Cereal Breeding

New methods and their practical use in wheat breeding

What is Plant Breeding?

1. Consciously generate variability by crossing two individuals with desired agronomic characters

2. Select the desired genotype as quickly as possible and propagate it as a homozygous strain

3. It is not about an evolutionary adaptation and not transgenetics but about genetically adapting of the crop value via crosses

What is Plant Breeding?

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Genpool (varieties and lines – genetic resources)

Phenotyping

Official test

Varieties

Seeds

Sales /turn over

Definition of breeding aims and selecting appropriate parents

Examination in a sufficient number of environments in which you can safely select for the target characteristics

Stocks maintanace and multiplication

Continuous maintenance breeding, seed production up to certified seed

Common Breeding Method for self pollinating crops

Pedigree Breeding (Combination Breeding)
relatively slow - 1 generation / year
At least 7 years up to the official BSA test

Pedigree Breeding is today the most common breeding method
Parents with desired characteristics are crossed and the
progenies are generated via the selection from the F2

Negative – takes a long time and success is often luck based on a lot of unknown factores like:

- Selected parents and their combination ability
- size of the progeny we select from must be high
- General field condtions to visible the breeding aims

A combintion with other methods is desirable and necessary

Methods to Speed up the Generation Cycles

SSD (Single Seed Descent)

Relative expensive (requires air-conditioned greenhouses and vernalization rooms)

fast

Up to 3 generations per year (14 months)

1 to 2 years faster than pedigree breeding

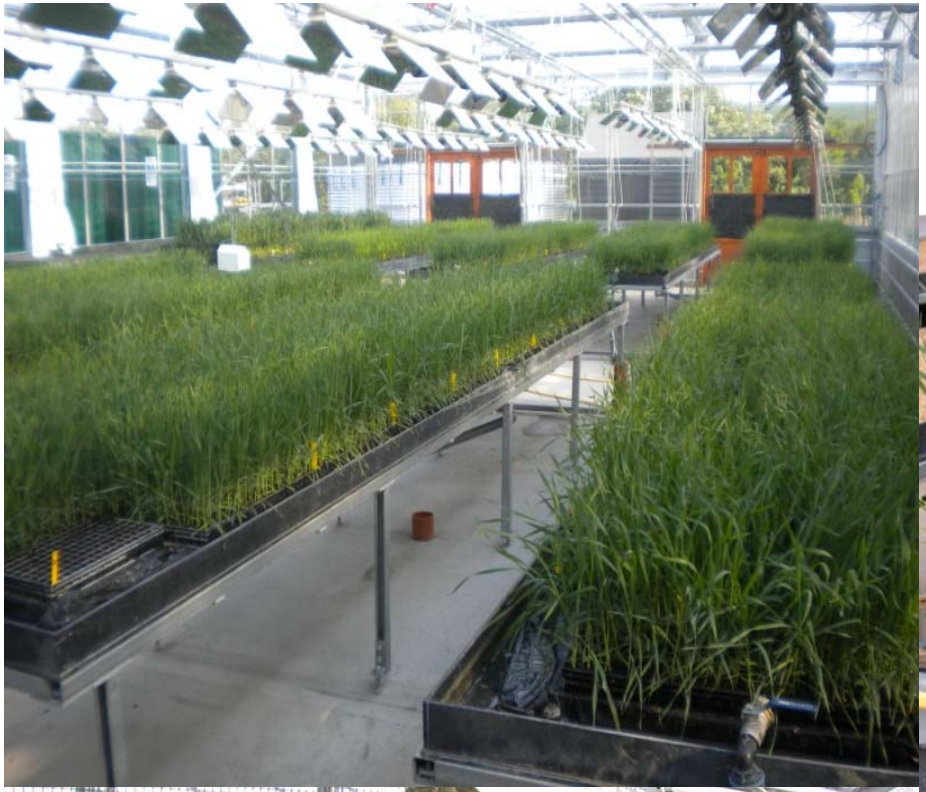
DH (double haploids)

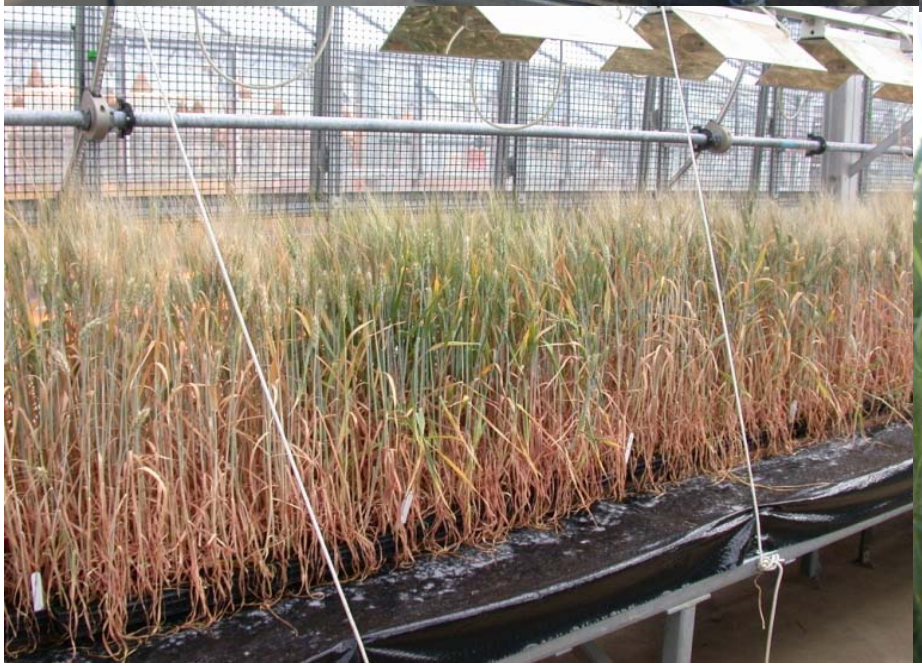
expensive (climate chamber, laboratory for tissue culture)

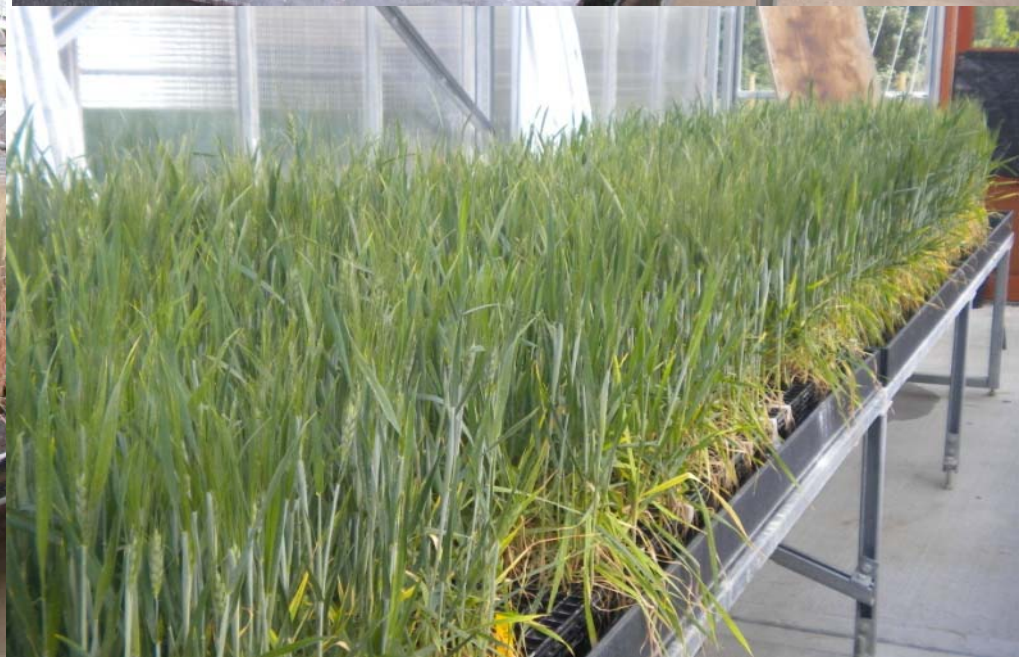
fastest method

Homozygosity in one cycle

Up to 3 years faster than pedigree breeding







Accelerating Breeding Process

	years from crossing to: official application	registration
normal Pedigree (RAGT)	7	10
Single Seed Descent (SSD)	6	9
accelarate SSD	5	8
Double Haploid	4-5	7-8

- Comparatively fast

  Example from SSD: variety Intro, approval 2011 was crossed in 2002

  But no speed at any price.

  lack of field selection in DH lines or fast SSD

- We need more efficient selections by molecular markers

Marker technology development

1990s	2000s	2009-	2014-
RFLPs (Restriction fragment length polymorphism)	SSRs (simple seqence repeat)	KASP-SNP (Single nucleotide polymorphism- competitive allele specific)	SNP Arrays (chip SNP)
1-10 of dp / line	Tens of dps / line	Hundreds of dps / line	Thousands of dps / line
Needs lots of HQ DNA	PCR – less DNA (polimerase chain reaction)	Fluorescent PCR – even less DNA	Moderate DNA quantity and quality

Molecular Markers 2

Genotyping characters with molecular markers

Clearly identifiable DNA segments, so-called marker genes, are always linked to the target genes

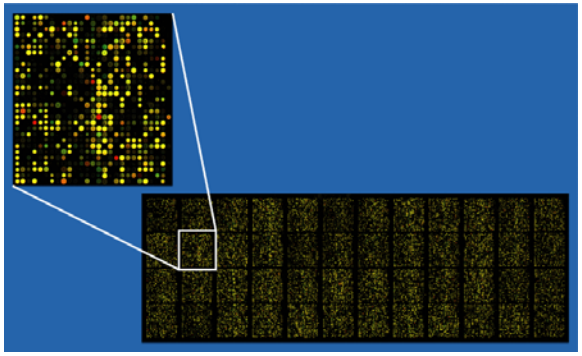
Features such as GR, BR, Fusarium soil-borne viruses, OBM, PCH1 eye spot, etc.

For difficultly inherited traits, the hit rate is currently just 10-20%

Development of the technology

DNA chip technology

e.g. 90K Infinium iSelect SNP chip



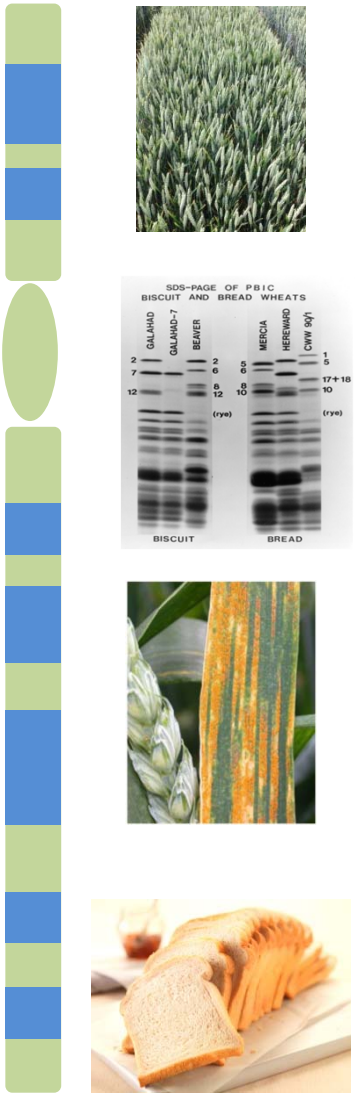
Marker assisted selection (MAS)

Capture of simple individual features (monogene) and

Quantitative features (QTL's) like yield, baking quality or Fusarium resistance

Genotyping analysis

Genome

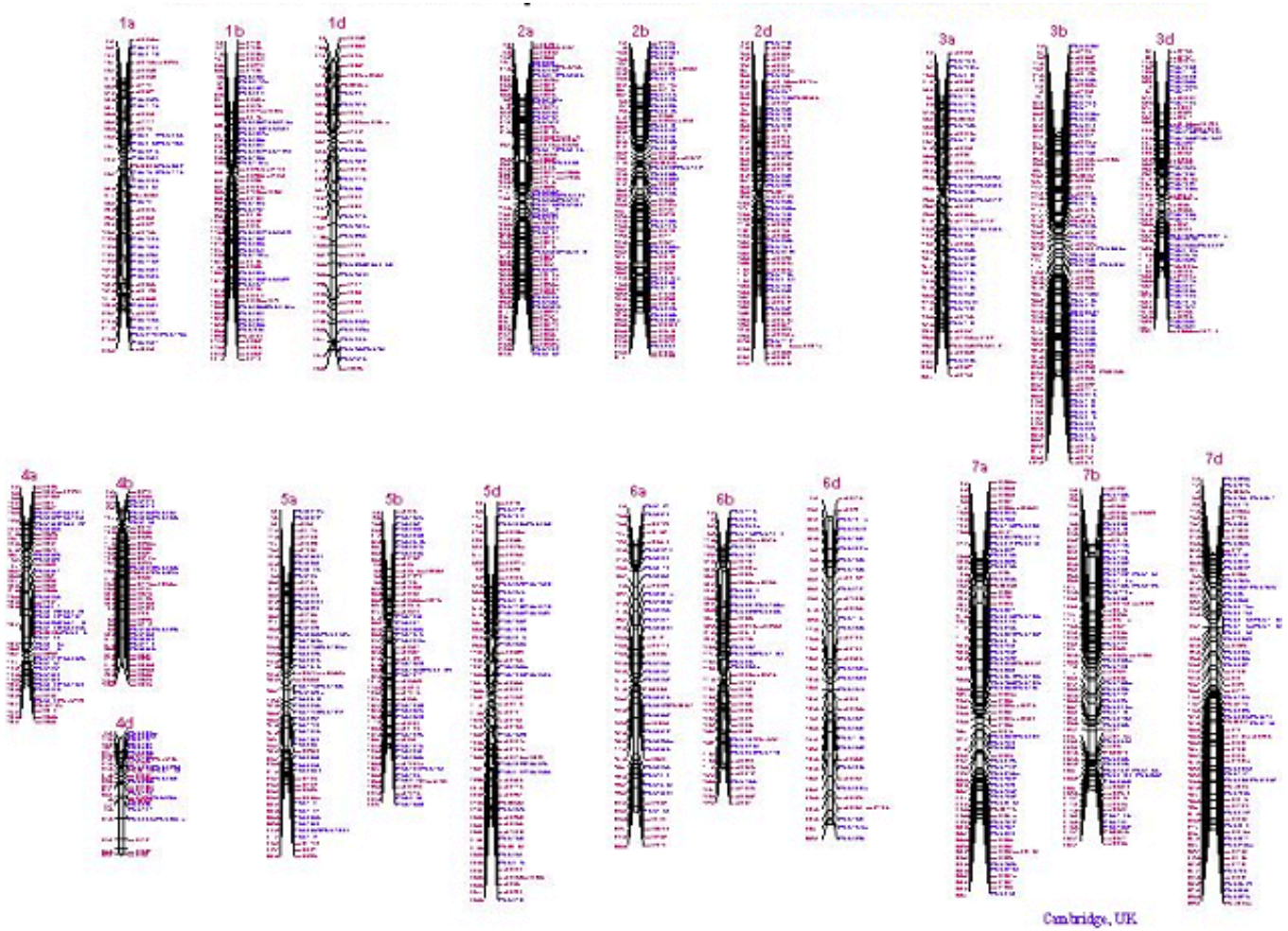


Molecular marker map

Traits

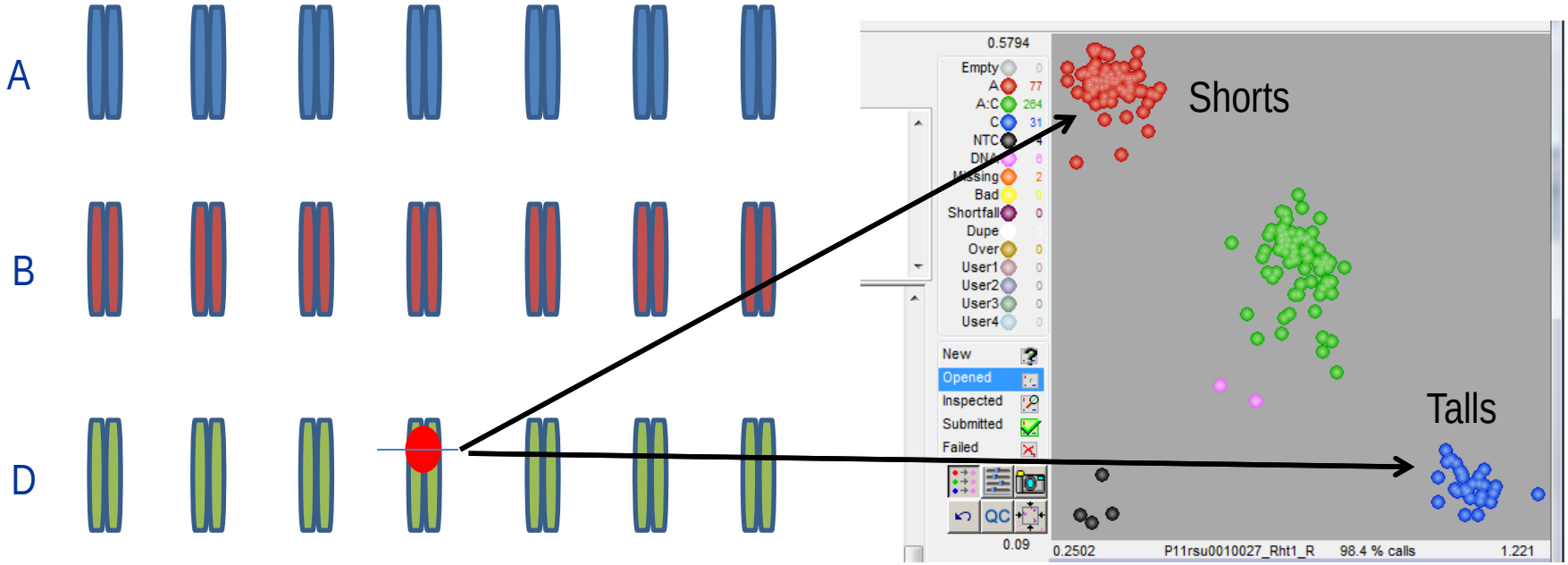
- Yield
- Height
- Disease
- Yield
- Quality
- Disease
- Quality
- Yield
- Maturity
- Disease

Genotyping analysis



Practical use -Marker Assisted Selection

- Use DNA markers to tag individual genes
- For example: height gene



Reduced Hight Genes Rht 1 und 2

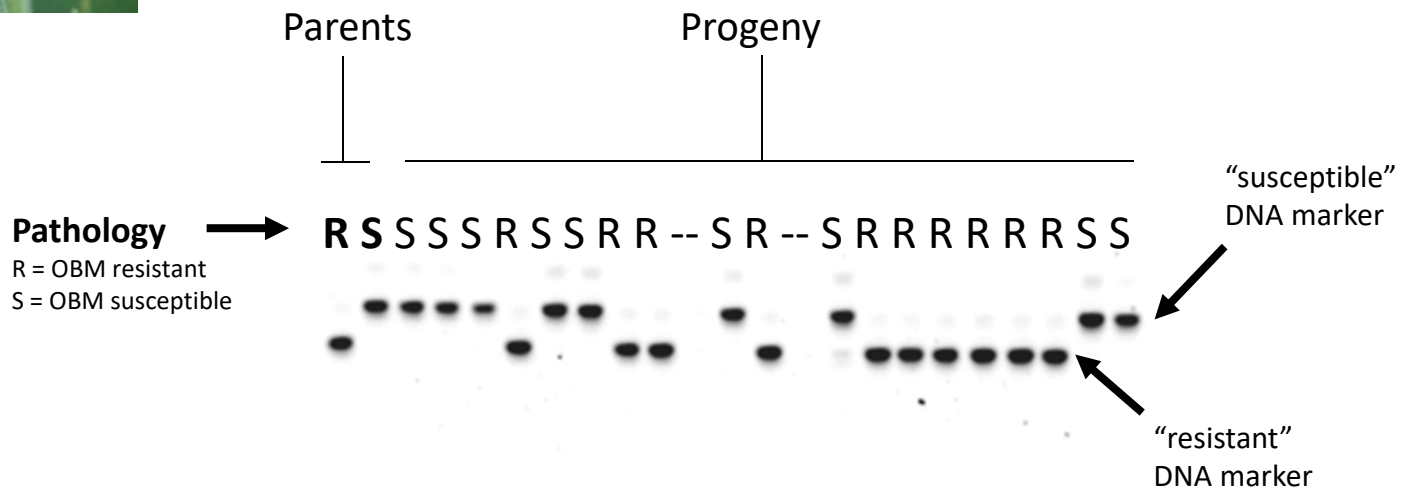
Phenotypes of *Rht-B1* (Rht1) and *Rht-D1* (Rht2) dwarfing alleles in NILs. Wheat NILs (var Mercia) were grown to maturity.
The photograph is from the John Innes Centre archives (produced by Tony Worland).

- Increased harvest index: up to 50% of the total mass is grains
- Increased grain count / spike
- But initially disadvantages:
  Poor grain filling
  Increased susceptibility to fusarium
  (HFN weakness?)
  (Frost resistance?)

Long-term breeding work could overcome these negative correlations

Resistance to Orange Blossom Midge (OBM)

Applied to genes of major effect that have a difficult phenotype to measure e.g. Resistance to Orange Blossom Midge (OBM)+



BUT WHAT ABOUT MULTIGENE RELATED FEATURES?

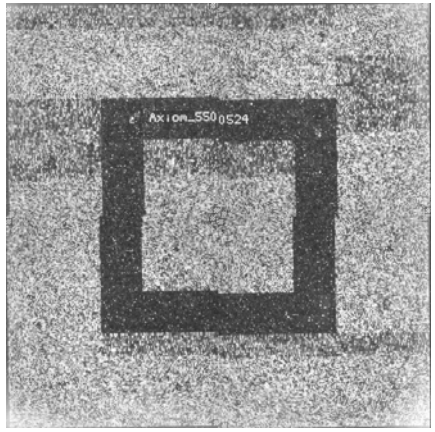
Genomic selection

The genomic selection promises a precise statement in the selection of features through the knowledge of gene interactions in complex inheritances.

1. Genomic selection
   Detection of complex features, such as
   e.g. Yield based on a variety of genes
   with a variety of markers
2. Allocation of the data volume
   with certain computer programs
   according to certain algorithms
3. Practical breeding
   Definition and selection of crossing partners
4. Paradigm change in breeding

Genomic Selection

Whole genome profiles



Performance data from multi-site field trials

Statistical models

Potentially predict yield, resistance and quality for any line based on genome profile

The SNP Revolution...

It is possible to identify genetic variation and associations to phenotypes with SNP's (single nucleotide polymorphism) !

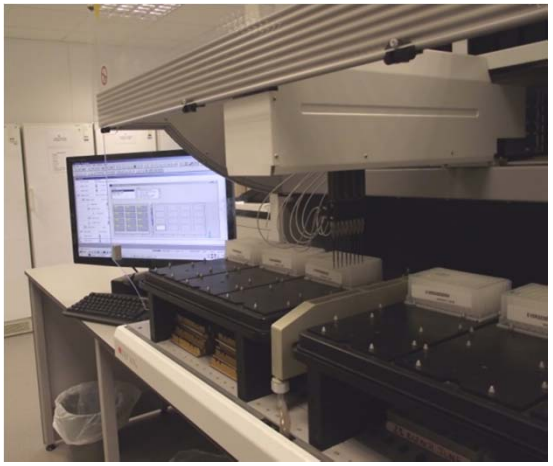
SNPs are abundant and cheap...

Provide possibilities for high throughput screening

Increases the numbers of traits that can be tracked at early generations

Increases possibilities for dissecting complex traits conferred by multiple QTL and understanding how these QTL function...

Genomic selections -Automation is essential

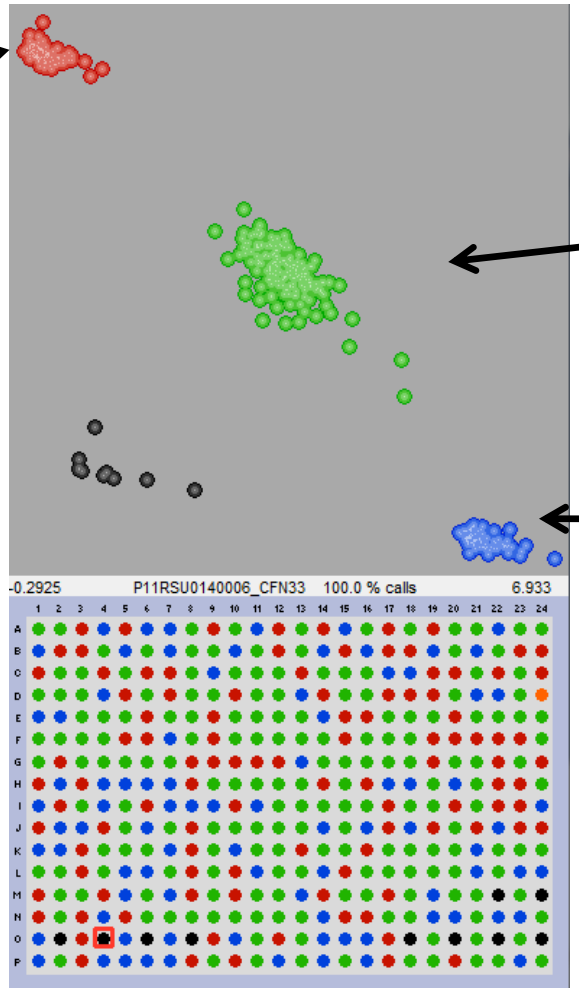


DNA Extaction → Liquid handling → High-throughput PCR → Allele calling

Practical use: SNP Selection for Yield

•Identifying lines with positive version (allele) of yield gene

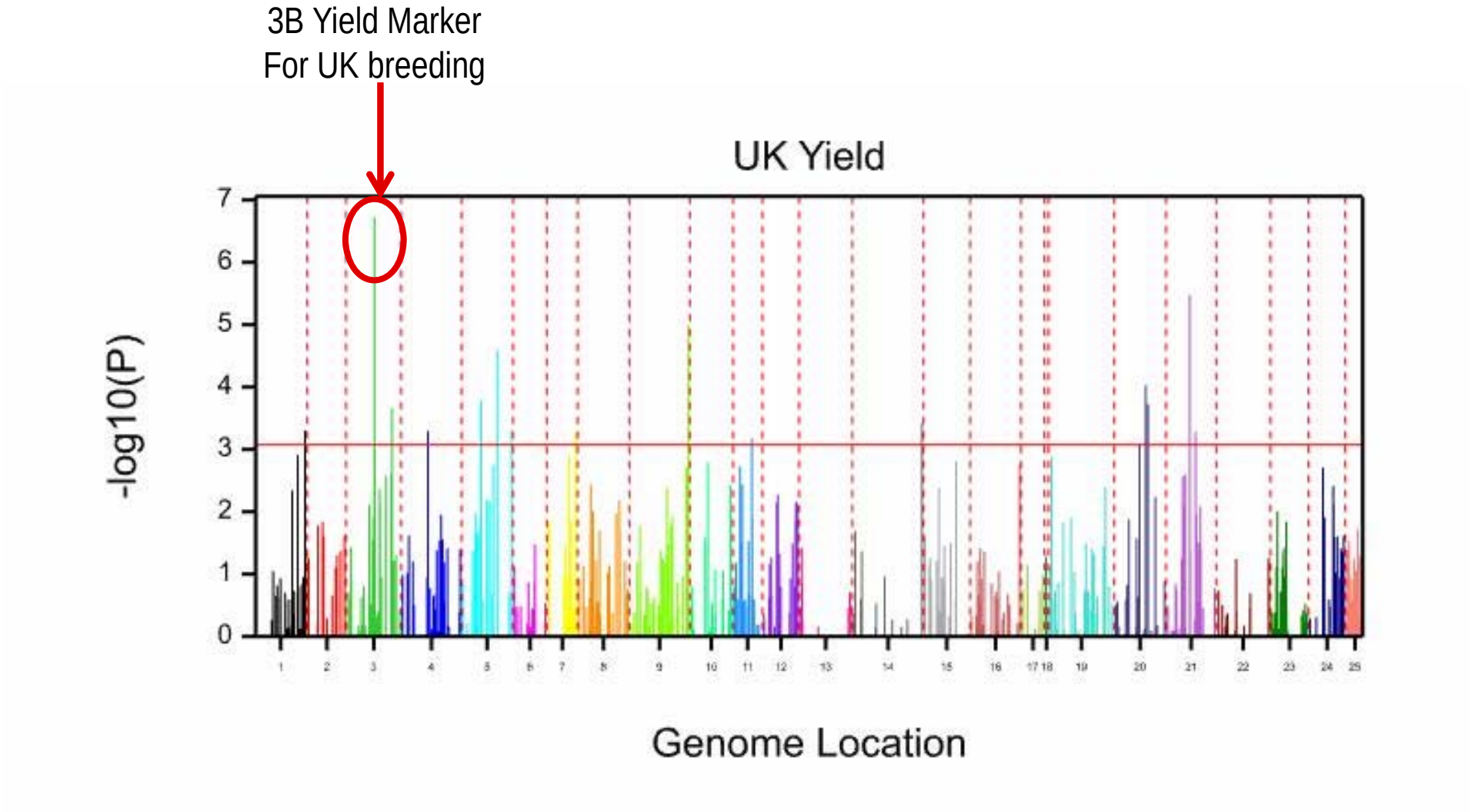
Positive Yield Gene
- Keep



Both genes
-Keep & select
in future generation

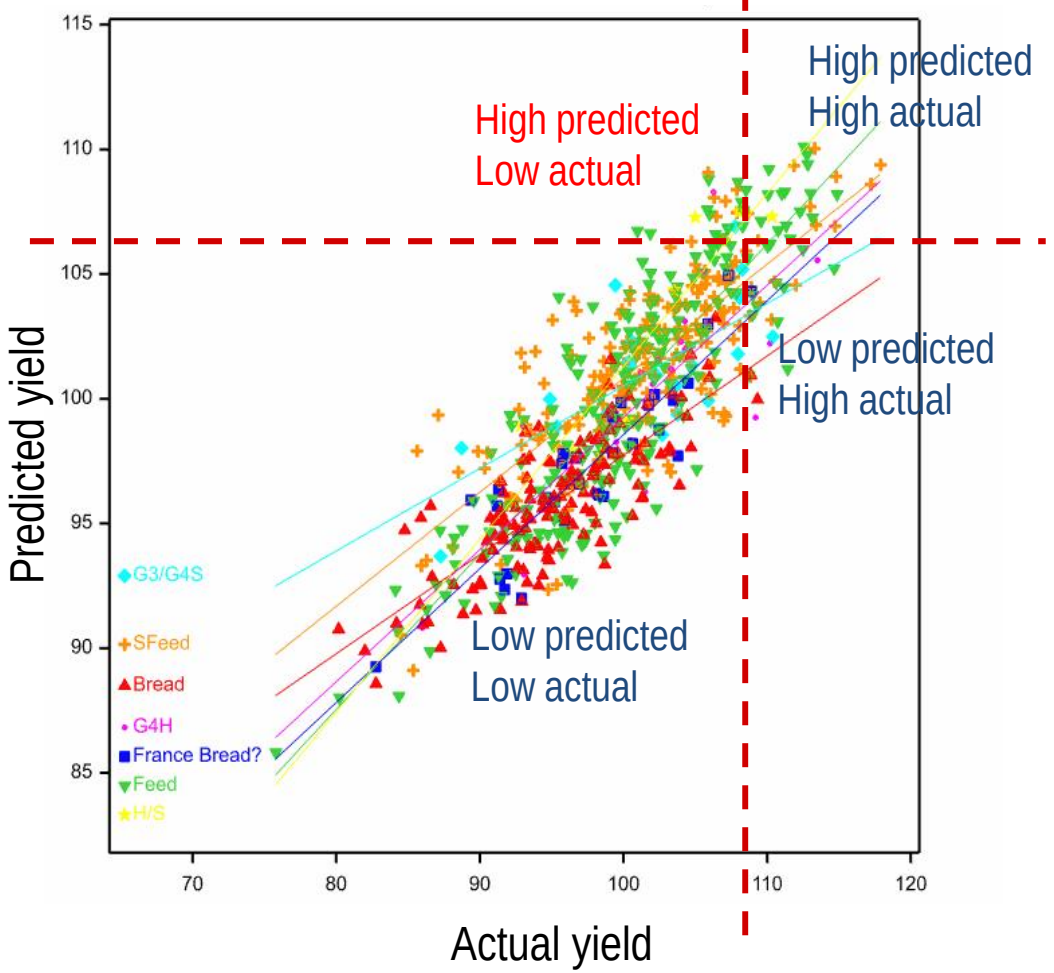
Negative Yield Gene
- Discard

Genome Wide Association Studies (GWAS)



Genomic selection can speed up plant breeding

Genomic selection pilot and future promise



Sourcing useful genes

1. Land races or varieties from around the world
   e.g. Sumai 3 - Chinese Landrace with Fusarium resistance
2. Material from crosses with closely related species
   e.g. with Thinopyrum intermedium (research project with JKI Quedlinburg)
3. Synthetic wheat
   Crossing of e.g. Triticum turgidum (AABB) with T.tauschii (DD)
4. CIMMYT Material (Mexico)
   world-wide center for wheat breeding for the third World
   greatest genetic diversity
5. Natural or induced mutations
6. Prebreeding (PD breeding); Parental Development




Sourcing useful genes

Exotic parent
with known trait of interest

x Elite adapted parent

F1 x Elite adapted parent

F1 BC1 x Elite adapted parent

F1 BC x Elite adapted parent

VALIDATION

Adaptation
Trait value
Yield impact
Genetic linkage

Resistance to Eye Spot (Oculimacula ssp.) by PCH1 und PCH2

Aegilops ventricosa

Backcrossing and MAS

Aegilops kotschyi

PCH1 +PCH2 Resistance Genes

First record in 60ties
1987 var. Rendezvous
Clarus 2002, Akzento 2005,
Linus 2010, Rebell 2013

Hybrids in Wheat

1. >10% additional yield possible, heterotic groups necessary

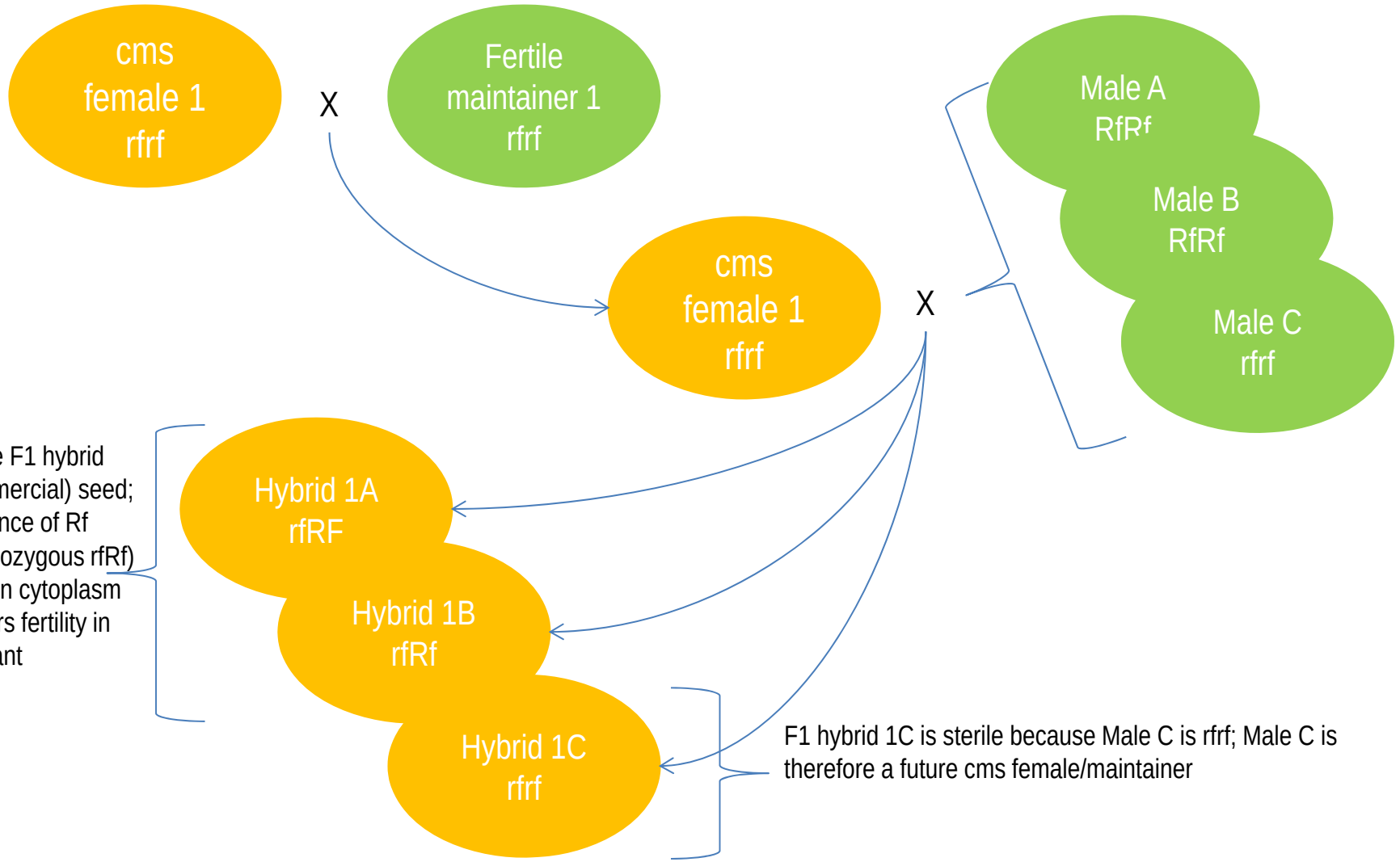
2. Practical hybridisation system not yet developed
   gametocides
   CMS Processes like rye and barley
   Hybrids in 5 - 10 years on the market

3. Agricultural practice
   Economy: Extra yield - seed price
   Safety of seed production

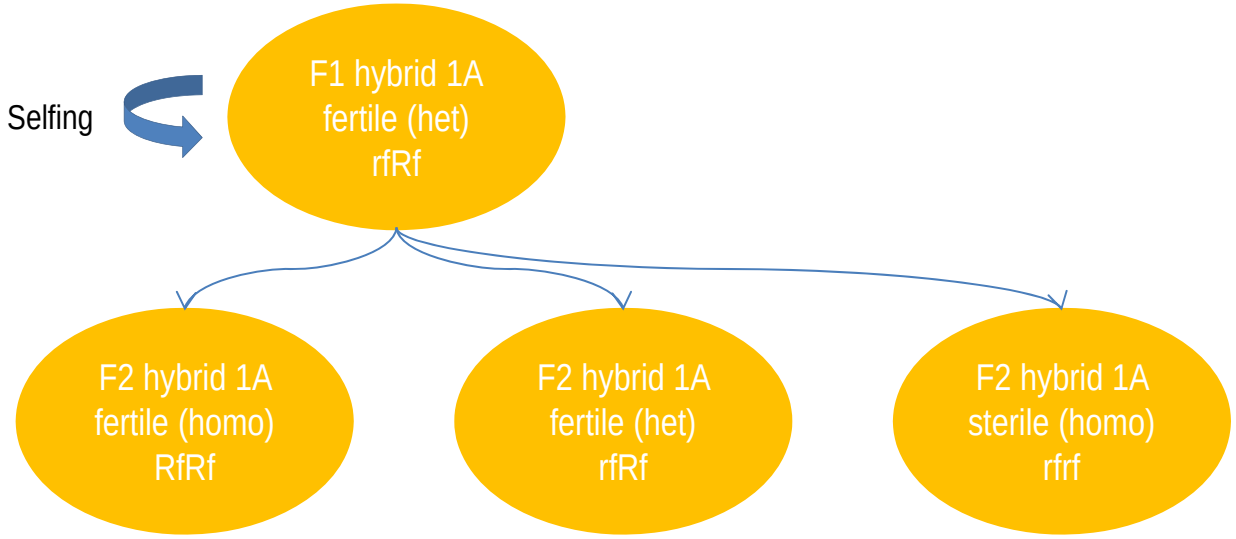
4. Safety of the restoration (fertile ears) in the cultivation, dominant restorer gene of the pollen donor (male)

5. Research project 'Breeding value' ('ProWeizen' initiative)
   RAGT involved

HYBRID WHEAT – DEVELOPMENT OF CMS

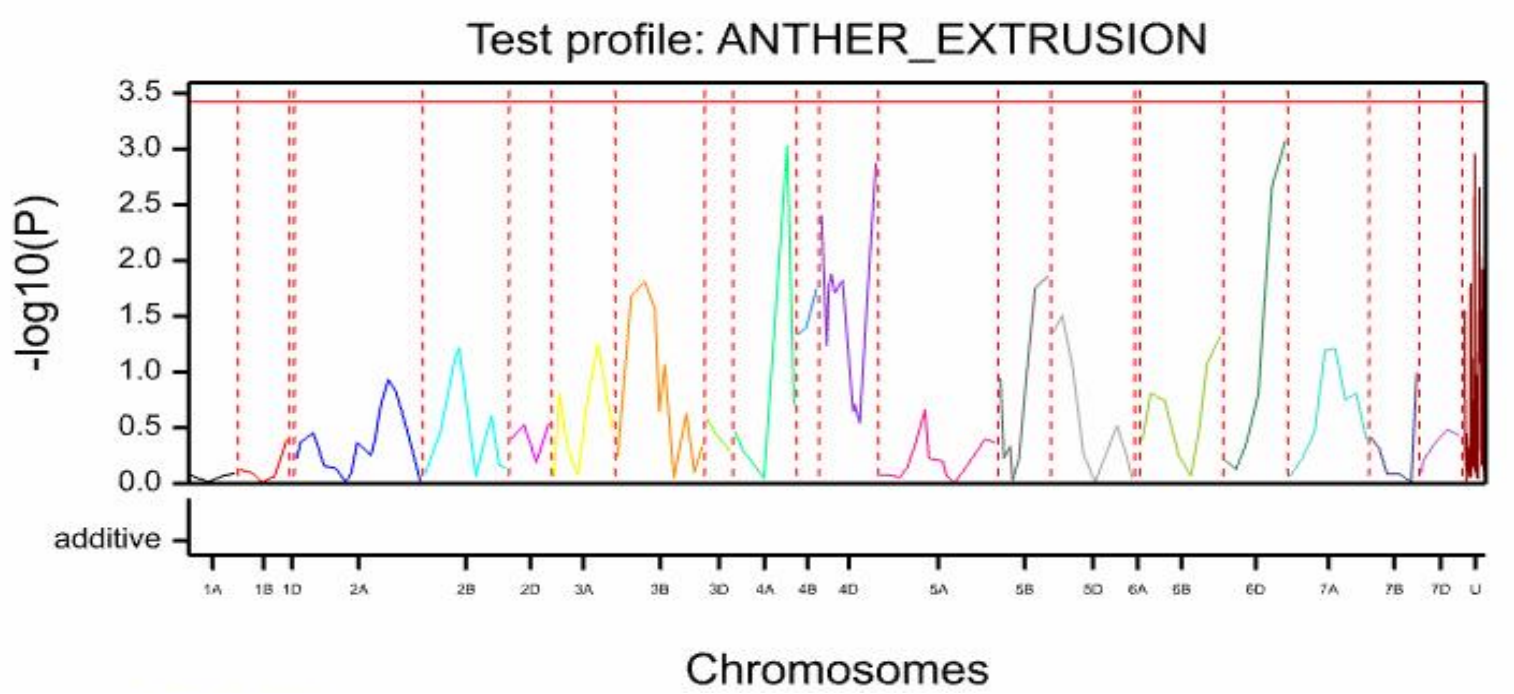


HYBRID WHEAT – DEVELOPMENT OF CMS



HYBRID WHEAT – IMPROVING MALES

- Anther extrusion and duration of pollen shed are important phenotypes for hybrid males
  - QTL discovery for these traits underway

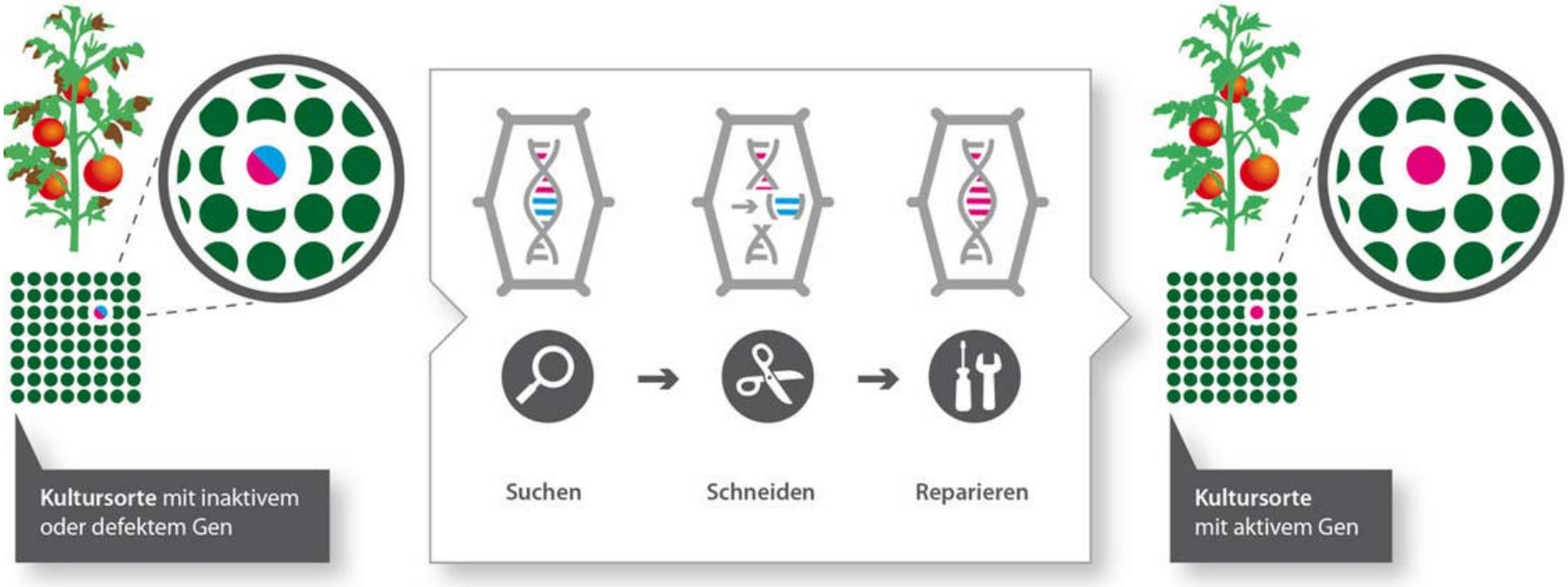


HYBRID WHEAT – IMPROVING MALES

- Diversity in males potentially limiting
  - Can we discover ‘diverse’ but competitve male lines?
  - How to improve the anthere extrusion
  - Find males with good restauration ability

CRISPR/CAS

Genome Editing



CRISPR/CAS

PANORAMA | CRISPR/Cas

Neuer Turbo für die Züchtung

Wie bei einem chirurgischen Eingriff können Pflanzenzüchter mithilfe der neuen Technologie CRISPR/Cas gezielt die DNS verändern. Dirk Schenke und Daguang Cai erklären, wie das funktioniert und wie sie als Forscher die neuen Möglichkeiten nutzen.

Eine wachsende Weltbevölkerung, die sich abwechslungsreich ernähren will, die abnehmende Flächenverfügbarkeit sowie die zunehmenden Probleme durch Klimaveränderungen: Die Anforderungen an die Landwirtschaft steigen. Dabei stoßen wir mit unseren Anbausystemen mehr und mehr an Grenzen. Um die enormen Herausforderungen zu meistern, werden unter anderem große Hoffnungen in die Pflanzenzüchtung gesetzt. Große Fortschritte versprechen dabei neue Züchtungstechniken wie CRISPR/Cas, mit denen man schnell und kostengünstig gezielte Veränderungen im Erbgut vornehmen kann.

In der konventionellen Züchtung wird versucht, mittels Strahlung oder chemischer Mutagenese eine gewünschte Veränderung in der Pflanze herbeizuführen. Nur ist dieser Ansatz leider nicht effizient. Man kann diese grobe Methode quasi mit einem Schrotschuss vergleichen, der zufällig irgendwo in den Genen der Pflanze Schaden anrichtet. Das bedeutet: Falls eine Pflanze durch diese Behandlung weniger anfällig für eine Krankheit sein sollte, so kennt man nicht sofort den Grund dafür. Und diesen kann man auch nur mit großem Aufwand herausfinden, weil es an sehr vielen Stellen im Erbgut der Pflanze zu Veränderungen kommt. Und das ist dann das eigentliche Problem: die vielen ungewollten Veränderungen (»Kollateralschäden«) bewirken unerwünschte Nebeneffekte wie z. B. geringere Erträge oder schlechtere Qualitäten. Das Produkt ist eben rein zufällig und nicht zielgerichtet entstanden.

Erschwerend kommt hinzu, dass in höheren Lebewesen jedes Gen mindestens in zwei Kopien vorliegt und es recht unwahrscheinlich ist, dass beide Kopien durch die Mutation verändert werden.

Eine neue Ära. Vor kaum vier Jahren entdeckte ein Team um die französische Molekularbiologin Prof. Emmanuelle Charpentier sowie die Amerikanerin Prof. Jennifer Doudna, dass sich ein Mechanismus des bakteriellen Immunsystems auch in höheren Organismen eignet, um ganz

This method opens unlimited possibilities but needs more knowlege

CRISPR/Cas ist vor allem deshalb so attraktiv für Züchter, weil die Technik schnell, effizient und kostengünstig ist.

- The procedure has to be compared with advanced pedigree breeding and not with mutation breeding
- Which gene acts as and in which place in the genome is still unknown
- It's no transgenic method (not clear yet)
- How is it turned on or off
- It's quick efficient and cheap
- Method is one of many possibilities

DLG Nachrichten 1/2017,
Dr.Dirk Schenke; Prof. Dr.Dagunag Cai

13th July 2012

R·A·G·T

R·A·G·T SEMENCES

European multi-species seed company

RAGT Cereal Breeding

European cereal breeding programme sites

- Supported from Ickleton site
- Quality screening
- Molecular marker screening
- Disease screening
- Glasshouses production
- Stock production

Ickleton (GB)

Premesques

Louville-la-Chenard

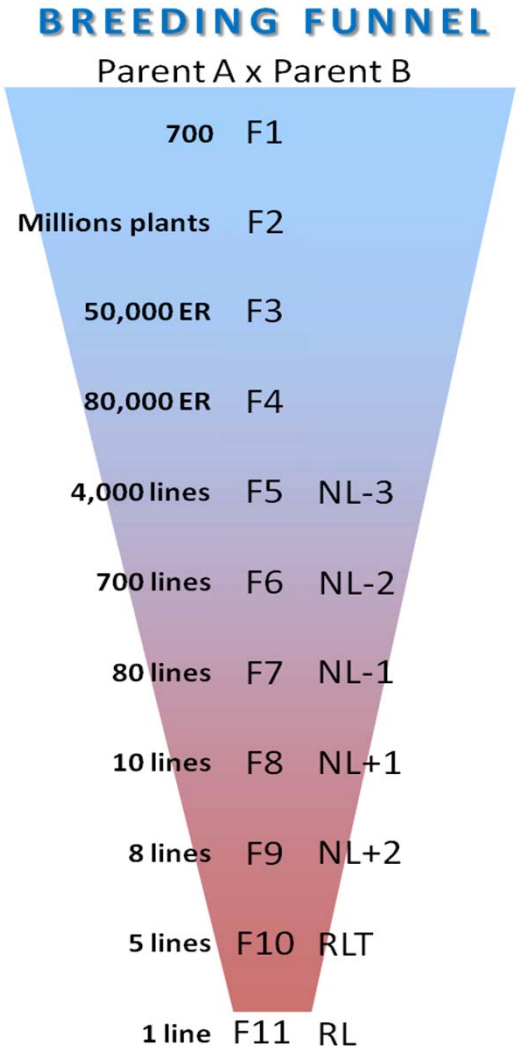
Montbartier

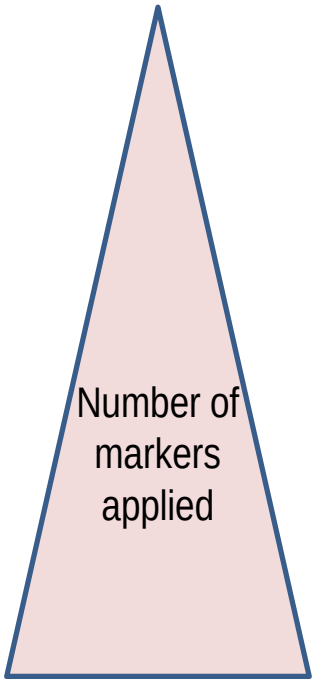
Druelle

Silstedt (Germany)

Branisovice (Czech Republic)

Marker Assistent Breeding in the Process





Major gene enrichment: Disease resistances

Accurate parent characterisation: major genes and QTL

Genetical Backgrounds of our actual WW varieties

variety	Rht-Gen	Ppd-Gen	Frost	VPM-res	Fusarium	Virus	Quality
	drawing	Day length	winterhardiness	Eye spot	FHB	Soil born virus	Backing quality
Boregar	+	-	0+	+	+	-	A
RGT Reform	0+	+	+		+	-	A
Meister	0	+	0+		+	-	A
Linus	+	+	+	+	0	-	A
Rebell	+	-	0	+	+	+	A
RGT Aktion	+	+	++	+	0	-	A

Future prospectives in RAGT Wheat Breeding

1. Acceleration of the breeding process
2. Use of new genetic resources
   - for the new development of potential crossing parents
   - Resistance sources from Aegilops, Thinopyrum, etc.
   - Yields from Synthetics (NIAB, CIMMYT)
3. Marker assisted selection
   - Molecular markers,
4. New methods of genomic selection
   - chip technology
   - 90,000 markers per genotype
   - Allocation with corresponding algorithms
5. Genome editing
   - CRISPR / CAS
6. Hybrid cultivation
   - 10% additional yield
   - New hybridization systems


Thank You for Your Attention