Host miRNA response in leaves of PVY infected tobacco plants

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Outlines of the talk

1. Host miRNAs and their targets responsive to virus – a brief introduction

2. Analysis expression levels of the selected miRNAs in PVY infected tobacco

Small RNAs in plants

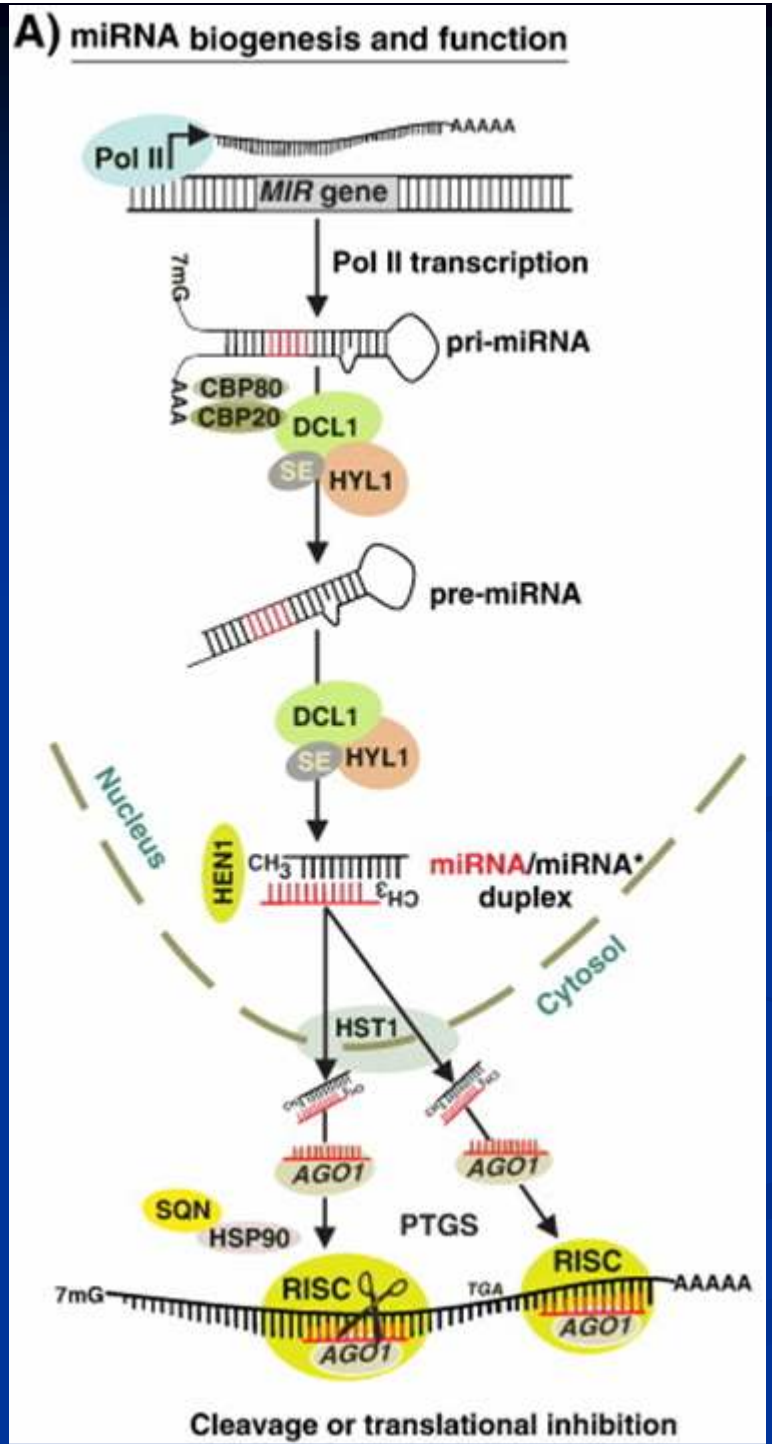
1. Small non-coding RNAs
   - 20-24 nt
   - Regulator of protein-coding gene expression
   - TGS (Transcriptional gene silencing)
   - PTGS (Posttranscriptional gene silencing)

2. In plants (based on biogenesis and function):
   - miRNAs (microRNA)
   - siRNAs (small interfering RNAs)

3. miRNAs (by PTGS)
   - Targeting specific mRNA for cleavage
   - Translational inhibition

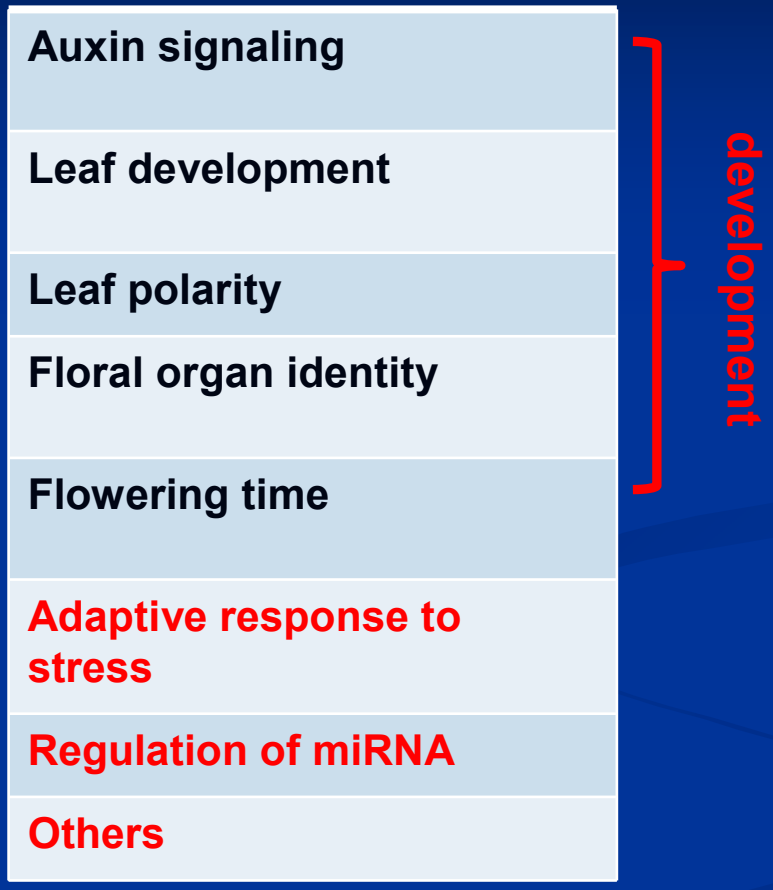
Carrington and Ambros 2003, Bartel 2004, Lanet et al. 2009, Voinnet 2009, Chen 2010, Khraiwesh et al. 2012

miRNA biogenesis in plants



Khraiwesh et al. 2012

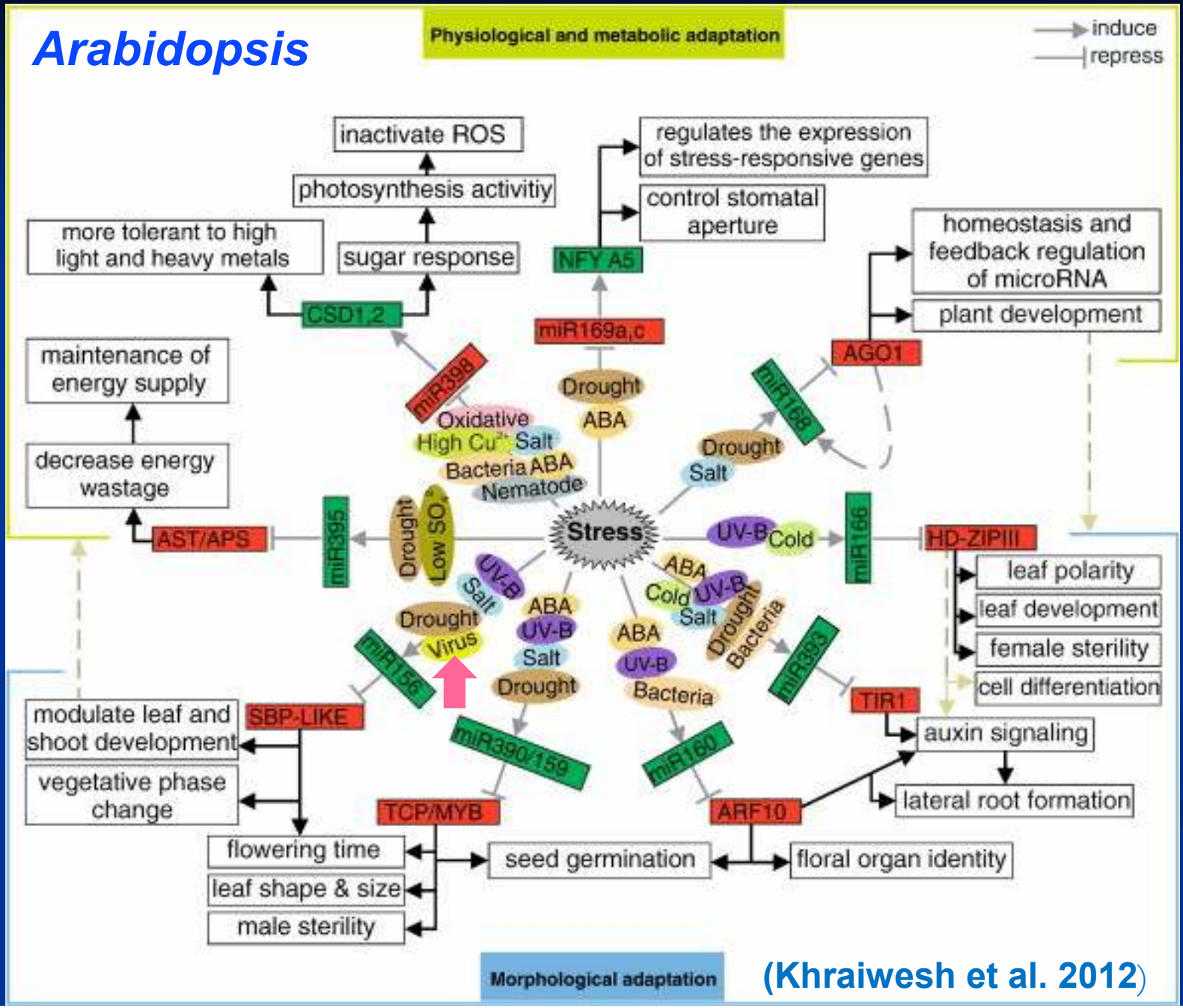
Function of conserved plant miRNAs



Others: e.g. nitrogen fixation (Katiyar-Agarwal and Jin 2010)

(Jones-Rhoades et al. 2006; Zhang et al. 2006; Shukla et al. 2008; Sunkar 2010, 2012; Khraiwesh et al. 2012)

Regulatory network of stress-responsive miRNAs



miRNAs in plant-virus interaction

Plant	Virus
Grapevine	*Grapevine vein clearing virus*
	Grapevine leafroll–associated virus
Tomato	*Cucumber mosaic virus*
	Tomato aspermy virus
	Tomato leaf curl New Delhi virus
Cotton	*Cotton leafroll dwarf polerovirus*
Rice	*Rice strip virus*
	Rice dwarf virus
	Southern rice black-streaked dwarf virus
	Papaya meleira virus
Carica papaya	*Hibiscus chlorotic ringspot virus*
Kenaf	*Tomato*
	Soybean mosaic virus
Soybean	*Begomovirus*
Nicotiana benthamiana	*Tobacco mosaic virus*, PVX, PVY
Tobacco	*Tomato mosaic virus*
	PVX, PVY, TMV,TEV, ToMV
Brassica rapa	*Turnip mosaic virus*
Arabidopsis	*Tobacco mosaic virus*

Summary from 40 publications 2007-2015

miRNAs in PVY infected plants

mRNAs/targets	PVY infection	
	N. tabacum	N. *benthamiana*
miR168	nd	↑
miR160	↓	nd
miR166	↑	nd
miR159	↑	nd
miR164	↓	nd
miR165	nc	nd
miR167	↑	nd
miR169	↓	nd
miR156	↓	↑
SPL9	nd	↑
miR171	↑	↑
miR171*	↑	nd
SCL6	nd	↑
miR398	nd	↑
CSD	nd	↑

Bazzini et al. 2007
Pacheco et al. 2012

CSD: Copper superoxide dismutase.
SCL: Scarecrow-like transcription factor.
SPL: Squamosa promoter binding protein-like transcription factor.

Aim of this work

To analysis expression levels of the selected miRNAs in tobacco plants infected by different PVY strains.

Materials and Methods

1. Plant material:
   Tobacco cv. Samsun (15 plants/treatment)
2. PVY isolates:
   PVY^{NTN}-B (PVY-3202)
   PVY^{NTN}-A (PVY-3303) (Biological PVY^{Z}-NTN)
   PVY^{N-Wi} (PVY-3411)
3. Treatment:
   Mock-inoculated (treated with water)
   PVY-inoculated (sap inoculation)
4. Sampling:
   upper non-inoculated leaves at
   3 and 14 days post-inoculation (dpi)
5. Real-time RT-qPCR quantification of miRNA (Xie et al. 2010)

Disease symptoms in PVY infected tobacco cv. Samsun

PVY-3202
NTN-A

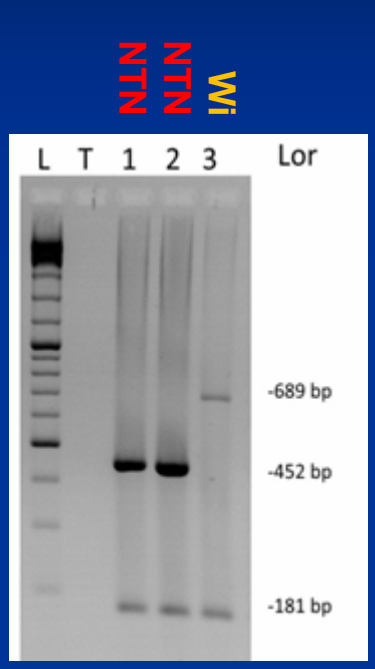
PVY-3411
Wi-B

PVY-3303
NTN-B (Z-NTN)

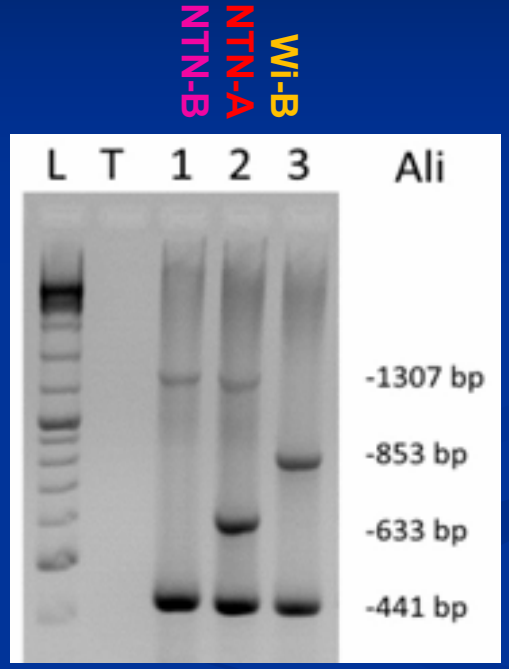
Healthy

Upper non-inoculated leaves, 14 dpi

Detection of PVY in PVY-3202, PVY-3303 and PVY-3411 infected tobacco at 14 dpi



PVY N:O (PVY^{N-Wi}) : 689 bp + 181 bp. (Lor)
PVY^{NTN} : 452 bp + 181 bp. (Lor)



PVYN-Wi (B): 853 bp + 441 bp (Ali)
PVY^{NTN} (A) : 1307bp + 633bp + 441bp (Ali)
PVY^{NTN} (B): 1307bp + 441bp (Ali)

Selected miRNAs tested in PVY infected tobacco

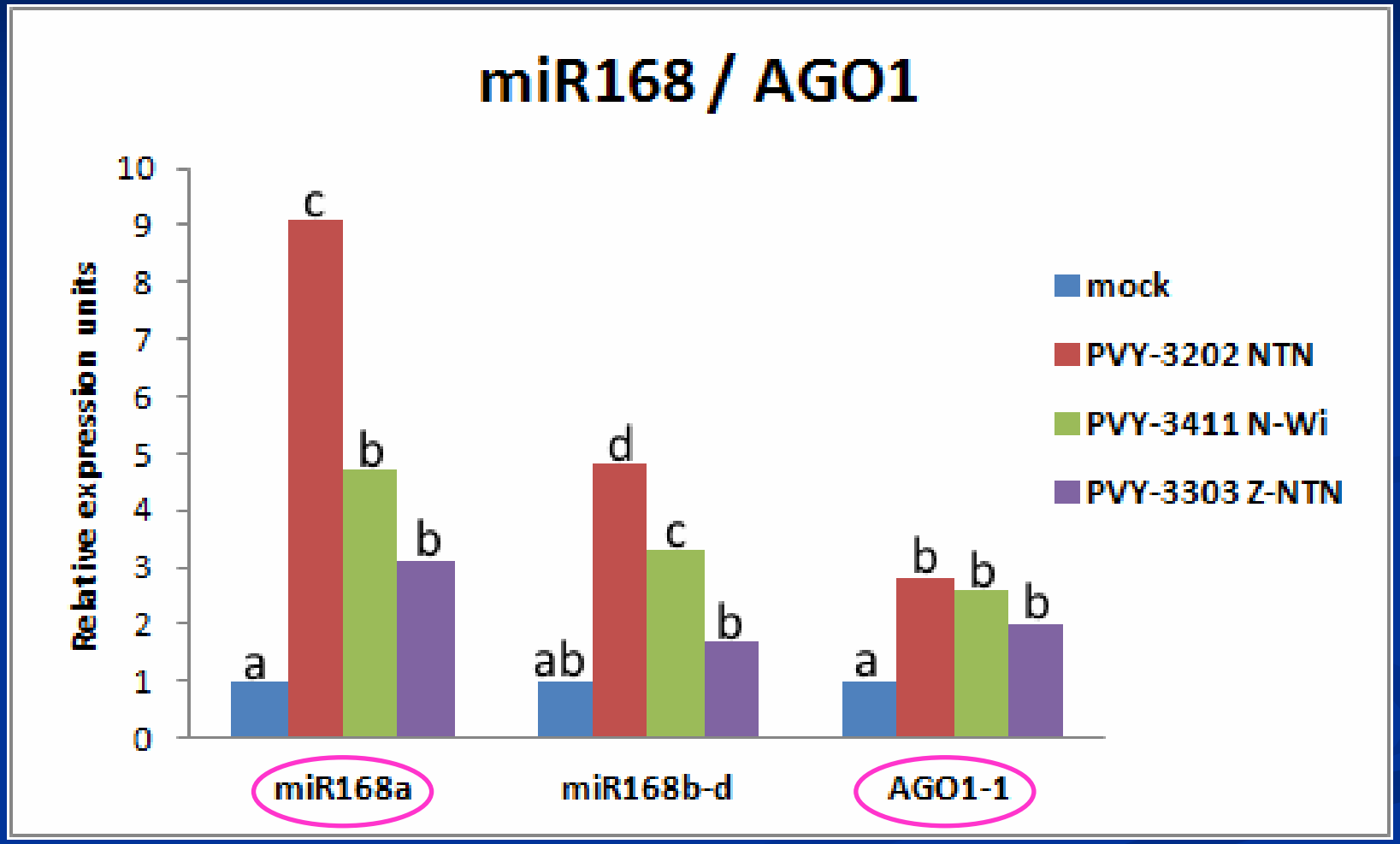
miRNA	Target genes	Target protein class	Function	Ref.
miR162	*DCL1*	RNAse III-like Dicer-like I endonuclease	miRNA biogenesis	This study
miR168	*AGO1-2*	Argonaute protein	miRNA function	This study
miR159	*Plastid*	Chloroplast protein	Chloroplast function	This study
miR172	*AP2*	Transcription factor	Development	This study
miR160	*ARF*	Auxin responsive factor	Auxin signaling	This study
miR164	SA induced protein 19	SA induced protein 19	Defense response	This study
miR166	*HD-ZIP III*	Transcription factor	Development	This study
miR390	*LRR*	*R* protein with leucine-rich repeat	Defense response	Bukhari et al. 2015
miR396	*GRF*	Growth regulation factor	Development	This study

miRNA levels in upper non-inoculated leaves PVY infected tobacco (14 dpi)

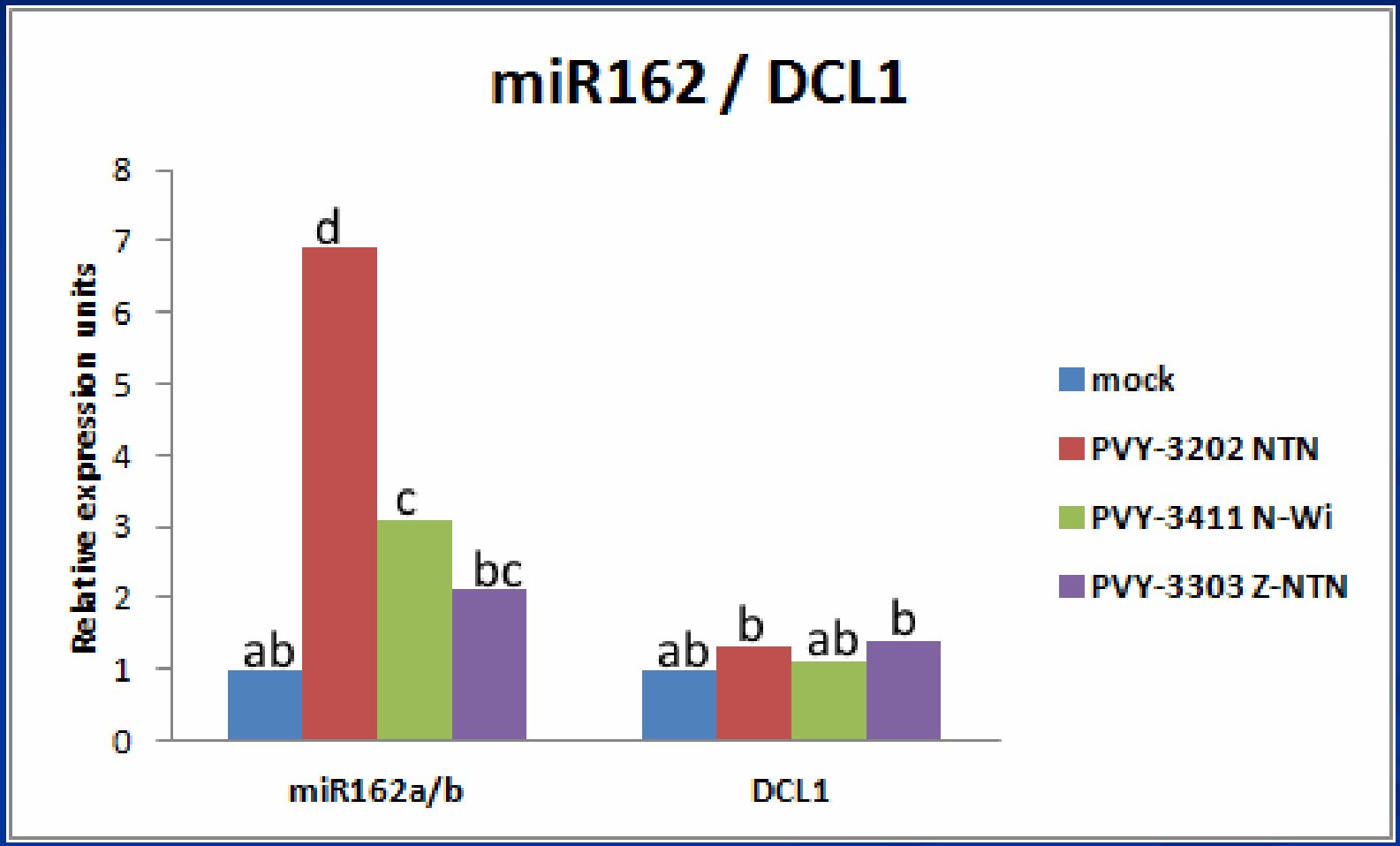
miRNA/target (upper leaves)	Mock	PVY-3202 (NTN-B)	PVY-3411 (Wi-B)	PVY-3303 NTN-A (Z-NTN)
nta-miR168a	1,0^{a}	9,1^{c} ↑↑	4,7^{b} ↑	3,1^{b} ↑
nta-miR168b-d	1,0^{ab}	4,8^{d} ↑↑	3,3^{c} ↑	1,7^{b}
AGO1-1	1,0^{a}	2,8^{b} ↑	2,6^{b} ↑	2,0^{b} ↑
nta-miR162a/b	1,0^{ab}	6,9^{d} ↑↑	3,1^{c} ↑	2,1^{bc}
DCL1	1,0^{ab}	1,3^{b}	1,1^{ab}	1,4^{b}
nta-miR172a-e/u/x/y	1,0^{ab}	3,8^{d} ↑↑	2,5^{c} ↑	1,7^{b}
nta-miR172f/w	1,0^{cde}	1,3^{e}	1,2^{de}	0,7^{bc}
nta-miR172h	1,0^{b}	1,8^{c} ↑	1,5^{c} ↑	0,6^{ab}
nta-miR172l	1,0^{bc}	1,5^{d} ↑	1,3^{bc}	0,8^{ab}
nta-miR166i	1,0^{abc}	1,2^{bc}	1,4^{c}	1,4^{c}
nta-miR166a-h	1,0^{c}	1,6^{e} ↑↑	1,3^{d} ↑	1,0^{c}
nta-miR390b	1,0^{a}	31,5^{c} ↑↑	11,7^{b} ↑	7,1^{ab}

No changes in the levels of miRNAs, i.e., nta-miR159a, nta-miR160a, nta-miR164a/c and nta-miR396b.

miRNA levels in upper non-inoculated leaves PVY infected tobacco (14 dpi)



miRNA levels in upper non-inoculated leaves PVY infected tobacco (14 dpi)



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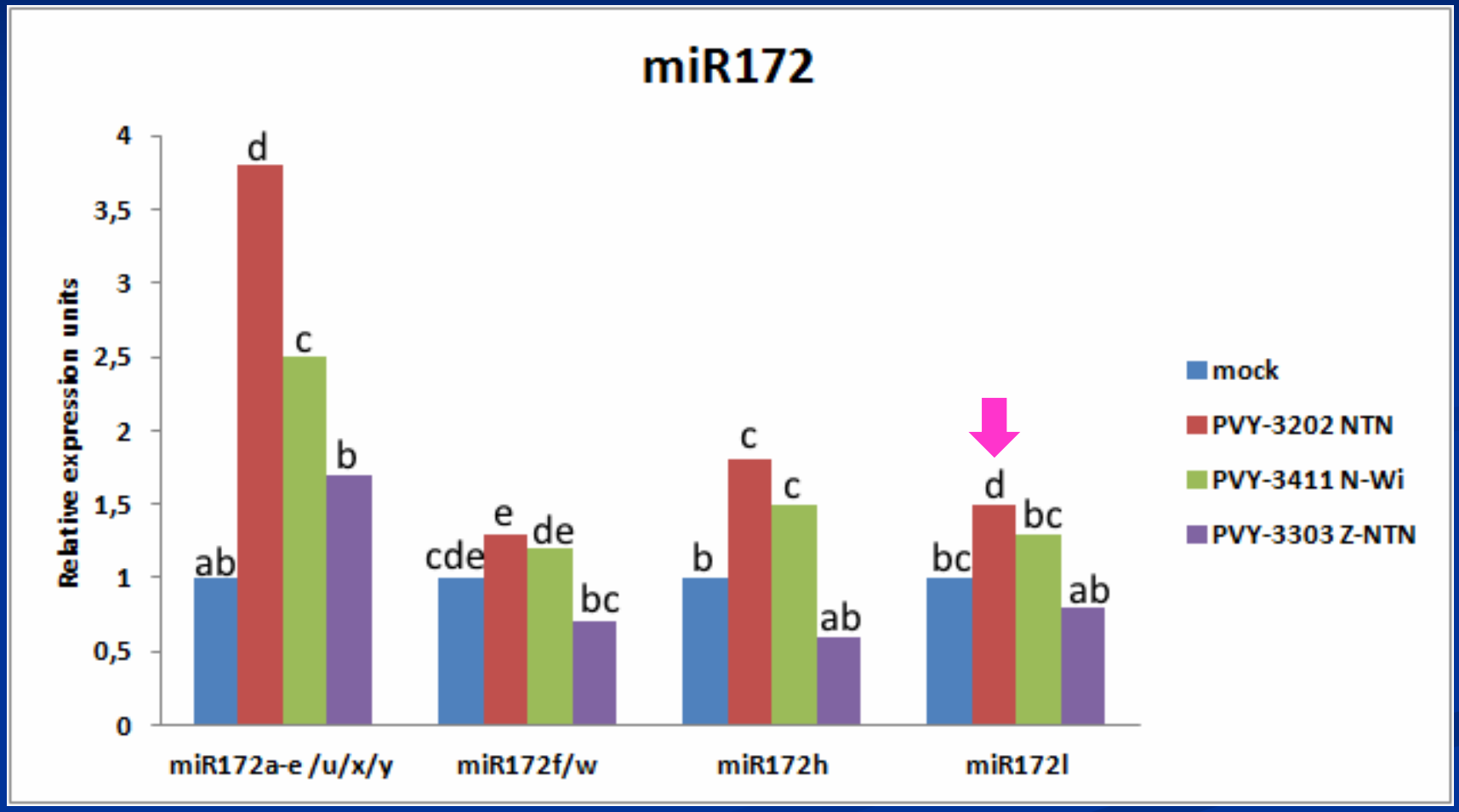

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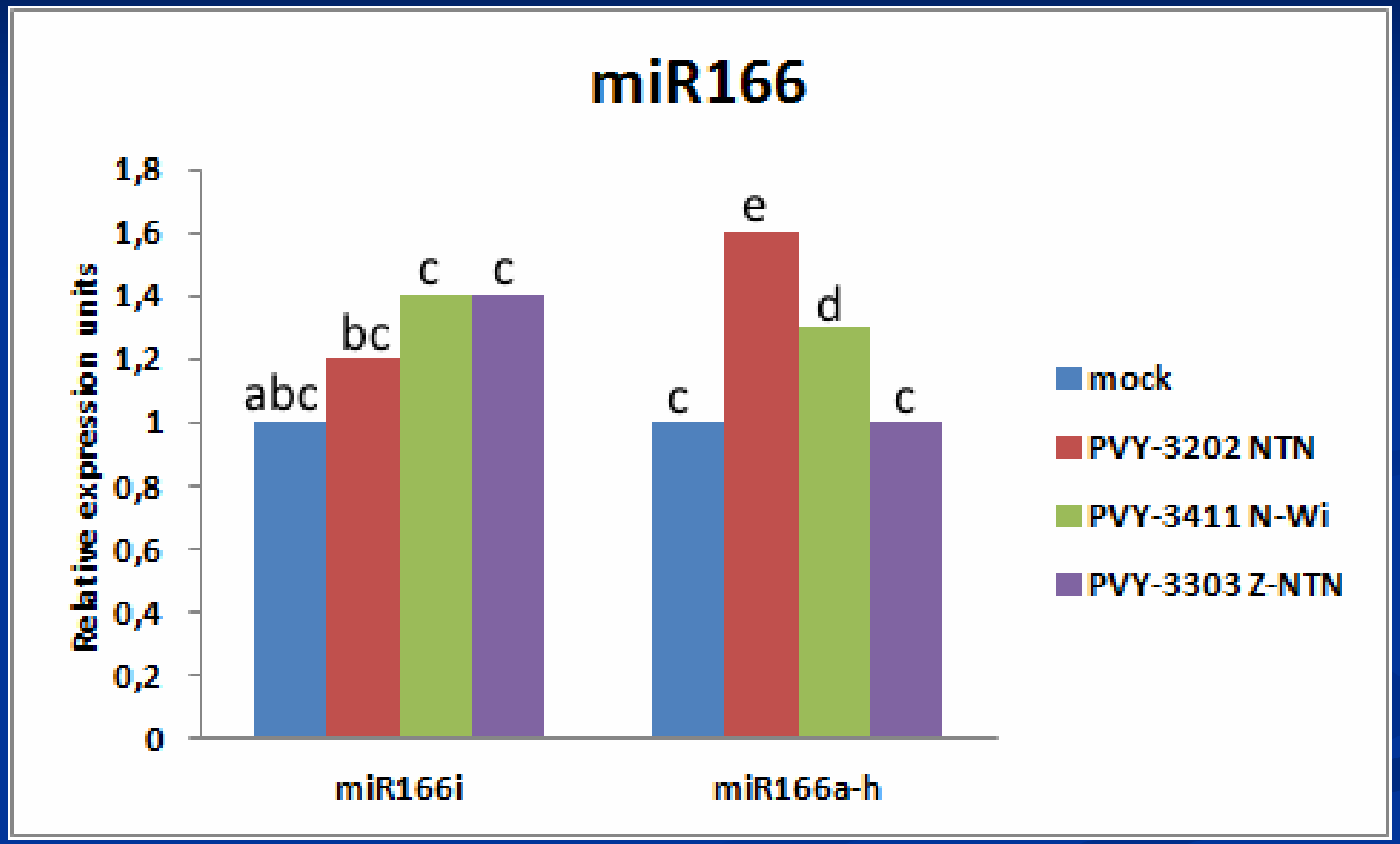

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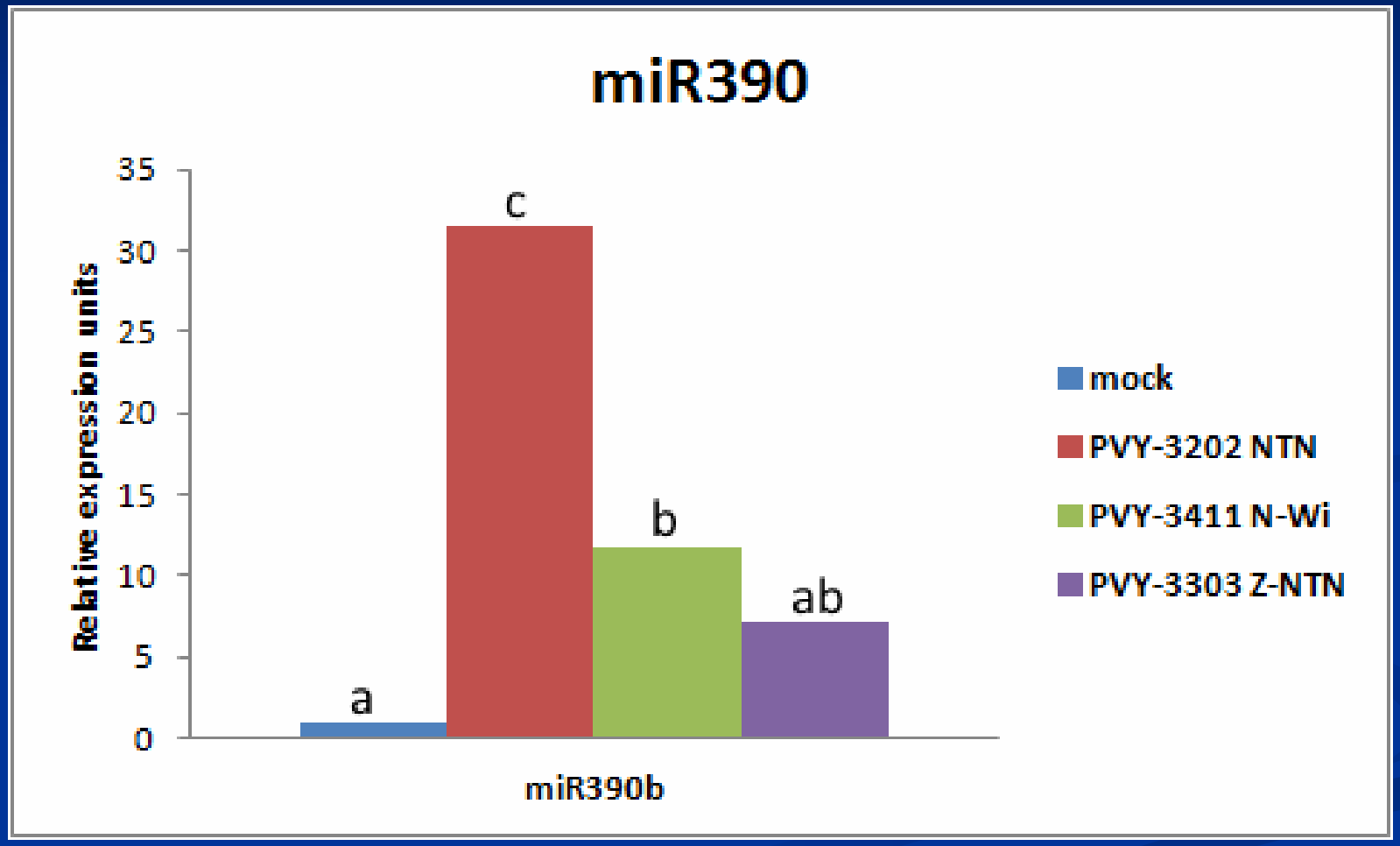

Conclusions

1. PVY infection caused up-regulation in 8 out 14 tested miRNAs.

   For miRNAs with multiple members, some members are responded to PVY.

Conclusions

2. **Parallel increase in miR168a and its target *AGO1-1* was observed irrespective of the PVY strains.**

Conclusions

3. Seven tested miRNAs showed strain-specific expression.

   Six of them (miR168b-d, miR162a/b, miR172a-e/u/x/y and h, miR166a-h, and miR390) were up-regulated by PVY^{NTN} and $PVY^{N\text{-}Wi}$ strains. PVY^{NTN} caused increase in the levels of the six miRNAs to a higher extent than did $PVY^{N\text{-}Wi}$.

   One of them (miR172l) was up-regulated only by PVY^{NTN}.

   However, PVY^{Z}-NTN strain did not cause any changes in the levels of the same set miRNAs.

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