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(1→3)-α-D-glucooligosaccharides as Elicitors Influencing the Activity of Plant Resistance Pathways in Wheat Tissues

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Abstract: *Laetiporus sulphureus* (Bull.: Fr.) Murrill is an arboreal species of the large-fruited Basidiomycota fungus from the *Polyporales*, family *Laetiporaceae*. The cell wall of this fungus is the source of many bioactive polymer compounds, including (1→3)-α-D-glucans. (1→3)-α-D-glucans can be hydrolyzed to shorter compounds, (1→3)-α-D-glucooligosaccharides (GOS), with different degrees of polymerization (DP). The use of GOS obtained from *L. sulphureus* (1→3)-α-D-glucans, as an elicitor of plant resistance, may be important for biological protection used in sustainable agriculture. In the presented study, GOS influenced the activity of antioxidant enzymes (Catalase—CAT, Ascorbate Peroxidase—APX, Guaiacol Peroxidase—GPX, and Superoxide Dismutase—SOD), lignin and flavonoids producing phenylpropanoids pathways (Phenylalanine Ammonia-Lyase—PAL and Tyrosine Ammonia-Lyase—TAL), and pathogen-related proteins (with Glucanase—GLUC and Chitinase—CHIT activity) in wheat (*Triticum aestivum* L.) seedling tissues. Other than that, the application of GOS increased the fresh weight of wheat stems and roots by 1.5–2-times, compared to the water control. The GOS at a concentration of 0.05% most strongly increased the activity of APX and GPX, where a 2-fold (up to 6000 U) and a 3-fold (up to 180 U) increase in enzymatic activity in wheat stems was observed, compared to the control. Simultaneously, 0.1% GOS significantly increased the activity of PAL (80 U in stems and 50 U in roots) and TAL (60 U in stems and 50 U in roots), where a 4–5-fold increase in enzymatic activity was observed, both in comparison to the water control and commercial elicitors (chitosan—CHI and laminarin—LAM). No effect of GOS on GLUC activity was observed, but a 1.5–2-fold increase in CHIT activity in plant tissues was noted. The complexity of the influence of GOS on the level of marker enzymes indicates the potential of their application in agriculture. This work is the first report of the successful use of (1→3)-α-D-glucooligosaccharides as an elicitor inducing resistance in the cereal plant (wheat).

Keywords: plant resistance markers; wheat; (1→3)-α-D-glucooligosaccharides; GOS; elicitors; catalase; peroxidase; phenylalanine ammonia-lyase; tyrosine ammonia-lyase; *Laetiporus sulphureus*

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1. Introduction

Membrane and especially cell wall polymers (CWP) of microorganisms (fungi, bacteria, and Protista), have become one of the most promising groups of substances for use in modern sustainable agriculture. This is in line with the principle of integrated agriculture and the latest methods that are safe for the environment and do not violate microbial diversity. These methods ensure the maximum reduction of the use of chemical pesticides, as well as the resignation from introducing live microbial cells, which is difficult due to numerous legal regulations [1–6]. The chemical composition of the fungal cell walls (FCW), the main ingredients of which are glucans, glycoproteins, chitin, and chitosan, is species-specific. [7–11]. Fragments of CWP can be released into the external

environment as exopolymers (EPS) as a result of the activity of cell wall degrading enzymes (CWDEs) [12,13]. CW glucans can be divided according to the anomeric conformation of glucose (α-glucans, β-glucans, and α,β-glucans) and the position of glycosidic bonds (1→3, 1→4, and 1→6) [11]. CWP fragments are inducers (elicitors) of plant resistance, i.e., a multi-stage process of biochemical changes carried out in many pathways [14,15]. Elicitors are defined as "agents or products which use natural mechanisms within the Integrated Pest Management (IPM). This includes macro-organisms (insects, nematodes), microorganisms (bacteria, fungi, and viruses), chemical mediators (pheromones, kairomones), and natural substances of plant, animal or mineral origin" [16].

The defense mechanisms of the plant are classified into three layers of defense: (1) pattern-triggered primary immunity (PTI), (2) effector-triggered secondary immunity (ETI), and (3) immunity associated with the exosome-mediated cross-kingdom RNA interference (CKRI) system [17]. Plants can detect elicitors at nanomolar concentrations leading to rapid activation of high transcription and strong defense responses but ones associated with growth–defense tradeoffs that reduce plant condition [18].

Elicitors act extremely quickly, triggering early immune-related events and physical changes in responsive cells, such as ion fluxes, oxidative burst, and phosphorylation cascade. The immune reaction initiated at the induction site is systemically transmitted to all parts of the plant. The plant resistance is classified into three types: (1) systemic acquired resistance (SAR), which involves the phytohormone salicylic acid (SA) as a signaling molecule, (2) induced systemic resistance (ISR) characterized by the production of jasmonic acid (JA) and ethylene (ET) as a signaling molecules, and (3) induced resistance (IR) activated by β-aminobutyric acid (BABA) and involves abscisic acid (ABA) as a signaling molecule. The main marker enzymes of plant resistance are phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL), which are responsible for the activation of the phenylpropanoid pathway [19,20].

The activation of resistance pathways by elicitors leads to reinforcement of plant CW, lignin accumulation, the production of plant defense compounds, antimicrobial enzymes, phytoalexins, and 17 classes of pathogen-related (PR) proteins with enzymatic activity, such as guaiacol and ascorbate peroxidase, ribonuclease, chitinase or glucanase [21,22]. Elicitor-induced pathways trigger the "priming" phenomenon in plants—the readiness to undertake a forceful defense upon contact with a phytopathogen, where a transcription factor (e.g., MYC2) mediates the enhanced response of these pathways to elicitation [23–25].

The elicitors have an inducing effect in very low, nanomolar concentrations. The effective and optimal doses of polysaccharide elicitors are usually in the range of 1.5–500 mg/L [20]. Strong elicitor properties have been demonstrated for FCW polymers, including chitin and chitosan (a deacetylated derivative of chitin) in which N-acetylglucosamine subunits are linked by (1→4)-β bonds. The second group of elicitors are (1→3)-β-glucans of various origins, in particular the widely used laminarin obtained from the brown alga Ochrophyta, *Laminaria digitata* [20,26]. Elicitor properties are also attributed to α-glucans, such as oligogalacturonides, in which (1→4)-α-linked galacturonosyl residues are esterified with methyl groups, and carrageenans of red seaweeds—galactans are composed of repeating dimmers of (1→4)-α-D-galactose, which are linked by alternating bonds of (1→3)-α and (1→4)-β, and substituted by one (κ-carrageenan), two (ι-carrageenan), or three (λ-carrageenan) sulfate ester groups [27,28]. However, it has been noticed that in FCW, α-glucans blocking access to (1→3)-β-D-glucans with strong elicitor properties, impair the plant defense response [11].

The elicitor activity of oligomers is strongly dependent on the degree of their polymerization (DP), i.e., the number of repeating sugar subunits [27]. Oligomers with DP 5 (e.g., glucopentose in potato leaves) are able to stimulate defense reactions in plants, but the most effective are oligomers composed of 7 or 8 units, but also in a wider range from 7 to 10 (grapevines), and even up to 12 units (cucumber) [29–31]. Small OGA (oligogalacturonide, DP 1–4) had a low elicitor activity [32] and induced early defensive reactions, while β-glucans with high DP was responsible for long-term defense effects [20,33]. Moreover,

very small OGA (DP 2–3) acted as a suppressor of plant-pathogen defense (in wheat) and accelerated the growth of phytopathogenic fungi [34].

Particularly promising sources of new, efficient, and reliable elicitors seem to be CWPs of Ascomycota and Basidiomycota fungi, including endo- and ectomycorrhizal fungi, as well as saprotrophs and pathogens that produce a huge mycelium biomass in the environment, such as wood hubs.

An excellent source of cheap and efficient elicitors seems to be the fruiting bodies of the sulfur shelf of *Laetiporus sulphureus* (Bull.) Murrill, also known as crab-of-the-woods or chicken-of-the-woods. *L. sulphureus* is a cosmopolitan fungus of the Basidiomycota phyla, commonly found in the Northern Hemisphere, especially in Europe. This fungus may be a saprotroph, but is usually a deciduous trees pathogen, causing brown wood rot [35].

The fruiting bodies of *L. sulphureus* are a rich source of (1→3)-α-D-glucans, the amount of which in dry matter reaches 75–88% [36]. Although (1→3)-α-D-glucans are widespread in the fungal kingdom, their role and the mechanism of biosynthesis have not been fully understood. According to the latest concepts of the fungal cell wall structure, the location of (1→3)-α-D-glucans is quite fluid and species-dependent [37]. They can be located in the outer layers of the cell wall (as in *Histoplasma capsulatum*) or constitute a component of a complex polysaccharide network located under the protein-polysaccharide layer (as in *Aspergillus fumigatus*). As shown by numerous studies, apart from the backup function, (1→3)-α-D-glucans play a crucial role in maintaining cell stiffness of many yeast species, including *Schizosaccharomyces pombe*. This is evidenced by the maintenance of the cylindrical shape of cells devoid of β-glucan layers [38]. For some species of pathogenic fungi, a relationship was also observed between the degree of their virulence and the presence of (1→3)-α-D-glucans in their cell wall.

We hypothesize that (1→3)-α-D-glucooligosaccharides (GOS) obtained *via* the acid hydrolysis of (1→3)-α-D-glucan from the fruiting body of Basidiomycota fungus *L. sulphureus* may have a beneficial effect on the development of wheat seedlings and induce an immune response in this plant, after seed inoculation with GOS.

The aim of the study is to determine the effect of (1→3)-α-D-glucooligosaccharides on the activity of defense marker enzymes in wheat seedlings during wheat seeds germination and seedling growth in the presence of GOS solutions.

2. Materials and Methods

2.1. Biological Materials

The fruiting bodies of *Laetiporus sulphureus* (Bull.: Fr.) Murrill was harvested from the various host trees in Lublin, Poland and the surrounding regions. Voucher specimens are deposited in the Department of Industrial and Environmental Microbiology, Maria Curie-Skłodowska University, Lublin, Poland.

2.2. Isolation of (1→3)-α-D-glucan

Isolation and purification of (1→3)-α-D-glucan from fruiting bodies of *L. sulphureus* were performed according to the procedure described by Wiater et al. [39]. Briefly, the dried fungal material was milled and the resulting powder was treated with Milli-Q water at 121 °C for 1.5 h (×3) to remove the water-soluble fraction. The remaining water-insoluble fraction was suspended in 1M NaOH under constant stirring. After overnight incubation at room temperature, the supernatant was neutralized with 1M HCl. The insoluble fraction was collected by centrifugation, washed with Milli-Q water (×3), and lyophilized to give a white powder ((1→3)-α-D-glucan). The obtained polysaccharides were analyzed by GC-MS, FT-IR, and ^{1}H NMR to confirm (1→3)-α-D-glucan purity (data not shown).

2.3. Attainment and Analysis of (1→3)-α-D-glucooligosaccharide Preparation

Crude oligosaccharides were obtained by partial hydrolysis of the (1→3)-α-D-glucan isolated from fruiting bodies of *L. sulphureus* in 0.1 M H_2SO_4 for 1h at 100 °C. The residues were removed by centrifugation (10 min; 12,000 rpm) and the supernatant was neu-

tralized with $CaCO_3$. After re-centrifugation, the soluble fraction of the hydrolysate was desalted with Amberlite MB3 (BDH Chemicals Ltd., Poole, UK). The desalted solution was concentrated at 40 °C using a Büchi Rotavapor R-205 rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) under vacuum and lyophilized in a freeze-dryer (Labconco, Kansas City, MI, USA). The preparation containing the mixture of (1→3)-α-D-glucooligosaccharides was analyzed by HPLC using the chromatographic system Prominence LC-20A (Shimadzu, Kyoto, Japan) connected to a refractive index detector (RID-10). The mobile phase (Milli-Q water) was run at a flow rate of 0.25 mL/min at 40 °C through a Rezex RSO-Oligosaccharide Ag+ column (1 cm × 20 cm, Phenomenex, Torrance, CA, USA). The column was calibrated using the following sugars: ALO-3038 maltooligosaccharide standard (light corn syrup) with a degree of polymerization from 1 to 14 (Phenomenex, Torrance, CA, USA), glucose (Merck, Darmstadt Germany), nigerose (Sigma-Aldrich, St. Louis, MO, USA), as well as nigerotriose and nigerotetraose (Megazyme, Wicklow, Ireland).

2.4. Wheat Seeds Elicitation and Determination of Plant Growth Parameters

The seeds of winter wheat (*Triticum aestivum* L. cv Arkadia) from DANKO Plant breeding (Choryń, Poland) were used in the experiment. The seeds were transferred to an Erlenmeyer flask (500 mL) and washed in tap water for 30 min. The grains were then transferred to a sterile Erlenmeyer flask (500 mL) and rinsed for 10 min in a $HgCl_2$ (0.1% *w*/*v*) solution. After this time, the $HgCl_2$ solution was removed and the seeds were washed 5-times with sterile distilled water for 15 min. Surface-sterilized seeds were then transferred to sterile Petri dishes. Wheat seeds were inoculated with each elicitor in triplicate (3 Petri dishes with wheat seeds; 20 seeds per Petri dish), separately for the 5th and 10th day of incubation. There were 120 wheat seeds per elicitor (6 Petri dishes), of which the seedlings from 3 Petri dishes were harvested on the 5th day of incubation, and the seedlings from the next 3 Petri dishes on the 10th day of incubation [26].

Seven experiment combinations were used:

Control:

1. Water control—C.

Oligosaccharides water solutions:

2. 0.05% (1→3)-α-D-glucooligosaccharides solution—GOS 0.05,
3. 0.1% (1→3)-α-D-glucooligosaccharides solution—GOS 0.1.

Commercial elicitor controls:

4. 0.05% salicylic acid—SAL (Sigma-Aldrich, Steinhim, Germany),
5. 0.05% laminarin from *Laminaria digitata*—LAM (Sigma-Aldrich, St. Louis, MO, USA),
6. 0.05% acibenzolar-S-methyl (benzo (1,2,3) thiadiazole-7-carboxylic acid S-methyl ester)—BTH (Acros Organics, New Jersey, NJ, USA),
7. 0.05% chitosan—CHI (Sigma-Aldrich, St. Louis, MO, USA).

1 mL of the prepared solutions were transferred separately to Petri dishes with wheat seeds and supplemented with 2 mL of sterile water. Seeds germinated in the dark at 20 °C in the Innova chamber (New Brunswick Innova 43 Incubator Shaker, Edison, NJ, USA) for 5 and 10 days.

The percentage of germinated seeds was determined for 120 wheat seeds on the 3rd day of incubation.

Stems and roots were harvested separately on the 5th (3 Petri dishes) and 10th (3 Petri dishes) day of incubation, and the average weight (milligram of fresh weight − mg/FW) of plant parts was calculated from the harvested stems and roots grown from seeds.

After 5 and 10 days of incubation, wheat stems and roots were harvested separately from 3 Petri dishes, pooled together, and frozen in liquid nitrogen for future experiments.

2.5. Enzymes Extraction

Plant defense enzymes were extracted from the wheat stems and roots according to the method described by García-Limones et al. [14] with some modifications. Collected

and frozen stems and roots was ground to a fine powder for 10 min in chilled mortar. The enzymes were extracted using 8 mL (per 1 g of FW) cold 50 mM phosphate buffer (pH 7.5) containing 1mM EDTA (Ethylenediaminetetraacetic acid, Sigma-Aldrich, Steinhim, Germany), 1 mM PMSF (Phenylmethylsulfonyl Fluoride, Sigma-Aldrich, Steinhim, Germany), and 5% PVPP (Poly(vinylpolypyrrolidone), Sigma-Aldrich, St. Louis, MO, USA), obtained by mixing for 5 min. The homogenates were centrifuged at 12,000 rpm for 15 min at 4 °C. Samples were carried out in the ice bath between every step of the procedure. Enzyme extracts were frozen in liquid nitrogen and stored at −80 °C [14,19,26].

2.6. Enzymes Activity

The enzymatic activity was expressed in terms of the protein content in the homogenate samples. Protein concentration was determined by the Bradford method [40]. All reagents were prepared in a buffer dedicated to the method. The concentration of the reagents in the following descriptions was the final concentration in the reaction mixture [14]. The negative control in all enzyme activity assays was heat-inactivated (100 °C, 10 min) in the plant extract.

2.6.1. Catalase (CAT) Activity

The CAT activity was determined based on decreased concentration of H_2O_2 in the mixture. The reaction was started by adding 20 µL of the extract sample to 980 µL of 50 mM phosphate buffer (pH 7.0) containing 20 mM H_2O_2 (POCH, Gliwice, Poland). The absorbance was measured at 240 nm for 1 min using the Varian Cary 50 UV–visible Spectrophotometer (Varian, Santa Clara, USA). The CAT activity was expressed at U − µM H_2O_2/min/mg protein.

2.6.2. Ascorbic Peroxidase (APX) Activity

The APX activity was determined based on decreased concentration of H_2O_2 in the mixture in the presence of sodium ascorbate. The reaction was started by adding 50 µL of extract sample to 950 µL of 50 mM phosphate buffer (pH 7.0) containing 5 mM H_2O_2 (POCH, Gliwice, Poland) and 0.25 mM sodium ascorbate (Sigma-Aldrich, Steinhim, Germany). The absorbance was measured at 290 nm for 1 min using the Varian Cary 50 UV–visible Spectrophotometer (Varian, Santa Clara, CA, USA). The APX activity was expressed at U − µM H_2O_2/min/mg protein.

2.6.3. Guaiacol Peroxidase (GPX) Activity

The GPX activity was determined based on transformation of guaiacol to tetra-guaiacol. The reaction was started by adding 10 µL of extract sample to 990 µL of 100 mM phosphate buffer (pH 6.5) containing 15 mM guaiacol (Sigma-Aldrich, St. Louis, MO, USA) and 0.05% H_2O_2 (POCH, Gliwice, Poland). The absorbance was measured at 470 nm for 1 min using the Varian Cary 50 UV–visible Spectrophotometer (Varian, Santa Clara, CA, USA). The GPX activity was expressed at U − µM tetra-guaiacol/min/mg protein.

2.6.4. Superoxide dismutase (SOD) Activity

The SOD activity was determined based on inhibition of the photochemical reduction of nitro-blue tetrazolium (NBT) in the presence of riboflavin. Various dilutions of the enzyme extract were prepared (×5, ×10, ×20, and ×40). 600 µL of 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA (Sigma-Aldrich, St. Louis, MO, USA), 13 mM methionine (Fluka, Steinhim, Germany), and 75 µM NBT (Sigma-Aldrich, Steinhim, Germany) was added to 200 µL of diluted extract. The reaction was started by adding 200 µL of 2 µM riboflavin. Reaction mixtures were incubated for 12 min at room temperature with a constant light source (lamp), 10 cm from the samples. The absorbance was measured at 560 nm using a UV-transparent 48-well plate and Infinite 200 PRO TECAN microplate spectrophotometer (Tecan, Grödig, Austria). The SOD activity was expressed as

the inhibition of the photochemical reduction of NBT. The SOD activity was expressed at U − inhibition/min/mg protein.

2.6.5. Phenylalanine Ammonia-Lyase (PAL) Activity

The PAL activity was determined based on the transformation of L-phenylalanine to trans-cinnamic acid. The reaction mixture contained 100 µL of enzyme extract, 650 µL of distilled water, and 500 µL of 100 mM L-phenylalanine in borate buffer (pH 8.8) (Sigma-Aldrich, Steinhim, Germany). The reaction mixture was incubated at 32 °C for 60 min. After the incubation, 0.5 mL of 5 N HCl was added to the mixture to stop the reaction. The absorbance was measured at 290 nm using a UV-transparent 96-well plate and Infinite 200 PRO TECAN microplate spectrophotometer (Tecan, Grödig, Austria). The transformation was based on the difference in absorbance measurements before and after incubation. The PAL activity was expressed at U − nM trans-cinnamic acid/min/mg protein [41,42].

2.6.6. Tyrosine Ammonia-Lyase (TAL) Activity

The TAL activity was determined based on the transformation of L-tyrosine to coumaric acid. The reaction mixture contained 100 µL of enzyme extract, 650 µL of distilled water, and 500 µL of 100 mM L-tyrosine (Sigma-Aldrich, Steinhim, Germany). The reaction mixture was incubated at 32 °C for 60 min. After the incubation, 0.5 mL of 5 N HCl was added to the mixture to stop the reaction. The absorbance was measured at 310 nm using a UV-transparent 96-well plate and Infinite 200 PRO TECAN microplate spectrophotometer (Tecan, Grödig, Austria). The transformation was based on the difference in absorbance measurements before and after incubation. The TAL activity was expressed at U − nM coumaric acid/min/mg protein [41,42].

2.6.7. Glucanase (GLUC) Activity

The GLUC activity was determined based on the transformation of laminarin from *Laminaria digitata* to glucose. The reaction mixture contained 500 µL of enzyme extract and 500 µL of 0.1% laminarin (Sigma-Aldrich, Steinhim, Germany) in acetate buffer (pH 5.6). The reaction mixture was incubated at 37 °C for 3 h with gentle agitation (80 rpm). After the incubation, glucose concentration was determined by the Somogyi [43] and Nelson [44] method modified by Hope and Burns [45]. The absorbance was measured at 520 nm using the Varian Cary 50 UV–visible Spectrophotometer (Varian, Santa Clara, CA, USA). The GLUC activity was expressed at U – nM glucose/h/mg protein.

2.6.8. Chitinase (CHIT) Activity

The CHIT activity was determined based on the transformation of chitin to N-acetylglucosamine. The reaction mixture contained 500 µL of enzyme extract and 500 µL 0.5% colloidal chitin (Sigma-Aldrich, Steinhim, Germany) in acetate buffer (pH 5.6). The reaction mixture was incubated at 45 °C for 20 h with gentle agitation (80 rpm). After the incubation, N-acetylglucosamine concentration was determined by DNS method [46]. The absorbance was measured at 550 nm using the Varian Cary 50 UV–visible Spectrophotometer (Varian, Santa Clara, CA, USA). The CHIT activity was expressed at U − nM N-acetylglucosamine/h/mg protein.

2.7. Statistical Analysis

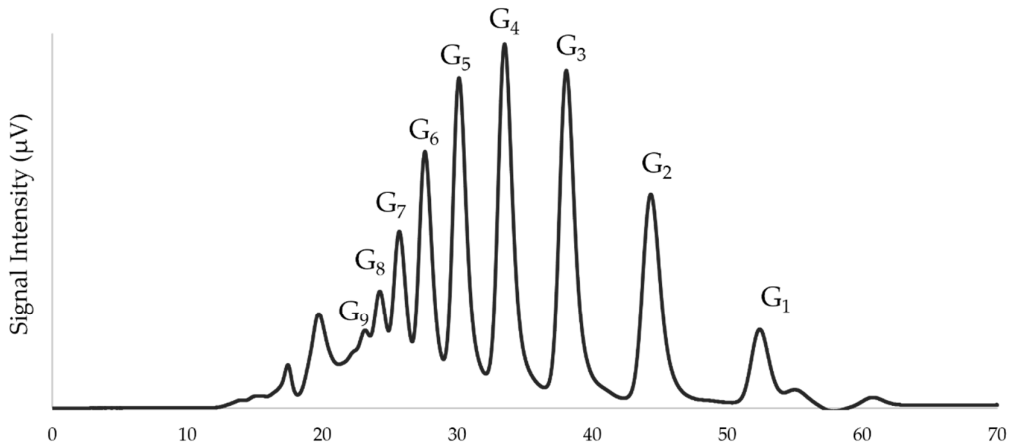
The data were presented as the mean value with standard deviation (SD) calculated using Microsoft Excel 365 (Microsoft Corp., Redmond, Washington, DC, USA). Statistical analyses were conducted using Statistica 13.3 (Stat Soft. Inc., Cracow, Poland). The data were subjected to an analysis of variance (one-way ANOVA) with a post-hoc Tukey's test at $p < 0.05$. The principal component analysis (PCA) was performed using Statistica 13.3 and Microsoft Excel 365. Heat maps were prepared using Microsoft Excel 365. Germination were performed from 120 seeds (n = 120). The plant fresh weight (FW) was calculated from the obtained 60 stems or roots (n = 60). The enzymatic activity was determined in plant

extracts (1 g of plan tissue was extracted in 8 mL of buffer) and measured in three biological replicates (n = 3), and then calculated on protein concentration.

3. Results

3.1. Preparation and Characterization of (1→3)-α-D-glucooligosaccharides

(1→3)-α-D-glucan used as the source of the oligosaccharides was isolated according to the method described by Wiater et al. [39] from the fruiting bodies of *L. sulphureus*. In our study, we used acid hydrolysis of polysaccharides as one of the methods for the production of potential biological activity of oligosaccharides. The (1→3)-α-D-glucooligosaccharides obtained *via* the acid hydrolysis of *L. sulphureus* (1→3)-α-D-glucan were characterized by HPLC analysis. The hydrolysate profile, shown in Figure 1, contained glucose (5.0%) and the mixture of (1→3)-α-D-glucooligosaccharides with a degree of polymerization (DP) of 2 to 9 (92.9%). Small amounts (2.1%) of oligosaccharides containing more than 9 glucose units were also noticed. The main products of (1→3)-α-D-glucan decomposition were nigerose (dimer, 13.0%), nigerotriose (trimer, 18.6%), nigerotetraose (tetramer, 19.3%), nigeropentaose (pentamer, 16.3%) and nigerohexose (hexamer, 11.7%).



Sugars (%)									
G1	G2	G3	G4	G5	G6	G7	G8	G9	>G9
5.0	13.0	18.6	19.3	16.3	11.7	7.4	4.5	2.1	2.1

Figure 1. Representative high performance liquid chromatography (HPLC) profile of (1→3)-α-D-glucooligosaccharides obtained by partial acid hydrolysis of *L. sulphureus* (1→3)-α-D-glucan. In the abbreviation Gn, "n" represents the degree of polymerization. G1: glucose, G2-G9: (1→3)-α-D-glucooligosaccharides with a DP of 2 to 9, >G9: (1→3)-α-D-glucooligosaccharides with a DP more than 9.

3.2. Influence on Plant Growth Parameters

After three days of incubation, the effect of the applied elicitor solutions on the amount of germinated wheat seeds was determined (Figure 2).

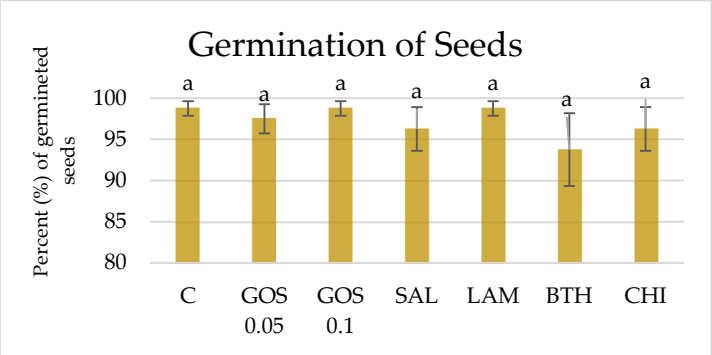


Figure 2. The average percentage (%) of germinated wheat seeds after inoculation with tested elicitors. C–water control, GOS 0.05–(1→3)-α-D-glucooligosaccharides 0.05%, GOS 0.1–(1→3)-α-D-glucooligosaccharides 0.1%, SAL–salicylic acid, LAM–laminarin, BTH–acibenzolar-S-methyl, CHI–chitosan. SD from germinated seeds (n = 120). One-way ANOVA, post hoc Tukey Test $p < 0.05$. Bars with the different letter are statistically significantly different from each other and control object.

No significant effect on the amount of germinated seeds was observed after the application of the tested elicitors. The average percentage values of germinated seeds ranged from 93% (after the application of BTH solution) to 98% (after the application of GOS 0.1 solution). The germination rate was in line with the manufacturer's information, which indicated 90% germination power.

On the other hand, the applied elicitors had a positive effect on the growth and development of wheat seedlings, increasing their fresh weight (FW) (Figure 3A,B). However, no significant effect of elicitors on the FW of the stems and roots was observed on the 5th day of incubation, compared to the water control (C). A significant increase in FW was observed after 10 days of seedling growth in the presence of elicitors, especially wheat stems. After the application of GOS 0.05, GOS 0.1, and CHI, a 2-fold increase in the FW of the stems was noted. Moreover, after SAL, LAM, and BTH stimulation, the fresh weight of the stems increased 1.5-times (Figure 3A). In turn, on the 10th day of incubation in the presence of GOS 0.05, GOS 0.1, CHI, and SAL, even a 1.5-fold increase in the FW of the roots was observed, compared to the C (Figure 3B).

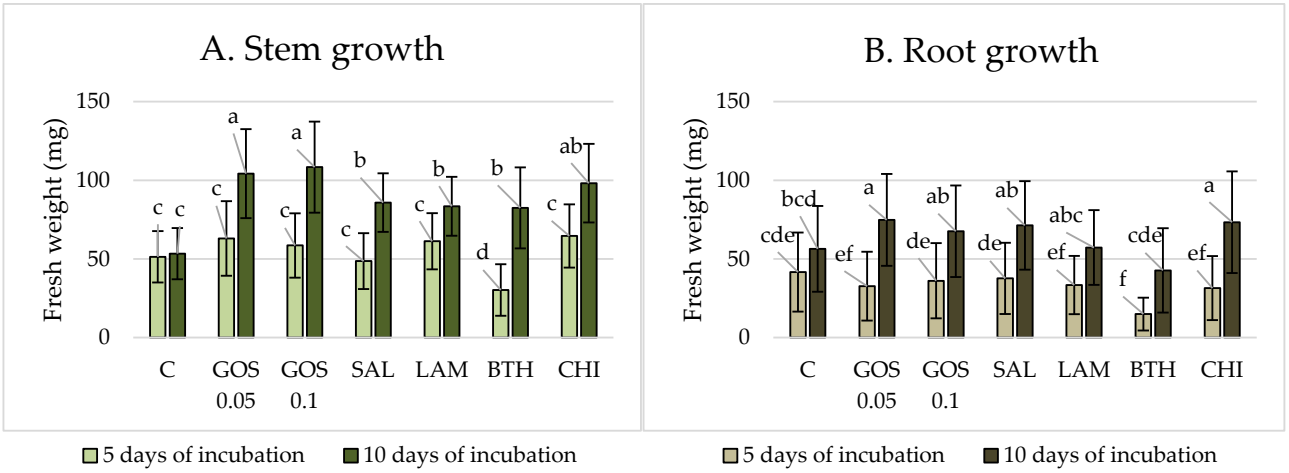


Figure 3. The fresh weight (FW) of (**A**) wheat stems and (**B**)roots after 5 and 10 days of incubation in the presence of elicitor solutions. C–water control, GOS 0.05–(1→3)-α-D-glucooligosaccharides 0.05%, GOS 0.1–(1→3)-α-D-glucooligosaccharides 0.1%, SAL–salicylic acid, LAM–laminarin, BTH–acibenzolar-S-methyl, CHI–chitosan. SD from FW of stems/roots (n = 60). One-way ANOVA, post hoc Tukey Test $p < 0.05$. Bars with the different letter are statistically significantly different from each other and control object.

3.3. The Activity of Defense Enzymes in Wheat Stems and Roots

The activity of crucial plant resistance marker enzymes in wheat stems and roots was determined. The first line of defense to lower ROS level in the plant tissues are enzymes such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and superoxide dismutase (SOD) (Figure 4).

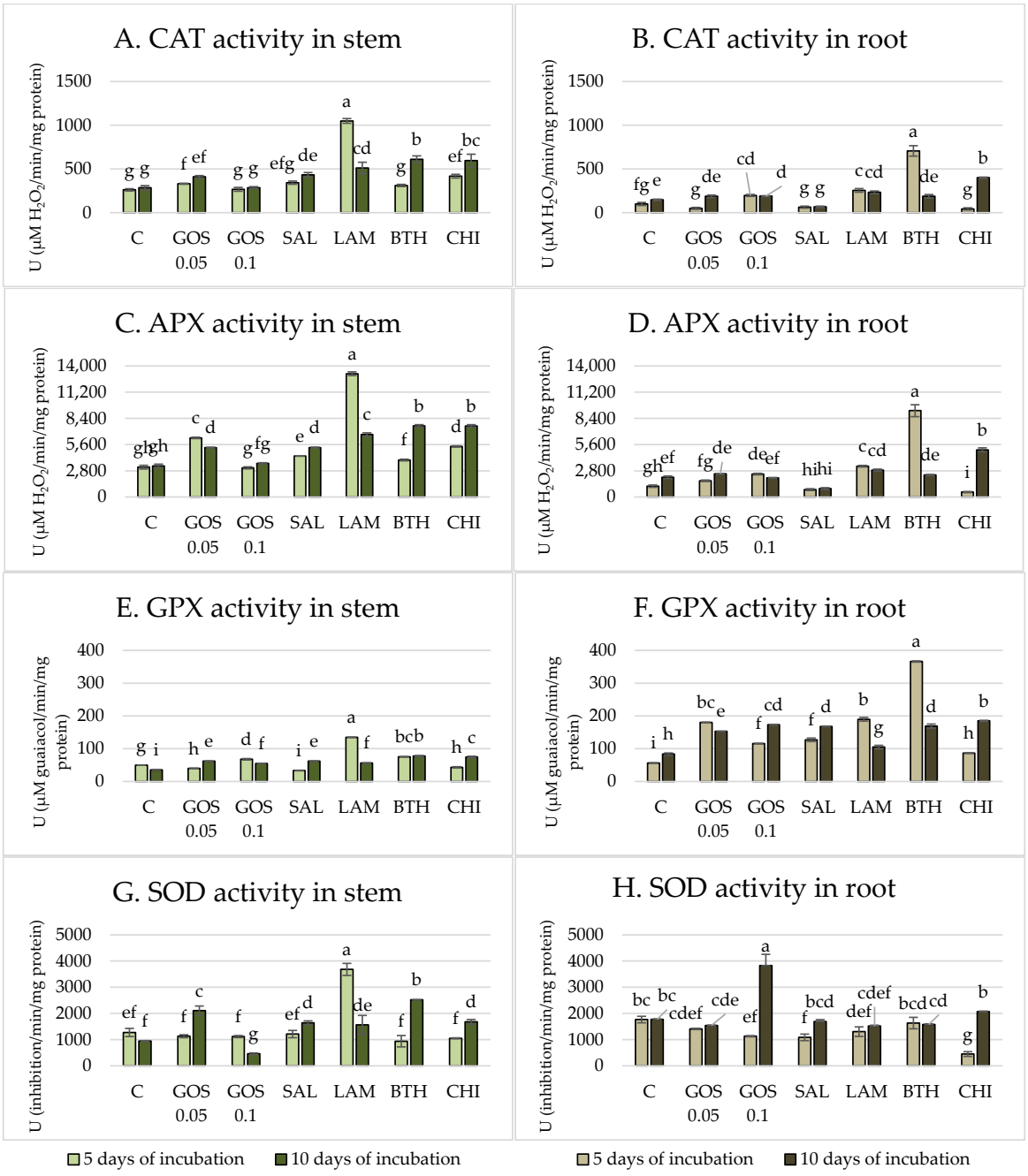


Figure 4. The activity of catalase (CAT), ascorbate (APX) and guaiacol (GPX) peroxidases, and superoxide dismutase (SOD) in wheat stems (**A**,**C**,**E**,**G**) and roots (**B**,**D**,**F**,**H**) after 5 and 10 days of incubation in the presence of elicitors. C–water control, GOS 0.05–(1→3)-α-D-glucooligosaccharides 0.05%, GOS 0.1–(1→3)-α-D-glucooligosaccharides 0.1%, SAL–salicylic acid, LAM–laminarin, BTH–acibenzolar-S-methyl, CHI–chitosan. One-way ANOVA, post hoc Tukey Test $p < 0.05$ (bars with the different letter are statistically significantly different from each other and control object). SD was measured in three biological replicates (n = 3) from plant extract sample (1g FW/8 mL of buffer). Bars with the different letter are statistically significantly different from each other and control object.

It was shown that the activity of CAT was twice as high in wheat stems than as in the roots (Figure 4A,B). After 5 days of incubation, the CAT activity in the plant stems was usually at the same level (260–280 U) as in the water control (C). Only the LAM elicitor had the most significant influence on CAT in wheat stems, where a 3-fold increase in the activity of this enzyme (up to 1049 U) was observed. A low increase in CAT activity in wheat stems on the 5th day of incubation was also noted in the case of using GOS 0.05 and CHI. In turn, on the 10th day of incubation, the activity of CAT in the stems was higher as a result of the action of commercial elicitors and GOS 0.05, which increased the activity of this enzyme almost twice, compared to the C (Figure 4A). On the other hand, in wheat roots, CAT activity was highest after seed inoculation with commercial elicitors, especially LAM, BTH, and CHI. The applied glucooligosaccharides had a significant effect on the CAT activity at a concentration of 0.1% (GOS 0.1), both on the 5th and 10th day of incubation. Moreover, GOS 0.1 influenced CAT activity in the wheat roots similar to the commercial elicitor LAM (Figure 4B).

A very similar mode of action of elicitors on APX activity in wheat stems and roots was observed as in the case of CAT activity (Figure 4C,D), however, the APX activity was even 12-times higher than CAT activity. Moreover, the APX activity was 3–4-times higher in the stems than in the roots after treatment with elicitors. The highest increase in APX activity (up to 13,000 U) was observed in wheat stems on the 5th day of incubation after the application of the LAM elicitor. Importantly, a 2-fold increase in APX activity in the stems caused GOS 0.05, both on the 5th and 10th day of incubation, compared to the C. Moreover, GOS 0.05 increased the APX activity in stems on the 5th day of incubation more strongly than the commercial elicitors SAL, BTH, and CHI. On the other hand, GOS 0.1 did not significantly increase the APX activity in wheat stems (Figure 4C). In turn, glucooligosaccharides had a significant effect on the APX activity in wheat roots only at a concentration of 0.1% (GOS 0.1) after 5 days of incubation, increasing the activity of this enzyme twice, compared to the C. However, the APX activity in wheat roots was highest after seed application with commercial elicitors, especially LAM, BTH, and CHI (Figure 4D).

In contrast to CAT and APX, the activity of GPX after inoculation of seeds with elicitors was even 2-times higher in wheat roots (Figure 4E,F). The GOS 0.05 and GOS 0.1 influenced the activity of GPX in wheat stems similar to commercial elicitors and water control (Figure 4E). On the other hand, after the application of GOS 0.05 and GOS 0.1, the activity of GPX in the wheat roots was even three times higher than in the C, both on the 5th and 10th day of incubation. A similar level of GPX activity was observed in the case of commercial elicitors SAL, LAM, and CHI (Figure 4F).

After the application of elicitors, the activity of SOD was similar in both the stems and the roots (Figure 4G,H). The increase in SOD activity in wheat stems was highest after LAM application (3681 U) on the 5th day of incubation. In turn, glucooligosaccharides increased SOD activity in wheat stems only at a concentration of 0.05% after 10 days of incubation. However, this activity was significantly higher than after the application of SAL and CHI (Figure 4G). Importantly, GOS 0.1 did not increase the activity of SOD in the stems, but it most strongly increased the activity of this enzyme in wheat roots on the 10th day of incubation, compared to the water control and commercial elicitors. After the application of GOS 0.01, the SOD activity in the wheat roots on the 10th day of incubation (3829 U) was even 2.5-times higher than after treatment with commercial elicitors (1520-2067 U) and water control (1774 U). The GOS 0.05, similarly to commercial elicitors, did not increase the SOD activity in wheat roots (Figure 4H).

The second important group of defense enzymes are phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL), which activate the phenylpropanoid pathway. It was shown that GOS 0.05 and GOS 0.1 significantly increased the PAL activity in both wheat roots and stems (Figure 5A,B). The PAL activity in the wheat stems was the highest on the 10th day of incubation after the application of GOS 0.1 (82 U), increasing the activity of this enzyme 3-fold compared to the water control (27 U). Moreover, the PAL activity

after the GOS 0.1 application was significantly higher than after treatment with commercial elicitors (Figure 5A). In turn, the activity of PAL in wheat roots was the highest on the 5th day of incubation after the application of both GOS 0.05 (52 U) and GOS 0.1 (67 U). Thus, the oligosaccharides increased the PAL activity in the roots even 4-fold compared to the water control (18 U) and commercial elicitors (15–42 U). However, the applied elicitors did not significantly increase the PAL activity in wheat roots on the 10th day of incubation (Figure 5B).

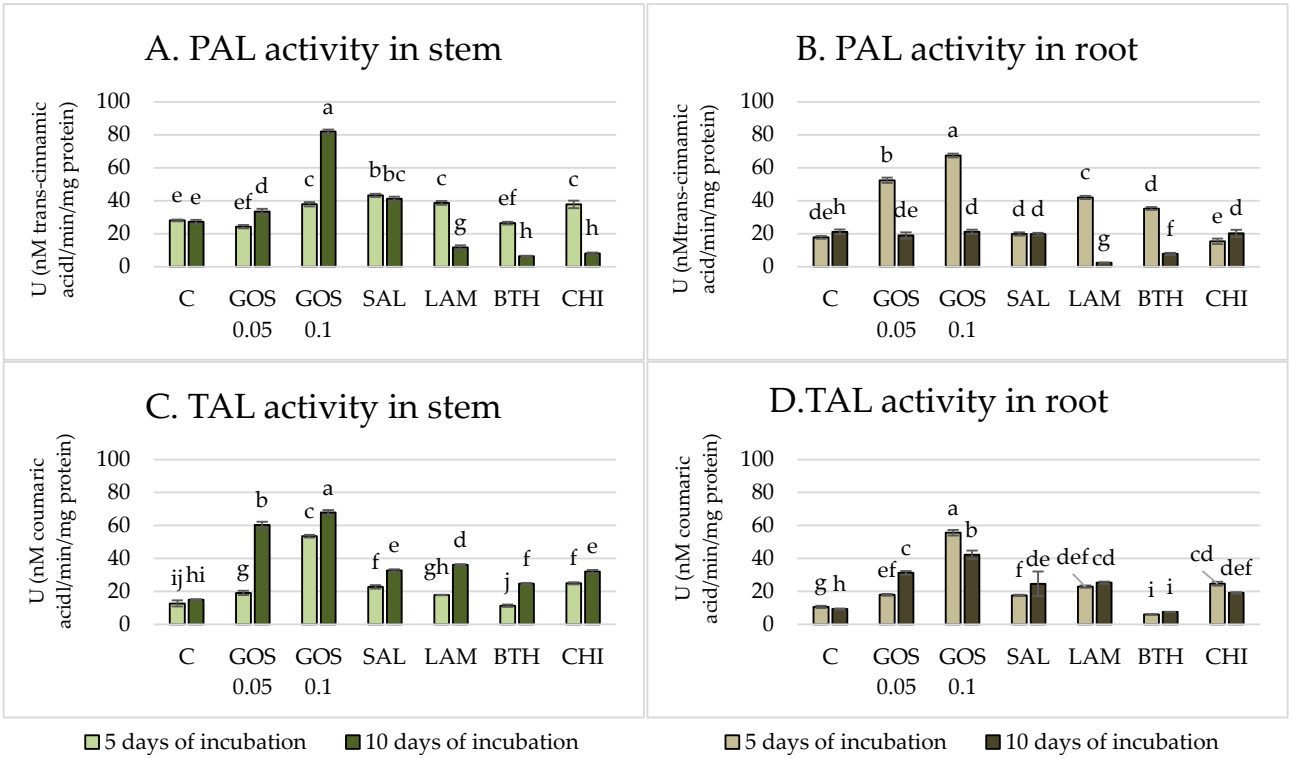


Figure 5. The activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) in wheat stems (**A**,**C**) and roots (**B**,**D**) after 5 and 10 days of incubation in the presence of elicitors. C–water control, GOS 0.05–(1→3)-α-D-glucooligosaccharides 0.05%, GOS 0.1–(1→3)-α-D-glucooligosaccharides 0.1%, SAL–salicylic acid, LAM–laminarin, BTH–acibenzolar-S-methyl, CHI–chitosan. SD in three replicants. One-way ANOVA, post hoc Tukey Test $p < 0.05$ (bars with the different letter are statistically significantly different from each other and control object). SD was measured in three biological replicates (n = 3) in plant extract samples (1g FW/8 mL of buffer). Bars with the different letter are statistically significantly different from each other and control object.

After the application of oligosaccharides, an increase in TAL activity in wheat stems and roots was also noted (Figure 5C,D). The highest increase in TAL activity was observed in wheat stems in the presence of GOS 0.1 (82 U) and GOS 0.05 (60 U) after 10 days of incubation. Moreover, the activity of TAL in stems after the application of GOS was even 4.5-times higher than in the presence of water control (15 U) and almost 3-times higher than after the application of commercial elicitors (25–36 U) (Figure 5C). On the other hand, in wheat roots, the TAL activity (56 U) was most strongly increased by GOS 0.1 on the 5th day of seedling growth. The GOS 0.1 also increased TAL activity in the roots most strongly after 10 days of incubation. Commercial elicitors (SAL, LAM, BTH, and CHI) had even 5-times lower influence on the enzymatic activity in the wheat roots than GOS 0.1. Importantly, the GOS 0.05 also caused a significantly higher increase in TAL activity in the roots than the water control and commercial elicitors, after 10 days of incubation (Figure 5D).

The activity of enzymes classified as PR proteins, glucanase (GLUC) and chitinase (CHIT), was also determined (Figure 6).

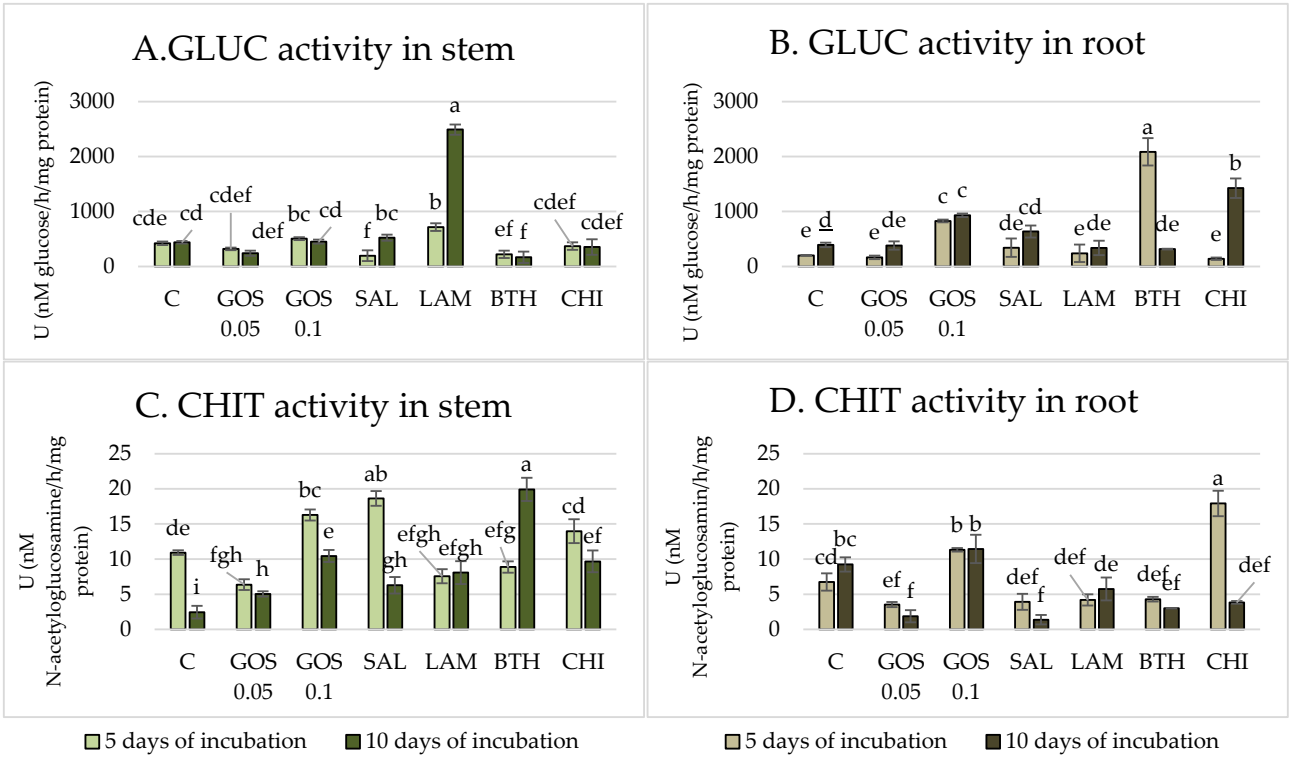


Figure 6. The activity of glucanase (GLUC) and chitinase (CHIT) in wheat stems (**A**,**C**) and roots (**B**,**D**) after 5 and 10 days of incubation in the presence of elicitors. C–water control, GOS 0.05–(1→3)-α-D-glucooligosaccharides 0.05%, GOS 0.1–(1→3)-α-D-glucooligosaccharides 0.1%, SAL–salicylic acid, LAM–laminarin, BTH–acibenzolar-S-methyl, CHI–chitosan. One-way ANOVA, post hoc Tukey Test $p < 0.05$ (bars with the different letter are statistically significantly different from each other and control object). SD was measured in three biological replicates (n = 3) in plant extract samples (1g FW/8 mL of buffer). Bars with the different letter are statistically significantly different from each other and control object.

No significant influence of GOS was observed on the GLUC activity in the wheat stems. It was also observed that the application of GOS 0.05 did not increase the GLUC activity in wheat roots. The highest increase in GLUC activity in the stems was caused by the LAM elicitor (2491 U) after 10 days of incubation. In contrast, the LAM did not increase the GLUC activity in the roots (Figure 6A,B). Moreover, the GOS at a concentration of 0.1% significantly increased the GLUC activity in wheat roots compared to the water control and commercial elicitors (SAM and LAM) after both 5 and 10 days of incubation, increasing the GLUC level up to 3-times (Figure 6B).

The CHIT activity in wheat tissues was 100-times lower than GLUC activity. Moreover, the CHIT activity after treatment with elicitors was higher in the stems than in the roots (Figure 6C,D). The GOS increased the CHIT activity in the wheat stems and roots at a concentration of 0.1%. After the application of GOS 0.1, the CHIT activity in the stems on the 5th day of incubation increased several times, compared to the water control and commercial elicitors LAM and BTH. In addition, GOS 0.1 acted on the CHIT activity in the stems similar to the SAL and CHI (Figure 6C). Other than that, after the application of GOS 0.1, the level of CHIT in wheat roots was significantly higher (11 U) than after the use of water control and all commercial elicitors, except for CHI, which increased CHIT activity most strongly (18 U) on the 5th day of incubation (Figure 6D).

3.4. The Principal Component Analysis (PCA) and Heat Map of Defense Enzyme Activity

The relationship between the inoculation of wheat seeds with commercial elicitors and GOS, and the increase in the activity of defense enzymes in the stems and roots of the plant

was determined *via* Principal Component Analysis (PCA) (Figure 7). The first two axes of the PCA based on the First Order Reaction (FOR) model explained 53.87% of data variability, with principal component 1 (PC1) accounting for 30.05% and principal component 2 (PC2) accounting for 23.82% of the variance. The PCA analysis showed a significant increase in the activity of all examined parameters as a result of the action of commercial elicitors and GOS. Importantly, after the application of GOS, the activity of these enzymes was comparable to the commercial elicitors and significantly higher compared to the water control. The samples on the left and lower side of the diagram had the highest values of tested parameters. Other than that, the increase in the activity of immune enzymes was particularly evident in wheat stems. A clear increase in the activity of APX, CAT, and CHIT was noted in 10-day-old wheat seedling stems. After seed inoculation with commercial elicitors and GOS 0.05, a significant increase in the mass of the stems was also observed on the 10th day of incubation. The PCA analysis showed an increase in PAL and TAL activity in 5-day-old wheat stems after seeds inoculation with SAL, CHI, GOS 0.05, and GOS 0.1. However, the activity of these parameters also increased in the 10-day-old wheat stems after the application of GOS 0.1. In turn, the values of the GPX, SOD, and GLUC were particularly high in wheat roots after the application of BTH (on the 5th day of incubation), as well as CHI and GOS 0.1 (on the 10th day of incubation).

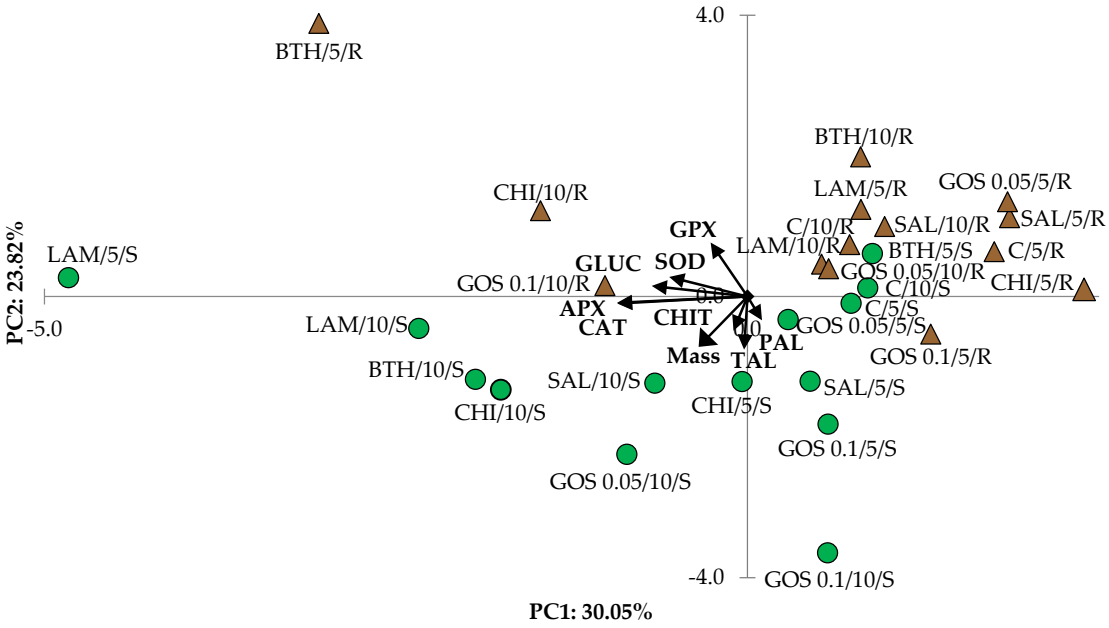


Figure 7. Diagram of the Principal Component Analysis (PCA), describing the activity of immune enzymes, CAT–catalase, APX–ascorbic peroxidase, GPX–guaiacol peroxidase, PAL–phenylalanine ammonia lyase, TAL–tyrosine ammonia-lyase, SOD–superoxide dismutase, GLUC–glucanase, and CHIT–chitinase in 5- and 10-day-old wheat tissue—stem (S) and root (R), after the application of water control (C), commercial elicitors (LAM–laminarin, BTH–benzo (1,2,3) thiadiazole-7-carboxylic acid S-methyl ester, and CHI–chitosan), and oligomers (GOS 0.05–0.05% (1→3)-α-D-glucooligosaccharides and GOS 0.1–0.1% (1→3)-α-D-glucooligosaccharides). Graphic designations: green circle—the stem, brown triangle—root. CAT, APX, GPX, PAL, TAL, SOD, GLUC, and CHIT—average activity expressed in the U unit; Mass—stems and roots fresh weight (in milligrams). Graphic designations: green circle—the stem, brown triangle—the root.

The increase in the activity of marker enzymes of the resistance pathways in wheat tissues after seed inoculation with GOS, commercial elicitors (SAL, LAM, BTH, and CHI) and water control was documented as a heatmap (Figure 8). This analysis illustrated a significant increase in the activity of marker enzymes in wheat tissues after the application of GOS, compared to the water control, especially in relation to the PAL, TAL, and GLUC activity. Other than that, the action of the GOS was often similar or stronger to the com-

mercial elicitors. The activity of the parameters studied was also dependent on the day of incubation and the plant tissue.

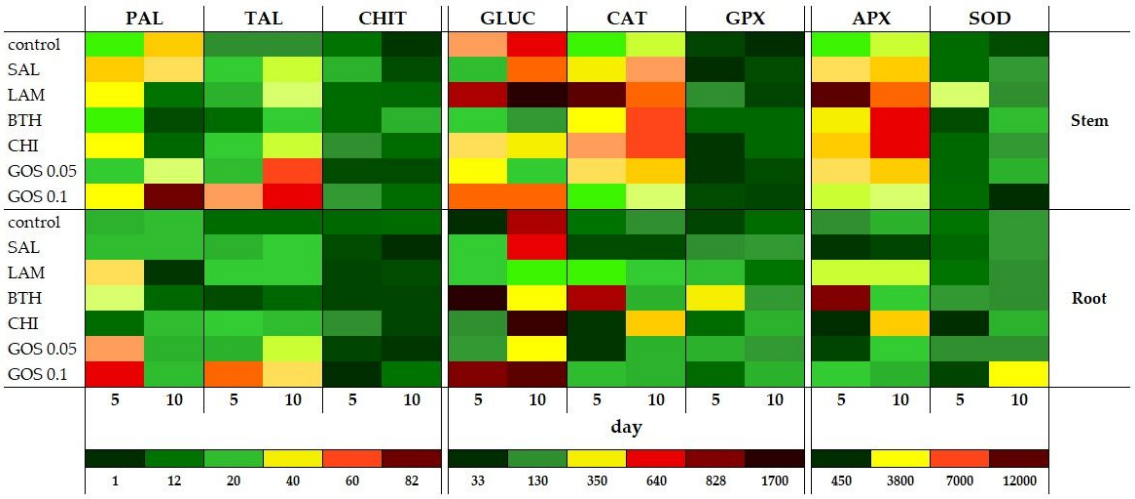


Figure 8. The heatmap of the immune enzymes, PAL–phenylalanine ammonia lyase, TAL–tyrosine ammonia-lyase, CHIT–chitinase, GLUC–glucanase, CAT–catalase, GPX–guaiacol peroxidase, APX–ascorbic peroxidase, and SOD–superoxide dismutase, showing an increase in the activity of these enzymes in 5- and 10-day-old wheat tissues (stem and root) after the inoculation of seeds with water control (control), commercial elicitors (LAM–laminarin, BTH–benzo (1,2,3) thiadiazole-7-carboxylic acid S-methyl ester, and CHI–chitosan), and oligomers (GOS 0.05–0,05% (1→3)-α-D-glucooligosaccharides and GOS 0.1–0.1% (1→3)-α-D-glucooligosaccharides). The activity of PAL, TAL, CHIT, GLUC, CAT, GPX, APX, and SOD presented in the heatmap is expressed in the U unit. The highest activity can be identified by red color and the lowest activity by green color.

4. Discussion

The main elicitor factors responsible for stimulating the induction of plant immunity are compounds with a structure of β-glucans with different types of glycosidic bonds and branches. Their properties are also influenced by the a different degree of polymerization [20,47]. The second group of important elicitor factors are chitin and chitosan, a heteropolymer of (1→4)-β-linked N-acetylglucosamine with a variable percentage of deacetylated glucosamine. For these factors, the degree of deacetylation is crucial [20,48]. However, important compounds with elicitor properties seem to be a short oligosaccharides (OS) linked by α-bonds. This group of compounds includes oligogalacturonides (OGAs) [27], fructooligosaccharides (FOSs) [49], mannan-oligosaccharides (MOSs) [50], Salecan oligosaccharides (ScOs) [51], ALG oligosaccharides (AOSs) [52], and chitosan-oligosaccharides (COSs) [53]. All these compounds can stimulate the plant's defense response.

In our research, we observed the effect of stimulation (up to 50–100%) of the growth of wheat seedlings after seed inoculation with the oligomer suspensions, similar to the studies described by Li et al. [53], in which a positive effect of the chitosan–oligosaccharide (COS) mixture mixture on the growth of maize seedlings after coating the seeds with this biopesticide was observed. This positive effect was attributed to the reduction in the respiration rate, an increase in photosynthetic pigments, and an increase in root vigor as a result of a reduction in the consumption of photosynthesis products (higher photosynthetic rate). Other than that, an increased shoot length of wheat seedlings was observed [54] after treatment with chitooligomers (chitobiose to chitoheptaose) characterized by a different degree of polymerization, and the best effect was achieved with a mixture of these oligomers. Randhir and Shetty [55] showed that the application of natural elicitors (protein hydrolysates, lactoferrin and oregano extract) enhanced the vigor of broad bean (*Vicia faba*) seeds and plant productivity, as well as the total phenol content and the induction of phenylpropanoid and shikimate pathways. The stimulation of germination probably

resulted from the increased activity of GPX and the high concentration of phenols, which are necessary for the lignification process and structural development during the plant growth [55].

Many studies indicate that low molecular mass substances (i.e., SA), sugars and sugar polymers act as signaling molecules in determining seed vigor and affect plant development by shaping the hormonal response and emphasize the different effects of sugars used at low and high concentrations [56–58]. It was observed that high levels of exogenous glucose caused ABA accumulation, which resulted in a delay of germination and the inhibition of seedling development by limiting energy and metabolites, whereas low or medium levels of the exogenous sugar monomer (glucose) mitigated the inhibition of seed germination induced by exogenous ABA. At high sugar concentrations in the seed germination medium, the imbibition process is disrupted by reducing the water potential of the growth medium and hindering the uptake of water by the seeds [59].

Treatment of tobacco with polymer led to increased activity of PAL, caffeic acid concentration, O-methyltransferase and lipoxygenase, SA accumulation, and transcriptional activation of PR proteins [22]. Laminarin, at a later stage of the plant growth, caused the expression of genes associated with octadecanoid and phenylpropanoid pathways, leading to significant protection of vine leaves against *Botrytis cinerea* and *Phomopsis viticola* [60]. Other polymeric compounds with elicitor properties are chitin and chitosan. Chitin stimulated the production of phenylpropanoids in *Hypericum perforatum* cell cultures. Chitin also showed a higher ability to stimulate the synthesis of hypericin and pseudohypererin compared to pectin and dextran [20,61] in soybean and parsley, mediated by the polymer (1→3)-β-D-glucan. In melon tissues cultured in the presence of this polymer, an increase in chitinase activity was observed, and in the case of wheat, lignin deposition and an increase in the level of phenolic acids in the leaves were noted [22].

Glucan elicitors from fungal cell walls belonging to the β-glucans group have been described extensively [20]. The data reported in this study showed that (1→3)-α-D-glucooligosaccharides (GOS) obtained from fungal (1→3)-α-D-glucan, induced multiple defense in wheat tissues. So far, only a few studies have shown the participation of α-glucans in the induction of plant resistance [62–65].

There is very little information as to whether α-glucans may be a factor recognized by plants as PAMPs that induce immunity. α-glucans from the cell wall of the non-pathogenic binucleate *Rhizoctonia* induced β-glucanase activity in potato sprouts, the primary site of the infection by *R. solani*. However, this α-glucan did not induce phytoalexin accumulation, which is commonly known as the crucial stage of the plant defense response [62]. The application of (1→3)-α-D-glucans isolated from a non-pathogenic *Rhizoctonia* induced a wide range of potato defense reactions and resulted in 40% protection against *Rhizoctonia* cancer and 60% protection against dry rot in potato plants and tubers. This (1→3)-α-D-glucan caused a strong increase in the level of PR proteins, glucanase and chitinase, and showed an increase in the cell wall deposition of callose and lignin [63]. Wolski et al. [64] observed the activity of α-glucanase in potato sprouts after the application of glucans from the cell wall of the pathogenic *R. solani* AG-3 isolate. However, this effect was not observed after treatment with glucans from the cell wall of non-pathogenic strains [60]. Lu et al. [62] showed that tomato fruits were able to recognize (1→3)-α-D-glucans and induce resistance against *B. cinerea* infection.

Different groups of oligosaccharides affect various immune pathways and the activity of defense enzymes [20]. One of the most important features of elicitor compounds is their activity at low concentrations. For laminarin, the influence on the activity of plant defense was observed at the concentration of 50 μg/mL [66], and in the case of OGA, at the concentration of 60 μg/mL [67]. The GOS used in our study showed elicitor activity at a concentration of 0.05% (500 μg/mL) in the case of CAT, APX, and GPX. On the other hand, the GOS at the concentration to 0.1% (1000 μg/mL) had a higher influence on the activity of the PAL, TAL, GLUC, and CHIT. Li et al. [56] achieved the effect of stimulating the activity of these enzymes at the concentration of 200 μg/mL in maize seedlings. MOS with a degree

of polymerization of 2–6 at a concentration of 500 µg/mL trigger the ROS level in rice and tobacco [50]. In contrast, GOS-OGA (FytoSave) preparation for foliar application required a concentration of 60 µg/mL to induce a protective response in potato and rice [68]. The dependence of changes in enzyme activity on the concentration of the elicitor compound is similar as in the case of chitosan, where reports on its elicitor properties refer to the concentration range from 500 to 2000 µg/mL [20].

Antioxidant enzymes, including CAT, GPX, APX, and SOD, play an important role in protecting plants against reactive oxygen species (ROS). ROS play a crucial role in the regulation of plant growth, but an increase in their concentration in tissues can damage the plant. Increasing the level of ROS is one of the first plant responses to pathogen attack [53,69]. The GOS has a significant influence on the level of CAT and APX in the stems and the activity of GPX in the roots. The activity of CAT and GPX (POX in the described study) was increased after the application of *P. glaucum* seedling with cell walls glucans (CWGs) from *Trichoderma harzianum* [70]. However, GOS doesn't influence the SOD activity in wheat tissues similar to CWG. AOS used together with *Meyerozyma guilliermondii* increases the activities of enzymes like: superoxide dismutase (SOD), catalase (CAT), peroxidase. (POD) [71]. Given that the activity of antioxidant enzymes is significantly related to the ability of plants to eliminate oxidative stress caused by biotic or abiotic stress, GOS can be expected to improve the stress resistance of wheat plants.

Phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) play a crucial and synergistic role in catalyzing the first stage of the transformation of the amino acid phenylalanine and tyrosine, respectively, in the phenylpropanoid pathway. These enzyme lead to the development of lignin and other phenolic compounds in the phenylpropanoid pathway, which are involved in reinforcing the cell structure against pathogens and the production of several types of phytoalexin [72–74]. The GOS at the concentration of 0.1% had the greatest influence on the activity of PAL and TAL enzymes. Laminaripentaose from *Laminaria digitata* caused an increase in the PAL level in tomato tissues [75]. The same activity was observed in soybean after treatment with AOS [76]. The COS-OGA (FytoSave) increased the expression of PAL genes in rice and potato [68]. Moreover, TAL activity was increased by chitin and chitosan oligomers in soybean leaves [54].

PR proteins with β-glucanase (PR2 and PR5 group) and chitinase (PR3, PR4, PR8, and PR11 group) activity were produced in plant tissues after pathogen infection or induction by elicitors of plant resistance [41]. These enzymatic activities were detected in fungal cultures supplemented with fragments of the pathogen's cell wall [12,13,26]. Proteins with β-glucanase and chitinase activity can degrade the cell walls of fungi and inhibit their growth [77]. Although GOS, which have been studied, provide a higher increase in chitinase activity (Figure 6). This may translate into the plant defense response during the hydrolysis of the pathogen's cell wall. In particular, these oligosaccharides are derived from the cell wall of the tree parasite fungus. On the other hand, the use of COS causes increased activity of both enzymes in wheat seedlings [78]. (1→3)-β-glucans from *Septoria tritici* induced glucanase and chitinase activity in two different strains of wheat. A higher response was observed in plants co-inoculated with pathogens [77].

The most important property of the examined GOS is their structure—they are glucose polymers linked by α-bonds. Most of the described and cited studies are based on polymers with β-bonds. The results obtained in our research proved that oligomers with α-bonds can be used in agriculture as plant resistance elicitors and effective biological plant protection agents.

5. Conclusions

Despite their different applications, mainly as compounds with probiotic potential, (1→3)-α-D-glucooligosaccharides can also be used to stimulate plant resistance against pathogens. The results presented in this study showed that GOS had a positive effect on the activity of antioxidative enzymes (CAT, APX, GPX, SOD), enzymes involved in the phenylpropanoid pathway and lignin synthesis (PAL, TAL), as well as pathogenesis-related

(PR) proteins with enzymatic activity (GLUC, CHIT). Other than that, GOS can replace commonly used commercial elicitors due to their stronger effect on increasing resistance in plants. Also interesting seems to be the variable influence of the concentration of (1→3)-α-D-glucooligosaccharides on the activity of individual enzymes. The obtained results indicate the complicated influence of elicitor compounds on the transmission and generation of immune signals in plants. This study demonstrated for the first time the effective use of (1→3)-α-D-glucooligosaccharides, obtained by acid hydrolysis of (1→3)-α-D-glucan from *L. sulphureus*, in the induction of the cereal plant (wheat) resistance.

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