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Screening Winter Wheat Genotypes for Resistance Traits against *Rhizoctonia cerealis* and *Rhizoctonia solani* Infection

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Abstract: *Rhizoctonia cerealis* and *Rhizoctonia solani* are considered to be among the most harmful soil-borne pathogens for crop plants globally. The lack of effective protection and the requirement to minimize the use of chemical pesticides necessitate the need to develop alternative protective methods. One such method is resistance breeding against biotic and abiotic stresses. Here, we present studies on the presence of resistance traits in winter wheat genotypes that evaluate the plants’ resistance to the above two pathogens, in both field and laboratory environments. In the field environment, the incidence and severity of sharp eyespot were studied using 132 winter wheat cultivars, where random samples at the BBCH 75–77 were collected for analysis. The degree of the intensity of sharp eyespot was determined, applying the 0–4° scale. The susceptibility of the 132 cultivars of winter wheat to *R. cerealis* (AG-D subgroup I) and *R. solani* (AG-5) was also studied under laboratory conditions. In the laboratory, test pieces of potato dextrose agar colonized by the test isolates were placed onto filter paper soaked with distilled water and then placed into Petri dish. Infection on the roots, coleoptiles and leaves was then assessed after 15 days for *R. cerealis* and after 10 days for *R. solani*. None of the tested winter wheat genotypes were found to be asymptomatic to the pathogens. A moderate susceptibility was observed for such genotypes as Anthus, Baryton, Bellenus, Borderland Benatka, Blonde, Cubus, Estero, and Flairway. However, the classification of those associated with moderate susceptibility in laboratory tests resulted in severe symptoms in field tests. Hence, field experiments provide the most reliable measurements to determine the effects of pathogens on the plants.

Keywords: winter wheat; *Rhizoctonia cerealis*; *Rhizoctonia solani*; resistance

1. Introduction

Many pathogenic fungi of soil origin, causing extensive losses in agroecosystems, threaten the cultivation of cereal plants. Pathogens affect the water and nutrient uptake and balance of plants, which translates into reducing the size and quality of the crop yield. Several pathogens of increasing importance, which include fungi of the genus *Rhizoctonia*, cause many problems in the cultivation of cereals, and especially in winter forms [1–3]. *Rhizoctonia solani* J.G. Kühn causes significant losses among crops by infecting the shoots, roots and stems of various plant species, including cereals [4].

Effective plant protection is currently based on the use of chemical products, often in the form of seed dressing, although these preparations are not able to guarantee full protection against both *R. cerealis* and *R. solani* in wheat production [1,2]. Moreover, the use of conventional chemical protection 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 methods of plant protection, and the main emphasis is on the search for
the determinants of wheat resistance to pathogens. This would make it possible to obtain material to grow varieties characterized by lower susceptibility or even resistance to sharp eyespot [1,2,5].

For many years, the issues of plants’ resistance to pathogens, pathogen penetration processes, methods of receiving and transmitting signals about infection, processes of induction of defense mechanisms, and their activation and course have been studied [6]. In the course of evolution, plants have developed many defense mechanisms against pathogens and pests feeding or parasitizing on them. The effectiveness of such mechanisms overlaps with a number of other induced reactions, e.g., with a genetic and biochemical background [6]. Proper recognition of the pathogen by the plant in the infection process is crucial for effective defense.

Wheat is the basic type of cereal crops grown in Poland, and worldwide it is the third-most produced cereal grain, next to rice and maize, taking into account the acreage and annual harvest. Of the total yield, 60% of wheat grain is used for flour [7]. Almost every year, the cultivation of common wheat dominates in the overall grain-sown acreage in Poland. However, this trend is also visible in other European countries, as well as in both Americas and Asia [6,8]. The sown area is systematically growing from year to year, illustrating how important wheat production has become. Importantly, the vast majority of wheat crops in Poland are winter crops. However, winter crops are more susceptible to losses caused by pathogens of the genus Rhizoctonia, owing to these pathogens having optimal growth and development at temperatures below 20 °C. Taking into account the scale of production and the share of winter cereals in the annual production, the need for appropriate management and protection of crops against pathogens becomes important.

Rhizoctonia fungi occur as pathogens, saprophytes and symbionts (e.g., as mycorrhizating fungi for some species of orchids), as well as living on plants considered to be weeds [9–14]. As pathogens, they damage crops of cereals, root crops, vegetables, and grassy plant communities, including those created for human use, such as golf courses, grasslands, and sports fields. Moreover, R. solani is a very serious problem for greenhouse crops [15]. Pathogens of this genus are polyphagous of cereal plants (wheat, triticale, barley, rye, oats, and corn), vegetables (potato, tomato, cucumber, sunflower, cucurbits, and legumes (peas and beans)), sugar beet, fruit trees, forest trees, ornamental plants, herbs, medicinal plants, and many others from an ever-expanding list of plants, because new host plants are noted every year [13,16–23].

Pathogenic fungi of the genus Rhizoctonia, characterized by an aggressive course of pathogenesis, inhibits the development of young plants and rots the stem base, leading to significant yield losses [3,6]. This results from disturbances in the transport of nutrients and water, internal tissue maceration, destruction of vascular bundles, disturbance of redox homeostasis, and disturbances in photosynthesis. Rhizoctonia infestation can result in yield losses of cereal grains in the range of 5% to 30% [3,6,24]. In addition, Rhizoctonia are able to survive long term on plant crop debris and “wait” for the eventual emergence of a suitable host plant. Both R. solani and R. cerealis can survive as saprotrophs inhabiting the root zone of plants. Furthermore, these microorganisms are able to produce sclerotia as a spore structure at a relatively fast pace, which is capable of producing hyphae even a few years after the sclerotia are formed. Importantly, these pathogens are cosmopolitan microorganisms that occur in almost every corner of the world, in all climatic zones, living not only on plants of economic utility or ornamental importance, but also even on plants recognized as weeds. Despite rational and correct management strategies, these pathogens pose a serious threat to crops worldwide. A major problem is our inability to effectively combat pathogens, in the form of interventional chemical treatments. As already mentioned, these microorganisms are soil-borne pathogens living on crop residues, often constituting their reservoirs and thus being a potential source of infection for subsequent crops [25,26]. That is why proper crop rotation, selection of appropriate varieties and general high level of agricultural technology are so important.
The aim of this study was to test the available wheat genotypes in terms of their sensitivity to *Rhizoctonia* fungi in order to select genetic material characterized by reduced susceptibility, which could then be used for possible resistance breeding and further research.

2. Materials and Methods

2.1. Susceptibility Tests of Wheat Cultivars Conducted under Field Conditions

In order to determine the susceptibility of wheat genotypes (132–Table S1) to infection by fungi of the genus *Rhizoctonia* (*Rhizoctonia cerealis* (E.P. Hoeven) R.T. Moore (*Ceratobasidium cereale* D.I. Murray et Burpee teleomorph) and *Rhizoctonia solani* J.G. Kühn (*Teleomorph Thanatephorus cucumeris* (A.B. Frank) Donk)), field experiments were conducted under favorable conditions in 2013–2014 and 2016–2017 (Figure S1). The experiments were carried out at the Research Station of the Faculty of Agriculture and Biotechnology of the Bydgoszcz University of Science and Technology (53° 13'14.5" N 17° 52'19.6" E, 53.220681, 17.872100), on typical Luvisol that had been formed of a sandy loam, valuation class 4a, with pH in KCl = 6.82, humus content: 10.9 g kg⁻¹, P₂O₅ = 26.4 mg·100 g⁻¹, K₂O = 21.2 mg·100 g⁻¹, Mg = 4.0 mg·100 g⁻¹. The previous crop was winter oilseed rape. The sowing date of winter wheat was in the third decade of September. In the field experiments manual sowing in three replications was used. Before sowing, mineral fertilization was applied in the form of ammonium nitrate (34 kg ha⁻¹ N), potassium salt (80 kg ha⁻¹ K₂O) and superphosphate (70 kg ha⁻¹ P₂O₅). In addition, in the BBCH 31 phase, the herbicide Mustang 306 SE (florasulam 6.25 g/L, 2.4-D 300 g/L) was sprayed at a dose of 0.6 L ha⁻¹. On a microplot with an area of 0.35 m² (0.35 × 1.0 m), 100 untreated kernels of each wheat variety were sown, and 15 g of *R. cerealis* (AG-DI) or *R. solani* (AG-5) inoculum grown on autoclaved millet grain was added. *Rhizoctonia* isolates (*R. cerealis* AG-D subgroup I Ww isolate 542, *R. solani* AG-5 Ww 11 isolate) were isolated from winter wheat by one co-author and characterized by PCR and ITS sequencing. *Rhizoctonia* isolates characterized by high virulence were selected for the study, which was confirmed in previous tests carried out in the laboratory and field conditions on various species of cereals. In the field experiments the conditions were favorable (20 ± 2 °C for *R. cerealis* and 25 ± 2 °C for *R. solani* and app. 20 kPa soil moisture; based on the previous experiments) for the development of *Rhizoctonia* pathogens. In order to obtain the inoculum, 50 g of millet seeds were weighed into Erlenmeyer flasks and then poured over with 30 mL of distilled water. The flasks were closed with cotton wool plugs, covered with aluminum foil and left for 24 h at room temperature (21–22 °C). Then, the grain was autoclaved for 60 min at 121 °C. Slices of PDA medium overgrown with mycelium of the tested *Rhizoctonia* isolates (*R. cerealis* AG-D subgroup I Ww isolate 542, *R. solani* AG-5 Ww 11 isolate) were inoculated on the prepared material. *Rhizoctonia* isolates characterized by strong virulence, obtained from the stem base of winter wheat, were selected for the study. The inoculum was incubated for 3 weeks at 20 °C until the millet grains were completely overgrown with the mycelium of individual *Rhizoctonia* isolates. After 7 days of incubation, the flasks were shaken daily to break up larger clusters. Field assessment was carried out at BBCH 12–13 (damping off evaluation—two-three lease unfolded) and the BBCH 75–77 stage (disease index and percentage of the population—from medium milk: grain content milky, grains reached final size, still green to late milk; the same period for all the genotypes). In this stage the most pronounced symptoms of the disease are observed. The plants were sampled randomly (30 plants per microplot) at this stage, shaken from the soli residue, and brought to the lab for further processing. The assessment was based on a 5-point evaluation scale of symptoms on the stem base (0-no symptoms, 1-lesser symptoms, 2-mild symptoms, 3-greater symptoms, 4-severe symptoms). The obtained results were converted into the disease index (DI) according to the Townsend–Heuberger transformation [27].
\[ DI = \sum_{i=0}^{i} n \times v \times \frac{1}{i \times N} \times 100\% \]

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 (%).

2.2. Susceptibility Study of Wheat Cultivars Conducted in the Paper Test

In the filter-paper test, the sensitivity of 132 wheat cultivars to fungi infection of the genus *Rhizoctonia* was investigated. For this purpose, agar discs overgrown with *R. solani* or *R. cerealis* mycelium were placed on Anumbra Petri plates (200 × 30 mm), lined with three wetted (16 mL sterile distilled water) filter papers (Munktell 75 g/m², filtration pace 8 s). The germinated (3 days old) wheat seeds were then placed on the agar discs. After 10 days for *R. solani* and 15 days for *R. cerealis*, the plants were assessed for disease symptoms on roots, stem base and leaves. The assessment of the severity of disease symptoms was performed on a different number of days from the inoculation due to the different rate of disease symptoms observed on *Rhizoctonia* species. In the case of *R. solani*, a high intensity of disease symptoms was observed 10 days after inoculation. During this period of time, no clear symptoms of *R. cerealis* were recorded. Thus, the evaluation was performed 15 days after inoculation. The assessment was based on a 5-point evaluation scale (0-no symptoms, 1-lesser symptoms, 2-mild symptoms, 3-greater symptoms, 4-severe symptoms). A total of 15 plants per replicate were assessed (three replicates per combination/cultivar). The obtained results were converted into the disease index (DI) according to the Townsend–Heuberger transformation as described earlier [27].

2.3. Statistical Analysis

Data acquired from the tests (years 2013–2014 and 2016–2017) were combined and averaged for further statistical processing in MS Excel (raw data: https://zenodo.org/record/7236021, accessed 21 October 2022). The statistical analysis was performed in R Core Team software (version 4.1.1) with the R Studio overlay (version 1.4.1717). The r-Pearson linear correlation coefficient was used to calculate the relationship between the values of disease indices for individual wheat cultivars and the tested pathogen and the dependence of the degree of infection between individual parts of plants. A hierarchical K-means analysis was performed with regard to the tested wheat genotypes for pathogens *R. solani* AG-5 and *R. cerealis* AG-DI in field and laboratory experiments. The number of required groups (clusters) was calculated using the “Gap statistic”, “Wss” and “Silhouette algorithms”, bootstrap 1000. Knowing the optimal number of groups, a grouping of wheat species of a similar nature was performed in a two-dimensional system according to Kassambara [28,29].

3. Results

The results obtained from the exposure of plants to two pathogens were analyzed for correlation between the variables (roots disease index, steam base disease index, leaves disease index). An observation of the intensity of symptoms in laboratory conditions was made on the roots, the base of the shoot and the leaves. In field tests, a general disease index was observed by pathogens as well as for seedling damping off. No plant genotype tested was found to be fully resistant to infection by both *R. cerealis* and *R. solani*. Therefore, in the studies, plants showing less infestation were classified as less susceptible or potentially resistant, since the use of just the term resistance would be misleading. The presented correlation matrix (Figure 1) indicates the presence of highly significant \((p < 0.0001)\) relationships between the studied variables (Figure 1 refers to both the field and the laboratory tests). There was a moderate negative correlation between the severity of
symptoms based on the shoot and the roots ($R = -0.501$). The strongest positive correlation of the severity of symptoms was observed between the roots and leaves ($R = 0.730$). In the case of the relationship between the infestation of leaves and the base of the shoot, the correlation was negative ($R = -0.426$). An important relationship is also the intensity of symptoms on the tested parts of plants in laboratory tests and the scale of seedling damping off. It was observed that the severity of symptoms on the roots and the aforementioned damping off are positively correlated to a moderate degree ($R = 0.366$), as are the severity of symptoms on the leaves and the observed damping off ($R = 0.350$). Importantly, the severity of symptoms on the shoot base and damping off are correlated to a moderately strong degree.

![Figure 1](image-url)  
**Figure 1.** Correlation matrix between the studied variables depending on the species of pathogen used (R. c.—red color—R. cerealis; R. s.—blue color—R. solani; x, y axes—percentage of disease severity caused by Rhizoctonia species, Roots—roots disease index (%), Stem base—stem base disease index (%), Leaves—leaves disease index (%), Disease index—the disease index of plants in field trials (%), Percentage—the percentage of infected population showing symptoms (%), Damping-off (%), *—significance level 0.05, **—significance level 0.001).

When considering the mean values of each of the tested parameters, some differentiation was observed (Table 1). Average root infection in laboratory variants for *R. cerealis* oscillated at the level of DI 32.1 and was almost twice as high as root infection by *R. solani*. On the other hand, when analyzing the severity of symptoms on the base of the shoot, it was observed that it was *R. solani* that had a preference for infecting these tissues, as the mean DI in laboratory conditions was 96.1%, while for *R. cerealis* it was 76.9%. In the case of intensification of symptoms on leaves, a higher virulence of *R. cerealis* (DI 52.3%) can be observed compared to *R. solani* (DI 18.1%). Under field conditions, we can also observe greater pressure from *R. cerealis*, as the mean disease index that was observed was 28% compared to *R. solani*, which was 7.9%. The scale of seedling damping off caused by the tested pathogens is also important. During the course of this study, it was observed that in
field conditions, *R. cerealis* was characterized by stronger pressure and greater virulence against the tested plant species and caused over 30% (30.8%) loss in population, whereas *R. solani* was responsible for a 12.8% loss of the studied population of plants.

Table 1. Average values for tested parameters (± standard deviation) for each pathogen.

| Pathogen   | Laboratory Test | Field Test |
|------------|----------------|------------|
|            | Roots          | Stem Base  | Leaves | Disease Index | Percentage | Damping Off |            |
| *R. cerealis* | 32.1 ± 0.8    | 76.9 ± 0.7 | 52.3 ± 1.3 | 28.0 ± 1.0 | 49.7 ± 1.5 | 30.8 ± 1.7 |
| *R. solani*  | 15.5 ± 1.2    | 96.1 ± 0.5 | 18.1 ± 2.1 | 7.9 ± 0.6  | 18.4 ± 1.2 | 12.8 ± 0.7 |

Under controlled laboratory conditions and in favorable field conditions, a differentiated reaction of plants to the tested pathogens was observed. The hierarchical K-means analysis of the data resulted in three clusters being separated in each experiment. The first cluster (green) corresponds to plants with low susceptibility to pathogens, yellow represents plants with moderate susceptibility/resistance, and red represents high susceptibility to the tested pathogens (Figure 2, Table 2). In the case of plant exposure to *R. cerealis* in laboratory conditions (Figure 3), it was observed that 57 of the studied genotypes showed potential resistance traits to the pathogen used, 45 were characterized by moderate susceptibility and 30 as highly susceptible. In the case of the same pathogen, but under field conditions (Figure 4), 38 genotypes were among the group with potential resistance traits, 33 were moderately susceptible, and 66 were susceptible. When analyzing individual plant genotypes for the presence of resistance traits (green cluster), it was not observed that any wheat genotypes were in the same group, i.e., no genotype was simultaneously in the green cluster in both laboratory and field tests. However, it was observed that low symptoms in laboratory tests were usually associated with moderate severity of disease symptoms, and moderate occurrence of symptoms in laboratory tests was often associated with severe infection of a given genotype in field tests (Figure 2). In the case of plant exposure to *R. solani*, a very large variation in plant responses was observed. Only six genotypes showed potential resistance traits under laboratory conditions (Figure 5). The vast majority (112) were in the group of having a moderate reaction to the pathogen, while only 14 wheat genotypes were highly susceptible. On the other hand, in the field (Figure 6) it was observed that 69 genotypes were characterized by reduced susceptibility to *R. solani*, 28 were moderately responsive to the pathogen, and 35 were highly susceptible. Belonging to a moderate susceptibility cluster was observed in both types of experiments for such genotypes as Anthus, Baryton, Bellenus, Borderland Benatka, Blonde, Cubus, Estero and Flairway. It was also observed that the classification of the genotype in a cluster with moderate susceptibility in laboratory tests resulted in severe symptoms in field tests (Figure 2).

Table 2. Cluster cardinality of each experiment variant according to disease severity.

| Type of Experiment-Cluster | Potentially Resistant (Green Cluster) | Intermediate (Yellow Cluster) | Susceptible (Red Cluster) |
|----------------------------|--------------------------------------|-------------------------------|--------------------------|
| *R. cerealis* Laboratory   | 57                                   | 45                            | 30                       |
| *R. cerealis* Field        | 38                                   | 33                            | 61                       |
| *R. solani* Laboratory     | 6                                    | 112                           | 14                       |
| *R. solani* Field          | 69                                   | 28                            | 35                       |
Figure 2. The heatmap of the infestation distribution of the tested plants in individual experimental variants, i.e., the type of pathogen and the type of experiment (laboratory, field). The colors successively correspond to the clusters to which the plants were assigned using the hierarchical K-means method: green—potentially resistant, yellow—moderate, red—susceptible/non-resistant. Rc.L—*R. cerealis* in the laboratory conditions, Rc.F—*R. cerealis* in the field conditions, Rs.L—*R. solani* in the laboratory conditions, Rs.F—*R. solani* in the field conditions.

Figure 3. Distribution of the studied genotypes of plants using the method of hierarchical K-means in laboratory conditions in the inoculation of *R. cerealis* (clustering in the form of a phylogenetic tree). The colors correspond to the assigned clusters according to the resistance type; red—susceptible, yellow—less susceptible, green—least susceptible.
Figure 4. Distribution of the studied genotypes of plants using the method of hierarchical K−means in field conditions with the inoculation of *R. cerealis* (clustering in the form of a phylogenetic tree). The colors correspond to the assigned clusters according to the resistance type; red—susceptible, yellow—less susceptible, green—least susceptible.

Figure 5. Distribution of the studied genotypes of plants using the method of hierarchical K−means in laboratory conditions with the inoculation of *R. solani* (clustering in the form of a phylogenetic tree). The colors correspond to the assigned clusters according to the resistance type; red—susceptible, yellow—less susceptible, green—least susceptible.
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The colors correspond to the assigned clusters according to the resistance type; red—susceptible, yellow—less susceptible, green—least susceptible.

4. Discussion

In the course of evolution, plants have developed complex defense mechanisms against pathogens. In parallel, humans have been continuously forced to improve them in terms of selected features, including resistance or more desirable resistance to pests. Smith et al. [30,31] examined spring wheat genotypes for the presence of resistance features to R. solani AG-8 under strict, controlled field conditions with strong and low pathogen pressure, where high values of infestation were observed both in artificially inoculated variants as well as in natural inoculations. Similarly, in our own research, a more comprehensive observation of the resistance trait under field conditions was recorded. Thus, the prognosis of field studies seems to be greater, confirming the reports of Smith et al. [5,30,31]. At the same time, it can be observed that in the case of R. solani, laboratory tests provide less information about the possible resistance of plants. Cromey et al. [32,33] observed that only about 17% of the examined genotypes of winter wheat among the cultivars tested showed features of resistance to R. cerealis. Two cultivars (Centaur and Regency) were characterized by noticeably lower susceptibility to R. cerealis (less than 15% of plants) and showed significantly fewer symptoms of sharp eyespot in field trials. Importantly, in field studies, it was observed that the sowing date had a significant influence on the occurrence of sharp eyespot, as the plants sown in early autumn were characterized by a greater degree of infection than those sown in late autumn. Additionally, Lemańczyk [20] observed differentiation in the occurrence of symptoms among the cultivars studied. He found that none of the tested cultivars was resistant to the fungi of the genus Rhizoctonia and some triticale cultivars were characterized by a similar reaction to R. cerealis and R. solani. For example, the Baltic and Zorro cultivars were characterized by being comparably partially resistant to Rhizoctonia. A correlation was observed between the infestation by R. cerealis and R. solani on the coleoptile and the infestation of the leaves, but a moderately negative correlation was observed between the infection of the roots and the root of the stalk, even when a strong correlation between the infestation of the roots and the leaves was recorded.
Interestingly, a negative correlation was observed between the symptoms of the stalk base and leaves. On the other hand, belonging to a moderate susceptibility cluster was observed in both types of experiments (i.e., laboratory and field experiments) for such genotypes as Anthus, Baryton, Bellenus, Benatka Kresowa, Blonde, Cubus, Estero, and Flairway in relation to exposure to \( R. solani \). Lemańczyk and Kwaśna [3] examined winter wheat cultivars for the presence of \( R. cerealis \) in the production fields of central Poland and found that the symptoms of sharp eyespot were visible on the base of the shoots in between 41% and nearly 70% of the tested plants, depending on the site. Despite the decrease in the quality of the grain in terms of consumption values, it was not observed that the pathogen’s activity led to a reduction in the biological properties of the kernels in terms of their ability to germinate.

The studies by Płonka and Roj [34] on the susceptibility of wheat cultivars also indicated the differentiation of susceptibility of plants to \( R. cerealis \) and \( R. solani \). In these studies, they did not observe that any of the studied genotypes were fully resistant to pathogens. Moreover, they found that \( R. cerealis \) caused a greater severity of lesions (i.e., a greater value of the disease index) among the plants tested, as well as most plants showing lesions, compared to plants inoculated with \( R. solani \). In our own laboratory studies, it was not possible to identify wheat varieties fully resistant to infection by \( R. cerealis \) and \( R. solani \), as none of the studied genotypes were characterized by a complete absence of disease symptoms. The tested pathogens differed in their infection site preferences. \( R. cerealis \) caused more severe damage to the base of the shoot and to the leaves, while \( R. solani \) caused a very strong infection of the stem base. It was also observed that \( R. cerealis \), in the general context of laboratory and field tests, was more virulent to plants in contrast to \( R. solani \). The use of the classification method through hierarchical clustering of K-means in two-dimensional terms made it possible to divide the studied genotypes into three groups with differentiated characteristics of resistance. Considering the occurrence of resistance to \( R. cerealis \) in laboratory conditions, it could be observed that 57 cultivars were identified as resistant to this pathogen and 45 as moderate. In the field, however, only 38 genotypes showed resistance traits and 33 were classified as moderate. Regarding \( R. solani \), under laboratory conditions it was observed that only 6 genotypes were potentially resistant, and the vast majority (112) of genotypes were classified as moderate. In contrast, under field conditions, 69 genotypes were resistant to the pathogen, and 28 showed a moderate susceptibility trait. The arsenal of plant defense capabilities is only partially known, although this partial knowledge may turn out to be useful in the selection of genetic material in breeding processes so that it shows the desired features. In addition, obtaining genetic material with the desired characteristics does not mean winning the war against pathogens, because pathogens will adapt over time and break the plant’s defense capabilities [35]. These opportunities can be expressed in various ways. All of this translates into a plant’s ability to resist biotic stress for a long time.

5. Conclusions

This study was aimed at presenting the nature of the insensitivity trait to selected pathogens highly pathogenic for the cereal plants \( R. cerealis \) (belonging to the AG-DI group) and \( R. solani \) (belonging to the AG-5 group). Neither strict laboratory tests under controlled conditions nor field tests with strong pathogen pressure showed that any wheat genotype was asymptomatic, which would be synonymous with the presence of resistance. Unfortunately, in the case of these studies, we can only talk about plant resistance to pathogens. Importantly, differences in the location of occurrence of symptoms caused by both pathogens were observed, i.e., they showed certain preferences as to the location of infestation. Furthermore, significant \((p < 0.0001)\) relationships between the location of occurrence of symptoms were observed. In the case of the studied pathogens, field experiments seem to be the most reliable, as the distribution of the trait seems to reflect the presence of the desired trait more strongly than in the case of laboratory experiments.
Further experiments should be conducted to examine the resistance genes occurrence in different germplasms.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture12121981/s1, Figure S1: Field experiment placement in faculty experimental station. Table S1: Winter wheat genotype names used in the research.

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References
1. Guo, Y.X.; Liu, Q.H.; Ng, T.B.; Wang, H.X. Isarfelin, a peptide with antifungal and insecticidal activities from Isaria felina. Peptides 2005, 26, 2384–2391. [CrossRef] [PubMed]
2. Hamada, M.S.; Yanni, Y.; Ma, Z. Sensitivity to iprodione, difenoconazole and fluidoxinol of Rhizoctonia cerealis isolates collected from wheat in China. Crop Prot. 2011, 30, 1028–1033. [CrossRef]
3. Lemańczyk, G.; Kwaśna, H. Effects of sharp eyespot (Rhizoctonia cerealis) on yield and grain quality of winter wheat. Eur. J. Plant Pathol. 2013, 135, 187–200. [CrossRef]
4. Al-Abdalall, A.H.A. Assessment of yield loss caused by root rots in wheat and barley. J. Gen. Plant Pathol. 2014, 80, 28–59. [CrossRef]
5. Shamim, M.D.; Deepak, K.; Deepti, S.; Pramila, P.; Singh, K.N. Evaluation of major cereal crops for resistance against Rhizoctonia solani under green house and field conditions. Indian Phytopathol. 2014, 67, 42–48.
6. Liu, J.; Mundt, C.C. Genetic structure and population diversity in the wheat sharp eyespot pathogen Rhizoctonia cerealis in the Willamette Valley, Oregon, USA. Plant Pathol. 2020, 69, 101–111. [CrossRef]
7. Rachoń, L.; Szumiło, G.; Stankowski, S. Porównanie wybranych wskaźników wartości technologicznej pszenicy zwyczajnej (Triticum aestivum ssp. vulgare), twardej (Triticum durum) i orkiszowej (Triticum aestivum ssp. spelta). Fragm. Agron. 2011, 28, 52–59.
8. Okabura, P.A.; Steber, C.M.; DeMacon, V.L.; Walter, N.L.; Paulitz, T.C.; Kidwell, K.K. Scarlet-Rz1, an EMS-generated hexaploid wheat with tolerance to the soilborne necrotrophic pathogens Rhizoctonia solani and collar rot disease caused by Rhizoctonia solani in sunflower from India. Australas. Plant Dis. Notes. 2010, 5, 11–13. [CrossRef]
9. Doornik, A.W. Effect of storage duration and temperature on the survival of Rhizoctonia solani in tulip and iris bulbs. Neth. J. Plant Pathol. 1982, 88, 185–190. [CrossRef]
10. Arakawa, M.; Inagaki, K. Molecular markers for genotyping anastomosis groups and understanding the population biology of Rhizoctonia species. J. Gen. Plant Pathol. 2014, 80, 401–407. [CrossRef]
11. Maculewicz, D. Binucleate Rhizoctonia spp. as a biocontrol agents against plant pathogens. Ecol. Chem. Eng. A. 2015, 22, 195–203. [CrossRef]
12. Jiang, J.H.; Tam, S.L.; Toda, T.; Chen, L.C. Controlling Rhizoctonia damping-off of chinese mustard by using endomycorrhizal Rhizoctonia spp. isolated from orchid mycorrhizae. Plant Dis. 2016, 100, 85–91. [CrossRef] [PubMed]
13. Choupannejad, R.; Sharifnabi, B.; Fadaei Tehrani, A.A.; Gholami, J. Rhizoctonia solani AG4 associated with foliar blight symptoms on barley in Iran. Australas. Plant Path. 2017, 12, 2. [CrossRef]
14. Inokuti, E.M.; Thierry-Lanfranchi, D.; Edel-Hermann, V.; Gautheron, N.; Fayolle, L.; Michereff, S.J.; Steinberg, C. Genetic and pathogenic variability of Rhizoctonia solani causing crown and root rot on sugar beet in France. J. Plant Pathol. 2019, 101, 907–916. [CrossRef]
15. Kazempour, M.N. Biological control of Rhizoctonia solani, the causal agent of rice sheath blight by antagonistics bacteria in greenhouse and field conditions. J. Plant Pathol. 2004, 3, 88–96.
16. Herr, L.J. Biological control of Rhizoctonia solani by binucleate Rhizoctonia spp. and hypovirulent R. solani agents. Crop Prot. 1995, 14, 179–186. [CrossRef]
17. Gutiérrez, S.A.; Cundom, M.A.; Barrera, V.; Gasoni, L. First record of Rhizoctonia zeae on corn in Argentina. Australas. Plant Dis. Notes. 2007, 2, 137–138. [CrossRef]
18. Rosa, D.D.; Ohto, C.T.; Basseto, M.A.; Furtado, E.L.; de Souza, N.L. First report of Rhizoctonia solani AG 4 HG-II attacking Gazania rigens plants in Brazil. Australas. Plant Dis. Notes 2008, 3, 1–2. [CrossRef]
19. Lakshmidevi, N.; Sudisha, J.; Mahadevamurthy, S.; Prakash, H.S.; Shekar Shetty, H. First report of the seed-borne nature of root and collar rot disease caused by Rhizoctonia solani in sunflower from India. Australas. Plant Dis. Notes. 2010, 5, 11–13. [CrossRef]
20. Lemańczyk, G. Susceptibility of winter triticale cultivars to Rhizoctonia cerealis (sharp eyespot) and R. solani. J. Plant. Prot. Res. 2012, 52, 421–433. [CrossRef]

21. Erper, I.; Kilicoglu M, Turkkan, C.M.; Onder, H. Characterization and pathogenicity of Rhizoctonia spp. isolated from winter squash in the Black Sea region of Turkey. Eur. J. Plant Pathol. 2016, 146, 683–697. [CrossRef]

22. Misawa, T.; Kayamori, M.; Kurose, D.; Sasaki, J.; Toda, T. First report of Rhizoctonia disease of lily caused by Rhizoctonia solani AG-11 in Japan. J. Gen. Plant Pathol. 2017, 83, 406–409. [CrossRef]

23. Moliszewska, E.; Nabrdalik, M.; Ziembik, Z. Rhizoctonia solani AG 11 isolated for the first time from sugar beet in Poland. Saudi J. Biol. Sci. 2020, 27, 1863–1870. [CrossRef] [PubMed]

24. Mahoney, A.K.; Babiker, E.M.; See, D.R.; Paulitz, T.C.; Okubara, P.A.; Hulbert, S.H. Analysis and mapping of Rhizoctonia root rot resistance traits from the synthetic wheat (Triticum aestivum L.) line SYN-172. Mol. Breeding 2017, 37, 130. [CrossRef]

25. Kannaiyan, S. Effect of certain fungicides on the production of enzymes by Rhizoctonia solani. Plant. Soil. 1988, 108, 299–302. [CrossRef]

26. Kurowski, T.P.; Marks, M.; Makowski, P.; Jaźwirska, E. Zdrowotność pszenicy ozimej w stanowiskach po różnych sposobach dwuletniego ugorowania. Fragm. Agron. 2009, 26, 102–108.

27. Townsend, G.R.; Heuberger, J.W. Methods for estimating losses caused by diseases in fungicide experiments. Plant Dis. Rep. 1943, 24, 340–343.

28. Kassambara, A. Practical Guide to Cluster Analysis in R. Unsupervised Machine Learning, 1st ed.; STHDA; CreateSpace Independent Publishing Platform: Scotts Valley, CA, USA, 2017.

29. Kassambara, A. Practical Guide to Principal Component Methods in R, 1st ed.; STHDA; CreateSpace Independent Publishing Platform: Scotts Valley, CA, USA, 2017.

30. Smith, J.D.; Kidwell, K.K.; Evans, M.A.; Cook, R.J.; Smiley, R.W. Assessment of spring wheat genotypes for disease reaction to Rhizoctonia solani AG-8 in controlled environment and direct-seeded field evaluations. Crop Sci. 2003, 43, 694–700. [CrossRef]

31. Smith, J.D.; Kidwell, K.K.; Evans, M.A.; Cook, R.J.; Smiley, R.W. Evaluation of spring cereal grains and wild Triticum germplasm for resistance to Rhizoctonia solani AG-8. Crop Sci. 2003, 43, 701–709. [CrossRef]

32. Cromey, M.G.; Butler, R.C.; Munro, C.A.; Shorter, S.C. Susceptibility of New Zealand wheat cultivars to sharp eyespot. N. Z. Plant Protect. 2005, 58, 268–272. [CrossRef]

33. Cromey, M.G.; Hide, C.C.L.; Meenken, E.D. Resistance to sharp eyespot in wheat. N. Z. Plant Protect. 2012, 65, 204–212. [CrossRef]

34. Plonka, K.J.; Roj, J. Wrażliwość pięciu odmian pszenicy ozimej na Rhizoctonia cerealis i R. solani. Prog. Plant Prot. 2012, 52, 657–662.

35. Bai, G.; Shaner, G. Management and resistance in wheat and barley to Fusarium head blight. Annu. Rev. Phytopathol. 2004, 42, 135–161. [CrossRef] [PubMed]