Tritordeum as a Habitat for the Development of the Rice Weevil *Sitophilus oryzae* L.—Analysis of Selected Properties of the Cereal Grains Determining Their Resistance Mechanisms

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Abstract

In the face of the global climate and ecological crisis, as well as growing consumer needs and demands, a transformation of the global food production and distribution system is necessary. The productivity and quality characteristics of Tritordeum make this cereal an effective tool in the sustainable modernization of the agricultural sector. However, this potential can be significantly limited in the supply chain by storage pests. This study aimed to assess the impact of Tritordeum resistance on the rice weevil (*Sitophilus oryzae* L.). The experiment used 11 Tritordeum breeding lines in comparison to three cereal species derived from conventional cultivation systems (common wheat *Triticum aestivum* L., durum wheat *Triticum durum* Desf., spring barley *Hordeum vulgare* L.). The research showed that Tritordeum may be a substrate on which *S. oryzae* feeds, although the intensity of the pest's development varied depending on the line. The study also demonstrated that the hardness of the Tritordeum seed coat did not directly influence the development intensity of the analyzed beetles. It was noted, however, that the degree of infestation by these insects depended on the chemical profile of the infested kernels. The increased total protein content and lower fiber content (compared to common wheat) likely influence the development of Tritordeum resistance. This study demonstrates that Tritordeum possesses inherent resistance traits linked to its grain chemistry, providing a basis for breeding more storage-resistant cereal cultivars.

Keywords: breeding lines; storage pests; sustainable agriculture; Tritordeum

1. Introduction

The contemporary model of agricultural development, despite systematically increasing production and economic efficiency, has failed to solve the complex problem of food security [1–3]. Previously used agrotechnical practices have not only failed to meet evolving market needs but also caused numerous ecological threats. In the face of these problems, ensuring access to high-quality food produced in an environmentally neutral manner is one of the most important challenges facing the modern world [4–6].

Stability and efficiency in the agricultural sector, adapted to the principles of sustainable development, can be achieved through intensified biological progress [7,8]. A

promising tool for implementing this new concept in agriculture is breeding aimed at enhancing the varietal characteristics of cultivated plants. The introduction of heterotic varieties into crop production, combining the most valuable traits of different parental lines, could revolutionize plant breeding and elevate it to a more advanced and sustainable level [5,7,8].

Tritordeum (×*Tritordeum martinii* A. Pujadas, nothosp. nov.) is a relatively new allopolyploid crop obtained by crossing diploid *Hordeum chilense* Roem. & Schult. (HchHch) with tetraploid *Triticum durum* Desf. (AABB) [9]. This genotypically improved hybrid is characterized by greater environmental flexibility (including higher drought and salinity resistance) compared to conventionally bred cereal varieties [10,11]. Tritordeum, with morphological and agrotechnical parameters similar to those of common wheat, also has a chemical composition more beneficial to consumer health. The above-standard nutritional parameters of Tritordeum are primarily manifested by a higher content of bioactive compounds, and micro- and macroelements (including carotenoids, polyphenols, fiber, magnesium, iron, calcium and potassium) [12–16]. Detailed studies have also shown that supplementing the diet with Tritordeum has a beneficial effect on the circulatory system, glycemic balance, and intestinal peristalsis, and also supports antioxidant processes in the body, exhibiting anti-inflammatory properties [17,18]. Among the numerous benefits of this grain, particularly noteworthy is its reduced gluten content, which is a cause of several systemic gluten-related diseases [19–23]. Due to its numerous health-promoting and cultivation properties, Tritordeum has the potential to become a pillar of a healthy and sustainable alternative to common varieties [24,25]. In recent years, Tritordeum has been introduced to European markets as an alternative to traditional cereals.

The safe and efficient flow of food products in the global food supply chain is the foundation of food security. A key role in this complex process is played by the storage of agricultural crops, with cereals being among the most frequently wasted raw materials [26,27]. During grain storage, grain losses can reach up to 60% of the harvested grain [26]. The primary cause of these losses is infestation by storage pests, the most significant of which is the rice weevil (*Sitophilus oryzae* L.) (Coleoptera: Curculionidae). This beetle can infest both whole and damaged grains of rice, corn, wheat, and barley, among others. The resulting quantitative losses (loss of stored grain mass) and qualitative losses (including contamination of grain with feces, exuviae, and dead individuals) lead to a reduction in the availability and commercial value of stored grain products [22,28–30].

Due to its wide adaptability and high reproductive potential, the rice weevil can pose a threat to stored grains of new cereal species. This study aimed to fill a gap in the research by assessing whether Tritordeum can serve as a suitable reservoir for the development of *S. oryzae* and whether, in the future, cereal variety breeding could create varieties that are more resistant to this pest during storage. The pest's development intensity was assessed based on the number of progenies, the mass of one progeny beetle, the mass of dust produced, and the grain mass loss during the beetle's development. An attempt was also made to determine the physicochemical characteristics of the grain that are associated with increased grain resistance to *S. oryzae* feeding. This information can be used in further breeding processes and, consequently, it would minimize storage losses of this raw material.

The following research hypotheses were formulated:

- The intensity of rice weevil development varies depending on the cereal species studied;
- The tested Tritordeum lines differ in the physicochemical properties of the grain;
- The intensity of *S. oryzae* development on the tested Tritordeum lines results from the different physicochemical characteristics of the grain.

2. Materials and Methods

2.1. Materials

Entomological material (*S. oryzae*) was obtained from a mass breeding grown on winter wheat cultivar Delawer at the Department of Entomology, Phytopathology and Molecular Diagnostics (University of Warmia and Mazury in Olsztyn, Poland). Observations on the feeding of this pest were carried out on kernels of 11 Tritordeum breeding lines (T1—line HT 440; T2—line HT 129; T3—line HTC 2060; T4—line HT 157; T5—line HT 352; T6—line JB3; T7—line HT 438; T8—line HTC 1324; T9—line HT 444; T10—line HTC 2083; T12—line '60' HTC 2083) which were obtained from Professor Antonio Martín of the Instituto de Agricultura Sostenible—CSIC, Spain, with the assistance of Petr Martinek, Eng., of the Agricultural Research Institute Kromeriz, Ltd., (Kromeriz, Czech Republic) [31]. The reference varieties were naked spring barley, Gawrosz (T11_K1), from the Strzelce-IHAR Plant Breeding Group (Strzelce, Poland), durum spring wheat, Duragold (T13_K2), from Saatzucht Donau GesmbH & CoKG, Probstdorf (Reichersberg, Austria) and winter common wheat, Mewa (T14_K3), from Smolice Plant Breeding (Smolice, Poland). The grain tested came from a strict field experiment located at the Agricultural Experiment Station in Bałcyny near Ostróda, Poland (53°360′ N, 19°510′ E), conducted by the Department of Genetics, Plant Breeding and Bioresource Engineering (University of Warmia and Mazury in Olsztyn, Poland). The experiment had a random block design with two replicates. The plot size was 9 m^2. NPK fertilizer was applied before sowing at a rate of 60/25/80 kg ha^{-1}. The seeds were not dressed, and fungicides and insecticides were not applied during the growing season. The grain for all analyses was harvested at the full maturity stage.

2.2. Bioassays

To achieve humidity and temperature equilibrium, the analyzed cereals were placed in an environmental chamber for 7 days. Conditioning of the cereals and subsequent entomological testing took place in the controlled space of a SANYO MLR 350-H (Sanyo, Ōizumi-mach, Gunma, Japan) chamber (temperature 30 °C; humidity 70%; complete darkness). Entomological observations were conducted in vinidur containers with a diameter of 80 mm and a height of 30 mm. Twenty g of plant material (T1; T2; T3; T4; T5; T6; T7; T8; T9; T10; T11_K1; T12; T13_K2; T14_K3) was weighed into the ventilated containers (10 mm opening secured with a chiffon mesh). Twenty individuals of *S. oryzae* were placed on each of these prepared containers. Adult beetles (3–4 days old) were used in the experiment in a 1:1 sexual ratio. The sex of young *S. oryzae* beetles was determined by the proportion of the rostrum [32]. On the 14th day of the experiment, adult insects were removed from the containers. Further observations concerning the development of the progeny generation in the infested material began after another 14 days. The experiment was conducted in 5 replicates for each combination (*n* = 5). The weight of the initial test material and further analyses concerning the weight of individual experiment components (weight of one progeny, weight of dust produced, weight loss of kernels) were measured using a WPS 220/C/2 laboratory scale (RADWAG, Radom, Poland). The duration of the study and its conditions were designed based on our own research and observations on the development of this pest [33–35].

2.3. Physicochemical Properties of Grain

The content of protein, fat, ash and crude fiber in the tested grain was determined according to the methodology described in the work by Suchowilska et al. [31]. Grain samples were milled using a Cyclotec 1093 sample Mill (FOSS, Hilleroed, Denmark). The ash content was calculated as the weight of the residue (ash) divided by the weight of the original sample, multiplied by 100 to express the result as a percentage. A sample was

placed in a previously ignited, cooled and tared porcelain crucible and then incinerated at 600 °C for at least 2 h. The crucibles were transferred and cooled in a desiccator, and the weight loss was used to determine the percentage of ash. Crude protein content (N × 5.7) [36] was determined in two replications, using the Buchi system (K-424 Digestion Unit and B-324 Distillation Unit, Flawil, Switzerland). Crude fat was extracted using the Soxhlet method (Buchi Extraction System B-811, Flawil, Switzerland) (solvent: diethyl ether (POCh Gliwice, Poland), extractor size 100 mL, 2.5 g analytical samples of air-dried ground grain). Extraction was carried out at 60 °C for 4 h, in two replications. After ether evaporation, solvent caps containing crude fat were dried for 2 h at 105 °C the exsiccator and weighed. Crude fiber content was determined using the Fibertec 2010 system (FOSS, Hilleroed, Denmark) and the Weende method. Ground samples of 2 g were placed in FOSS crucibles with P2 porosity (40–100 lm). The samples were placed in a hot extraction unit, immersed in 1.25% H_2SO_4 (POCh, Gliwice, Poland) and boiled for 40 min. Sulfuric acid was removed, the samples were rinsed three times with hot demineralized water, placed in a cold extraction unit and rinsed with acetone (POCh Gliwice, Poland). The samples were dried at 105 °C for 3 h, and the amount of fiber was determined in a quantitative analysis. Grain hardness analyses were performed in the single kernel characterization system (SKCS 4100, Perten Instruments, Springfield, IL, USA).

2.4. Statistical Analysis

The obtained data describing the developmental parameters of *S. oryzae* and the chemical composition and physical characteristics of the Tritordeum lines studied were evaluated for their distribution using the Shapiro–Wilk W test. Data that were not normally distributed (progeny, mass of 1 progeny beetle, ash content, fiber, protein, fat, and kernel hardness) were logarithmically transformed (ln x + 1), and a one-factorial ANOVA was used to assess the significance of differences in the variables across the cereal varieties. The Tukey HSD test was used to assess the significance of mean differences in the developmental parameters and physicochemical characteristics of the kernels. The results allowed the combination of the means into statistically indistinguishable groups and the assignment of the same letter index (a, b, c). Nonmetric multidimensional scaling (NMDS) analysis, using the Bray–Curtis measure of similarity, was employed to identify differences between the examined grains of Tritordeum breeding lines and other cereal species, based on their chemical composition and kernel hardness. The nonparametric ANOSIM test [37] was used to evaluate these differences statistically. A Bray–Curtis matrix-based dissimilarity analysis was performed to assess the similarity of physical features (hardness) and chemical composition of the hybrid cereal varieties, including total protein, crude ash, dry matter, and crude fat. Dendrograms were then created using the Ward method. A redundancy analysis (RDA) technique [38] was used to visually assess the relationships between parameters describing the development of *S. oryzae* (progeny, mass of one progeny beetle, mass of dust, and loss of mass) and selected physicochemical features of the grains from the examined varieties and cereal lines. The RDA method was selected based on the calculation of gradient lengths (SD) for the analysis. Statistical calculations and their graphical interpretation were performed using Statistica 13.3, Canoco 4.51, and PAST 2.17b.

3. Results

3.1. Developmental Parameters of S. oryzae

The development intensity of *S. oryzae* feeding on the tested species and cereal lines was assessed by determining the number of progeny of the pest (F = 4.01, *p* = 0.00), the mass of one progeny (F = 9.62, *p* = 0.00), the mass of produced dust (F = 13.77, *p* = 0.00), and the loss of kernel mass (F = 8.97, *p* = 0.00). One-factorial ANOVA revealed that the

differences in means describing the pest's development across different cereal varieties were statistically significant (Table 1).

Table 1. Results of one-factorial analysis of variance, ANOVA, for the tested *S. oryzae* progeny, mass of produced dust, loss of grain mass, and mass of 1 progeny of beetles on the Tritordeum lines and control combinations.

	df *	ANOVA F Value	p **
Progeny of beetles	13	4.01	0.00
Mass of 1 progeny beetle	13	9.62	0.00
Mass of dust	13	13.77	0.00
Loss of grain mass	13	8.97	0.00

* Degrees of freedom; ** Test probability value *p*.

The best conditions for the development of *S. oryzae* were found on the grain of the T2 line and T14_K3 control combination, on which an average of 325.6 and 324.0 individuals, respectively, completed their development (Figure 1a).
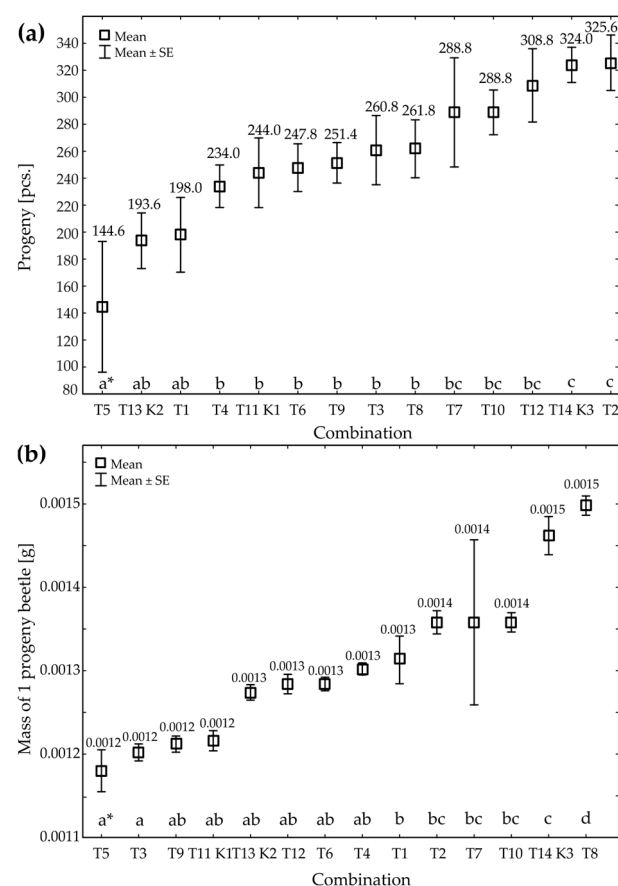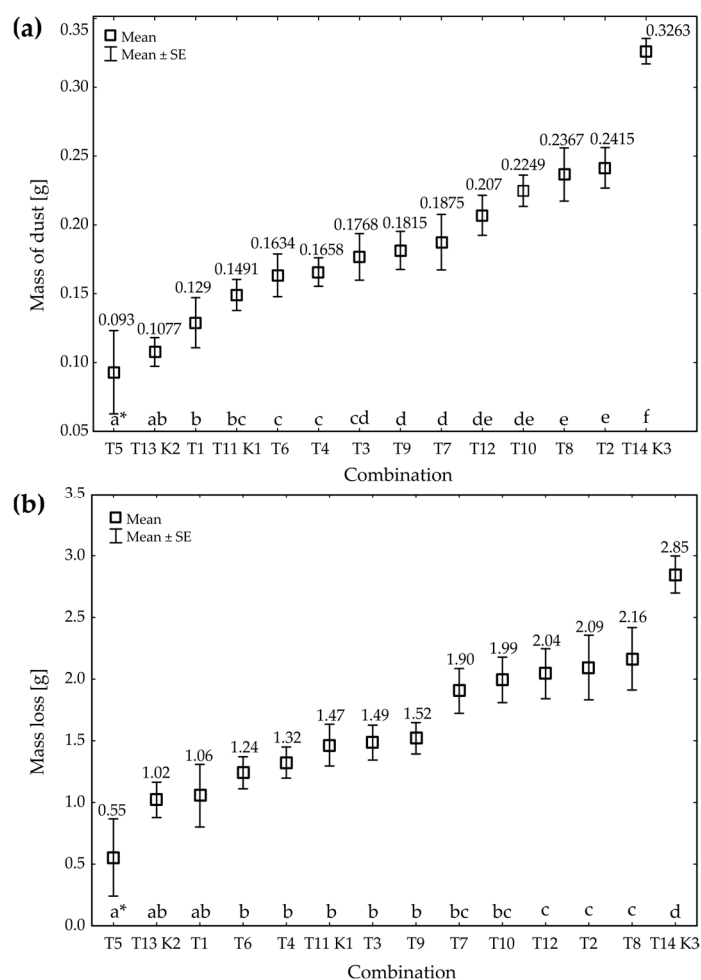

Figure 1. Mean number of progeny beetles of *S. oryzae* (**a**) and mass of 1 progeny beetle (**b**) observed on Tritordeum lines and control combinations. * Means in columns followed by the same letter do not differ—Tukey's HSD test.

Similarly high progeny population numbers, although constituting a different homogeneous group (Tukey's HSD test), were observed in combinations with grain lines T12, T10 and T7 (avg. 308.8, 288.8 and 288.8). In combinations T4, T6, T9, T3 and T8, and the T11_K1 control, the pest achieved progeny population numbers that averaged between 234.0 and 261.8 individuals. The most resistant to *S. oryzae* feeding was the T5 grain line, on

which only 144.6 indiv., on average, completed development. The varieties with the lowest progeny beetle population numbers also included combinations with grain lines T1 and the T13_K2 control (avg. 198.0 and 193.6 indiv.) (Figure 1a).

The highest average mass of a single *S. oryzae* progeny beetle was observed on combinations T8 and T14_K3 (mean 0.0015 g) (Figure 1b). A lower average mass of a single progeny beetle was observed on combinations with grain lines T2, T7 and T10 (mean 0.0014 g). On combinations T13_K2, T12, T6, T4 and T1, the average mass of an individual oscillated around 0.0013 g. The lowest mass of *S. oryzae* progeny beetle (0.0012 g) was observed on combinations T5, T3, T9 and T11_K1 (Figure 1b).

The following two parameters, studied to describe the intensity of *S. oryzae* development, were very strongly and positively correlated with the size of the progeny generation. In the case of dust mass produced by feeding beetles, the highest amount was recorded in the control combination T14_K3 (avg. 0.3263 g) and in the T2 line (avg. 0.2415 g) and T8 (avg. 0.2367 g). The lowest dust mass was recorded in combination with grain of the Tritordeum line T5 (avg. 0.093 g) and in the T13_K2 control (avg. 0.1077 g) (Figure 2a).

Figure 2. Mean mass of dust (**a**) and mass of grain loss (**b**) observed on Tritordeum lines and control combinations. * Means in columns followed by the same letter do not differ—Tukey's HSD test.

Analyzing the last parameter associated with the development of *S. oryzae*—grain mass loss—it can be concluded that the highest and lowest values of this parameter were almost identical to those for dust mass produced by feeding beetles. The highest grain mass losses were observed in the control combination T14_K3 (avg. 2.85 g) and combinations T8, T2 and T12 (avg. 2.16 g, 2.09 g, and 2.04 g). The lowest kernel mass losses were recorded in combinations T5 (avg. 0.55 g), T13_K2 (avg. 1.02 g), and T1 (avg. 1.06 g) (Figure 2b).

3.2. Physicochemical Properties of the Hybrid Varieties of the Tested Grain

Kernels of the tested Tritordeum breeding lines and reference grain were examined for selected chemical properties and hardness. The results of a one-factorial ANOVA revealed that the tested cultivars differed in these respects (Table 2).

Table 2. Result of one-factorial ANOVA analysis of variance chemical parameters for tested Tritordeum lines and control combinations.

	df *	ANOVA F Value	p **
Crude ash	13	468.74	0.00
Fiber	13	103.79	0.00
Total protein	13	2154.65	0.00
Crude fat	13	395.33	0.00
Hardness	13	574.77	0.00

* Degrees of freedom; ** Test probability value *p*.

Crude ash content (F = 468.74, p = 0.00), fiber (F = 103.79, p = 0.00), total protein (F = 2154.65, p = 0.00), crude fat (F = 395.33, p = 0.00), and hardness (F = 574.77, p = 0.00) were significantly different between the tested combinations with grain from the Tritordeum lines and the control (Table 2).

Ash content in the tested grain was highest in grain from lines T5 (avg. 3.04%) and T2 (avg. 2.79%), as well as in lines T3 (avg. 2.71%), T6 (avg. 2.68%), and T12 (avg. 2.63%) (Table 3). Low values of this component were recorded in the control grain T14_K3 (avg. 1.40%) and lines T8 (avg. 1.89%) and T7 (avg. 2.06%). In the case of fiber content, the highest content of this component was found in the control grains T14_K3 (avg. 3.21%) and line T12 (avg. 2.93%). Low levels of this component were characterized by the control varieties T11_K1 (avg. 1.67%) and T13_K2 (avg. 1.94%). Protein was the component whose lowest amounts were found in the grain of the two control varieties T14_K3 and T11_K1 (avg. 14.37 and 14.81%) and the Tritordeum T8 line (avg. 14.89%). Its highest content was recorded in control grain T13_K2 (18.12%) and in lines T1 (18.29%), T2 (18.54%), and T5 (18.73%). The last tested chemical component of grain, crude fat, was the compound whose lowest content was found in control grain T14_K3 and T13_K2 (avg. 0.59%, 1.99%) and in the Tritordeum T7 line (1.97%). However, its high content was found in control grain T11_K1 (avg. 2.67%) and in lines T3, T1 and T2 (avg. 2.62%, 2.59%, and 2.52%). The hardness of kernels of the tested control and hybrid cultivars also varied. The highest hardness was observed in kernels of the two control cultivars, T13_K2 and T14_K3 (avg. 84.5 and 82.4), while the lowest hardness was observed in kernels of the Tritordeum lines: T5, T2 and T1 (avg. 40, 40, and 41) (Table 3).

A valuable tool for identifying differences in the physicochemical composition of the tested Tritordeum grain, grouping lines based on the similarity of these traits, and graphically illustrating the obtained results is the use of non-parametric multidimensional scaling (NMDS) techniques. NMDS analysis grouped the tested grain varieties into three distinct clusters, clearly separated from each other in the ordination diagram (Figure 3). The first group included the three Tritordeum lines T1, T2 and T5, which the NMDS algorithm placed in the left part of the diagram. The middle and lower parts of the diagram grouped most of the tested lines (T3, T4, T6, T7, T8, T9, T10, and T12) and the control combination T11_K1. The upper right part of the diagram contains two control wheat combinations: T13_K2 and T14_K3 (Figure 3).

Table 3. Average values of the chemical composition of grains Tritordeum lines and control combinations. Mean values (±SD) of the tested chemical compounds [% of dry matter] and the hardness of grains.

Combinations	Crude Ash	Fiber	Total Protein	Crude Fat	Hardness Score
T1 HT 440	2.52 ± 0.5 e *	2.11 ± 0.06 bc	18.29 ± 0.10 i	2.59 ± 0.05 cd	41 ± 1.0 a
T2 HT 129	2.79 ± 0.03 f	2.14 ± 0.05 c	18.54 ± 0.06 j	2.52 ± 0.01 cd	40 ± 1.0 a
T3 HTC 2060	2.71 ± 0.02 ef	2.52 ± 0.04 d	17.64 ± 0.03 h	2.62 ± 0.05 d	55 ± 1.0 b
T4 HT 157	2.11 ± 0.02 dc	2.15 ± 0.07 c	15.06 ± 0.06 c	2.15 ± 0.05 b	67 ± 1.0 d
T5 HT 352	3.04 ± 0.01 g	2.15 ± 0.01 c	18.73 ± 0.07 j	2.42 ± 0.07 c	40 ± 1.0 a
T6 JB3	2.68 ± 0.03 ef	2.25 ± 0.07 c	17.09 ± 0.01 g	2.33 ± 0.01 bc	58 ± 1.0 c
T7 HT 438	2.06 ± 0.08 c	2.28 ± 0.06 cd	16.25 ± 0.06 f	1.97 ± 0.01 b	53 ± 1.0 b
T8 HTC 1324	1.89 ± 0.02 b	2.22 ± 0.05 c	14.89 ± 0.04 b	2.05 ± 0.01 ab	71 ± 1.0 e
T9 HT 444	2.20 ± 0.04 cd	2.46 ± 0.05 d	15.46 ± 0.01 d	2.20 ± 0.03 b	69 ± 1.0 de
T10 HTC 2083	2.13 ± 0.06 cd	2.27 ± 0.03 cd	16.00 ± 0.08 e	2.18 ± 0.02 b	67 ± 1.0 d
T11 Gawrosz K1	2.23 ± 0.01 cd	1.67 ± 0.08 a	14.81 ± 0.07 b	2.67 ± 0.01 d	60 ± 1.0 c
T12 '60' HTC 2083'	2.63 ± 0.01 ef	2.93 ± 0.02 e	16.19 ± 0.05 f	1.90 ± 0.05 b	60 ± 1.0 c
T13 Duragold K2	2.26 ± 0.01 d	1.94 ± 0.01 b	18.12 ± 0.02 i	1.99 ± 0.5 ab	84.5 ± 0.5 f
T14 Control K3	1.40 ± 0.01 a	3.21 ± 0.18 f	14.37 ± 0.04 a	0.59 ± 0.06 a	82.4 ± 1.0 f

* Means in columns followed by the same letter do not differ—Tukey's HSD test.
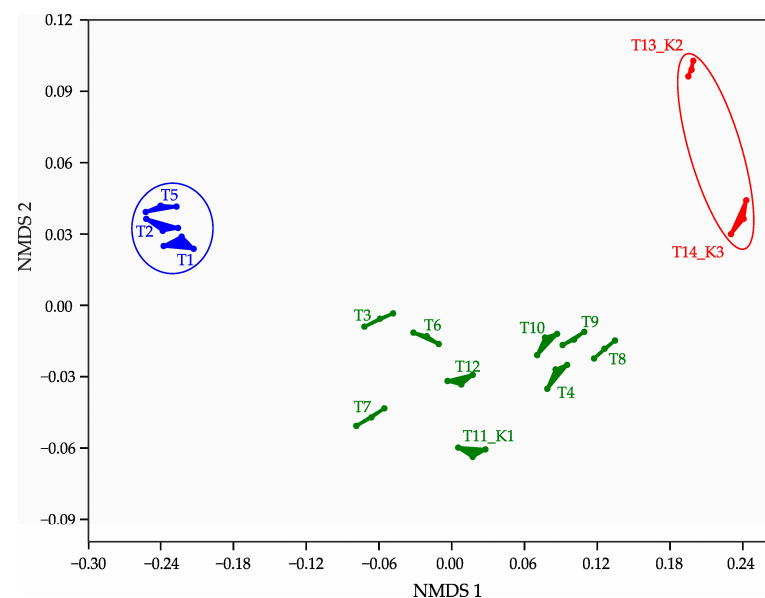

Figure 3. NMDS diagram describing similarities of tested Tritordeum lines with respect to chosen chemical and physical characteristics of grain.

Differences in the susceptibility of the tested Tritordeum lines and control combinations may result, among other things, from the physicochemical properties of the kernels. Cluster analysis was used to identify these factors. This analysis enabled a comparison of the tested cereal grains in terms of their chemical composition similarity (Figure 4). In terms of total protein content, cluster analysis divided the grain of the tested cereal varieties into two main groups. The first group consisted of the two control combinations, T11_K1 and T14_K3, and the Tritordeum lines T4, T8, and T9. The second group included the remaining combinations, including the T13_K2 control, whose grain was very similar to the grain of lines T1 and T5 in this parameter. The grain of these three lines proved to be the most resistant to *S. oryzae* feeding. The dendrogram below divides the grain of the tested varieties into two groups based on ash content. One includes the Tritordeum lines T1, T2, T3, T5, T6 and T12, while the other includes the remaining lines and grain from the control varieties. Among them, the grain from the control variety, T14_K3, stands out the most.

The control combinations, T11_K1 and T13_K2, and the T9 line, were very similar in terms of ash content (Figure 4).
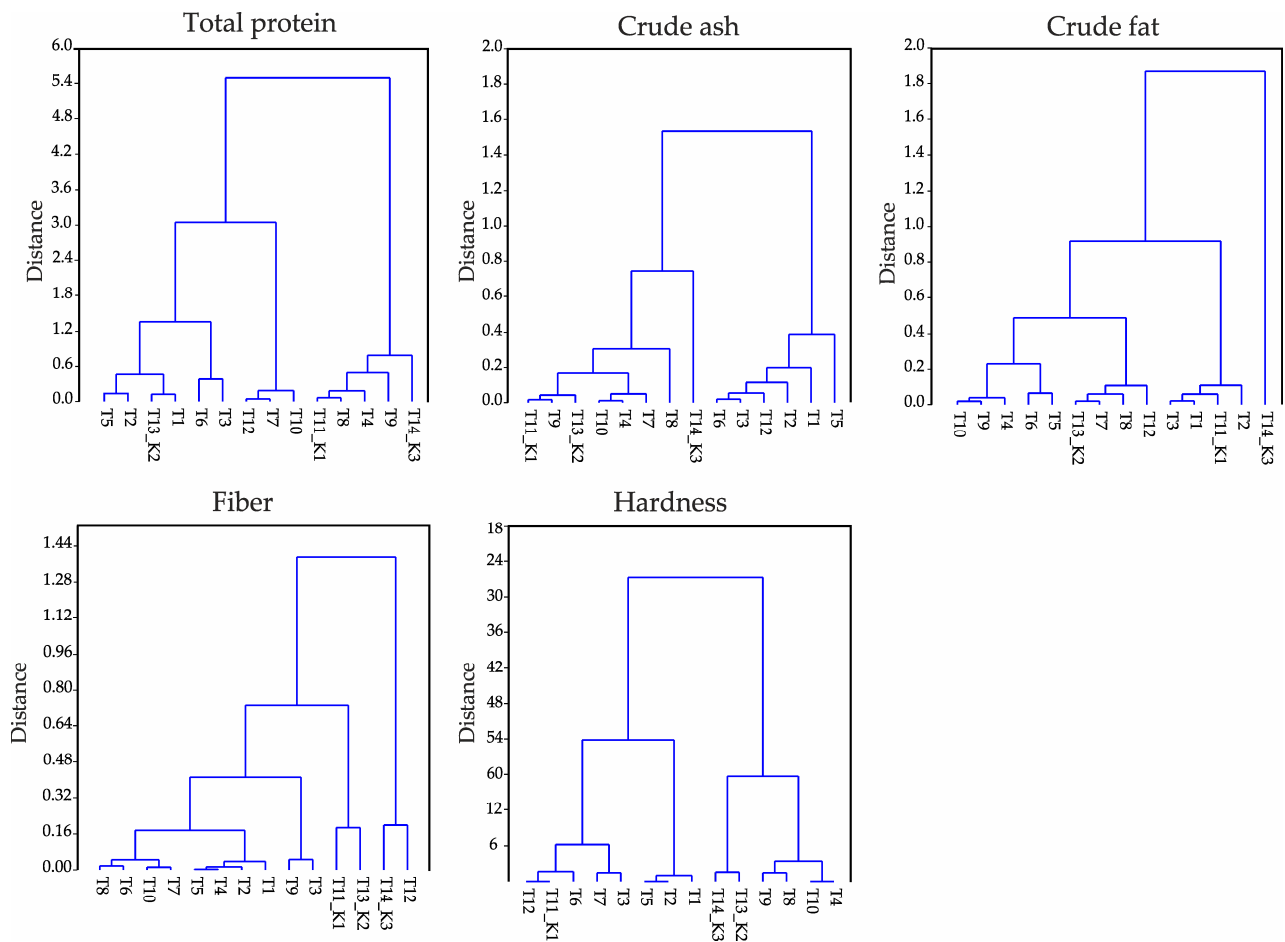
Figure 4. Dendrograms show the general similarity of the examined Tritordeum lines depending on the tested chemical (total protein, crude ash, crude fat, fiber) and physical (hardness) compounds. A dissimilarity matrix was prepared based on the Word technique with the Bray–Curtis similarity measure.

In terms of crude fat content, the dendrogram clearly distinguishes the T14_K3 control, constituting it an independent group. The remaining lines were divided into three principal subgroups. The first includes grain from the T11_K1 control, and T1, T2 and T3. The second subgroup includes varieties T7, T8, T12 and the T13_K2 control. The final subgroup comprises the remaining Tritordeum lines T4, T5, T6, T9 and T10. In terms of crude fiber content, the dendrogram distinguishes the grain from the T14_K3 control and the grain from the T12 line as very similar, indicating that these lines have low natural resistance to *S. oryzae* feeding. However, there were no further clear correlations between the content of this grain component and increased resistance to feeding by the tested pest species. The only physical factor studied describing Tritordeum grain hardness divided it into two main groups, shown in the dendrogram (Figure 4). In the first group, two subgroups were distinguished. One included grain from the control cultivars T13_K2 and T14_K3, and the second subgroup included lines T4, T8, T9, and T10. In the second leading group, the dendrogram also distinguished two subgroups. The first group included grain from lines T1, T2, and T5. The second group included the remaining Tritordeum lines and grain from the control cultivar K1 (Figure 4).

To determine the relationship between *S. oryzae* developmental parameters and the examined physicochemical characteristics of grain from the Tritordeum line, a correspondence analysis (RDA) was performed. The resulting ordination graph presents a graphical interpretation of these correlations (Figure 5). Crude fiber content was a factor significantly correlated (F = 12.22, *p* = 0.004) with the first ordination axis, and with the size of the *S. oryzae* progeny generation. The non-resistant Tritordeum lines T10, T7, and T12, and the control combination T14_K3, were located close to these vectors. Kernel hardness also correlated with low resistance. Tritordeum lines characterized by high resistance to *S. oryzae* feeding (T1 and T5) were located in the lower left quadrant of the ordination plot, close to the vector describing the total protein content in grain (F = 6.887, *p* = 0.02) and the vectors describing the crude fat content (F = 1.286, *p* = 0.256) and ash content (F = 0.777, *p* = 0.4). However, it should be noted that the T2 cultivar, which showed low resistance to *S. oryzae* feeding, was also located close to the vector describing the total protein content.
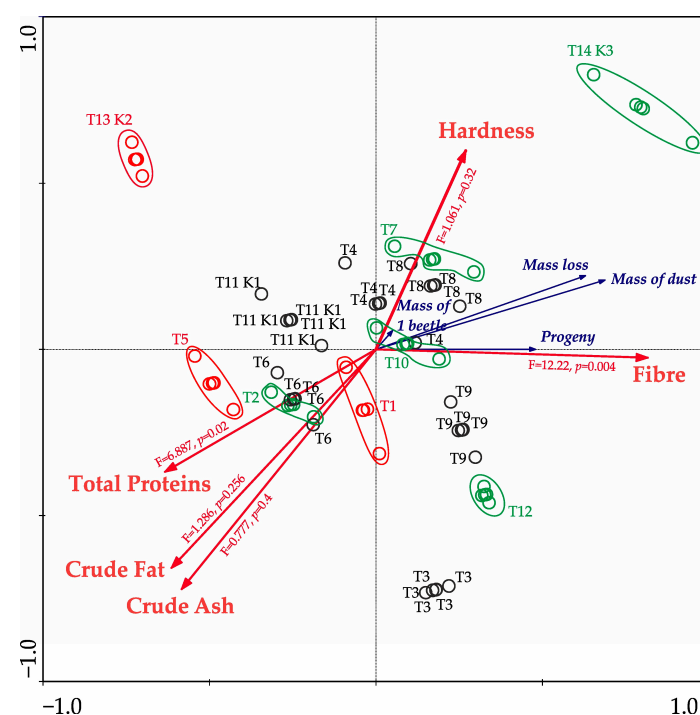
Figure 5. Redundancy analysis (RDA) diagram presenting correlations between analyzed parameters for the development of *S. oryzae* and the chemical and physical characteristics of Tritordeum lines. Statistical significance (Monte Carlo test) is indicated on red vectors representing the studied variables.

4. Discussion

Thanks to genetic selection and modification, hybrid cereals are characterized by high yield and quality, increased resistance to unfavorable environmental conditions, and improved pest resistance [39–41]. The increased resistance of Tritordeum to insect feeding has been confirmed primarily for crop pests [40,42]. However, cereal plantations and the phytophagous insects that feed on them are not the only threat to various cereal species. A key determinant of global food and nutritional security is the safe and efficient storage of food at all stages of the supply chain [43–47]. Therefore, for Tritordeum to become a pillar of future agriculture, knowledge of its kernels' defense strategies against storage pests, which cause the most significant losses during storage, is essential [48,49].

The degree of cereal infestation by storage pests depends primarily on the type of raw material infested. Previous studies have shown that *S. oryzae* develops in large numbers on wheat [35,50] and barley [51], among other hosts. Entomological observations conducted

on Tritordeum showed that the tested breeding lines also provide favorable conditions for its development, although with varying intensities (Table 1, Figure 1).

The most intensive development of the pest was observed on the HT 129 line (T2—avg. 326.6 indiv.) and common wheat (T14_K3—avg. 324.0 indiv.) (Figure 1). Among these combinations, Tritordeum, despite having a progeny population size comparable to wheat, was distinguished by a 26.7% lower grain mass loss, a 26.0% lower dust mass, and a 6.7% lower mass of a single progeny (Figures 1 and 2). Additionally, slower development of *S. oryzae* was observed in the T5 line, where the number of progeny was more than twice as low (avg. 144.6 indiv.) and the weight of lost grain was more than five times lower (0.55 g) than in the combination with common wheat (T14_K3—avg. 324.0 indiv., 2.85 g) (Figure 2). Therefore, storing Tritordeum grain may result in lower direct losses (reduced mass of stored kernels) and indirect losses (lower amounts of dust produced, which promotes the growth of molds, a source of mycotoxins) [49].

The varied susceptibility of grains to feeding by harmful organisms in storage is due to the physical, biochemical and physiological heterogeneity of cereals, which shapes their resistance mechanisms [52–54]. Seed coat hardness has been considered one of the primary structural factors influencing the development dynamics of stored-product pests [55–57]. Our previous studies on *S. granarius* showed, however, that an increase in the hardness of the outer layer of kernels is not always correlated with an increase in grain resistance to these organisms [54]. The highest level of seed coat hardness of the kernels used in the experiment with *S. oryzae* was observed in the preferential varieties (T13_K2 and T14_K3). Common wheat and durum wheat kernels were more than twice as hard (T14_K3—82.4, T13_K2—84.5) as Tritordeum kernels with the thinnest seed coat layer (T1—41, T2—40, T5—40) (Table 3). Graphical RDA correspondence analysis (Figure 5) revealed a correlation between combinations with low resistance to *S. oryzae* feeding (particularly with common wheat—T14_K3) and kernel hardness. However, the intensity of pest development on control varieties with similar hardness value was classified as one of the highest (T14_K3—mean 324) and lowest (T13_K2—mean 193.6) (Figure 1, Table 3). In contrast, Tritordeum breeding lines with the same hardness level (40 ± 1.0) were the habitat in which *S. oryzae* developed the most intensively (T2—326.6) and the least intensively (T5—144.6) throughout the experiment (Tables 2 and 3). While statistical analyses revealed statistically significant differences in the pericarp hardness of the analyzed kernels, this factor was not clearly related to the developmental intensity of the tested pest. The lack of a clear correlation between the developmental intensity of the progeny of the rice weevil and the hardness of the rice kernels was also demonstrated by, among others, Sahoo & Sahoo [58].

The chemical composition of the kernels also contributes to their resistance to storage pests [57,59]. This factor significantly determines the developmental intensity of the analyzed insects, as observed in our previous studies on *S. granarius*, *T. confusum*, and *R. dominica* [52,60,61]. The statistical analysis conducted in the rice weevil experiment revealed that the chemical profile of the tested cereals varied significantly (Table 2). NMDS analysis also confirmed significant differences between the physical and chemical structures of common wheat and durum wheat, as well as those of Tritordeum and barley kernels (Figure 3).

Crude fat was an important factor influencing the development of stored-food pests in our previous observations on *S. granarius* [53] and *T. confusum* [60]. Similar findings were observed by Mebarkia et al. [57] and Nawrot et al. [59]. The experiment on Tritordeum also demonstrated the significant effect of these organic fractions on rice weevil feeding. Common wheat, on which the rice weevil developed most intensively, contained the lowest

values of this parameter (T14_K3—0.59%) and, in terms of similarity analysis, formed a separate, single group (Table 3, Figure 4).

Detailed RDA correspondence analysis showed that high filial abundance correlated with fiber content and corresponded to the T10, T7, T12 and T14_K3 varieties, i.e., varieties susceptible to *S. oryzae* feeding (Table 3, Figure 5). Moreover, combinations T14_K3 and T12 were characterized by the highest values of this component, which cluster analysis further classified as a separate subgroup (Table 3, Figure 4). Highly resistant cultivars were correlated on the RDA graph with total protein, crude fat and ash (Figure 5). The Tritordeum breeding line, on which the rice weevil developed the least (T5), had the highest ash content among the tested combinations and the highest total protein content (Table 3). However, common wheat (T14_3) was the combination with the lowest protein and ash values throughout the experiment (Table 3). RDA also showed a correlation of protein with the cultivars most resistant to storage pest feeding (T1, T5). Correlation analysis of the chemical composition of barley, maize, millet, rice, sorghum, and wheat cereals in the study conducted by Abedi et al. [62] also showed a correlation between the total protein content and the fecundity of *S. oryzae*. The study by Mebarka et al. [57] revealed that the susceptibility of *T. aestivum* to *S. granarius* feeding increases with increasing protein content [57].

Analysis of the results from our study, together data from other authors, refs. [35,63] indicates that in the case of *S. oryzae*, establishing a correlation between grain chemical composition and the intensity of this pest's development is ambiguous. Arya et al. [64] emphasized in their study on the development of *S. oryzae* that the high physiological flexibility of this species necessitates multifactorial analyses. The lines that proved most resistant to *S. oryzae* (T1 and T5) and the least resistant line (T2) had very similar grain chemical composition, particularly regarding protein, crude ash and fiber content. Analyzing only the most resistant line (T5) and the most susceptible line (T2), it is evident that they differ significantly in crude fat content. The amount of this chemical component may therefore be crucial in determining the intensity of *S. oryzae* feeding and development. However, analysis of the chemical composition of the T1 line (high resistance to *S. oryzae* feeding) indicates that the correlation between increased crude ash content and high pest resistance in the T5 line is not linear. This resistance in the studied Tritordeum lines is probably also dependent on the content of other chemical components of the kernels, such as lower crude ash and protein content. This is indicated, for example, by studies on the development of the grain weevil and the grain hooded moth, which showed that the intensity of feeding by these pests resulted primarily from the presence of specific protein fractions (albumin, globulin and glutenin) in the kernels [65].

The influence of the varying chemical composition of grains of different cereal species and varieties on the development of storage pests results, of course, from the nutritional requirements of a given species, but also from the presence of specific gut microflora in their digestive tracts [34,35,63]. Observations on the composition of the gut microbiomes of *S. oryzae* and *Rhyzopertha dominica* F. revealed the influence of the internal microbiomes of the studied insects on the development and functioning of these pests, as well as on the shaping of their food preferences [33–35]. Therefore, further detailed analysis of the factors influencing the intensity of Tritordeum colonization by *S. oryzae* is necessary.

5. Conclusions

1. Tritordeum grain is a habitat in which *S. oryzae* can develop, although with varying intensity.
2. The Tritordeum lines studied differed in their physicochemical grain characteristics.

3. The physical properties of the kernels (seed coat hardness) did not influence the resistance of the studied cereals to *S. oryzae* feeding. Resistance is therefore determined by chemical factors or a combination of chemical factors.

4. Resistance of Tritordeum to rice weevil feeding appears to result primarily from the grain's chemical composition, particularly protein or associated fractions, rather than hardness.

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