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Jasmonic and Salicylic Acids Influence Potato Anionic Peroxidase

Antonina V. Sorokan, Guzel F. Burhanova, Bulat R. Kuluev, Igor V. Maksimov*

Laboratory of Biochemistry of Plant Immunity, Institute of Biochemistry and Genetics, Ufa, Russia

Abstract

The effect of salicylic acid (SA; 1 μM) and jasmonic acid (JA; 0.1 μM) added to the culture medium of tube-grown potato (*Solanum tuberosum* L.) plants on the activity of genes encoding peroxidase M21334 and PR-1 and PR-6 proteins during the development of the defense response against *Phytophthora infestans* (Mont.) de Bary was studied. It was shown in our previous works, that *M21334* gene encodes anionic peroxidase with p*I*~3.5 which participates in lignin accumulation around infected sites and thereby in formation of mechanical barrier on the pathogen way into plant tissues. Transcription of this gene and enzymatic activity of its product rather than JA-dependent PR-6 gene were significantly activated by jasmonate and *P. infestans* inoculation. Combination of SA and JA was still more efficient than JA individually. Decrease of leaf area with symptoms of late blight on the leaves of plants treated by SA and JA indicated immune modulatory action of their composition.

Keywords

Salicylic Acid, Jasmonic Acid, *Phytophthora infestans, Solanum tuberosum*, Peroxidase

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1. Introduction

Terrestrial green plants are exposed to pervasive attack by diverse attackers, such as pathogens and herbivores. The vast majority of attackers are relatively specialized in terms of the strategy of nutrition. So, biotrophic pathogens exist in plant tissues for a long time and suppress immune response of host plants. Necrotrophic pathogens can kill plant cells and feed on dead tissue. It is known that the majority of pathogens combine properties of both biotrophs and necrotrophs in a varying degree. This group is named hemi-biotrophs. Over the past 470 million years plants have evolved effective inducible defence systems to fight with attackers realizing these diverse strategies [1]. But depending on the pathogen trophicity plants often activate radically different defence mechanisms. Characterization of the regulation of plant response to pathogens with different lifestyles is a focus of intense research among plant scientists. The role of plant hormones is widely discussed. It is considered that salicylic (SA) and jasmonic (JA) acids [2] induce, respectively, systemic acquired resistance (SAR) to biotrophs [3; 4] and induced systemic resistance (ISR) to phytophages (insects) and necrotrophs [5]. It is repeatedly reported in the literature that signals inducing SAR and ISR are strictly individual and often suppress the effects of each other. So, JA treatment many times activates peroxidase in the leaves of wild-type Arabidopsis, but in *cep1* mutant with constitutively increased SA production, this activation isn't observed [6]. This so-called hormone crosstalk, as is commonly believed, provides the plant with a powerful capacity to finely regulate its immune response to the invaders with different strategy. According to this point of view, combine addition of SA and JA lead to decrease of plant resistance to pathogens [7; 8]. However, data on additive or even synergistic effects of these

*Corresponding author
E-mail address: phyto@anrb.ru (I. V. Maksimov)

signal compounds in SAR and ISR are also known [9; 10]. Potato resistance to late blight is developed under the influence of both SA and JA [1]. In the early 1980's it was first established that SA is involved in the development of SAR, detected by the expression of the marker gene encoding PR-1 [3]. Some isogenes of class III peroxidases are also sensitive to SA [11]. JA is involved in ISR triggering by activation of genes encoding enzymes of phenolic metabolism, including some isoperoxidases [12] and proteinase inhibitors (for example, PDF1.2 and PR-6) [5]. In particular, JA activates two SA-insensitive isogenes of peroxidases in *Stylosanthes humilis* plants [13]. A diversity of responses of peroxidase genes during the development of SAR and ISR is evidently related to a broad spectrum of molecular forms of this enzyme. Identification of physiological functions of its particular isoforms is very important [12; 14] because peroxidases, especially anionic peroxidases, are often referred as key factors in disease resistance of plants [15; 16].

Earlier we observed that in transformed potato plants containing antisence-consruction of *M21334* gene production of anionic peroxidase with p*I* ~ 3.5 was reduced [17]. Yet phenolic compounds accumulated intensely in leaves of transformed *m21334* plants infected with the late blight agent, as our cytochemical experiments showed. It is noteworthy that phenol compounds concentrated in the guard cell area of stomata in infected plants. Healthy leaves displayed only a weak blue staining, far lower in intensity than in infected plants. Lignin fluorescence in cell walls of infected leaves of clone *m21334* was substantially lower than in wild type plants. Thus, *m21334* plants were not deficient in phenol compounds necessary for lignin synthesis. A lower lignin accumulation was apparently due to lack of anionic peroxidase with p*I* ~ 3.5, which catalyzes lignin synthesis. It should be noted that our transgenic potato line with inhibited synthesis of anionic peroxidase with p*I* ~ 3.5 did not dramatically differ in phenotype from control plants, but was highly sensitive to late blight. So, we have supposed that this peroxidase is one of the key players in plant resistance to the late blight pathogen.

The aim of this work was to determine the effects of SA and JA added together or individually to basal medium for potato growth on activity of the gene *M21334* and activity of its protein product during the development of the defense reaction against the late blight pathogen. Transcriptional activity of "marker" genes encoding PR-1 and PR-6 proteins during the development of defense responses against late blight pathogen was investigated for verification of principal role of SA and JA in the development of plant defense reaction.

2. Methods

2.1. Plant Material

Investigation was carried out with using tube-grown aseptic potato (*Solanum tuberosum* L.) plants of cv. Nevsky susceptible to late blight agent *P. infestans* (Mont.) de Bary, cultivated *in vitro* an illuminance of 4 klx with a 16-h photoperiod and 26/20°C (day/night). Potato plants were grown on a Murashige-Scoog basal medium with addition of 1 μM SA or 0.1 μM JA or its combination in the given concentrations chosen on the basis of [18].

Wild type and transformed *m21334*-deficient plants were used. *Agrobacterium tumefaciens* strain AGL0 was used for plant transformations. Agrobacterium used for co-cultivation carried the binary vector pCambia 1305.1, containing CaMV35S promoter, the gene of resistance to hygromycin (Hyg) and antisense-sequence of fragment of *M21334* gene, encoding a peroxidase. The *GUS* gene was cut out of a binary vector pCambia 1305.1 by digesting it with NcoI and PmlI restrictases, and the sticky ends were filled in with T4 DNA polymerase. Within the pCambia 1305.1 vector, the target gene fragment was cloned instead of the GUS reporter gene (1305.1/AntiPerM21334) Competent *A. tumefaciens* cells were electroporated by Micropulser (Bio-Rad, USA). Stem explants of tube-grown potato plants were cultured with suspension of transformed Agrobacterium and then were planted on the MS medium with hygromycin (100 μg/l) and Augmentin© (700 mg/l) for generation of calli. After 20 days calli were transferred on medium containing 1.5 mg/l trans-zeatin, 0,5 mg/l IAA and 0,1 mg/l ferulic acid for shoot regeneration. 25 % of explants regenerated. Shoots were planted on MS medium for cultivation of potato plants and propagation [17]. Soluble proteins of clones were extracted and IEF and gel is coloured on peroxidase activity.

2.2. Microbial Material

For plant inoculation high virulence strain 1.2 of *P. infestans* was used, which was kindly presented us by Prof. Yu.T. D'yakov (Faculty of Biology, Moscow State University). Non-infected plants were used as a control.

Before the experiment, pathogen aggressiveness was regained by sterile potato micro tubers (from tube culture) inoculation; 2-day- old infected tissue was placed on agar medium and was grown for 7 days. To obtain zoospores the surface of 7-day-old culture of *P. infestans* was poured with 10 ml of sterile distilled water and kept at 40C for 30 min and then at room temperature for another 30 min. Zoospores were counted in the Fuchs-Rosenthal chamber.

2.3. Plant Inoculation and the Assessment of Late Blight Symptoms Caused by *P. infestans*

After 30 days of culturing, some plants were infected by application of 5 µl of zoospore suspension (10^5 spore/ml, spore concentration was counted with the Fuchs–Rosenthal chamber). Leaves of control plants were treated with distilled water. Visual disease symptoms were followed for 7 days, and the degree of infection of each plant was evaluated using a 5-point scale. No symptoms corresponded to 0 points, the lesion of 1 to 25% of the leaf area corresponded to 1 point, of 26 to 50% - 2 points, of 51 to 75% - 3 points, and lesion of more than 75% - 4 points.

2.4. Plant Protein Extraction, Peroxidase Activity Assay and Isoelectrofocusing

Potato stems were removed from infected and non-infected plants and homogenized at 4°C using phosphate buffer (PB), pH 6.2, in a ratio of 1:5 (mg of plant tissues per ml of buffer) for plant proteins extracting on the 0.25, 1 and 2 dpi. The extracts were centrifuged at 16000 g for 10 min at 40 C, and the supernatants were used for isoelectrofocusing (IEF) and peroxidase activity tests. The concentration of protein in the supernatant was evaluated by the method of Bradford (1976).

The method based on the oxidation of o- phenylenediamine as a substrate for peroxidase was used. 75 µl of the plant extract, preliminarily diluted by 30 times, and 25 µl of 0.08 mg/ml of substrate were added to the wells of flat bottomed microtiter plates for immunoanalysis (Nunc, United States). On the 2 min after the addition of 25 µl of 0.43 mM H_2O_2, colour development was stopped by the addition of 50 µl of 4 N H_2SO_4. Optical density of tested samples was measured with the Benchmark Microplate Reader spectrophotometer (BioRad, United States) at 490 nm. Enzyme activity was showed in units per gram fresh weight of plant tissue.

IEF and peroxidase isoform spectrum analysis in protein extracts were performed with the AVGE-1 installation (Hiiu-Kalur, Estonia), using 7% PAAG and 2.5% isolytes (ICN, protein pI from 3.0 to 5.0).

The aliquots of the supernatant (5 µl) were dialyzed against 5 ml of distilled water and loaded on the surface of PAAG. Isoelectric points (pI) of potato peroxidases were measured using the diagnostic standards (Sigma, United States). After IEF, the presence of isoperoxidases in the gel was detected with 0.01% 3,3-diaminobensidine-HCl in 0.1 M phosphate buffer in the presence of 0.016% H_2O_2. PAAG were scanned with a Scanjet G4050 scanner (HP, United States). The images obtained were analysed using a Total Lab v. 2.01 software (UVP, United States).

Table 1. Primers used in this study.

Gene	Gene product	Primers	Sourse
M21334	Peroxidase	F: 5'-ttcgacaacaagtactacttcga-3'R: 5'-cggatctctcccgcgctgc-3'	[19]
AY050221	PR-1 protein	F: 5'-tgggtggtggttcatttcttgt-3'R: 5'-catttaattccttacacatcataag-3'	[20]
SGN-U313509	PR-6 protein	F: 5'-gggaaagaatatgctcaagttat-3'R: 5'-aattctccatcatcttccactg-3'	[21]
X55749.1	actin	F: 5'-gatggtgtcagccacac-3'R: 5'-attccagcagcttccattcc-3'	[22]

2.5. Total RNA Isolation and Semi-quantitative PCR (Sq-PCR)

Total RNA was isolated from potato shoots fixed in liquid nitrogen on the 0.25, 1, or 2 days after inoculation (dpi), using "TRIzol Reagent" according to manufacturer instruction (Invitrogen, USA). The concentrations of all samples were levelled by RNA optical density measurement using a spectrophotometer BioSpec-mini DNA-RNA-Protein analyser (Shimadzu, Japan). Reverse transcription (RT-PCR) was carried out in 20 µl total volume of a mixture containing 10 mg of total RNA, 1 µl of M-MuLV reverse transcriptase (Thermo Scientific, Lithuania), 9 µl of 1x reverse transcription buffer, 32 mM MgCl2, 1 µl of oligonucleotide primers, 5 µl of 1.25 mM dNTP. Primer annealing on the RNA template was carried out for 5 min at 65°C, and cDNA synthesis was performed on RNA template for 1 h at 37°C. The resulting cDNA was used in the amplification reaction. Sq-PCR was carried out in a TA4-PCR-01-Tertsik thermocycler (DNA Technology, Russia), using primers to sequences *M21334* gene encoding peroxidase and genes encoding PR-1 and PR-6 potato proteins (Table 1).

PCR products were separated in 7% PAAG, using a Mini-Protean II Electrophoretic Ct-II chamber (BioRad, US). For visualization of DNA after electrophoresis the gel was incubated for 10 min in a solution of ethidium bromide (0.5 mg/mL), viewed in the Gel Doc XR transilluminator (Bio Rad, US), photographed in a Gel Camera System, and the data were processed with a LabWorks 4.6 and TotalLab v. 2.01 (UVP, UK) computer programs. Amplicon sizes were determined using a GeneRulerTM 100 bp DNA Ladder (Thermo scientific, Latvia). Transcription of investigated genes was aligned to actin gene X55749.1 transcription and expressed in percent's.

2.6. Statistical Processing

Experiments were performed in triplicate with at least four recordings each; each treatment was performed with 20 tube plants. Analysis of variance (ANOVA) for experimental datasets was performed using Statistica version 7.0 (Stat Soft)/

Significant effects of treatment were determined by the magnitude of F-value (P=0.05). Enter your own text here. This section should contain sufficient information to allow others to repeat the research. For well-known methods and their minor variants, it is sufficient to summarise them and provide key references. It is also critical to provide detailed descriptions of the study design and the statistical analyses performed to evaluate all focal hypotheses.

3. Results

3.1. Visual Late Blight Symptoms

On the leaves of plants untreated with SA and JA, symptoms of late blight manifested on the second day as light-brown spots with yellow rims, which spread gradually and converted by the 7th day post inoculation (dpi) into light-brown necrotic lesions, corresponding to an average 2.7 lesion points (Table 2). Plant treatment with SA, JA, or their combination reduced the degree of the development of late blight. We observed only small dark-brown spots on the leaves in this case. Treatment with the mixture of SA and JA was the most efficient in disease suppression. It is essential that used SA and JA concentrations did not cause any visual changes in parameters of uninfected plants.

Table 2. Means (± SEM) of disease severity of late blight on leaves of potato plants treated by 1μM SA, 0.1 μM JA and their composition on the 7th dpi.

Treatment	Disease severity (0-4)
Water	2.50 ± 0.64
1μM SA	2.00 ± 0.51
0.1 μM JA	1.80 ± 0.46
1μM SA, 0.1 μM JA	1.20 ± 0.42

3.2. Activity of Peroxidase

Control level of peroxidase activity decreased during the experiment. In infected potato plants peroxidase activity was equal to control ones on the 0.25 and 1st dpi and decreased on 1/3 on the 2nd dpi (fig. 1). SA-treatment of plants reduced peroxidase activity, but in combination with JA the SA-effect wasn't observed, as well as in JA-treated plants. Infection of plant treated by SA, JA, or their mixture activated the enzyme equally in early period of the experiment (0.25 – 1 dpi). On the 2 dpi in SA-treated plants peroxidase activity was higher than in control ones, and in JA or SA+JA treated this parameter significantly decreased. The fact that in infected plants the influence of these compounds promoted increase of peroxidase activity on the 0.25 dpi agreed with the date about peroxidase as a "vanguard" of defense reaction.

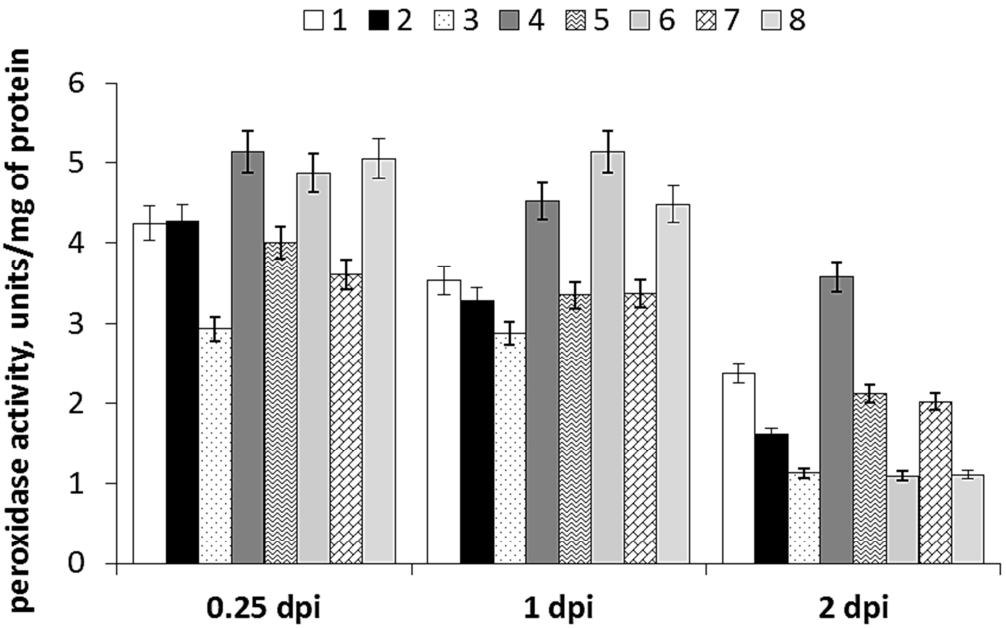

Figure 1. Influence of 1μM SA, 0.1 μM JA and their composition added to the basal medium for cultivation of tube-grown potato on peroxidase activity in health and infected by *P. infestans* plants.

1 – control; 2- *P. infestans* inoculation; 3 – SA treatment; 4 – SA + *P. infestans*; 5 – JA treatment; 6 – JA + *P. infestans*; 7 – SA + JA treatment; 8 – SA + JA + *P. infestans*.

3.3. Transcription Activity of Gene *M21334* of Peroxidase and Genes of PR-1 and PR-6 Proteins of Potato

It is well known that the treatment of plants by SA and JA triggers specific signalling pathways, which can be marked by transcription activity of genes, encoding PR-1 and PR-6 proteins respectively. As showed above, SA and JA induce the activity of peroxidase, participating in the development of plant resistance. To investigate the mechanism of the

participation of these compounds in regulation of potato resistance to late blight pathogen we analysed transcription activity of the genes encoding PR-1 and PR-6, specific for SAR and ISR respectively [19].

PR-1 transcripts didn't accumulate in non-treated with SA and JA health and infected plants (Fig. 2). We supposed that this fact could be explained by susceptibility of cv. under study. So, susceptible plants didn't develop an active defense reaction on the early stage of pathogen attack, and transcription of PR-genes could be late or absent. Its transcription significantly activated in SA-treated infected plants but not in JA-treated ones, as expected. But it's worth noting that combination of SA and JA 1.5 fold increased activity of *PR-1* gene after infection, and in this case its activity was maximal. Under the influence of JA transcript level of the *PR-6* gene triply activated both in health and infected plants, while SA activated accumulation of PR-6 transcripts after pathogen invading 3.8 times. Combination of JA and SA stimulated transcription of this gene but in a less degree than JA individually.

Earlier the peroxidase gene *M21334* of potato was defined by [18] due to the method of peptide sequencing. Using data about nucleotide sequence of the gene, encoding peroxidase M21334, we analysed transcription activity of this gene under the influence of SA and JA in health and infected by *P. infestans* potato plants. It was shown that the expression of *M21334* gene in infected plants increased significantly (by 40%) on the 1st dpi but on the 2nd dpi was rather equal to means in control non-treated health plants (Fig. 2). In infected plants treated by SA and JA transcript level of this gene was higher in compared with the control ones by 20-30% and 60-80% respectively.

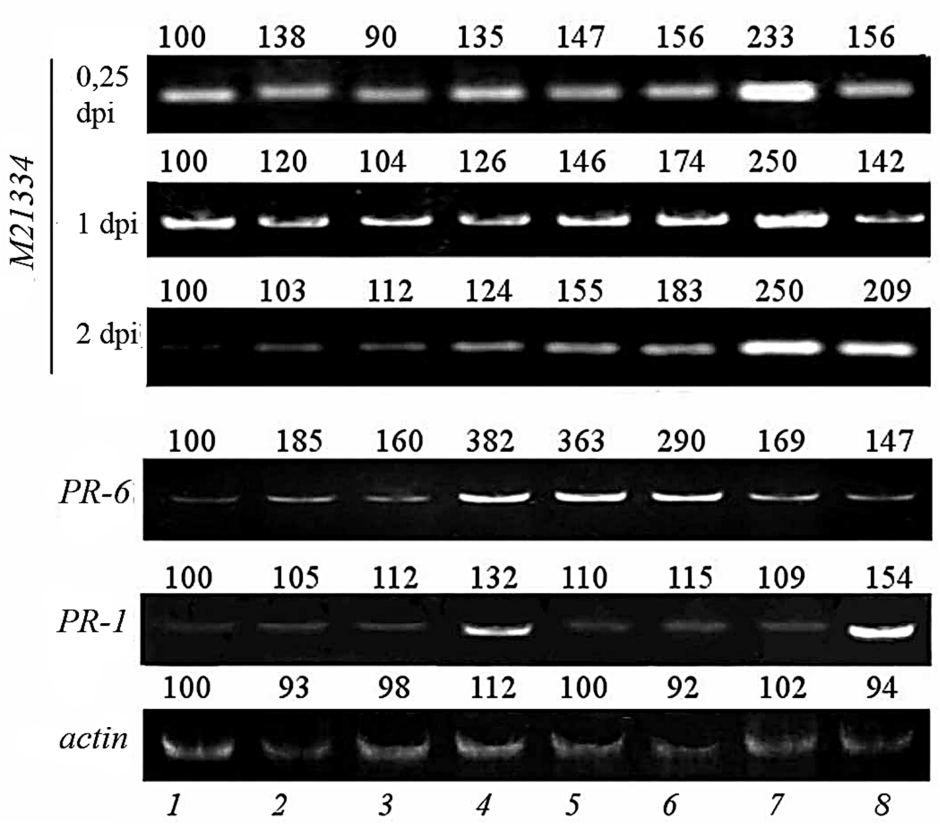

Figure 2. Influence of 1μM SA, 0.1 μM JA and their composition added to the basal medium for cultivation of tube-grown potato plants on transcriptional activity of *M21334* gene, encoding anionic peroxidase, in health and infected by *P. infestans* potato plants.

1 – control; 2 - *P. infestans* inoculation; 3 – SA treatment; 4 – SA + *P. infestans*; 5 – JA treatment; 6 – JA + *P. infestans*; 7 – SA + JA treatment; 8 – SA + JA + *P. infestans*. Levels of mPRA of *PR-1* and *PR-6* genes were investigated on the 2nd dpi, *M21334* – on the 0.25, 1st and 2nd dpi. Numerals means normalized amount of transcripts of the investigated genes (%) related to actin gene transcription.

3.4. Activities of Potato Anionic Peroxidases

Earlier we have shown that increase of plant resistance to this strain of *P. infestans* was accompanied with peroxidase activation [16]. It was demonstrated that both anionic isoperoxidases and cationic isoforms of this enzyme responded the infection. It was shown in Fig. 3, isoform with p*I* ~ 3.5 was sensitive to pathogen attack and influence of the examined. In infected plants their activity was 2-times higher than those found in control plants respectively. Activity of ~ 3.5 isoform in health and infected plants was 130 and 220% of those found in the water-treated control plants under SA

influence, and 150 and 250% under JA influence. It's worth noting that in infected plants the combination of SA and JA increased the activity of anionic peroxidases with p*I* ~ 3.5 more significantly than its components individually (Fig. 3).

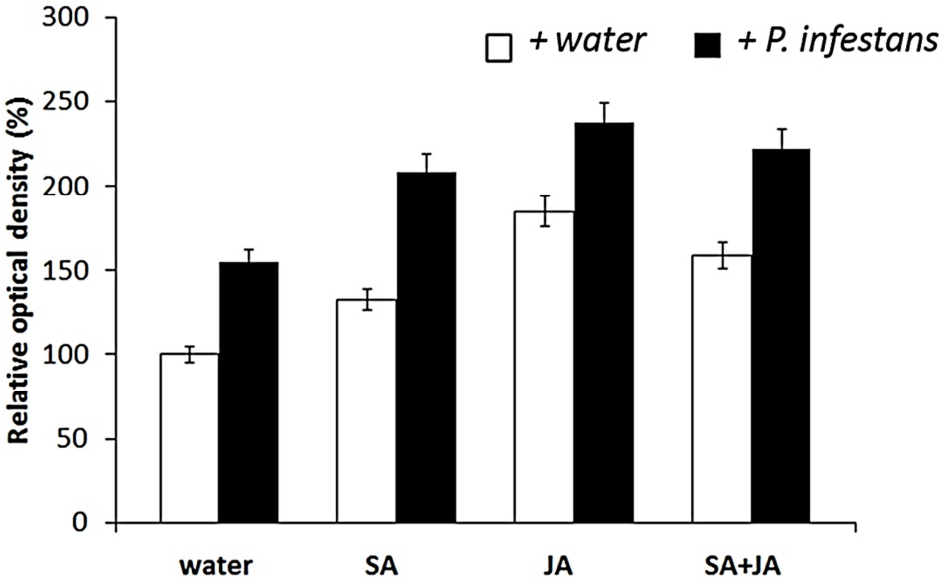

Figure 3. Influence of 1μM SA, 0.1 μM JA and their composition added to the basal medium for cultivation of tube-grown potato plants on anionic peroxidase activity in health and infected by *P. infestans* potato plants on the 2nd dpi.

4. Discussion

Alteration of peroxidase activity in plants exposed to different stress factors, especially pathogenic attacks, is one of the most universal and quickly detected reactions [23]. It is known that plant peroxidases characterized by a broad spectrum of isoforms, some of which are susceptible to various stressors [12]. So, anionic peroxidases come into notice due to their participation in development of the resistance to hyper- and hypothermia, pH changes, high salinity and pathogenic attacks [24]. Hyper expression of anionic peroxidase in tobacco led to increase of its resistance to feeding by insects [15]. Tobacco plants with antisense-construction of this gene had deficient lignin accumulation in sites under attack of phytophages [26]. This isoperoxidases participate in formation of suberine layer in damaged zone [27].

Earlier we showed that peroxidases which can interact with cell walls of oomycete *P. infestans* play an important role in defense reactions of potato against late blight pathogen. Similarly, among anionic isozyme pattern high-sensitive to the pathogen invasion isoenzyme were demonstrated [16]. In this paper we have shown that the anionic peroxidase p*I* ~ 3.5 of potato is activated by infection and influence of SA or JA (Fig. 4). This isoperoxidase is encoded by *M21334* gene, as we demonstrated with using of plants with antisense-construction of this gene. Treatment of plants by JA individually or in combination with SA stimulates transcription activity of *M21334* gene more significantly than SA, and it is important that combination of SA and JA promotes this parameter maximally (Fig. 2). It correlates with increase of anionic peroxidase activity and increase of plant resistance to late blight (Table 2). In other words in this case repeatedly described interference between SA- and JA- induced reactions [7] isn't observed. This data point out the priority of JA-dependent pathway in triggering of anionic isoperoxidase during the development of plant resistance.

Although *PR-6* gene is JA-inducible, its transcription activity significantly increases under SA-influence in infected plants (Fig. 2). Presumably, SA can sensitize genome of plants to the future infection and, possibly, to perception of endogenic JA accumulated during *P. infestanse* penetration. Interesting that *Fusarium graminearum* infection of *Arabidopsis* is accompanied with transcription both SA- (PR-1) and JA- (PDRF1.2) induced genes [5]. It should be taken into account that in the majority of papers reporting direct interference between SA and JA transgenic plants with substantial alterations of hormonal balance were used. In these plants some mutations might be pleiotropic [2]. It's well known that hormonal regulation of different processes in plants depends on ratio of hormones. However, it should be noted that a few papers report concentration correlations of SA-JA cross-talk [10; 28]. Thus, some of them show that antagonism is observed only in variants with using of high, exceeding physiological levels, concentration of this compounds, whereas low (~ 0.1 μM) concentration of JA stimulates transcriptional activity of SA-induced PR-1 gene [10]. Expression of JA-induces PDF1.2 gene of Arabidopsis is increased by injection of 350 μM SA into apoplast [7]. Higher concentrations of SA or JA inhibit transcriptional activity of

marker genes of ISR and SAR, respectively. This "fine-tuning" of SA-JA interaction, probably, provides flexible responses of plants on invasion of pathogens of different trophycal groups. Given the predominance of JA-signalling in the development of potato defence reaction against oomycete of *P. infestans*, reported in this paper, we can propose of predominance of necrotrophic components in its life cycle and effectiveness of ISR against its attacks. Besides, it has been shown by [28] that peptide Pep-13, comprised in this oomycete, induced accumulation of both endogenic SA and JA.

Thus, activation of izoperoxidase gene *M21334* transcription and activation of anionic peroxidases in potato can be a non-specific stress-reaction. This gene unlike genes, encoding PR-1 и PR-6, can be sensitive both SA and JA, and we can propose that anionic peroxidase participating in realization of resistance against both biotrophic and necrotrophic pathogens it must be emphasized that this property of peroxidase genes is very important during the development of the defense reactions to attacks of hemi- biotrophic oomycete *P. infestans*.

5. Conclusions

1 SA and JA reduced the intensity of lesion formation on the infected potato leaves, and their combination shown greater effect.

2 Participation of lignin-synthetizing isoperoxidase in the defence reactions of potato against *P. infestans* was demonstrated.

3 It was shown that the ability of JA to induce the expression of anionic peroxidase with p*I* ~ 3.5. plays an important role in its "immunostimulatory" action.

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Contributions by Authors

/a/ biotechnological manipulations with plants, measuring of peroxidase activity, izoelectrofocusing, translation of the article;

/b/ bioinformatics search, investigations of transcriptional activity of genes;

/c/ obtaining of *Agrobacterium* including antisense-construction;

/d/ discussion, preparation of the manuscript.

References

[1] Thaler J.S., Humphrey P.T., Whiteman N.K. (2012). Evolution of Jasmonate and Salicylate Signal Crosstalk. *Trends Plant Sci.* 17, 260–270.

[2] Glazebrook J. (2005). Contrasting Mechanisms of Defense against Biotrophic and Necrotrophic Pathogens. *Annu. Rev. Phytopathol.* 43. 205–227.

[3] Park S.-W., Kaimoyo E., Kumar D., Mosher S., Klessig D.F. (2007). Methyl Salicylate Is a Critical Mobile Signal for Plant Systemic Acquired Resistance. *Science.* 318, 113–116.

[4] Maksimov I., Troshina N., Surina O., Cherepanova E. (2014). Salicylic Acid Increases the Defense Reaction against Bunt and Smut Pathogens in Wheat Calli. *J. Plant Int.* 9, 306-314.

[5] Makandar R., Nalam V., Chaturvedi R., Jeannotte R., Sparks A.A., Shah J. (2010). Involvement of Salicylate and Jasmonate Signaling Pathways in *Arabidopsis* Interaction with *Fusarium graminearum*. *Mol. Plant–Microbe Interact.* 23. 861–870.

[6] Traw M.B., Kim J., Enright S., Cippolini D.F., Bergelson J. (2003). Negative Cross-Talk between Salicylate- and Jasmonate-Mediated Pathways in the Wassilewskija Ecotype of *Arabidopsis thaliana*. *Mol. Ecol.* 12, 1125–1135.

[7] Smith J.L., de Moraes C.M., Mescher M.C. (2009). Jasmonate- and Salicylate-Mediated Plant Defense Responses to Insect Herbivores, Pathogens and Parasitic Plants. *Pest. Manag. Sci*. 65, 497–503.

[8] Robert-Seilaniantz A., Grant M., Jones J.D. (2011). Hormone Crosstalk in Plant Disease and Defense: More than Just Jasmonate–Salicylate Antagonism. *Annu. Rev. Phytopathol*. 49, 317–343.

[9] Wees S., Swart E., Pelt J.A., van Loon L.C., Pieterse C.M.J. (2000). Enhancement of Induced Disease Resistance by Simultaneous Activation of Salicylate- and Jasmonate-Dependent Defense Pathways in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA*. 97, 8711–8716.

[10] Mur L.A.J., Kenton P., Atzorn R., Mierch O., Wasternack C. (2006). The Outcomes of Concentration-Specific Interactions between Salicylate and Jasmonate Signaling Include Synergy, Antagonism, and Oxidative Stress Leading to Cell Death. *Plant Physiol.* 140, 249–262.

[11] Almagro L., Gómes Ros L.V., Belchi-Navarro S., Bru R., Ros Barceló A., Pedreño M.A. (2009). Class III Peroxidases in Plant Defence Reactions. *J. Exp. Bot.* 60, 377–390.

[12] Cosio C., Dunand C. (2009). Specific Function of Individual Class III Peroxidase Genes. *J. Exp. Bot.* 60, 391–408.

[13] Curtis M.D., Rae A.L., Rusu A.G., Harrison S.J., Manners J.M. (1997). A Peroxidase Gene Promoter Induced by Phytopathogens and Methyl Jasmonate in Transgenic Plants. *Mol. Plant–Microbe Interact*. 10, 326–338.

[14] Mathé C., Barre A., Jourda C., Dunand C. (2010). Evolution and Expression of Class III Peroxidases. *Arch. Biochem. Biophys.* 500, 58–65.

[15] Dowd P.F., Johnson E.T., Pinkerton T.S. (2010). Identification and Properties of Insect Resistance-Associated Maize Anionic Peroxidases. *Phytochem.* 71, 1289–1297.

[16] Maksimov I.V., Sorokan' A.V., Cherepanova E.A., Surina O.B., Troshina N.B., Yarullina L.G. (2011). Effects of Salicylic and Jasmonic Acids on the Components of Pro/Antioxidant System in Potato Plants Infected with Late Blight. *Russ. J. Plant Physiol*. 58, 299–306.

[17] Sorokan A. V., Kuluev B. R., Burhanova G. F., Maksimov I. V. (2014). RNA Silencing of the Anionic Peroxidase Gene Impairs Potato Plant Resistance to *Phytophthora infestans* (Mont.) de Bary. *Molecular Biology,* 48, 709–717.

[18] Vasyukova, N.I., Chalenko, G.I., Gerasimova, N.G., Valueva, T.A., and Ozeretskovskaya, O.L. (2008). Activation of Elicitor Defensive Properties by Systemic Signal Molecules during the Interaction between Potato and the Late Blight Agent. *App. Biochem. and Microbiol*. 44, 213–217.

[19] Roberts E., Kutchan T., Kolattukudy P.E. (1988). Cloning and Sequencing of cDNA for a Highly Anionic Peroxidase from Potato and the Induction of Its mRNA in Suberizing Potato Tubers and Tomato Fruits. *Plant Mol. Biol.* 11, 15–26.

[20] Evers D., Ghislain M., Hoffmann L., Hausman J.F., Dommes J. (2006). A Late Blight Resistant Potato Plant Overexpresses a Gene Coding for α-Calactosidase upon Infection by *Phytophthora infestans*. *Biol. Plant.* 50, 265–271.

[21] Ozaki S., Ogata Y., Suda K., Kurabayashi A., Suzuki T., Yamamoto N., Iijima Y., Tsugane T., Fujii T., Konishi C., Inai S., Bunsupa S., Yamazaki M., Shibata D., Aoki K. (2010). Coexpression Analysis of Tomato Genes and Experimental Verification of Coordinated Expression of Genes Found in a Functionally Enriched Coexpression Module. *DNA Res.* 17(2), 105–116.

[22] Drouin G., Dover G.A. (1990). Independent Gene Evolution in the Potato Actin Gene Family Demonstrated by Phylogenetic Procedures for Resolving Gene Conversions and the Phylogeny of Angiosperm Actin Genes. *J. Mol. Evol.* 31, 132–150.

[23] Nenova V. & Bogoeva I. (2014). Separate and Combined Effects of Excess Copper and *Fusarium culmorum* Infection on Growth and Antioxidative Enzymes in Wheat (*Triticum aestivum* L.) Plants. *J. Plant Interact.* 9, 259-266.

[24] Leãoa G. A., de Oliveirab J. A., Felipea R., Farnesea F.S., Gusmana G.S. (2014). Anthocyanins, Thiols, and Antioxidant Scavenging Enzymes Are Involved in *Lemna gibba* Tolerance to Arsenic. *J. Plant Int.* 9, 143-151.

[25] Zhang L.X., Lai J.H., Gao M., Ashraf M. (2014). Exogenous Glycinebetaine and Humic Acid Improve Growth, Nitrogen Status, Photosynthesis, and Antioxidant Defense System and Confer Tolerance to Nitrogen Stress in Maize Seedlings. *J. Plant Int.* 9, 159-166.

[26] Spoel S.H., Koornneef A.,. Claessens S. (2003). NPR1 Modulates Cross-Talk between Salicylate- and Jasmonate-Dependent Defense Pathways through a Novel Function in the Cytosol. *The Plant Cell.* 15, 760–770.

[27] Keren-Keiserman A., Tanami Z., Shoseyov O., Ginzberg I. (2004). Peroxidase Activity Associated with Suberization Processes of the Muskmelon (*Cucumis melo*) Rind. *Physiol. Plant*. 121. 141–148.

[28] Halim V.A., Altmann S., Ellinger D., Eschen-Lippold L., Miersch O., Scheel D., Rosahl S. (2009). PAMP-Induced Defense Responses in Potato Require Both Salicylic Acid and Jasmonic Acid. *Plant J.* 57, 230–242.