Accelerating the development and utilization of new-type *Brassica napus* gene pool by genomics-based approaches

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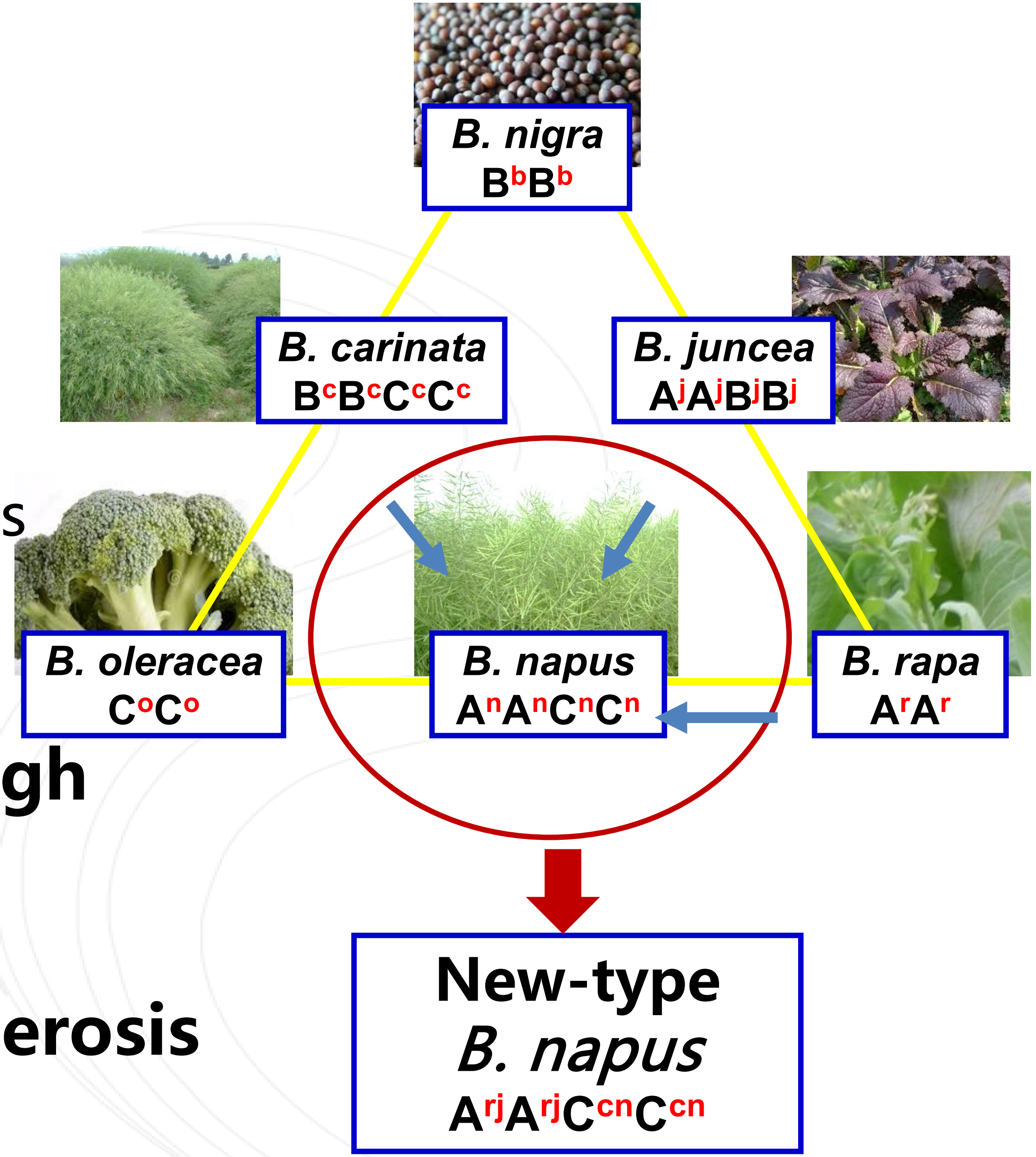
- *Brassica napus* is an important oilseed crop and heterosis utilization is most important approach for rapeseed production
- However, it has narrow genetic diversity which also affects the utilization of heterosis

◆ **Broadening its genetic basis through subgenome reconstruction**

◆ **Utilization of intersubgenomic heterosis**



B. nigra
B^bB^b

B. carinata
$B^cB^cC^cC^c$

B. juncea
$A^jA^jB^jB^j$

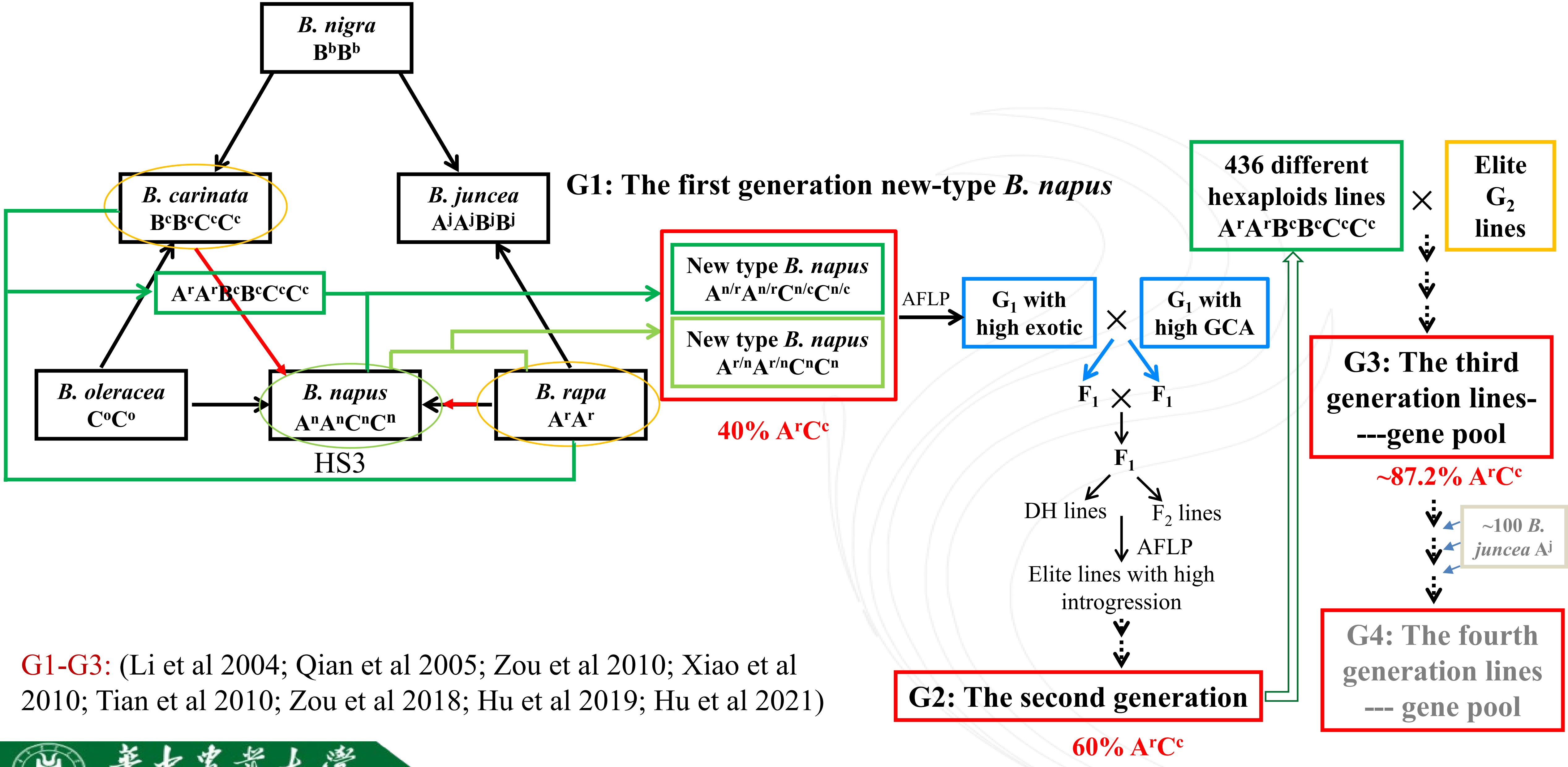
B. oleracea
C^oC^o

B. napus
$A^nA^nC^nC^n$

B. rapa
A^rA^r

New-type
B. napus
$A^{rj}A^{rj}C^{cn}C^{cn}$

The development of new-type *B. napus* gene pool



B. nigra
B^bB^b

B. carinata
$B^cB^cC^cC^c$

B. juncea
$A^jA^jB^jB^j$

$A^rA^rB^cB^cC^cC^c$

B. oleracea
C^oC^o

B. napus
$A^nA^nC^nC^n$

HS3

B. rapa
A^rA^r

G1: The first generation new-type *B. napus*

New type *B. napus*
$A^{n/r}A^{n/r}C^{n/c}C^{n/c}$

New type *B. napus*
$A^{r/n}A^{r/n}C^nC^n$

40% A^rC^c

AFLP

G_1 with high exotic

×

G_1 with high GCA

F_1 × F_1

F_1

DH lines

F_2 lines

AFLP

Elite lines with high introgression

G2: The second generation

60% A^rC^c

436 different hexaploids lines
$A^rA^rB^cB^cC^cC^c$

×

Elite G_2 lines

G3: The third generation lines---gene pool

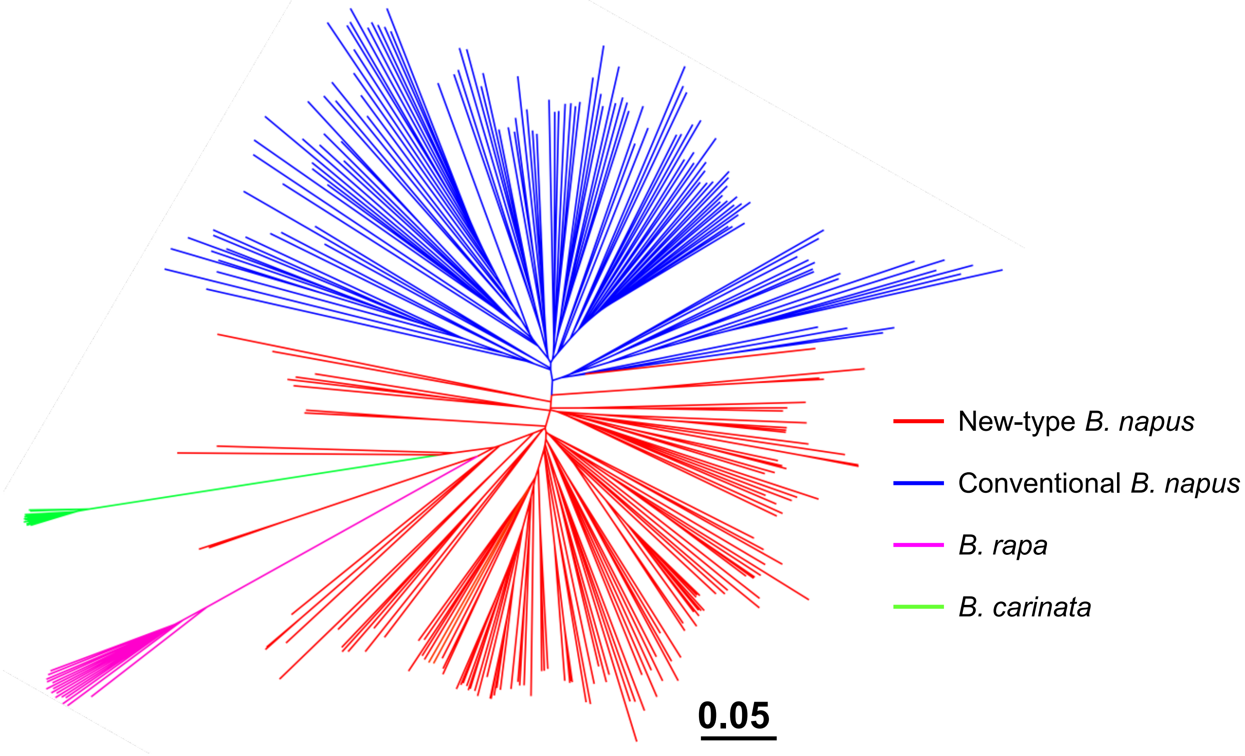
~87.2% A^rC^c

~100 *B. juncea* A^j

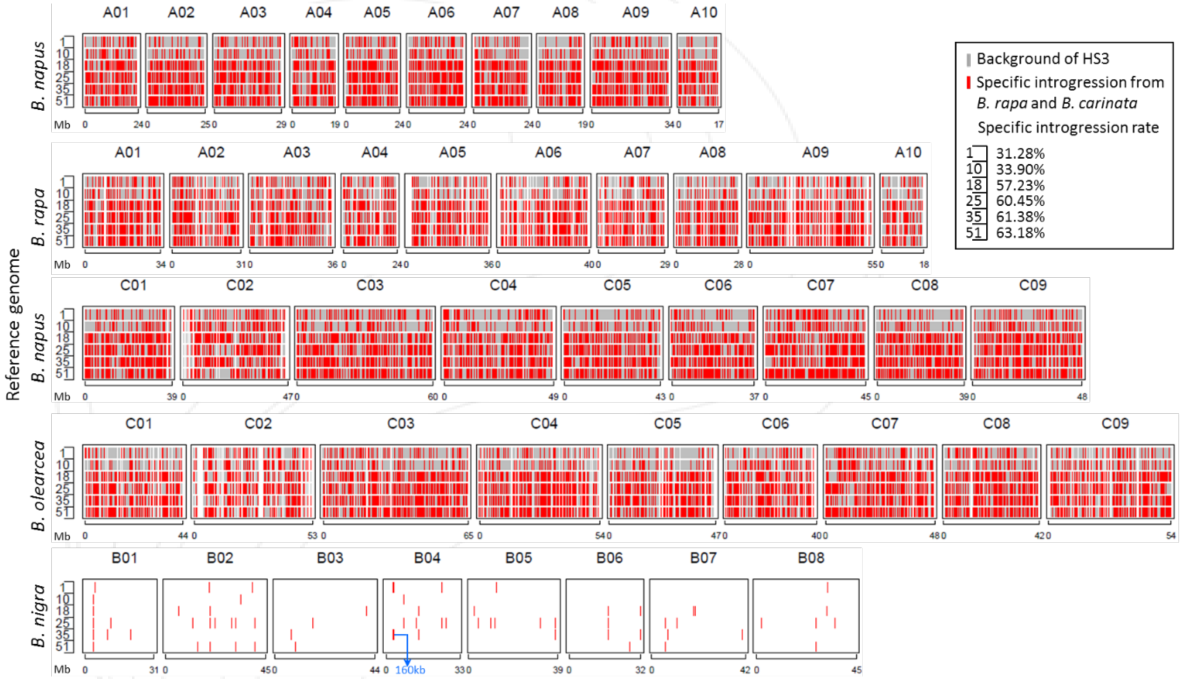
G4: The fourth generation lines --- gene pool

G1-G3: (Li et al 2004; Qian et al 2005; Zou et al 2010; Xiao et al 2010; Tian et al 2010; Zou et al 2018; Hu et al 2019; Hu et al 2021)

New-type *B. napus* is an independent subgroup



Inbred lines evaluated by *Brassica* 60K-SNP array



Rich introgression components from *B. napus* relatives

(Zou *et al*., 2018; Hu et al., 2019)

Excellent variations and breeding application

ZS11 (control) 3.50g

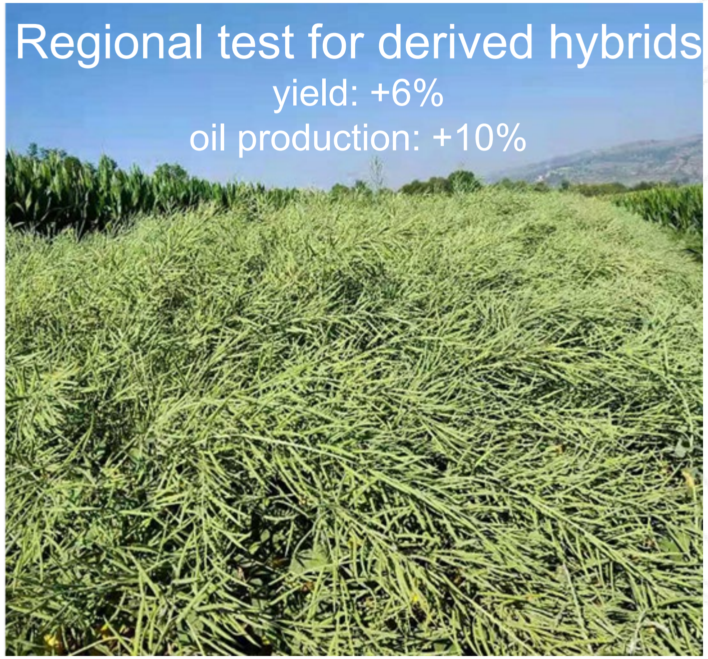
AM032 7.10g

AM140 Dark brown

19N940 Brown

105 pods / 85cm, density:1.24

36 seeds / pod



Regional test for derived hybrids

yield: +6%

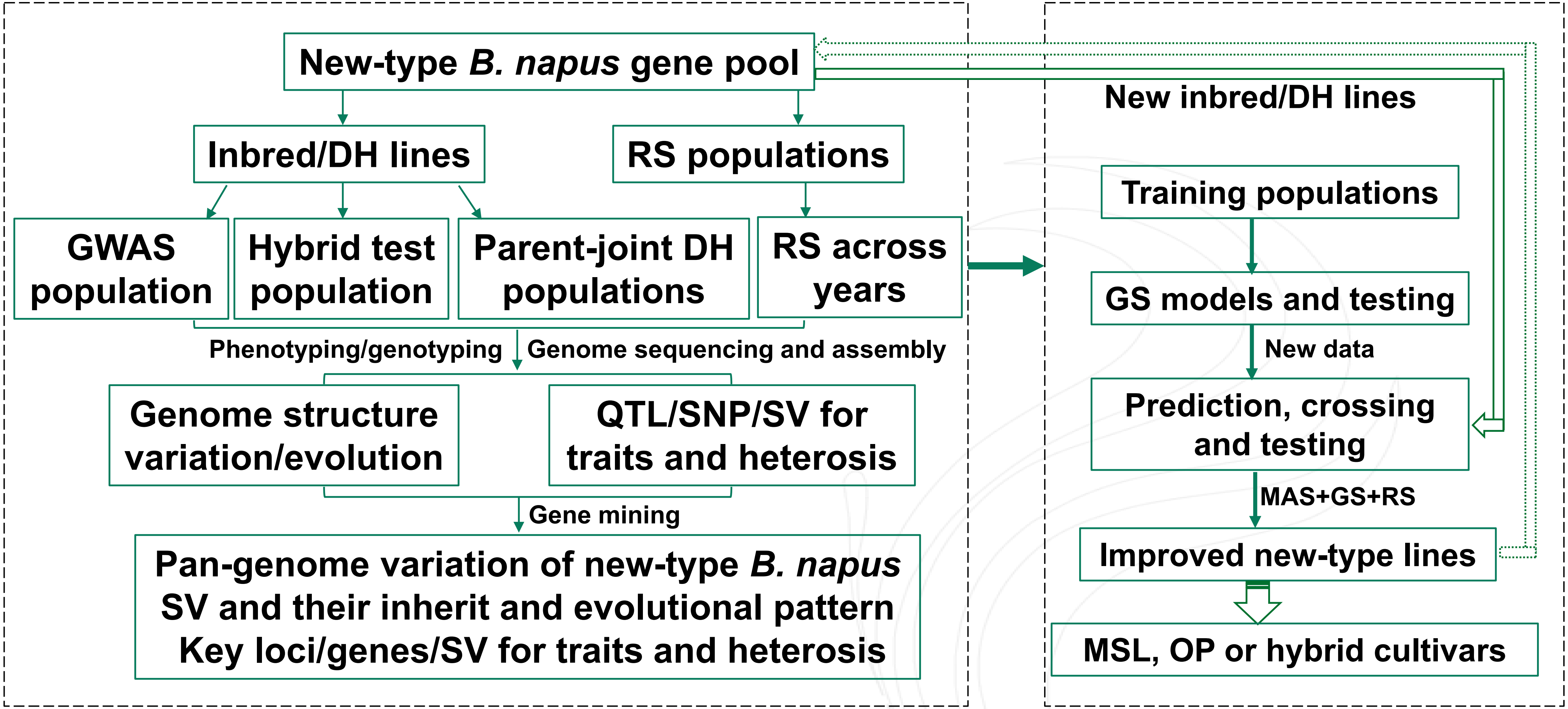
oil production: +10%

C18:2 35%

C18:3 15%

Questions

- What's the genome-wide novel variation of new-type *B. napus* population and which are favorable for breeding?
- How are these abundant variations fixed following *de novo* domestication and intensive selection?
- How should we accelerate the development of the gene pool and the application of elite variations with genomic tools for practical breeding ?



New-type *B. napus* gene pool

Inbred/DH lines

RS populations

GWAS population

Hybrid test population

Parent-joint DH populations

RS across years

Phenotyping/genotyping

Genome sequencing and assembly

Genome structure variation/evolution

QTL/SNP/SV for traits and heterosis

Gene mining

Pan-genome variation of new-type *B. napus*
SV and their inherit and evolutional pattern
Key loci/genes/SV for traits and heterosis

New inbred/DH lines

Training populations

GS models and testing

New data

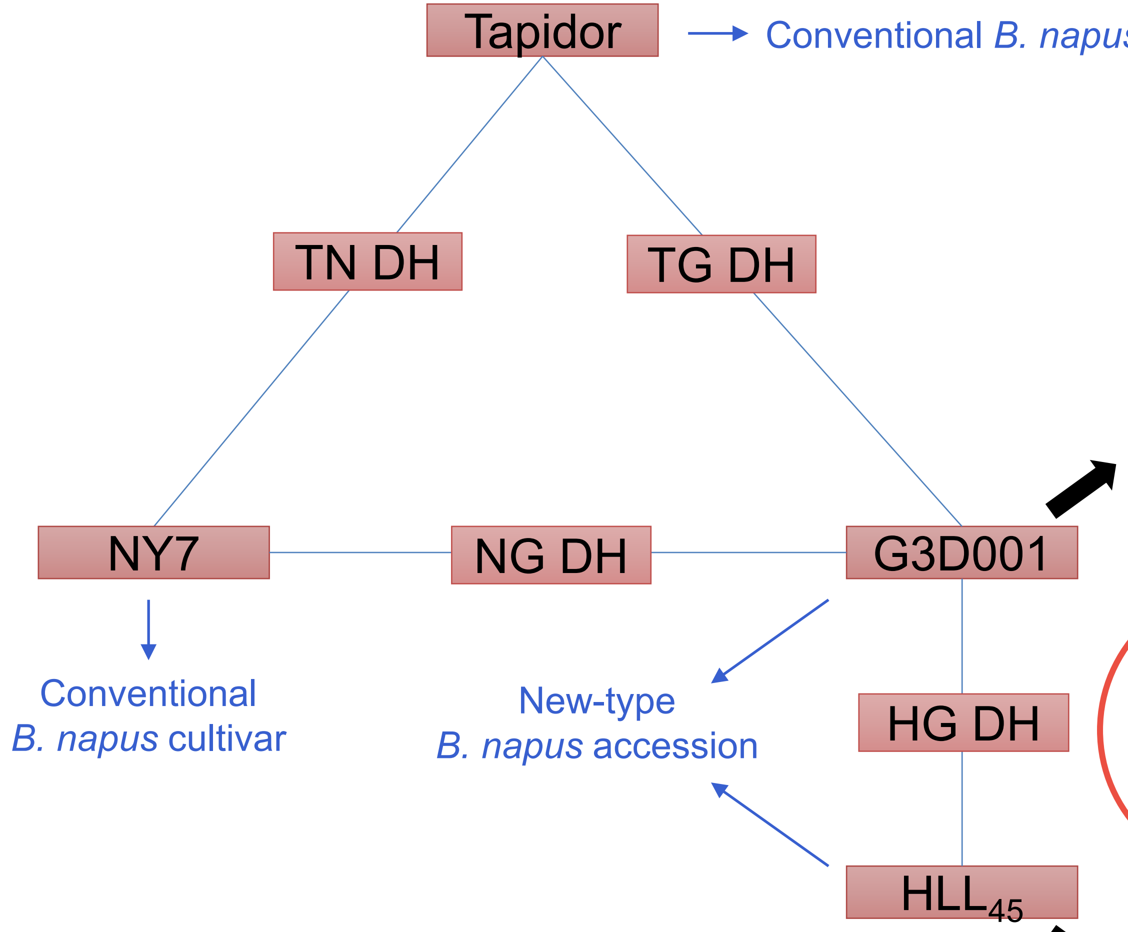
Prediction, crossing and testing

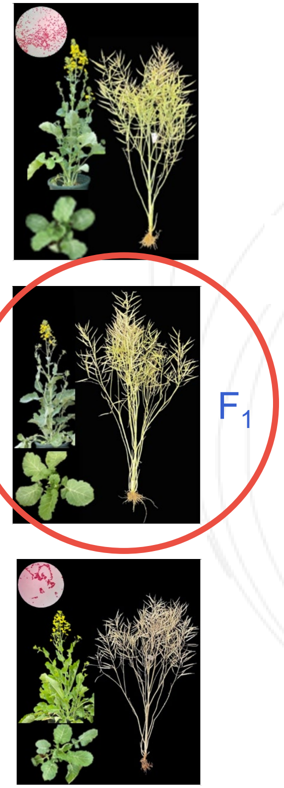
MAS+GS+RS

Improved new-type lines

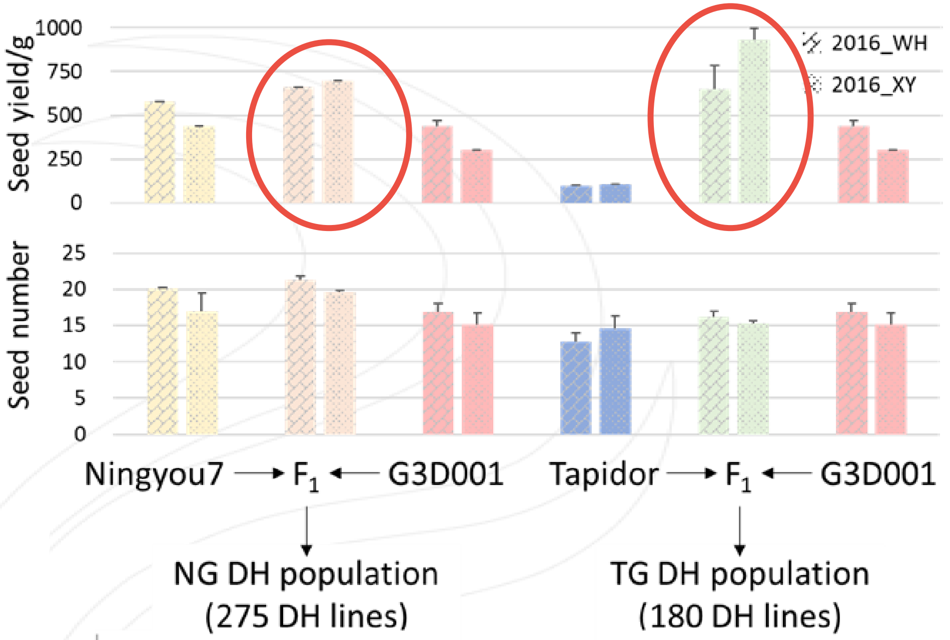
MSL, OP or hybrid cultivars

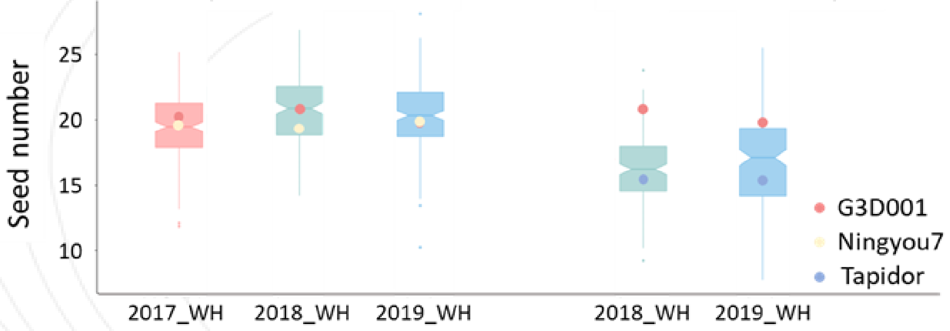
Mapping populations for variation identification





Construction of tetra-parent mapping populations





Phenotyping of F_1 and DH lines

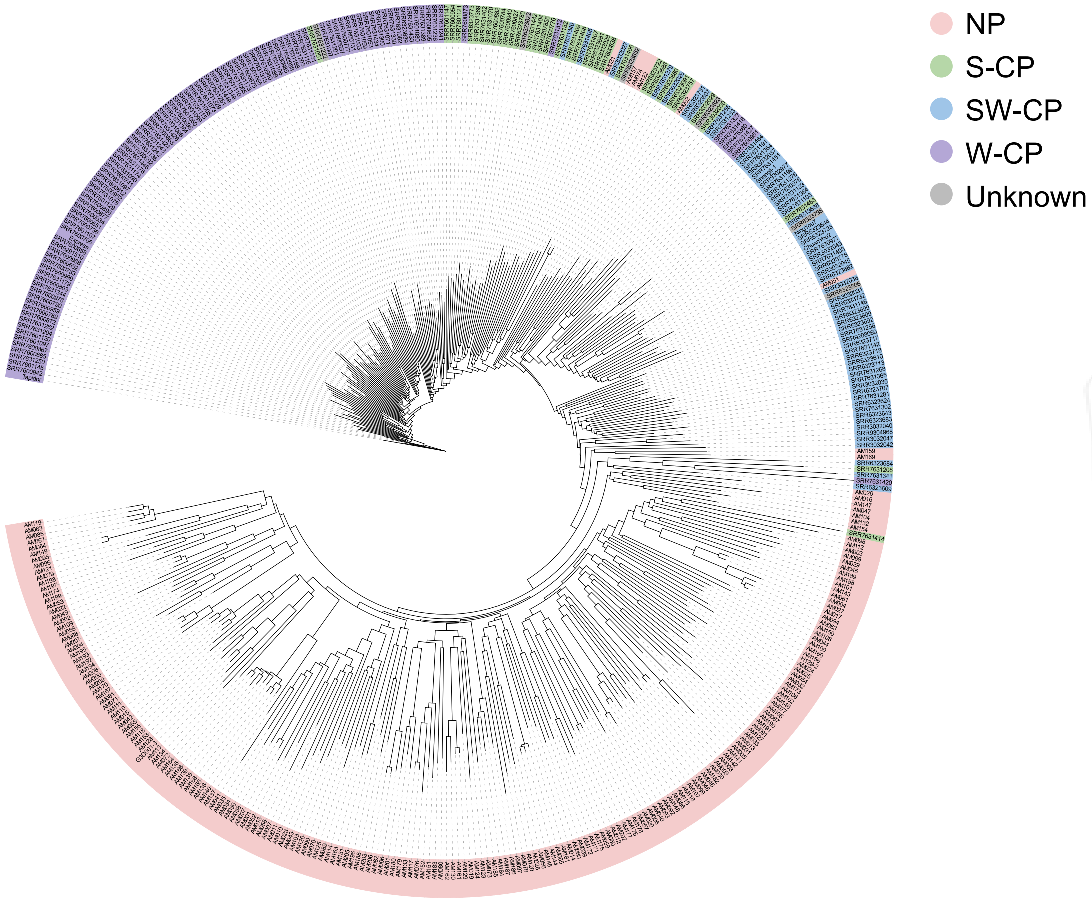
(Hu et al., unpublished data)

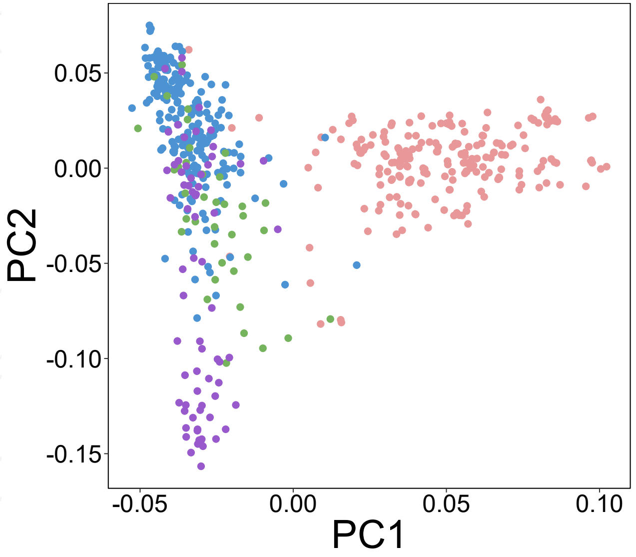
GWAS population: a panel of ~300 diverse lines

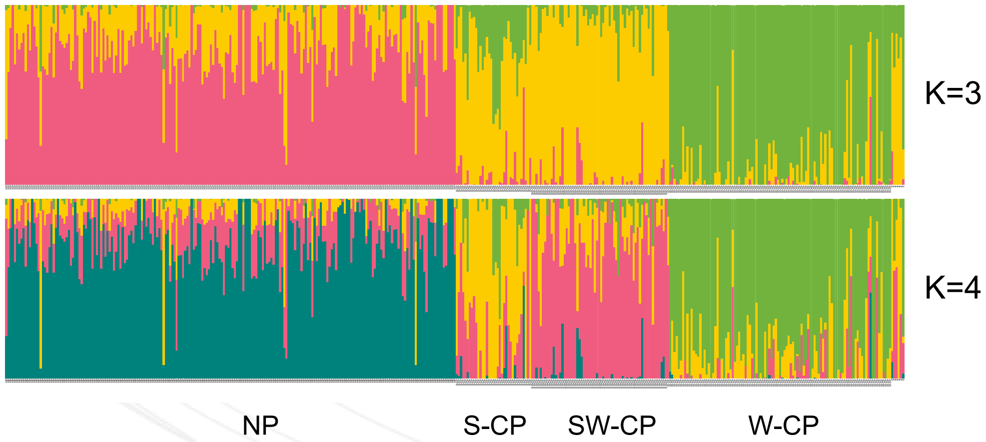



Progress 1 Comprehensive SV in new-type *B. napus*

Substantial sub-population differentiation of new-type *B. napus* population (NP) revealed by whole-genome sequencing

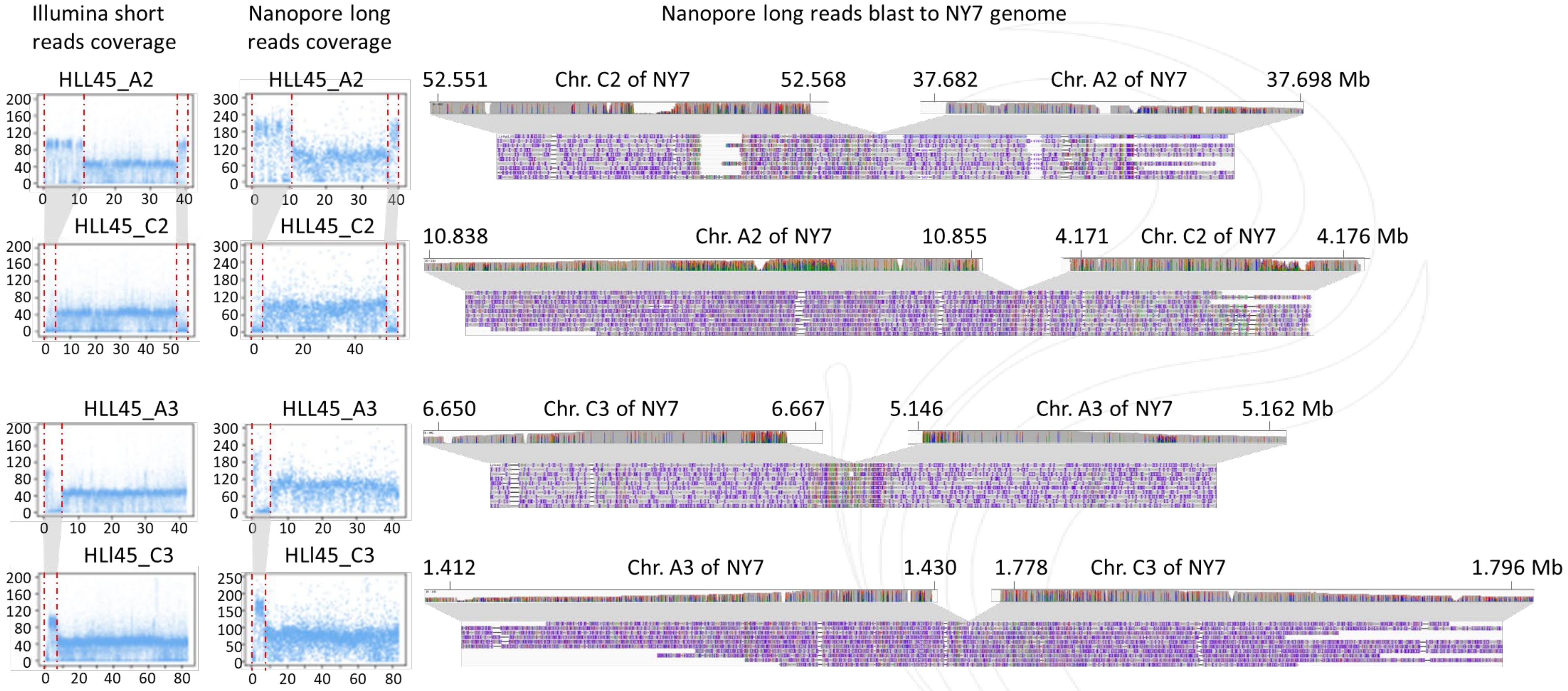






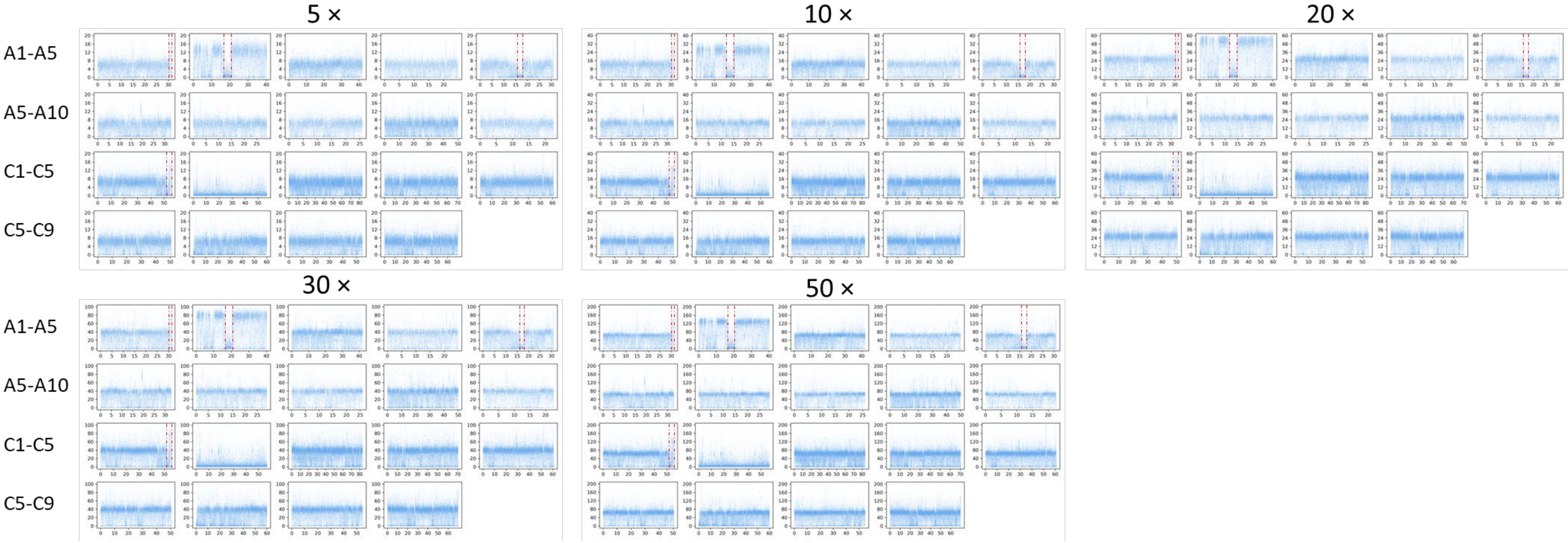
(Han Qin, unpublished data)

Extensive large-scale (>500 kb) SVs fixed in new-type *B. napus* lines



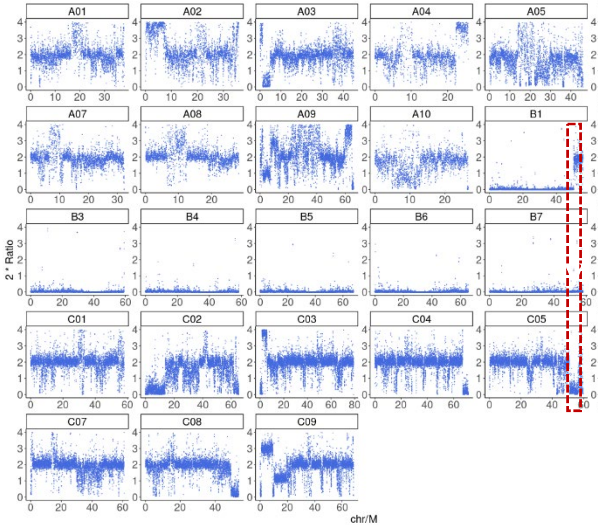
(Hu et al., unpublished data)

The SVs detected by different coverage of sequence data were same, and the SVs detected by 5× genomic sequence data were reliable.

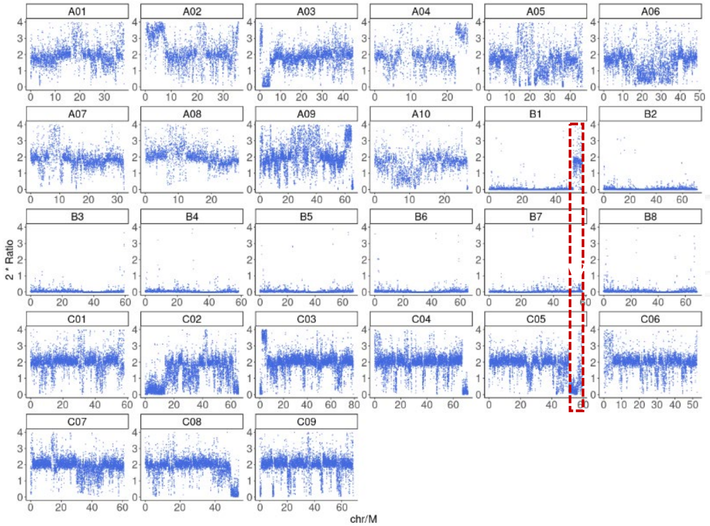


(Unpublished data)

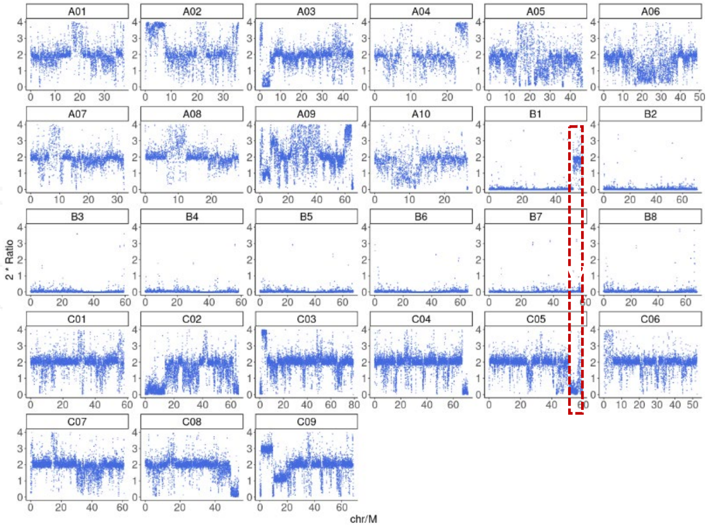
Stable inheritance of large SVs in new-type *B. napus*: an example on C5



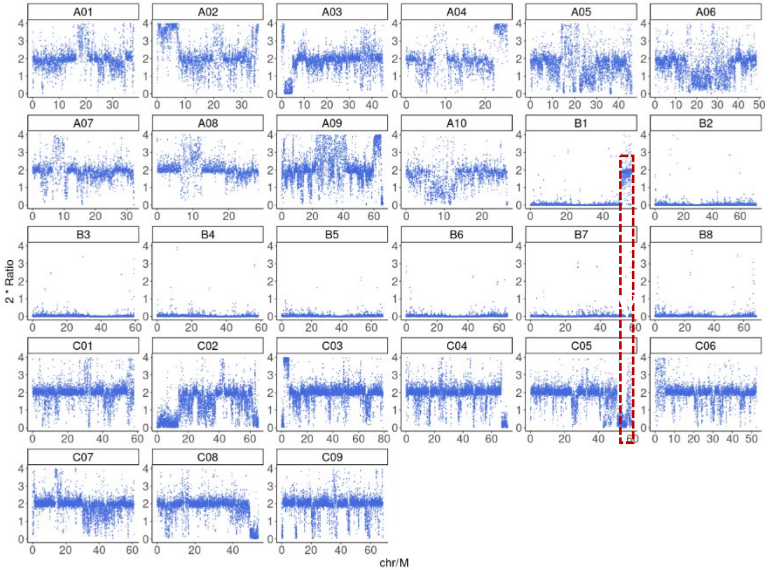
F_{12}



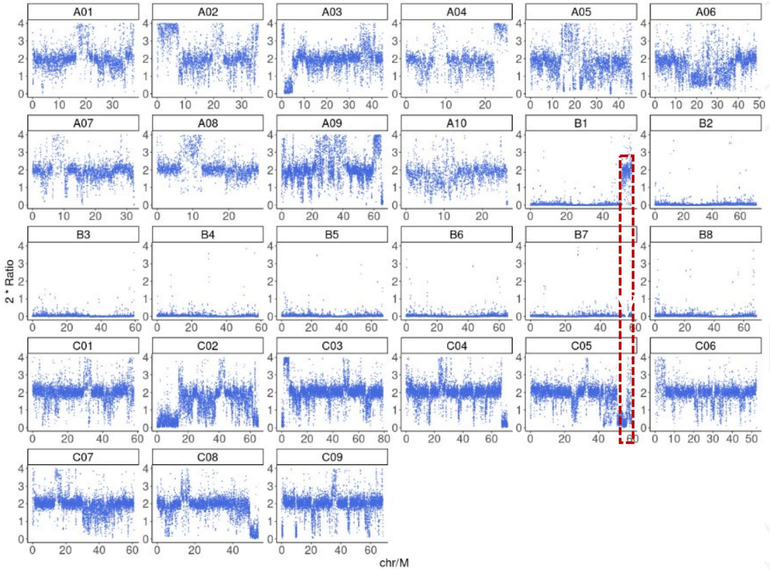
F_{14-1}



F_{14-3}

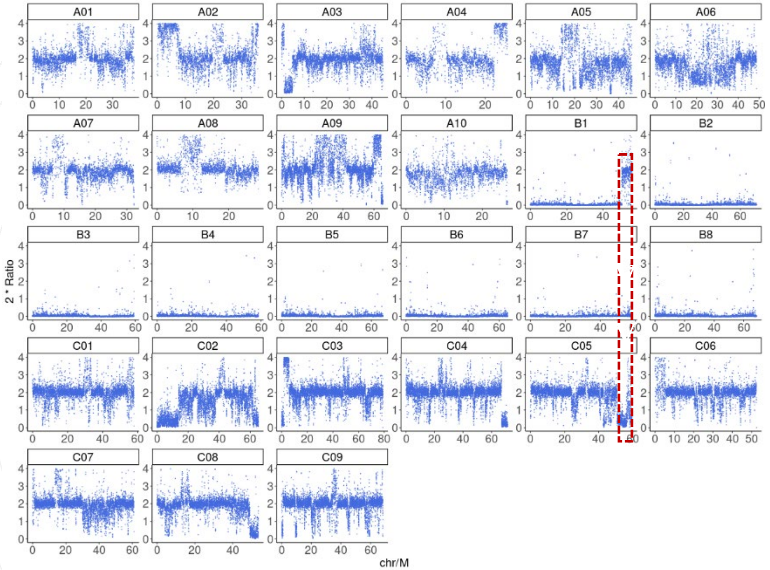


F_{16}



F_{17}-1

(Unpublished data)



F_{17}-2

Complex pattern of the polymorphism markers presented in the DH mapping populations: SV and exotic introgressions

Population	Reference genome	Polymorphic marker
HG DH	A^nC^n	1,326,409
	$A^rB^{c/ni}C^{c/o}$	468,277
	Total	1,794,686
NG DH	A^nC^n	1,420,556
	$A^rB^{c/ni}C^{c/o}$	326,414
	Total	1,746,970
TG DH	A^nC^n	1,479,851
	$A^rB^{c/ni}C^{c/o}$	564,099
	Total	2,043,950

⇨

Specific

Marker type	Parental genotype	
	G3D001	H129-2
Normal	0/0	1/1
	1/1	0/0
PAV	./.	1/1
	./.	0/0
	./.	0/1
	0/0	./.
	0/1	./.
	1/1	./.
Hemi	0/0	0/1
	0/1	1/1
	0/1	0/0
	1/1	0/1

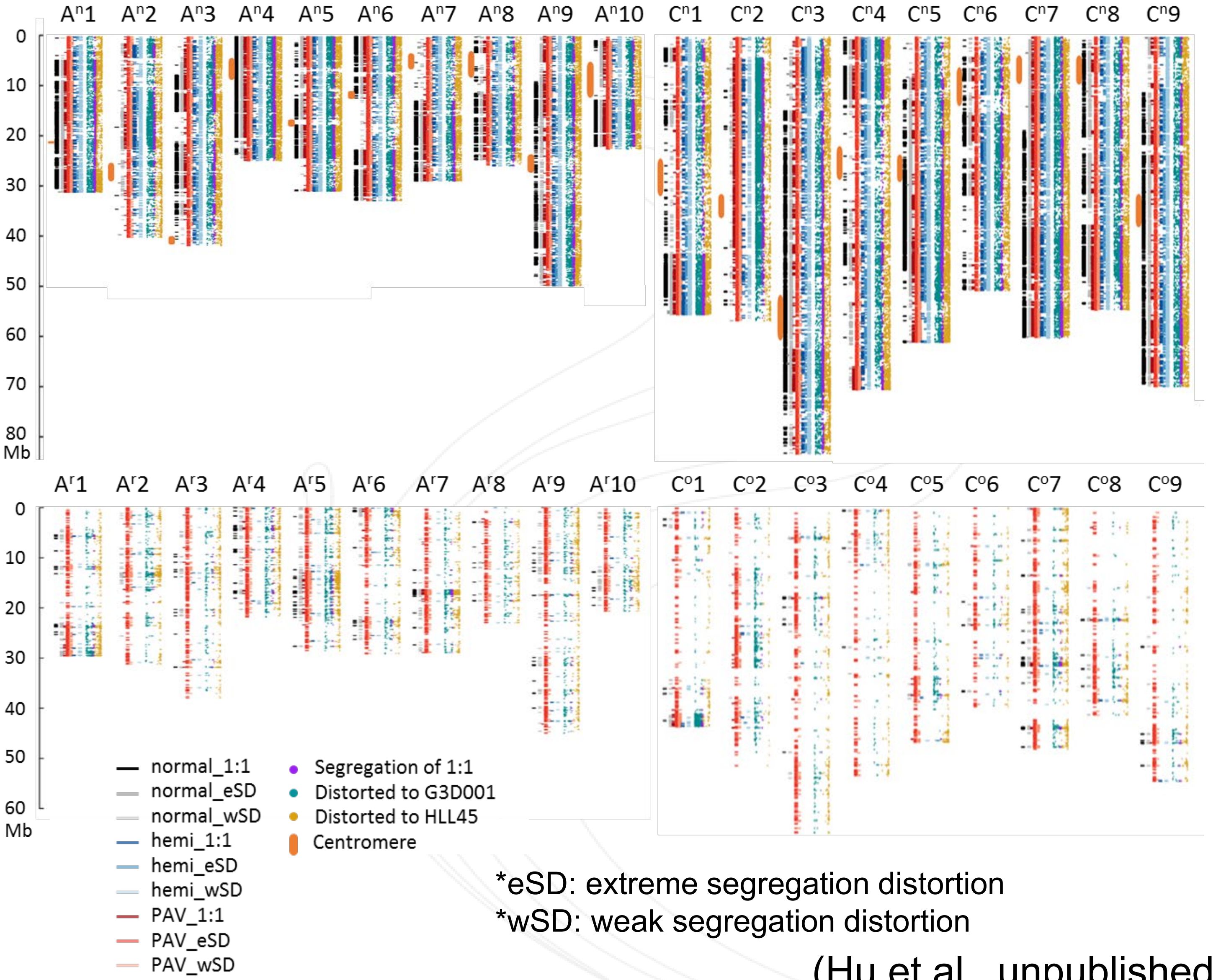
(Hu et al., unpublished data)

Distribution of various markers across the whole genome

Conventional markers with *B. napus* ($A^nA^nC^nC^n$) genome as the reference

Introgressive markers with *B. rapa* (A^rA^r) and *B. oleracea* (C^oC^o) genome as the reference

PAV/Hemi markers

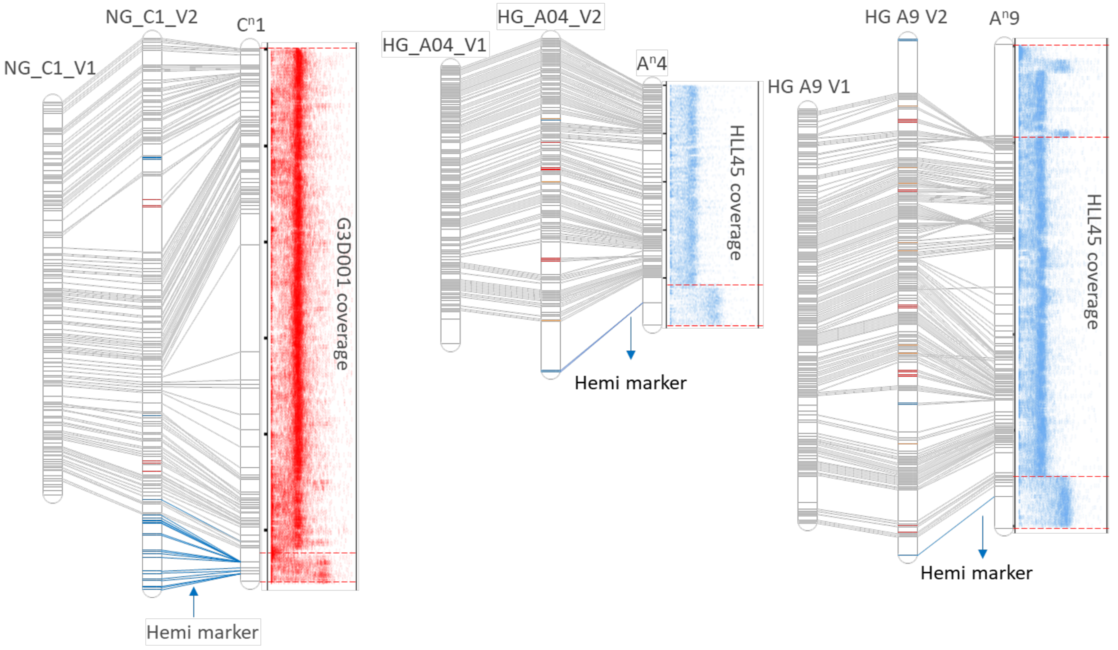


*eSD: extreme segregation distortion

*wSD: weak segregation distortion

(Hu et al., unpublished data)

Mapping more regions for SV and exotic introgressions

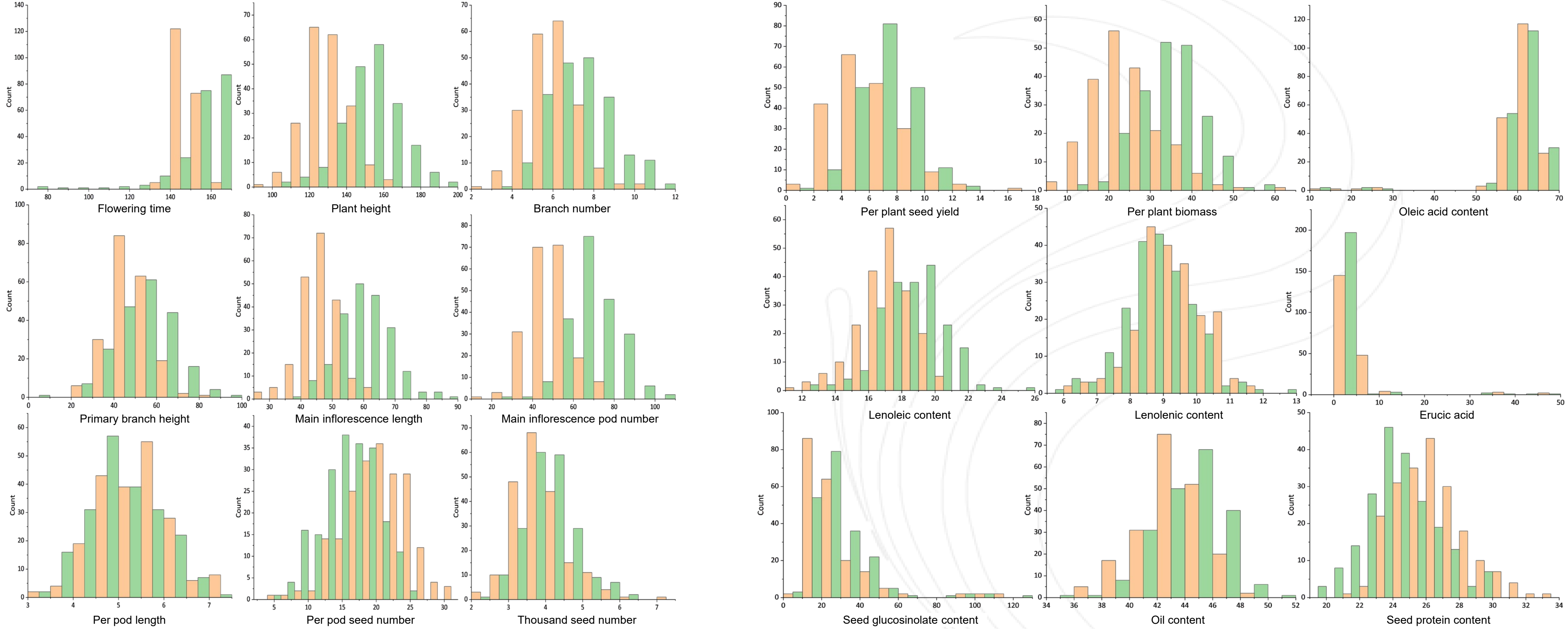


▬ Normal markers ▬ Pav markers ▬ Hemi markers ▬ A^r genomic markers ▬ C^c genomic markers

(Hu et al., unpublished data)

Progress 2 Trait-SV/marker associations for NP

- Phenotyping on three DH populations and GWAS populations: >3 years, 3 replications, important agronomic and quality traits



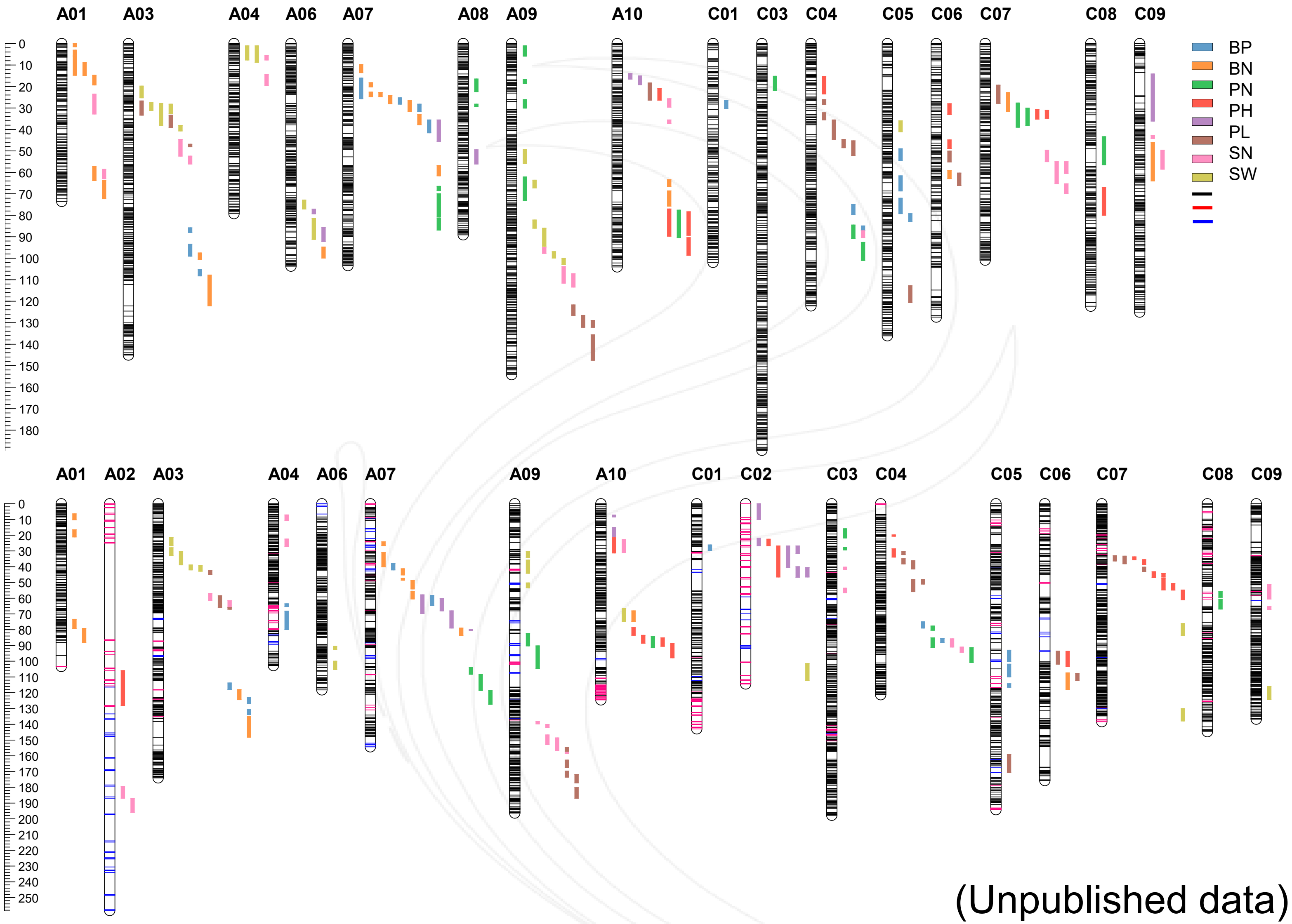
The new-type *B. napus* GWAS population has abundant phenotypic variations

(Han Qin, unpublished data)

Hundreds of QTL mapped for important traits

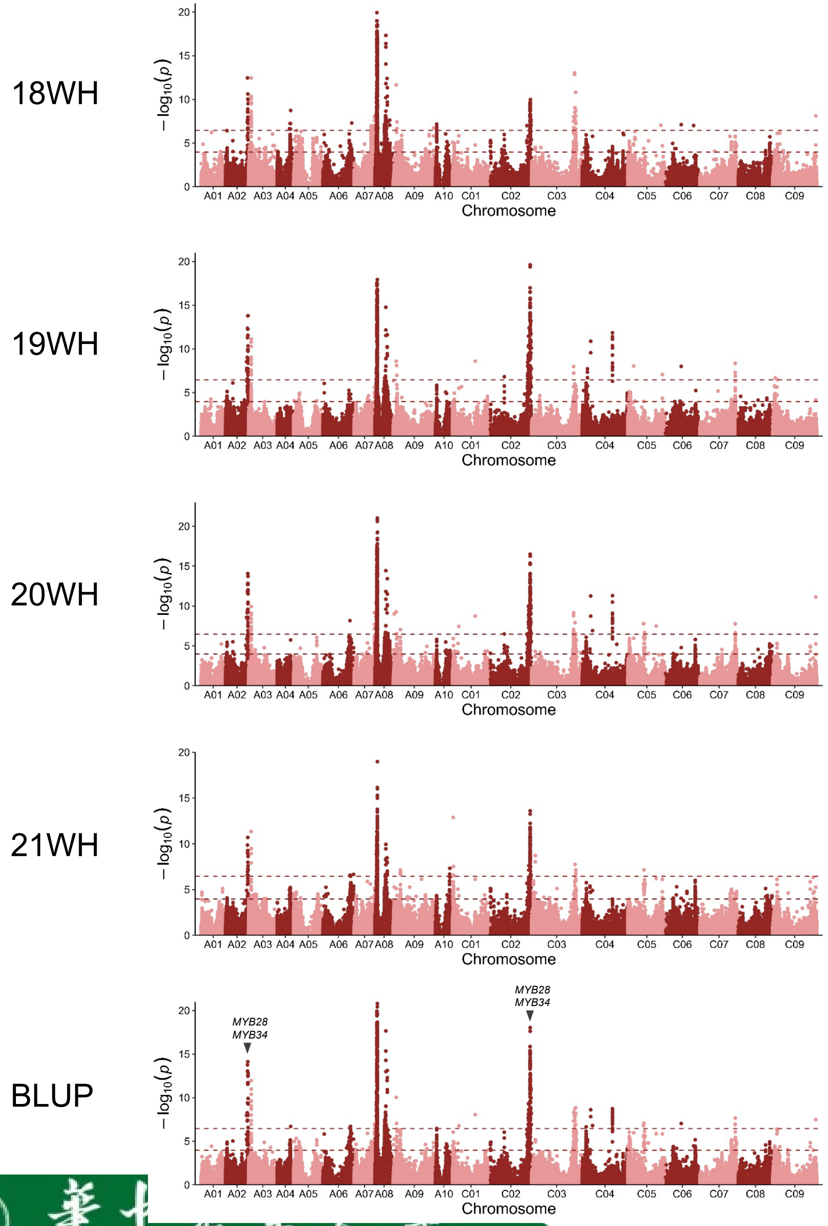
- ~ 500 QTL identified in the three DH mapping populations and diverse GWAS population accounting for development, yield traits, and seed quality traits

- ~150 possible novel QTL different with that detected in conventional *B. napus* populations



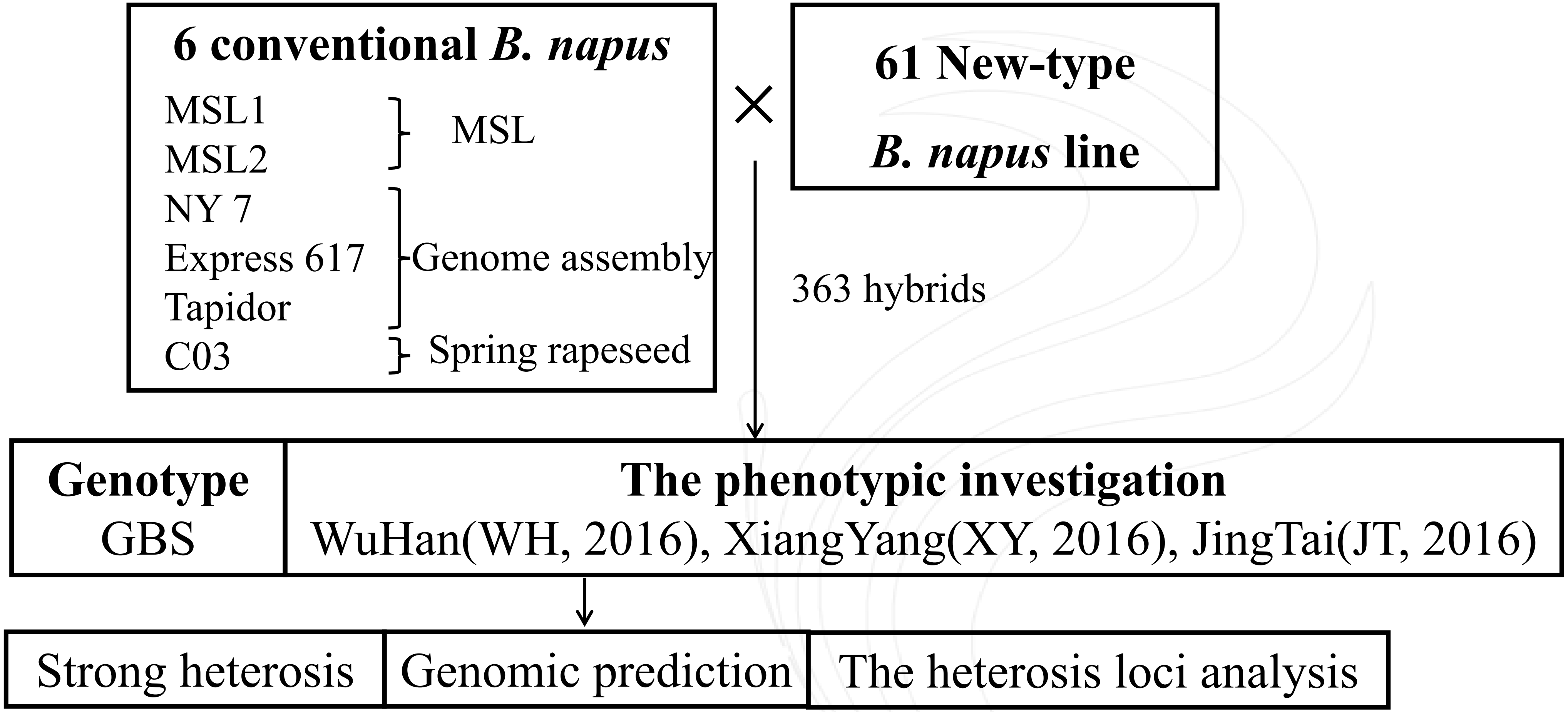
(Unpublished data)

An example: novel QTL for seed glucosinolate content



- Two major QTL were identified on chromosome A02 and C02 , which is related to major genes *MYB28* and *MYB34*.
- Five possible novel QTLs were identified. Especially the QTL on A08 can explain ~60% of the phenotypic variation.

Progress 3 Heterosis-SV/marker associations and GS



6 conventional *B. napus*

MSL1
MSL2 } MSL
NY 7
Express 617 } Genome assembly
Tapidor
C03 } Spring rapeseed

×

61 New-type
***B. napus* line**

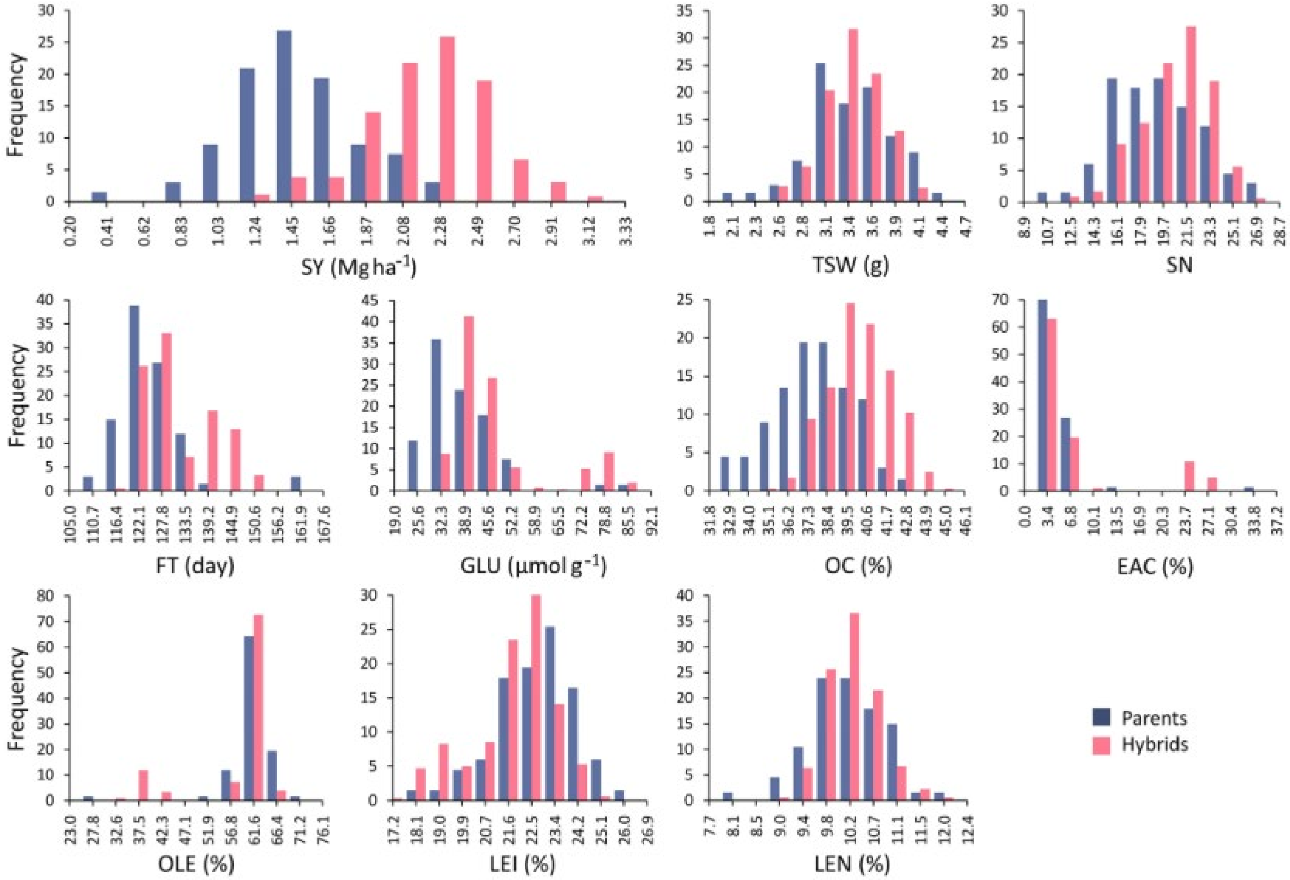
363 hybrids

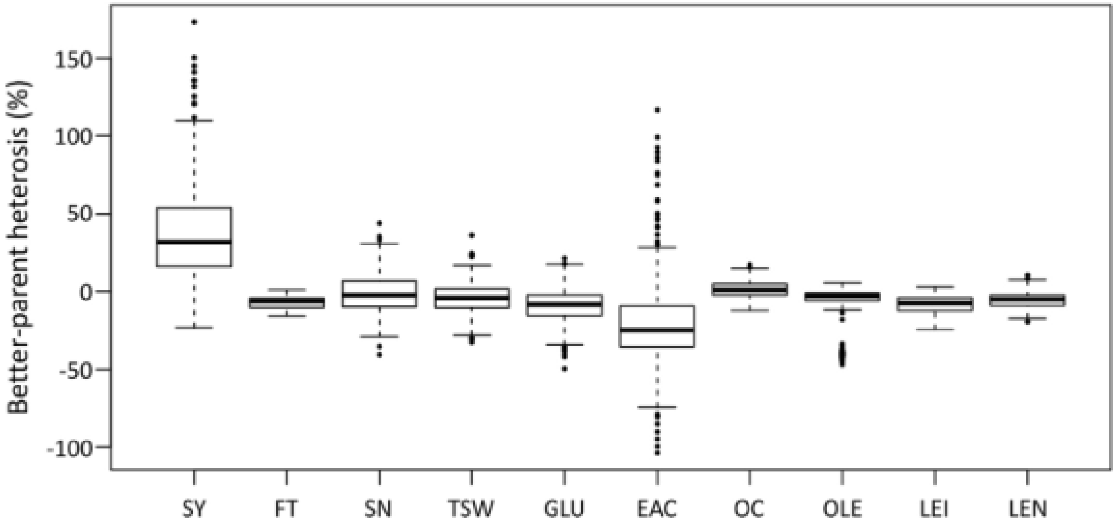
Genotype GBS	**The phenotypic investigation** WuHan(WH, 2016), XiangYang(XY, 2016), JingTai(JT, 2016)

Strong heterosis	Genomic prediction	The heterosis loci analysis

(*Hu et al.*, 2021, Crop Journal)

Strong heterosis in hybrids made between new-type *B. napus* and conventional *B. napus* testers



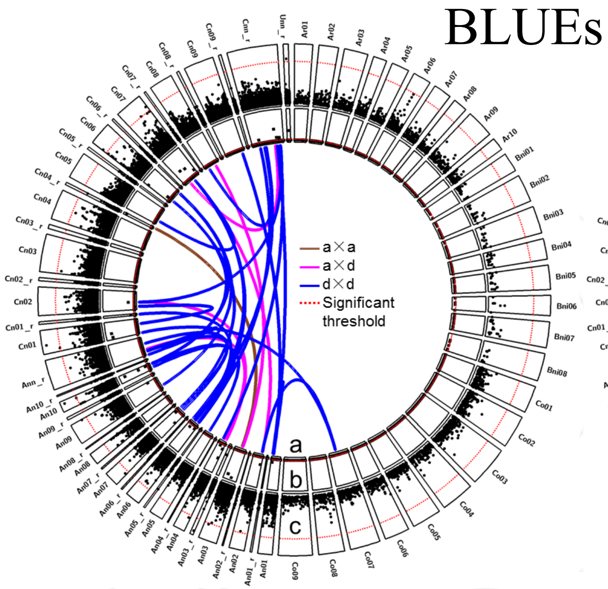


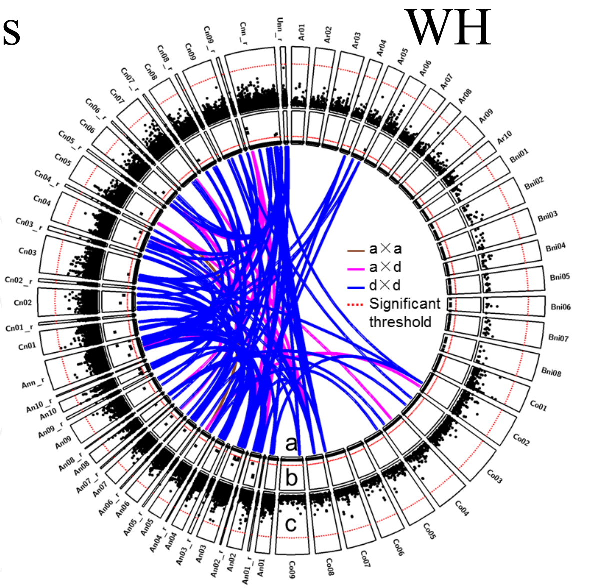
(*Hu et al*., 2021, Crop Journal)

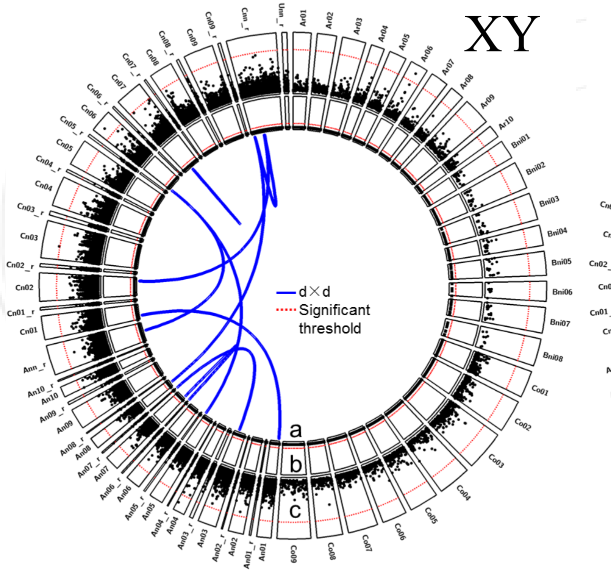
Detection of heterosis loci for seed yield

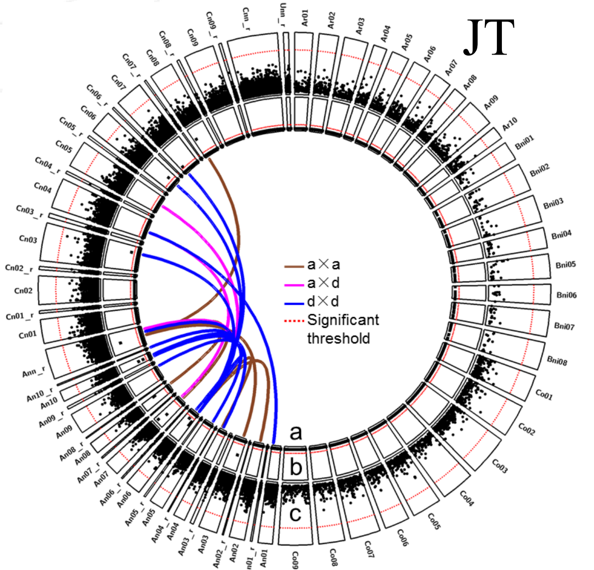
Significant sites	cross	WH	XY	JT
No. significant d effects	13	0	5	0
No. significant a×a effects	1	1	0	9
No. significant a×d effects	7	39	0	4
No. significant d×d effects	31	136	10	26
No. heterotic effects	33	47	18	24

More heterosis sites and d×d and a×d effects were detected in WH environment



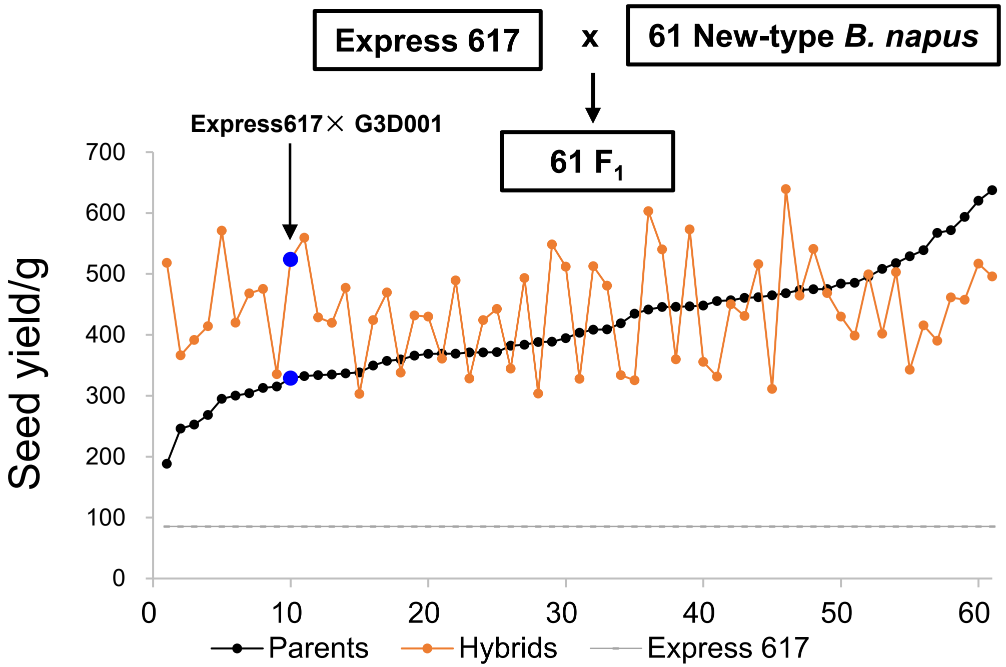






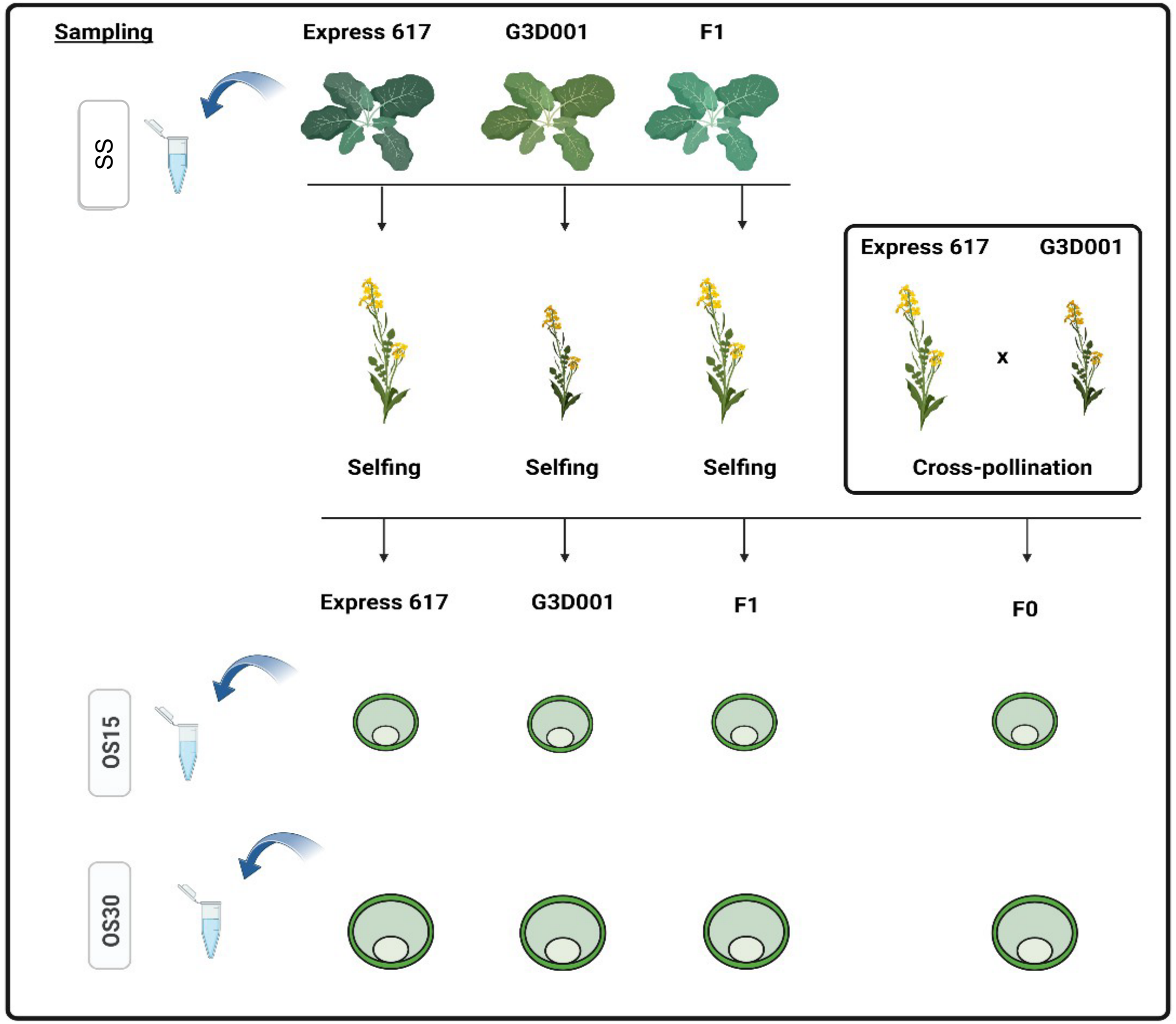
(*Hu et al.*, 2021)

Understanding genomic-basis of heterosis with omics-approach



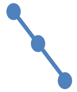
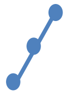
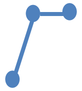
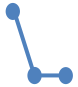
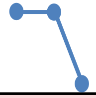
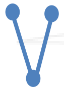
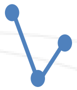
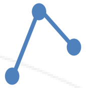
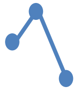
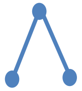
Express 617 x 61 New-type *B. napus*

61 F_1

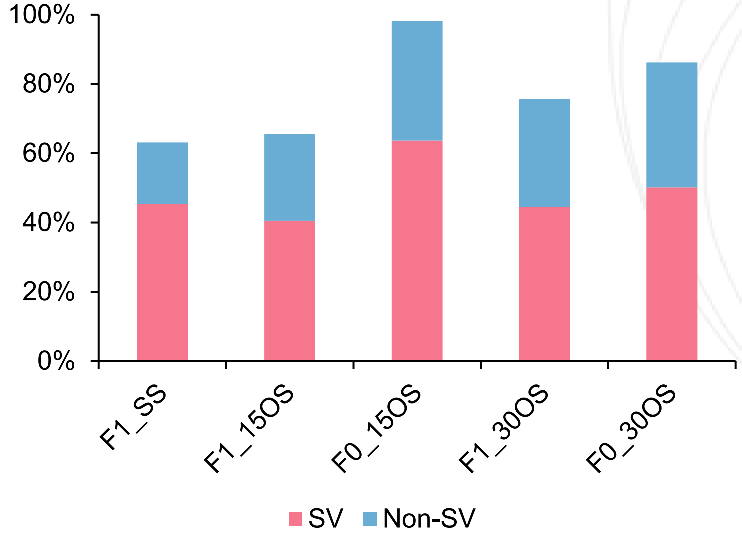


Cooperation with Prof. Rod Snowdon on NSFC-DFG cooperation project (2019-2021)

Predominant maternal dominant expression in DEGs

Type	Feature	Additivity		ELD-G3D001		ELD-Express 617		Heterotic down-regulation			Heterotic up-regulation		
		I	XII	II	XI	IV	IX	III	VII	X	V	VI	VIII
		E-F-G	E-F-G	E-F-G	E-F-G	E-F-G	E-F-G	E-F-G	E-F-G	E-F-G	E-F-G	E-F-G	E-F-G
													
mRNA	DEG_F1_SS	25.20%	8.68%	9.01%	20.34%	15.06%	18.75%	0.08%	0.77%	0.19%	0.13%	0.18%	1.61%
	DEG_F1_15OS	9.79%	6.44%	9.59%	18.16%	13.20%	24.58%	0.89%	3.64%	0.26%	0.49%	1.53%	11.43%
	DEG_F0_15OS	0.55%	0.08%	0.09%	1.04%	46.34%	50.78%	0.09%	0.13%	0.00%	0.00%	0.50%	0.40%
	DEG_F1_30OS	8.75%	5.45%	5.49%	11.72%	20.69%	37.91%	0.81%	1.74%	0.06%	0.28%	2.32%	4.80%
	DEG_F0_30OS	7.37%	2.18%	1.93%	3.21%	32.68%	48.52%	0.34%	0.53%	0.01%	0.05%	1.38%	1.81%

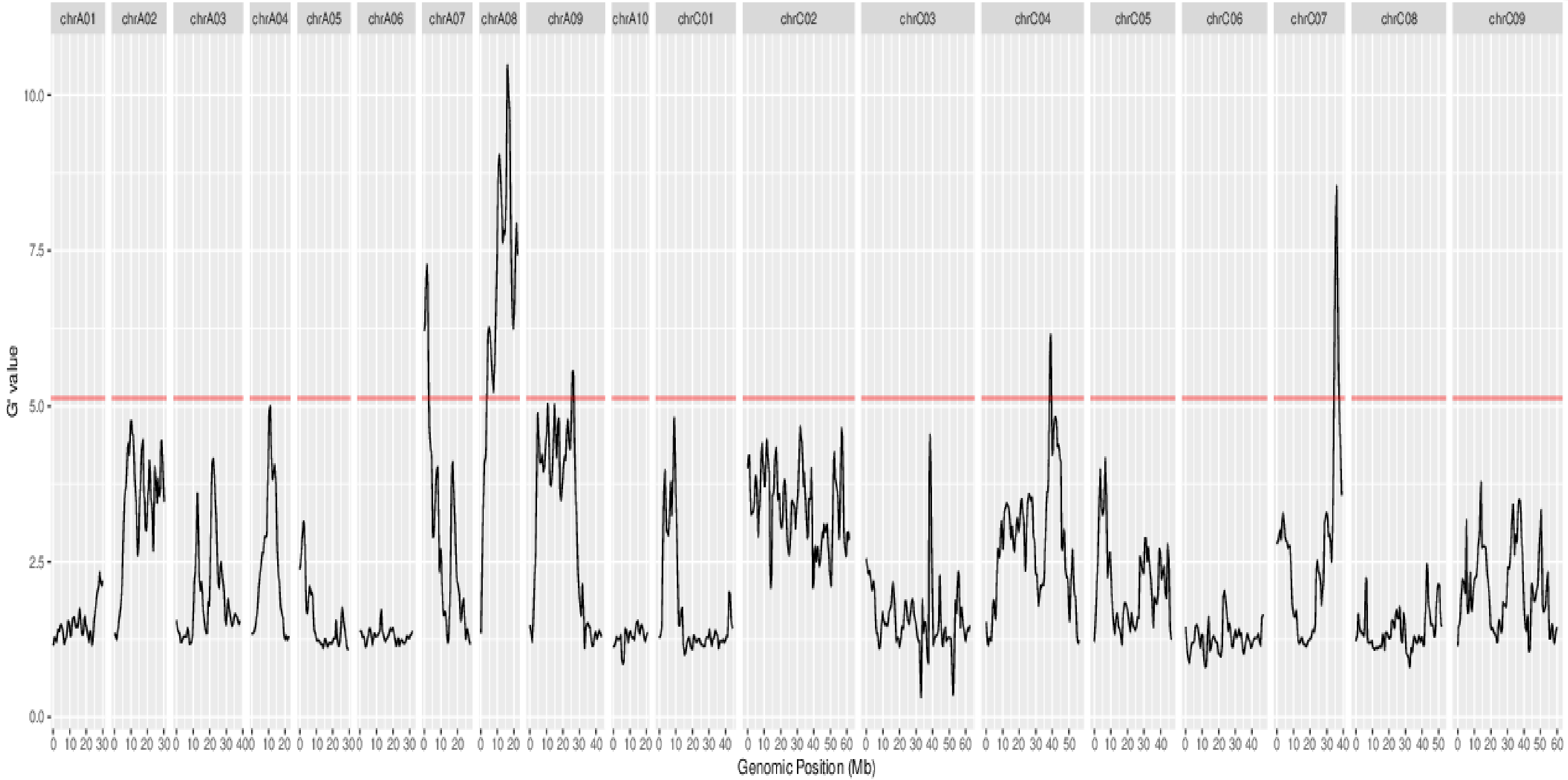
(Orantes-Bonilla et al., 2023, TAG)



The dominant expression level of SV-DEGs were higher than that of the non-SV DEGs

(Hao Wang, unpublished data)

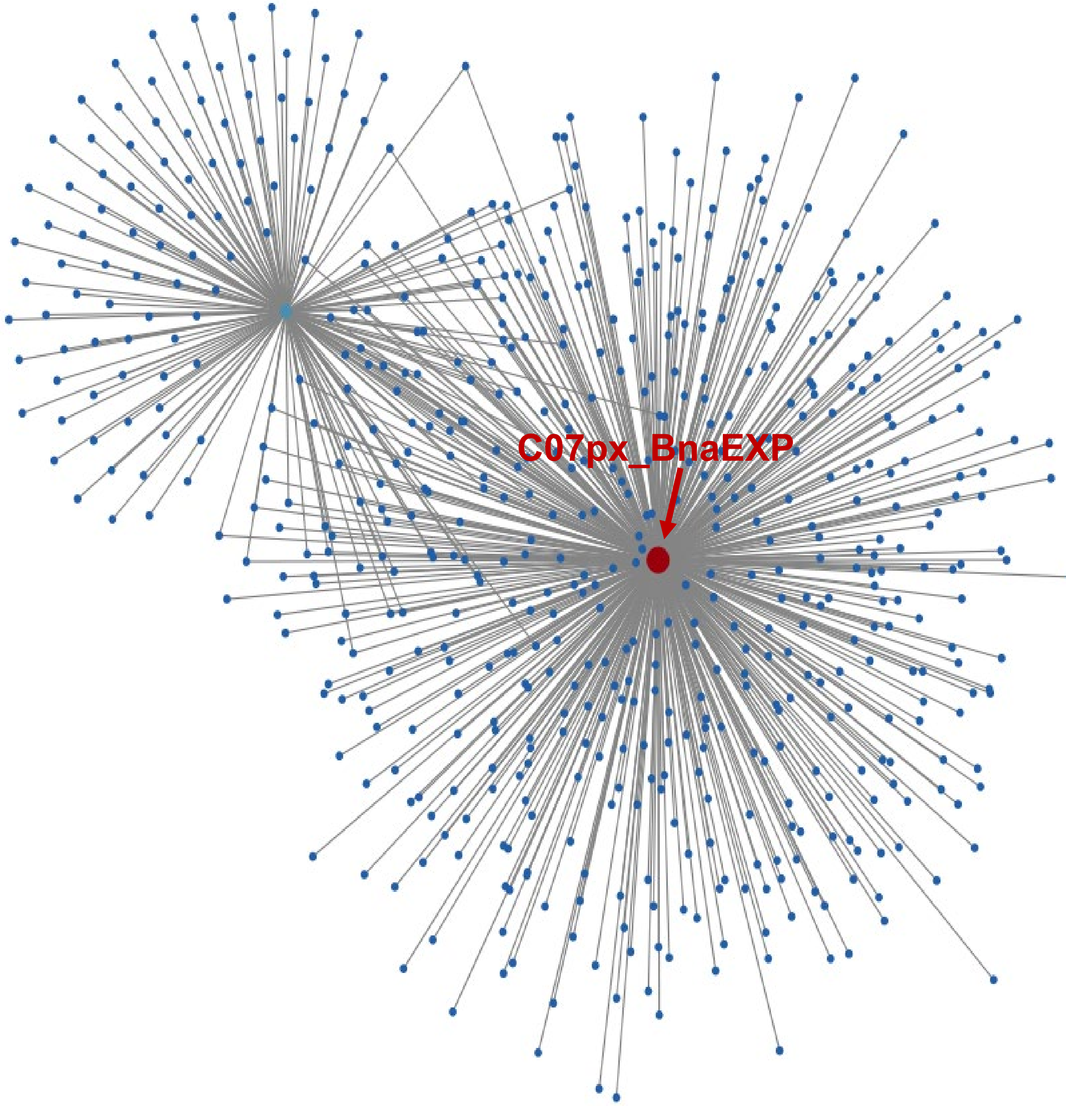
Further QTL mapping in EGF$_{2:3}$ population revealed an exotic introgression of *B. carinata* increasing seed weight

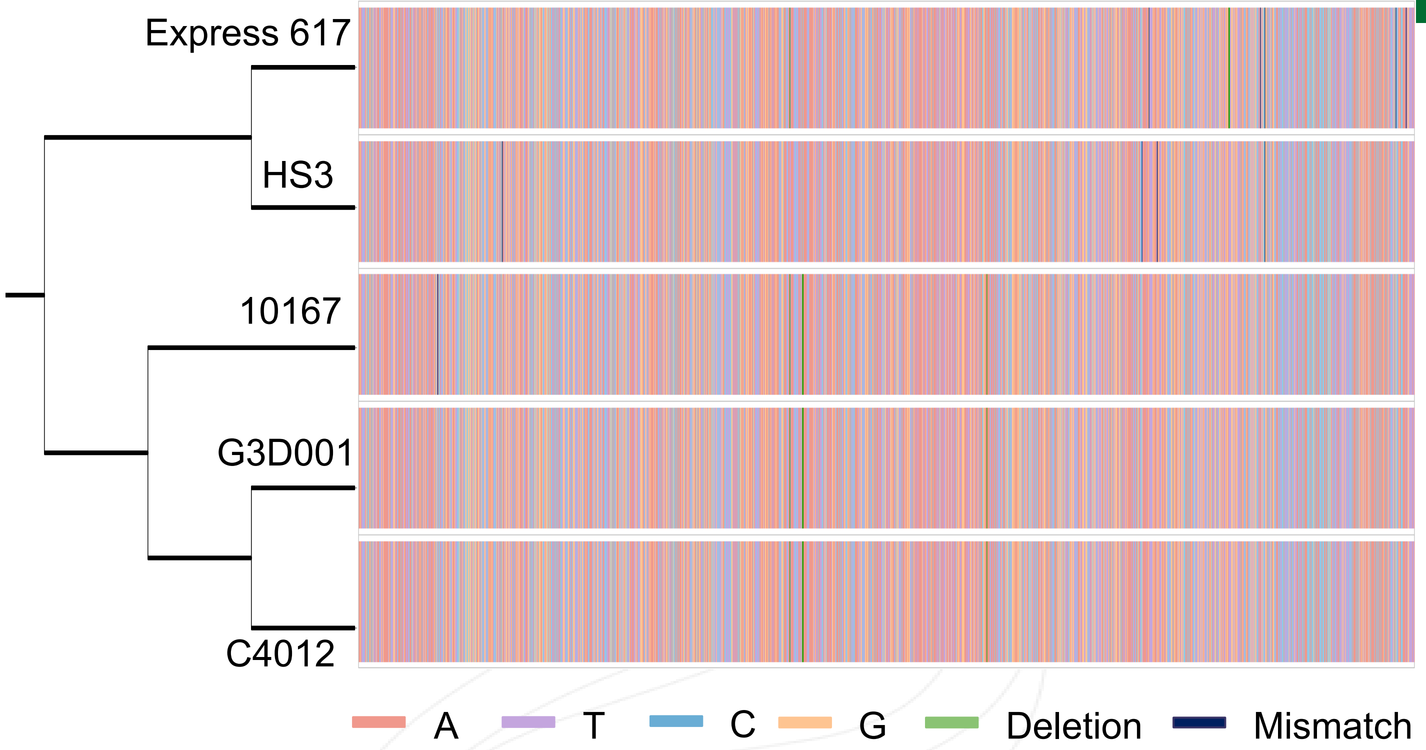


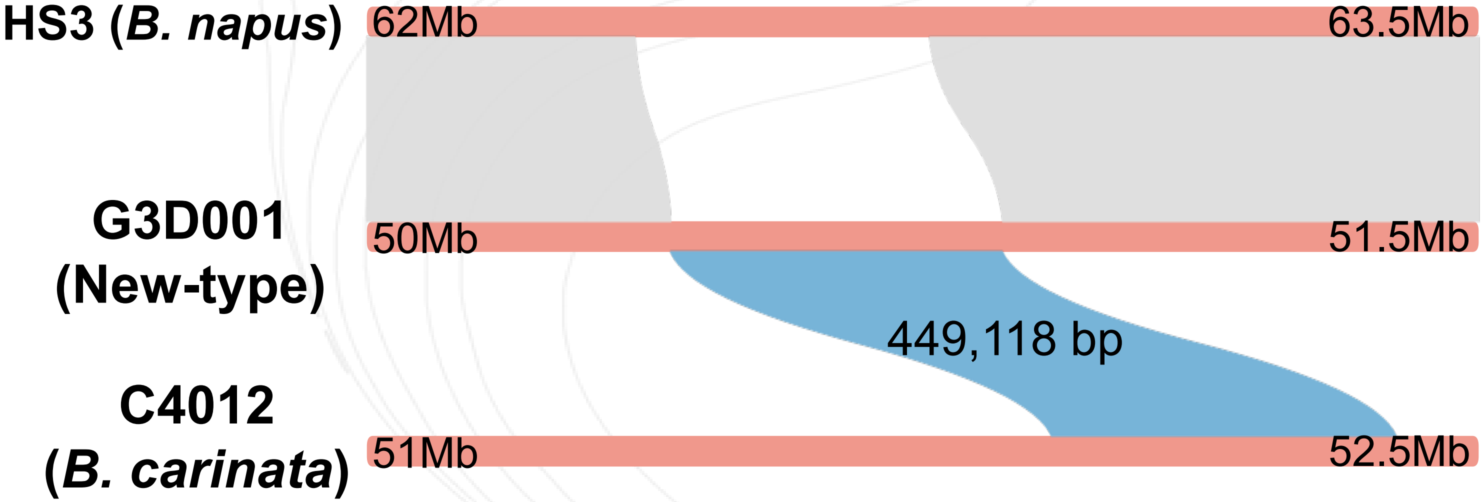
Chr	C07
Position(cM)	130
Effect.a	-0.0968
Effect.d	-0.2146
LOD	5.7164
Left marker	33897194
Right marker	35451143_pav
R2(%)	13.1106

This QTL on C07 were detected by both of the BSA and QTL mapping

(Hao Wang, unpublished data)

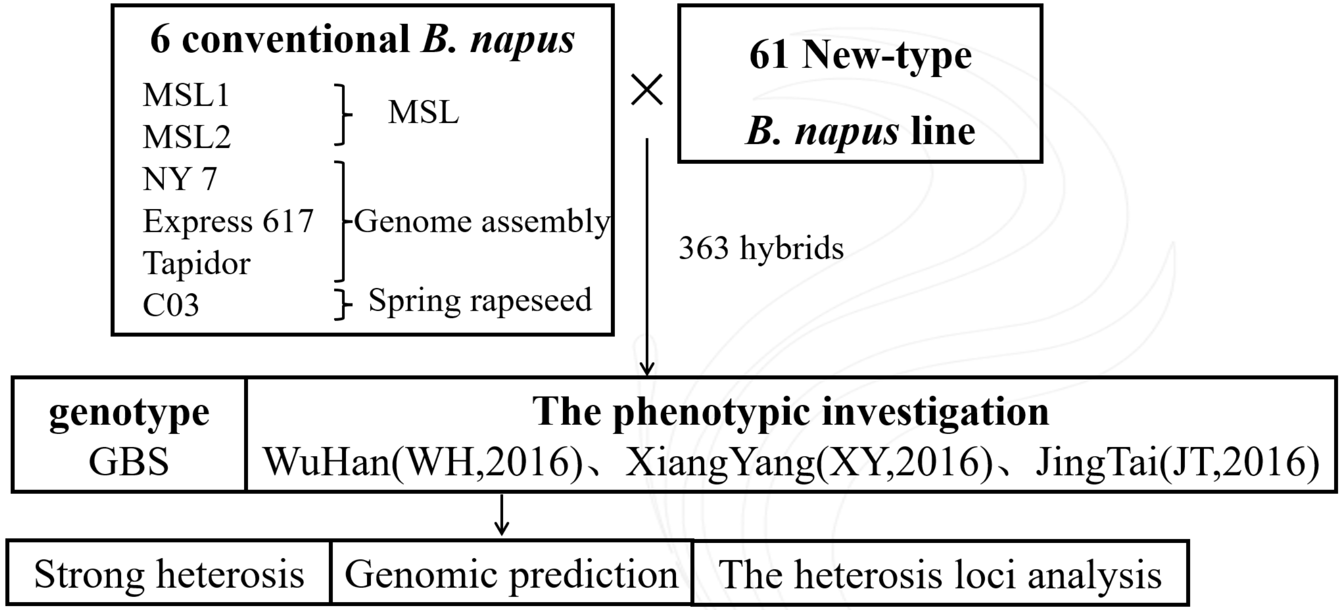






(Hao Wang, unpublished data)

GS test: using the 363 hybrids as training population



6 conventional *B. napus*
MSL1
MSL2 } MSL
NY 7
Express 617
Tapidor } Genome assembly
C03 } Spring rapeseed

×

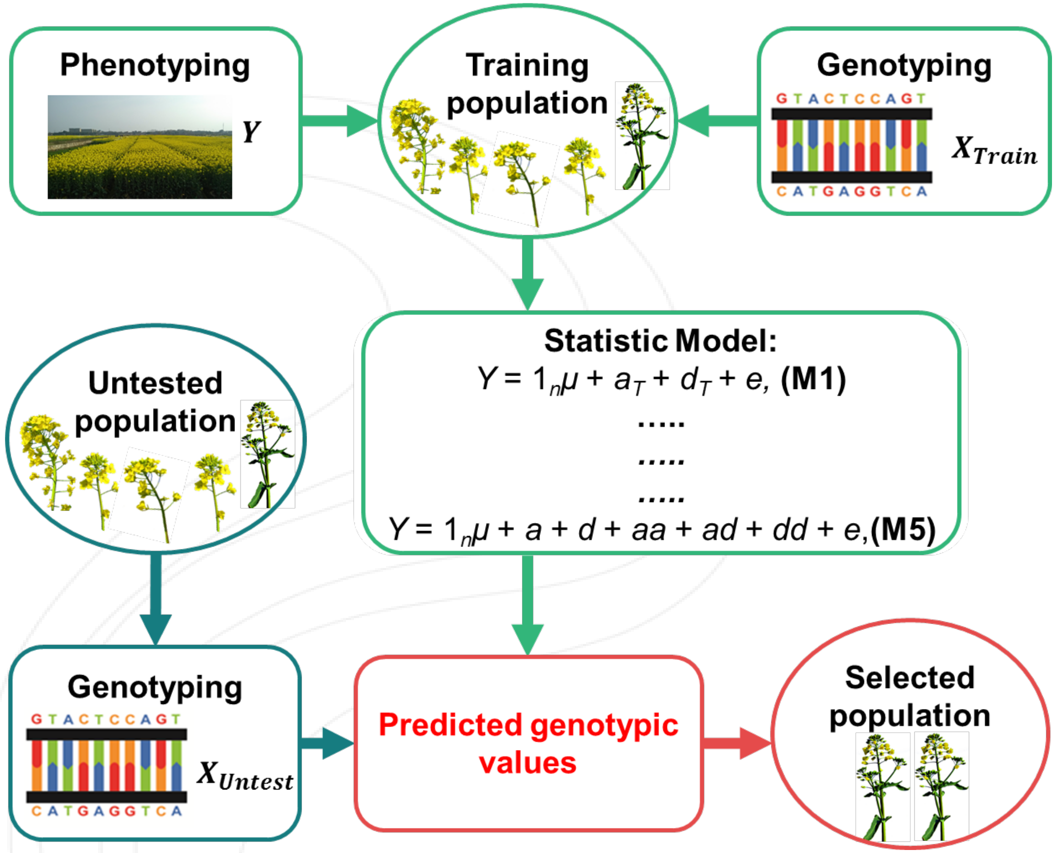
61 New-type *B. napus* line

363 hybrids

genotype
GBS

The phenotypic investigation
WuHan(WH,2016)、XiangYang(XY,2016)、JingTai(JT,2016)

Strong heterosis | Genomic prediction | The heterosis loci analysis



Statistic Model:

$Y = 1_n\mu + a_T + d_T + e$, **(M1)**

.....

.....

.....

$Y = 1_n\mu + a + d + aa + ad + dd + e$,**(M5)**

Test accuracy by constructing models incorporating different markers and genetic effects

(*Hu et al*., 2021, Crop Journal)

The prediction accuracy was improved for 7 traits by adding exotic introgression-related markers

$$Y = 1_n\mu + a_T + d_T + e, \quad \text{(M1)}$$

$$Y = 1_n\mu + a_T + a_S + d_T + d_S + e, \quad \text{(M3)}$$

Test pop.	Model	SY	Ft	SN	SW	GLSC	EAC	OC	OLC	HLC	HLLC
TS2	M1	0.660	0.758	0.591	0.589	0.635	0.691	0.585	0.626	0.760	0.669
	M2	0.561	0.742	0.621	0.585	0.690	0.711	0.561	0.677	0.748	0.693
	M3	0.637	0.759	0.631	0.604	0.672	0.718	0.592	0.631	0.751	0.682
	M4	0.649	0.768	0.637	0.609	0.648	0.717	0.609	0.656	0.756	0.692
TS1	M1	0.460	0.759	0.503	0.515	0.597	0.611	0.443	0.582	0.630	0.537
	M2	0.377	0.737	0.499	0.497	0.658	0.642	0.436	0.625	0.636	0.575
	M3	0.442	0.760	0.513	0.515	0.636	0.645	0.451	0.581	0.627	0.540
	M4	0.462	0.770	0.515	0.500	0.609	0.639	0.445	0.605	0.625	0.548
TS0	M1	0.173	0.583	0.179	0.392	0.076	−0.250	0.106	−0.145	0.142	0.137
	M2	0.006	0.530	0.129	0.394	0.223	−0.117	0.069	−0.055	0.212	0.190
	M3	0.127	0.552	0.176	0.402	0.104	−0.170	0.107	−0.189	0.184	0.135
	M4	0.129	0.574	0.221	0.383	0.025	−0.195	0.095	−0.163	0.150	0.129

The best models for different traits varied, and the predication accuracy was the highest for multi-environment intergrated data

Env.	M	SY	FT	SN	SW	GSLC	EAC	OC	OLC	HLC	HLLC
Mul-integrated	M1	0.728	0.972	0.751	0.649	0.971	0.990	0.690	0.987	0.910	0.828
	M2	0.685	0.964	0.769	0.650	0.964	0.988	0.693	0.986	0.906	0.822
	M3	0.727	0.971	0.765	0.654	0.970	0.989	0.700	0.987	0.911	0.825
	M4	0.734	0.973	0.754	0.651	0.971	0.990	0.701	0.987	0.913	0.829
WH	M1	0.574	0.959	0.771	0.569	0.953	0.980	0.484	0.975	0.853	0.746
	M2	0.581	0.952	0.780	0.591	0.948	0.978	0.512	0.973	0.853	0.722
	M3	0.585	0.959	0.782	0.586	0.951	0.979	0.501	0.974	0.859	0.738
	M4	0.578	0.961	0.775	0.569	0.954	0.980	0.475	0.975	0.858	0.745
XY	M1	0.347	0.947	0.692	0.694	0.980	0.989	0.426	0.989	0.912	0.687
	M2	0.245	0.939	0.702	0.740	0.978	0.987	0.424	0.988	0.913	0.681
	M3	0.318	0.947	0.705	0.733	0.980	0.988	0.441	0.989	0.915	0.688
	M4	0.337	0.951	0.699	0.716	0.979	0.989	0.420	0.990	0.912	0.687
JT	M1	0.619	0.849	-	0.581	0.967	0.988	0.771	0.980	0.890	0.804
	M2	0.564	0.801	-	0.541	0.963	0.985	0.735	0.977	0.881	0.804
	M3	0.614	0.843	-	0.572	0.967	0.987	0.760	0.978	0.884	0.802
	M4	0.611	0.847	-	0.584	0.967	0.987	0.771	0.980	0.887	0.803

Cooperation with Prof. Joch C. Reif (IPK)

Summary

- Frequent large-scale SV drives the genome diversity and plasticity of *de novo* domesticated *B. napus*
- SVs were not randomly generated and could be fixed through influence on phenotypes during artificial selection
- Hundreds of QTL were identified in new-type *B. napus* and their derived hybrid populations
- The novel QTL identified in new-type *B. napus* population would provide valuable genetic resource for rapeseed breeding
- The GS accuracy would be further improved by adding SV-related markers
- Further genome-based approach will be performed to explore and add the breeding value of the novel gene pool

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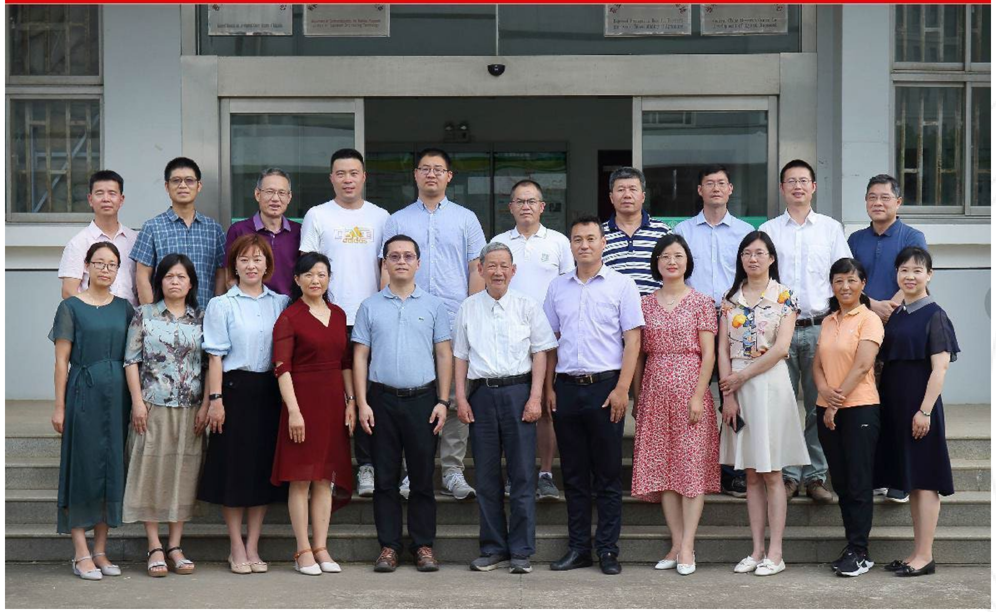

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