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The development of express method for detection viral diseases in plants

Abstract: Nowadays there are numerous methods in identifying viral infections. Many of them require the use of radioactive isotopes and application raised against virus derived proteins. In this research we aimed to develop new inexpensive methods of quick identification of viral infections.

Keywords: express method, P19, viral diseases, RNA silencing, Tomato bushy stunt virus

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Introduction. Viruses are the most widespread agents of economical damage in farming agriculture. Although the number of methods to protect the plants from pathogens has been implemented, the virus infections are still damaging agriculture by decreasing its productivity throughout the world [1]. The infection of plants by viruses starts from spreading through the cellular pathways, which continuously damages cells and tissue. Consequently, it leads to development of viral symptoms such as significant growth inhibition, fading of leaves, visible damage of tissues and apical necrosis [2]. Therefore, disease detection is one of the valuable ways to reduce the system damage in plants [3]. However, the detection of viruses requires well-equipped laboratory that is not always economically affordable. Thus, it is suggested that the development of low-cost methods in detection of virus infections may play an important role in earlier detection. In this work the research object is a close relative of tobacco *Nicotiana benthamiana* and the type species of the tombusvirus family Tomato bushy stunt virus (TBSV). They are well-confirmed exemplary for the research of plant viruses in which TBSV can affect systemic infection [4]

Materials and Methods *Plant conditions*

N. benthamiana plants were grown under dark condition for 8 h at 22 ^{0}C and under lighting for 16 h at 25 ^{0}C with 70-75% relative humidity during 35-40 days. Temperatures varied from 21 to 28 ^{0}C. The spectrum of lamps which were used for illumination of growth room was 2700 K and 6400 K [5].

Transformation. For transformation DNA plasmid pUC-19 with inserted virus genome and the competent cells of *Escherichia coli* (*E.coli*) JL-109 strain were used. Transformation process was conducted by the heat shock method. Firstly, the combination of chemically competent bacteria and DNA was incubated in ice for short time and then the blend was located at 42 ^{0}C for 3 min (heat shock) and placed back in ice. Super Optimal broth (SOB) medium was added in order to promote effectiveness transformation of plasmids. Secondly, the transformed cells were incubated at 36-37 ^{0} C for 30 min with shaking. *E.coli* JL-109 strains were grown overnight at 28 ^{0} C in Lauria Bertani (LB) broth supplemented with 50 μg/ml ampicillin. Bacterial cultures were then centrifuged at 4000 rpm/min for 5 min and supernatant was discarded. The cells were plated in solid medium in Petri dish. They were incubated for 24 h at 37 ^{0} C [6].

*Extractions and purification of plasmids.*The competent cells were resuspended in 250-350 μl of the resuspension solution and then transferred in a microcentrifuge , added 250 μl lysis solution. It was mixed 4-6 times. After that 350 μl neutralization solution was added. The solution was centrifuged for 5 min at 10 000 rpm/min. The next step was the purification of plasmids through transportation the supernatant to the supplied Gene Jet spin column. It was pelleted during 1 min at 10 000 rpm and then discarded the flow-through and peace the column back. It was followed by adding the washing solution and centrifuging for 30-60 sec. Then this step was repeated twice. After transferring the spin column into a fresh 1.5 ml tube 50 μl of the elution buffer was added and centrifuged. Then column was discarded and the purified plasmid was stored at -20 ^{0} C [7].

In vitro transcription. Before in vitro transcription the restriction Sma1 enzyme digest linearized at the 3'-end of the viral cDNA sequence of inserts which was included in plasmids. After that, transcripts were synthesized via T7 RNA polymerase. These transcripts were used for inoculation of the plants [8].
Plant inoculation
N. benthamiana was inoculated by wild type TBSV and Δp19 TBSV (mutant with changes p19 genes). 9 For plants inoculation in vitro generated transcripts of full-length TBSV cDNAs were used. Control plants were mock-inoculated by using phosphate buffer without viral RNA. Healthy and infected plants were grown separately in the same conditions [9].
Detection of viral infection in inoculated plants. The leaves of *N. benthamiana* were analyzed for the presence of TBSV virions. There were three samples from three different plants. Control plants, samples from wild type TBSV and inoculated by its mutant type without P19 were severally homogenized in TRIS/EDTA (TE) buffer in ratio 1/2 (sample/buffer) on ice. The next step was centrifuging all samples at 10 000 rpm ($4\,^0$C) for 20 min, and then 15 μl of each sample were mixed with 6 $\times$ Loading buffer. Separation of macromolecules was in 1% agarose gel with ethidium bromide for 45 min with Tris/Borate/EDTA (1 $\times$ TBE) buffer. UV light used to detect viral particles in agarose gel. It is followed by capillary transfer onto nitrocellulose membrane with TBSV virion-specific polyclonal antibodies as the evidence there were TBSV virions. Membrane blocking and determination of phosphatase activity after incubation with antibodies, were performed like it was described for Nothern blotting [5].

Results and discussion

Detection of viral infection in inoculated plants
N. benthamiana plants infected with TBSV wild type and its mutant type without P19 (Δ P19 TBSV) transcripts developed typical severe symptoms at 1 week post inoculation (Fig. 1 B, C).Visual investigation of infected plants disclosed morphological signs of viral infection: significant retardation in growth, visible damage of tissues, withering of leaves and apical necrosis [5]. Usually the plants which was infected by TBSV grew weaker of at 2 weeks post inoculation than plants was infected by Δ19 TBSV transcripts.

Figure 1 – The effect of TBSV infection on symptoms development in *N. Benthamiana*. (A)Healthy plant, (B) inoculated with Wt TBSV plant at 1 week postinoculation, (C) inoculated with Δ19 TBSV at 1 week postinoculation

Moreover, plants were tested for the presence of viral particles. For this aim, samples extracted from inoculated and healthy plants were subjected to electrophoresis in 1% agarose gel with subsequent staining with ethidium bromide. The presence of virus particles in agarose gel was visualized by UV light. TBSV virions were readily detectable as a major band in samples extracted from virus infected leaves. On the contrary, samples extracted from healthy plants did not contain such particles (Fig. 2).
To verify that separated bands represent TBSV virions, a capillary transfer onto nitrocellulose membrane was performed followed by immuno-staining with TBSV virion-specific polyclonal antibodies. Analysis with antibodies, revealed a presence of the viral protein in upper leaves, indicating systemic infection (Fig. 3).

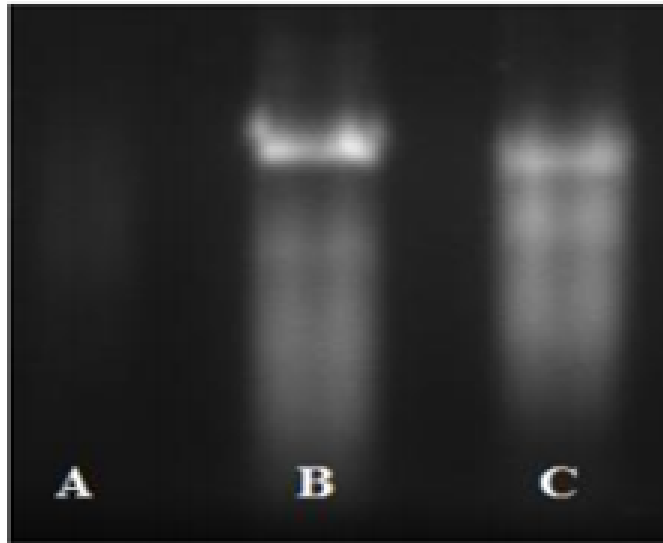

Figure 2 – Detection of viral particles in *N. benthamiana* (A) Healthy plant, (B) infected with TBSV, (C) infected with △19 TBSV

Figure 3 – Nothern blot assay for detection TBSV virions. (A) Healthy plant, (B) infected with TBSV, (C) infected with △19 TBSV

Conclusion. This methodological approach could be successfully realized for express detection of virions in inoculated plants. This procedure could be particularly used in cases when visual morphological signs of viral infection are not very clear. This was evident for plants infected with TBSV P19-null mutant which causes relatively mild symptoms compared to the wild type due to the lack of silencing suppressor expression [10]. This work was supported by grant № AP05135013, № BR05236574 of the MES RK.

Список литературы

1 Savary, S.; Ficke, A.; Aubertot, J.; Hollier, C. Crop losses due to diseases and their implications for global food production losses and food security // Food secur. - 2012. - Vol. 4, № 4. - P. 519-537.
2 Marc H.V. van Regenmortel, Brian W.J. Mahy. Emerging Issues in Virus Taxonomy // Emerg. Infect Dis. - 2004. - Vol. 10, № 1. - P. 8-13.
3 Fraser, R.S.S., Whenham, R.J., Abscisic acid metabolism in tomato plants infected with Tobacco mosaic virus: relationships with growth, symptoms and the Tm-1 gene for TMV resistance // Physiol. Mol. Plant Pathol. - 1989. - Vol. 34, № 3. - P. 215-226.
4 Yamamura, Y; Scholthof, H. B. Tomato bushy stunt virus: a resilient model system to study virus-plant interactions // Molecular plant pathology. - 2005. - Vol. 6, № 5. - P. 491-502.
5 T.M. Yergaliyev et al. The involvement of ROS producing aldehyde oxidase in plant response to Tombusvirus infection // Plant Physiology and Biochemistry. - 2016. - Vol. 109, № 10. - P. 36-44.
6 Xiaotian T. Fang, Dag Sehlin, Lars Lannfelt. Efficient and inexpensive transient expression of multispecific multivalent antibodies in Expi 293 cells // Biological Procedures Online. - 2017. - Vol. 19, № 11. - P. 60-67.
7 Birnboim H. C., Doly, J. A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nucleic Acids // Nucleic Acids Research. - 1979. - Vol. 7, № 6. - P. 1513-1523.
8 Scholthof, H. B., Morris, T. J., and Jackson, A. O. The capsid protein gene of Tomato bushy stunt virus is dispensable for systemic move ment and can be replaced for localized expression of foreign genes // Mol. Plant-Microbe Interact. - 1993. - Vol. 6, № 3. - P. 309-322.
9 Scholthof, H. B., Scholthof, K.-B. G., and Jackson, A. O. The tomato bushy stunt virus replicase proteins are coordinately expressed and membrane-associated // Virology. - 1995. - Vol. 208, № 1. - P. 365-369.
10 Omarov, R., Sparks, K., Smith, L., Zindovic, J., Scholthof, H.B. Biological rele-vance of a stable biochemical interaction between the tombusvirus-encoded P19 and short interfering RNAs // J. Virol. - 2005. - Vol. 80, № 6. - P. 3000-3008.

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Өсімдіктерде вирустық ауруларды анықтаудың жедел әдісін жасау

Аннотация: Қазіргі таңда вирустық ауруларды анықтаудың көптеген әдістері бар. Олардың көбісі радиоактивті изотоптарды және спецификалық антиденелерді талап етеді. Бұл мақалада әдістемелігі жағынан қарапайым және аз жабдықталған зертханаларда жүргізуге мүмкіндік беретін вирустық инфекцияларды анықтауда тез әрі қолжетімді әдістер қарастырылды.
Түйін сөздер: жылдам әдіс, P19, вирустық аурулар, РНК-интерференция, TBSV.

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Разработка экспресс-метода для выявления вирусных заболеваний в растениях

Аннотация: В настоящее время существует множество методов определения вирусных заболеваний. В данной статье описаны доступные экспресс-методы определения вирусных инфекций, которые можно использовать в простых и малооборудованных лабораториях.
Ключевые слова: экспресс-метод, P19, вирусные заболевания, РНК-интерференция, вирус кустистой карликовости томатов.

References

1 Savary, S.; Ficke, A.; Aubertot, J.; Hollier, C. Crop losses due to diseases and their implications for global food production losses and food security, Food secur., 4(4), 519-537 (2012).
2 Marc H.V. van Regenmortel, Brian W.J. Mahy. Emerging Issues in Virus Taxonomy, Emerg. Infect Dis., 10(1), 8-13 (2004).
3 Fraser, R.S.S., Whenham, R.J., Abscisic acid metabolism in tomato plants infected with Tobacco mosaic virus: relationships with growth, symptoms and the Tm-1 gene for TMV resistance, Physiol. Mol. Plant Pathol., 34(3), 215-226 (1989).
4 Yamamura, Y; Scholthof, H. B. Tomato bushy stunt virus: a resilient model system to study virus-plant interactions, Molecular plant pathology., 6(5), 491-502 (2005).
5 T.M. Yergaliyev et al. The involvement of ROS producing aldehyde oxidase in plant response to Tombusvirus infection, Plant Physiology and Biochemistry., 109(10), 36-44 (2016).
6 Xiaotian T. Fang, Dag Sehlin, Lars Lannfelt. Efficient and inexpensive transient expression of multispecific multivalent antibodies in Expi 293 cells, Biological Procedures Online., 19(11), 60-67 (2017).

7 Birnboim H. C., Doly, J. A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nucleic Acids, Nucleic Acids Research., 7(6), 1513-1523 (1979).

8 Scholthof, H. B., Morris, T. J., and Jackson, A. O. The capsid protein gene of Tomato bushy stunt virus is dispensable for systemic move ment and can be replaced for localized expression of foreign genes, Mol. Plant-Microbe Interact., 6(3), 309-322 (1993).

9 Scholthof, H. B., Scholthof, K.-B. G., and Jackson, A. O. The tomato bushy stunt virus replicase proteins are coordinately expressed and membrane-associated, Virology., 208(1), 365-369 (1995).

10 Omarov, R., Sparks, K., Smith, L., Zindovic, J., Scholthof, H.B. Biological rele-vance of a stable biochemical interaction between the tombusvirus-encoded P19 and short interfering RNAs, J. Virol., 80(6), 3000-3008 (2005).

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