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RECENT DATA ON THE CAUSATIVE AGENT OF PALE GREEN DWARF (ACHOLEPLASMA LAIDLAWII VAR. GRANULUM INCERTAE SEDIS) IN UKRAINE: PATHOGENICITY AND VIRULENCE FACTORS AND HOST REACTIONS

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Contemporary state of the distribution of mycoplasma diseases of cultivated crops in Ukraine was analyzed. The changes of the physiological state of plant cells under the impact of mollicutes were investigated. It was demonstrated that there is temporary increase in the activity of peroxidase, catalase, polyphenoloxidase, phenylalanine-ammonia-lyase at the early stages of interaction. The adhesive properties are changed in the mollicutes under the impact of plant lectin; there is synthesis of new polypeptides. It was determined that the phytopathogenic acholeplasma is capable of producing a complex of proteolytic enzymes into the culture medium. It was concluded that when plant cells are infected with acholeplasma, a number of signaling interactions and metabolic transformations condition the recognition of pathogenesis and ensure the aggregate response of a plant to stress in the form of defense reactions. It was assumed that some specificities of the biology of phytopathogenic acholeplasma determine their avoiding the immune mechanisms of plants and promote long-term persistence of mollicutes.

Key words: cultivated crops, mycoplasmosis, phytopathogenic acholeplasma, persistence, enzymes.

The elaboration of balanced agroecosystems and the maintenance of conditions for their stable development is rather a complicated process, related to a wide scope of issues. These include the determination of physical, chemical and biological processes in soil, the elaboration of modern agrotechnologies, the improvement of the specialization of agrarian production systems, the optimization of the structure of agricultural landscapes and the organization of the territory of land-utilization [1, 2]. Ukraine has witnessed some movement in economic relations with Western countries which has a positive impact on the general state of agrarian production; at the same time there are some objective reasons, like privatization of land resources, restructuring of agricultural enterprises, which often impair the technological foundation of crop cultivation.

In addition, there is a change in climatic conditions, remarkable for specific zones of cultivating crops which conditions the fluctuation of biodiversity in ecosystems [3]. All this increases the danger of the microorganisms, not registered before, penetrating the territory of Ukraine and spreading therein. It is noteworthy that in these conditions mycoplasma, plant disease agents, enhance their aggression and harmfulness, accelerating the penetration of phytomycoplasma, regulated by the European and Mediterranean Plant Protection Organization, into Ukraine.

The apple tree proliferation disease, grape yellowing, stolburs, wheat light green dwarf disease are quite common in the territory of Europe and conditioned by plant damage with phytopathogenic mycoplasma. The harmfulness of mycoplasma diseases is a common problem, as the infections, caused by mycoplasma, are related to catastrophic diseases, which often become epiphytoties [4].

Mycoplasmas (mollicutes) are the smallest prokaryotic microorganisms, deprived of a cellular wall and capable of independent existence and restoration [5]. It was described that the mollicutes, parasitizing on plants, are capable of avoiding the non-specific defense reaction of plants – oxidative stress, which leads to the persistence of mollicutes. The scientists even assumed that this reaction of plants may somehow promote the viability of mycoplasma, but the mechanisms of mycoplasmosis emergence are yet to be elucidated [5–7].

According to recent notions, the symptomatic manifestation of infectious processes depends considerably both on the infection agent and the specificities of the interaction of genetically determined signaling pathways in the parasite-host system [8–10]. As for mollicutes, a close connection to the membrane of the eukaryotic cell may result in masking their antigens and in direct biochemical effects which cause local damage of the target cell structures. The inhibition and control of mycoplasma infections of plants are related to the study of molecular mechanisms of mycoplasma interaction with plant cells and the persistence principles for these microorganisms and the phytopathogenesis, conditioned by latter.

Fine mechanisms of mycoplasmosis emergence are yet to be studied, as the interaction of cells is a complicated and multi-component process. Therefore, our efforts have been directed at the investigation of transformations in the living parasite-host complex on the model of plant cells, infected with phytopathogenic strains of acholeplasma [11]. The dominant of these investigations is the analysis of specificities of signaling and metabolic relations in the network of molecular interactions of mollicutes and cells of the host macroorganism.

Specific virulence factors have not been determined for plant mycoplasma, which are the agents of catastrophic epiphytoties, and the phytopathogenesis is caused by mycoplasma persistence and related phytoimmunity reactions. The damage of plants with phytoplasmosis may be manifested in the form of single symptoms of mycoplasmosis, and not as epiphytoty, for a long time [4–7, 12, 13]. Therefore, it is extremely important and urgent to conduct a detailed study of the agents of such dangerous diseases and the mechanisms of manifestation of their phytopathogenic properties to prevent the occurrence of mycoplasma diseases of plants, new to Ukraine.

Acholeplasma laidlawii is a mollicute, widely spread in the environment, which is found in soil, manure, wastewater, cell cultures, tissues of humans, animals, and plants [5]. The phytopathogenic representative of these mollicutes, *A. laidlawii* var. *granulum* st. 118 is the agent of wheat mycoplasmosis which is capable of causing the outbreaks of the disease and the decrease in the yield of infected fields [12, 13].

Among famous biologically active wheat substances a relevant role is attributed to wheat germ agglutinins (WGA) – the lectin, which may perform the function of a plant signaling molecule [14–18]. It promotes the growth and development of wheat with simultaneous change in the metabolism of symbiotic bacteria as a communication factor in the plant-microbe symbiosis. At the same time there are data on the increase in WGA level in *Triticum vulgare* L. plants after the abiogenous stresses under the impact of drought, osmotic and heat shock. Taking this into consideration one of the tasks of our work was to study the impact of this lectin on the agent of wheat light green dwarf disease – *A. laidlawii* var. *granulum* st. 118. It was demonstrated that the introduction of WGA to the culture medium of acholeplasma causes the pleiotropic effect: there is the activation of growth processes, the increase in the total amount of protein compared to the control, the decrease in hemagglutinating activity which leads to the attenuation of adhesive properties of the pathogen. We believe that after the direct contact of mollicute glycopolymers and a wheat germ lectin the signal is transmitted inside the cell, due to which metabolism is enhanced, there is expression of new proteins, and the biomass of the microorganism is increased. On the other hand, under the impact of WGA the investigated microorganism demonstrates a change in adhesive properties, which may be a manifestation of non-specific plant protection due to the intrusion of the pathogen. It was assumed that it promotes the transition of the infection into the latent state and long-term persistence of acholeplasma in wheat plants.

Cell cultures are widely used as a model to study many physiological and biochemical processes in plants as it allows having adequate estimation of exchange processes in plant cells and their response to various irritations of the environment in controlled conditions [9, 11, 19–21]. In our opinion the system of joint cultivation of the pathogen and cultures of target plant cells is a convenient model in the study of specificities of plant cell response to the infection with phytopathogenic mycoplasma, thus it was further used in the investigations of stress condition of plants in conditions of developing artificial mycoplasmosis.

The infestation of plants with obligate pathogens is accompanied with the increase in the energy content of cellular processes, in particular, there is the increase in breathing intensity which is related to the activation of enzymatic systems of peroxidase, catalase, polyphenoloxidase which perform the functions of terminal oxidases. The protection of biological molecules and cell organelles is carried out by enzymes that catalyze the biotransformation of primary reactive oxygen species (ROS) and low molecular weight compounds of different chemical nature that react with these substances [8, 10, 19, 22–25].

There are numerous data in the study of oxidation-reduction enzymes in conditions of oxidative stress in plants, infected with different fungal or bacterial pathogens [19, 22–26]. Taking into account the insufficient amount of publications on the issues of investigating these enzymes while infecting plants with mollicutes, we analyzed the impact of the phytopathogenic mollicute *A. laidlawii* var. *granulum* st. 118 on the change in the activity of peroxidase and catalase of plant cells in standard *in vitro* conditions (unpublished data).

For this reason we used callus cultures of sugar beet to infect them with the mollicute *A. laidlawii* var. *granulum* st. 118. During the initial contacts of the plant and the pathogen the activity of peroxidase and catalase relative to the control is increased. Several hours after the infection there was a decrease in enzymatic activity, after which the indices were stable. The changes, revealed in the activity of oxidative enzymes, are related to the induction of defense mechanisms of plant cells as a response to the stress.

It is assumed that polyphenoloxidase (PPO) is activated in case of infesting plant cells with phytopathogenic organisms. Its function is to oxidize phenols to highly toxic quinones, inactivating foreign exoenzymes, destroying the cells of pathogens and the host-plant, *i.e.* it is involved in the reaction of plant hypersensitivity [8, 22]. The analysis of PPO activity in callus tissues of sugar beet demonstrated that *A. laidlawii* var. *granulum* strain 118 has a considerable impact on the change in enzyme activity which may be compared to the effect of hydrogen peroxide activity.

Thus, it may be concluded that the infection of sugar beet calluses with the cells of phytopathogenic acholeplasma leads to the stimulation of defense reactions of plant cells – temporary increase at the early stages of the activity of peroxidase and catalase enzymes, participating in the development of systemic acquired sensitivity as well as polyphenoloxidase enzymes. It confirms the literature data, according to which the ratio in the activity of different oxidative systems has adaptive meaning and may be considered as a necessary condition for the manifestation of plant resistance and the content of enzymes of the system of antioxidative protection and low molecular antioxidants in different organisms may be considerably different [8, 22–26]. Thus, as seen from the abovementioned, the impact of mollicutes on the plant organism may be considered to be a biotic stress, as its consequence is a considerable disbalance of antioxidants and prooxidants to the benefit of the latter. The oxidative stress results in the accumulation of reactive oxygen species, which may act as inducers of corresponding defense mechanisms in plant cells.

Therefore, immediately after being infected with a phytopathogenic mollicute the plant tissues are in conditions of severe stress, total metabolism is inhibited. On the other hand, the stress-induced activation of the enzyme indicates its specific role in overcoming this stress.

The dynamics in the activity of enzymes of phenolic plant metabolism, in particular, phenylalanine-ammonia-lyase (PAL), when they are infected with the representatives of *Mollicutes* class, has not been studied yet and is necessary to disclose the specificities of pathogenesis of plant mycoplasmosis. It was determined that PAL activity of a plant culture under the impact of *A. laidlawii* var. *granulum* st. 118 starts increasing one hour after the infection, and in two hours its level increases 4-fold compared to the control. With further cultivation of calluses in the presence of the pathogen, the level of PAL activity decreases and 6 hours after the infection it is only 50 % higher than the indices of the control non-infected samples. After 24 h of cultivation the PAL activity decreases to the initial level and remains the same during the whole experiment. Compared to other microorganisms, this “outbreak” of activity is non-permanent [27, 28]. Acholeplasma may be capable of overcoming the defense activity of non-specific mechanisms of plant cells and/or avoiding hypersensitivity reactions, which is a likely constituent of the adaptation of phytopathogenic mollicutes to long-term persistence. The increase in PAL activity during the infection of sugar beet calluses with *A. laidlawii* var. *granulum* st. 118 may also be viewed as a manifestation of defense reactions of plant cells in response to the activity of the pathogen.

Along with other enzymes the phytopathogenic microorganisms produce extracellular proteases. In many cases there is some correlation between the activity of extracellular proteases of the phytopathogen and the intensity of the plant disease. The proteases of phytopathogenic microorganisms are capable of cleaving the antimicrobial proteins of plants and playing a relevant role in the destruction of cellular wall proteins [29].

It was assumed that the ability of producing extracellular enzymes may be one of pathogenicity factors for phytopathogenic mollicutes. It was determined that the mollicute *A. laidlawii* var. *granulum* st. 118 is capable of producing proteolytic enzymes along with nucleases and some hydrolases into the cultivation medium. It was also demonstrated that the complex of proteolytic enzymes, which are a part of the preparation, obtained by us, is rather manifold and capable of cleaving such protein substrates as casein, hemoglobin, and fibrin.

The comparative analysis of structural and functional specificities of proteases of phytopathogenic mollicutes and related representatives of bacilli allowed determining a certain level of affinity of their physical and chemical properties and substrate specificity. It is anticipated that in this case the purpose of proteolytic enzymes is overcoming defense barriers of plants rather than providing mollicutes with peptides and aminoacids, required for the growth and development. Further studies will allow defining the activation mechanisms and determining the source of inhibition of the activity of proteolytic enzymes of phytopathogenic mollicutes.

Thus, it may be concluded that when plant cells are infected with acholeplasma, a number of signaling interactions and metabolic transformations condition the recognition of the pathogen and ensure the aggregate response of a plant to stress in the form of defense reactions. Some specificities of the biology of phytopathogenic acholeplasma determine their avoiding the immune mechanisms of plants and promote long-term persistence of mollicutes.

The results of our work may be used as a foundation for the elaboration of the concept of decreasing the harmfulness of mycoplasma diseases of plants to maintain ecological balance in agroecosystems, which is in good agreement with modern trends of developing sustainable agriculture in Ukraine.

Нові дані про збудника блідо-зеленої карликовості зернових (Acholeplasma laidlawiivar.granulum incertae sedis) в Україні: фактори патогенності, вірулентності та реакції рослини-господаря

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Зроблено аналіз сучасного стану розповсюдження мікоплазмових хвороб сільськогосподарських культур в Україні. Досліджено зміни фізіологічного стану клітин рослин під впливом молікутної інфекції. Показано, що на ранніх етапах взаємодії тимчасово збільшується активність пероксидази, каталази, поліфенолоксидази, фенілаланін-аміак-ліази. У молікутів під дією рослинного лектину змінюються адгезивні властивості, відбувається синтез нових поліпептидів. Встановлено здатність фітопатогенної ахолеплазми продукувати у культуральне середовище комплекс протеолітичних ферментів. Зроблено висновок, що низка сигнальних взаємодій і метаболічних перетворень за інфікування клітин рослин ахолеплазмою обумовлюють розпізнавання патогену і забезпечують сумарну відповідь рослини на стрес у вигляді реакцій захисту. Висунуто припущення, що певні особливості біології фітопатогенних ахолеплазм визначають уникання дії механізмів імунітету рослин і сприяють тривалій персистенції молікутів.

Ключові слова: мікоплазмоз, фітопатогенні ахолеплазми, персистенція, ферменти.

Новые данные о возбудителе бледно-зеленой карликовости зерновых (Acholeplasma laidlawiivar. granulum incertae sedis) в Украине: факторы патогенности, вирулентности и реакции растения-хозяина

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Проанализировано современное состояние распространения микоплазменных болезней сельскохозяйственных культур в Украине. Исследованы изменения физиологического состояния клеток растений под влиянием молликутной инфекции. Показано, что на ранних этапах взаимодействия временно увеличивается активность пероксидазы, каталазы, полифенолоксидазы, фенилаланин-аммиак-лиазы, у молликутов под действием растительного лектина изменяются адгезивные свойства, происходит синтез новых полипептидов. Установлена способность фитопатогенной ахолеплазмы продуцировать в культуральную среду комплекс протеолитических ферментов. Сделан вывод о том, что ряд сигнальных взаимодействий и метаболических превращений при инфицировании клеток растений ахолеплазмой определяют распознавание патогена и обеспечивают суммарный ответ растения на стресс в виде реакций защиты. Выдвинуто предположение, что определенные особенности биологии фитопатогенных ахолеплазм обусловливают избежание действия механизмов иммунитета растений и способствуют длительной персистенции молликутов.

Ключевые слова: микоплазмоз, фитопатогенные ахолеплазмы, персистенция, ферменты.

REFERENCES

1. *Patyka VP, Tihonovich IA, Filip'ev ID, Gamajunova VV, Andrusenko* II. Microorganisms and alternative agriculture. Kyiv, Urozhaj.1993;176 p.

2. *Petrichenko VF, Tihonovich IA, Kots' S. Ya, Patyka MV, Mel'nichuk TM, Patyka VP*. Agricultural microbiology and balanced development of agroecosystems *Visnyk agrarnoi nauky*.2012;(8):5–11.
3. *Patyka M V, Patyka VP*. Modern problems of biodiversity and climate fluctuations. *Visnyk agrarnoi nauky*. 2014;(6):5–10.
4. *Vlasov Yu I, Gevorkjan Z G*. Mycoplasma diseases of agricultural plants. Erevan, Izd-vo ANArm. SSR.1981;125p.
5. *Borhsenius SN, Chernova OA, Chernov VM, Vonskij VM*. Mycoplasma. St Petersburg,Nauka.2002;320p.
6. *Chernov VM, Chernova OA, Tarchevskij IA*. Phenomenology of Mycoplasma infection of plants. *Fiziologija rastenij*.1996;**43**(5):694−701.
7. *Chernov VM, Mukhametshina NE, GogolevYuV, Nesterova TN, Chernova OA*. Mycoplasma adaptation to adverse growth conditions: Nanotransformation and phytopathogenicity of *Acholeplasma laidlawii* PG8. *Doklady Akad Nauk*.2007;**413**(2):271−5.
8. *Kolupaev JuJe*. Stress response of plants (molecular-cellular level): monograph. Kharkiv, Khar. derzh. agrar. Univ. Publ.2001;173 p.
9. *Ozereckovskaja OL, Varlamov VP, Vasjukova NI, Chalenko GI, Gerasimova NG, Panina JaS*. Effect of systemic signalling molecules on the rate of spreading the immunizing effect of elicitors over potato tissue. *Prikl biokhimija i mikrobiologija*.2004;**40**(2):252−6.
10. *Tarchevskij IA*. Plant cell signaling systems. Moscow, Nauka.2002;296p.
11. *Korobkova KS, Onishhenko AM, Panchenko LP, Mamchur OYe, Dmitruk OO, Red'ko* VI. Elaboration of the *in vitro* model system to study the interactions of phytopathogenic mollicutes with plant cells. *Mikrobiol Zh.* 2009;**71**(4):58−62.
12. *Skripal' IG, Malinovskaja LP*. Medium SM IMV-72 for isolation and cultivation of phytopathogenic mycoplasma. *Mikrobiol Zh*.1984**;46**(2):71−5.
13. *Onishchenko AN, Kozar FE, Kraeva GV*. Mycoplasma-like bodies in the vascular bundle cells of wheat infected with light green dwarf. *Mikrobiol Zh*. 1973;**35**(4):500−2.
14. *Sadovnikova YuN, Bespalova LA, Antonjuk LP*. Wheat germ agglutinine is a growth factor for the bacterium *Azospirillum brasilense. Dokl akad nauk SSSR*.2003; **38**9(4):544−6.
15. *Trifonova TV, Maksjutova NN, Timofeeva OA, Chernov VM*. Change in lectin specificity of winter wheat seedlings in the course of interaction with mycoplasma. *Prikl biokhimija i mikrobiologija*.2004;**40**(6):675−9.
16. *Antonyuk LP, EvseevaNV*. Wheat lectin as a factor in plant-microbial communication and a stress response protein. *Mikrobiologiia*.2006;**75**(4):544−9.
17. *Oka Y, Chet I, Spiegel Y*. An immunoreactive protein to wheat-germ agglutinin antibody is induced in oat roots following invasion of the cereal cyst nematode *Heterodera avenae*, and by jasmonate. *Mol Plant Microbe Interact*.1997;**10**(8):961−9.
18. *Singh PH, Bhaglal P, Bhullar SS*. Wheat germ agglutinin (WGA) gene expression and ABA accumulation in the developing embryos of wheat (*Triticum aes-ivum*) in response to drought. *Plant Growth Reg*.2000;**30**(2):145−50.
19. *Kirillova NV*. Changes in the activity of superoxide dismutase in the *Rauwolfia serpentine Benth*. callus cultures grown under standard conditions and heat shock. *Prikl biokhimija i mikrobiologija*.2004;**40**(1):89−93.
20. *Maksimov IV, Surina OB, Troshina NB*. Assessment of resistance of wheat plants to phytopathogens with use of joint cultures. *Trudy Nikitskogo Bot. sada*. 2009;**131**:160−7.
21. *Persijanova EV, Kiselev KV, Bulgakov VP, Timchenko NF, Chernoded GK, Zhuravlev JuN*. Induction of protective reactions in the callus cultures of a ginseng at interaction with human pathogen *Yersinia pseudotuberculosis*. *Fiziologija rastenij*. 2008;**55**(6):834−41.
22. *Zenkov NK, Men'shhikova EB*. Activated oxygen metabolites in biological systems*. Usp sovrem biol*.1993; **113**(3):286−96.
23. *Komov VP, Troitskaya LA, Kirillova NV*. Isolation and purification of peroxidase from a callus tissue cultu-re of ginseng. *Prikl biokhimija i mikrobiologija*. 1998;**34**(5):495−8.
24. *Dat J, Vandenabeele S, Vranova E, VanMontagu M, Inze D, Van Breusegem F*. Dual action of the reactive oxygen species during plant stress responses. *Cell Mol Life Sci*.2000;**57**(5):779−95.
25. *Temple MD, Perrone GG, Dawes IW*. Complex cellular responses to reactive oxygen species. *Trends Cell Biol.* 2005;**15**(6):319−26.
26. *Sies H*. Strategies of antioxidant defense. *Eur J Biochem*. 1993;**215**(2):213−19.
27. *Adamovskaya VG, Molodchenkova OO, Ciselskaya LY, Bezkrovnaya LYa*. The change of phenylalanine-ammonia-lyase activity, content of total phenolic compounds and lignin in the seedlings of spring barley at the influence of fusariose infection and salicylic acid. *Visnyk Kharkivs'koho Natsional'noho agrarnoho Univ. Serija* Biologija.2007;**1**(10):50−8.
28. *Evtushenko EV, Saprykin VA, GalitsynMYu, Chekurov VM*. The effect of biologically active substances from coniferous plants on the L-phenylalanine ammonia lyase and peroxidase activities in wheat leaves. *Prikl biokhimija i mikrobiologija*.2008;**44**(1):123−8.
29. *Rubin BA, Arcihovskaja EV, Aksenova VA*. Biochemistry and physiology of plant immunity. Moscow, Vysshaya shkola.1975;318 p.