Book of abstracts

EAPR – EUCARPIA CONGRESS

Potato Breeding after completion of the DNA Sequence of the Potato Genome

27-30 June 2010, Wageningen, the Netherlands

Citation: Abstract book of the EAPR-EUCARPIA congress "Potato Breeding after completion of the DNA Sequence of the Potato Genome", 27-30 June, 2010, Wageningen, the Netherlands. Edited by H.J. van Eck & L. Bouwman. Potato Res. (2010) 53: 199. https://doi.org/10.1007/s11540-010-9157-y

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Preface

At the moment of writing the news media have assured that the Icelandic volcano Eyjafjallajökull has become dormant again. This is a relief, because ash clouds have caused considerable disruption to air travel across Western Europe and worries among conference participants. The organisers would have been in sackcloth and ashes if our airport is closed at the start of this EAPR / EUCARPIA section meeting.

While the ashes have disappeared, the sackcloth has remained as background for this meeting. This strong and natural material is still used to wrap precious seed potatoes. Five Dutch potato breeding companies Agrico, Averis, HZPC, Meijer and VanRijn-KWS are greatfully acknowledged by generously sponsoring this meeting, and therefore you will see their potato bags of jute sackcloth in the conference hall. The aim of displaying these bags is also to remind us that returns on investments of public and private research grants into potato genomics will happen in the end by selling seed potatoes in these jute bags.

On behalf of the EAPR section 'Breeding and Varietal Assessment' and the EUCARPIA Section 'Potatoes' the organisers would like to welcome you to Wageningen for the 16th joint section meeting. These joint section meetings are amongst our favourite meetings to attend, bringing together a unique mixture of breeders and biologists which one seldom encounters at other potato related meetings. We think of ourselves as working at the "interface" of biology and breeding, and this meeting brings together exactly that cross-section of the potato community that needs to spend time together in meeting rooms (and bars!) to ensure the continued flow of useful information across that interface.

While the volcano has become dormant, there is a tremendous eruption of DNA sequence data that will never stop. This year, our favourite crop species has reached an important milestone, with the release of the first draft sequence of the potato genome. The timing of the meeting yields an excellent opportunity to allow the Potato Genome Sequencing Consortium (PGSC) members to describe some of the most interesting aspects of the project to an audience which is effectively the end-user community. However, as always, scientific communication is at its most exciting and useful as a dialogue, and so we hope that the section members will take the opportunity to interact with each other and the PGSC to help illuminate the way forward in best exploiting this potentially powerful new resource. The new generation DNA sequencing machines will change genomics and potato breeding. Breeders and scientists have to become familiar with an entirely new mode of navigation – genome browsers - to be able to make use of the genomics data eruption. Hopefully the specific discussion session will be equally beneficial to potato breeders as well as genome scientists.

So welcome to the meeting, we hope you enjoy the interesting array of talks and posters, but remember, don't keep your thoughts to yourself; comment, discuss, argue, generate new ideas, and help to contribute to a feeling that this was a meeting you were really glad you came to.

Herman J. van Eck chairman EAPR Section 'Breeding and Varietal assessment'

Dan Milbourne chairman EUCARPIA Section 'Potatoes'

Acknowledgements

In the first place I wish to thank those colleagues that have contributed to the program of this conference. Their oral and poster presentations resulted into an interesting program that has attracted a great number of participants, far more than I could have anticipated. In particular the contributions by colleagues from the potato genome sequencing consortium (**PGSC**) offered a unique interaction between genome scientist and practical breeders. In addition I wish to thank the keynote speakers: David Douches, Glenn Bryan, Christiane Gebhardt and Jan Draaistra. A special thanks to Martin Kropff, the rector magnificus of Wageningen University who completed the official opening by delivering a very inspiring presentation.

In the second place I wish to thank a number of sponsors:

- The Dutch breeding companies – **Agrico, Averis, HZPC, Meijer** and **VanRijn-KWS** have made a significant donation which offered me the much needed financial security that was unclear from the beginning
- The **EAPR** and **EUCARPIA** also made a significant donation. The EAPR will grant a free of charge membership for 2010 for all non-member EAPR or Eucarpia participants, including a subscription to the journal Potato Research. EAPR hopes that the participants will continue their membership in 2011 which will allow to attend the general EAPR conference in Oulu at a reduced congress fee.
- **Illumina** has sponsored the meeting in return for the opportunity to display their products for DNA sequencing and SNP assays. I have never viewed this as a commercial advertising, but as a much needed interaction between scientist, breeders and Illumina product developers to bring genotyping products to the market that perform well. Due to the high level of sequence polymorphism and the tetraploid level of commercial potato the situation is somewhat different from other model species used by the scientific community. The Illumina representative Jack Peart will participate in the entire conference and please contact him for any of your genotyping questions.
- **CBSG** (the Centre for BioSystems Genomics) has made a donation allowing us to offer drinks and bites during the poster session on Monday evening. The Centre for BioSystems Genomics (www.cbsg.nl) is a public-private partnership of major Dutch and international companies and top plant scientists working on potato, tomato, Arabidopsis and Brassica. CBSG was established in 2002 as a Centre of Excellence under the auspices of the Netherlands Genomics Initiative with a total research budget of 53 M€. In 2008, CBSG2012 entered its second 5 year phase with an equivalent budget. Several CBSG projects will report their results in this meeting.
- Two publishers are acknowledged for their willingness to display their books with relevance to the participants. **Wageningen Academic Publishers** contributes with a portfolio of titles on potato agronomy and **Elsevier** has granted a 40% conference discount on their titles.
- The **NIVAP** (Nederlands Instituut voor Afzetbevordering van Pootaardappelen / Netherlands Potato Consultative Foundation / www.potato.nl) will donate to participants the next issue of 'The Netherlands Catalogue of Potato Varieties' (in preparation). Furthermore the book table will also have their book 'Potato Diseases' on display. Furthermore I appreciate the kind suggestion by Hans M.G. Peeten to offer their beautiful potato necktie to the speakers.

I wish to thank the scientific committee for many helpful suggestions towards the selection of keynote speakers and the composition of the program. Furthermore I wish to thank the many colleagues who have been involved in the excursion to labs and trial fields in Wageningen.
Finally **Wageningen UR - Plant Breeding** and **BioExploit** are acknowledged for allowing the local organisers such an investment of working hours to complete job of organising this meeting. In particular I wish to thank **Liesbeth Bouwman** (BioExploit) for her skilful help of the entire administrative and financial part of the organisation.

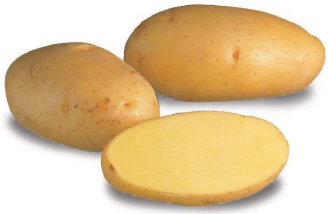
Herman J. van Eck chairman EAPR Section ‘Breeding and Varietal assessment’

Program overview EAPR - Eucarpia section meeting, Wageningen 2010

Sunday 27 June	Monday 28 June	Tuesday 29 June	Wednesday 30 June
		8.00 Coffee	8.00 Coffee
	8.00 Registration and coffee	**Late Blight**	**Pathogen Resistance continued**
	9.00 Welcome	8.30 Geert Kessel	8.30 Ewa Zimnoch-Guzowska
	9.15 Opening by Martin Kropff rector magnificus WU	8.50 Vivianne Vleeshouwers	8.50 Anna Finkers Tomczak
	9.45 Keynote 1 - SOLCAP David Douches	9.10 Dennis Halterman	**Quality Traits**
		9.30 Denis Griffin	9.10 Mark Taylor
		9.50 Walter Verweij	9.30 David de Koeyer
			9.50 Claude Urbany
		10.10 Coffee break	10.10 Coffee break
	10.30 Coffee break and Registration	10.40 Keynote 3 - MAB at ENZA Jan Draaistra	**Quality Traits continued**
	11.00 Keynote 2 PGSC Glenn Bryan		10.40 Paula Hurtado Lopez
			11.00 Gefu Wang-Pruski
	PGSC session	11.25 Keynote 4 - Precision Breeding Christiane Gebhardt	11.20 Dominika Czyzewska
	11.45 Kåre Lehmann Nielsen		11.40 Anna Camila Nader-Nieto
	12.05 Jan de Boer		12.00 Jiwan Palta
	12.30 Lunch	12.15 Lunch	12.20 Lunch
		Marker Assisted Breeding	13.15 Section Meeting
	PGSC session continued	13.15 Dan Milbourne	
	13.30 Erwin Datema	13.35 Roeland Voorrips	**Omics Studies**
	13.50 Sanjeev Sharma	13.55 Jan Uitdewilligen	13.40 Alessandra Traini
	14.10 Daniel Bolser	14.15 Tony Slater	14.00 Erin Bakker
	14.30 Isaak Tecle	14.35Nithya Subramanian	14.20 Bjorn Kloosterman
	15.00 Tea break	15.00 Tea break	14.40 Closure and tea break
	15.30 Discussion From sequence to breeding	**Pathogen Resistance**	
		15.30 Jari Valkonen	
	Genetic Modification	15.50 Sathiyamoorthy Meiyalaghan	
	16.00 Jeanne Jacobs	16.10 Excursion	
	16.20 Ronald Hutten		
	Abiotic Stress		
	16.40 Gerard van der Linden		
	17.00 Break		
	Abiotic Stress continued		
	17.30 Sergio Feingold		
	17.50 Ankush Prashar		
	18.10 Jiwan Palta		
	19.00 Dinner	19.00 Conference Dinner	
19.45 Registration	20.00 Posters; book table; Illumina		
20.30 Welcome Reception with drinks and bites	21.30 Drinks and bites **Sponsored by CBSG**		

Arrival

Departure



EAPR2011

The 18th Triennial Conference of the European Association for Potato Research

Oulu, Finland July 24th – 29th 2011

We are pleased to inform you that the 18th Triennial Conference of the EAPR will be arranged in Finland, in the charming city of Oulu. The conference venue is Oulu City Theatre.

The overall schedule of the conference will be as follows:

	Morning	**Afternoon**	**Evening**
Sunday, July 24th		Registration	Get-together party
Monday, July 25th	Scientific program	Scientific program	
Tuesday, July 26th	Scientific program	Scientific program	
Wednesday, July 27th	Excursions		
Thursday, July 28th	Scientific program	Scientific program	Conference dinner
Friday, July 29th	Scientific program	Scientific program	

The official web site of the Conference will be launched later. The link for the official site will be added here as soon as it is launched.

Further information (click title to move to the corresponding homepage):

- City of Oulu: http://www.oulutourism.fi/en/etusivu.aspx
- Travel information: http://www.congressoulu.fi/index.html
- EAPR homepage: www.eapr.net

Chairman of the organizing committee:

Academy Professor ***Jari Valkonen***
Department of Applied Biology
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Mobile: +358 40 7432 479
Fax: +358 9 1915 8727
e-mail: *jari.valkonen@helsinki.fi*

Registration open for SOL2010 www.sol2010.org

SOL2010

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Dundee

September 5-9

2010

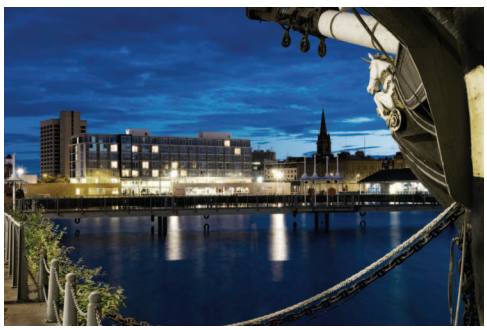
SCRI and UK-SOL are delighted to announce that they will host SOL2010 from September 5-9 in Dundee, Scotland. The tomato and potato genomes are nearing completion and this will be a stimulating and exciting meeting

Conference Hotel

The Apex City Quay Hotel and Spa is located in the heart of Dundee and guests will have free access to the luxurious gym and spa facilities

Early bird registration deadline

May 31, 2010 – Register NOW for a great rate!



Abstract Submission

If you would like to have an abstract considered for inclusion, please go to the website for details www.sol2010.org Deadline for abstracts to be submitted is June 30, 2010.

Keynote lecture:

Professor Sir David Baulcombe FRS

Sessions:

- SOL Biodiversity and Evolution
- Plant Growth and Development
- SOL Genomes
- Biotic Stress
- Abiotic stress
- Translational Genomics and Molecular Breeding
- Informatics and Computational Biology
- Tools and Emerging Technologies
- Parallel sessions (tomato, potato, pepper, tobacco, coffee etc)
- Metabolomics /Proteomics
- Functional Genomics and Systems Biology

	Before May 31	After May 31
Delegate Package	£450	£550
Student rate	£325	£425
Accompanying Delegate Rate	£50	£50
Conference Dinner	£50	£50

Delegate Package
Four day conference, includes wine and cheese civic welcome and tour, all lunches and daytime refreshments, one dinner in the hotel, and one excursion.

Student rate
Four day conference, includes wine and cheese civic welcome, lunches and daytime refreshments, one dinner in the hotel, and one excursion.

Accompanying Delegate Rate
Includes wine and cheese civic welcome, one dinner and one excursion.

Conference Dinner
The conference dinner will be held in historic 15th century Guthrie Castle, a uniquely Scottish venue.

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CBSG2012: a Public-Private Partnership

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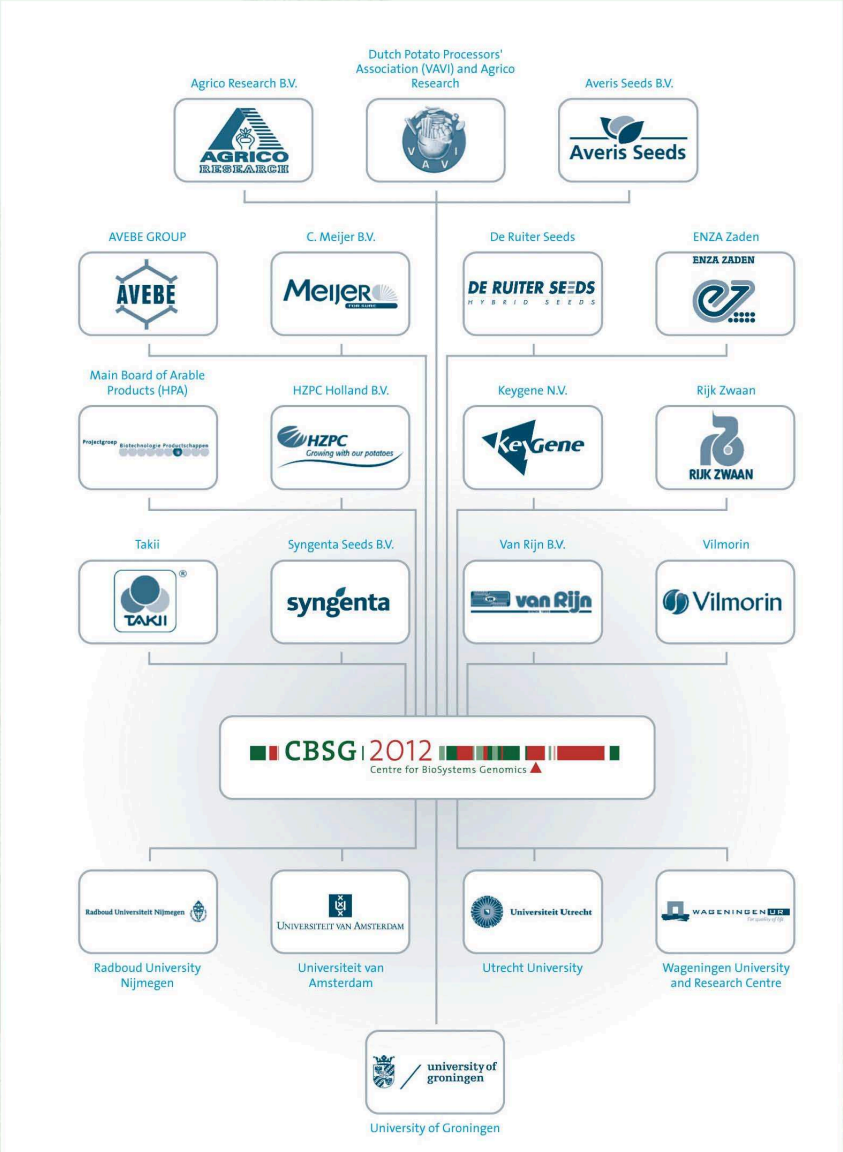
Centre for BioSystems Genomics

The Centre for BioSystems Genomics is a consortium of major Dutch and international companies and top plant scientists working on potato, tomato, Arabidopsis and Brassica. It is a unique public-private partnership in plant genomics involving universities, research institutes, (inter)national companies and branch organisations active in potato, tomato and Brassica research and exploitation

CBSG2012 carries out plant genomics research using the latest, state-of-the-art technologies. Our limited choice of crops has been made to maintain focus and to cover the species of greatest importance to Dutch Agro-industry. Our consortium covers the entire production chain from (pre)breeders to processors in both the food and non-food industries.

CBSG2012 aims to exploit the full potential of a broad range of genomics approaches in order to create new opportunities for sustainable agro-production systems for potato, tomato and Brassica which shall have socio-economic implications for producer, processor and consumer alike, through crop production, enhanced food quality and reduced environmental impact.



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Programme EAPR – EUCARPIA CONGRESS
Potato Breeding after completion of the DNA Sequence of the Potato Genome

27-30 June 2010, Wageningen, the Netherlands

Sunday June 27

19.45h Registration
20.30h Welcome reception

Monday June 28

8.30h Coffee and registration
9.00h Welcome and announcements by organisation committee
9.15h Official opening by Martin Kropff, rector magnificus Wageningen University

Keynote lectures
Chair: Herman van Eck
9.45h *SOLCAP: SNP Development for Elite Potato Germplasm*
David Douches, Michigan State University, East Lansing, United States of America

10.30h Coffee break and registration

11.00h *PGSC: "The potato genome sequence"*
Glenn Bryan, Scottish Crop Research Institute, Dundee, United Kingdom

PGSC session
Chair: Christian Bachem
11.45h *Gene expression analysis to identify those genes important for making the potato out of the potato*
Kåre Lehmann Nielsen, Aalborg University, Aalborg, Denmark
12.05h *The integrated cytogenetic, physical, genetic and sequence map of potato chromosome 5*
Jan de Boer, Plant Breeding, WUR, Wageningen, the Netherlands

12.30h Lunch

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Carrera


Mozart


Chopin


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Marilyn

Vivaldi

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PGSC session continued

13.30h *Anchoring and comparative analysis of the homozygous DM and heterozygous RH genome sequences*
Erwin Datema, Plant Research International, WUR, Wageningen, the Netherlands

13.50h *Anchoring the potato genome*
Sanjeev Sharma, Scottish Crop Research Institute, Dundee, United Kingdom

14.10h *Anchoring the potato genome: in-silico approaches*
Daniel Bolser, Dundee University, Dundee, United Kingdom

14.30h *QTL analysis and linking QTLs to Tomato and Potato genomes at SGN Database*
Isaak Tecle, Boyce Thompson Institute for Plant Research, Cornell University, Unites States of America

15.00h Tea break

15.30h Discussion session (please see description on page 27)
From DNA sequence to potato breeding

Genetic Modification

Chair: Ronald Hutten

16.00h *T-DNA minicircles for Agrobacterium-mediated delivery of potato genes without vector backbone sequences*
Jeanne Jacobs, The New Zealand Institute for Plant & Food Research Christchurch, New Zealand

16.20h *Selection of "true to type" GMO potatoes*
Ronald Hutten, Plant Breeding, WUR, Wageningen, the Netherlands

Abiotic Stress

Chair: Gerard van der Linden

16.40h *QTL analysis of drought tolerance in a diploid mapping population*
Gerard van der Linden, Plant Research International, WUR, Wageningen, the Netherlands

17.00h Coffee break

Abiotic Stress continued

17.30h *P450 genes revisited (in the light of the potato genome sequence)*
Sergio Feingold, INTA - EEA Balcarce, Balcarce, Argentina

17.50h *Water use efficiency in potatoes: traits to phenotype*
Ankush Prashar, Scottish Crop Research Institute, Dundee, United Kingdom

18.10h *Breeding for frost tolerance in Potato: Merging physiological, biochemical and genetic approaches*
Jiwan Palta, University of Wisconsin, Madison, United States of America

19.00h Dinner

20.00h Poster session

21.00h Drinks and bites **sponsored by CBSG**

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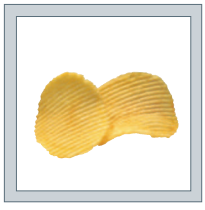
Lady Anna

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Tuesday June 29

8.00h Coffee

Late Blight

Chair: Denis Griffin

8.30h *Durable deployment of late blight resistance genes from an epidemiological perspective*
Geert Kessel, Plant Research International, WUR, Wageningen, the Netherlands

8.50h *Exploiting Phytophthora infestans effectors and deployment of R genes*
Vivianne Vleeshouwers, Plant Breeding, WUR, Wageningen, the Netherlands

9.10h *Molecular interactions determining Rpi-blb1 mediated late blight resistance*
Dennis Halterman, University of Wisconsin, Madison, United States of America

9.30h *Recent changes in Phytophthora infestans populations in Ireland challenge available cultivar resistance*
Denis Griffin, Teagasc, Crops Research Centre, Carlow, Ireland

9.50h *Identifying late blight resistance genes in Solanum accessions*
Walter Verweij, the Sainsbury Laboratory, Norwich, United Kingdom

10.10h Coffee break

Keynote lectures

Chair: Evert Jacobsen

10.40h *Marker Assisted Selection at a major vegetable breeding company – cost effective technologies and MAS strategic considerations*
Jan Draaistra, ENZA zaden, Enkhuizen, the Netherlands

11.25h *'Precision breeding' in tetraploid potato: Where are we and where do we want to go?*
Christiane Gebhardt, Max Planck Institute for Plant Breeding Research, Cologne, Germany

12.15h Lunch

Marker Assisted Breeding

Chair: Dan Milbourne

13.15h *Characterisation of the major disease resistance locus on potato chromosome 4 allows the development of diagnostic markers for resistance breeding*
Dan Milbourne, Teagasc, Carlow, Ireland

13.35h *Analysis of tetraploid cultivars with GoldenGate markers: Identification of 5 genotypic classes and trait associations*
Roeland Voorrips, Plant Research International, WUR, Wageningen, the Netherlands

13.55h *Genetic variation at the StGWD locus is associated with starch-bound phosphate levels of tetraploid potato cultivars*
Jan Uitdewilligen, Plant Breeding, WUR, Wageningen, the Netherlands

14.15h *Developing molecular genetic marker technology capability to enhance Australian potato breeding*
Tony Slater, Department of Primary Industries, Victoria, Australia

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Say potato, say

AGRICO

14.35h *Exploiting Genetic Variation for Elevated Mineral Concentrations in Potatoes*
Nithya Subramanian, Scottish Crop Research Institute, Dundee, United Kingdom

15.00h Tea break

Pathogen Resistance
Chair: Jari Valkonen

15.30h *Additional genetic factors behind the responsiveness and higher levels of virus resistance expressed by potato genotypes carrying virus-specific R genes*
Jari Valkonen, University of Helsinki, Helsinki, Finland

15.50h *Exploring intragenic approaches towards disease resistance in potatoes*
Sathiyamoorthy Meiyalaghan, The New Zealand Institute for Plant & Food Research Christchurch, New Zealand

16.10h Excursion (please see description on page 24)

19.00h Conference Dinner at Doorwerth Castle

Wednesday June 30

8.00h Coffee

Pathogen Resistance continued

8.30h *Breeding research for main virus resistance in potato*
Ewa Zimnoch-Guzowska, Plant Breeding and Acclimatization Institute, Młochów, Poland

8.50h *Physical map and comparative genomics of the potato cyst nematode resistance locus H1 at three haplotypes in potato*
Anna Finkers-Tomczak, Nematology, Wageningen University, Wageningen, the Netherlands

Quality Traits
Chair: Richard Visser

9.10h *Identification of genes that impact on potato tuber colour, flavour and texture using transcriptomic and transgenic approach*
Mark Taylor, Scottish Crop Research Institute, Dundee, United Kingdom

9.30h *Mapping and validation of QTL for after-cooking darkening*
David De Koeyer, Agriculture and Agri-Food Canada**,** Fredericton, NB, Canada

9.50h *Potato Tuber Bruising: Some are more hurt than others*
Claude Urbany, Max Planck Institute for Plant Breeding Research, Cologne, Germany

10.10h Coffee break

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Quality Traits continued

10.40h *Dynamic of senescence-related QTLs in potato using time series data*
Paula Hurtado Lopez, Plant Breeding, WUR, Wageningen, the Netherlands

11.00h *A comprehensive approach to study quantitative traits using After-Cooking Darkening as a model*
Gefu Wang-Pruski, Nova Scotia Agricultural College, Truro, Canada

11.20h *Identification of alternative oxidase marker alleles associated with reducing sugar content in diploid potato tubers*
Dominika Czyzewska, Plant Breeding and Acclimatization Institute, Młochów, Poland

11.40h *From marker to function: The role of natural variation of starch phosphorylase in cold sweetening*
Anna Camila Nader-Nieto, Max Planck Institute for Plant Breeding Research, Cologne, Germany

12.00h *Breeding for improved tuber internal quality and processing quality traits*
Jiwan Palta, University of Wisconsin, Madison, United States of America

12.20h Lunch

13.15h Section Meeting

Omics Studies

Chair: Glenn Bryan

13.40h *Interpreting and exploiting genome data based on suitable integrated bioinformatics platforms*
Alessandra Traini, University Federico II of Naples, Naples, Italy

14.00h *An integrated genome wide genetic map of sequenced NBS-LRR disease resistance gene homologues (RGH) and resistance loci in potato*
Erin Bakker, Nematology, Wageningen University, Wageningen, the Netherlands

14.20h *An integrative -omics approach for studying potato tuber quality traits*
Bjorn Kloosterman, Plant Breeding, WUR, Wageningen, the Netherlands

14.40h Closure and tea break

High Density Potato Genotyping Chip

USDA-NIFA Agriculture and Food Research Initiative (AFRI) sponsored Solanaceae Coordinated Agricultural Project (SolCAP)

1. **How many SNPs will be on the SolCAP Potato Panel?** SNP content is still being finalized. The tool will be designed using 10,000 Attempted BeadTypes. The final SNP number will therefore depend upon how many beadtypes are used per SNP (some require two, most require only one) and the final content that passes Illumina's quality control for internal redundancy and representation. We expect final SNPs between 8,000 and 9,000 SNPs.
2. **For which lines in potato are these SNP considered appropriate?** SNP discovery was completed using Sanger-based SNPs from Kennebec, Bintje and Shepody ESTs. They also include SNPs identified from Illumina transcriptome sequencing of Atlantic (high solids chip-processor), Snowden (low reducing sugar storage chip processor) and Premier Russet (low reducing sugar frozen processing) lines.
3. **What are the goals for spacing and gene region coverage? How much of the genome is represented?** We expect approximately 25% of the SNPs will be mapped to candidate genes, 10% to SNPs from known genetic markers, and65% to genes distributed across scaffolds, primarily those anchored to the DM1-3 516R44 *S. phureja* draft genome.
4. **How are the SNPs discovered? How are the SNPs selected?** Final SNP content will be selected from 69,011 SNPsthat pass the filtering and design criteria for the Infinium® platform using the following criteria: a) SNP discovery methods with a minimum of 20 reads, b) SNPs are biallelic, c) have a minimum of 50 bp from predicted exon/intron junctions, and d) Target SNPs have not more than 1 adjacent SNP within 50 bp of targeted SNP. It is estimated that 500 candidate genes have a SNP that passes the filtering criteria.
5. **What do I need (reagents, etc) to run the chips?** Participants will receive all reagents necessary to run the chips included in their order (at the consortium per sample price). They will need a minimum of 200ng of DNA in a minimum concentration of 50ng/ul as measured by a fluorescent method of quantification. Access to an Illumina BeadArray Reader or iScan is also required for processing the chips. For customers with an automation setup for their instrument, a 24 sample tip guide is also required to use automation. If you need help finding a local service provider or core facility contact, please contact Illumina.
6. **How are the content validated? How many of the SNPs can I expect to be segregating in my germplasm?**
Validation work is ongoing. For the most up to date information, check at http://solcap.msu.edu/data.shtml
7. **What is the minimum order I can place?** Minimum orders are for 48 samples (the smallest reagent kit configuration), provided shipment is requested immediately. A minimum of 288 samples are required for subsequent orders.
8. **What is the consortium price?** Consortium pricing will depend upon the number of samples achieved worldwide by June 15, 2010. The per-sample price is expected to be between $65 and $85 USD. The chip may be available to order at non-consortium pricing for those who missed the June 15 deadline - please contact Illumina (see below) to discuss.
9. **What are the benefits and responsibilities of accessing this pricing?** Members of the consortium will have reorder privileges for 1 year from the date of manufacture of the

beadpool at the consortium price. The minimum sample number for a re-order is 288 samples. The responsibility of consortium members is to reference the SolCAP/USDA20100406v2 AFRI content for any publications, public presentations, press releases, or public announcements resulting from the use of products

10. **Is there a limit to how many samples I can order at the consortium price?** There is no limit to the number of samples that can be ordered for initial shipment. Any requests for a second ship date (in the next year or the life of the beadpool) must meet a minimum order of 288 samples.

11. **Can I re-order additional chips?** The beadpool is manufactured in liquid phase. Once the beadpool is manufactured, the life of the liquid phase is 1 year from beadpool manufacture. When orders are filled (with a minimum order of 288 samples), the liquid beadpool is stabilized on chips, chips are put through a quality control and the chips are under warranty for a minimum of 6 months from date of chip manufacture. This means that the effective time period during which experiments can be run is 18 months. Reorders with a minimum of 288 samples can be submitted during the life of the beadpool.

12. **I am in competition with other interested parties and do not want my interest in running samples widely publicized. Is it a requirement to being involved that other members know about my experiments?** Any information on sample numbers you order will be kept confidential. You can decide whether you are comfortable revealing the goals of your experiment to the SolCAP participants. Illumina will only divulge the total samples numbers pooled by all participants that will lock in the consortium price.

13. **Will SolCAP or Illumina be providing a cluster file for the genotyping positions?** SolCAP will provide a cluster file, suitable for use in GenomeStudio which will allow for the calling of three cluster positions at each SNP locus.

14. **Does the genotyping chip discriminate between the three heterozygotes and the two homozygotes in tetraploid potato?** In initial validation assays with 96 SNPs x 192 samples, SolCAP was able to manually score 5 expected clusters in 56% of the data, 4 clusters in 16% of the data, 16% with 3 clusters (as with a diploid).

15. **How do I participate?** Please direct initial enquiries to Jack Peart (Regional Agrigenomics Specialist) at jpeart@illumina.com .

Excursion Program

At the moment of printing this book, the organisation of the excursions is not yet complete. Therefore the excursion items are tentative and the names of those who guide the excursion are partly unknown. Nevertheless the time schedule and tentative items can be given.

The excursion fits between an oral presentation and the conference dinner. Three busses will take the entire group to **Wageningen Campus**, parking site near Unifarm Greenhouse facilities.

!!! KEEP IN TIME!!!

Use the 15:00 – 15:30 coffee break if you want to bring your party dress, dancing shoes, coat and/or umbrella from your hotel room, because time is limited before departure after the last oral presentation. After the last oral presentation on Tuesday at 16.10 you are expected to go immediately from the auditorium to the busses. After the excursions the same busses will proceed from the Campus to the Conference Dinner.

16:15	**Departure from Hof van Wageningen** 20 minutes driving time to Wageningen Campus – Unifarm
16:35	Arrival at Unifarm 10 minutes to find your group 1st session 5 minutes walking time to field, lab, building
16:50 – 17:10	**Excursion 1st round** 5 minutes walking back
17:15 – 17:30	Drinks at Unifarm 5 minutes to find your group 2nd EXCURSION session 5 minutes walking time to field, lab, building
17:40 – 18:00	**Excursion 2nd round** 5 minutes walking back
18:10	Departure from Campus to Doorwerth Castle
18:30	Arrival - Group picture in front of Doorwerth Castle
18:30 – 20:30	Conference dinner
21:30 – 22:30	Party time
21:30 – 23:00	**Return to hotel** (Bus will regularly shuttle back and forth)

Your excursions
During the conference you can put your name on lists until booked full. First come, first choice. From the excursion items listed below you can pick two items. So please sign up for one excursion during the first round and one excursion during the second round. You cannot opt-out from the bus-ride from the conference venue to the conference dinner, but hypothetically you can stay in or near the bus if you wish to opt-out for the excursions. Those who have their own means of transport and do not want to use the bus should inform the organisers.

Tentative excursion items (max. number of participants)

- Greenhouse excursions
    - Effector Genomics (10 persons) – In the restricted GMO compartments of our greenhouse, you will see co-infiltration experiments of **Alireza Salami,** a postdoc in the group of Vivianne Vleeshouwers where *in planta* transient expression of effector and R-gene molecules allow to study their recognition.
    - BioImpuls (10 persons) – in the crossing greenhouse **Christel Engelen** will explain the goals and progenitors of a new project 'BioImpuls' to breed material for the organic market.

- Field excursions
    - Late blight trial field (20 persons) – **Ronald Hutten** will demonstrate introgression breeding material and GMO's carrying the *Rpi-blb1* and *Rpi-blb2* transgenes.
    - Nitrogen Use Efficiency (20 persons) – Trials grown with different levels of fertilisation will be demonstrated by **Marjolein Tiemens-Hulscher**. The aim is to obtain insight in the genetics and breeding for NUE.

- Lab excursions
    - Potato starch physicochemical analysis (10 persons) – **Richard Visser** will introduce you to various equipment in our starch lab and will explain the phenotypic measurements of starch related traits in its research context.
    - Marker genotyping laboratory (10 persons) – A variety of equipment for genotyping will be demonstrated by **Jan Uitdewilligen**, with emphasis on high resolution melting assays for mutant detection and haplotyping studies.
    - Metabolomics (10 persons) – Within Plant Research International a metabolomics platform for large-scale, non-targeted metabolomic analyses is present, comprising an MS –based system where both GC (Gas chromatography) and LC (Liquid Chromatography) are used for the initial separation of complex extracts. **Geert Stoopen** and **Sjaak van Heusden** will explain the technology and examples in tomato breeding respectively.

- University library in Forum building (15 persons)
    - A brand new building of splendid architecture hosts the major lecture halls and practical rooms. Due to exams we have to limit the excursion to the most beautiful part of the building. The university library has the shape of a giant globe. Some unique or old potato literature will be shown by the staff for special collections.

Discussion session – From DNA sequence to potato breeding

The Monday's program includes a session where genomics scientists and bioinformaticians active within PGSC (the Potato Genome Sequencing Consortium) will release their latest results. After the tea break 15:00 – 15:30 a discussion is scheduled to offer an opportunity for interaction between conference participants.

Have you ever seen good examples of a discussion with such a diverse group of participants? Sometimes these discussions hardly offer anything of interest. To maximize the chance for a useful discussion I will try to explain the format and the goals. This is the best possible preparation I can think of.

Proposed format for the discussion session:
During the tea break (15:00 – 15:30) you can hand over your written questions. In addition provocative propositions can be postulated. These questions and propositions will be addressed by those speakers who are invited to sit on stage. Speakers may invite other specialists in the audience to give their input. Of course the audience can also contribute answers to questions or to the defence or the rejections of the propositions.

Proposed questions or goals for the discussion session:

- How much has changed or will change for geneticists and potato breeders due to the release of the DNA sequence?
- What is still missing, before DNA sequence information can become a useful resource to potato breeders. Identification of missing information could end in setting a future research agenda. What data / experiments are needed? What tools or databases to make use of such data.
- Not only PGSC has offered its presentations, but also David Douches has presented SOLCAP. In simple terms SOLCAP and PGSC represent two complementary dimensions (sequence width and sequence length) of the potato genome. The sequence width in terms of SNP variation is being disseminated via Illumina Infinium array to the potato community.
- For breeders or geneticist the association between allelic variation and phenotypic variation is vital. SNP variation is only partially informative with respect to allelic variation, because most SNPs will be unphased (without linkage phase, and thus without knowledge of underlying haplotypes). Is there a way to present SNPs along with haplotype information on generic genome browsers?
- ….. etc.

Anticipated outcomes:
I am confident that the discussion will expose that the participants represent a tremendous variation in knowledge. Some issues may be very obvious to a breeder, but a revelation for a genomics scientist and vice versa. Some issues can be solved by bio-informaticians in one hour, whereas other issues my take years of data collection. Some issues may still lie beyond the horizon of the technically achievable.

Abstracts Presentations

Keynote lectures

SNP Development for Elite Potato Germplasm

David Douches[1], David M. Francis, Allen Van Deynze, John Hamilton, Walter De Jong, Lukas Mueller and C. Robin Buell
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There is a need to reduce the gap between genomics research and applied potato breeding. Our project aims to provide infrastructure to link sequence variation in genes with valuable traits in potato. In the first year, we have focused on the identification of SNPs within US cultivated potato germplasm. Normalized cDNA libraries from three tetraploid potato varieties were prepared from pooled RNA from callus, tuber, leaf and flower tissue. Libraries were sequenced to 2-2.5 Mb depth per variety using Illumina GAII "next generation" sequencing technology. The reads, single and paired end, were assembled using Velvet generating on average 38 Mb of transcriptome sequence. Due to the high quality and depth of sequence coverage, we were able to identify > 150,000 high quality SNPs in potato. To identify the genomic context of the assemblies and permit higher level analyses, we aligned the potato transcriptome to the doubled monoploid *Solanum phureja* DM1-3 516R44 (CIP801092) draft genome sequence released by the Potato Genome Sequencing Consortium. An Illumina Infinium 10,000 SNP array will be used to detect SNPs in potato. For design of the potato genotyping platform, we identified about 60,000 high quality SNPs that meet Infinium design specifications and are single copy in the genome. Over 500 candidate genes in potato will be evaluated for 1-20 SNPs each. In total, we will evaluate 2769 SNPs in candidate genes, 508 SNPs in genetic markers, and 6723 SNPs distributed throughout the genome (based upon reference genome scaffolds). We estimate that our SNPs cover about 650Mb of genome scaffolds. Access to the potato reference genome has made SNP detection and selection much more rational. A validation study with 96 SNPs is under way to evaluate Infinium design score, ability to score for tetraploid dosage, SNP polymorphism in the germplasm and parents of diploid and tetraploid mapping populations. Numerous outcomes for breeding are envisioned using this genome-wide set of markers.

The Potato Genome Sequence

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www.potatogenome.net

Potato is the world's most important vegetable crop, and a key member of the Solanaceae. The 840 Mb genome of potato has been sequenced by the global Potato Genome Sequencing Consortium (PGSC). Initially, the sequencing effort employed a chromosome by chromosome, BAC by BAC sequencing strategy of the diploid 'RH89-039-16' (RH) clone. In addition to RH, a doubled monohaploid clone 'DM1-3 516R44' (DM) has been used to generate a high quality draft sequence using Next Generation Sequencing. Progress towards generation of draft sequences of the two genotypes has been rapid, with high genome coverage of both genotypes. Currently version 3.0 of the DM assembly is available (www.potatogenome.net). Resources developed include fosmid and BAC libraries, improved physical maps, and an anchored physical/genetic reference map, onto which more than 80% of the DM genome assembly has been mapped. We are currently generating annotation of the genes, examining the transcriptome, and performing analysis of genes critical to potato biology. The timely release of the potato genome sequence provides the entire Solanaceae research community an opportunity to exploit the genome sequence for fundamental and applied biological studies, including plant breeding. The potato genome sequence will serve to accelerate potato improvement and help to meet the challenges facing food production in the 21st century.

Marker Assisted Selection at a major vegetable breeding company. Cost effective technologies and MAS strategic considerations

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Enza Zaden is a leading, independent breeder of innovative vegetable varieties. With a team of 1200 employees on all continents of the world, we provide new vegetable varieties for the professional grower. Marker Assisted Selection (MAS) has dramatically enhanced our efficiency and reliability of selection in the past decade and reduced the costs. This cost reduction is most apparent in a heated greenhouse crop like tomato, cucumber or pepper. Especially for traits which are expressed only at later stages of development, such as fruit and seed characteristics. Another example of cost reduction is identification in an early stage of the best donor plants for double haploid (DH) induction. We use MAS also to improve the efficiency in recurrent backcross programs, were it reduces the rounds of backcrosses needed. To decrease linkage drag after introduction of resistance from wild species, DNA markers flanking the introgression are used to pre-select individuals that are recombinant in the vicinity of the gene. The most difficult assessable quantitative traits with low heritability will clearly have the greatest benefits of MAS. Obviously, marker development for these characteristics is also most complicated and needs simplifications, like separation of the phenotype into genetic components and separating the effect of each QTL by generating Near Isogenic Lines (NILs). Methods for assessing the allelic variation at these agronomically important loci such as yield, nutritional content, quality and disease resistances are now available. The next step in the application of markers in breeding is the understanding of the genetics basis of *all* agronomically important characters and the allelic variation at those loci. With this 'Breeding by Design' concept [1], the breeder would be able to design superior breeding lines 'in silico'.

1 Peleman, J.D. and Rouppe van der Voort, J. (2003). Breeding by Design. *Trends Plant Sci.* 8, 330-334

'Precision Breeding' in tetraploid potato: Where are we and where do we want to go?

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Around 40 phenotypic characters are relevant for potato breeding, most of which are complex, meaning that they are controlled by multiple genetic and environmental factors. Knowing the genes and their allelic variants that underlay agronomic traits allows the development of molecular diagnostic tools for selecting improved potato cultivars. Diagnostic DNA-based markers are either derived directly from polymorphisms in genes causal for a trait of interest or are in linkage disequilibrium with those genes. They can be used to identify superior genotypes among parents and progeny in breeding programs (Precision Breeding). Diagnostic markers can be identified by a combination of QTL (quantitative trait locus) mapping, candidate gene mapping and association mapping using functional and positional candidate genes as markers. This approach was successfully used to identify loci, which contribute to the natural variation of pathogen resistance or tuber traits in tetraploid breeding populations. Statistical epistasis between candidate loci was found for tuber starch content and starch yield. Candidate genes associated with field resistance to late blight or tuber quality traits are currently tested for their diagnostic power in marker-assisted selection experiments. Further analysis of candidate genes includes allele mining and comparative functional analysis of alleles associated with a phenotypic trait versus non-associated alleles. Comparative sequencing of candidate gene alleles reveals an amazing degree of molecular diversity in potato.

PGSC session

Gene expression analysis to identify those genes important for making the potato out of the potato.

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As part of the potato genome sequencing project undertaken by the Potato Genome Sequencing Consortium, deep transcript sequencing using the short read EST technology, mRNASeq, was carried out on both the DM and the RH potato variety. The purpose was several fold: i) to assist/validate gene prediction; ii) to annotate untranslated regions of predicted genes; and iii) characterize the transcriptome of different tissues and under biotic and abiotic stress. Mapping of reads from both DM and RH to the DM reference sequence could be done with very high efficiency, indicating a very high sequence conservation within the coding regions of the two genomes.
The focus of this presentation will be analysis of the transcriptome of tuber derived tissues, identifying transcripts that set apart the tuber from the rest of the plant tissues to shed light on how this unique organ is functioning at transcript level. Special attention will be given to the gene expression in the carbohydrate metabolism.

The integrated cytogenetic, physical, genetic and sequence map of potato chromosome 5

J.M. de Boer[1], X. Tang[1,3], E. Datema[2], T.J.A. Borm[1], B. te Lintel-Hekkert[2], R.C.H.J. van Ham[2], H. de Jong[3], C.W.B. Bachem[1], and R.G.F. Visser[1].
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Chromosome 5 of the heterozygous diploid potato genotype RH89-039-16 (RH) has been sequenced to near-completion using the BAC by BAC approach. The overlapping BAC sequences have been joined into larger sequence scaffolds, comprising total length of 55 Mbp. The scaffold sequences are embedded in the BAC fingerprint physical map of genotype RH, and are anchored via AFLP markers to the ultradense genetic map of chromosome 5. The cytogenetic distribution of the BAC sequences has been examined in detail by multi-color BAC ladder FISH on pachytene chromosomes. This analysis showed the relation between genetic, cytogenetic and physical distances across the chromosome, and enabled further ordering of BAC scaffolds beyond the resolution of the genetic map. Our integrated BAC sequence map of potato chromosome 5 is unique in that it includes much the pericentromeric heterochromatin. Estimates of repeat content and gene content in euchromatin versus heterochromatin will be presented. The chromosome 5 sequence map provides a resource for the identification of candidate genes that are linked to traits of agronomic importance, and for molecular marker development in regions of interest.

Anchoring and comparative analysis of the homozygous DM and heterozygous RH genome sequences.

E. Datema[1], J. de Boer[2], T. Borm[2], B. te Lintel-Hekkert[1], C. Bachem[2], R. van Ham[1] and R. Visser[2]
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In order to overcome the technical difficulties of assembling the highly heterozygous RH potato genome, the genome sequencing effort of the PGSC was extended to include a homozygous, doubled monoploid (DM) wild potato cultivar. Nonetheless over 1,600 partially overlapping BAC clones of the RH genome have been sequenced to draft quality within the PGSC consortium, representing approximately 20% of the genome. While these BACs are unevenly distributed over the 12 potato chromosomes, their sequences provide both a genetically anchored backbone for organizing the DM whole-genome assembly and an important source for studying the sequence variation within potato. Using next-generation Illumina sequencing, we have corrected single base errors and ordered these sequences into non-redundant scaffolds, allowing us to align the corresponding DM scaffolds. These data have then been used to gain insight into both the sequence and structural variation between the DM and RH potato alleles, as well as the differences between the two heterozygous RH alleles. In coding regions, the sequence identity between DM and RH was 98.4%. Remarkably, we found that the overall sequence identity between DM and RH (97.5%) was higher than the sequence identity between the two genetic phases of RH (96.5%).

Anchoring the potato genome

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Potato, the world's most important vegetable crop and a key member of the Solanaceae, is being sequenced by the multi-national Potato Genome Sequencing Consortium (PGSC, see www.potatogenome.net). Using a whole genome shotgun approach the PGSC has generated a high quality draft sequence of a completely homozygous 'doubled monoploid' clone (DM1-3 516R44 or CIP 801092) of *S. tuberosum* group Phureja complementing their earlier efforts using the heterozygous RH89-039-16 clone. In order to augment the genetic and physical anchoring of the sequenced DM genome, a segregating backcross population between the DM clone and a heterozygous diploid *S. goniocalyx* clone (CIP No. 703825) as the recurrent parent was established. The polymorphism across 169 progeny clones was assessed using a total of 4836 STS markers including 2174 DArT^{TM} (Diversity Arrays Technology Pty Ltd), 378 SSR (simple sequence repeat) alleles and 2304 SNP (single nucleotide polymorphism) marker types. SSR and SNP markers were designed directly to scaffolds, whereas polymorphic DArT marker sequences were searched against the scaffolds for high quality unique matches. The data from 2619 polymorphic STS markers was analysed using JoinMap®4 and a DM genetic map containing the expected 12 potato linkage groups was developed *de novo*. The mapped STS markers, because of their known unique position and/or sequence on the genome, were directly anchored to the DM super-scaffolds. This in turn assisted in physical anchoring of DM super-scaffolds on to the DM/DI//DI linkage map. In addition to this, *in silico* approaches involving the RH genetic and physical map, as well as tomato map data from SGN (http://solgenomics.net/) were also exploited to further enhance the anchoring of DM genome. Overall, we are able to genetically anchor 623 Mb (85.7%) of the assembled 727 Mb genome arranged in 651 super-scaffolds to an approximate location onto one of the twelve potato linkage groups. In the post potato sequencing era, this integrated sequence and genetic reference map will form an important resource for linking to all future genetic mapping efforts by the potato community.

Anchoring the potato genome: *in-silico* approaches

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The potato genome has been assembled into 622 super-scaffolds larger than 250 kb that together constitute 90 percent the total assembled genome sequence (727.4 Mb). Positioning and orientating these super-scaffolds into their 12 linkage groups is an important next step for the assembly. A variety of genetic and sequence resources can be combined to achieve this goal. We have used the Ultra High Density potato genetic map and the associated BAC End Sequences from *S. tuberosum* and the tomato EXPEN 2000 genetic map to construct two '*in-silico*' physical maps for the sequenced strain. A genetic map developed for the sequenced strain anchors approximately 50 percent of the genome (393 Mb in 336 super-scaffolds). This figure can be increased to 86 percent (623 Mb in 651 super-scaffolds) by combining data from the UHD and tomato *in-silico* maps. In my talk I will briefly describe some of the underlying bioinformatics steps that were used obtain and combine these two *in-silico* physical maps. This work has been done as part of the Potato Genome Sequencing Consortium.

QTL analysis and linking QTLs to Tomato and Potato genomes at the SGN Database

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Quantitative trait loci (QTL) analysis is still an important approach in elucidating the genetic basis underlying complex traits. However, as entire genomes are being sequenced a challenge remains: linking phenotypic variation of complex traits to the underlying genomic variation. At the Sol Genomics Network (SGN) (http://solgenomics.net), we have developed a QTL analysis software (http://solgenomics.net/qtl/) which allows QTL researchers to submit their raw QTL data, perform on-the-fly QTL analysis, visualize QTL map locations and do comparative analysis between QTLs and corresponding regions on genetic maps and genomes at SGN. The QTL analysis is based on R/QTL (http://www.rqtl.org) and users can decide using a web interface what statistical parameters to employ for the analysis. The QTL mapping output fully integrates with other SGN analysis tools and is cross-referenced with relevant datasets at SGN and external databases. For example, using the Comparative Map Viewer (http://solgenomics.net/cview/), users can compare predicted QTL regions to genetic maps from the same or different Solanaceae species. When peak or flanking markers match genome regions, users can use SGN's genome browser (GBrowse, http://solgenomics.net/gbrowse/) to identify corresponding sequences and annotationshttp://solgenomics.net/gbrowse/. At this stage, for tomato, corresponding genome regions are annotated with ESTs, unigenes, gene and protein models provided by the International Tomato Annotation Group (ITAG) and experimentally characterized genes annotated by the Solanaceae community. This close data integration can facilitate identification of candidate genes, elucidation of the underlying variation at the molecular level and marker assisted breeding for a trait of interest. Currently, the QTL software is implemented for backcross and F2 intercross diploid QTL populations. In the near future, it will expand to include 4-way cross (out-crossing) populations. When the annotated potato genome is available at SGN and in GBrowse, we envision its application for the potato research community. We invite the community to investigate the tool and provide feedback

Genetic Modification

T-DNA minicircles for *Agrobacterium*-mediated delivery of potato genes without vector backbone sequences

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Minicircles are supercoiled DNA molecules devoid of plasmid backbone sequences, formed as a product of *in vivo* excision by site-specific recombination. They can be induced to form in bacterial systems, but due to the absence of bacterial replication origins and selectable marker genes, they do not persist. Minicircles offer a method to reduce DNA molecules to a simple, well-defined expression cassette for transformation. They offer an important tool for effective delivery of cisgenes, intragenes and transgenes through transformation without the inadvertent integration of vector backbone sequences, an important limitation of current technology. The induction of minicircles from T-DNA regions in *Agrobacterium* immediately prior to co-cultivation with plant tissues provides a means to limit T-strand formation to a well-defined region intended for transfer to plant cells. The careful assembly of the minicircle region using plant-derived sequences for site-specific recombination and a T-DNA border, provides a means to assure the absence of foreign DNA during plant transformation using intragenic approaches. Applications of this approach for delivery of potato genes to elite potato cultivars will be described.

Selection of "true to type" GMO potatoes.

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Within the "DuRPh" (Durable Resistance to Phytophthora) project true to type late blight resistant GMO potato clones are selected to perform resistance management field trials. The first set of GMO potato clones used for this study are transformants of the varieties Désirée and Première, using four different constructs containing single late blight resistance genes (Rpi-blb1, Rpi-blb2, Rpi-blb3, R3a) in combination with the nptII gene (kanamycine resistance). Selection was carried out at three stages. Firstly two *in vitro* plantlets per transformant were grown in the greenhouse. Evaluation on morphology, insert number, and late blight resistance (detached leaf test) was performed. Secondly 25 *in vitro* plantlets per selected transformant were grown in a screenhouse to produce seed tubers. Plant morphology and tuber appearance were evaluated. Finally the transformants were field grown in a yield trial (2x4 plants) and two late blight trials (2x6 plants). In all field trials morphology was evaluated. In the yield trial several tuber traits were investigated. Transformants that were regarded "not true to type" were excluded from consecutive evaluation stages. The percentages of not true to type transformants in the three different stages were found to be 12.5%, 5.3% and 38.4% respectively, resulting overall in 51% true to type transformants. The number of inserts in the transformants varied from 1 to 15. Over 50% of the transformants contained only 1 or 2 inserts. The percentage of not true to type transformants containing 1 insert was 40%. From the results it was concluded that an extra insert generates approximately a 4-5% higher percentage of not true to type transformants. This suggests that the vast majority of deviations from the wild type in the production of GMO potatoes is not caused by the insert itself but rather by a combination of the transformation/regeneration method and the inherent instability in the wild type genotype.

Abiotic Stress

QTL analysis of drought tolerance in a diploid mapping population

A.M. Anithakumari[1], R.G.F.Visser[2] and C.G. van der Linden[2]
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Potato is an important food crop, yet it is relatively susceptible to drought. As a first step towards identifying the genetic basis for drought tolerance in potato, we make use of diploid potato populations that have been genetically well characterized (CxE, SHxRH). The CxE population has been extensively evaluated for drought tolerance in two successive years (2008, 2009) under greenhouse conditions by measuring traits like Relative Water Content of leaf, Δ13C as a measure of Water Use Efficiency, Chlorophyll Fluorescence, Chlorophyll Content, shoot and root biomass and tuber yield. The progeny displayed a wide contrast for drought tolerance, with individuals surviving and recovering completely after 3 weeks of drought, and others completely wilted beyond recovery. Most of the traits had high heritabilities. For optimal QTL mapping, we expanded the CxE and SHxRH genetic maps with 499 SNP markers (384 and SNP arrays, enriched for putative stress tolerance candidate genes). The SNPs were discovered in public EST databases using QualitySNP software and detected with the Illumina GoldenGate assay. About 300 SNPs served as bridge markers between CxE and SHxRH maps.
QTLs effective in multiple environments and years were detected for tuber number, tuber weight, plant height, Shoot fresh and dry weight. Other QTLs were found to be dependent on the environment: QTL x Environment interaction was found for instance for leaf δ13C under drought conditions. Several QTLs but not all were linked to maturity type indicating that the timing of tuber formation is an important determinant for drought tolerance and yield, but that other factors play an important role as well.
Future studies are zooming in on the drought tolerance QTLs that we identified in CxE using expression studies and candidate gene identification and analysis, and validation of the QTLs in field drought trials.

P450 genes revisited (in the light of the potato genome sequence)

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Plant P450 enzymes are monooxygenases that catalyze oxidations on different substrates and have been shown to be involved in the biosynthesis of secondary metabolites with roles in pigmentation, antioxidant activity and defense to UV light, insects and xenobiotics. Present in prokaryotes and eukaryotes, P450 genes have greatly expanded in plants, constituting the largest plant gene family. *Arabidopsis thaliana* contains 272 P450 genes, while potato seems to hold one of the richest P450 gene sets.
Early identification of potato P450s was performed from 1998 to 2004 by querying the former TIGR potato and tomato EST databases (now "The Gene Index Project"; http://compbio.dfci.harvard.edu/tgi/) with the 11 known *Arabidopsis thaliana* sequences that represent most of the plant P450 clades. Out of the 486 sequences retrieved (238 from potato and 248 from tomato) 106 putative non-redundant P450 genes, were mapped across all 12 potato chromosomes in interspecific potato segregating populations (BCB & BCT) by means of single strand conformation polymorphism, producing P450-SSCP functional markers.
With the complete potato sequence unveiled by the Potato Genome Sequencing Consortium (PGSC; http://www.potatogenome.net), we used the Pfam (http://pfam.sanger.ac.uk) P450 Hidden Markov Model to search the protein database (PGSC-DMv3). This search identified 393 P450 domains with an e-value $\leq 10.e^{-100}$. Some of these genes were found in clusters on the super-scaffolds, indicating tandem duplication of genes during evolution.
A BLAST search of the original 106 mapped sequences was performed against the P450 containing super-scaffolds. Genes corresponding to all the original sequences were found in the assembly. Out of the 71 P450 containing super-scaffolds anchored to the PGSC recombination map, map locations for 67 were congruent with our mapped data, with 4 misplaced sequences. Additionally, 8 sequences showed dual locations, suggesting potential duplications in chromosomes 2 & 10; 5 & 11; 3 & 4 and 4 & 9. A few (7) linked P450-SSCPs were ascribed to different parts of the same gene, while others (3) were possible allelic variants of the same gene that were not assembled in the original databases. This fact was not unexpected since PCR primers were designed based on available sequences, often partial, at the time of the initial study.

Water use efficiency in potatoes: traits to phenotype.

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High yielding potato cultivars are generally sensitive to low soil moisture and may suffer reduction in tuber yield due to a sparse and shallow root system and other aspects of the plant's physiology. Our research aims to breed drought tolerant varieties and the current focus is on developing phenotypic screens for water use efficiency (WUE) to allow us to explore the genetic basis of key WUE traits. Ten genotypes with contrasting transpiration efficiencies based on leaf $\delta^{13}C$ values were cultivated under controlled glasshouse conditions. After emergence, plants were grown for 36 days in soils watered to field capacity (30% volumetric content, -5 kPa water potential) before being divided into three groups irrigated to 30%, 20% (-300kPa, slight stress) and 12% (-1500kPa, wilting point) volumetric content. Preliminary data show that transpiration-efficient genotypes, as indicated by low leaf $\delta^{13}C$ values, have consistently lower stomatal conductance at 12% volumetric soil moisture than do transpiration-inefficient genotypes. Stomatal density varies significantly with watering treatment ($p=0.002$), being denser in genotypes watered to 12% volumetric content than in those at 20% or soil field capacity; there were also significant differences among genotypes. Tuber yield and morphological characters are also significantly different ($p<0.01$) at different moisture levels.

Breeding for frost tolerance in Potato: Merging physiological, biochemical and genetic approaches

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Potato is a cool season crop, cultivated in the temperate zone in North America, Europe and the Andean highlands of South America. In these areas frost injury is one of the factors limiting potato production. In the Andean region of South America, it has been estimated that potato productivity can be doubled by improving frost tolerance of cultivated potatoes. The cultivated potato species *S. tuberosum* is very frost sensitive and is often killed when tissue temperatures fall below – 3°C. Several non-cultivated tuber-bearing species such as *S. acule* and *S. commersonnii* can survive temperatures as low as -6°C while growing under normal conditions (non-acclimated frost tolerance, NAFT). In addition, some of these species acclimate in response to cold temperatures and increase their frost tolerance (acclimation capacity, ACC), while the commonly cultivated species fails to cold acclimate. To understand the genetics of freezing stress resistance, we performed an interspecific hybridization of two diploid potato species that vary in NAFT and ACC. The species were *Solanum commersonii*, which is freezing tolerant and able to cold acclimate and *Solanum cardiophyllum*, which is freezing sensitive and unable to cold acclimate. Analysis of the backcross progenies shows that non-acclimated freezing tolerance and acclimation ability are genetically distinct traits that segregate independently. Thus in order to improve frost tolerance both traits must be selected individually and recombine to develop frost tolerant potatoes. This can be achieved only by precise selection under controlled conditions and not by field selection. The plasma membrane is a key site of alteration by freeze-thaw stress and cold acclimation. Our results show that an alteration in the function of plasma membrane ATPase is one of the earliest manifestations of stress. We have provided evidence that these alterations could be mediated by perturbation of cellular/membrane Ca^{2+} and/or changes in membrane lipid composition. Based on these biochemical, physiological and genetic studies we have used a systematic approach to move frost hardy traits (NAFT, ACC) from the wild to cultivated potatoes. Using precise screening techniques under controlled conditions we have been able to improve frost hardiness of cultivated type.

Late Blight

Durable deployment of potato late blight resistance genes from an epidemiological perspective

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DuRPh is a 10-year research project which aims to develop potato cultivars with a durable resistance against late blight. This will offer opportunities to drastically reduce pesticide use in the potato cultivation. DuRPh researchers want to provide existing potato cultivars with additional resistance genes from related species, that can also be crossed, through cisgenic genetic modification.
Under the assumption that all R-genes can be overcome by *P. infestans*, the DuRPh team is developing R-gene cassettes. DuRPh also researches the possibility of creating so called dynamic cultivars: modified existing potato cultivars containing several lines with a different R-gene cassette each. As the composition of flu vaccins changes annually, also these cassettes can be changed regularly. Moreover, the DuRPh team will look to the best possible use of different sets of resistance genes in space and time to create late blight buffering potato fields and regions. A mix of field and simulation studies is used to determine e.g. the optimal number of lines within a dynamic cultivar. Simulations are conducted with variables such as distance between fields, field size, mixing of cassettes within or between fields, weather and disease control strategy. The most promising options are put to the test in multiple year field experiments.

Exploiting *Phytophthora infestans* effectors and deployment of *R* genes

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The Irish famine pathogen *Phytophthora infestans* causes late blight, the most destructive disease of cultivated potato (*Solanum tuberosum*). Durable management of late blight necessitates the deployment of multiple resistance (*R*) genes into potato. However, current approaches to isolate *R* genes are inefficient and innovative phenotyping methods are required to detect whether individual *R* genes are functionally active. We are developing new strategies to accelerate the cloning of potato *R* genes and optimize functional characterization by effector genomics. During early infection stages of potato, R proteins initiate defence responses when *P. infestans* secretes the cognate avirulence (Avr) effector, typically a member of the RXLR family of host-translocated effector proteins. We used the sequenced *P. infestans* genome T30-4 to select a genome-wide repertoire of expressed effectors. These effectors are functionally profiled on resistant *Solanum* species for initiation of defence responses. Candidate R-Avr pairs have been identified. The candidate *Avr* genes are further investigated for their ability to induce defence responses in resistant offspring of segregating populations. Identified *Avr* genes are subsequently used for functional detection of the *R* gene in potato. The current status of effector genomics screenings will be presented, and strategies how to exploit effectors in *R*-gene based resistance management will be discussed.

Molecular interactions determining Rpi-blb1 mediated late blight resistance

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The potato late blight resistance (*R*) gene *Rpi-blb1* belongs to the valuable class of plant *R* genes that confer resistance to a broad spectrum of pathogen isolates. Rpi-blb1 protein recognizes the presence of members of the *Phytophthora infestans* effector family IPI-O to elicit resistance. We have studied *IpiO* diversity from 40 different *P. infestans* isolates collected from Guatemala, Thailand, and the United States. We have found that all of the isolates contain IPI-O variants that can be recognized by Rpi-blb1. However, some of these isolates contain an extraordinarily large number of variants. A few isolates also contain an IPI-O variant (IPI-O4) that is not recognized by Rpi-blb1. Isolates containing IPI-O4 are able to overcome resistance in Rpi-blb1-containing potato leaves to cause significantly more disease than isolates that do not contain IPI-O4, even when other IPI-O proteins are present. We show that the presence of IPI-O4 blocks the ability of Rpi-blb1 to recognize the presence of other IPI-O variants through direct interaction with the resistance protein, thereby preventing programmed cell death related to the resistance response.

Recent changes in *Phytophthora infestans* populations in Ireland challenge available cultivar resistance

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Following the introduction of the A2 mating type into Europe in the late 1970s changes within European *P. infestans* populations have occurred. Comparable to elsewhere in Europe, the Irish population has remained relatively stable throughout the 1990s. Since 2007 the Irish population has undergone a rapid change, with the A2 genotype, Blue-13, now dominating throughout the main potato growing regions. Analysis of data from the Teagasc Oak Park late blight variety resistance trials which rely on natural inoculum, prior to and during this change (between 2006 and 2009) indicate the late blight resistances of some commercial varieties have been affected. The most notable variety affected in these trials was the Oak Park bred variety Setanta. In 2006 this variety showed relatively little disease, with infections slow to develop and a subsequent late blight resistance rating of 8. In 2009 under naturally high disease pressure caused by the *P. infestans* genotype Blue-13 Setanta showed a poor ability to resist disease development, and had a subsequent late blight resistance rating of 3. This dramatic change in resistance rating has highlighted the need to re-evaluate the Irish commercial variety resistance ratings in light of the recent population changes. Setanta was previously thought to have high levels of horizontal resistance as the variety always exhibited low levels of disease which developed slowly. The rapid deterioration of this resistance has behaved in a similar way to the breakdown of an R gene. Whether this change in resistance ratings resulted from Blue-13 being more virulent or aggressive towards Setanta and related varieties is currently unknown and may have consequences for future late blight resistance breeding strategies. Once again this raises a question over the classical definition between vertical and horizontal resistance mechanisms.

Identifying late blight resistance genes in *Solanum* accessions

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Potato is the fourth ranked crop in world food production and despite the extensive use of agrochemicals, late blight, caused by the oomycete *Phytophthora infestans*, is responsible for substantial crop losses. In the past, late blight resistant genes (R genes) from *Solanum demissum* have been introgressed in cultivated potato but these were quickly overcome by new races of the pathogen. Wild type potato species are a good source of R genes, which could be deployed to achieve durable resistance in the popular potato varieties. We use a collection of wild species of *Solanum* from all over the world to search for resistance against *P. infestans.* In a detached leaf assay multiple accessions are tested and resistant and sensitive strains are crossed to create a segregating population. To identify polymorphism in loci that confer resistance, the transcriptome of bulked resistant (BR) and bulked susceptible (BS) individuals is sequenced using the Illumina GA2 instrument.

Currently, we are following this approach to identify resistant genes in *Solanum berthaultii,* a wild type potato variety from Bolivia. We use several programs to align the BR and BS Illumina reads to the sequence data available on http://www.potatogenome.net/. Perl programs are deployed to identify the polymorphism in an R gene that confers resistance. We are also characterizing additional resistances in *S.venturii* and *S.nigrum*.

We suggest that eventually multiple R genes will be simultaneously introduced in popular potato varieties in order to achieve durable resistance.

Marker Assisted Breeding

Characterisation of the major disease resistance locus on potato chromosome 4 allows the development of diagnostic markers for resistance breeding

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Complete sequence based characterisation of resistance gene loci offers a route for the development of diagnostic markers and haplotypes for use in resistance breeding. We are currently in the process of characterising the major disease resistance locus on potato chromosome 4 in the two genotypes (RH and DM) that are the basis for the potato genome sequencing initiative. Our earliest efforts to demonstrate the utility of this sequence information for breeding have revolved around a large effect quantitative trait locus (QTL) on linkage group IV ($GpaIV_{adg}^{s}$) conferring resistance to *G. pallida* pathotype Pa2/3. The QTL was originally mapped in the tetraploid breeding line 12601ab1. We have shown that $GpaIV_{adg}^{s}$ is also present in a breeding line called C1992/31 via genetic mapping in an F1 population produced by crossing C1992/ 31 with the cultivar Record. C1992/ 31 is relatively divergent from 12601ab1, confirming that $GpaIV_{adg}^{s}$ is an ideal target for marker-assisted selection in currently available germplasm. To generate markers exhibiting diagnostic potential for $GpaIV_{adg}^{s}$ sequence from several bacterial artificial chromosome (BAC) clones from the resistance gene region was exploited to develop multiple primer sets generating single-copy amplicons, which were examined for polymorphisms exhibiting linkage to $GpaIV_{adg}^{s}$ in C1992/31. Eight such polymorphisms were found. Testing and validation in two panels of potato germplasm of direct relevance to commercial potato breeding programmes allowed the identification of four markers (one In/Del and three single nucleotide polymorphisms). Using these markers and others (derived from the available literature), we are now considering how best to practically implement marker assisted selection for resistance in a commercial potato breeding programme.

Analysis of tetraploid cultivars with Golden Gate markers: Identification of 5 genotypic classes and trait associations

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A panel of 224 tetraploid potato cultivars was analyzed with a Golden Gate assay of 384 SNPs. The Golden Gate platform returns intensity scores for the two alternative alleles at the SNP position. The challenge in tetraploids is to convert these two quantitative scores into one of five possible discrete genotypes: from nulliplex to quadruplex. We use a mixture model approach, where for each SNP the distribution of the ratio of the signal intensities is fitted to a mixture model consisting of five distributions, one for each of the five genotype classes. In cases where five peaks occur in the frequency histogram the assignment of each peak to one of the genotype classes is straightforward. In many cases however the allele frequency is far from 50%, resulting in the absence of expected genotypes and less than five peaks. In order to correctly deal with those cases we use a model that imposes constraints on the positions of the means based on the known allele ratios in the five possible genotypes, and facultatively also on the frequencies of each genotype based on a Hardy-Weinberg equilibrium ratio. The developed algorithm only produces a genotype for samples with a high probability of belonging to only one of the five peaks, and it discards the SNPs that produce too few reliable scores. The genotypes resulting from this approach were used in association mapping of two phenotypic traits: flesh colour and eye depth. The genotyped SNPs allowed us to study the relationships between associated SNPs and their respective traits in detail.

Genetic variation at the *StGWD* locus is associated with starch-bound phosphate levels of tetraploid potato cultivars

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The chemical and functional properties of starches differ widely between plant species. A special feature of potato starch is the high amount of phosphate groups esterified to the amylopectin fraction. Several candidate genes involved in this trait can be proposed. The most promising candidate gene is Glucan Water Dikinase (GWD), a key enzyme in starch breakdown that catalyses the transfer of phosphate to the C-6 position of glucosyl residues of the amylopectin fraction. Werij et al (unpublished results) have demonstrated the co-localisation of the GWD locus and a QTL for starch-bound phosphate levels on potato chromosome 5, using the diploid potato mapping population CxE. The BC_1 structure of the CxE offspring allows the identification of only three GWD haplotypes in four possible combinations of alleles (ab × bc → ab, ac, bb, bc). Re-sequencing of GWD in a collection of 220 commercial tetraploid cultivars and progenitor lines revealed ten different haplotypes. Upon identification of linkage phase known tagSNPs within these haplotypes, the allelic composition of each tetraploid cultivar could be deduced. The alleles observed in cultivars matched with expectations based on pedigree information. The distribution of certain alleles at the time and place of cultivar introduction provides insight in commercial cultivar development. Linkage drag associated with the introgression of disease resistance could be traced. Significant associations have been detected between specific GWD-alleles and starch-bound phosphate levels. (JU and A-MW are supported by grant WPB7926 of the Dutch Technology Foundation STW).

Developing molecular genetic marker technology capability to enhance Australian potato breeding

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Potatoes grown for a range of products in Australia are worth c. AU$480m *per annum* at the farm gate. Cultivation occurs across a range of environments, including cool-temperate, Mediterranean, hot semi-arid and warm tropical. As potatoes show a significant genotype x environment response, different cultivars are grown in different locations. The potato industry is hence seeking new cultivars with superior performance in these different environments, and are resistant to relevant pest and disease pressures. The potato breeding program has distinct pre-breeding and commercial cultivar development phases. Commercial cultivar development currently uses a conventional potato breeding strategy, and relies on outcrossing and screening of a large number of derived lines in order to identify improved cultivars. Pre-breeding research is focused on developing the capacity to implement marker-assisted selection. As in other regions, potato cyst nematode (PCN) resistance is a priority for Australia, although incidence of only *G. rostochiensis* Ro1 has been recorded. The parental genotype collection has been phenotypically screened for resistance and assessed for ability of the TG689 marker to predict presence of the corresponding H1 resistance gene. Concordance of 98% was observed, which may be used in association with knowledge of pedigree. Tomato spotted wilt virus (TSWV) can be a significant local problem, requiring development of a reliable phenotyping method, which has proven challenging. Grafting and mechanical inoculation methods have been assessed to determine the most reliable method for challenge of pair cross-derived populations for linked genetic marker development. Potato virus Y (PVY) resistance is the next focus area, currently at the stage of phenotypic assessment for evaluation of 3 potential diagnostic markers. Activities in this program will permit adoption of molecular genetic marker-based breeding technology for commercial potato germplasm improvement in Australia.

Exploiting genetic variation for elevated mineral concentrations in potatoes

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At least 22 mineral elements are required for the well-being of humans and these can be supplied by a balanced diet. Mineral deficiencies are common in both developing and developed countries and the enhancement of mineral elements in food crops represents a possible strategy to increase the human dietary mineral intake. To determine the prospects for this approach in potatoes (*Solanum tuberosum* L.), we screened a diverse set of cultivated potato germplasm, including a Phureja-Tuberosum Core Collection and a Neotuberosum population (derived from Andean tetraploids). Concentrations of mineral elements were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on freeze-dried tuber samples. Mineral elements measured included those of human dietary significance such as iron, zinc, copper, calcium, magnesium and manganese in addition to others of more importance to the plant such as phosphorus, potassium and sulphur. Significant differences were found between diploid Phureja and tetraploid Tuberosum clones. Neotuberosum clones showed a greater variation for most mineral elements and have a broader genetic background. The genetic basis for variation in concentrations of mineral elements was further studied by Quantitative Trait Loci (QTL) analysis, using an F1 tetraploid mapping population, 12601ab1 x Stirling. Statistical analysis showed a significant variation between clones for tuber concentrations of mineral elements. A total of 30 QTLs were detected for nine mineral elements by interval mapping using TetraploidMap software. Several QTLs associated with maturity were identified on Stirling Linkage group V. Identification of candidate genes underlying mineral traits is currently underway and this will enable marker-assisted breeding to enrich potatoes with essential dietary minerals.

Pathogen Resistance

Additional genetic factors behind the responsiveness and higher levels of virus resistance expressed by potato genotypes carrying virus-specific R genes

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Limited durability of dominant, monogenic, R gene mediated resistance to pathogens in the field has raised needs to better utilize and combine different types of resistance to pathogens in cultivars. However, breeding for multigenic resistance could be a tedious task without knowledge of the genes involved, or without tangible tools for screening the genes in breeding populations. The aim of this study was to better understand the genetic factors which may play a role in the responsiveness and higher levels of virus resistance expressed by potato genotypes carrying virus-specific R genes. Gene expression was compared by microarray analysis and real-time RT-PCR between the F1 progeny genotypes which react with i) hypersensitive response to PVA (HR), ii) allow PVA accumulation in inoculated leaves but restrict PVA infection to the inoculated leaf by blocking systemic movement (nnr), or iii) are susceptible and systemically infected with PVA (S) (Vuorinen et al. 2010, Ann. Appl. Biol., in press). Several defense-related genes (DRGs) were autoactivated in HR genotypes at an early stage of plant growth in the absence of PVA infection, which was not observed in the phenotypic groups nnr and S. Autoactivation of DRGs was also not evident *in vitro* and up to 2 weeks of growth in soil in a controlled growth cabinet, but was apparent 2 weeks later. A number of other DRGs were induced in the inoculated leaves of HR genotypes as a response to infection with PVA, which was not observed in nnr and S genotypes. Data suggest that part of the higher responsiveness and resistance in the HR genotypes is attributable to additional 'minor' genes induced by environment, growth stage and/or infection. Some genotypes might also be more responsive to chemical induction of pathogen and pest resistance, which could be screened for in plant breeding programs.

Exploring intragenic approaches towards disease resistance in potatoes

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The use of resistant cultivars in potato breeding programmes is an important tool for disease management. Recent advances in plant molecular genetics have identified several genes for resistance to potato diseases from within the germplasm pool available to potato breeders. Antimicrobial peptides, such as Snakin-1 (StSN1) and Snakin-2 (StSN2), have been isolated from potato tubers. Over-expression of the *StSN1* and *StSN2* genes in potato is known to provide broad spectrum activity against a wide range of bacterial and fungal pathogens.
We have investigated the use of intragenic gene transfer technology towards disease resistance in potatoes. An intragenic expression cassette was constructed with the 5' promoter and 3' terminator regions of a potato gene for chlorophyll a/b binding protein (*StLhca3*). The coding regions of the potato *StSN1* and *StSN2* genes were cloned individually between these regulatory regions. The resulting intragenes were cloned into the binary vector pMOA33 and transferred to potato cultivar Iwa using *Agrobacterium*-mediated transformation. Over 30 independently derived transformed lines for each intragene were screened using quantitative RT-PCR to identify those with high expression. Pathogenicity bioassays on these lines showed that over-expression of either *StSN1* or *StSN2* conferred resistance to *Pectobacterium atrosepticum* (previously called *Erwinia carotovora* subsp. *atroseptica).* The cab5'-*StSN*-cab3' chimeric intragenes are being transferred into potato using intragenic vectors/minicircles to develop disease-resistant intragenic potato plants.

Breeding research for main virus resistance in potato

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The meaning of resistance breeding against viruses has a different weight in the context of environmental conditions for potato production, the development of seed sector in the region, and availability of cheep, healthy seeds for the potato growers.
In Poland, potatoes are grown on the whole territory of the country under strong viral infection pressure, and seed exchange is not a common habit in small farms, which are predominant in our agricultural structure.
Currently, breeding research for virus resistance is focused on three main viruses: PVY, PLRV and PVM, of which PVY is the most destructive in Poland. Recent decades yielded enormous variation of the PVY population. Since 2004 a rapid increase of the PVY^{NTN} was noted in potato fields, and last year this strain reached 66% of the Polish population of PVY. Unpredictable variations in serological, biological and molecular features of PVY isolates might have evidenced high rate of recombination events in present PVY population. Breeding for PVY resistance was initiated in Poland in early 1950-ties within a program for development of the parental lines with multiple resistances to viruses. The goal was to introduce into parental forms the resistances to six viruses: PVY, PLRV, PVM, PVS, PVX, PVA.
Two independent sources of Ry_{sto} gene were utilized in the program: Ry_{sto} associated with male sterility originated form MPI (Ross, 1986) and $Ry\text{-}f_{sto}$ (fertile) originated from VIR, St. Petersburg. The gene $Ry\text{-}f_{sto}$ provides a comprehensive resistance to all known strains of PVY and PVA and it was mapped to chromosome XII. We have developed CAPS marker GP122 linked to the resistance gene and successfully used for identification of forms extremely resistant to PVY. Nowadays, this marker is used for MAS. Recently, the novel, hypersensitivity gene *Ny-1* found in cv. Rywal has been mapped. The expression of HR was temperature – dependent. Major PVY strains were effectively localized when *Ny-1* plants were grown at 20°C, while at 28°C plants were systemically infected.
PLRV resistance introduced into *4x* parental lines originated from cvs. Aquila and Apta and MPI 49/540/2. Complimentary, on diploid level were utilized highly resistant lines originated from *S. chacoense* and *S. tuberosum*. Genes *PLRV.1 (chc)* and *PLRV.4 (tbr)* of diploid sources were localized on chromosome XI, although the found markers associated to them are not exploited in MAS, and selection of resistant forms based on phenotyping.
PVM is an important virus frequently found in Poland and East European countries. Resistance to PVM is rare in cultivars. Up to now three cvs originated form IHAR's parental lines expressed high resistance to PVM. Sources of resistance exploited in parental line breeding originated from *S. megistacrolobum* (governed by *Rm* gene, HR type) or *S. gourlayi* (governed by *Gm* gene, resistance to multiplication type). Both genes were mapped to potato chromosomes in clusters of resistance genes: *Rm* to chromosome IX and *Gm* to chromosome XI. DNA markers for both genes were identified. First parental lines with the *Rm* gene were delivered to breeders in 1985, and with the *Gm* gene in 1993. In 2002 the gene *Ns* was mapped to chromosome VIII, and PCR markers for this gene were also developed. Since 1967 the breeding lines with an increasing complexity of virus resistances have been offered to Polish breeders. Out of 271 parental lines offered, 58 cultivars were developed.
The input of Parental Lines Program in enhanced resistance to viruses of Polish cultivars has been discussed.

Physical map and comparative genomics of the potato cyst nematode resistance locus *H1* at three haplotypes in potato

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The *H1* locus confers resistance to the potato cyst nematode *Globodera rostochiensis* pathotypes 1 and 4. It is positioned at the distal end of chromosome V of the diploid *Solanum tuberosum* genotype SH83-92-488 (SH) on an introgression segment derived from *S. tuberosum* ssp. *andigena*. Markers from a high-resolution genetic map of the *H1* locus were used to screen a BAC library to construct a physical map covering a 341 Kb region of the resistant haplotype coming from SH. For comparison, physical maps were also generated of the two haplotypes from the diploid susceptible genotype RH89-039-16 (*S. tuberosum* ssp. *tuberosum/ S. phureja*), spanning syntenic regions of 700 and 319 Kb. Gene predictions on the genomic segments resulted in the identification of large cluster consisting of variable numbers of the CC-NB-LRR type of *R* genes for each haplotype. Furthermore, the regions were interspersed with numerous transposable elements and genes coding for an extensin-like protein and an amino acid transporter. Comparative analysis revealed a major lack of gene order conservation in the sequences of the three closely related haplotypes. Our data provide insight in the evolutionary mechanisms shaping the *H1* locus and will facilitate the map-based cloning of the *H1* resistance gene.

Financial support was obtained from CBSG and the EU IP BIOEXPLOIT (FOOD-CT-2005-513959)

Quality Traits

Identification of genes that impact on potato tuber colour, flavour and texture using transcriptomic and transgenic approaches

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A wide range of metabolites impact on potato tuber nutritional and sensory quality. Research at SCRI has focussed on tuber isoprenoid metabolites particularly carotenoids. Transgenic experiments have clearly illustrated the potential for enhancing potato tuber carotenoid content. However, equally clear is that the further development of the potato tuber as a platform for carotenoid biosynthesis requires a greater understanding of this pathway in terms of its regulation and storage organelle capacity. Thus we have applied a genetic approach combined with the use of the 44K element POCI microarray to give us new insights into how the pathway is regulated. As the nutritional value of flavonoids such as anthocyanins becomes more apparent, tuber flavonoid metabolism is also attracting more research interest. From comparative transcript profiling of potato germplasm differentiated in tuber anthocyanin content several novel candidate genes likely to impact on this process have been identified. Flavour and texture are also important factors in driving consumer choice. However these traits are generally only assessed in the later stages of a breeding programme after selection for more easily quantifiable traits. In order to understand potato tuber flavour and texture we have compared metabolite and transcript profiles in potato germplasm clearly differentiated in these traits. A range of volatile and non-volatile metabolites are associated with potato flavour and candidate genes associated with the biosynthesis of these molecules have been identified. Using parallel approaches, genes that impact on potato tuber texture have also been characterised.

Mapping and validation of QTL for after-cooking darkening.

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After-cooking darkening (ACD) of potato tubers is a critical quality trait for fresh market and processing sectors of the potato industry. A diploid population comprised of 138 F1 hybrids was characterized for ACD and other traits in six environments. The population was genotyped using simple sequence repeat and high-resolution DNA melting (HRM) markers. A linkage map was generated and used for quantitative trait loci (QTL) analysis. Four major QTL regions were detected on chromosomes 2, 4, 6, and 10. Two additional populations – one diploid and another tetraploid were used to validate these and other minor QTLs. Three of the four larger QTLs were significant in the diploid validation population and an additional QTL was detected. Dosage-sensitive HRM markers linked to the ACD QTLs were developed and tested in a small tetraploid population. The identified QTLs for ACD have confirmed the role of chlorogenic acid biosynthesis genes and have also suggested that genes in the anthocyanin biosynthesis pathway may be important for this trait. These HRM probe assays will be useful for breeding tetraploid potatoes with low ACD.

Potato Tuber Bruising: Some are more hurt than others.

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Important agronomic plant traits mostly rely on complex molecular networks and the action of multiple genes. Tuber bruising, a crucial problem concerning tuber value, is a quality trait of cultivated potato (*Solanum tuberosum*) and characterized by an internal tissue discoloration initiated by mechanical impact. The existing natural diversity for bruising is exploited in order to elucidate its molecular basis. An association mapping study was conducted, using a candidate gene approach to identify the genes contributing to tuber bruising and the enzymatic browning component. A population of 205 tetraploid potato genotypes related by descent was evaluated for two years at six environments for bruising as well as other relevant tuber quality traits such as starch content. A total of 362 polymorphic DNA fragments, including 157 SSR alleles, derived from 33 candidate genes and 29 SSR loci were scored and tested for association with the evaluated traits using a mixed model approach. Several markers like polyphenoloxidase (PPO) alleles associated with bruising and other tuber characteristics. Further genetic and biochemical analysis of total tuber PPO activity revealed a large natural enzymatic variation within the population and a correlation between apparent *Vmax* values and bruising susceptibility of the tested potato varieties. Furthermore, *Genomics* in combination with 2D-Gel analysis (*Proteomics*) of bruising specific tuber protein variation as well as time-dependent damage induced proteomic remodeling led to the identification of novel candidate genes and/or bruising biomarkers. The observed trait correlations and the significant and robust associations will facilitate diagnosis and combination of superior alleles in breeding programs. This project is a joint endeavor between *Omics* and potato breeding and is funded by the InnoNet program of the German Ministry for Economy and Technology (BMWi).

Dynamics of senescence-related QTLs in potato using time series data

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The study of the expression of quantitative traits over time helps to understand developmental processes which occur during the growing season. Temperature and other environmental factors play an important role. The dynamics of haulm senescence was observed in a diploid potato mapping population in two consecutive years (2004 and 2005) under field conditions in Finland. The available time series data were used in a smoothed generalized linear model to characterize the curves describing the senescence development in terms of onset, mean and maximum progression rate and inflection point. These characteristics together with the individual time points were used in a Quantitative Trait Loci (QTL) analysis. Although QTLs occurring early in the senescence process coincided with QTLs for onset of senescence, the analysis of the time points made it difficult to study senescence as a continuous trait. Characteristics estimated from the senescence curve allowed us to study it as a developmental process and give a meaningful biological interpretation to the results. Stable QTLs in the two experimental years were identified for progression rate and year specific QTLs were detected for onset of senescence and inflection point. Interesting pleiotropic effects and epistatic interactions between QTLs were also detected when two-way interactions were studied.

A comprehensive approach to study quantitative traits using after-cooking darkening as a model

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Over a decade of research on after-cooking darkening in potato tubers, the author and her collaborators have generated a series of genetic, biochemical and agronomic data, illustrating the complexity of the trait. However, some controlling factors, either genomic or environmental, have always shown significance regardless the methods of the analysis. Therefore, it is believed that quantitative traits can be effectively analyzed in a timingly fashion, once clear objectives and strategies are identified. This presentation will use after-cooking darkening as a model to demonstrate the road map designed and the subsequent investigations took place, and the combined outcomes of current understanding of the trait. The author will also propose some most effective downstream approaches for cultivar development/improvement based on the findings.

Identification of alternative oxidase marker alleles associated with reducing sugar content in diploid potato tubers

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Two *AOX* (alternative oxidase) gene families have been described in higher plants. The *AOX1* genes are induced by environmental stress, whereas *AOX2* are usually constitutively or developmentally expressed. The AOX1 activity seems to play a protective function against cell damage in response to the reactive oxygen species production under environmental stresses.New candidate gene *AOX1a* for reducing sugar content in diploid potato tubers was identified. We found for the first time an allelic variation in the loci AOX1a which was significantly correlated with reducing sugar content in potato and explained up to17.5% of the variability of the trait. Our results provide indirect data that *AOX1a* is the genetic factor involved in sugar content control in potato tubers. However, stimulation of respiration and alternative oxidase activity in potato tubers upon storage at 4°C is well established. *AOX1a* mapped on potato chromosome VIII.

From marker to function: The role of natural variation of starch phosphorylase in cold sweetening

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The broad genetic diversity of potato cultivars and wild type relatives offers a wealth of natural resources from which breeders can select the best performing genotypes for their varieties. New breeding dynamics call not only for the development of markers, but also for the better understanding of the molecular basis underlying physiological processes in crop plants. In this direction, previous association mapping experiments identified polymorphisms in genes of the carbohydrate metabolism which are associated with tuber starch content and chip quality before and after cold storage. Two of these genes encode starch phosphorylases (*Stp*). To elucidate the possible role of *Stp* for natural variation in tuber starch and sugar content, full length Stp cDNAs were cloned and an allele corresponding to the associated polymorphisms was identified. The association of this allele was further confirmed for tuber sugar accumulation upon cold storage in a population of cultivars selected for superior versus inferior chip quality. The expression of the associated *Stp* allele was shown to be constant during cold storage and independent of the dosage, suggesting that the alleles are not subjected to transcriptional regulation. Sequencing data showing novel amino acid exchanges in addition to preliminary observations at the protein level suggest post-transcriptional modifications. The knowledge gained about the performance of the natural alleles in cold storage and future prospects on this research are summarized.

Breeding for Improved Tuber Internal Quality and Processing Quality Traits.

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Tuber internal quality is a major limiting factor for the US potato industry. Breeders invest time and money in producing advanced selections which, in the end, often fail because of tuber internal defects, tuber bruising or storage quality issues. In-season fertilization with calcium is known to result in an increase in tuber calcium and lowered incidence of tuber internal defects, bruise susceptibility and reduced storage rot. Cultivated potato tubers are generally deficient in calcium. Our studies are aimed at investigating the genetic potential for improving tuber calcium accumulation ability and to determine if this improvement will lead to improved tuber quality. We have found significant genetic variations in tuber calcium accumulation ability among the major US potato cultivars. Atlantic, a popular chip cultivar, suffers from poor internal quality and high scab susceptibility. Superior on the other hand has good tuber internal quality and scab tolerance as well as high tuber calcium. In an attempt to improve tuber internal quality of Atlantic we have developed a population, from reciprocal crosses between Atlantic and Superior, that is segregating for calcium accumulation ability, specific gravity, yield, hollow heart, bruise and pitted scab. We are evaluating and screening these hybrids to identify clones that combine the desired traits of Atlantic (high gravity and high yield) and Superior (high calcium and high internal quality), with agronomic traits such as yield, tuber size and tuber appearance. By simultaneous evaluation of these hybrids for disease and pest resistance and performance under commercial production, we hope to make a rapid progress towards developing better chipping cultivars with enhanced tuber internal quality and tuber storage quality. In addition we are utilizing wild species *S. microdontum* and *S. kurtizianum*, that represents the extremes for tuber calcium traits, to understand the genetics of tuber calcium uptake and the potential role of tuber calcium in tuber quality.

Omics Studies

Interpreting and exploiting genome data based on suitable integrated bioinformatics platform.

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The long-term goal of the International Solanaceae Genome Project is to exploit the information generated from '-omics' on Solanaceae species, in order to analyze the genome organization, the functionality and the molecular evolution of the entire *Solanaceae* family. This encouraged the production of an overwhelming amount of molecular data requiring advanced computational technologies to properly offer bioinformatics resources for suitable investigation and data mining. In this frame, we designed and implemented the multilevel computational environment ISOL@, an Italian SOLAnaceae resource. In its early life, ISOL@ was exclusively focused on the analysis of the BAC-based tomato genome sequence, but it was constantly updated and evolved to adapt to novel technologies and to other Solanaceae species. At present, ISOL@ is made up of different databases integrated into a unique platform including tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) genome sequences and the publically available Solanaceae transcriptomes, offering tools for comparative analyses within Solanaceae. ISOL@ can be accessed through two convenient gateways. The *genome gateway* allows the annotations of tomato and potato genome sequences to be browsed; the *transcriptome gateway* provides an access point to explore the catalogue of transcripts from different species collected in the SolEST database and their association with the genome references. The platform includes accessory applications to improve cross referencing among data and to profit from the integration of various, heterogeneous collections from different species. The gathering and convergence of data generated by high-throughput technologies, the effective integration of different collections and the analysis of the information content based on comparative approaches represent the challenges that ISOL@ attempts to solve. Indeed, we believe they represent key approaches to support '-omics' efforts and meaningful interpretations, useful to exploit and understand biological relevant information from intriguing data sources.

An integrated genome wide genetic map of sequenced NB-LRR disease resistance gene homologues (RGH) and resistance loci in potato

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Like all plants, potato has evolved a surveillance system consisting of a large array of genes encoding for immune receptors that confer resistance to pathogens and pests. These so-called resistance or R proteins are composed of functional modules involved in pathogen recognition and the activation of a defence response. The majority of resistance genes identified to date belongs to the class of genes encoding nucleotide binding (NBS) and leucine rich repeats (LRR) domains. To date, twelve functional resistance genes have been identified in potato. However, at more than twenty regions in the potato genome, one or more resistance loci have been mapped, but none of the underlying genes have been identified. Therefore, we screened the RH BAC library with NBS probes and a representative set of 288 unique BACs was selected for sequencing and mapping. This resulted in the identification of 767 NB-LRR genes and gene fragments, which could be grouped into forty-five discrete NB-LRR clusters that were distributed throughout the potato genome. Ten RGH clusters are syntenic to previously identified functional *R* genes, whereas thirty-five clusters are novel. Integration of functional resistance loci described in literature revealed that they often co-localise with the RGH clusters. Hence, this integrated whole genome RGH map provides a rich source to employ marker assisted selection and/or a candidate gene approach for the identification of resistance genes in potato.

An integrative -omics approach for studying potato tuber quality traits

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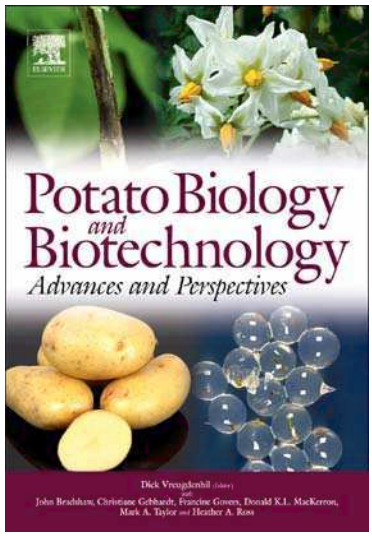
Utilization of the natural genetic variation in traditional breeding programs remains a major challenge in crop plants. The development of high throughput –omics technologies has brought the potential of population wide data collection in fields like transcriptomics, metabolomics and proteomics. As a result, large amounts of data have become available which need to be associated with observed trait variation. We have screened a diploid potato population for gene-expression and secondary metabolite content using a microarray and LC-MS approach respectively. Variation within these data sets was treated as a quantitative trait and resulted in the identification of many expression and metabolite quantitative trait loci (eQTL and mQTL's). However, the integration of the data sets together with phenotypic and marker data is still problematic. Here we present approaches to study the various ~omics datasets to allow the construction of networks integrating gene expression, metabolites and phenotypic data. We used univariate regression and modern regression methods to select subset of the metabolites and transcripts that showed association with potato tuber flesh colour. Network reconstruction approaches after data integration can be used to visualize a pathway of individual components associated with a trait of interest for which the functional genes and metabolites are still unknown. We present such a network approach targeting tuber flesh colour as a quality trait.



POTATO BIOLOGY AND BIOTECHNOLOGY: ADVANCES AND PERSPECTIVES

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Description
In the past 15-20 years major discoveries have been concluded on potato biology and biotechnology. Important new tools have been developed in the area of molecular genetics, and our understanding of potato physiology has been revolutionized due to amenability of the potato to genetic transformation. This technology has impacted our understanding of the molecular basis of plant-pathogen interaction and has also opened new opportunities for the use of the potato in a variety of non-food biotechnological purposes. This book covers the potato world market as it expands further into the new millennium. Authors stress the overriding need for stable yields to eliminate human hunger and poverty, while considering solutions to enhance global production and distribution. It comprehensively describes genetics and genetic resources, plant growth and development, response to the environment, tuber quality, pests and diseases, biotechnology and crop management. **Potato Biology** is the most valuable reference available for all professionals involved in the potato industry, plant biologists and agronomists.

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I. The markets 1. The fresh potato market Iain McGregor 2. Global markets for processed potato products Michael A. Kirkman 3. The seed potato market Cees D. van Loon II. Genetics and genetic resources 4. Molecular taxonomy Ronald G. van den Berg and Mirjam M.J. Jacobs 5. Molecular markers, maps and population genetics Christiane Gebhardt 6. Genetics of morphological and tuber traits Herman J. van Eck 7. Genetics of resistance to pests and disease Ivan Simko, Shelley Jansky, Sarah Stephenson and David Spooner 8. Potato breeding strategy John E. Bradshaw 9. Genomics Glenn J. Bryan 10. Potato cytogenetics Tatjana Gavrilenko III. Plant growth and development 11. Above-ground and below-ground plant development Paul C. Struik 12. Signalling the induction of tuber formation David J. Hannapel 13. Photosynthesis, carbohydrate metabolism and source-sink relations Daniel Hofius and Frederik A. J. B rnke 14. Dormancy and sprouting Jeffrey C. Suttle 15. Molecular physiology of the mineral nutrition of the potato Marcel Bucher and Jens Kossmann IV. Responses to the environment 16. Water availability and potato crop performance Jan Vos and Anton J. Haverkort 17. Potato crop response to radiation and daylength A.J. Haverkort 18. Response to the environment: temperature Paul C. Struik 19. Response to the environment: carbon dioxide Ludwig De Temmerman, Karine Vandermeiren, Marcel van Oijen 20. Towards the development of salt tolerant potato D.J. Donnelly , S.O. Prasher and R.M. Patel V. Tuber quality 21. The harvested crop Mike Storey 22. Skin-set, wound-healing and related defects Edward C. Lulai 23. Internal physiological disorders, nutritional and compositional factors that affect market quality Joseph R. Sowokinos 24. Potato Flavour and Texture Mark A. Taylor, Gordon J. McDougall and Derek Stewart VI. Pests and diseases 25. Insect pests in potato Edward B. Radcliffe and Abdelaziz Lagnaoui 26. The nematode parasites of potato Didier Mugni ry and Mark S. Phillips 27. Bacterial pathogens of potato Jan M. van der Wolf and Solke H. De Boer 28. Viruses: economical losses and biotechnological potential Jari P.T. Valkonen 29. Fungal and fungus-like pathogens of potato Aad J. Termorshuizen VII. Biotechnology 30. Developments in transgenic biology and the genetic engineering of useful traits Steve Millam 31. Field-testing of transgenic potatoes A. J. Conner 32. Soil-free techniques Steve Millam and Sanjeev K. Sharma VIII. Crop management 33. Agronomic practices D.M. Firman and E.J. Allen 34. Minerals, soils and roots Philip J. White, Ron E. Wheatley, John P. Hammond and Kefeng Zhang 35. Mathematical models of plant growth and development D.K.L. MacKerron 36. Decision support systems in potato production B. Marshall

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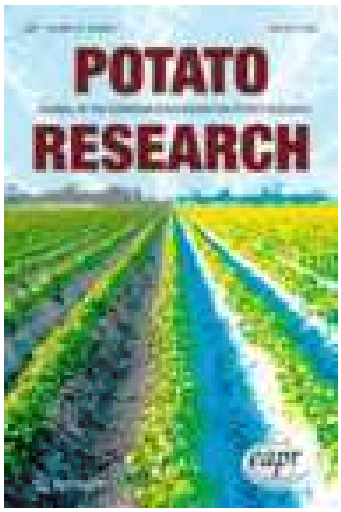

POTATO RESEARCH

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Aims and Scope

Research, the journal of the European Association for Potato Research (EAPR), promotes the exchange of information on all aspects of this fast-evolving global industry. It offers the latest developments in innovative research to scientists active in potato research. The journal includes authoritative coverage of new scientific developments, publishing original research and review papers on such topics as:

- Molecular sciences
- Breeding
- Physiology
- Pathology
- Nematology
- Virology
- Agronomy
- Engineering and Utilization

In addition, Potato Research publishes reports and papers from the Triennial Conferences of the EAPR, along with accounts of meetings of the Sections of the Association, book reviews, and more.

Editorial Board:

Abstracts Poster presentations

1 - PAPACLIMA: A project to identification and exploitation of candidate genes for adaptation to abiotic stresses caused by climate change for potato breeding.

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Abiotic stresses caused by climate change represent a critical limitation and a mayor threat for sustainable agriculture and food security. It is necessary to develop new cultivars with tolerance to abiotic stresses by exploiting the existing biodiversity of species. In the PAPACLIMA project we are developing molecular markers for the characterization and identification of commercial potato varieties and useful wild species for breeding which are adapted to the threats of climate change. For this purpose we are evaluating in these materials the adaptation to different abiotic stresses (heat, coldness, drought) by means of field and greenhouse trials. Some promising accessions have already been identified. We are also detecting candidate genes (CG) for tolerance to these stresses using different molecular tools. On the other hand we are exploiting existing genomic resources in potato and other plant species to detect new CG or for validating known candidate genes. We are developing molecular markers based on the sequence information of CG for analyzing their allelic variability in our germplasm and for associating specific phenotypic expression levels with particular alleles. A Knowledge Base is being established with all results and a molecular marker set will be selected for analysing stress adaptation in potatoes and related species which can be used for marker assisted breeding in potato.

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2 - Characteristics of potato tuber proteins of selected cultivated potato species

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Four untraditional potato species with potential in potato breeding were evaluated for basic tuber protein characteristics – concentration of total nitrogen substances (on dry matter basis), concentration of "pure" protein (on dry matter basis), relative abundance of patatin proteins and number of patatin mass isoforms. *Solanum tuberosum* cv. Desireé was used as control system. All of the evaluated potato species genotypes (*S. tuberosum* cv. Desireé, *S. andigena*, *S. phureja*, *S. goniocalyx*, *S. stenotomum*) were obtained from the genebank of Potato Research Institute Ltd., Havlíčkův Brod. Because of low dormancy ability of *S. phureja* (Griola negra, genebank accession 703295) tubers, both variant of the *S.phureja* tubers – germinated and ungerminated, were taken into account. Ungerminated tubers of *S. phureja* exhibited extremely high level of N matters (33.6 %) and high level of proteins (14.6 %). Interestingly high levels of tuber protein were also examined for species *S. stenotomum* (10.7 %) and *S. andigena* (7.5 %). Patatin represents nutritiously improving part of the potato tuber protein with genetically highly fixed occurrence in potato tubers. Increasing of patatin relative abundance is one of the breeding possibilities for enhancing of potato tuber protein quality. Detection of patatin proteins isoforms and their quantification was performed using chip electrophoresis system Experion (BioRad, USA). Patatin relative abundance of 44.5 % was examined for the species *S. andigenum*; high levels of patatin proteins were also examined for species *S. stenotomum* (37.7 %) and *S. goniocalyx* (38.7 %). Storage function of patatin proteins was obvious from intensive declined of this protein system during *S. phureja* tubers germination. Germinated tubers contained 9.2 % of patatin in total tuber protein in contrast to 28.2 % of patatin proteins detected in ungerminated tubers of the same species accession. All of the presented results were statistically evaluated using one-way ANOVA method with significance difference at $P \leq 0.05$. The work was supported by project NAZV QI 91A069 and MSM 6007665806.

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3 - Using the DArT marker platform and tomato/potato reference genome sequence for comparative genomics in *Solanum*

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A DArT array for use with wild potato species has been developed. Comprised of approximately 16,000 elements, it is optimized to study the potato tertiary genepool. To date, DArT-linkage maps have been constructed for *Solanum commersonii* and *S. bulbocastanum.* To build the maps, we used F_1 mapping populations. At LOD score 4.0, the *S. commersonii* map consisted of 13 linkage groups, whereas 12 linkage groups were generated for *S. bulbocastanum*. Length and density of the *S. bulbocastanum* map were higher than those of *S. commersonii*. DArT markers correspond to bacterial clone libraries. Plasmid inserts of DArT markers were sequenced and compared to the available sequenced genomes of tomato and potato. Analysis revealed that most of DArT markers originate from expressed regions. To date, based on sequence similarity scores, *S. bulbocastanum* mapped DArT markers have been assigned to locations on the tomato and potato physical maps. This comparative mapping approach confirmed marker order along *S. bulbocastanum* linkage groups. Moreover, it allowed assignment of *S. bulbocastanum* linkage groups to specific tomato or potato chromosomes. Our results established the utility and power of comparative mapping for the study of wild *Solanum* species. In addition, the resources we have generated will facilitate access to economically important traits.

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4 - Markering of the major QTL regulating the osmotic stress tolerance in tetraploid potato

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Osmotic stresses such as drought or salinity are major limiting factors to plant productivity and crop production. Molecular control mechanisms of osmotic stress tolerance are based on the activation and regulation of specific genes playing role in signaling, transcriptional control, protection of membranes and proteins, free-radical and toxic-compound scavenging. Identifying genomic regions (QTLs) that regulate different stress tolerance can help to develop new tolerant potato cultivars through marker-assisted breeding.
A tetraploid potato mapping population of 88 F_1 genotypes derived from a cross between cv. White Lady (WL) and breeding line S440 was analysed using different molecular techniques to map the major QTLs playing role in osmotic stress tolerance. The population was tested for osmotic stress tolerance in-vitro, using mannitol as selective agent. Two root parameters including average root length and average root number per plant were measured. The genotypes showed significant variation in their osmotic stress tolerance under stress condition. F_1 genotypes showed transgressive segregation in their average root length.
Partial linkage maps of different markers (SSR, IT, ISSR, RAPD) were constructed for both parents. The major QTL (LOD>5) which explained about 50% of the total variance was mapped a linkage group consisting seven markers in the parent WL.

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5 - Evaluation of salinity tolerance traits in a diploid potato mapping population

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Soil salination is a primary factor in crop yield losses especially in irrigated agriculture. Nearly 7% of the world's total land area and more than 25% of the world irrigated lands is affected and the problem is further increasing.
Potato is a major field crop that is relatively sensitive to salt. Breeding for salt tolerance is not straightforward since it is a complex trait. The most severe effect of salt stress is ionic cytotoxicity, which is caused by replacement of K^+ by Na^+ in the cytoplasm. To cope with salt stress, plants try to maintain high K^+/Na^+ ratio in the cytosol of the cells, especially in the shoot.
We evaluated salt tolerance of a diploid potato population under controlled conditions on hydroponics in the greenhouse. During a period of salt stress several physiological traits were measured. The plants were harvested after two weeks and growth parameters were determined, as well as ion contents. High variation for salt tolerance was observed in the progeny, with some plants hardly surviving while others seemed relative unaffected. Important traits like leaf area, shoot length, plant fresh weight and chlorophyll content were affected by salt treatment, and had a high heritability.
Correlation analyses were done with the collected trait data and positive correlations were found under saline conditions of K^+ concentration in roots, stems and most notably leaves with total leaf area, plant weight and shoot length. Na^+ content was negatively correlated with these growth parameters, as well as with chlorophyll content in older leaves. This might indicate that tissue tolerance to high Na^+ especially in older leaves and the ability to maintain low Na^+ in younger leaves are mechanisms that can confer salinity tolerance in potato.
We are currently performing QTL analysis to identify areas of the potato genome that are responsible for traits that enhance salt tolerance.

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6 - Marker driven breeding for the rapid pyramiding and multiplexing of genes underlying disease resistance traits

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Most potato breeding programmes aimed at commercial cultivar development follow the same basic breeding scheme which typically takes up to 15 years to produce a variety. The breeding scheme comprises two phases, a single round of crossing and over a decade of phenotypic selection and advanced trialling to produce a new variety. The goal of potato breeding programmes is to produce high-yielding, uniform varieties that possess a combination of disease resistance and tuber quality traits. Significantly however, the implementation of such a breeding scheme makes production of varieties that combine all these traits extremely difficult to achieve, because each 15 year breeding cycle is based on only a single round of crossing, limiting the number of traits that can be combined in this period. Over the last decade, DNA-based molecular markers which allow diagnosis for the presence or absence of the genetic components of traits without the need for laborious multiplication and testing have gradually become available for several disease resistances and quality characteristics, raising the possibility of accelerated breeding programmes. We have initiated a small scale experimental breeding programme to investigate the potential for the routine deployment of marker-assisted selection (MAS) as part of the potato breeding programme at Oak Park. In this scheme, MAS is being used to allow the use of recurrent selection in order to rapidly pyramid and multiplex multiple traits into single genotypes over three successive annual rounds of crossing. A limited amount of phenotypic selection for breeders preference is also being implemented. At the end of the three year period, suitably high performing material will be advanced to the main breeding programme.

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7 - Assessing genetic diversity in wild potato species via SSRs

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Genetic resources of cultivated potato (*Solanum tuberosum* L. subsp. *tuberosum*) constitute an important gene reservoir for valuable agronomic traits like resistances to diseases or pests. In a project aiming at the development of molecular markers for alleles confering resistance to potato wart (*Synchytrium endobioticum* (Schilb.) Perc.), wild potato accessions from the IPK Genebank were examined in order to assess their degree of intra- and inter-accession variability, as well as their interspecific diversity (where applicable). Twelve species from nine taxonomic series of tuber-bearing potatoes were selected because of earlier publications on their resistance to potato wart. In a first step, tubers from appr. 800 individual genotypes coming from 82 genebank accessions were produced and (partially) tested for resistance against *S. endobioticum* (race 18) at JKI Kleinmachnow according to Glynne-Lemmerzahl. Simultaneoulsy, leaf material of all individuals was harvested and used for the extraction of genomic DNA. SSR analyses were conducted in order to elucidate the applicability and power of resolution of microsatellite markers from different sources (genomic DNA/EST sequences) of cultivated potato. Here, 14 SSRs were employed in four multiplex PCR reactions and separated on an automated fragment analysis system using fluorescence labeling. Samples were loaded as two combined multiplexes containing amplification products from six or eight SSR primer pairs, respectively.The evaluation of the generated banding patterns shed a light on the degree of diversity within accessions - e.g. mainly low levels in selfers like *S. acaule* Bitter or *S. demissum* Lindley vs. higher levels in outcrossers like *S. sparsipilum* (Bitter) Juz. & Bukasov or *S. trifidum* Correll - and to a certain extent also within and between species. In combination with resistance data for the respective genotypes, this diversity assessment will provide the basis for the planned identification of novel resistance alleles against potato wart.

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8 - SNP markers and their associations in tetraploid potato

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With Illumina's GoldenGate SNP genotyping platform, 384 SNP assays were tested on a panel of 224 tetraploid cultivars. For 206 successful assays the ratio of fluorescent signals from the alleles (theta value) was analysed with specific software to classify the cultivars according to the five possible genotypes expected for tetraploids. Both the theta values and the SNP genotypes were used for a genome-wide association study. Three datasets with trait values were analyzed: (1) a three-locations field trial in 2008, (2) a replicated two-locations field trial in 2006, and (3) highly unbalanced multi-year multi-location trait observations from five Dutch breeding companies. Association analyses were performed using mixed models. Comparison of the significance of marker-trait associations (p-values) obtained with the two SNP data types resulted in a high correlation: r = 0.65. This suggests that estimation of genotypes from theta values is of limited value to identify additional marker-trait associations. But, predicted genotypes support the genetic interpretation of allelic substitutions on the trait value. Comparison of the detected associations between each phenotypic dataset resulted in the persistent identification of 26% of the marker loci.

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9 - Polyploiditation effects on trascriptomic and metabolic traits in synthetic polyploids of 2x *Solanum commersonii*

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The common occurrence of polyploidy suggests an evolutionary advantage of having multiple sets of genetic material for adaptive evolution. Polyploidy produces anatomical, biochemical and genetic changes that affect physiological processes in plants. Biochemical effects include changes in the amounts of gene products (gene dosage effects of polyploidy) and changes in biochemical diversity. Potato species provide excellent model systems for studies on polyploidy. The aims of this study were to produce 4x genotypes from 2x *Solanum commersonii* and to characterize synthetic polyploids from the biochemical and transcriptomic standpoint. For metabolite profiles, the analysis of 4x genotypes showed that leaf content of total alkaloids was significantly lower in the 4x genotypes than in the 2x progenitor. By contrast, 4x genotypes showed an increased amount of some phenol compounds, as caffeine and ferulic acids and rutin. To elucidate the molecular events associated to polyploidization, gene expression studies are being carried out. To reach this goal we have designed a CustomArray 90K Combimatrix which allows to analyse up to 30000 gene sequences using the 25000 potato TC present in the SolEST database. Moreover, we designed this array to study gene expression of 6000 genes with unknown orientation. We have analysed differences in gene expression between the parental and three different tetraploids. After hybridization, scan and signal analyses, now we are identifying genes whose expression is different in the two levels of ploidy. Bioinformatic analyses (annotation, clustering and mapping analyses) and Real-time PCR experiments are in progress.

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10 - Structural analysis of 77 full length *Rx/Gpa2* sequences derived from 10 distinct *Solanum* species

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The highly homologous resistance genes *Rx1* and *Gpa2* (88% sequence similarity) are located in a small *R* gene cluster on ChrXII in potato and confer resistance to two completely unrelated pathogens, respectively potato virus X (PVX) and the potato cyst nematode *Globodera pallida*. This suggests that subtle changes in R proteins can result in novel recognition specificities and makes these two genes an excellent model system to study the co-evolution between plants and pathogens driving *R* gene diversity and to unravel pathogen-specific patterns in the structure of R proteins. Therefore, full length genomic DNA sequences of 77 *Rx/Gpa2* homologues from 10 distinct *Solanum* species were obtained in an allele mining study to resolve the evolutionary patterns at this *R* gene cluster. In addition, the sequence information of the *Rx/Gpa2* homologues was used for computer-aided 3D modelling of the NB-ARC and LRR domain to investigate their structural features in more detail. Here, we show how primary sequence information can be converted into structure predictions of different R protein domains. These structural models facilitate the identification of potential target sites involved in pathogen recognition and R protein activation.

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11 - Tolerance to blackspot bruise of diploid potato hybrids and cultivars

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The objective of our study was to determine variability in tolerance to blackspot bruise among 12 potato cultivars and interspecific diploid hybrids originated from wild *Solanum* species and dihaploids of *S. tuberosum*. The thirty one diploid hybrids belonged to different groups: useful for direct consumption, chip processing, with high starch content, resistant to soft rot and to late blight. Cultivars were represented by seven table and five starch forms. For susceptibility to blackspot bruise 20 undamaged tubers were tested per genotype. Before the test tuber samples were stored by 5 months in storage at 5-10ºC then conditioned at 11ºC for 12 h. Blackspot bruise reaction in tubers was induced by impacts in hexagon plywood drum followed by incubation of tubers at 20ºC for 72 hours. Evaluation of blackspot bruises was performed directly after peeling of the tubers. Each tuber was scored separately. Two parameters of blackspot bruise reaction were described: (1) mean surface of blackspot bruises (scale 1-9, where 1= >80% surface of tuber covered by bruises, 9= lack of bruises), (2) discoloration of tuber surface according to Danish Coloured Cards (scale 1-9, 9- lack of discoloration). Additionally, blackening of fresh tuber flesh after 4 h of air exposition was evaluated.
We observed variability of diploid clones and cultivars in blackspot bruise reaction. Among tested hybrids we found some, useful for direct consumption and chipping, with high tolerance to blackspot bruise. The most susceptible were diploid clones with high starch content. Starch cultivars were also susceptible but not so much like diploids with high starch content. Several diploid clones with high tolerance to blackspot bruise able to produce 2*n* gametes are potential donors of this trait for tetraploid breeding pool.

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12 - Documentation of research data sets for long-term institutional archives associated with the sequencing of the potato genome

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Traceability and transparency on how hypotheses and conclusions are obtained are among the basic principles enabling scientific advance. However, as data generation increases exponentially, these principles are often left behind and valuable resources are lost. In recognition of this, international organizations like the OECD and science communities (e.g. minimum information standards at MIBBI.org) reinforce the need to standardize data documentation through guidelines and minimum standards including the management of versions and auditing of changes. The lack of consistency and standard tools becomes a major issue within the context of an institutional archive and research community. We explored the combination of five tools to derive an organizational scheme for an institutional archive and community resource. They are: a) the Dublin core list of generic bibliographical descriptors to describe any digital asset; b) the set of seven classical questions (who, when, why, where, what, what way, with what) serving at the same time as a unique key and an informative 'headliner' or name; c) the OECD classification of data types (tables, text, images, sounds); d) the use of minimal standards (ASCII) for text and tabular data files; and e) the use of best practices from documenting software source code to manage raw and final data, manage versions and audit changes made to digital documents by multiple users over time. The resulting scheme or structure – called MIDAP (minimum dataset platform) -can house community standards for data content. It can be implemented with a file browser. We developed and provide templates and applications for microsatellite and sequence data of the Potato Genome Sequencing Consortium for data pertaining to the potato genetic linkage map and mapping population. MIDAP is intended primarily for archival uses; it may be linked into databases allowing for full traceability of published data to original documentation.

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13 - Dynamics of senescence-related QTLs in potato using time series data

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The study of the expression of quantitative traits over time helps to understand developmental processes which occur during the growing season. Temperature and other environmental factors play an important role. The dynamics of haulm senescence was observed in a diploid potato mapping population in two consecutive years (2004 and 2005) under field conditions in Finland. The available time series data were used in a smoothed generalized linear model to characterize the curves describing the senescence development in terms of onset, mean and maximum progression rate and inflection point. These characteristics together with the individual time points were used in a Quantitative Trait Loci (QTL) analysis. Although QTLs occurring early in the senescence process coincided with QTLs for onset of senescence, the analysis of the time points made it difficult to study senescence as a continuous trait. Characteristics estimated from the senescence curve allowed us to study it as a developmental process and give a meaningful biological interpretation to the results. Stable QTLs in the two experimental years were identified for progression rate and year specific QTLs were detected for onset of senescence and inflection point. Interesting pleiotropic effects and epistatic interactions between QTLs were also detected when two-way interactions were studied.

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14 - Glycoalcaloid contents in a family from a cross between a diploid *S. tuberosum* and a diploid S. *sparsipilum* clone are under polygenic control.

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Breeders often exploit wild species to introgress new traits into potato varieties. In addition to the favorable traits, undesirable ones like "high content of glycoalcaloids in the tubers" can be introduced. That is why it is very important to identify the different chromosomic regions involved in the glycoalcaloid content in order to be able to detect eventual colocalisation of favorable QTL and undesirable traits, and to select against these last ones. In the present study, tubers of 95 clones from a diploid population between the dihaploïd Caspar H3 and a clone of the diploid wild species *Solanum sparsipilum* were analyzed for their α-chaconine and α-solanine contents by HPLC. The 2 glycoalcaloid contents were quantitatively distributed. QTL analysis performed according CIM methods allowed to detect nine QTLs involved in the character expression. They are present on both parents. For the α-chaconine, 3 QTLs were identified on the map of the *S. sparsipilum* clone on chromosomes I, V and VIII and 2 QTLs on the map of Caspar H3 on chromosomes IV and VI. For the α-solanonine, 2 QTLs were identified for each parent. They were mapped on the *S. sparsipilum* chromosomes V and XII and on the Caspar H3 chromosomes IV and VI. Each QTL explained between 8 and 18% of the total variance. In Caspar H3, two QTLs for α-chaconine and α-solanine contents colocalized and for *S. sparsipilum* the QTLs were mapped on the same position on chromosome V.

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15 - Genetic Variation in Potato (*Solanum tuberosum* L.) Canopy Development: A Model Approach using Standard Cultivars and a Segregating Population

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We investigated the potential of a model-based approach to assist in the genetic analysis of the environment-sensitive, quantitative crop trait canopy cover in potato (*Solanum tuberosum* L.). We used a model based on beta functions to analyze the genotype × environment interactions related to the dynamics of canopy cover. The model equations describe three phases of canopy growth: build-up phase (*P*1), maximum cover phase (*P*2), and decline phase (*P*3). The model has five parameters: t_{m1} indicates the transition from accelerating to diminishing growth during *P*1, t_1 marks the end of *P*1 when canopy cover attains its maximum level v_{max}, t_2 marks the end of *P*2 when canopy cover starts to decline, and t_e represents the end of the crop cycle when canopy cover has declined to nil. Values of these parameters were estimated for 100 individuals of an F1 population, their parents, and five standard cultivars differing in maturity type, using data collected in six field experiments. The model successfully described differences in canopy dynamics among individual genotypes across environments. Model parameters were used to derive several secondary variables: D_{P2} (duration of *P*2), D_{P3} (duration of *P*3), and A_{sum} (area under the canopy cover curve reflecting the crop's capacity to intercept incoming radiation). The length of *P*1 (i.e. t_1) was relatively conservative, but $D_{P2,}$ and D_{P3} varied greatly. Later genotypes had higher A_{sum} because they had longer $D_{P2,}$ and D_{P3}. Genotypic and phenotypic variance components of the F1 population were estimated for all traits across environments and almost all of them proved significant ($P<0.01$). For most traits, genetic variability and heritability were high. There are opportunities, therefore, for future potato breeding programmes to exploit the genetic variability available in the F1 segregating population and to select for highly heritable traits in order to improve radiation interception efficiency.

Key words: Potato (*Solanum tuberosum* L.), canopy dynamics, beta function, components of variance, genotype-by-environment interaction (G×E), genetic variability, heritability, maturity type.

16 - The role of molecular markers in the Bioimpuls organic potato breeding program

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The organic potato sector urgently needs better adapted, non GMO varieties to deal with the constraints of the low-input, organic farming system. Besides late blight resistance the program focuses on resistance against rhizoctonia, scab, alternaria and PVY. Special attention is also paid to early tuber bulking, dormancy and nutrient-efficiency. Since 2008 a joint breeding program "Bioimpuls" was initiated including Louis Bolk Institute, Wageningen University, six breeding companies, and several farmer breeders. The approach is based on three parallel goals: a) to develop new progenitors through classical introgression breeding with new late blight resistance genes, b) to provide the breeding sector with plant material (seeds/seedlings) for selection and c) to stimulate farmer breeder participation in the selection process, by giving training courses on potato breeding and technical support. A breeding program has been set up with approximately 35.000 seedlings a year, including the whole range from wild species hybrid to commercial crosses. To manage new late blight resistance genes in a sustainable way one of the strategies will be to combine two to three resistance genes from different genetic sources. To be able to select for those genotypes with combined resistance genes molecular markers are indispensable. During a workshop by Bioexploit and Eucarpia's Section Organic and Low-input Agriculture in 2009 the role of molecular markers in organic breeding programs was evaluated by applying a SWOT analysis. One of the conclusions was that more interaction between the conventional and organic research communities would be fruitful and examples of good practices with respect to marker assisted selection (MAS) for organic plant breeding would improve the better understanding of the potential contribution of MAS in organic breeding programs. Also 'cleaner' protocols e.g. by replacing harmful chemicals would also contribute to better acceptance of MAS. Molecular markers for late blight resistance was considered as an example of useful application.

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17 - Transcript profiling potato-late blight interaction

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Two Solanum genotypes, a wild relative of cultivated potato *S. cajamarquense* (Cjm) and an advanced tetraploid clone B3C1 (B3) were inoculated with two *Phytophthora infestans* isolates and leaves were sampled at 72 and 96 h after inoculation. Gene expression in the inoculated versus non-inoculated samples was monitored using the TIGR 10K potato array. This is study number 83 of the TIGR expression profiling service project, and all data is publicly available in Solanaceae Gene Expression Database at ftp://ftp.tigr.org/pub/data/s_tuberosum/SGED
Differentially regulated cDNA clones were selected separately for each isolate-time point interaction by significant analysis of microarray (SAM) and differentially regulated clones were classified into functional categories by MapMan. The results show that the genes activated in B3 and Cjm have largely the same biological functions and are commonly activated when plants respond to pathogen attack. The genes activated within biological function categories were considerably different between the genotypes studied, suggesting that the defence pathways activated in the two genotypes during the tested conditions may involve unique genes. Expression kinetics of a number of candidate genes was monitored by real-time RT-PCR. Some of the genes thought to be genotype specific may be activated across genotypes at other time points during disease development. The candidate genes activated in B3 and Cjm were used to search other gene expression profiles during late blight potato interactions. Comparisons of the expression of these genes in DM transcriptome (Potato Genome Sequencing Consortium) and other TIGR studies will be discussed.

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18 - Validation of Marked-Assisted Selection for resistance in a potato breeding program

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Many PCR markers linked to important traits have been identified in potato. Their potential benefits in breeding programs have been highlighted for many years, however, practical applications are limited. We have evaluated the presence of SCAR *RysC3* from *Solanum andigena* and CAPS marker $GP122_{564}$ from *Solanum stoloniferum for resistance to PVY*, H1 for resistance to *Globodera rostochiensis* and HC (*QRL Pa 2/3*) for resistance to *Globodera pallida* in the accessions of our germplasm collection used as progenitors in the potato breeding program. Innovator was the only variety revealing the HC marker, but other markers were present in several cultivars. Subsequently, crosses were performed between resistant varieties containing a selective marker and susceptible cultivars. Progenies were phenotypically evaluated for resistance against the considered pathogens and were tested for the presence of the marker. Parental varieties of progeny Pirola x 7XY-I revealed resistances to PVY descending from *S. andigena* and *S. stoloniferum,* as well as resistance to *G. rostochiensis*. There was a good agreement between resistance and the presence of the corresponding marker(s) in the progeny genotypes, confirming the value of these markers for marker assisted breeding to accelerate the generation of improved potato varieties with resistances in breeding programs.

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19 - Detection of QRL to late blight on chromosomes IV and IX in tetraploid potato

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Non specific resistance to *Phytophthora infestans* has become an objective for our breeders. To be able to deal with such a complex polygenic trait in breeding programs, one can use molecular markers linked to quantitative resistance loci (QRL). In this context, tetraploid families have been obtained using source of resistance used delivered by CIP in the 80ies (Population A). Resistant parents of the families were obtained by Inra after several years of additional selection for adaptation to European conditions of culture. They were used in subsequent crosses by breeders. Material was evaluated under natural conditions of contamination in Ploudaniel, France during several years (2005 to 2009). 104 markers chosen from bibliographic sources were tested on one family. They are located on nearly all the chromosomes but some of them are concentrated on regions where late blight QRL were previously described. We performed analysis of variance to detect markers linked to the variation of variables representing resistance such as rAUDPC. Depending of the year and the variable, we were able to explain up to 50% of the variation using 2 to 3 markers. One major region is located on chromosome IX and another one seems to be around a well known QRL of chromosome IV. Perspectives of this work are to strengthen position of the QRL and compare QRL detected across the progenies. We also plan to evaluate the proportion of QRL detected in this generation of material which is also efficient in "BC" type families currently under phenotypic evaluation. We also want to determine the efficiency of this resistance factors in front of the changes observed in the pathogen population (A1/A2 mating type).

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20 - High Resolution Melting for potato hybrid genotyping

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Single nucleotide polymorphisms (SNPs) are the most abundant type of variation in genomes, and have the potential to provide the basis for plant genotyping assay. Although several methods for SNPs detection in plants have been described, most of them are imprecise and expensive. High-resolution melting (HRM) is a simple and low-cost technology widely used for polymorphism scanning and human genotyping. In plants, HRM has been successfully used in biodiversity assessment studies. Wild potato species provide a tremendous germoplasm resource for potato breeding. Among them diploid *Solanum commersonii* is a very interesting one for its resistance to biotic and abiotic stresses. Indeed *S. commersonii* genome has been introgressed, in combination with others *Solanum* species, in many potato hybrids over the last years. The aim of this study was the development of *S. commersonii* chromosome-specific markers, and to use them in HRM assays to determine the chromosome dosage of potato hybrids. Single-copy, conserved orthologous markers (COSII) were used to identify SNPs among the *Solanum* species tested. Out of 45 COSII markers tested 24 gave a single PCR amplicon and 6 of them were sequenced. Mutation analysis identified 81 SNPs specific to *S. commersonii* chromosomes I-VI. Primers flanking these SNPs were designed and used in HRM assay. The melting curves obtained with *S. commersonii* specific primers designed on chromosome II allowed to successfully determine the genome dosage of triploid hybrid with a 2:1 *S. commersonii*:*S. tuberosum* genome ratio. Our preliminary data confirmed the potential of HRM in plant genotyping to discriminate chromosomes and to assess genomic ratios. Further studies will be performed to confirm and implement our results.

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21 - Allele Diversity at *Allene Oxide Synthase 2* (*AOS2*) in *Solanum phureja*, a Candidate Gene for Late Blight Resistance

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Late blight disease caused by the oomycete *Phytophthora infestans* is a major threat to potato cultivation worldwide. Resistance to late blight has been extensively studied in *Solanum tuberosum*, but, little is known about the resistance in *S. phureja,* which is an important diploid crop cultivated in Andean mountains. Polymorphism at *StAOS2* locus has been shown to be associated with resistance to late blight in *S. tuberosum* without compromising plant maturity. Specific questions addressed in this study were: what is the extent of allele diversity at *SpAOS2* locus? And, does allelic diversity at *SpAOS2* correlates with resistance to late blight? For answering 84 individuals were analyzed. 18 individuals of them are related by descent and were sampled from breeding programs, 55 accessions belong to Colombian *S. phureja* collection and five are commercial cultivars. Resistance to late blight was evaluated under field conditions and genetic diversity indexes and simulations were used to analyze data. The results indicate that (1) an excess of haplotypes at *SpAOS2* in comparison with *StAOS2*, and (2) a lack of correlation between *SpAOS2* polymorphism and plant resistance to late blight. Hence, this study has shown that *S. phureja* is a reservoir of important genetic variation at *AOS2* locus, but no correlation of any of these alleles with resistance to late blight could be found. This fact can be explained by a differential genetic selection pattern. There has been a selection pressure in potatoes grown in temperate climate to be resistant without compromise to late maturity, but it has not been a selection force for Andean potatoes. To our knowledge this is the first analysis of a candidate gene for late blight resistance in a collection of *S. phureja*. The relevance of introgression, population structure, genetic drift, ancestral variation and low genetic polymorphism remains to be analyzed in the species.

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22 - Breeding studies of pigmented tuber flesh in potatoes

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A diploid (2x) breeding project, based primarily on hybrids between phu and stn and tbr haploids, was conducted at the Potato Research Centre. Studies were conducted to assess progeny segregating for several traits including adaptation, tuber type and size, yield, fertility and pigmented flesh. Later, in response to scientific and public interest in the purported health benefits derived from the consumption of flavonoid- rich foods, research and development of cultivars with pigmented flesh was renewed.

Initial efforts focused on the diploid germplasm with breeding and selection for elevated levels of antioxidant activity, tuber type and agronomic performance. The chromosome number of a deeply pigmented pigmented selection, 9970-02 (2x) was doubled using colchicine to permit breeding with proven tetraploid parents. Promising selections were offered to the Canadian potato industry for their evaluation through the Agriculture and Agri-Food Accelerated program. Similarly, selections derived from this work are under investigation for their nutraceutical and functional food properties by scientists within the BioPotato Network.

In complementary research, 12 potato clones with different colour characteristics were used as parents. Twenty-one F1 populations segregating for tuber flesh colour were produced and 419 progeny from these populations were studied. A method was developed for collecting and analysing images of tuber flesh using a flat bed scanner and customized software using the Intel open CV image processing library .NET wrapper EmguCV.

The parent and progeny clones were genotyped using high-resolution DNA melting (HRM) analysis which produces characteristic melting- temperature curves according to DNA sequence composition. The melting temperature patterns are indicative of allelic dosage. Scanned images of tubers from a population 15505 (Adirondack Blue x AC Red Island) were grouped according their digital colour analysis. These colour groupings could be matched with results obtained from HRM analysis employing the LS030 (*dfr*) probe for red pigmentation. In this manner it was possible to genotype the parents according to which melting curve line they were associated. The parents did not produce similar curves; AC Red Island had a genotype 69/69/71/71 (ratio of alleles with melting peaks in degrees C) and Adirondack Blue had a genotype designated 69/69/69/71. The 71C peak represents the allele at the dfr locus that produces red pigmentation. Therefore, it was concluded that AC Red Island has 2 copies of the red pigment allele while Adirondack Blue has 1 copy.

This work will contribute to the development of marker-assisted selection strategies for anthocyanin-enriched potato germplasm.

23 - The Potato Breeding and Variety Development Program at Aberdeen, Idaho, USA: Overview of Program Research and Variety Releases

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Established in 1949, the potato breeding and variety development program at Aberdeen, Idaho has emphasized the development of potato varieties for the irrigated production of the western United States. The program is a joint collaboration between the Agricultural Research Service and the University of Idaho and is a partner in the regional Northwest (Tri-State) Potato Variety Development Program comprised of federal and state scientists in the states of Idaho, Oregon, and Washington. In 2009, four of the top ten potato varieties grown in the fall production states of the U.S. were bred and developed at Aberdeen. Research objectives of the program include increased sustainability by the reduction of: 1) pesticides (via host-plant resistance), 2) fertilizers (nitrogen and phosphorus), and water inputs. Emphases for resistance breeding include late blight, potato virus Y, potato leafroll virus, corky ringspot (pathogen being tobacco rattle virus), zebra chip (putative pathogen being '*Candidatus* Liberibacter' spp.), and wireworm. Enhanced nutritional qualities (increased protein and vitamin C concentrations) and resistance to cold-induced sweetening are also primary objectives of the program. The Aberdeen program also is contributing to the research efforts of the Solanaceae Coordinated Agricultural Project (SolCAP) by the generation and maintenance of the tetraploid mapping population used by SolCAP, and by collecting phenotypic data on this population to allow the association of molecular markers with traits of interest.

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24 - Use of POCI microarray platform in qualitative characterization of early potato varieties in Italy

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Off-season potato (*Solanum tuberosum* L.) is harvested at different time points compared to conventional potato. Besides, it is consumed soon after harvest, due to its poor preservation upon cold storage.
In Italy, one-third of the area devoted to the potato crop (19,000 ha in 2008), is cultivated with off-season potato, that represents the most important export item in the potato marketable sector (more than 60% of the total; Germany is the main importing country). In Italy, the off-season cycle is employed in specific geographic areas in Southern Italy, among which Sicily and Apulia are the most important. Tipipapa is an applied-research initiative, funded by the Italian Ministry of Agriculture (MiPAF), aimed at providing scientific bases and promoting the traceability, characterization and valorization of potato varieties produced off-season in some regions of southern Italy. Tubers of the varieties Spunta and Sieglinde were harvested in different areas of Sicily and Apulia, immediately freeze-dried, and total RNA was extracted from tuber tissue. RNA was labelled and hybridized to POCI potato chip (Agilent, One-color protocol). The data obtained indicate that an average of 457 genes (two comparisons) were regulated (up- or down) when different varieties cultivated in the same location are compared, an average of 86 were regulated when the same variety was cultivated in different regions (Sicily and Apulia, 7 comparisons), and an average of only 26 genes were regulated when the same variety was cultivated in different locations of the same region (5 comparisons). The genes up- or down-regulated have been classified according to their functional class. Among the most regulated genes in the between-varieties comparisons, a nuclear RNA binding protein and a nucleosome assembly protein 1-like protein, while in the comparison between cultivation areas, a rhamnosyl transferase gene, a cinnamyl alcohol dehydrogenase and a putative MYB transcription factor were found.

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25 - Genome wide identification and mapping of NBS-encoding resistance genes in *Solanum phureja*

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Most of plant disease resistance proteins identified to date includes those that encode the nucleotide-binding site (NBS) domain. We were able to find 526 NBS-encoding resistance (R) gene homologs in the genome of the recently sequenced *Solanum phureja* using hmm models and manual curation. Highly similar homologues for most of the *Solanaceae* R genes cloned were discovered. We used four distinct draft physical maps of potato (two from *Solanum tuberosum* and two from *Solanum phureja*) plus data from a physical map of potato generated with tomato markers (EXPEN 2000) to anchor as many R-genes as possible. 326 R gene (61.98%) homologs were mapped to its relative position in each chromosome. At this stage we see that NBS-genes are well distributed across linkage groups.

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26 - Natural Variation of enzymes involved in starch degradation pathway in *Solanum tuberosum*

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The development of diagnostic markers by exploiting the natural allelic variation of candidate genes is a powerful tool in tetraploid potato varieties for precision breeding regarding complex agronomic traits. Multiple genetic and environmental factors are the basis for important quality traits like long-term storage and processing characteristics. The latter can rely on the balance between tuber starch and sugars that affects potato processing due to enzymatic reactions at low temperature (cold-sweetening). The major aim of this project was to screen for superior natural alleles of candidate genes involved in starch degradation - one of the major sources of reducing sugars. Besides starch phosphorylases, two strong functional candidate genes are the α-Glucan-Water Dikinase *(GWD)* and the Phosphoglucan-Water Dikinase *(PWD)*, which trigger the initial breakdown of starch in *Solanum tuberosum.* Genomic structures and cDNA sequences were deduced for both genes by means of BAC-library screening and sequencing. To determine whether natural variation at these loci contributes to processing quality, single nucleotide polymorphisms (SNPs) were screened in a population of 220 individuals and tested for association with chip quality, tuber starch content, yield and starch yield. Some SNPs in conserved domains of *GWD* and *PWD* explained between 14% and 22% of the phenotypic variation of several tuber traits i.e. chip quality before and after cold-storage and yield. Allelic variation of *GWD* and *PWD* may provide suitable markers for marker-assisted breeding.

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27 - Development of a robust screening method for tomato spotted wilt virus infection in potato

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Tomato spotted wilt virus (TSWV) is a tospovirus transmitted by thrips. Primary TSWV infection causes necrotic spots on terminal leaves, stem necrosis and occasional plant death, while secondary infection in plants grown from TSWV-infected tubers causes leaf, stem and tuber necrosis, and severe stunting. TSWV was first reported in 1915 in Australia and has caused serious outbreaks in potato crops with a disease incidence of up to 60%. Screening potato cultivars for resistance to TSWV is problematic. Glasshouse screening is limited to the summer months, due to ineffective inoculation during the shorter day lengths, and the virus is difficult to maintain in infected potato plants. Ideally, a molecular marker closely linked to the gene(s) for TSWV resistance would assist the Australian breeding program. A previous study screened 40 parental cultivars for TSWV resistance using grafting and mechanical inoculation. Both methods yielded congruent results for 20 cultivars, with 10 resistant and 10 susceptible cultivars identified. To test a high throughput inoculation method, 22 small F1 families were spray inoculated. Results were inconsistent between the resistant and susceptible controls, suggesting that the spray inoculation method required further optimisation. Once a reliable phenotyping protocol has been established, development of molecular markers diagnostic for, or closely linked to, TSWV resistance genes can begin. Within tomato, the single, dominant TSWV resistance gene, *Sw-5*, has been cloned and sequenced. Based on synteny between tomato and potato genomes, the TSWV resistance gene in potato may be located in a similar chromosomal position. Initial efforts will therefore focus on screening all previously mapped SSRs across chromosome 9 for linkage with the TSWV resistance trait. In parallel, a comparative genomics approach using the *Sw-5* sequence and the *S. phureja* genome draft will be used to identify new SSRs from *S. tuberosum* ESTs, also potentially linked with TSWV resistance.

28 - Development and optimisation of a genetic identity kit for Australian potato germplasm

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The understanding of genetic diversity within a breeding program or germplasm collection to maximise genetic divergence, exploit hybrid vigour and minimise inbreeding depression can be an efficient method of obtaining genetic gain in cultivar production. Currently, potato cultivar identification is based on plant and tuber morphology and can be subjective and unreliable. DNA fingerprinting can be applied to accurately identify cultivars and assess levels of genetic diversity. Simple sequence repeats (SSRs) are currently the marker system of choice within potato for assessing genetic diversity. Initially 27 SSRs were screened for amplification efficiency across 92 commercially important cultivars, including clonal variants. From this initial suite of SSRs, 15 generated acceptable amplification products using a standardised touchdown PCR program, of which 12 were selected for further analysis. These were able to discriminate all but 2 of the commercial cultivars. A further 463 genotypes from the Australian breeding program were then screened. One SSR, STM2028, was excluded due to variable performance. While not all cultivars were discriminated with 11 SSRs, there was sufficient variation to separate the breeding lines into small clades of 2-4 individuals. Clonal variants of 3 cultivars were not differentiated, but clonal variants of Sebago, Kennebec and Atlantic differed by 1-2 alleles. Importantly, the SSRs were able to identify genetically divergent parents for future crosses in the breeding program. Discrimination of all commercial cultivars is essential, and the test will be refined to deliver this outcome. In parallel, DNA from a range of starting material will be extracted and screened to extend utility and determine transferability across different potato tissues. Once the reproducibility of the genetic identity kit has been proven, this service will be provided to the Australian potato industry.

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29 - Evaluation and optimisation of the T G689 marker linked to PCN resistance

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Potato cyst nematode (PCN) is a major problem in potato growing regions throughout the world. Australia has had limited detections of only one pathotype, *G. rostochiensis* Ro1, which is currently controlled by stringent quarantine and regulatory procedures. Identification and breeding of resistance to PCN is essential. The resistance gene *H1* provides resistance to *G. rostochiensis* pathotypes Ro1 and Ro4. It has been linked without recombination to RFLP marker CP113, although, the CP113 marker has proven to be unreliable as a diagnostic marker. TG689 was identified as tightly linked to the *H1* gene, although in a few instances has lost the association with the *H1* gene, generating false results. Screening for PCN resistance was carried out under strict quarantine glasshouse conditions, and 246 cultivars have been phenotyped. Primers for TG689 were designed and a semi-nested PCR approach was adopted, amplifying a conserved 196bp fragment in all samples as a positive control, and amplification of a 139bp fragment in samples with *H1*-mediated PCN resistance. Due to lack of resolution in the agarose gels the assay was redesigned using fluorescently labeled primers and resolved through an ABI3730xl capillary electrophoresis platform. A total of 384 cultivars were screened with the TG689 marker. The cultivars assessed genotypically included all that had undergone the phenotypic screen. With the co-incident cultivars, there was 98% congruence between the phenotypic and genotypic data. Three cultivars displayed false negative results (resistant phenotype, TG689 *H1* band absent) and 2 cultivars exhibited false positive results (susceptible phenotype, TG689 *H1* band present). The high level of congruence between phenotype and genotype data suggests, provided pedigree is taken into consideration, that the optimised TG689 marker assay can be used for diagnostic screening of PCN resistance.

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30 - Characteristics of potato somatic hybrids between *Solanum michoacanum* (Bitter.) Rydb. resistant to *Phytophthora infestans* and *S. tuberosum* L.

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The objective of this work was to produce interspecific somatic hybrids resistant to *Phytophthora infestans* (Mont.) de Bary by means of protoplast fusion of the wild potato species *Solanum michoacanum* (2x, 1EBN, source of resistance) and *S. tuberosum* (2x, 2EBN; 4x, 4EBN). *S. michoacanum* is reproductively isolated from *S. tuberosum* and cannot be crossed directly with potato. In cooperation with Julius Kühn Institute, Germany, somatic hybrids were produced by protoplast electrofusion of two accessions of *S. michoacanum,* with three diploid clones of S. *tuberosum* and the cultivar Rywal, which are susceptible to late blight. For eight genotype combinations somatic hybridization was achieved and viable calluses and shoots obtained. The hybrid nature of 169 regenerated plants obtained from the fusion of *S. michoacanum* with two diploid breeding clones was confirmed by three SSR markers (ST13ST, STI057, STM1049). Using flow cytometry tetraploid, hexaploid and octoploid plants were identified among the 41 somatic hybrids. From this material more than 1000 plants (1 plant per callus) were regenerated from all fusion combinations in Poland. Two *in vitro* copies were preserved for each of them.

Greenhouse grown plants were characterized in terms of their phenotypic traits, like vigour, habit, shape of leaves, flowering and pollen fertility. The ploidy level was estimated by counting the chloroplast number in guard cells. Fifty of the 160 plants analyzed were identified as hybrids based on two CAPS markers (C2_At5g51970, C2_At2g14260) and three RAPD markers (OPA03, OPA09, OPA11). Simultaneously, 300 plants were evaluated for resistance to foliage blight using the detached leaflet assay (scale 1-9, 9 = the most resistant). One somatic hybrid was resistant to foliage blight. The work is to be continued.

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31 - Mapping Phytophthora infestans resistance in two Solanum populations

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With the aim to localize new late blight resistance genes two different mapping populations were created. The first population resulted from a cross of diploid *S. caripense* parental genotypes C and K where C carries the resistance while K is susceptible to late blight. For the fast and efficient construction of a genetic framework map SNP-based allele-specific primers derived from COSII sequences were created. With these markers the resistance was mapped to chromosome XI in proximity to the tomato's Sw5 locus conferring resistance to spotted wilt virus. Amplicons of PCR markers displaying close linkage to the prospected resistance locus were cloned and sequenced. Based on the the synteny with other Solanum species including the tomato and potato, more markers were designed and mapped on the CK map to narrow down the site of the new resistance locus.
A second mapping population resulted from a cross of tetraploid *S. tuberosum* group tuberosum clonal accession MF-II with clone TPS67 of group andigena (Neotuberosum). Both parents carry monogenic late blight resistance and therefore, two resistance loci were mapped in this population; Rpi-tbrM1 from MF-II on chromosome XI and RPi-adgT1 from TPS67 on chromosome IV. The mapping was facilitated by applying syntenic tomato and potato markers of known map position. Markers closely linked to the resistance loci were modified for use as tools in selection. Two closed-tube techniques were evaluated, the KASPar assay (KBiosciences) and the high resolution melting method with unlabeled probes. Both techniques resulted to be fast, easy to adapt, and cost-efficient. Both techniques advantageously allow for the simultaneous detection of more than a single marker allele gaining in that way security on the functionality of both PCR and correct allele detection. As an example, one marker, denominated At1g07960, linked to the RPi-tbrM1 locus was tested with both methods. This marker's "allele 2" that is linked in cis to this resistance was found by both techniques to be present in a wide range of breeding materials even when these are unrelated to MF-II. The significance of this finding and the characteristics of the two one-step marker techniques are discussed.

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32 - Development of potato markers for processing ability based on genotype-specific expression differences

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Processing ability and starch content are important quantitative traits in potato breeding. With the objective to develop methods more effective than the current selection based on the phenotype we explore the possibility to use phenotype-associated gene expression for selection. For this purpose a 5322-probe microarray representing 4331 different cDNAs was developed. These probes were assembled from a library of *P. infestans* infected potato cultivar Yungay, from the TIGR EST collection, and from cloned PCR products. The microarray is enriched for signaling and metabolism related elements in defense-, light-, photoperiod-, stress-, hormone-, nitrogen-, sugar-, starch- and tuber- related pathways. Gene expression in tubers after cold storage was investigated by triplicate microarray analyses of cDNA from 14 selected cultivars. Genes associated with starch metabolism formed groups with synchronized expression across different potato cultivars. This points to a well-regulated co-expression of specific gene complexes within the starch metabolism in tubers. Several candidate markers displaying strong negative correlation with processing ability and starch content but also candidates with positive correlation were detected. Thirty genes whose expression appeared indicative of processing ability (discoloration after frying), high starch and low glucose content were chosen and their expression was validated by qRT-PCR on 14 cultivars. Finally a subset of these genes with high potential to become expression markers were selected on the good reproducibility in qRT-PCR of microarray results, as well as on their lasting expression after another two months of tuber storage.

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33 - From sequence diversity to functional variation in the molecular network of tuber bruising.

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Potato tuber black spot bruising, an increasing issue in breeding programs is an internal discoloration of tuber tissue initiated by mechanical impact. Bruising susceptibility is a complex trait, which depends upon many genes and is influenced by environmental factors. As bruising susceptibility is cultivar dependent, the natural variation in a population consisting of 205 tetraploid potato varieties and breeding clones related by descent was exploited in order to identify the genes and proteins underlying this trait. In the course of this project, a combination of state-of-the-art *Omics* tools like association mapping (*Genomics*) and 2D-gel analysis (*Proteomics*) accompanied by mass-spectrometry gave insight in the molecular networks controlling the trait and the existing sequence diversity at given candidate loci. As polymorphic markers derived from lipase and polyphenoloxidase (PPO) genes significantly associated with tuber bruising as well as other quality traits, the allelic variation within the population was assessed. The given natural variation concerning coding and non-coding DNA regions as well as qualitative and quantitative protein information were integrated in the phenotypic range of the investigated population. Further biochemical and molecular analysis of associated candidate gene alleles might provide an answer to the questions why polymorphic markers associate with traits and furthermore if and how sequence diversity results in functional differences and thereby explains phenotypic variation. The present study marks a joint effort between breeding industries and research and punctuates the interest of applied science and basic research to redirect the genetic progress and the molecular knowledge back into the field. Funding is provided by the InnoNet program of the German Ministry for Economy and Technology (BMWi).

34 - Identification of alleles of carotenoid pathway genes important for zeaxanthin accumulation in potato tubers

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We have investigated the genetics and molecular biology of orange flesh colour in potato (*Solanum tuberosum* L.). To this end the natural diversity in three genes of the carotenoid pathway was assessed by SNP analyses. Association analysis was performed between the SNP haplotypes and flesh colour phenotypes in diploid and tetraploid potato genotypes. We observed that among eleven beta-carotene hydroxylase 2 (*CHY2*) alleles only one dominant allele has a major effect, changing white into yellow flesh colour. In contrast, none of the lycopene epsilon cyclase (*LCYe*) alleles seemed to have a large effect on flesh colour. Analysis of nine zeaxanthin epoxidase (*ZEP*) alleles showed that all (diploid) genotypes with orange tuber flesh were homozygous for one specific *ZEP* allele. This *ZEP* allele showed a reduced level of expression. The complete genomic sequence of the recessive *ZEP* allele, including the promoter, was determined, and compared with the sequence of other *ZEP* alleles. The most striking difference was the presence of a non-LTR retrotransposon sequence in intron 1 of the recessive *ZEP* allele, which was absent in all other *ZEP* alleles investigated. We hypothesise that the presence of this large sequence in intron 1 caused the lower expression level, resulting in reduced ZEP activity and accumulation of zeaxanthin. Only genotypes combining presence of the dominant *CHY2* allele with homozygosity for the recessive *ZEP* allele produced orange-fleshed tubers that accumulated large amounts of zeaxanthin.

35 - Triple *R*-gene stacking for sustainable resistance to late blight in potato

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In order to achieve more durable late blight resistance in potato, a strategy of stacking *R* genes was proposed. Three out of four resistance genes (their corresponding Avr genes were available) were selected after testing 28 *Phytophthora infestans* isolates collected from China. The second step was to stack these 3 *R* genes into pBINPLUS vector in order to transform them into the susceptible potato cultivar Desiree. Around 500 rooted shoots were obtained from kanamycin containing medium, with a transformation efficiency (# of rooted shoots/ # of explants) of ~62%. There were 128 NPTII containing shoots detected after PCR giving resistance to Kanamycin. PCR analysis showed that 69% of these shoots were containing all 3 *R* genes, and ~13% of them were missing all *R* genes; ~7% of the shoots possessed 2, and another 7% only 1 of the *R* genes. Twenty-eight out of 128 plants were selected for DLA with specific isolates, which could confirm separately the biological activity of at least 2 of 3 *R* genes; in addition, all these plants were tested with the corresponding *Avr* genes for HR (Hypersensitiviy Reaction) by ATTA (*Agrobacterium tumefaciens* Transient Assay). The 28 DLA and ATTA tested plants showed resistance or susceptibility corresponding to their PCR result. Furthermore, 5 transformants containing all three *R* genes, and 5 transformants without *R* genes were tested with previously used Chinese isolates, giving as expected fully resistance or susceptibility, respectively. These results suggest that 1. NptII gene is a reliable selection marker during triple gene transformation; 2. High ratio of transformants received 3 *R* genes with a proper biological activity; 3. this *R* gene combination did not show epistatic effects.

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History on EAPR and EAPR/EUCARPIA section meetings

Informal conferences

1951 1st informal conference, Aalborg, Denmark, on storage problems
1953 2nd international informal conference on potatoes, Sutton Bonington, England
1955 3rd international informal conference on potatoes, Wageningen, Netherlands
1957 4th international informal conference on potatoes, Lund, Sweden

At 14th August 1957 the European Association for Potato Research was formed. Lund, Sweden

Sections

1959 Potato Research 2(2):147-151. Section Varieties installed by the council.
Joint section meetings with Eucarpia

Triannual conferences

1960	1st	Braunschweig-Völkenrode, Germany
1963	2nd	Pisa, Italy
1966	3rd	Zurich, Switserland
1969	4th	Brest, France
1972	5th	Norwich, United Kingdom
1975	6th	Wageningen, Netherlands
1978	7th	Warsaw, Poland
1981	8th	Munchen, Germany
1984	9th	Interlaken, Switserland
1987	10th	Aalborg, Denmark
1990	11th	Edinburgh, United Kingdom
1993	12th	Paris, France
1996	13th	Veldhoven, Netherlands
1999	14th	Sorento, Italy
2002	15th	Hamburg, Germany
2005	16th	Bilbao, Spain
2008	17th	Brasov, Romania
2011	18th	Oulu, Finland

Section Meetings
Breeding and Varietal Assessment

1962	1st	Cambridge, United Kingdom
1968	2nd	Gross-Lusewitz, Germany
1970	3rd	Dublin Ireland - joint meeting with Utilisation section
1971		Warsaw, Poland
1973	4th	Wageningen, The Netherlands
1977	5th	Lund, Sweden
1980	6th	Edinburgh, United Kingdom
1983	7th	Aarhus, Denmark
1985	8th	Cambridge, UK proceedings
1988	9th	Wageningen, Netherlands
1992	10th	Landerneau, France
1994	11th	Freising, Germany
1997	12th	Viterbo, Italy
2000	13th	Warsaw, Poland
2003	14th	Oulu, Finland
2006	15th	Carlow, Ireland
2010	16th	Wageningen, Netherlands