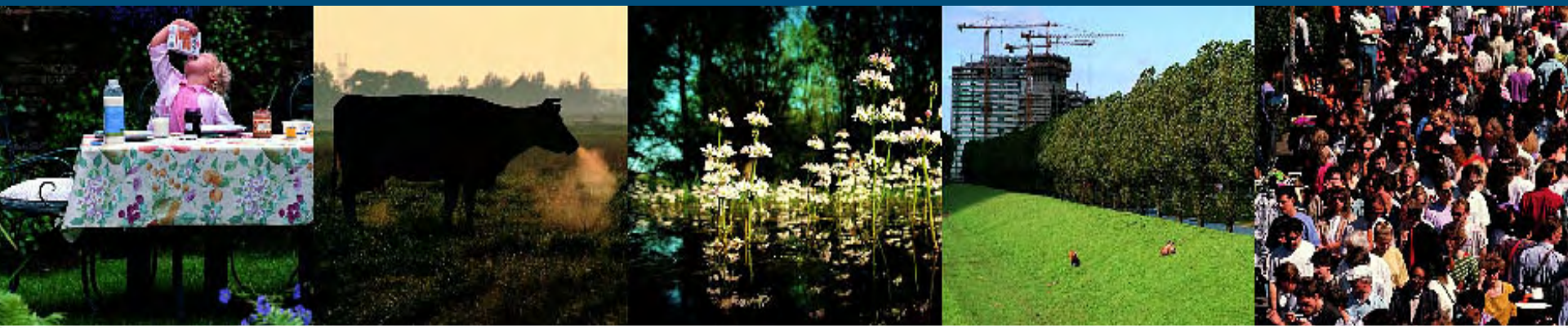
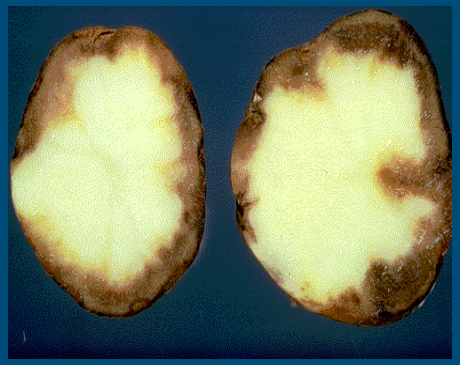
Future prospects for potato genome technology

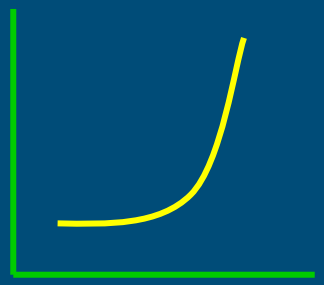
Prof. Dr. Richard G.F. Visser



PLANT BREEDING
WAGENINGEN UR

The phenotype





Physiology

Phenotype

Candidate
Gene approach

Metabolome

Proteome

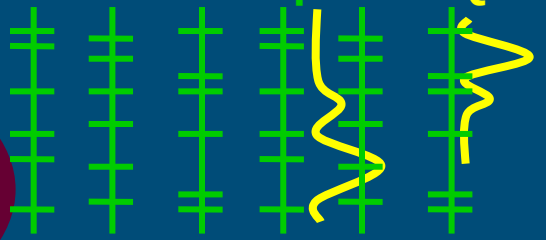
Transcriptome

Genome

G x E

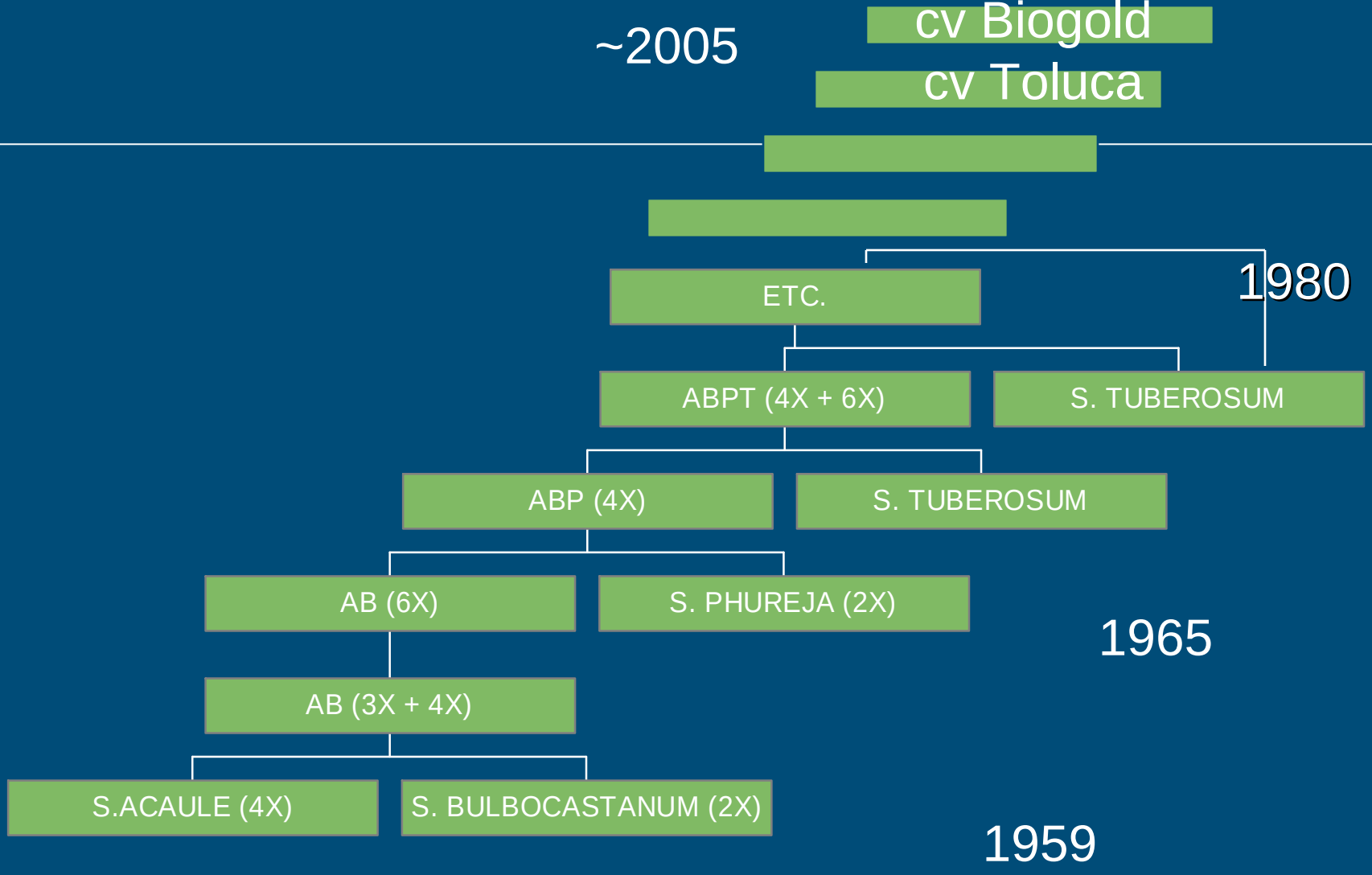
Genetic map

QTLs



Metabolic
routes

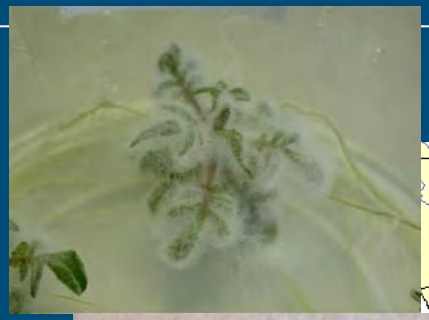
Genotype



Breeding for durable Phytophthora resistance (Prof Hermsen)

Phenotyping

Novel sources of LB resistance



Screening of 1,000 *Solanum* accessions

Solanum resistance screening

Initial HTP set up

- 1,000 accessions sown
- 5,000 genotypes tested
- 1,677 genotypes resistant in HTP vitro screen

Further screening

- detached leaves with isolates 90128 and IPO-C
- Field trials with isolate IPO-C

Selection

- Core collection 280 genotypes (end 2007)

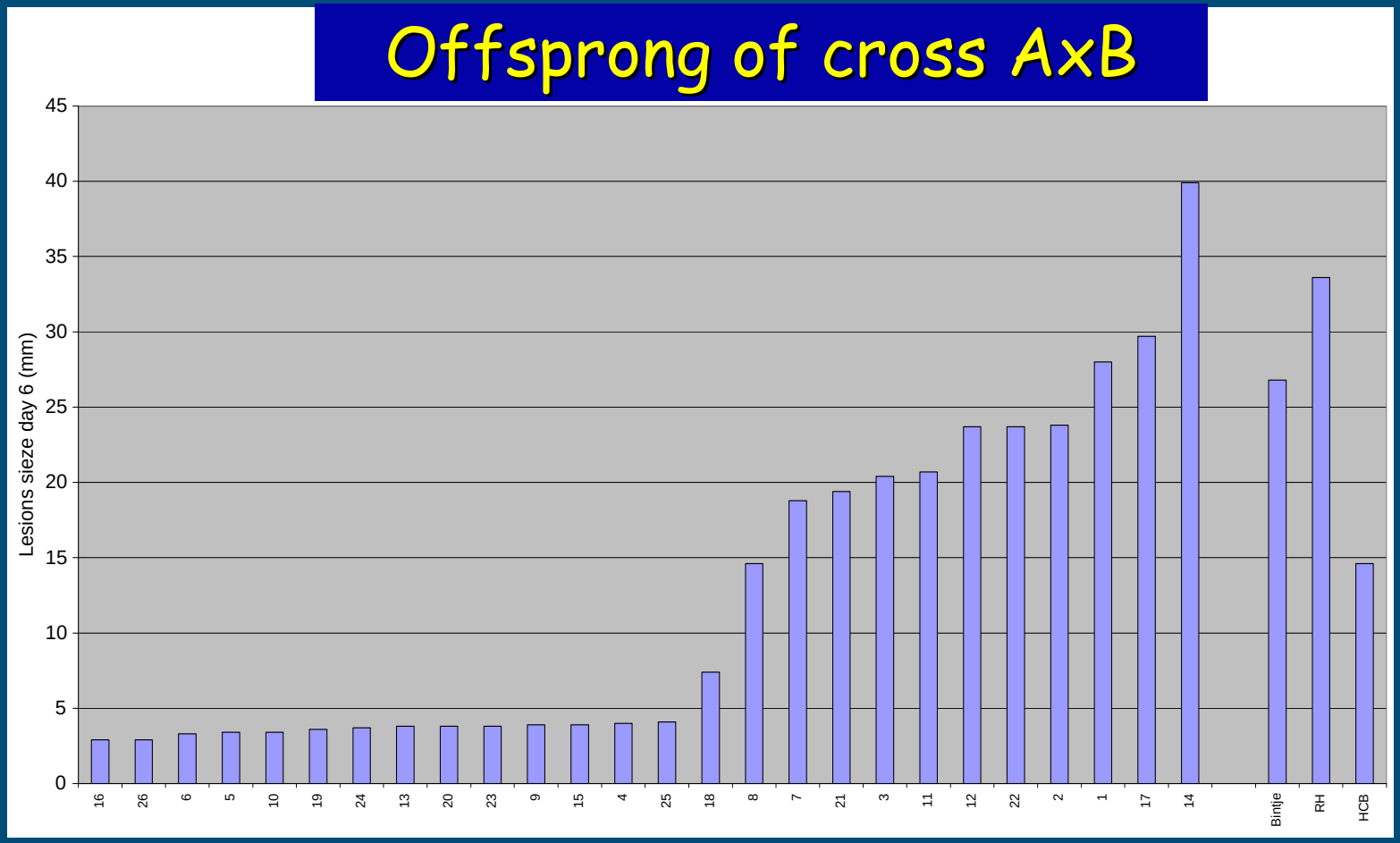
Population analyses

- 232 populations tested, ca 40 seeds sown for initial screen

Segregation category	# populations 2007
All resistant	61
BW segregation	36
Variation	39
Gradient	54
All susceptible	42
Total	232

75 BW/Var segregating

Genetic basis of resistance: 1:1 segregation of resistance in F1 progeny



Phenotyping Quality traits

- Mostly done in segregating populations
- Sizes between 100 and 250 genotypes
- Different years and different environmental conditions
- Lots of work and quite expensive
- Not easily translated to 'real' varieties

Rate enzymatic blackening

5 minutes

30 minutes

Chip color scale: 1 (black) to 8 (no browning)

Phenotyping

In the genomics era phenotyping is the limiting factor or bottleneck!

Different programmes on assessing HTP phenotypes (diseases and quality traits)!

All available characterized material can be easily genotyped and –omics analysis can be performed at various levels

Leads to increased speed for isolating genes coding for important traits and speeds up breeding

Genotyping

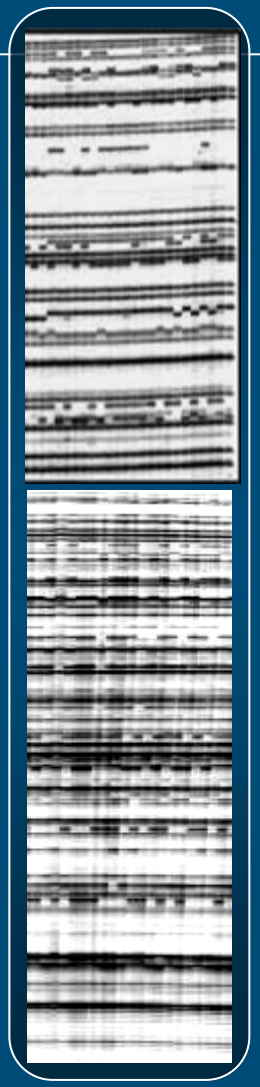
Technology development

- Marker technology
  - AFLP
  - cDNA - AFLP (for trait mapping & gene expression analysis)
  - SSR's
  - Domain profiling (R-gene profiling, MADS-box profiling, etc.)
  - SNP detection and htp analysis
  - Tilling

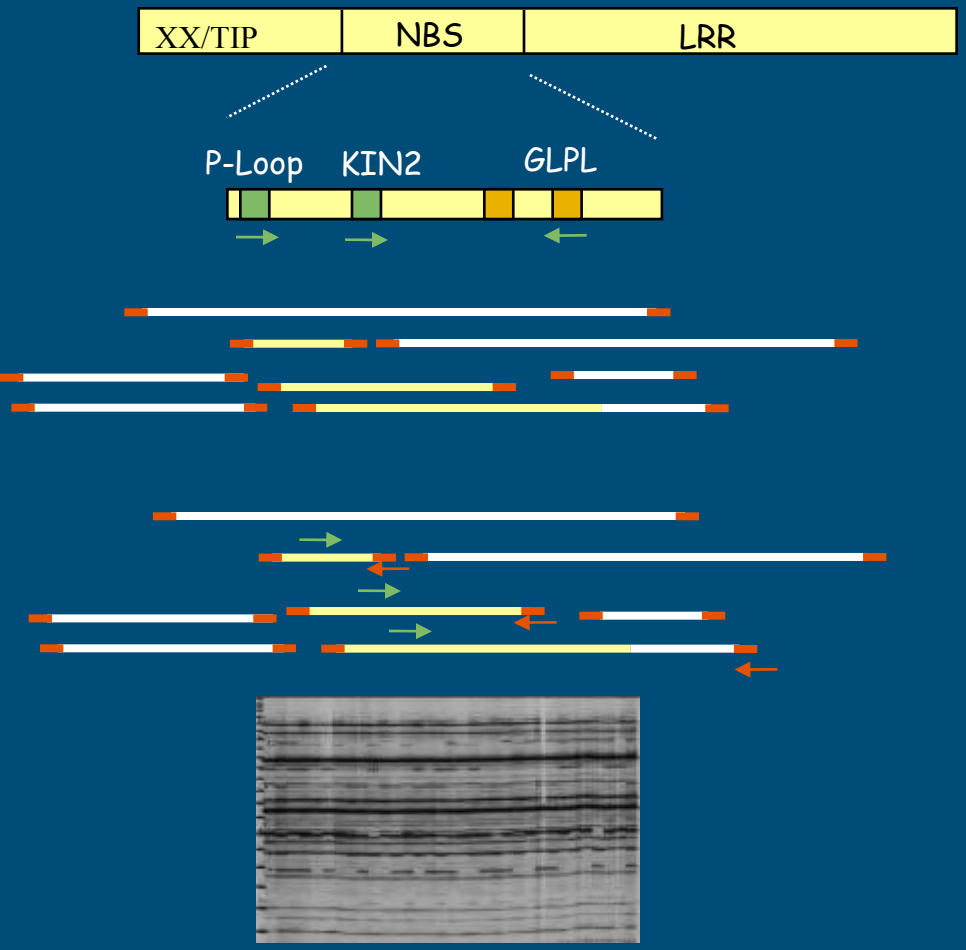
- Physical map construction
- Micro arrays (cDNAs; 2000 element), oligo array (60 mers; POCI ~44.000 element)
- Sequence information and software

Genotyping:

- Neutral markers:
  - SSRs (anchoring CxE and SHxRH)
  - AFLP™
- Functional markers
  - Candidate gene SNPs (gene by gene)
    - Requires sequence knowledge of genes
  - Motif-directed Profiling
    - Targets members of gene families

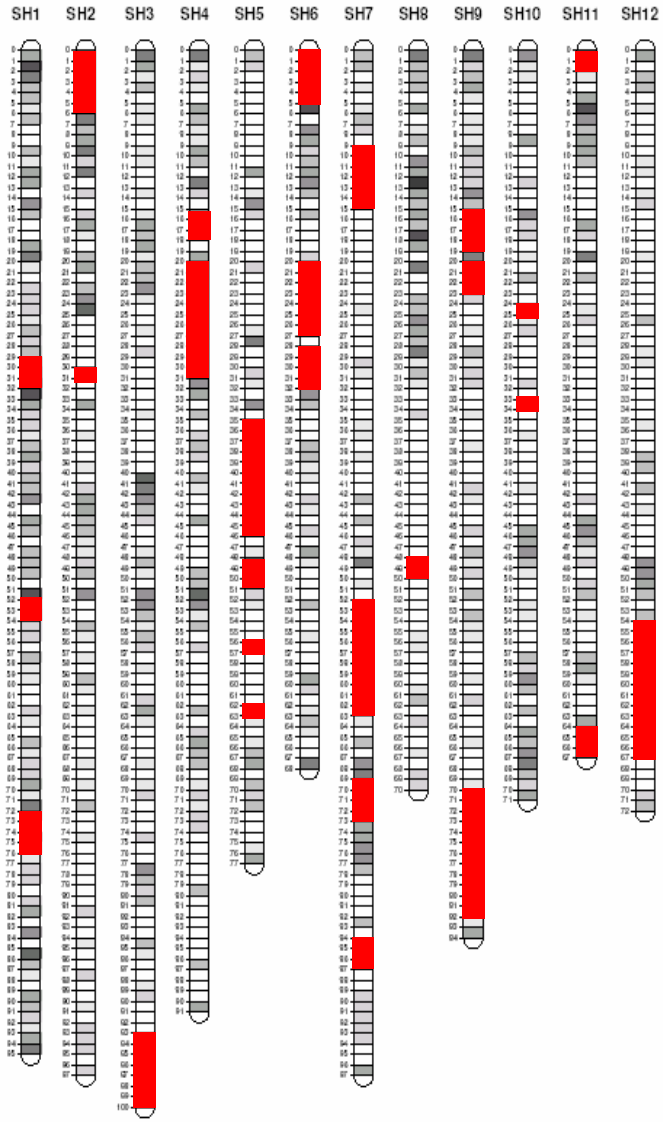


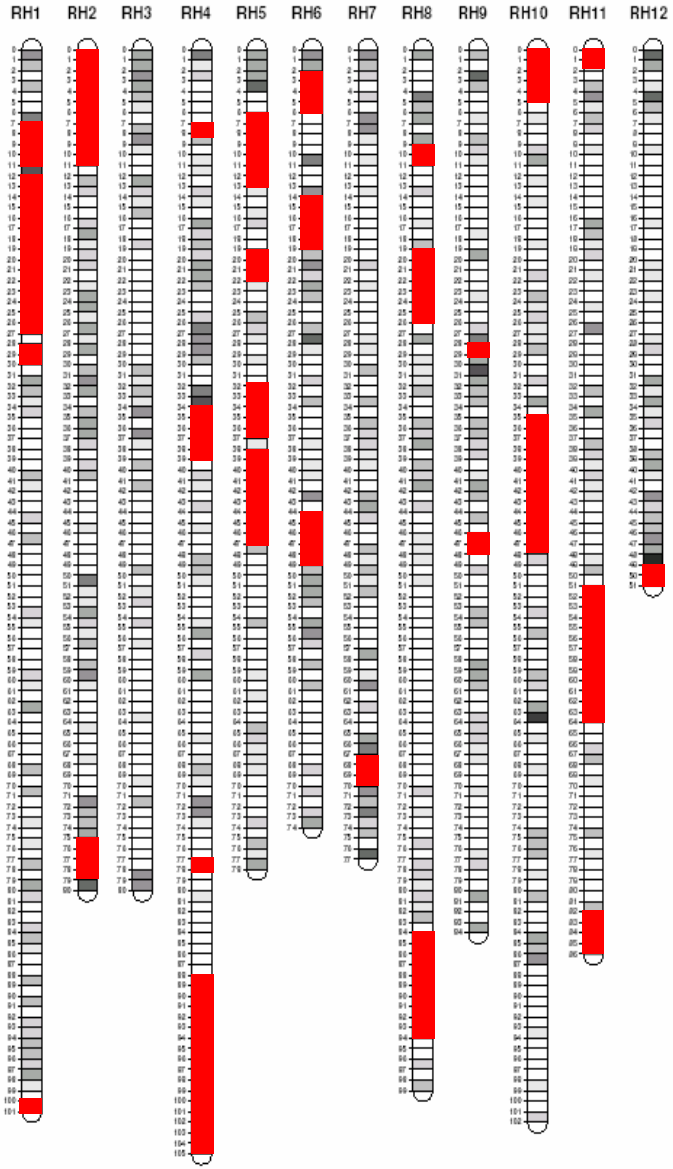
Domain profiling (e.g. NBS-directed profiling)



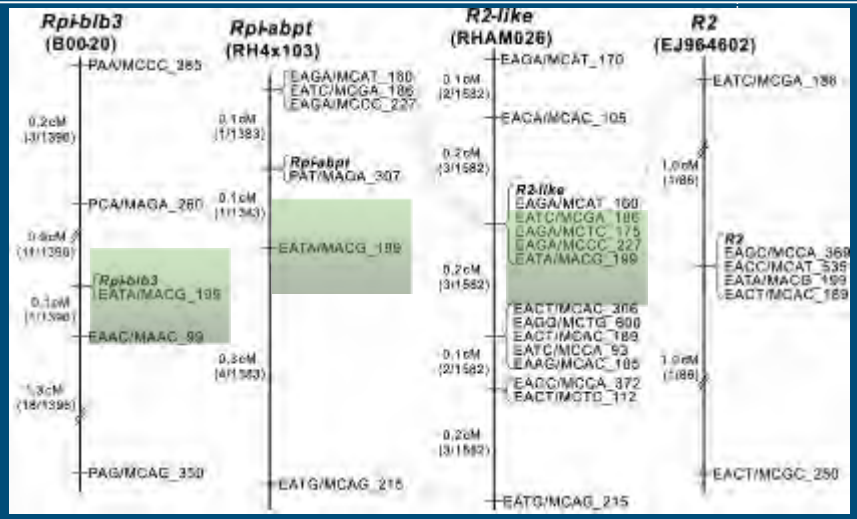
- Design (degenerate) primers for conserved motifs
- Restriction/adapter ligation
- Labelled PCR with adapter and motif-specific primers
- PAGE (multiple marker profile)
- Sofar for *Myb* genes, peroxidases, R genes & WRKY genes

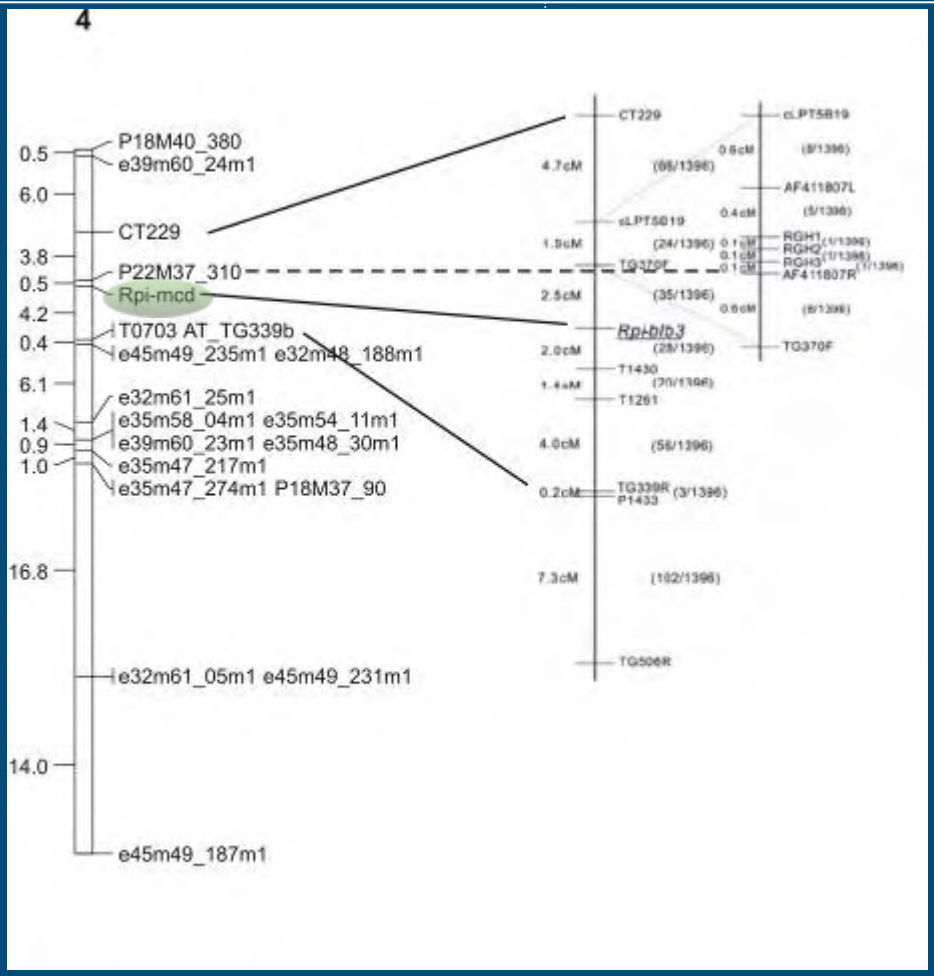
Genetic positions of R-gene clusters in SHxRH





Allele mining at the MLB-locus on chr. IV





- *Rpi-blb3*
- *Rpi-abpt*
- *R2 (Rpi-dms2)*
- *R2-like*

- *Rpi-mcd1*

Dissection of Potato genetics for Drought tolerance

- **Mapping population: CxE**
  - Wide contrast
  - Large number of progeny
  - Growth and yield data from multiple environments

Phenotyping

- In-vitro
- Green house
- Field

Genotyping

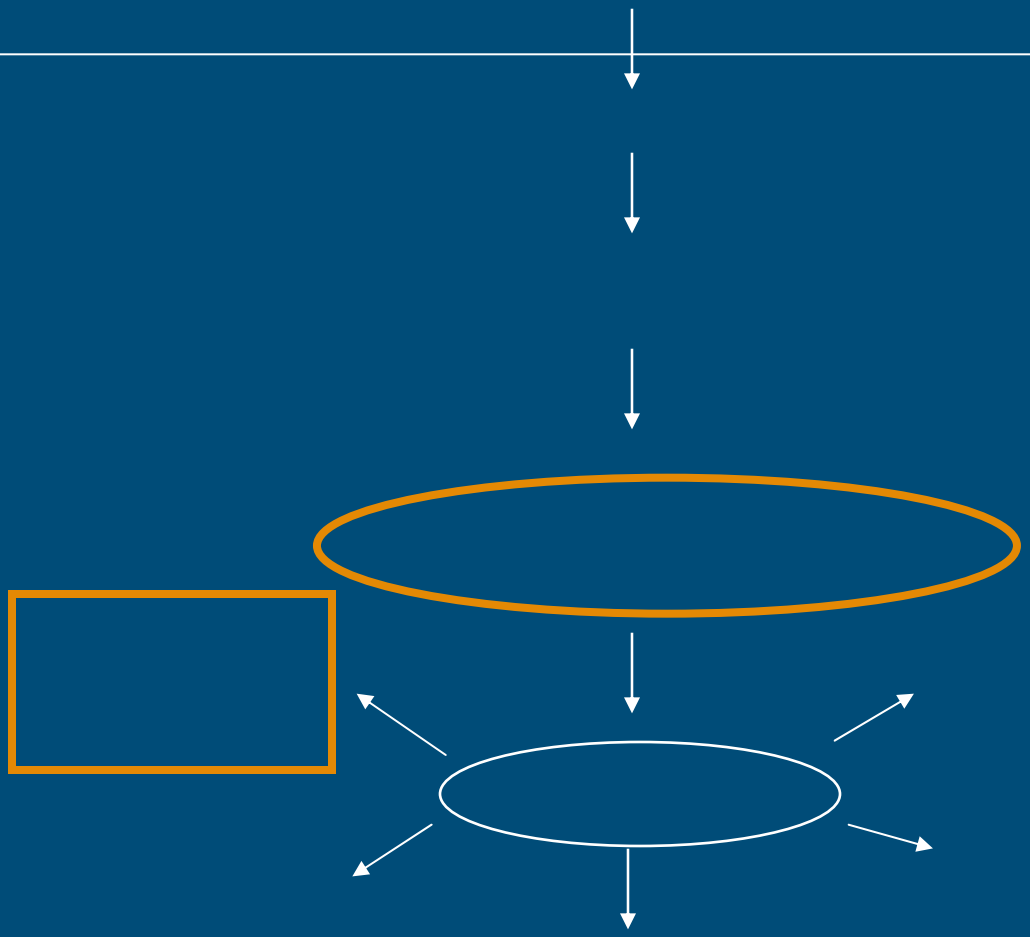
- Molecular markers

QTL analysis

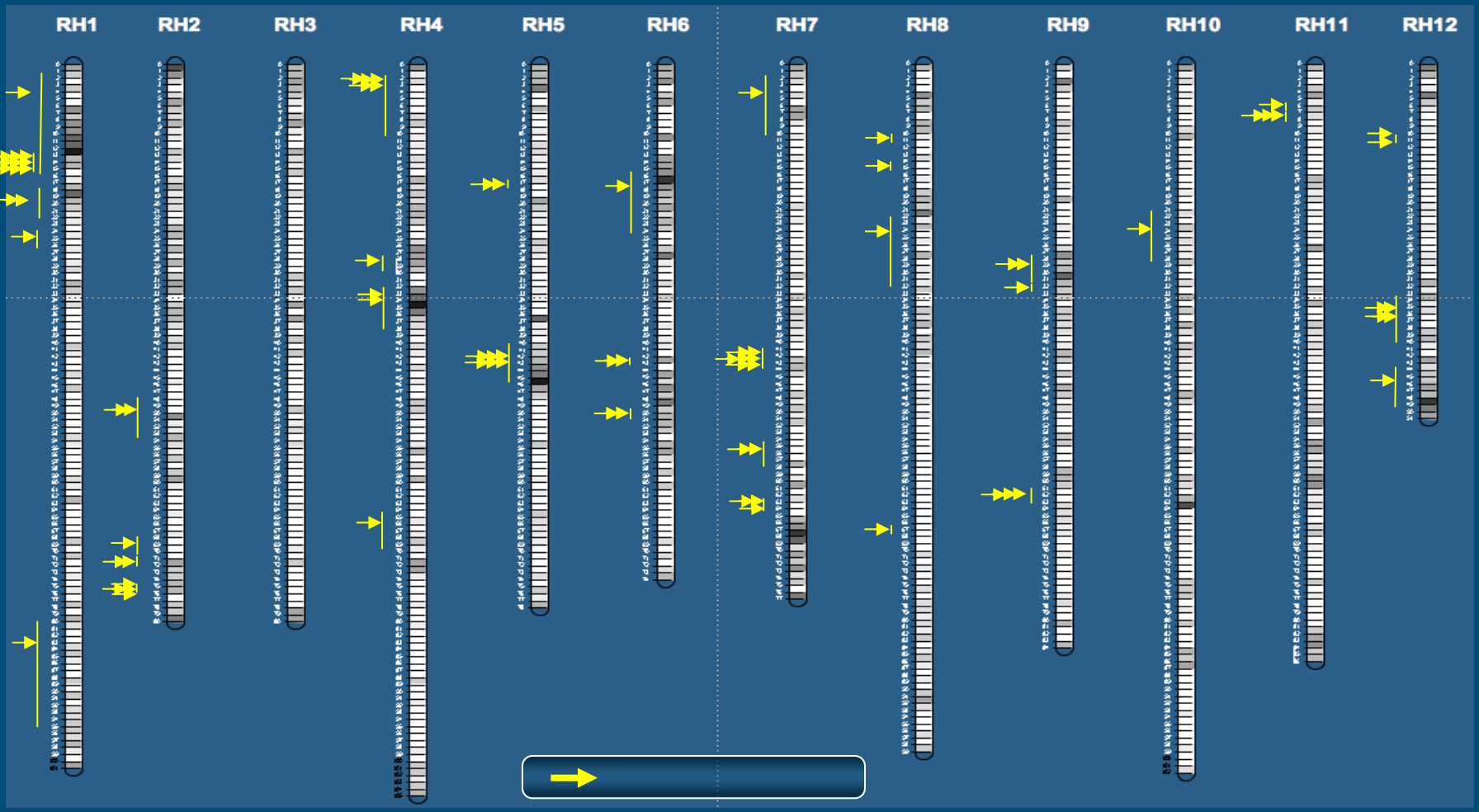
- **Reference population SHxRH**
  - Ultra-high density map
  - Physical map
  - Genome sequence

Varieties?

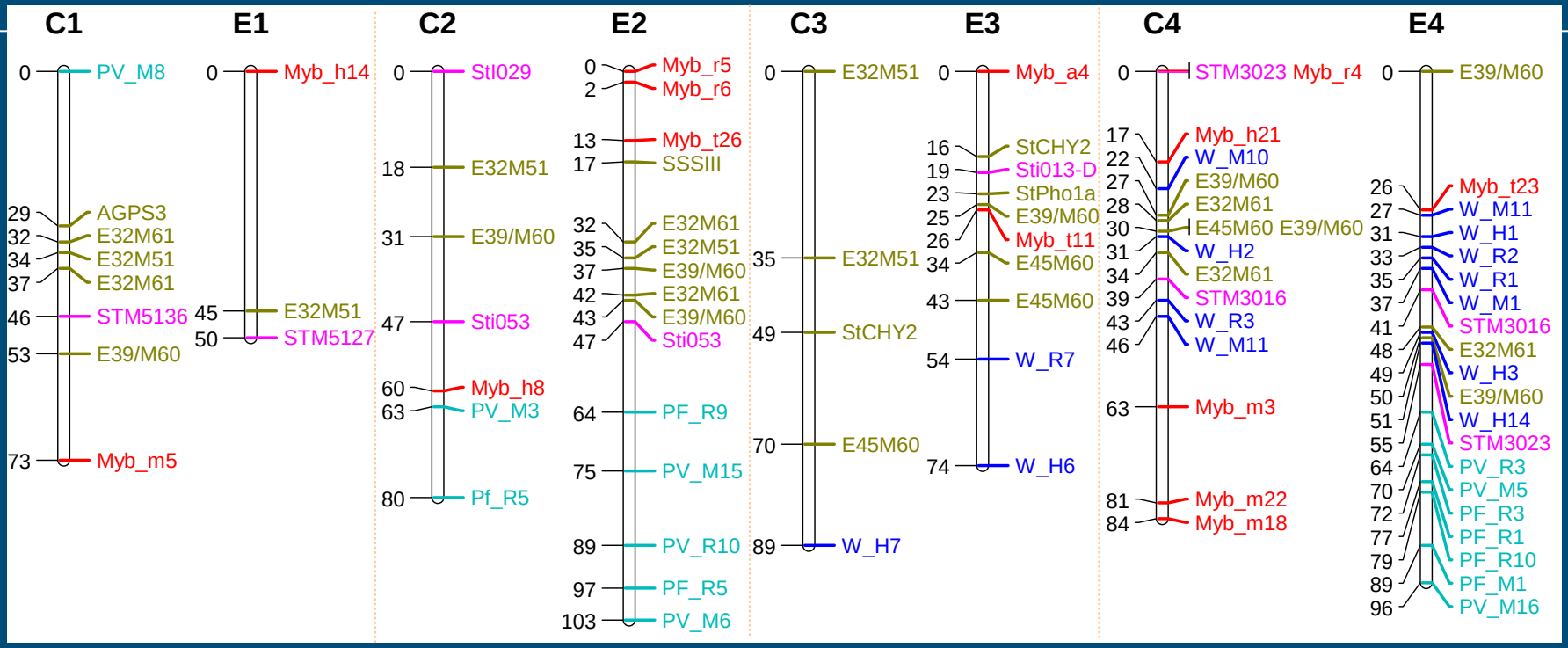
Drought (Salinity, UV
High/low Temperature, pathogen,



Myb markers can be easily mapped:



C X E genetic map



VVUv

P|ev

DIOSv

Z UN\v

Shur{lgdvhv

Combine with phenotypic studies on drought in gh and field

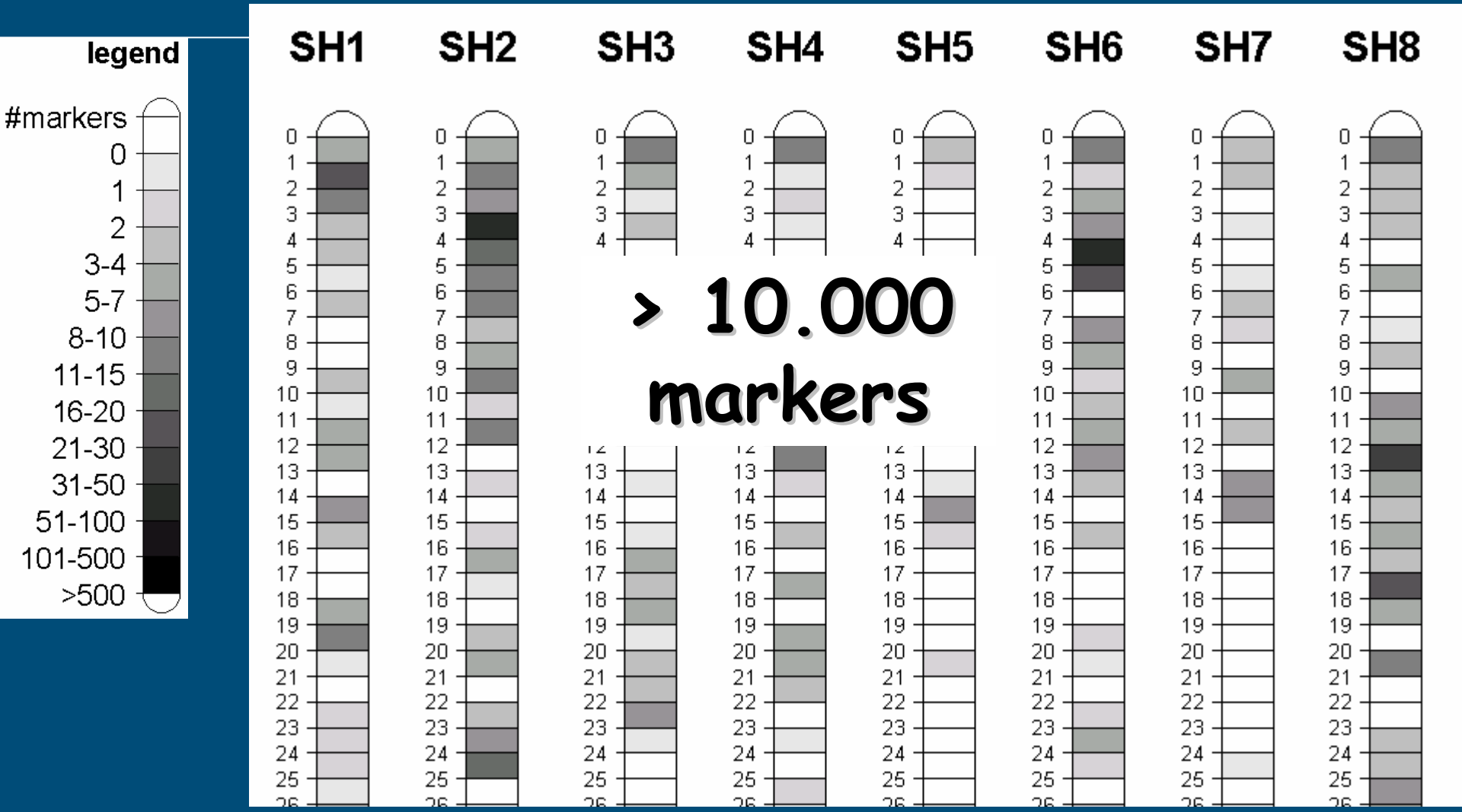
Potato Structural Genomics

- An ultra dense linkage map
  - Exploitation of this map - map alignment
  - Exploitation of this map - map based cloning (*H1, Sen1B, R3*)
  - The mapping of R-gene clusters by domain profiling
  - The cloning of other genes involved in Resistance and Quality
  - The mapping of centromeres

- A physical map of potato
  - Source of markers for marker assisted selection
  - Will facilitate map based cloning efforts
  - Selection of a minimal tiling path - prepare for a potato DNA sequencing effort-→ has started; all 12 chromosomes accounted for by International effort in Potato Genome Sequencing Consortium

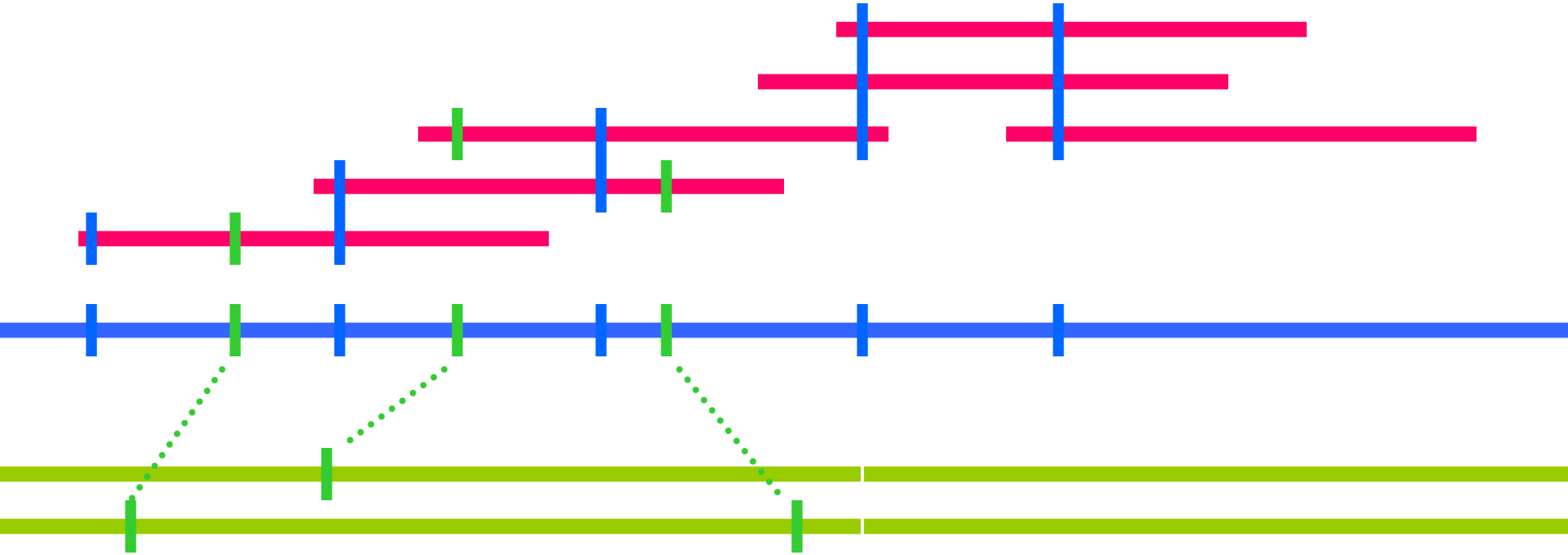
- BAC pool mapping and potato genome sequencing

Potato Ultra Dense Map in SH x RH population



- Recombination events instead of pair wise distances

Physical map of diploid potato RH 89-039-16

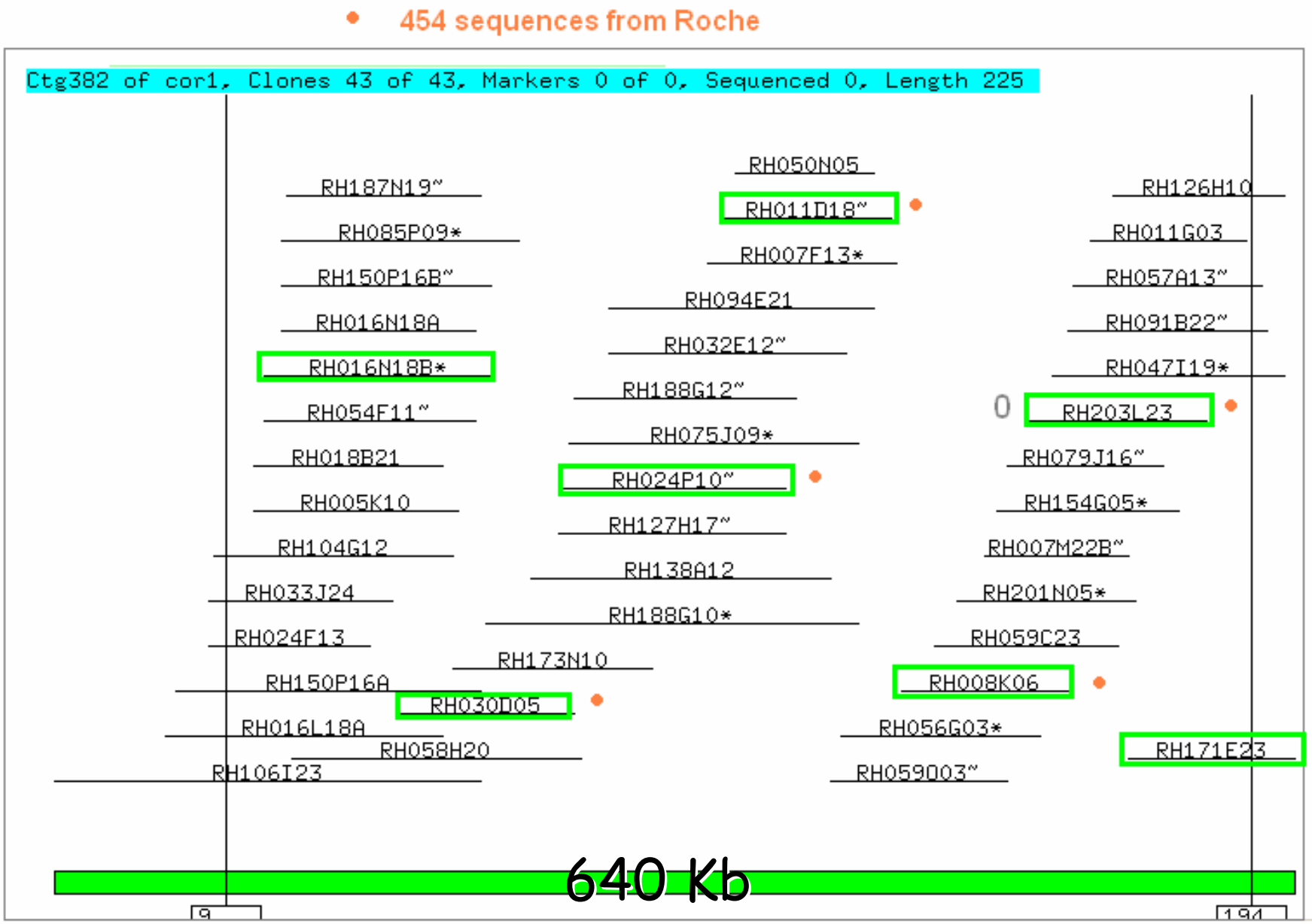


Potato Physical Map & Potato Genome Sequencing Consortium (PGSC)

- BACs fingerprinted >78,000
- Contig construction -> First draft in January 2004, second draft in September 2004 as good as the finalized tomato map. 'Final' version in Dec 2008. About 1750 genetically anchored contigs.
- Provides a BAC library-backed map with an extremely high marker density (> 20000 AFLP markers; avg distance 0.03 cM (43 kbp)
- BAC ends sequenced (~ 130.000 by TIGR in 2007)
- Sequencing started with different partners (Incl. SCRI)
- Chromosme 5 (NL) should be finished in October 2008 (>600 BACs)

http://potatogenome.net

Chr. 5 fingerprint contig with sequenced BACs



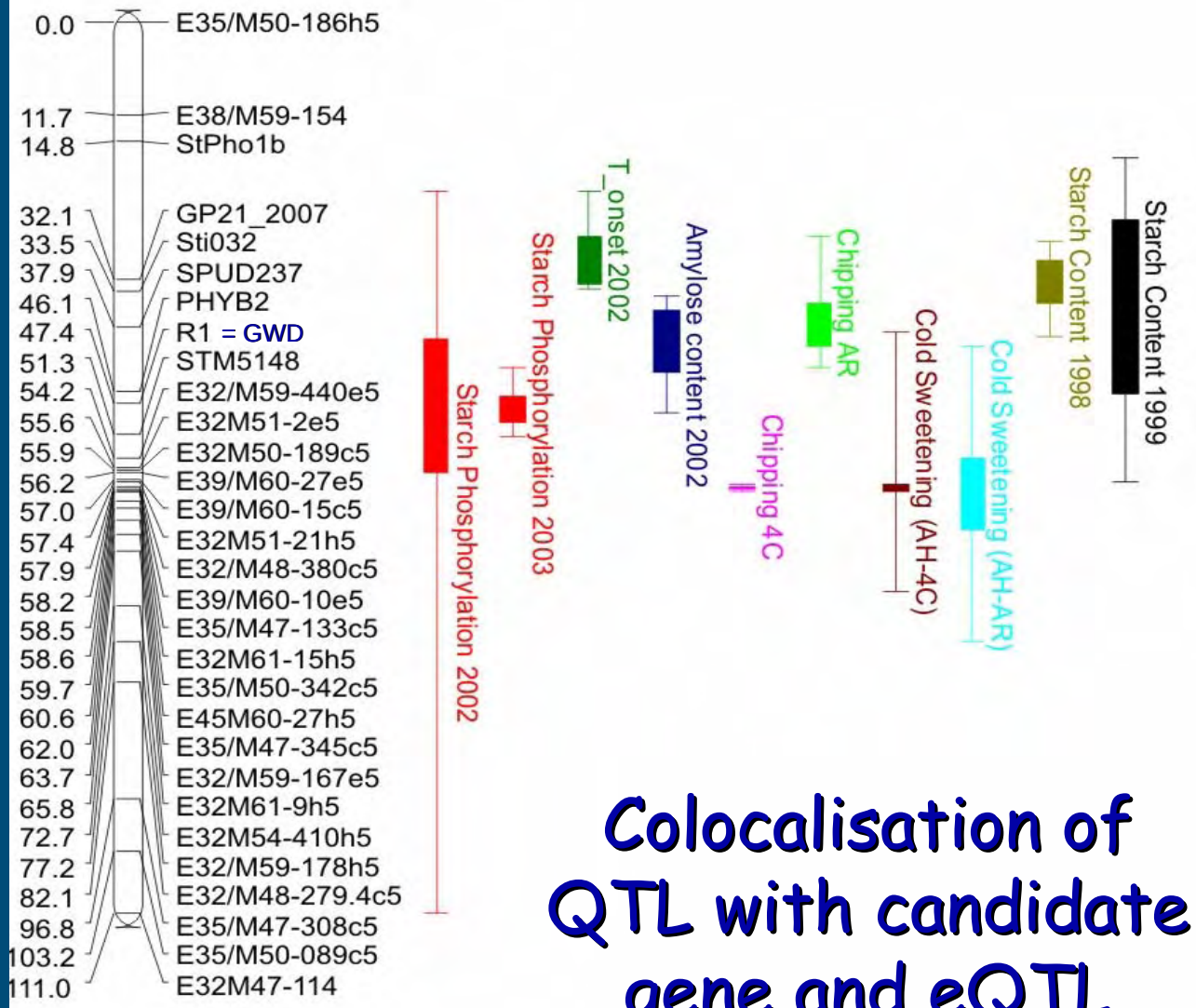
BAC sequencing statistics June 2008 (NL)

- Chromosome 1
  - BACs currently sequenced: 255
  - Total sequenced by end 2008: 350
  - In progress by end 2008: 150
  - Anticipated chromosome size: 1000

- Chromosome 5
  - BACs currently sequenced: 316
  - Longest contig 28 BACs ~2.5 Mb
  - First draft sequence completed by October 2008: 600

Identification of QTLs related to Quality and tuberisation/agronomy related processes

- Different QTLs:T-QTLs & D-QTLs & E-QTLs
- Important = difficult traits like Adapatation, yield stability
- Translation from population to varieties

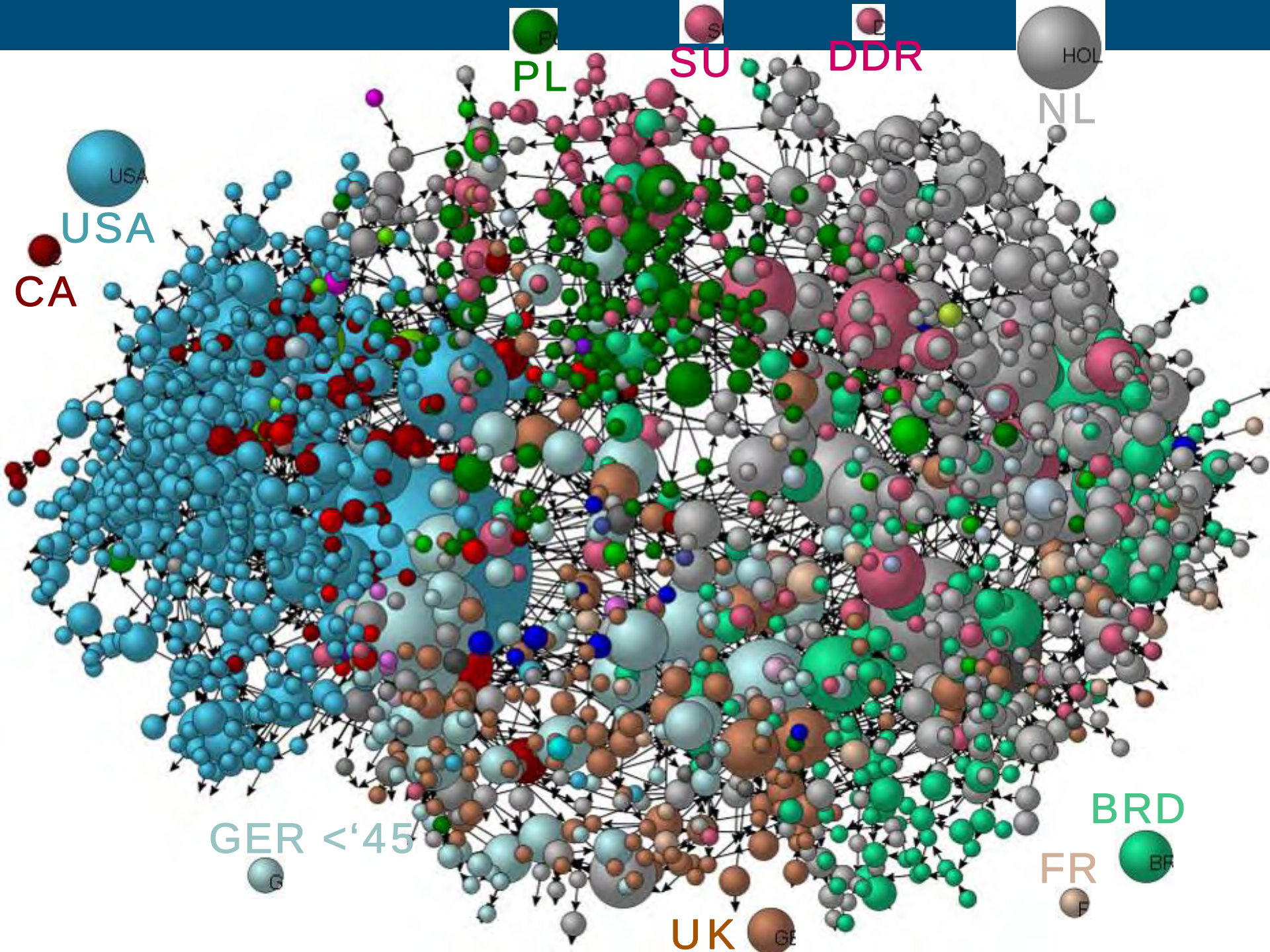


Colocalisation of QTL with candidate gene and eQTL

Potato pedigree database

Can be consulted via internet:

- 7000 genotypes in potato pedigree database
- of 6400 genotypes: one parent known
- 4500 genotypes with two parents
- 3700 in one big cluster


PL
SU
DDR
HOL
NL
USA
USA
CA
GER <‘45
BRD
FR
UK

Potato pedigree database

Conclusions:

Strong clustering per country

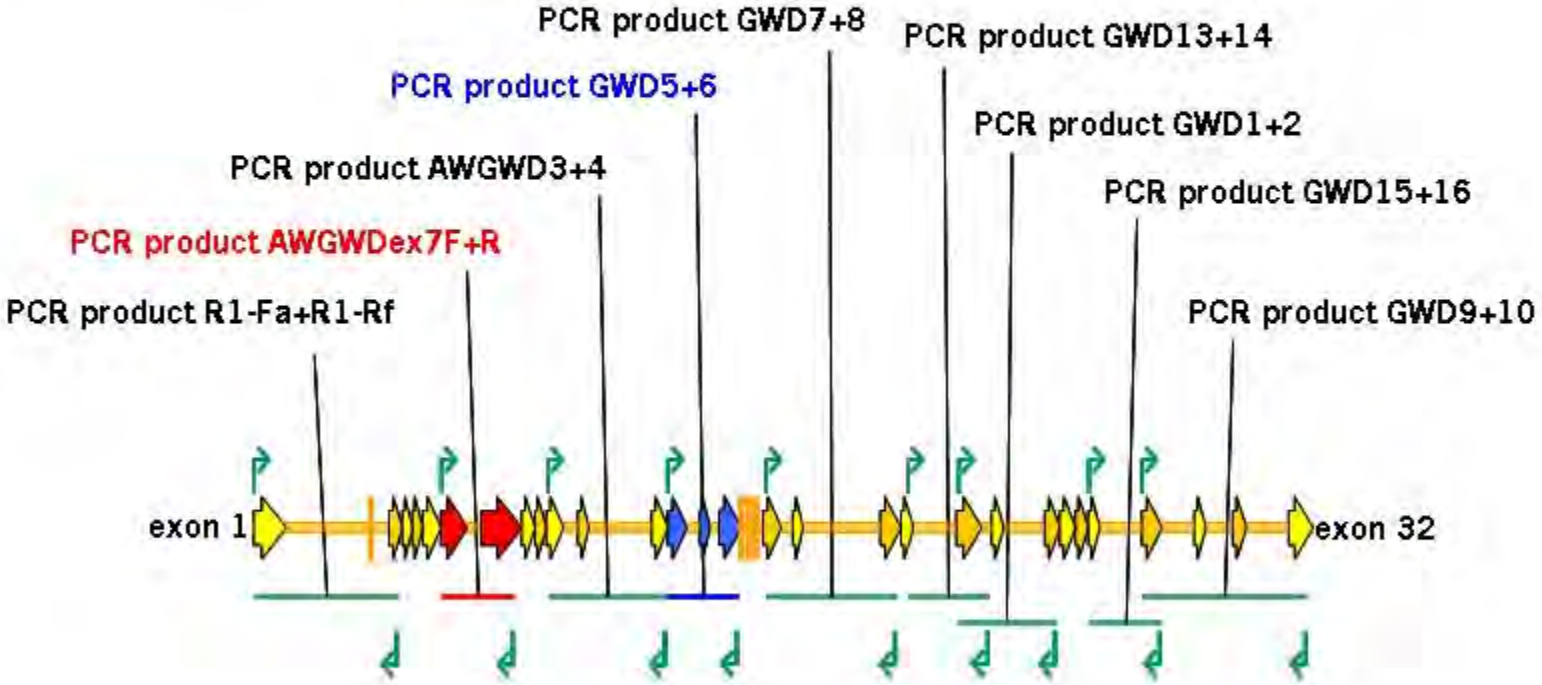
May point to:
- little exchange of clones between countries,
- conservatism of breeders,
- strong adaptation to different environments.

Statistical set up is also used for association studies. Identity by descend and relation between different alleles

LD mapping in varieties: translate molecular genetic data to improve potato breeding efficiency

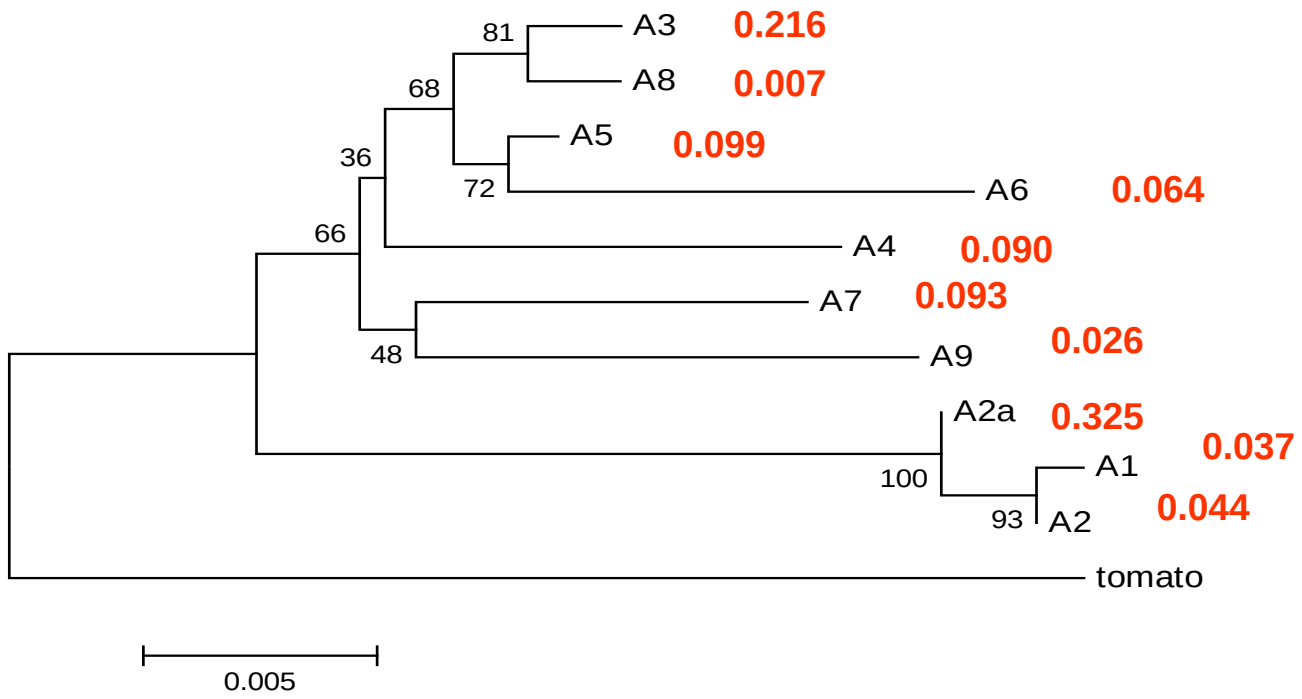
- Germplasm
  - 225 cultivars selected
  - Represent worldwide potato gene pool
- Phenotypic data
  - Focus on quality traits
  - Repeated field trial performed in 2006
  - Multi-year multi-location data provided by breeding companies
- Challenges:
  - Autotetraploid → extract maximal information
  - Example GWD (involved in cold sweetening)

Partial genomic sequence GWD gene in haploid potato clone 7322 (8958 bp)



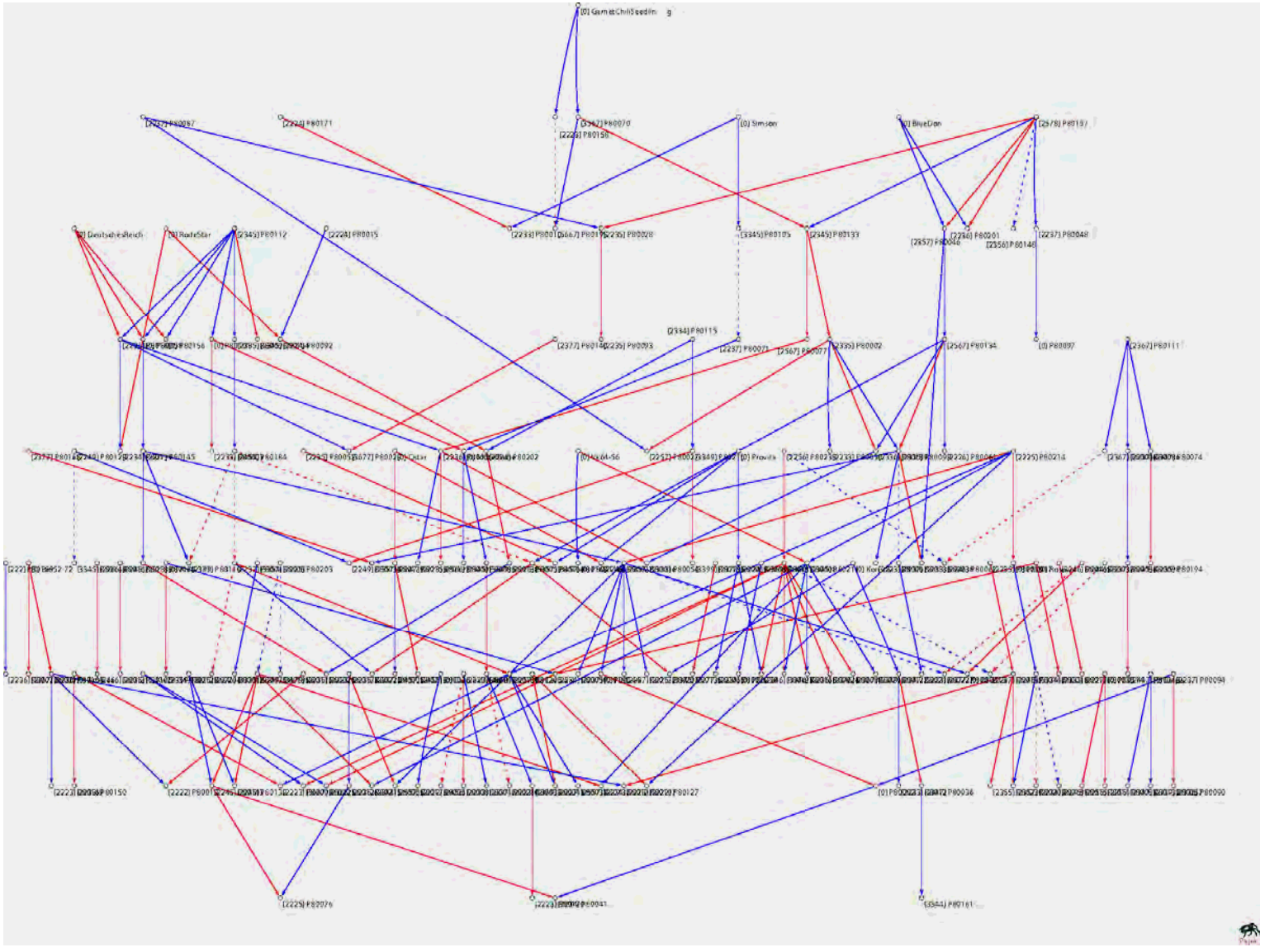
Total gene 32 exons ~1500 aa

Dendrogramme of 10 alleles of GWD (red is %)

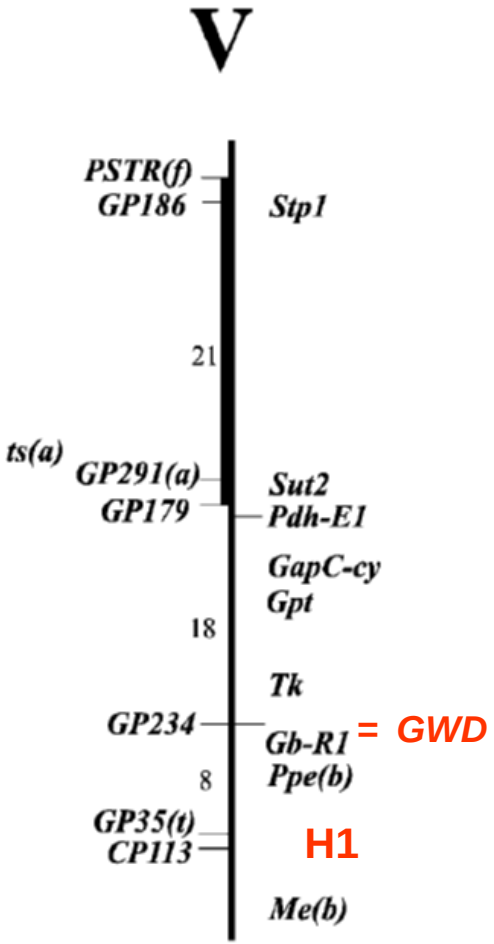


Which allele is correlated to cold sweetening ?

Relationship in varieties of GWD alleles



Chromosomal location of GWD & specifics of alleles



- One allele disappeared in course of time (# 8).

- Other alleles are newly introduced (alleles 1 and 9). One of these (allele 1) is present in the genepool since 1963 (in cultivar 'Maris Piper'). This allele is linked to the nematode resistance H1.

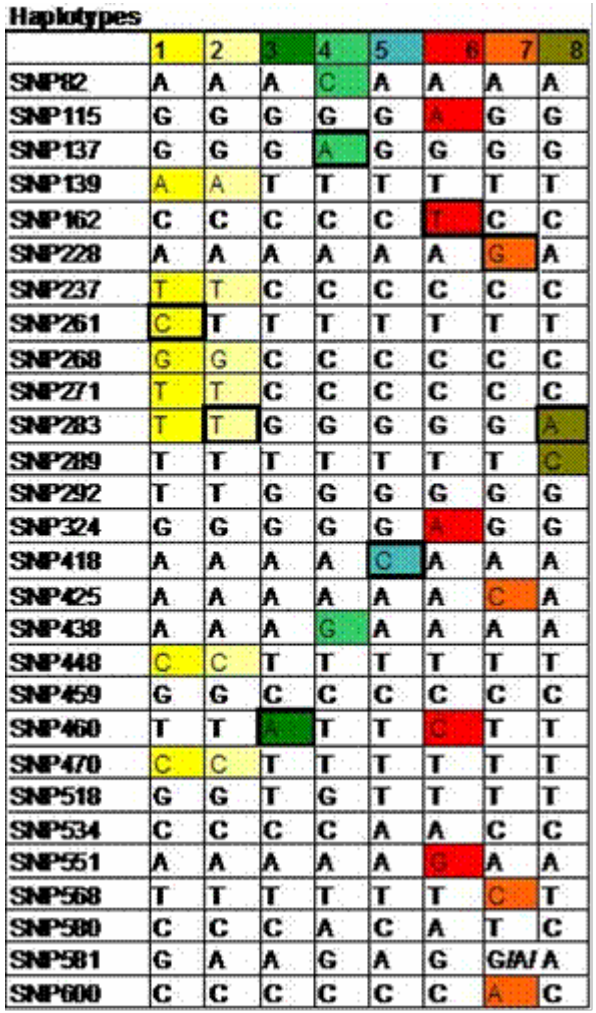
- Allele 9 is present in the genepool as of 1946. Varieties which contain this allele are 'Craigs Bounty', 'Kennebec', 'Maritta' en 'Sirtema'.

GWD haplotype identification & structure

- Identified eighth GWD haplotypes by:
  - Linked (co-segregation) SNPs
  - Sequence database haplotypes
  - Monoploid sequences

Verified by cloning

- Identified haplotype specific tagging SNPs and identify the allele



Haplotypes	1	2	3	4	5	6	7	8
SNP82	A	A	A	C	A	A	A	A
SNP115	G	G	G	G	G	A	G	G
SNP137	G	G	G	A	G	G	G	G
SNP139	A	A	T	T	T	T	T	T
SNP162	C	C	C	C	C	T	C	C
SNP228	A	A	A	A	A	A	G	A
SNP237	T	T	C	C	C	C	C	C
SNP261	C	T	T	T	T	T	T	T
SNP268	G	G	C	C	C	C	C	C
SNP271	T	T	C	C	C	C	C	C
SNP283	T	T	G	G	G	G	G	A
SNP289	T	T	T	T	T	T	T	C
SNP292	T	T	G	G	G	G	G	G
SNP324	G	G	G	G	G	A	G	G
SNP418	A	A	A	A	C	A	A	A
SNP425	A	A	A	A	A	A	C	A
SNP438	A	A	A	G	A	A	A	A
SNP448	C	C	T	T	T	T	T	T
SNP459	G	G	C	C	C	C	C	C
SNP460	T	T	A	T	T	C	T	T
SNP470	C	C	T	T	T	T	T	T
SNP518	G	G	T	G	T	T	T	T
SNP534	C	C	C	C	A	A	C	C
SNP551	A	A	A	A	A	G	A	A
SNP568	T	T	T	T	T	T	C	T
SNP580	C	C	C	A	C	A	T	C
SNP581	G	A	A	G	A	G	G/A	A
SNP600	C	C	C	C	C	C	A	C

Concluding remarks

- Availability of genome information very helpful
  - easy to find chromosomal position and putative identity of genes
- Search for allelic versions which determine a trait can start
  - Challenge how to use this in breeding

Acknowledgements

Ronald Hutten, Herman van Eck, Hans van Os, Bart Brugmans, Jan de Boer, Theo Borm, Ralph van Berloo, Tae-Ho Park, Vivianne Vleeshouwers, Carolina Celis, Fred van Eeuwijk, Evert Jacobsen, Christian Bachem, Adillah Tan, Bjorn Kloosterman, Edwin van der Vossen, Anoma Lokossou, Anitha Kumari, Gerard van der Linden, Bjorn d'Hoop, Annemarie Wolters, Jan UitdeWilligen

And many other colleagues in WUR, EU, India, China and US

Many interesting Traits… (too many bulks)

Texture after cooking

- Enzymatic Discoloration
- PSD
- Starch Gelling Temp
- Flesh Color (Carotenoids)
- Cold sweetening (chip color)
- Amino Acids (Methionine)
- etc…

Hybridization of individual clones on the array might be more efficient

(do *In silico* bulking) ⟶

Leads to candidate genes to be analysed in varieties