The 16th International Rapeseed Congress

A systematic dissection in oilseed rape provides insights into the genetic architecture and molecular mechanism of yield heterosis

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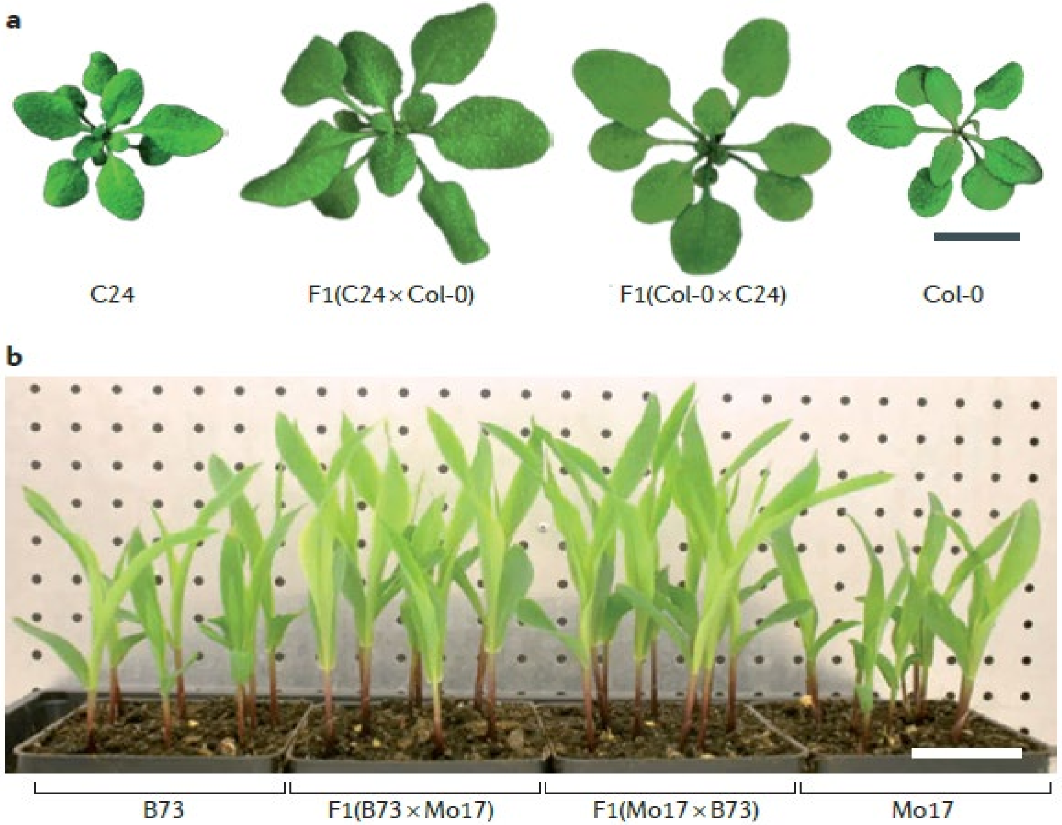
Outline

1. **Research background**
2. **Phenotypic analysis of heterosis in rapeseed**
3. **Dissecting the genetic basis of yield heterosis**
4. **Cloning the gene for yield heterois**
5. **Dissecting the regulatory mechanism of yield heterosis**
6. **Summary and conclusions**

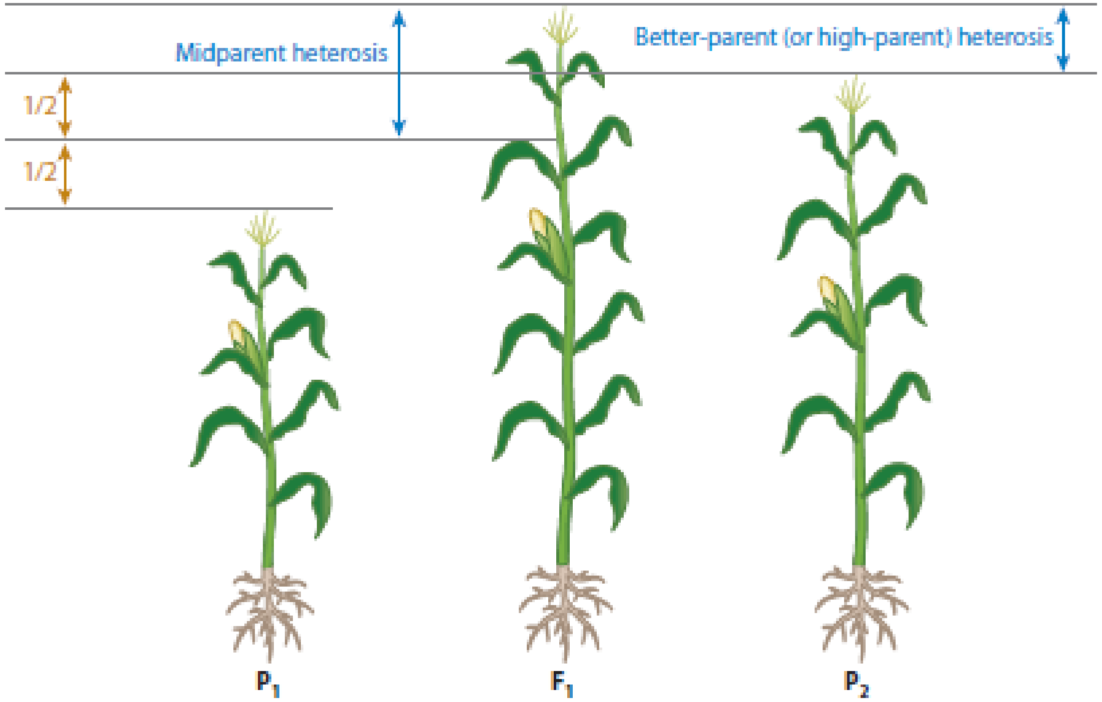
1. Research background

(1) What is heterosis?



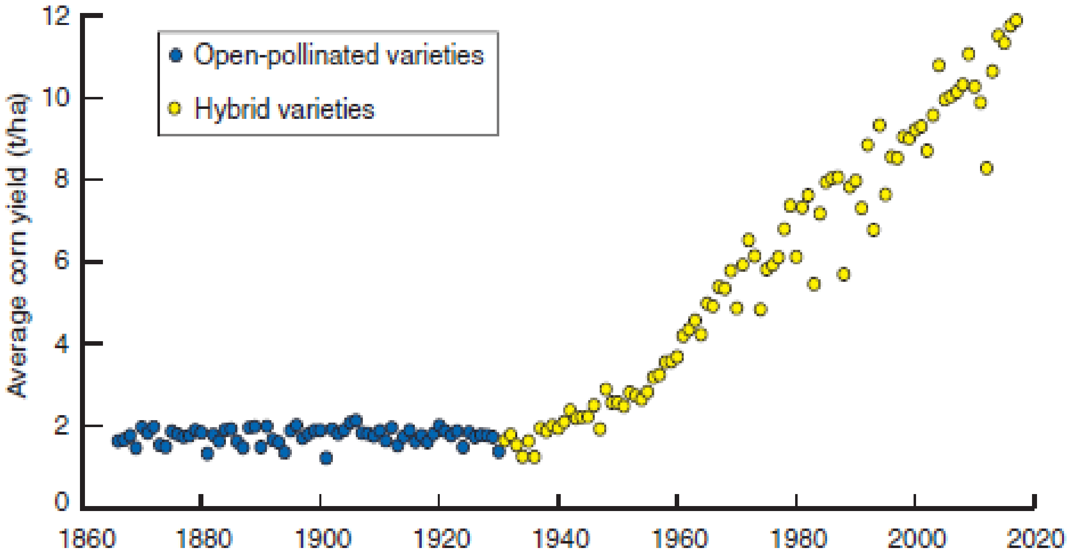
- Heterosis is an universal biological phenomenon in which hybrid offspring outperform parents in terms of growth, fitness, resistance, and yield etc.

(2) How to measure heterosis?



- Mid-parent heterosis value: $F1-(P_1+P_2)/2$
- MPH rate (%): [F1-(P1+P2)/2]/[(P1+P2)/2]
- Better-parnet heterosis value: F1-BP
- BPH rate(%): (F1-BP)/BP.

(3) How importance of heterosis?

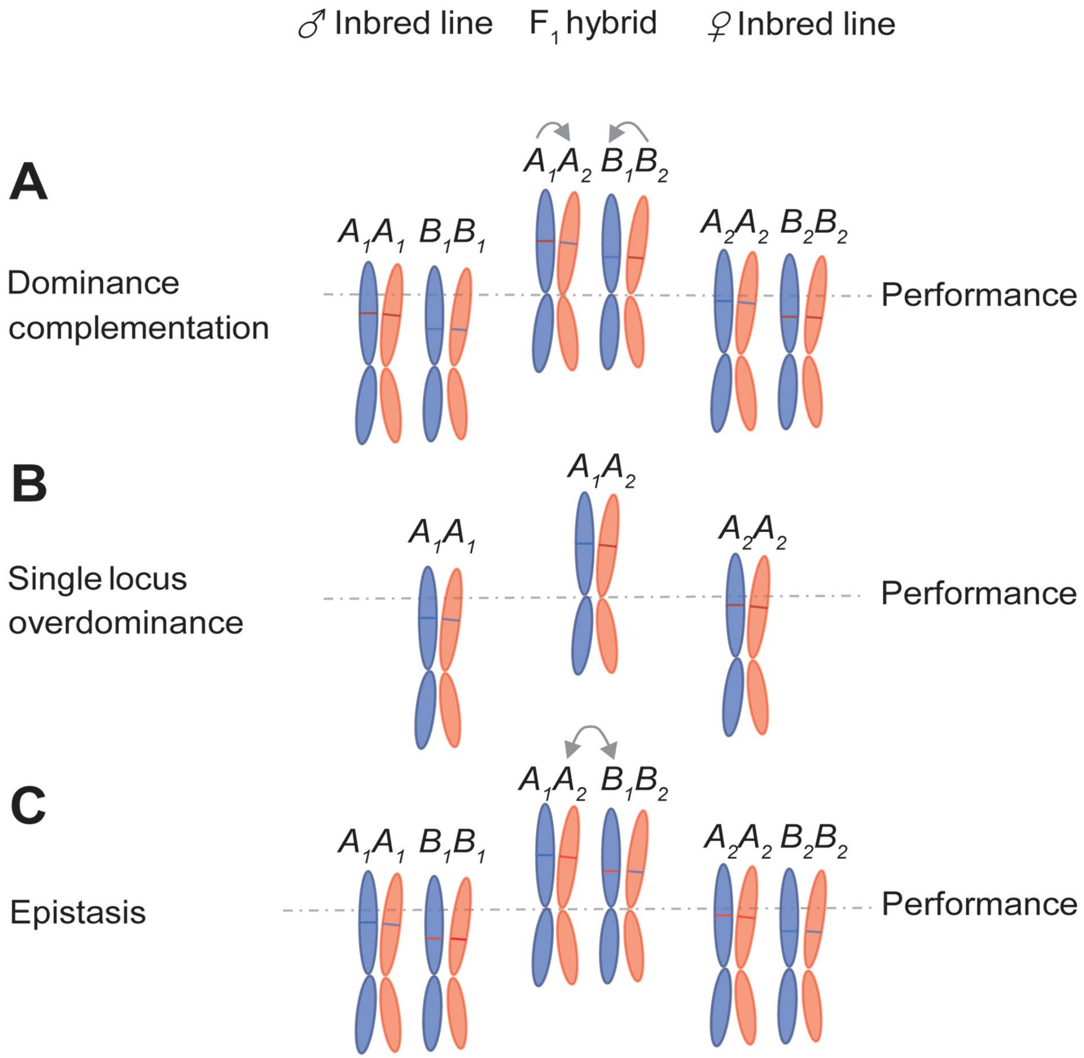


Data source: United States Department of Agriculture, National Agricultural Statistics Service (www.nass.usda.gov).

Share of hybrid varieties for main crops:

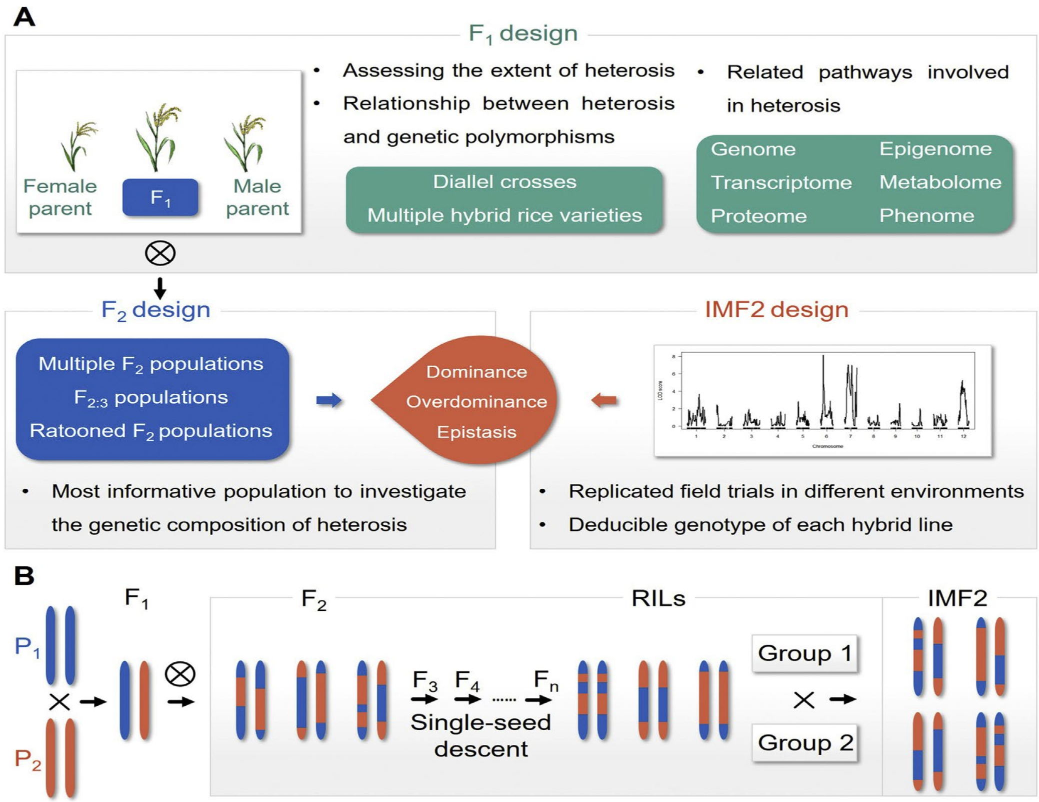
- Maize: ~100% in the world.
- Rice: >70% in China.
- Rapeseed: >90% in Europe and Canada, >70% in China.
- Cotton: >80% in India.
- Sugar beet: ~100% in Europe.

(4) The genetic hypotheses of heterosis



- Several classical models have long been proposed to explain the genetic basis of heterosis.

(5) The previous genetic studies on heterosis



① The detailed dissection of specific examples is limited.

② A few of genes for heterosis have been identified and regulation mechanisms remain largely unclear.

③ A comprehensive and systematic view on the genetic architecture of yield heterosis is unavailable.

(6) Our research plans

ZS11 ZS11×73290 73290×ZS11 73290

① How is heterosis formed in this cross?

② What genes are responsible for heterosis?

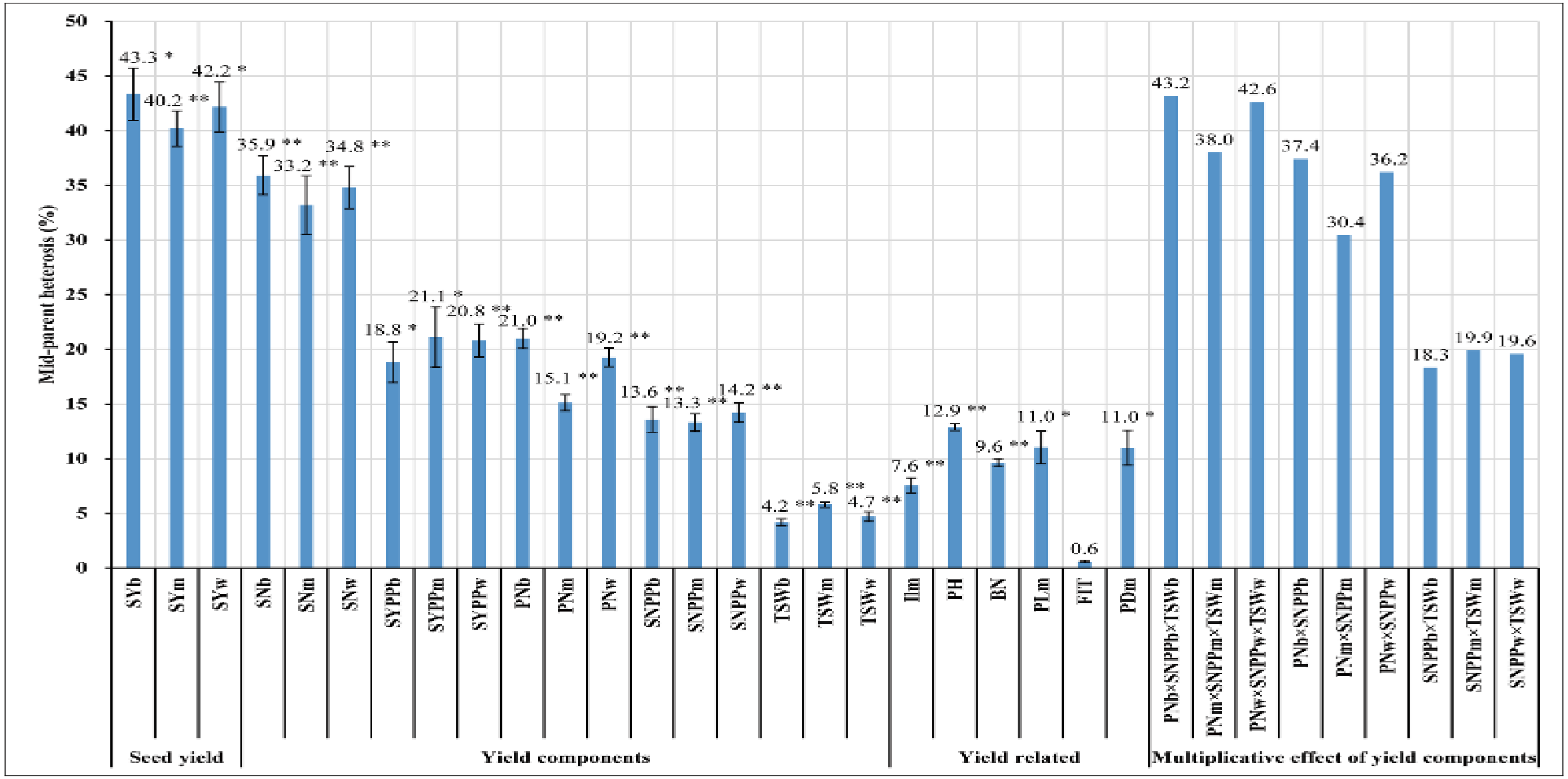
③ How heterosis genes work?

- ➢ This rapeseed cross was used as an example to systematically dissect the yield heterosis at the phenotypic, genetic, and molecular levels.

2. Phenotypic analysis of yield heterosis

➢ First, the mid-parent heterosis (MPH) of 24 traits were investigated in the cross of ZS11×73290 in 3 years.

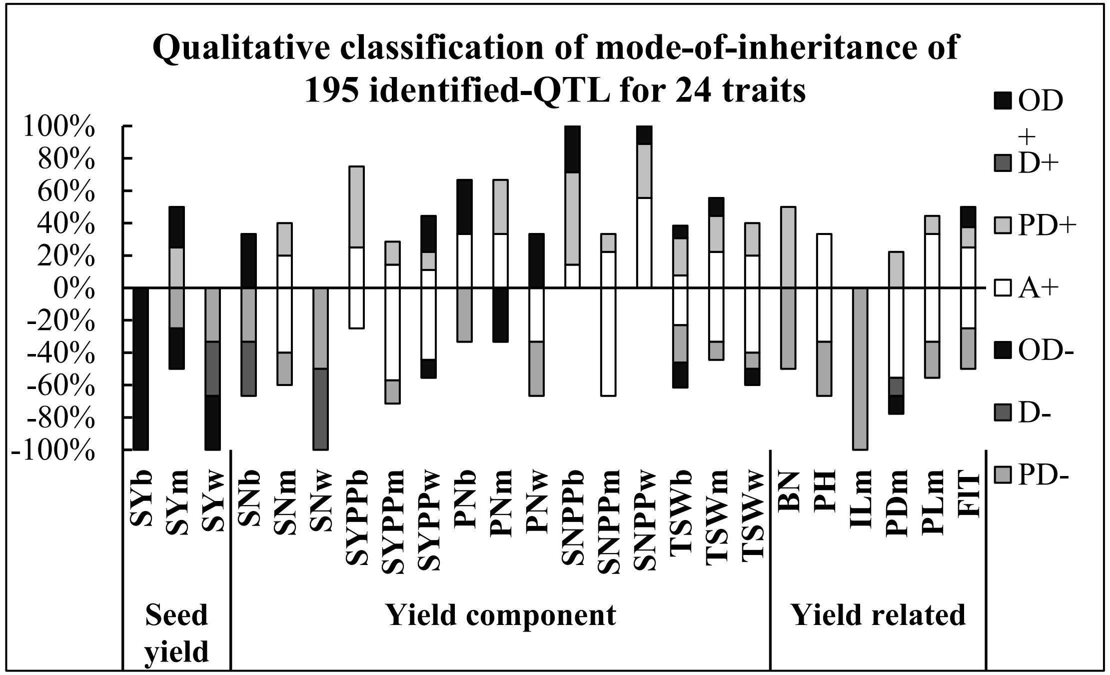
- The MPH of composite traits (such as SY) was higher than that for its component traits.
- The MPH of composite traits (such as SY) was basically equal to the multiplicative effect of that for its component traits (PN, SNPP and TSW).

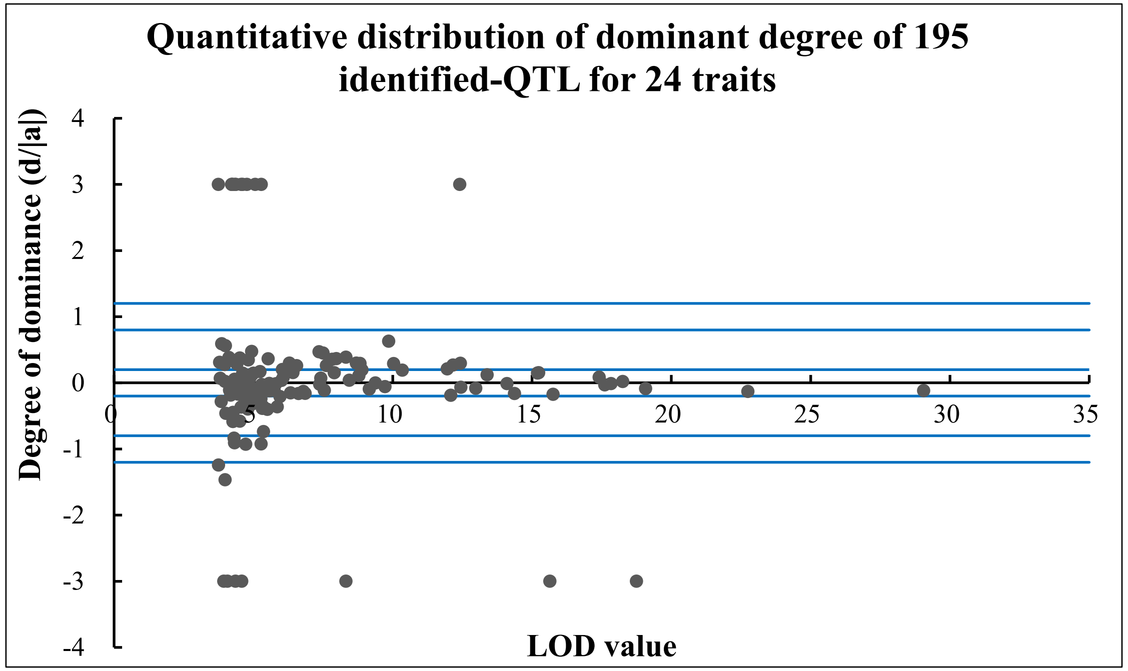


✓ **Strong heterosis of yield results from the multiplicative effect of moderate heterosis of its component traits**

3. Dissecting the genetic basis of yield heterosis

- To further identify the heterotic QTL in this cross, the corresponding F_2 and $F_{2:3}$ were constructed and used for QTL mapping in multiple environments, and a total of 195 QTL were identified.





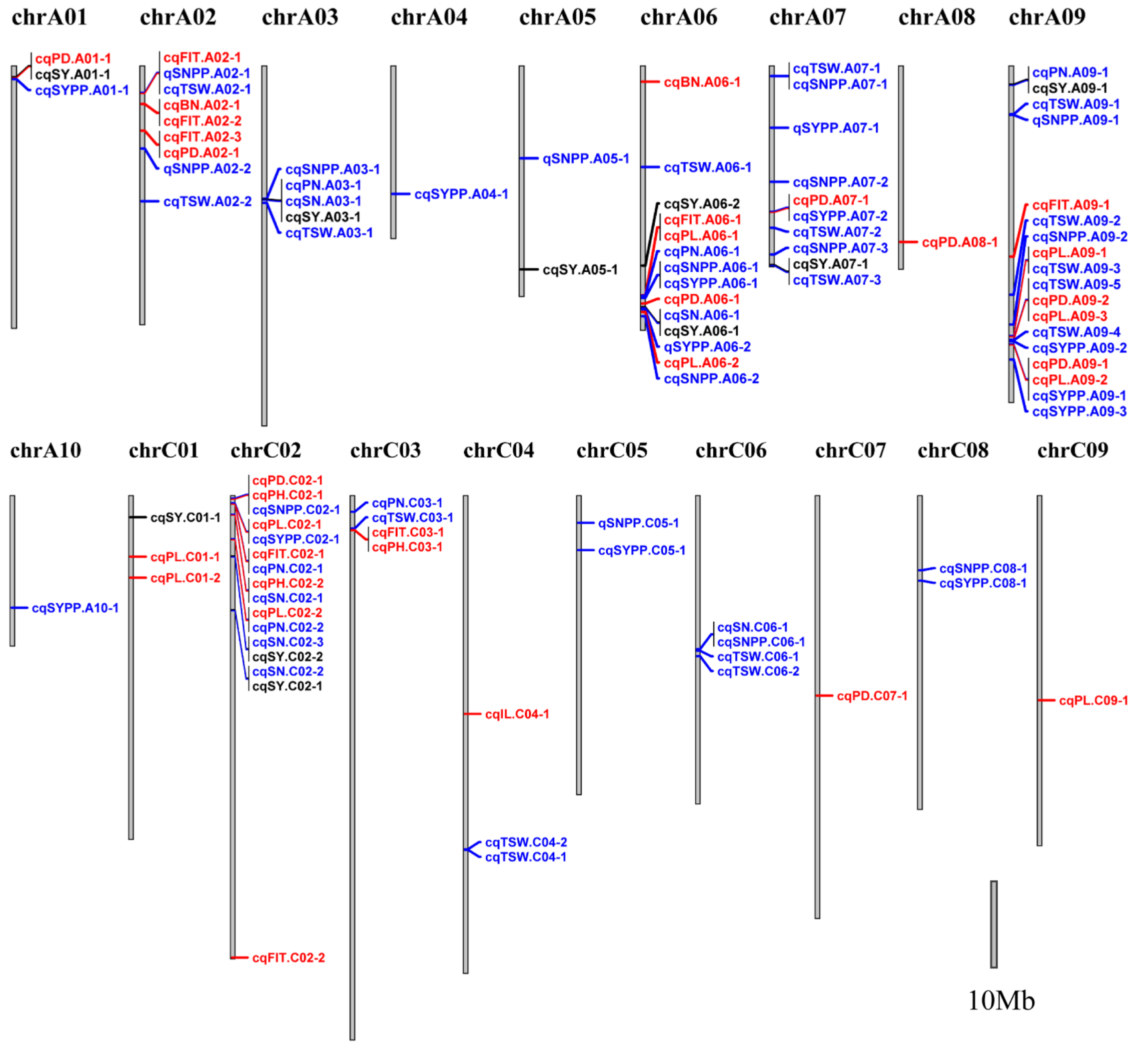
- Additive (|D/A|<0.2): 96 QTL (49.2%)
- Partial-dominant (0.2≤|D/A|<0.8): 72 QTL (36.9%)
- Full-dominant (0.8≤|D/A|<1.2): 5 QTL (2.6%)
- Over-dominant (|D/A|≥1.2): 22 QTL (11.3%)

- OD QTL were detected only in one environment
- OD QTL drastically decreased with LOD value
- Most OD QTL had small additive effects

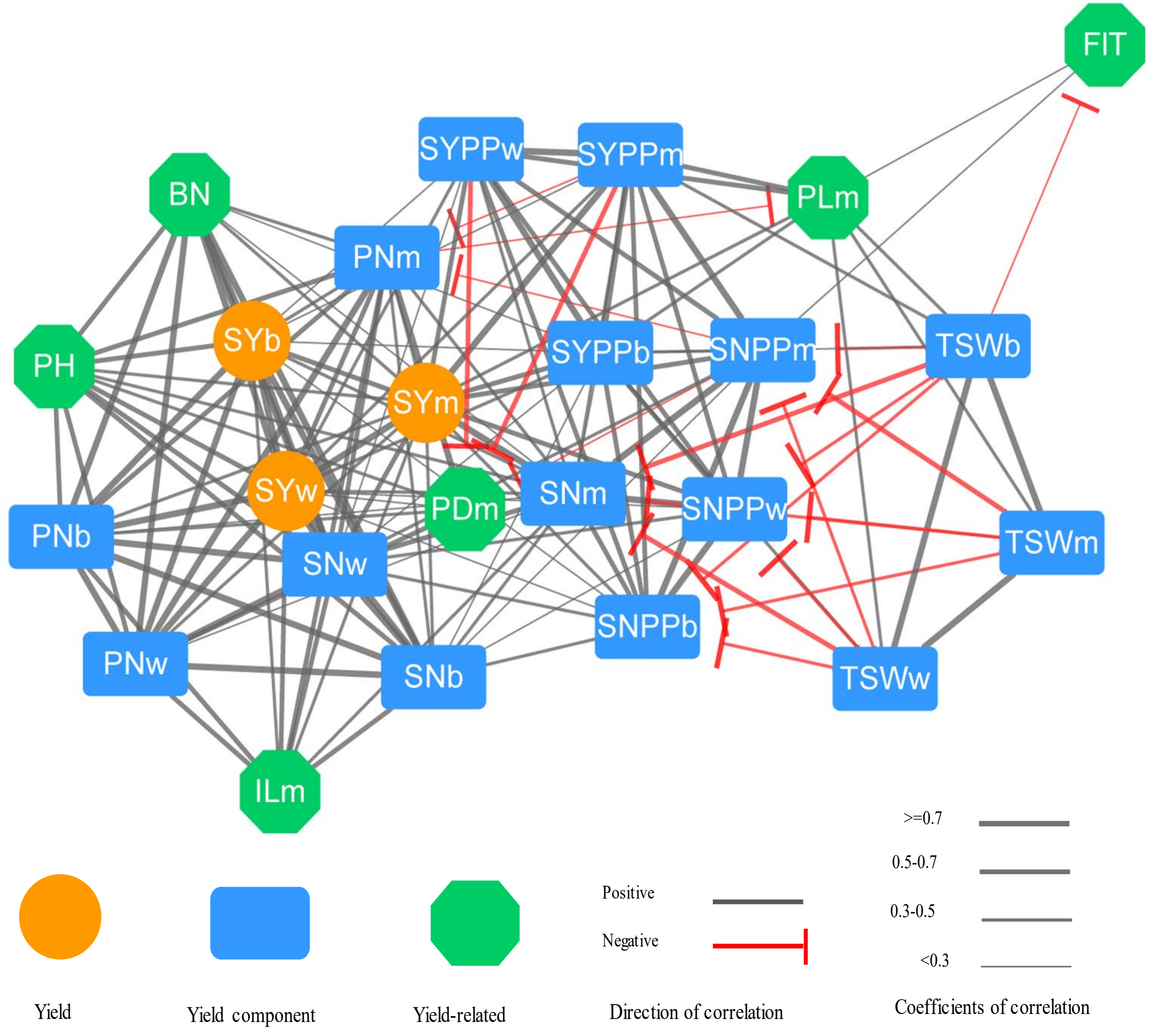
Therefore, OD QTL likely result from false positive

✓ Partial-dominance might play a major role in rapeseed heterosis at the single-locus level.

- The physical mapping of these heterotic QTL showed that they were clustered rather than distributed randomly across the genome.



- This was highly accordant with the result of trait network analysis that all the 24 investigated traits were linked directly or indirectly.



➢ To characterize the genetic architecture of yield heterosis, a trait-QTL network was constructed.

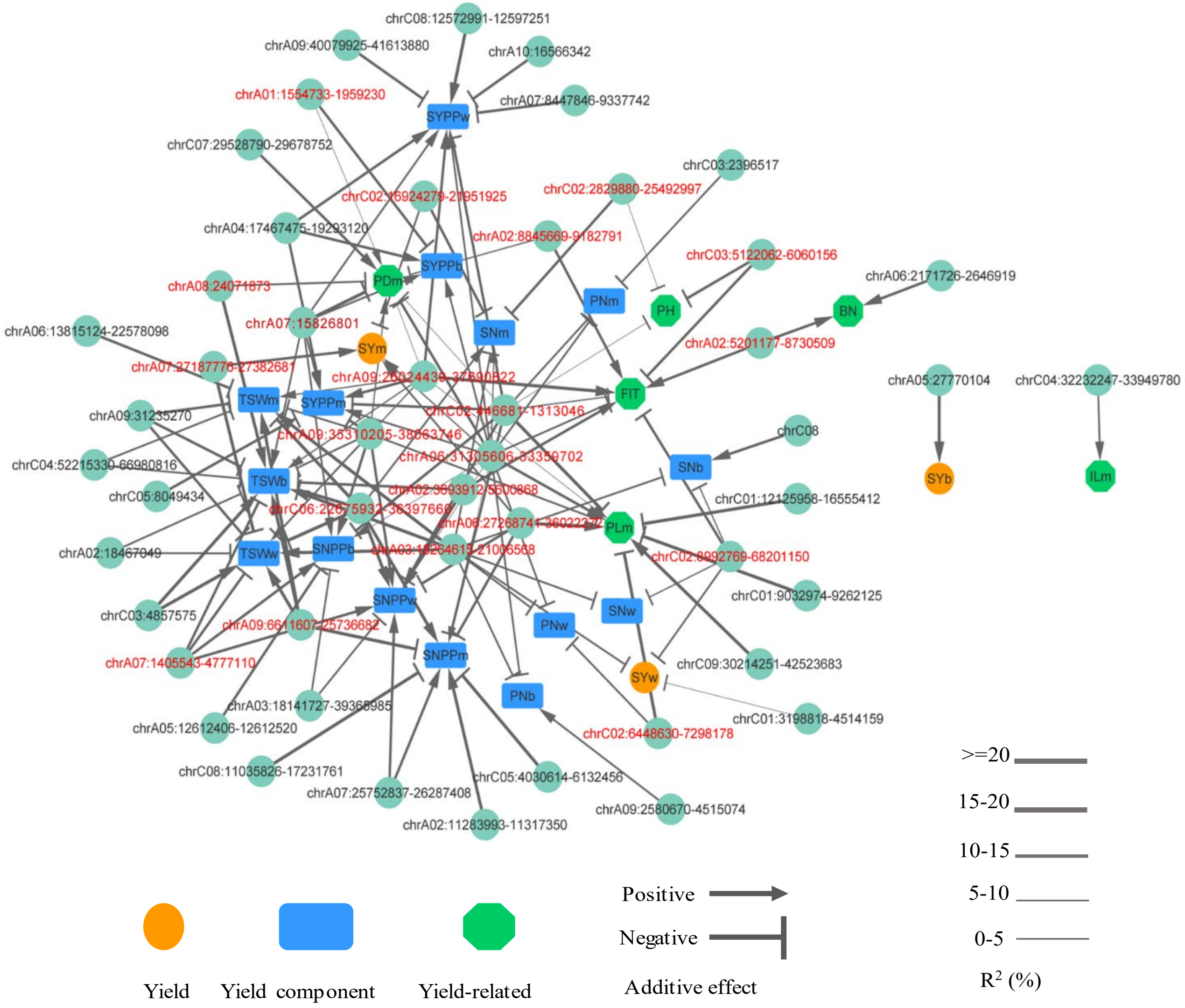
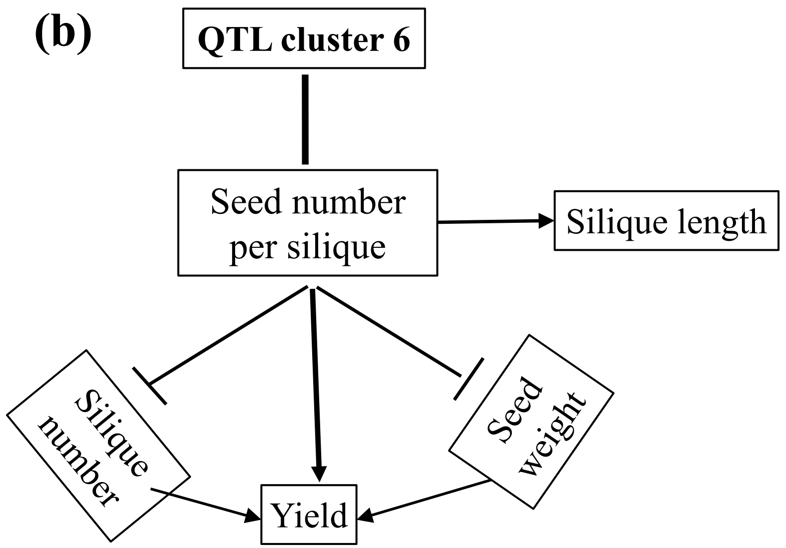


Figure 3(a) Trait-QTL network for yield heterosis. Traits and QTL (or QTL clusters) were treated as nodes and linked when the LOD of QTL exceeded the threshold value. The QTL were labelled with chromosome number and corresponding location, and those marked with the red colour indicated the 21 QTL clusters. The abbreviation of all traits was described in the section of Methods.

- **Almost all heterotic QTL were linked within a network with obvious center-periphery structure.**
- **Obviously, several hub heterotic-QTL that controlled multiple traits with relatively large effects were located in the center of network.**

- Of these hub heterotic-QTL, QC6 and QC14 were selected to further dissect the causal trait relationship in the formation of yield heterosis.



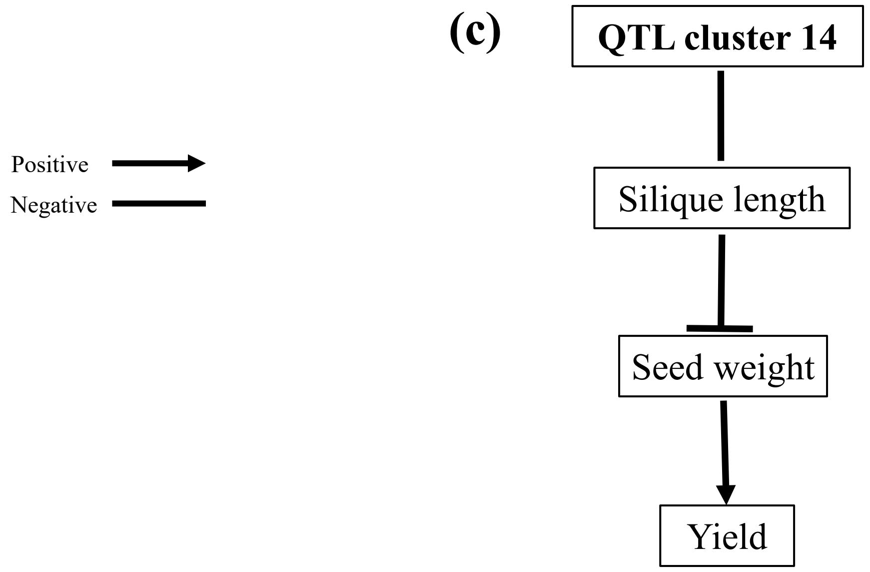
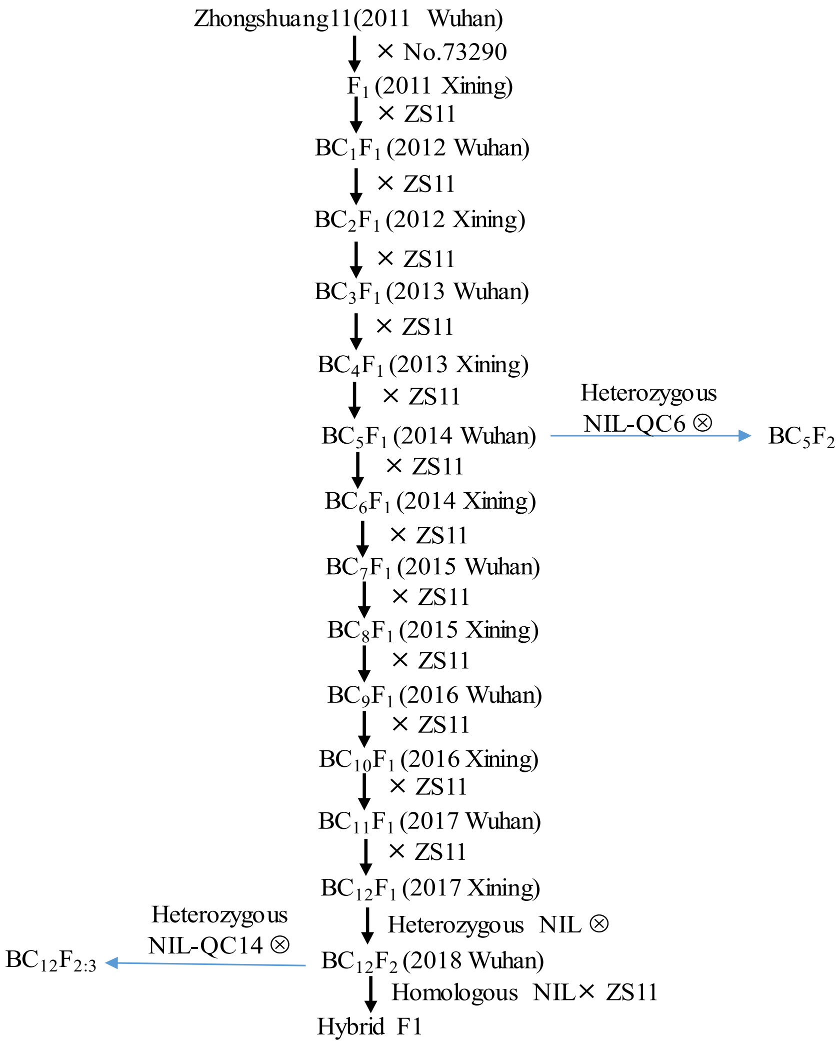


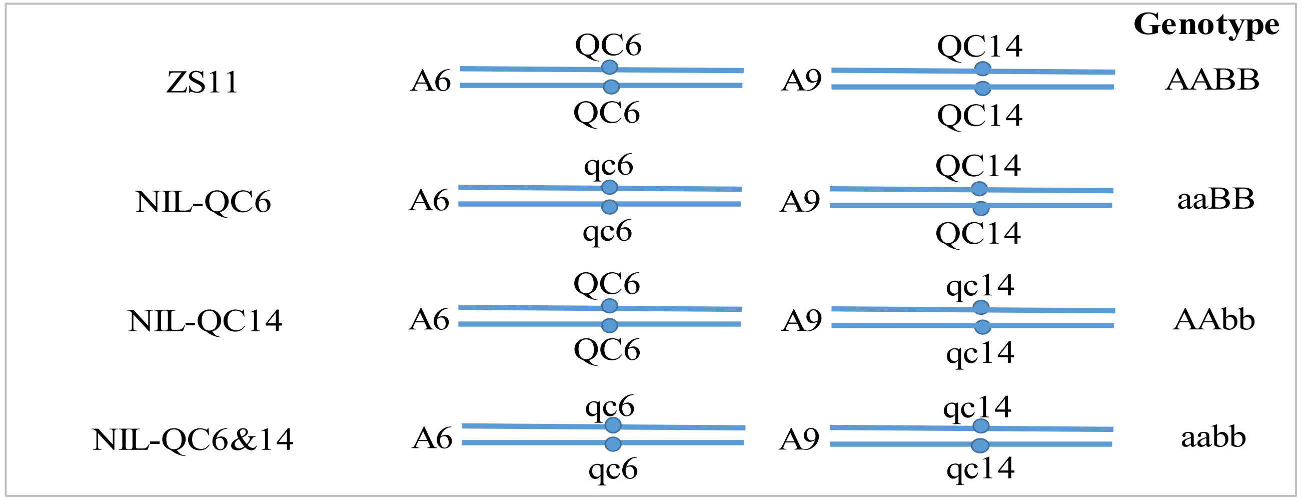
Figure 3(b) A direct path to yield heterosis. QTL. QC6 had a heterotic effect on seed number per pod and then affected yield heterosis. Simultaneously, QC6 had a pleiotropic effect on pod length as well as a antagonistic pleiotropic effect on pod number and seed weight.

Figure 3(c) Another indirect path to yield heterosis. QTL cluster 14 (QC14) had a heterotic effect on one yield-related trait (pod length), which had a smaller heterotic effect on seed weight and then affected yield heterosis.

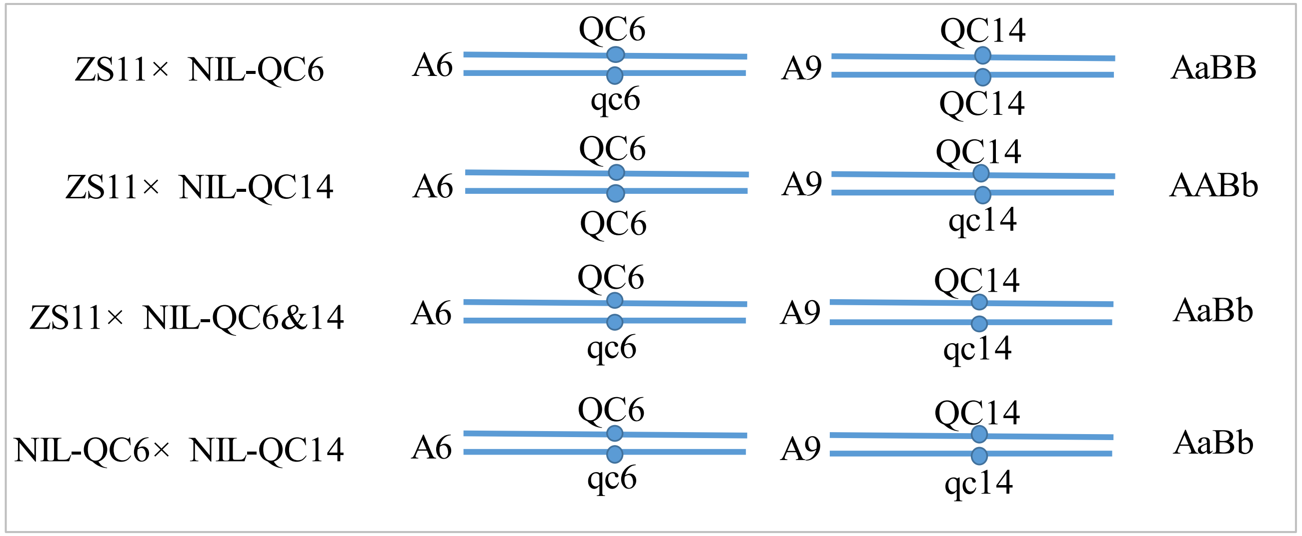
- **The results showed pleitropy of heterotic-QTL, up/down-stream and positive /negative feedback between traits, direct and indirect path to determine heterosis.**
- ✓ **This demonstrated the transmission of heterotic effects between traits, and the complexity of the genetic archtecture of yield heterosis.**

- **To accurately estimate their heterotic effects, QC6 and QC14 were also subjected to develop high-generation NILs for individual QTL and both, followed by designed crossing between them.**


Zhongshuang11(2011 Wuhan)
↓ × No.73290
F_1 (2011 Xining)
↓ × ZS11
BC_1F_1 (2012 Wuhan)
↓ × ZS11
BC_2F_1 (2012 Xining)
↓ × ZS11
BC_3F_1 (2013 Wuhan)
↓ × ZS11
BC_4F_1 (2013 Xining)
↓ × ZS11
BC_5F_1 (2014 Wuhan) → Heterozygous NIL-QC6 ⊗ → BC_5F_2
↓ × ZS11
BC_6F_1 (2014 Xining)
↓ × ZS11
BC_7F_1 (2015 Wuhan)
↓ × ZS11
BC_8F_1 (2015 Xining)
↓ × ZS11
BC_9F_1 (2016 Wuhan)
↓ × ZS11
$BC_{10}F_1$ (2016 Xining)
↓ × ZS11
$BC_{11}F_1$ (2017 Wuhan)
↓ × ZS11
$BC_{12}F_1$ (2017 Xining)
↓ Heterozygous NIL ⊗
$BC_{12}F_2$ (2018 Wuhan) → Heterozygous NIL-QC14 ⊗ → $BC_{12}F_{2:3}$
↓ Homologous NIL× ZS11
Hybrid F1



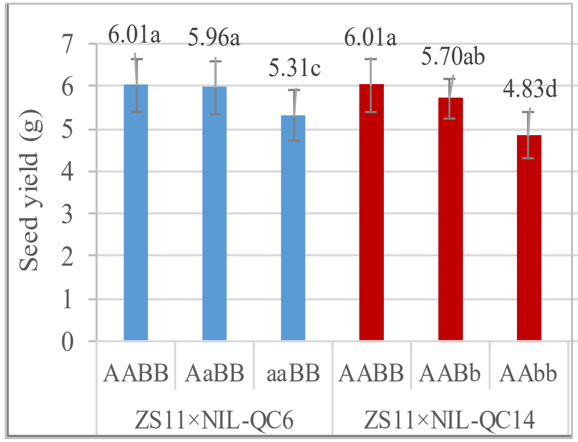
					Genotype
ZS11	A6	QC6 QC6	A9	QC14 QC14	AABB
NIL-QC6	A6	qc6 qc6	A9	QC14 QC14	aaBB
NIL-QC14	A6	QC6 QC6	A9	qc14 qc14	AAbb
NIL-QC6&14	A6	qc6 qc6	A9	qc14 qc14	aabb

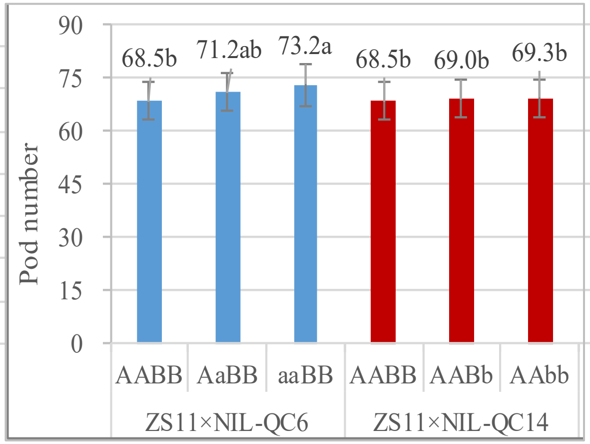


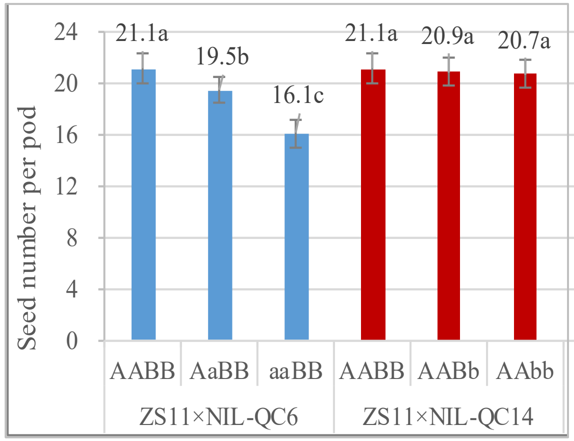
ZS11× NIL-QC6	A6	QC6 qc6	A9	QC14 QC14	AaBB
ZS11× NIL-QC14	A6	QC6 QC6	A9	QC14 qc14	AABb
ZS11× NIL-QC6&14	A6	QC6 qc6	A9	QC14 qc14	AaBb
NIL-QC6× NIL-QC14	A6	QC6 qc6	A9	QC14 qc14	AaBb

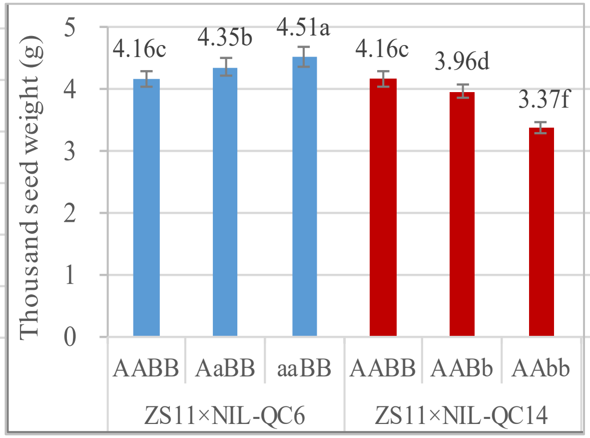
- **Seven traits affected by QC6 and QC14 were measured for the four crosses.**

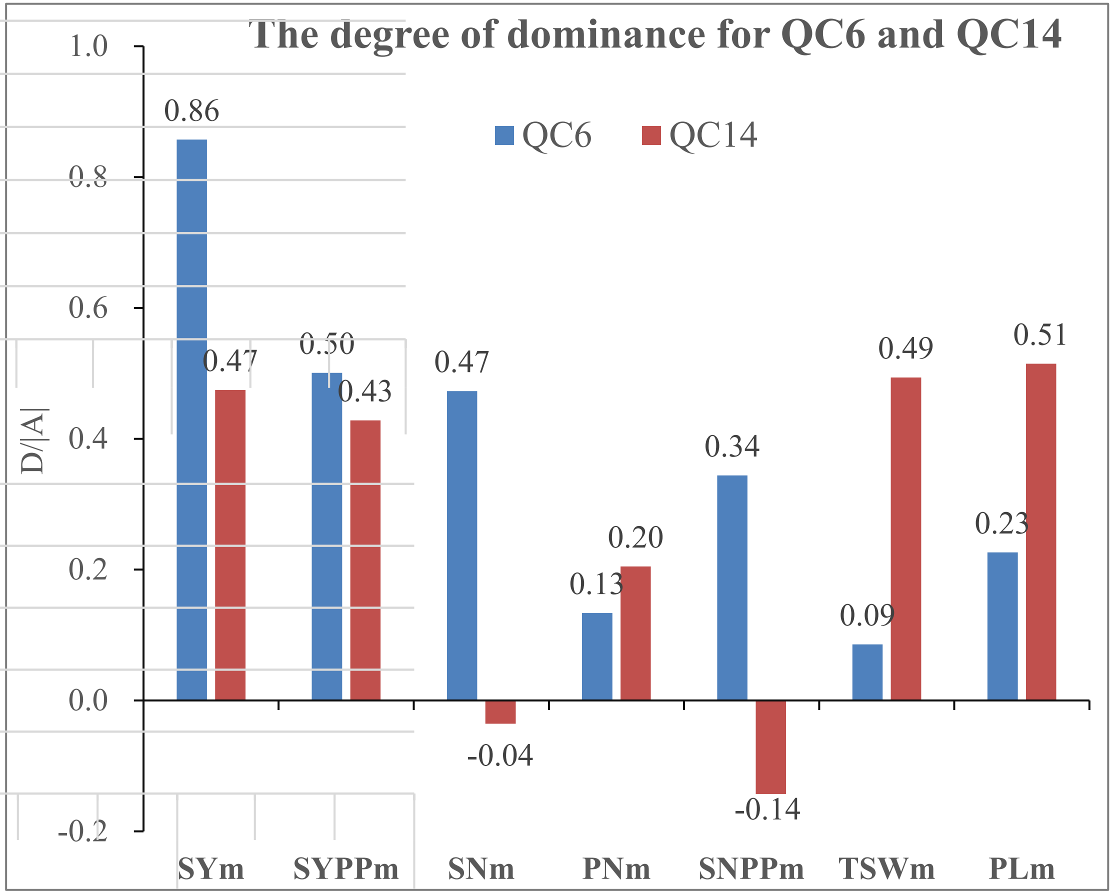
- For the **single heterozygote** obtained by crossing ZS11 and NIL-QC6/NIL-QC14, their **performance were between the two homozygotes** for all investigated traits, and the calculated **dominant degree were all <1**.





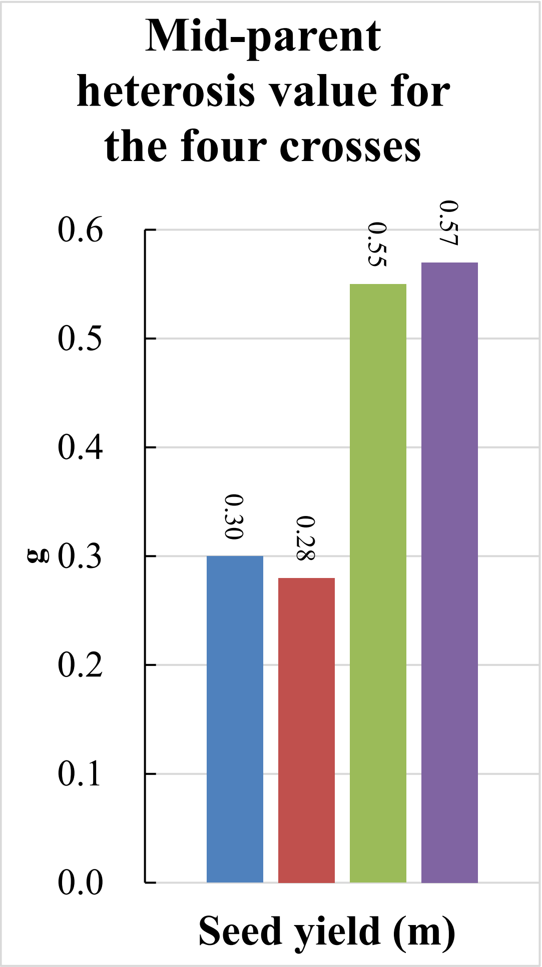


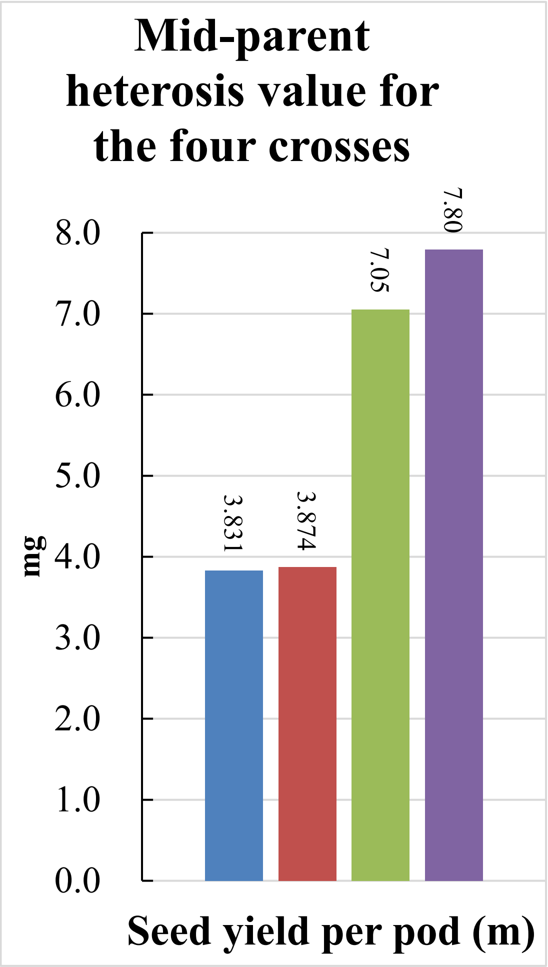


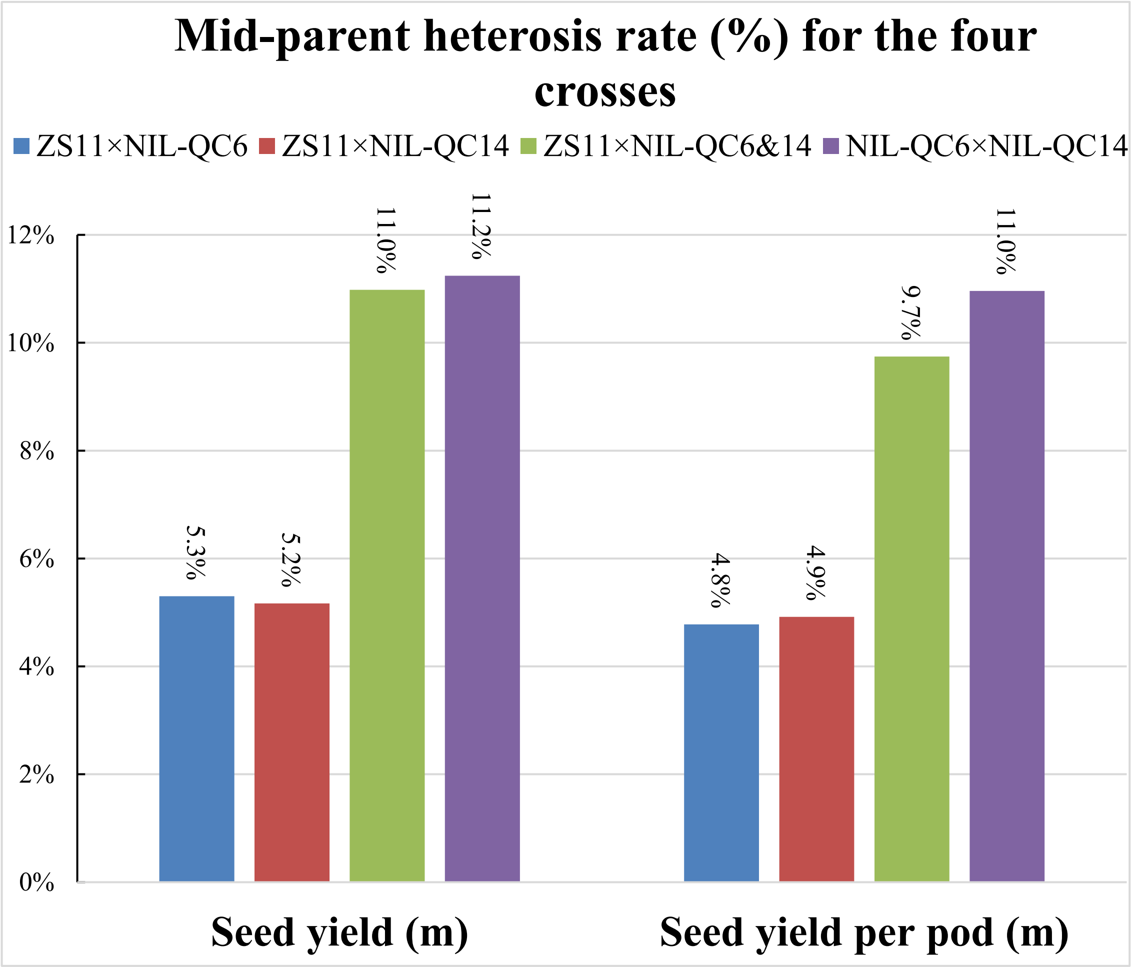


✓ These results indicated the **partial-dominance mode-of-inheritance for both QTL**, according well with the results of primary QTL mapping.

- **For the double heterozygote from the crossing between parental/complementary homozygotes, both the mid-parent heterosis value and rate (%) of QC6 and QC14 had an accumulative effect.**

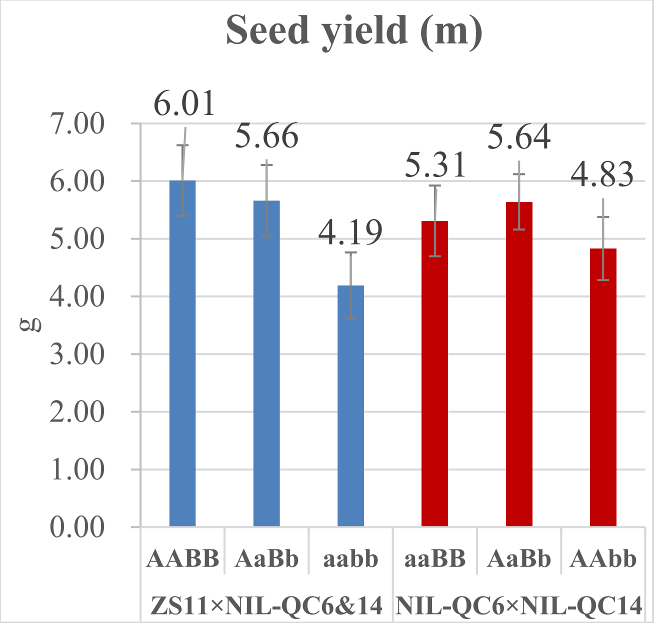

Mid-parent heterosis value for the four crosses

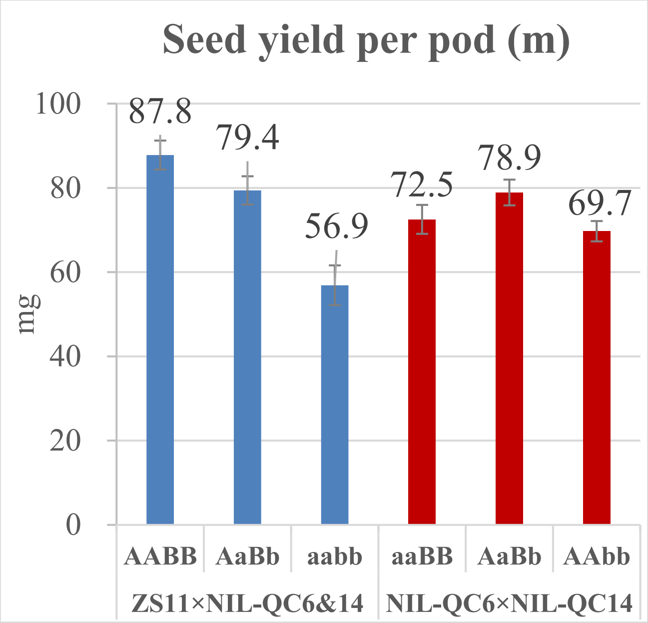

Mid-parent heterosis value for the four crosses


Mid-parent heterosis rate (%) for the four crosses

✓ **This indicated the pyramiding of two heterotic QTL produces higher mid-parent heterosis.**

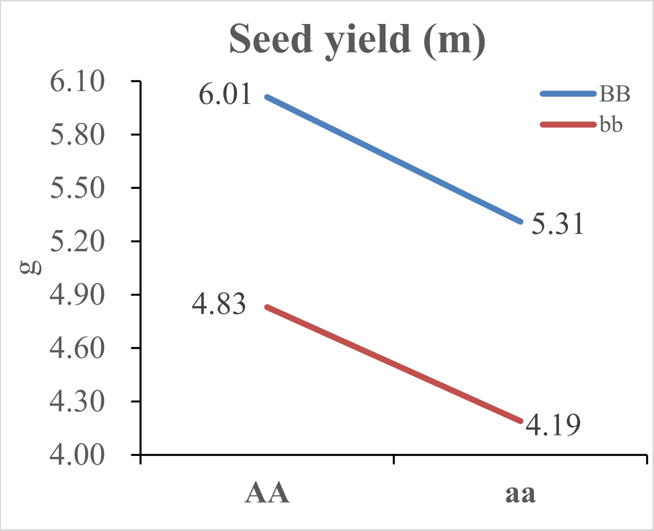
- For the crossing between two parental homozygotes (favorable allele from same parent), the hybrid did not show better-parent heterosis for all investigated traits.
- For the crossing between two complementary homozygotes (favorable allele from different parents), the hybrid showed better-parent heterosis for seed yield(m) and seed yield per pod (m).

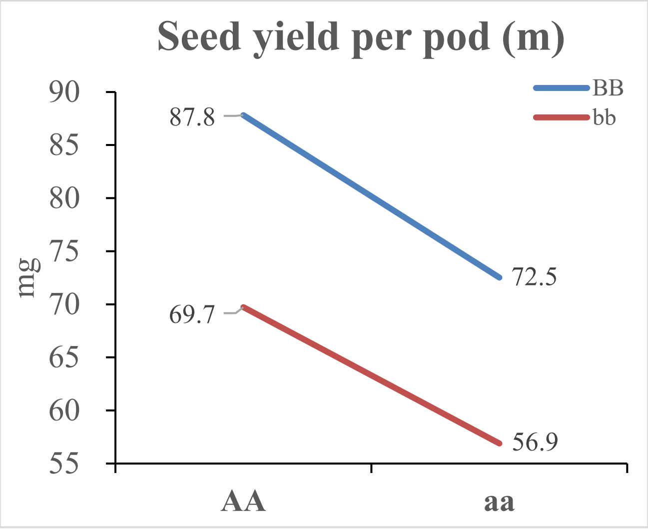




- The phenotypic analyses of these NILs showed that QC6 and QC14 has no epistasis for all investigated traits.

✓ Therefore, their genotypic values are determined by additive and dominant effects.



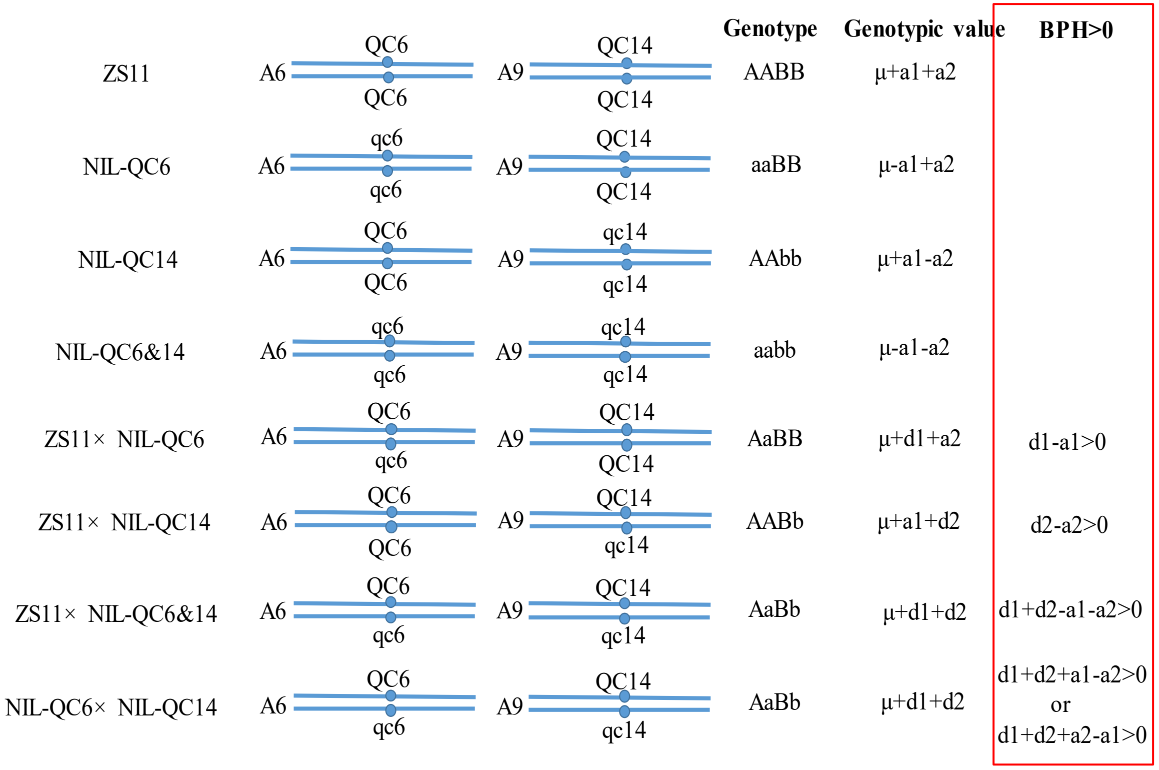


- **In the absence of epistasis, the better-parent heterosis of the four crosses can be calculated from the genotypic value of their parents.**

- **For single heterozygote:** better parent heerosis is positive only if it's over-dominant (i.e., over-dominance hypothesis).
- **For double heterozygotes, two situations:**

① **If the two advantageous alleles are from the same parent,** positive BPH require at least one QTL being over-dominant.

② **If the two advantageous alleles are from the different parents,** positive BPH require d1+d2>|a1–a2|, which is certain for two full-dominant loci (i.e., dominance complementation hypothesis) but uncertain for partial-dominant loci.



					Genotype	**Genotypic value**	**BPH>0**
ZS11	A6	QC6 QC6	A9	QC14 QC14	AABB	μ+a1+a2	
NIL-QC6	A6	qc6 qc6	A9	QC14 QC14	aaBB	μ-a1+a2	
NIL-QC14	A6	QC6 QC6	A9	qc14 qc14	AAbb	μ+a1-a2	
NIL-QC6&14	A6	qc6 qc6	A9	qc14 qc14	aabb	μ-a1-a2	
ZS11× NIL-QC6	A6	QC6 qc6	A9	QC14 QC14	AaBB	μ+d1+a2	d1-a1>0
ZS11× NIL-QC14	A6	QC6 QC6	A9	QC14 qc14	AABb	μ+a1+d2	d2-a2>0
ZS11× NIL-QC6&14	A6	QC6 qc6	A9	QC14 qc14	AaBb	μ+d1+d2	d1+d2-a1-a2>0
NIL-QC6× NIL-QC14	A6	QC6 qc6	A9	QC14 qc14	AaBb	μ+d1+d2	d1+d2+a1-a2>0 or d1+d2+a2-a1>0

- In fact, this precondition can also be extended to more than three loci, with a slight modification.

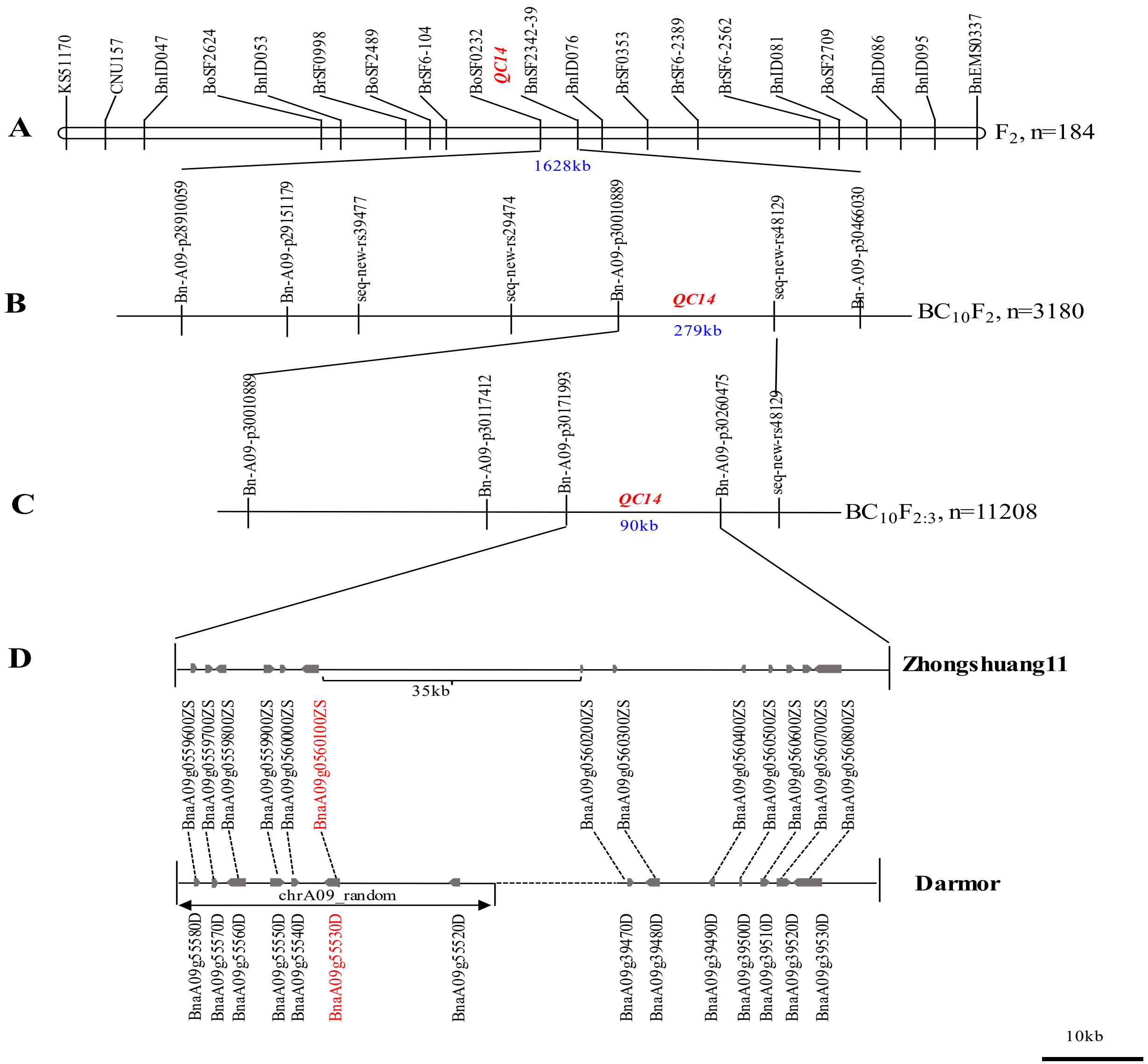
✓ **The model derivation further proves theoretically that the complementation of two partial-dominant loci in hybrid can also produce better-parent heterosis under certain condition.**

4. Cloning the genes responsible for yield heterosis

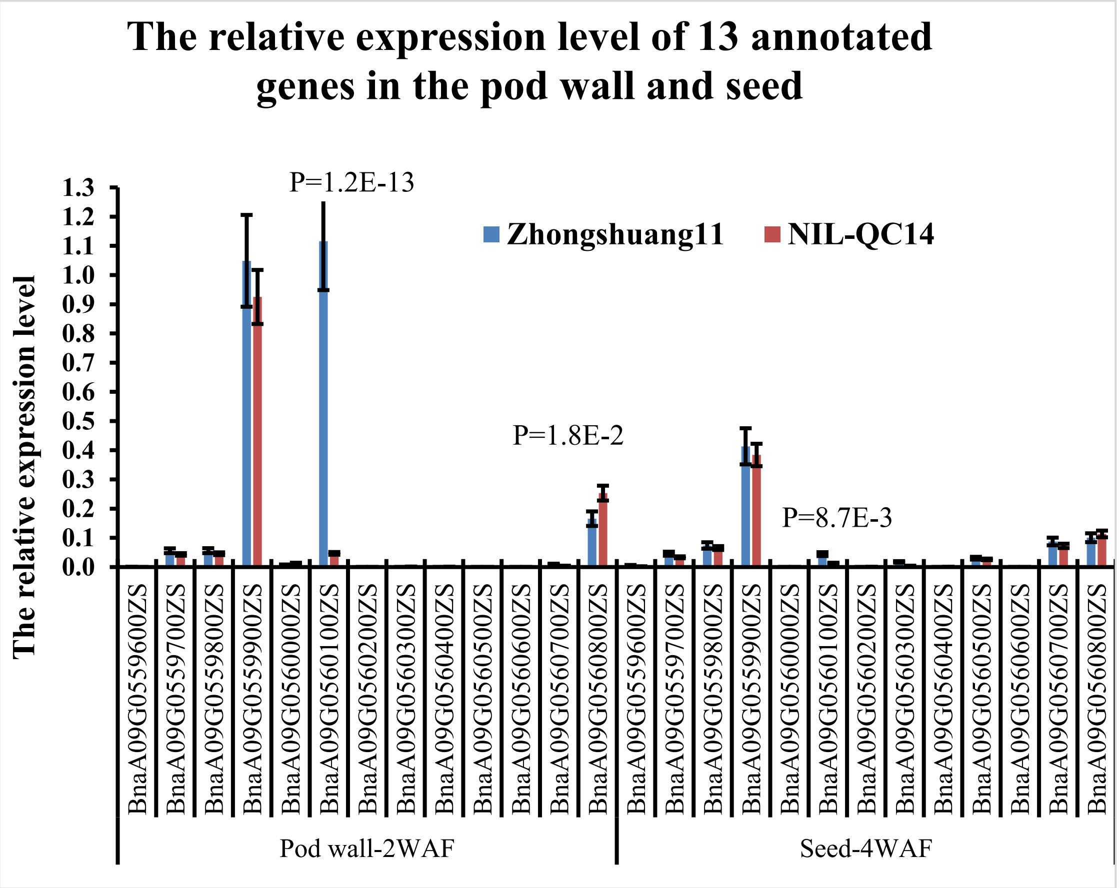
- To identify the heterosis genes, the NILs heterozygous at QC6 or QC14 were self-crossed to develop the corresponding segregation population for fine-mapping.

- QC6 was fine-mapped to a large region (Yang et al., 2016) with recombination suppression in a large population of 37 976 individuals (Zhang, 2017), which made it very difficult to identify the causal gene.

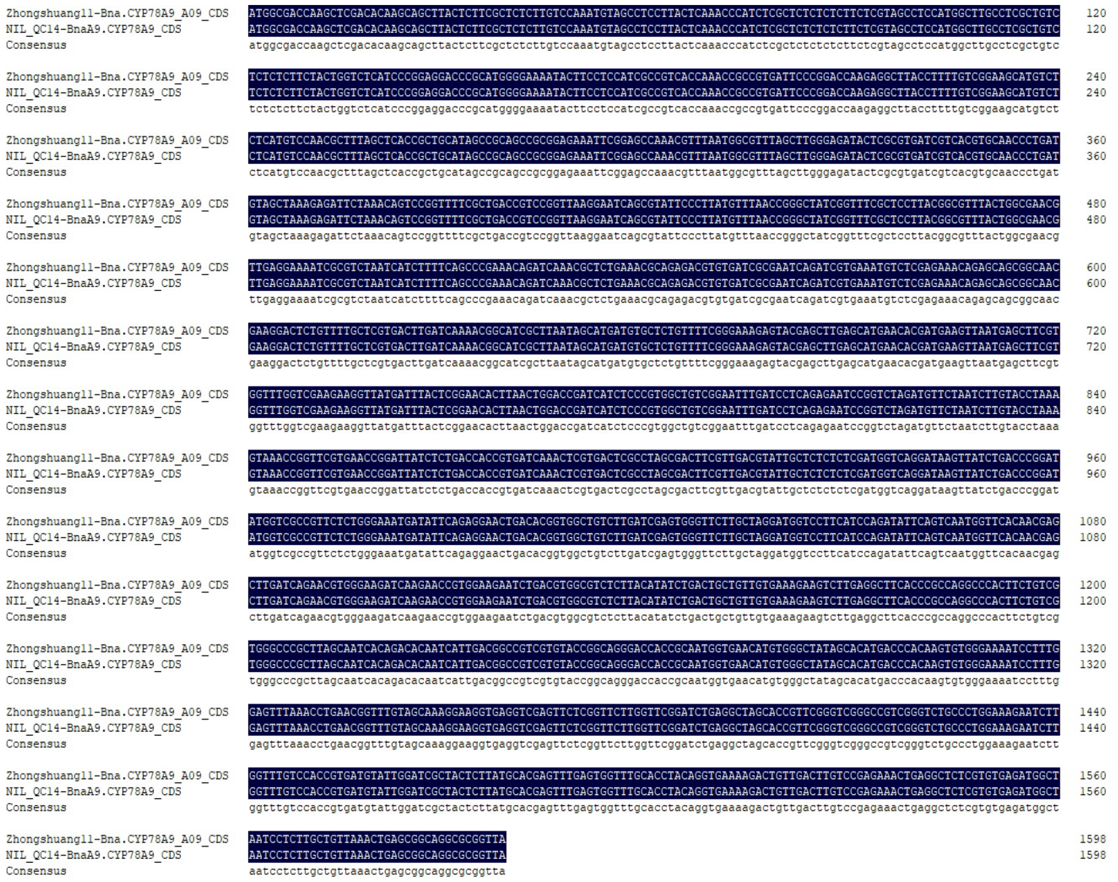
- However, QC14 was successfully delimited to a small 90 kb region containing only 13 annotated genes in the reference genome of Zhongshuang11.



- The detailed functional annotation of their homologues in *Arabidopsis* showed that only *CYP78A9* (*BnaA09G0560100ZS*) was involved in silique and seed development.



The relative expression level of 13 annotated genes in the pod wall and seed

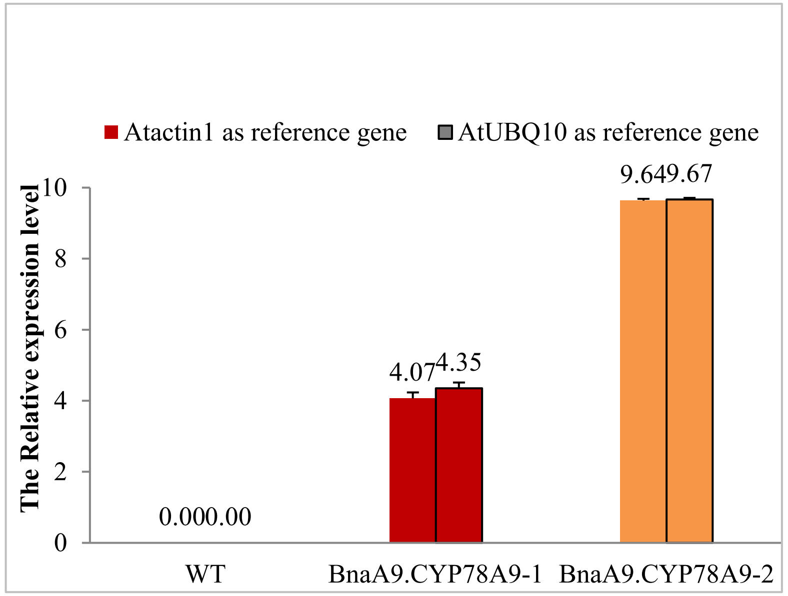


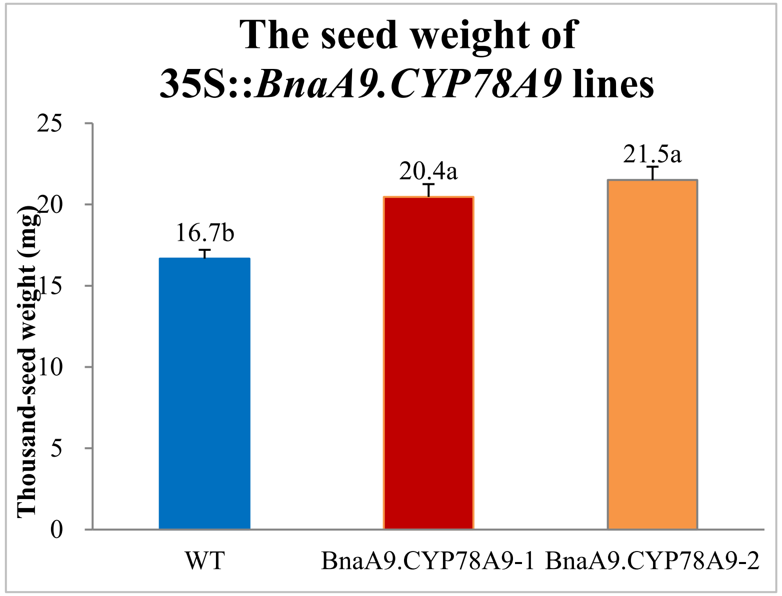
```
Zhongshuang11-Bna.CYP78A9_A09_CDS  ATGGCGACCAAGCTCGACACAAGCAGCTTACTCTTCGCTCTCTTGTCCAAATGTAGCCTCCTTACTCAAACCCATCTCGCTCTCTCTCTTCTCGTAGCCTCCATGGCTTGCCTCGCTGTC   120
NIL_QC14-BnaA9.CYP78A9_CDS         ATGGCGACCAAGCTCGACACAAGCAGCTTACTCTTCGCTCTCTTGTCCAAATGTAGCCTCCTTACTCAAACCCATCTCGCTCTCTCTCTTCTCGTAGCCTCCATGGCTTGCCTCGCTGTC   120
Consensus                          atggcgaccaagctcgacacaagcagcttactcttcgctctcttgtccaaatgtagcctccttactcaaacccatctcgctctctctcttctcgtagcctccatggcttgcctcgctgtc

Zhongshuang11-Bna.CYP78A9_A09_CDS  TCTCTCTTCTACTGGTCTCATCCCGGAGGACCCGCATGGGGAAAATACTTCCTCCATCGCCGTCACCAAACCGCCGTGATTCCCGGACCAAGAGGCTTACCTTTTGTCGGAAGCATGTCT   240
NIL_QC14-BnaA9.CYP78A9_CDS         TCTCTCTTCTACTGGTCTCATCCCGGAGGACCCGCATGGGGAAAATACTTCCTCCATCGCCGTCACCAAACCGCCGTGATTCCCGGACCAAGAGGCTTACCTTTTGTCGGAAGCATGTCT   240
Consensus                          tctctcttctactggtctcatcccggaggacccgcatggggaaaatacttcctccatcgccgtcaccaaaccgccgtgattcccggaccaagaggcttaccttttgtcggaagcatgtct

Zhongshuang11-Bna.CYP78A9_A09_CDS  CTCATGTCCAACGCTTTAGCTCACCGCTGCATAGCCGCAGCCGCGGAGAAATTCGGAGCCAAACGTTTAATGGCGTTTAGCTTGGGAGATACTCGCGTGATCGTCACGTGCAACCCTGAT   360
NIL_QC14-BnaA9.CYP78A9_CDS         CTCATGTCCAACGCTTTAGCTCACCGCTGCATAGCCGCAGCCGCGGAGAAATTCGGAGCCAAACGTTTAATGGCGTTTAGCTTGGGAGATACTCGCGTGATCGTCACGTGCAACCCTGAT   360
Consensus                          ctcatgtccaacgctttagctcaccgctgcatagccgcagccgcggagaaattcggagccaaacgtttaatggcgtttagcttgggagatactcgcgtgatcgtcacgtgcaaccctgat

Zhongshuang11-Bna.CYP78A9_A09_CDS  GTAGCTAAAGAGATTCTAAACAGTCCGGTTTTCGCTGACCGTCCGGTTAAGGAATCAGCGTATTCCCTTATGTTTAACCGGGCTATCGGTTTCGCTCCTTACGGCGTTTACTGGCGAACG   480
NIL_QC14-BnaA9.CYP78A9_CDS         GTAGCTAAAGAGATTCTAAACAGTCCGGTTTTCGCTGACCGTCCGGTTAAGGAATCAGCGTATTCCCTTATGTTTAACCGGGCTATCGGTTTCGCTCCTTACGGCGTTTACTGGCGAACG   480
Consensus                          gtagctaaagagattctaaacagtccggttttcgctgaccgtccggttaaggaatcagcgtattcccttatgtttaaccgggctatcggtttcgctccttacggcgtttactggcgaacg

Zhongshuang11-Bna.CYP78A9_A09_CDS  TTGAGGAAAATCGCGTCTAATCATCTTTTCAGCCCGAAACAGATCAAACGCTCTGAAACGCAGAGACGTGTGATCGCGAATCAGATCGTGAAATGTCTCGAGAAACAGAGCAGCGGCAAC   600
NIL_QC14-BnaA9.CYP78A9_CDS         TTGAGGAAAATCGCGTCTAATCATCTTTTCAGCCCGAAACAGATCAAACGCTCTGAAACGCAGAGACGTGTGATCGCGAATCAGATCGTGAAATGTCTCGAGAAACAGAGCAGCGGCAAC   600
Consensus                          ttgaggaaaatcgcgtctaatcatcttttcagcccgaaacagatcaaacgctctgaaacgcagagacgtgtgatcgcgaatcagatcgtgaaatgtctcgagaaacagagcagcggcaac

Zhongshuang11-Bna.CYP78A9_A09_CDS  GAAGGACTCTGTTTTGCTCGTGACTTGATCAAAACGGCATCGCTTAATAGCATGATGTGCTCTGTTTTCGGGAAAGAGTACGAGCTTGAGCATGAACACGATGAAGTTAATGAGCTTCGT   720
NIL_QC14-BnaA9.CYP78A9_CDS         GAAGGACTCTGTTTTGCTCGTGACTTGATCAAAACGGCATCGCTTAATAGCATGATGTGCTCTGTTTTCGGGAAAGAGTACGAGCTTGAGCATGAACACGATGAAGTTAATGAGCTTCGT   720
Consensus                          gaaggactctgttttgctcgtgacttgatcaaaacggcatcgcttaatagcatgatgtgctctgttttcgggaaagagtacgagcttgagcatgaacacgatgaagttaatgagcttcgt

Zhongshuang11-Bna.CYP78A9_A09_CDS  GGTTTGGTCGAAGAAGGTTATGATTTACTCGGAACACTTAACTGGACCGATCATCTCCCGTGGCTGTCGGAATTTGATCCTCAGAGAATCCGGTCTAGATGTTCTAATCTTGTACCTAAA   840
NIL_QC14-BnaA9.CYP78A9_CDS         GGTTTGGTCGAAGAAGGTTATGATTTACTCGGAACACTTAACTGGACCGATCATCTCCCGTGGCTGTCGGAATTTGATCCTCAGAGAATCCGGTCTAGATGTTCTAATCTTGTACCTAAA   840
Consensus                          ggtttggtcgaagaaggttatgatttactcggaacacttaactggaccgatcatctcccgtggctgtcggaatttgatcctcagagaatccggtctagatgttctaatcttgtacctaaa

Zhongshuang11-Bna.CYP78A9_A09_CDS  GTAAACCGGTTCGTGAACCGGATTATCTCTGACCACCGTGATCAAACTCGTGACTCGCCTAGCGACTTCGTTGACGTATTGCTCTCTCTCGATGGTCAGGATAAGTTATCTGACCCGGAT   960
NIL_QC14-BnaA9.CYP78A9_CDS         GTAAACCGGTTCGTGAACCGGATTATCTCTGACCACCGTGATCAAACTCGTGACTCGCCTAGCGACTTCGTTGACGTATTGCTCTCTCTCGATGGTCAGGATAAGTTATCTGACCCGGAT   960
Consensus                          gtaaaccggttcgtgaaccggattatctctgaccaccgtgatcaaactcgtgactcgcctagcgacttcgttgacgtattgctctctctcgatggtcaggataagttatctgacccggat

Zhongshuang11-Bna.CYP78A9_A09_CDS  ATGGTCGCCGTTCTCTGGGAAATGATATTCAGAGGAACTGACACGGTGGCTGTCTTGATCGAGTGGGTTCTTGCTAGGATGGTCCTTCATCCAGATATTCAGTCAATGGTTCACAACGAG   1080
NIL_QC14-BnaA9.CYP78A9_CDS         ATGGTCGCCGTTCTCTGGGAAATGATATTCAGAGGAACTGACACGGTGGCTGTCTTGATCGAGTGGGTTCTTGCTAGGATGGTCCTTCATCCAGATATTCAGTCAATGGTTCACAACGAG   1080
Consensus                          atggtcgccgttctctgggaaatgatattcagaggaactgacacggtggctgtcttgatcgagtgggttcttgctaggatggtccttcatccagatattcagtcaatggttcacaacgag

Zhongshuang11-Bna.CYP78A9_A09_CDS  CTTGATCAGAACGTGGGAAGATCAAGAACCGTGGAAGAATCTGACGTGGCGTCTCTTACATATCTGACTGCTGTTGTGAAAGAAGTCTTGAGGCTTCACCCGCCAGGCCCACTTCTGTCG   1200
NIL_QC14-BnaA9.CYP78A9_CDS         CTTGATCAGAACGTGGGAAGATCAAGAACCGTGGAAGAATCTGACGTGGCGTCTCTTACATATCTGACTGCTGTTGTGAAAGAAGTCTTGAGGCTTCACCCGCCAGGCCCACTTCTGTCG   1200
Consensus                          cttgatcagaacgtgggaagatcaagaaccgtggaagaatctgacgtggcgtctcttacatatctgactgctgttgtgaaagaagtcttgaggcttcacccgccaggcccacttctgtcg

Zhongshuang11-Bna.CYP78A9_A09_CDS  TGGGCCCGCTTAGCAATCACAGACACAATCATTGACGGCCGTCGTGTACCGGCAGGGACCACCGCAATGGTGAACATGTGGGCTATAGCACATGACCCACAAGTGTGGGAAAATCCTTTG   1320
NIL_QC14-BnaA9.CYP78A9_CDS         TGGGCCCGCTTAGCAATCACAGACACAATCATTGACGGCCGTCGTGTACCGGCAGGGACCACCGCAATGGTGAACATGTGGGCTATAGCACATGACCCACAAGTGTGGGAAAATCCTTTG   1320
Consensus                          tgggcccgcttagcaatcacagacacaatcattgacggccgtcgtgtaccggcagggaccaccgcaatggtgaacatgtgggctatagcacatgacccacaagtgtgggaaaatcctttg

Zhongshuang11-Bna.CYP78A9_A09_CDS  GAGTTTAAACCTGAACGGTTTGTAGCAAAGGAAGGTGAGGTCGAGTTCTCGGTTCTTGGTTCGGATCTGAGGCTAGCACCGTTCGGGTCGGGCCGTCGGGTCTGCCCTGGAAAGAATCTT   1440
NIL_QC14-BnaA9.CYP78A9_CDS         GAGTTTAAACCTGAACGGTTTGTAGCAAAGGAAGGTGAGGTCGAGTTCTCGGTTCTTGGTTCGGATCTGAGGCTAGCACCGTTCGGGTCGGGCCGTCGGGTCTGCCCTGGAAAGAATCTT   1440
Consensus                          gagtttaaacctgaacggtttgtagcaaaggaaggtgaggtcgagttctcggttcttggttcggatctgaggctagcaccgttcgggtcgggccgtcgggtctgccctggaaagaatctt

Zhongshuang11-Bna.CYP78A9_A09_CDS  GGTTTGTCCACCGTGATGTATTGGATCGCTACTCTTATGCACGAGTTTGAGTGGTTTGCACCTACAGGTGAAAAGACTGTTGACTTGTCCGAGAAACTGAGGCTCTCGTGTGAGATGGCT   1560
NIL_QC14-BnaA9.CYP78A9_CDS         GGTTTGTCCACCGTGATGTATTGGATCGCTACTCTTATGCACGAGTTTGAGTGGTTTGCACCTACAGGTGAAAAGACTGTTGACTTGTCCGAGAAACTGAGGCTCTCGTGTGAGATGGCT   1560
Consensus                          ggtttgtccaccgtgatgtattggatcgctactcttatgcacgagtttgagtggtttgcacctacaggtgaaaagactgttgacttgtccgagaaactgaggctctcgtgtgagatggct

Zhongshuang11-Bna.CYP78A9_A09_CDS  AATCCTCTTGCTGTTAAACTGAGCGGCAGGCGCGGTTA                                                                                     1598
NIL_QC14-BnaA9.CYP78A9_CDS         AATCCTCTTGCTGTTAAACTGAGCGGCAGGCGCGGTTA                                                                                     1598
Consensus                          aatcctcttgctgttaaactgagcggcaggcgcggtta
```

- *BnaA09G0560100ZS* also showed the largest expression difference in pod walls and seeds, which was chosen as most likely candidate gene.

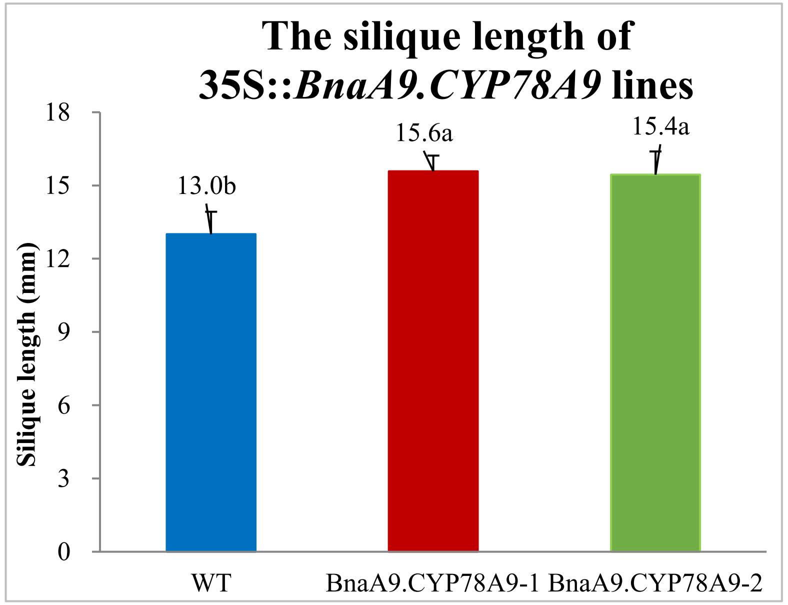
- *BnaA09G0560100ZS* had no difference in coding sequence, suggesting that QC14 might be generated by the expression difference of this gene.

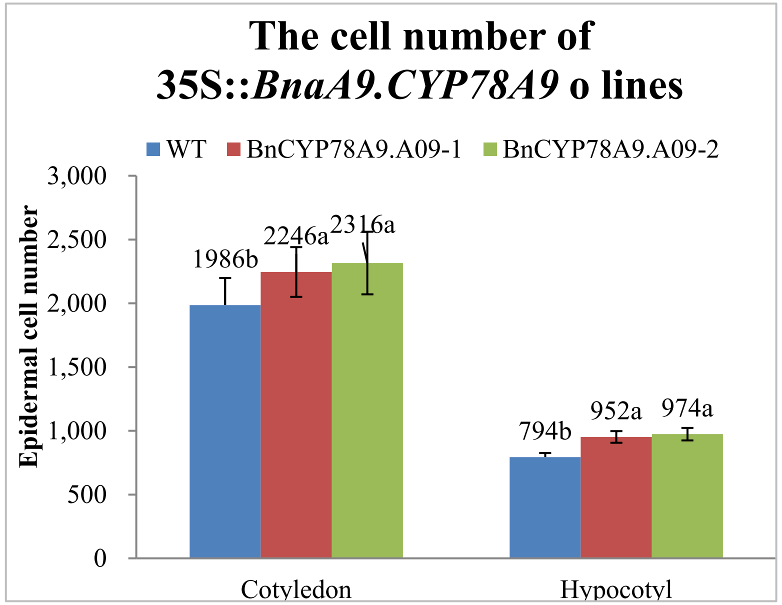
- To test this speculation, the CDS of *BnaA09G0560100ZS* (thereafter designated as *BnaA9.CYP78A9*) was first over-expressed in *Arabidopsis*.



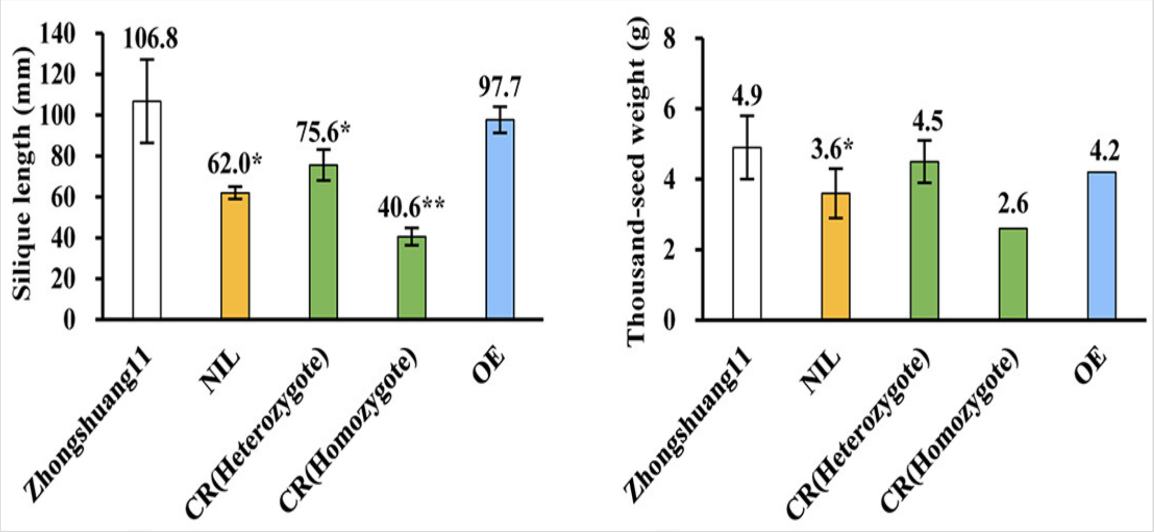

The seed weight of 35S::*BnaA9.CYP78A9* lines

- This resulted in a significant increase in *BnaA9.CYP78A9* expression level was observed in transgenic positive plants, thus resulting in an increase in cell number, eventually leading to the rise in silique length and seed weight.


The silique length of 35S::*BnaA9.CYP78A9* lines


The cell number of 35S::*BnaA9.CYP78A9* o lines

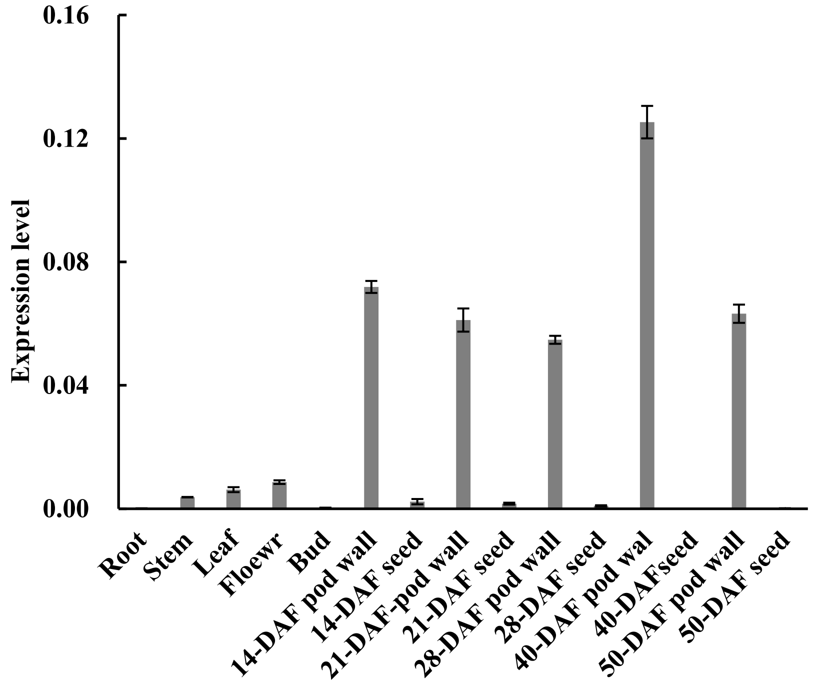
➢ To further verify the function of *BnaA9.CYP78A9* in rapeseed, it was over-expressed in NIL-QC14 and edited in the parent Zhongshuang11 respectively.

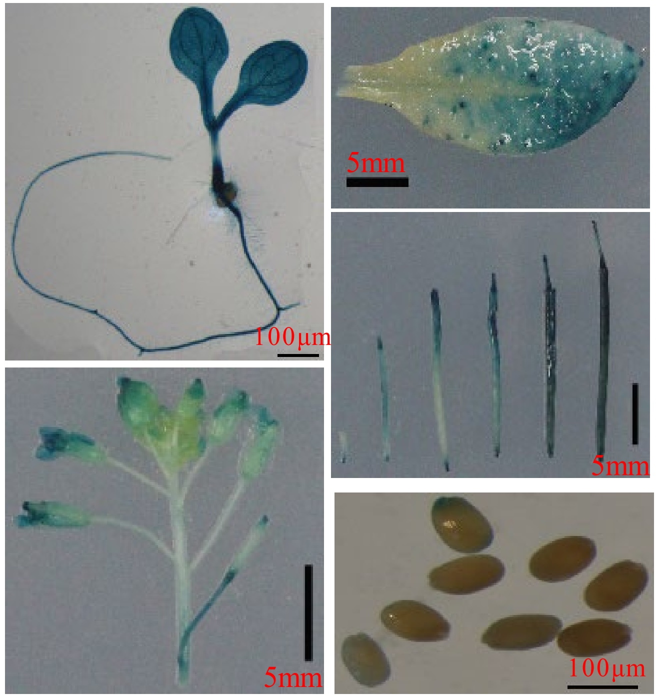


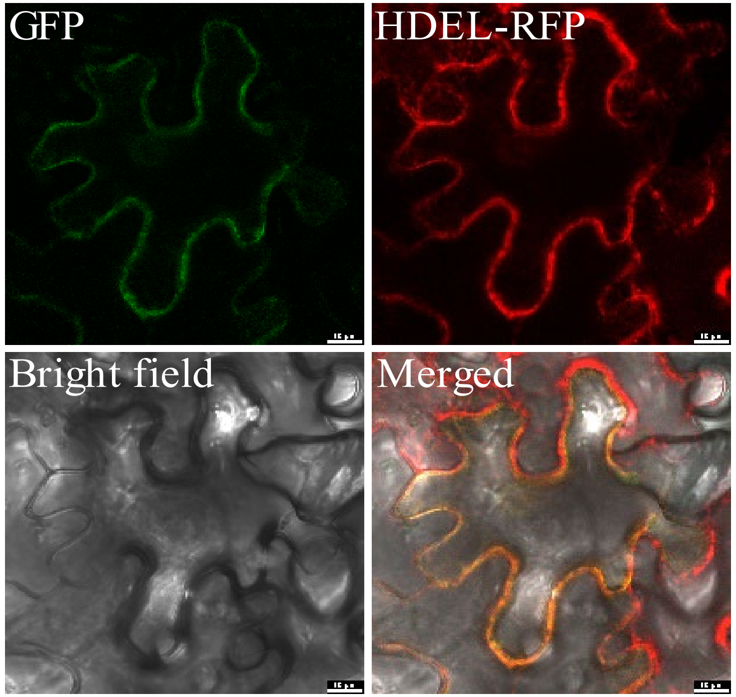
- The silique length and seed weight of several over-expressed plants was significantly increased, whereas those of gene-edited plants were significantly reduced.

✓ **These results confirmed that *BnaA9.CYP78A9* was the causal gene of QC14.**

- To explore the expression pattern of *BnaA9.CYP78A9*, qRT-qPCR and GUS staining were perofored in rapeseed and *Arabidopsis*, respectively.



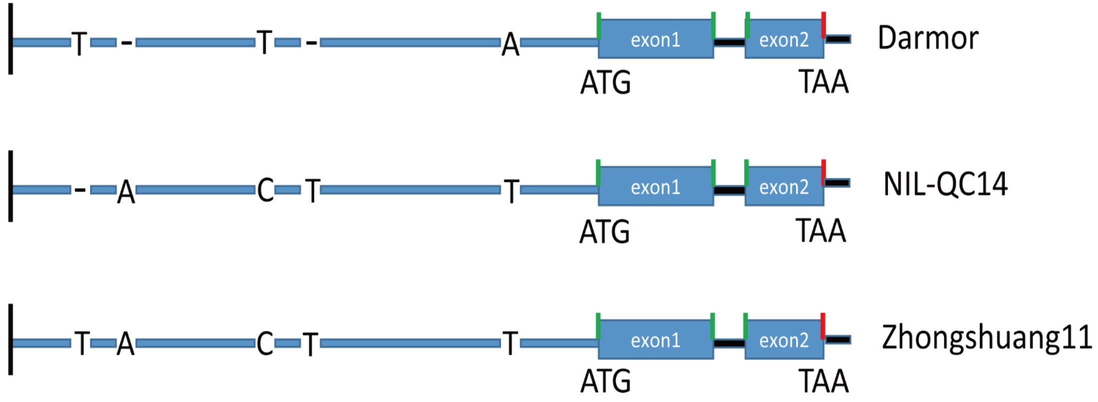




- Both experimental results showed that *BnaA9.CYP78A9* was preferentially expressed in green tissues.
- Its expression level was high in silique walls, middle in flowers, buds, leaves and stems, low in roots and seeds.

- A subcellular localization analysis showed that *BnaA9.CYP78A9* was localized to the endoplasmic reticulum in tobacco.

- To further find causal variation of *BnaA9.CYP78A9*, its full-length in ZS11 and NIL-QC14 was cloned and sequenced, but no difference was found.



- To determine whether QC14 was also resulted from this TE, the corresponding PAV marker was used to genotype NIL-QC14 and Zhongshuang11.

Num.	Ave. of PLm (mm)	PAV markers	
		TEp-F/TE-R	TEa-F/TE-R
19	76.1	1	1
28	88.5	1	0
40	55.1	0	1

- This TE was present in Zhongshuang11 but absent from NIL-QC14, and completely co-segregated with the silique lengths of these NILs.

- A recent study showed that a CACTA-like TE inserted into its upstream region can increase silique length and seed weight.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A CACTA-like transposable element in the upstream region of *BnaA9.CYP78A9* acts as an enhancer to increase silique length and seed weight in rapeseed

	SYm (g)	SYPPm (g)	SNm	PNm	SNPPm	TSWm (g)	PLm (mm)
TEp	7.04	97.6	1565	72.1	21.7	4.5	88.5
TEa	5.74	77.4	1573	74.2	21.2	3.65	55.1
TEaTEp	6.74	92	1570	73.3	21.5	4.29	77.1
MPH(%)	5.5	5.1	0.1	0.2	0.2	5.3	7.4

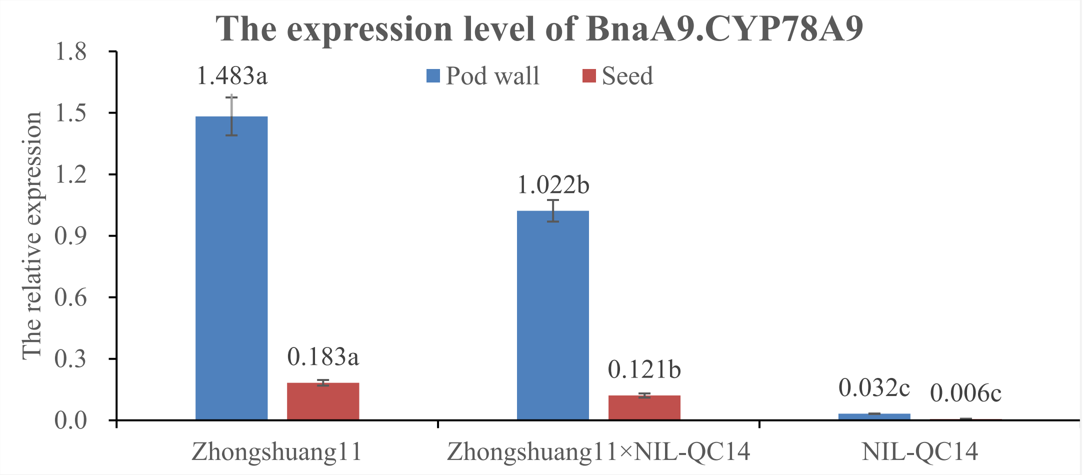
- For these traits, the calculated MPH of PAV marker of this TE was basically consistent with that of QC14.

✓ **These results supported that heterotic effect of QC14 was resulted from heterozygous status of TE**

5. Dissecting the regulatory mechanism of heteorsis

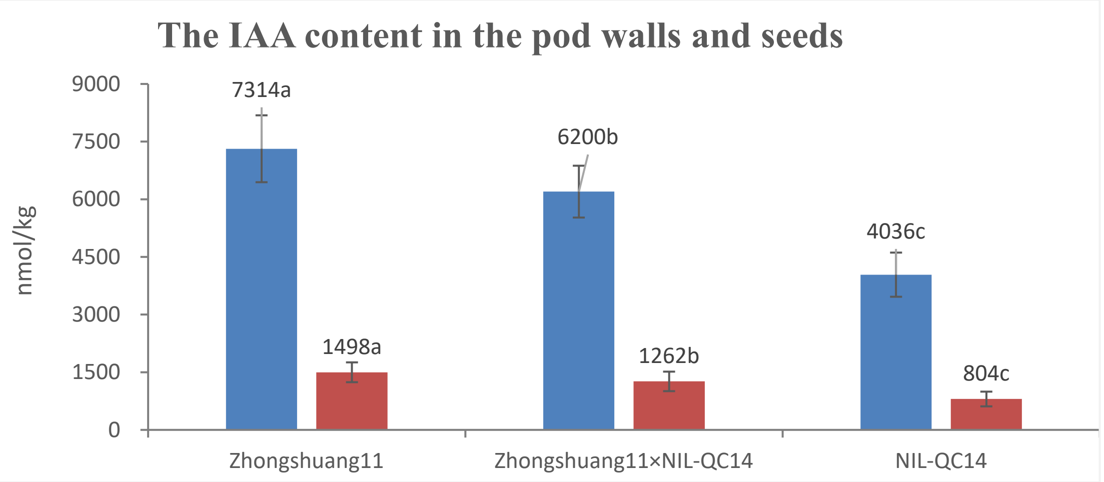
- To further reveal the underlying regulatory mechanism, a systematic comparative study was conducted using this cross.

(1) The *BnaA9.CYP78A9* expression was investigated



- The *BnaA9.CYP78A9* expression level in hybrid F1 was significantly higher than means of two parents, but lower than Zhongshuang11.

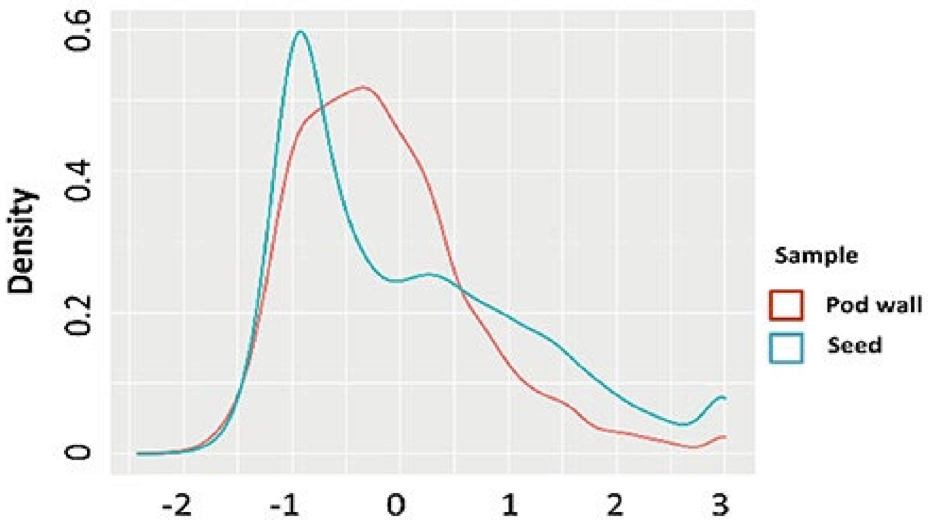
(2) The phytohormones contents were measured



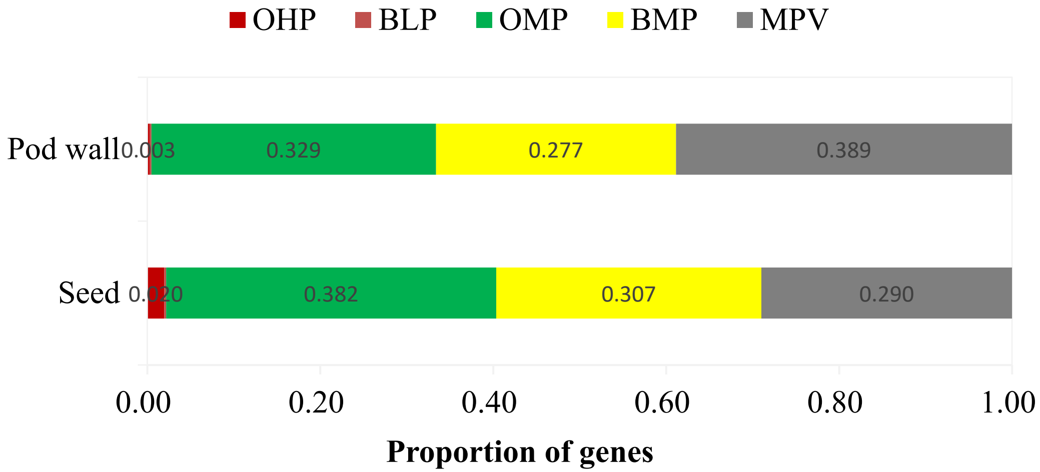
- The IAA content of hybrid F1 was significantly higher than the mean of two parents, but lower than Zhongshuang11.

✓ **The heterozygous status of TE in the upstream region of *BnaA9.CYP78A9* could lead to the partial-dominance expression of this gene and over mid-parent content of IAA.**

(3) The expression pattern of the downstream responsive genes was investigated by RNA-seq



The quantitative frequency distribution of D/|A| value



The qualitative classification of five types of DEGs pattern: over-high-parent (OHP), below-low-parent (BLP), over-middle-parent (OMP), below-middle-parent (BMP) and middle-parent value (MPV).

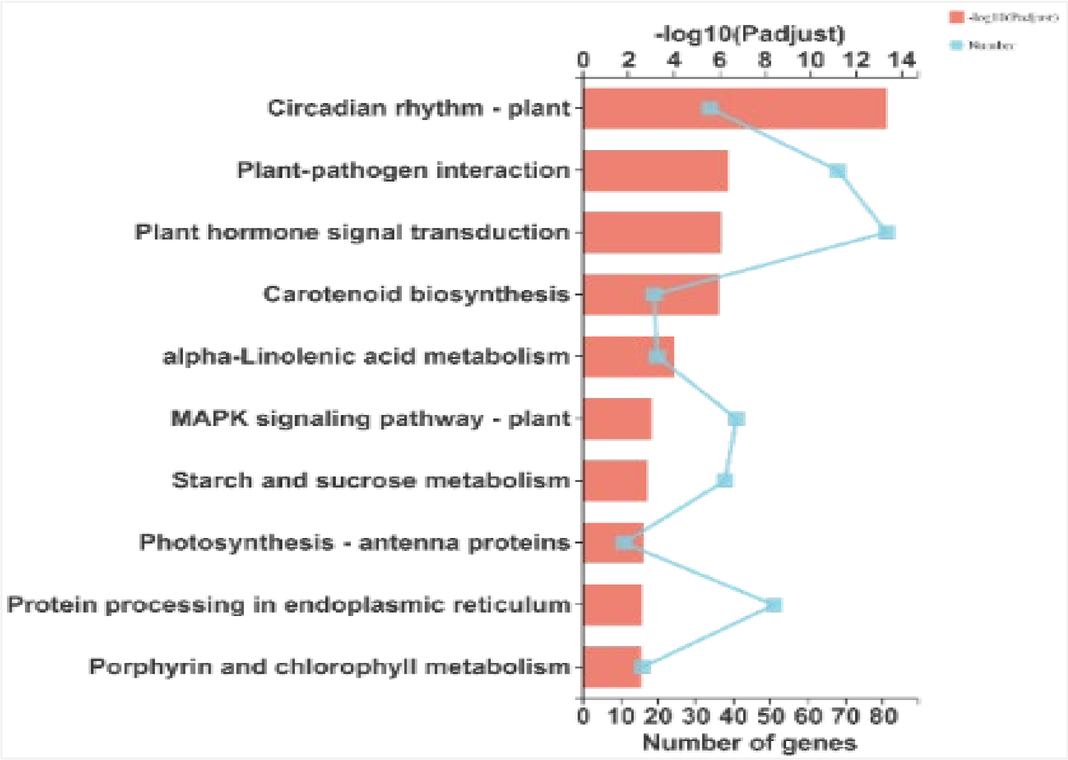
- Most DEGs (>80%) showed non-additive expression and the dominance effects of expressive aboundance of most DEGs were negative.

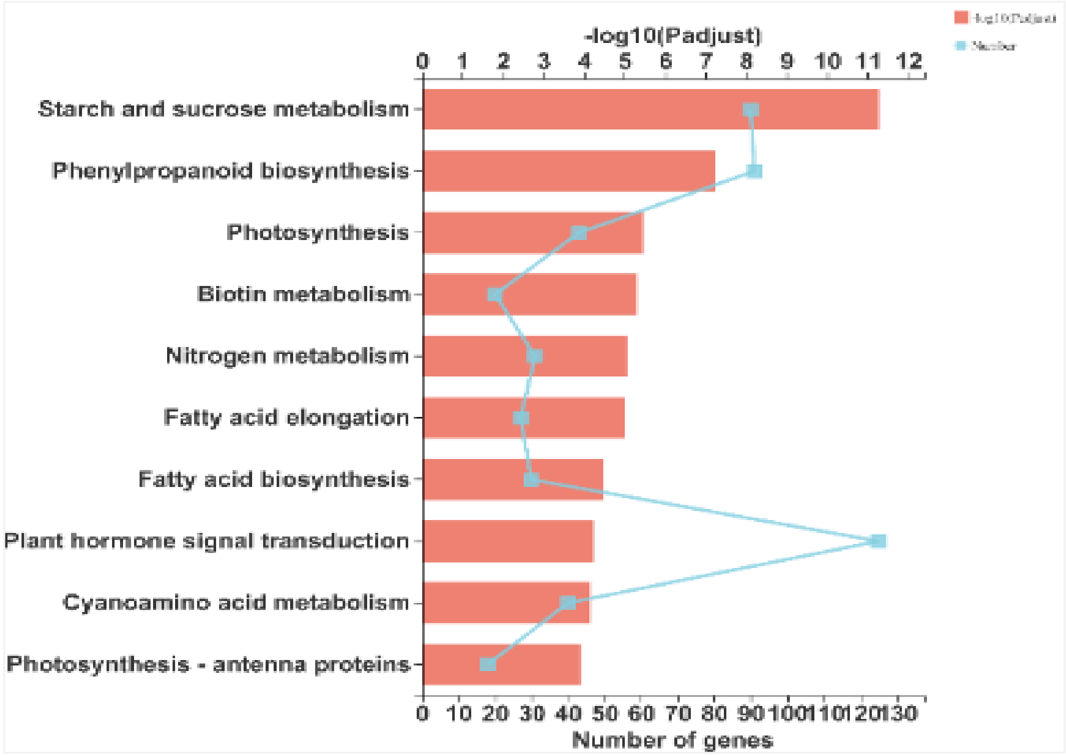
◆ The previous study showed that there were more genes negatively regulating silique and/or seed size (Hussain et al., 2020).

- The OHP and BLP pattern accounted for lowest proportion (<1%) in both pod wall and seed, highly accordant with previous reports.
- These results suggested that over-dominance was also rare at gene expression level.

✓ **The non-additive expression of down-stream responsive genes might be responsible for the heterotic phenotype of this cross.**

- To reveal the functional association between DEGs and heterotic phenotype, all of these DEGs was subjected to KEGG analysis.





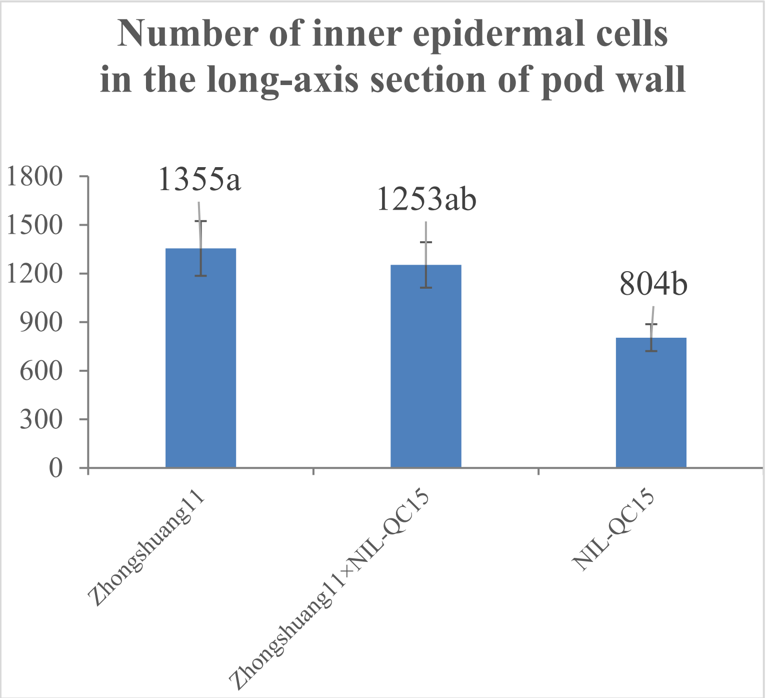
- The DEGs in both pod wall and seed were significantly enriched in phytohormone signal transduction pathway.

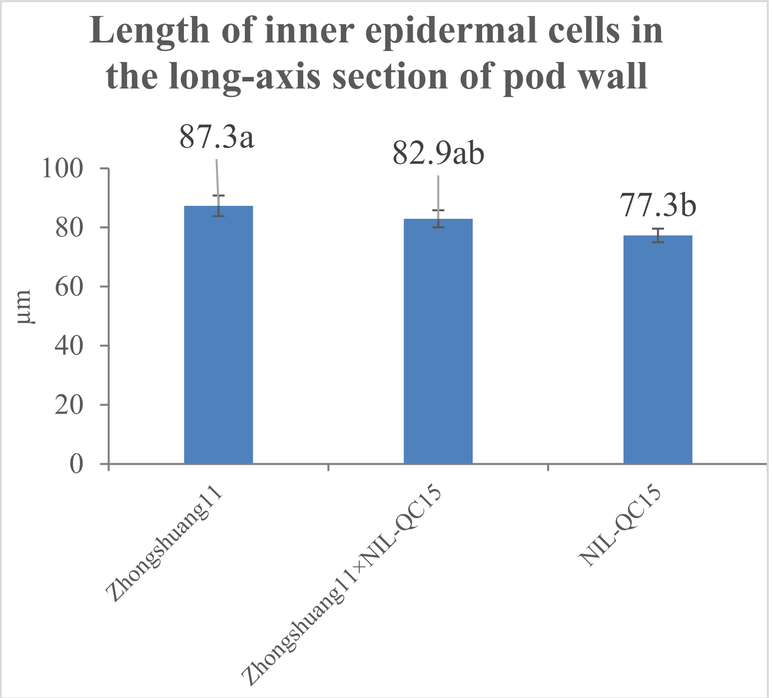
① Of these, auxin-related DEGs accounted for the highest propportion.

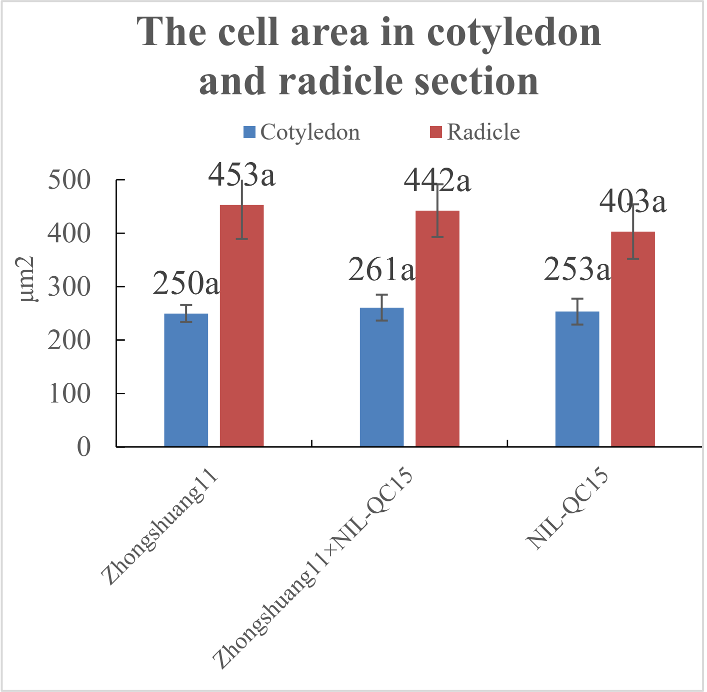
② A total of 36 and 86 DEGs were homologous to the known silique and seed size-regulating genes, respectively.

✓ **The identified DEGs were highly associated with AUX pathway as well as silique and seed size.**

(4) The cell number and/or size in both silique wall and seeds of this cross were investigated

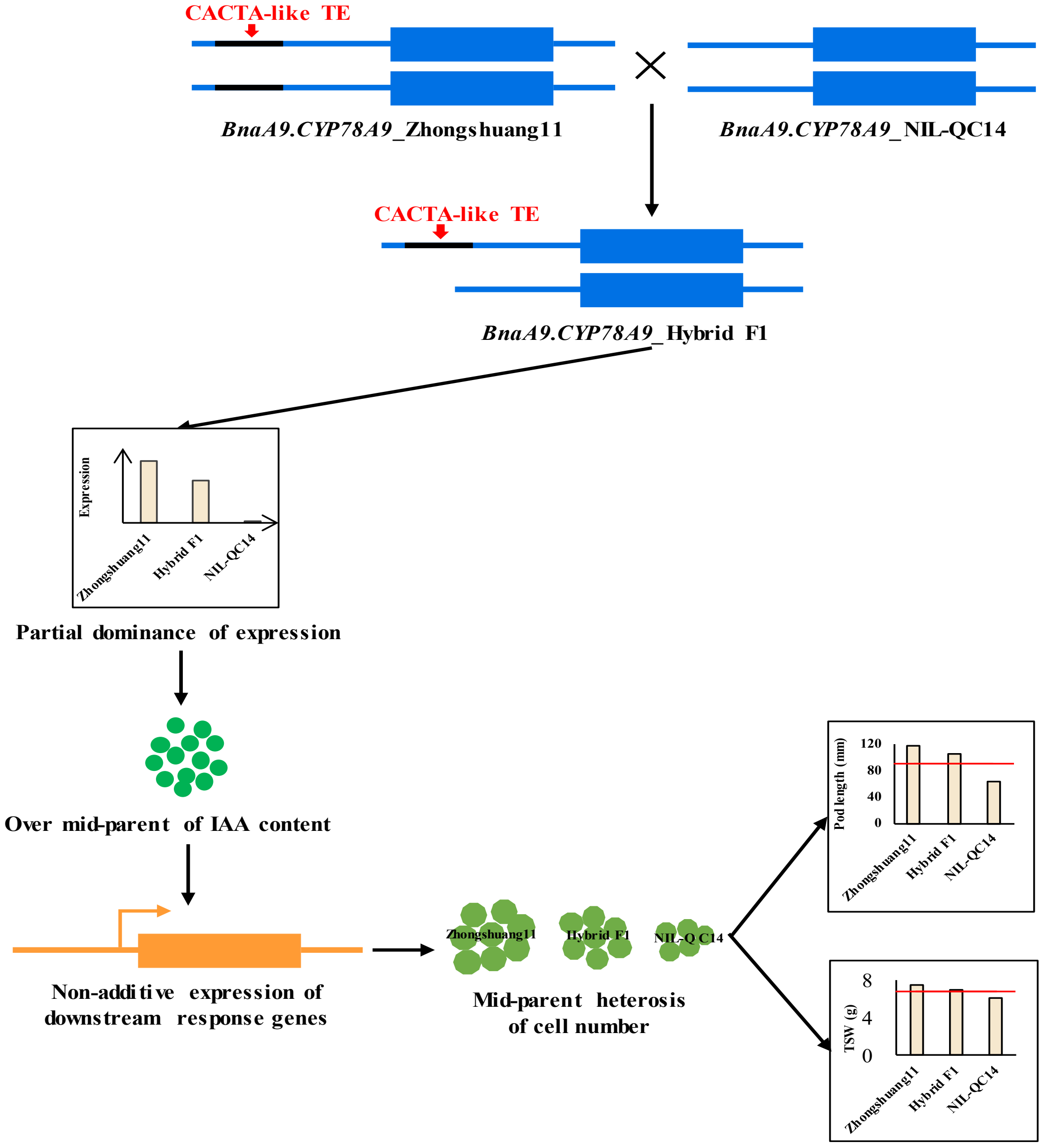

Number of inner epidermal cells in the long-axis section of pod wall


Length of inner epidermal cells in the long-axis section of pod wall


The cell area in cotyledon and radicle section

- The pod length and seed size difference between Zhongshuang11 and NIL-QC14 was **mainly attributable to the variation in cell number,** followed by cell size.
- The **cell number in the pod wall of hybrid F_1 showed significant mid-parent heterosis**, cell length in the pod wall and cell area in both cotyledon and radicle exhibited no significant mid-parent heterosis.

✓ **The heterotic effect of QC14 on silique length&seed weight was due to MPH of cell number**



Partial dominance of expression

Over mid-parent of IAA content

Non-additive expression of downstream response genes

Mid-parent heterosis of cell number

Mid-parent heterosis of pod length and seed weight

Zhongshuang11
(TE in upstream of *BnaA9.CYP78A9*)

×

NIL-QC14
(no TE)

Hybrid F1
(The heterozygous status of TE)

Partial dominant expression of BnaA9.CYP78A9

Over mid-parent content of IAA

Non-additive expression of downstream responsive genes

Mid-parent heterosis of cell number

MPH of silique length, seed weight and yield

6. Summary and conclusion

1. The strong heterosis of composite traits (such as yield) results from the multiplicative effects of moderate heterosis of its component traits.
2. The genetic architecture of yield heterosis is very complex, which is characterized by obvious center-periphery structure, hub-QTL, up/downstream and positive/negative feedback between traits.
3. At single-locus level, partial-dominance seemed to be the major genetic basis of yield heterosis.
4. To obtain excellent hybrids, the most important principle is to accumulate advantageous alleles (whether in homozygous or heterozygous status) as most as possible rather than to increase the overall genetic distance between inbred parents.
5. We demonstrate the first case of better-parent heterosis produced by complementation of partial-dominant loci, provided the theoretical proof and found the precondition:$d1+d2>|a1-a2|$, representing an important supplement to dominance complementation hypothesis.
6. We cloned the first heterotic-QTL in oilseed rape and reveal its molecular regulation mechanism.
7. The accumulative effect of heterotic QTL and the multiplicative effect of component traits could well explain yield heterosis in rapeseed, which support pyramiding heterosis theory.

Thanks for your Listening