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Functional Analysis of PhasiRNA in Soybean Anther

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Functional analysis of anther phasiRNA in soybean

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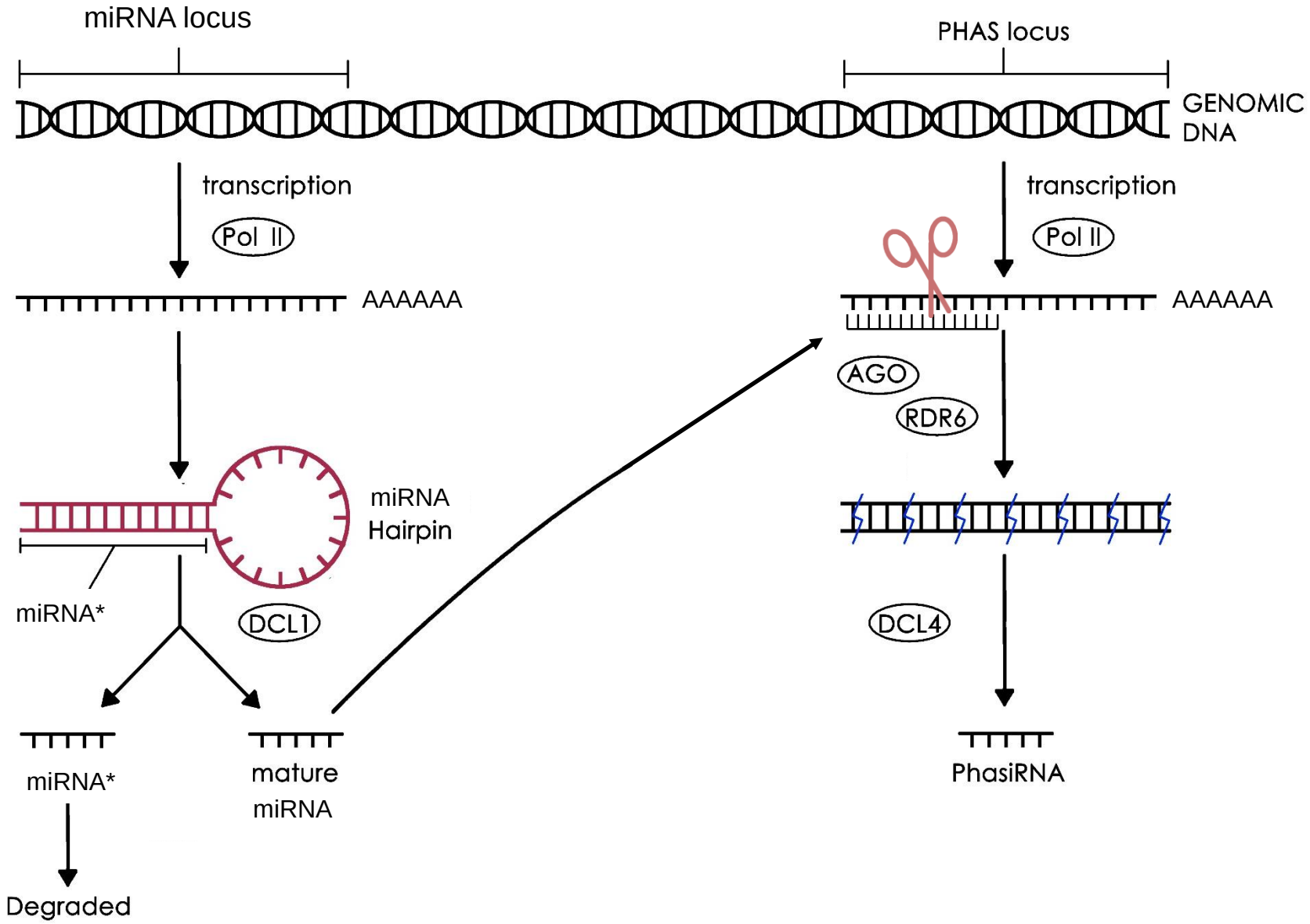
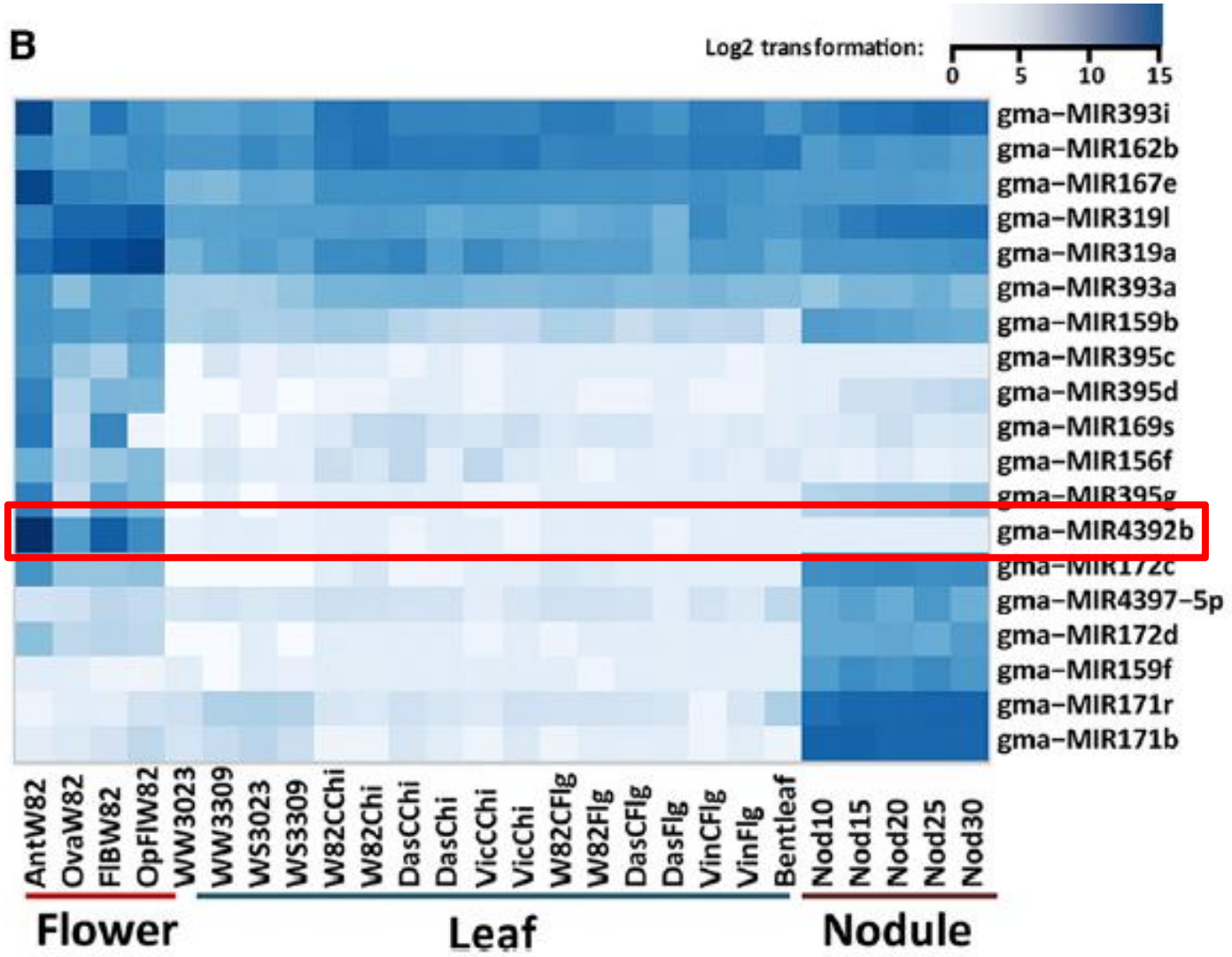
Biogenesis of phasiRNA

- miRNA binds to a PHAS precursor to trigger the synthesis of dsRNA. DCL proteins then cut phasiRNA
- Phased, secondary, small, interfering RNA
- Key regulators



miR4392 is highly abundant in soybean flowers, especially in anthers



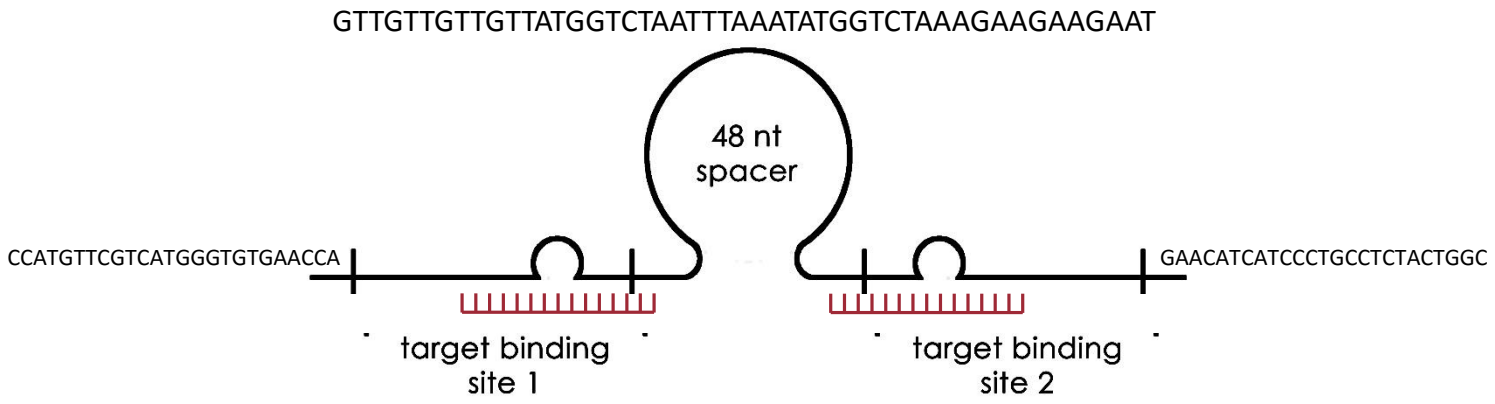
Arikit, S., et al. "An atlas of soybean small RNAs identifies phased siRNAs from hundreds of coding genes." *The Plant Cell* 26.12 (2014): 4584-4601.

What is the function of miR4392 triggered phasiRNA?

Is miR4392 necessary for anther and pollen development?

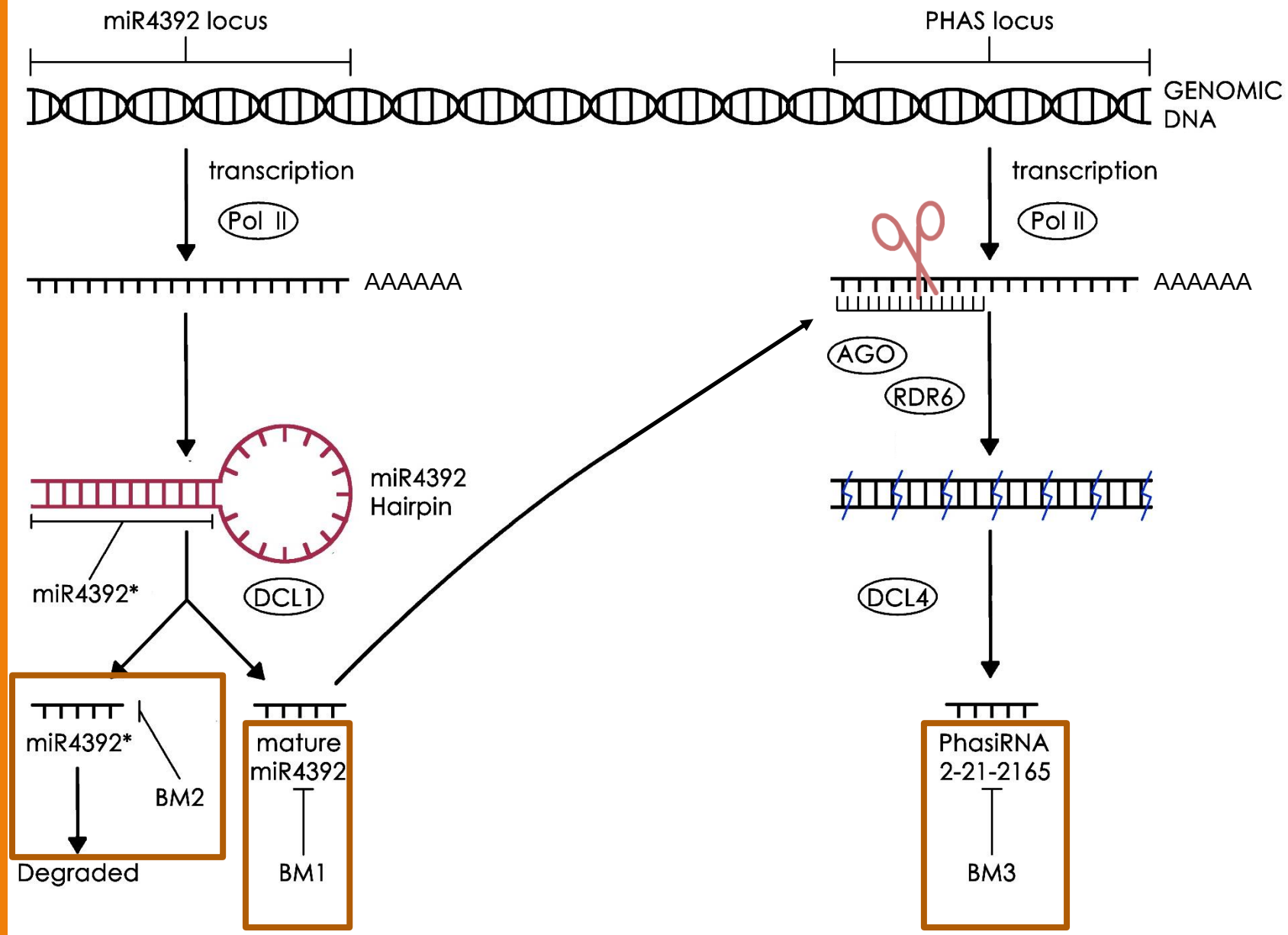
Knock down miR4392 and phasiRNA function with STTM

Short tandem target mimic (STTM)

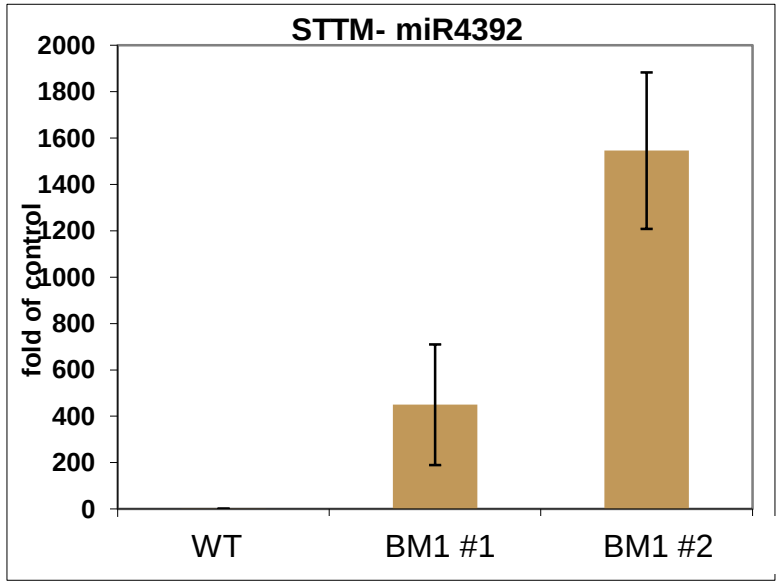


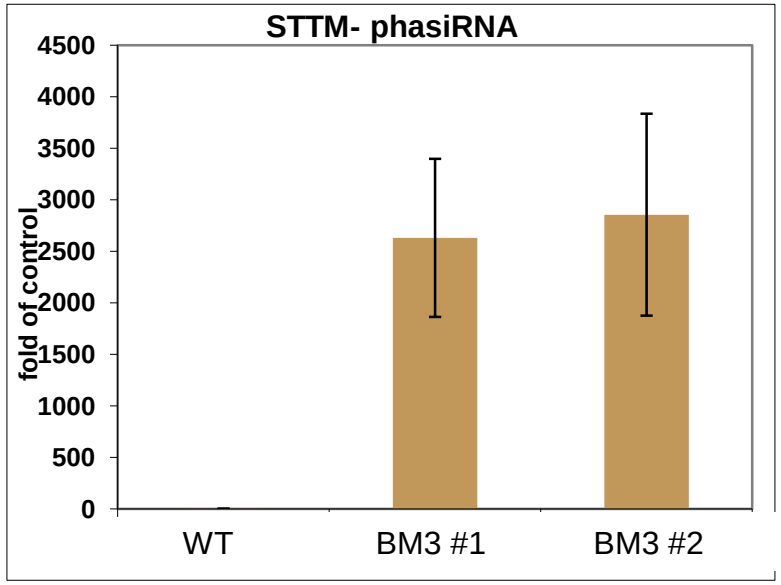
STTM Target Binding Site Sequences		
	Target	Insert sequence
BM1	**miR4392**	TCCGAAATCACActaTTTTCGCAGA
BM2	**miR4392***	TCTATGTGAAActaATGTGACTCG
BM3	**phasiRNA 2-21-2165**	GTCGGATCTAActaAACTGCATTG
BM4	**Empty vector**	AACACGACGTActaAAGATGGAAT

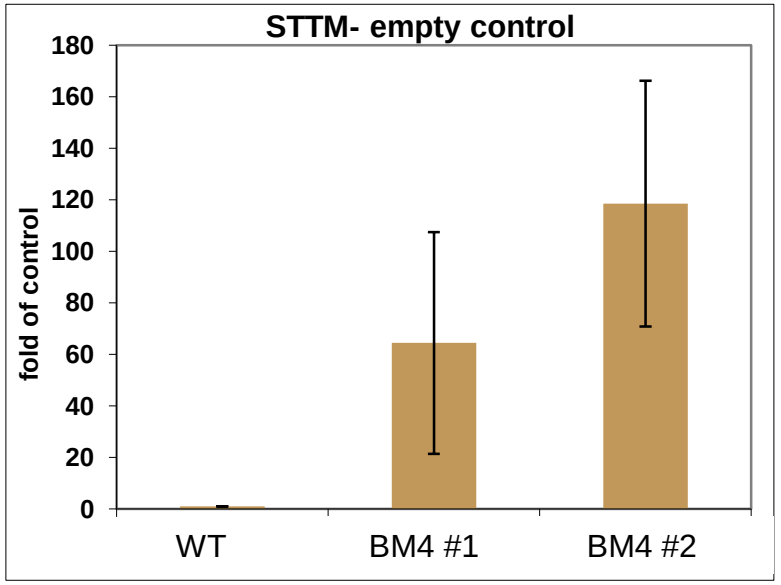
STTM in soybean miR4392-phasiRNA pathway



STTM highly expressed in the following transgenic soybean



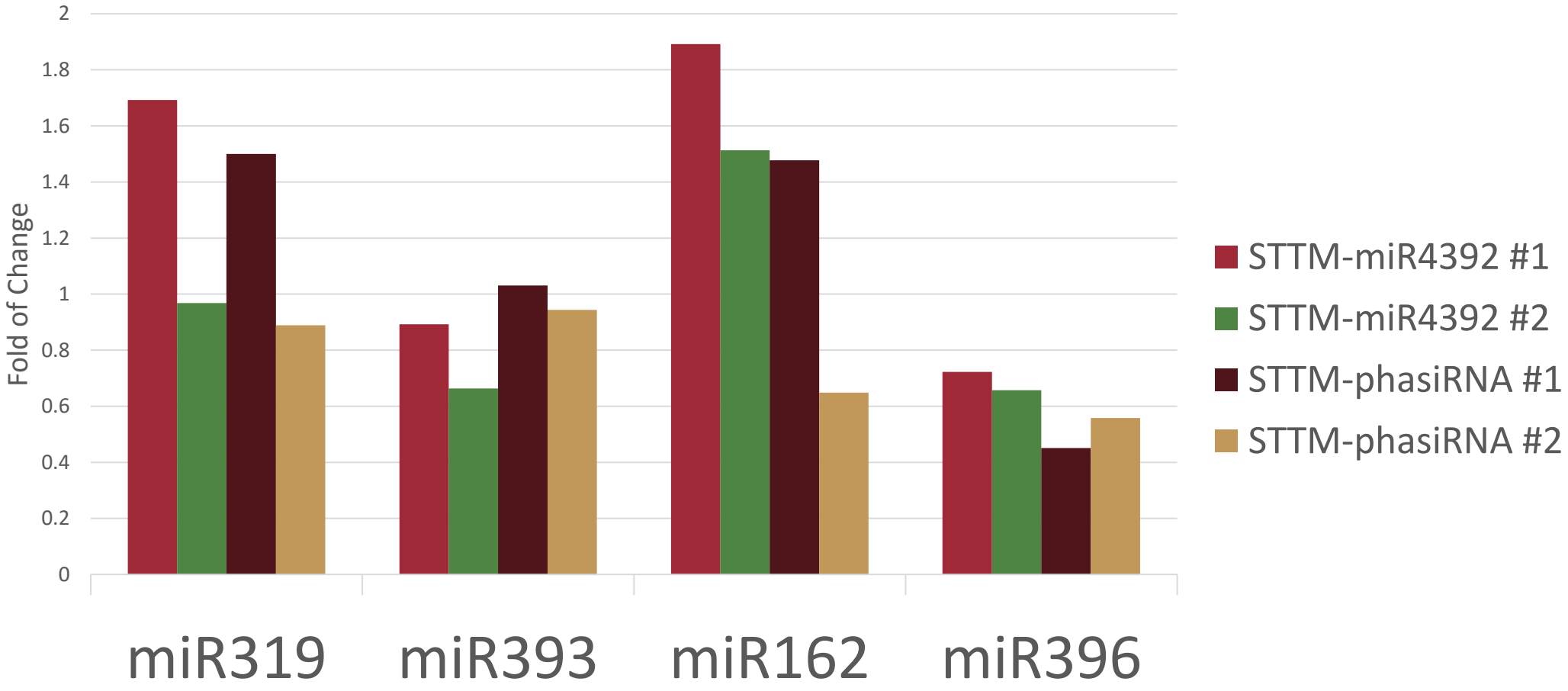




Data from STTM-miR4392* was excluded from analysis due to lack of STTM expression in cDNA

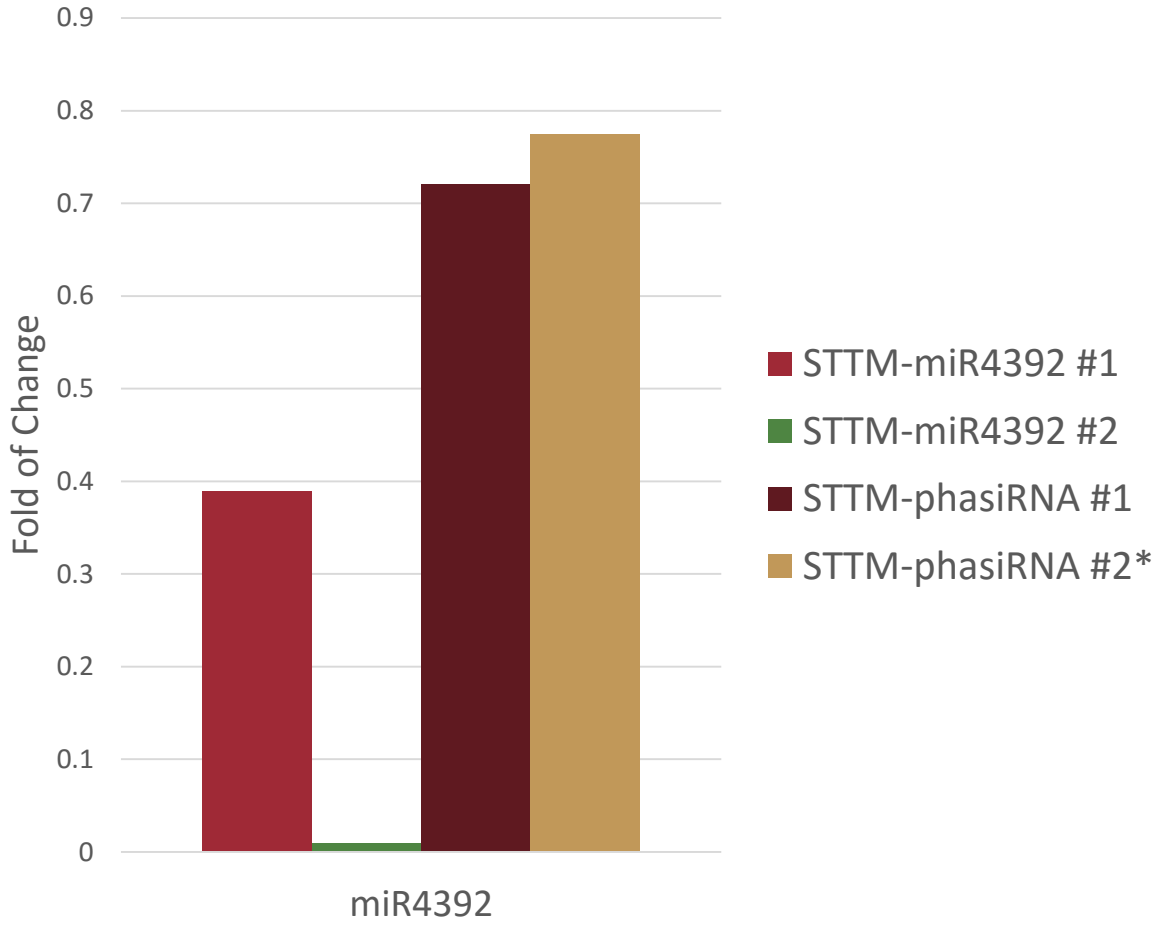
sRNA deep sequencing strategy for soybean STTM

Other abundant miRNA in soybean were unaffected by STTM



miRNA4392 abundance is decreased in STTM-miR4392

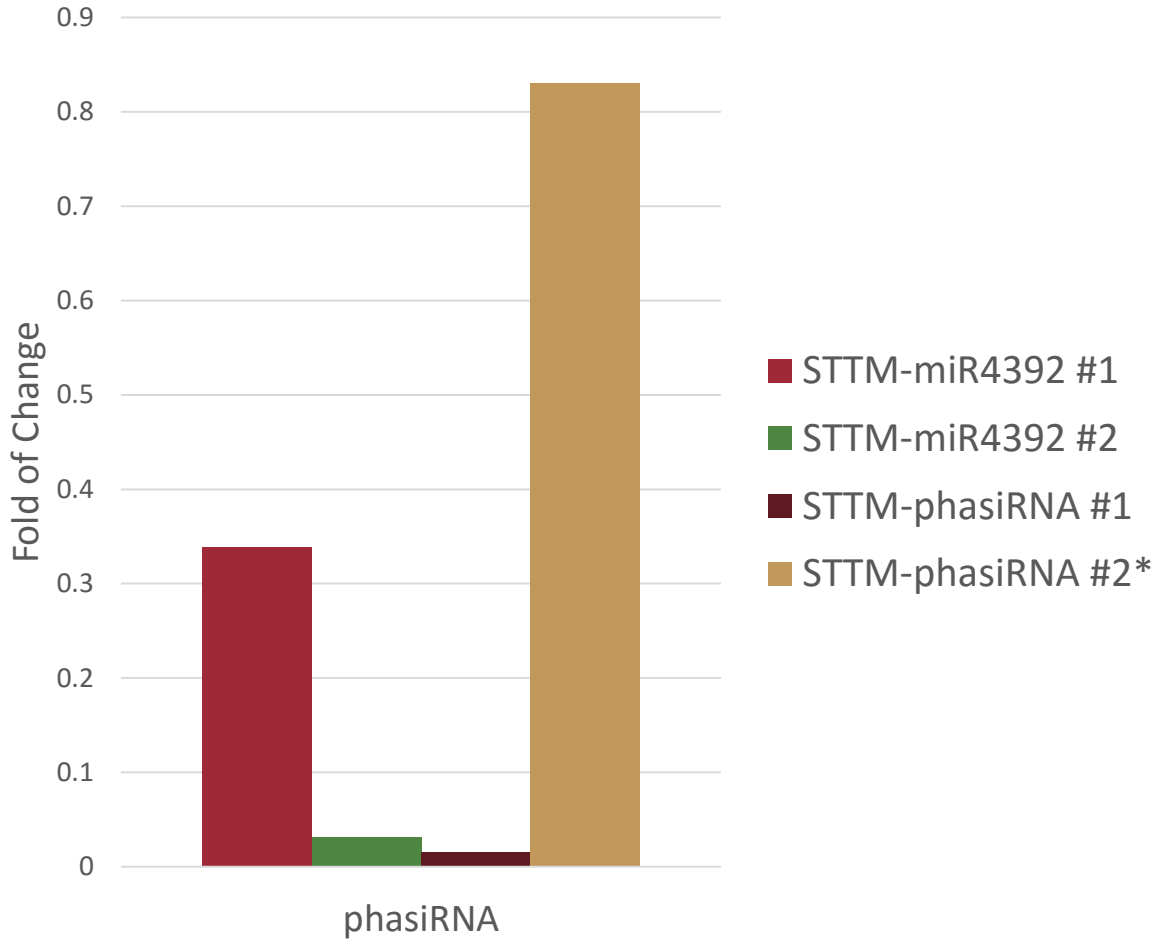
	MATCHED READS
Library ID	miR4392
STTM-MIR4392 #1	1185
STTM-MIR4392 #2	29
STTM-PHASIRNA #1	2196
CONTROL	2359



*STTM-phasiRNA #2 is treated as a control due to STTM silencing

phasiRNA abundance decreased in STTM-miR4392 and STTM-phasiRNA

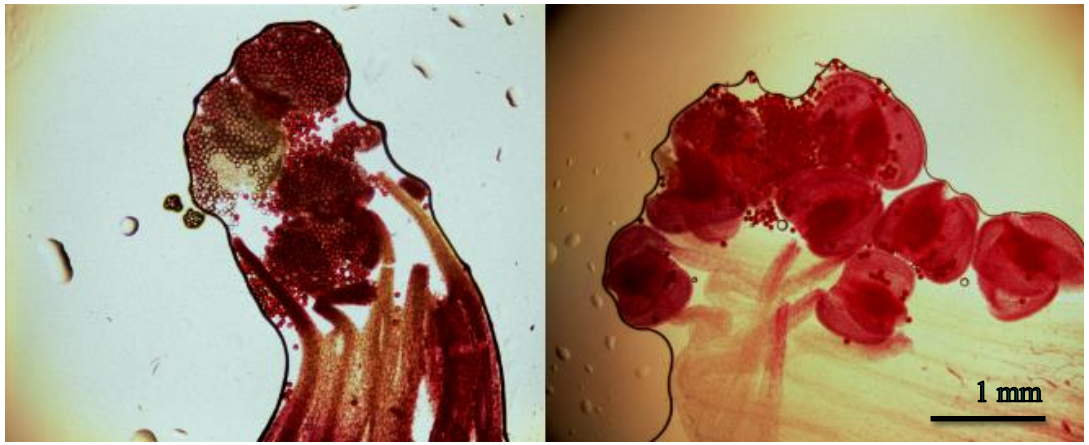
	MATCHED READS
Library ID	phasiRNA
STTM-MIR4392 #1	22
STTM-MIR4392 #2	2
STTM-PHASIRNA #1	1
CONTROL	54



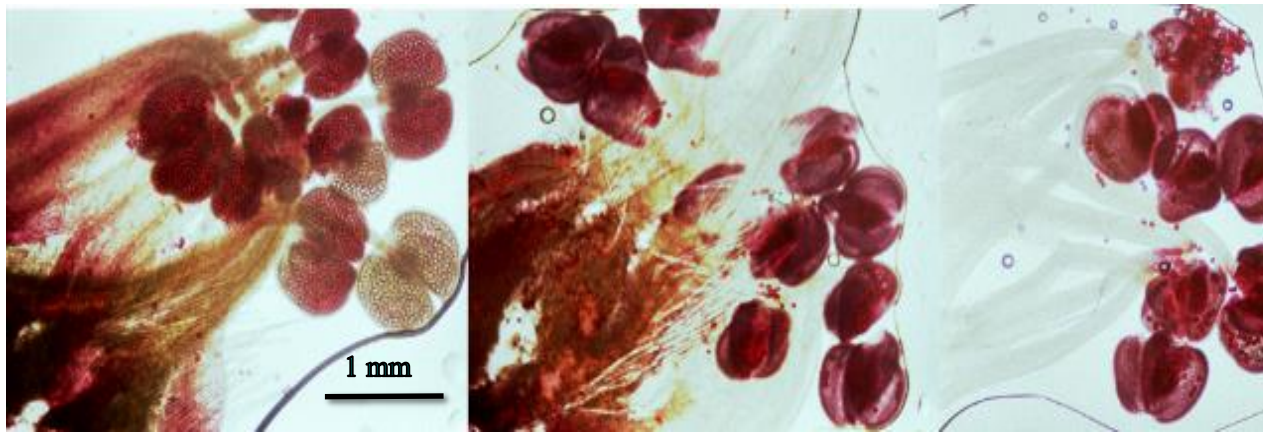
*STTM-phasiRNA #2 is treated as a control due to STTM silencing

Soybean anthers are unaffected by phasiRNA abundance

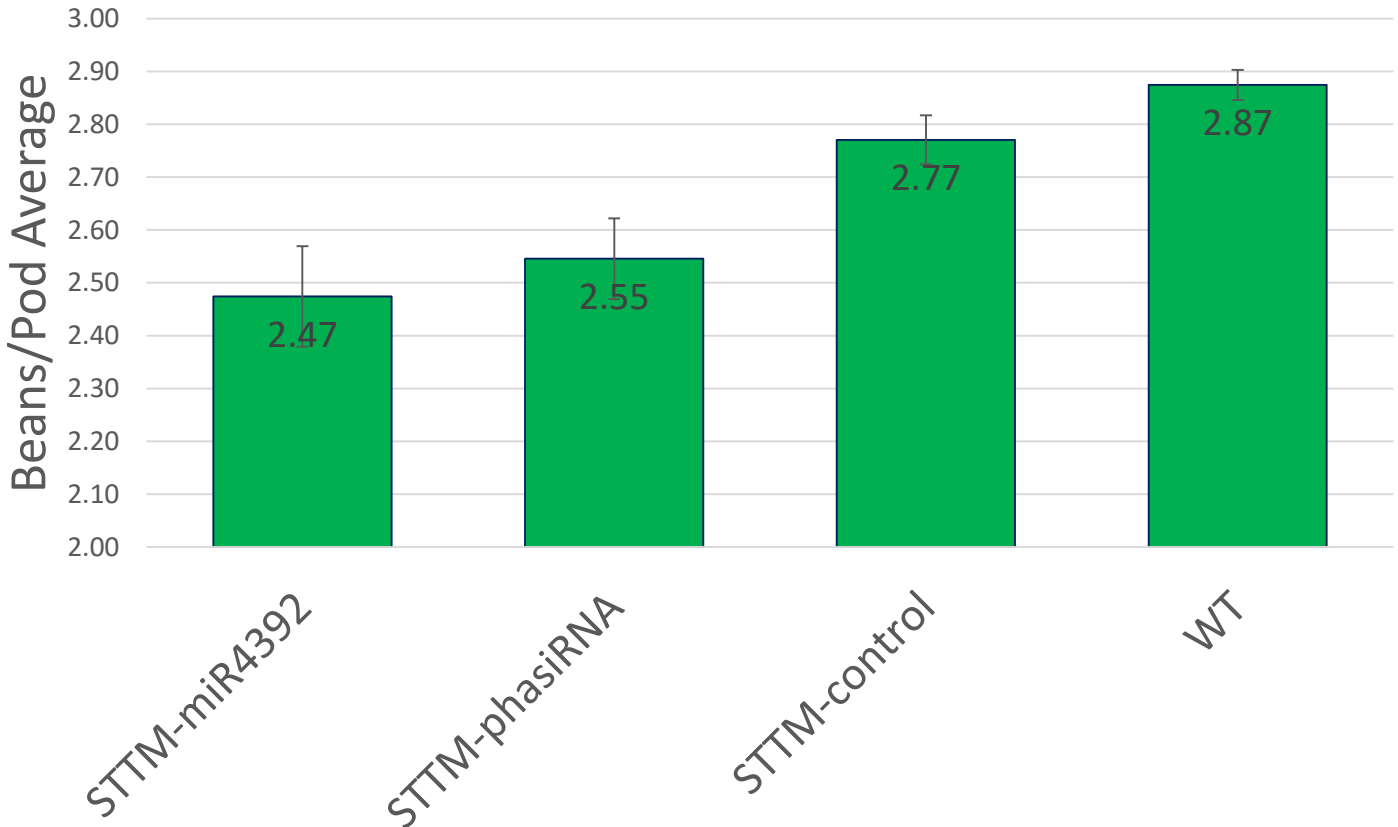
ANTHERS FROM CONTROL



ANTHERS FROM STTM MIR4392



Beans per pod decrease in STTM-miR4392 and STTM-phasiRNA



Conclusion

- In soybean the presence of miRNA and phasiRNA in reproductive tissues plays a critical role in successful seed production.
- miR4392 is the first phasiRNA found in soybean to directly influence reproductive development.

Future Work

- In-depth investigation of the staging of soybean flowers and how that affects the abundance and location of miRNA and phasiRNA in the anther and ovary
    - In situ hybridization
- Target prediction for phasiRNA triggered by miR4392

Acknowledgments

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