Development of viral infection in model system ‘Nicotiana tabacum – Tobacco Mosaic Virus’ under the influence of endophytic bacteria

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We present a study of endophytic bacteria influence on the development of viral infection in model system ‘Nicotiana tabacum – tobacco mosaic virus’. It appears that the application of Pantoea aglomerans causes a 41 ± 5,3 % decrease of viral antigen content in plant tissues and results in suppression of phytoviral infection.

Key words: *Endophytic bacteria, tobacco mosaic virus (TMV), phytopathogens*

Viral diseases of plants introduce a relevant problem in agriculture because traditional methods of virus spread control may be not effective enough. Widely used chemical substances are potentially dangerous for people and the environment. Therefore, a search for biological agents that could protect plants from virus invasions is a promising area of research [1].

We suggest endophytic bacteria as possible plant protectants. They are non-pathogenic microorganisms that colonize plant tissues under the natural conditions. Unlike symbiotic and pathogenic inhabitants, endophytic bacteria are not specific to a plant and do not cause any anatomical formations, such as root nodules. However, comparing to symbiotic and non-symbiotic microorganisms, endophytic bacteria might cooperate more intensively with the host [2].

Biological control of plant pathogens bases on induced systemic resistance (ISR) that occurs in plants as a response to stimulation factor. Plants possess different types of defensive mechanisms against pathogen attack. Considering the latest evidence, a basic resistance depends on the structure of the cell wall while ISR involves a pathogenic stimulus or the application of chemicals or natural substances [3].

The investigation of endophytic bacteria as possible triggers of ISR might be implied for creating the antiviral biological preparations with no use of chemicals.

Aim of Research. Study of endophytic bacteria influence on plants growth and the development of viral infection in model system '*Nicotiana tabacum* - tobacco mosaic virus'.

Materials and Methods. As objects of study we used four groups of tobacco plants grown under the standard conditions. Sterilized seeds were cultivated with bacterial suspension at 25ºC for 24 hours. The second application of bacteria was conducted directly on the plants leaves 3 days prior to inoculation with the virus. We infected plants with TMV on a three-leaf stage using carborundum as an abrasive.

According to a research scheme, uninfected plants from the first, control, group were untreated with bacteria. The second group presented untreated plants but infected with virus. On the contrary, plants from the third group were not inoculated with virus but carried bacteria. And finally, the fourth group included infected plants, previously treated with studied microorganisms (fig.1).

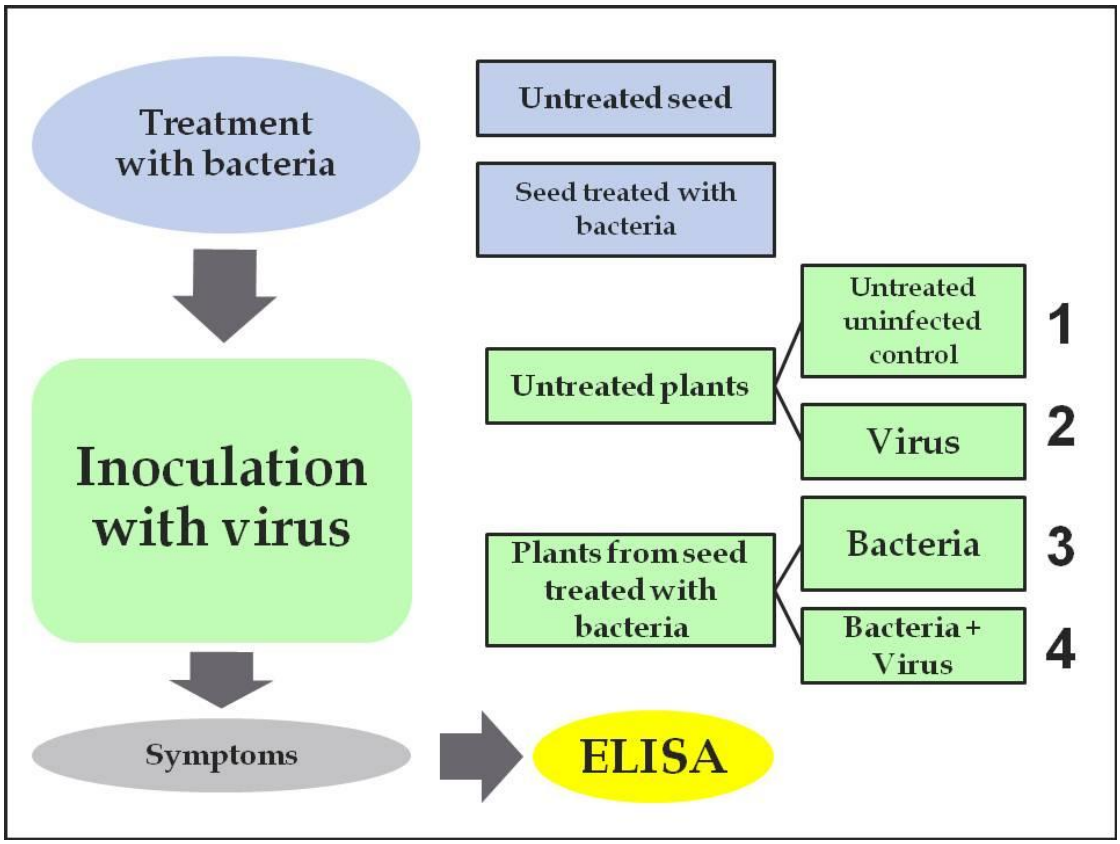

Fig.1. Study of endophytic bacteria influence on the development of viral infection in model system '*Nicotiana tabacum* – tobacco mosaic virus'

In the experiment we used *Lactobacterium plantarum, Methylibacterium radiotolerans, Pseudomonas* sp., *Pantoea aglomerans* and a bacterial-fungal association, called EM, as possible inductors of plant defence. EM included *Lactobacillus casei, Rodopseudomonas palustris, Sacharomyces cerevisiae.*

A content of viral antigen in infected plant material was determined by non-direct ELISA, on 405 nm wave length.

Results. To investigate timing and severity of symptoms, we performed a visual diagnostics of the viral infection in plants treated with endophytic bacteria. It should be mentioned that there may be no correlation between the time when symptoms occur and the concentration of viral antigen in plant tissues.

Such symptoms as leaves deformation and system mosaics, appeared in 20 days post inoculation.

Plants treaated with *Lactobacterium plantarum* and infected with TMV, demonstrated better growth level than untreated infected plants and those from control group. Moreover, we noticed differences in the development of rootage.

As for *Methilobacterium radiotolerans*, EM and *Pseudomonas sp*, investigated plants showed severe symptoms of viral infection and the suppression of growth and development comparing to untreated infected plants. (fig. 2).

Figure 2. Symptoms of viral infection in plants treated with bacteria and infected with tobacco mosaic virus

1 – untreated infected tobacco; 2 – EM + virus; *Lactobacterium plantarum* + virus

According to our observations, infected plants previously treated with *Pantoea aglomerans* illustrated a moderation in the development of viral infection. The symptoms on plants treated with this endophytic bacteria were less severe. Moreover, we also indicated an acceleration in plants growth (fig. 3).

Figure 3. Influence of endophytic bacteria *Pantoea aglomerans* on the development of viral infection

1 – *Pantoea aglomerans* + virus; 2 – untreated infected tobacco; 3 – *Pantoea aglomerans*; 3 – untreated uninfected tobacco

To confirm the results of visual diagnostics we conducted the non-direct ELISA. This analysis estimated a similar content of viral antigen in infected plants treated with EM, *Lactobacterium plantarum*, *Methilobacterium radiotolerans* and *Pseudomonas sp*. However, we observed a 41± 5,3% decrease of viral antigen content in plant material that carried *Pantoea aglomerans.* It affirmed a moderation of TMV infection. A diagram below shows the results obtained in the experiment

(fig.4).

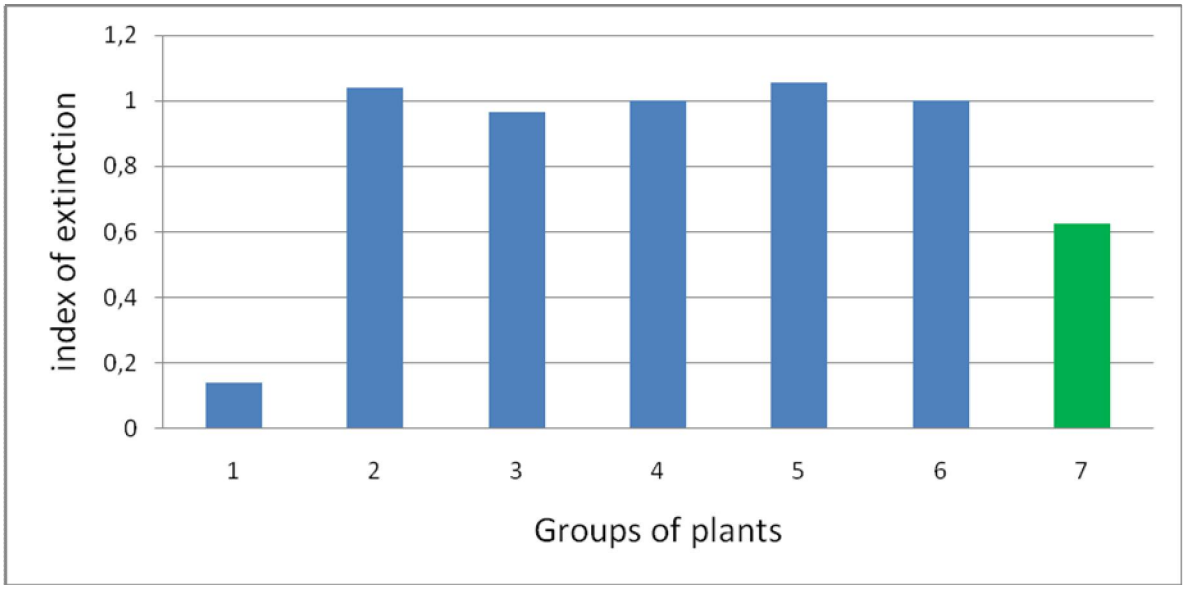

Fig. 4 TMV content in infected plants treated with endophytic bacteria

1 – untreated uninfected plants; 2 – untreated infected plants; 3 – EM + virus; 4. *Lactobacterium plantarum* + virus; 5 – *Methilobacterium radiotolerans* + virus ; 6 – *Pseudomonas sp.* + virus; 7 – *Pantoea aglomerans* + virus

Due to ELISA results, the amount of TMV in plant tissues did not change under the influence of EM, *Lactobacterium plantarum*, *Methilobacterium radiotolerans* and *Pseudomonas sp*

However, *Pantoea aglomerans* demonstrated a priming impact on plant organism. The application of this bacteria prior to inoculation with virus resulted in suppression of infection in model system *Nicotiana tabacum* - tobacco mosaic virus. The mechanism of such activity is possibly dependent on the formation of defensive response in plants. Considering this, we propose *Pantoea aglomerans* as a perspective object for further research.

Conclusions. We investigated the influence of endophytic bacteria on the development of viral infection in model system *Nicotiana tabacum* – tobacco mosaic virus. Such bacteria as *Lactobacterium plantarum*, *Methilobacterium radiotolerans*, *Pseudomonas sp* and EM – association, did not suppress the development of viral infection. However, treatment with *Pantoea aglomerans* stimulated growth of

tobacco plants and decreased the amount of virus in infected plants by 41 ±5,3%. The mechanism of suppression is probably based on the activation of plants resistance mechanisms.

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BIBLIOGRAPHY

1. Помазков Ю.И. Биологическая защита растений (краткий курс). Для студентов III курса специальности «Агрохимия» / Ю.И. Помазков, В.Г. Заец– М.: РУДН, 1997. – 116с.

2. Овчаренко Л.П. Метагеномний аналіз організмів довкілля / Н.О. Козировська – Київ: Спринт Принт, 2008. – с.193-202.

3. Бронин А.М. Ризосферные бактерии рода Pseudomonas, способствующие росту и развитию растений // Соросовский образовательный журнал. – 1998. – №10. – с.25 – 31.

4. Crowther J. ELISA. Theory and practice Humana Press, N.Y. – 1995. - P.38-39.

5. Антитела 1. Методы / Под ред. Кэти Д.- М: Мир.- 1991a.- с.68-92. 6. Антитела 2. Методы / Под ред. Кэти Д.- М: Мир.- 1991b.- с.152-165.

РОЗВИТОК ВІРУСНОЇ ІНФЕКЦІЇ У МОДЕЛЬНІЙ СИСТЕМІ «*Nicotiana tabacum* – ВІРУС ТЮТЮНОВОЇ МОЗАЇКИ» ПІД ВПЛИВОМ ЕНДОФІТНИХ БАКТЕРІЙ

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Викладено результати досліджень впливу ендофітних бактерій на розвиток вірусної інфекції у модельній системі «Nicotiana tabacum - вірус тютюнової

мозаїки». Показано, що обробка рослин ендофітною бактерією Pantoea aglomerans призводить до зниження вмісту вірусного антигена на 41±5% у рослинному матеріалі і пригнічення розвитку фітовірусної інфекції.

Ключові слова: *Ендофітні бактерії, вірус тютюнової мозаїки, фітопатогени*

РАЗВИТИЕ ВИРУСНОЙ ИНФЕКЦИИ В МОДЕЛЬНОЙ СИСТЕМЕ «Nicotiana tabacum – ВИРУС ТАБАЧНОЙ МОЗАИКИ» ПОД ВЛИЯНИЕМ ЭНДОФИТНЫХ БАКТЕРИЙ

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Изложены результаты исследования влияния эндофитных бактерий на развитие вирусной инфекции в модельной системе «Nicotiana tabacum – вирус табачной мозаїки». Показано, что обработка эндофитной бактерией Pantoea aglomerans приводит к снижению содержания вирусного антигена на 41(±5,3)% в растительном материале и подавлению развития фитовирусной инфекции.

Ключевые слова: *Эндофитные бактерии, вирус табачной мозаики, фитопатогены*