



Biodieselanlage



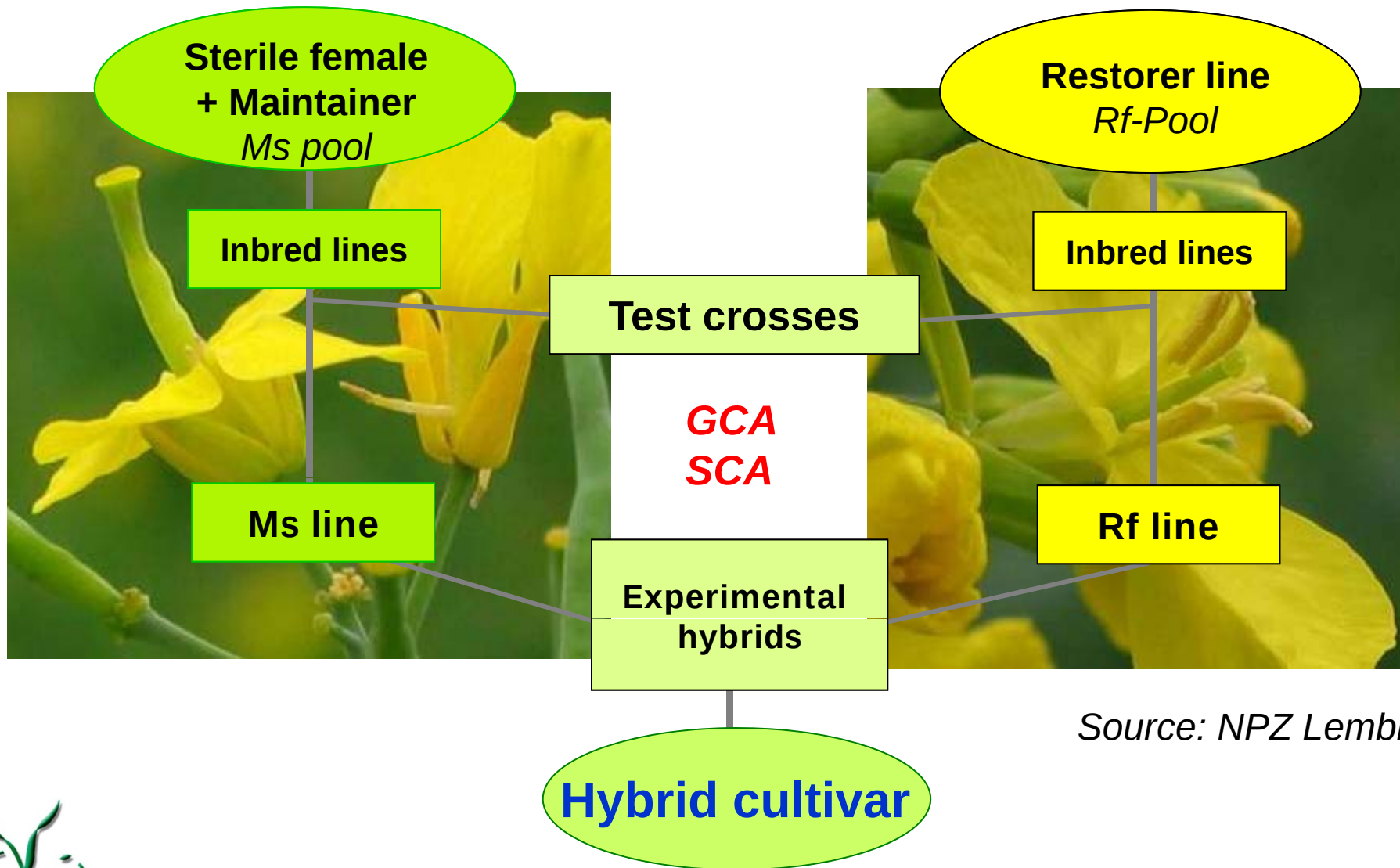
Advances in Biotech- and Marker-Assisted Breeding of Oilseed Rape (*Brassica napus*)



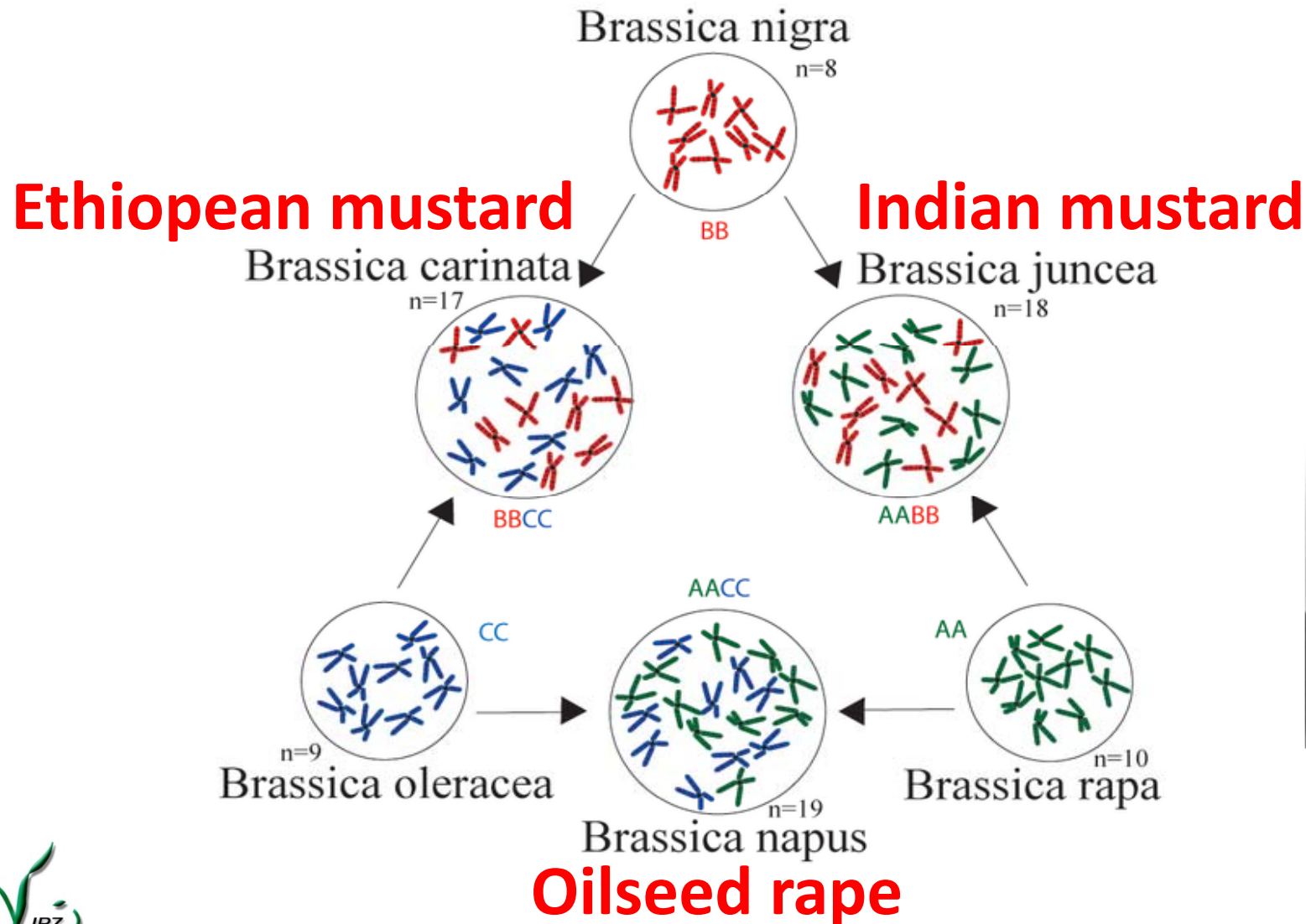
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Hybrid breeding scheme for rapeseed



Creating diversity: Natural allopolyploids by hybridization and speciation in Brassicaceae



Woo Jang-Choon
1898 -1959

Doubled haploids of novel trigenomic Brassica derived from various interspecific crosses

X. X. Geng et al. (2013) Plant Cell Tiss Organ Culture

unique interspecific crosses:

- *Brassica rapa* (genome AA) x *B. carinata* (BBCC)
- *B. nigra* (BB) x *B. napus* (AACC),
- complex cross between *B. juncea* (AABB), *B. napus* and *B. carinata*, relatively stable chromosome number ($2n=54$)



Ploidy level in microspore-derived plants

by flow cytometry based on DNA content of controls with known DNA content. Plants were classified as hexaploid (6x), triploid (3x) mixed, or unknown, and doubling frequency (%) is the proportion of hexaploids in the total (Geng X.X. et al. 2013)

Hybrid code	Hexa-ploid	Triploid	Mixed	Un-known	Total	Doubling frequency (%)
H08-1	23	16	4	11	54	42.6
H11-2	370	298	6	19	693	53.4
H16-1	241	185	3	7	436	55.3
H24-1	21	19	2	14	56	37.5



Broadening genetic variation via „Resynthesis“ of rapeseed



<https://www.google.de/search?q=cabbage>

B. oleracea
 $2n=2x=18$

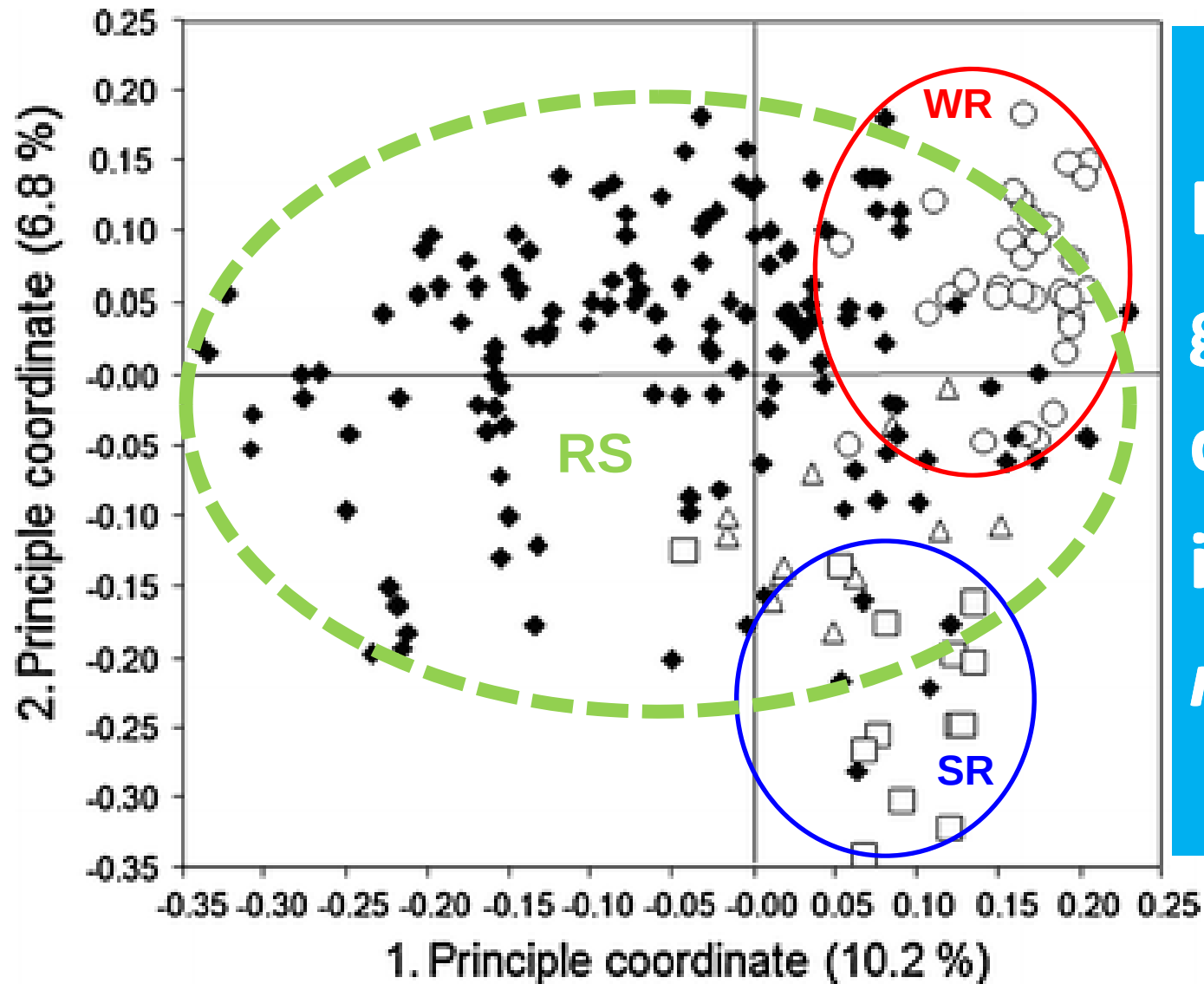
X



<http://international.stockfood.com>

B. rapa
 $2n=2x=20$

Brassica napus
 $2n=4x=38$



Extending
genetic
diversity
in *Brassica
napus*

Principle coordinate analysis of 142 resynthesized lines and 57 *B. napus* L.
genotypes of European and Asian origin. Symbols are for winter (circle), for spring-type (square),
for Asian *B. napus* (triangle) and for resynthesized lines (filled diamond);

Girke et al., GRACE (2012)

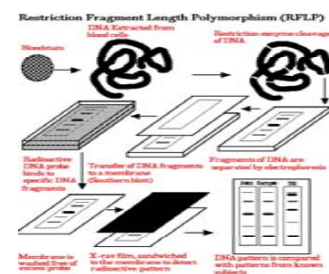


New marker types for high-throughput MAS

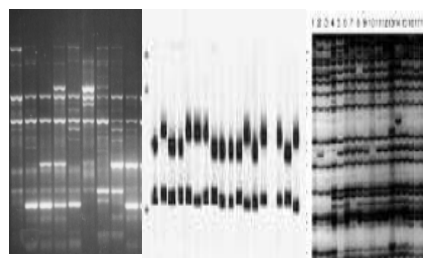
RFLP Restriction fragment length polymorphism
 RAPD Random amplification of polymorphic DNA
 SSR Simple sequence repeats (microsatellite markers)
 AFLP Amplified fragment length polymorphism

DArT Diversity arrays technology
 SNP Chips e.g. Infinium Bead Chips, Affymetrix GeneChips
 RAD Restriction-site associated DNA
 GBS Genotyping-By-Sequencing

**Single nucleotide
polymorphism (SNP)
markers**



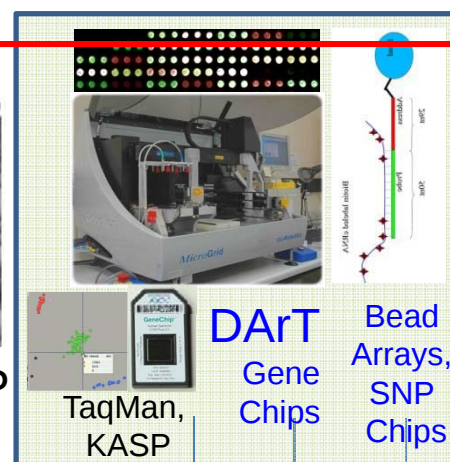
RFLP



RAPD

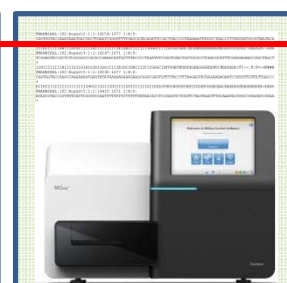
SSR

AFLP



DArT
Gene
Chips

Bead
Arrays,
SNP
Chips



RAD GBS

1st generation

2nd generation

3rd generation

4th generation

1974

Hybridization of
labeled probe
to size-separated
restricted DNA

Hybridization-based,
gel-based

1990

PCR-based,
gel-based

1992

1995

2002

PCR-based,
gel-free
(fluorescence)

2004

Genotyping
by
hybridization

2006

2008

Genotyping
by
sequencing

2011

Library cloning

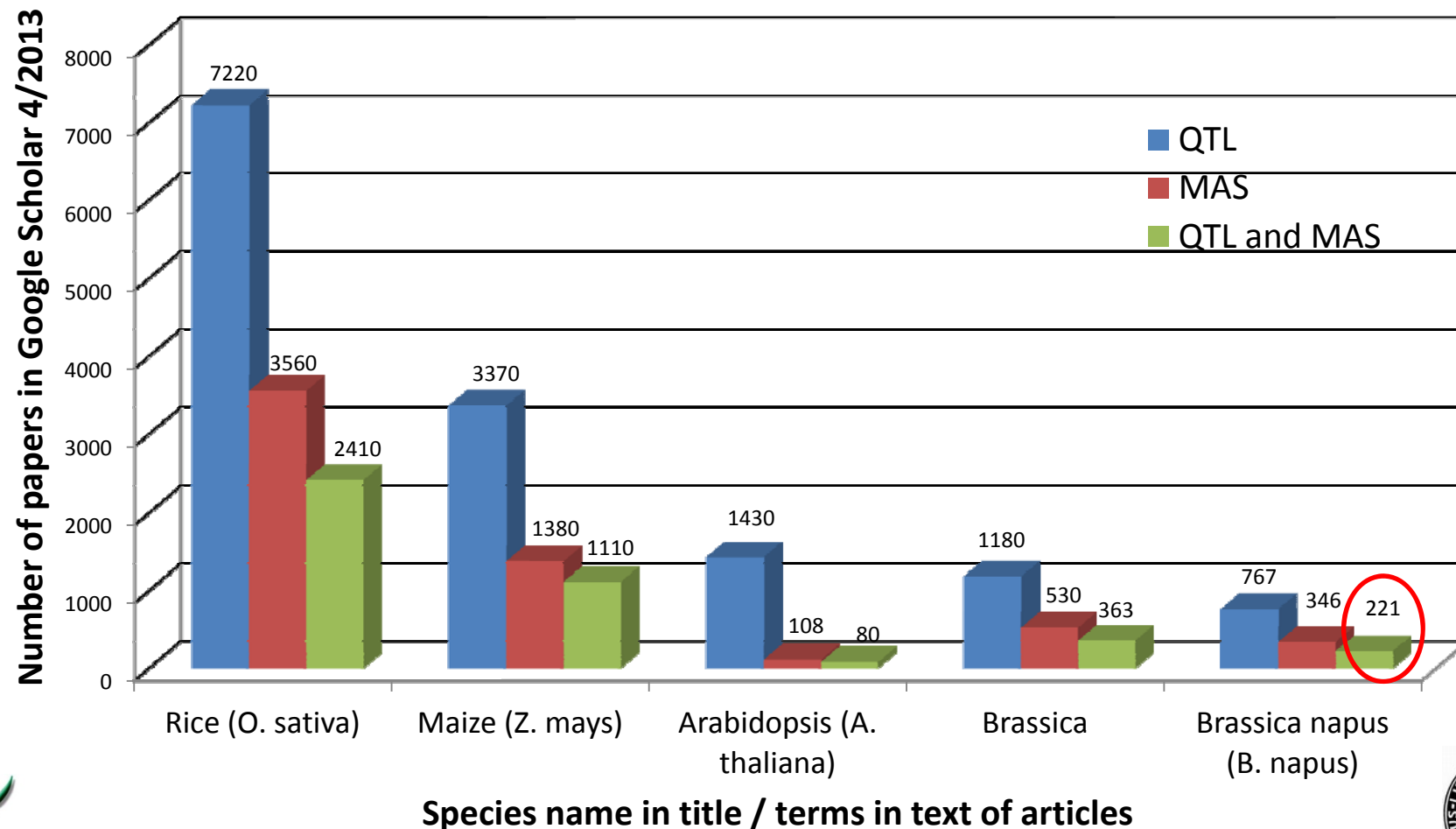
(probe design from sequences required)

Complex data
processing required

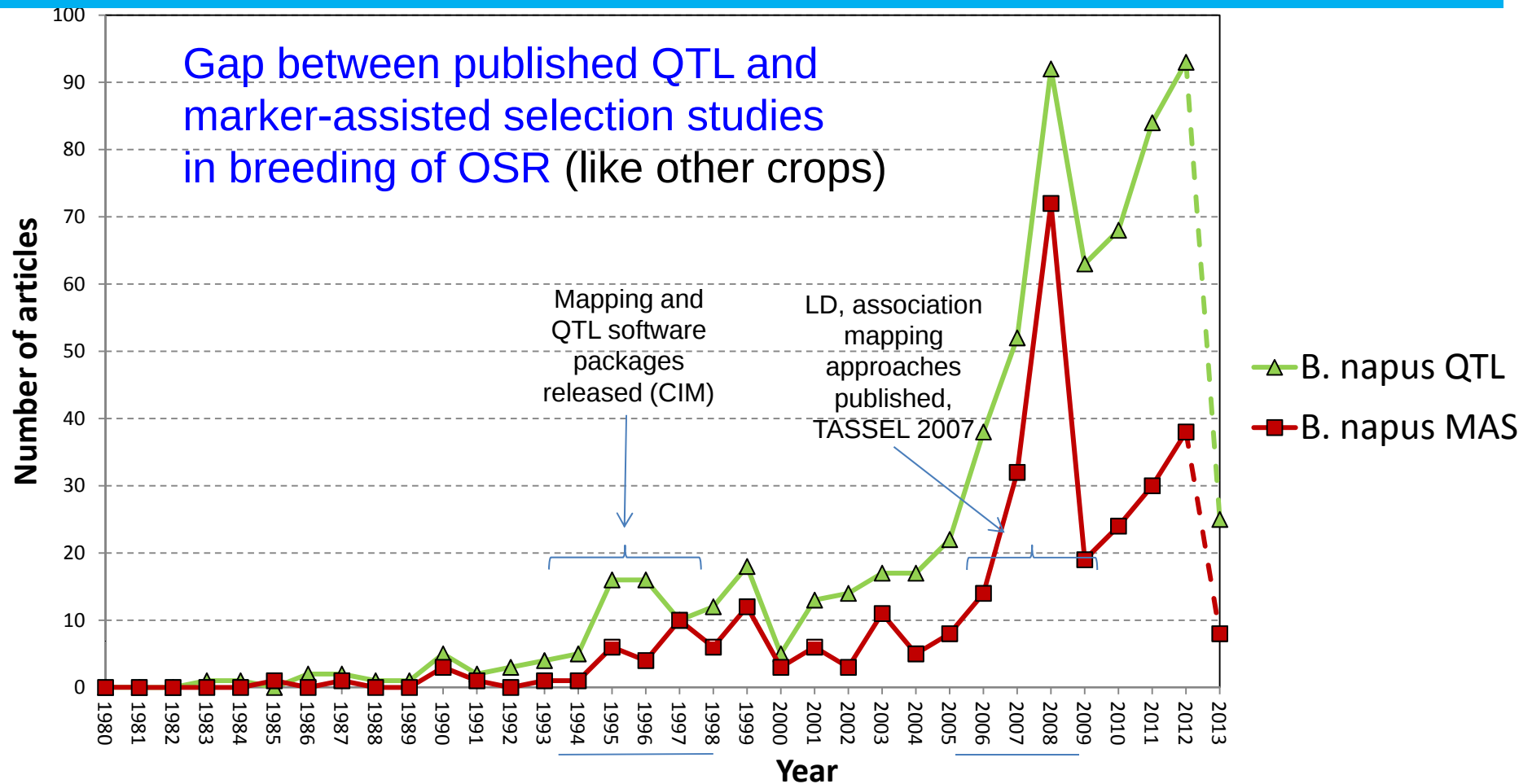


From QTL mapping to marker-assisted selection (MAS) in OSR breeding

Papers listed in Google Scholar: 10 April 2013. Note: Google Scholar indexes papers, not journals. It includes journal and conference papers, theses and dissertations, academic books, pre-prints, abstracts, technical reports and other scholarly literature from all broad areas of research and thus includes doubles and overestimates the relevant literature.



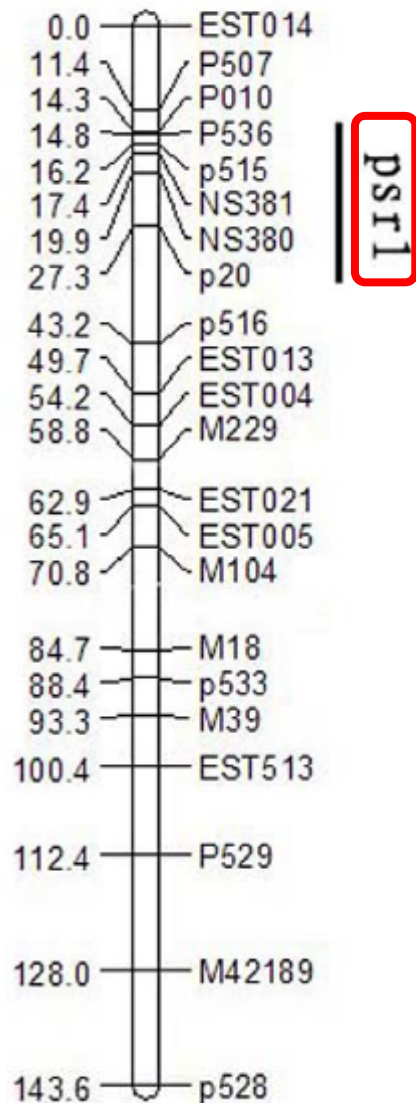
From QTL mapping to marker-assisted selection (MAS) in OSR breeding



Numbers of articles with the terms "*Brassica napus*" in title and "quantitative trait locus" or "quantitative trait loci" (QTL) and "marker-assisted selection" (MAS) or similar **somewhere within the document** (excluding citations) by years (1980–2013) from **Google Scholar** (9 April 2013).



SNP Discovery in Rapeseed: Pod Shatter-Resistant Associated SNPs



Genetic linkage map of chromosome A09 from rapeseed.

The numerals in left column indicate the genetic distance [cM]. SNP or SSR marker names are indicated in the right column. **Psr1** indicates the QTL region of pod shatter-resistance.*)

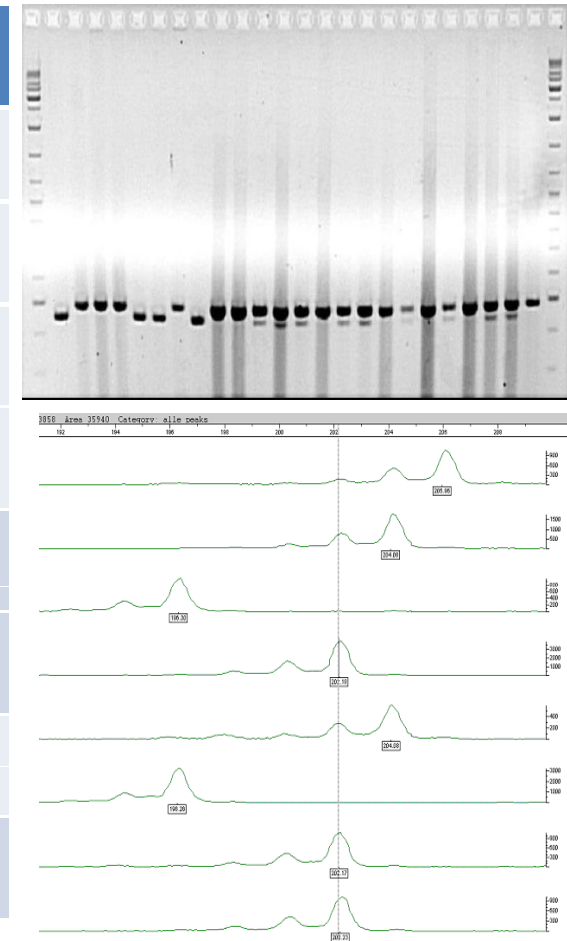
*) genetic contribution rate 47%.

Hu Z et al. (2012) PLoS ONE 7(4): e34253.



Major OSR traits for which molecular markers are currently used by (German) Breeders

Trait of interest	Type of marker
Genetic distance	SSR, SNP, DArT
Clearfield® HT	SNP
MSL hybrid system	InDel, SNP, SSR
Ogu-Rf hybrid system	SNP, InDel, SSR
Dwarf growth type	SNP
High oleic/Low linolenic	SNP
Phoma resistance	SSR, InDel
Clubroot resistance	SSR



➔ Markers for quantitative traits to be used for MAS



QTL mapping of resistance to fungal diseases

Pathogen	Brassica species	Consensus QTL (number of populations)
<i>Leptosphaeria maculans</i> (Phoma stem canker, blackleg)	<i>B. napus</i> <i>B. napus</i> <i>B. napus</i> <i>B. napus</i>	A7, A2, A10 (race-specific) A4 (race non-specific) A1 (4 of 5), A10 (3 of 5), C1 (2 of 4) A10
<i>Plasmodiophora brassicae</i> (clubroot)	<i>B. rapa</i> <i>B. rapa</i> <i>B. napus</i> <i>B. oleracea</i>	A1, A6, A8 A2, A3 A2, A3, A8, C3, C5, C3,C5
<i>Albugo candida</i> (white rust)	<i>B. napus</i> <i>B. rapa</i>	A2 (2 of 2) A4 (race non-specific), A2 (race-sp.)
<i>Sclerotinia sclerotiorum</i> (Sclerotinia rot)	<i>B. napus</i> <i>B. napus</i> <i>B. napus</i>	A3 (2 of 3) C7 (3 of 3) C12 (2 of 3)
<i>Verticillium longisporum</i> (Verticillium disease)	<i>B. napus</i>	C5, C4 (major QTL) A6, C8, C1 (minor QTL)



Breeding for resistance to *Verticillium longisporum* and marker development



1. **Screening** in *B. rapa* and *B. oleracea* for *Verticillium* resistance
2. Creation of **resynthesized *B. napus* (RS)**
3. Production of **DH mapping populations** (elite lines x RS lines)
4. **Genetic mapping** and analysis of resistance
5. **Comparative QTL analysis:** identification of common QTL
6. Development of new **QTL-linked markers**
7. **Oil quality** analysis of DH lines

=> Obermeier C. et al., Poster

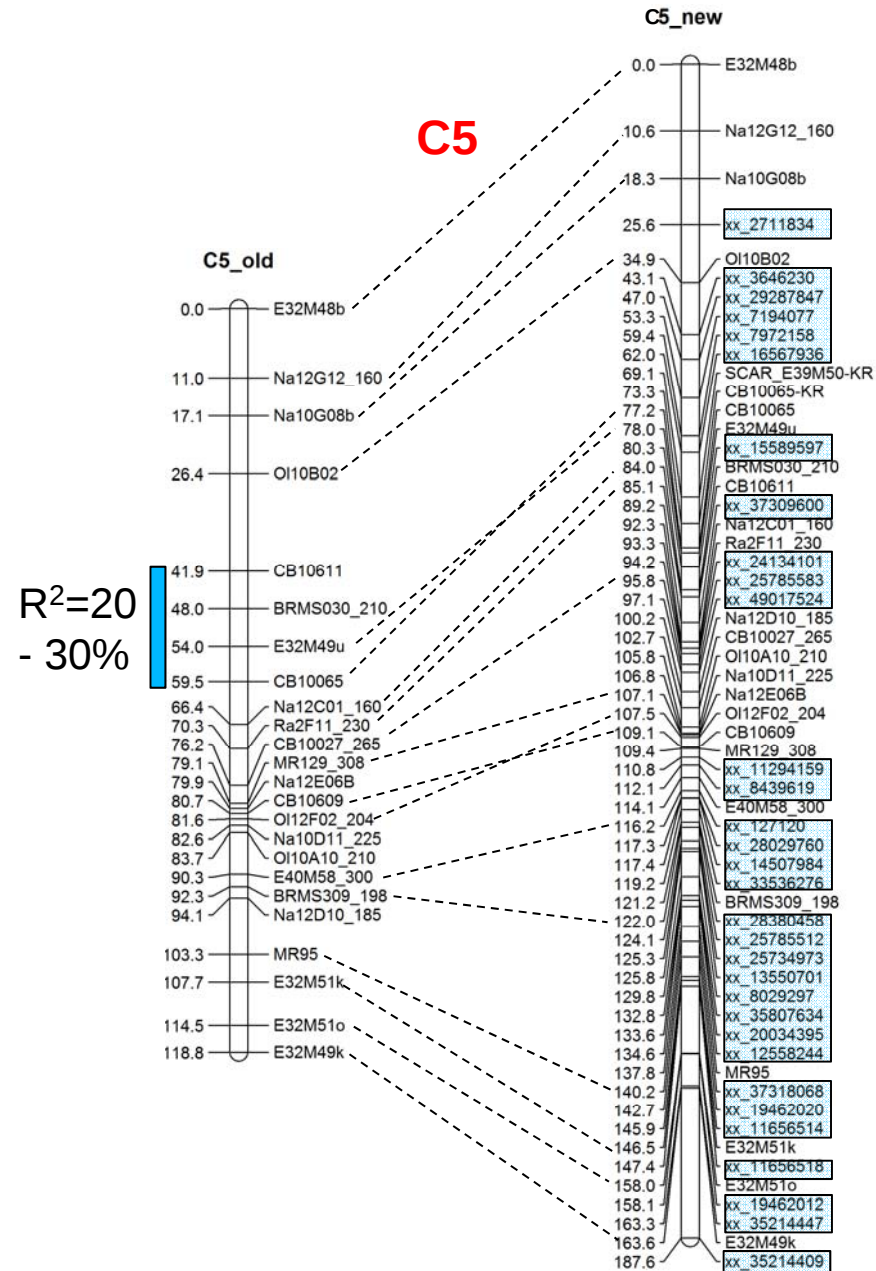
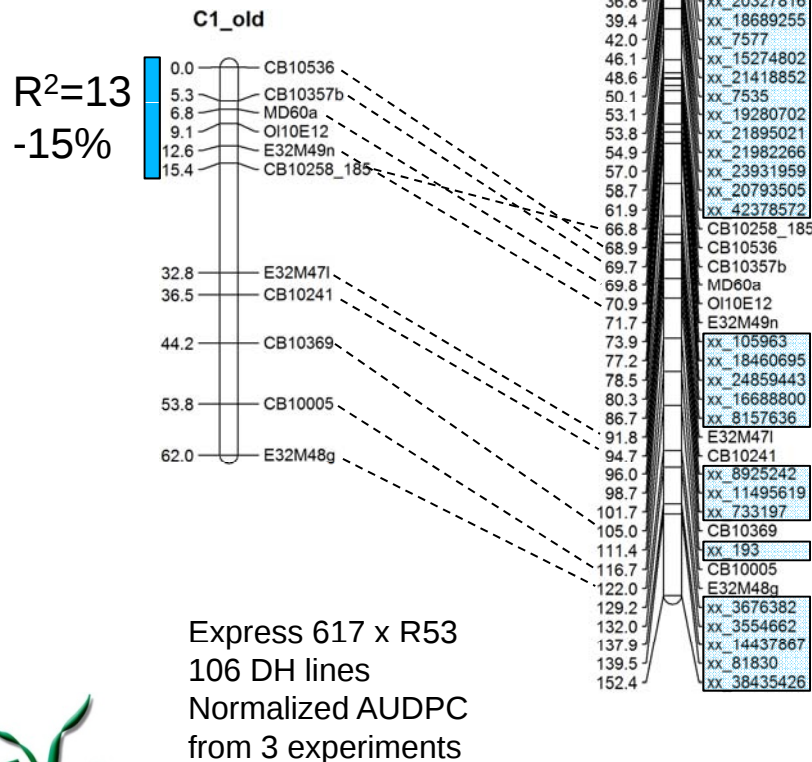


=> Obermeier C. et al., Poster



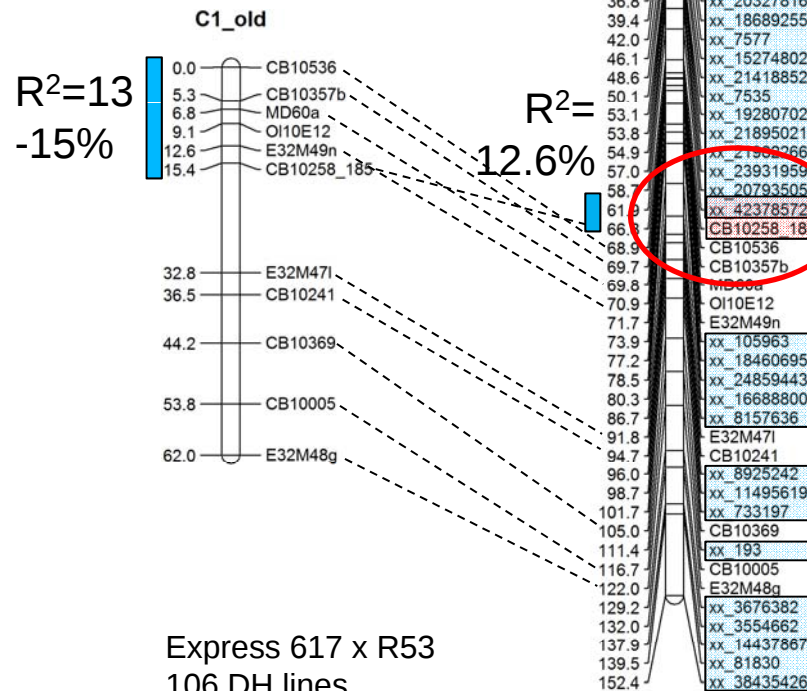
QTL mapping of *V. longisporum* resistance

QTL for *V. longisporum* resistance

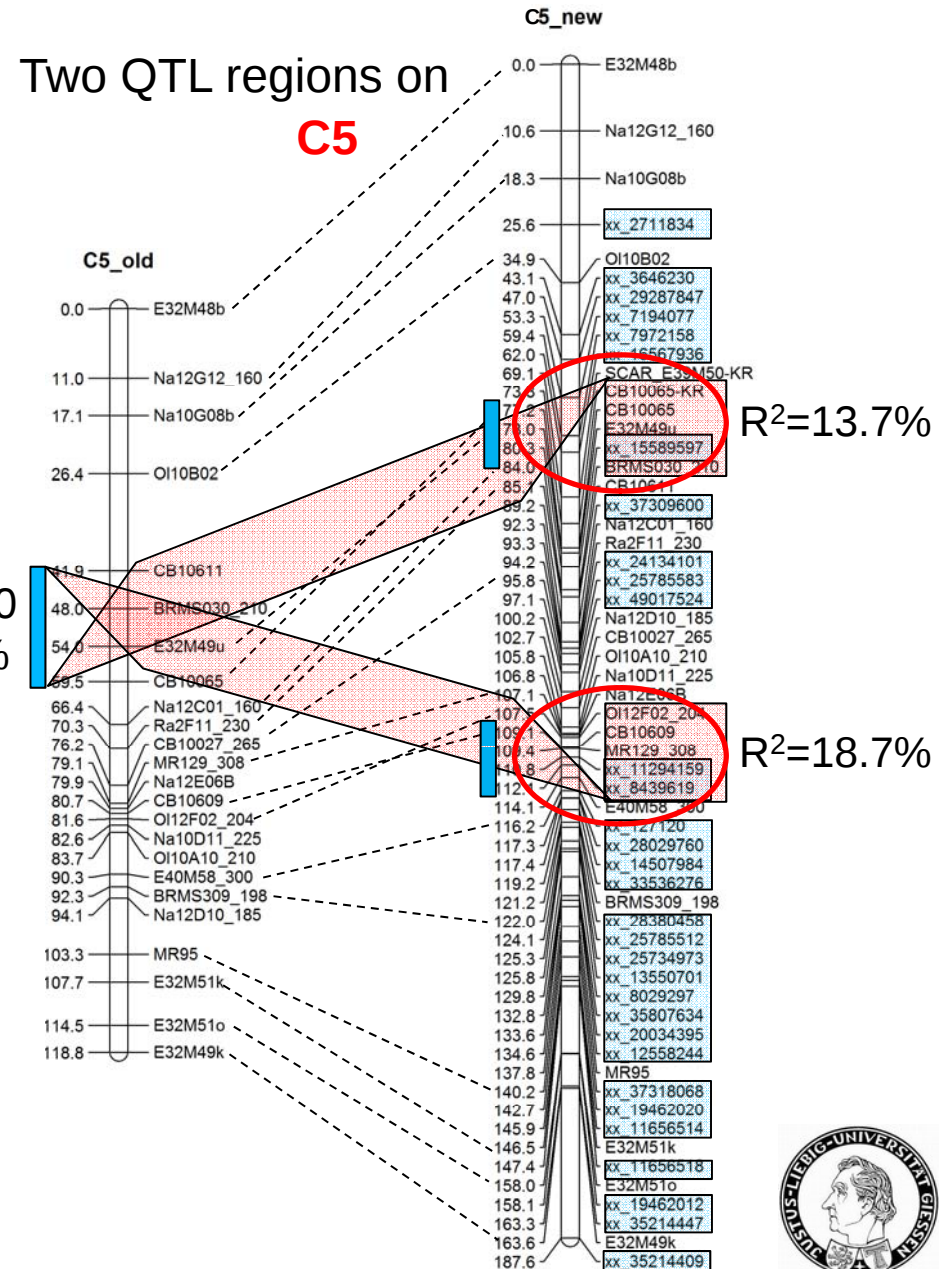


Development of new markers for marker-assisted selection

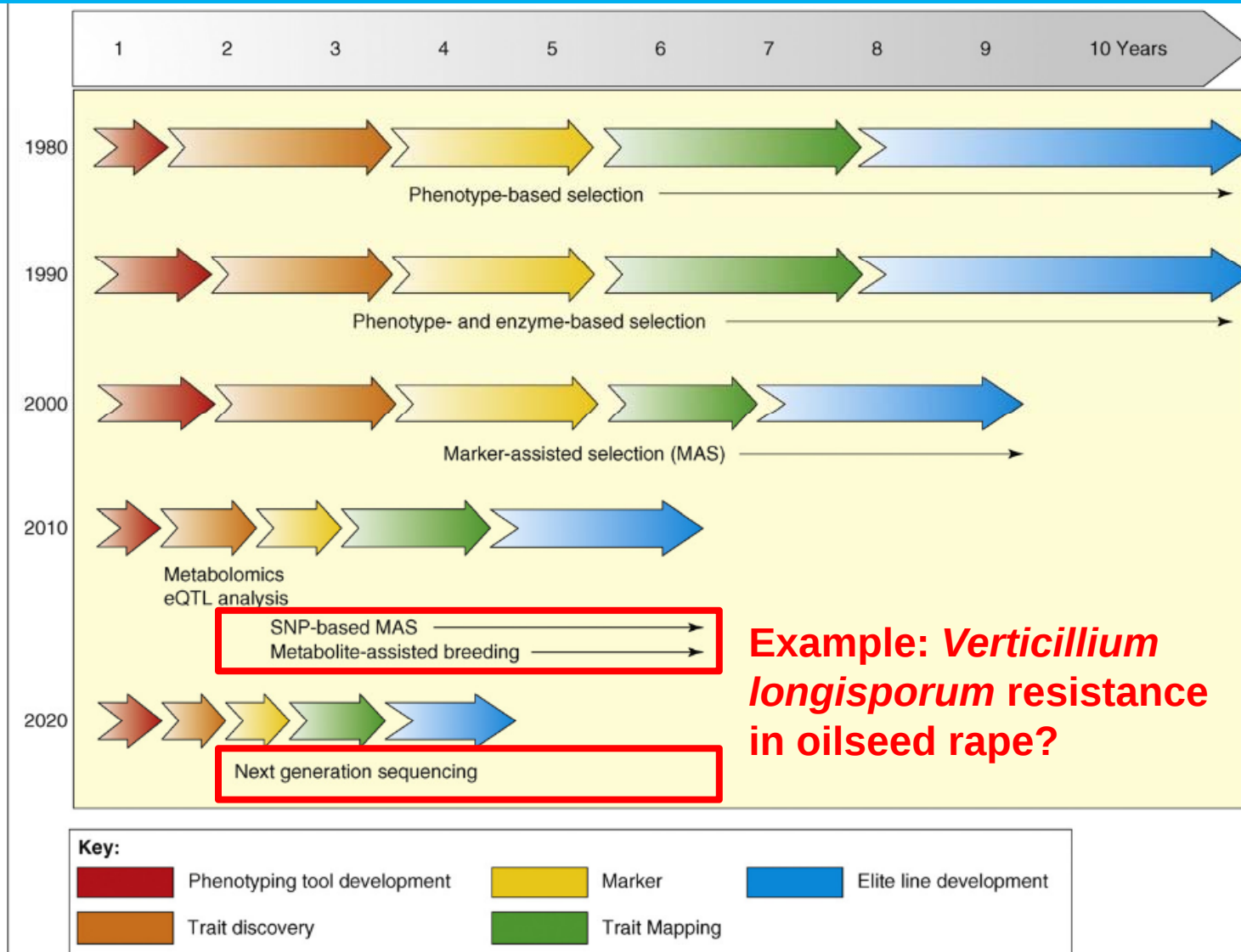
QTL for *V. longisporum* resistance



Express 617 x R53
106 DH lines
Normalized AUDPC
from 3 experiments



Breeding technology pipeline from past to present to future



Example: *Verticillium longisporum* resistance in oilseed rape?

Adapted from
Fernie & Schauer
(2008)



Fernie AR, Schauer N (2008). Metabolomics-assisted breeding: a viable option for crop improvement? Trends in Genetics 25:39-48



Stress tolerance and yield stability due to strong root system



- Better availability and acquisition of water and **nutrients**
- **Stress** (drought) and disease **resistance** (e.g. Verticillium), better crop stability
- Adequate **yield** (output) at lower input (fertilization, pesticides, etc.)





Thanks:

German Breeders (DSV, NPZ, KWS, GFP)

Dr. Christian Obermeier, M. Hossain, a.o.

Prof. A. v. Tiedemann, U Göttingen (Germany)

**Thank you for your
attention!**

