



Role of miR164c under combined stress



***ath-miR164c* influences plant responses to the combined stress of drought and bacterial infection by regulating proline metabolism**

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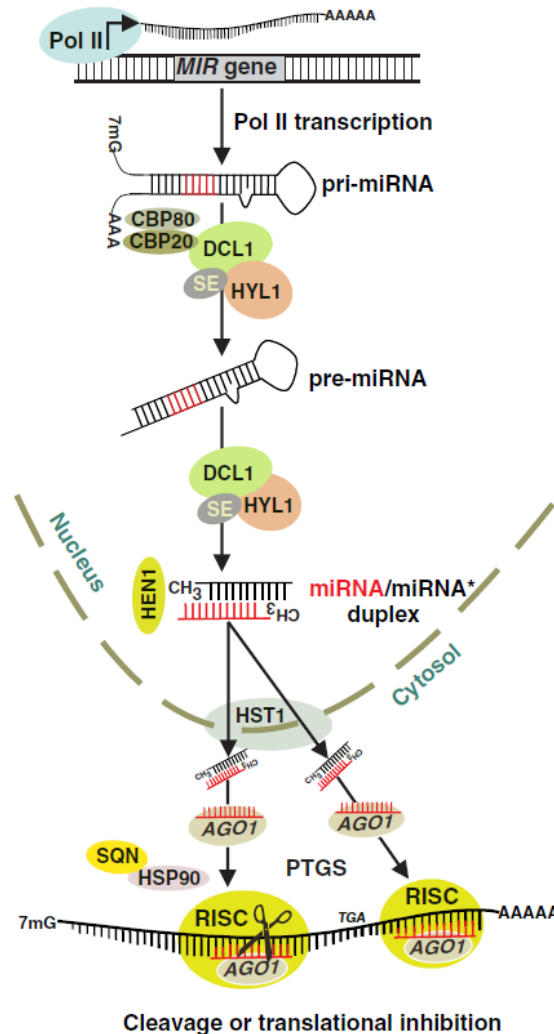
Plants response to stress



- In nature plants are exposed combination of stresses during its life cycle which affects its growth and productivity
- To combat stress plants have adapted several morpho-physiological & molecular mechanisms
- Molecular mechanisms- regulation of gene expression, protein synthesis and degradation etc.
- Among the key molecular players, miRNAs known play a vital role in stress response

miRNAs biogenesis & function

miRNAs are small regulatory RNAs of 20–22 nucleotides that are encoded by endogenous MIR genes, function by causing TGS or PTGS



DCL1- DICER LIKE 1
HYL1- HYPONASTIC LEAVES 1
SE- SERRATE
HEN1- HUA ENHANCER 1
HST1- HASTY1
AGO1- AGONATE 1
RISC- RNA INDUCED SILENCING COMPLEX

Stress-regulated small RNAs and their target families

Biotic stress

Abiotic stress

Viral infection	
AL _{Ms} miR156	SBP-LIKE
Nt-miR160	ARF
AL _{Ms} miR164	NAC
Br-miR1885	TIR-NBS-LRR
Br-miR158	PPR
Nematode infection	
Az-miR160	ARF
Az-miR164	NAC
Az-miR167	ARF
Az-miR171	SCL
Az-miR396	GRF
Az-miR398	CSD
Az-siRNA9	At5g18900
Az-siRNA32	RC/Helitron
Az-siRNA41	At1g50890
Az-siRNA46	At1g72860
Symbiotic nitrogen fixation	
Gm-miR159	MYB
Gm-miR160	ARF
Gm-miR164	NAC
Gm-miR166	HD-ZIPIII
Gm-miR168	AGO
Gm-miR169	MIHAP2-1
Gm-miR172	AP2-LIKE
Gm-miR393	TIR1
Gm-miR396	GRF
Gm-miR482	Gm1g28730
Gm-miR1521	Gm0g12340
Mt-miR169	MIHAP2-1

Bacterial infection	
Az-miR160	HD-ZIPIII
Az-miR167	ARF
Az-miR393	TIR1/AFB
Az-miR398	CSD
Az-miR825	Zinc finger
Az-natsRNA	TGB2-PPRL
Az-lsiRNAs	ATRAP
Fungal infection	
Pta-miR156	SBP-LIKE
Pta-miR160	ARF
Ta-miR156	SBP-LIKE
Ta-miR159	MYB
Ta-miR164	NAC
Ta-miR171	SCL
Ta-miR393	TIR1
Ta-miR396	GRF

Oxidative stress	
At-miR398	CSD

Mechanical stress	
Pt-miR156	SBP-LIKE
Pt-miR162	DCL
Pt-miR164	NAC
Pt-miR406	Plastocyanin
Pt-miR475	PPR
Pt-miR480	PDOT
Pt-miR481	Unknown

Hypoxia stress	
Zm-miR159	MYB
Zm-miR166	HD-ZIPIII
Zm-miR167	ARF
Zm-miR171	Spry-2
At-miR395	APS
Os-miR396	WRKY
Pt-miR474	Acyl-CoA thioesterase
Os-miR526	Aldehyde dehydrogenase
At-lasiR289 (TAS1a,b,c)	PPRs

Nutrient homeostasis	
High N	
At-miR167a	ARF
Low PO ₄ ³⁻	
At-miR399	E2-UBC
Low SO ₄ ²⁻	
Bn-miR160	ARF
Bn-miR164	NAC
Bn-miR394	F-box
At-miR395	AST/APS
Low Cu ²⁺	
At-miR396	CSD
At-miR397	Laccase
At-miR408	PCL
At-miR857	Laccase
Heavy metal (Cd ²⁺)	
Bn-miR160	ARF
Bn-miR164	NAC
Bn-miR394	F-box
Os-miR602	XET
Os-miR604	WAK-LIKE

ABA stress	
At-miR319/159	TCP/MYB
At-miR160	ARF
Os-miR167	ARF
AL _{Os} miR169	NFY
AL _{Pv} miR393	TIR1/AFB
At-miR397	Laccase
At-miR398	CSD
At-miR369a	Unknown
At-miR402	HhH-GPD
At-miR417	RDRP
Pv-miR2118	U170K related
Pv-miR159.2	Chlatriin heavy chain
Pv-miRS1	bHLH
Pv-miR1514	PseME1 related
Pv-miR2119	ADH1
Pv-miR1026	bHLH

UV-B radiation	
At-miR156	SBP-LIKE
At-miR159	MYB
AL _{Pt} miR160	ARF
AL _{Pt} miR166	HD-ZIPIII
AL _{Pt} miR167	ARF
Pt-miR168	AGO
At-miR169	NFY/MIHAP2-1
At-miR171	SCL
At-miR172	AP2-LIKE
At-miR393	TIR1/AFB
AL _{Pt} miR398	CSD
At-miR401	Unknown

Cold stress	
At-miR166	HD-ZIPIII
AL _{Bd} miR169	NFY/MIHAP2-1
AL _{Bs} miR172	AP2-LIKE
At-miR393	TIR1/AFB
At-miR396	GRF
AL _{Ba} miR397	Laccase
At-miR408	PCL
Pt-miR168	AGO
Pt-miR477	GRAS
Pt-miR156	SBP-LIKE
Pt-miR475	PPR
Pt-miR476a	PPR
At-miR402	HhH-GPD
Ta-siRNA 005047_0654_1904.1	

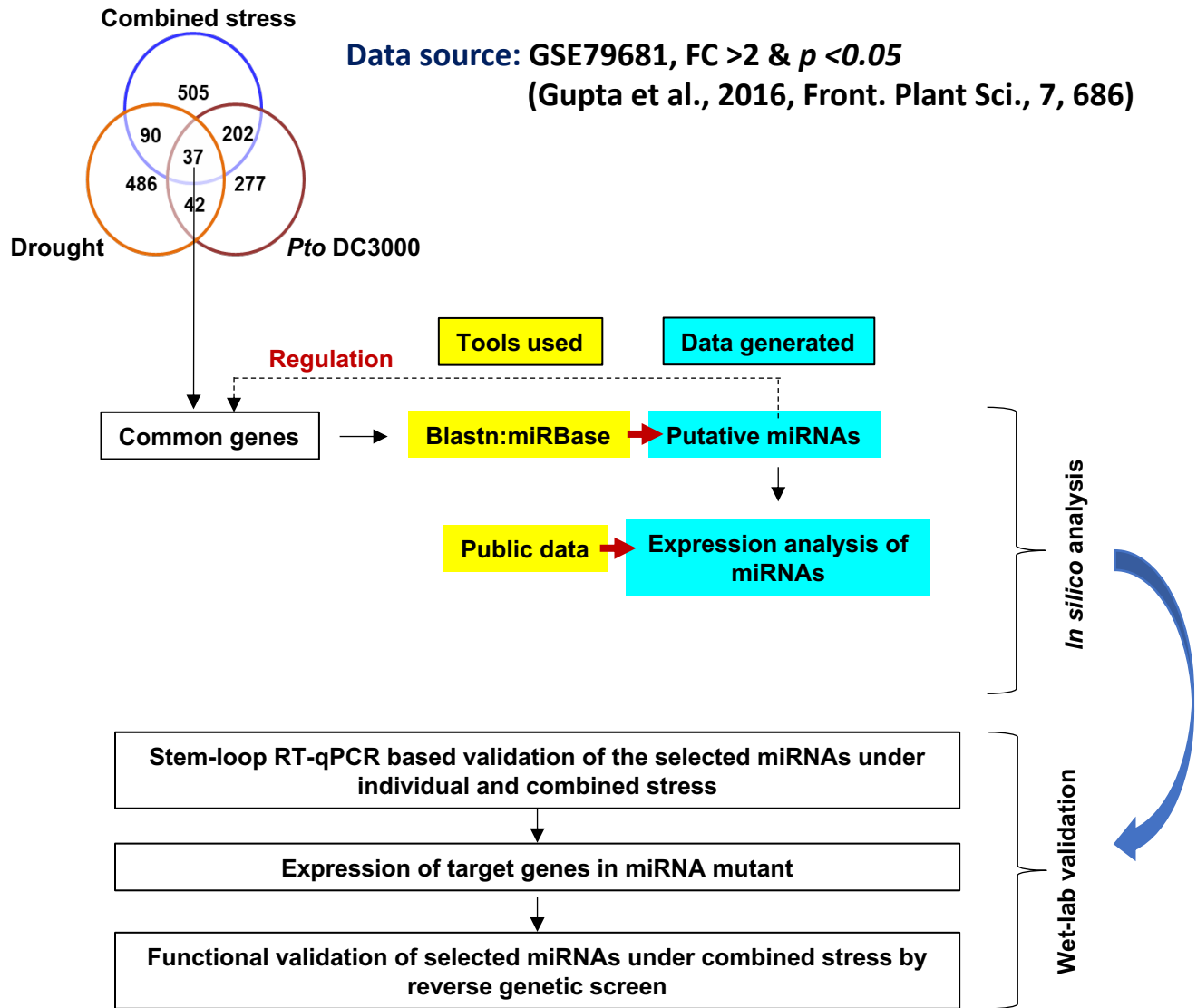
Heat stress	
Ta-miR156	SBP-LIKE
Ta-miR159	MYB
Ta-miR160	ARF
Ta-miR166	HD-ZIPIII
Ta-miR168	AGO
Ta-miR169	MIHAP2-1
Ta-miR172	AP2-LIKE
Ta-miR393	TIR1
Ta-miR827	Unknown
Ta-SiRNA 002061_0636_3054.1	
Ta-SiRNA 005047_0654_1904.1	
Ta-SiRNA 080621_1340_0096.1	

Salt stress	
At-miR156	SBP-LIKE
Zm-miR156	SBP-LIKE
At-miR158	PPR
Zm-miR162	DCL
At-miR167	ARF
Zm-miR167	ARF
At-miR168	AGO
AL _{Zm} miR169	NFY/MIHAP2-1
At-miR171	SCL
At-miR319/159	TCP/MYB
AL _{Pv} miR393	TIR1/AFB
At-miR394	F-box
Zm-miR395	AST/APS
At-miR396	GRF
Zm-miR396	GRF
At-miR397	Laccase
At-miR398	CSD
Pt-miR482.2	DRP
Pt-miR1450	L-RTMK
Pt-miR171	SCL
Pt-miR530a	Zinc knuckle (CCHC-type)
Pt-miR1445	DHPM
Pt-miR1446	GRML
Pt-miR1447	Ankyrin repeat
Pt-miR1447	Ankyrin repeat
Pv-miRS1	bHLH
Pv-miR159.2	Chlatriin heavy chain
SRO5-P5CDH-natsRNA	P5CDH
Ta-SiRNA 002061_0636_3054.1	

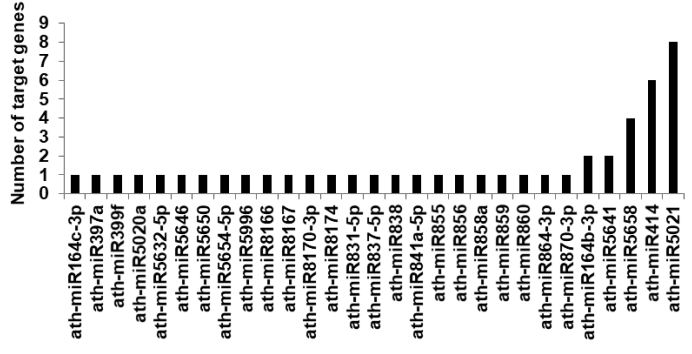
Drought stress	
At-miR157	SBP-LIKE
At-miR167	ARF
At-miR168	AGO
At-miR169	NFY
Os-Mi-miR169	MIHAP2-1
At-miR171	SCL
At-miR319/159	TCP/MYB
At-miR393	TIR1/AFB
At-miR396	GRF
At-miR397	Laccase
Mt-miR398	CSD
AL _{Mt} miR408	Plastocyanin
Os-miR156	SBP-LIKE
Os-miR168	AGO
Os-miR172	AP2-LIKE
Os-miR319	TCP
Os-miR396	GRF
Os-miR397	Laccase
Os-miR408	Plastocyanin
Pt-miR1446	GRML
Pt-miR1444	Polyphenol oxidase
Pt-miR1447	Ankyrin repeat
Pt-miR1450	L-RTMK
Pv-miR2118	U170K related
Pv-miR159.2	Chlatriin heavy chain
Ta-SiRNA 002061_0636_3054.1	
Ta-SiRNA 005047_0654_1904.1	
Ta-SiRNA 007927_0100_2975.1	

No miRNAs under combined stress

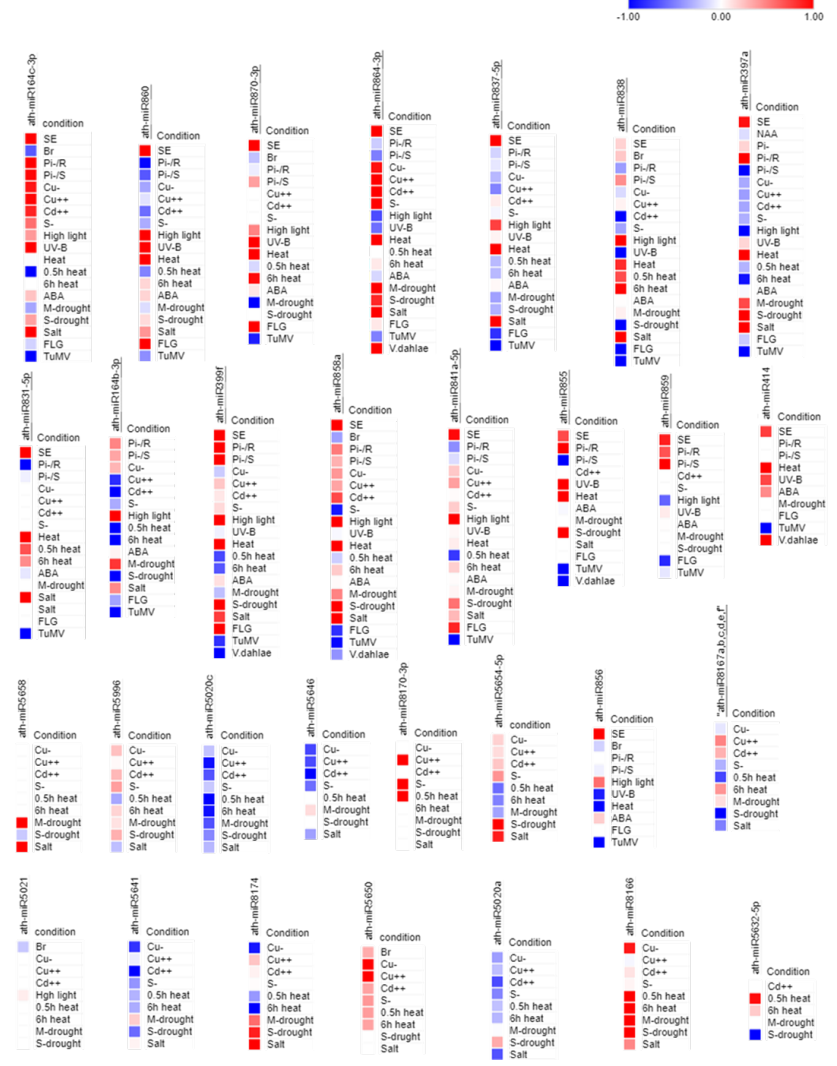
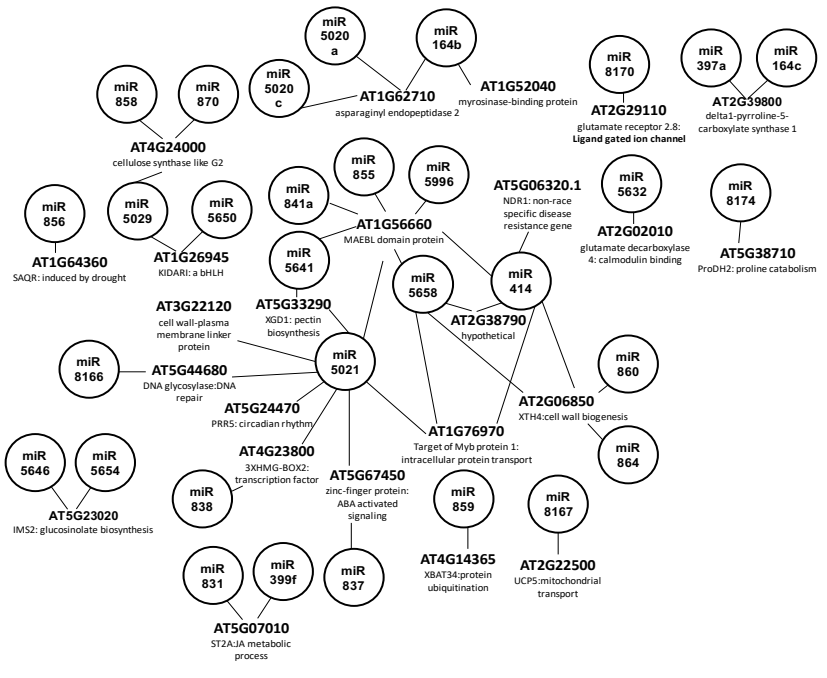
Methodology



miRNA and mRNA association network

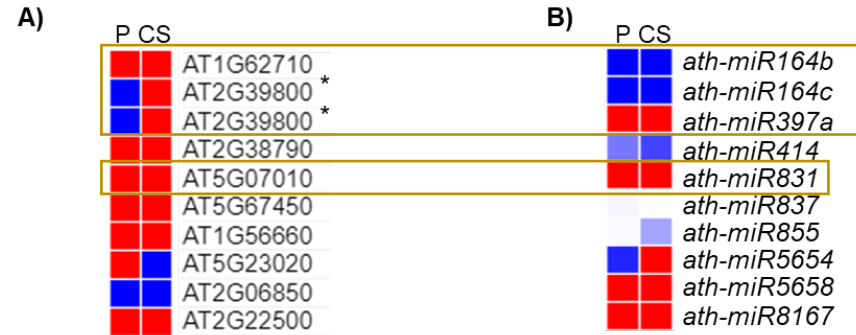
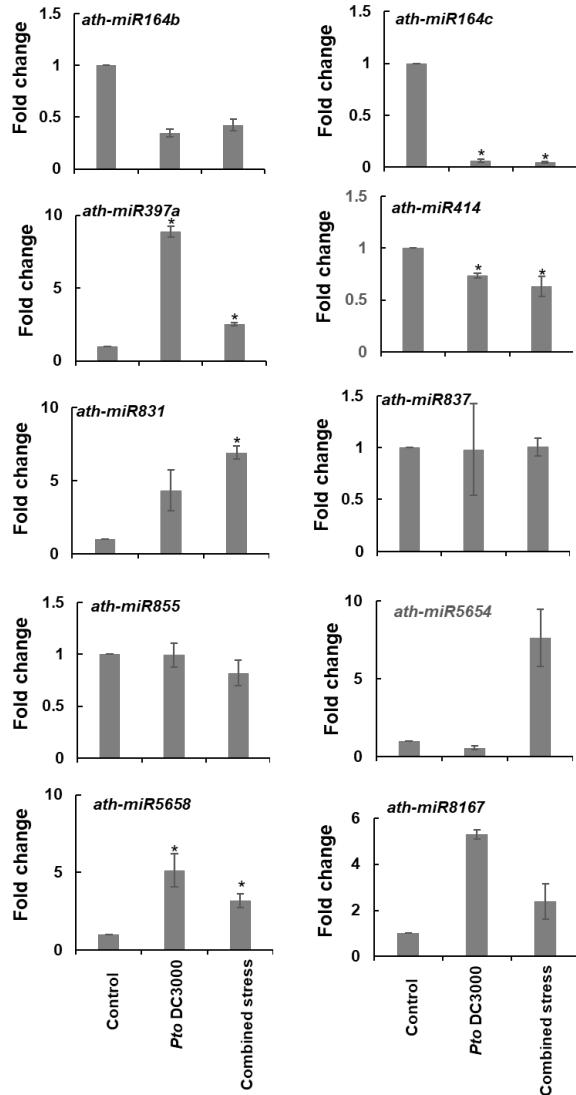


mi-RNA families



10 miRNAs were selected based on their expression under stress and hybridization energy

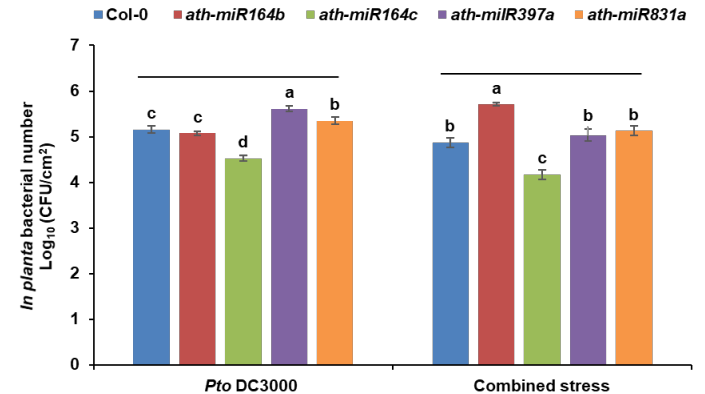
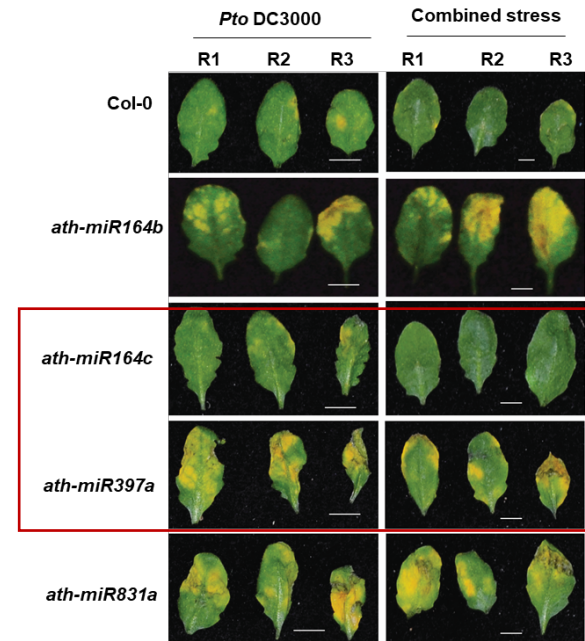
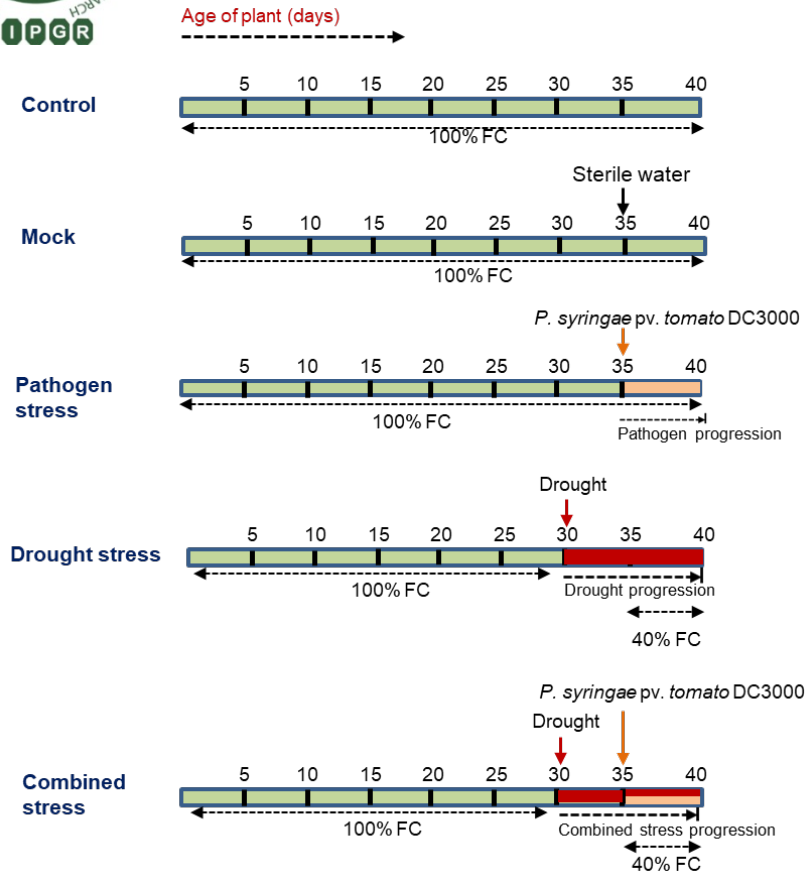
Expression analysis



ath-miR164b, ath-miR64c, ath-miR397a, ath-miR831 were selected for further analysis

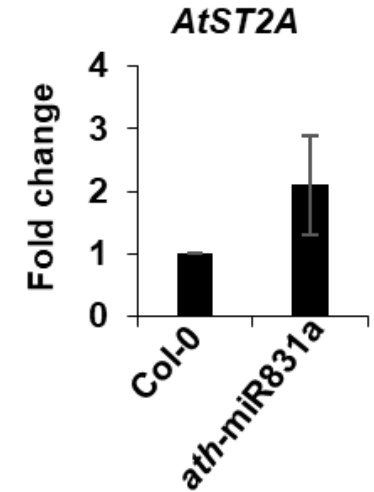
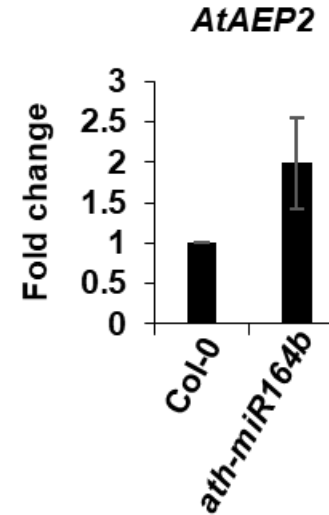
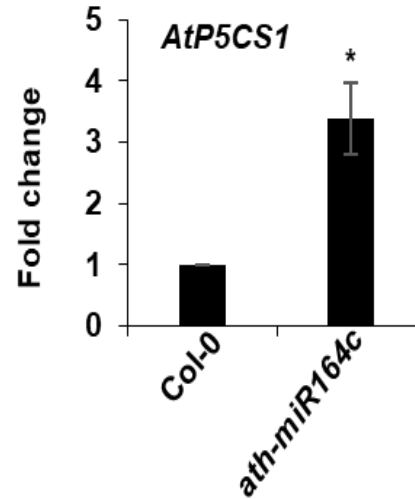
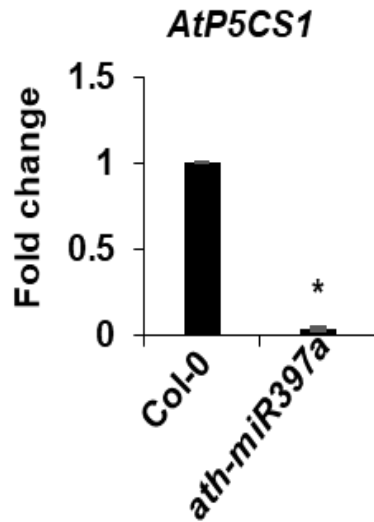
Combined stress response of miRNA mutants

Combined stress protocol



Why *ath-miR164c* & *ath-miR397a* mutants showed differential stress response of ??

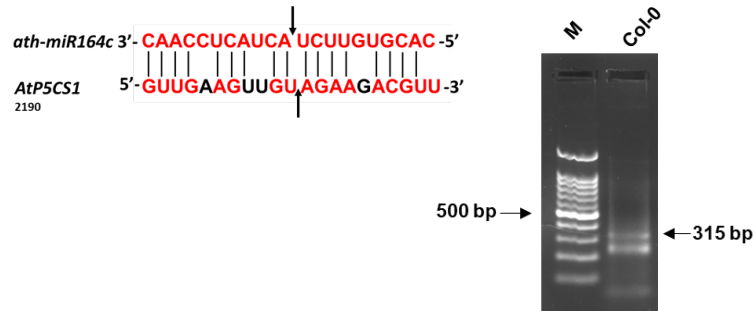
Target gene expression analysis in mutants



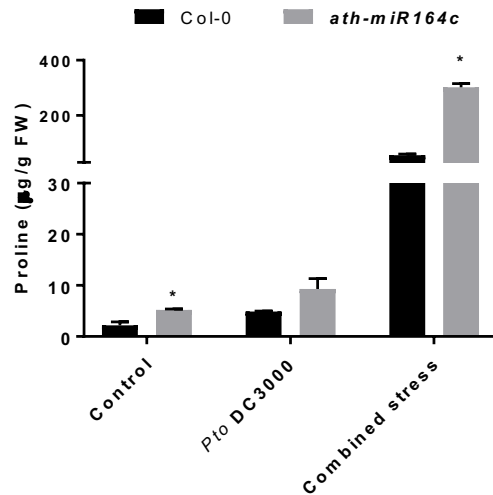
- *ath-miR164c* & *ath-miR397a* differentially regulate *AtP5CS1* gene expression and that could be the reason for differential stress response

Is there change in proline level in *ath-miR164c* mutant ??

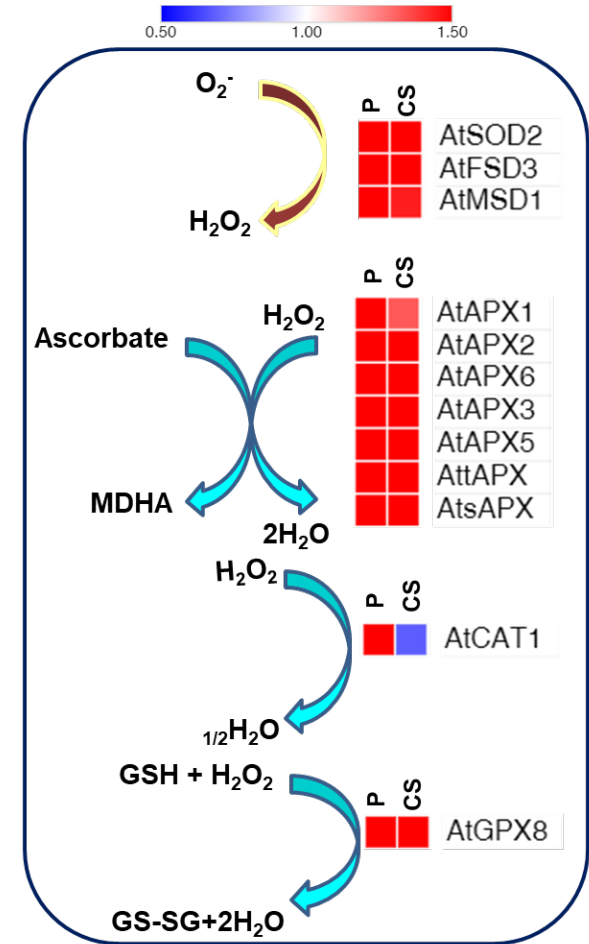
Cleavage assay- 5'RLM-RACE



Proline quantification

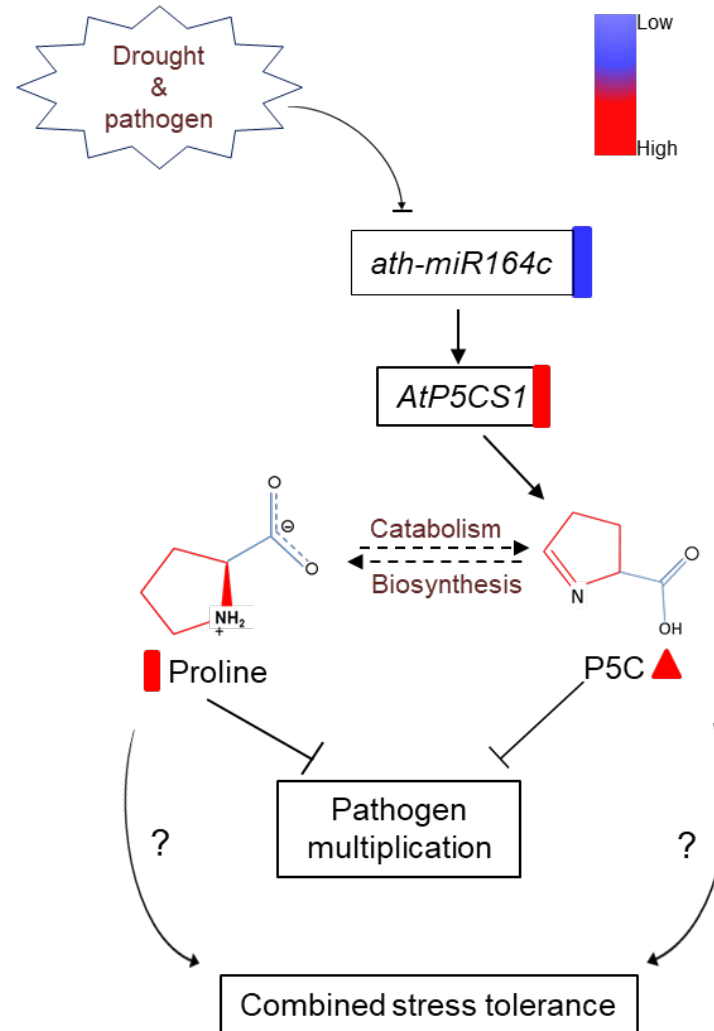


Expression analysis of mitochondrial antioxidant genes



➤ *ath-miR164c* showed increased proline accumulation and enhanced antioxidant genes expression under pathogen and combined stress which resulted in improved resistance

A hypothetical model depicting *ath-miR164c*-mediated *AtP5CS1* regulation in *A. thaliana* under combined stress





Outcome of the studies



- ❑ *ath-miR164c* acts as a negative regulator of *AtP5CS1* and indirectly regulates proline-P5C levels
- ❑ miRNA-mediated regulation of proline pathway act as convergent point for both biotic and abiotic stress and further, that can be exploited for the crop improvement.

Future prospective

- ❑ Proline pathway is known to be regulated by more than one miRNA, further studies are needed in this area for better understanding of proline pathway regulation under different combined stresses.



References



- **Gupta A, Patil M, Qamar A, Senthil-Kumar M. ath-miR164c influences plant responses to the combined stress of drought and bacterial infection by regulating proline metabolism. Environmental and Experimental Botany. 2020 Apr 1;172:103998.**
- **Khraiwesh B, Zhu JK, Zhu J. Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms. 2012 Feb 1;1819(2):137-48.**



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