Influence of Bacillus Subtilis and Stress Phythormones on the Content of H$_2$O$_2$, Expression of Protective Proteins Genes and Proteome of Potato Leaves When Infected with *Phytophthora infestans* Mont de Bary in Conditions of Soil Dry [†]

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Abstract: The effect of bacteria *Bacillus subtilis* strain 26D in combination with stress phytohormones—salicylic (SA) and jasmonic (JA) acids, on the content of H$_2$O$_2$, transcriptional activity of genes of pathogen-induced (PR) proteins (PR-1, PR-6, PR-9) and a change in the spectrum of potato (*Solanum tuberosum* L.) leaves proteins in connection with development of resistance to the causative agent of late blight—oomycete *Phytophthora infestans* (Mont.) de Bary against the background of a lack of moisture in soil have been investigated. Plants grown from microtubers on a soil substrate treated with *B. subtilis* suspension (10^8 cells/mL) and with a mixture of bacteria with SA (10^{-6} M), JA (10^{-7} M), SA + JA (1:1 ratio), then were infected with *P. infestans* zoospores (10^7 spores/mL) and cultivated under artificial drought conditions. Some of treated plants were left uninfected. A significant decrease in degree of *P. infestans* leaf infection was revealed under influence of *B. subtilis* treatment in combination with JA. An increase in potato resistance was mediated by a stimulating effect on the concentration of H$_2$O$_2$ and on the transcriptional activity of PR-protein genes in plant tissues. Using two-dimensional electrophoresis we have revealed the differences in the presence of 19 polypeptides in the pI range 4.0 to 9.0 and MW range 30 to 125 kDa. It was shown that treatment of *B. subtilis* plants most significantly changes the spectrum of individual proteins in both healthy and infected plants. Probably, stress phytohormones activate plant defense mechanisms aimed at the generation of H$_2$O$_2$ and preventing the change in the spectrum of protective proteins induced by symbiotic bacteria. The most significant factor determining the change in the proteome of *P. infestans* infected potato plants against the background of drought is the combination of *B. subtilis* with JA.

Keywords: *Solanum tuberosum*; *Phytophthora infestans*; *Bacillus subtilis*; salicylic and jasmonic acids; PR-proteins; proteome; systemic resistance

1. Introduction

Increasing plant resistance to pathogens and unfavorable agro-climatic conditions are topical issues in crop production. In this regard, the most promising are microbiological approaches and techniques that are based on using the potential of plants and soil microorganisms. The basis of

environmentally friendly preparations for protecting plants from stresses of a biotic and abiotic nature is plant growth promoting bacteria (PGPB) [1–3]. In this regard, highly effective bacteria of the genus *Bacillus* that retain their viability for a long time are especially attractive. Among the abiotic factors that form the productivity of plants, the availability of water is the most important [4]. Potato growth and development, the accumulation of crop tubers is highly dependent on soil moisture. This dependence in potatoes is much more pronounced than in other types of agricultural crops [5]. The mechanism of the protective action of biological products based on *Bacillus* spp. may be due to increased production of H_2O_2 and its mediated participation in the enhancement of gene expression of PR-proteins [6,7]. It is assumed that transcription factors that control gene expression in response to biotic stresses and drought are included in the same group [8]. Possibly, by promoting the generation of ROS, bacteria of the genus *Bacillus* induce the transmission of signals that trigger other defense mechanisms. It has been shown that treatment of plants with *Bacillus subtilis* promotes the development of systemic induced resistance (SIR), mediated by the action of jasmonic acid [9]. However, the formation of wheat resistance to pathogens under the influence of *Bacillus* can also develop through salicylate signaling by the type of systemic acquired resistance (SAR) [10]. Among the studied genes of PR-proteins that determine the development of SAR, the PR-1 gene was regulated by the SA-dependent signaling cascade [11]. SA responses are dependent on a regulatory protein called Non-Expressor Genes Associated with Pathogenesis 1 (NPR1). The NPR1 gene is activated via redox pathways through the accumulation of SA and is transferred to the nucleus; however, it does not bind to DNA directly, but acts through transcription factors. Exposure to necrotrophic pathogens phytophages and CRPP leads to enhanced biosynthesis of jasmonic acid (JA) and to the development of SIR, the developmental marker of which is expression of the PR-6 gene [12]. For a long time, it was believed that SA- and JA-mediated signaling pathways have an antagonistic effect on each other [13,14]. However, the separation of signaling pathways is very arbitrary and not final; new data appear on the presence of other possible alternative inductors and participants in the formation of plant resistance to stress [15,16]. It has been shown that exogenously applied salicylic acid, in addition to increasing resistance to biotrophic pathogens, enhances plant resistance to a number of physiological stresses [17], similar data were obtained during treatment with jasmonates [15]. Thus, despite the large amount of data on the increase in plant resistance to pathogens and abiotic stresses under the influence *Bacillus*, the mechanisms of modulation of defense reactions under their influence in combination with signaling molecules remain unclear.

The aim of this work is to study the effect of *Bacillus subtilis* bacteria in combination with signaling system mediators (salicylic and jasmonic acids) on the H_2O_2 content, transcriptional activity of PR-genes, and changes in the spectrum of proteins in *Solanum tuberosum* tissues upon infection with *Phytophthora infestans* and moisture deficiency.

2. Experiments

We used potato plants grown from microtubers of the susceptible variety Early Rose. The tubers were planted in containers with soil (TerraVita, high moor peat of varying degrees of decomposition, cleaned river sand, perlite, complex mineral fertilizer, vermicompost; pH 6.0–6.5) to a depth of 3–4 cm. Plants were grown on a light platform for 15 days, then they were treated with a suspension culture of bacteria *Bacillus subtilis* strain 26D from the commercial biological product Fitosporin-M (Bashinkom, Russia) with a final titer of 10^8 cells/mL, a mixture of bacteria with SA (10^{-6} M), JA (10^{-7} M), SA + JA, at the rate of 5 mL per 1 plant. The bacteria were cultured in a liquid LB medium for 24 h, then the suspension was diluted with distilled water to the required concentration.

On the 3rd day after infection, we began to create a moisture deficit by reducing watering. During drought, the relative humidity of the soil was 35–40%. For molecular biochemical studies, plant leaves were fixed 10 days after inoculation.

The development of the disease was assessed by the % of the affected area to the area of the leaf blade (damage degree) 7 days after infection. The leaves were photographed, the images were analyzed using the ImageJ software (NIH, USA).

2.1. Determination of H_2O_2 Content

Leaves were homogenized in 0.025 M phosphate buffer, pH 6.2, in a 1:3 ratio, and centrifuged for 20 min at 10,000× *g*. The supernatant was used to determine the H_2O_2 content. The H_2O_2 content was measured at 560 nm using xylenol orange. The reagent contained 0.074% Mohr's salt in 5.81% sulfuric acid and 0.009% xylenol orange in 1.82% sorbitol (in a ratio of 1:100).

2.2. Assessment of the Transcriptional Activity of Pathogen-Induced Protein Genes

RNA was isolated from plants using Trizol (Molecular Research Center, Inc., USA). To obtain cDNA based on mRNA of the studied samples, a reverse transcription reaction was performed using M-MuLV reverse transcriptase according to the supplier's protocol. The analysis of the accumulation of transcripts of the genes PR-1 (GenBank number AY050221), PR-6 (GenBank number JX683427) and PR-9 (GenBank number M21334) was carried out by quantitative real-time PCR on an iCycler iQ5 Real-Time PCR Detection System (Bio-Rad, USA) using the SYBR Green I intercalating dye (Syntol, Russia). Changes in gene transcriptional activity (estimate of the number of mRNA copies for each gene) were determined by the relative reference gene St_act ("housekeeping gene", actin, GenBank number X55749) using the "iCycler iQ5 Real-Time Detection System software" ("Bio-Rad", USA). The conditions for PCR were selected experimentally. Analysis of amino acid and nucleotide sequences was performed using the Lasergene software package from DNASTAR, Inc. (USA).

2.3. Two-Dimensional Electrophoresis

The leaf homogenate was resuspended in a buffer solution (0.7 M sucrose, 0.5 HEPES-KOH (pH 7.5), 0.1 M KCl, 2% mercaptoethanol, 1 mM EGTA, 1 mM PMSF, 0.1 mM sodium orthovanadate), incubated for 30 min at 4 °C. Proteins were extracted with a phenol solution according to the described method [18]. To 1 mL of a protein solution in acetone was added 2 mL of phenol saturated with Tris-HCl, the resulting mixture was incubated at −20 °C for 30 min, then centrifuged for 30 min at 200× *g*. Proteins from the phenolic phase were precipitated with a fourfold volume of 0.1 M ammonium acetate in ethanol at −20 °C for 10 h.

The resulting precipitate was washed three times with ammonium acetate and dissolved in a lysis buffer (8 M urea, 2 M thiourea, 1% CHAPS, 30 mM DTT, 20 mM Tris, 0.3% ampholyte solution.

Isoelectric focusing of proteins was performed on a Protean IEF system (Biorad, USA). To separate proteins by the isoelectric point, we used ready-made 7-cm strips (Biorad, USA), pH range 3–10. Before focusing, passive rehydration was performed for 12 h at 20 °C. Focusing was carried out at a voltage of 4000 V (20,000 V h) for 22 h, then the voltage was maintained at 500 V until the end of the process. After isoelectric focusing, the strips were kept for 15 min successively in solutions of 2% dithiothreitol and 2.5% iodoacetamide in buffer solutions with 25% glycerol, then washed in 0.025 M Tris-glycine buffer, pH 8.3.

SDS-electrophoresis was performed in 10% PAGE. Strip and marker proteins on filter paper were placed on a polyacrylamide gel and spilled in 1% agarose on Tris-glycine buffer solution. Electrophoresis was carried out at a voltage of 90–120 V, the gels were stabilized in 50% ethanol for 10 min, then stained with 0, 1% Coomassie G-250 solution.

2.4. Statistical Processing

The experiments included at least 3 biological repeats in the analysis of biochemical parameters and at least 15 repeats in the analysis of transcriptional activity. The histograms show sample means and their 95% confidence intervals.

3. Results and Discussion

3.1. Development P. infestans on Potato Leaves and the Content of H_2O_2 in Them during Treatment with Bacillus subtilis in Combination with SA and JA during Soil Drought

A comparative analysis of the development of the late blight pathogen on the leaves of a susceptible potato variety against the background of soil drought revealed differences in the growth rate of oomycete *P. infestans* between the control and variants with pretreatment with bacteria and signaling molecules (Figure 1a). Thus, in the control, the degree of leaf damage was 64%; pretreatment with *B. subtilis*, including in combination with SA and JA, significantly reduced the damage. Pretreatment with *B. subtilis* in combination with JA produced the most effective protective effect, which is consistent with the data obtained on test tube plants [19] and on *S. tuberosum* tubers [20].
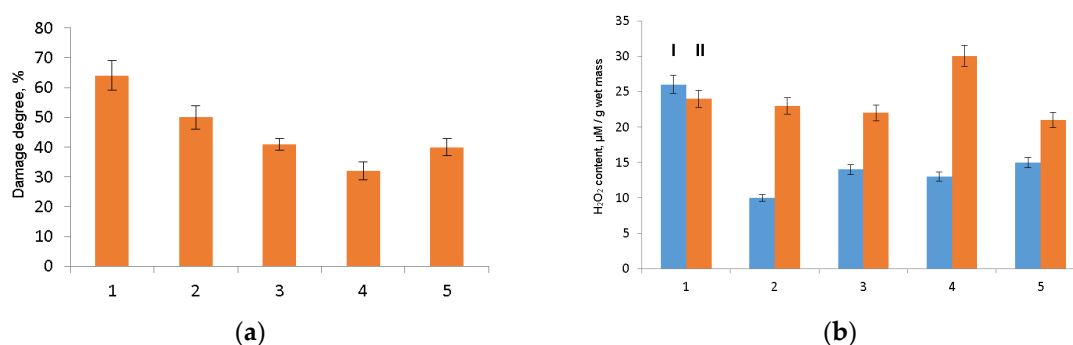

Figure 1. (**a**) The effect of treatment with *B. subtilis* and signaling molecules on the infection of potato leaves by the causative agent of late blight *P. infestans* during soil drought. (**b**) Influence of *B. subtilis* treatment and signaling molecules on the H_2O_2 content in potato leaves during *P. infestans* infection and soil drought. 1—control (infection with *P. infestans*); 2—*B. subtilis* + *P. infestans*; 3—*B. subtilis* + SA + *P. infestans*; 4—*B. subtilis* + JA + *P. infestans*; 5—*B. subtilis* + SA + JA + *P. infestans*. I—uninfected, II—infected plants.

Mechanisms for increasing of *S. tuberosum* resistance to *P. infestans* under the influence of the bacterial complex *Bacillus* spp. with signaling molecules could be associated with changes in the concentration of H_2O_2 in plant tissues [6,7]. Studies have shown that the concentration of hydrogen peroxide in pretreated plants was lower than in untreated plants when exposed to soil drought (Figure 1b). This may be due to the antistress effect of metabolites of *B. subtilis*. It is known that under the influence of *Bacillus*, the activity of antioxidant enzymes is induced and the level of proline in plants increases [21]. At the same time, in plants pretreated with bacteria and signaling molecules upon infection with *P. infestans* under conditions of soil drought, the level of hydrogen peroxide in the leaves increased markedly, especially when treated with *B. subtilis* + JA (Figure 1b). Changes in the concentration of H_2O_2 in plant tissues during pathogenesis can occur as a result of many metabolic processes, but to a greater extent this occurs as a result of changes in the activity of antioxidant enzymes.

H_2O_2 can be considered as the most important molecule involved in the transmission of intracellular signals that regulate gene expression and the activity of defense systems [22], including an increase in the concentration of calcium ions in the cytosol, which plays an important role in the transmission of signaling information to the plant genome [22]. It was shown that H_2O_2 is involved in the activation of gene expression of stress proteins [6]. Probably, the combination of *Bacillus* with signaling molecules enhances the generation of ROS generation and the transmission of signals that trigger the work of defense mechanisms that prevent the development of pathogens.

3.2. Influence of Bacillus subtilis Bacteria and Signaling Molecules on the Transcriptional Activity of PR-Protein Genes in Potato Plants during P. infestans Infection and Soil Drought

The systemic resistance of plants to diseases, which is nonspecific, is based on the expression of many protective genes [8]. It is of considerable interest to elucidate possible ways of induction of a protective response in potato plants under the influence of *B. subtilis* bacteria in combination with signaling molecules to the hemibiotrophic pathogen *P. infestans* and drought.

As can be seen at Figure 2, infection and treatment of *B. subtilis*, including in combination with signaling molecules, stimulated the accumulation of transcripts of the PR-1, PR-6, and PR-9 genes in potato plants. Treatment with *B. subtilis* and signaling molecules in all variants of the experiment led to a significant increase in the level of PR-6 gene transcription in infected plants as compared to uninfected ones. The highest level of expression of this gene was observed during treatment with *B. subtilis* in combination with JA of both infected and uninfected plants. Similarly, the PR-1 gene was most intensely expressed in plants treated with bacteria in combination with SA.
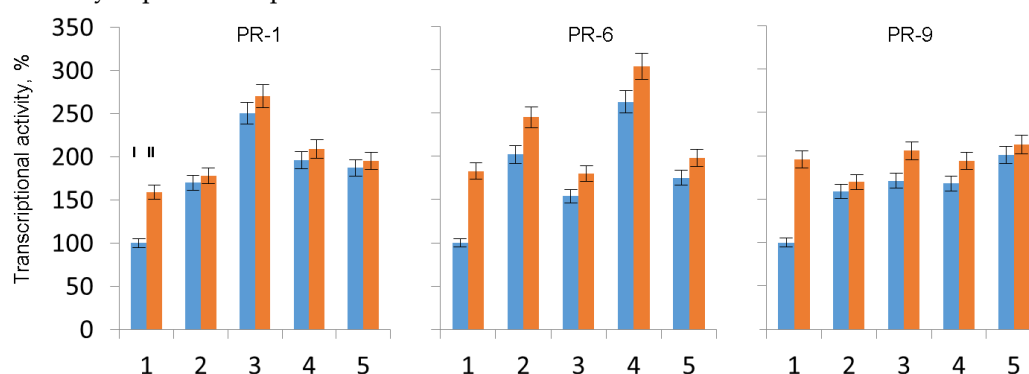

Figure 2. The effect of *B. subtilis* and signaling molecules treatment on transcriptional activity of PR-proteins genes. 1—control (infection with *P. infestans*); 2—*B. subtilis* + *P. infestans*; 3—*B. subtilis* + SA + *P. infestans*; 4—*B. subtilis* + JA + *P. infestans*; 5—*B. subtilis* + SA + JA + *P. infestans*. I—uninfected, II—infected plants. The activity of the tubulin gene is taken as 100%.

Treatment with *B. subtilis* in the absence of signaling molecules made a significant contribution to the increase in the expression level of the studied PR-proteins in uninfected plants. The combination of SA and JA with bacterial treatment had a generally smaller, and in some cases even a negative effect, for example, the expression of PR-6 decreased in the presence of SA. In infected plants, treatment with bacteria in the absence of SA and JA significantly increased the expression of PR-6 and decreased the expression of PR-9.

Expression of the PR-9 gene was significantly increased in infected plants as compared with uninfected plants when treated with *B. subtilis* in combination with both SA and JA. In infected plants, treatment with *B. subtilis* in combination with signaling molecules did not lead to an increase in PR-9 expression. In uninfected plants, the expression of PR-9 increased equally when treated with *B. subtilis* both in the absence and in combination with SA or JA, but the combined effect of SA and JA caused a greater increase in PR-9 expression.

3.3. Influence of Bacillus subtilis Bacteria and Signaling Molecules on the Proteome of Potato Leaves during P. infestans Infection and Soil Drought

One of the tools for identifying the specificity of changes in gene expression under the influence of plant infection with pathogens or exposure to signaling molecules and metabolites of endophytic bacteria can be the study of the proteome. By the method of two-dimensional electrophoresis of *S. tuberosum* leaf proteins, 19 proteins were identified, the presence of which in leaves differed depending on the variant of the experiment (Figure 3).

The largest amount of proteins was observed in the leaves of plants treated with *B. subtilis*, and four proteins (Mr/pI 100/6.5, 80/7, 40/7.5, 40/8) were present only in this variant of the experiment. The protein with Mr/pI 35/8 was observed only at treatment with *B. subtilis* + SA in the absence of JA,

the protein with Mr/pI 45/9 was observed both in the absence and in the presence of JA. The protein with Mr/pI 40/8.5 was present only in plants treated with *B. subtilis* and plants treated with *B. subtilis* together with both signaling molecules.
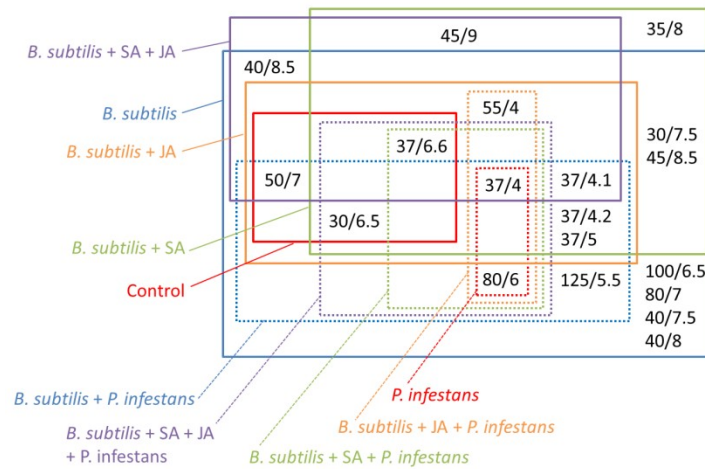

Figure 3. Potato leaf proteins (Mr/pI), present in different variants of the experiment.

During infection with *P. infestans*, the largest amount of proteins was also observed at treatment with *B. subtilis* in the absence of signaling molecules. The protein with Mr/pI 55/4, which was present in all uninfected plants, was observed during infection only at *B. subtilis* + JA treatment. Protein with Mr/pI 50/7 in infected plants was present only at *B. subtilis* treatment without SA nor JA.

As a result of cluster analysis, all plants infected with *P. infestans*, as well as uninfected and untreated plants, turned out to be the closest in the spectrum of proteins (Figure 4a). Among these variants, the strongest differences were observed in infected plants treated with *B. subtilis*. Plants treated with *B. subtilis* together with SA, JA, or their mixture were combined into another group with similar protein spectra. Uninfected plants treated with *B. subtilis* in the absence of SA or JA showed the strongest differences in the protein spectrum. Thus, treatment with *B. subtilis* in the absence of resistance stimulants significantly changes the spectrum of plant proteins, which is especially pronounced in non-infected plants. It can be assumed that infection with *P. infestans* and treatment with signaling molecules activates plant defense mechanisms, preventing changes in the spectrum of proteins caused by symbiotic bacteria.
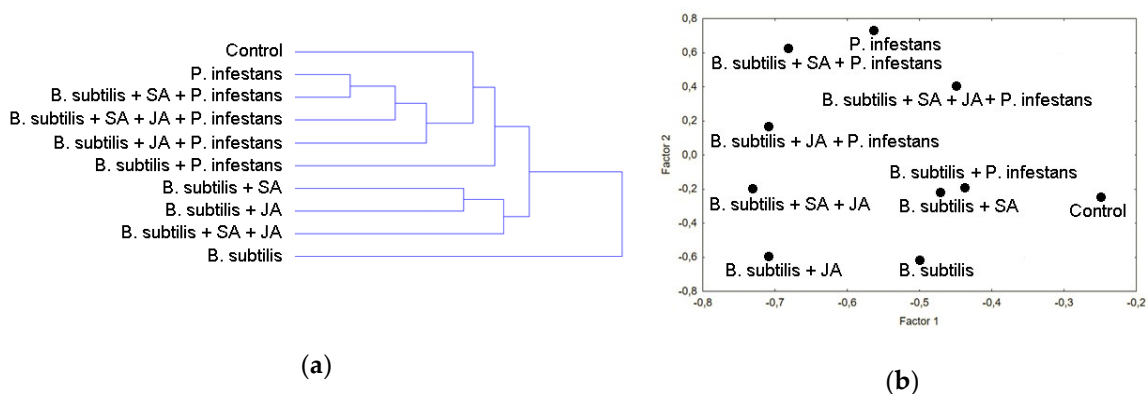

(**a**)

(**b**)

Figure 4. (**a**) Clustering of experimental variants according to the presence of various proteins in plant leaves. (**b**) Results of factorial analysis of experimental variants based on the spectrum of proteins in plant leaves.

In infected plants treated with *B. subtilis* mixed with signaling molecules, the greatest changes in the protein spectrum were caused by JA; plants treated with SA or a mixture of SA and JA differed to a lesser extent from the control infected plants. As is known, jasmonates regulate the processes of plant development and adaptation to external biotic and abiotic stressors [23].

According to the results of factor analysis (Figure 4b), it is not possible to unambiguously compare any of the treatments to the obtained factors. A significant correspondence between the presence of JA treatment with the first factor, and *P. infestans* infection with the second factor, can be noted, and the greatest difference from the control was observed during JA treatment. Thus, FA treatment and infection with *P. infestans* appear to be the main factors causing changes in the spectrum of plant proteins. In general, it can be concluded that infection with the late blight pathogen, treatment with endophytic bacteria and signaling molecules cause complex responses in plants, in which many proteins are involved.

4. Conclusions

Thus, the obtained research results indicate that the mechanism of activation of defense systems in potato plants by endophytic bacteria of the genus Bacillus and signaling molecules—salicylic and jasmonic acids—is mediated by the accumulation of H_2O_2 and an increase in the activity of PR-proteins. The development of protective reactions leads to significant changes in the spectrum and relative content of individual proteins in both healthy and infected plants. The revealed differences in the activation of the transcriptional activity of PR-protein genes under the influence of *B. subtilis* bacteria and signaling molecules suggest differential pathways for the formation of resistance to *P. infestans* in potato plants with their participation.

Author Contributions: L.Y. conceived and designed the experiments; V.T., G.B., E.C., and A.S. performed the experiments and analyzed the data; L.Y. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

JA	Jasmonic acid
PGPB	Plant growth promoting bacteria
PR-proteins	Pathogen related proteins
SA	Salicylic acid
SAR	Systemic acquired resistance
SIR	Systemic induced resistance

References

1. García-Gutiérrez, M.S.; Ortega-Álvaro, A.; Busquets-García, A.; Pérez-Ortiz, J.M.; Caltana, L.; Ricatti, M.J.; Manzanares, J. Synaptic plasticity alterations associated with memory impairment induced by deletion of CB2 cannabinoid receptors. *Neuropharmacology* **2013**, *73*, 388–396, doi:10.1016/j.neuropharm.2013.05.034.

2. Maksimov, I.V.; Veselova, S.V.; Nuzhnaya, T.V.; Sarvarova, E.R.; Khairullin, R.M. Plant growth-promoting bacteria in regulation of plant resistance to stress factors. *Russ. J. Plant Physiol.* **2015**, *62*, 715–726, doi:10.1134/S1021443715060114.

3. Verma, P.; Yadav, A.N.; Kumar, V.; Singh, D.P.; Saxena, A.K. Beneficial plant-microbes interactions: Biodiversity of microbes from diverse extreme environments and its impact for crop im-provement. In *Plant-Mi-Crobe Interactions in Agro-Ecological Perspectives*; Singh, D.P., Singh, H.B., Prabha, R., Eds.; Springer: Singapore, 2017; pp. 543–580, doi:10.1007/978-981-10-6593-4_22.

4. Vurukonda, S.S.K.P.; Vardharajula, S.; Shrivastava, M.; SkZ, A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiol. Res.* **2016**, *184*, 13–24.

5. Burton, W.G. Challenges for stress physiology in potato. *Am. Potato J.* **1981**, *58*, 3–14, doi:10.1007/BF02855376.

6. Pfannschmidt, T.; Bräutigam, K.; Wagner, R.; Dietzel, L.; Schröter, Y. Potential regulation of gene expression in photosynthetic cells by redox and energy state: Approaches towards better understanding. *Ann. Bot.* **2009**, *103*, 599–607.

7. Yarullina, L.G.; Sorokan, A.V.; Burkhanova, G.F.; Cherepanova, E.A.; Maksimov, I.V. Influence of chitooligosaccharides with different acetylation degrees on the H_2O_2 content and the activity of pathogenesis-related proteins in potato plants infected with Phytophthora infestans. *Appl. Biochem. Microbiol.* **2018**, *54*, 528–534, doi:10.1134/S0003683818050174.

8. Kuluev, B.; Mikhaylova, E.; Ermoshin, A.; Veselova, S.; Tugbaeva, A.; Gumerova, G.; Gainullina, K.; Zaikina, E. The ARGOS-LIKE genes of Arabidopsis and tobacco as targets for improving plant productivity and stress tolerance. *J. Plant Physiol.* **2019**, *242*, 1530–1533, doi:10.1016/j.jplph.2019.153033.

9. Veselova, S.V.; Nuzhnaya, T.V.; Maksimov, I.V. *Jasmonic Acid: Biosynthesis, Functions and Role in Plant Development*; Series Plant Science Research and Practices; Nova Science Publishers: USA, 2015; pp. 33–66, doi:10.1134/S0003683817030048.

10. Burkhanova, G.F.; Veselova, S.V.; Sorokan', A.V.; Blagova, D.K.; Nuzhnaya, T.V.; Maksimov, I.V. Strains of Bacillus ssp. regulate wheat resistance to Septoria nodorum Berk. *Appl. Biochem. Microbiol.* **2017**, *53*, 346–352, doi:10.1134/S0003683817030048.

11. Gimenez-Ibanez, S.; Solano, R. Nuclear jasmonate and salicylate signaling and crosstalk in defense against pathogens. *Front. Plant Sci.* **2013**, *4*, 72, doi:10.3389/fpls.2013.00072.

12. Durrant, W.E.; Dong, X. Systemic acquired resistance. *Annu. Rev. Phytopathol.* **2004**, *42*, 185–209, doi:10.1146/annurev.phyto.42.040803.140421.

13. Mur, L.A.J.; Kenton, P.; Atzorn, R.; Miersch, O.; Wasternack, C. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiol.* **2006**, *140*, 249–262, doi:10.1104/pp.105.072348.

14. Koornneef, A.; Pieterse, C.M.J. Cross talk in defense signaling. *Plant Physiol.* **2008**, *146*, 839–844, doi:10.1104/pp.107.112029.

15. Chanda, B.; Xia, Y.; Mandal, M.K.; Sekine, K.T.; Gao, Q.M.; Selote, D. Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. *Nat. Genet.* **2011**, *43*, 421–427, doi:10.1038/ng.798.

16. Fu, J.; Zhang, D.F.; Liu, Y.H.; Ying, S.; Shi, Y.S.; Song, Y.C. Isolation and Characterization of Maize PMP3 Genes Involved in Salt Stress Tolerance. *PLoS ONE* **2012**, *7*, e31101, doi:10.1371/journal.pone.0031101.

17. Hayat, Q.; Hayat, S.; Irfan, M.; Ahmad, A. Effect of Exogenous Salicylic Acid under Changing Environment: A Review. *Environ. Exp. Bot.* **2010**, *68*, 14–25, doi:10.1016/j.envexpbot.2009.08.005.

18. Vincent, D.; Wheatley, M.; Cramer, G. Optimization of protein extraction and solubilization for mature grape berry. *Electrophoresis* **2006**, *27*, 1853–1865, doi:10.1002/elps.200500698.

19. Maksimov, I.V.; Sorokan', A.V.; Chereoanova, E.A.; Surina, O.B.; Troshina, N.B.; Yarullina, L.G. Effects of salicylic and jasmonic acids on the components of pro/antioxidant system in potato plants infected with late blight. Russian. *J. Plant Physiol.* **2011**, *58*, 299–306, doi:10.1134/S1021443711010109.

20. Yarullina, L.G.; Kasimova, R.I.; Ibragimov, R.I.; Akhatova, A.R.; Umarov, I.A.; Maksimov, I.V. Qualitative and Quantitative Changes of Potato Tuber Proteome under the Influence of Signal Molecules and Infection with Phytophthora infestans. *Appl. Biochem. Microbiol.* **2016**, *52*, 71–78, doi:10.1134/S0003683816010154.

21. Lastochkina, O.V.; Aliniaeifard, S.; Seifikalhor, M.; Yuldashev, R.; Pusenkova, L.; Garipova, S.Plant growth-promoting bacteria: Biotic strategy to cope with abiotic stresses in wheat. In *Wheat Production in Changing Environments. Responses, Adaptation and Tolerance*; Singapore, 2019; pp. 579–614, doi:10.1007/978-981-13-6883-7_23.

22. Galvez-Valdivieso, G.; Fryer, M.J.; Lawson, T.; Slattery, K.; Truman, W.; Smirnoff, N.; Asami, T.; Davies, W.J.; Jones, A.M.; Baker, N.R. The high light response in Arabidopsis involves ABA signaling between vascular and bundle sheath cells. *Plant Cell* **2009**, *21*, 2143–2162, doi:10.1105/tpc.108.061507.

23. Glazebrook, J. Contrasting Mechanisms of Defense against Biotrophic and Necrotrophic Pathogens. *Annu. Rev. Phytopathol.* **2005**, *43*, 205–227, doi:10.1146/annurev.phyto.43.040204.135923.

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