ҚР БҒМ ҒЫЛЫМ КОМИТЕТІНІҢ «МИКРООРГАНИЗМДЕРДІҢ РЕСПУБЛИКАЛЫҚ КОЛЛЕКЦИЯСЫ» РМК

«Л.Н. ГУМИЛЕВ АТЫНДАҒЫ ЕУРАЗИЯ ҰЛТТЫҚ УНИВЕРСИТЕТІ» ҚеАҚ

Қазақстан Республикасы Тәуелсіздігінің 30 жылдығына арналған «Микробиология, биотехнология және биоалуантүрліліктің өзекті мәселелері» атты Халықаралық ғылыми-тәжірибелік конференциясының МАТЕРИАЛДАРЫ

МАТЕРИАЛЫ
Международной научно-практической конференции «Актуальные проблемы микробиологии, биотехнологии и биоразнообразия», посвященной 30-летию Независимости Республики Казахстан

MATERIALS
of the International Scientific and Practical Conference "Actual Problems of Microbiology, Biotechnology and Biodiversity", dedicated to the 30th anniversary of the Independence of the Republic of Kazakhstan

Нұр-Сұлтан
2021

Қазақстан Республикасының Білім және ғылым министрлігі

Ғылым Комитеті «Микроорганизмдердің Республикалық Коллекциясы» РМК
«Л.Н. Гумилев атындағы Еуразия Ұлттық Университеті» ҚеАҚ

РГП «Республиканская коллекция микроорганизмов»
Комитет науки Министерства образования и науки Республики Казахстан
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RSE «Republican collection of microorganisms»
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Международная научно-практическая конференция «Актуальные проблемы микробиологии, биотехнологии и биоразнообразия», посвященная 30-летию Независимости Республики Казахстан. - 17 сентября 2021 г. - г. Нур-Султан: 192 -стр.

В сборник вошли тезисы научных статей научных работников, преподавателей ВУЗов, студентов, магистрантов, докторантов, участвовавших в Международной научно-практической конференции «Актуальные проблемы микробиологии, биотехнологии и биоразнообразия», посвященной 30-летию Независимости Республики Казахстан по следующим научным направлениям: сохранение биоразнообразия - микроорганизмы, растения и животные; микробные и «зеленые» технологии; молекулярная биология, генная инженерия и геномика микроорганизмов; антибиотики, биофармацевтика и фармакология; биотехнология в сельском хозяйстве, пищевой промышленности и медицине; образовательная деятельность в высших учебных заведениях области биологических наук; биоинформатика и биостатистика.

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The effect of complete or partial inactivation of p19 and p41 proteins with nuclease activity on the level of viral infection of TBSV

Annotation. The TBSV P19 protein is a plant RNA interference suppressor. The study of the mechanism of action of the TBSV P19 protein as a protective strategy of the virus has an important role in understanding the interaction of two organisms in the struggle for survival. The P19 protein, encoded by the *Tomato bushy stunt virus* (TBSV), is a potent suppressor of RNA-interference and plays an important role in the infection of plants with *Nicotiana benthamiana* virus. Expression of the P19 protein leads to complete plant collapse, which leads to more pronounced virus damage.

Keywords. *Tomato bushy stunt virus* (TBSV), virus, capsid protein, RNA interference, *Nicotiana benthamiana*, virion, suppressor protein, recombination.

TBSV is a soil-borne pathogen, but it is also easily tolerated by mechanical leaf inoculation of a wide variety of experimental plants. It is believed that the spread of TBSV occurs in several ways: 1) it enters the drainage waters of soils where plant crops are grown; 2) with wastewater - when people eat contaminated raw vegetables, the virus passes unharmed through the human digestive tract into the sewer. Despite outbreaks and subsequent crop losses caused by some tombus viruses, TBSV is generally not considered an economically dangerous plant pathogen. This may partly explain why tomatoes do not have genetic resistance against TBSV, although gene versus gene resistance has been observed in some experimental host plants of other genera.

Since the early 2000s, the biological, physicochemical, ultrastructural and molecular properties of TBSV and other tombus viruses have been studied in detail. Such reviews prove that TBSV and other tombus viruses have served as very useful models in structural biology to study the expression and replication of genes of viruses with + RNA, as well as to explain the biochemical principles of viral movement and its pathogenicity. Studies of the structural aspects of TBSV gene silencing suppressors demonstrate a possible biochemical mechanism of some of the biological and pathogenic properties of TBSV.

From previous studies, it was found that the symptoms of infection with wild-type TBSV and P19 mutants (157) had similar phenotypic characteristics, however, the complete or partial absence of P19 leads to plant recovery after inoculation due to delayed accumulation of sgRNA 2.

Earlier, the derivatives of GFP TBSV - RMJ-1, RMJ-3 were obtained [1], where the expression of the GFP protein, which was replaced by the capsid protein

CP - P41 TBSV, is an indirect indicator of the development of infection in inoculated local leaves of Nicotiana benthamiana, which clearly illustrates the

dependence of expression viral proteins on the dose-dependent level of P19 (Figure 1) [2,3].

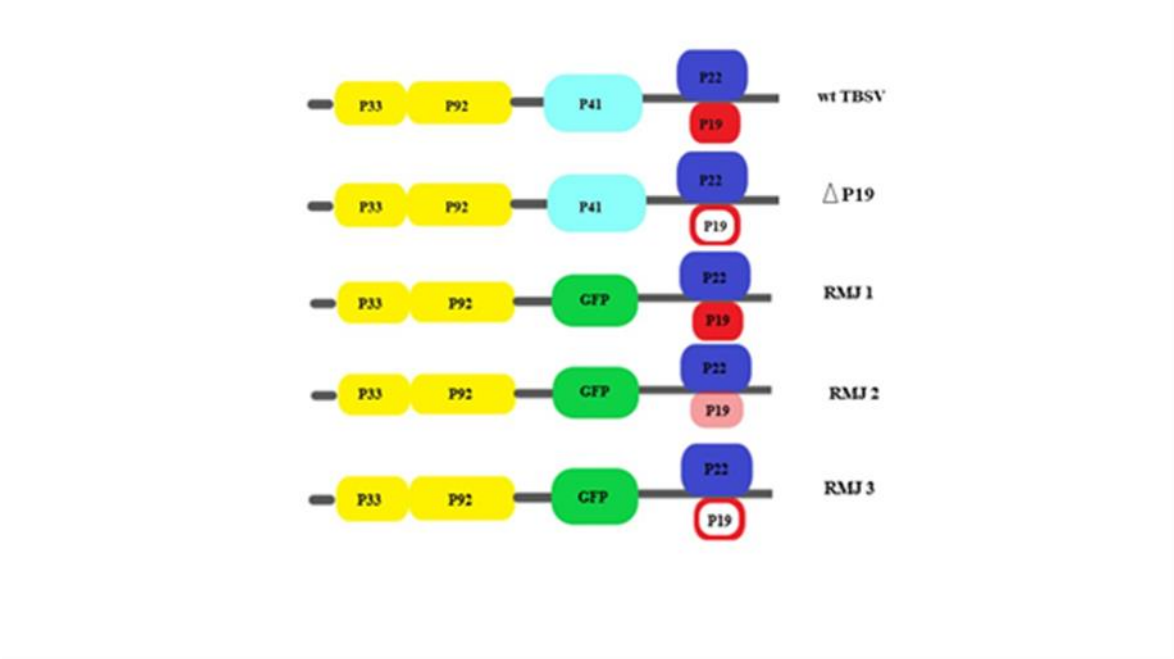

Figure 1 - Genome organization of the wild-type *Tomato Bushy Stunt Virus* and its mutants.

Materials and methods. The object of the study was the wild-type TBSV (*Tomato Bushy Stunt Virus*) phytopathogenic virus from the *Tombusviridae* family, as well as the model plant *Nicotiana benthamiana*.

Plant growing conditions

N. benthamiana plants will be grown in a greenhouse under long day photoperiod conditions (16 hours light / 8 hours dark), at a relative humidity of 75-80%. Temperature fluctuations - from 20 to 27 0C. To illuminate the growth room, lamps with a spectrum of 2700 K and 6400 K will be used. To illuminate the growth chamber, the observation was made at a resolution of 2, 3, 7 and 10 dpi using a portable ultraviolet lamp. Photos will be taken with a Nikon camera in both normal and ultraviolet light. The GFP signal will be quantified using ImageJ software. Leaf samples will be collected at 7 and 15 dpi for further molecular analysis.

In vitro transcription

In vitro transcription was performed according to the protocol kit (T7 polymerase transcription, Promega). A reaction mixture was prepared containing 5 µl of transcription buffer, 0.5 µl of RNAzin, 1.5 µl of rNTP mix, 1 µg of linear DNA, 15 µl of water without RNase, 1 µl of T7 RNA Polymerase. This mixture was incubated for 1 hour at 37 ° C. Detection of transcripts was carried out using agarose gel electrophoresis using ethidium bromide, followed by visualization under a Vilber Lormat gel documenting device (France).

Inoculation of plants with viral material

At the age of 30-35 days, the selection of plants was carried out, the main criterion of which is the same size of plants to comply with equal experimental conditions. Selected groups of plants were photographed for documentation.

Viral transcripts and buffer for inoculation were applied to the surface of the leaves of the middle layer in a volume of 100 μl for each plant. The inoculation buffer consisted of 10 mM sodium phosphate buffer pH 6.9 and saltite. On the surface of the leaves, with the help of silite, light movements were applied to mechanical damage, through which the viral RNA penetrated into plant cells.

After completion of inoculation, control and infected plants were kept under the same conditions, but in separate locations to avoid contamination.

Conclusion. This work aimed to analyze the effect of the restoration of the P19 and P41 genes in *Nicotiana benthamiana* plants by double inoculation with RNA transcripts of the recombinant constructs TBSV - 157 and RMJ-1, RMJ-3. In the course of experiments, it was established that the lack of energy consumption for the assembly of the complete capsid protein allows an increase in the speed and strength of infection in inoculated leaves. A truncated fragment of P41 in RMJ-1, RMJ-3, when co-inoculated with wt TBSV and 157, leads to the spontaneous assembly of virus-like capsid particles. Therefore, co-inoculation has a recombinant effect resulting in the restoration of the capsid protein in TBSV-derived GFPs.

References

1 Shamekova M., Mendoza M. R., Hsieh Y. C., Lindbo J., Omarov R. T., Scholthof H. B. Tombusvirus-based vector systems to permit over-expression of genes or that serve as sensors of antiviral RNA silencing in plants // Virology. – 2014. – Vol.452. – P. 159-165.

2 Manabayeva S. A., Shamekova M., Park,J. W., Ding X. S., Nelson R. S., Hsieh Y. C. Differential requirements for Tombusvirus coat protein and P19 in plants following leaf versus root inoculation // Virology. – 2013. – Vol.439(2). – P. 89-96.

3 Matsuura K. Dressing up artificial viral capsids self-assembled from C-terminal-modified β-annulus peptides // Polymer Journal. – 2020. – Vol. 52(9). – P. 1035-1041.

4 Adhab M., Angel C., Rodriguez A., Fereidouni M., Király L., Scheets K., Schoelz J. E. Tracing the lineage of two traits associated with the coat protein of the tombusviridae: silencing suppression and HR elicitation in Nicotiana species // Viruses. - 2019. – Vol.11(7). – P. 588.

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