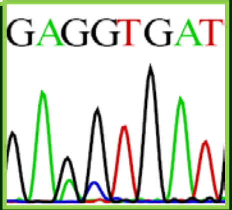
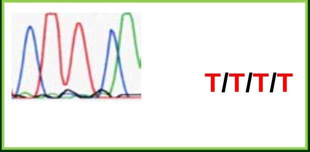
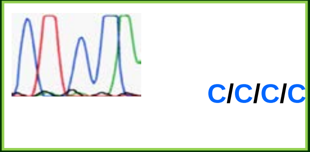
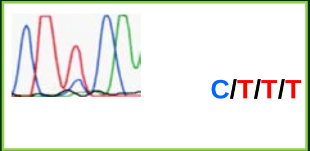
The development of potato genome analysis at the Max-Planck-Institute for Plant Breeding Research in the context of potato breeding in Middle Europe.

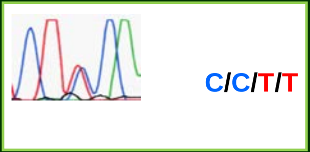
Christiane Gebhardt
MPI for Plant Breeding Research
Cologne, Germany

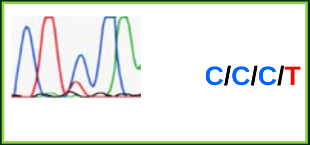


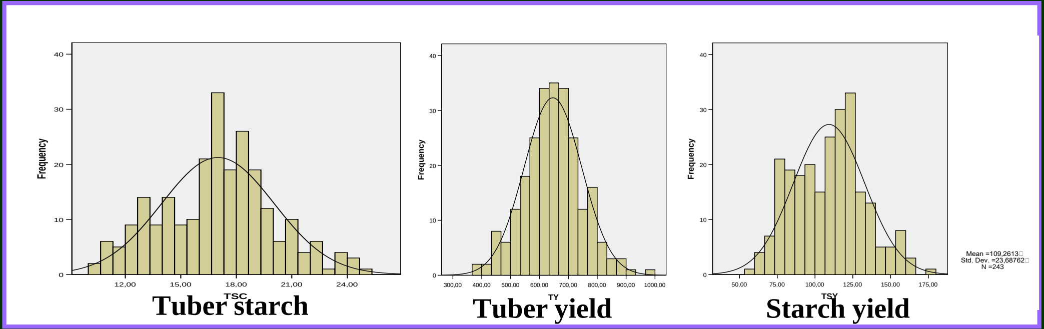








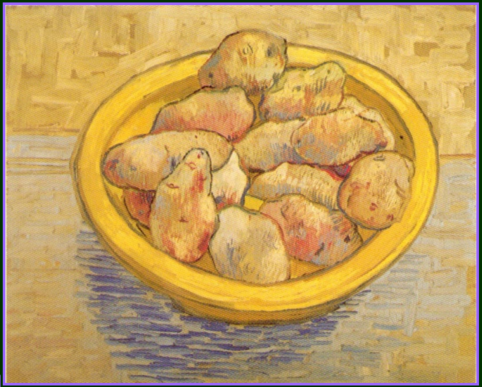




Nairobi
7. June 2018

Solanum tuberosum-Ipomoea batatas

- **Polyploid crops with polysomic inheritance, difficult genetics.**
- **Non-inbred due to self-incompatibility and severe inbreeding depression.**
- **Vegetative propagation, low multiplication rate, planting material propagates diseases (e.g. viruses)**



Vincent van Gogh

http://en.wikipedia.org/wiki/File:Sweet_potatoes,_Padangpanjang.jpg#filelinks

Potato breeding and seed production in Western Europe

- Variety breeding and seed production are performed by private industry (mostly small and medium enterprises – SME‘s).
- Income is generated by the production and selling of certified seed potatoes.
- Investments are made for the development of new varieties.
- Essential for the business: Legislation to protect varieties and breeders rights.

Example for uses of the potato crop in Germany (11.4 million tons in 2016/17, 45 t/ha)

- **Processed food products: 18.2%**
- **Fresh consumption: 17,8%**
- **Industrial starch: 9.2%**
- **Seed potatoes: 5.4%**
- **Energy (biogas), feed, waste: 9.0%**
- **Export (fresh, processed, starch): 45.4%**

Source: P. Sutor, R. Riester, Agrarmärkte 2017, LfL

Varietal maintenance and pre-basic seed production

Clonal maintenance

In vitro culture

Minituber production (PBTC)

PBTC > PB1 propagation

PB1 > PB2 propagation

Separated in lots

PBTC = Pre-basic tissue culture, PB = Pre-basic

Source and copyright: Dr. Jens Lübeck (Solana Research)

Seed potato production and certification

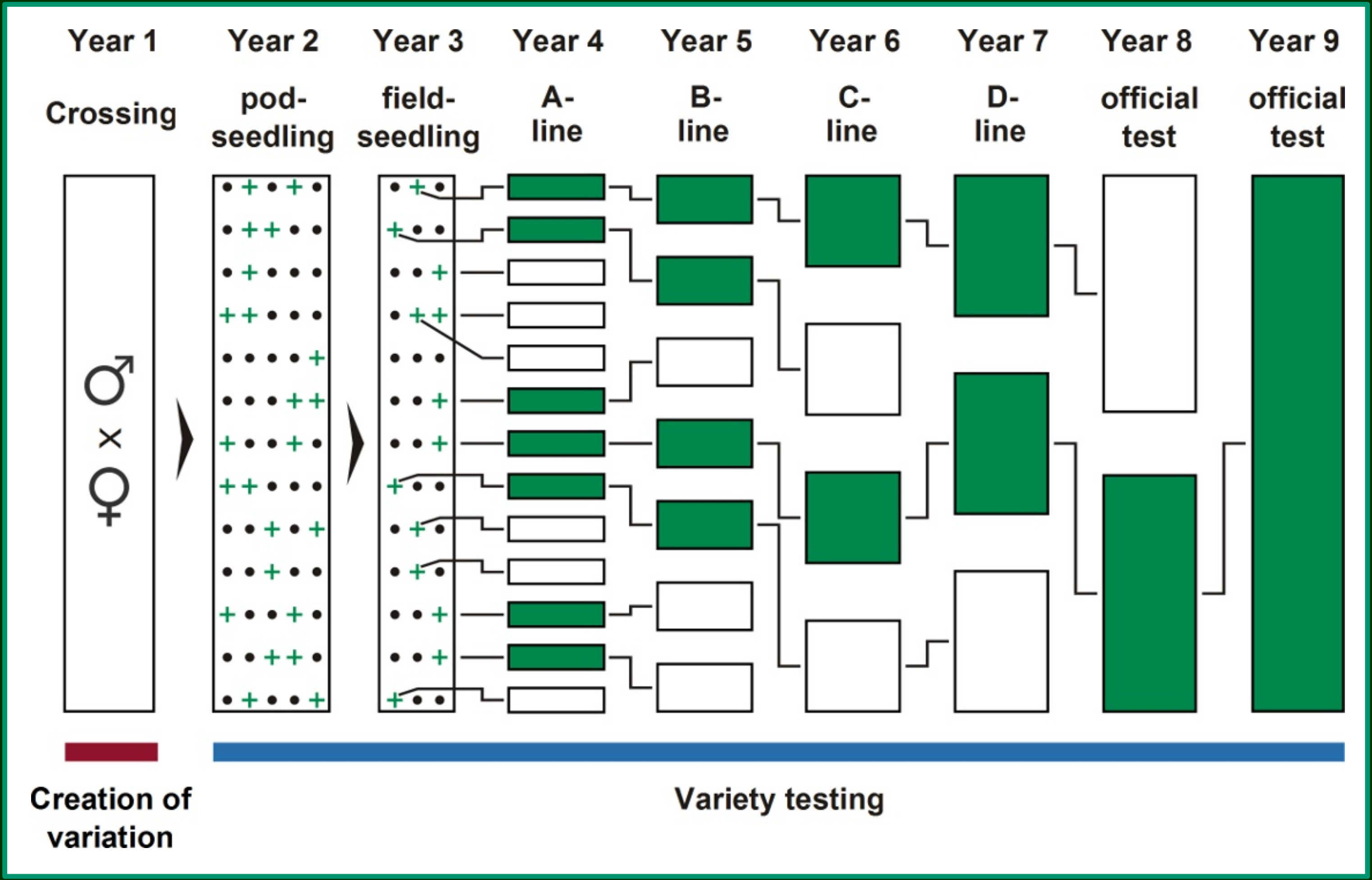
Category	Class
Pre-basic seed	PBTC
	PB
Basic seed	S
	SE
	E
Certified seed	A
	B

in vitro

8-9 field generations

Certified seed potatoes:
threshold levels for defects and diseases (e.g. viruses 8-10%)

Variety breeding – Breeding scheme



Source and copyright: Dr. Jens Lübeck (Solana Research)

Basic plant research over the last 70 years resulted in three concepts, which impact on ‚traditional‘ plant breeding

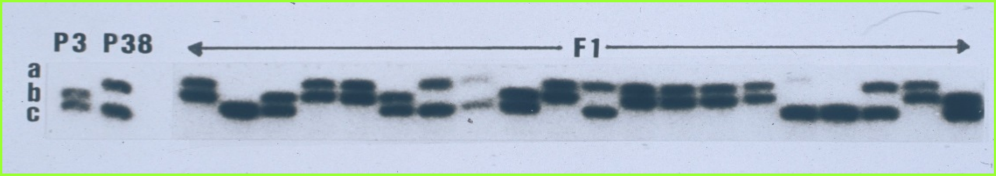
- *In vitro* cell and tissue culture.
- Genetic engineering (gene therapy).
- Marker-assisted selection (gene diagnosis).

Potato research at the Max-Planck Institute for Plant Breeding Research

- ~ 1960 – 1990: Prebreeding: Introgression of pathogen resistance and other traits from wild potato species.

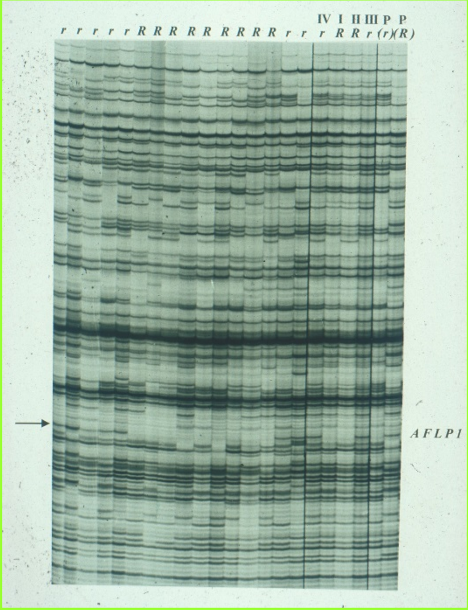
- ~ 1970 – 1990: Cell and tissue culture: Ploidy reduction to diploid level by anther culture; somatic hybridization.
- 1985 – 2005: Genetic engineering (e.g. virus resistance).
- 1985 – 2016: Genome analysis: Molecular linkage maps, mapping and cloning of resistance genes, QTL mapping, association mapping.

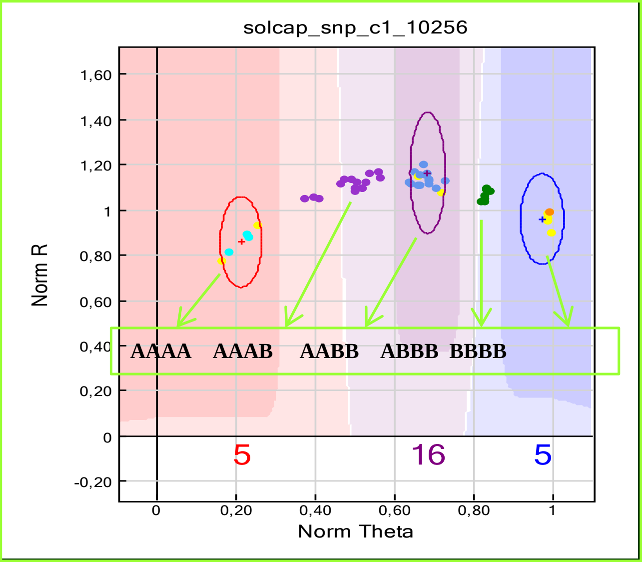
The evolution of DNA marker technology



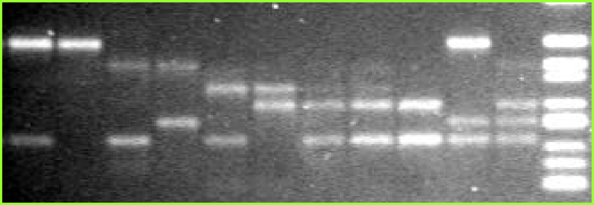
RFLP

AFLP

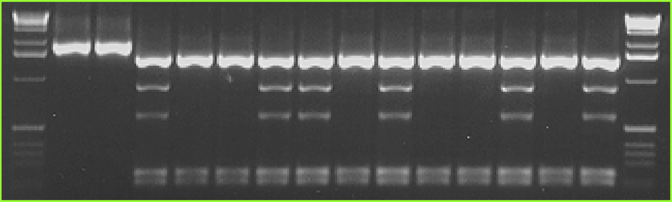




SNP array

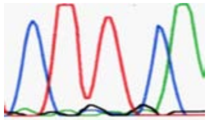


SSR

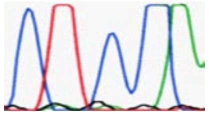


CAPS

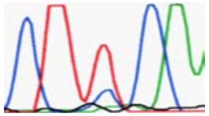
SNP



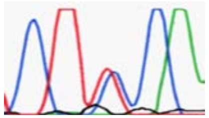
T/T/T/T



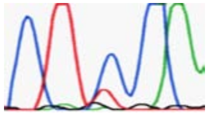
C/C/C/C



C/T/T/T

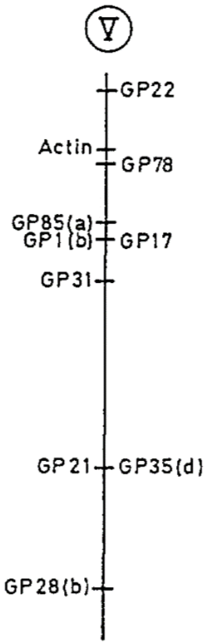


C/C/T/T

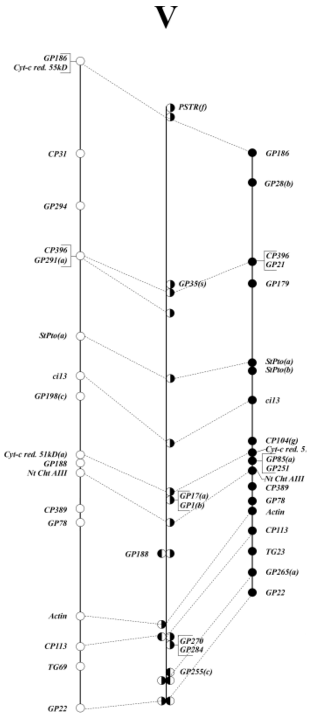


C/C/C/T

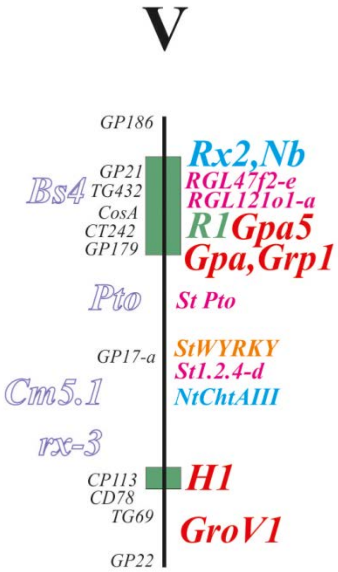
The evolution of potato maps



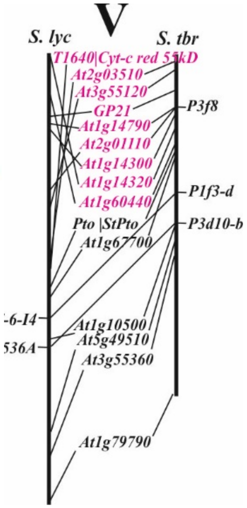
1989



1994



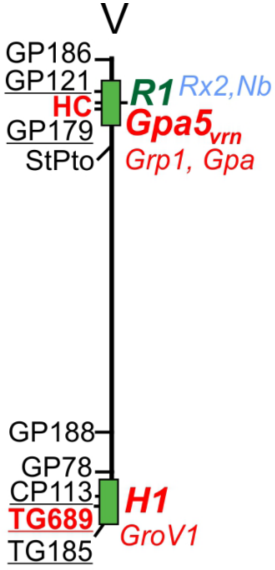
2003



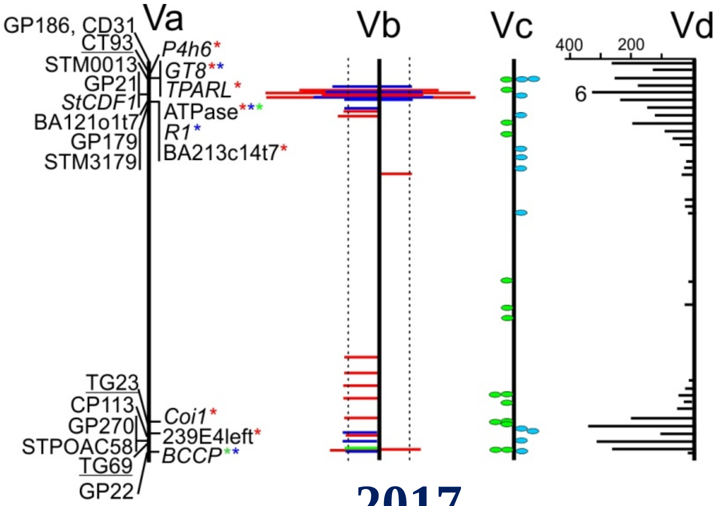
2012

From genetic to physical maps

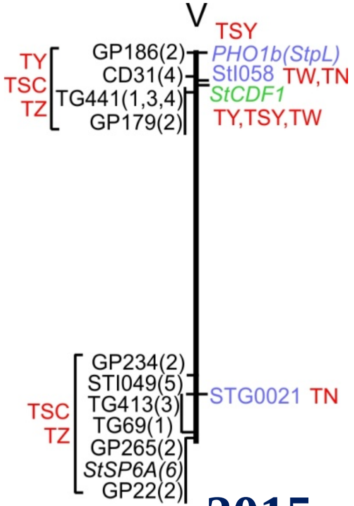
PGSC (2011). Genome sequence and analysis of the tuber crop potato. Nature 475: 189-195



2013

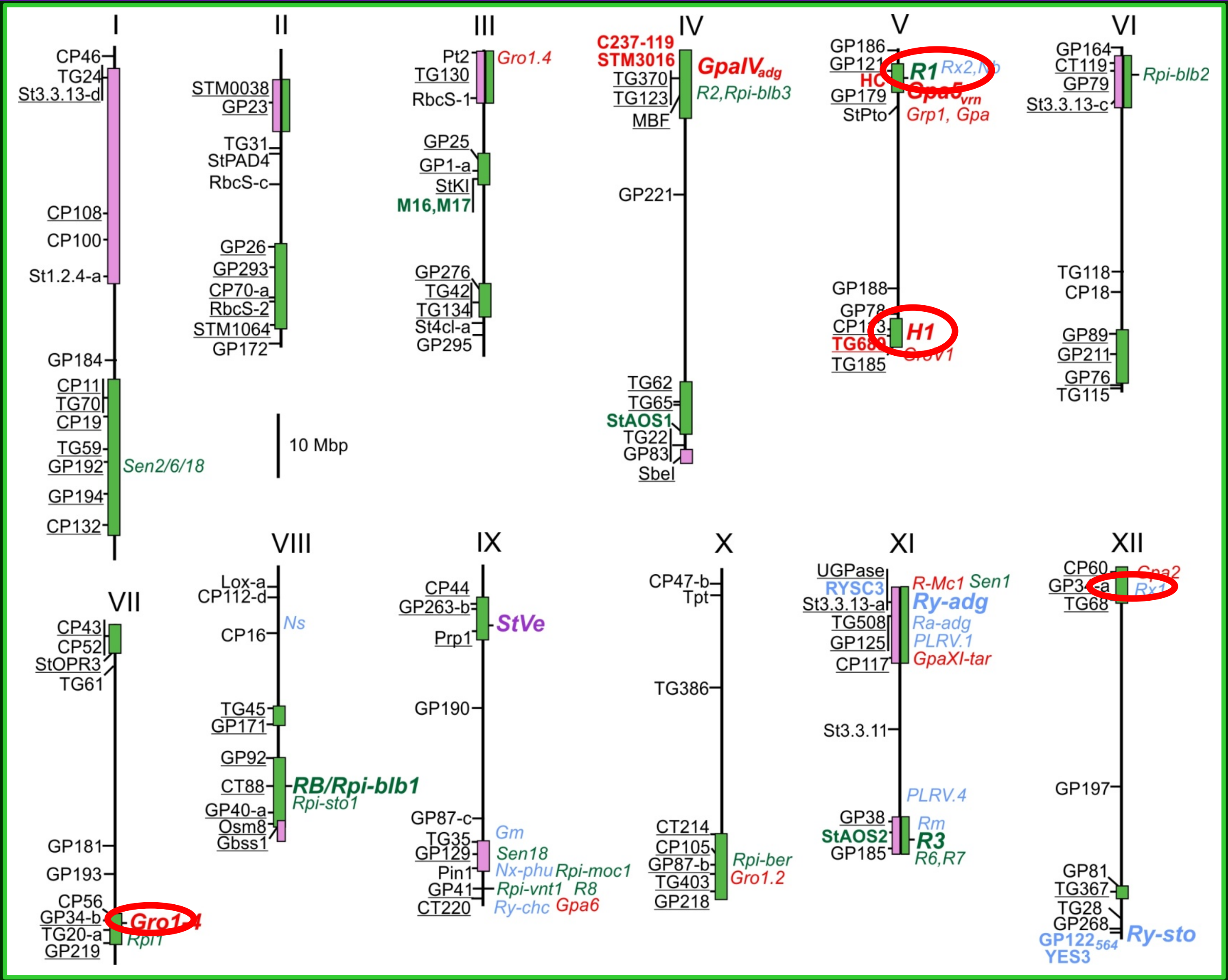


2017



2015

Research in the 90ties of last century: Linkage mapping in experimental, diploid populations of major genes and QTL for resistance, cloning of *R* genes.



The gap between impact factor and impact

Basic
‚curiosity driven‘
science

?

Applied
plant breeding

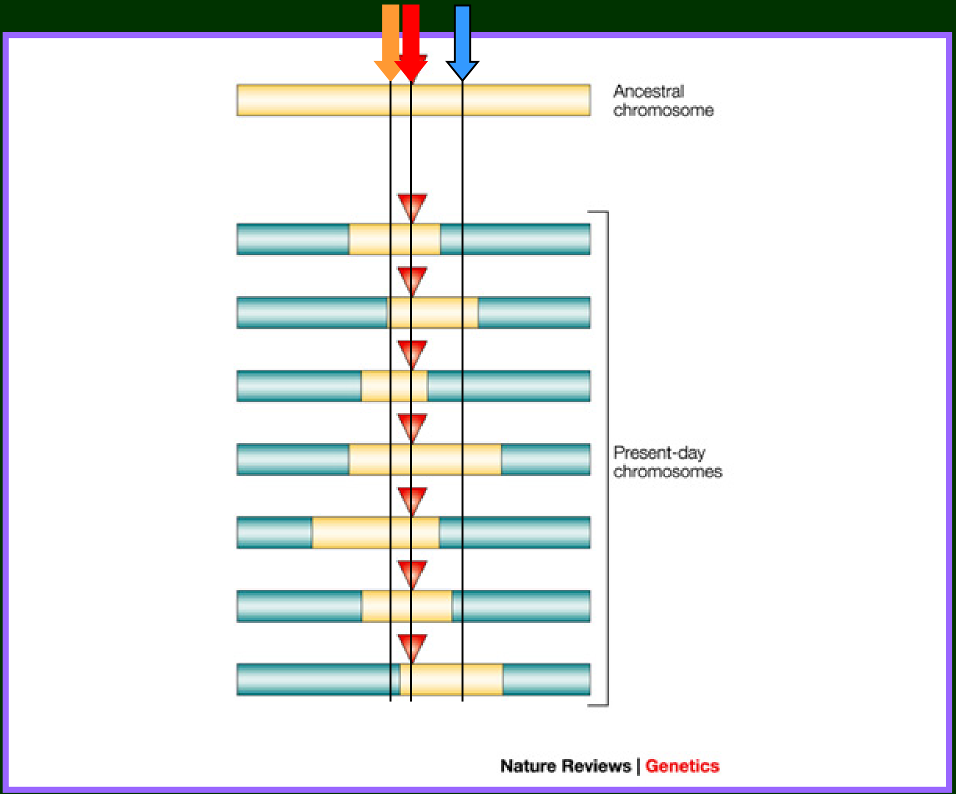
In our case: Understanding the molecular basis of the natural variation of disease resistance and tuber quality traits in potato.

Developing commercially successful varieties using efficient and cost effective selection methods.

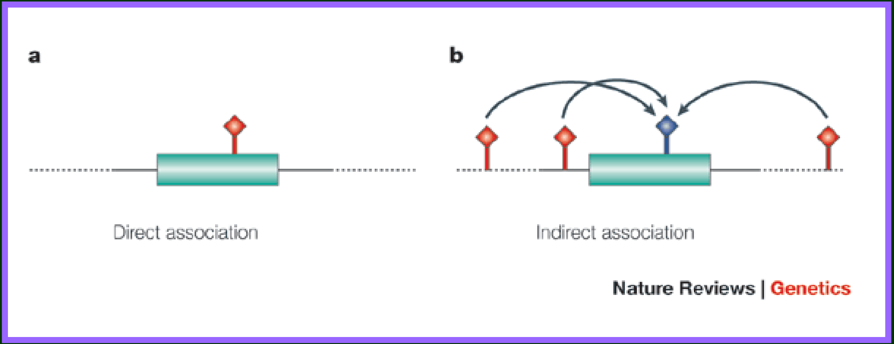
Translation:

Developing diagnostic DNA-based markers for increased efficiency and precision of selection (Precision Breeding).

Diagnostic DNA markers are in linkage disequilibrium (LD) with a specific trait allele distributed in a population of individuals related by descent.



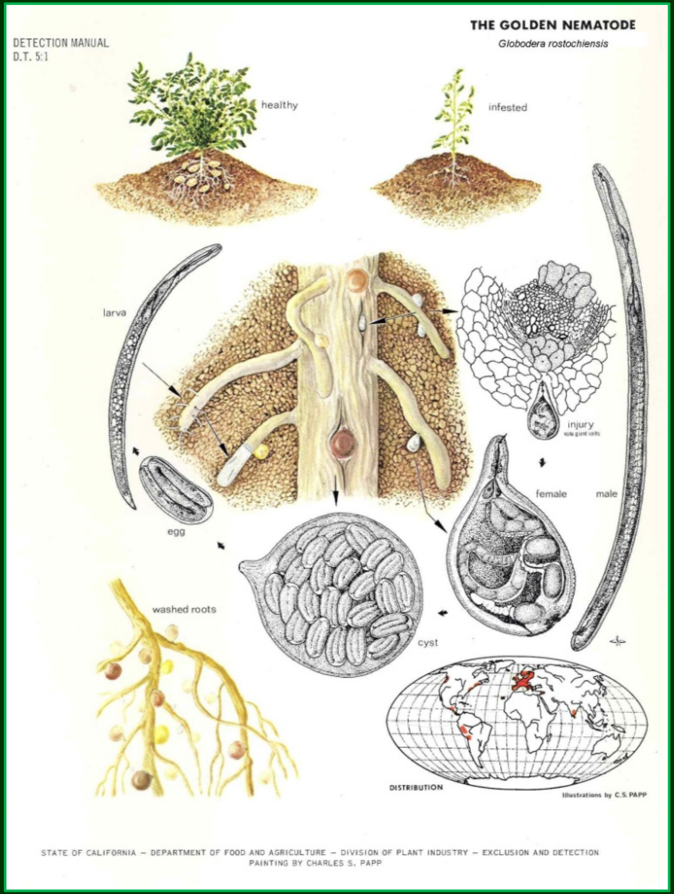
Nature Reviews | Genetics



Nature Reviews | Genetics

LD is a function of the recombination frequency and the number of meiotic generations separating the individuals in a breeding population from their ancestors.

Example: A diagnostic DNA marker for resistance to the root cyst nematode *Globodera pallida*

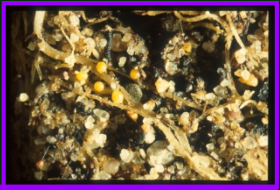


Why ?

- Quarantine pest
- Phenotypic assay costly in time and labor
- Quantitative resistance

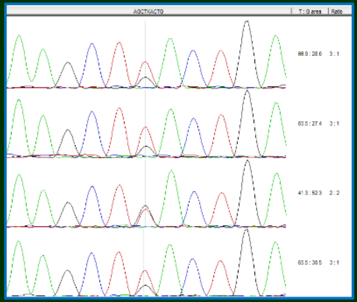
http://plpnemweb.ucdavis.edu/nemaplex/images/Papp%20Golden%20Orig.jpg

The strategy

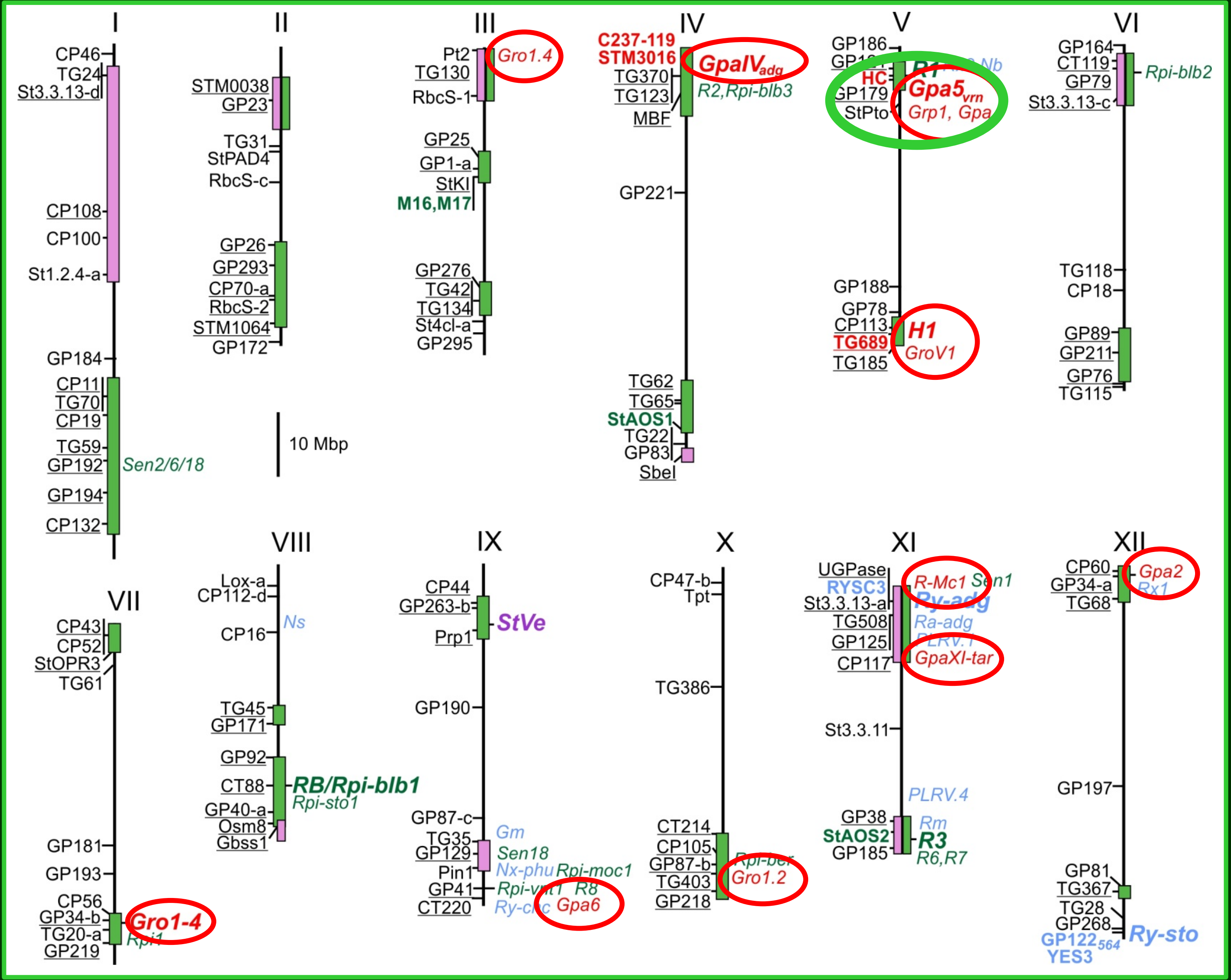


- A tetraploid variety resistant to *G. pallida* pathotype Pa2/3 was crossed with a elite susceptible variety (breeding company).
- 200 F1 progeny were phenotypically evaluated for resistance to *G. pallida* (breeding company).
- The 23 most resistant and the 23 most susceptible F1 clones were selected.
- The 46 clones were genotyped for 96 SNP (single nucleotide polymorphism) markers by amplicon sequencing (Sanger) in candidate loci chosen based on known map positions of nematode resistance loci (selective genotyping).
- The SNP markers were tested for linkage with nematode resistance (simple chi-square test).

Sattarzadeh/Achenbach et al. 2006, Mol. Breed 18: 301.



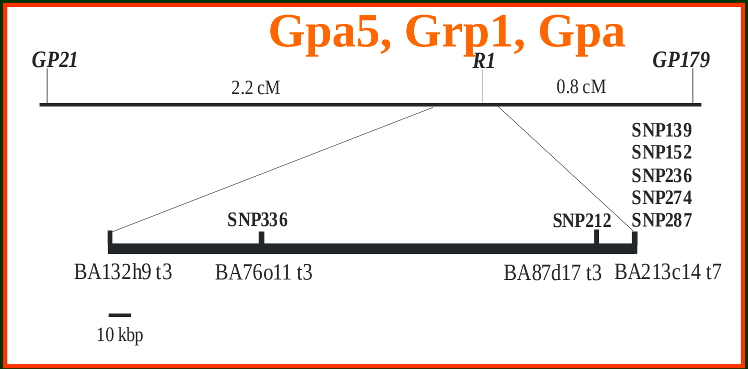
Molecular linkage mapping in various experimental populations in several labs had identified a number of nematode resistance loci.



Several cloned plant genes for pathogen resistance shared the same sequence motifs: NBS – LRR type genes

Result: A major resistance QTL was linked to SNPs on potato chromosome V

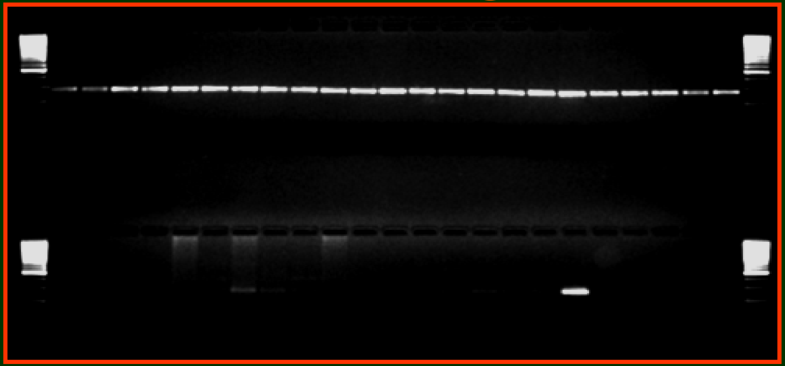
Local physical map around the *R1* late blight resistance locus on chromosome V was available



Haplotype	BA76o11t3	BA87d17t3	BA213c14t7				
	snp336	snp212	snp287	snp274	snp236	snp152	snp139
a	C	C	T	A	C	T	G
b	**G**	C	**C**	A	**T**	**A**	G
c	C	**T**	T	**T**	C	T	**A**

Haplotype *C* (HC) was linked to resistance

23 most resistant F1 plants



23 most susceptible F1 plants

Assessment of diagnostic value of the ‚HC‘ marker for resistance to *G. pallida* Pa2/3

	Pathotype	No. varieties	HC positive
Susceptible varieties	-	21	0
Resistant varieties	Pa2	6	0
	Pa2/3	23	21
	Pa2/3 Partial resistance	5	2

Most agronomic characters are complex traits, controlled by multiple genetic and environmental factors

Genetic factors

Environmental factors

Quantity and activity of enzymes of secondary metabolism

Higher amounts of antioxidants reduce bruise susceptibility

Climate, soil, fertilizer

Quantity and activity of enzymes of starch metabolism

Higher tuber starch content increases bruise susceptibility

Handling during harvest, transport, storage

Quantity and activity of enzymes/proteins influencing membrane stability, cell shape and number

Higher amounts of phenolic compounds may increase bruise susceptibility

Gebhardt (2013) TIGS 29:248-256

Complex agronomic traits in potato

Polygenic

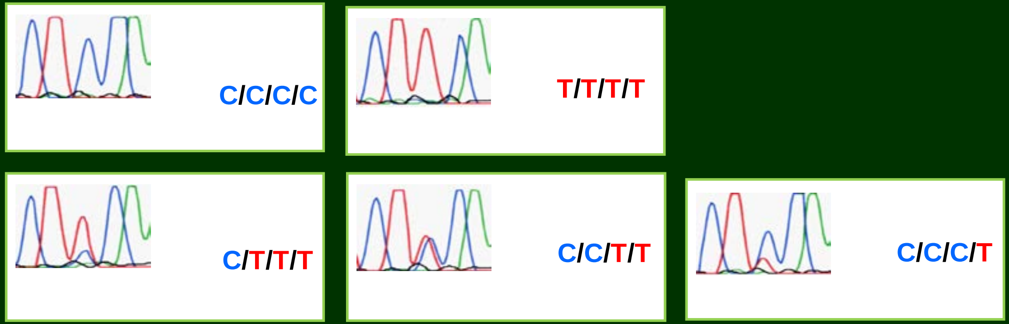
- Tuber yield
- Tuber starch content and composition
- Resistance to bruising, enzymatic discoloration
- Processing quality (reducing sugars)
- Tuberization
- Tuber dormancy
- Plant maturity, earliness
- Field resistance to late blight
- Resistance to Erwinia/Pectobacterium
- Resistance to potato wart (*Synchytrium endobioticum*)
- Abiotic stress (drought, heat, salt)

Oligogenic

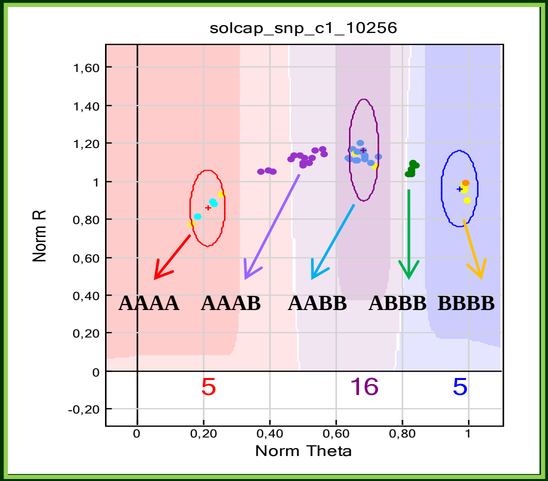
- Resistance to *G. pallida*
- Resistance to PLRV
- Glycoalkaloid content
- Tuber shape

Strategies for identifying diagnostic DNA markers for complex traits

1. Association mapping in populations of tetraploid varieties and breeding clones from breeders using as markers DNA variation in candidate genes (genes functionally related to the trait of interest).
2. Novel candidate genes: Using comparative proteomics and/or transcriptomics for identification of differential proteins/transcripts; testing the corresponding genes for diagnostic value by association mapping.
3. Genome wide association study (GWAS) using as markers genome wide distributed, random SNPs (Illumina array technology or genotyping by sequencing).



Sanger sequencing of PCR amplicons



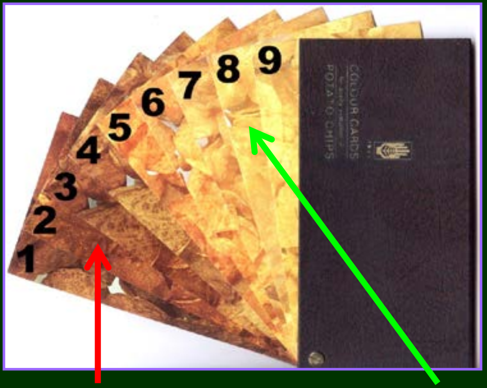
SolCAP SNP 8.3k array

Association versus linkage mapping

- Natural population resulting from random mating of individuals related by descent.
- Genetic distance estimated by linkage disequilibrium (LD)
- Segregation of multiple alleles per locus
- Allele and genotype frequencies depend on Hardy-Weinberg principle, selective constraints or random genetic drift.

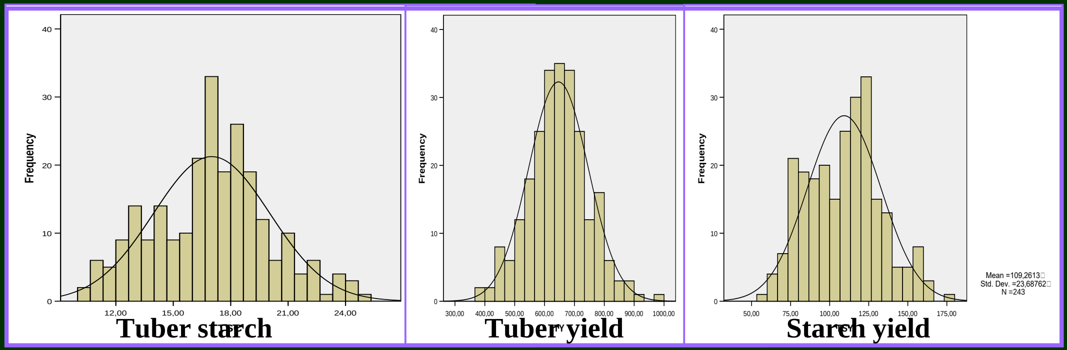
- Experimental population (F1, F2, BC, RILs, Ils) derived from crossing two parents.
- Genetic distance estimated by the recombination frequency
- Segregation of 2 to 4 alleles per locus
- Allele and genotype frequencies obey Mendelian segregation ratios.

Complex tuber traits:
Chip color, starch content, yield, starch yield.



High content of reducing sugars glucose and fructose, bad processing quality

Low content of reducing sugars glucose and fructose, good processing quality



Tuber starch

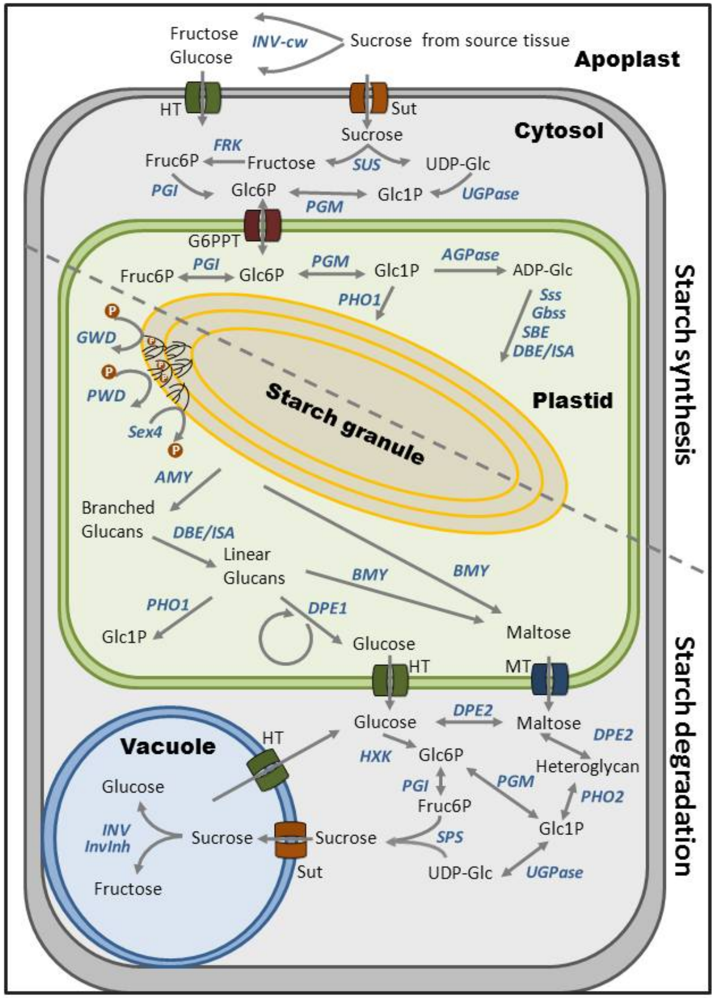
Tuber yield

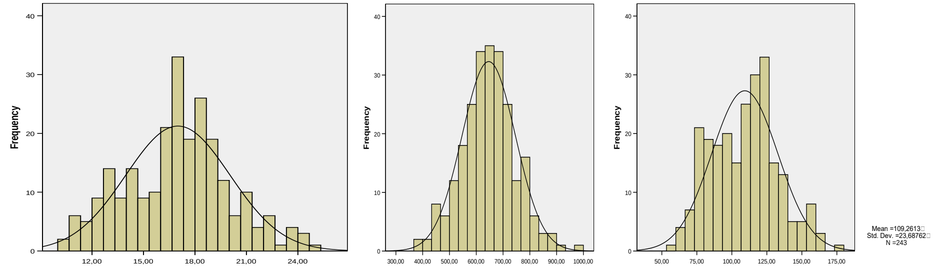
Starch yield

	CQS	TSC	TY	TSY
CQA	0.715***	0.586***	- 0.310***	0.301***
CQS		0.678***	- 0.269***	0.390***
TSC			- 0.182**	0.730***
TY				0.528***

Chipcolor after harvest (CQA) and 3 months storage at 4°C (CQS), tuber starch content (TSC), Tuber yield (TY) and starch yield (TSY) are correlated.

Candidate genes for tuber starch and sugar content (chip color): Genes functional in starch-sugar interconversion



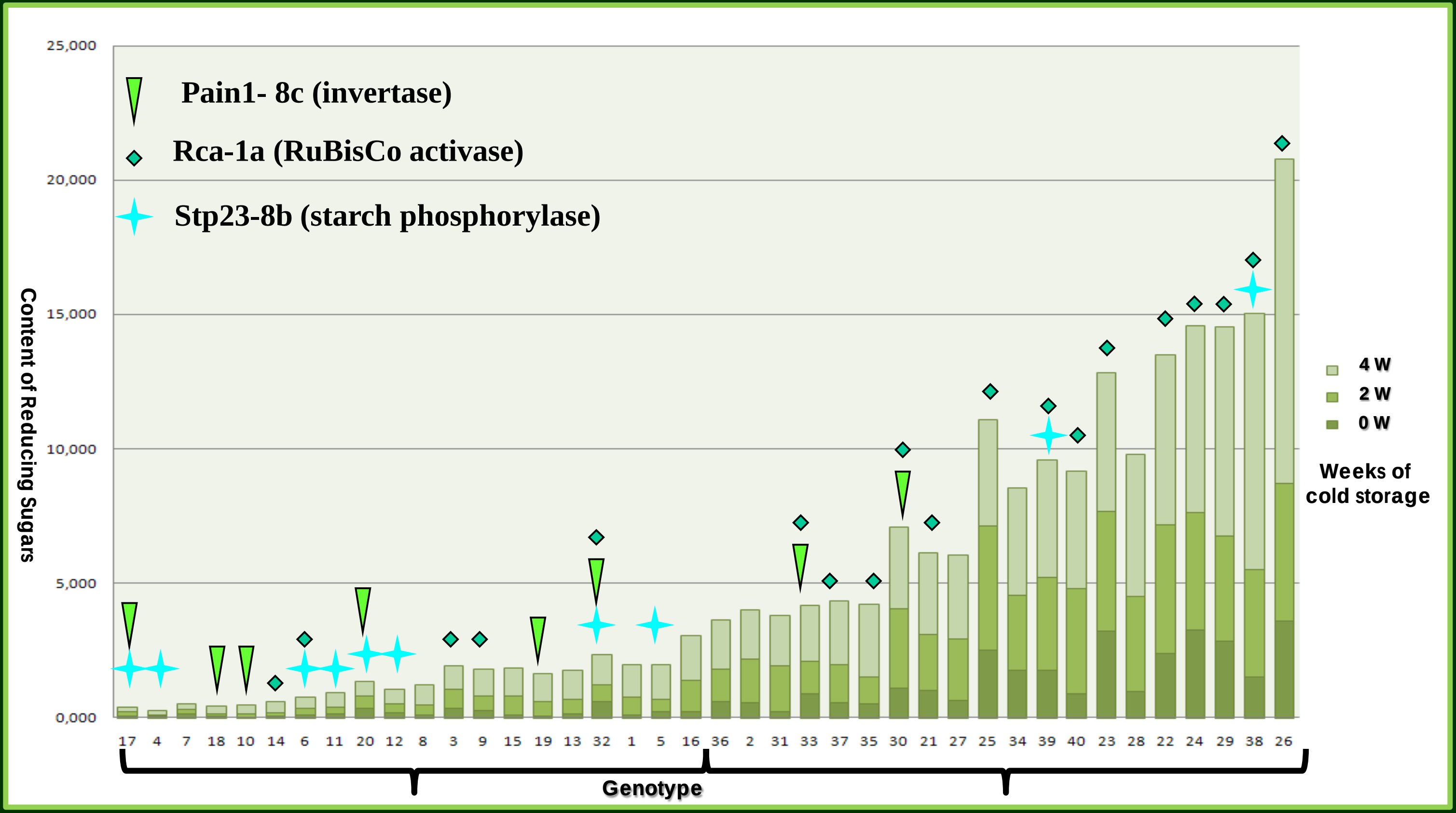


Tuber starch

Tuber yield

Starch yield

Diagnostic markers for chip color from candidate genes in a panel of 20 good and 20 bad processing varieties.

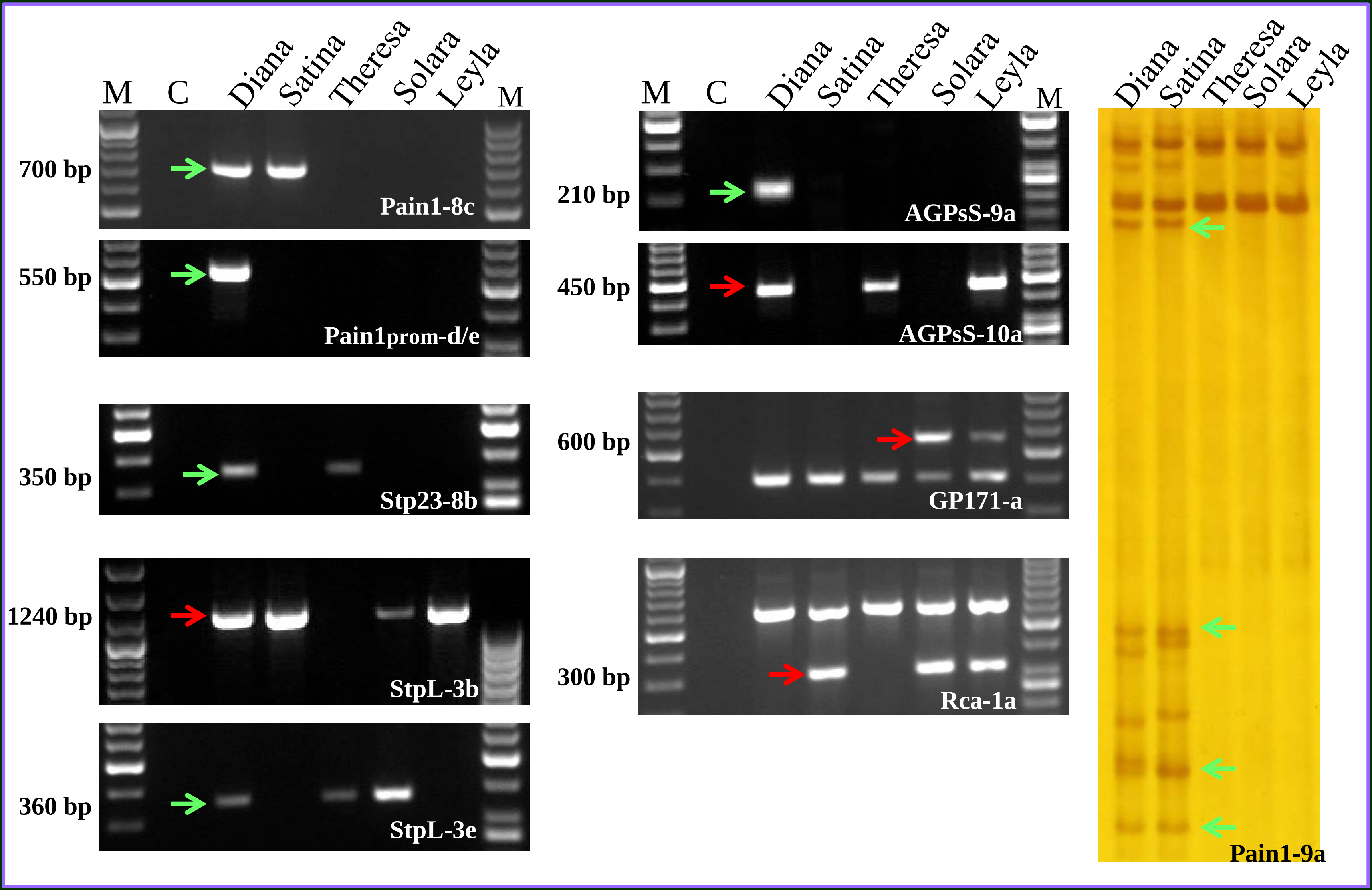


Good processing varieties

Bad processing varieties

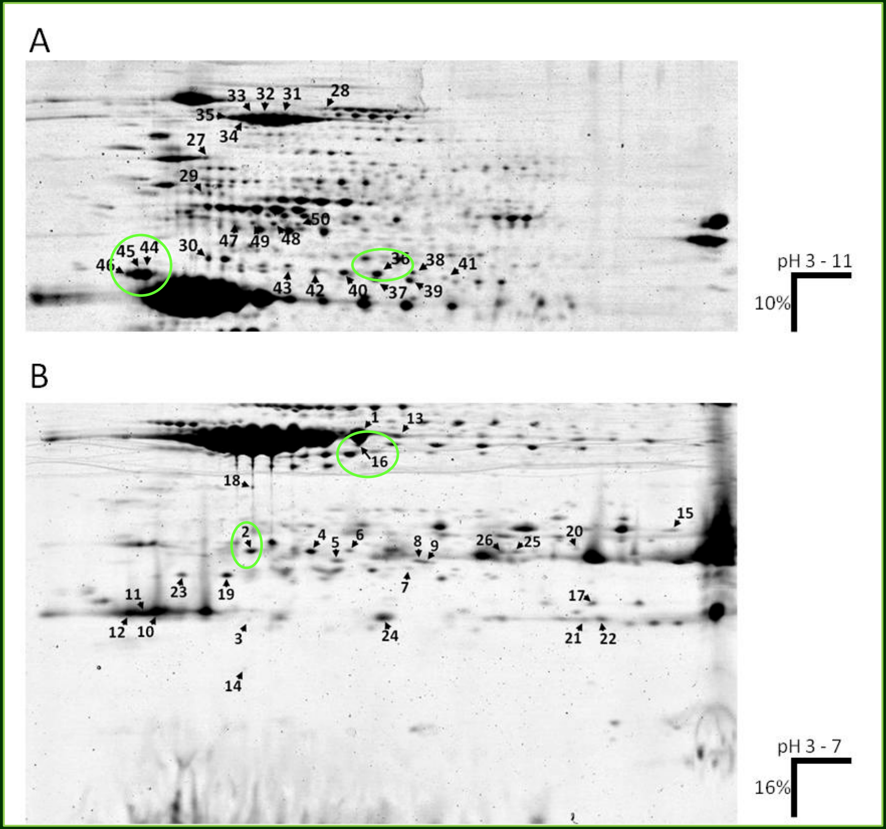
Schreiber et al. (2014), G3 4:1797

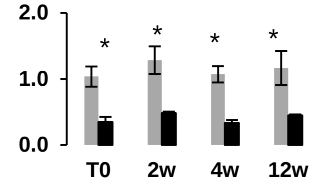
Conversion of diagnostic markers into easy to use PCR assays for screening in breeder's lab.



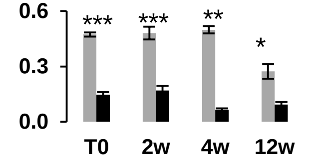
Li et al. (2013) TAG 129: 1039

Comparative 2-dimensional gel electrophoresis of tuber protein of good and bad processing varieties over 12 weeks of cold storage identified novel candidate genes for processing quality.

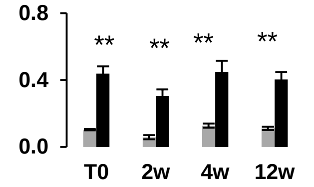




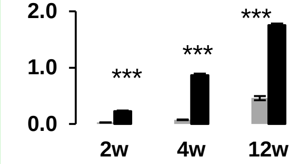
Spot 45: Patatin



Spot 36:
Phospholipase A1



Spot 2: Serine protease inhibitor

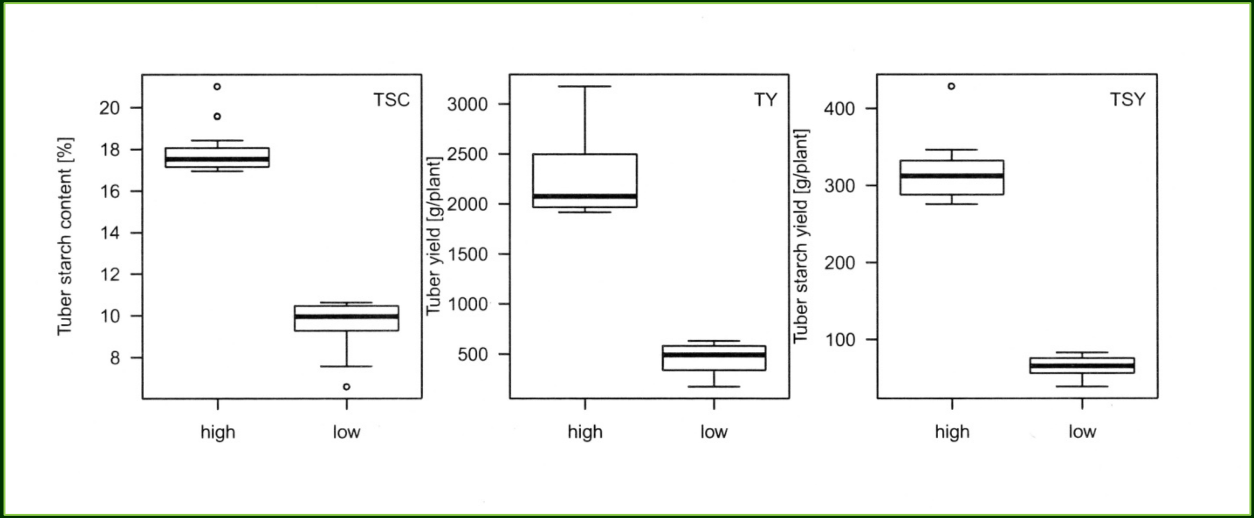


Spot 16:
Unknown protein

Fischer et al. (2013), BMC Plant Biology 13: 113

Example: Genome wide SNP genotyping of case-control populations by the 8.3k SolCAP SNP array and selective genomic sequencing (restriction site associated DNA (RAD) sequencing)

Case-control populations: 24 genotypes per population selected for maximum phenotypic difference from an association panel of 284 clones.



Tuber starch content (TSC)

Tuber yield (TY)

Starch yield (TSY)

Schönhals et al. (2017) BMC Genomics 18:642

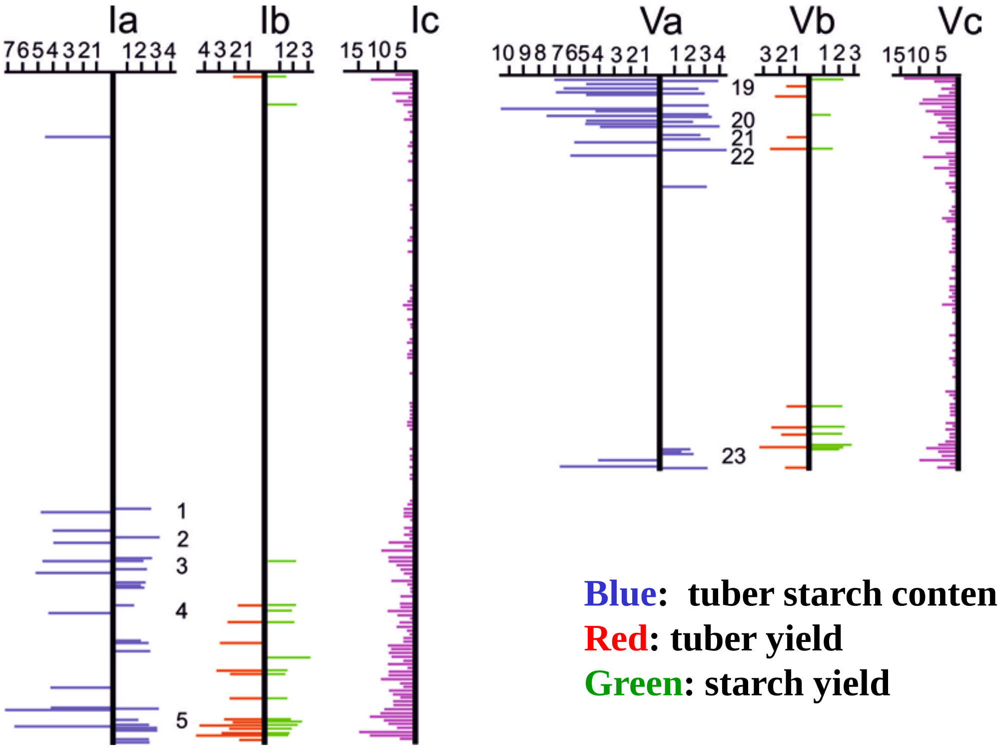
Differential SNP allele frequencies between cases and controls

	SolCAP SNP array (~ 7400 SNPs)	RAD sequencing (~2 200 000 SNPs)
No of differential SNPs	306	25 501
No of genes with differential SNPs	275	6664

72 genes detected by both genotyping methods.

Schönhals et al. (2017) BMC Genomics 18:642

Physical chromosome maps of genes with differential SNPs (SolCAP array: a, b; RADseq SNPs: c)



Blue: **tuber starch content**
Red: **tuber yield**
Green: **starch yield**

Schönhals et al. (2017) BMC Genomics 18:642

Conclusions and Outlook

- **It took 15 to 20 years of potato research before *in vitro* culture and molecular techniques were adopted by the practical breeding business.**
- **Collaborative research projects with breeders were essential for narrowing the gap between basic science and application.**
- **For quantitative traits, association mapping in populations of advanced breeding materials was superior to linkage mapping for narrowing the gap between basic science and application.**
- **Genomic selection based on genome wide SNP markers will eventually close this gap.**
- **Important in the past and future: The availability, phenotypic and genotypic analysis, and introgression of diverse genetic resources.**

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