Maternal control of seed weight in rapeseed: the causal link between the size of silique and seed

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Outline

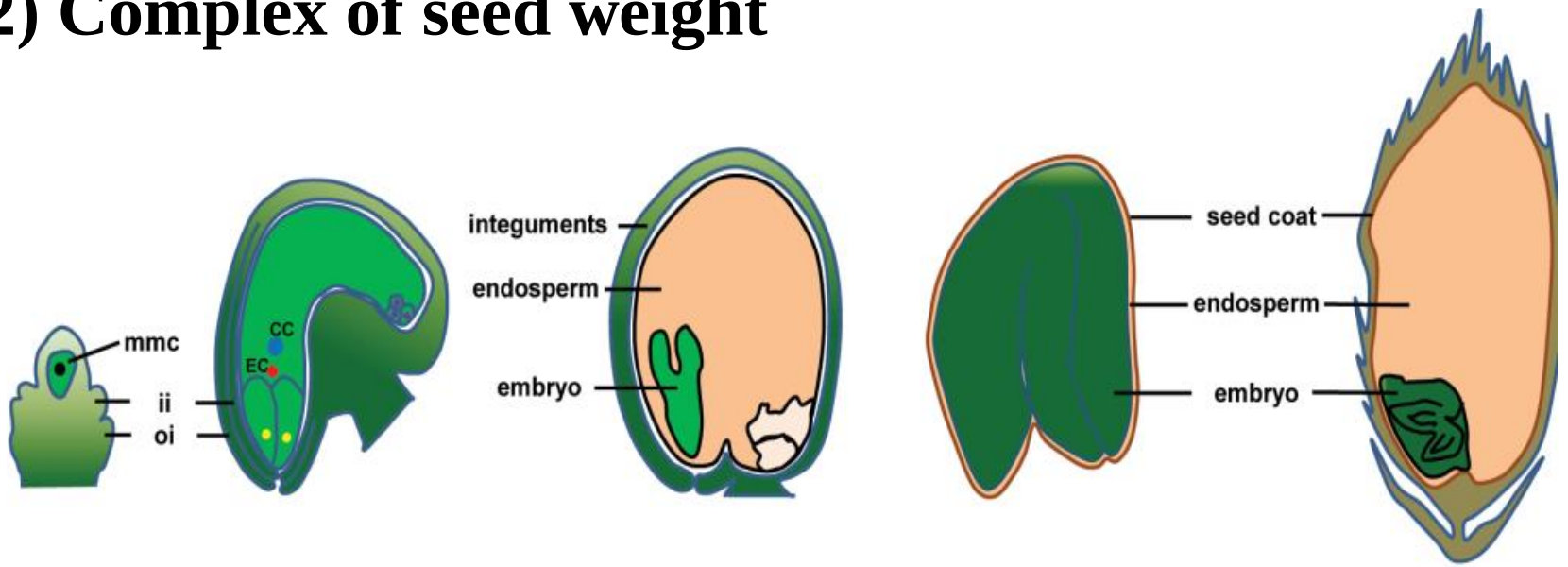
1. **Research Background**
2. **Maternal control of seed weight in rapeseed**
3. **The causal link between the size of silique and seed**
4. **Summary and conclusion**

1. Research Background

(1) Importance of seed weight

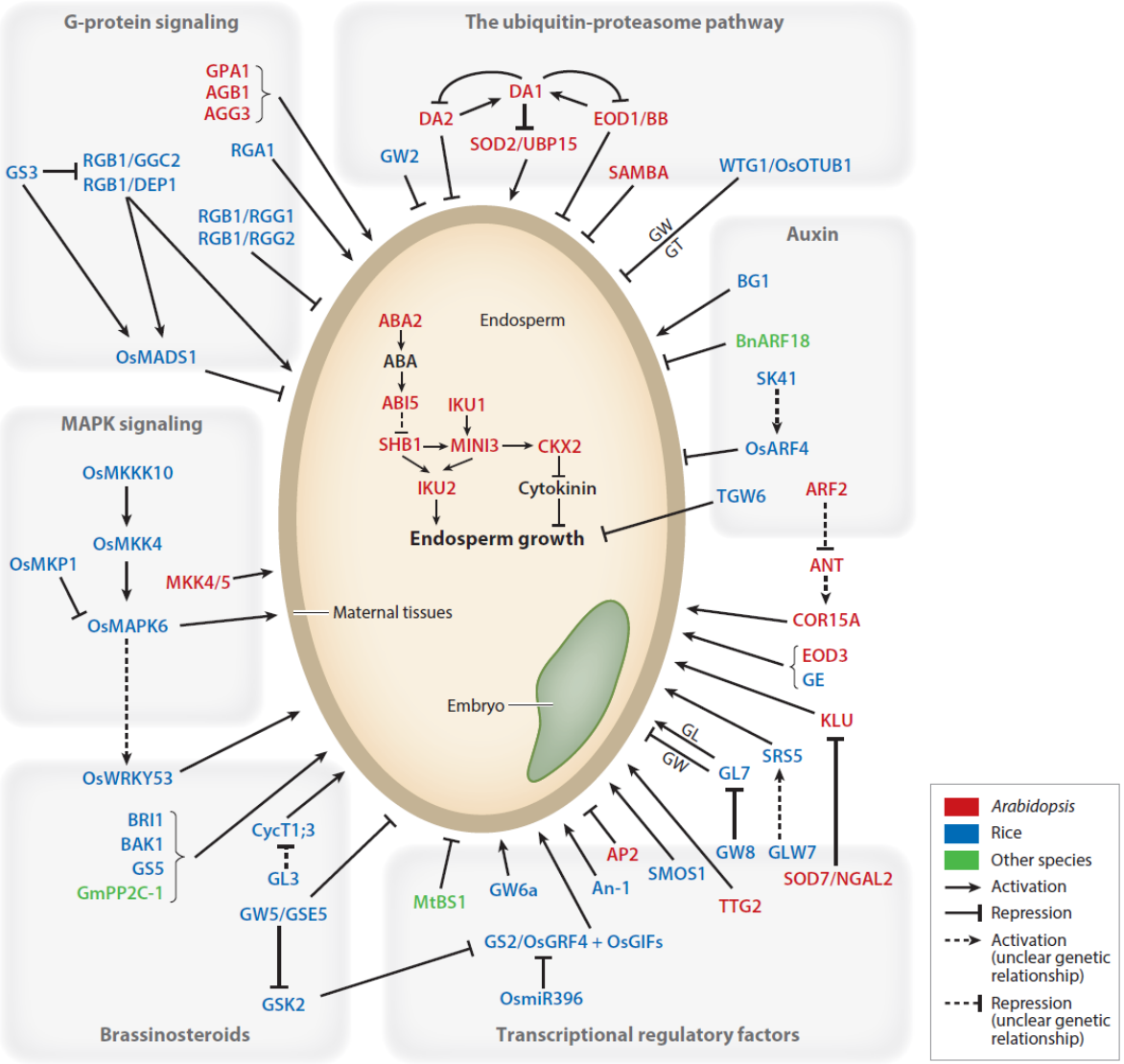
A character tightly related to the evolution and fitness of plants and a target agronomical trait for crop domestication and improvement (Li and Li, 2015; 2016）。

(2) Complex of seed weight



- **Belong to the seed traits, whose genetic model include the effects of embryo, endosperm, cytoplasm and maternal genotype (Zhu, 1995).**

(3) Known genes and network for seed weight regulation

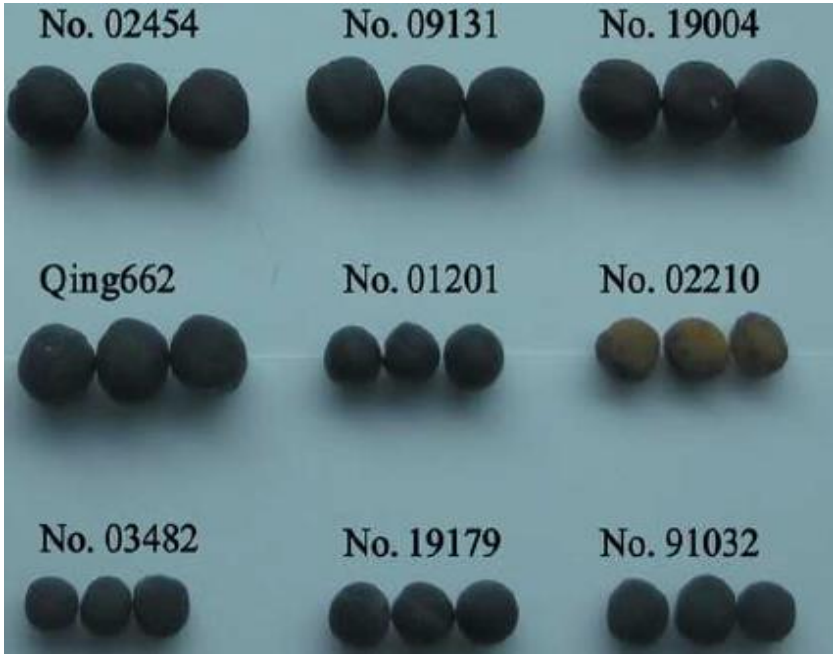


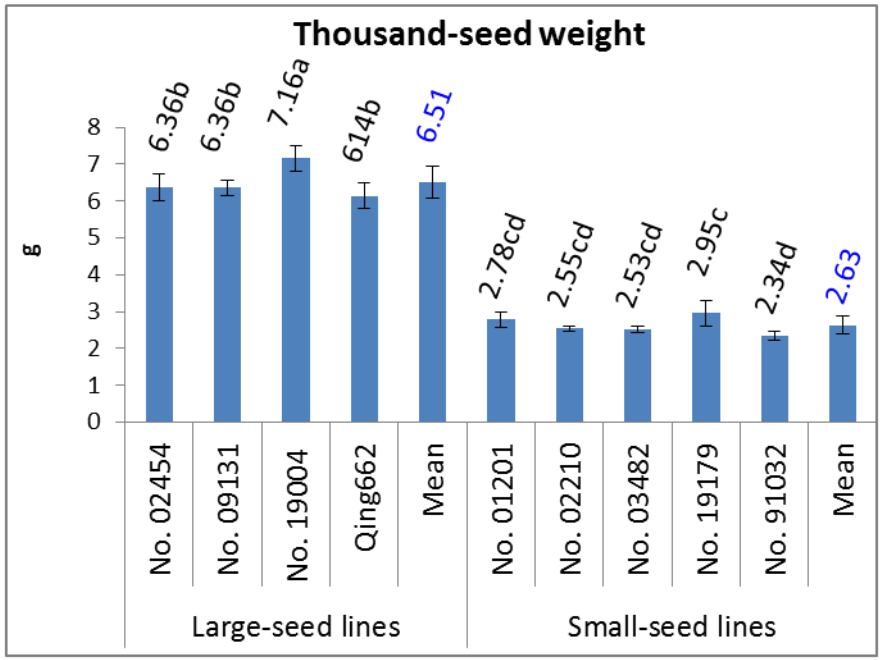
- **The present research into seed weight was focused on seed (such as embryo and endosperm) itself， whereas the impact of mother plant is unclear。**

2. Discovery of the maternal control of seed weight in rapeseed (*Brassica napus* L.)

(1) Representative lines of extreme seed weight

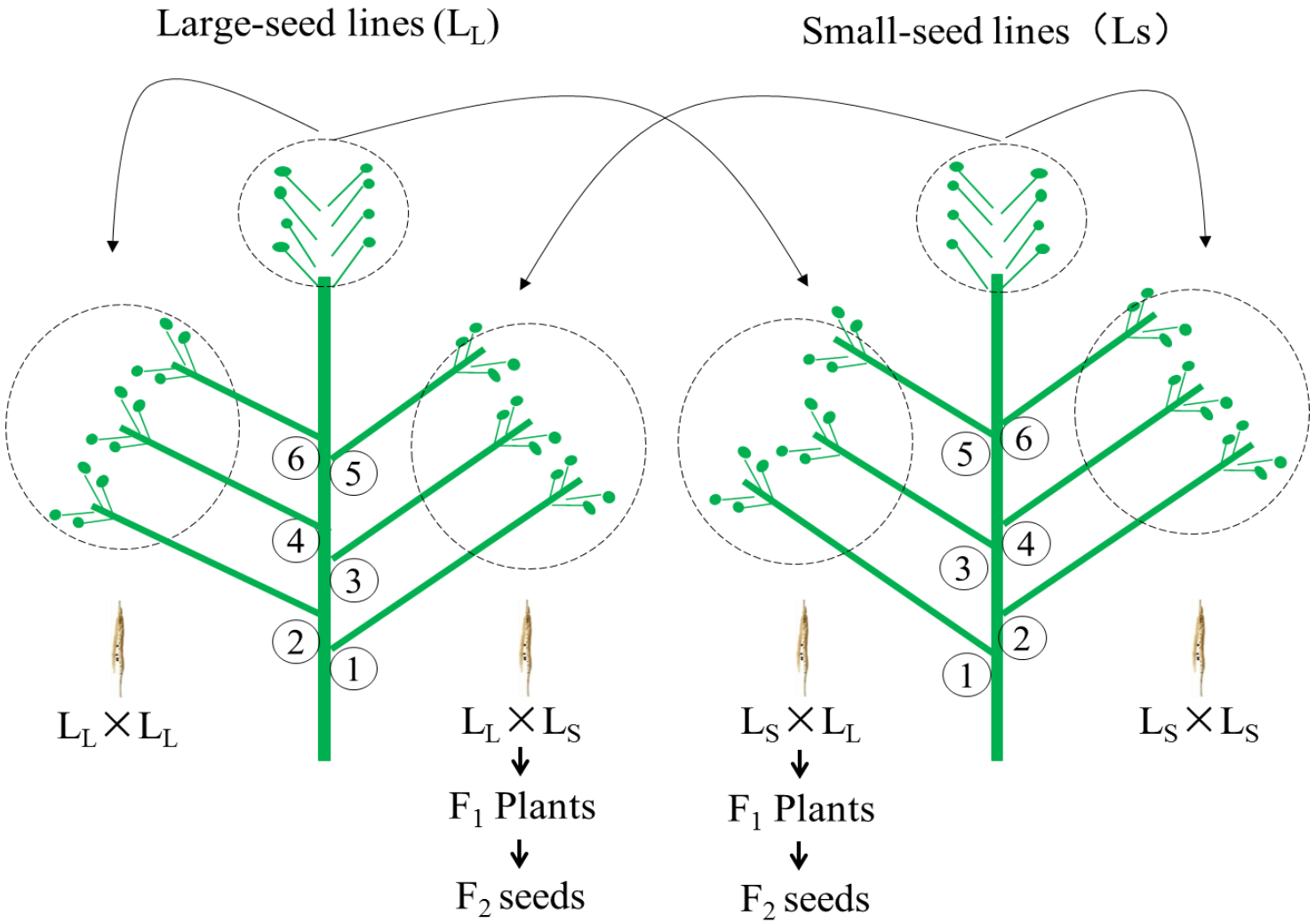
- We analysed of the phenotypic and genotypic diversity of 1083 inbred lines of rapeseed from all over the world.





- **We obtained four large-seed (Thousand-seed weight > 6 g) and five small-seed (TSW < 3 g) lines with large genetic distance and similar flowering time.**

(2) Genetic experiment design



① **The different branches on one pair of plants were alternatively self- and cross-pollinated, and the weight of obtained seeds were investigated;**

② **The reciprocal F_1 seeds were planted and the weight of F_2 seeds were investigated ad compared.**

（3）Maternal VS xenia effects on seed weight

- For all lines, the weights of seeds obtained from self and cross-pollination showed no significant difference, that means xenia effect is not significant.

Parental lines (L-S)	F_1(L×L)	F_1(L×S)	Maternal	Xenia	F_1(S×S)	F_1(S×L)	Materna	Xenia
No. 02454- No. 01201	6.55±0.40A	6.61±0.66A	1.00	0.00	4.19±0.24B	4.92±0.32B	0.70	0.30
No. 02454- No. 03482	6.14±0.13A	6.15±0.59A	0.93	0.07	4.99±0.42B	4.95±0.58B	1.00	0.00
No. 02454- No. 19179	6.79±0.44A	6.46±0.49A	0.86	0.14	4.29±0.68B	4.42±0.84B	0.96	0.04
No. 02454- No. 91032	6.51±0.37A	6.37±0.83A	0.99	0.01	4.93±0.72B	5.06±0.19B	0.83	0.17
No. 02454-No. 02210	6.22±0.45A	6.12±0.70A	0.95	0.05	4.33±0.29B	4.57±0.49B	0.85	0.15
No. 09131- No. 01201	7.29±0.15A	7.07±0.50A	0.92	0.08	4.56±0.14B	5.11±0.24B	0.80	0.20
No. 09131- No. 02210	7.01±0.20A	7.32±0.52A	1.00	0.00	5.04±0.44B	5.05±0.69B	0.99	0.01
No. 09131- No. 03482	7.53±0.58A	7.84±0.18A	1.00	0.00	4.89±0.29B	5.40±0.56B	0.82	0.18
No. 09131- No. 19179	7.42±0.15A	7.62±0.09A	1.00	0.00	5.40±0.98B	5.12±0.50B	1.00	0.00
No. 09131- No. 91032	6.96±0.25A	6.87±0.25A	0.94	0.06	4.58±0.52B	4.90±0.91B	0.90	0.10
No. 19004- No. 01201	7.36±0.52A	7.70±0.35A	1.00	0.00	4.50±0.31B	5.12±0.42B	0.80	0.20
No. 19004- No. 02210	6.76±0.28A	6.88±0.16A	1.00	0.00	3.65±0.37B	3.83±037B	0.94	0.06
No. 19004- No. 03482	6.85±0.38A	6.98±0.48A	1.00	0.00	3.60±0.16B	3.88±0.10B	0.91	0.09
No. 19004- No. 19179	7.66±0.17A	7.73±0.22A	1.00	0.00	4.15±0.08B	4.25±0.15B	0.97	0.03
No. 19004- No. 91032	7.03±0.27A	7.19±0.22A	1.00	0.00	4.15±0.33B	4.35±0.17B	0.92	0.08
Qing662- No. 01201	5.66±0.35a	5.47±0.60a	0.82	0.18	4.44±0.11b	4.43±0.58b	1.00	0.00
Qing662- No. 02210	5.84±0.61a	5.67±0.66a	0.93	0.07	4.25±0.44b	4.51±0.18b	0.82	0.18
Qing662- No. 03482	8.53±0.08A	8.32±0.17A	0.95	0.05	4.84±0.44B	5.02±0.49B	0.95	0.05
Qing662- No. 19179	7.30±0.53A	7.30±0.44A	0.97	0.03	4.99±0.44B	5.08±0.43B	0.97	0.03
Qing662- No. 91032	8.09±0.30A	7.33±0.42A	0.82	0.18	4.36±1.44B	4.94±0.44B	0.81	0.19

$m=\sum(F_1-P_2)(P_1-P_2)/\sum(P_1-P_2)^2$ (Cong, 1996)

- The mean of maternal effects for all crosses is 0.93, which showed that seed weight difference of these lines was dominantly controlled by maternal effect.

（4）Maternal genotypic vs Cytoplasmic effects

- **The weights of F_2 seeds from most reciprocal F_1 were similar, that means cytoplasmic effects is generlaly not significant.**

Parental lines (L-S)	F_2(L×S)[a]	F_2(S×L)
No. 02454- No. 02210[b]	3.97±0.49a	3.78±0.32a
No. 02454- No. 91032	4.31±0.40a	4.38±028a
No. 02454- No. 03482	4.72±0.62a	4.72±0.32a
No. 09131- No. 02210	3.66±0.22a	3.61±0.16a
No. 09131- No. 91032	3.92±0.20a	3.99±0.58a
No. 09131- No. 03482	4.39±0.35a	4.57±0.36a
No. 02454- No. 19179	4.74±0.51a	4.52±1.19a
No. 02454- No. 01201	4.31±0.39a	4.24±0.19a
No. 09131- No. 19179	4.50±0.50a	4.63±0.28a
No. 09131- No. 01201	4.05±0.19a	4.00±0.19a
No. 19004- No. 19179	4.54±0.37a	4.47±0.36a
No. 19004- No. 01201	4.17±0.40a	4.12±0.28a
No. 19004- No. 02210	3.95±0.31a	3.75±0.40a
No. 19004- No. 91032	4.23±0.22a	4.08±0.27b
No. 19004- No. 03482	4.31±0.37a	4.32±0.22a
Qing662- No. 19179	4.15±0.27a	4.38±0.37b
Qing662- No. 01201	4.08±0.18a	4.13±0.34a
Qing662- No. 02210	3.93±0.44a	3.85±0.51a
Qing662- No. 91032	3.87±0.23a	3.85±0.30a
Qing662- No. 03482	4.07±0.16a	4.14±0.19a

- **The results of two genetic experiments further showed that seed weight difference of these lines is mainly determined by maternal genotype.**

（5）Maternal vs Embryonic vs Cytoplasmic effects

- **Estimation of the variance components using the diploid seed embryo-cytoplasmic-maternal effects (2nGoCGm）model（Zhu，1996）.**

Table 4. Estimation of genetic variance components for seed weight in rapeseed.

Parameter	Variance	Parameter	Variance
V_A	0.070**	V_{AE}	0.014
V_D	0.015	V_{DE}	0.024**
V_C	0.001**	V_{CE}	0.000
V_{Am}	0.483**	V_{AmE}	0.000
V_{Dm}	0.179**	V_{DmE}	0.026**
		V_e	0.188**

V_A, embryo additive variance; V_D, embryo dominance variance; V_C, cytoplasmic variance; V_{Am}, maternal additive variance; V_{Dm}, maternal dominance variance; V_{AE}, embryo additive interaction variance; V_{DE}, embryo dominance interaction variance; V_{CE}, cytoplasmic interaction variance; V_{AmE}, maternal additive interaction variance; V_{DmE}, maternal dominance interaction variance; V_e, residual variance

** Significantly different at the 0.01 level.

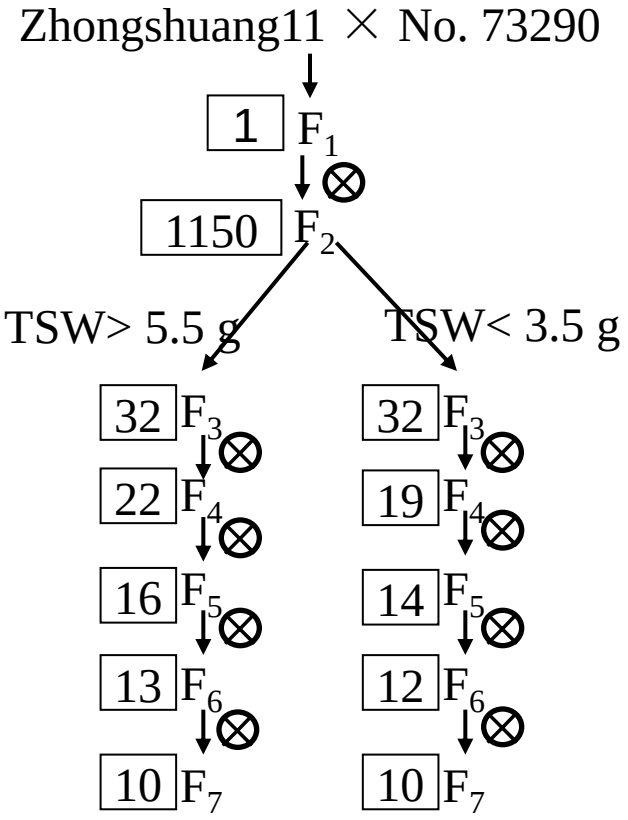
The maternal, embryonic and cytoplasmic genotype explained the variances of 68.8%, 12.3%和0.2% respectively 。

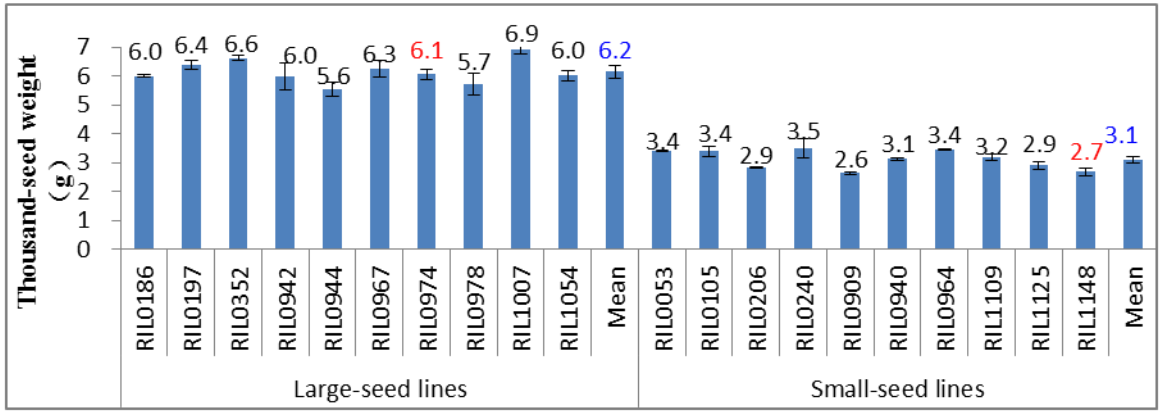
- **This result also showed that seed weight difference of these lines is mainly controlled by maternal genotype.**

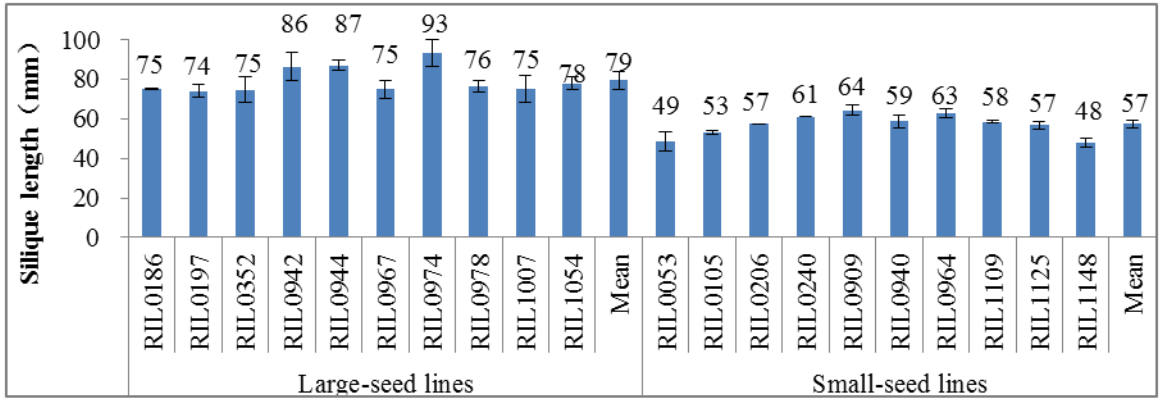
Li et al. (2015). The natural variation of seed weight is mainly controlled by maternal genotype in rapeseed (*Brassica napus* L.). PLoS ONE 10(4): e125360

3. Causal link between the size of silique and seed

（1）RILs with extreme seed weight



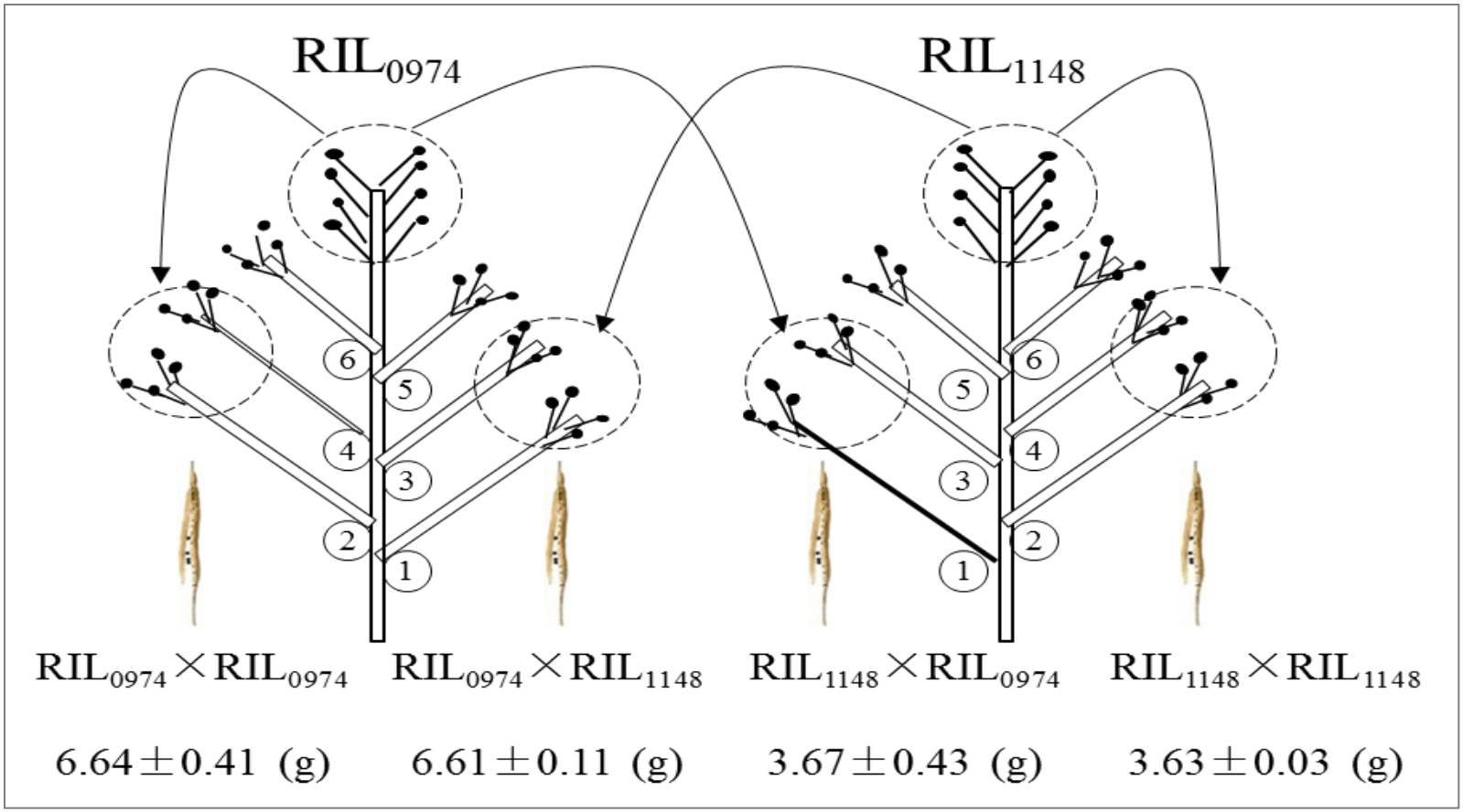




- **Interestingly, silique length of large-seed lines was all longer than small-seed lines, indicating association between silique length and seed weight.**

➢ To dissection the causes of seed weight difference between these lines, one pair of lines with extreme difference was chosen for comparative study.

（2）Genetic cause of seed weight difference between RIL_{0974} and RIL_{1148}

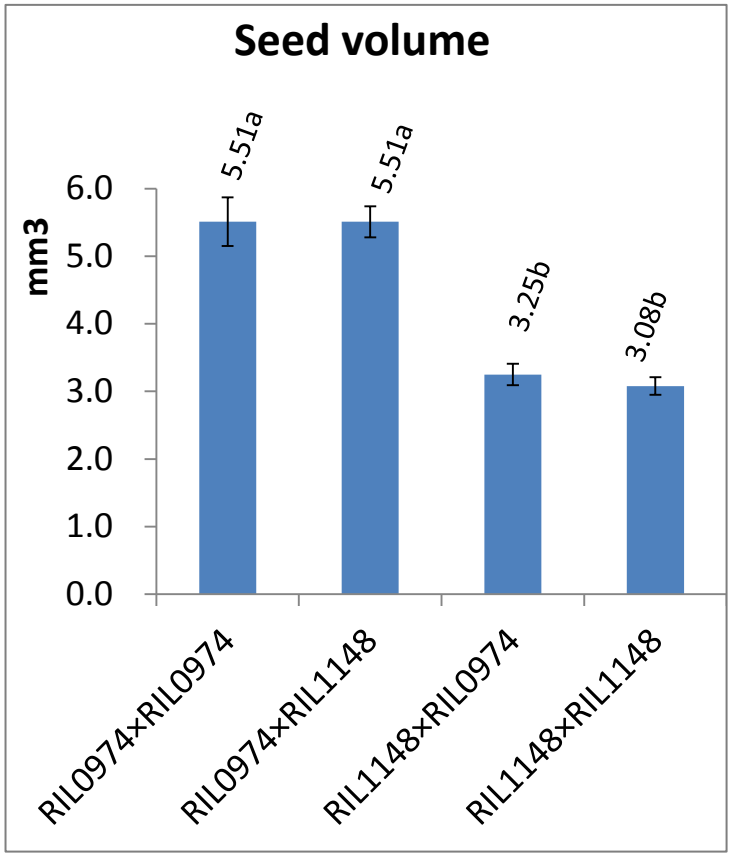


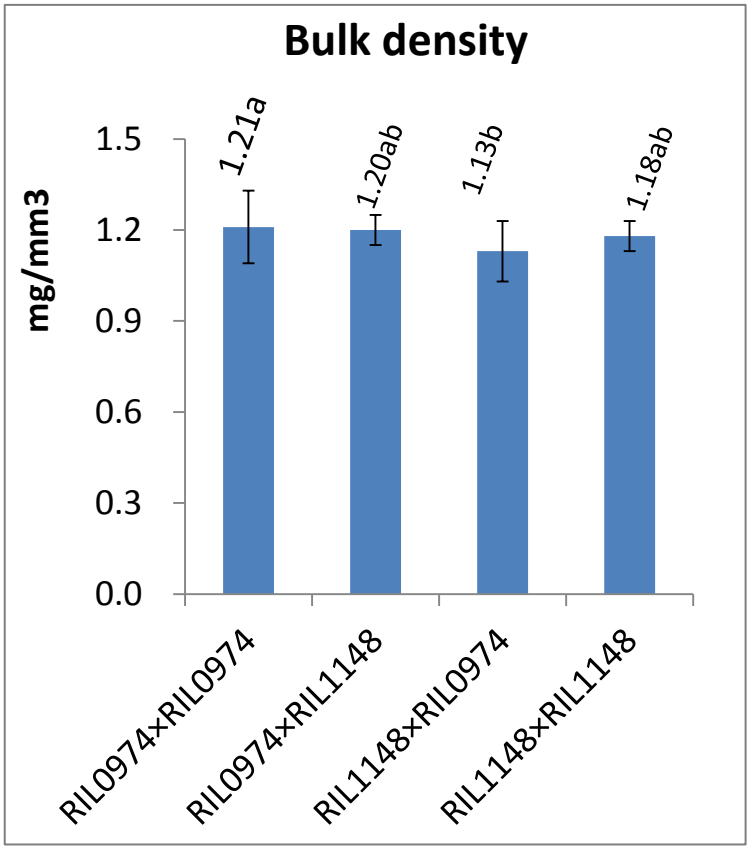
For both RIL_{0974} and RIL_{1148}, the weight of seeds from cross-pollination was near to that from self-pollination, the maternal effect was 0.97 and 0.88, respectively。

- **This result improved that seed weight difference between RIL_{0974} and RIL_{1148} was controlled by maternal genotype.**

（3）Morphological cause of seed weight difference between RIL_{0974} and RIL_{1148}

- Seed volume and bulk density were compared between RIL_{0974} and RIL_{1148}

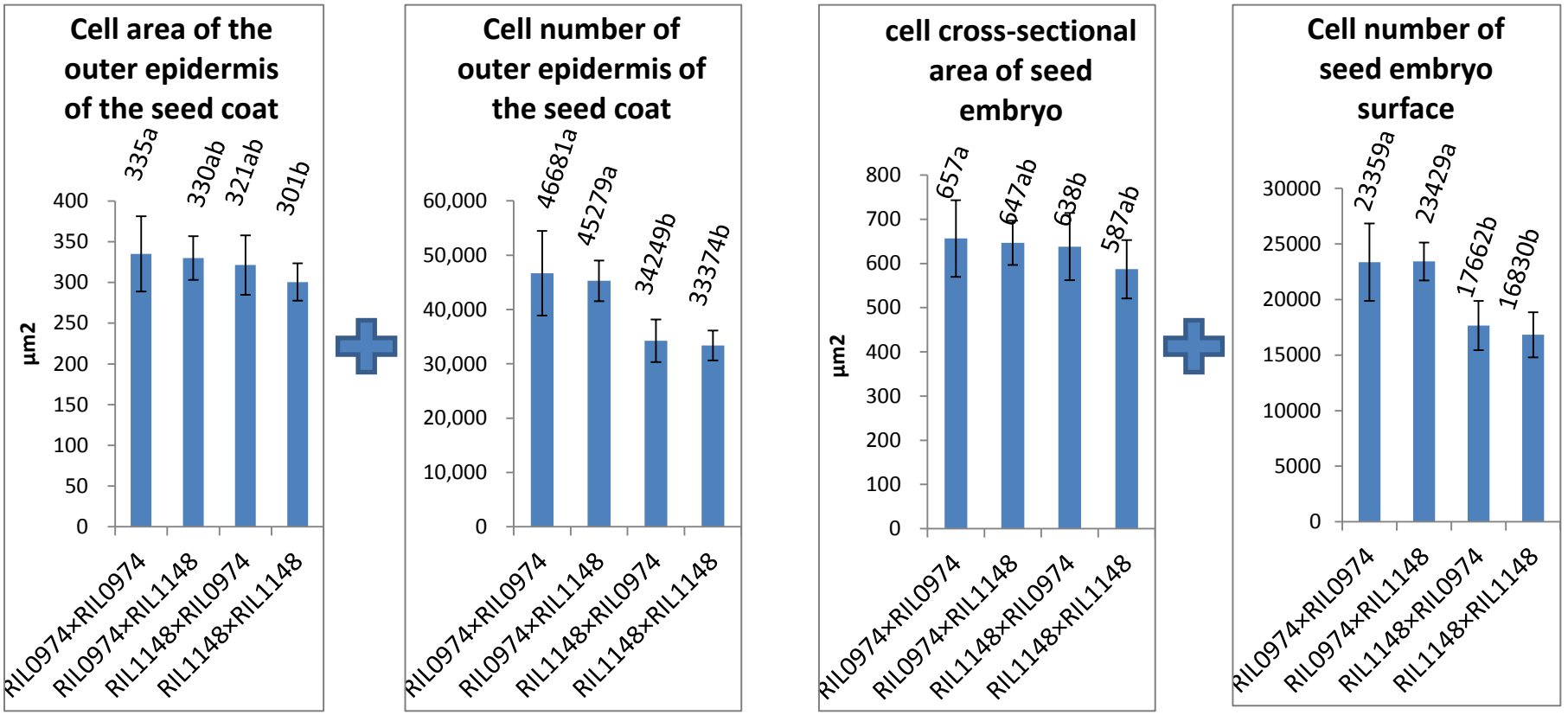




- RIL_{0974} VS RIL_{1148}: 1.74 and 1.04 times for seed volume and bulk density, respectively. Therefore, seed weight difference between RIL_{0974} and RIL_{1148} was caused by seed size rather than bulk density.

（4）Cytological cause of seed size difference between RIL_{0974} and RIL_{1148}

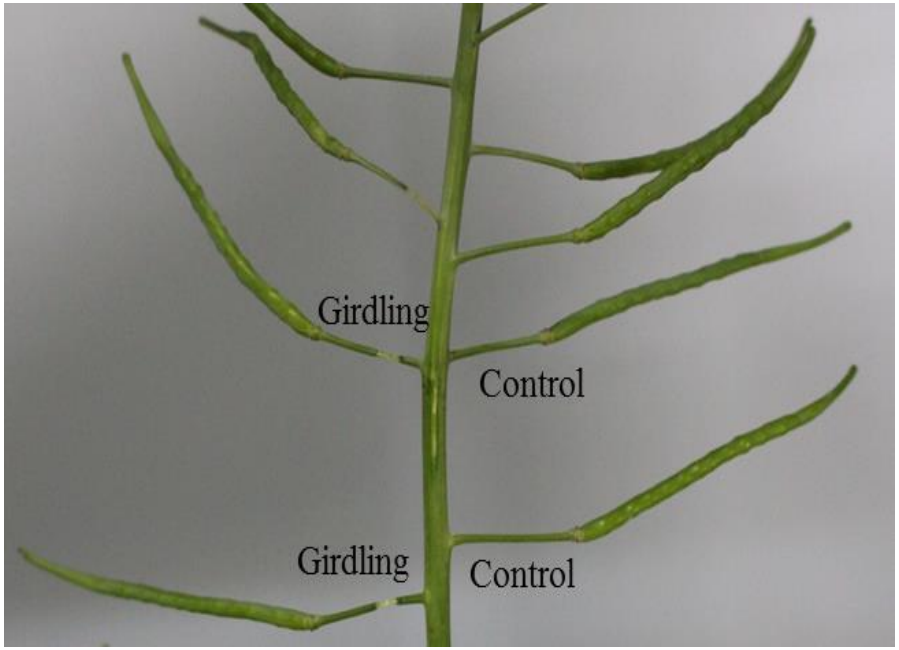
- Cell number and size were compared between RIL_{0974} vs RIL_{1148}

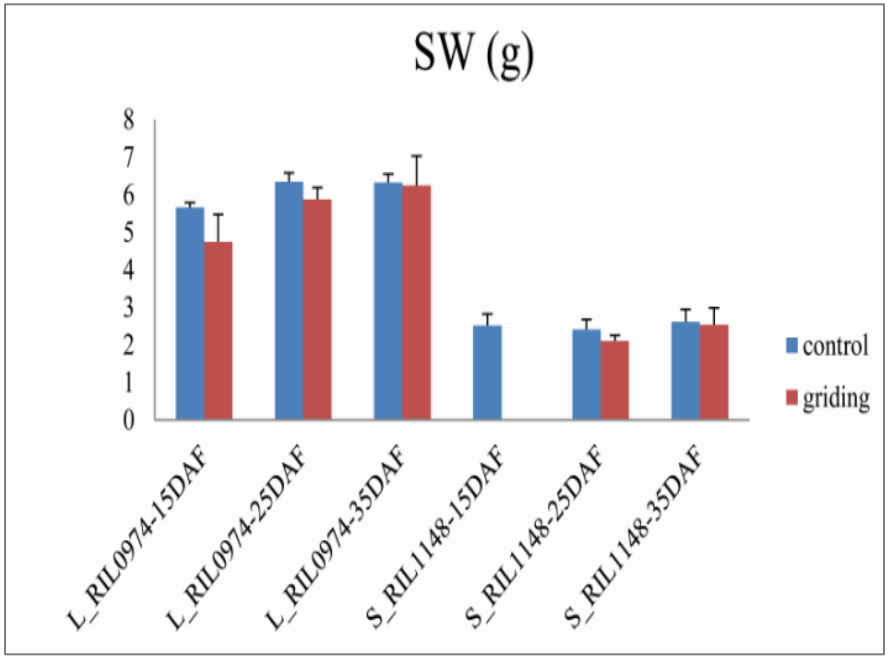


- RIL_{0974} vs RIL_{1148}: 1.36/1.36 and 1.07/1.06 times for seed coat/embryo surface cell number and area, respectively. This result showed that seed size difference between two RILs was mainly due to cell number followed by size。

（5）Physiological cause of seed weight difference between RIL_{0974} and RIL_{1148}

- To distinguish the relative role of silique and other photosynthetic organs on seed weight, the girding experiment was conducted on phloem.



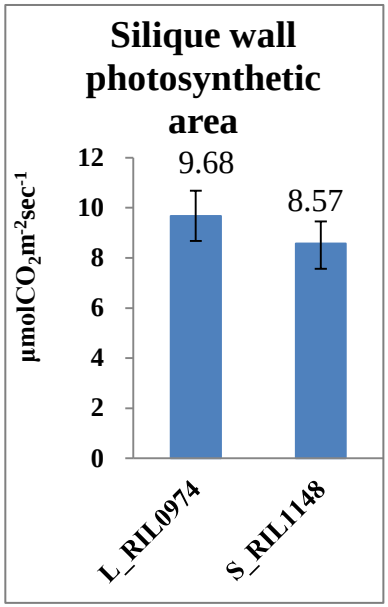


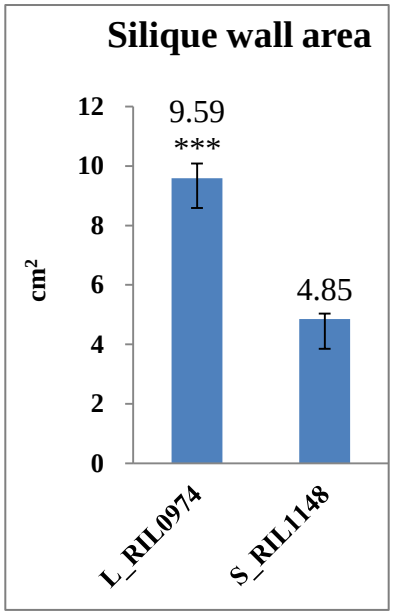
After girding of phloem, the falling range of seed weight for both large- and small-seed RILs declined from ≈20% to ≈10% and ≈2%, as the silique develop.

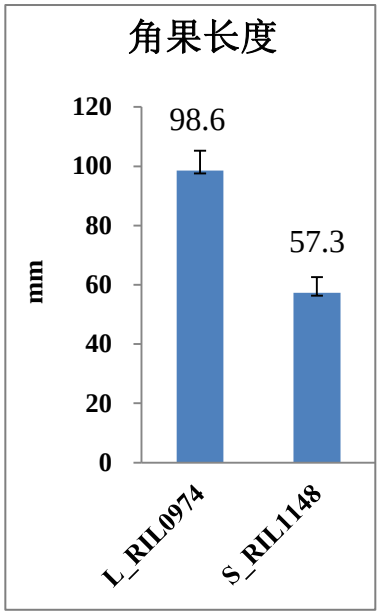
- **Photosynthate from pod wall is the major contributor to seed weight in rapeseed**

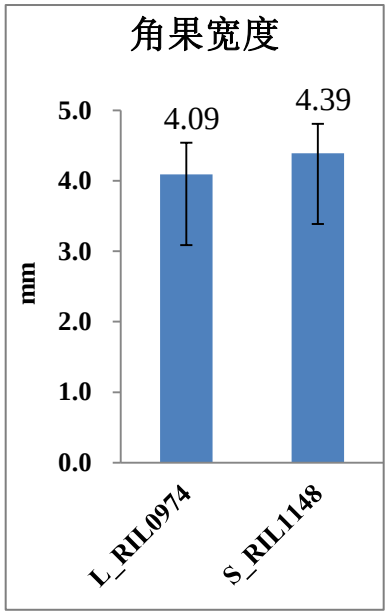
- To dissect the influence way of silique wall photosynthesis on seed weight, silique wall photosynthetic rate and area were investigated.

- To find the cause of silique wall area difference between two RILs, the silique length and width were compared.







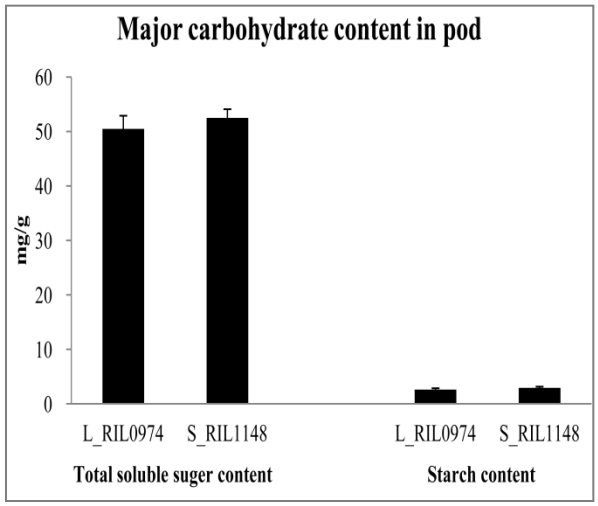


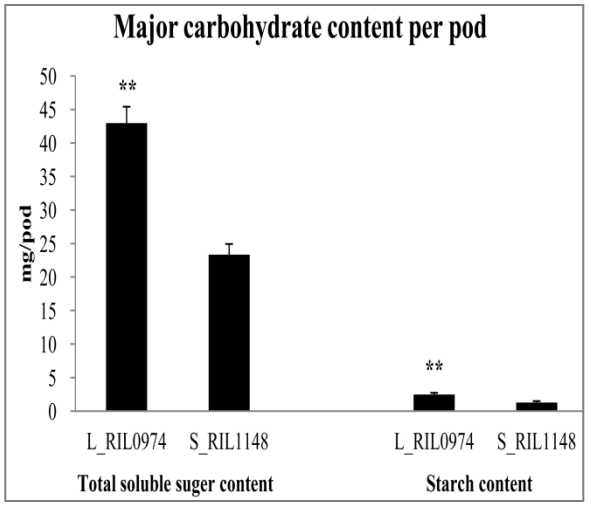
The photosynthetic rates of RIL_{0974} and RIL_{1148} were basically the same, but the silique wall area of the former is larger than latter.

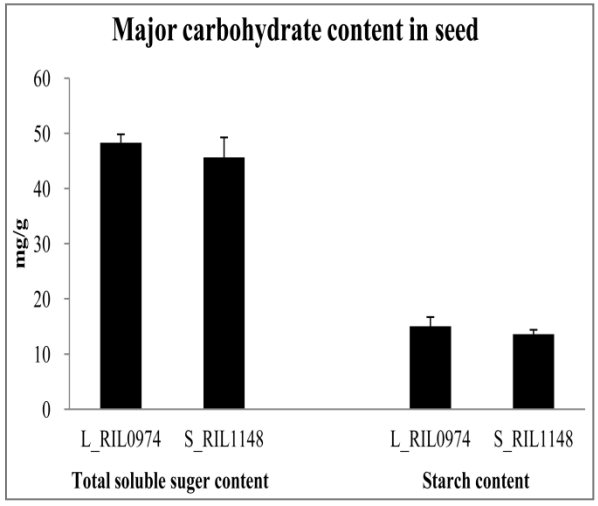
The width of RIL_{0974} and RIL_{1148} have no significant difference, but the length of former is larger than that of the latter.

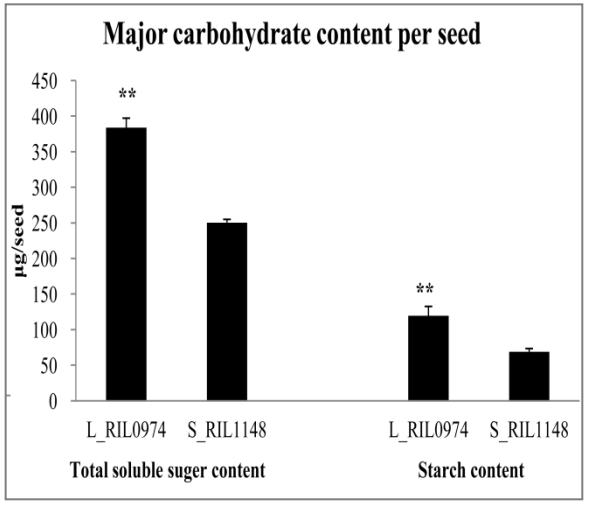
- Although silique wall photosynthetic rates of the large- and small-seed RILs have no significant difference, but the difference in silique length could lead to the difference in silique wall area and carbon assimilation.

- To further dissect the impact of carbon assimilation difference on the accumulation of photosynthate, the total soluable sugar and starch were compared between the two RILs
- The difference of photosynthate gross between RIL_{0974} and RIL_{1148} lead to the difference of its transport and storage in the seeds





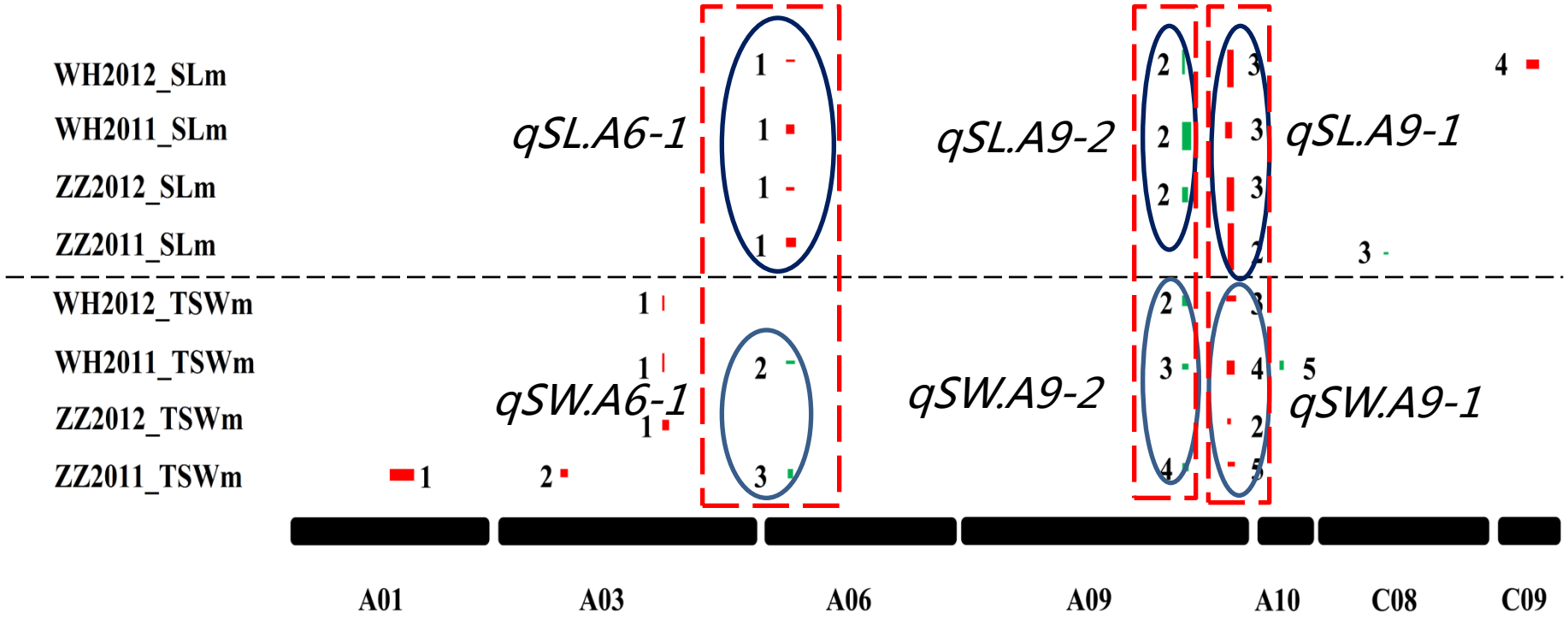




- The above physiological and biochemical results indicated：silique length→photosynthetic area and carbon assimilation → photosynthate accumulation in silique wall→photosynthate transport and storage in the seeds→seed filling, size and weight。

（6）Molecular mechanism of seed weight difference between RIL_{0974} and RIL_{1148}

- To find the molecular evidence of silique length affecting seed weight, QTL mapping and comparison were conducted using the BnaZNRIL population

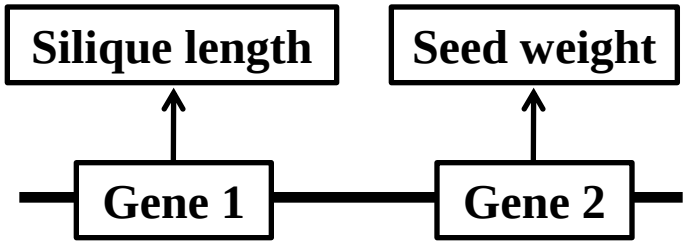


Footnote: WH and ZZ represent Wuhan and Zhengzhou respectively; red and green indicate the positive alleles from Zhongshuang11 and No.73290, respectively.

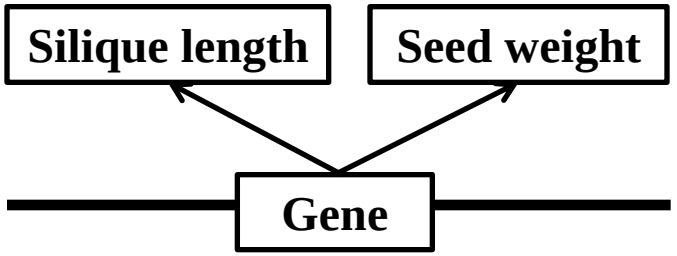
- A6: one pair of co-localized QTL, opposite direction on two traits; A9: two pair of co-localized QTL, same direction on two traits。

Several putative models underlying the co-localization of QTLs for silique length and seed weight

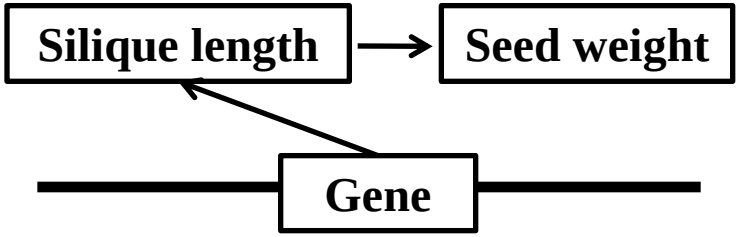
① Tight-linkage

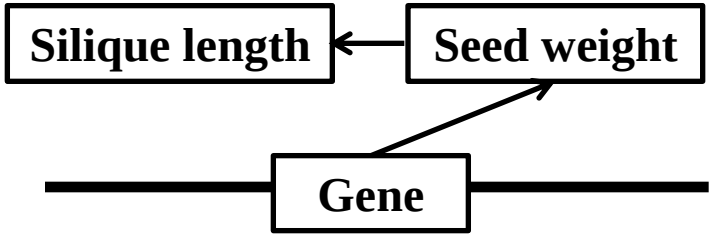


② Pleiotropy

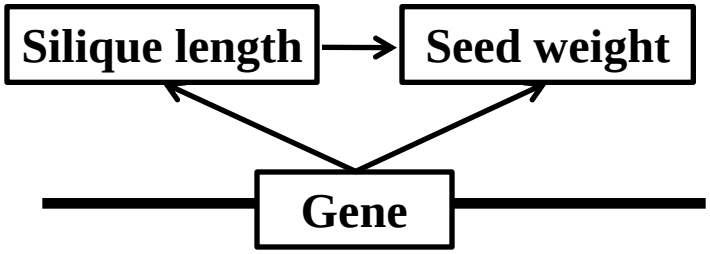


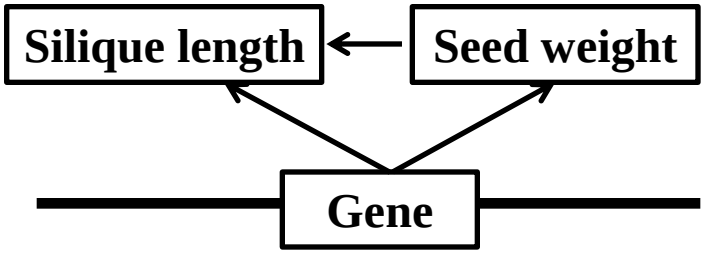
③ Physiological interaction



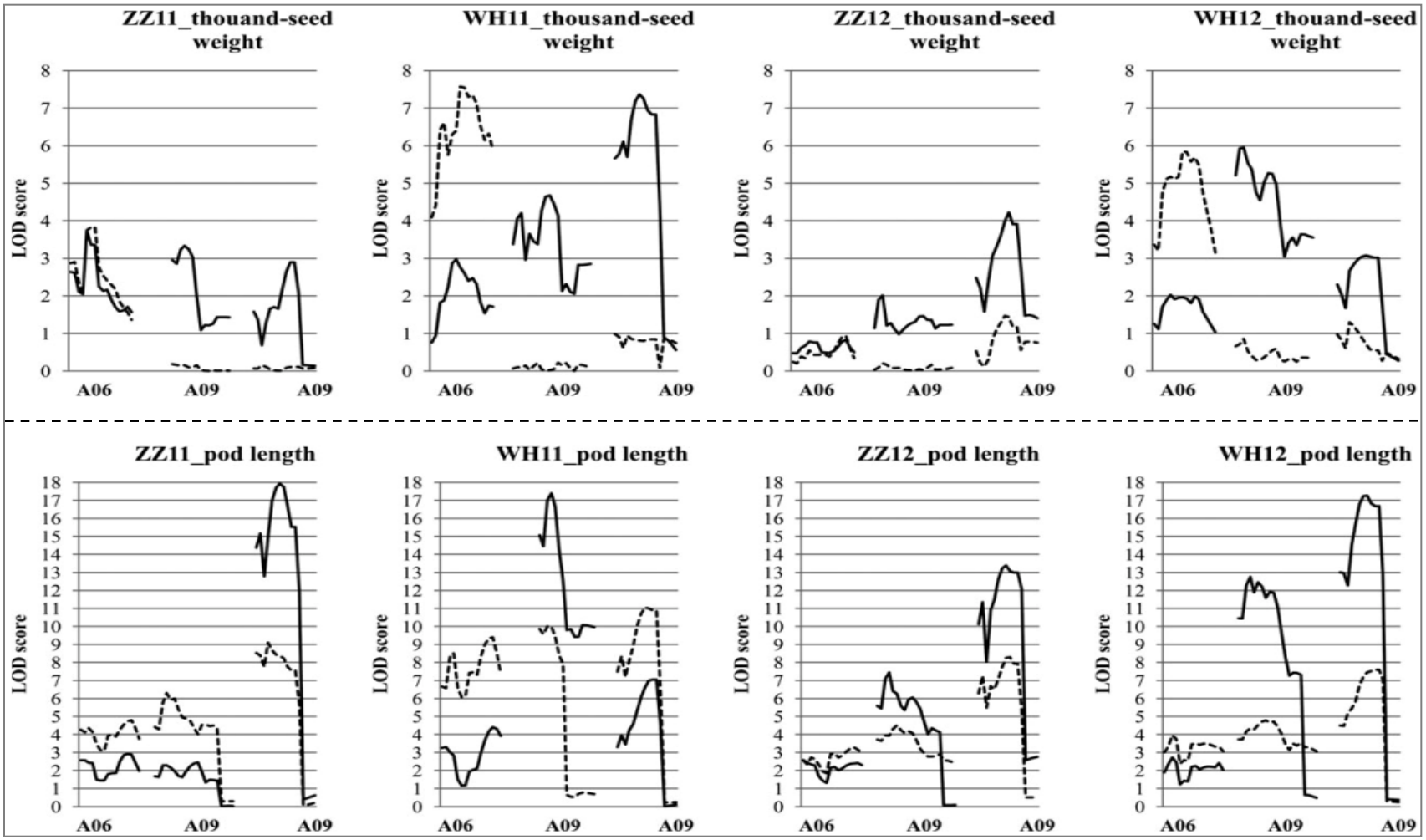


④ Combination of pleiotropy and physiological interaction





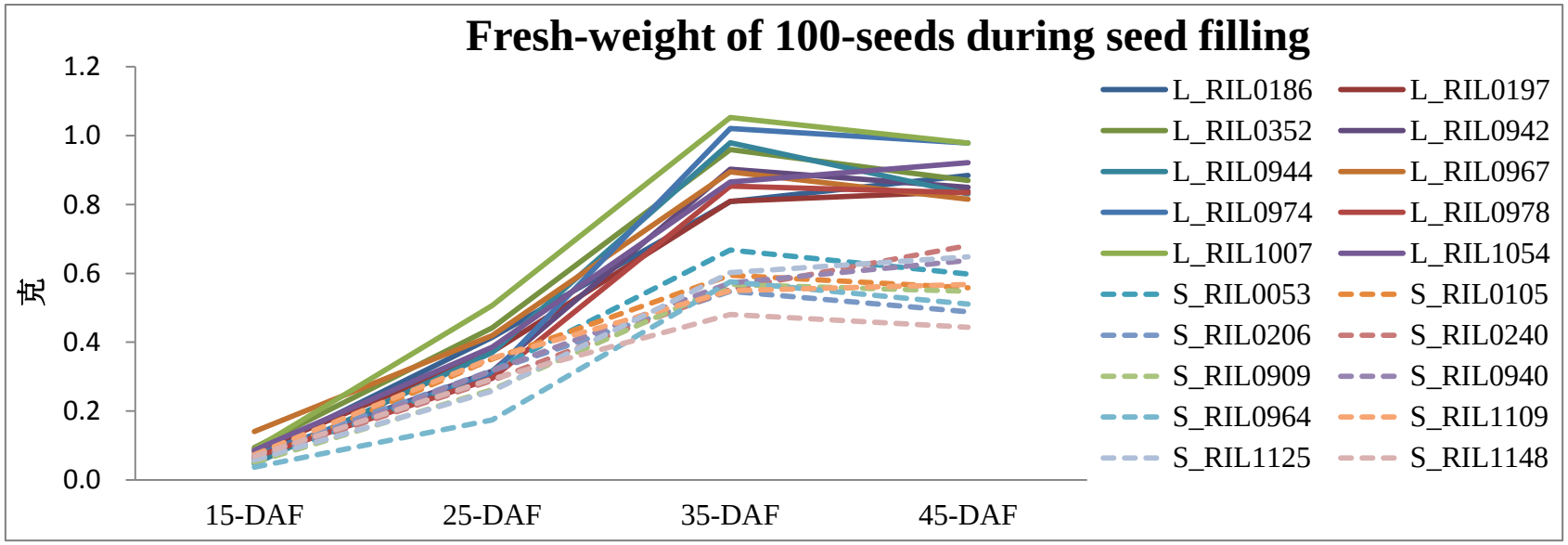
- Reciprocal conditional QTL analysis was used to dissect the genetic cause of three co-localized QTL pairs.

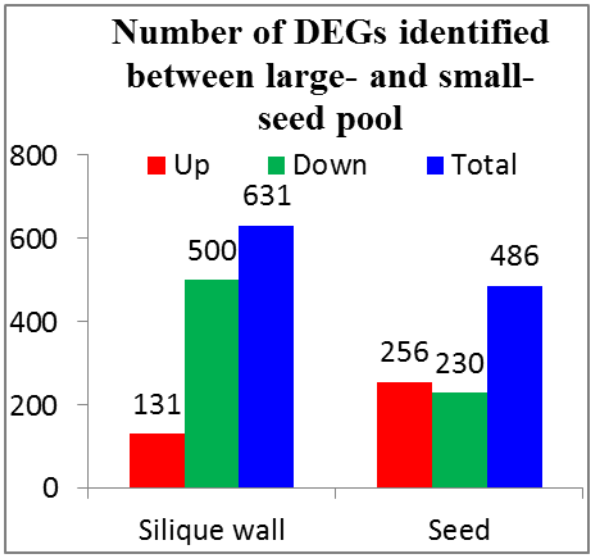


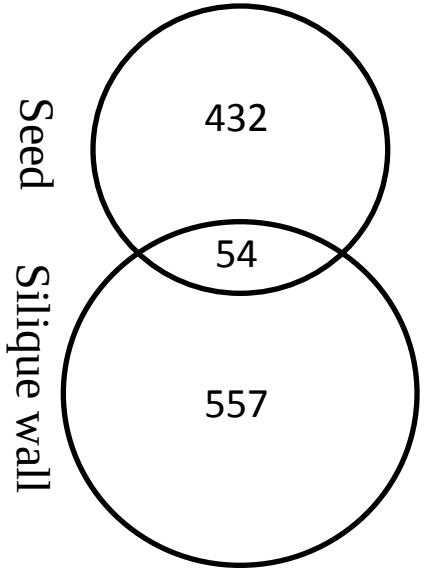
Footnote：solid and dotted lines represent the LOD score of before and after conditional QTL

- A6: LOD scores increase after SL | SW and SW | SL, indicating negative pleiotropy; A9: LOD scores become non-significant when SW | SL; while LOD scores decrease when SL | SW, indicating physiological interaction where SL as upstream of SW。

➢ To obtain more information on how silique wall regulating seed weight, silique wall and seed of the large- and small-seed RILs pool were subjected to transcriptomic analysis (at 25-DAF)



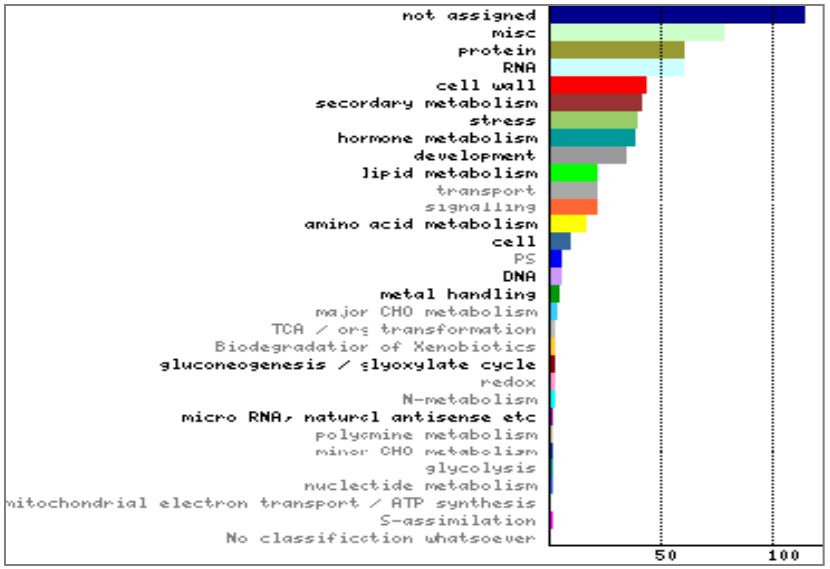




- A total of 631 and 486 DEGs were identified from silique wall and seed, respectively; only 54 were common, indicating large difference on the regulatory network of two traits.

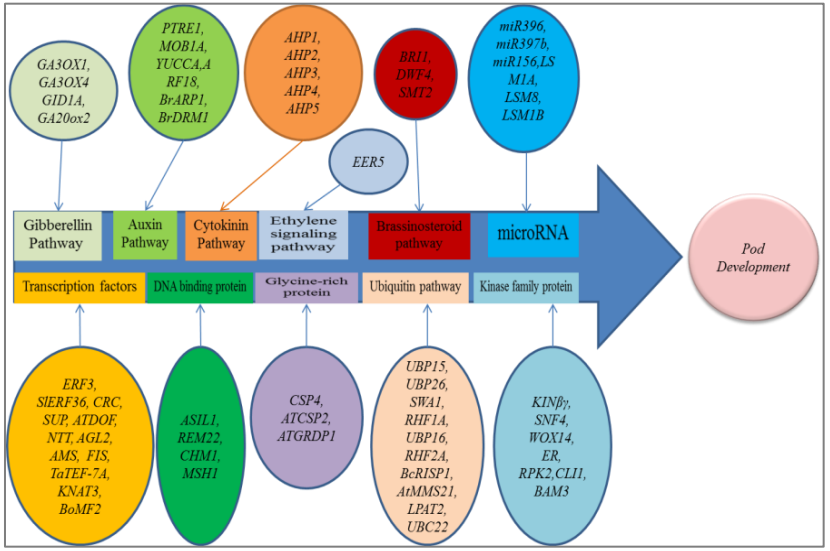
➢ KEGG of DEGs in silique wall:

- Nine enriched pathway: protein, cell wall, DNA, development, secondary, hormone, lipid and amino acid metabolism, most are involved in cell division and expansion; CYP450 and flavonoid exhibited high proportion, indicating important role in silique development



➢ Comparison of DEGs in silique wall and known Genes for silique size:

- Two were homologous to CYP78A9 and CYP72C1; 62% DEGs was involved the known pathway of silique size: such as hormone, transcription factor, receptor-kinase and G-protein signaling, ubiquitin-proteasome, cell organization and cell wall protein。

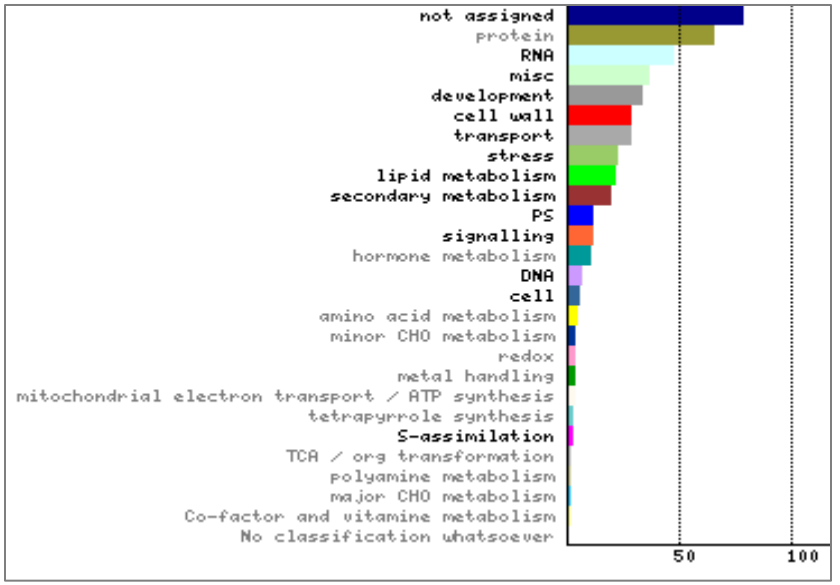


DEGs in silique wall are highly associated with silique development

Quaid et al. (2019) Genetic and signal pathways of fruit size in plants. PBI-00440-2019

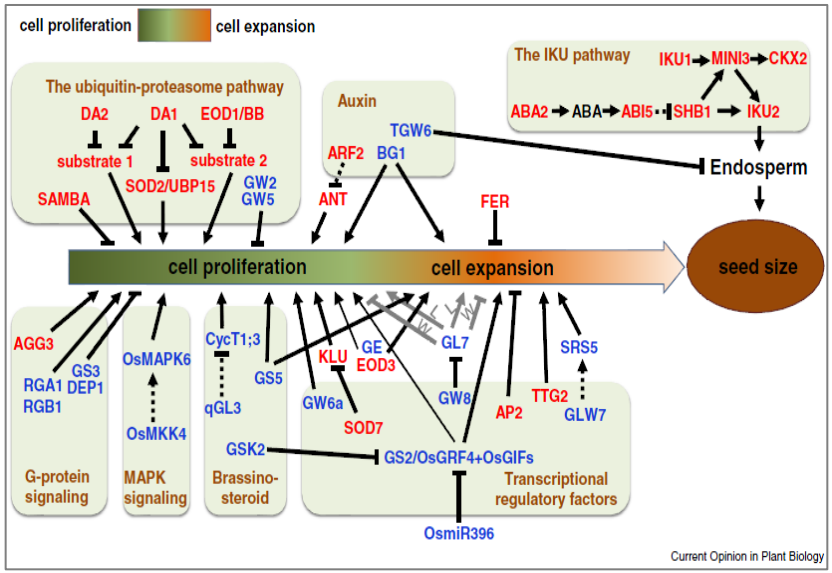
➢ **KEGG of DEGs in the seeds:**

- **Eight enriched pathway:** Cell wall and DNA synthesis provide the structural and material basis for cell division and expansion; while transport, storage protein, lipid and secondary metabolism are related to seed metabolism.



➢ **Comparison of DEGs in seeds and genes for seed size:**

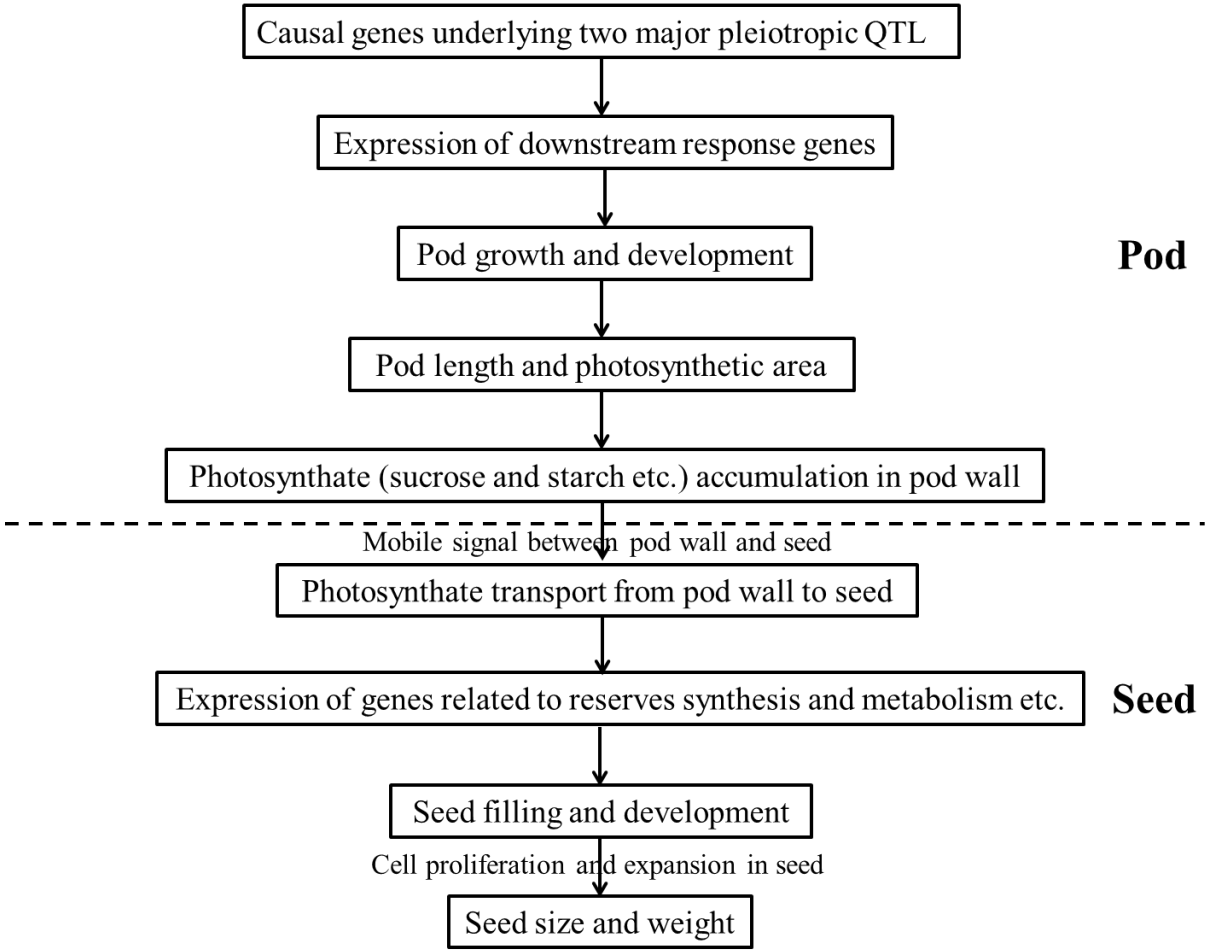
- 11 DEGs were homologous to known seed size genes (AEP4, AGL62, CHS, CRA1, CRB, EMS1 and ETO1); 55% DEGs were involved in the known pathway of seed size, such as hormone, transcription factor, receptor-kinase and G-protein signaling, lipid metabolism, ubiquitin-proteasome, cell cycle and division.



DEGs in seeds are highly associated with seed filling and development

New pathway for maternal control of seed weight in rapeseed

- The above systematic comparative analyses (including genetic, physiological, cytological and molecular)，revealed that silique photosynthetic area could regulate seed size in a maternal fashion. The mechanism model as follow:



- These RIL lines harbour two major QTL for silique length，they can regulate the expression of downstream genes related to silique development, then affect silique length and photosynthetic area，photosynthate and its transport and storage in seeds, finally affect seed filling, size and weight

Li et al. (2018) Maternal control of seed weight in rapeseed: the causal link between the size of pod (mother, source) and seed (offspring, sink). https://doi.org/10.1111/pbi.13011

4. Summary and conclusion

1. We discovered the maternal control of seed weight in rapeseed.

- This original discovery overturns the potential cognition in the past, breaks through the limitation that the original research focuses on seeds themselves, and points out the direction for the research on seed weight of rape.

2. We discovered a new mechanism for the maternal control of seed weight through silique photosynthetic area

- This original achievement opened up a new field of seed weight research and provided a new theory and approach for the improvement of rape yield and traits.

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3. Research assistant：Qing Yang, Li Jin, Jiepeng Zhan
4. Graduate student：Na Li, Dongji Song, Wei Peng

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Thanks for your attention!