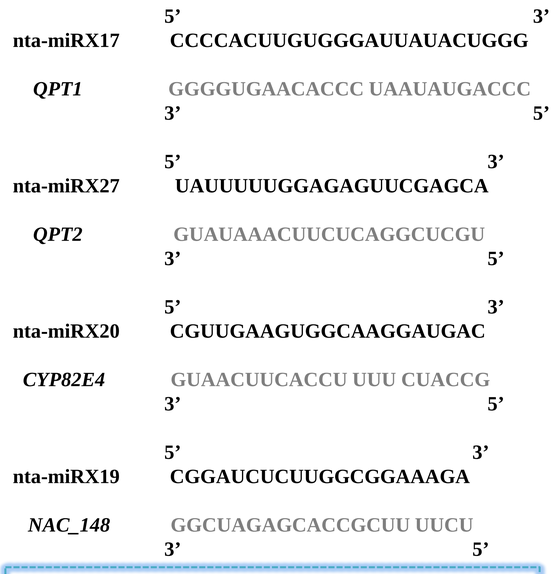
Regulation of Nicotine Biosynthesis by an Endogenous Target Mimicry of miRNA in Tobacco

Fangfang Li[1], Weidi Wang[1], Nan Zhao, Bingguang Xiao, Peijian Cao, Xingfu Wu, Chuyu Ye, Enhui Shen, Jie Qiu, Qian-Hao Zhu, Jiahua Xie, Xueping Zhou[*], and Longjiang Fan[*]

Plant Physiology, 2015

The interaction between non-coding endogenous target mimicry (eTM) and its corresponding miRNA is a newly discovered regulatorymechanism and plays pivotal roles in various biological processes in plants. Tobacco (*Nicotiana tabacum*) is a model plant for studying secondary metabolite alkaloids, of which nicotine accounts for ~90%. In this work, we identified four novel tobacco-specific miRNAs that were predicted to target key genes of the nicotine biosynthesis and catabolism pathways, and an eTM, nta-eTMX27, for nta-miRX27 that targets *QPT2* encoding a quinolinate phosphoribosyltransferase. Our results demonstrated that enhanced nicotine biosynthesis in the topping treated tobacco plants is achieved by nta-eTMX27-mediated inhibition of the expression and functions of nta-miRX27. To our knowledge, this is the first report about regulation of secondary metabolite biosynthesis by a miRNA-eTM regulatory module in plants.

Identification of Nicotine Biosynthesis Related miRNAs in *N. tabacum*



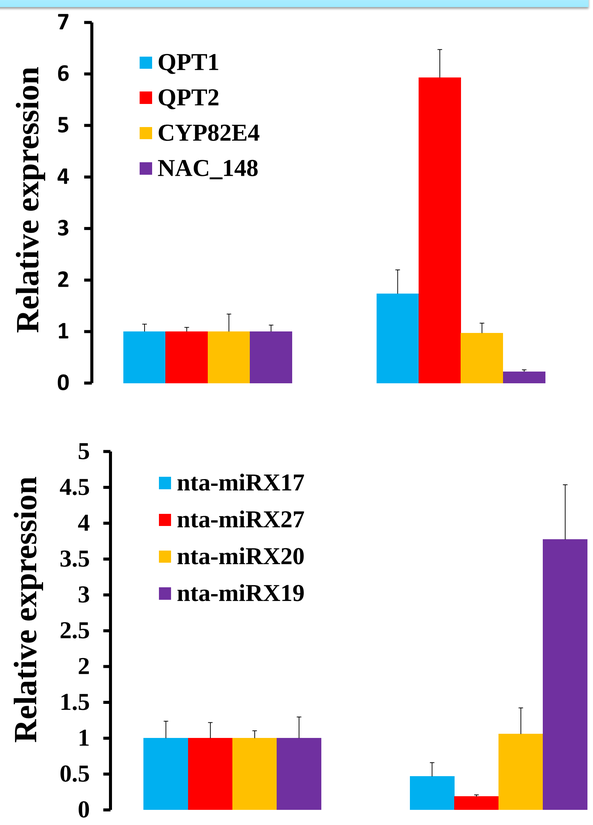


Figure 1. Genes involved in nicotine biosynthesis in tobacco and expression changes of miRNAs and their nicotine-related targets at 48 hours (h) after topping.

Identification and Expression Analysis of eTMs for nta-miRX27

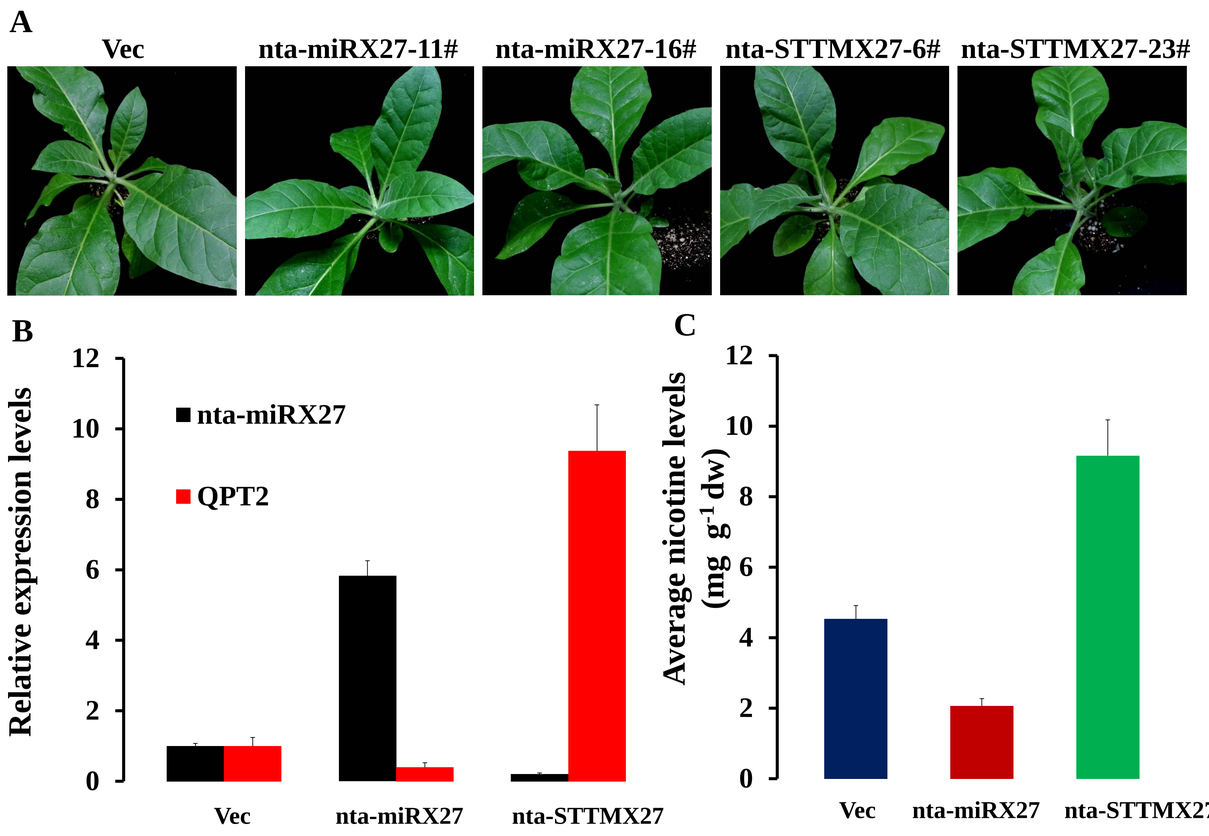
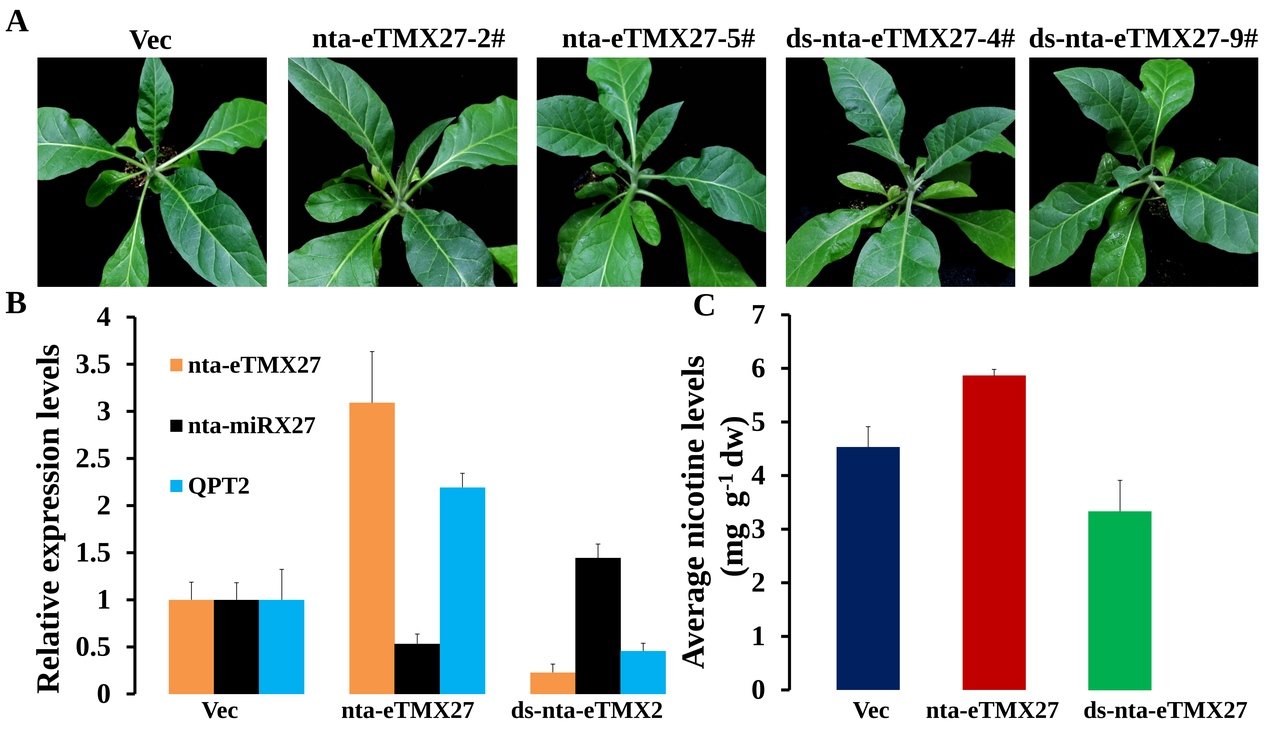


Figure 2. Nta-miRX27 repressed QPT2 expressions and reduced nicotine contents. A, Phenotypes of 30-day-old transgenic plants containing nta-miRX27 or nta-STTMX27 compared with that of plants transformed with an empty vector (Vec). B, Relative expression levels of nta-miRX27 and its target gene QPT2 in ntamiRX27 and nta-STTMX27 transgenic plants compared with that of the Vec plants. C, Average nicotine contents in leaves sampled from the Vec, nta-miRX27 and nta-STTMX27 plants.

Nta-eTMX27 Induced miRNA Degradation and Repressed miRNA Function



Evolution of nta-miRX27 and nta-eTMX27

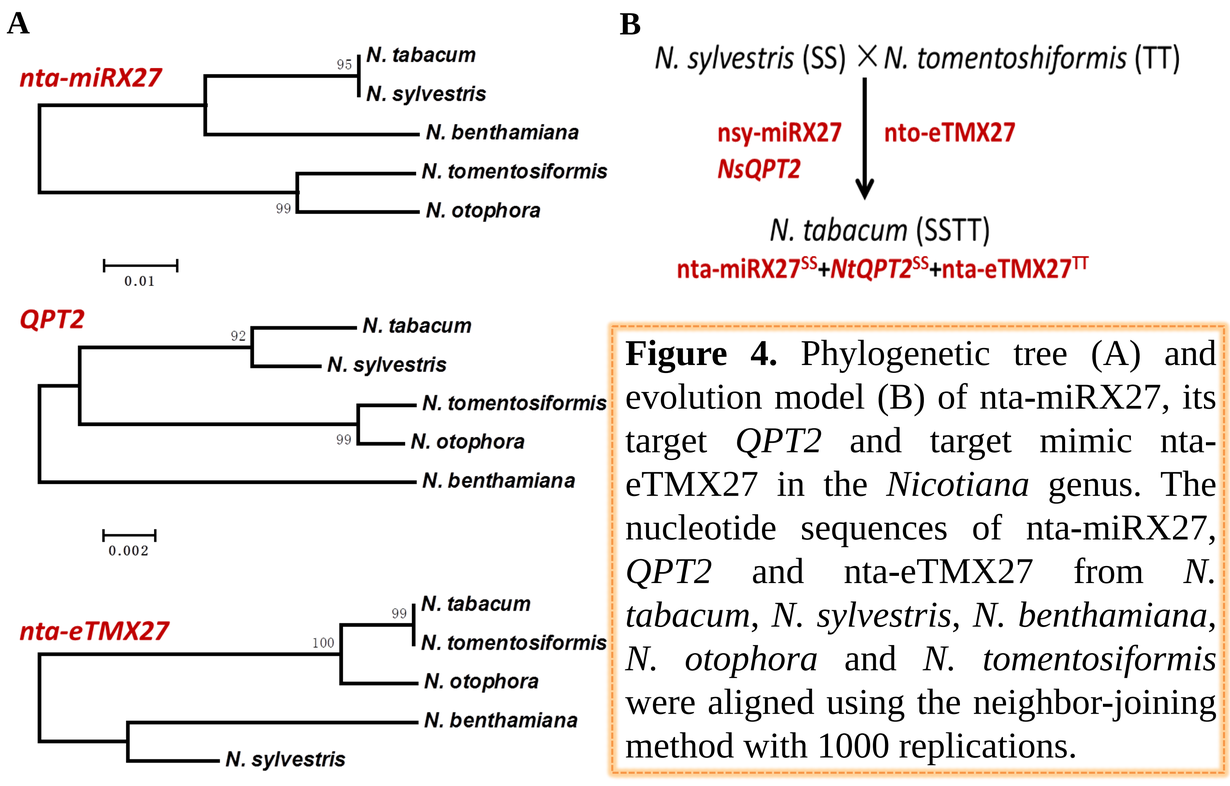


Figure 4. Phylogenetic tree (A) and evolution model (B) of nta-miRX27, its target *QPT2* and target mimic nta-eTMX27 in the *Nicotiana* genus. The nucleotide sequences of nta-miRX27, *QPT2* and nta-eTMX27 from *N. tabacum*, *N. sylvestris*, *N. benthamiana*, *N. otophora* and *N. tomentosiformis* were aligned using the neighbor-joining method with 1000 replications.

Figure 3. Functional analyses of nta-eTMX27. A, Phenotypes of nta-eTMX27 overexpression and silencing plants. B, Relative expression levels of nta-eTMX27, nta-miRX27 and QPT2 in nta-eTMX27 overexpressing and silencing transgenic plants compared with that of the Vec plants. C, Average leaf nicotine contents of the Vec, nta-miRX27 and nta-STTMX27 lines. D, Expression levels of nta-eTMX27 in roots of transgenic plants transformed with the Vector (Vec) or dsnta-eTMX27 construct in response to topping. E, Relative expression levels of nta-eTMX27 in roots of transgenic plants transformed with the Vec or ds-nta-eTMX27 after topping treatment at 48 h. F, Relative expression levels of QPT2 in the Vec and ds-nta-eTMX27 transgenic plants after topping treatment at 48 h.