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Binucleate *Rhizoctonia* Strain: A Potential Biocontrol Agent in Wheat Production

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Abstract: As a polyphagous organism, *Rhizoctonia* is one of the most infectious soil-borne pathogens for many plant species. To reduce this threat to plants and hence provide good quality plant products for the end-user, it is crucial to develop sophisticated and environmental friendly plant protection methods. One such method is biological control using beneficial agents, e.g., microorganisms such as bacteria and fungi, but also mites or insects. To investigate the potential of this control, we present here a study involving AG B0 (BNR), which is a binucleate, non-pathogenic *Rhizoctonia* as a control against pathogens from the same genus, namely, *Rhizoctonia cerealis* (AG DI) and *Rhizoctonia solani* (AG5 and AG 1IC). This is novel because the relationship between plant-pathogen and plant-non-pathogen interactions has received only limited attention. Once the relative activities of the various plant defense mechanisms and the overall plant conditions were taken into account, the non-pathogenic binucleate strain was found to lower the impact of the pathogenic strain. We conclude that the response of plant genotype within the context of the protective activity of the non-pathogenic *Rhizoctonia* is race-specific. The research showed the ability to successfully protect wheat plants from *R. cerealis* negative impact, as well as in some cases from *R. solani*.

Keywords: pathogens; wheat; biological control; enzymes

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1. Introduction

Taking into account the growing food production needs of the human population, the protection of crops against pests, as well as maintaining high quality produce for human consumption, is crucial. Unfortunately, despite the principles of integrated pest management having been implemented in 2014, which assume priority use of non-chemical methods (e.g., agrotechnical, biological, and physical) over chemical protection, the use of conventional protection products still dominates, either in the form of preventive seed treatment or in the form of emergency spraying [1–3]. Owing to their differences in karyotype and genotype, *Rhizoctonia* fungi pose a very complex problem for crop protection. The multitude of anastomosis groups and the related genetic complexity present a great global problem. Unfortunately, despite intensive work on this problem, a sufficiently effective method of interventional and preventive control of the pathogen has not been obtained. An increasingly important alternative to conventional chemical control of *Rhizoctonia* pathogens is the use of biological plant protection agents [3]. Depending on the type of pathogen, i.e., whether it is bacteria, actinomycetes, or fungus, etc., the protective measures vary. There are many reports in the literature dealing with the biological control of *Rhizoctonia* spp., from the fight for a niche through competition, in which a stronger, more expansive microorganism wins, to direct combat of pathogens by antibiosis or parasitism [1,2]. Batemann et al. [2] indicate the importance of the problem and the lack of effective conventional plant protection products in the fight against *Rhizoctonia*, which has prompted many researchers to use microorganisms as a method of biological protection in the fight against plant pathogens. The microorganisms that can be used in such protection

include bacteria of the *Bacillus* genus, characterized by a fast growth rate and activity limiting the occurrence of pathogenic fungi on the basis of direct control by, among others, insecticides, lytic and antibiotic [3–5], or the ability to synthesize indole-3-acetic acid and stimulate plant growth [6,7]. Other promising biological plant protection bacteria are strains of the genus *Pseudomonas* (*P. fluorescens, P. aureofaciens, P. aeruginosa* and others), which are characterized by some effectiveness in reducing the occurrence of *Rhizoctonia* [8–12]. Another beneficial microorganism in reducing *Rhizoctonia* is the Gram-negative endophytic bacteria *Pantoea agglomerans*, which can limit the growth of *R. solani* [7,13,14]. In turn, Yin et al. [15] suggest that the biodiversity of bacteria in the rhizosphere, and especially in the presence of *Chryseobacterium soldanellicola*, is effectively able to limit the growth of *R. solani* AG 8. Kasiamdari at al [16], Berta et al. [17], and Buysens et al. [18] report on the possibility of using mycorrhizal fungi of the genus *Rhizophagus* (formerly *Glomus*) to reduce the negative effects caused by fungi of the genus *Rhizoctonia.* As the habitat of these fungi is the rhizosphere, they can effectively prevent the development of pathogens by competitive interaction and displacing a weaker competitor (pathogen) by occupying an appropriate niche, thereby blocking access to nutrients. Another group of microorganisms that is of interest as a potential biological plant protection agent is yeast. It is possible to use some yeasts to control soil-borne pathogens that exhibit antagonistic activity against such pathogens as *R. cerealis* or *Gaeumannomyces graminis*. Wachowska and Borowska [19] indicate the possibility of using *Aureobasidium pullulans* var. *pullulans* and *Sporobolomyces roseus* in limiting the development of *R. cerealis* in winter wheat crops. Moreover, some yeast species are able to survive adverse environmental conditions, such as residues of conventional plant protection products. Thus, yeast can perform a protective function for crops for better and longer than other microorganisms [20]. The literature is littered by reports in which *Trichoderma* fungi are used to limit the development of pathogens. They are common with a parasitic model of action against other fungi (mycoparasites), including plant pathogens, and induce defense processes in plants. As these fungi are faster growing than *Rhizoctonia*, they are able to reduce rapidly the population of these pathogens. Their fungistatic properties in relation to pathogens were observed already in the 1930s. Chet et al. [21] in in vitro studies observed that *Trichoderma hamatum* effectively inhibited the development of mycelium of *R. solani* and *Pythium* spp. There are also reports of the use of hypovirulent (non-pathogenic) *Rhizoctonia* strains to control pathogenic *Rhizoctonia* strains as well as other harmful pathogens such as *Fusarium* and *Pythium* [22–24]. Depending on the affiliation to the anastomosis group of hypovirulent microorganisms, it is possible to limit the occurrence and development of pathogens [25]. This model of action can take various forms. A competitive interaction between a pathogen and a non-pathogen is assumed in order to occupy the same ecological niche and food resources. It was also observed that non-pathogenic strains of *Rhizoctonia* grew over the outer epidermal layer of the root, without a tendency to grow into the sub-epidermal cells of the root, which further intensified the effects' competition between microorganisms, as well as preventing pathogens from penetrating inside the roots [26]. In addition, the induction of defense mechanisms in plants treated with non-pathogenic strains is believed to make it difficult, or even impossible, for pathogens to invade the plant and limit their development. These mechanisms include the induction of the chitinolytic apparatus, the induction of lignification mechanisms and the general strengthening of the cell wall structure by deposition of cellulose, hemicellulose, phenolic radicals and others [1]. Sharon at al [26] indicate that the model of action of non-pathogenic, hypovirulent *Rhizoctonia* is based on inducing the chitinolytic apparatus in the form of enzymes from the group of β-1, 3-glucanases, chitinases, and enzymes from the group of oxidoreductases (including e.g., peroxidases), or L-phenylalanine ammonia lyase (PAL) as a mechanism for strengthening cell walls, as well as stimulating metabolic pathways related to the deposition of pectins and suberins. The common denominator in all of the cited examples that use biological plant protection agents is the inducing systemic resistance (SAR) within the organism. Xue at al [27] proved that an early introduction into a non-pathogenic (hypovirulent) environment of the *Rhizoctonia* strain caused the induc-

tion of mechanisms of acquired systemic immunity in the form of increased enzymatic activity of endo- and exo-chitinases, glucanases and peroxidases in bean plants. Thus, the presence of such a microorganism resulted in a reduction of disease symptoms and a several-fold increase in the activity of the tested enzymes. Hassan et al. [28] proved that it is possible to use non-pathogenic *Rhizoctonia* strains to reduce the occurrence of *R. solani* and the losses caused by it in cucumber cultivation. It was observed that early treatment of seedlings with a non-pathogenic strain allowed for a significant reduction of disease symptoms. It was noted that the use of such a strain significantly reduced the production of infectious structures (appresorium) by pathogenic *R. solani*. Similar conclusions were drawn by Elsharkawy et al. [29] by treating cucumber seedlings with a non-pathogenic binucleate *Rhizoctonia* AG A isolate. They proved that already after 12 h the outer layer of the root was colonized by the hypovirulent strain, thus preventing the development of the pathogenic *R. solani* AG 4 isolate. Numerous deformations in the structure of the *R. solani* mycelium were also found when a non-pathogenic microorganism was introduced earlier and increased production of pectins by the host plant, which included methylated pectins and a significant content of uronic acids, showing a lytic effect on fungal cell walls, as well as increased transport of calcium ions. In turn, Harris and Adkins [25] observed that non-pathogenic, binucleate *Rhizoctonia* isolates effectively inhibited the growth of *R. solani* in greenhouse experiments in both sterile and non-sterile media. Moreover, it was observed that in the presence of the pathogenic *R. solani* isolate, the non-pathogenic strain resulted in the stimulation of growth in the hair root zone of pepper roots. Webb et al. [30] used two non-pathogenic *R. zeae* isolates against sugar beet in biological protection against soil-borne pathogens. In in vitro studies, they observed that both isolates effectively inhibited the development of *R. solani* AG 2-2IIIB, *F. oxysporum* f. sp. *betae*, *Phoma betae* and *Pythium aphanidermatum.*

Unfortunately, biosecurity has its limitations. The use of a live microorganism to combat another and more dangerous one requires familiarization with the nature of the pathogen itself, its development cycle, habitat and nutritional requirements, and the relationship with the host plant. The introduction of new microorganisms into the environment may be associated with the disturbance of the existing balance in a given agrocenosis. An additional problem is the sheer multitude of anastomose groups among *Rhizoctonia*, which is associated with the likelihood of fusion between the target pathogen present in the environment that we want to combat, and the used biological plant protection factor, which may show some biocompatibility. Another limitation is the requirement that the microorganism used should not show any pathogenic features, even in poor conditions in terms of the availability of nutrients. In other words, it cannot be opportunistic in order to achieve basic benefits from the relationship with the plant and have a negative impact on the host plant by inhibiting its growth and development, causing developmental deformities and disturbances in metabolic pathways, or showing similar effects to pathogenic phenomena in relation to the plant. Thus, the habitat in which such a biological agent is to be used cannot induce such characteristics. In addition, it is important to select the right seed material for a given habitat, so that the development of plants is as optimal as possible. This involves the need to classify the soil complex in the place of cultivation and on this basis to match the appropriate varieties. In addition, the effective operation of a biological plant protection product in a given habitat is related not only to the interaction with a biotic basis (pathogen-non-pathogen, plant-pathogen, etc.), but also strictly depends on the amount and intensity of abiotic factors, i.e., temperature, water availability, pH [31]. The aim of our research was to assess where the use of hypovirulent, non-pathogenic *Rhizoctonia* strain as a biological control agent can be possible, and if so, whether there are any limitations in the case of cereal protection from pathogens from the same genus.

2. Materials and Methods

2.1. Experiment Conditions and Plant Material

A strict experiment in controlled environment was set up in order to study the response of wheat genotypes to the researched microorganisms. Five wheat genotypes were used in the experiments: *Triticum aestivum* ssp. *aestivum* cv. Toras, *Triticum turgidum* ssp. *durum* (Desf.) Husn. cv. Karmadur, *Triticum aestivum* ssp. *spelta* L. cv. Rokosz, *Triticum sphaerococcum* (Percival), and *Triticum persicum* Vavilov. These were used as the first experimental factor. In order to facilitate the notation in this work, the following abbreviated names of the species have been adopted: *T. aestivum*, *T. durum, T. spelta, T. sphaerococcum*, and *T. persicum*. The listed plant genotypes came from the collection of plant genotypes of the Department of Agronomy, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology. Different genotypes of fungi of the genus *Rhizoctonia* (*R. cerealis* AG DI isolate Ww 542 (*Rc* AG DI), *R. solani* AG5 isolate Rs11 (*Rs* AG5), *R. solani* AG 1IC (*Rs* AG 1IC), and binucleate *Rhizoctonia* sp. AG B0 (*R* AG B0)) were used as the second experimental factor. All fungal strains used in the research came from the collection of pathogens of the Laboratory of Department of Biology and Plant Protection, Bydgoszcz University of Science and Technology. The tested plants were grown on a soil substrate consisting of the peat substrate Gramoflor Profisubstrat with the composition of NPK: 100–300 mg N L^{-1}, 100–300 mg P_2O_5 L^{-1}, 100–300 mg K_2O L^{-1}, pH = 6.5. The substrate was mixed with quartz sand in a ratio of 4:1 (by volume). 20 wheat grains were sown in four repetitions in each pot. The sown grains were covered with a layer of quartz sand, on which an inoculum of 2 g of millet grain overgrown with *Rhizoctonia* mycelium was placed. Then the layer with the inoculum was covered with the basic soil substrate. The experiment was carried out in a growth chamber (phytotron Pol-Eko KK1200) under the following conditions: photoperiod 16:8 (day/night), PAR radiation 125 µmol m^{-2} s^{-1}, temperature 20 °C day and night, air humidity at least 80%. From the moment of emergence, the plants were counted daily and assessed for possible disease symptoms. After 15 days, the plants were gently removed from the substrate, washed, weighed and assessed on the basis of a 5-point rating scale (0—no symptoms, 1—lesser symptoms, 2—mild symptoms, 3—greater symptoms, 4—severe symptoms). The obtained results were converted into a disease index (DI) according to the Townsend-Heuberger [32] transformation:

$$DI = \frac{\sum_{0}^{i} n \times v}{i \times N} \times 100\%$$

where i is the highest level of infection, n refers to the number of plants infected for a given infection level, v is the infection level (from 0 to i), and N corresponds to the total plant number sampled. The disease index is presented as a percentage (%). The damping-off severity is also presented as a percentage t (%). They were then preserved for further analysis by freezing at −80 °C.

2.2. Determination of the Activity of Selected Markers

Material from growth chamber experiments was used to determine the occurrence and activity of selected resistance markers. The tested markers included: enzymes of the class of hydrolases involved in the direct fight against pathogens (chitinases and glucanases), enzymes of the class of oxidoreductases involved in the oxidative burst and maintaining redox homeostasis—superoxide dismutase. The collected material was also used to determine the energy state of plant tissues by measuring the content of free sugars. PR proteins were extracted in 50 mM Sorensen buffer pH 7.0 with 1 M NaCl, 1% PPVP, 1 mM EDTA and 1 mM sodium ascorbate. 250 mg of fresh plant tissue was ground in a chilled mortar with 2 mL of ice-cold buffer with addition of digested sterile quartz sand. The homogenate was centrifuged at 15,000× g for 25 min at 4 °C. The supernatant was decanted from the sediment. The pellet was reconstituted in 0.5 mL extraction buffer,

centrifuged again at 15,000× g for 15 min. The obtained supernatant was decanted and combined with the previously obtained one. The obtained extract was treated as a source of cytosolic, apoplastic and ionically bound proteins to the cell wall.

SOD superoxide dismutase activity (EC 1.15.1.1) was determined according to the method of Beauchamp and Fiodorovich [33,34], where the measure of enzymatic activity is the ability to inhibit the photochemical reduction of tetrazolium blue. The reaction mixture (generating O_2^-) consisted of 50 mM phosphate reaction buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.01 mM EDTA and from 0.02 to 0.03 mL of enzyme fraction. The reaction was started after adding riboflavin to the reaction mixture and placing it at a distance of 20 cm from a 15 W UV radiation source. Samples without the enzyme fraction were used as controls. The reaction was carried out for 10 min, after which the absorbance value was read at 560 nm. The unit of activity was the reaction of inhibiting the reduction of NBT by 50% in relation to the control sample as defined by McCord and Fridorovich [34]. Enzyme activity was determined per 1 g of fresh weight and 1 mg of protein.

The activity of chitinases (CHI) and β-1,3-glucanases (GLU) was determined using the modified method of Abeles [35,36] based on the Miller reaction [37] and Zhang [38]. 200 µL of the reaction mixture consisted of 100 µL of enzyme fraction and 100 µL of a solution of colloidal chitin (2 mg·mL^{-1} for chitinases) or laminarin (1 mg·mL^{-1} for glucanases) suspended in acetate buffer at pH 4.5. The reaction was carried out at 37 °C for 60 min. After incubation, the samples were centrifuged for 5 min at 5500× g. Then, 100 µL of DNS reagent was added to 100 µL of the supernatant and heated at 95 °C for 5 min, then cooled to 25 °C and measured at 550 nm. The value of released glucose equivalents was read from the standard curve in the range of 20–100 µg. Enzyme activity was determined in U units (1 U = 1 nM) per 1 h, and 1 g of fresh weight or per 1 mg of protein.

Protein concentration was determined by the method of Bradford [39]. As a standard for the calibration curve, bovine albumin was used in a concentration range of 0–50 µg.

The content of free sugars was determined according to the method of DuBois et al. [40] modified by Bacete et al. [41]. The extracts (100 µL) were mixed with 100 µL of 5% phenol solution and vortexed, then 500 µL of 96% sulfuric acid was added and vortexed again. After cooling, the reaction mixture in a volume of 250 µL was applied to microliter plates and read at 490 nm. The content of free glucose equivalents was determined using a calibration curve for glucose in the range of 0–40 µg. The content of free sugars was converted to g of fresh weight.

2.3. Statistical Analysis

Data preparation was carried out in MS Excel. The data were subjected to a statistical analysis performed in R Core Team (version 4.0.3) with the R Studio add-on [42]. Normalization of the distribution of empirical data was achieved using the Box-Cox transformation. For the results describing the degree of plant infection, biomass both for a single plant and as a whole, the activity of chitinases, β-1,3-glucanases, SOD, and the content of free sugars, a one-way analysis of variance was carried out using the post-hoc Tukey's HSD for the significance level of $p = 0.05$, in order to determine significantly different objects. The r-Person linear correlation coefficient was used to calculate the relationship between the traits. The multidimensional exploration technique of principle component analysis (PCA) was used to explain the multi-trait differentiation of winter wheat species in relation to each of the tested pathogens and to classify wheat genotypes in terms of the first two components. In order to determine the adequacy of the selection of input variables for factor analysis, the KMO (Kaiser-Meyer-Olkin) test was performed. An HCPC (hierarchical clustering on principal component) analysis was performed to divide the combinations from each plant genotype into three clusters, according to Euclidean distance and ward method. All statistical calculations were performed according to Kassambara [43,44].

3. Results

3.1. Basic Biometric Parameters

The results from the research indicate a varied response of selected plant species to the tested strains of *Rhizoctonia* (this is a species feature). Among the tested strains, the least parasitic trait was found in *R. cerealis* AG DI (*Rc* AG DI), and the most in *R. solani* AG 1IC (*Rs* AG 1IC).

In the case of *T. aestivum*, the plants had the highest GI value when exposed to *Rc* AG DI 14.51 (Table 1), with the control plants having a GI value of 13.99, and the lowest value was found in plants exposed to both *Rhizoctonia* AG B0 (*R* AG B0) and *Rs* AG 1IC (10.61). It was observed that when *T. aestivum* was exposed to a single strain of the microorganism, the plants were stimulated to grow. In the case of the achievable biomass, *T. aestivum* reached the highest values for plants treated with *R* AG B0 and *R. cerealis* AGDI (*Rc*)—13.28 g and 0.76 g per plant.

Table 1. One-way Anova results for each plant genotype's basic biometric parameters including the germination index (GI) in experimental combinations. Different letters (a to g) indicates a statistical different group as determined by the mean value of the parameter.

Genotype	Combination	GI *	Total Biomass [g]	Biomass Per Plant [g]
T. aestivum	Control	13.99 ab	13.10 ab	0.70 abc
	Rs11	14.01 ab	8.53 cd	0.49 d
	Rc	14.51 a	12.53 abc	0.67 abc
	AG B0	13.61 ab	13.13 ab	0.72 ab
	1IC	12.30 abc	9.85 a–d	0.59 a–d
	Rc-Rs	13.82 ab	9.28 a–d	0.56 bcd
	Rc-B0	13.36 ab	13.28 a	0.76 a
	Rs-B0	13.99 ab	8.33 d	0.54 cd
	Rc-1IC	11.76 bc	9.63 a–d	0.60 a–d
	Rs-1IC	12.54 abc	9.03 bcd	0.57 bcd
	1IC-B0	10.61 c	9.88 a–d	0.67 abc
	Rc-Rs-B0	13.29 abc	8.20 d	0.55 cd
	Rc-Rs-1IC	13.10 abc	8.85 cd	0.59 a–d
	Rc-B0-1IC	12.06 abc	8.83 cd	0.64 a–d
	Rs-B0-1IC	13.06 abc	10.55 a–d	0.61 a–d
	Rc-Rs-Bo-1IC	13.05 abc	8.63 cd	0.58 bcd
T. durum	Control	7.14 cde	9.65 ab	0.49 abc
	Rs11	7.80 bcd	6.28 cde	0.37 c
	Rc	8.56 a–d	10.18 a	0.51 ab
	AG B0	9.72 ab	10.73 a	0.54 a
	1IC	4.42 f	4.55 e	0.37 c
	Rc-Rs	8.77 abc	5.93 cde	0.41 bc
	Rc-B0	9.60 ab	10.33 a	0.52 ab
	Rs-B0	10.67 a	8.05 a–d	0.43 abc
	Rc-1IC	4.48 f	5.65 de	0.38 c
	Rs-1IC	4.49 f	5.63 de	0.39 bc

Table 1. *Cont.*

Genotype	Combination	GI *	Total Biomass [g]	Biomass Per Plant [g]
	1IC-B0	5.15 ef	5.63 de	0.41 abc
	Rc-Rs-B0	8.03 bcd	9.03 abc	0.46 abc
	Rc-Rs-1IC	4.72 f	3.83 e	0.38 c
	Rc-B0-1IC	6.42 c–f	6.55 b–e	0.44 abc
	Rs-B0-1IC	5.38 ef	4.28 e	0.37 c
	Rc-Rs-Bo-1IC	6.24 def	5.33 de	0.43 abc
T. persicum	Control	8.15 ab	4.68 ab	0.29 ab
	Rs11	7.71 ab	1.80 d–g	0.22 b
	Rc	7.88 ab	3.38 a–d	0.25 ab
	AG B0	7.57 ab	5.05 a	0.34 a
	1IC	7.68 ab	2.95 b–f	0.27 ab
	Rc-Rs	7.57 ab	1.50 d–g	0.25 ab
	Rc-B0	8.74 a	4.20 abc	0.26 ab
	Rs-B0	6.56 ab	1.43 efg	0.22 b
	Rc-1IC	5.95 ab	2.35 c–g	0.22 b
	Rs-1IC	5.57 ab	1.60 d–g	0.22 b
	1IC-B0	6.27 ab	3.13 b–e	0.28 ab
	Rc-Rs-B0	6.69 ab	1.50 d–g	0.22 b
	Rc-Rs-1IC	5.01 b	0.78 g	0.19 b
	Rc-B0-1IC	5.52 ab	2.30 d–g	0.25 ab
	Rs-B0-1IC	6.28 ab	1.23 fg	0.21 b
	Rc-Rs-Bo-1IC	6.70 ab	1.25 efg	0.21 b
T. sphaerococcum	Control	10.53 ab	9.83 a	0.52 ab
	Rs11	9.28 ab	5.43 cd	0.39 bcd
	Rc	10.97 a	9.33 ab	0.48 abc
	AG B0	9.83 ab	10.15 a	0.55 a
	1IC	7.70 ab	4.48 d	0.36 bcd
	Rc-Rs	9.85 ab	5.93 bcd	0.38 bcd
	Rc-B0	10.36 ab	8.78 abc	0.48 abc
	Rs-B0	9.84 ab	5.83 bcd	0.43 a–d
	Rc-1IC	8.52 ab	5.70 bcd	0.33 cd
	Rs-1IC	7.28 b	4.88 d	0.35 cd
	1IC-B0	8.28 ab	6.68 a–d	0.44 a–d
	Rc-Rs-B0	8.77 ab	6.90 a–d	0.40 a–d
	Rc-Rs-1IC	7.64 ab	3.88 d	0.31 d
	Rc-B0-1IC	10.30 ab	7.05 a–d	0.44 a–d
	Rs-B0-1IC	8.60 ab	6.18 bcd	0.44 a–d
	Rc-Rs-Bo-1IC	10.41 ab	6.68 a–d	0.43 a–d

Table 1. *Cont.*

T. spelta	Control	9.74 ab	11.50 a	0.58 abc
	Rs11	8.73 abc	9.45 a–d	0.54 abc
	Rc	8.08 a–d	9.68 abc	0.50 a–d
	AG B0	9.47 ab	12.30 a	0.62 a
	1IC	6.79 bcd	7.00 cde	0.47 bcd
	Rc-Rs	9.47 ab	6.93 cde	0.47 bcd
	Rc-B0	10.29 ab	11.03 ab	0.56 abc
	Rs-B0	10.58 a	10.03 abc	0.58 ab
	Rc-1IC	5.80 cd	4.58 e	0.35 d
	Rs-1IC	5.84 cd	5.83 de	0.47 bcd
	1IC-B0	5.69 cd	5.70 de	0.53 abc
	Rc-Rs-B0	8.46 abc	6.45 cde	0.48 a–d
	Rc-Rs-1IC	4.80 d	5.75 de	0.45 bcd
	Rc-B0-1IC	4.68 d	3.75 e	0.43 cd
	Rs-B0-1IC	8.16 a–d	8.55 a–d	0.57 abc
	Rc-Rs-Bo-1IC	7.78 a–d	7.40 b–e	0.51 bc

* Germination Index.

T. durum was found to be a very susceptible genotype to pathogen pressure. It was characterized by slow growth and lower achievable biomass. In the case of this genotype, a slightly stimulating effect of microorganisms was also observed. The highest GI was found in plants exposed to *R. solani* AG5 (*Rs* AG5) and *R* AG B0—10.67. The lowest values were found for the variants *Rs* AG 1IC (4.42) and *Rc* AG DI, *Rs* AG5 and *Rs* AG 1IC (4.72). In the context of achievable biomass, it was observed that variants *R* AG B0; *Rc* AG DI—*R* AG B0 and *Rc* AG DI were characterized by the highest values of 10.73, 10.33 and 10.16 g, respectively (Table 1). The same applies to a single plant weight.

For *T. persicum* (Table 1) the lowest GI variation was observed, although the highest value was achieved by plants in the variants *Rc* AG DI and *R* AG B0 (8.74). In the case of the achievable biomass, a very high susceptibility to the tested microorganisms was observed, both in a negative and positive aspect. *T. persicum* plants responded well to the exposure of *R* AG B0, because in this variant the achievable total plant biomass was 5.05 g, and the control variant only reached 4.68 g. Similarly, for the variant *Rc* AG DI—*R* AG B0, a stimulating effect (4.20 g) was observed compared to exposure to only *Rc* AG DI (3.38 g). A similar effect can be seen in the variants of *Rs* AG 1IC—*R* AG B0 (3.13) and *Rs* AG 1IC (2.95 g). However, this trend was not observed for *Rs* AG5.

Low variability was observed for *T. sphaerococcum* (Table 1) in the context of the GI. Plants under pressure from *Rc* AG DI (10.97) and control plants (10.53) sprouted the strongest. The lowest value was observed for the variants *Rs* AG5—*Rs* AG 1IC (7.28), followed by *Rc* AG DI—*Rs* AG5—*Rs* AG 1IC (7.64) and for the single variant *Rs* AG 1IC (7.70). For achievable plant biomass, the highest value was observed for plants exposed to *R* AG B0 (10.15 g), control plants (9.83 g), and exposed to *Rc* AG DI (9.33 g). Similar trends were observed for the values per single plant.

T. spelta plants (Table 1) were characterized by the highest germination index for plants in two-fungus variants, i.e., *Rs* AG5—*R* AG B0 (10.58), *Rc* AG DI—*R* AG B0 (10.29) and controls (9.74). In the case of achievable biomass, the highest value was achieved by plants treated with *R* AG B0 only (12.30 g), followed by the control (11.50 g). The lowest value was observed for the variants *Rc* AG DI—*R* AG B0—*Rc* AG 1IC (3.75 g) and *Rc* AG DI—*Rs* AG 1IC (4.58 g).

3.2. Disease Severity

The occurrence of disease symptoms on plants of selected genotypes was observed in the context of exposure to the studied microorganisms. It should be noted that no disease symptoms were observed on any of the plant genotypes in the context of exposure to *R* AG B0. It did not cause any pathological changes or any inhibitory effects, as was the case with basic biometric parameters.

In the case of *T. aestivum* (Table 2), the strongest pressure was characterized by the variant *Rc* AG DI AG DI—*R* AG B0—*Rs* AG 1IC (consecutively 86.64% of the stock infested, DI 45.45%, average degree of infestation 1.82). The positive effect of the non-pathogenic strain was observed only for variants with *Rc*, i.e., *Rc* AG DI (57.66% of the infected stock, DI 27.89%, average degree of infection 1.12) and the variant *Rc* AG DI—*R* AG B0 (50.44% infested stock, DI 25.28%, average degree of infestation 1.01). A similar effect was not observed for pathogens of the species *Rs*.

Table 2. One-way Anova results for each plant genotype in the context of disease severity parameters in experimental combinations. Different letters (a through to f) indicate a statistically different group defined by the value (mean).

Genotype	Combination	Infected Plant Cast	Disease Index	Mean Infection Degree
T. aestivum	Control	0.00 e	0.00 e	0.00 e
	Rs11	68.09 a–d	35.52 a–d	1.42 a–d
	Rc	57.66 cd	27.89 bcd	1.12 bcd
	AG B0	0.00 e	0.00 e	0.00 e
	1IC	76.22 a–d	32.36 a–d	1.29 a–d
	Rc-Rs	63.80 bcd	32.68 a–d	1.31 a–d
	Rc-B0	50.45 d	25.28 bcd	1.01 bcd
	Rs-B0	68.79 a–d	40.05 abc	1.60 abc
	Rc-1IC	91.99 a	50.18 a	2.01 a
	Rs-1IC	50.99 d	19.05 de	0.76 de
	1IC-B0	84.06 abc	38.56 a–d	1.54 a–d
	Rc-Rs-B0	68.99 a–d	38.49 a–d	1.54 a–d
	Rc-Rs-1IC	73.31 a–d	38.42 a–d	1.54 a–d
	Rc-B0-1IC	86.64 ab	45.45 ab	1.82 ab
	Rs-B0-1IC	69.72 a–d	26.46 bcd	1.06 bcd
	Rc-Rs-Bo-1IC	50.45 d	24.05 cd	0.96 cd
T. durum	Control	0.00 c	0.00 e	0.00 e
	Rs11	85.88 ab	71.94 abc	2.88 abc
	Rc	8.75 c	2.19 e	0.09 e
	AG B0	0.00 c	0.00 e	0.00 e
	1IC	85.83 ab	66.90 a–d	2.68 a–d
	Rc-Rs	90.78	64.49 a–d	2.58 a–d
	Rc-B0	0.00 c	0.00 e	0.00 e
	Rs-B0	89.52 a	65.78 a–d	2.63 a–d
	Rc-1IC	100.00 a	76.77 ab	3.07 ab
	Rs-1IC	90.99 a	77.76 ab	3.11 ab

Table 2. *Cont.*

Genotype	Combination	Infected Plant Cast	Disease Index	Mean Infection Degree
	1IC-B0	90.52 a	71.46 a–d	2.86 a–d
	Rc-Rs-B0	65.69 b	48.89 d	1.96 d
	Rc-Rs-1IC	96.88 a	80.07 ab	3.20 ab
	Rc-B0-1IC	89.49 a	57.69 bcd	2.31 bcd
	Rs-B0-1IC	93.56 a	82.91 a	3.32 a
	Rc-Rs-Bo-1IC	89.94 a	52.19 cd	2.09 cd
T. persicum	Control	0.00 c	0.00 d	0.00 d
	Rs11	93.30 a	76.38 ab	3.06 ab
	Rc	82.82 ab	54.55 bc	2.18 bc
	AG B0	0.00 c	0.00 d	0.00 d
	1IC	90.06 ab	68.68 ab	2.75 ab
	Rc-Rs	82.71 ab	65.64 ab	2.63 ab
	Rc-B0	62.70 b	26.19 cd	1.05 cd
	Rs-B0	95.83 a	71.35 ab	2.85 ab
	Rc-1IC	90.10 ab	59.15 bc	2.37 bc
	Rs-1IC	96.43 a	75.20 ab	3.01 ab
	1IC-B0	81.86 ab	49.70 bc	1.99 bc
	Rc-Rs-B0	87.71 ab	61.67 b	2.47 b
	Rc-Rs-1IC	100.00 a	97.00 a	3.88 a
	Rc-B0-1IC	92.86 a	50.84 bc	2.03 bc
	Rs-B0-1IC	80.63 ab	65.52 ab	2.62 ab
	Rc-Rs-Bo-1IC	84.38 ab	63.02 b	2.52 b
T. sphaerococcum	Control	0.00 d	0.00 f	0.00 f
	Rs11	90.06 a	57.34 abc	2.29 abc
	Rc	43.01 c	22.51 def	0.90 def
	AG B0	0.00 d	0.00 f	0.00 f
	1IC	88.28 a	56.94 abc	2.28 abc
	Rc-Rs	93.98 a	68.66 ab	2.75 ab
	Rc-B0	52.57 bc	19.39 ef	0.78 ef
	Rs-B0	67.13 abc	40.09 cde	1.60 cde
	Rc-1IC	75.41 abc	41.69 cde	1.67 cde
	Rs-1IC	89.54 a	70.08 ab	2.80 ab
	1IC-B0	67.23 abc	31.52 cde	1.26 cde
	Rc-Rs-B0	71.82 abc	48.87 bcd	1.95 bcd
	Rc-Rs-1IC	97.92 a	79.43 a	3.18 a
	Rc-B0-1IC	76.59 ab	49.69 bc	1.99 bc
	Rs-B0-1IC	85.88 a	56.52 abc	2.26 abc
	Rc-Rs-Bo-1IC	70.34 abc	51.52 bc	2.06 bc

Table 2. *Cont.*

Genotype	Combination	Infected Plant Cast	Disease Index	Mean Infection Degree
T. spelta	Control	0.00 a	0.00 e	0.00 e
	Rs11	90.15 ab	57.79 abc	2.31 abc
	Rc	76.22 ab	45.48 bcd	1.82 bcd
	AG B0	0.00 a	0.00 e	0.00 e
	1IC	93.35 ab	49.62 a–d	1.98 a–d
	Rc-Rs	96.67 a	68.01 ab	2.72 ab
	Rc-B0	72.37 b	26.44 d	1.06 d
	Rs-B0	80.07 ab	44.46 bcd	1.78 bcd
	Rc-1IC	88.46 ab	64.42 ab	2.58 ab
	Rs-1IC	87.28 ab	61.83 ab	2.47 ab
	1IC-B0	80.15 ab	36.77 cd	1.47 cd
	Rc-Rs-B0	84.27 ab	58.36 abc	2.33 abc
	Rc-Rs-1IC	95.23 a	73.47 a	2.94 a
	Rc-B0-1IC	91.67 ab	71.50 a	2.86 a
	Rs-B0-1IC	94.36 ab	59.36 abc	2.37 abc
	Rc-Rs-Bo-1IC	85.60 ab	62.12 ab	2.48 ab

In the case of *T. durum* (Table 2), a very high susceptibility to pathogens was observed in both the single and mixed exposures. A very good effect of the *R* AG B0 strain was observed, which in the case of co-exposure of plants to *Rc* AG DI caused the inhibition of disease processes—no disease changes were found on plants of this variant. It should also be mentioned that the plants were only slightly infected by *Rc* AG DI (8.75% of the infected stock, DI 2.19%, average degree of infection 0.09). We should consider the simultaneous action of two features—the innate resistance of *T. durum* to *Rc* and the additional stimulation by *R* AG B0. Unfortunately, no inhibitory effect was observed on both *Rs* strains.

For *T. persicum* (Table 2), the inhibitory effect of the *R* AG B0 strain on the development of *Rc* AG DI was again observed as in the previous cases: 82.82% of infected stocking rate, DI 54.55%, average infestation rate 2.18 for the *Rc* AG DI variant, and 62.70% infested stocking, DI 26.19%, average infestation rate 1.05 for the *Rc* AG DI variant—*R* AG B0. In addition, lower inhibitory capacity was observed for *R*. AG B0 in the context of plant protection against *Rs* 81.86% infested stock, DI 49.70, average infestation 1.99 for *Rs* AG 1IC—*R* AG B0.

T. sphaerococcum plants (Table 2) were characterized by resistance to the tested pathogens similar to that of *T. aestivum*. No trends were observed from previous genotypes in the context of inhibiting the development of *Rc* AG DI, although in the case of this genotype of plants it was observed that the presence of *R* AG B0 inhibited the development of both strains of *Rs* For *Rs* AG5 (90.06% infested stock, DI 57.34%, average infection rate 2.29) the presence of *R* AG B0 inhibited the occurrence of disease symptoms on plants (67.13% of infected stock, DI 40.09%, average infection rate 1.60). In turn, for *Rs* AG 1IC, 88.28% of the infected stock was observed, DI was 56.94%, the average degree of infection was 2.28, where the addition of the inoculum containing *R* AG B0 inhibited the development of the disease in the form of 67.23% of the infected stock observed, DI 31.52%, mean infection rate 1.26.

For *T. spelta* (Table 2), inhibitory trends were observed for all plant pathogens tested. For *Rc* AG DI, the addition of *R* AG B0 reduced the tested parameters from 76.22% infested stock, DI 45.48%, average degree of infestation 1.82 to 72.37% infested stock, DI 26.44%, average degree of paralysis 1.06. For *Rs* AG5, a reduction in parameters from 90.15%

infested stock, DI 57.79%, mean infestation 2.31 to 80.07% infested stock, DI 44.46%, and mean infestation 1 was observed, 78, for the *Rs* AG5 variant—*R* AG B0. Inhibition of disease development was also observed when plants were exposed to the strain *Rs* AG 1IC. From single variant (*Rs* AG 1IC alone) 93.35% infested stock, DI 49.62%, mean infestation 1.98 to 80.15% infested stock, DI 36.77%, 1.47 in average infestation for the combination *Rs* AG 1IC—*R* AG B0.

3.3. Enzymatic and Metabolic Parameters

The activity of selected enzyme complexes and the content of free sugars in plant tissues of the tested combinations were analysed. In the case of *T. aestivum* plants (Table 3), chitinase activity was observed at a similar level, except for plants exposed to *Rs* AG5—*R* AG B0 (999.89 U/gFW), *Rc* AG DI—*Rs* AG5 (700.68 U/gFW), and *Rc* AG DI—*R* AG B0 (407.02 U/gFW). In the case of glucanases, no influence of the tested microorganisms on enzyme activity was observed. Superoxide dismutase was also not significantly different between variants. There was very little variation in free sugar content. The highest content was observed for plants exposed to *Rs* AG 1IC—*R* AG B0 (7.07 g/gFW), the lowest for the control variants (4.34 g/gFW), *Rc* AG DI (4.53 g/gFW) and *Rs* AG5 (4.62 g/gFW).

Table 3. One-way Anova results for each plant genotype basic enzymatic and metabolic parameters in experimental combinations. Different letters by the value (mean) indicates a statistical different group.

Genotype	Combination	U CHI/gFW	U CHI/mg	U GLU/gFW	U GLU/mg	U SOD/gFW	U SOD/mg	Free Sugars mg/gFW
T. aestivum	Control	196.45 c	36.32 c	181.58 a	34.51 a	642.39 a	126.74 a	4.34 b
	Rs11	165.77 c	20.63 c	240.06 a	33.41 a	624.77 a	89.83 a	4.62 b
	Rc	216.33 c	31.19 c	167.64 a	28.07 a	661.91 a	115.82 a	4.53 b
	AG B0	240.56 c	35.35 c	184.10 a	27.78 a	700.94 a	104.65 a	5.25 ab
	1IC	209.62 c	42.45 bc	166.06 a	33.19 a	723.11 a	152.94 a	4.57 b
	Rc-Rs	700.68 ab	78.77 ab	165.53 a	18.91 a	743.00 a	86.46 a	6.28 ab
	Rc-B0	407.02 bc	50.83 bc	196.23 a	23.96 a	593.32 a	75.11 a	6.19 ab
	Rs-B0	999.89 a	118.20 a	202.50 a	23.72 a	805.34 a	94.71 a	6.21 ab
	Rc-1IC	240.59 c	27.98 c	250.14 a	28.30 a	724.62 a	83.66 a	5.65 ab
	Rs-1IC	267.93 c	28.73 c	246.97 a	27.53 a	749.82 a	89.97 a	5.73 ab
	1IC-B0	221.77 c	36.57 c	273.13 a	33.50 a	670.62 a	82.76 a	7.07 a
	Rc-Rs-B0	197.96 c	22.98 c	225.16 a	20.73 a	765.17 a	89.70 a	6.10 ab
	Rc-Rs-1IC	321.38 c	45.76 bc	232.97 a	28.83 a	733.72 a	105.67 a	6.52 ab
	Rc-B0-1IC	254.40 c	31.67 c	174.55 a	23.67 a	685.02 a	100.67 a	6.00 ab
	Rs-B0-1IC	195.05 c	23.82 c	184.77 a	22.84 a	576.14 a	71.45 a	6.96 a
	Rc-Rs-Bo-1IC	155.38 c	18.93 c	227.17 a	26.33 a	666.45 a	80.15 a	6.94 a
T. durum	Control	2141.34 ab	335.59 ab	283.16 cd	45.41 ab	723.70 a	116.18 a	13.85 a
	Rs11	671.76 b	103.14 ab	364.81 a–d	58.18 ab	816.85 a	130.56 a	13.49 a
	Rc	611.49 b	88.36 b	345.91 a–d	51.06 ab	827.05 a	122.31 a	13.21 a
	AG B0	700.12 b	99.65 b	335.74 bcd	48.09 ab	774.73 a	110.10 a	13.52 a
	1IC	1087.08 ab	136.93 ab	455.89 ab	56.07 ab	778.42 a	99.10 a	15.48 a
	Rc-Rs	1723.51 ab	236.56 ab	462.46 ab	61.49 ab	841.10 a	111.08 a	15.21 a
	Rc-B0	490.67 b	74.91 b	245.24 d	40.04 b	687.08 a	112.45 a	12.20 a
	Rs-B0	701.66 b	91.58 b	316.06 bcd	40.39 b	776.54 a	99.65 a	14.73 a
	Rc-1IC	1567.84 ab	226.21 ab	432.85 abc	57.16 ab	737.74 a	96.86 a	14.83 a
	Rs-1IC	1115.28 ab	150.55 ab	421.06 abc	60.75 ab	727.32 a	106.40 a	15.27 a
	1IC-B0	1133.93 ab	157.11 ab	399.10 a–d	60.07 ab	746.43 a	115.97 a	13.50 a

Table 3. *Cont.*

Genotype	Combination	U CHI/gFW	U CHI/mg	U GLU/gFW	U GLU/mg	U SOD/gFW	U SOD/mg	Free Sugars mg/gFW
	Rc-Rs-B0	1070.20 ab	168.65 ab	372.07 a–d	54.44 ab	675.21 a	99.96 a	12.61 a
	Rc-Rs-1IC	785.43 b	117.36 ab	497.03 a	67.56 a	762.35 a	99.68 a	15.62 a
	Rc-B0-1IC	964.93 ab	127.31 ab	421.60 abc	53.58 ab	653.78 a	81.68 a	14.67 a
	Rs-B0-1IC	3075.88 a	381.35 a	398.52 a–d	52.03 ab	648.86 a	85.80 a	15.91 a
	Rc-Rs-Bo-1IC	2960.02 a	317.89 ab	372.93 a–d	41.79 ab	720.08 a	80.99 a	15.81 a
T. persicum	Control	126.80 a	12.95 ab	172.50 e	18.00 g	799.08 a	84.33 abc	5.59 c
	Rs11	205.46 a	19.17 ab	422.70 bcd	39.14 c–g	914.20 a	86.79 abc	7.45 abc
	Rc	230.56 a	30.38 ab	355.49 b–e	44.87 b–f	881.42 a	113.47 a	7.68 abc
	AG B0	177.09 a	23.39 ab	193.48 de	24.18 fg	842.20 a	105.24 abc	6.19 bc
	1IC	258.10 a	27.18 ab	381.62 b–e	41.06 b–g	866.01 a	97.37 abc	7.64 abc
	Rc-Rs	109.42 a	8.14 b	382.91 b–e	28.43 efg	800.84 a	59.77 c	7.24 bc
	Rc-B0	178.30 a	13.98 ab	255.29 cde	20.06 g	780.55 a	62.60 bc	7.19 bc
	Rs-B0	162.73 a	12.45 ab	416.96 b–e	32.42 d–g	911.47 a	70.86 abc	7.47 abc
	Rc-1IC	250.66 a	19.89 ab	344.89 b–e	27.85 efg	875.18 a	70.35 abc	7.95 abc
	Rs-1IC	304.71 a	34.33 a	470.28 bc	58.08 bc	857.62 a	107.72 ab	8.28 abc
	1IC-B0	145.03 a	17.07 ab	454.92 bc	51.11 b–e	863.67 a	99.69 abc	8.15 abc
	Rc-Rs-B0	136.34 a	14.68 ab	478.19 bc	51.89 b–e	825.42 a	90.44 ab	8.57 ab
	Rc-Rs-1IC	302.38 a	34.86 a	751.06 a	86.72 a	939.76 a	108.95 abc	10.04 a
	Rc-B0-1IC	155.19 a	20.00 ab	412.56 b–e	52.83 bcd	840.84 a	108.42 ab	7.27 abc
	Rs-B0-1IC	128.68 a	14.15 ab	567.02 ab	64.09 ab	900.35 a	106.34 ab	8.35 abc
	Rc-Rs-Bo-1IC	135.67 a	16.22 ab	458.79 bc	54.59 bcd	826.98 a	99.13 abc	8.07 abc
T. sphaerococcum	Control	2831.85 ab	678.04 ab	168.31 de	37.91 b–e	749.10 bc	178.89 ab	17.46 a
	Rs11	3652.35 ab	636.31 ab	107.64 e	18.43 de	893.28 ab	151.49 bc	13.77 ab
	Rc	3035.88 ab	713.08 ab	86.13 e	18.99 de	848.33 abc	187.43 ab	12.76 ab
	AG B0	5360.02 a	1245.91 a	58.18 f	13.73 e	804.91 abc	186.04 ab	14.35 ab
	1IC	2702.07 ab	483.09 ab	209.50 de	36.90 b–e	845.29 abc	148.32 bc	17.41 a
	Rc-Rs	2051.73 ab	516.78 ab	227.14 cde	55.11 abc	959.07 a	239.49 a	17.77 a
	Rc-B0	785.75 b	159.28 b	163.86 de	34.04 cde	711.32 c	147.43 bc	12.68 ab
	Rs-B0	549.38 b	116.51 b	234.87 b–e	50.35 a–d	889.37 ab	194.12 ab	13.13 ab
	Rc-1IC	1736.20 b	270.34 b	355.55 a–d	54.86 abc	830.53 abc	127.64 bc	11.93 b
	Rs-1IC	556.23 b	88.71 b	467.43 a	77.05 a	835.09 abc	137.02 bc	14.98 ab
	1IC-B0	459.73 b	70.74 b	453.02 ab	69.35 ab	854.63 abc	132.43 bc	13.60 ab
	Rc-Rs-B0	1557.61 b	267.82 b	488.68 a	80.19 a	807.73 abc	129.06 bc	14.15 ab
	Rc-Rs-1IC	1329.03 b	203.94 b	509.71 a	76.45 a	871.13 abc	126.60 bc	14.24 ab
	Rc-B0-1IC	873.40 b	95.97 b	447.25 abc	49.06 a–d	796.66 abc	90.49 c	12.83 ab
	Rs-B0-1IC	995.63 b	157.97 b	447.22 abc	68.62 ab	820.11 abc	128.40 bc	14.05 ab
	Rc-Rs-Bo-1IC	1117.10 b	162.52 b	454.08 ab	63.31 abc	730.00 bc	104.38 c	12.92 ab
T. spelta	Control	107.77 ab	14.48 ab	137.11 b	18.30 ab	685.51 a	90.78 a	3.67 b
	Rs11	130.53 ab	14.02 ab	203.56 ab	20.17 ab	542.03 a	57.90 a	4.69 b
	Rc	57.00 b	5.88 b	148.24 b	15.63 b	611.31 a	68.85 a	3.70 b
	AG B0	140.47 ab	16.47 ab	223.54 ab	26.85 ab	705.86 a	83.87 a	4.55 b
	1IC	125.30 ab	12.38 ab	186.04 ab	18.30 ab	667.01 a	66.01 a	4.46 b
	Rc-Rs	214.15 ab	23.64 ab	307.79 ab	34.20 ab	644.81 a	72.73 a	7.30 ab
	Rc-B0	132.20 ab	13.05 ab	130.49 b	14.96 b	646.25 a	74.74 a	4.70 b
	Rs-B0	87.45 b	10.53 ab	145.67 b	18.38 ab	621.79 a	80.32 a	4.97 b

Table 3. *Cont.*

Genotype	Combination	U CHI/gFW	U CHI/mg	U GLU/gFW	U GLU/mg	U SOD/gFW	U SOD/mg	Free Sugars mg/gFW
	Rc-1IC	153.70 ab	14.16 ab	301.47 ab	30.92 ab	615.83 a	66.33 a	5.72 b
	Rs-1IC	217.64 ab	20.58 ab	257.58 ab	25.64 ab	479.34 a	52.18 a	5.60 b
	1IC-B0	304.44 a	27.91 a	276.39 ab	25.56 ab	639.67 a	60.28 a	6.83 ab
	Rc-Rs-B0	113.51 ab	11.37 ab	269.42 ab	26.25 ab	604.52 a	62.23 a	6.31 b
	Rc-Rs-1IC	102.01 ab	9.10 ab	317.11 ab	28.25 ab	521.07 a	47.58 a	6.67 ab
	Rc-B0-1IC	241.28 ab	24.48 ab	380.16 a	39.30 a	604.93 a	65.22 a	10.09 a
	Rs-B0-1IC	157.91 ab	16.26 ab	323.85 ab	33.25 ab	643.99 a	71.52 a	6.79 ab
	Rc-Rs-Bo-1IC	104.25 ab	11.07 ab	272.87 ab	29.35 ab	575.95 a	64.80 a	6.13 b

In the case of *T. durum*, greater responsiveness was observed in terms of enzymatic activity of chitinases and glucanases (Table 3). The highest value of chitinolytic activity was observed in the variant containing both strains of *R. solani* and AG B0 (3075.88 U/gFW) and with the highest pressure from microorganisms, i.e., the variant containing all tested *Rhizoctonia* strains (2960.02 U/gFW). In the case of glucanases, the variant containing three pathogens (*Rs* AG5—*Rs* AG 1IC—*Rc* AG DI) with an activity of 497.03 U/gFW was characterized by the highest value. No significant differences were observed in terms of superoxide dismutase activity and the content of free sugars.

For *T. persicum* (Table 3) no significant differences in chitinolytic activity were observed among the tested variants. In terms of glucanase activity, the highest activity was observed only for the combination containing the three tested pathogens (*Rc* AG DI—*Rs* AG5—*Rs* AG 1IC) with a value of 751.06 U/gFW. In the context of superoxide dismutase activity, no significant differences were observed per gram of fresh weight, and the highest specific activity of this enzyme was observed for plants treated with *Rc* AG DI (113.47 U/mg). In the case of the content of free sugars, the highest value was found in plants treated with three pathogens (*Rc* AG DI—*Rs* AG5—*Rs* AG 1IC).

For *T. sphaerococcum*, the highest chitinolytic activity was observed among all tested plant genotypes, both in the control variants and those treated with a single microorganism. Moreover, increasing pressure from microorganisms often resulted in an observed decrease in chitinase activity. Importantly, in the case of this enzyme, the combination with *R* AG B0 (5360.02 U/gFW) was characterized by the highest activity. In the case of glucanases, the highest activity value was found in plants exposed to three pathogens *Rc* AG DI—*Rs* AG5—*Rs* AG 1IC (509.71 U/gFW), and *Rc* AG DI—*Rs* AG5—*R* AG B0 (488.68 U/gFW). In the context of superoxide dismutase activity, the highest values were observed in the variant *Rc* AG DI—*Rs* AG5: 959.07 U/gFW. In the case of free sugars, no greater variation was observed, although the highest content was observed in the variants *Rc* AG DI—*Rs* AG5 (17.77 mg/gFW), control (17.46 mg/gFW) and *Rs* AG 1IC (17.41 mg/gFW).

For *T. spelta* plants (Table 3), the highest chitinolytic activity was observed in the tissues of the plants for the variant *Rs* AG 1IC—*R* AG B0 (304.44 g/gFW). For the activity of glucanases, the highest activity was observed in the plants for the variant *Rc* AG DI—*R* AG B0—*Rs* AG 1IC (380.16 U/gFW). In the case of superoxide dismutase activity, no significant differences were observed within the tested combinations. Similarly, in the case of the content of free sugars, the variation was small, although the highest content in the tissues of the *Rc* AG DI—*R* AG B0—*Rs* AG 1IC variant (10.09 mg/gFW) was observed.

3.4. Principal Component Analysis

The results obtained were subjected to a multidimensional analysis of principal components. Each of the plant genotypes was spread on a two-dimensional plane in order to determine the relationships between variables and factors. After obtaining the derived matrix, all tested combinations were additionally classified by HCPC analysis using the Euclidean distance and the Ward method to obtain three different clusters. Thanks to

this, it was possible to identify clusters with different pressure from microorganisms, or to mitigate this pressure by the strain *R* AG B0.

In the case of *T. aestivum*, variants with the lowest cluster 1 pressure (Figures S1 and S2) were observed to be oriented on the 2-dimensional plane and correlated with achievable plant biomass and superoxide dismutase activity. Importantly, this cluster included control variants, *Rc* AG DI, *Rs* AG 1IC, *R* AG B0 and *Rc* AG DI—*R* AG B0. The variants with the highest pressure from pathogens (cluster 2) were oriented on the plane of the first dimension and were correlated with the activity of glucanases and the content of free sugars. Importantly, the addition of the *R* AG B0 strain to the plants inoculated with *Rs* AG5 resulted in the intensification of defense processes in the form of chitinolytic activity and SOD. This variant is within cluster 3 and is strongly correlated with the activity of the listed defensins.

For *T. durum*, variants with the lowest pressure were oriented within cluster 3 (Figures S3 and S4) on the plane of the first dimension and were correlated, as in the previous case, with the achievable plant biomass and the germination index (GI). Within this cluster, variants such as control, *Rc* AG DI, *R* AG B0 and mixed pathogen-non-pathogen variants (*Rc* AG DI—*R* AG B0, *Rs* AG5—*R* AG B0, and *R* AG DI—*Rs* AG5—*R* AG B0). It can be concluded that the addition of a non-pathogenic microorganism reduced the pressure of the pathogen on the plants. Cluster 2, on the other hand, was oriented on the plane of dimension 2 and was correlated with the enzymatic activity of glucanases, superoxide dismutase and the content of free sugars.

For *T. persicum*, the cluster with the least pressure was cluster 1 correlated with achievable biomass and GI, within dimension 2 (Figures S5 and S6). This cluster included variants such as control, *Rc* AG DI, *R* AG B0, *Rs* AG 1IC and *Rc* AG DI—*R* AG B0.

For *T. sphaerococcum* (Figures S7 and S8) an analogous situation was observed as in previous plant genotypes. Cluster 3, correlated with the achievable biomass of plants and the germination index, was oriented on the plane of dimension 1. Again, this cluster included control plants, *R* AG B0, *Rc* AG DI and *Rc* AG DI—*R* AG B0. However, the addition of the non-pathogenic strain to plants inoculated with *Rc* AG DI shifted the variant in plane 1 closer to the high pressure cluster.

In the case of *T. spelta*, the strongest effect of *R* AG B0 on plant health was observed. Cluster 1 (Figures S9 and S10) included such variants as control, *Rc* AG DI, *R* AG B0 and, importantly, *Rc* AG DI—*R* AG B0 and *Rs* AG5—*R* AG B0. This cluster was oriented on plane 2 and was correlated with parameters such as achievable plant biomass, germination index and SOD activity in plant tissues. The addition of the non-pathogenic strain caused a shift for plants inoculated with *Rc* AG DI and *Rs* AG5 from the high pressure cluster to the low pressure cluster.

4. Discussion

Under favorable conditions, *Rhizoctonia* fungi can pose a serious threat to crops [45]. The use of conventional chemical protection is not very effective, is environmentally unfriendly, and at best can only provide a short-term solution owing to the common phenomenon of microorganisms acquiring immunity to the active substances within the fungicides. There is an urgent need for alternative plant protection methods, of which biological protection is an important one in modern agriculture. It is even a requirement to meet the requirements of modern agriculture, which is an inseparable element of sustainable development. In the era of high chemicalization of agricultural production, it provides an excellent alternative to protect plants and agricultural products. The possibility of using hypovirulent *Rhizoctonia* in plant protection is a tempting alternative in combatting pathogenic strains of the same genus [26,46], as well as other important pathogens [1,22–24]. Combatting or limiting the development of pathogenic microorganisms when using the hypovirulent binucleate strain of *Rhizoctonia* (BNR) can have a multifaceted effect, including the prevention of colonization or displacement of pathogens from the rhizosphere [47], the induction of SAR mechanisms in the plant system (as well as the synergistic effect of

both mechanisms [23,24]), and inducing effects through the secretion of elicitors that are a product of BNR secretion enzymes [48]. In the context of the studied pathogens, it can be seen that the greatest threat to plants is *R. solani*, which is polyphagous with high virulence. This dependence was visible in our research, where *R. solani* of both anastomosis groups was responsible for the greatest infection of the tested plant genotypes. In the case of *R. solani* AG5, the addition of BNR did not always result in a noticeable reduction in plant infestation. For *T. aestivum*, the infection index actually increased. Only for *T. sphaerococcum* was a reduction in the disease index observed. In the case of *R. solani* AG 1IC, a significant reduction in the disease index was observed only for two plant genotypes, *T. persicum* and *T. sphaerococcum*. In terms of infection caused by *R. cerealis* AG DI, more success was achieved in reducing disease symptoms. Apart from *T. sphaerococcum*, where the addition of BNR caused an increase in the disease index, in the case of the rest of the tested genotypes a noticeable reduction to even a complete inhibition of the disease was observed (*T. durum*). This confirms the reports on the possibility of using BNR as an effective form of plant protection [28,29,49]. In the case of induction of SAR mechanisms, processes of cell wall strengthening as well as direct pathogen control were induced. Induction of the production and increase in the activity of chitinases and glucanases in the presence of a non-pathogenic strain of *Rhizoctonia* has been observed in bean plants (*Phaseolus vulgaris* L.) and other types of plants [27,50,51]. During our research, the induction of chitinase and glucanase activity was also observed in the presence of the *Rhizoctonia* AG B0 strain. The model of action seems to be very similar to the effect exerted by many other biocontrol agents that are used in the biological protection of plants [5,52].

As part of the research, it was observed that the non-pathogenic *Rhizoctonia* strain is able to inhibit the development of some pathogens. With multidimensional analysis of principal components, a reduction in the pressure from pathogens, activation of the tested defense mechanisms, or an impact on the achievable biomass of plants were observed. For plants exposed only to the non-pathogenic strain, a greater achievable plant biomass was observed over the course of the experiment. In the case of *T. aestivum*, it was observed that plants exposed to *R. cerealis* and the non-pathogenic strain resulted in an even greater induction and the ability to achieve higher biomass than in the control variants. Therefore, there is an assumption that *R* AG B0 may colonize the root zone and thus positively affect the development of plants [45,53]. However, in the context of the germination index, a slightly stimulating effect of the BNR strain could be observed, unlike the Khan et al. report [54], where the addition of a hypovirulent strain inhibited soybean germination.

5. Conclusions

Biological plant protection is becoming a requirement of modern agriculture. Rational targeting of interactions between organisms can contribute to the effective protection of crops against pathogens. Studies have shown that in the case of pathogens such as *Rhizoctonia*, both the less severe *R. cerealis* and the polyphagous *R. solani* can be controlled in a non-chemical way. However, it is important to conduct further experiments in the case of the tested BNR from the AG B0 group, if only because of the genotypic response of plants to BNR alone. It was observed that this is a racially specific feature and not every genotype of the plant was effectively protected. The tested BNR can effectively inhibit the development of *R. cerealis*, so it is possible to develop an effective strategy to protect cereals against this particular pathogen. The most effective method may be to encapsulate or treat the seed material with the tested BNR strain.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13020523/s1.

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