

## #060

## Suppression of bna-miR168a improving multiple yield-related traits greatly by targeting BnaAGO1s in allotetraploid rapeseed

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**Background:**

Rapeseed is an indispensable oilseed crop worldwide, improving rapeseed yield is an eternal issue. But the yield-related traits are all complex quantitative traits that is controlled by environmental factors and polygenes. Additionally, Rapeseed is an allotetraploid species which own a large number of redundant homologous genes. All these leading to a difficulty to improving Rapeseed yield greatly by a single gene.

**Objective:**

MicroRNAs are known as multifunctional factors in regulating complex quantitative traits by controlling the post-transcriptional regulation of targets expression. We want to screen a single regulator (miRNA) which own the powerful functions in regulating multiple yield-related traits greatly, and further elucidate their mechanism.

**Methods:**

76 sets miRNA-Seq data among 4 pairs of yield-related materials were used to screen the core differentially expressed miRNAs (bna-miR168a). The stem loop of bna-miR168a and the modified IPS1 were cloned into the 35S-pEarleyGate 101 vector to create transgenic plants with overexpression and target mimic lines. Bioinformatics analysis was performed to elucidate target genes (AGO); qRT-PCR in vivo and Luciferase system in vitro were performed to verify the relationship between bna-miR168a and BnaAGO1s. miRNA-Seq analysis was performed to screen the target miRNAs (miR403, miR166f) based on the transgenic bna-miR168a lines. Further transgenic validation with miR403, miR166f and miR168a/miR403, miR168a/miR166f were used to prove the module of "miR168a-AGO1-miRNAs". All transgenic materials were planted in multiple environments for more than 2 years continuously, and all the yield-related traits were investigated.

**Results:**

Bna-miR168a expressed higher in low yield-related materials, including thousand-seed weight, silique length and harvest index, whereas show lower expression in high yield materials. Three bna-miR168a target mimic (M168a) and overexpression (OE168a) transgenic lines were created. OE168a displayed inhibition on plant growth, whereas M168a exhibited positive regulatory effects in multiple agronomically traits greatly, including branching and silique numbers, silique length, seeds-size, thousand-seed weight, etc. Especially suppression of bna-miR168a can harvest significantly more seeds than control, and the results were verified in two environments (up to 80-93% higher in Chongqing and 90-113% higher in Guizhou environments), and species (rapeseed and Arabidopsis). qRT-PCR and LUC results show all the four BnaAGO1s exhibit negative expression level with bna-miR168a. The miRNA-Seq show bna-miR403 and bna-miR166f own opposite expression patterns in both OE168a and M168a lines. The transgenic verification of OE403, M403, OE166f, M166f, OE168a/OE403, M168a/OE403, OE168a/OE166f, and M168a/OE166f lines further proved their "miR168a-AGO1s-miR403/166f" modules.

**Conclusions:**

Bna-miR168a was a core regulator influencing complex yield-related traits, which exhibited significantly differential expression level among multiple yield-related materials. Bna-miR168a own the powerful functions in regulating multiple agronomically traits greatly and stably during the whole growth stage in allotetraploid rapeseed, especially the traits of branching and silique numbers, silique length, seed-size and thousand-seed weight. Suppression of bna-miR168a can improve the rapeseed yield greatly by targeting to all the four BnaAGO1s simultaneously, which play essential roles in the RNA-induced Silencing Complex. And the module of "miR168a-AGO1-miR166f-HD ZIP" and "miR168a-AGO1-miR403-AGO2" can influence the formation of branching numbers and the development of silique in rapeseed respectively.