



# Differential expression of miRNAs and their targets in wax-deficient rapeseed

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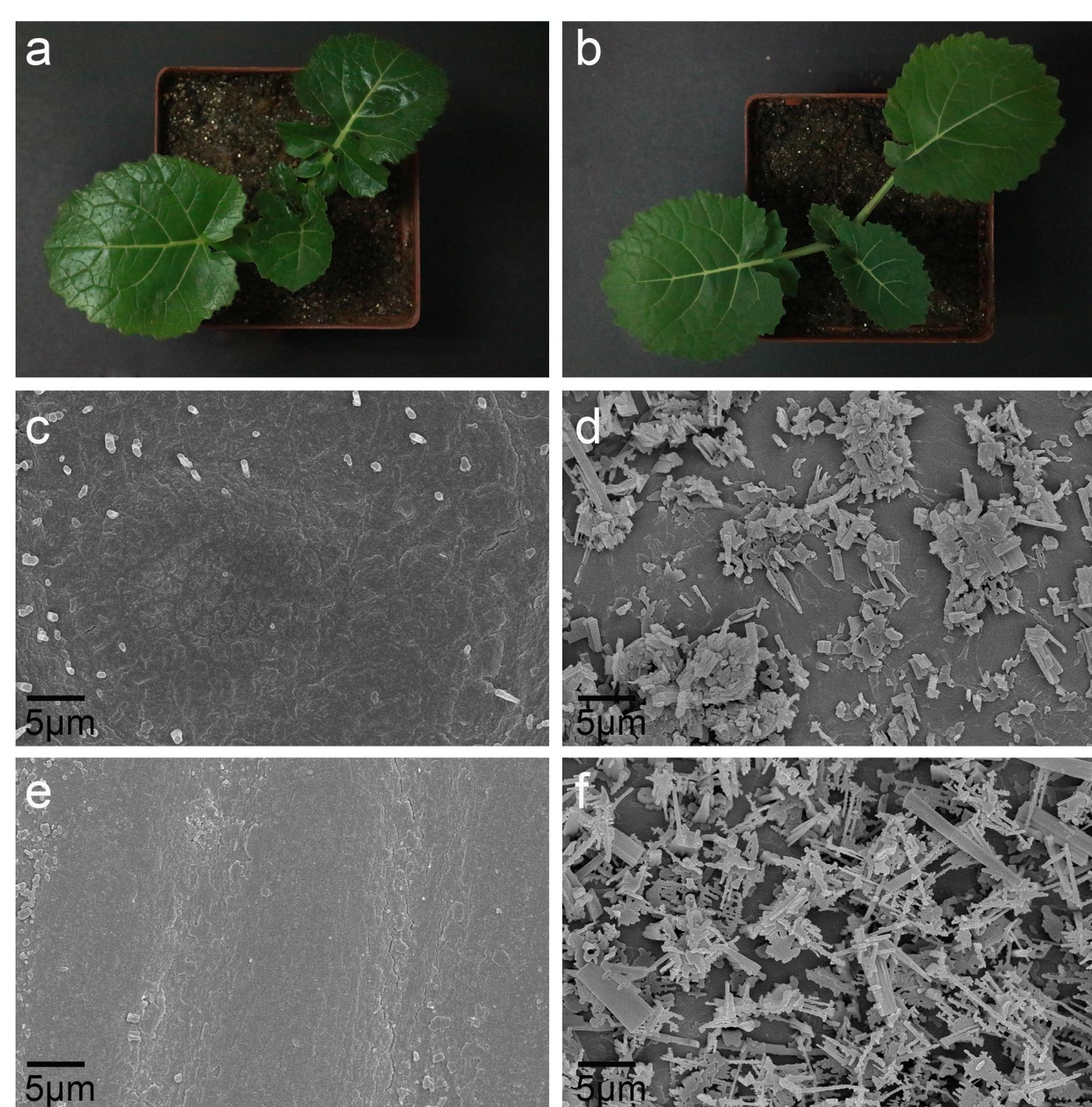
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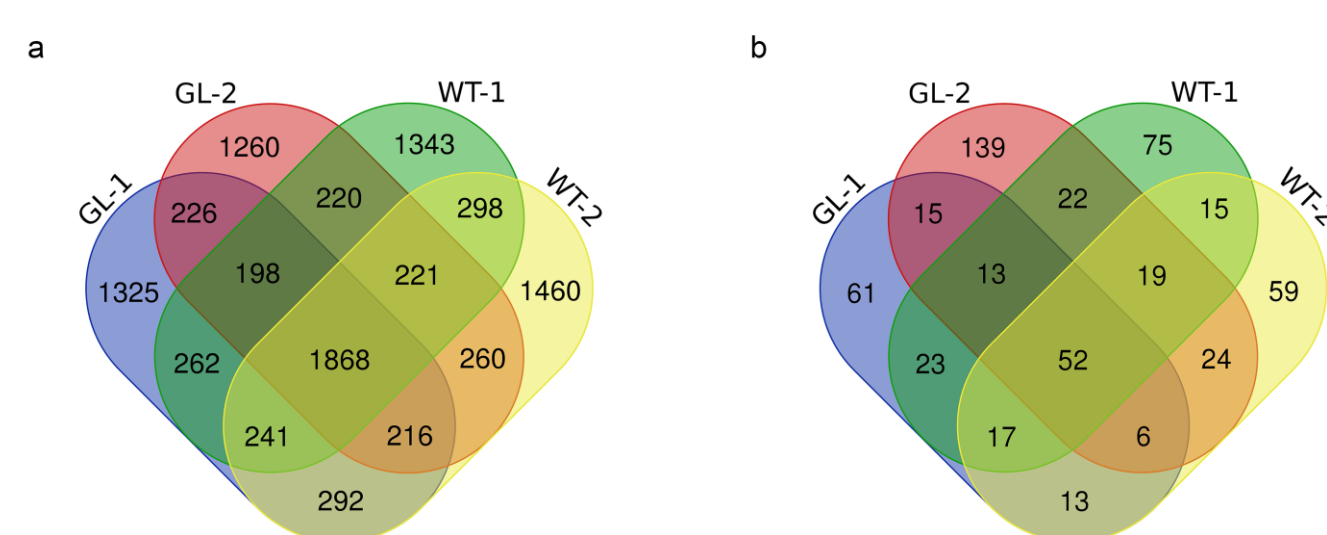
## Abstract

The cuticle of the plant, composed of cutin and wax, is the outermost hydrophobic layer covering the epidermis of all its aerial organs, protecting it from many abiotic and biotic stresses. To investigate the function of small RNAs in wax biosynthesis in *B. napus*, we constructed four small RNA libraries from the stem epidermis of wax-deficient and wild-type plants for sequencing. Totally, 300 unique known miRNAs were identified and eight of them showed differential expression. Stem-loop qRT-PCR verified that the expression of *bna-miR165a-5p* was increased in the mutant leaves, while its putative target, *BnaA06g40560D* (*CYP96A2*), was down-regulated. In addition, 5' RACE experimental data showed that *bna-miR827a* cleaves three *NITROGEN LIMITATION ADAPTATION* (*NLA*) genes (*BnaC08g45940D*, *BnaA10g01450D* and *BnaC05g01480D*). The down-regulation of *bna-miR827a* resulted in decreased cleavage on its targets, and led to the up-regulation of *BnaC08g45940D* and *BnaA10g01450D* genes.

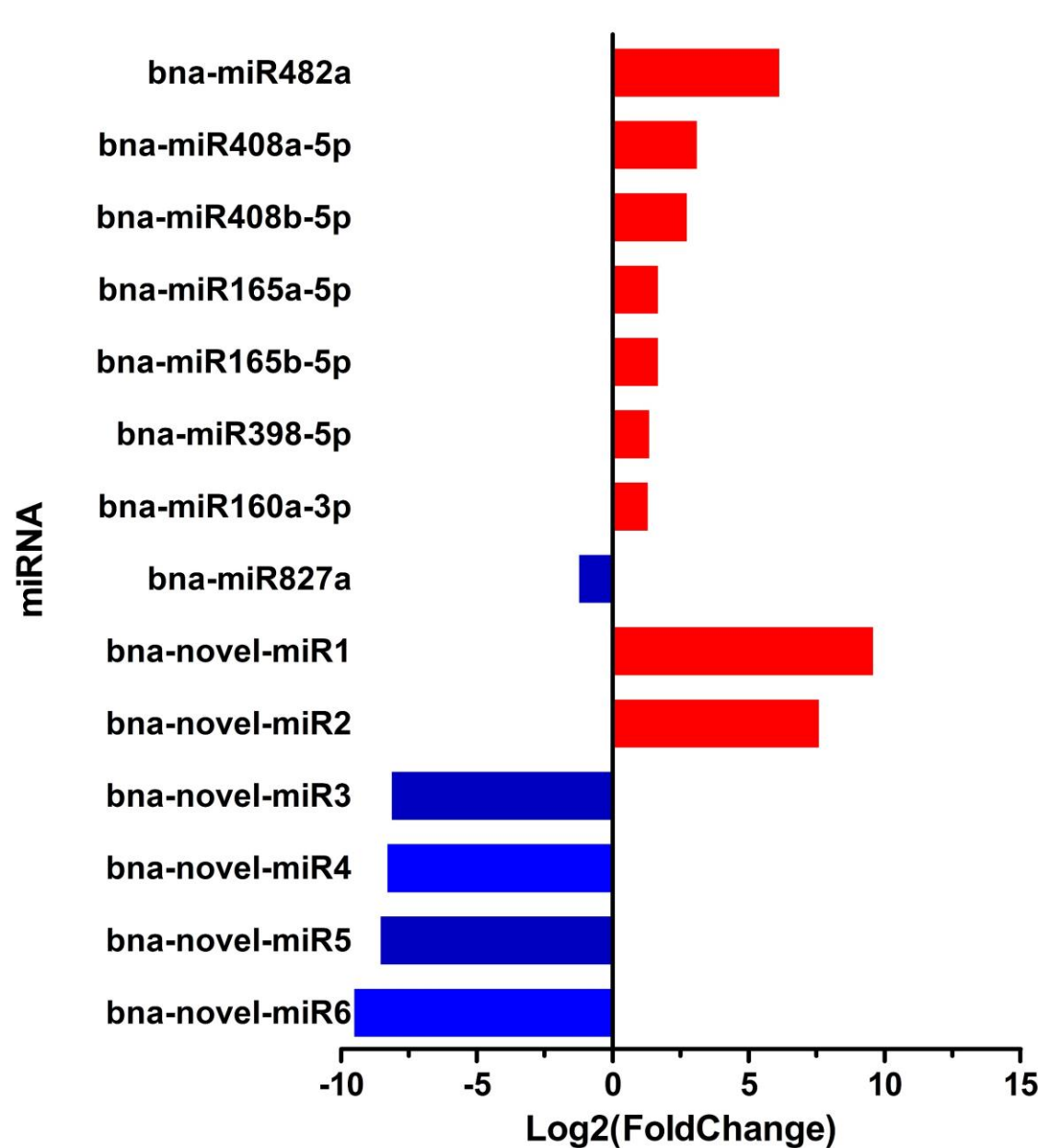
## Results



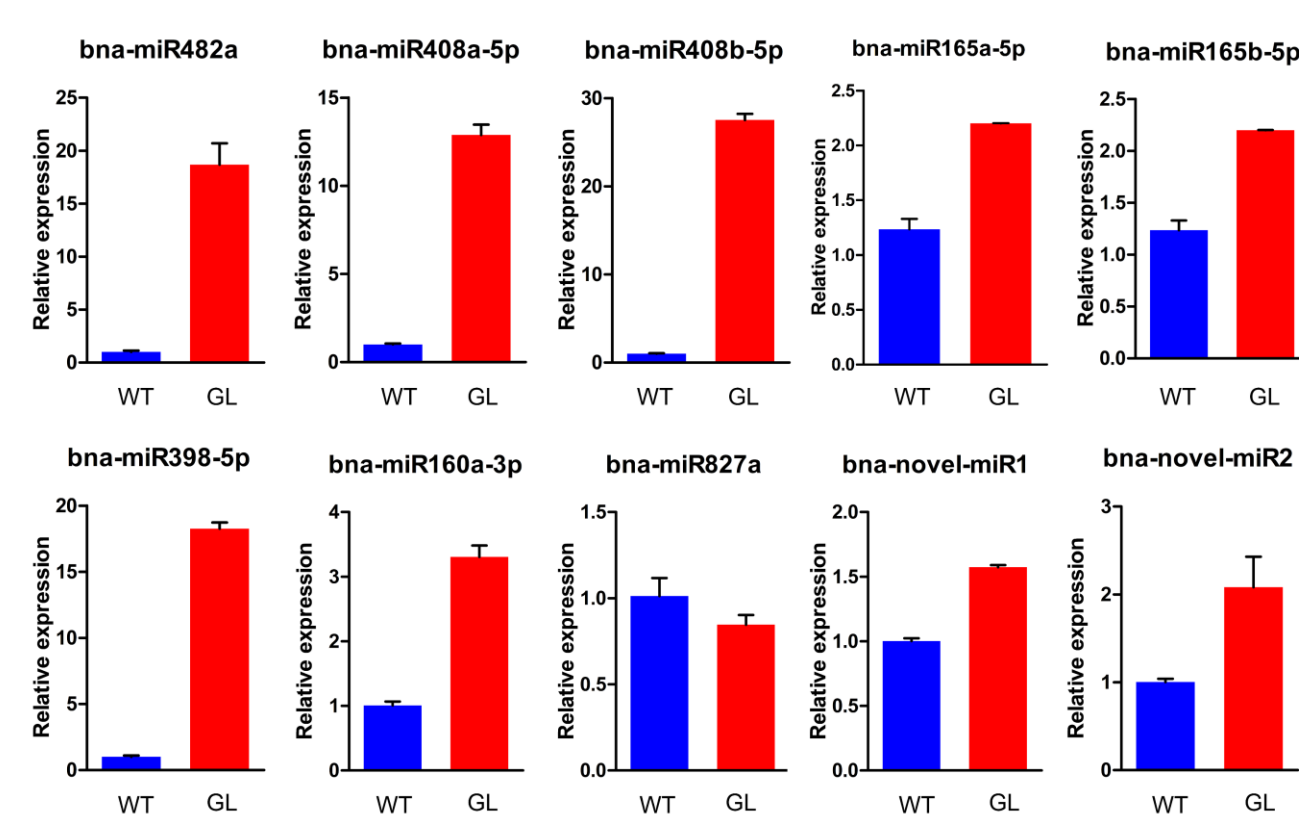
**Fig. 1** Phenotypic characterization of glossy and waxy epidermis. (A-B) Phenotype of glossy and wild type materials (C) Wax crystals on glossy leaf are bare and columnar. (D) Wax crystals on wild-type leaf are dense, with high proportion of sheet wax crystals. (E) Epidermis of glossy stem is smooth, with nearly no wax crystals. (F) Wax crystals on wild-type stem are abundant and intact. Scale bar = 5  $\mu$ m



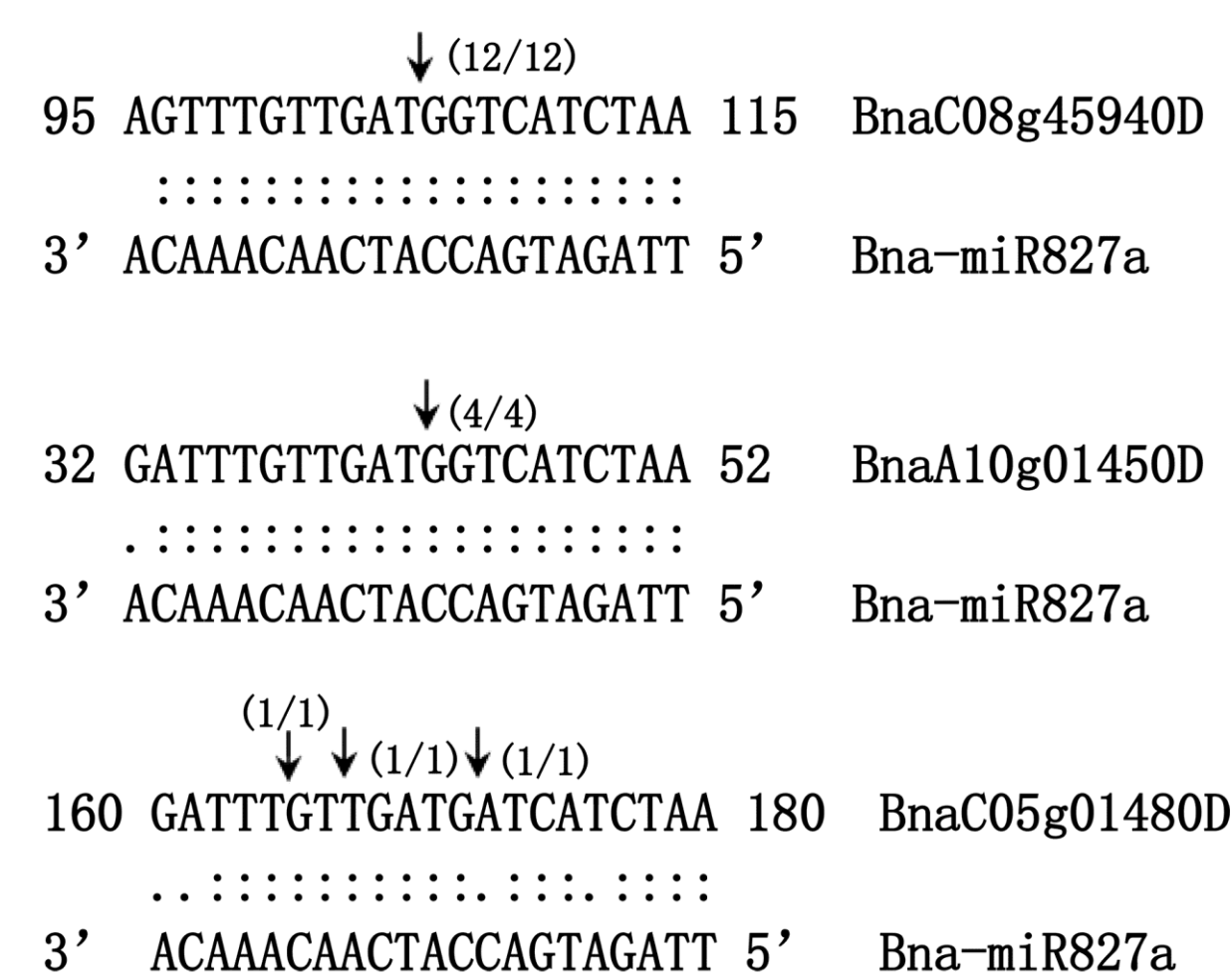
**Fig. 2** Overlap of reads mapped to known miRNAs and novel miRNAs in four libraries.



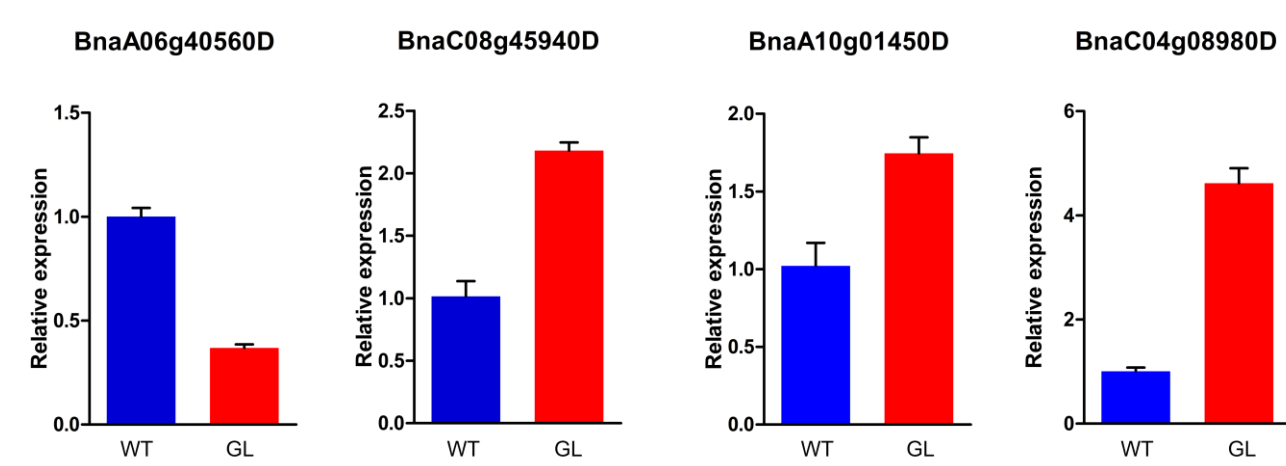
**Fig. 3** Differentially-expressed miRNAs in four libraries. Six known miRNAs and one known miRNAs were up-regulated and down-regulated, respectively. Two and four novel miRNAs were up-regulated and down-regulated, respectively



**Fig. 4** Validation of differentially-expressed miRNAs by stem-loop qRT-PCR. The bars indicate standard error (SE) of the mean (n=3)



**Fig. 5** The verified cutting sites of *bna-miR827a* on its target genes by 5' RACE in *Brassica napus*. *BnaC08g45950D*, *BnaA10g01450D* and *BnaC05g01480D* genes are the predicted targets of *bna-miR827a*. The cleavage sites of *BnaC08g45950D* and *BnaA10g01450D* are precisely mapped from the 10th position of the complement of *bna-miR827a* 5' end. Three different breakpoints on *BnaC05g01480D* were detected by 5' RACE, which are broken at the 9th, 14th, and 16th position from *bna-miR827a* 5' end, respectively.



**Fig. 6** Relative expression level change of predicted target genes verified by qRT-PCR in wild-type and mutant plants.

## Conclusion

These results showed that *bna-miR165a-5p* might participate in wax biosynthesis process by regulating its putative target *BnaA06g40560D* (*CYP96A2*). The expression of a Pi-related miRNA, *bna-miR827a*, and its target genes was affected in wax-deficient rapeseeds. These results will promote the study of post-transcriptional regulation mechanisms of cuticle biosynthesis in *B. napus* and provide new directions for further research.

The sequencing raw data are available in Gene Expression Omnibus repository with the accession number GSE115073