Suppression of *bna-miR168a* improving Rapeseed yield greatly by targeting to *BnaAGO1s*

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Sydney, 26 September, 2023

Background

Rapeseed is a major oil crop worldwide

Improving the rapeseed yield is an eternal topic!

Background

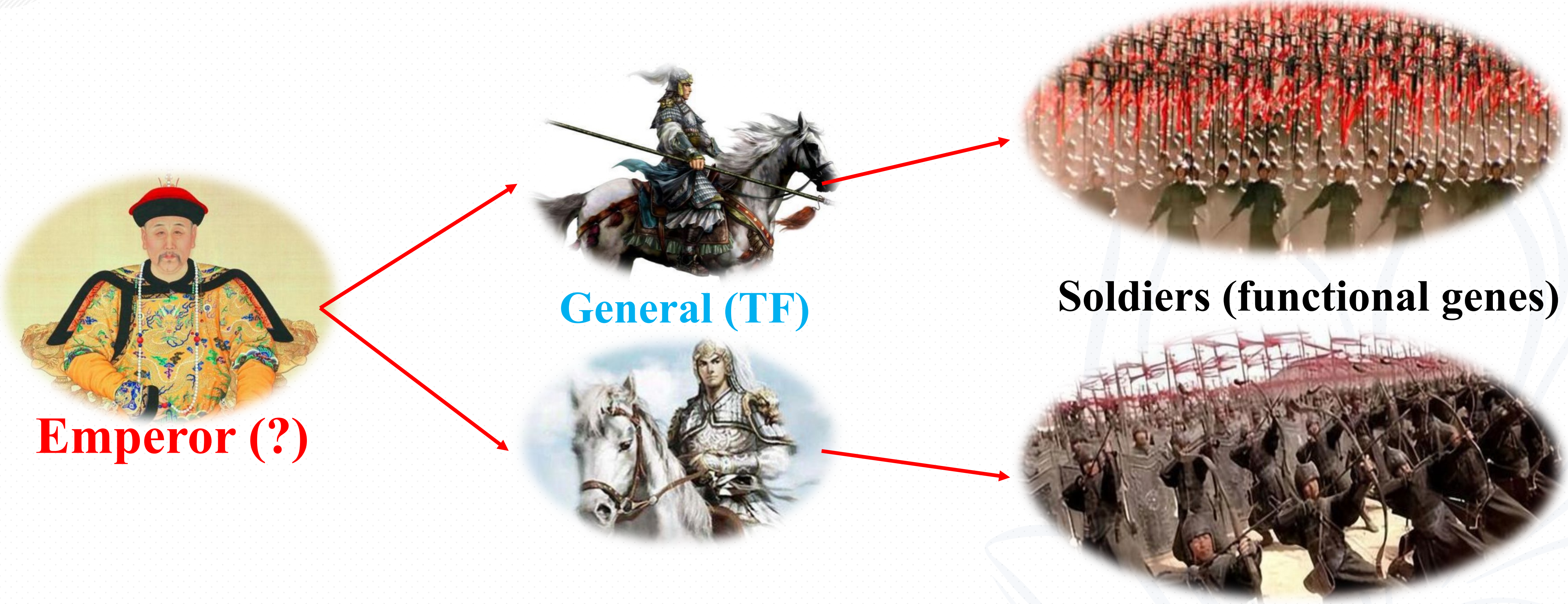
- Rapeseed yield is controlled by three mainly yield determined factors: SPP, silique number per plant; SPS, seed number per silique; TSW, thousand seed weight.

- Unfortunately, almost all the yield-related characters are quantitative traits controlled by poly-genes and multiple environmental factors.

- Rapeseed is a polyploid specie, which own abundant homologous genes.

All of these leading to a difficulty to improve Rapeseed yield greatly by a single gene!

Object – find “Emperor” in Rapeseed



Emperor (?)

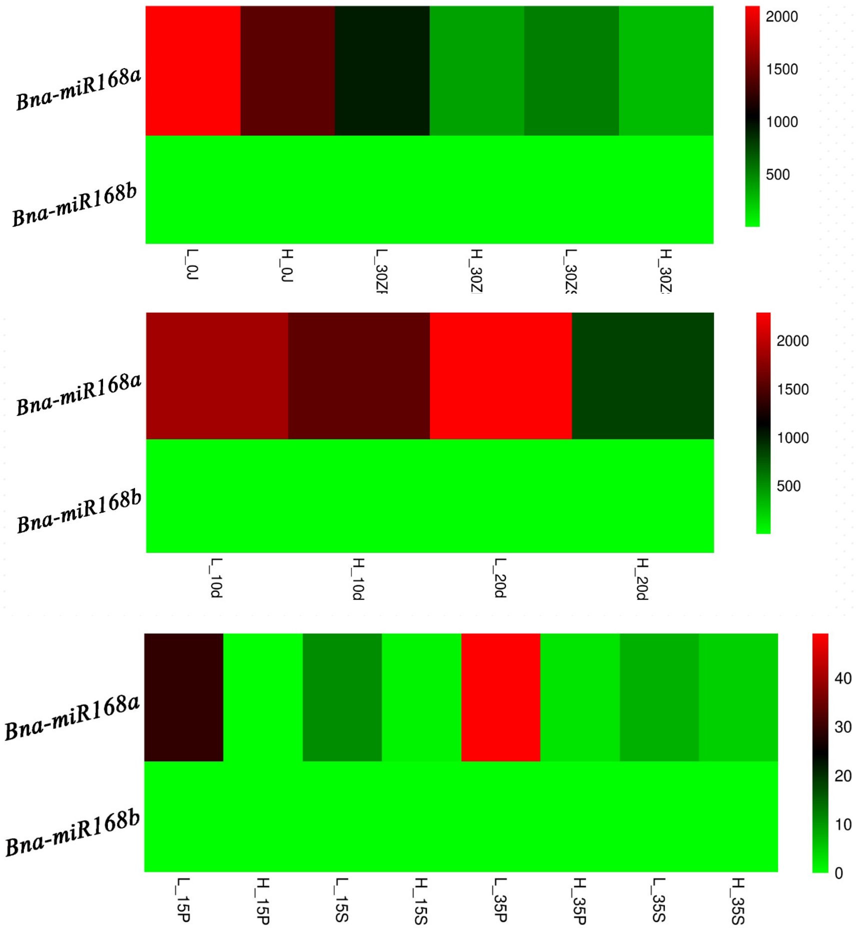
General (TF)

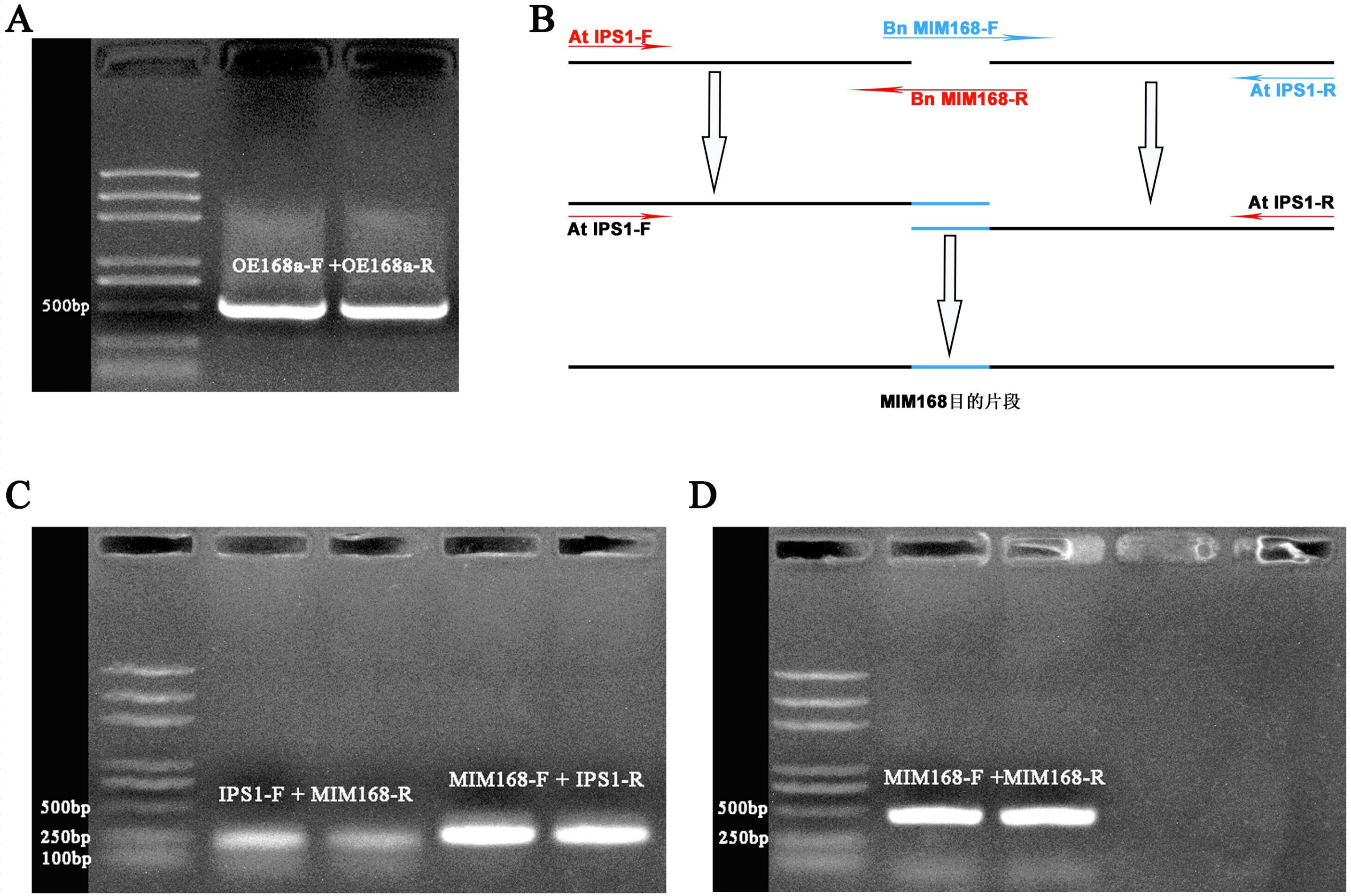
Soldiers (functional genes)

MicroRNAs are known as multifunctional factors in regulating complex quantitative traits.

We considered if there is a miRNA which own powerful functions like a general or even emperor in rapeseed?

Screening and cloning of key miRNAs





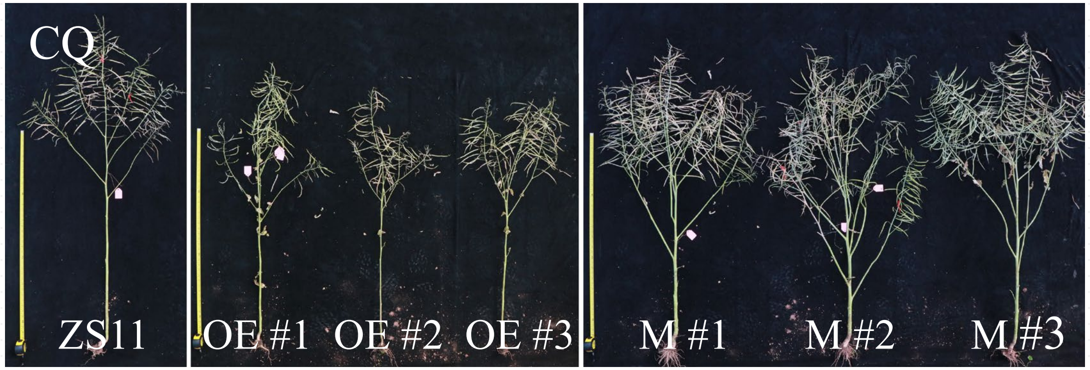
bna-miR168a expressed lower in high yield material, but expressed higher in low yield materials

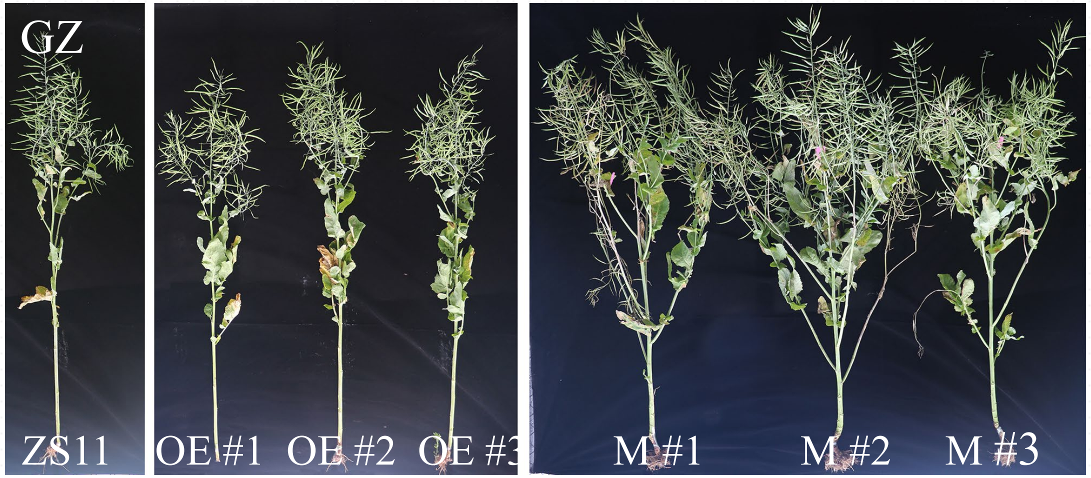
We cloned the mature and stem sequence of *bna-miR168a*; and create transgenic plants with overexpressed and target mimic of *bna-miR168a*

Part I.

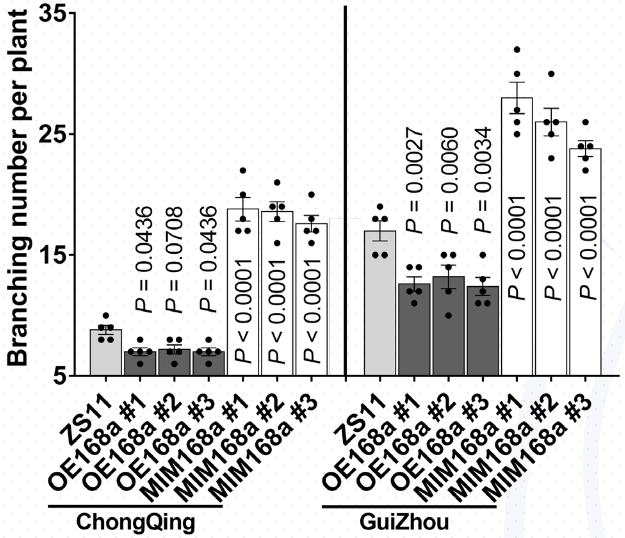
Suppression of *bna-miR168a* own the powerful function in improving **multiple** yield-related traits **greatly** in Rapessed

bna-miR168a regulating the trait of SPP

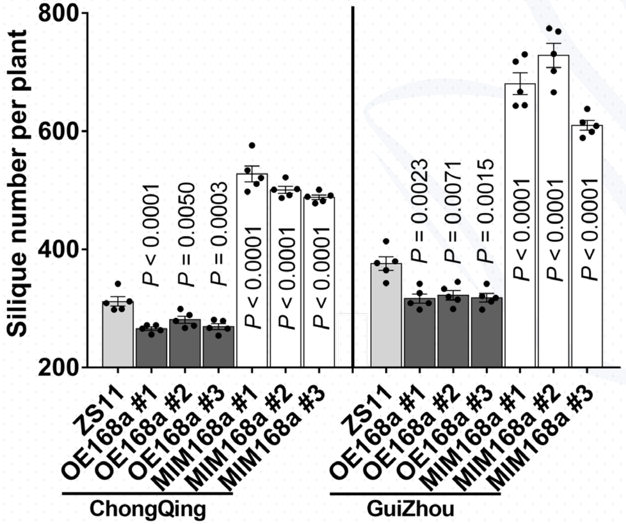




Overexpressed miR168a can inhibit plant growth; Whereas suppression of *miR168a* can promote plant growth and development



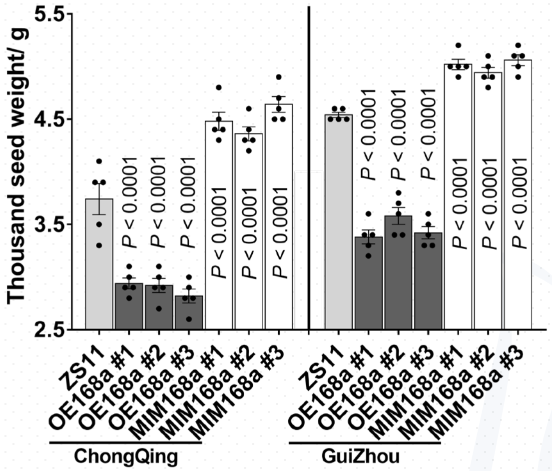
Suppression of *bna-miR168a* can increase branching numbers per plant

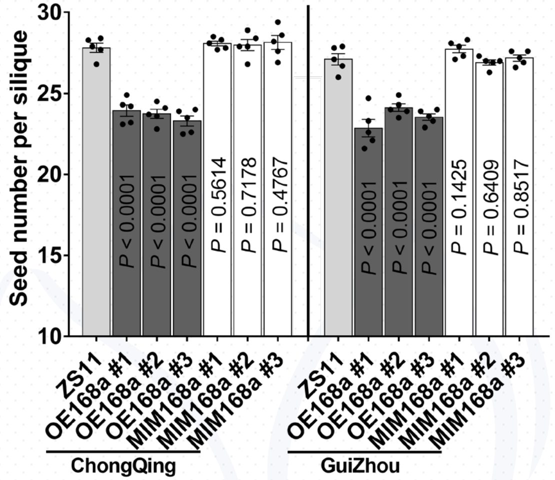


The enhancing of branching numbers resulting in a greatly increase of silique number per plant, which was one of the most essential yield determined factor

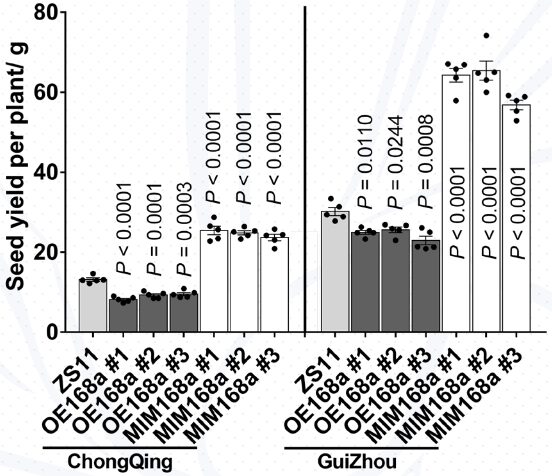
bna-miR168a regulating the traits of SPS and TSW

Suppression of miR168a own bigger and longer silique than control and overexpressed lines



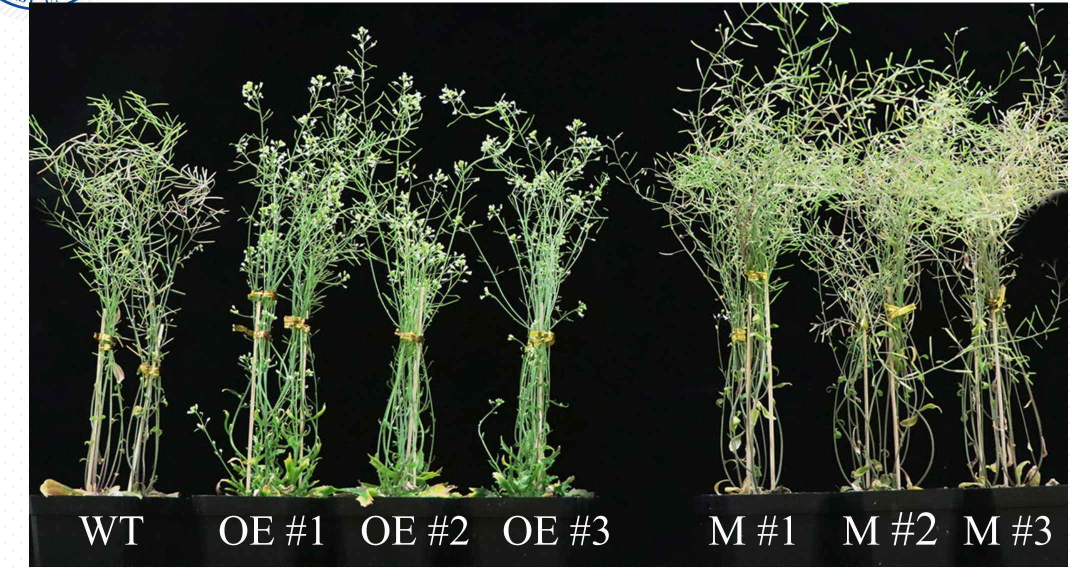


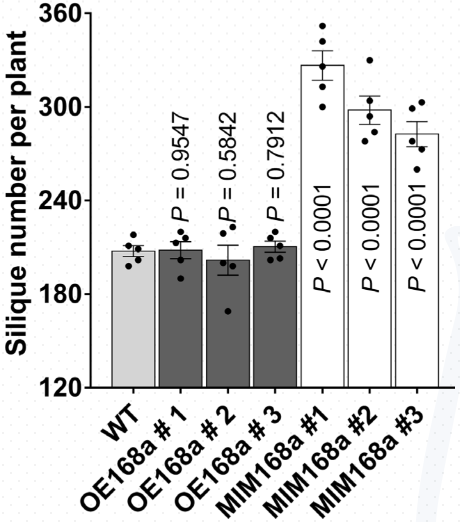
SPS and TSW show significant difference between transgenic and control plants

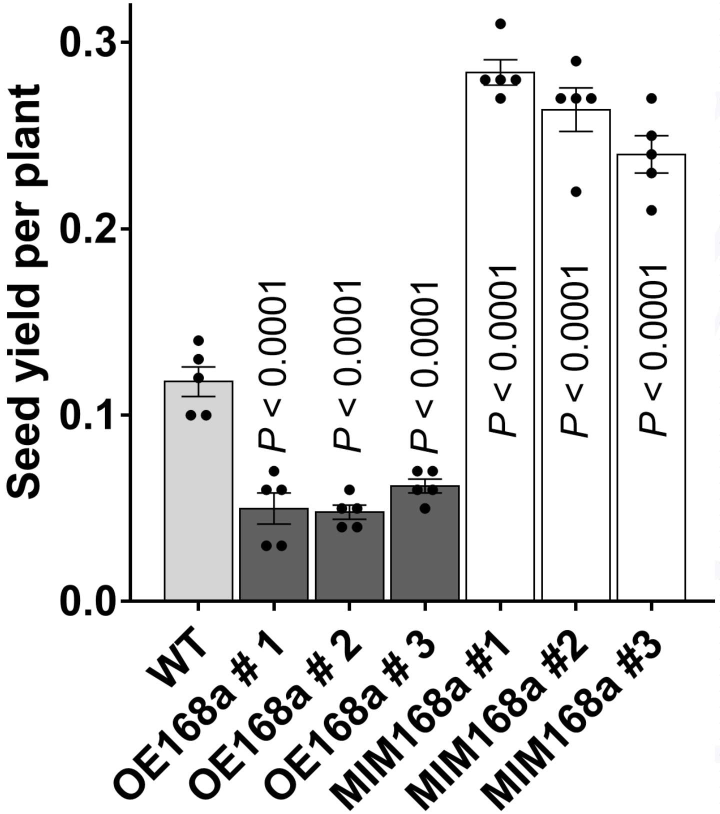


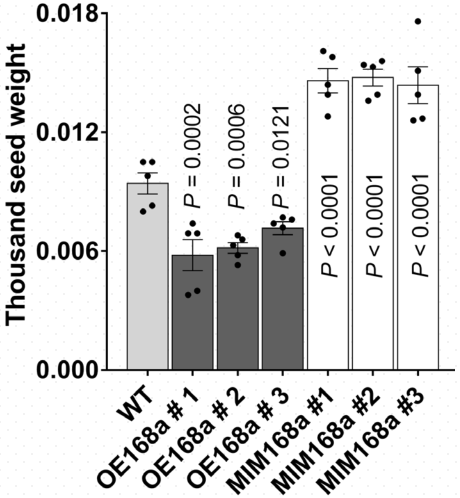
All the changes of SPP, SPS and TSW resulting in a higher seed yield with suppression of *bna-miR168a*

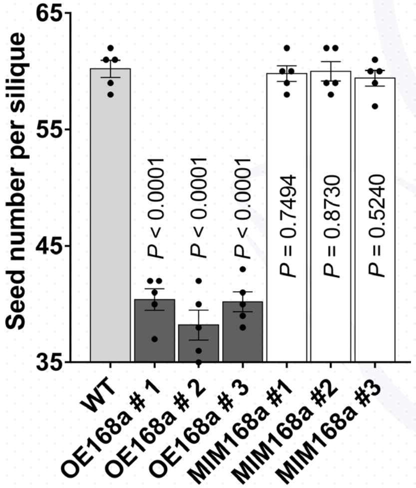
bna-miR168a can also regulate the seed yield greatly in *Arabidopsis*









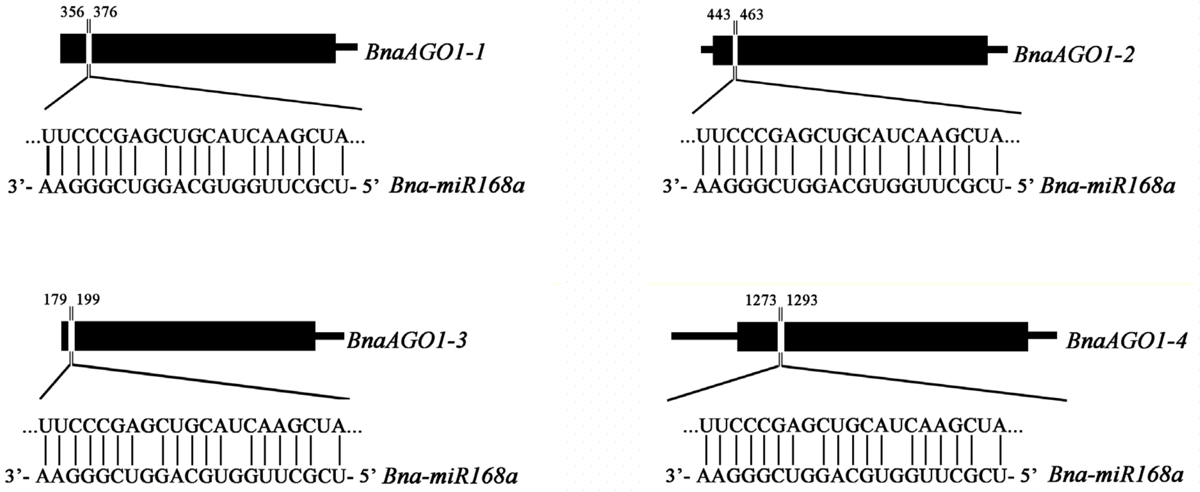


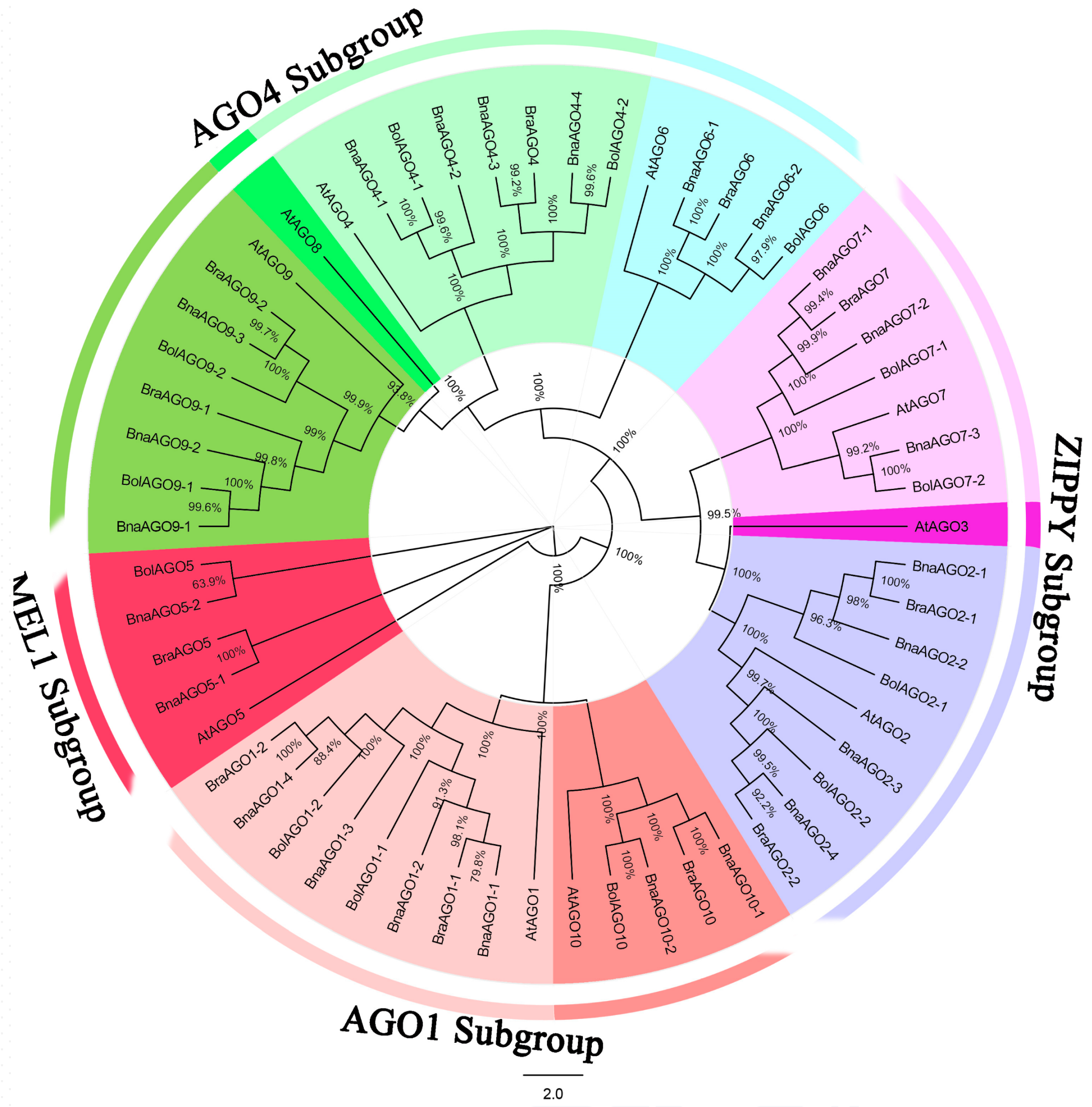
Similar to Rapeseed, we also found the powerful function of *bna-miR168a* in *Arabidopsis*

Part II.

Why *bna-miR168a* own the powerful function in regulating Rapeseed / Arabidopsis yield?

bna-miR168a targets to all the four BnaAGO1s members



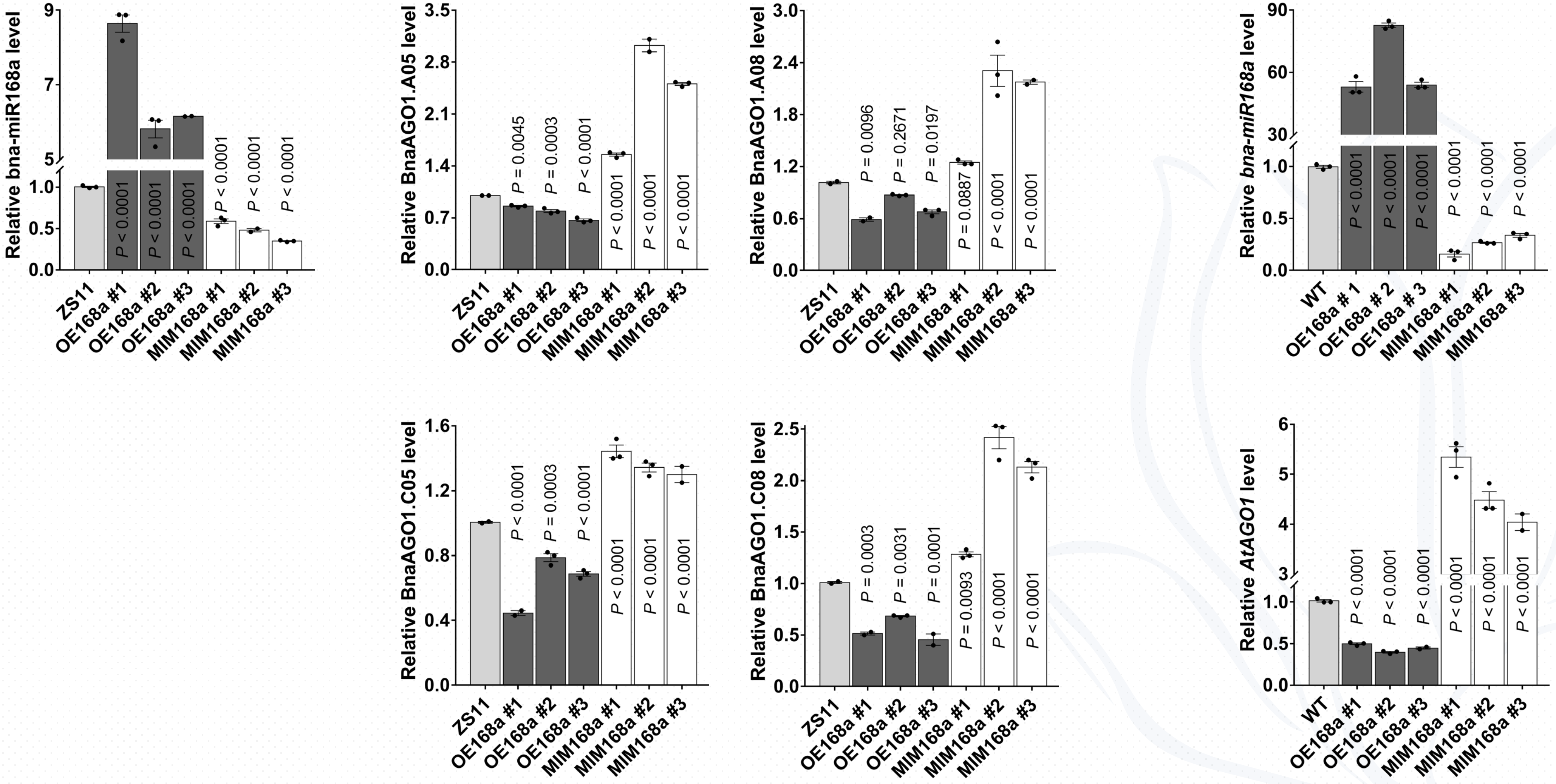


Targets predication online show *bna-miR168a* own four AGO1s targets with same target site

Genome-wide identification show that AGO1 possess four copies in rapeseed

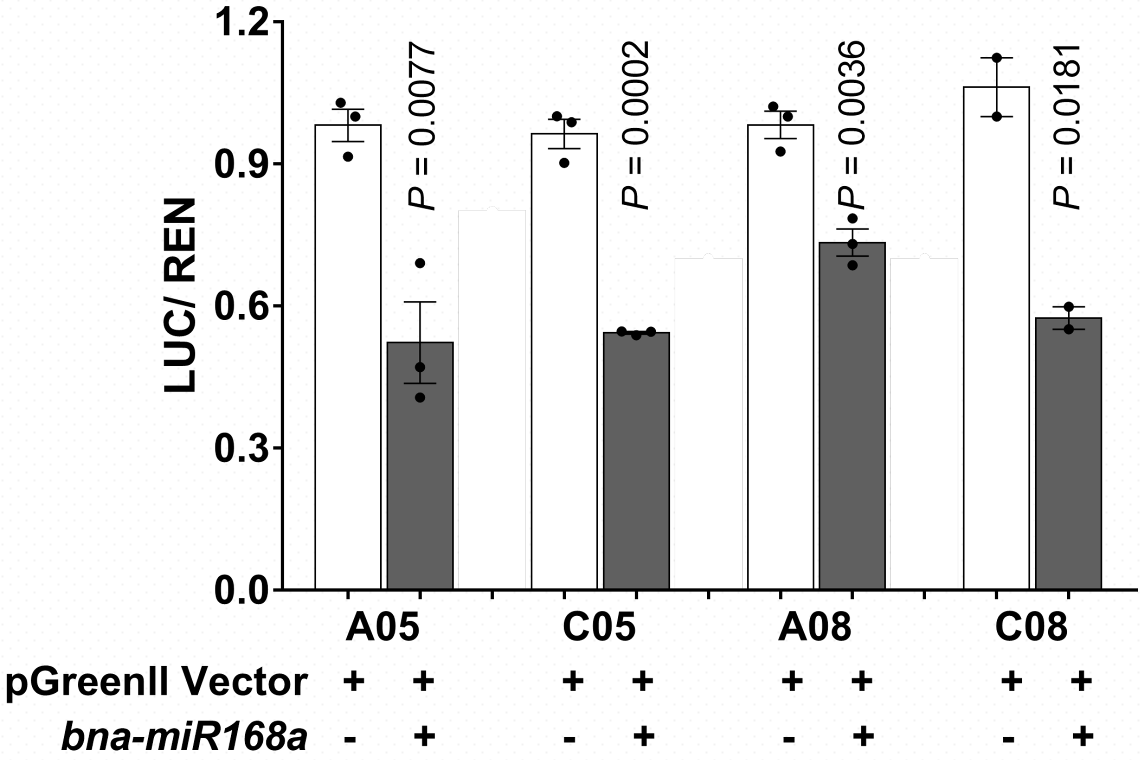
Zhang *et al.*, *Int. J. Mol. Sci*, **2023**

bna-miR168a targets to all the four *BnaAGO1s* members

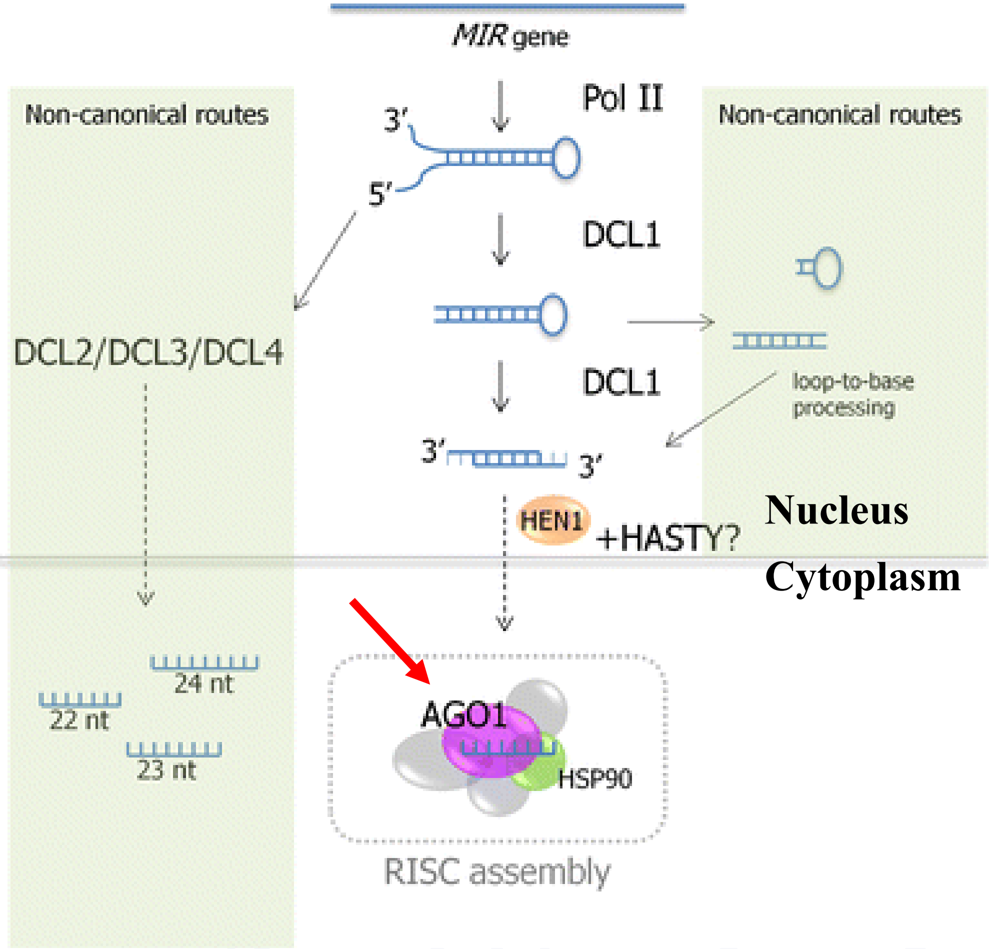


Vivo qRT-PCR in rapeseed and Arabidopsis both proved our prediction

bna-miR168a targets to all the four *BnaAGO1s* members



Vitro analysis with LUC also proved the relationship between *bna-miR168a* and the four target AGO1s

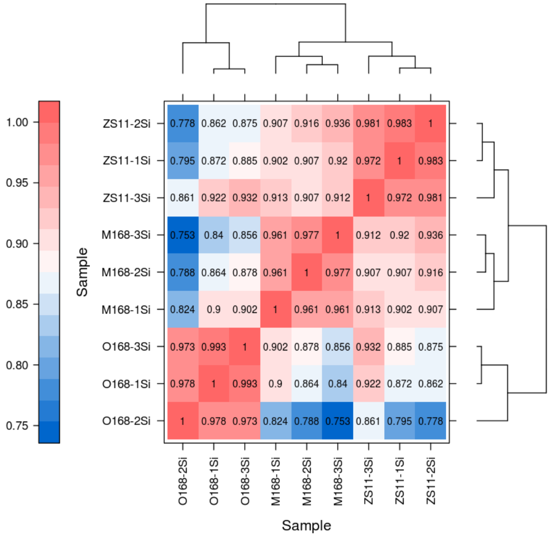


AGO1 is the most essential component of RNA-induced silencing complex (RISC), which all miRNAs must combine with it to perform their functions

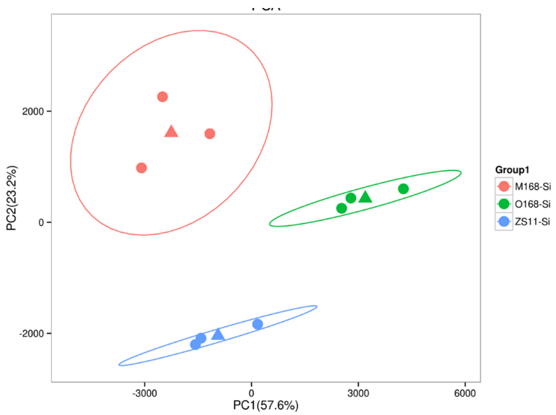
Part III.

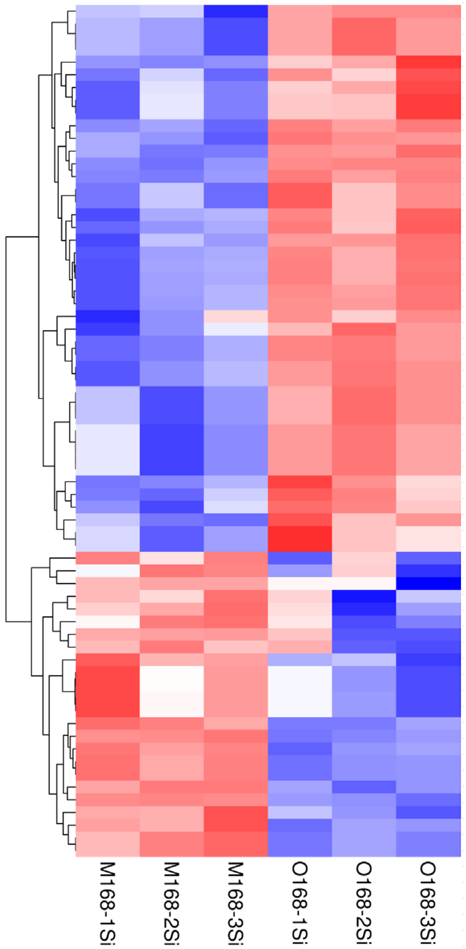
If “miR168a-AGO1-miRNAs-Targets” module occurred and play essential roles in regulating rapeseed yield?

miRNA/mRNA-Seq analysis(miR168a-Si)

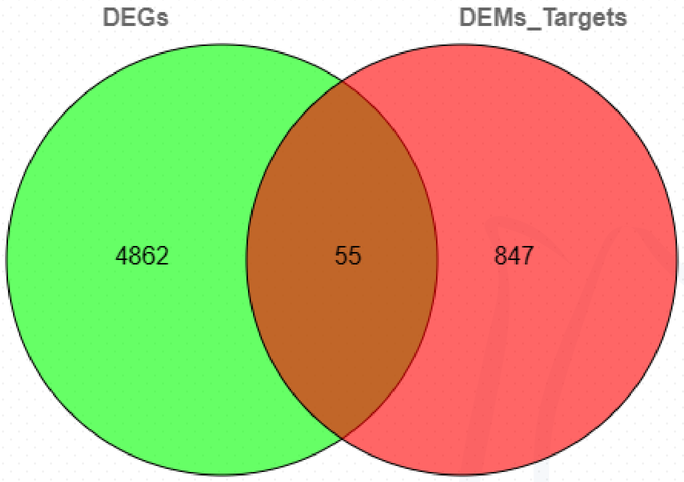


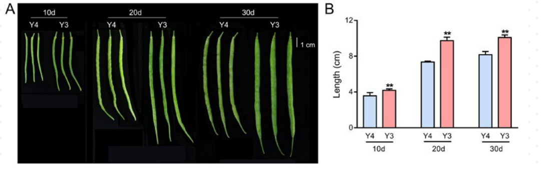
	O168-2Si	O168-1Si	O168-3Si	M168-1Si	M168-2Si	M168-3Si	ZS11-3Si	ZS11-1Si	ZS11-2Si
ZS11-2Si	0.778	0.862	0.875	0.907	0.916	0.936	0.981	0.983	1
ZS11-1Si	0.795	0.872	0.885	0.902	0.907	0.92	0.972	1	0.983
ZS11-3Si	0.861	0.922	0.932	0.913	0.907	0.912	1	0.972	0.981
M168-3Si	0.753	0.84	0.856	0.961	0.977	1	0.912	0.92	0.936
M168-2Si	0.788	0.864	0.878	0.961	1	0.977	0.907	0.907	0.916
M168-1Si	0.824	0.9	0.902	1	0.961	0.961	0.913	0.902	0.907
O168-3Si	0.973	0.993	1	0.902	0.878	0.856	0.932	0.885	0.875
O168-1Si	0.978	1	0.993	0.9	0.864	0.84	0.922	0.872	0.862
O168-2Si	1	0.978	0.973	0.824	0.788	0.753	0.861	0.795	0.778

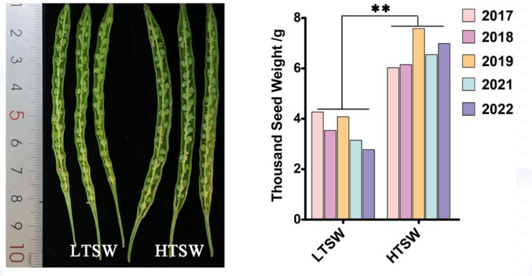


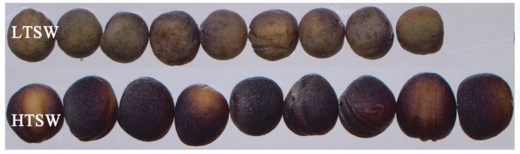


Total 90 DEMs and 4917 DEGs were identified between transgenic lines and control ZS11







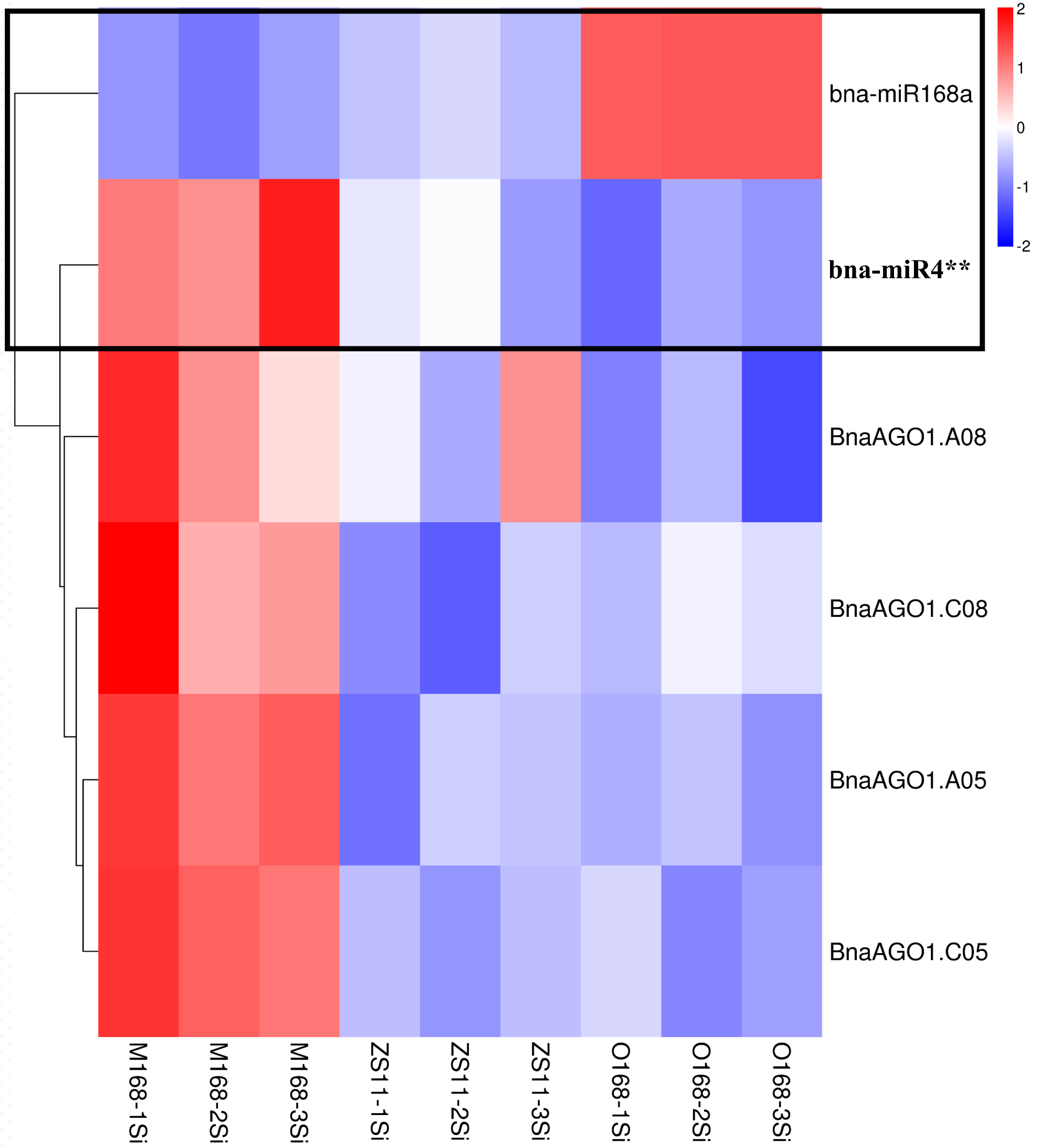


Bna-miR4**

Bna-miR1**

Combining with the DEGs and Targets of DEMs, 55 common DEGs were regulated by 33 miRNAs (9 miRNA subfamily): 9 MIRNAs-39Targets + 6 novel_miRNAs-16 Targets

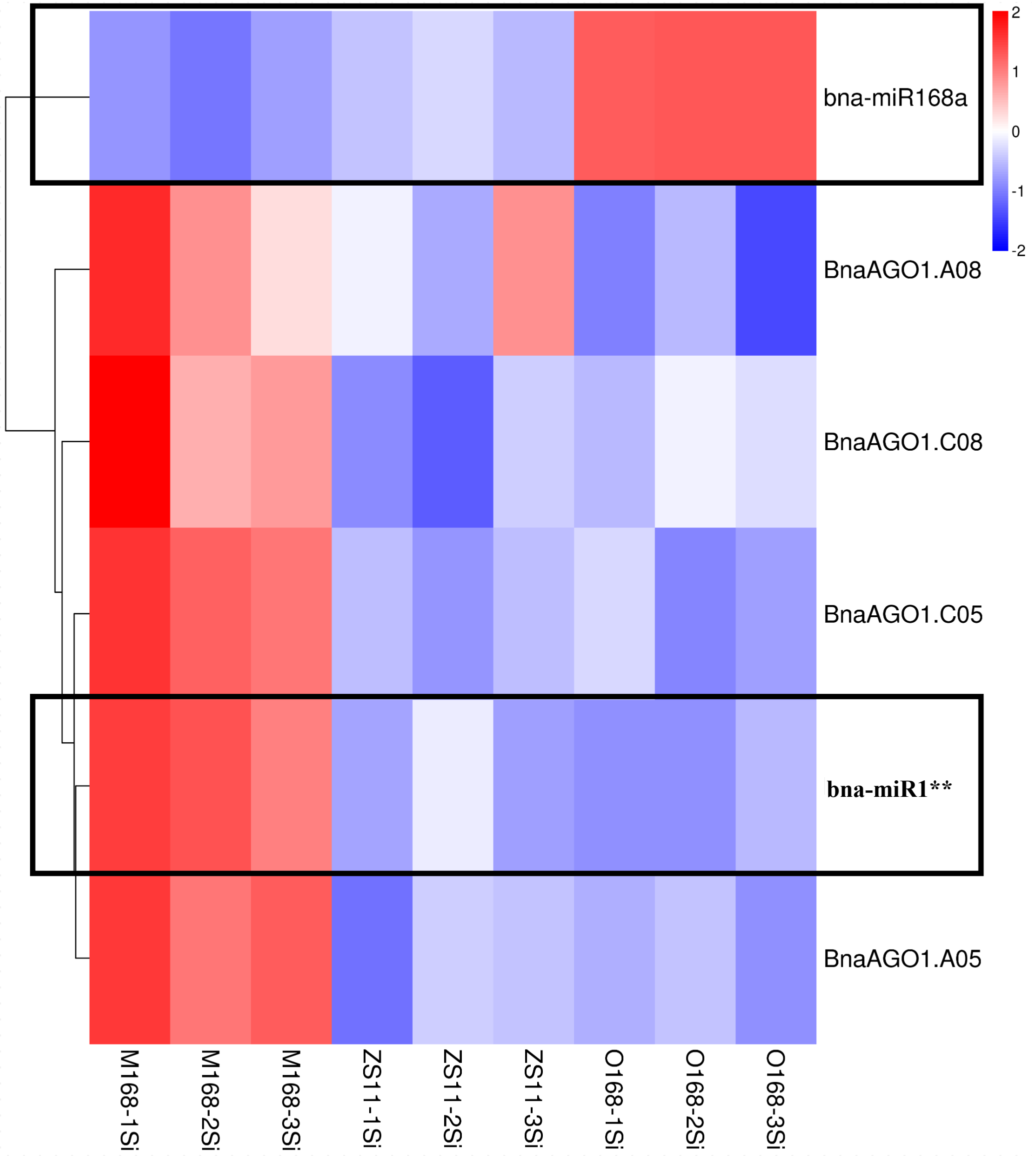
Functional research of Target miRNAs

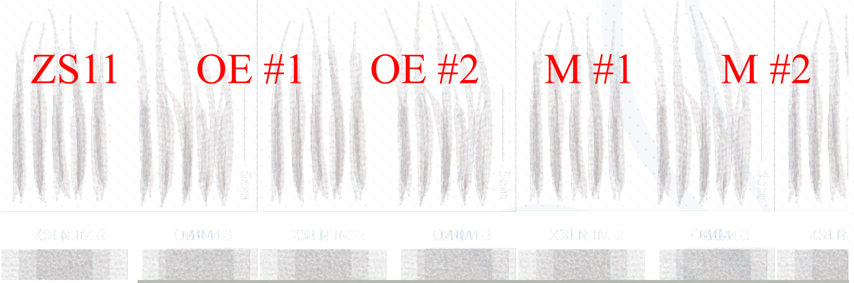


We identified one miRNA which was down-regulated by *bna-miR168a*

This miRNA own the opposite phenotypic characters with miR168a in regulating silique development

Functional research of Target miRNAs

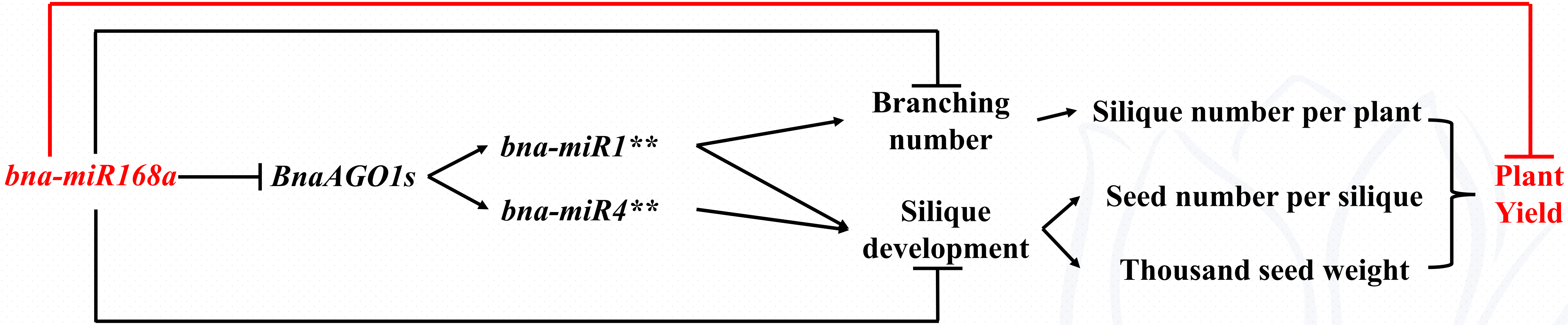




Another miRNA which was also down-regulated by miR168a

This miRNA own the opposite phenotypic characters with miR168a in regulating silique and plant development

Construction of "BnamiR168a-AGO1s-miRNAs-Targets"



- *bna-miR168a* own a powerful function in regulating multiple yield-related traits greatly in both rapeseed and *Arabidopsis*.

- *bna-miR168a* is a special miRNA targeting to all the four *BnaAGO1s*, which is the most essential component of RISC

- Module of "miR168a-AGO1-miRNAs-Targets" play an essential roles in regulating multiple characters, including but not limited to yield, seed color, flowering time, etc.

Successfully identified and clone “Né zhā” in Rapeseed

Completed: Yield Traits

Completing: Flowering time

Né zhā: Little Child

possessing immense power

Future completion: Other characters…

Completing: Seed Color

Acknowledge


Chao Zhang


Zhongchun Xiao


Jingsen Liu


Huan Chen


Bo Yang


Si Chen


Sirou Xiang

Screening of candidate genes

Functional research

Prof: Jiana Li

NSFC
National Natural Science
Foundation of China

Thanks for your listening