

**Molecular Regulation of Host Defense Responses Mediated by Biological Anti-TMV Agent  $\epsilon$ -poly-L-lysine****Научный руководитель – Liu He***he liu**PhD*

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Microbes and microbial metabolites induced resistance is known to protect plants from several invaders, including fungus, bacteria and viruses etc. [1]. Our laboratory has isolated and purified  $\epsilon$ -poly-L-lysine ( $\epsilon$ -PL) from *Streptomyces microflavus* var. *liaoningensis* (unpublished results) with independent intellectual property rights (acceptance number 201910330737.2), and has reported its antifungal effect and its action mechanism[2]. The latest research in our laboratory also found that  $\epsilon$ -PL has a good potential for anti plant viral diseases[3], but the antiviral mechanism was not to be further explored. In this study, the results showed that  $\epsilon$ -PL can effectively inhibit the infection of tobacco mosaic virus (TMV) in *Nicotiana glutinosa* and *N. tabacum* cv. NC89, and can effectively delay TMV in *N. benthamiana*. The inactivation effect and preventive effect on TMV was 90.6% and 79.3% when the *N. glutinosa* was treated with 500 $\mu$ g/ml  $\epsilon$ -PL (Fig. 1). Fluorescence detection of *N. benthamiana* seedlings inoculated with GFP-TMV showed that the fluorescent spots in the inoculated leaves, upper leaves or parietal leaves were significantly reduced compared with the control group. The top leaves of *N. benthamiana* treated with 100  $\mu$ g/ml  $\epsilon$ -PL showed no fluorescent spots, while the green fluorescent areas of the control leaves were obvious. RNA accumulation of TMV-infected tobacco leaves and tobacco protoplasts treated with different concentrations of  $\epsilon$ -PL were analyzed by Northern blot. The results showed that the TMV-RNA accumulation in the sample treated by  $\epsilon$ -PL was significantly reduced, indicating that  $\epsilon$ -PL affects the synthesis and accumulation of viral nucleic acid. Observation by transmission electron microscopy revealed that  $\epsilon$ -PL can induce cleavage of virions (Fig. 2). Systemic infection and significant inhibition of virus accumulation in tobacco BY-2 protoplasts. The results of RNA-seq showed that approximately 620 and 900 differently expressed genes (DEG) were significantly up-regulated and down-regulated after treatment of BY-2 protoplasts with 5 $\mu$ g/ml  $\epsilon$ -PL (Fig. 3). In addition, KEGG analysis indicated that various DEGs are involved in endoplasmic reticulum (ER) protein processing, suggesting a possible correlation between ER homeostasis and viral resistance. RT-qPCR was performed to verify gene expression of key DEGs related to defense, stress response, signal transduction and plant hormones, which is consistent with the results of RNA-seq. The existing reports mainly focused on  $\epsilon$ -PL application in food preservatives, but its mechanism of action as a biocontrol agent against plant diseases has not been elucidated[4]. Our work showed for the first time that  $\epsilon$ -PL can be used as a microbial pesticide against plant virus diseases and provides valuable insights into the antiviral mechanisms of  $\epsilon$ -PL.

**References**

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- 3) Hu, G. Dong, Y. Zhang, J. Fan, X. Ren, F. Progress in plant viroid elimination techniques // Zhiwu Baohu Xuebao (Journal of Plant Protection). 2017, 44(2). p.177-184
- 4) Liu, H. Chen, J. Xia, Z. An, M. Wu, Y. Effects of  $\epsilon$ -poly-L-lysine on vegetative growth, pathogenicity and gene expression of *Alternaria alternata* infecting *Nicotiana tabacum* // Pestic Biochem Physiol. 2020. 163. p.147-153.

### Illustrations



Рис. 1. Anti-TMV effect on *N. glutinosa* treated with 500  $\mu\text{g}/\text{ml}$   $\epsilon$ -PL

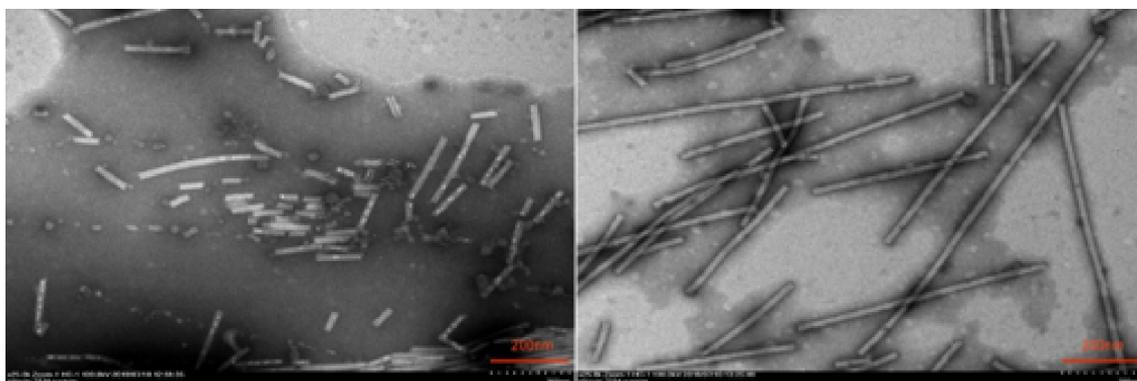


Рис. 2. Morphological changes of TMV virions under  $\epsilon$ -PL treatment. The left figure was treated with 5  $\mu\text{g}/\text{ml}$   $\epsilon$ -PL, the right one was treated with PBS.

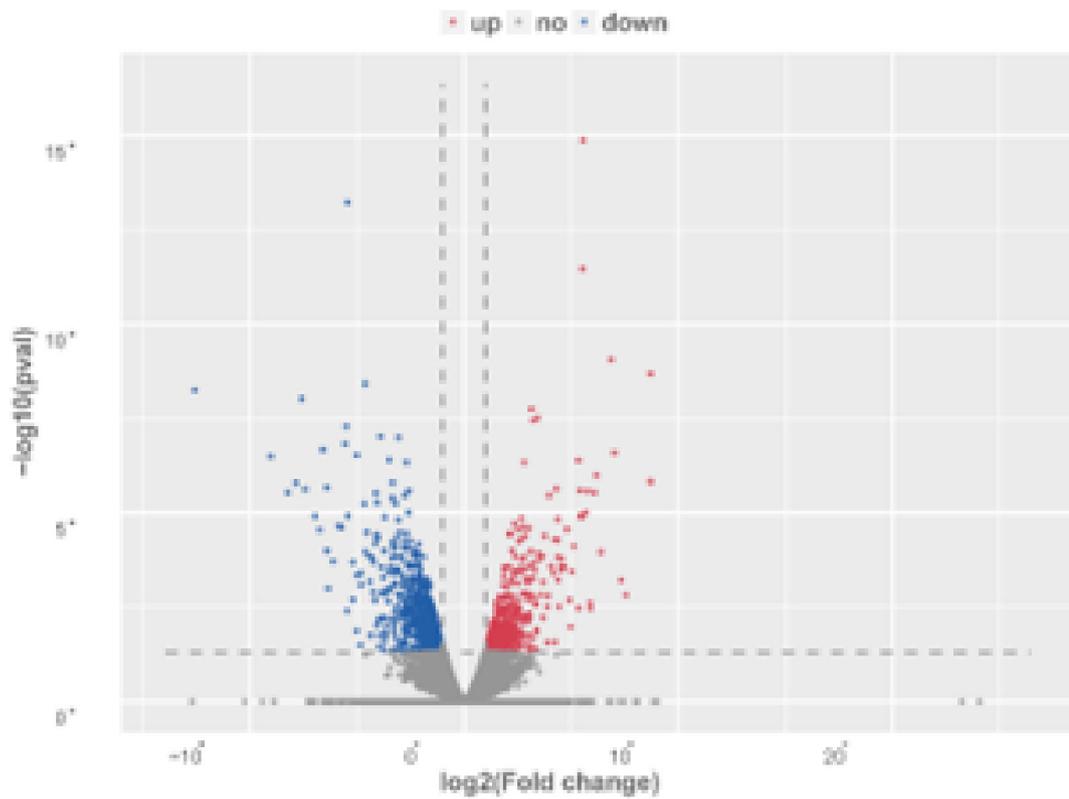


Рис. 3. Analysis of volcano maps of differential gene expression levels treated with  $5\mu\text{g} / \text{ml}$   $\epsilon$ -PL.