital image sequence processing (DISP) based non-invasive technique for visualising and quantifying spatio-temporal dynamics of leaf growth has been established for *Arabidopsis thaliana*. This technique now enables to characterise spatio-temporal leaf growth in mutants and transgenic plants for analysis of the molecular control underlying diel growth.
Arabidopsis thaliana leaves showed highest relative growth rates (RGR) at dawn and a minimum growth rate at the beginning of the night. Along the lamina, a basipetal gradient of growth rate distribution was found, similar to other dicotyledonous species. Growth of mutants in starch metabolism, with an endogenous change in the diel sugar availability, revealed altered temporal growth patterns with reduced nocturnal growth. These mutants are known to be retarded in growth dependent on the day length. The sugar-sensing mutant *gin2-1* does not show any changes in spatio-temporal growth, indicating that the glucose-sensor hexokinase 1 (AtHXK1) does not control wild-type diel leaf growth under the chosen conditions.
Diel growth pattern of *Arabidopsis* leaves are controlled by the growing leaf tissue, independent of the whole plant context, as shown by temporal growth analysis of leaf discs, reproducing the detected growth pattern for wild-type plants and starch mutants.

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Improvement of nutritional quality of rice

Session: Improving plant product quantity and quality
Food and feed

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Rice is an important cereal and central part of the diet in asian and developing countries. As most crops rice exhibits deficiencies in essential amino acids such as tryptophan, lysine and the sulphur containing amino acids cysteine and methionine. In industrialised countries these compounds are additives for feed, produced by bacteria in an energy-consuming process, being subsequently converted to meat. WHO suggests that by increasing the plant's sulphur-containing amino acid contents by a factor of eight, malnutrition of humans could be avoided. Screening natural varieties with respect to amino acid contents revealed that none of the current grown elite cultivars have the potential to contribute to solve this dilemma.
Following this idea, key genes of the sulphur assimilation pathway (serine acetyl transferase) and methionine biosynthesis (cystathionine gamma-synthase) were expressed in transgenic rice plants. In both cases the projects were successfully resulting in increases in contents of free cysteine and methionine up to 4fold and 15fold, respectively, and even the protein-bound methionine content was increased up to 2.5fold. Thus, it was possible to approach the WHO given threshold for methionine. Moreover, the increase in cysteine led to an increase in glutathione, a known compound reducing oxidative stress in plants and thus combining different beneficial properties for nutritional value as well as plant and human health.

The synthesis of chlorogenic acid in artichoke: comparison of two newly isolated *hqt* genes

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Session: Improving plant product quantity and quality
Food and feed

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Artichoke (Cynara cardunculus var. scolymus L.) is used not only as an edible vegetable, but also for its beneficial medical properties. Various potential pharmacodynamic effects have been observed in vitro for mono- and dicaffeoylquinic acids (e.g. chlorogenic acid, cynarin), caffeic acid and flavonoids (e.g. luteolin-7-O-glucoside) which are the main phenolic constituents of artichoke extracts. The polyphenolic fractions are abundant in artichoke plant organs and bioavailable to humans by oral consumption.
The aim of our study is to acquire new knowledge in the metabolism of caffeoylquinic acids in artichoke, by isolating and studying the genes involved in the synthesis of these compounds. In particular, we are focusing on the genes coding for HQT (hydroxycinnamoyl-CoA quinate:hydroxycinnamoyl transferase), a BAHD acyl transferase synthesing chlorogenic acid in other plants. We report on the isolation and characterization of two full-length *hqt* cDNAs from artichoke leaves. These sequences showed a high level of similarity to *hqt* genes from other plants. A phylogenetic analysis of the putative HQT protein sequences from artichoke together with other acyltransferase sequences, showed that the two artichoke HQTs cluster together and belong to a bigger group of HQT-encoding genes from tobacco, tomato, potato, and coffee. On the other hand, the sequences of another acyl transferase, HCT, form a separate cluster.
The two artichoke *hqt* cDNAs were cloned in an S-TAG vector and expressed in *E. coli*, to confirm HQT activity. To better characterize their biochemical properties, kinetic analyses were performed using the recombinant HQT proteins with different substrates. Moreover, gene expression was evaluated by real time PCR in leaves and flower heads of some genotypes belonging to the IGV artichoke collection.

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TILLING feasibility in Citrus as tool for genetic crop improvement

Session: Improving plant product quantity and quality
Food and feed

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Citrus is one of the most important and widely grown fruit crop throughout the word. It is an economically valuable fruit crop plant and a source of important health and nutrition benefits to people. Nevertheless, the citrus has a low level impact of traditional breeding approaches to genetic improvement mainly of its reproductive biology that generate a slowing breeding cycles and of the cost of large population caused by the large size and the slow development of the plants.
In the present study we report our preliminary data on the TILLING feasibility in Citrus as tool for genetic improvement. TILLING technique combines chemical mutagenesis with high-throughput methods for point mutation discovery and is being used successfully in a large number of species.
In a pilot experiment we mutagenized citrus seeds (cv Carrizo) with two different concentrations (0,5% and 0,7%) of the chemical mutagen EMS (ethyl methane sulfonate). DNA of 24 M1 plants (14 and 10 plants from 0,5% EMS and 0,7% EMS respectively) was extracted and AFLP analysis were performed to estimate the mutation frequency and thus the efficiency of EMS treatment. Our molecular data showed that the EMS doses utilized for producing our mutant plant material can be used to produce a large citrus TILLING population.
A citrus TILLING population will be useful for functional studies and for analysing key genes involved in physiological processes of high agronomical relevance. The identification of new allelic variants will provide resource both for basic functional genomic research and commercial crop improvement.

Exploiting the diversity of form in *Miscanthus* for increased Biomass

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Session: New products: Plant based biofuels: how to improve them?

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There is an urgent need to breed new, higher yielding *Miscanthus* varieties in order to deliver lignocellulosic biomass whilst improving land use efficiency. Understanding the genetic control of biomass performance traits is of vital importance for the acceleration of breeding higher yielding varieties. Plant architecture is important for biomass yield and is under genetic control. IBERS curates a unique and comprehensive collection of *Miscanthus* which includes plants with very divergent architecture including *M. sinensis* which is compact with numerous thin stems and *M. sacchariflorus* which is tall with few thicker stems.
A thorough phenotypic characterisation of this UK *Miscanthus* collection is being carried out at IBERS in order to identify desirable idiotypes. The European *Miscanthus* Improvement (EMI) project demonstrated that no single genotype performed optimally at all latitudes in Europe and so different genotypes will be required for different locations. Linking genotype to phenotype and generating molecular markers for desirable traits will accelerate the breeding cycle and thereby allow more rapid development of lines adapted for their environments and end usage. To this end orthologues of candidate genes encoding morphological characteristics are being identified and cloned in *Miscanthus* with the aid of bacterial artificial chromosome (BAC) libraries, and association studies being performed to link genotype to phenotype. Alleles conferring improved characteristics for biomass will be identified and made available for use in the *Miscanthus* breeding programme based at IBERS.

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A handy techniques for a promising biofuel crop

Session: New products: Plant based biofuels: how to improve them?

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Miscanthus is a genus of "woody" rhizomatous grasses, growing to 3-4 m in height. It produces new shoots annually which produce erect, robust stems and appear similar to thin bamboo cane, having a diameter of approximately 10 mm. Most *Miscanthus* species are native to subtropical and tropical regions of Africa and southern Asia, with one species (*M. sinensis* Anderss. (Chinese silvergrass)) extending north into temperate eastern Asia.
The sterile hybrid between *M. sinensis* and *M. sacchariflorus*, *Miscanthus giganteus* (Giant Chinese Silver Grass), has been trialed as a biofuel in Europe since the early 1980s. Its dry weight annual yield can reach 25t/ha.
These high yields prove that this *Miscanthus* has the potential to make an important contribution to the energy generation from renewable sources. Commercial generation projects using biomass power have now commenced around the UK and EU with more due to come on stream over the next few years. However, it doesn't produce a viable seed. In order to propagate large numbers of plantlets for several thousand acres of biomass to be planted, tissue culturing is being used and some companies have already patented some exclusive micropropagation processes.
We present here a simple micropropagation method that was used successfully with the ornamental *M. sinensis* "Yakushima" for commercial purposes. It is hoped that this method could also be tested for the giant variety.

Oligolignol profiling of *Arabidopsis thaliana* cell cultures

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Session: New products: Plant based biofuels: how to improve them?

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Lignin amount and composition impact on a range of industrial processes, among others the production of paper and bio-ethanol. Removing lignin from lignocellulosic plant tissues is a laborious and expensive process. Understanding how lignification occurs can open new perspectives for the genetic engineering or selection of plant varieties with improved processing properties.

Lignin is present in the secondary cell wall. It confers rigidity to the plant and allows the transport of water and nutrients. In angiosperms, lignin is mainly composed of two aromatic units, guaiacyl (G) and syringyl (S), that are derived from the monolignols coniferyl and sinapyl alcohol. Upon their oxidation, these monomers couple with each other and with lignin in a combinatorial way, yielding a variety of interunit linkages of which the main types are β-O-4, β-β and β-5.These linkages are present as β-aryl-ethers, resinol and phenylcoumaran bonding structures. Although lignin polymerisation is well understood, still little is known about the transport of the monolignols to the cell wall and the initiation of the polymerisation process, including so-called lignin nucleation sites.

A. thaliana cultures that produce coniferyl alcohol and coniferyl alcohol-based oligomers were used to gain a better understanding about the initial stages of the lignification process. The advantage of cell cultures is that cells and cell culture medium can be analysed separately.

We have profiled the oligolignol composition across the life cycle of these cell cultures analysing the medium. Maximal oligolignol concentrations were observed at day 7 after subculture. Analysing the composition of the oligolignol pool at day 7 lead to the following observations:

β-aryl ether and phenylcoumaran bonding structures were predominant, and the few β-β linkages observed were lariciresinol-like instead of resinol bonding structures. These lariciresinol bonding structures can only arise following the reduction of resinol bonds. This indicates that post-coupling enzymatic reactions occur or that sufficient amounts of reductantia are present in the culture medium to perform the reaction purely chemically. Coniferin, the glucosylated form of coniferyl alcohol and the suggested transport form of coniferyl alcohol through the plasma membrane, was also detected opening perspectives to study the transport of monolignols to the cell wall.

Finally, some dimers and trimers with units derived from ferulic acid were detected. These units were sometimes further derivatized. These derivates might hint at lignin initiation points and, therefore, are further structurally analysed.

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Analysis of *Brachypodium distachyon* cell walls and comparison with other Poales using novel glycan microarrays

Session: New products: Plant based biofuels: how to improve them?

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Brachypodium distachyon has been proposed as a model plant for grasses, due to its small genome size (123 Mbp, comparable to *Arabidopsis thaliana*), relatively short life cycle (about 15 weeks) and relatively small size at maturity. In addition, *Brachypodium* is a member of the Poaceae family and Pooideae subfamily, which also includes grasses as *Lolium perenne* and some important temperate cereals, such as *Hordeum vulgare*, *Triticum aestivum* and *Secale cereale*. As well as the interest of these species as forage grasses and in the food industry, the lignocellulosic biomass derived from them is considered an important potential bioethanol feedstock. However the cell walls of these plants are recalcitrant to enzymatic degradation to fermentable sugars. A greater understanding of the detailed composition and architecture of Pooideae cell walls may provide a basis for improved energy crop design and more effective down stream processing.
We have analyzed for the first time cell walls from different organs of *Brachypodium distachyon*, and compared them to other Poales counterparts. We have used a recently developed technique, Comprehensive Microarray Polymer Profiling (CoMPP, Moller *et al.*, 2007), which combines the specificity of monoclonal antibodies with the high-throughput capacity of microarrays. As expected, we found *Brachypodium* cell walls to be similar to those from wheat, barley and *Miscanthus*. They contain relatively low levels of both non-esterified and esterified pectins and arabinan and galactan side were detected in all studied organs. The main hemicelluloses were xylans and arabinoxylans, abundant in both leaves and stems. Some cell wall glycoproteins (extensins and AGPs) were also present in all the organs studied.

Moller, I., Sørensen, I., Bernal, A.J., Blaukopf, C., Lee, K., Øbro, J., Pettolino, F., Roberts, A., Mikkelsen, J.D., Knox, J.P., Bacic, A. and Willats, W.G. (2007) High-throughput mapping of cell-wall polymers within and between plants using novel microarrays. The Plant Journal, 50(6),1118-1128

Microbial fuel cell produces electricity from plant root exudates.

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Session: New products: Plant based biofuels: how to improve them?

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The world needs sustainable, efficient, and renewable energy production. We present a new concept, the Plant Microbial Fuel Cell (plant-MFC), for direct and continuous *in situ* conversion of solar energy into electricity. MFC's convert chemical energy, available in a bio-convertible substrate, directly into electricity. Under anaerobic conditions the bacteria in the bio-anode function as a catalyst to oxidize the substrate into electrons and protons and CO_2. The electrons are transferred to the anode and the protons diffuse through a proton-permeable membrane to the cathode compartment.
In the plant-MFC the plant is placed with its root system in de bio-anode of the MFC close to the electrogenic bacteria. The plant roots produce exudates, mainly consisting of organic acids and carbohydrates in the bio-anode. The exudates are then converted into electrical energy by the bacteria.
The proof of principle of the Plant-MFC was demonstrated using Reed Manna grass (*Glyceria maxima*). Eight MFC's were constructed. In six MFC's a Reed Manna grass plant was placed in the bio-anode; the other two MFC's did not contain a plant and served as control. An incubation period of about 60 days proved necessary to start the Plant-MFC. After this period the Reed manna grass all six Plant-MFC's produced electricity during a period of 40 days with a maximum production of 67mW per m^2 anode surface. The MFC's without a plant did not produce electricity.
Based on these data we estimate that the Plant-MFC has a potential production of 21 GJ electrical power ha^{-1} $year^{-1}$ in Europe. This makes the Plant-MFC a good candidate as a novel sustainable bioenergy source characterized by (1) non-destructive, *in situ* harvesting of solar energy; (2) energy efficient carbohydrate production by plants; (3); and (4) carbon neutral and low nutrient input operation.

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Biofuels and polluted soils – a double winning combination

Session: New products: Plant based biofuels: how to improve them?

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Due to the absence of protective measures since many decades, soils in many areas in Romania are polluted with heavy metals (especially Cd and Pb) originating from smelters. Because after December 1989 land was given back to the former owners, without anybody carrying about their pollution state, the new farmers are supposed to cultivate and earn their living on these soils, with all the incumbent health risks.
A possible solution is to cultivate these soils with non-edible plants having enough commercial value to allow the farmer to sell it and buy clean food. Among these plants are sunflower, rapes and soybeans, for Biodiesel production, and short rotation coppice plants (salix and miscanthus) to be used as biomass in cogeneration plants. Because soils in most cases are acidic, the use of red mud is a feasible solution in both increasing the pH value, and for retention of heavy metals in soils.
The paper presents the encouraging first results (in terms of cultivation possibilities, yields and amount of heavy metals in plant parts and products) of our researches in Copsa Mica area, considered to be one of the most polluted in Europe, as well as our further intentions to cultivate "plants for the future".

Seed-specific expression of influenza A (H5N1) hemagglutinin subunit HA1 in barley for oral bird immunization

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Session: New products: Biomaterials, biopharmaceuticals and other new products

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Since the year 2002 several outbreaks of highly pathogenic avian influenza A (H5N1) virus killed millions of wild and domestic birds in Asia. Single human fatalities caused by the H5N1 strain have also been reported recently. The H5N1 strain has spread further, and animals infected by the virus, probably through contact with migratory birds, have been found in Europe. The development of a cost-effective vaccine for the immunization of both domestic and wild birds is mandatory. Furthermore, control of H5N1 through vaccination in the avian population will greatly reduce the risk of virus transfer across species. It is of great interest that a major outbreak in humans, as was observed in 1918, will be avoided. Our strategy to generate a vaccine against the H5N1 influenza A virus is based on the expression of hemagglutinin HA1 subunit, a major virus surface antigen, in plant tissue that may be used for massive oral immunization of birds. Various transient and stable plant expression systems were tested. Among those, a codon-optimized HA1 antigen driven by the seed specific α-gliadin promoter of wheat resulted in the highest expression. Representative molecular and biochemical analyses of transgenic barley have been performed. Western blot analysis revealed a particularly high expression of HA1 in the seeds of two out of 84 transgenic lines. Immunological evaluations of recombinant H5N1 hemagglutinin antigen are in progress.

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Preparation of recombinant nucleases with anti-cancerogenic potential, their molecular analysis and production in plants for medicinal utilization

Session: New products: Biomaterials, biopharmaceuticals and other new products

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Plant nuclease I (bifunctional nuclease) (E.C.3.1.30.x) is an unspecific endonuclease, which belongs to enzymes capable of degrading double and single stranded nucleic acids. These enzymes have been reported to comprise major nuclease activity in number of plant species. It plays various functions in plants including induction of senescence and apoptosis.
Previously we described anticancerogenic effects of plant bifunctional nucleases (Souček, J. e.a. Neoplasma 53:402, 2006; Lipovová, P. e.a. Neoplasma 55:158, 2008). Antiproliferative effects were reached at approximately ten-times lower protein concentrations in comparison to studied animal RNases and simultaneously, side-effects were much lower than caused by animal RNases. This makes plant nucleases perspective anticancerogenic agents that could be, similarly to onconase, selected for clinical trials in the future. This is especially true for new recombinant TBN1 nuclease that we originally cloned from petioles of viroid-infected tomato showing plant "paralyzing" pathogenicity (Matoušek, J. e.a. Biol. Chem. 388: 1, 2007). TBN1 exhibited practically no immunosuppressivity as assayed in CLM *in vitro* system, as well as low ebryotoxicity and aspermatogenicity in comparison to, for instance, widely investigated animal BS-RNase. Recently we cloned other homologues of plant bifunctional nucleases, HBN1 from *H. lupulus* pollen and ABN1 from *A. brassica* leaves and developed *in planta* system for nuclease production. While all these recombinant enzymes seem be toxic to be produced in bacteria, leaf infiltration system including suppressors of PTGS appears to be very efficient for large-scale nuclease production. Nuclease is extracted four-five days post infiltration before apoptotic processes inactivate proteosynthesis. Usually 10 mg of ultra pure TBN1 nuclease can be prepared from 100 g of infiltrated *N. benthamiana* leaves; this is amount of enzyme TBN1 that completely hydrolyzes 1.2 g of highly polymerized dsDNA or ssRNA to mononucleotides within one minute at 37°C. *In planta*-produced nucleases appear to be modified posttranslationally (PM), at least by N-glycosylation. For instance, mature TBN1 contains about 14% of sugar having 36 kDa. It is probable that PM of nuclease that occurs in plants leads to an improvement of its anticancerogenic properties, similarly as found for additional N-glycosylation of onconase. In order to verify this possibility, we aim to modify cloned bifunctional nucleases by site-directed mutagenesis, cDNA shuffling, as well as by *in vivo* PM using the "leaf factory" system.

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Elaboration of the technological procedure and chemical composition of a special confectionary product with enhanced antioxidant activity

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Session: New products: Biomaterials, biopharmaceuticals and other new products

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Antioxidants are essential in the neutralisation of the reactive oxygen species and free radicals. The intake of functional food products with enhanced antioxidant content, antioxidants might be regarded as efficient tools for the prevention of chronic diseases. The object of our research was to develop a new functional biscuit with enhanced antioxidant-activity. Improved methods were applied for the precise establishment of the antioxidant-content of biscuits was prepared with lysine and the effect of diverse saccharides on the activity was studied as well.

By the reaction of carbohydrates and proteins Maillard-reaction occurs, compounds with high antioxidant-activity are produced. Heating of saccharides with lysine at diverse temperatures for various times were accomplished. Functional biscuits were prepared with lysine and four different kinds of saccharides: saccharose, fructose, glucose, isosweet.

Two methods were chosen for our studies out of the many well known methods that are suitable for determining antioxidants. FRAP (ferric reducing ability of plasma) assay is based on the reduction of Fe(III) ions to Fe(II) by the antioxidants. DPPH (diphenylpicryl-hydrazyl) is a stable free radical with purple colour which intensity decrease with the antioxidant-activity. Improvement of the methods was accomplished in order to the reach optimal applicability for accurate estimation of the antioxidant activity in distinctive food matrices. To achieve the highest effectivity variable compositions of the reaction liquid as well as the ratio of the reaction solutions (FRAP/DPPH) and the antioxidant samples were optimised. The following conditions proved to be the most effective in terms of producing the most appropriate calibration and detectability: FRAP reagent consist of 25.0ml buffer solution, 2.5ml $FeCl_3$-solution, 2.5ml TPTZ-solution. 2.9ml FRAP reagent was added to 0.1ml sample having antioxidant activity. In case of DPPH the ratio of the reagent to the sample was 1:5. The methods were tested on functional biscuits having different saccharide content.

The antioxidant activity was the highest in case of the biscuit prepared with glucose and isosweet and fructose (1500-1700mg ascorbic acid/kg), saccharose containing biscuit was less pronounced, and the lowest values were measured in case of biscuits without lysine (0.016-0.034mg ascorbic acid/kg). One peace of biscuit (approx.3g) has as antioxidant activity as 0.048-0.0140mg ascorbic acid has, while the activity of one biscuit prepared with lysine and glucose was equal with 12.687mg ascorbic acid. Enhanced antioxidant-content of the developed new functional biscuits prepared with lysine have been confirmed by the improved methods, so they might play a considerable role in health protection and prevention of several chronic diseases.

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Development of a new functional food product and novel methods to reveal the thermal degradation mechanism and the prebiotic effect of inulin

Session: New products: Biomaterials, biopharmaceuticals and other new products

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Inulin-type fructans can be found in more than 36.000 plant species and they are among the most abundantly occurring carbohydrates in nature. Inulin is a polydisperse substance with linear chains of fructose monomers having a terminal glucose moiety. The number of monomers is typical for the plant comprising inulin, like chicory root with an average degree of polymerisation (DP) of 10.

Major goal of our study was to develop a new, inulin-containing functional food (biscuit) by applying novel analytical and microbiological methods to map both heat degradation pathway and the change of prebiotic impact of inulin. Relevance of the studies is enhanced by the fact that major prebiotic effect might be attributed to fructans depending substantially on the degree of polymerization.

The basic principle of the developmemt of the new functional foodstuff is that thermal degradation of inulin results in the formation of degradates activating 5 times more the *Bifidobacterium* species than the non-treated inulin. Inulin has been added the pastry and the dependancy of microbiological activity on the time-interval of thermal treatment was examined in cases of *E. coli, Bifidobacterium* and *Enterococcus.* 12 min of treatment at 190^{o}C was the most efficient in order to acquire the most pronounced prebiotic effect. Subsequent to the treatment 90% of the original amount of inulin mixed into the pastry has been decomposed leading to a new product (biscuit) with multiplicated bacterium activating effect. The biscuit's prebiotic impact exceeds significantly that of a normal product without inulin.

Comparison of distinctive extraction and sample preparation protocols has been performed. Throughout our studies inulin was acquired from *Chicorium intybus L*. (chicory), *Dahlia species* (dahlia), *Helianthus tuberosus* (Jerusalem artichoke), and the samples were treated at 8 different temperatures for 9 distinctive time periods. Thermal treatments were carried out from 150^{o}C up to 230^{o}C (10^{o}C increments each sample) to characterise thermal degradation of the inulin, and determine all the yielded oligo- and polymers.

By now the polymerisation degree of inulin's decomposition products was determined just up to DP12 (Ronkart,S.N.2007). By means of HPLC-ELS-MS technique spectra of oligo-, and polymers deriving from inulin's heat degradation were obtained ranging from DP3 up to DP31. We also identified and isolated various fructan oligomers as degradates. From alteration of retention times and molecular weights exact number of fructose units might be concluded, thus the entire decomposition pathway was revealed.

Major output of our study is that a new functional foodstuff with enhanced prebiotic effect might be produced by the application and thermal treatment of inulin. The formed fructans may be analysed directly, without prior enzymatic or chemical hydrolysis with the application of simple sample preparation procedures and HPLC-ELS technique.

A toolkit for engineering multi-enzyme pathways into higher plants

P 125

Session: New products: Biomaterials, biopharmaceuticals and other new products

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Despite growing interest in the use of plants as green factories for the production of high-value bioactive compounds, the *de novo* engineering of multi-enzyme pathways in plants has been limited to a few success stories. The lack of a rapid *in planta* system for assessing functionality of expression constructs and for determining an optimal transgene pool has hampered engineering projects.

Glucosinolates are defence-related plant secondary metabolites whose cancer-preventive and antibacterial activities promise their future use as therapeutic agents. The biosynthesis of glucosinolates from amino acids involves at least five enzymatic steps. Several intermediates in the pathway are highly reactive or toxic, which stresses the need for coordinated expression of the genes.

By using two 2A-polycistronic open reading frames (coding for five enzymes altogether) and transient co-transformation of *Nicotiana benthamiana*, we have produced a glucosinolate in a heterologous organism for the first time. The identification of an accumulating by-product – evidence of a metabolic bottleneck – led to screening of candidate genes for a missing activity. Co-expression of an uncharacterized gene led to a 17-fold increase in glucosinolate accumulation and a drastic decrease in accumulation of the by-product. Incorporation of the new gene into one of the two 2A-polycistronic open reading frames (now coding for a total of 6 enzymes) gave similar results in terms of resolution of the bottleneck.

The combination of methods used provides a toolkit for engineering multi-enzyme pathways into plants. The toolkit allows rapid *in planta* assessment of functionality of expression constructs as well as fast optimization of a transgene pool – including screening of candidate genes for desired biosynthetic activities – before the stable transfer to a host plant.

P 126

Ariltetralin lignans from in vitro cultures of *Linum tauricum* ssp. *linearifolium* and their cytotoxic activity

Session: New products: Biomaterials, biopharmaceuticals and other new products

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Lignans are a large class of phenolic compounds characterized by the coupling of two phenylpropane (C_6C_3) units. This group of natural products has drawn the attention due to their tumour-inhibitory activity. Podophyllotoxin is the most used lignan. The strong cytotoxic effect of podophyllotoxin resulted in the introduction of 3 clinically useful medicines: Etoposide, Teniposide and Etopophos. In continuation of our research on lignans in Linum species, we have established several callus and suspension cultures from single sterile seedlings from *L. tauricum ssp.linearifolium*, endemic species in the Balkan area and checked for the occurrence of lignans.

The two main lignans: podophyllotoxin (PTOX) and 6-methoxypodophyllotoxin (MPTOX) were identified in the cultures. Since PTOX is the preferred precursor for the semi-synthesis of anti-cancer drugs like etoposide and etopophos®, the accumulation of predominantly PTOX in this subspecies is especially interesting.

The both compounds, isolated for the first time from the intact plant were identified by HPLC, UV and 1H NMR. As a result of more than 3 years maintenance of the cultures, and optimisations of growth media, a stable growth and production of the both compounds was achieved. Suspension cultures synthesized 5.38 mg/g dw PTOX and 1.7 mg/g dw MPTOX respectively.

The antiproliferative action of the extracts was tested against malignant cell lines (the chronic myeloid leukemia – derived cell lines K-562 and LAMA-84, the Hodgkin lymphoma-derived HD-MY-Z and the human urinary bladder carcinoma-derived EJ cells) with etoposide as a positive control. The tested extracts reduced the viability of tumor cells in a concentration-dependent manner, whereby their relative potency was comparable or even superior to that of the referent drug etoposide. The extract from L. tauricum ssp. linearifolium showed a moderate cytotoxicity to all tested cell lines with IC50 in the range from 0,031 to 0,912 μg/ml.

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In planta tailoring of pectin properties for application on medical devices

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Session: New products: Biomaterials, biopharmaceuticals and other new products

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The term pectin covers a divers group of associated galacturonic-acid rich polysaccharides that are major components of the plant cell wall. Pectin is composed of three major polysaccharide domains: homogalacturonan (HGA), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II).
In the European research project Pecticoat (www.pecticoat.net) enzymatically modified RG I was used as an innovative nanocoating for medical devices. It was shown that cell attachment and spreading on the surface of the device can successfully be modulated by grafting surfaces with the different types of RG I obtained after the various enzymatic treatments.
An alternative to the enzymatic treatment would be to modify the RG I structure *in planta*. At the start of the project, transgenic potato lines expressing pectin modifying enzymes were available. They contain genes encoding one of the following enzymes: rhamnogalacturonan lyase, endo-1,4-beta-D-galactanase, endo-alpha-1,5-L-arabinanase, beta-galactosidase, UDP-Glc 4-epimerase and pectin acetyl esterase. These enzymes influence the RG I backbone, RG I side chain composition, acetyl esterification or the availability of nucleotide-sugars necessary for pectin biosynthesis. Development of new types of potato pectins is performed by crossing the available potato lines, thereby combining the action of two pectin modifying enzymes in a single plant. Crosses were performed and nearly all combinations were successful, yielding over 17000 seeds. The F1 offspring was characterised both molecularly and biochemically. Results of the phenotypic characterisation will be presented. As an alternative, expression of two pectin modifying enzymes in equimolar ratio was attempted by transformation of a fusion protein of galactanase and arabinanase into potato. The work will result in novel pectic biomaterials that can be applied in medical devices and other products.

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Modification and improvement of a plasmid vector for the production of antigenic molecules in GM tobacco, for veterinary use

Session: New products: Biomaterials, biopharmaceuticals and other new products

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The production of important molecules (as subunit vaccines) in plants is increasingly considered for relevant advantages: low costs of production, purification and delivery, no risks of contamination by pathogens and high scale production, but improvement and enhancement of transformation techniques are needed. MAR/SARs (Matrix/Scaffold Attachment Regions) have been reported as a network of proteinaceous fibrils that permeates the nucleus and organizes chromatin into a series of topologically isolated loop domains of 5-200 kb. These sequences may influence the structure of transgenes and their expression possibly reducing or eliminating some forms of gene silencing.
Our research is addressed at the production of plant derived antigens to be used in veterinary prophylaxis. In this field, the optimisation of transgene expression is crucial, also because of the necessity of plant containment during the whole cultivation period.
In particular, we sub-cloned *Rb7,* a MAR sequence from *Nicotiana tabacum,,* in the binary vector pAMPAT, inside t-DNA close to LB and RB terminations, in its two possible orientations. The vector expression cassette carries a 511 bp portion of *Fib*, encoding Fibrinogen Binding Protein, from *Staphylococcus aureus,* under the control of 35SS constitutive promoter. The Fib protein fragment was proved to be effective against *S. aureus* mastitis in dairy cattle. *Nicotiana tabacum,* var. Samsun was transformed *via Agrobacterium tumefaciens* with the four constructs carrying *Rb7* elements in all their possible combinations. Statistical analysis was performed after four different experiments, showing an enhanced transformation efficiency for MAR containing constructs (higher shoot number and shorter shooting time). Molecular and immunological analysis on transformed plants are now in progress, to define the transgene copy number and the resulting protein expression level.

Improved immunogenicity of plant-derived vaccines against RHD

P 129

Session: New products: Biomaterials, biopharmaceuticals and other new products

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Vaccines against the Rabbit hemorrhagic disease virus (RHDV), a highly infectious pathogen of the European rabbit (*Oryctolagus cuniculus*), are commercially produced by experimentally infected rabbits. VP60, the only structural capsid protein of RHDV, seems to be an appropriate subunit vaccine and offers possibilities to develop an alternative vaccine production strategy. Transgenic plants demonstrate a promising production platform for veterinary vaccines, up to now a number of viral and bacterial antigens were expressed by higher plants -amongst others VP60 (1, 2). However, low expression levels and non satisfying immunogenicity of plant-derived VP60 prevented commercialisation of a plant-derived RHD-vaccine up to now.
In order to develop suitable plant-derived RHD-vaccines we are studying the enhancement of the expression and the immunogenicity of VP60 in different plant species. Different genetic modifications (codon adaptation, integration of regulatory and putative stabilizing sequences, and the addition of the well-known adjuvant *ctb*) led to a higher expression level as well as to a tremendous enhancement of the immunogenicity of plant-derived VP60 (3). This was only possible in tobacco and pea but not in potato, carrot or canola. Tobacco and pea derived CTB::VP60 demonstrated at least a 100fold to 400fold higher immunogenicity compared to VP60-vaccines of potato tubers (1, 4). Rabbits immunised with pea-derived CTB::VP60 were fully protected against RHDV.

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A preliminary investigation into the efficacy of TransBacter strains for transforming food and ornamental crops

Session: New products: Biomaterials, biopharmaceuticals and other new products

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Plant transformation techniques have been available to researchers for over twenty years. During that time patent associated cost issues have, due to their associated royalties and legal entanglements greatly hindered the application of biotechnology products to agriculture, medicine etc. and slowed innovation in this field. This is regrettable as biotechnology has a lot to offer in terms of solving some of the world's problems (e.g Golden Rice). Intellectual property rights are a serious issue that need to be considered when embarking on any research program. In 2005 the non-profit biotech company CAMBIA demonstrated the transformation of *Nicotiana tabacum* L. cv. Wisconsin 38 (tobacco) using non-*Agrobacterium* species (TransBacter strains). These TransBacter strains are available under an open source licence agreement to non-profit organisations and illustrates a new wave of thinking in this business (see www.cambia.org). In our lab a number of students are working on developing efficient transformation systems for 1) *Solanum tuberosum,* 2) *Musa acuminate* and 3) *Pelargonium x hortorum* using these TransBacter strains. To date we have recorded successes with all of the crops mentioned above using these alternative strains where transformation efficiency has reached almost 71 % for Pelargonium and similar efficiencies have been achieved for potato. Our study has major implications for the biotech industry where high transformation rates can be achieved when using non-*Agrobacterium* patent-free strains.

Anti-inflammatory potential of thymol and carvacrol: cyclooxygenase-2 *in vitro* assay

P 131

Session: New products: Biomaterials, biopharmaceuticals and other new products

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Phenolic monoterpenes are important constituents in essential oil of numerous aromatic plants and spices such as oregano (*Origanum vulgare*), marjoram (*Origanum majorana* L.) savory (*Satureja thymbra*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare*) and black cumin (*Nigella sativa*). These species are widely used in traditional medicine against various microbial diseases and gastrointestinal and inflammatory disorders (1). Many of their biological activities are attributed to oxygen derivatives of p-cymene, such as phenols (thymol and its isomer carvacrol) or quinones (thymoquinone, dithymoquinone). Antimicrobial, antiangiogenic, antioxidative and analgesic activity of these compounds was also confirmed by recent studies (1, 2, and 3). Inhibition of cyclooxygenase–1 (COX-1) and COX-2 isoform by mentioned quinones as well as thymol has been published (4). The comparison of inhibitory potential of phenolic monoterpenes thymol and carvacrol against COX-2 enzymatic activities is reported here.

The anti-inflammatory assay was based on inhibition of conversion of [^{14}C] radioactive arachidonic acid to its products prostaglandins catalyzed by COX–2. The inhibition was monitored as concentration of prostaglandin E_2 and D_2 as the main products of the COX reaction in our conditions. The identification and quantification of the metabolites were performed by HPLC on C18 reversed phase column with an on-line radioactivity flow detector. IC_{50} values and percentage inhibition of different thymol and carvacrol concentrations were compared with standard COX-2 inhibitors indomethacin and NS-398 as control samples. Student's two tailed t-test was used for calculation of statistical significance and IC_{50} values were determined by regression analysis.

Carvacrol and thymol showed similar inhibition activity against COX-2. Difference between IC_{50} of both tested phenolic compounds was negligible (0.8 uM and 0.9 uM for carvacrol and thymol, respectively). Inhibitory effect of both phenols and control substances was almost identical; there was almost no difference between IC_{50} values of phenols, indomethacin (0.7 uM) and NS-398 (0.8 uM). These results indicate relatively strong inhibition of COX activity by both tested phenols, which is comparable with commercially used drugs.

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Cultivation of ginseng root cultures in various bioreactor systems

Session: New products: Biomaterials, biopharmaceuticals and other new products

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Different systems of large-scale cultivation of multiple ginseng adventitious roots of *Panax ginseng* C. A. Meyer comparing to common cultivation in Erlenmayer flasks were established. Roots were isolated from plantlets regenerated from somatic embryos and cultivated separately in liquid media. Formation of adventitious roots was reached in liquid Schenk and Hildebrandt medium supplemented with 24.6 μM indole-3-butyric acid.
The best saponin yields were achieved in partially or temporary immersion systems (22.81 ± 0.15 mg.g^{-1} of dry weight in "Mafe" bioreactor, 22.33 ± 0.17 mg.g^{-1} in RITA TIS and 21.5510 ± 0.21 mg.g^{-1} in TIS – rocking bioreactor). Saponin production in standard conditions in Erlenmayer flasks placed on rotary shaker was considerably lower (11.63 ± 0.86 mg.g-1 of dry weight).
However the best production of biomass was achieved in Erlenmayer flasks followed by RITA TIS and "Mafe" bioreactors. We suppose that close growth values in RITA and "Mafe" bioreactors are related to similar media mixing and aeration conditions in both systems. The same situation in biomass production was observed in "simple airlift bubble reactor" and LifeReactor™. In both, air is sparged below the root flowing afloat the bioreactor funnel or bag. We observed that ginseng adventitious roots are inhibited in growth by shaking and saponin production is decreased in immersed systems with low aeration of tissues.
Higher biomass growth in standard cultivation system (rotatory shaker) can be explained by well-established adaptation of cultures after long-term cultivation in Erlenmayer flasks.
We concluded that the most effective and promising system for production of ginsenosides in adventitious roots is RITA TIS or "Mafe" bioreactors - systems with high aeration (partially or temporary immersion) and stationary cultivation.

This work was supported by KJB400550705 and ME08070 project.

Bowman-Birk inhibitors from lentil: heterologous expression, characterization and anti-tumoral properties

P 133

Session: New products: Biomaterials, biopharmaceuticals and other new products

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The Bowman-Birk inhibitors (BBIs) represent the most widespread class of serine proteinase inhibitors, and are widely found in legume seeds as well as in other legume organs or other plant families.
BBIs are generally double-headed and their inhibitory domains are associated primarily with inhibition of the digestive enzymes, trypsin and chymotrypsin.
The BBI trypsin inhibitor site has the ability to inhibit animal digestive enzymes and has been associated with the negative effect on bioavailability of dietary proteins and protein digestibility. The role of the trypsin proteinase inhibitors in the plant seems to be related to plant defence from attacks by insects, pathogens and other predators.
On the other hand, many reports highlight the involvement of BBI chymotrypsin inhibitor site to prevent or suppress carcinogen-induced transformation *in vitro* and carcinogenesis in animal model systems. As a result, soybean extracts enriched in BBI have attained investigational new drug status with the US Food and Drug Administration and is being studied in the prevention of cancer.
Two BBI gene classes have been reported in lentil, one coding for a trypsin/trypsin inhibitor, the other encoding a trypsin/chymotrypsin inhibitor, the sequence of the latter one being incomplete at the 3' end.
In the present study, we report on the isolation of a complete cDNA sequence coding for lentil trypsin/chymotrypsin BBI. Two forms of the inhibitor were identified: a mature form, corresponding to the protein isolated from lentil seeds, and its C-terminal unprocessed form. In order to understand the implications of particular C-terminal amino acid residues for the specificity and potency of inhibition of key target proteases, the two forms were expressed in the methylotrophic yeast *Pichia pastoris.* After purification, recombinant molecules were analysed by MALDI-TOF mass spectrometry, and their inhibitory activities evaluated, by means of enzymatic assays using specific substrates for trypsin or chymotrypsin. K_i both for trypsin and chymotrypsin were comparable to other K_i observed for BBI proteins.
The ability of lentil trypsin/chymotrypsin BBI to modulate the viability of human colorectal adenocarcinoma HT29 cells *in vitro* was assessed.

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Production of foreign proteins bearing a functional signal peptide from a potyviral vector

Session: New products: Biomaterials, biopharmaceuticals and other new products

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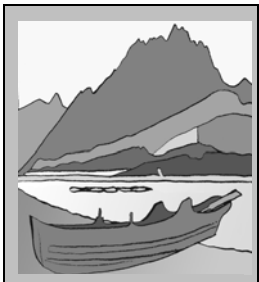
Plant viral vectors are increasingly being used for several purposes such as production of heterologous proteins, viral-induced gene silencing (VIGS) and phenocopying mutations. Viral vectors can be derived from viruses expressing their genes through subgenomic promoters or virus-encoded polyproteins. In the former case, the inclusion of a signal peptide (SP) intended for subcellular targeting of the heterologous protein generates a canonical mRNA, made from a (usually) duplicated subgenomic promoter. In the case of polyprotein-based vectors, proteolysis can be co- or post-translational, depending on the particular cleavage site and the proteinase involved, but in any case, the protein does not carry the signal peptide immediately after the initiation codon of its mRNA, as it normally happens in SPs recognized by Signal Recognition Particles (SRPs). We have tested if the inclusion of SPs right after a polyprotein cleavage site will lead to the efficient production of a protein matured from a SP-carrying pre-protein. We have found that this type of constructs can direct the production of an important amount of functional foreign protein if flanked by the foot-and-mouth disease virus 2A catalytic peptide, as exemplified by the production of horseradish peroxidase from a turnip mosaic virus vector. The results obtained will be presented and discussed.



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