Variation in miRNA expression between TRV-infected tobacco plants is correlated with symptom severity and *TRV-16K* transcripts abundance

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In the present work, expression of certain miRNAs and their targets were investigated in three tobacco plants infected with *Tobacco rattle virus* (TRV) isolate Deb57 collected from Northern Poland in 2008.

Materials and Methods

Nicotiana tabacum cv. Samsun plants in 3-4 leaf stage were mechanically inoculated with leaf sap from the TRV-infetced tobacco plants. Systemic leaf samples were take at 0-, 5-, 9-, and 16- days post inoculation (dpi). Tobacco miR168a, miR159a and miR172a, as predicted by Frazier et al. (2011), were detected and quantified by Applied Biosystems TaqMan microRNA assay according to the manufacturer's instructions from 2 µg of the total RNA. Eukaryotic 18S rRNA endogenous control (Applied Biosystems) was used as a reference gene to normalize miRNA expression value. Following, from the same RNA preparation, the transcript levels of *TRV 16K* gene, and the miRNA targeted tobacco genes *AGO1* and *MYB1*, were quantified using Power SYBR Green RNA-Ct 1-step kit (Applied Biosystems). The apple mitochondrial *nad5* gene encoding NADH dehydrogenase subunit 5 was used as a reference gene to normalize mRNA value (Menzel et al. 2002).

Results

TRV infection resulted in alteration of tobacco miRNAs and their targets, correlating with the disease symptom severity and transcripts accumulation of the TRV-encoded silencing suppressor 16-kDa *TRV 16K* gene (Table1, Figures 1-3). Inter-plant variability was observed. In plant 1, showing no symptoms at 5dpi, the most severe symptoms and a dramatic increase of *TRV-16K* transcripts at 16dpi were observed, along with a sharp decrease of miR168a and miR159a at 5dpi in comparison to the non-inoculated control (i.e. 0 dpi sample). At 9dpi and 16dpi, the levels of miR168a and miR159a gradually increased, however still below the non-inoculated control. In plant 2, severe symptoms and high expression of *TRV-16K* occurred at 5dpi and remained consistent up to 16dpi. The expression of miR168a and miR159a was down-regulated compared to the control. The plant 3 showed no symptoms at 5dpi, but the most severe symptoms and highest expression of *TRV-16K* transcript appeared at 9dpi. The levels of miR168a showed a sharp decrease at 5dpi and then increased again, while that of miR159a decreased gradually. In plant 1 and plant 2, the transcript levels of targets *AGO1* and *MYB1* were negatively regulated by the corresponding mi168a and miR159a, showing an elevated expression compared to the non-inoculated control. In plant 3, the expression levels of *AGO1* and *MYB1* were below that of the control. Expression of miR172a was decreased in all plants tested.

Figure 1. TRV symptoms on the potato tuber (cv. Elanda) (on the left) and tobacco plant (cv. Samsun nn).

Table 1. Disease symptom severity of tobacco plants inoculated with TRV isolate Deb57

Plant	0 dpi	5 dpi	9 dpi	16 dpi	21 dpi	28 dpi
1	-	-	+	++	+	++
2	-	++	++	+	+	++
3	-	-	+++	++	+	++

dpi: days post inoculation

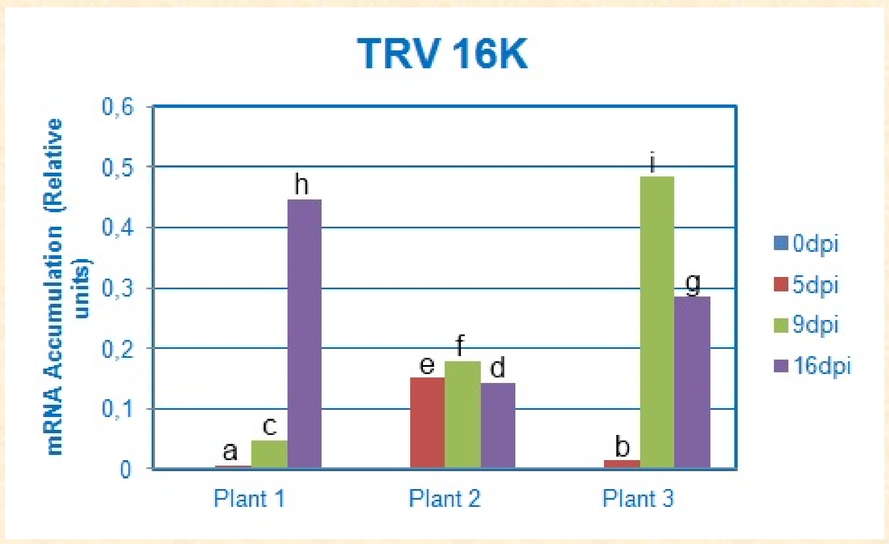
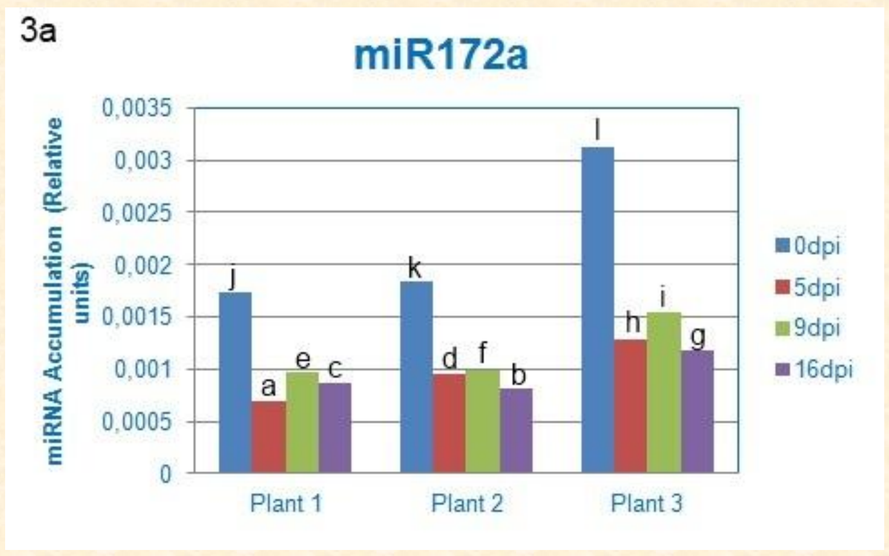
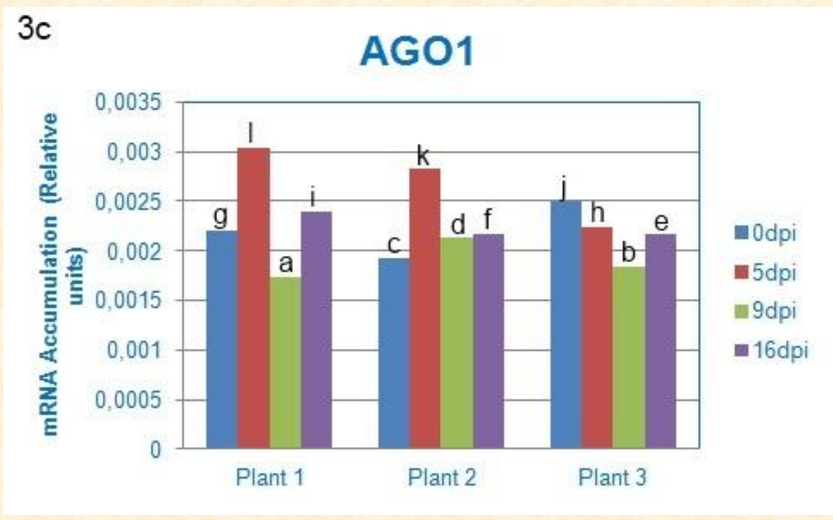
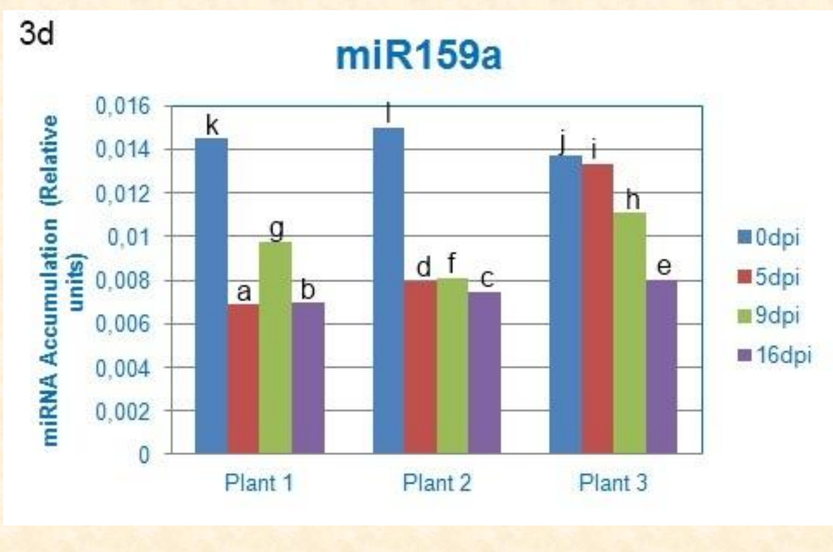


Figure 2. Relative systemic accumulation of the transcripts of TRV-encoded silencing suppressor 16-kDa gene *TRV 16K* at each time point. dpi: days post inoculation. The letters above the columns indicate statistical significant difference between samples.







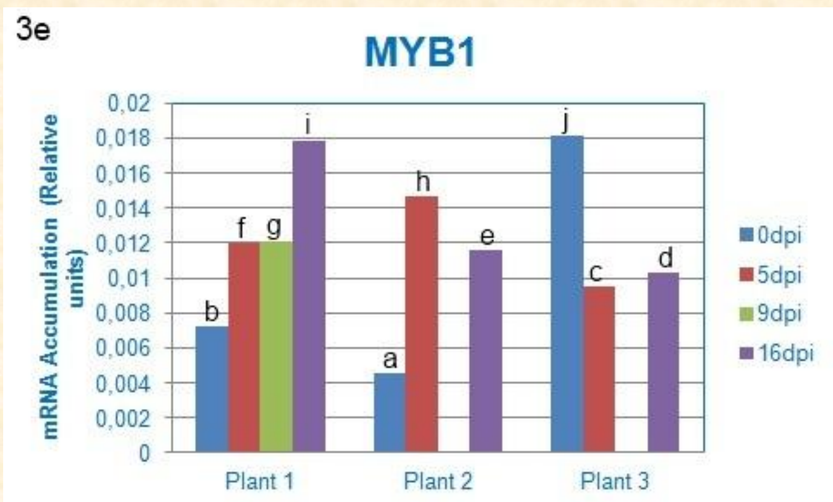


Figure 3. TRV infected tobacco plants miRNAs and mRNA targets accumulation levels at each time point by qPCR. dpi: days post inoculation. The letters above the columns indicate statistical significant difference between samples.

Conclusion

Inter-plant variability in miRNA and its target expression was observed in TRV infected tobacco plants. Such variation was correlated with the disease symptom severity and transcripts accumulation of the TRV-encoded silencing suppressor 16-kDa gene.

Acknowledgments

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References

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