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VIRUS INFECTION COURSE IN DIFFERENT PLANT SPECIES UNDER INFLUENCE OF ARBUSCULAR MYCORRHIZA

The results of the research aimed to study the influence of plants root colonization by arbuscular mycorrhizal fungus Glomus intraradices on virus infection development are presented in the paper. Tobacco mosaic virus (TMV) – Nicotiana tabacum model was used for the experiment. TMV – Lycopersicon esculentum and Cucumber green mottle mosaic virus (CGMMV) – Cucumis sativus were used as important agricultural plants. Microscopy, molecular and serological methods were used in the experiment. It was shown that arbuscular mycorrhiza inhibits the plant virus infection development in Nicotiana tabacum and Cucumis sativus plants but not in Lycopersicon esculentum.

K e y w o r d s: TMV (Tobacco mosaic virus), CGMMV (Cucumber green mottle mosaic virus), Glomus intraradices, arbuscular mycorrhiza.

Symbiotic interactions between the plants and microorganisms in rhizosphere is one of the major factors of plant health and soil fertility. Arbuscular mycorrhiza (AM) is a very common type of symbiotic interactions for the majority of the plants. Arbuscular mycorrhizal fungi (AMF) are widely spread and characterized by vast host range. The plants colonized by AMF demonstrate higher growth rate than plants without AM [13, 14]. Moreover AM increases resistance of the plants to stress factors and soilborne pathogens [6]. Activation of plant resistance to pathogens can be explained by anatomical or pathogenic changes in root system or alterations in rhizosphere microbial associations caused by arbuscular fungi [11]. The facts about the role of AM in inhibition of virus infection can be used to improve efficiency of antiviral preventive measures [7, 8]

The aim of this research was to study the influence of root colonization of plants by arbuscular mycorrhizal fungi on development of virus infection, caused by TMV in model plant *Nicotiana tabacum* and in important agricultural plants such as *Lycopersicon esculentum,* and by CGMMV in *Cucumis sativus* plants. *Glomus intraradices* was used in this experiment as one of the most widespread AMF that forms symbiosis with a large number of plant species. Furthermore *Glomus* mycelium can be cultivated at industrial scale and can be used in soil mixtures for the greenhouses.

Materials and methods

The plants used in the experiment are characterized as having systemic reaction in response to phytoviral infection. *Nicotiana tabacum –TMV* was used as a classic model, and *Lycopersicon esculentum* and *Cucumis sativus* were choosen as the plants important for agriculture, particularly for the greenhouses. These systems differed in the inoculation method. For the *Nicotiana tabacum cv. trapesond*, TMV viral preparation was poured in soil at concentration of 24.41 μg/ml, the final concentration of virus in soil constituted 500 ng/ml. The plants in *Cucumis sativus* – CGMMV system were infected by soaking seeds in viral preparation (concentration of virus was 200 μg/ml) overnight. The plants in *Lycopersicon esculentum* – TMV system were infected mechanically in two upper level leaves. Concentration of viral preparation was 250 μg/ml [5].

All plants were divided in four groups: the intact plants, the virus-infected plants without AM, non-infected plants with AM, and the plants with both virus and *Glomus intraradices*.

An incubation mixture was used to accumulate the infectious inoculum of AMF in *Tagetes patula* plants. The quantitative analysis of root colonization rate was performed in 80 days [3]. The obtained inoculum was used in the mixture with zeolite. The final proportion of soil and zeolite was 5:1.

Light microscopy of roots was carried out to control the process of AMF colonization. For the quantative characterization of root mycorrhization the following parameters were used: F – colonization frequency (the percent of roots with fungal structures), M – colonization intensity (percent of colonized cortex in each root), A – percent of arbuscules in colonized roots, a – percent of arbuscules in the whole root system. The minimal value of these parameters in the case of successful colonization is 50% [3].

DNA from plant material was extracted using the phenol-chlorophorm method. [15]. PCR analysis was carried out to determine AMF in roots [16]. For PCR, primers complementary to internal transcribing spacers of rRNA genes of *Glomus intraradices* were used. The nucleotide sequence of primers: ITS1- TCCGTAGGTGAACCTGCGG; ITS4- TCCTCCGCTTATTGATATGC [16]. The results of PCR were visualized using electrophoresis in 1% agarose gel. Gene Ruler 100 bp DNA ladder (100-3000 bp, MBI Fermentas) was used as markers set.

Indirect ELISA with polyclonal rabbit antibodies was used to detect the viral antigens in plant samples. For ELISA the samples from each plant were collected. The leaves with strongly pronounced macroscopic symptoms, such as mosaic, deformation of leaf blade and necrosis were taken. The samples from the plants not showing the symptoms of virus infection including dried-up leaves were also collected [5].

Results and discussion

The efficiency of colonization was determined by counting the fungal structures in roots of the colonized plants. The number of arbuscules as primary structures playing the role in nutrients exchange shows the rate of transport between the fungus the and the plant. Light microscopy analysis of the colonized roots showed the presence of both arbuscles and vesicles in the root cortex of the plants that confirms successful colonization. The results of the quantitative analysis of plant roots colonization rate are shown in Table.

Table

Quantitative analysis of AM colonization

Experimental plant	Virus presence	F%	M%	A%	a%
Nicotiana tabacum	+	73.33	53.30	65.94	35.20
	-	76.67	37.67	96.90	36.50
Lycopersicon esculentum	+	76.67	23.53	97.18	22.87
	-	94.00	32.50	93.74	30.34
Cucumis sativus	+	93.56	35.84	94.23	29.05
	-	95.46	48.09	96.37	33.75

The rates of root colonization, shown in the table, were considerably higher than the minimal rate of 50% confirming successful formation of symbiotic interactions between the plants and AMF.

The PCR analysis of colonized roots was carried out as well to detect the presence of *Glomus intraradices* nucleic acid. The expected fragment approx of 450 bp has been detected (Fig. 1).

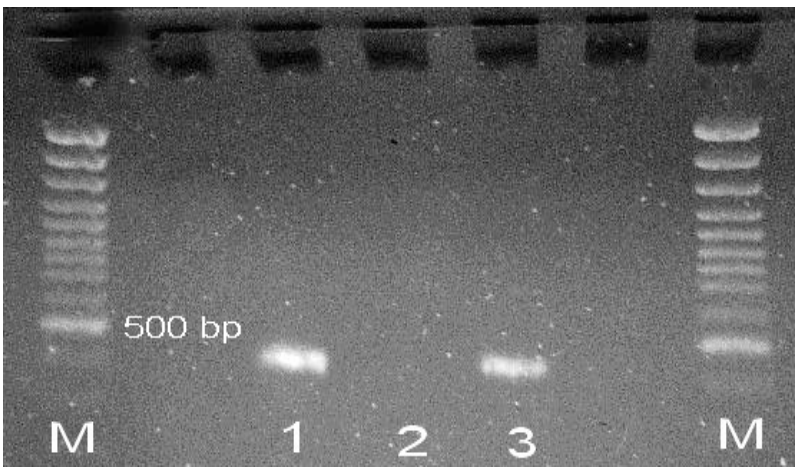

Fig. 1. PCR results for *Nicotiana tabacum* (1) and *Lycopersicon esculentum* (3) plants, 2-negative control, M - markers
(Gene Ruler 100bp DNA ladder plus, 100 — 3000bp)

The following pattern was revealed for the *Nicotiana tabacum cv. trapesond* — TMV model after visual observations and ELISA. The infected plants without AM had clear symptoms in 80 days, such as mosaic and leaf blade deformation. No symptoms on further stages were observed for the plants colonized with AMF, so virus infection did not develop in such plants in contrast to the plants without AM. Intact plants and the plants colonized by AM but not infected served as the control groups. The content of viral antigens in infected plants without AM permanently increased, as revealed ELISA test. At the early stages of virus infection in infected

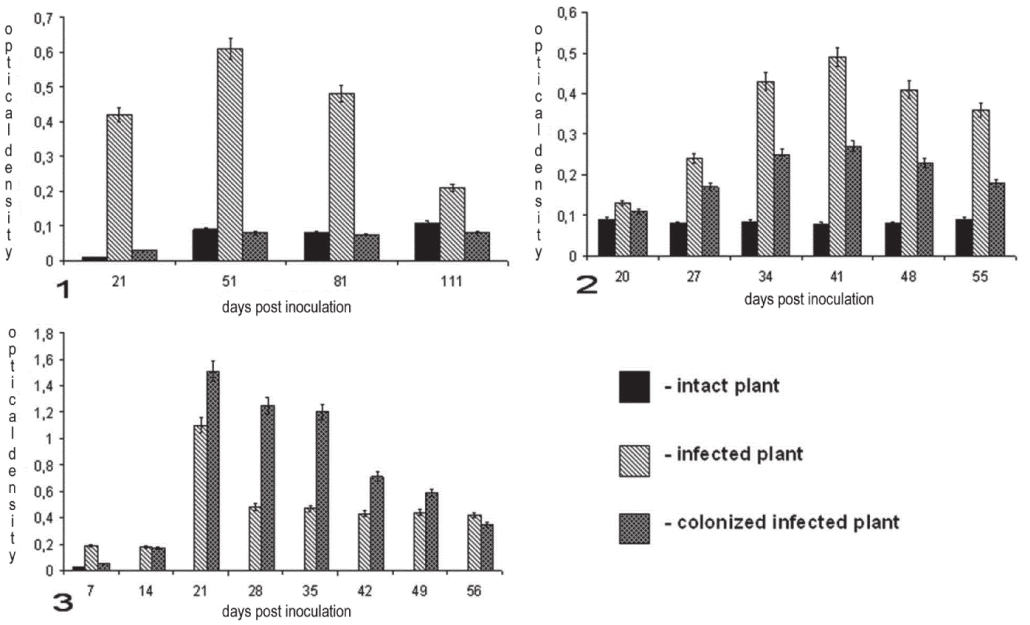

Fig. 2. Dynamics of viral antigen accumulation in *Nicotiana tabacum cv. trapesond TMV* (1), *Cucumis sativus - CGMMV* (2) and *Lycopersicon esculentum - TMV* (3) systems

plants with AM the content of viral antigens was four times less as compared with infected plants without AM. The final content of viral antigens in such plants was 2.5 times less (Fig. 2).

The symptoms of virus infection on *Lycopersicon esculentum* plants were observed as green or yellow mosaic. Content of viral antigens in infected plants with and without AM was equal at the early stages of virus infection up to 14 days post inoculation, and then it increased in the plants colonized by AM. At the subsequent stages of virus infection the content of viral antigens in colonized plants subsequently decreased (Fig. 2). This fact can be explained by the way of virus penetration to the plant. It was shown previously that AM can lead to increase of disease severity of foliar pathogens [13].

For *Cucumis sativus* — CGMMV system, visual observations revealed that infected plants had clear symptoms, such as decrease in leaf blade size, growth inhibition, mosaic and leaf blade deformation. The plants colonized by AMF and infected by virus did not manifest pronounced symptoms. ELISA test revealed that infected plants with AM accumulated less amount of viral antigen comparing to infected plants without AM (Fig. 2).

These results can be compared with data about interaction of AMF and non-viral pathogens, such as bacteria and root-infecting fungi. The arbuscular mycorrhiza formation leads to increase of phosphate uptake by the plants, and that nutritional status can be related to the enhanced resistance of the plants to soilborne pathogens such as *Aphanomyces euteiches* [9]. Also it was shown that not only nutritional status can affect the severity of the disease. AM can activate defense-relative genes, such as PR family genes prior to pathogen penetration [1]. The other way of controlling the pathogen proliferation is the competition between arbuscular mycorrhizal and pathogenic fungi to colonize root tissues [4, 10].

As it can be seen, the effect of AM colonization of the plants is different, but it is still difficult to estimate the reasons of such differences. The most obvious reason is the way the virus infects the plant as was already shown for non-viral soilborne pathogens [13]. Obtained data show that for viral pathogens that are introduced through soil the effect is still the same. But in the case when virus is introduced mechanically into the leaf blade the effect of myccorhization is opposite that may be caused by increased nutrition rates that promote more favorable conditions for virus replication. This fact is very important, because it makes the usage of AM in open field conditions difficult. But in the greenhouses where virus mechanical transmission can be controlled and soil mixture composition can be easily changed, AM can be used to control virus transmission via soil, moreover it increases nutritional rates of the plants and their resistance to non-viral pathogens being of major importance especially for the greenhouses.

It is estimated that AM inhibits plant virus infection development caused by TMV in *Nicotiana tabacum* plants and by CGMMV in *Cucumis sativus* plants if the virus is introduced through root system. It is also shown, that there is no such effect on *TMV – Lycopersicon esculentum* system, where the plants are infected mechanically into the leaf. These facts show that AM may play the role in activating plant responses to soilborne pathogens but promote higher susceptibility to foliar deseases, although the mechanisms of such effects are still unclear and need further research.

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ПРОТІКАННЯ ВІРУСНОЇ ІНФЕКЦІЇ У РІЗНИХ ВИДІВ РОСЛИН ПІД ВПЛИВОМ АРБУСКУЛЯРНОЇ МІКОРИЗИ

Реферат

В статті наведено результати досліджень, присвячених вивченню впливу колонізації коренів рослин арбускулярним мікоризним грибом *Glomus intraradices* на протікання фітовірусної інфекції. У експерименті використовували такі модельні системи як ВТМ—*Nicotiana tabacum*, ВТМ—*Lycopersicon esculentum* та ВЗКМО—*Cucumis sativus*. Мікроскопічними, молекулярно-біологічними та серологічними методами дослідження показано, що арбускулярна мікориза інгібує розвиток фітовірусної інфекції в рослинах *Nicotiana tabacum* та *Cucumis sativus,* але не в *Lycopersicon esculentum*.

К л ю ч о в і с л о в а: ВТМ (вірус тютюнової мозаїки), ВЗКМО (вірус зеленої крапчастої мозаїки огірка), *Glomus intraradices*, арбускулярна мікориза.

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ПРОТЕКАНИЕ ВИРУСНОЙ ИНФЕКЦИИ У РАЗНЫХ ВИДОВ РАСТЕНИЙ ПОД ВЛИЯНИЕМ АРБУСКУЛЯРНОЙ МИКОРИЗЫ

Реферат

В статье приведены результаты исследований, посвященных изучению влияния колонизации корневой системы растений арбускулярным микоризным грибом *Glomus intraradices* на протекание фитовирусной инфекции. В эксперименте использовали такие модельные системы как ВТМ—*Nicotiana tabacum*, ВТМ—*Lycopersicon esculentum* а также ВЗКМО—*Cucumis sativus*. Микроскопическими, молекулярно-биологическими и серологическими методами исследований показано, что арбускулярная микориза ингибирует развитие фитовирусной инфекции в растениях *Nicotiana tabacum* и *Cucumis sativus*, но не в *Lycopersicon esculentum*.

К л ю ч е в ы е с л о в а: ВТМ (вирус табачной мозаики), ВЗКМО (вирус зеленой крапчатой мозаики огурца), *Glomus intraradices*, арбускулярная микориза.