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Increasing Potato Sustainability to PVY under Water Deficiency by *Bacillus* Bacteria with Salicylic Acid and Methyl Jasmonate

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Abstract: The protective effect of *Bacillus* bacteria against viruses can be significantly expanded by combination with salicylic acid (SA) or methyl jasmonate (MeJ). In soil water deficiency conditions, potato leaves were sprayed with *Bacillus subtilis* strain 47 combined with MeJ and MeJ + SA displayed a decrease in PVY and preservation of the shoot's growth. Signaling molecules with *Bacillus subtilis* mitigated the adverse effect of PVY under water deficiency by manipulating enzymatic/non-enzymatic antioxidant levels and activity in treated plants. The application mixtures increased the mass and number of mini-tubers during the microclonal propagation of plants. Treatment with bacteria in combination with signaling molecules significantly changed the content and phosphorylation status of a number of hydrolases, catalase, phosphorylase, annexin, and protease inhibitor. Based on the analysis of changes in the proteome, the key mechanisms mediating the induction of plant resistance to change in the tuber proteome aimed at enhancing the expression of protective protein genes that increase resistance to pathogens and abiotic stress.

Keywords: Potato virus Y (PVY); water deficiency; *Bacillus subtilis*; salicylic acid; methyl jasmonate; *Solanum tuberosum* L.; biochemical characters; mini-tuber productivity and quality

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1. Introduction

According to the FAO, the potato (*Solanum tuberosum* L.) is the fourth largest food crop in the world. It serves as an important source of carbohydrates, antioxidants, and vitamins and also as a raw material for starch production [1].

However, under production conditions, potatoes are affected by many viral and fungal pathogens. In addition, plants are simultaneously or sequentially exposed to adverse abiotic effects that can significantly impact the formation of protective reactions to stress factors [2–5].

Among the abiotic stresses that affect plant productivity, water scarcity is the most severe environmental factor for potato cultivation in the world. The growth and development of potatoes and the yield of tubers largely depend on soil moisture. Even brief exposure to water stress can have noticeable effects. The problem of water supply also arises in regions with sufficient but uneven during the growing season annual precipitation [6]. Virus diseases of potatoes are one of the main issues for the deterioration of the tuber quality and decrease in plant productivity. Currently, about 40 pathogenic viruses have been identified in potatoes [7,8].

One way of environmentally safe increasing plant resistance to adverse biotic and abiotic effects is the application of non-pathogenic rhizospheric bacteria (PGPR—plant

growth promoting rhizobacteria) [9,10]. It has been shown that PGPRs induce plant systemic resistance to various types of pathogens [11] and to a wide range of abiotic stresses, including drought, salinity, and heavy metals [12]. Induced Systemic Resistance (ISR) activated by PGPM is modulated by signal transduction networks in which salicylic acid (SA) [13,14] and jasmonic acid (JA) [15,16] can take part.

These signaling molecules emerge as important chemical signals that, in addition to governing internal processes, are instrumental in the multidirectional communication between plants and their associated microbial communities as the most (functionally) diverse entity of their living environment [17]. Information on their impact on microbiomes (particularly the endosphere), however, is only just emerging. SA is involved in the assembly of epiphytic and endophytic root microbial communities [17] and can modulate the physiological status of the whole plant–endophyte system and improve the biocontrol potential of endophytic strains [18].

The direct, independent of plant signals, effect of SA on microbes in bulk soil has been established. Collectively, these results suggest that SA may act via canonical signaling pathways, via interaction with other hormones, such as JA or directly on community members to promote or inhibit their growth. [17].

Exogenous JA application, used to activate JA signaling, has been shown to increase the alpha diversity of the Arabidopsis rhizosphere along with the enrichment of several potentially beneficial microbial taxa [16]. Results are not always consistent across plant species and tissues. A contrasting role of JA in epiphytic Arabidopsis leaf communities and wheat (*Triticum aestivum*) root endosphere community composition has been reported [17].

In this regard, it is important to study ways to increase the effectiveness of microbiological preparations to protect food crops from a wide range of pathogens by combining them with signaling molecules [19–21].

Effective adaptation of plants to biotic and abiotic stress factors depends on the formation of specific regulatory mechanisms. It is believed that the overlapping of different induced signaling pathways in plants promotes the minimizing of energy costs, creating a flexible signaling network, and the rearrangement of the protective response [22].

In this regard, the purpose of this work is to elucidate the mechanisms of the formation of complex resistance in potato plants to PVY under moisture deficiency and assessment the regulatory role of bacteria of the genus *Bacillus* in combination with signaling systems mediators (salicylic acid and jasmonic methyl ether) in modulating the plant's protective response on biotic and abiotic stress factors.

2. Materials and Methods

Materials

Bacteria. The gram-positive aerobic *Bacillus subtilis* (*B. subtilis*) 47 strain (brand name Karphil) from the collection of the Institute of Microbiology National Academy of Sciences of Belarus (https://mbio.bas-net.by/prod/karphil, accessed on 27 January 2023) was used.

Virus. We used the natural isolate Potato virus Y (PVY^0) that was obtained from the Research and Practical Centre of the National Academy of Sciences of Belarus for potato, fruit and vegetable growing (Republic of Belarus). The virus was maintained by propagating potato plants with tubers in an insect-proof greenhouse with an ambient temperature of 20–21 °C, illumination of 12,000 lux and photoperiod of 16/8 h (day/night). The presence of viruses in the sap was checked by ELISA, as outlined below. The proof of PVY infection was a growth disorder of potato plants, chlorosis and necrotic spots on the leaves, which led to the death of the lower leaves.

Plants. Microclonal propagated virus-free potato plants (*Solanum tuberosum* L.) cultivar Breeze of the Belarusian selection, which was obtained from Research and Practical Centre of National Academy of Sciences of Belarus for potato, fruit and vegetable growing (Republic of Belarus).

Experimental Design. The potato plant's microclonal propagation was carried out on an MS (Murashige–Skoog) agar media. The adaptation of in vitro-produced plantlets

to ex vitro conditions was accomplished in containers on peat-growing potting media with nutrients in the form of mineral salts. The plants were grown in an insect-proof greenhouse at a temperature of 20–21 °C, illumination of 12,000 lux and a photoperiod of 16/8 h (day/night) for a 3rd week and then treated by spraying the leaf surface: *Bacillus subtilis* strain 47 (Karfil, Minsk, Belarus) at 1×10^7 CFU, methyl jasmonate (jasmonic methyl ether (mixture of isomers), purity of 99.1% (HPC Standart, GmbH, Borsdorf, Germany), Germany–10^{-7} Mol/L and/or salicylic acid, purity of 98% (Toronto Research Chemicals, Toronto, ON, Canada), Canada–10^{-6} Mol/L.

Artificial infection of PVY was carried out by mechanical inoculation with donor plants sap [23] with modification using fine-grained sandpaper for wounding leaves 3 days after treatment with mixtures.

Water deficiency application. Modeling of soil water deficiency conditions was started 3 days after artificial infection of PVY of leaves and continued for 3 months until the appearance of mini-tubers. The moisture content of the peat growing media (40–45% soil water capacity) was achieved by reducing the watering of plants. In the control, the plants were grown at an optimal humidity of 80–85% of soil water capacity. The regulation of optimal soil humidity and water deficiency conditions was carried out by the weight method, as well as using a moisture meter TR 001 (China). Samples of leaves were collected 14 days after infection with the appearance of damage symptoms to the leaves. Mini-tubers were obtained 3 months after PVY infection.

ELISA. Detection of PVY was carried out by enzyme-linked immunosorbent assay (ELISA). The presence of viruses in plant tissue was assessed using ELISA kits produced by the Federal Potato Research Center named after A. G. Lorch (Russia). Fresh leaf samples (0.2 g) were crushed in a mortar with a buffer for samples and conjugates in a ratio of 1:10 (weight/volume). Diluted antibodies (1:500) specific rabbit IgG were adsorbed on the surface of the wells of polystyrene multi-well plates and incubated overnight at +(4–8) °C. After incubation, excess antibodies were washed out with 200 µL washing buffer per well 3 times. Then, each 100 µL of homogenized plant samples' extracts was added to the wells of the multi-well plate, covered with a lid and incubated overnight at +(4–8) °C. After incubation, non-binding material was washed off, and 100 µL of diluted conjugate (1:500) was added to each well; the plate was covered with a lid and incubated for 1 h at 37 °C. After thorough washing of the unbound conjugate, the enzymatic reaction was started by adding 100 µL of a freshly prepared pNPP (para-nitrophenylphosphate) substrate solution (1 mg/5 mL) to each well. The plate was incubated at room temperature for 40–50 min, after which the reaction was stopped by adding 50 µL of 3 M H2SO4 to each well. The results of ELISA were evaluated using an EIA analyzer photometer V-300 (Vityaz, Belarus) at λ = 492 nm.

Measurements of physiological traits. Shoots length was measured using a ruler.

The mini-tubers' weight was determined by weighing on analytical scales "Ohaus Adventurer RV64" (China).

Total dry matter. Plant samples were put in weighing bottles and kept in a drying oven for 4 h at 103 ± 2 °C. Then samples were taken out from the drying oven and kept in desiccators for 30 min to reach room temperature. After being weighed, samples were put again in a drying oven for 1 h to dry. These drying periods (1 h) were performed until reaching the constant weighing (7 h). The dry matter percentage (DM, %) of samples was expressed as DM = (dry biomass weight/wet biomass weight) × 100%. The dry biomass of each plant sample was expressed as wet biomass weight × dry matter percentage/100.

The starch content. The starch content in fresh tubers was calculated according to [24] the formula: Starch, % = 17.55 + 0.891 × C − 24.182, where C is the dry matter content in the mini-tubers, %.

Chlorophyll (Chl) pigment estimation. Chlorophylls were extracted from potato leaves using 96% ethanol as solvent. Briefly, 0.1 g of fresh leaves sample was homogenized in 2.0 mL of 96% chilled ethanol (v/v). The absorbance was taken at 655 nm, 649 nm and 470 nm in a Jasko V-630 spectrophotometer (Japan) for Chl a and Chl b, and carotenoids,

respectively, and chlorophyll contents (mg/g DW) were calculated using the equations suggested by [25].

Measurements of biochemical characters. Quantitative estimation of biochemical contents, including polyphenol oxidase (PPO), phenolic compounds, proline, soluble proteins, and activities of enzymes, such as ascorbate peroxidase (APX) and glutathione reductase (GR), were investigated by using Jasko V-630 spectrophotometer (Japan).

The protein content in the extract was determined by the Bradford method [26].

Determination of Proline. Total proline content was quantified according to Bates et al. with modifications [27]. Leaves 0.3 g was ground in 3% sulfosalicylic acid and centrifuged for 15 min at 12,000× *g*. Glacial acetic acid and ninhydrin reagent were added to an aliquot of the supernatant in a ratio of 1:1:1 and heat for 60 min at 90 °C on a thermal shaker with constant stirring (300 rpm). The optical density was measured at a wavelength of 515 nm. The proline content of each sample was obtained using a standard curve based on mg/g DW.

Determination of total phenolic content. The total phenolic content in leaves was determined by the method of Folin-Ciocalteu described by [28], using chlorogenic acid as standard. Wet samples of leaves 0.2 g were homogenized in 10 mL of 70% ethanol and incubated in a thermal shaker at 90 °C for 45 min. After cooling to room temperature, the supernatant was poured into clean 15 mL tubes. The rest of the plant material was once again filled with 1 mL of 70% ethanol, and the procedure was repeated; the extracts obtained were combined. For determination of the phenolic content, 0.1 mL of the sample extract was followed by 0.4 mL sodium carbonate solution and Folin–Ciocalteu reagent (1 mL). The phenol content was expressed as chlorogenic acid equivalents in 1 mg dry mass (DM).

Determining the polyphenol oxidase activity. Polyphenol oxidase (PPO) activity was measured using catechol as the exogenous substrate, according to Kumar et al. [29], with some modifications. A sample of leaves 0.2 g frozen in liquid nitrogen was homogenized in 5 mL of 50 mM K-Na-phosphate buffer (pH 7.2) in the cold. Then the homogenate was centrifuged for 20 min at 4500× *g* at 4 °C. The PPO activity was judged by the increase in optical density at 560 nm in the reaction mixture of K-Na-phosphate buffer (50 mm, pH 7.2), 0.02% paraphenylenediamine, 1% catechol, and supernatant. The change in optical density at 560 nm was recorded for 3 min. The enzyme activity was calculated in conventional units/min/mg protein.

Determination of ascorbate peroxidase activity. A sample of leaves 0.2 g frozen in liquid nitrogen was homogenized in 1.5 mL (0.1 M potassium phosphate extraction buffer (50 mM phosphate buffer, pH 7.8 + 1 mM EDTA + 10 mM sodium L-ascorbate). Centrifuge the homogenate at 12,000 g for 15 min in a temperature-controlled centrifuge at 4 °C. Ascorbate peroxidase (APX) activity was determined spectrophotometrically by recording the decrease in absorbance at 290 nm because of ascorbate oxidation in 2 mL of reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.1 mM hydrogen peroxide, 0.1 mM EDTA and a suitable amount of enzyme extract [30]. One unit of APX activity was assumed as the amount of the enzyme which oxidized 1 nmol ascorbate min mg protein.

Determination of glutathione reductase activity. Glutathione reductase activity was assayed by [31]. A sample of leaves 0.2 g frozen in liquid nitrogen was homogenized in 1.5 mL (0.1 M potassium phosphate extraction buffer (50 mM potassium phosphate buffer, pH 7.8 + 1 mM EDTA + 10 mM sodium L-ascorbate). Centrifuge the homogenate at 12,000× *g* for 15 min in a temperature-controlled centrifuge at 4 °C. To measure the activity of GR, 200 µL of the extract was added to 2100 µL of the measurement buffer (pH 7.8) and 75 µL NADPH (5 mM); the reaction was started by adding 100 µL of G-SS-G (10 mM), which was added immediately before the measurement. In the control sample, HSSG was replaced with a buffer. The kinetics of NADPH consumption was recorded for 5 min at 340 nm. The enzyme activity can be calculated using the molar extinction coefficient (absorbance of one molar solution) of 6.2 mM $^{-1}$ cm $^{-1}$. GR activity was assumed as 1 nmol/h/mg protein.

Ascorbic Acid Determination. The ascorbic acid concentration was assayed by [32] with modifications. Fresh mini-tubers of 0.5 g were homogenized with 0.6 mL of metaphosphoric acid. Then the extract was centrifuged at 4500 rpm. To measure the control sample, 0.200 µL of 0.025% 2,6-dichlorophenol indophenol sodium (DCPIP sodium salt) (12.5 mg +50 mL of warm water (45 °C) +1 drop of 0.01 n NaOH) was added to 2000 µL with 2% metaphosphoric acid and reactions were recorded for 35 s at a wavelength of 520 nm. In the experimental sample, 0.200 µL of 0.025% 2.6 DCPIP was added to 2000 µL of the extract, and the reaction against 2% metaphosphoric acid was measured for 35 s. The calculation was carried out according to the calibration curve by ascorbic acid (µg/mL).

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. 2 mL of phenol saturated with Tris-HCl were added to 1 mL of a protein solution in acetone, 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, Hercules, CA, 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 [33].

Statistical analysis. All biochemical and physiological experiments were performed with three biological and three analytical replicates. The data were presented as the mean ± standard error (SEM). The data were analyzed by Student t-test ($p < 0.05$) to determine any statistically significant differences, and treatments were compared using the LSD test ($p < 0.05$) with control (health plants) and PVY (infected with PVY plants); treatments were not compared with each other.

3. Results

All treatments reduced the viral infection degree in potato leaves under optimal growing conditions. PVY infection decreased most significantly by 24% by using *B.subtilis* + MeJ. The *B.subtilis* bacteria application did not affect the PVY infection under soil water deficiency. However, *B.subtilis* + MeJ and *B.subtilis* + MeJ + SA mixtures decreased of infection rate by 16% and 12%, respectively (Figure 1).

The PVY infection caused shoot growth inhibition and decreased length compared to the control in soil moisture deficiency conditions by 23.2%. Mixtures of *B.subtilis* with MeJ and *B.subtilis* with MeJ + SA provided the preservation of plant growth at the level of control uninfected plants (Figure 2).

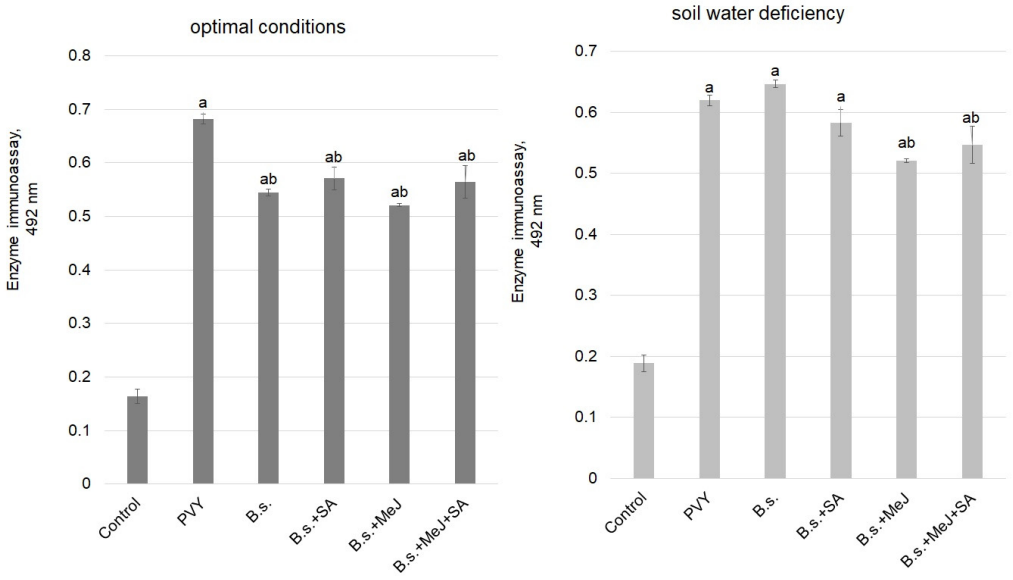

Figure 1. Detection of PVY by ELISA in the sap of potato leaves after application mixture of *B. subtilis* with SA and MeJ. Control—health plants; PVY—infected with PVY plants; B.s.—*B. subtilis* + PVY; B.s. + SA—*B. subtilis* + salicylic acid + PVY; B.s. + MeJ—*B. subtilis* + methyl jasmonate + PVY; B.s. + MeJ + SA—*B. subtilis* + salicylic acid + methyl jasmonate + PVY. a—indicates means that are statistically significantly different from the control, b—indicate means that are statistically significantly different from the PVY.

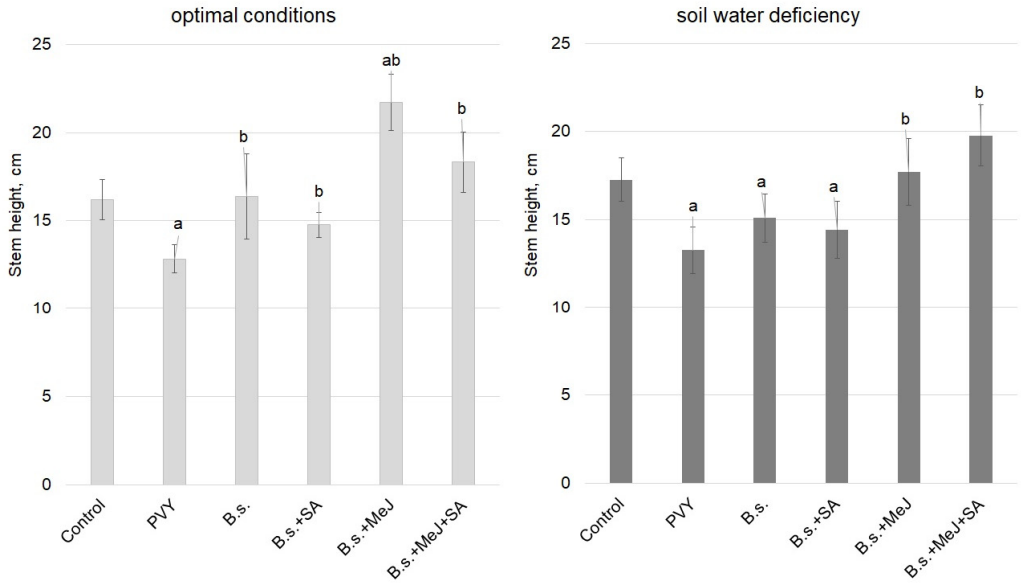

Figure 2. Effect of PVY infection and the mixture of *B. subtilis* with SA and MeJ application under soil water deficiency on potato stem height. Control—health plants; PVY—infected with PVY plants; B.s.—*B. subtilis* + PVY; B.s. + SA—*B. subtilis* + salicylic acid + PVY; B.s. + MeJ—*B. subtilis* + methyl jasmonate + PVY; B.s. + MeJ + SA—*B. subtilis* + salicylic acid + methyl jasmonate + PVY. a—indicates means that are statistically significantly different from the control, b—indicate means that are statistically significantly different from the PVY.

The content of photosynthetic pigments decreased in infected potato plants compared to control under soil moisture deficiency. However, all treatments increased the chlorophyll content, especially *B. subtilis* + MeJ and *B. subtilis* + MeJ + SA (Table 1).

Table 1. Effect of PVY infection and the mixture of *B. subtilis* with SA and MeJ application on total dry matter and chlorophyll contents.

	Optimal Condition		Water Deficiency	
Treatment	**Dry Matter %**	**Chlorophylls Content, mg/g DM**	**Dry Matter %**	**Chlorophylls Content, mg/g DM**
Control	12.8 ± 0.39	12.7 ± 0.61	13.7 ± 0.37	20.1 ± 0.61
PVY	13.5 ± 0.11 ^{a}	11.2 ± 0.71	13.7 ± 0.22	13.1 ± 0.71 ^{a}
B. subtilis	13.3 ± 0.12 ^{a}	11.5 ± 0.15	13.8 ± 0.24	15.5 ± 0.15 ^{ab}
B. subtilis + SA	13.4 ± 0.13 ^{a}	10.3 ± 0.56 ^{a}	14.5 ± 0.08 ^{ab}	15.7 ± 0.56 ^{ab}
B. subtilis + MeJ	12.2 ± 0.18 ^{b}	11.7 ± 0.34	15.3 ± 0.10 ^{ab}	16.8 ± 0.34 ^{ab}
B. subtilis + MeJ + SA	12.8 ± 0.25 ^{b}	10.9 ± 0.71	14.5 ± 0.25 ^{ab}	18.7 ± 0.71 ^{b}

a—indicates means that are statistically significantly different from the control, b—indicate means that are statistically significantly different from the PVY

The plant infection with the PVY under soil water deficiency led to a decrease in the proline content. The maximum proline accumulation by 89% in relation to control plants and almost 3 times compared to infected plants were noted in the mixture of *B. subtilis* + SA. Applying *B. subtilis* with MeJ + SA increased proline by 39% in relation to control plants and 2.2 times to infected ones. The proline content remained at the uninfected plants level with treatments of *B. subtilis* and *B. subtilis* + MeJ (Figure 3).

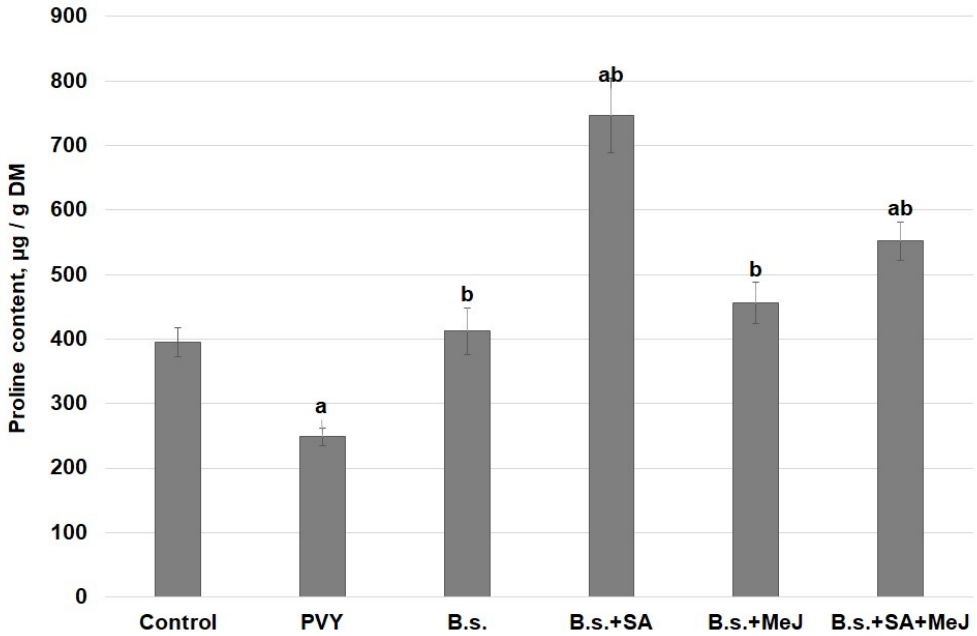

Figure 3. Effect of PVY infection and the mixture of *B. subtilis* with SA and MeJ application under soil water deficiency on proline content. Control—health plants; PVY—infected with PVY plants; B.s.—*B. subtilis* + PVY; B.s. + SA—*B. subtilis* + salicylic acid + PVY; B.s. + MeJ—*B. subtilis* + methyl jasmonate + PVY; B.s. + MeJ + SA—*B. subtilis* + salicylic acid + methyl jasmonate + PVY. a—indicates means that are statistically significantly different from the control, b—indicate means that are statistically significantly different from the PVY.

Viral infection was no effect on the accumulation of phenolic compounds with insufficient soil moisture supply. *B. subtilis* and a mixture of *B. subtilis* + SA contributed to a significant increase in their content; to a lesser extent, the accumulation of phenolic compounds was facilitated by the treatment of *B. subtilis* + MeJ. The application of the *B. subtilis* + SA + MeJ has not affected the phenolic compounds content (Figure 4).

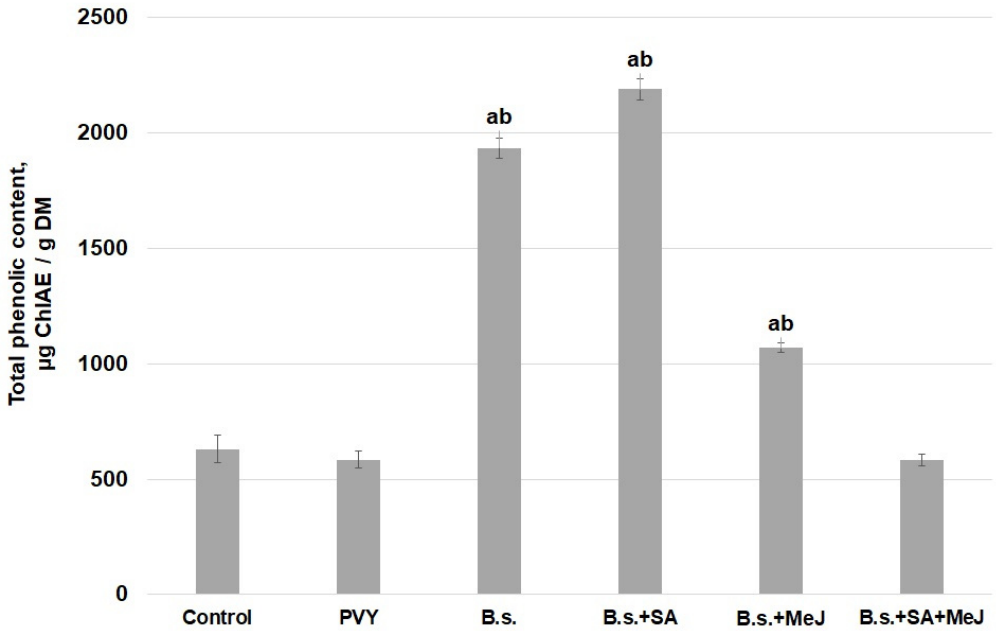

Figure 4. Effect of PVY infection and the mixture of *B. subtilis* with SA and MeJ application under soil water deficiency on total phenolic contents. Control—health plants; PVY—infected with PVY plants; B.s.—*B. subtilis* + PVY; B.s. + SA—*B. subtilis* + salicylic acid + PVY; B.s. + MeJ—*B. subtilis* + methyl jasmonate + PVY; B.s. + MeJ + SA—*B. subtilis* + salicylic acid + methyl jasmonate + PVY. a—indicates means that are statistically significantly different from the control, b—indicate means that are statistically significantly different from the PVY.

The infection with PVY under soil water deficiency increased polyphenol oxidase (PPO) activity. PPO activity decreased by an average of 20% in all treatment options relative to the infected plants, in *B. subtilis* and *B. subtilis* + MeJ + SA variants relative to the control. *B. subtilis* + MeJ increased PPO activity (Figure 5).

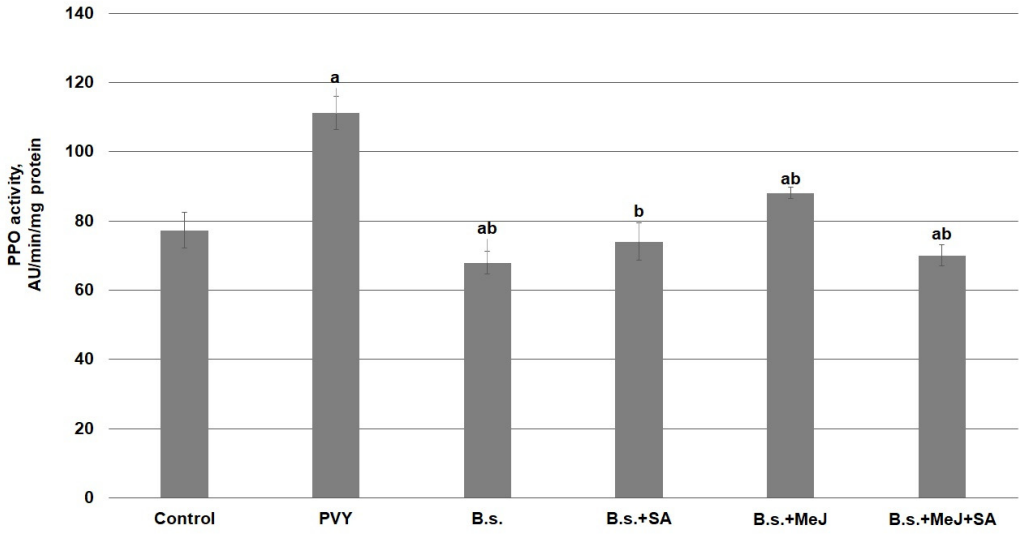

Figure 5. Effect of PVY infection and the mixture of *B. subtilis* with SA and MeJ application under soil water deficiency on PPO activity. Control—health plants; PVY—infected with PVY plants; B.s.—*B. subtilis* + PVY; B.s. + SA—*B. subtilis* + salicylic acid + PVY; B.s. + MeJ—*B. subtilis* + methyl jasmonate + PVY; B.s. + MeJ + SA—*B. subtilis* + salicylic acid + methyl jasmonate + PVY. a—indicates means that are statistically significantly different from the control, b—indicate means that are statistically significantly different from the PVY.

Infection of plants and treatment with *B. subtilis* under soil water deficiency increased the ascorbate-glutathione cycle enzyme activity. Applying mixtures of *B. subtilis* + SA, *B. subtilis* + MeJ, and *B. subtilis* + MeJ + SA reduced the activity of the enzyme (ascorbate peroxidase and glutathione reductase), which declined up to the level of the control plants under moisture deficiency conditions (Figure 6).

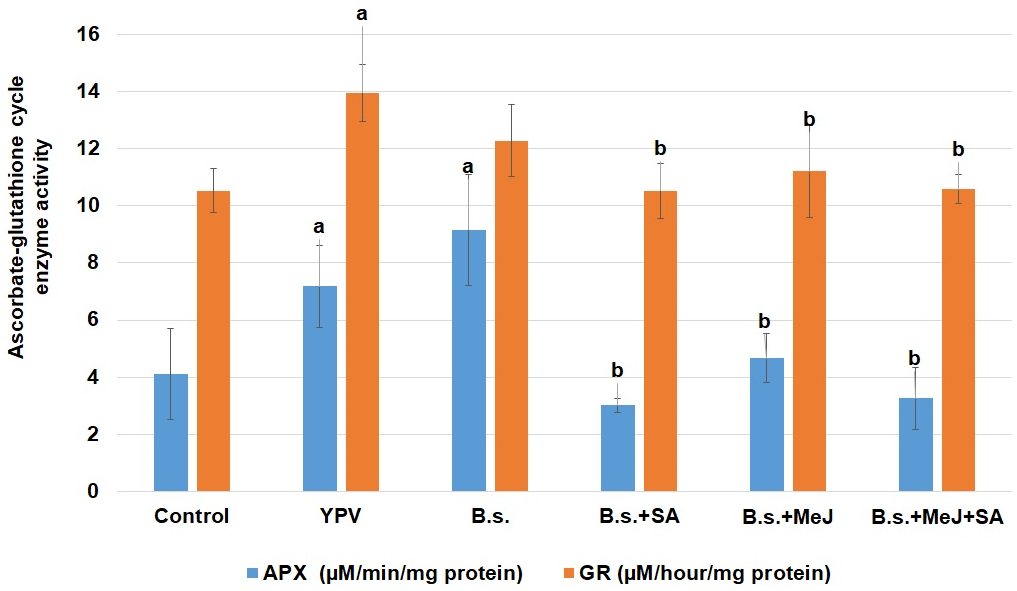

Figure 6. Effect of PVY infection and the mixture of *B. subtilis* with SA and MeJ application under soil water deficiency on ascorbate-glutathione cycle enzyme activity. Control—health plants; PVY—infected with PVY plants; B.s.—*B. subtilis* + PVY; B.s. + SA—*B. subtilis* + salicylic acid + PVY; B.s. + MeJ—*B. subtilis* + methyl jasmonate + PVY; B.s. + MeJ + SA—*B. subtilis* + salicylic acid + methyl jasmonate + PVY. a—indicates means that are statistically significantly different from the control, b—indicate means that are statistically significantly different from the PVY.

The plant PVY contagion had no obvious systematic effect on the potato mini-tubers' weight and number under soil water deficiency. The investigated treatments stimulated a significant number, but not the weight of mini-tubers in comparison with infected control plants. A significant increase in the number of mini-tubers was noted in the *B. subtilis* + MeJ + SA. PVY caused a decrease in the ascorbic acid and starch contents in the mini-tubers, but treatments increased their accumulation to the control level and above. Mixtures of bacteria with SA + MeJ reduce the starch content (Table 2).

Table 2. Effect of PVY infection and the mixture of *B. subtilis* with SA and MeJ application under soil water deficiency on productivity and quality of mini-tubers.

Treatment	Number of Minitubers, pcs/Container	Weight of Minitubers, g/Container	Starch Content, %	Ascorbic Acid Content, μg/g WM
Control	28.5 ± 2.2	365.0 ± 23.7	12.9 ± 0.78	26.3 ± 3.3
PVY	31.0 ± 2.5	337.9 ± 26.1	12.6 ± 0.10	21.4 ± 3.1
B. subtilis	39.5 ± 3.2	351.6 ± 26.4	14.0 ± 0.29	27.5 ± 4.2
B. subtilis + SA	36.0 ± 3.4	360.0 ± 25.5	13.3 ± 0.69	32.0 ± 6.1
B. subtilis + MeJ	38.5 ± 2.8	358.8.0 ± 27.3	14.4 ± 0.20	26.5 ± 5.0
B. subtilis + MeJ + SA	42.5 ± 3.7	361.2 ± 25.3	12.0 ± 0.11	30.1 ± 5.8

Different proteins (14 or their isoforms) with isoelectric points from 3.5 to 9 and molecular weights of up to 100 kDa were identified by two-dimensional electrophoresis, the content of which in the tubers differed depending on the variant of the experiment (Table 3, Figure 7).

Table 3. Effect of PVY infection and mixture of *B. subtilis* with SA and MeJ application in optimal conditions and under soil water deficiency on different proteins content in mini-tubers.

No.	The Name of the Protein According to Uniprot	pI	Protein Content, µg/g Fresh Weight											
			Optimal Conditions						Soil Water Deficiency					
			Control	Infection PVY	Infection + *B. subtilis*	Infection + *B. subtilis* + SA	Infection + *B. subtilis* + MeJ	Infection + *B. subtilis* + SA + MeJ	Control	Infection	Infection + *B. subtilis*	Infection + *B. subtilis* + SA	Infection + *B. subtilis* + MeJ	Infection + B. subtilis + SA + MeJ
1	Alpha-glucan phosphorylase	4	- *	-	-	-	-	0.1	-	-	-	-	-	-
2		7	-	-	-	-	-	0.1	-	-	-	-	-	-
3		9	-	-	-	-	-	-	-	-	0.1	-	-	0.1
4	Stromal 70 kDa heat shock-related protein, chloroplastic	4.5	-	-	0.1	0.1	-	-	-	-	-	-	-	-
5	Elongation factor 2	6	-	-	-	-	-	-	-	-	0.1	-	-	-
6	Kinesin-like protein KIN-5D	7	-	-	-	-	-	-	-	-	-	6	-	-
7	Catalase isozyme 2	8	-	-	-	-	-	0.2	-	-	-	0.1	0.1	0.1
8	Patatin	3.5	-	-	0.1	0.1	-	0.1	-	-	4.3	-	-	-
9		4	-	2.1	-	0.1	0.1	2.5	-	2.3	3.5	2.5	-	0.1
10		5	-	3.2	-	-	-	-	-	-	-	2.6	-	0.1
11		6	-	-	-	-	-	-	-	-	2.4	3.4	-	-
12	Aspartic protease inhibitor 8	7	-	-	3.6	5.7	3.1	-	0.2	-	-	2.8	0.1	-
13	Annexin D2-like	5	5.5	5.6	3.5	-	6.1	6.0	-	5.8	-	4.1	-	-
14	Formate dehydrogenase, mitochondrial	8	-	0.2	-	-	-	-	-	3.0	-	-	0.1	-

* "-"—protein is present in a concentration insufficient to detect.

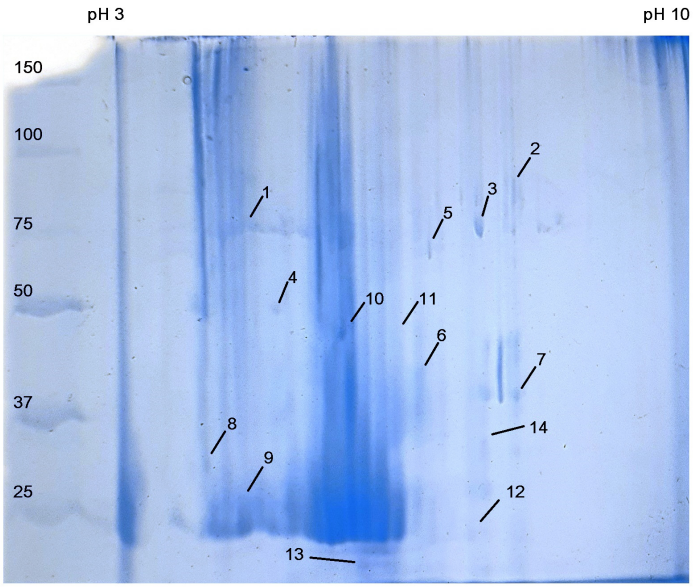

Figure 7. The two-dimensional electrophoresis of potato proteins representative gel. The spot numbers show proteins as listed in Table 2.

The greatest number of different proteins were detected under normal conditions when treating *B. subtilis* together with both signaling molecules and in soil water deficiency conditions—when treating *B. subtilis* together with salicylic acid.

4. Discussion

It was found *B. subtilis* strain 47 suppressed the PVY virus development by 20.1% under optimal growing conditions. Previously in [34], a comparative study of the antiviral activity of a new bio preparation based on bacteria *B. subtilis* (strain 47) and commercial biopesticides (beta-protectin, phyto-protectin, frutin) in potato plants of Belarusian selection was carried out in vivo and ex vitro. The obtained results indicate that pretreatment of potato plants with the *B. subtilis* 47 prevents virus infection, inducing the antiviral resistance of the potato, and is accompanied by redox enzyme activity changes.

However, in our study, *B.subtilis* 47 did not show antiviral activity under insufficient soil water supply conditions. Application of bacteria with MeJ or MeJ + SA mixture reduced the viral damage degree to potato leaves, probably only due to the signaling molecules' action. Thus, it was previously shown that exogenous SA at a concentration of 2 mM induced tomato resistance in response to viral post-inoculation. The plants treated with SA had a normal growth phenotype [35].

It was reported that exogenous treatment with different concentrations of MeJ resulted in a decrease in the MDA level on legume plants infected with the mungbean yellow mosaic India virus; it is assumed that the protective role of MeJ is associated with restoring the membrane stability, thereby preventing the penetration of the virus [36].

One of the ways to induce resistance of potato plants to the pathogen of late blight and drought by the bacteria treatment with signaling molecules is the generation of hydrogen peroxide, increased expression of PR protein genes and changes in the plant proteome [37].

The most common effect of viral infection and lack of soil moisture on plant physiology is growth inhibition, which is crucial for plant survival under stress [38].

Treatments with *B. subtilis* + MeJ and *B. subtilis* + MeJ + SA stimulated the potato plant's growth. The shoot length increases in *B. subtilis* mixture treatment were probably due to the regulation of the synthesis of phytohormones responsible for growth processes. Most PGPR can enhance plant vigor directly due to the synthesis of various metabolites of hormonal and signaling nature, such as auxins and cytokinins [39,40].

Soaking corn seeds in a MeJ solution had a stimulating effect on growth parameters under water stress conditions due to increased levels of IAA and cytokinins [38].

It was reported that combined treatment with a mixture of MeJ and SA contributed to the greatest increase in the shoot's length and root's length of corn in drought conditions, compared with individual MeJ or SA [41], which is consistent with our obtained experimental data.

Chlorophylls are critical in photosynthesis for the production of food in the form of carbohydrates. The structural and functional state of photosynthetic pigments can serve as an indicator of unfavorable conditions during virus-plant interaction under water stress conditions. PVY infection with soil moisture deficiency caused a decrease in the chlorophylls a and b content in potato leaves in relation to uninfected control, which may be caused by activation of chlorophyll synthetase system components, such as chlorophyllase or by direct exposure of the virus to pigment synthesis. Similar results of reducing photosynthetic pigments during viral infection were previously recorded in tomato plants [42] and cucumbers [43].

Application of *B. subtilis* and their mixtures with MeJ or a MeJ + SA enhanced the accumulation of photosynthetic pigments. In the study [44], the influence of virus infection on chlorophyll content was noticeable in infected tobacco plants. The increased leaf chlorophyll content in *Paenibacillus lentimorbus* B-30488 inoculated tobacco plants resulted in increased photosynthetic efficiency (Fv/Fm).

It has been shown that SA treatment improves membrane permeability, facilitating the absorption and use of mineral nutrients, such as Mg and Fe, which are necessary for chlorophyll biosynthesis and assimilate transport [45].

In addition, there is evidence of the effect of MeJ on photosynthetic activity. Thus, treatment with jasmonates led to an increase in the pool of chlorophylls and an increase in the efficiency of photosynthesis of FS II by activating two enzymes, such as protochlorophyllide reductase and α-aminolevulinic acid dehydratase [46].

Thus, growth stimulation and an increase in the photosynthetic pigments content by *B. subtilis* + MeJ + SA is associated with a positive synergistic interaction of all components of the mixture.

It is known that the regulation of proline is crucial for maintaining the osmotic potential of tissues. Additional synthesis of this amino acid increases the general resistance of plants to abiotic stresses since proline protects the membranes, macromolecules, and structural elements of the cell, thus leading to an increase in nonspecific resistance. Proline considerations a metabolic signal regulating redox homeostasis and the expression of some stress response genes [47–49].

In addition, many studies have shown that the proline content increases during the formation of plant protective reactions against viral pathogens [50].

In our study, infection PVY of plants with soil water supply caused a decrease in the proline content (Figure 3). In [51], it was shown that artificial infection with turnip mosaic virus (TuMV) affects the stomata closure and improves the water balance in arabidopsis, which is due to a decrease in gas exchange in infected plants and a decrease in water loss. At the same time, an increase in the content of SA and abscisic acid in TuMV-infected plants was revealed. It can be assumed that PVY is also able to somewhat optimize the water balance of potato plants, thereby not causing the accumulation of osmolites.

However, treatments of *B. subtilis* + SA and *B. subtilis* + MeJ + SA caused significant proline accumulation. Studies have shown [44] modification of proline level by *Paenibacillus lentimorbus* B-30488 and CMV in different treatments proved to act as an indicator of ROS status in a plant; lowered proline and ROS in B-30488 and higher in B-30488 + CMV treatments shows the ability of the bacteria to maintain ROS balance as per the requirement of the plant.

Exogenous SA treatment increased the leaf proline content of plants under 150 mM salt stress by 23.1%, suggesting that SA enhances the accumulation of proline, which acts as a compatible solute that helps preserve the leaf's water status under salt stress [52,53].

Significant indicators of the functioning of the plant's protective system are the polyphenol oxidase activity (PPO), as well as the phenolic compounds content that play an important role in the defense of the plants from the introduction of the pathogen [54]. It was found that under stressful conditions (irradiation, mechanical damage, changes in the chemical composition of the environment), the activity of phenol oxidase enzymes in the cell increases, preventing the spread of ROS [55,56]. However, in our study, the PPO activity decreased under the influence of treatments for viral infection and insufficient water supply. Inhibition of the ascorbate-glutathione cycle enzymes (APX and GR) activity under PVY infection and soil water deficiency was revealed when treating plants with mixtures of bacteria with SA, MeJ and SA + MeJ. Oxidative stress was mitigated through reduced activities of ascorbate-glutathione cycle enzymes as compared to the control.

It has been reported [57] in the study of the role of plant growth-promoting rhizobacteria (PGPR) strains viz. *Burkholdera cepacia* SE4, *Promicromonospora* sp. SE188 and *Acinetobacter calcoaceticus* SE370 in counteracting salinity and drought stress to cucumber plants. Oxidative stress was mitigated by PGPR through reduced activities of catalase, peroxidase, polyphenol oxidase, and total polyphenol as compared to the control.

SA suppressed the polyphenol oxidase induction in wheat plants and thereby participated in the utilization of ROS [58]. Treatment MeJ of broccoli leaves caused inhibition of PPO activity, thereby reducing phenols degradation [59].

The phenolic compounds content increased significantly with the use of *B. subtilis* and a mixture of *B. subtilis* + SA under PVY infection and soil water deficiency.

During the study of the growth-promoting potential and ability of PGPR species (*Pseudomonas fluorescens* WCS417 r and *Bacillus amyloliquefaciens* GB03) to increase the drought tolerance in peppermint, it was established negative effects of drought were mitigated in plants exposed to PGPR inoculation, resulting in significantly less reduction in the above growth traits related to plants not treated with PGPR, regardless of the severity of the drought treatment. In addition, drought-stressed plants treated with PGPR had a significantly higher total phenolic content than water-stressed plants without PGPR [60].

It has been reported that some strains of rhizobacteria affect the number and mass of potato tubers produced [61,62]. In addition, an increase in potato yield is possible due to the use of growth regulators with anti-stress properties and contributing to increased resistance to abiotic and biotic factors. [63,64].

The yield and quality of potatoes were improved by *B. subtilis* strain Bv17 earlier it was shown [61].

It is known that MeJ has induction activity of tuber formation. One way to favorable influence of MeJ on the tuber initiation is the direction of cell division to the radial growth of the stolon tips. It was found that MeJ orients cortical microtubules similarly to uniconazole, and its participation in the division of stolon cells is opposite to the action of gibberellic acid [65].

It was found that an increase in the ascorbic acid content in transgenic potato plants was accompanied by an increased tolerance to various abiotic stresses [66]. Thus, under salt stress conditions tomato plant's foliar spray by SA contributed to the accumulation of ascorbic acid and reduced its adverse effects [67]. In addition, ascorbic acid has shown a positive effect on the induction of tuber formation [68], which is generally consistent with our results.

Thus, the accumulation of ascorbic acid during treatment with immunostimulants probably contributes to reducing the stress load in potato tubers by exerting an antioxidant effect and increasing the reproduction coefficient.

Fourteen different proteins or their isoforms were identified by two-dimensional electrophoresis, the content of which in the tubers differed depending on the experiment variant (Table 3). The greatest number of different proteins were detected under normal conditions after treatment by *B. subtilis* together with both signaling molecules and in drought conditions—by *B. subtilis* combined with salicylic acid. For all identified proteins, the role in protection from abiotic or biotic stresses is known. Thus, alpha-glucan phosphorylase is associated with resistance to moisture deficiency [69] and temperature stress [70]. Stromal 70 kDa heat shock-related protein is a chaperone; its role in protecting plants from pathogens is also known [71]. Elongation factor 2 takes part in the formation of resistance to root nematodes and cold resistance [72]. The protective function of patatin is realized through lipidacylhydrolase and acyltransferase activity [73]. Increased expression of kinesin-like protein KIN-5D causes protective reactions of tomato plants against the tobacco mosaic virus [74]. Catalase isozyme 2 participates in SA-mediated repression of auxin accumulation and jasmonic acid biosynthesis in arabidopsis pathogenesis, causing resistance to biotrophic and susceptibility to necrotrophic pathogens [75]. Formate dehydrogenase is involved in response to abiotic stress and the SA- and JA-mediated response to bacterial pathogens [76]. Aspartic protease inhibitor 8 is associated with potato resistance to Y and A viruses [77]. Annexin participates in reactions to injury and eating by phytophages and is a central regulator of plant growth and signaling under stress [78]. Thus, the treatment of potato plants with a mixture of bacteria with signaling molecules causes a change in the tuber proteome aimed at enhancing the expression of protective protein genes that increase resistance to pathogens and abiotic stress.

5. Conclusions

The results showed that in optimal growing greenhouse conditions, the foliar application of *B. subtilis* strain 47 in combination with SA or MeJ before PVY inoculation was the effective treatment for decreasing the infection of potato plants. A mixture of *B.subtilis* + MeJ and *B.subtilis* + MeJ + SA reduced the infection development under soil water deficiency and enhanced potato plant development. The application of the *B. subtilis* 47 strain, in combination with SA or MeJ, decreased the activity of antioxidant enzymes (PPO, APX and GR) and raised proline accumulation, which indicates mitigation of oxidative stress in treated plants. Under soil water deficiency *B. subtilis* and *B. subtilis* + SA treatments increased significantly total phenolic content, did not decline the infection degree and did not stimulate plant growth. The application of *B. subtilis* in combination with SA or MeJ led to an increase in a significant number, but not the weight of mini-tubers and endogenous ascorbic acid content. Numerous protein content or their isoforms in the tubers differed depending on the experiment variant, and the role of which in protection from abiotic or biotic stresses is known. Signaling molecules with *B. subtilis* strain 47 mitigated the adverse effect of PVY under water deficiency by manipulating enzymatic/non-enzymatic antioxidant levels and activity in treated plants.

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