

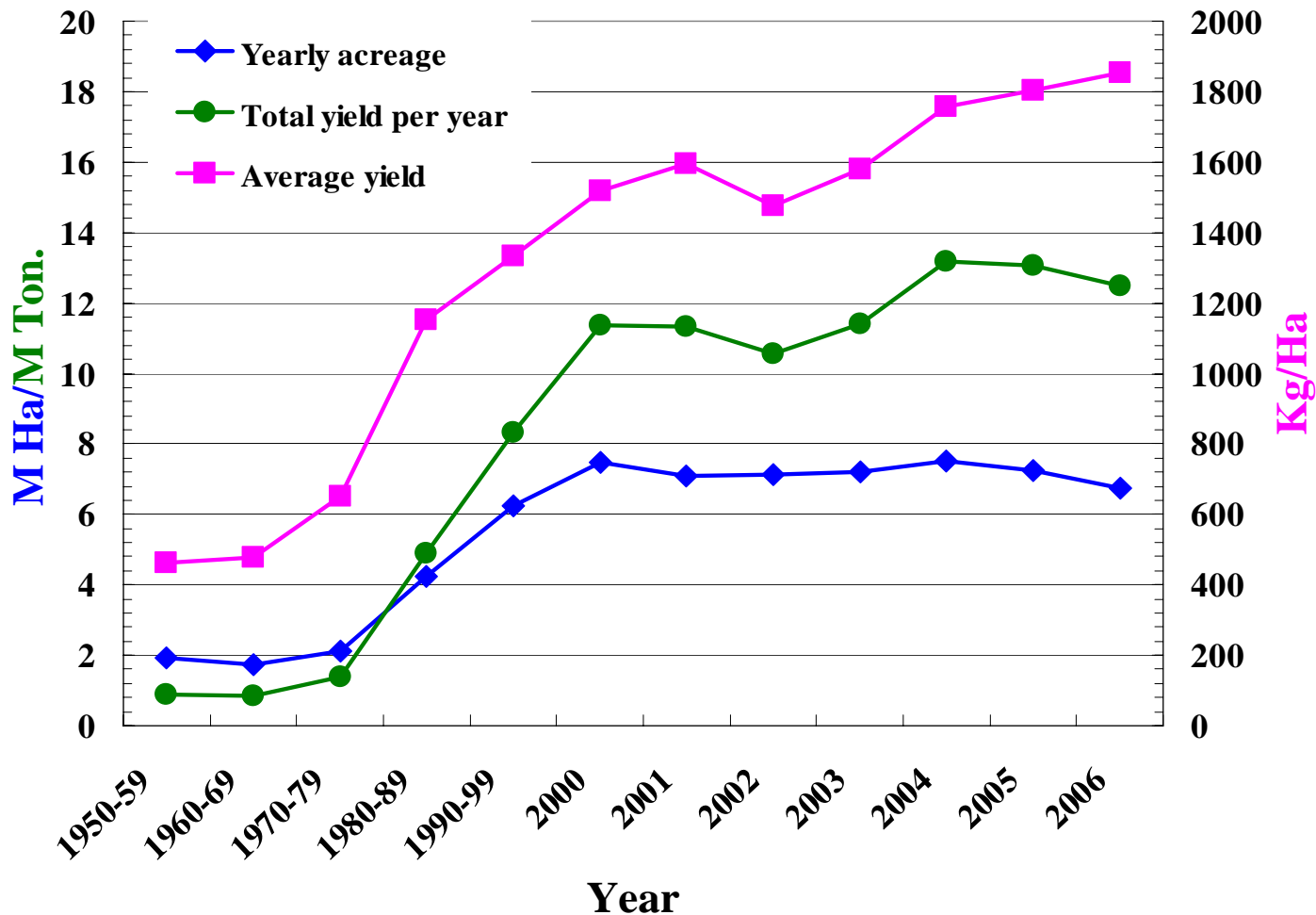
Genomics approaches toward genetic improvement of Brassica oilseeds

4th Applying Genomics to Calona Improvement Workshop
December 13, 2007
Saskatoon, Canada

Yongming Zhou
Huazhong Agricultural University
Wuhan, China

- **Rapeseed production and breeding in China**
- **Brassica genomics programs and resources in China**
- **Genomics approaches for rapeseed improvement at Huazhong Agricultural University (HZAU)**

Total production, planting areas, and average yield of rapeseed in China



Rapeseed production in China

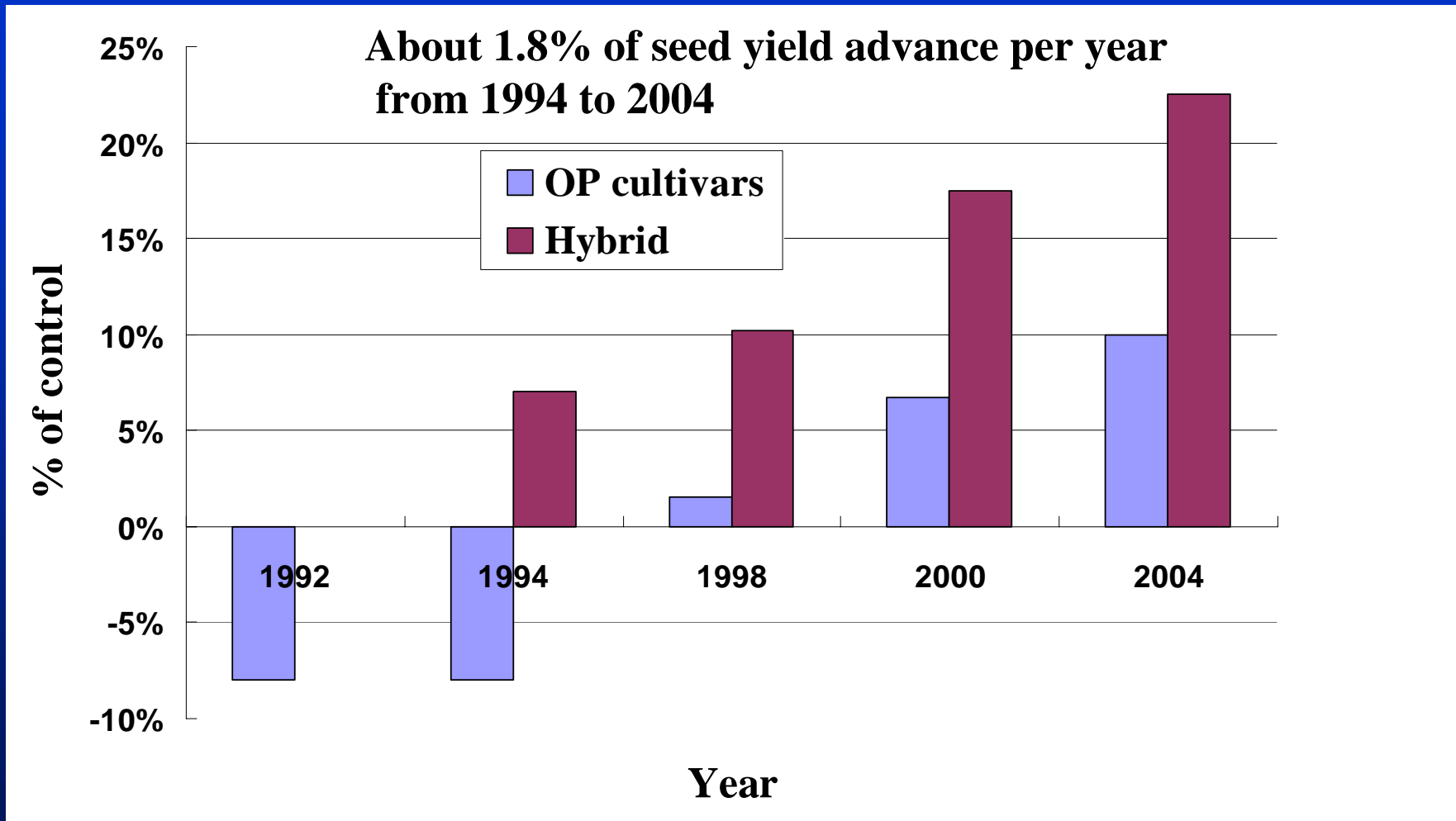
Over 85% of rapeseed is grown in the Yangtze River Basin

Over 90% of Brassica oil seeds is *B. napus* in China

Hubei: the largest rapeseed producer in China



Seed yield improvement through breeding



Data collected from the yield trials of DL cultivars in Hubei province
Control=Zhongyou821

Types of cultivars registered during 2000-05

Total	OP cultivars	Hybrids				
		CMS	GMS	EMS	GC	Sub-total
217	47	107	47	12	4	170

Pollination-control systems for hybrid production

CMS (Pol CMS and Shan 2A CMS) : 63%

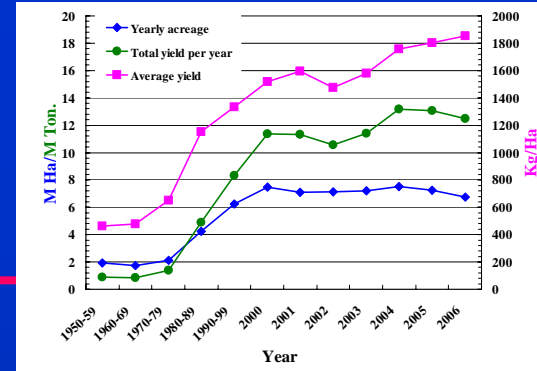
GMS (Dominant/recessive) : 28%

Planting areas of double low and hybrid cultivars

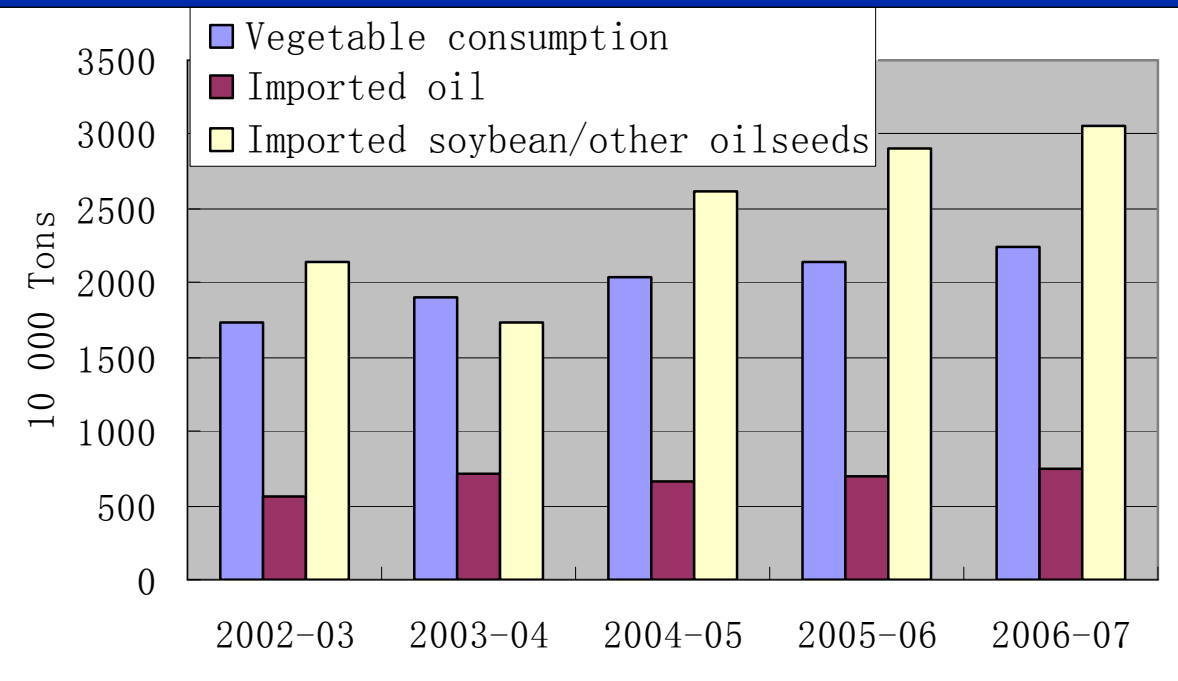
Year	Planting areas				
	Total (mil ha.)	DL cultivar		Hybrid cultivar	
		Area (mH)	Ratio %	Area (mH)	Ratio %
2000	7.495	3.890	51.9	2.933	39.6
2001	7.095	4.016	56.6	3.443	48.5
2002	7.143	4.572	64.0	3.549	49.6
2003	7.221	5.000	69.2	3.600	49.8
2004	7.838	5.598	71.42	5.331	68.01
2005	7.921	5.765	72.78	5.642	71.23

DL: low erucic acid and low glucosinolate

Breeding for future rapeseed



Consumption and supply for vegetable oil in Chinese market



2006-07

China produced 8.85mT

Vegetable oil

40~50% from rapeseed

Imported about 7.5 mT vegetable oil

Processed from imports (soybean etc) ~6 mT

Breeding for future rapeseed

To increase the total production

✓ **Enlarge growing areas**

-**potential:** spare winter lands

-**challenges:** changing cultivation practice
price issue

✓ **Enhance oil production per unit**

-Development of hybrids with increased seed yield potential

-Higher oil content

-Disease resistance

-High efficient nutrition uptake

-Traits required for changing cultivation practice: lodging-resistance; shattering resistance; herbicide resistance etc

**Brassica genomics programs
and resources in China**

Genomics resources under development



GENOMICS OF BRASSICA OIL CROPS ON FATTY ACID

National Basic Research Program of China

<http://www.geboc.org>

SSR
Comparative mapping data

- Mapping populations
- Tilling populations
- EST
- Mutant populations

Shanghai RAPeseed Database

Welcome to Shanghai RAPeseed Database

SEARCH

gene information

Line ID:	B0014011
GeneBank Accession Number:	EL622406
Full length cDNA clone:	Yes

The gene expression profiles during seed development

7DAP	9DAP	12DAP	17DAP	19DAP	21DAP	25DAP	31DAP	E-value	Score
1.382	1.863	1.580	2.352	1.202	1.374	0.839	0.756	2E-102	349.80

The relative expressions are compared to that of 3 DAP (day after pollination), and ratios were received by setting that at 3 DAP as 1. The E-value and Score are the results that got from the 'BLAST'.

Annotation: APETALA2 protein [Arabidopsis thaliana]

Flanking Sequence: GGAAAGAAAAGTTT TAGAACCAAGAAAATGTGGGATCTAAACGAC TCACACACCAGACAGTCAGACAAGAAATCTGAAGATTTTGTATTCTTCACGGGTAAACGGGTCGGATCTTCTCAAACTAAAGTTCATCTGCTGTAGTCATTGAAGATGGATCCGATGATGAACCTAACCGGG ITAGAGCCAACAACCCCTTATAACCCATCAGTCTTCCGGAGATG

Back

Unique EST 8,462
Full length cDNA 3,526
Unique SAGE tags 23,895
Tag-to-gene
502 tags to B.napus
860 tags to Brassica
EMS mutant lines
(in M3 generation)14,700

Wu et al., 2007

<http://rapeseed.plantsignal.cn>

**Genomics approaches for rapeseed
improvement at RGBL-HZAU**

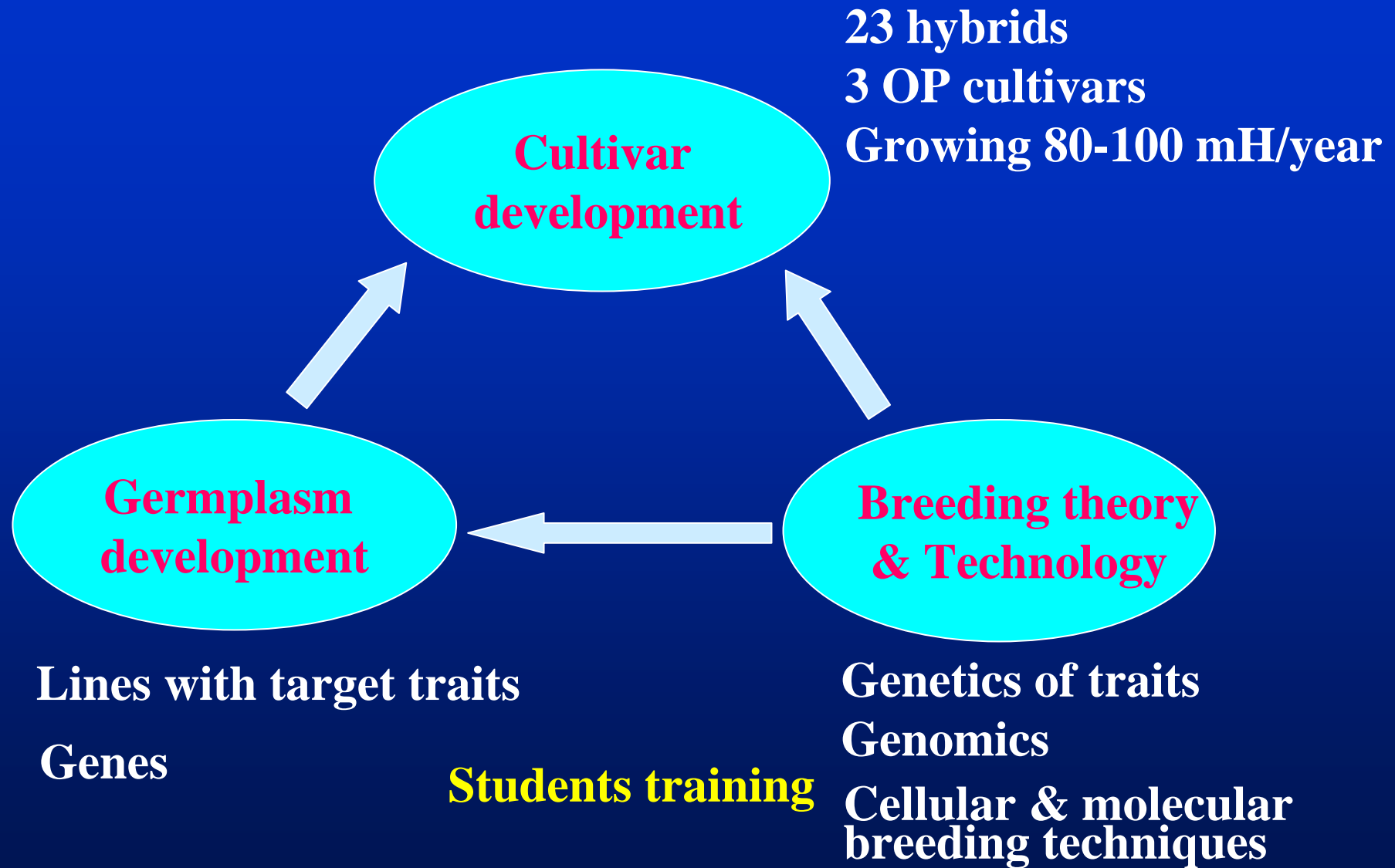
Rapeseed genetics and breeding at HZAU

◆ Starting in early 1950's : One of the pioneer institutes of rapeseed genetics and breeding in China

◆ Programs in basic researches and breeding applications



Rapeseed genetics and breeding at HZAU



Genomics approaches for rapeseed improvement

- ◆ Heterosis utilization
- ◆ QTL mapping and marker assisted selection
- ◆ Biotic and abiotic stress resistance

Heterosis utilization

- ◆ **Pol cms system and its utilization**
- ◆ **Development of alternative pollination control systems**
- ◆ **Mapping and isolation of genes for heterosis utilization**
- ◆ **Understanding the biological basis of heterosis**

Heterosis utilization

◆ Pol cms system and its utilization

-Over 60% of 3-line cms hybrids developed in China using the system

-Over 20 hybrids developed in HZAU Using the system and its derivative

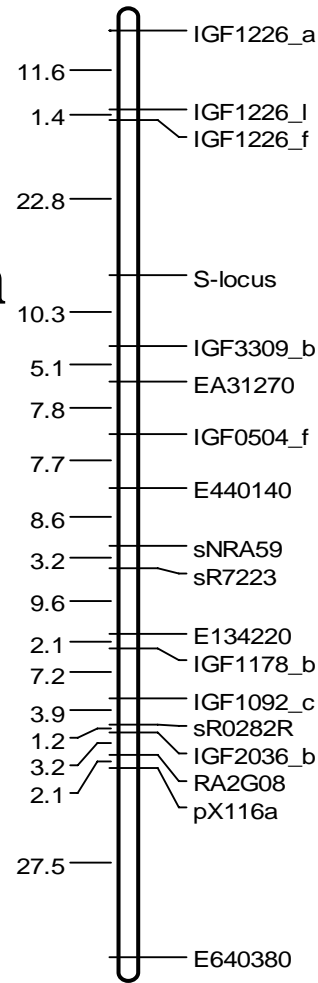


◆ *Hau* CMS: a novel CMS from *B. juncea*



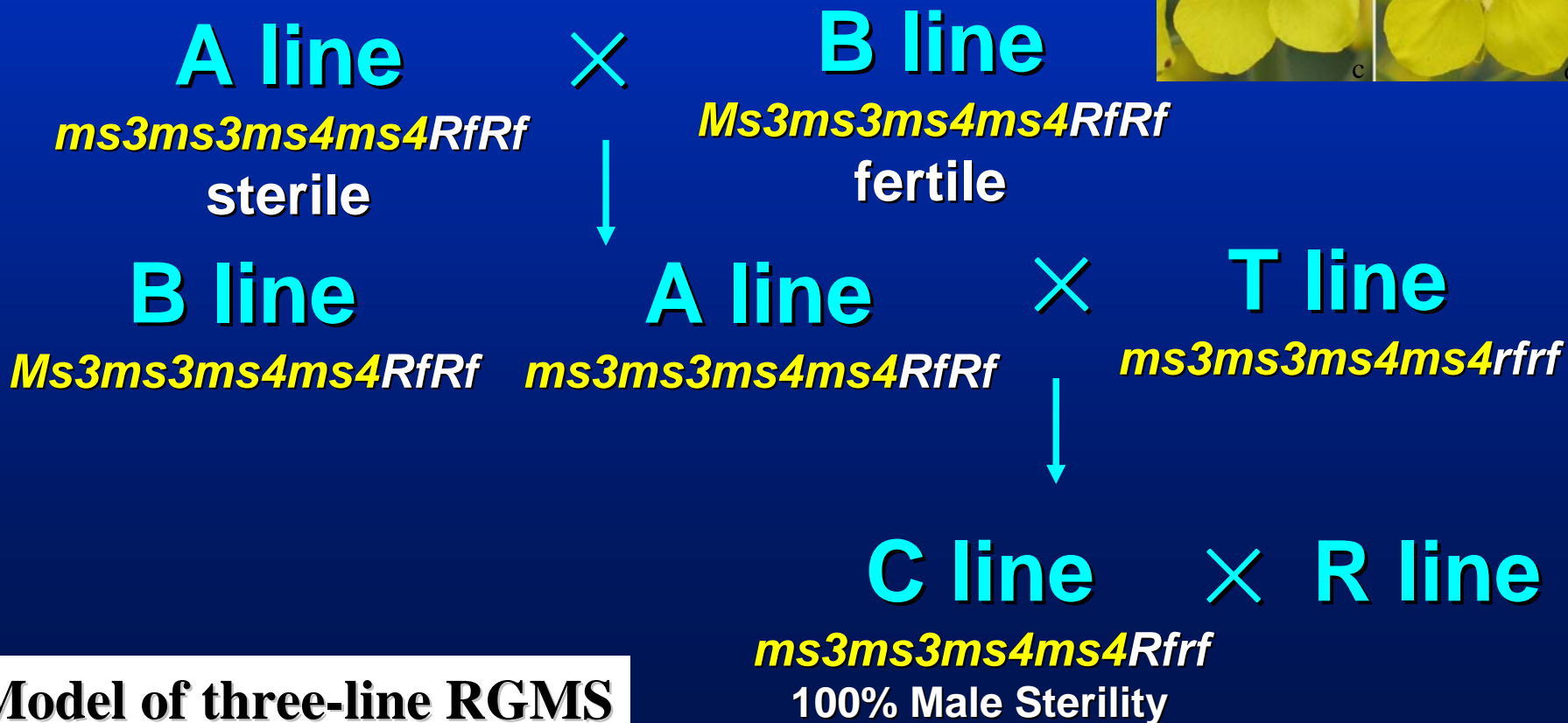
Self-incompatibility system

- ◆ First developed by Fu in 1975
- ◆ Three line system in *B. napus*
- ◆ Techniques for seed multiplication using salt-solution
- ◆ Hybrids in yield trial



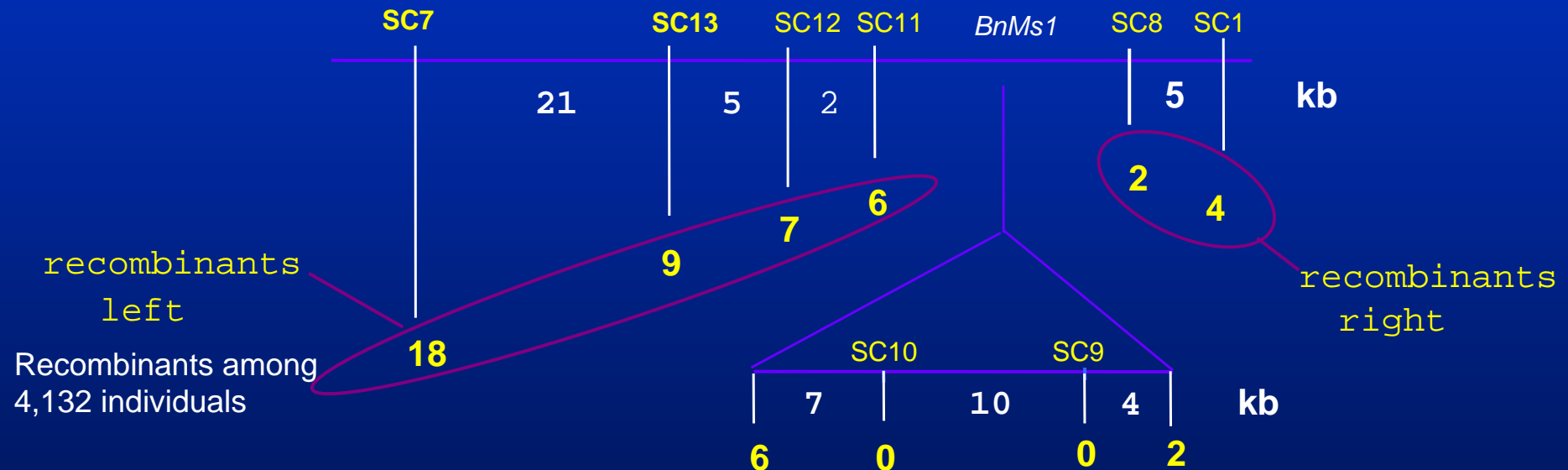
The rGMS system:

utilizes a temporary-maintainer to obtain
a 100% sterile rate in GMS lines (Tu et al, 2000)

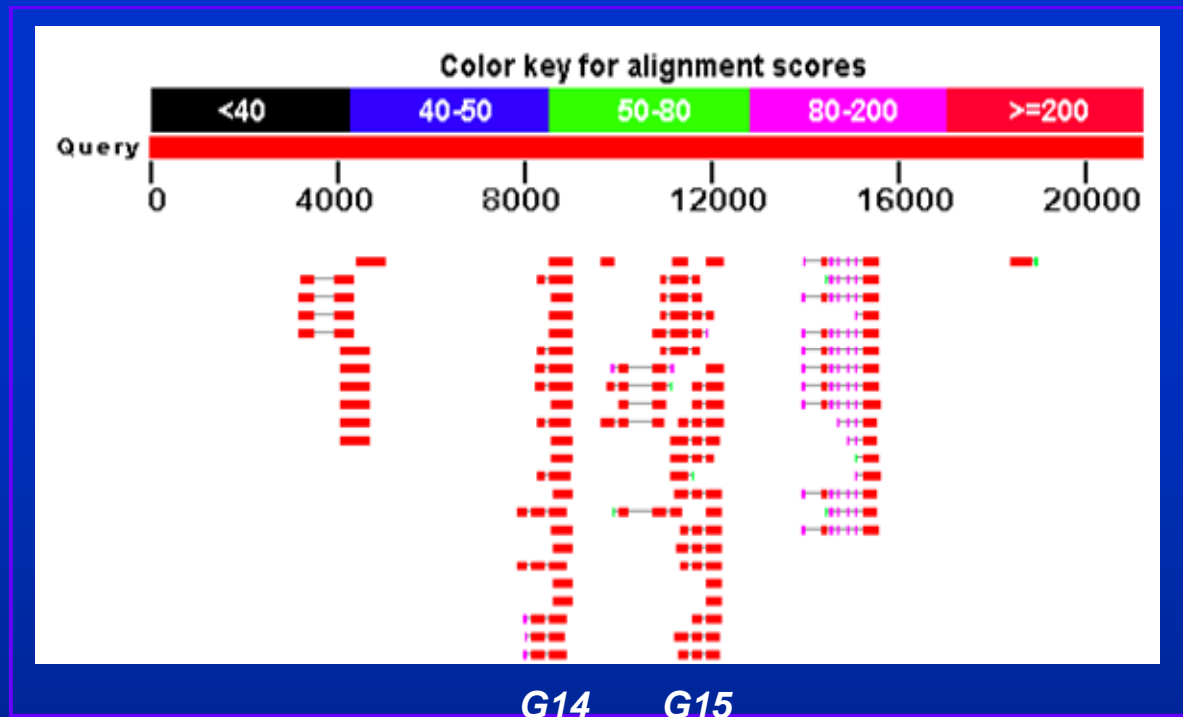


Model of three-line RGMS

physical delimitation of the *BnMs1* gene to a 21-kb DNA segment



BnMs1 was located between SC11 and SC8, the distance between two markers is 21kb. SC9 and SC10 were co-segregated with the *BnMs1* gene.

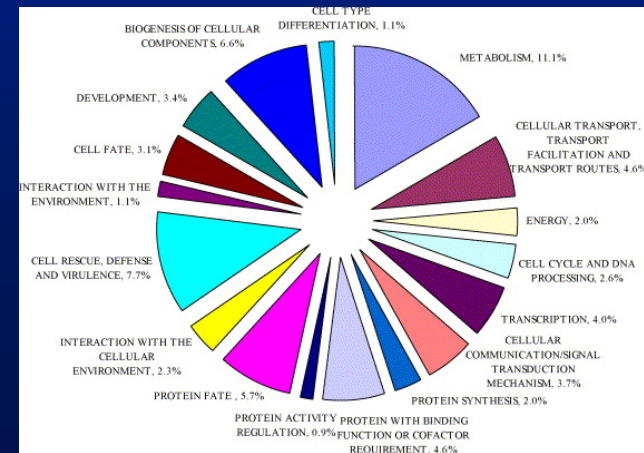


✓ Four EST homologues found within the 21 kb region in both *Aarabidopsis* and *Brassica* databases

✓ Comparative sequencing of S45AB identified 2 missense mutations for both candidate genes on *Bnms1* locus, V to A and A to S for G14 and G to R and V to A , for G15, respectively.

The dGMS system: Isolation of differentially expressed genes

- About 1200 differentially expressed clones were isolated between fertile and sterile plants in DGMS-line Rs1046AB, using SSH and cDNA microarray
- 216 unigenes out of over 400 clones sequenced identified, among which 212 up-regulated in fertile buds and 4 in sterile buds
- 178 out of 211 up-regulated homologous sequences belong to 17 groups, and 34 gene had no homologues in database



Intersubgenomic heterosis in *B.napus*

- ✓ *B. napus* were developed through crossing natural *B.napus* with *B. rapa* or *B. carinata*
- ✓ Molecular marker analysis identified new types of *B. napus* with partial A-genomic or/and C-genomic components from two napus relatives
- ✓ The resynthesized materials showed greater genetic distance from natural *B. napus* genotypes
- ✓ The materials could be beneficial for enlarging gene pool in hybrid breeding

$A^r A^r C^n C^n \times A^n A^n C^n C^n$

$A^r A^r C^c C^c \times A^n A^n C^n C^n$



Hybrids between the resynthesized and natural *B.napus* showed significant heterosis (Qian et al., 2005)

QTL mapping and marker assisted selection

- ◆ **Development of markers for various traits**
- ◆ **Identification of QTL and genes important for yield and yield components**
- ◆ **Identification of QTL for oil content and seed development**

Markers for traits related to seed quality and productivity

✓ Yellow seeds

✓ Seed weight

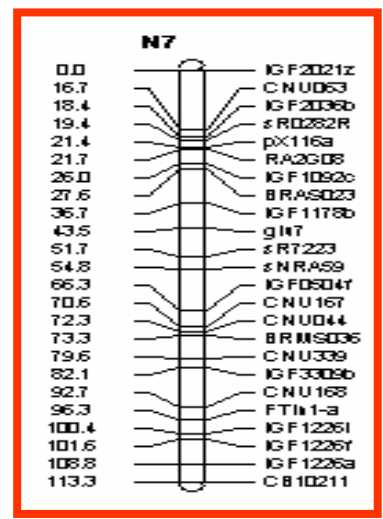
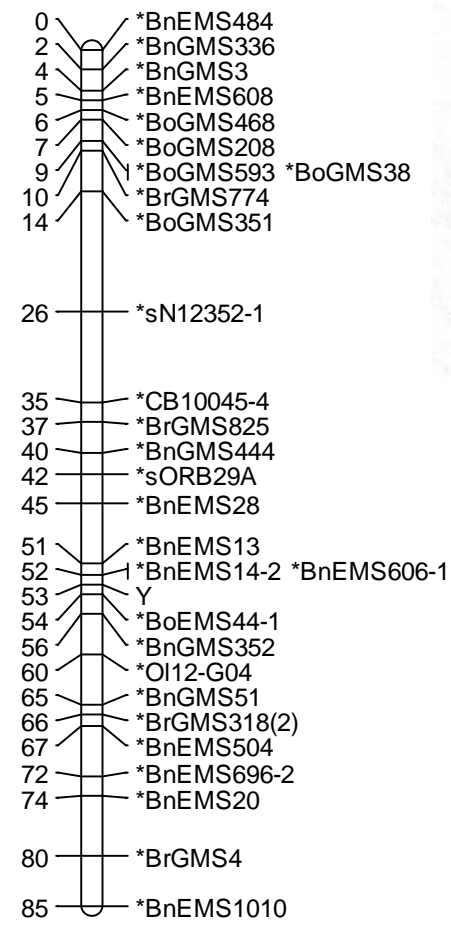
P1



F1

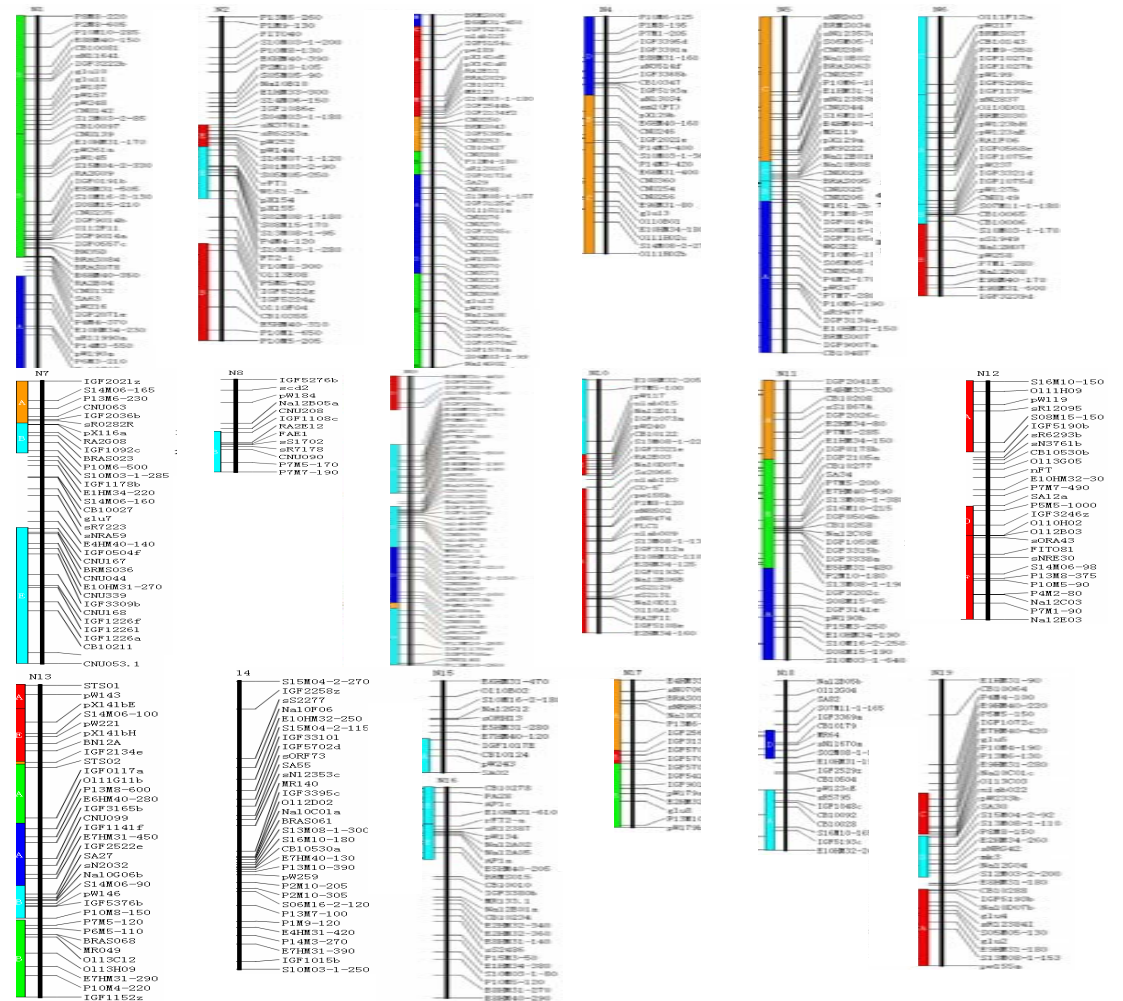


P2



TN genetic map: a recent map includes 621 loci developed based on previous version of the map with 354 loci (Qiu *et al.*, 2006)

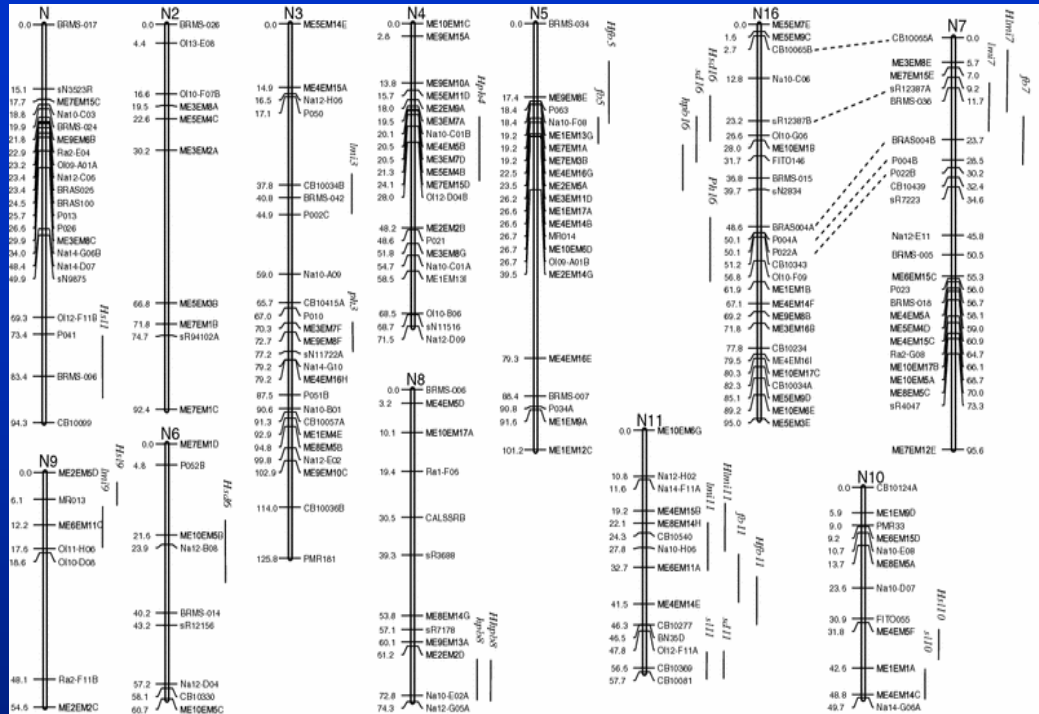
Comparative mapping identified 40 conserved segments and 84 conserved islands between *B.napus* and *Arabidopsis*, based on 229 STS anchor markers.



- 拟南芥第1染色体
- 拟南芥第2染色体
- 拟南芥第3染色体
- 拟南芥第4染色体
- 拟南芥第5染色体

Long et al., 2007

Dissection of genetic bases of seed yield component in *B. napus*



- 11 traits and heterosis analyzed
- 18 out of 88 QTL repeatedly detected
- 44 heterosis loci for yield
- 118 epistatic QTL
- AA interaction predominant

Two populations:

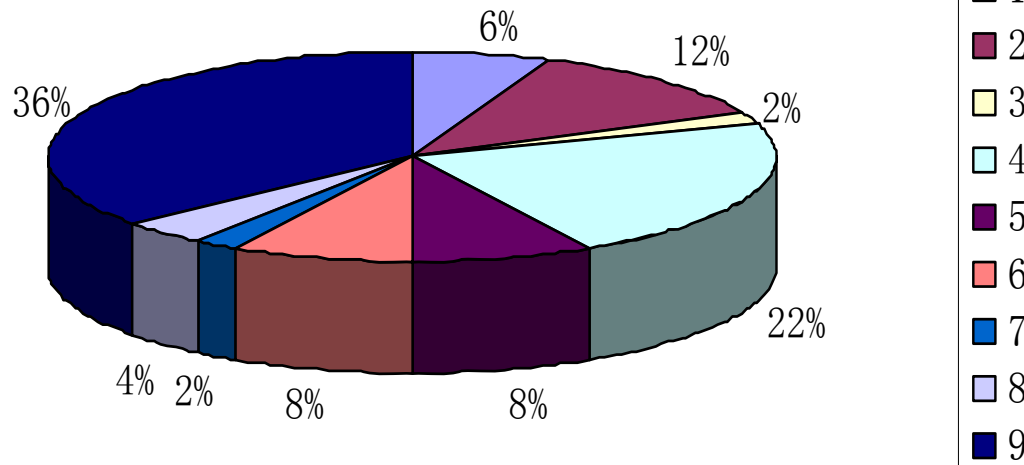
- ✓ 258 DH lines
- ✓ Immortalized F2 comprising 258 crosses from randomly intermating of the DH lines

Biotic and abiotic stress resistance

- ◆ Mapping QTL related to Sclerotinia resistance
- ◆ Identify genes related to disease resistance by cDNA array
- ◆ Tolerance to poor soil nutrition, such as boron deficiency
- ◆ Drought resistance
- ◆ Low and high temperature stresses

Screening of differential expressed genes from sclerotinia-challenged *Brassica napus* tissues

Unigene 52,
Known genes 32,
Disease defense related 23
(71.8%)



✓ 182 differentially
Displayed clones from 2928
Clones

✓ 62 sequences obtained

1: primary metabolism; 2: energy metabolism; 3: secondary metabolism;
4: disease defense; 5: cell structure; 6: transcription factors;
7: protein synthesis; 8: signal transduction; 9: unknown

Brassica is one of the most sensitive crops to boron (B) deficiency



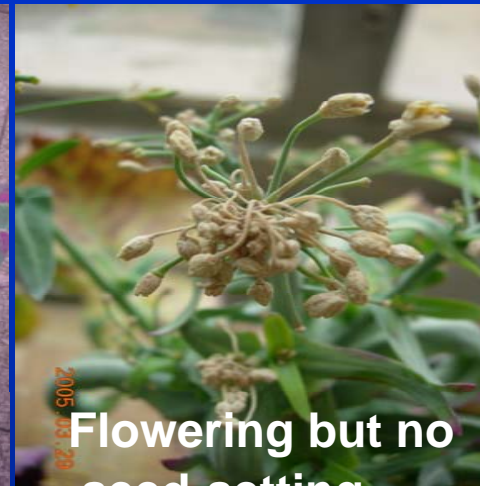
Stunted root
Curled leaf



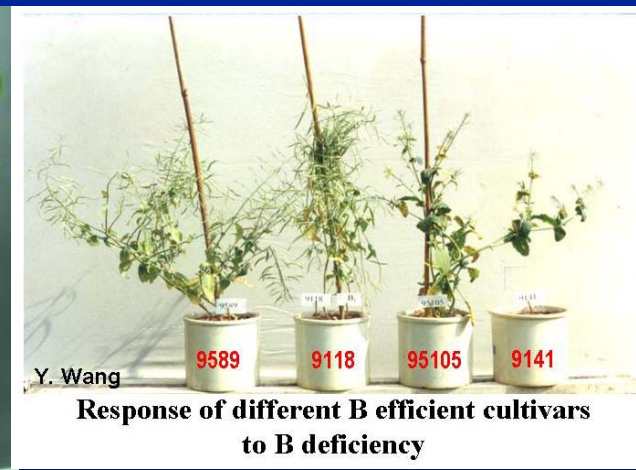
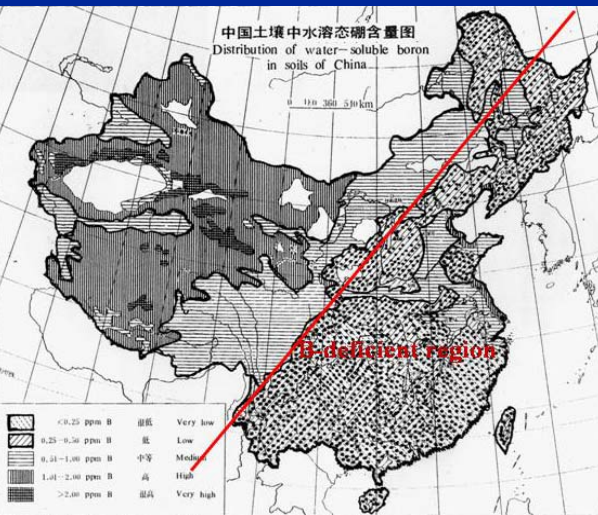
Brittle petiole
chapped stem



Dark-redish leaf



Flowering but no
seed-setting

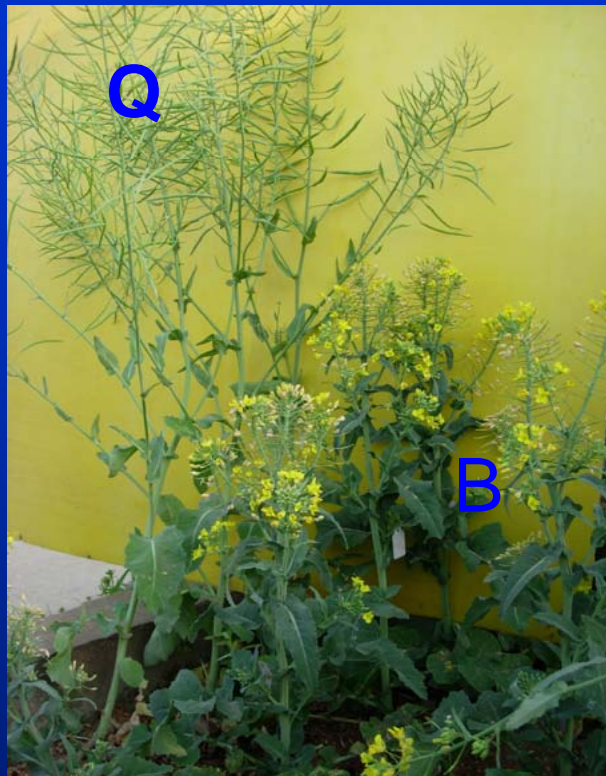


Response of different B efficient cultivars to B deficiency

33 million hectare cultivated land
Soil available B < 0.5 mg/Kg

Genotypic variation in response to B deficiency within *Brassica napus*

QTL for B efficiency in *Brassica napus*



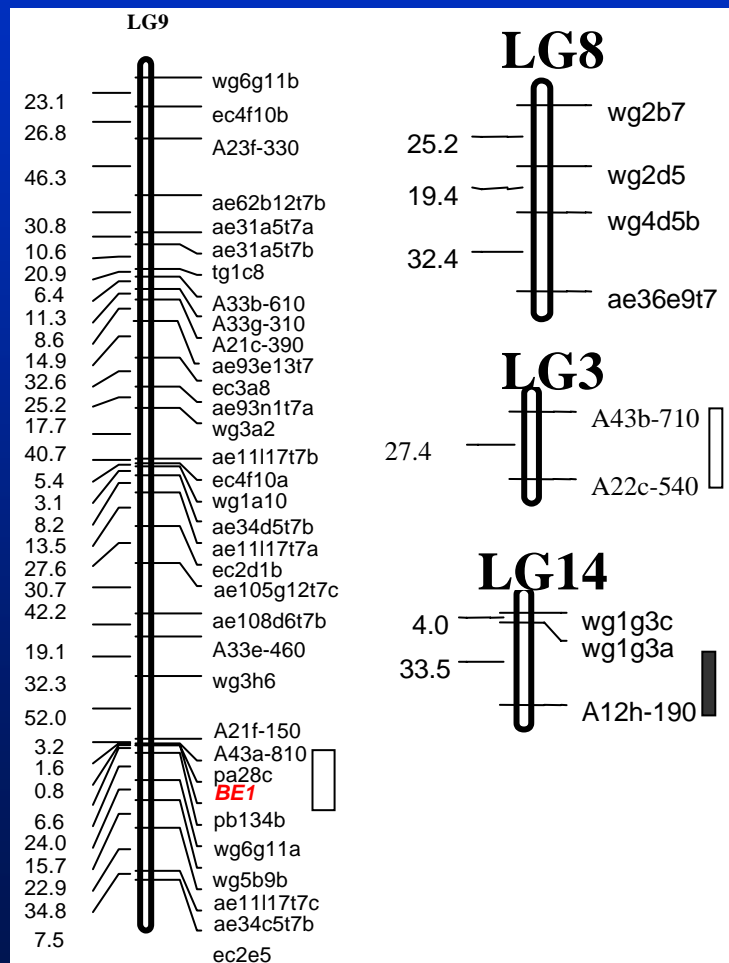
Four QTLs for seed yield under low B condition were detected in a F₂ population

Qingyou 10 (Q)



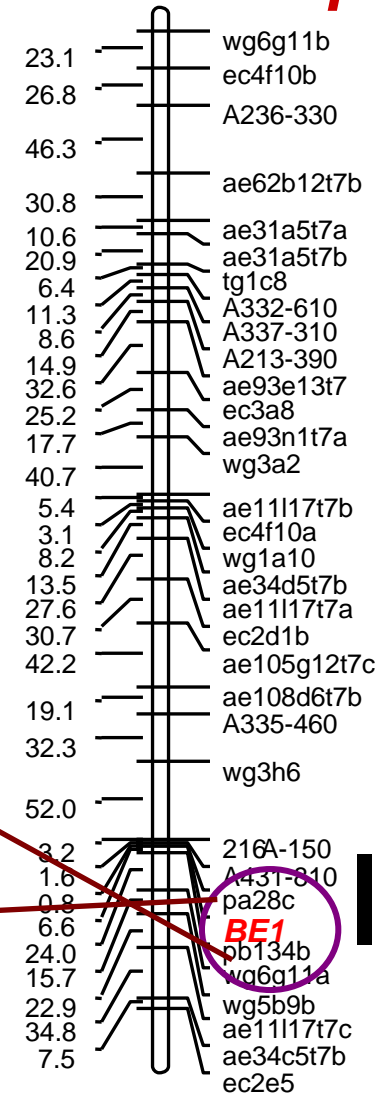
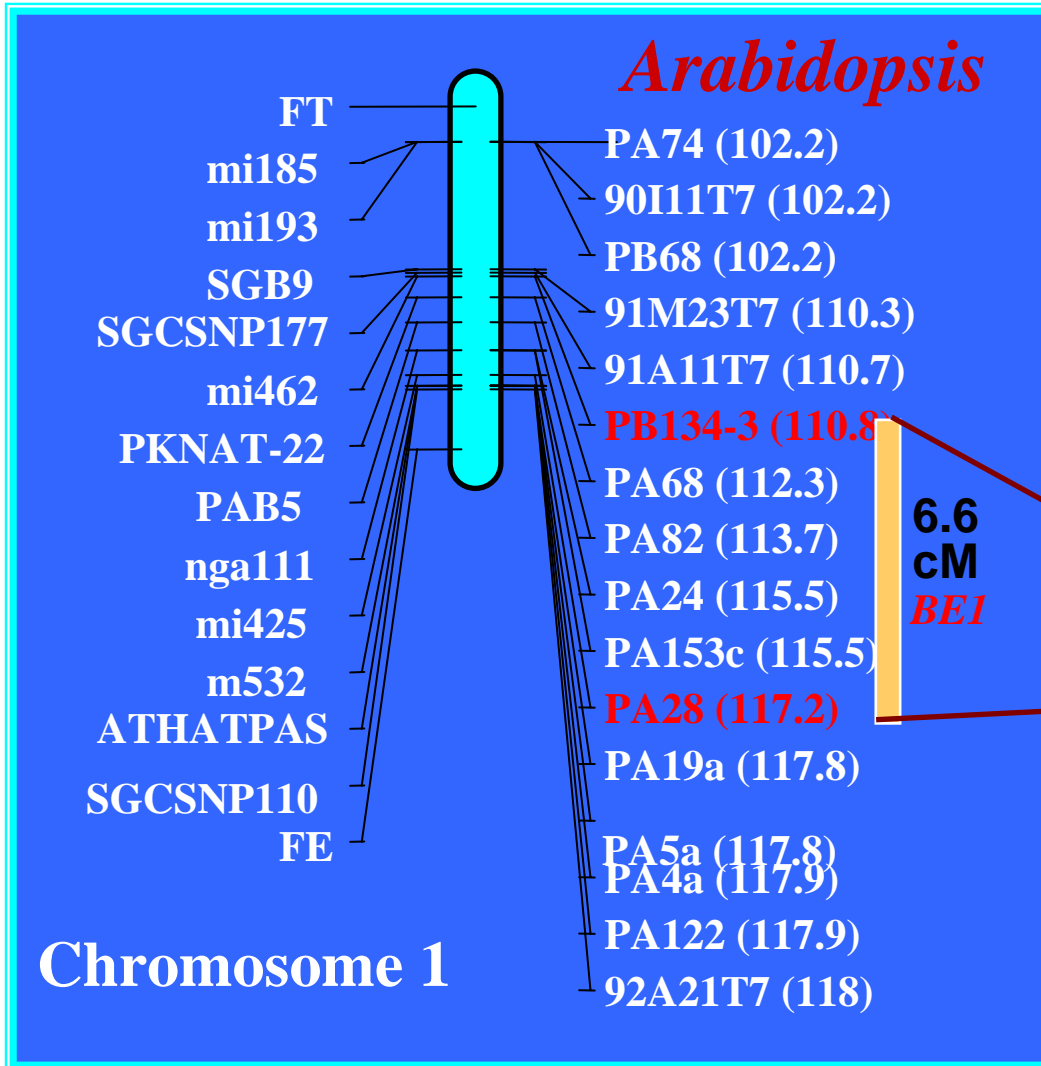
Bakow (B)

Xu et al., 2001



Comparative mapping between *B. napus* and *Arabidopsis*

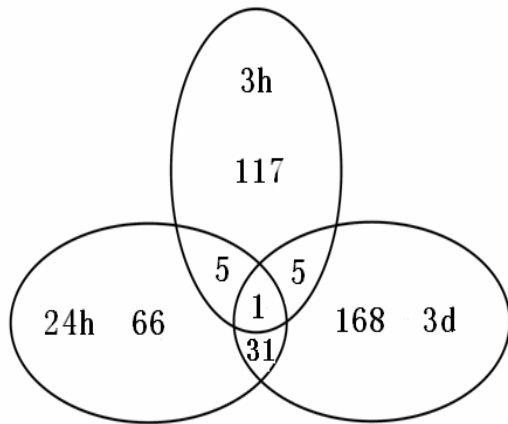
Brassica napus



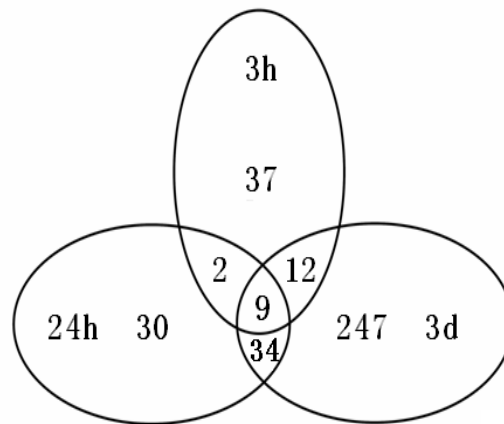
Wang et al., 2002

Arabidopsis gene expression profile induced by low B with *Arabidopsis* Affymetrix microarray with 22,810 genes

Up-regulated (393)

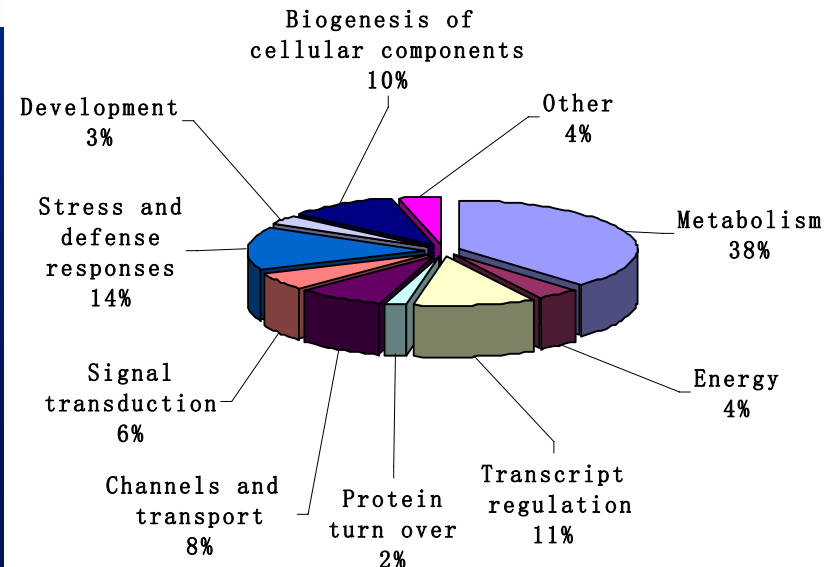


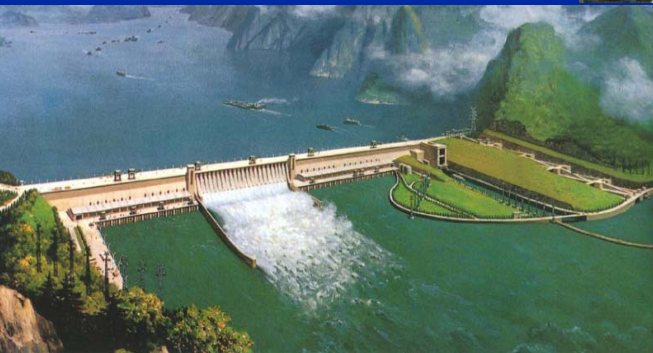
Down-regulated (371)



Three time points examined are 3 h, 24 h and 3 d after B starvation

Function classification for the differential expression genes





Acknowledgment

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(863) Programs

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