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Expression of *PR* genes and genes of heat shock proteins in potato plants *in vitro* under infection with ring rot and heat stress

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Aim. The changes in expression of the HSP101, HSP60, and HSP17.8 genes in tissues of potato plants of varieties Lukyanovsky under *in vitro* heat treatment and infection with a ring rot pathogen *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*) were investigated. **Methods.** These changes were assessed at the transcript and protein levels. **Results.** It was shown that under heat treatment at 39 °C for 2 h, the maximum accumulation of HSP101 was observed. In control experiments, the plants of the two varieties showed neither synthesis of HSP101, HSP60 and HSP17.8 proteins nor expression of the *HSP101*, *HSP60* and *HSP17.8* genes. Infection without heat treatment induced HSP60 expression. Infection suppressed activation of HSP genes upon heat stress. Additionally, infection of potato plants by *Cms* caused an increase in transcription of *PR-2* and *PR-4* genes. **Conclusions.** Potato plants under biotic and abiotic stress, both independently and combined, activate the expression of a wide range of the protective proteins, including HSP and PR families.

K e y w o r d s: heat shock proteins, PR, potato, *Clavibacter michiganensis* ssp. *sepedonicus*

Introduction

Plants are exposed to various stress factors of both abiotic and biotic nature. To maintain homeostasis in plants under stress conditions, nutrient and energy redistribution between growth, development processes and protective reactions takes place [1].

Heat shock proteins (HSPs) are synthesized in plants in response to the increase in temperature to protect from damages induced by extremely high temperature. HSPs act as chaperones, preventing from protein denaturation and aggregation, and promoting the restoration of protein activity after the exposure to high temperature [2, 3]. HSPs (i) provide for temporary binding and folding facilitation of immature peptides in course of translation, (ii) disassemble oligomeric protein complexes, (iii) control biological activity of regulatory proteins (including the transcription factors), (iv) facilitate protein transport through plant cell membranes, (v) prevent against aggregation of partially denatured proteins due to intermolecular

interactions [4]. It is known that HSPs are synthesized not only in response to high temperature, but also to a wide range of stress factors, e.g., salinity and dehydration [2, 5].

HSPs are divided into several classes depending on molecular weight: HSP100, HSP90, HSP70, HSP60 and low molecular weight HSP (sHSP) [6]. They differ by the functions and capability to accumulate under stress and normal conditions. Protein HSP101 plays a leading role in the development of plant thermotolerance [7], therefore, investigation of the HSP101 synthesis level in plants is of interest. A number of HSPs accumulate in plant cell in the absence of stress, and their expressions only slightly changed under stress, for example, HSP60 involved in the folding of newly synthesized proteins [8]. In some cases, the HSP accumulation, in particular sHSPs, is observed under biotic stress [9–11].

The expression of the *HSP101* gene is sensible to various stresses [7]. Therefore, the protein is convenient for monitoring the influence of different stress factors on the *HSP* expression. HSP60 may be used as a control variant. HSP17.8 belongs to the sHSP family, so it can be induced under pathologies [6].

The available literary data on the influence of thermal action on the plants subjected to biotic stress are highly controversial. In some cases, a preliminary thermal treatment led to an increase in susceptibility of plants to infection. For example, the temperature rise suppressed the defense responses of *Arabidopsis thaliana* seedlings against *Pseudomonas syringae* pathogen infection [12]. An increased temperature at tobacco cultivation suppressed the hypersensitivity reaction (HR) and promoted the enhanced penetration of mosaic virus into

the plants [13]. The preliminary thermal impact at 36 °C for 30–120 min intensified the infection of barley with the powdery mildew (fungus *Erysiphe graminis* f. sp. *Hordei*) [14] and with the pathogen *Blumeria graminis* f. sp. *Hordei* [15]. Short-term heat treatment of soybeans at 44 °C induced their susceptibility to *Phytophthora* [16]. Similar situation was observed in case of infecting *Coffea arabica* L. with the pathogens *Colletotrichum kahawae* and *C. gloeosporioides*, and the development of susceptibility in this case correlated with the induction of HSP70 synthesis [17]. On the contrary, the 50 °C heat treatment for 30–60 s suppressed infecting barley seedlings with powdery mildew. Similarly, the heat treatment of potato plants at 40 °C for 48 h inhibited their infecting with powdery mildew [11].

Currently, various infection diseases of cultivated crops, including potato, are widely spread. Most of such infections are represented by pathogenic fungi and bacteria [18]. For example, in the Nordic countries and Canada, up to 50 % of the potato crops are regularly lost owing to the ring rot potato disease caused by gram-positive bacterium *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*). There is no information about the conditions provoking HSP synthesis in potato under heat stress and pathogenesis, or in case of superposition of the two stress factors. The data available are either indirect or quite contradictory. The tomato plant cultivation at 15 °C suppressed manifestation of the symptoms of *C. michiganensis* infecting [19]. In contrast, planting the wild potato species *S. acaule* at 15 °C stimulated colonization of the plants with *Cms*, and only at 21 °C – made the plants resistant to the pathogen [20]. However, in these experiments, the plants were grown (i) all the time at elevated

temperature, (ii) in the temperature conditions (21–23 °C), when there was no synthesis of HSPs in plants, and (iii) the species peculiarities were not taken into account [2].

So, a role of HSPs in a cell under combined effect of the two stress factors on plant – infection and heat shock – is not clear. The aim of our investigation was to study the character of variations in the content of different HSP families (HSP101, HSP60, and HSP17.8) and PR proteins in potato tissues *in vitro* under heat stress and infection with the ring rot pathogen.

Materials and Methods

Our investigations were conducted with the potato plants *Solanum tuberosum* L. of species Lukyanovsky *in vitro*. The plants of this species are characterized by susceptibility to a number of pathogens, including ring rot [21]. That is why this species was chosen to obtain a vivid picture of the gene expressions changes. Microcloning of *in vitro* plants was performed by grafting. The seed pieces (grafts) were placed into the agar-based nutrient Murashige and Skoog (MS) medium with the addition of sucrose 30 g/l, pyridoxine 1 mg/l, thiamine 1 mg/l, ferulic acid 1 mg/l, pH 5.8–6.0, at the depth of the internodes. The seed pieces were cultivated under factorostatic conditions at 26±1 °C, illumination 32W/m², photoperiod duration – 16 h.

Potato tissues were infected with *Cms*, strain Ac 14 05, obtained from All-Russian collection of microorganisms (Moscow). The bacterial culture was grown in agar-based medium placed in Petri dishes and containing dialysate solution of yeast extract (Sigma-Aldrich, Inc., USA) 10 g/l, glucose 15 g/l, agar-agar (Biotechnovatsiya, Inc., Russia) 10 g/l, CaCO₃ (Reachem,

Inc., Russia) 5 g/l, pH 7.0. The bacteria were cultivated in a thermostat at 25 °C, in darkness.

To reveal the temperature of maximum HSP synthesis the potato plants *in vitro* were heated in an air-drying thermostat during 2 h at 26, 35, 37, 39, 42, 45, 50 °C. Next, the total protein was isolated, and the content of HSP101 in the samples was determined by PAGE, Western blotting and staining nitrocellulose membranes with antibodies.

A series of the following experiments was conducted to investigate the effect of the ring rot infection upon the accumulation of the *HSP* gene transcripts and content of HSPs in potato plants. The potato plants were infected with *Cms* and after 48 h of incubation at 26 °C underwent heat stress (39 °C, 2 h). The changes in expression of the genes were registered at both levels – transcript accumulation and protein synthesis. The time of coincubation was chosen due to our previous observations [22], which showed that after 24 h of coincubation the bacteria penetrated into the root and stem zones of potato plants.

We also investigated the process of accumulation of the studied genes' transcripts under the conditions of heat treatment and infection with the pathogen using reverse transcription real-time PCR. Earlier, in order to exclude possible false positive results, PCR was conducted with primers of the potato *HSP* genes on the matrix of plasmid and chromosomal DNA of *Cms*. As known, in the process of co-evolution of the pathogen and the host plant, acquisition and transfer of genes from one organism to the other (horizontal gene transfer) may occur [23]. As a result of the PCR test conducted on the *Cms* DNA matrix, no products of amplification were found, that evidenced for the absence of

genetic sequences, to which the primers were selected, in the bacteria.

In experiments on the influence of *Cms* bacterium infection (in combination with heat shock) upon *HSP* expression, the suspension with *Cms* (titer = 1×10^9 CFU/ml) was introduced into the growth medium of potato plants *in vitro*. The plants were incubated under factorostatic conditions for 48 h. Next, the plants underwent heat treatment in an air-drying thermostat at 26 or 39 °C for 2 h, afterwards the total protein and RNA were separated. The time of coincubation was chosen according to our previous experiments on the intensity of potato plant colonization with *Cms* by plating tissue homogenates of root, stem and apical zones. It has been found [22] that 2 days after infecting the potato plants *in vitro* were completely colonized with the pathogen.

For isolation of the total protein we used 0.5 g sample of the total plant (leaves, stems and roots). A buffer was added to the sample to extract the protein (0.1 M Tris-HCl, 0.003 M DDS-Na, 0.001 M β-mercaptoethanol, 4 % polyvinylpyrrolidone, pH 7.4–7.6) and a 1 mM solution of phenylmethylsulfonyl fluoride (PMSF) for inhibiting proteases. A sample with quartz sand was thoroughly ground to powder in a mortar with liquid nitrogen. Coarse cellular components of the sample were removed by centrifugation at 15,000 rpm (Centrifuge Allegra 64 R, USA) for 15 min. The protein from the supernatant was precipitated with a three-fold volume of cooled acetone. The protein pellet was dissolved in the buffer prepared for the sample (0.625 M Tris-HCl, 0.008 M DDS-Na, 0.1 M β-mercaptoethanol, 10% glycerol, pH 6.8), incubated at 100 °C for 5 min and centrifuged at 5000 rpm (Centrifuge

MiniSpin, Germany) for 15 min. The samples obtained were boiled in the water bath at 95 °C for 3 min. The protein was diluted in the sample buffer with bromophenol blue (0.625 M Tris-HCl, 0.008 M DDS-Na, 10 % glycerol, 0.001 % bromophenol blue, pH 6.8) and used for electrophoresis fractionation of 10 μg of protein per track [24]. Electrophoresis in PAAG was conducted according to the modified Laemmli system, on the Mini-PRONEAN III Electrophoretic Cell, Bio-Rad (USA). 13 % PAAG was used to the end of simultaneous fractionation of high-molecular weight and low-molecular weight proteins. The protein molecular weight was determined by the path length of the corresponding protein in the gel with the aid of a standard set of protein markers (SibEnzyme). In order to determine the protein load upon the track and to equalize the volume of application of the samples, a special program Gel Analysis (Russia) was used.

The transfer of proteins onto the nitrocellulose membrane (Sigma, USA) was carried out by a "wet" technique in a special blotting device (Bio-Rad, USA). In this work, antibodies against HSP101 (Agrisera AS 07253, Sweden), HSP60 (US Biological H1830-77B, USA), HSP17.6 TTP 2 (Agrisera As 07255, Sweden), class I, were used. The antibodies against HSP17.6 specific for *Arabidopsis thaliana* were used because HSP17.8 (present in potato tissues) is homologous to HSP17.6 of Arabidopsis. Visualization of antibodies was conducted with the use of secondary antibodies conjugated with alkaline phosphatase (Sigma, USA), in the presence of BCIP and NBT (Sigma, USA). After that, the nitrocellulose membranes were dried at indoor temperature and scanned.

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Isolation of plasmid DNA (miniprep) from bacteria was carried out by the technique of alkaline lysis using polyethylene glycol (PEG 6000). Chromosomal DNA of the bacteria was obtained with commercial kits GenFlute Bacterial Genomic DNA Kit (USA). In PCR on the matrices of plasmid and chromosomal DNA we used ReadyMix Tag PCR Reaction Mix kits (Sigma, USA). PCR was conducted on the GENE CYCLER (Bio-Rad, USA) according to the following program: 1 cycle 94 °C – 5 min; 25 cycles of 94 °C – 1 min; 56 °C – 1 min; 72 °C – 1 min; 1 cycle 72 °C – 7 min. For electrophoretic separation of the amplification products, 1.2 % agarose gel in TAE buffer (242.2 g/L TRIS, 89.6 ml/L glacial acetic acid, 18.62 g/L disodium EDTA pH 7.6) was used with the addition of 5 µg/ml ethidium bromide. The voltage was 80 to 110 V. The gel was photographed in the ultraviolet spectrum with the gel-documentation system (Bio-Rad, USA).

Isolation of RNA from plant tissues was carried out with TRI-Reagent (Sigma-Aldrich, USA) according to the manufacturer's protocol. Since small volumes of the samples were used in the experiments, for RNA isolation the tissues of leaves were taken from the middle tier of the potato plants. Homogenization of the TRI-Reagent material was carried out in a TissueLyser II homogenizer (Qiagen, USA) for 2 min at the frequency of 30 cycles per second. The proteins were denatured with bromochloropropane (Sigma-Aldrich, USA). The nucleic acids were precipitated with 2.5 volumes of 96 % ethanol at – 20 °C during the night. Next, the samples were centrifuged at 14,000 g and +4 °C for 10 min, the residual nucleic acid pellet was dried at the room temperature, resuspended in 25–40 µl of deionized

sterilized water and used to synthesize the first cDNA strand. The amount (normalized multiplicity of expression) and purity of the isolated RNA were evaluated spectrophotometrically (NanoPhotometer NP80 spectrophotometer, Implen GmbH, Germany) via the optical density indicator at 260/280 nm. Furthermore, the quality of isolated RNA was monitored by the technique of electrophoresis in 1 % agarose gel under non-denaturing conditions.

In case of cDNA synthesis, 1 µg of RNA was taken for one reaction. When the first cDNA strand was created, RNA previously treated with DNA-ase I (Fermentas, Lithuania) was used as the matrix. Synthesis was conducted using oligo (dT) 15 primer and reverse-transcriptase Rever-tAid H Minus M-MuLV (Fermentas, Lithuania) according to the manufacturer's protocol (with minor modifications).

The sequences of potato genes *HSP17.8*, *HSP101*, *HSP60* and *EF-1α* (the reference gene) were taken from the Spud DB database (http://solanaceae.plantbiology.msu.edu/) and tested in Blast NCBI GenBank (http: // www. ncbi.nlm.nih.gov/genbank/). The following primers for the analyzed genes were chosen. For the *HSP17.8* gene, the pair *HSP17.8L*: TCCAAAGGAAGAGGTGAAGAAACC and *HSP17.8R*: CGACTCAGCATAAGACACAG GCA. For the *HSP101* gene, the pair *HSP101L*: AGGAGGTGGTTGGAGAAGAAAGT and *HSP101R*: CCCAGTAGCAGCATTCACAAGC. For the *HSP60* gene, the pair *HSP60L*: GTAG AGGGTGCTGTCGTGGT and *HSP60R*: GCTGTCCTAATCACTTTCACTGGAT. For the *EF-1α* gene, the pair *EF-1αL* ATGGTTGT TGAGACCTTTGCTGA and *EF-1αR*: GCACT GTTCACTTTCCCTTCTTCTG. For the *PR4* gene, the pair *PR4L*: GGCTGGACCGCTTTTTG

TGG and *PR4R*: CTGTTTCTTGTGTTCCTGT TCCTGT. For the *PR2* gene, the pair *PR2L*: GCTGCGATGGAACGAACAGGA and *PR2R*: CCAGGCTTTCTCGGACTACCT.

The reverse-transcriptase PCR was conducted in real time using a ready-made mixture of qPCR-mix-HS SYBR reagents (Eurogen, Russia) and the proper equipment C 1000 Thermal Cycler CFX 96 Real-Time System (Bio-Rad, USA). The volume of the reaction mixture was 10 μl. PCR was conducted according to the following protocol: warming up to 50 °C for 2 min, one denaturing cycle (95 °C, 5 min), 36 amplification cycles (95 °C, 20 sec – 60 °C, 30 sec – 72 °C, 30 sec).

Software MS Excel and Statistica was used for statistical analysis of the data obtained.

Results and Discussion

It is known that HSP101 plays a leading role in thermotolerance in plants [7], therefore, in the first series of experiments we have chosen the conditions, under which maximum synthesis of HSPs *in vitro* under heat treatment is observed in potato plants. The maximum amount of HSP101 in potato plants *in vitro* was found under heat treatment at 39 °C, 2 h (Fig. 1).

Then we have investigated the process of accumulation of transcripts of the studied genes under the conditions of heat treatment and infection with the pathogen. It was revealed that the greatest accumulation of HSP101 (Fig. 1) and a high content of the *HSP101* gene transcripts (Fig. 2) were observed at 39 °C. As far as the genes *HSP17.8* and *HSP60* are concerned, a multiple increase in the level of transcripts after heat treatment of the plants was revealed with respect to the control plants (without heat treatment). The smallest changes in the level of transcripts (do-

zens-fold) were observed for the gene *HSP60*, the largest changes (thousands-fold) – for the gene *HSP17.8* (Fig. 2). No valid expression level for all these genes was observed in the control plants and in case of infecting the plants with the phytopathogen. In the case of superposition of the two stress factors (heat treatment of the plants infected with *Cms*) a 4-fold decrease in the number of the *HSP101* gene transcripts was observed. Noteworthy, a level of the *HSP60* gene expression in potato tissues at heat stress and infection was substantially lower (18 c.u. (conditional units)) than that at heat treatment only (35 c.u.). The combined treatment of potato plants resulted in [an] increase of the number of the *HSP17.8* gene transcripts by 1.5 times as compared to heat treatment only.

At the next stage of the research the content of tested HSPs was measured. The data are presented in Fig. 3. Synthesis of HSP60 and HSP101 in the control plants was observed in trace amounts. Heat treatment of potato plants at 39 °C induced the synthesis of HSP101, HSP60 and HSP17.8. Infecting potato plants with *Cms* significantly induced the synthesis of HSP60, and insignificantly – HSP101 (Fig. 3). However, infecting potato plants under heat
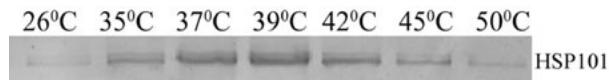

Fig. 1. The content of the HSP101 protein in potato tissues *in vitro* under various thermal effects in the range of 26–50 °C. Results of Western blotting. The stacking gel was prepared with a 13 % polyacrylamide concentration by weight, 10 μg of protein was applied to the track.

Data of a typical experiment are shown. The experiments were conducted in the form of at least 3 independent rechecks. 3 plants in each sample were used in order to extract protein (for immune blotting).
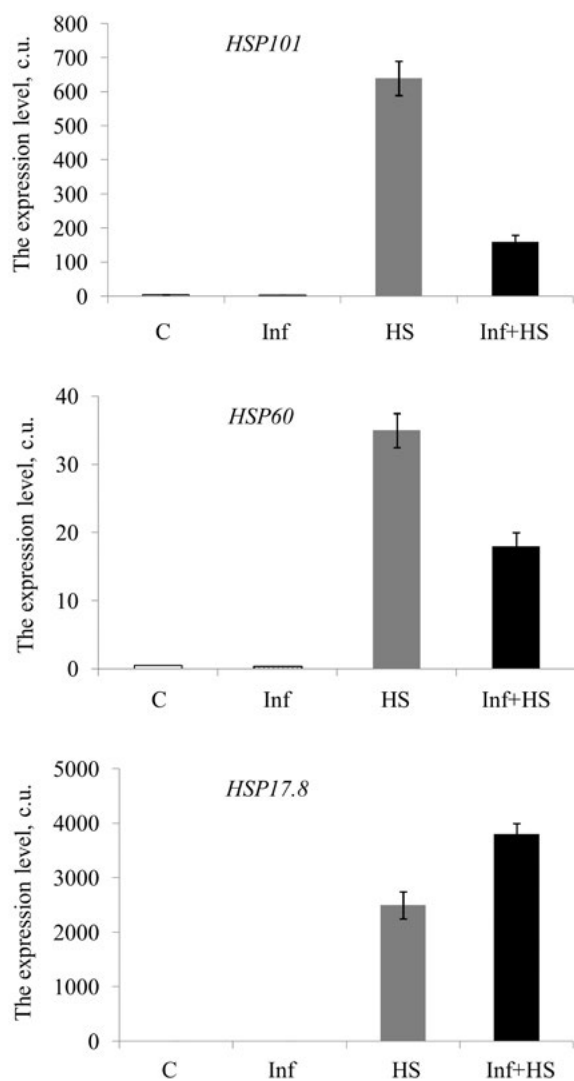
Fig. 2. Variations in the level of transcripts of the genes *HSP101*, *HSP60* and *HSP17.8* in potato leaf tissues *in vitro* under heat stress and infection.

Data of a typical experiment are shown. C – check samples; HS – heat shock conditions corresponding to heat stress (39 °C, 2 h); Inf – conditions of inoculation of potato plants with *Cms*; Inf+HS – conditions, when potato tissues are inoculated with *Cms* and subjected to heat stress (39 °C, 2 h). The experiments, which presumed isolation of RNA (for real-time PCR), were conducted in the form of at least 3 independent rechecks. 3 plants in each sample were used. M±SD, n=3.

stress at 39 °C enhanced the synthesis of HSP101 (Fig. 4). Such an impact also resulted in an increase of HSP17.8 and induction of HSP60.

In present work both the largest content of HSP101 in potato plants *in vitro* (Fig. 1, 3), and a large number of the *HSP101* gene transcript (Fig. 2) were revealed under heat treatment at 39 °C (2 h). All the available data only indirectly indicate the temperature range influencing the HSP synthesis in potato plants. Meanwhile, there are no clear data on the time and temperature ranges of thermal exposure inducing the HSP synthesis in potato plants [25–27]. So, it was revealed for the first time that the heat treatment of potato plants *in vitro* at 39 °C for 2 h induced maximum synthesis of HSPs. The temperature of maximum synthesis of HSPs in *A. thaliana* is known to be 37–38 °C [28], in *Sorghum bicolar* – 45 °C [29], in yeast *S. cerevisiae* – 37–39 °C [30].

Infecting potato plants *in vitro* with *Cms* actually did not influence the *HSP* expression at both the *HSP* gene transcript level (Fig. 2) and the protein level (Fig. 3). Infection induced the HSP synthesis only in trace amounts (Fig. 3). It is likely that, because of the lack of specific receptors to *Cms* pathogen, the pathogen susceptible potato plants react to *Cms* bacteria like to any other stress factors, and respond to this pathogen with a such nonspecific reaction as the HSP synthesis. Owing to the presence of specific *Cms* receptors, recognition of the pathogen takes place in the cells of resistant variety plants, and a cascade of signaling reactions is initiated [31]. This process leads to the regulation of gene expression and probably to obtaining synthesized proteins of direct antimicrobial activity.

However, the results of measuring the studied HSP gene transcripts indicate that the

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combination of *Cms* infecting and heat treatment of potato plants inhibits the thermal activation of the *HSP101* and *HSP60* gene expression. Meanwhile, heat stress stimulates the *HSP17.8* gene expression.

These results evidence that biotic stress is able to initiate and transform the protective response of plants to heat influence. The fact of such response correlates with the data related to variations in the expression level of the genes encoding pathogen-related (PR) proteins. In this work, it was shown that the expression levels of the genes *PR2* (1,3-β-glucosidase) and *PR4* (hevein-like protein) in tissues of the susceptible potato variety Lukyanovsky were amplified under the *Cms* infection, and the expression level of the *PR2* gene under biotic stress was twice higher than that of *PR4* (Fig. 4). The growth of the number of transcripts of these genes by 2–3 times is considered to be substantial. According to literary data, the infecting of tomato plants with *Clavibacter michiganensis* ssp. *michiganensis* (tomato pathogen) is known to amplify the contents of such proteins as 1,3-β-glucosidase (*PR2*), endochitinase (*PR3*), hevein-like protein (*PR4*), thaumatin/osmotin (*PR5*), cucumisin-like serine protease [32]. The induction of expression of the *PR* genes (*PR1*, *PR2*, *PR3*, *PR4*, *PR5*, *PR6*) was also shown in the tomato plants infected with bacteria *Pectobacterium carotovorum* [33].

Noteworthy, in our investigation, when potato plants were heat treated, the levels of transcripts of the *PR2* and *PR4* genes were also increased in comparison with contral samples. The *PR4* gene expression was 3-fold higher under heat stress compared to the control sample. There are the reports that expression of this gene in tissues of other plants can increase
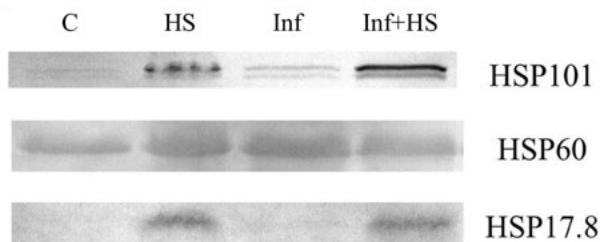

Fig. 3. Results of Western blotting of the HSP101, HSP60 and HSP17.8 proteins of potato plants under the conditions of heat exposure and infecting with *Cms*.

Data of a typical experiment are shown. C – control samples; HS – heat stress (39 °C, 2 h); Inf – inoculation of potato plants with *Cms*; Inf+HS –potato tissues are inoculated with *Cms* and subjected to heat stress (39 °C, 2 h).

Potato plants *in vitro* were inoculated with *Cms*. After 2 days of co-incubation, the plants were heat treated at 39 °C (2 h) and the content of HSPs was determined.

The experiments were conducted in the form of at least 3 independent rechecks. 3 plants in each sample were used in order to extract protein (for immune blotting).
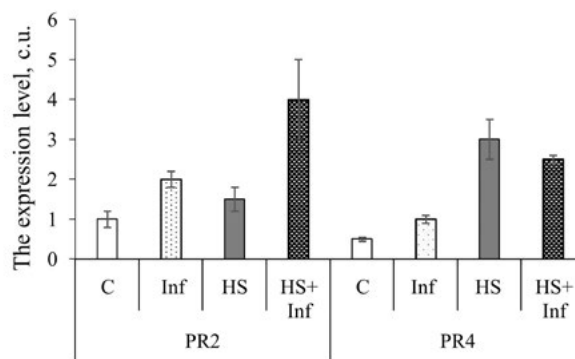

Fig. 4. Variations in the level of transcripts of the *PR-2* and *PR-4* genes in potato leaf tissues *in vitro* under heat stress and *Cms* infection.

Data of a typical experiment are shown. C – control samples; HS – heat stress (39 °C, 2 h); Inf – inoculation of potato plants with *Cms*; Inf+HS – potato tissues are inoculated with *Cms* and subjected to heat stress (39 °C, 2 h).

The experiments, which presumed isolation of RNA (for real-time PCR), were conducted in the form of at least 3 independent rechecks. 3 plants in each sample were used. M±SD, n=3.

under abiotic stresses. For example, it was observed in rice under ultraviolet treatment, temperature effect (4 °C, 12 °C), salinity, exposure to abscisic and jasmonic acids [34], and in arabidopsis – when treated with ethylene [35].

Under the combined influence of the two stress factors (*Cms* infection and heat treatment), there was a 4-fold growth of the *PR2* and *PR4* gene transcripts number (Fig. 4). The changes in the number of transcripts of the *HSP* genes may be explained by changes in the PR-protein genes expression. For example, under biotic stress, one can observe an increase in the *PR* gene expression (Fig. 4), while the *HSP* gene expression is not intensive. Under heat treatment, it is possible to observe some increase in the *HSP* and *PR* gene expression, which may be explained by intensification of the protective proteins synthesis as a non-specific protective response of the plant to stress factors. In case of superposition of two stress factors, the number of transcripts of the majority of analyzed *HSP* genes is smaller than that under only heat treatment; furthermore, on the contrary, the number of transcripts of the *PR* genes grows.

Conclusions

Therefore, for the first time we have shown the following. (1) High-temperature exposure (39 °C for 2 h) of potato plants cultivated *in vitro* provokes the greatest increase of the *HSP101* gene transcript level and maximum induction of the HSP101 synthesis. (2) *Cms* infecting does not affect the expression of *HSPs* and activates the expression of *PR2* and *PR4* genes in potato plants. (3) Heat stress combined with infection leads to an increase in the expression of all the genes studied, whereas the maximum expression of the *HSP17.8* and *PR2* genes

is observed. The obtained results indicate that the plants under stressful influences activate the protective programs, including a wide range of the protective proteins.

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Експресія генів *PR* та білків теплового шоку в картоплі *in vitro* при інокуляції кільцевою гниллю й тепловому впливі

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Мета. Дослідити зміну експресії білків теплового шоку БТШ101, БТШ 60 і БТШ 17.8 в тканинах картоплі сорту Лук'яновський *in vitro* при тепловому впливі та зараженні збудником кільцевої гнилі *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*). **Методи.** Зміну експресії вивчено на двох рівнях: кількості транскриптів та вмісту протеїну. **Результати.** Показано, що при тепловому впливі 39 °C протягом 2 годин *in vitro* спостерігається максимальне накопичення HSP101. В контрольних варіантах у рослин було не було відмічено ані синтезу білків HSP101, HSP60 й HSP17.8, ані утворення транскриптів генів *HSP101*, *HSP60* та *HSP17.8*. Зараження без теплового впливу індукувало експресію білку HSP60. Зараження пригнічувало активацію експресії БТШ при тепловому стресі. При зараженні рослин картоплі *Cms* підвищувалась кількість транскриптів генів *PR-2* та *PR-4*. **Висновки.** Рослини картоплі при біотичних і абіотичних стресах, як окремо, так і спільно, активують експресію широкого спектру захисних білків, включаючи білки сімейства HSP і PR.

К л ю ч о в і   с л о в а: білки теплового шоку, PR, картопля, *Clavibacter michoganensis* ssp. *sepedonicus*.

Экспрессия генов *PR* и белков теплового шока в картофеле *in vitro* при заражении кольцевой гнилью и тепловом воздействии

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Цель. Исследовать изменение экспрессии БТШ101, БТШ60 и БТШ17.8 *in vitro* в тканях картофеля сорта Лукьяновский при тепловом воздействии и заражении возбудителем кольцевой гнили *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*). **Методы.** Изменение экспрессии изучено на двух уровнях: количества транскриптов и содержании белка. **Результаты.** Было показано, что при тепловом воздействии 39 °C 2 часа в картофеле наблюдается максимальное накопление HSP101. В контрольных вариантах у растений не было отмечено ни синтеза белков HSP101, HSP60 и HSP17.8, ни образования транскриптов генов *HSP101*, *HSP60* и *HSP17.8*. Заражение без тепловой обработки индуцировало экспрессию белка HSP60. Заражение подавляло активацию экспрессии БТШ при тепловом стрессе. При заражении растений картофеля *Cms* повышалось количество транскриптов генов *PR-2* и *PR-4*. **Выводы.** Растения картофеля при биотическом и абиотическом стрессах, как по отдельности, так и совместно, активируют экспрессию широкого спектра защитных белков, включая белки семейства HSP и PR.

К л ю ч е в ы е   с л о в а: белки теплового шока, PR, картофель, *Clavibacter michoganensis* ssp. *sepedonicus*.