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CHANGES OF SEVERAL METABOLIC PARAMETERS OF SOYA INOCULATED WITH PHYTOPATHOGENS AT APPLICATION NANOCHELATES

SUMMARY

In greenhouse experiments changes in several metabolic parameters soybeans plants after inoculation by *P. savastanoi* pv. *glycinea* 9190, *X. axonopodis* pv. *glycines* 9192, BCMV, *A. laidlawii* on background pre-treatment by nanochelates (NHs) of V, Ge, Cu, Mo have been shown. The phenolic compounds content increased in leaves at inoculation by phytopathogens on background pre-treatment by NHs and without it. The content of H_2O_2 in leaves increased in variants: BCMV (+VNHs, GeNHs, CuNHs), 9192 (+VNHs, CuNHs), 9190 (+GeNHs, CuNHs), *A. laidlawii*+MoNHs. But it decreased in the variants: BCMV + MoNHs, *A. laidlawii* + VNHs, 9190 + VNHs, 9192 (+ GeNHs, CuNHs) compared to controls. The peroxidase activity increased in variants: *A. laidlawii*, 9190, GeNHs + 9190, GeNHs + 9192, CuNHs, CuNHs (+ phytoplasmas, 9192), MoNHs (+ BCMV, phytoplasmas, 9190, 9192) with decreased catalase activity. The content of ABA in the leaves increased in variants: 9190, 9192 (+ VNHs, CuNHs), GeNHs + 9190, but the IAA content reduced. However, on variants MoNHs (+9190, 9192), GeNHs + 9192, *A. laidlawii*, *A. laidlawii* (+ GeNHs, CuNHs, MoNHs) the content of both phytohormones was decreased. The content of IAA and ABA in BCMV-infected leaves without treatment was increased, but on background of NHs – it decreased

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significantly. The F_v/F_m-value increased in almost all variants, except for CuNHs + BCMV. The photosynthetic rate (R_{Fd}-value) at inoculation plants with viral, bacterial and phytoplasmas pathogens on background treatment of MoNHs; bacterial strain on background of pre-treatment of GeNHs; phytoplasmas on background of VNHs have been increased. Thus, pre-treatment by nanochelates had regulatory effect on soybean plant metabolism helping increase its resistance to phytopathogens was shown. Thus, pre-treatment by nanochelates had regulatory effect on soybean plant metabolism but the pre-treatment of MoNHs was more effectiveness thanks not only intensification resistance against pathogens but and the assimilation of CO_2 increased.

Keywords: *Xantomonas axonopodis pv. glycines, Pseudomonas savastanoi pv. glycinea, Acholeplasma laidlawii var. granulum,* BCMV, nanochelates

INTRODUCTION

Soybeans are one of the most popular oilseeds grown worldwide. Among the world's soybean producers are Brazil, the United States and Argentina (Shahbandeh, 2022).

Factors that negatively affect the yield and quality of soybeans, in addition to risky climate change, are infections with fungi, bacteria, viruses, phytoplasmas and other phytopathogens. In addition, the gradual expansion of sown areas and production of this crop has led to a sharp increase in the number of diseases (Mammadov *et al*., 2018). It should be noted that various phytopathogens effect on plant metabolism by including specific virulence factors. Thus, it is known that effector proteins with enzymatic functions, such as the SUMO XopD protease, play an important role in the interaction of *Xanthomonas* with host plants. In addition, *Xanthomonas* expresses a unique class of type III effectors, including the AvrBs3 family, which simulation plant transcriptional activators and manipulate plant transcripts (Kay & Bonas, 2009; Büttner & Bonas, 2010). AlgU has been shown to play a critical role in the pathogenesis of *Pseudomonas savastanoi* by regulating many virulence factors (Nguyen *et al*., 2021). Meanwhile, the existence of a common regulatory mechanism for different species of bacteria has been reported. Thus, the pathogen *Pseudomonas savastanoi* enters to the host plant through a secretion system of type III, which is regulated by a two-component system RhpRS. This system depends on the mechanisms of phosphorylation and nutrition and can switch between the regulation of virulence and metabolism, which is widespread among different species of bacteria (Xie *et al.*, 2019).

Other harmful pathogens transmitted by insect vectors and have limited metabolism – phytoplasmas. There is a wide range of phytoplasmas, and they cause many symptoms in different host plants, but legumes often have weak symptoms on the leaves and infertility of flowers (Sharman, 2016). Genomic analysis of phytoplasmas isolated from soybean plants with signs of "flower bud deformation" showed the presence of genes such as auxin response factor 9 (ARF9) and the transcription factor domain of the Forkhead family (FHA), which

are directly involved in plant development in various ways such as hormonal regulation, plant morphology, embryogenesis and DNA repair (Kale *et al*., 2016).

Plant viruses are submicroscopic obligate parasites that consist of RNA or DNA particles coated with a protein coat. Their reproduction depends on the cellular mechanism of their hosts and cannot occur outside it (Soybean viruses, 2019; Gergerich & Dolja, 2006). Viral infections also pose a serious threat to the productivity and quality of soybeans. It is known that main passes of plants virus diseases transmitted are insects: soybean aphids, thrips, bean leaf beetle and infected seeds (Soybean viruses, 2019). About twenty-seven viruses are known to be potentially dangerous to plants today. Soybeans often have viruses such as Soybean mosaic virus (SMV), Bean pod mottle virus (BPMV), Alfalfa mosaic virus (AMV), Soybean dwarf virus (SbDV), Soybean vein necrotic virus (SVNV), Tobacco ringspot virus (TRSV), and Tobacco streak virus (TSV) *et al* (Soybean viruses, 2019). Some of these viruses have a wide range of host plants. Yep, Bean common mosaic virus (BCMV) causes significant loss of bean productivity but can also infecting of soybeans (Lee *et al*, 2015; Kyrychenko & Prylipko, 2020). In research by Zhou *et al*. (2014) identified 30 isolates of BCMV that are capable of infecting soybeans. The main tactics for controlling viral infections are natural or artificial resistance to viruses, the use of uninfected seeds, control of weed reservoirs of viruses, control of insect vectors of plant viruses (Hill & Whitham, 2014, Gergerich & Dolja, 2006). Plants induce a multicomponent protective response to pathogenic infection, involving the expression of many protective genes encoding enzymes and protective metabolites. During pathogenic infection, there is a noticeable accumulation of protein associated with pathogenesis (PR), in a place away from the source of infection. At the same time there is an accumulation of salicylic acid and H_2O_2 at the site of infection to regulate systemic acquired resistance (SAR) in plants. The multistage process of the plant protection mechanism against phytopathogens includes host cell death, necrosis, accumulation of phenolic compounds, cell wall modification, and synthesis of specific antimicrobial compounds (Chowdhary *et al.*, 2021).

It should be noted that all the above pathogens can significantly inhibit the productivity of soybean plants. However, there are currently no effective chemical measures to control the diseases caused by these pathogens. The treatment of biologically active substances created with the use of nanotechnologies can be modern alternative methods of control of these pathogens. Nanoparticles of Au, Ag, alumina (Al), Se, titanium dioxide (TiO_2), silicon dioxide (SiO_2), copper oxide (CuO), zinc oxide (ZnO), calcium oxide (CaO) and magnesium oxide (MgO) have a proven antimicrobial action (Karimi, 2019; Filipović *et al*., 2021). Antimicrobial activity of Ag-Cu, complex Co-Cu-Zn-Fe-Mn-Mo-Mg, biological substance of I-Se, and each nano chelates of V and Ge, have been shown (Huliaieva *et al*., 2020). Known that MoO_3 nanoparticles has high antimicrobial activity against gram-negative and gram-positive bacteria, *Candida albicans* and *Aspergillus niger* and the ability to remove free radicals

(Fakhri & Nejad, 2016). However, the effect of different nanoparticles on the metabolism of cultivated plants requires in-depth study. Therefore, the aim of our work was to study the effect of pre-sowing treatment with nanochelates Ge, V, Cu, Mo and inoculation of soybean plants with phytopathogenic bacteria, mycoplasmas and viruses on its several metabolic parameters.

MATERIAL AND METHODS

Experimental soybean plants of the variety Artemida were grown in a greenhouse on the Institute of Microbiology and Virology of NASU of D.K. Zabolotny territory. Before sowing, soybean seeds were treated by soaking in a solution of nanochelates: V (2.25 μg / l), Ge (3.75 μg / l), Cu (4.5 μg / l) and Mo (8 μg / l). Nanoparticles were obtained by erosion-explosive method, producer: "Nanomaterials and Nanotechnologies" society (Ukraine).

In research used pathogens: *Xantomonas axonopodis* pv. *glycines* IMV B-9192 (soyabean bacterial pustule), *Pseudomonas savastanoi pv. glycinea* IMV B-9190 (bacterial blight of soybean), *Acholeplasma laidlawii* var. *granulum* 118 (IMV BM – 34) (pale-green dwarf of cereals), Bean common mosaic virus (BCMV). Artificial inoculation of plants with *A. laidlawii* was conduct by prick of suspension in soybean stem. Artificial inoculation of plants with bacterial pathogen conducted by applying by brush of bacterial suspension on the leaves repeatedly pierced with a needle in 4–6 leaf phase. Bacterial and phytoplasmas strains was obtained from off Ukrainian collection of microorganisms of Institute of Microbiology and Virology of NASU of D.K. Zabolotny. BCMV-inoculation of soybean plants was carried out in the phase of two leaves, by applying a brush on soybean leaves freshly prepared virus-containing material after pre-dusting with carborundum. Sheme of experiments: 1 – Control; 2 – *A.laidlawii* (phytoplasmas) inoculation; 3 – BCMV-inoculation; 4 – *X. axonopodis* pv. *glycines* 9192 inoculation; 5 – *P. savastanoi pv. glycinea* 9190 inoculation; 6 – pre-sowing treatment VNHs; 7 – VNHs+phytoplasmas; 8 – VNHs+BCMV; 9 – VNHs+ *X. axonopodis* pv. *glycines* 9192; 10 – VNHs+ *P. savastanoi pv. glycinea* 9190; 11 – GeNHs; 12 – GeNHs+phytoplasmas; 13 – GeNHs+BCMV; 14 – GeNHs+ *X. axonopodis* pv. *glycines* 9192; 15 – GeNHs+ *P. savastanoi pv. glycinea* 9190; 16 – CuNHs; 17 – CuNHs+phytoplasmas; 18 – CuNHs+BCMV; 19 – CuNHs+ *X. axonopodis* pv. *glycines* 9192; 20 – CuNHs + *P. savastanoi pv. glycinea* 9190; 21 – MoNHs; 22 – MoNHs+phytoplasmas; 23 – MoNHs+BCMV; 24 – MoNHs+ *X. axonopodis* pv. *glycines* 9192; 25 – MoNHs+ *P. savastanoi* pv. *glycinea* 9190. Repetition in the experiment triple.

The content of phytohormones – IAA and ABA were determined by quantitative Spectro densitometric thin layer chromatography (Biliavska *et al.*, 2017). Sampling of plant material (leaves) was performed 12 days after inoculation with phytoplasmas and BCMV and 6 days after inoculation by bacterial strains 9091 and 9192. Plant specimens of common bean with symptoms BCMV were tested by RT-PCR for the presence of BCMV. Total RNA was extracted from 0.5 g of leaf tissue by a previously reported method. The RT was

performed using Reverta kits (AmpliSens), according to the manufacturer's instruction. The work used primers that allow to identify a fragment of the nucleotide sequence encoding the protein gene of the envelope with the following nucleotide sequences: direct primer 5'-ttcggacgtcgtgagtgtta-3 'and reverse 5'-cccgagtccacattaattcc-3', the size of the amplification product was 391 bp (Kyrychenko & Kovalenko, 2018; Kyrychenko *et al*., 2019; Kyrychenko & Prylipko, 2020). For PCR, 2 μL of the template genomic DNA was amplified in a 25-μL of total volume containing 1 × reaction buffer, 5 pmol of each primer, 0.3 mM dNTPs, 1.25 U of TaqDNA polymerase and nuclease-free water. Reactions were performed under the following conditions: 3 min denaturation at 95°C, thermal cycling for 35 cycles (1 min at 94°C, 1 min at 60°C and 1 min 30 s at 72°C), ending with the final extension at 72°C for 5 min. PCR products of amplification were visualized in 1.5 % agarose gels with TBE buffer and ethidium bromide (0.5 mg mL^{-1}).

Quantitative detection of phytohormones was performed using a scanning spectrodensitometer "Sorbfil". Determination of the content of soluble polyphenols was performed by the method of Folin and Ciocalteu (Folian & Ciocalteu, 1927) in the modification of Singleton and Rossi (Singleton & Rossi, 1965), which is based on the reaction of phenols with Folin & Ciocalteu reagent (it produces a blue color which absorbs at 765 nm). The total content of phenol was determined 27 days after inoculation with phytopathogens. The activity of antioxidant enzymes – catalase (EC 1.11.1.6) and non-specific peroxidases (EC 1.11.1.7) in seedlings was determined in one sample. The catalase activity expressed in the amount of O_2 formed by the action of the enzyme for 1 min per 1 g of wet wt. (ml of $O_2 \cdot g^{-1} \cdot min^{-1}$). The activity of nonspecific peroxidases was studied by the method of Boyarkin. Peroxidase activity was expressed in arbitrary units per 1 $g^{-1} \cdot c^{-1}$ of wet wt. (Huliaieva *et al.*, 2018). Analysis of the enzymatic activity of catalase and peroxidase was performed 27 days after inoculation with phytopathogens. The hydrogen peroxide content was determined by the method of Bellincampi *et al*. (2000), estimating the color intensity of compounds formed with xylenol orange spectrophotometrically.

The photochemical activity of soybean leaves was measured by the biophysical method of induction of chlorophyll fluorescence using a portable device "Floratest" (Huliaieva *et al*., 2018). Measurements were performed one month after inoculation with experimental strains.

Induction fluorescence of parameters: minimum chlorophyll fluorescence (F_0); $F_v = F_m - F_0$ – the variable fluorescence; $\frac{Fv}{Fm}$ – maximum quantum yield of primary photochemistry; decrease ratio $R_{Fd} = \frac{Fm-Ft}{Ft}$, which, when measured at saturation irradiance is directly correlated to the net CO_2 assimilation rate (PN) of leaves (Lichtenthaler *et al*., 2007; Misra *et al*., 2012).

For statistical analysis was used computer programs of Microsoft Excel. On diagrams shown the arithmetic mean errors.

RESULTS AND DISCUSSION

In greenhouse experiments we observed the appearance of symptoms at artificial infection: yellowing at the edges leaf (on phytoplasmas-infected soybean plants) (fig. 1A), the green vein banding, which become slightly darker than the areas between the veins and leaf malformation, which usually exhibit a downward curling or cupping (on BCMV-infected soybean plants) (fig. 1B, 1C). Symptoms soybean bacterial pustule begin as small, light green spots with raised centers and gradually the lesions can grow together into large irregular brown areas (fig. 1E, 1F). Symptoms bacterial blight of soybean have look angular lesions, which begin as small yellow to brown spots on the leaves, witch centers of the spots will turn a dark reddish-brown and dry out, yellowish green "halo" will appear around the edge (fig. 1F, 1G).

By the RT-PCR analysis of BCMV viruses was conducted in leaves common bean with BCMV-symptoms (fig. 1D), that used for inoculation soybean plants (fig. 2). The expected sizes of amplifying DNA fragments for BCMV were 391-base pair (bp) (Kyrychenko & Prylipko, 2020).

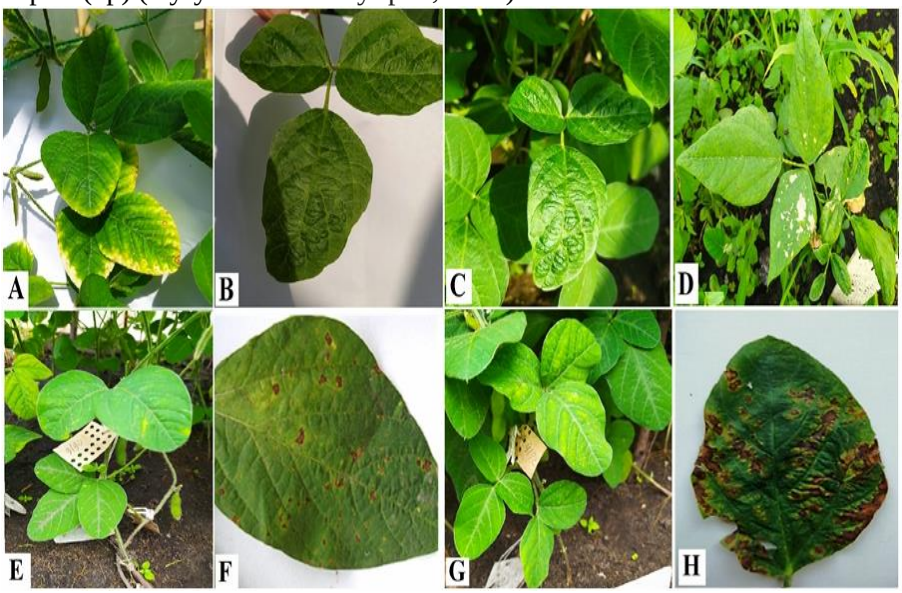


Figure 1. Appearance of symptoms

It is known that the infection of plants with phytopathogens and action of other stressors in cellular compartments (chloroplasts, mitochondria, peroxisomes) form active forms of oxygen (ROS), which serve as signaling molecules and participate in protection against pathogens (Shetty *et al*., 2008), but with excessive accumulation and cause cell death (Sharma *et al*., 2012; Suzuki *et al.*, 2012; Anjum *et al.*, 2016; Huang *et al*., 2019). The formation of ROS occurs not only under stressful conditions, but also in a result of electronic transport activity, in PSI and PSII (Foyer, 2018; Sharma *et al*., 2012). It should be noted that ROS are also involved in the regulation of important metabolic

processes associated with growth and development (Anjum *et al*, 2016; Damiani *et al*., 2016; Suzuki *et al.*, 2012; Dvořák *et al*., 2021). Research by Damiani *et al.* (2016) established the participation of ROS and NO in the establishment of symbiosis between legumes and rhizobia. Due to the above, the regulation of ROS in cells is an important metabolic process. It is known that ROC is regulated by so-called enzymatic (superoxide dismutase (SOD), catalase (CAT), guaiacolperoxidase (GPX), ascorbate-glutathione cycle enzymes (AsA-GSH), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR)) or non-enzymatic antioxidants (Ascorbate (AsA), glutathione (GSH), carotenoids, tocopherols, phenols) (Huang *et al*., 2019; Sharma *et. al*., 2012; Das & Roychoudhury, 2014). Typically, an increase in antioxidant activity indicates on the activation of ROS neutralization by increasing its content under oxidative stress, as well as increasing the resistance of plants to stressors (Sharma *et al*., 2012; Podgórska *et al*., 2017). Non-enzymatic antioxidants such as phenolic compounds are known to protect plants from phytopathogens due their antimicrobial properties and other important functions: involved in growth and development, including hormone production, osmoregulation, UV protection, etc. (Wallis & Galarneau, 2020; Chowdhary *et al*., 2021). Plants are accumulated phenols to delay the growth of microbial pathogens and limit their spread in the infected area (Chowdhary *et al.*, 2021). Research by Maddox *et al*. (2010) indicated inhibition of the growth of gram-negative phytopathogenic bacteria, which causes diseases of many crops – *Xylella fastidiosa*, under the action of minimal inhibitory concentrations of 12 phenolic compounds. The antiviral activity of phenolic compounds has also been confirmed (Chowdhary *et al*., 2021). However, the induction of phenolic metabolism in plants may be a response to multiple stresses (Sharma *et al*., 2012).

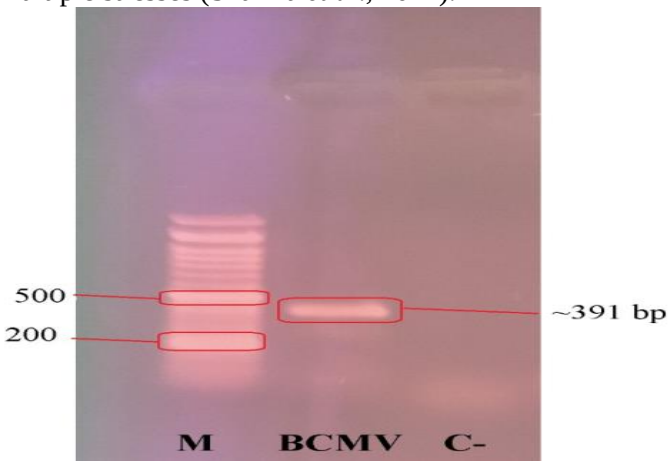


Figure 2. RT-PCR detection of BCMV viruses in common bean plants have been grown in greenhouse condition: M-molecular marker (CSL-MDNA-50BP DNA Ladder RTU), BCMV (from common bean leaves with BCMV symptoms), C- – negative control

Our studies have shown an increase in the content of phenolic compounds as at inoculated by phytopathogens: *P. savastanoi pv. glycinea* 9190 (by 52,6%) > *X. axonopodis* pv. *glycines* 9192 (by 43,9%) > B3MK (by 37,2%) > phytoplasmas (by 25,9%), so and at pre-sowing treatment by nanochelates: CuNHs (by 52,6%) > GeNHs (by 35,4%) > MoNHs (by 22,4%) > VNHs (by 9,7%) (fig. 3a). Moreover, content of phenolic compounds increased most significantly on variants: inoculation by bacteria on background of pre-sowing treatment of GeNHs, MoNHs, CuNHs; the BCMV-inoculation on background of pre-treatment of GeNHs, CuNHs, MoNHs; inoculation soybean by phytoplasmas on background of pre-treatment of CuNHs, VNHs, MoNHs (see fig. 3a). Thus, increase in phenolic compounds in leaves experimental variants are indicates on plant resistance increased to pathogenic infecting.

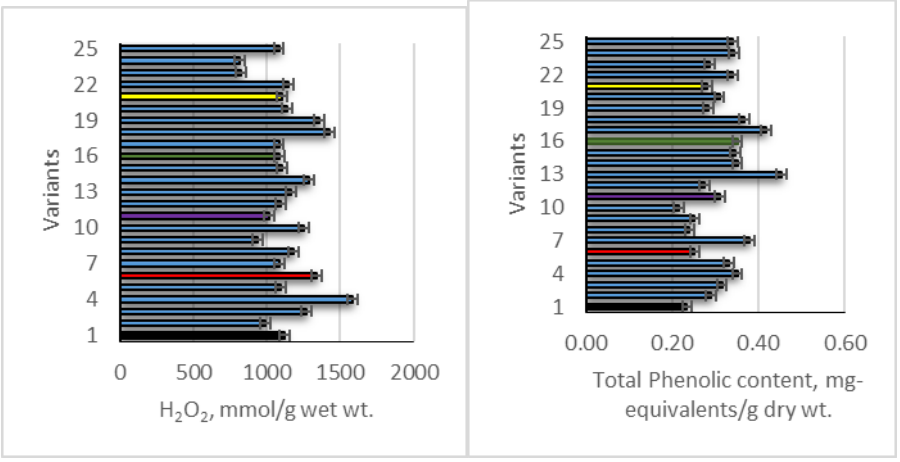
Another well-known ROS that does not belong to free radicals and performs important regulatory, protective and signaling functions is H_2O_2. Generated in cells in a result of various stressors, including the penetration of pathogens, as well as in the process of metabolic transformations (redox reactions, photorespiration, electron transport chain), hydrogen peroxide easily penetrates biological membranes (Slesak *et al*., 2007; Sharma *et al*., 2012; Niu & Liao, 2016; Smirnoff & Arnaud, 2019; Petrov & Breusegem, 2012). It is also known that H_2O_2 at low concentrations acts as a signaling molecule, participating in the regulation of certain biological processes and can increase plant resistance to pathogens. However, at high concentrations hydrogen peroxide – can inactivate enzymes, including the Calvin cycle – up to 50% (at concentrations of 10 μm), as well as SOD, protein kinases, phosphatases, transcription factors containing thiolate residues, oxidize amino acids, cysteine methionine and others (Sharma *et al*., 2012).

According to our data, the content of H_2O_2 decreased in the leaves at pre-treatment of GeNHs and increased at pre-treatment of VNHs, while at pre-treatment of MoNHs was at the control level (fig. 3b).

Research by Shin *et al*. (2004) indicated that the level of H_2O_2 in Arabidopsis in the leaves increased slightly after 6 hours but doubled 30 hours after potassium starvation. Similar tendencies were observed in maize plants (Shin *et al*., 2004). However, even low concentrations of non-essential metals, such as Cd, Pb, Hg, can disrupt physiological and biochemical processes in plants and cause oxidative stress. It is also known that metals that have redox activity (Fe, Cu) increase the level of ROS directly, and those that do not its (Cd, Hg, Zn) increase ROS indirectly (Cuypers *et al*., 2016). However, in our study we considered the dual function of ROS in plant metabolism and their role in increasing plant resistance, particularly at infection with phytopathogens. Because it is known that one of the early responses to pathogen infecting under the conditions of its recognition is an oxidative burst with significant formation of ROS and the product of its dismutation – H_2O_2 in the apoplast of plants (Sharma *et al*., 2012; Shetty *et al*., 2007). Research by Shetty *et al*. (2007) showed that hydrogen peroxide (H_2O_2) increases the resistance of wheat plants depending on

the biotrophic or the necrotrophic of phase life cycle: inhibits it's at the biotrophic faze cycle but promotes its transition to necrotrophic phase or development of necrotrophic pathogens.

The inoculation soybean by pathogens influenced on the content of hydrogen peroxide in different ways: its content increased at the inoculation of BCMV and *P. savastanoi* pv. *glycinea* 9190 and it decreased – at the inoculation *of X. axonopodis* pv. *glycines* 9192 and phytoplasma compared to control (fig. 3b). This may be due to different sensitivity of plants to these pathogens.



a b

Figure 3. Total phenolic content (a) and H_2O_2 content (b) in soybean leaves under the influence of various factors:1 – Cntrol; 2 – phytoplasmas; 3 – BCMV; 4 – 9190; 5 – 9192; 6 – VNHs; 7 – VNHs+phytoplasmas; 8 – VNHs+BCMV; 9 – VNHs+9190; 10 – VNHs+9192; 11 – GeNHs; 12 – GeNHs +phytoplasmas; 13– GeNHs +BCMV; 14 – GeNHs +9190; 15 – GeNHs +9192; 16 – CuNHs; 17 – CuNHs +phytoplasmas; 18 – CuNHs +BCMV; 19 – CuNHs +9190; 20 – CuNHs +9192; 21 – MoNHs; 22 – MoNHs +phytoplasmas; 23 – MoNHs +BCMV; 24 – MoNHs +9190; 25 – MoNHs +9192.

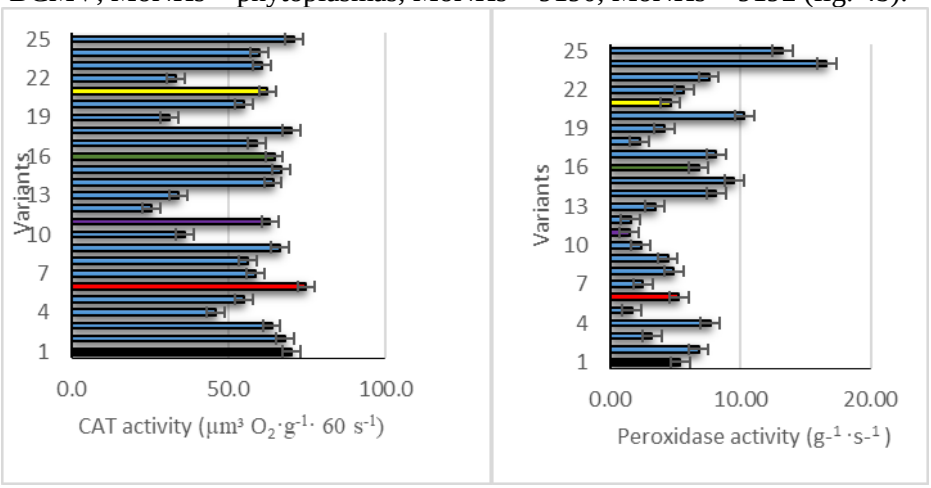
The largest decrease in the content of hydrogen peroxide in the leaves was observed on variants: the pre-treatment of MoNHs + BCMV, the pre-treatment of VNHs + phytoplasmas, the pre-treatment by VNHs + *P. savastanoi* pv. *glycinea* 9190, the pre-treatment both GeNHs, CuNHs with inoculation of *X. axonopodis* pv. *glycines* 9192.

It has been shown that, especially, flavonoids and phenylpropanoids are oxidized by peroxidase, and act in H_2O_2-scavenging, phenolic/AsA/POD system. But main enzymes that regulate the content of H_2O_2 in plants are catalase (CAT), ascorbate peroxidase (APX) and peroxidase type III (PRX) (Sharma *et al*., 2012; Smirnoff & Arnaud, 2019).

From a month after inoculation with phytopathogens increased in the CAT activity only in the variant pre-treatment of VNHs was shown. Known,

CAT activity may increase or decrease in depending on intensively and type of stress. It known that stresses that reduce the rate of protein turnover also may reduce CAT activity. (Sharma *et al*., 2012).

In all other variants decreased CAT activity except for the variants: inoculation with phytoplasma, VNHs + 9190, GeNHs + 9192, CuNHs + BCMV. While, on variant MoNHs + 9192 its value was equal the control level (fig. 4a). The peroxidase activity increased on variants: phytoplasmas, 9190, GeNHs + 9190, GeNHs + 9192, CuNHs, CuNHs + phytoplasmas, CuNHs + 9192, MoNHs + BCMV, MoNHs + phytoplasmas, MoNHs + 9190, MoNHs + 9192 (fig. 4b).



a b

Figure 4. CAT (a) and peroxidase activity (б) in soybean leaves under the influence of various factors: 1– Control; 2– phytoplasmas; 3– BCMV; 4 – 9190; 5 – 9192; 6 – VNHs; 7 – VNHs+phytoplasmas; 8– VNHs+BCMV; 9 – VNHs+9190; 10 – VNHs+9192; 11– GeNHs; 12– GeNHs +phytoplasmas; 13– GeNHs +BCMV; 14 – GeNHs+9190; 15– GeNHs +9192; 16– CuNHs; 17– CuNHs +phytoplasmas; 18 – CuNHs +BCMV; 19 – CuNHs +9190; 20 – CuNHs +9192; 21– MoNHs; 22– MoNHs +phytoplasmas; 23– MoNHs +BCMV; 24– MoNHs +9190; 25– MoNHs +9192.

The role of reactive oxygen species (ROS) as a secondary messenger in the responses of several plant hormones, including stomatal closure (ABA), root gravitropism (IAA), seed germination (GA), lignin biosynthesis (JA), programmed cell death (GA), hypersensitivity reactions (SA), and osmotic stress (SA), is known. Particularly, it is known that ABA-induced increase of H_2O_2 cytosolic levels cause to stomatal closure (Sharma *et al*., 2012). Abscisic acid (ABA) is a known phytohormone that regulates various aspects of plant metabolism, including adaptive responses to abiotic and biotic stresses as well as in the regulation of seed development and germination (Seo & Marion-Poll, 2019). The basal ABA is essential for proper chloroplast biogenesis, central metabolism, and expression of cell-cycle genes on the cellular level (Brookbank

et al., 2021). Auxin is also an important phytohormone involved in the regulation of plant growth and development, as well as in the signaling pathways of interaction between viruses and plants, affecting the development of the disease (Zhao & Li, 2021). It has been shown that plant viruses belonging to different families have developed different strategies to disrupt auxin signaling (for replication, systemic trafficking, transmission and development of viral symptoms), namely: (a) changing the intracellular localization of Aux/IAA, (b) preventing degradation of Aux/IAA by stabilizing or (c) inhibiting the transcriptional activity of ARF (Müllender *et al.*, 2021).

In our investigation was detected the decrease ABA content in soybean leaves at inoculation by phytoplasmas both of plants without treatment and plants with pre-treatment by NHs. The IAA content at inoculation by phytoplasmas decreased (by 85,0%) on variant without treatment and with treatment by GeNHs (by 25,9%), CuNHs (by 99,7%), MoNHs (by 94,4%), except for pre-treatment of VNHs, where concentration it increased by 85,5% (fig. 4). At inoculation by BCMV the content IAA and ABA increased by 5,8 and 75,3% accordingly. On variants with inoculation BCMV on background of pre-treatment by NHs both IAA and ABA content decreased in accordingly: VNHs (by 90,9 and 21,4%), GeNHs (11,1 and 21,4%), CuNHs (by 99,8 and 99,9%), MoNHs (71,9 and 92,3%) (fig. 5).

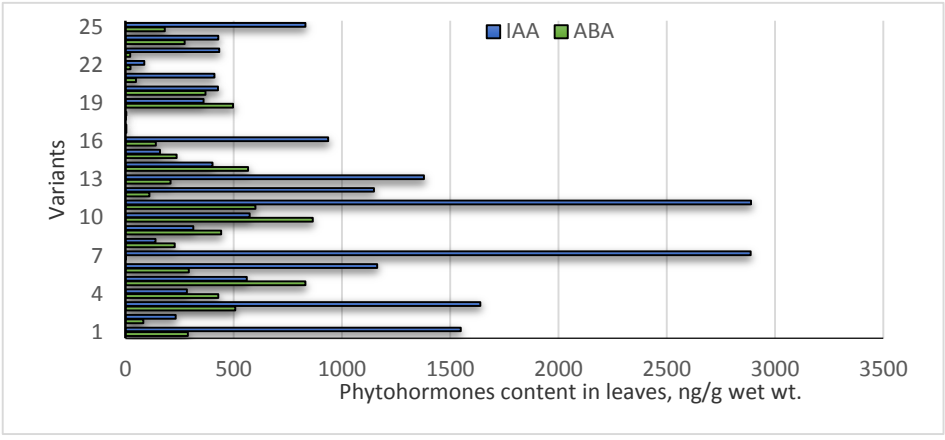


Figure 5. IAA and ABA content in soybean leaves under the influence of various factors: 1 – Control; 2 – phytoplasmas; 3 – BCMV; 4 – 9190; 5–9192; 6 – VNHs; 7 – VNHs+phytoplasmas; 8 – VNHs+BCMV; 9 – VNHs+9190; 10 – VNHs+9192; 11 – GeNHs; 12 – GeNHs +phytoplasmas; 13– GeNHs +BCMV; 14 – GeNHs +9190; 15 – GeNHs +9192; 16 – CuNHs; 17 – CuNHs +phytoplasmas; 18 – CuNHs +BCMV; 19 – CuNHs +9190; 20 – CuNHs +9192; 21 – MoNHs; 22 – MoNHs +phytoplasmas; 23 – MoNHs +BCMV; 24 – MoNHs +9190; 25 – MoNHs +9192

At inoculation with bacterial strains 9190 i 9192 the ABA content in leaves increased by 48,3 and 187,8% accordingly, hereat IAA content decreased

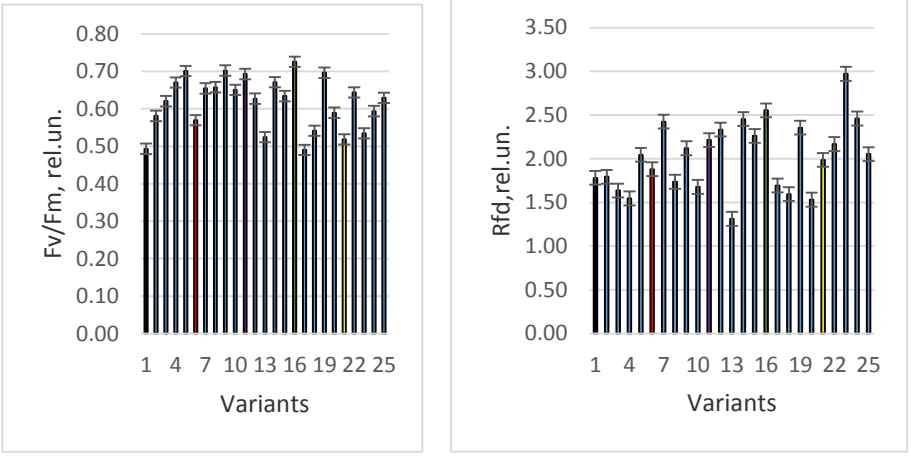
by 81,7 and 63,9% accordingly. At inoculation soybean plants by bacterial strains 9190, 9192 with pre-treatment VNHs, CuNHs and on variant with 9190+GeNHs similarly tendency changed to phytohormones content was shown. But in leaves on variants: GeNHs+9192 and MoNHs (+9190, 9192) content both IAA (by 89,7%) and ABA (18,1%) decreased.

It is known that ABA has remarkable impacts on plant defense against various pathogens, particularly bacteria, fungi, viruses. In particles, increased ABA at viral infection in plant suggested callose accumulation at PD or cell walls, RNA silencing. ABA generally affects several genes in the RNA silencing pathway, perhaps representing an important tool by which ABA tunes plant responses to different incentives (Alazem & Lin, 2017; Zhao & Li, 2021). Though ABA is active player in plant antiviral immunity, Pasin *et al*. (2020) reported, there the genus *Potyvirus* comprises a self-controlled RNA plant virus that may is evaded antiviral response of plants by controlled to the release of a downstream functional RNA-silencing suppressor and viral replication. It is also known that viral infection causes the simultaneous formation of synergistic or antagonistic phytohormones, disrupting hormonal induction, which correlates with the appearance of symptoms, active replication, movement, and systemic viral infection (Zhao & Li, 2021). It has been shown that at Arabidopsis–Pseudomonas interaction infection ABA double play – a positive role in pre-invasive stomatal immunity, but it plays a negative role in post-invasive PAMP triggered immunity and effector-triggered immunity. *P.syringae* TTSEs can upregulate ABA biosynthesis and/or signaling, potentially to suppress post-invasive immunity (Cao *et al*., 2011).

Thus, increased ABA content in leaves at BCMV-infection are indication suggested to promotion resistibility soybean plant. In same time, increased ABA at bacterial infected plants suggested to post-invasive exasperation of disease. However, we observed changes in phytohormonal status at pre-sowing treatment of plants NHs without infecting and with inoculation with phytopathogens, which indicates regulatory function NHs in plant metabolism.

The method of chlorophyll *a* fluorescence induction (CFI) is an important diagnostic tool in biological research, which reflects the influence of biotic and abiotic factors on the plant and its tolerance to stress (Lichtenthaler *et al*., 2007; Misra *et al*., 2012, Maxwell & Johnson, 2000; Rahimzadeh-Bajgiran *et al*., 2017; Stirbet *et al*., 2018; Guidi *et al*., 2019; McAusland *et al*., 2019; Zavafer *et al*., 2020; Huliaieva *et al*., 2018; Huliaieva *et al*., 2020a). First, we focused on determining changes in the F_v/F_m parameter is the maximum quantum yield of PSII. This parameter is widely used as an indicator of photosynthetic efficiency of plants, their response to stress. Its suppression is associated with photoinhibition of PSII activity due to inhibition of PS II repair (inhibition of protein synthesis) by ROS, formed by excessive both reduction of the primary electron acceptor of PSII plastoquinone Q_A, or recombination of charges between the acceptor and donor sides of PSII (Guidi *et al.*, 2019).

The investigation of changes in the parameters of the maximum quantum yield of PSII under the action of the studied factors was shown an increase in its value in almost all experimental variants, except CuNHs + BCMV, where its value was equal to the control (fig. 6a). Therefore, the increase in the value of the parameter is indicates the effective control and utilization of free radicals in the photosynthetic apparatus of experimental plants.



a b

Figure 6. FCI-parameters Fv/Fm (a) i R_{Fd} (b) in soybean leaves under the influence of various factors: 1 – Control; 2 – phytoplasmas; 3 – BCMV; 4 – 9190; 5 – 9192; 6 – VNHs; 7 – VNHs+phytoplasmas; 8 – VNHs+BCMV; 9 – VNHs+9190; 10 – VNHs+9192; 11 – GeNHs; 12 – GeNHs +phytoplasmas; 13 – GeNHs +BCMV; 14 – GeNHs +9190; 15 – GeNHs +9192; 16 – CuNHs; 17 – CuNHs +phytoplasmas; 18 – CuNHs +BCMV; 19 – CuNHs +9190; 20 – CuNHs +9192; 21 – MoNHs; 22 – MoNHs +phytoplasmas; 23 – MoNHs +BCMV; 24 – MoNHs +9190; 25 – MoNHs +9192

We determined another indicator parameter that is closely correlated with net CO_2 assimilation – the chlorophyll fluorescence decrease ratio – R_{Fd} (Lichtenthaler *et al*., 2007). The R_{Fd} parameter used in any investigation for evaluation different effects on the rate of photosynthesis: salt-induced changes in the photosynthetic apparatus (Stefanov *et al*., 2022), for an evaluation of hot air stressed on photosynthetic apparatus of maize (Park *et al*., 2016), for assessed in low and high temperatures resistance in a seasonal study of the acclimation of *Pterocephalus lasiospermus* (Perera-Castro *et al*., 2018), effect high-light stress on photosynthetic apparatus (Lichtenthaler & Burkart, 1999), in monitoring of salinity, temperature, and drought stress in grafted watermelon (Shin *et al*., 2021), for assessors of waterlogging stress on the photosynthetic apparatus of mulberry leaves (Rao *et al*., 2021) and other. It is worth noting that, in investigation of Perera-Castro *et al*. (2018) under influenced the lethal temperatures (LT50) on *Pterocephalus lasiospermus*, R_{Fd} resulted to be a more sensitive indicator

parameter for low and high temperature treatments, since thermic resistance estimated with R_{Fd} parameter was never higher than those estimated with F_v/F_m was shown (Perera-Castro *et al*., 2018).

Our studies revealed inhibition of R_{Fd} in the leaves of BCMV and 9190 infected plants. The photosynthetic rate (R_{Fd}-value) was equal to control variant at inoculation by phytoplasmas and lightly increased at inoculation with 9192 (fig. 6b). The R_{Fd}-value on variants with background of pre-sowing treatment by NHs increased in accordance: CuNHs (43,5%) > GeNHs (24,3%) > MoNHs (11,6%) > VNHs (5,6%).

The R_{Fd}-value changed in variants: in BCMV-infected soybean leaves on background of pre-treatment of VNHst was equal to control, at pre-treatment of GeNHs, CuNHs decreased and at pre-treatment of MoNHs it increased. In plants infected with phytoplasmas with background pre-treatment of VNHs, GeNHs i MoNHs the photosynthetic rate (R_{Fd}-value) increased, but at pre-treatment of CuNHs made tendency to decreased.

The Chl fluorescence decrease ratio (R_{Fd}) (net CO_2 assimilation) increased with infection by both bacterial pathogens with background pre-treatment of GeNHs and MoNHs. The R_{Fd}-value in soybean plant leaves on the background both pre-treatment of VNHs and CuNHs with 9190 infection – increased and with 9192 infection – decreased (see fig. 6b).

Thus, the infection soybean plants with phytopathogens in different ways influenced on Chl fluorescence decrease ratio (R_{Fd}): at BCMV and *P. savastanoi* pv. *glycinea* 9190 – decreased, but at inoculation by phytoplasmas equal to control and at inoculation with *X. axonopodis* pv. *glycines* 9192 lightly increased. This is indicating that R_{Fd}-value dependents on the phase of infection, plant resistance and the type of pathogen. In same time, pre-treatment of soybean by nanochelates contributed to increased R_{Fd}-value in variants of soybean plants without infected with pathogens. The R_{Fd}-value on background of MoNHs application with BCMV, bacterial, phytoplasmas infected soybean plants, on background pre-treatment of GeNHs at infection by both bacterial and phytoplasmas pathogens, on background pre-treatment of VNHs at infected with phytoplasmas have been increased. Thus, the use of nanochelates of Mo was the most effective.

CONCLUSIONS

The phenolic compounds content in soybean leaves increased in all variants: at pre-treatment by nanochelates, at inoculation plants by phytopathogens, at infected by phytopathogens on background pre-treatment by nanochelates.

The inoculation soybean with pathogens influenced on the content of hydrogen peroxide in different ways: its content increased at the inoculation of BCMV and *P. savastanoi* pv. *glycinea* 9190 and it decreased – at the inoculation of *X. axonopodis* pv. *glycines* 9192 and phytoplasma compared to control.

The CAT activity in tissue leaves increased only on variants pre-treatment VNHs. In same time, peroxidase activity of tissue leaves increased in variants: phytoplasmas and 9190 inoculation, GeNHs + 9190, GeNHs + 9192, CuNHs, CuNHs + phytoplasmas, CuNHs + 9192, MoNHs + BCMV, MoNHs + phytoplasmas, MoNHs + 9190, MoNHs + 9192.

The content ABA increased on variants: with BCMV-inoculation, *P. savastanoi* pv. *glycinea* 9190-inoculation, *P. savastanoi* pv. *glycinea* 9190-inoculation with pre-treatment of VNHs, CuNHs, GeNHs, *X. axonopodis* pv. *glycines* 9192-inoculation, *P. savastanoi* pv. *glycinea* 9190-inoculation with pre-treatment of VNHs, CuNHs, pre-treatment VNHs. But ABA content decreased on variants: BCMV-inoculation on background of pre-treatment nanochelates, *A.laidlawii*-inoculation, *A.laidlawii*-inoculation with pre-treatment of nanochelates, 9192-inoculation on background of pre-treatment of GeNHs, with inoculation both 9190, 9192 on background of pre-treatment of MoNHs.

The IAA content increased on variants: with BCMV-inoculation, at phytoplasmas-inoculation on background of pre-treatment VNHs. But IAA content decreased on variants: with 9190 and 9192 -inoculations (and with background pre-treatment of nanochelates), phytoplasmas-inoculation (with background pre-treatment by GeNHs, CuNHs, MoNHs), with BCMV-inoculation with background pre-treatment of nanochelates.

Besides the photosynthetic rate (R_{Fd}-value) increased at infection bacterial pathogens on background pre-treatment of GeNHs and MoNHs. The bacterial infection on background of pre-treatment by VNHs and CuNHs had a different effect on the R_{Fd}-value. It increased in *P. savastanoi* pv. *glycinea* 9190 infection plants and decreased in *X. axonopodis* pv. *glycines* 9192 infection plants.

Thus, pre-treatment by GeNHs, MoNHs, VNHs, CuNHs had regulatory effect on soybean plant metabolism and helping to increase its resistance to infecting with phytopathogens were shown.

In same time, using of pre-treatment of MoNHs before inoculation of soybean plants by pathogenic bacteria, BCMV and *A.laidlawii* promoted increase not only resistance to pathogens but increased the assimilation of CO_2.

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