



Tapetal Expression of *BnaC.MAGL8.a* Causes Male Sterile in *Arabidopsis*

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Abstract

Monoacylglycerol lipase (MAGL) hydrolyzes monoacylglycerol to release free fatty acid from the glycerol backbone. Although this enzyme has been shown to play important roles in mammals, its potential biological function in plants remains poorly understood. In a survey of MAGLs in *Brassica napus*, we have found that overexpression *BnaC.MAGL8.a*, a homolog of *AtMAGL8*, in tapetum leads to male sterility in *Arabidopsis*. Tapetal cells of the transgenic lines became vacuolated at stage 10 in transgenic plants and then degraded with microspores in stage 11. Defected exine wall was observed on the pollen of transgenic plant. Transcriptome analysis identified 398 genes differentially expressed between transgenic plants and wild type (WT). *ABORTED MICROSPORES (AMS)* and its regulated pollen wall biosynthesis genes were down-regulated, while genes in reactive oxygen species (ROS) homeostasis and jasmonates signaling pathway were up-regulated. These results suggested that expression of *BnaC.MAGL8.a* in tapetum invoked stress response and impaired pollen development.

Results

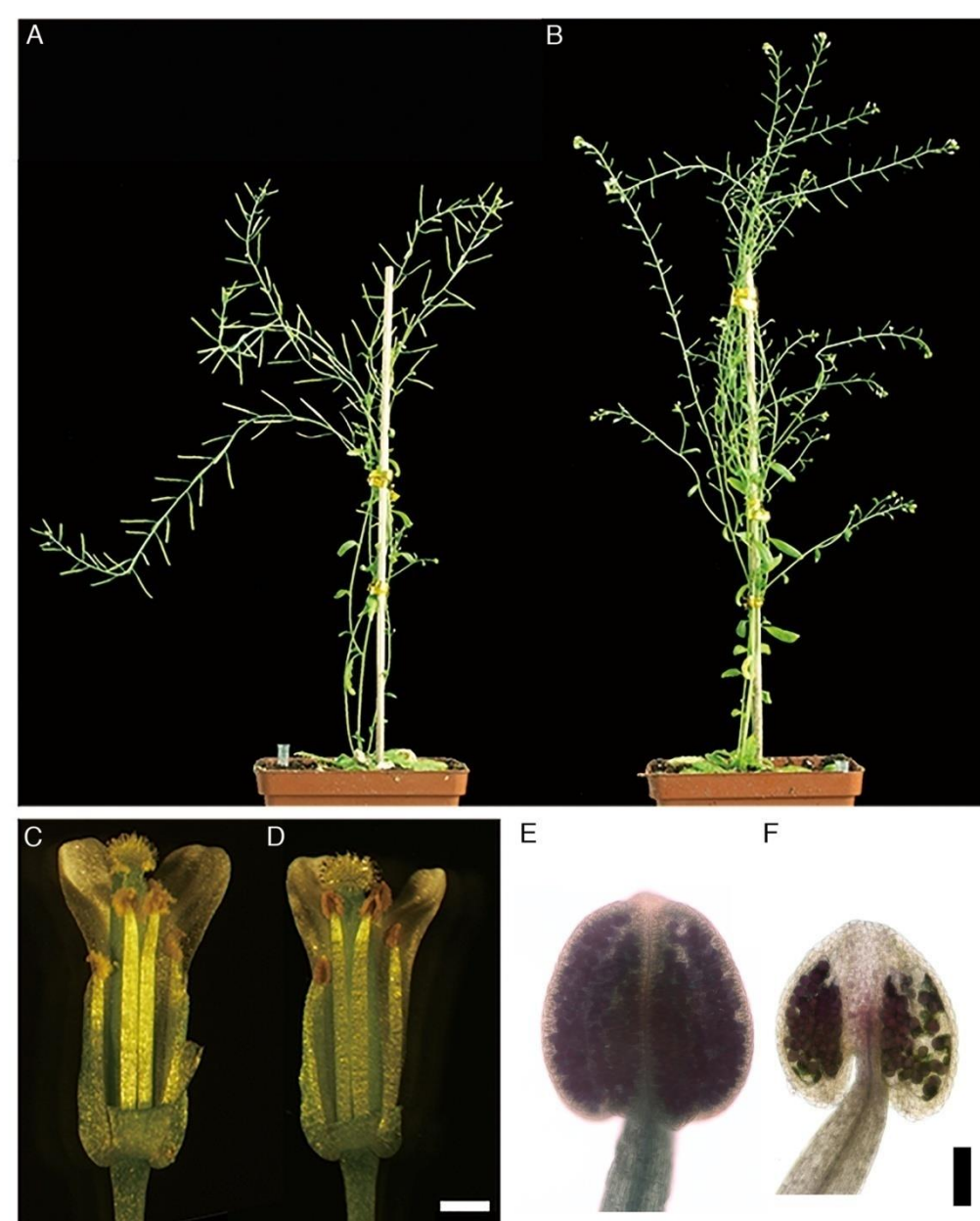


Fig. 1 *Bna9::BnaC.MAGL8.a* plants were male sterile. WT (A) and *Bna9::BnaC.MAGL8.a* plants (B) with siliques. WT (C) and *Bna9::BnaC.MAGL8.a* (D) flowers, bar = 1 mm. Alexander stained anthers from WT (E) and *Bna9::BnaC.MAGL8.a* plants (F), bar = 100 μm.

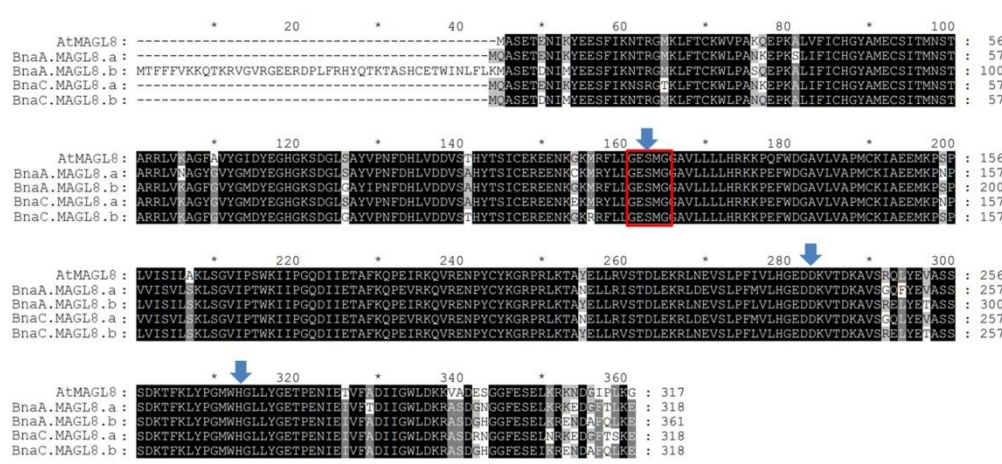


Fig. 2 Alignment of deduced amino acid sequences of *BnaC.MAGL8.a*, *BnaA.MAGL8.a*, *BnaC.MAGL8.b*, *BnaA.MAGL8.b* and *AtMAGL8*.

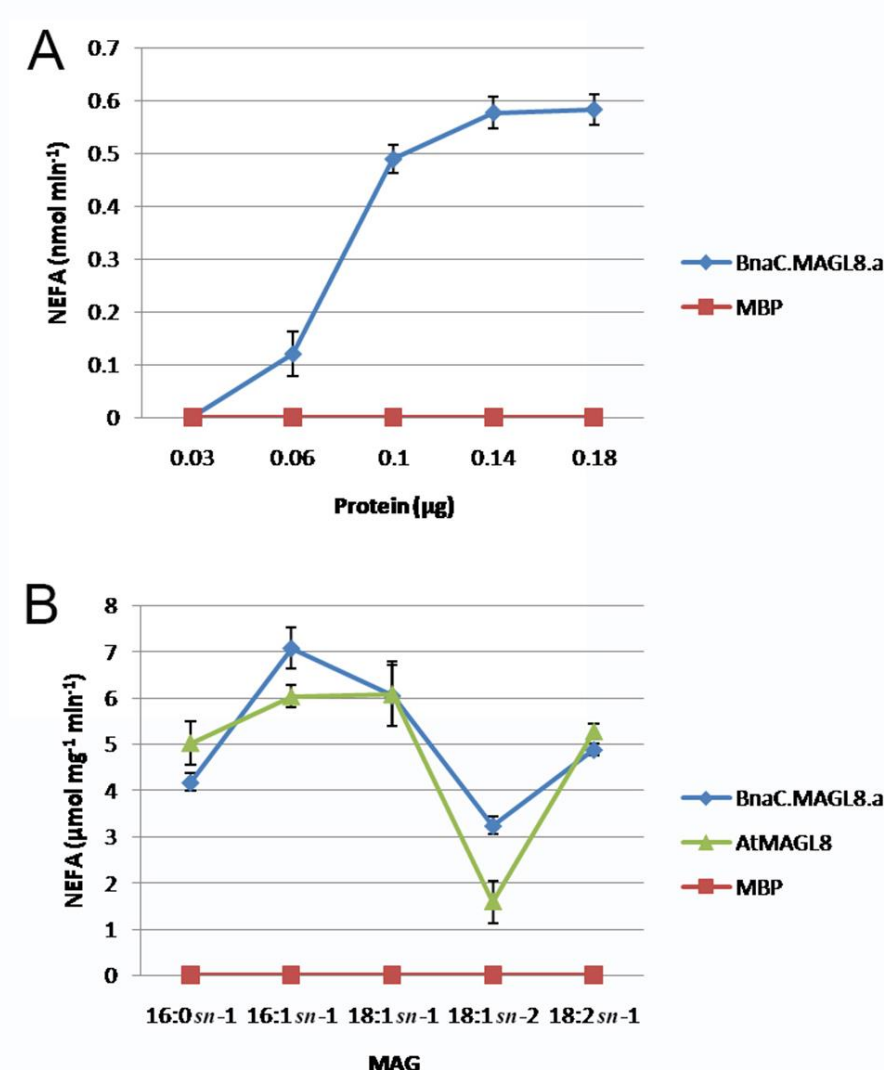


Fig. 3 *In vitro* enzyme assays of MBP::*BnaC.MAGL8.a* recombinant protein.

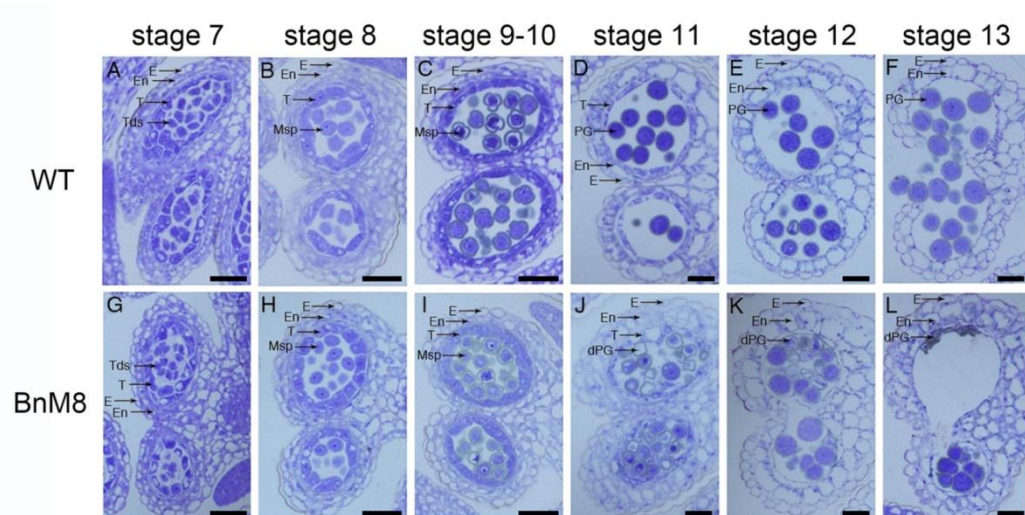


Fig. 4 Semi-thin transverse sections of anthers from WT and *Bna9::BnaC.MAGL8.a* plants. dPG, degenerated pollen grains; E, epidermis; En, endothecium; Msp, microspore; PG, pollen grains; T, tapetum; Tds, tetrads. Bar = 25 μm.

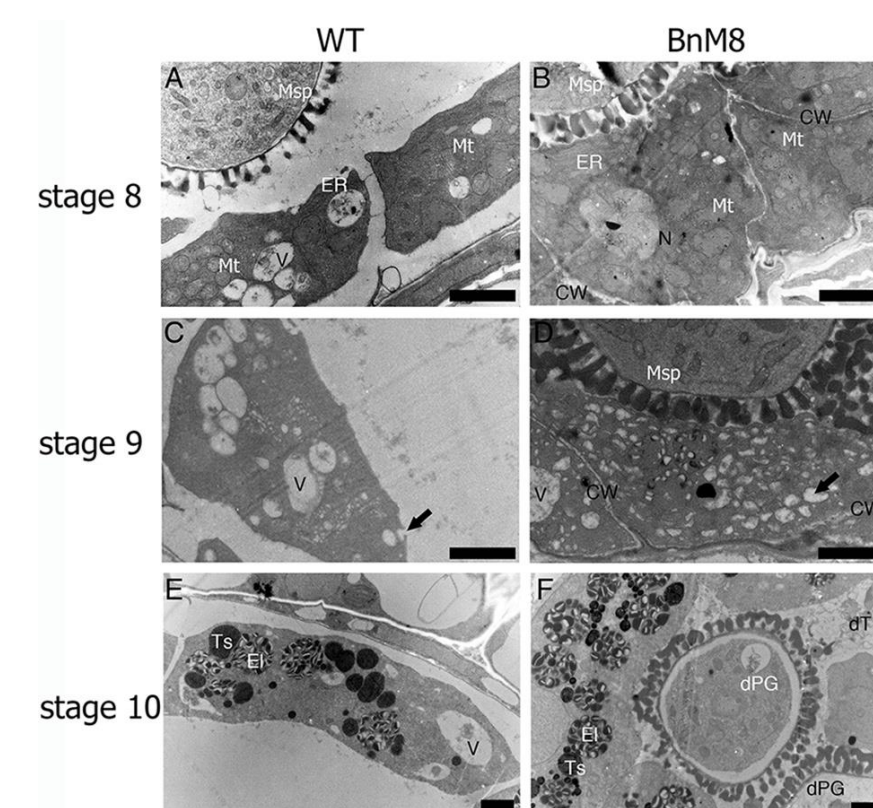


Fig. 5 Transmission electron micrographs of the tapetum from WT and *Bna9::BnaC.MAGL8.a* plants. CW, cell wall; dPG, degenerated pollen grain; dT, degenerated tapetum; EI, elaioplast; ER, endoplasmic reticulum; Ex, exine; In, intine; Mt, mitochondria; N, nucleus; Ta, tapetosome; V, vacuoles. Bars = 2 μm.

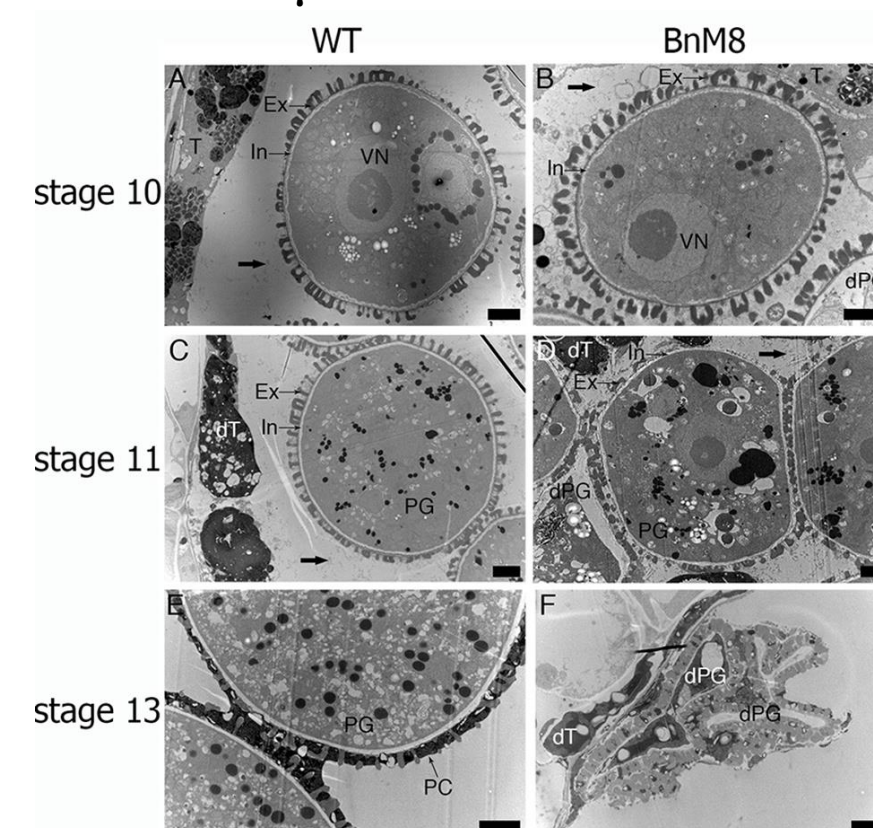


Fig. 6 Transmission electron micrographs of microspores and pollen grains from WT and *Bna9::BnaC.MAGL8.a* plants. dPG, degenerated pollen grain; dT, degenerated tapetum; Ex, exine; In, intine; PC, pollen coat; PG, pollen grain; VN, vegetative nucleus. Bars = 2 μm.

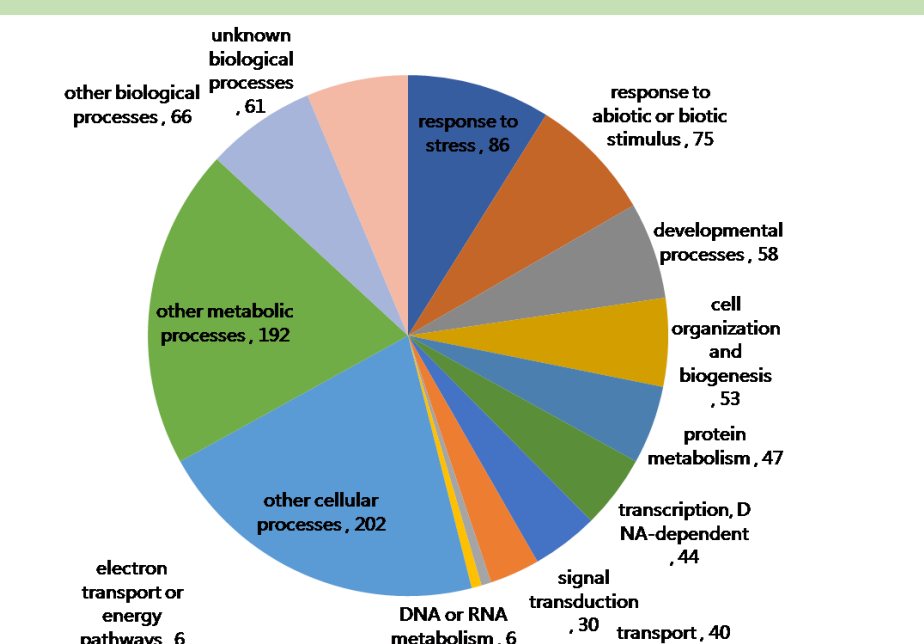


Fig. 7 Functional categorization of differentially expressed genes on GO biological processes.

Table 1 Genes related with pollen wall formation were down-regulated in transgenic plants

TAIR_ID	NAME ^a	Description	log2FC
AT2G16910	AMS	bHLH transcription factor	-0.76
AT3G26125	CYP86C2	a protein with cytochrome P450 domain	-1.58
AT1G69500	CYP704B1 ^b	participate sporopollenin synthesis	-1.53
AT1G28430	CYP705A24	member of CYP705A	-1.53
AT1G75920	EXL5 ^a	pollen coat protein	-1.43
AT5G53190	SWEET3	nodulin MN3 family protein	-1.35
AT1G33430	KNS4/UPEX1	involved in pollen exine formation	-1.28
AT4G14815		seed storage 2S album superfamily	-1.25
AT1G22015	DD46	galactosyltransferase family protein	-1.04
AT4G29250		HXXD-type acyl-transferase family	-0.99
AT5G07530	GRP17	the most abundant pollen coat protein	-0.99
AT5G16960		Zinc-binding dehydrogenase family	-0.92
AT1G26710		transmembrane protein	-0.90
AT1G71160	KCS7 ^a	participate sporopollenin synthesis	-0.90
AT4G20050	QRT3 ^a	degrading the pollen mother cell wall	-0.86
AT1G74540	CYP98A8 ^a	participate pollen wall synthesis	-0.74
AT1G75940	ATA27	similar to the BGL4 beta-glucosidase	-0.69
AT1G61110	NAC25	NAC transcription factor related with GA signal	-0.68
AT5G49070	KCS21 ^a	participate sporopollenin synthesis	-0.57
AT5G48210		prolamins-like protein (DUF1278)	-0.56
AT1G67990	TSM1	essential for phenylpropanoids synthesis	-0.56

Conclusion

In this work, we studied *BnaC.MAGL8.a*, which encodes a MAG lipase, from oil seed rape cultivar 'Zhongshuang 11'. Based on *AtMAGL8* being preferentially expressed in developing pollen and germinating seeds, we used *Bna9* promoter and *CaMV35S* (35S) promoter to drive *BnaC.MAGL8.a* expression in *Arabidopsis* to explore its potential biological function. Though 35S::*BnaC.MAGL8.a* transgenic plants showed similar phenotype as wild type, overexpression of *BnaC.MAGL8.a* in tapetum resulted in impaired pollen development and male sterility (Fig. 1). Alexander staining of anthers from transgenic plants revealed degenerated pollen grains adhered with round pollen grains in the locules. Although microspores released from tetrads as WT, the tapetum became vacuolated and degenerated with microspores. The elaioplasts and tapetosomes were observed in tapetum from both WT and *Bna9::BnaC.MAGL8.a* plants. Pollen wall formation was also perturbed in the transgenic lines. In addition to *BnaC.MAGL8.a*, transcriptome analysis uncovered 398 genes differentially expressed between WT and *Bna9::BnaC.MAGL8.a* plants, composing 157 up-regulated genes and 240 down-regulated genes.