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## Host miRNA response in leaves of PVY infected tobacco plants

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Plant microRNAs (miRNAs) are short (20-24 nt) non-coding RNAs that regulate gene expression by sequence-specific cleavage or translational repression of target transcripts (Bartel 2004). They play important roles in developmental processes and in plant response to abiotic and biotic stresses (Jones-Rhoades et al 2006, Chen 2010, Khraiweh et al 2012). Alteration of host miRNAs in virus-infected plants has been reviewed (Yin et al. 2014<sup>1</sup>).

In this work, the expression levels of a set of miRNAs in PVY-infected tobacco were studied by stem-loop real-time RT-qPCR. Two recombinant PVY<sup>NTN</sup> isolates were used: 12-94 (AJ889866) causing veinal necrosis (VN) in tobacco, and Gr99 (AJ890343) which contains R-400 and D-419 in the HC-Pro cistron unable to induce VN in tobacco. For each isolate, five plants (4-5 leaf stage) of *Nicotiana tabacum* cv. Samsun were mechanically inoculated with the infectious leaf sap. Five mock-inoculated plants were used as controls. Samples from the upper non-inoculated leaves were taken at 3- and 14-days post inoculation (dpi) and stored at -80 °C for RNA extraction. All the plants were kept in a growth chamber under controlled conditions (22 °C, photoperiod 16 h light). Real-time qPCR was performed in LightCycler480 instrument with SYBR method. The relative expression value for each miRNA was normalized to the best reference gene selected based on analysis of 13 tobacco candidate genes.

Of the fourteen tested miRNAs, no changes in their expression levels were observed at 3 dpi. At 14 dpi, nta-miR168a together with its target transcript *AGO1-1*, were highly induced by PVY irrespective of isolates. The levels of six miRNAs were not altered in plants inoculated by 12-94 and Gr99. However, the expression levels of the remaining seven miRNAs were increased 1.5- to 31.5-fold only in the plants infected with 12-94 showing VN. The levels of the same set of miRNAs remained unchanged in Gr99 infected plants which did not exhibit VN. Our results indicated that these seven miRNAs might specifically relate to the VN in tobacco infected with PVY<sup>NTN</sup> isolate 12-94.

<sup>1</sup>Yin Z, Chrzanowska M, Michalak K, Zimnoch-Guzowska E (2014) Alteration of host-encoded miRNAs in virus infected plants - experimentally verified. In: Guar RK, Hohn T, Sharma P, eds. *Plant Virus-Host Interaction*. Elsevier, Chapter 2, pp17-55.