DOI 10.18699/SBB-2022-010

Isolation of high quality RNA from *Phytophthora infestans* for transcriptome analysis

Ivanov A.[1, 2]*, Golubeva T.[1, 2]

[1] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

[2] *Novosibirsk State University, Novosibirsk, Russia*

* *a.ivanov2@g.nsu.ru*

Key words: late blight, plant pathogen, transcriptome analysis, RNA isolation

Motivation and Aim: *Phytophthora infestans* is one of the main pests of agricultural plants of the *Solanaceae* family, especially potatoes: the cost of combating against pathogen can be up to 30 % of the product's final price. Studies of its transcriptome, especially transcriptome changes during the colonization of the host plant, can be very useful for the development of new highly selective and eco-friendly drugs for pest control. For such studies, it is necessary to develop methods for isolating high-quality RNA from *P. infestans* samples, which is significantly complicated by the presence of a rigid cell wall and aggressive RNAses. The aim of our work was to study the effect of various parameters on the quality of total RNA isolated from samples of *P. infestans*.
Methods and Algorithms: To achieve this goal, we identified several key parameters that affect the integrity of total RNA during isolation: this is the process of sample preparation and grinding, as well as the use of RNA stabilizing solutions. The Plant MiniKit and Qiazol (Qiagen) were used to purify the RNA fraction. To optimize the protocol, we varied the above parameters and examined the obtained RNA using an RNA analyzer, the quality of the RNA was assessed based on the RNA integrity number (RIN).
Results: RNA isolation should be carried out immediately after collection of *P. infestans* samples. Grinding *P. infestans* is best done with Tissue Lyser. However, the original Qiagen lysis buffers are insufficient to prevent RNA degradation during the homogenization. Best results were achieved with Qiazol added to the samples prior to the homogenization. The samples were then proceeded according to Qiazol protocol. Qiagen spin columns were used for RNA isolation after phase separation step. Tissue Lyser grinding time is critical: RIN was decreased with longer grinding.
Conclusion: We have developed a protocol that allows isolate the total RNA from *P. infestans* with a sufficiently high quality, which is suitable for RNA-seq analysis (RIN $\geq$ 7).
Acknowledgements: The work was supported by a grant from the President of the Russian Federation for state support of young Russian scientists (MK-4311.2022.5).