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# Searching for Novel Oat Crown Rust Resistance in Diploid Oat *Avena strigosa* Schreb. Reveals the Complexity and Heterogeneity of the Analyzed Genebank Accessions

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**Abstract:** Crown rust, one of the most destructive diseases of oat, regularly occurs worldwide and leads to significant yield losses. The constant evolution of the *Puccinia coronata* f. sp. *avenae* pathogen causes a rapid decline in the effectiveness of currently used crown rust resistance genes, so new ones are urgently needed. In this study, 39 accessions of *Avena strigosa* Schreb. from ten countries gathered from the Polish National Genebank were evaluated at the seedling stage for crown rust reaction using a detached leaf assay and five isolates of *P. coronata* with diverse virulence profiles. Ten plants of each accession were tested, and 28 diverse infection profiles (IPs) were defined. One hundred and sixty-eight out of 390 plants revealed an IP of unidentified resistance. Thirty-eight (97%) of the accessions studied showed a heterogeneous infection pattern, none of the accessions displayed homogeneous susceptibility, and one (51887) was homogeneously resistant to all races used. The obtained results confirmed the complexity and heterogeneity of the accessions gathered in the genebanks. *A. strigosa* preserved as complex populations could be a valuable source of resistance to crown rust and potentially other pathogens. The variability of the analyzed populations was ascertained by a detailed diversity analysis of the transformed resistance/susceptibility data. The averaged resistance rating for the genebank specimens available in the databases may be an obstacle in revealing the beneficial alleles of genes hidden among the plants representing accessions preserved as complex populations. Potential donors of effective resistance may be discovered even in accessions with general susceptibility, which is a promising alternative at a time when making new collections of wild and weedy accessions is under threat from agricultural practice and climate change.

**Keywords:** *Avena strigosa*; *Puccinia coronata* f. sp. *avenae*; crown rust; accessions heterogeneity; resistance diversity



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## 1. Introduction

Oat is a cereal cultivated worldwide with a high range of applications from animal feed to pharmaceuticals and the food industry [1]. The global production of oat is severely affected by rust diseases [2]. Crown rust caused by *Puccinia coronata* f. sp. *avenae* regularly occurs worldwide and poses a great threat to oat yield and quality. The use of fungicides is common to prevent crown rust development; however, it is becoming more and more unaffordable for consumers. Moreover, the number of registered fungicide active ingredients for the control of *P. coronata* is often limited. Therefore, genetic resistance is one of the most important modern oat breeding goals. The complex life cycle of *P. coronata* leads to the emergence of new rust races and causes high virulence dynamics and phenotypic diversity [3,4]. The efficient adaptability of the fungus is resulting in a rapid breakdown of crown rust resistance genes in existing oat varieties, hence the need for new sources

of *P. coronata* resistance genes for the introgression and genetic enhancement of existing oat varieties.

While most *Pc* genes have been identified in the wild hexaploid oat *A. sterilis* [5], some have come from lower ploidy species, although their introduction into hexaploid *A. sativa* is much more difficult and demanding due to the lack of chromosome homology and the special technical requirements [6,7]. *Pc91* is the only crown rust resistance gene successfully introduced into cultivated oat from a wild tetraploid. This gene was transferred from the accession *A. magna* CI 8330 [8] and remains effective in Europe, although virulence to *Pc91* has already been recorded in Canada [9–11], Australia [12], and the USA [13]. Slightly better results were obtained in the case of the diploid black oat *A. strigosa* Schreb. often called black, sand, or bristle oat. *Pc23* and *Pc94* have been incorporated into a stable *A. sativa* background and used in oat resistance breeding [6,14]. *Pc94* originating from *A. strigosa* accession RL1697 is still in use in the modern varieties 'Leggett' and 'Stride' [15,16]. Recently in the studies of Rines et al. [17], a new and highly effective source of adult plant resistance to oat crown rust was identified in the diploid oat *A. strigosa* accession PI 258731 and introgressed into the hexaploid cultivated oat.

According to Vavilov [18], the origin of *A. strigosa* is northwestern and western Europe with the diversity center in northern Portugal and northwestern Spain, where a whole complex of endemic forms of this species was found [19]. *A. strigosa* was widely cultivated for grain fodder in many European countries and is currently grown to a limited extent on the marginal soils of Scotland and Lithuania [20–22]. The grain of this species is distinguished from common oat by its high nutritional value, manifested in a higher content of polysaccharides (38–72% more), fat (14–27% more), and protein (27–52% more) as well as health-promoting ingredients such as phenolic alkaloids, phenolic acids, tocopherols, tocotrienols, and β-glucan [23]. *A. strigosa* has also been reported as a carrier of genes for resistance to *Ustilago avenae* (Pers.) Rostr. (smut), *Puccinia graminis Pers*. f. sp. *avenae* Eriks. & E. Henn. (stem rust), and *Heterodera avenae* Woll. (cereal cyst nematode) [24–26].

Many researchers have proven that *A. strigosa* is a rich source of useful genes and has a high potential for oat variety improvement. Despite the crossing barriers, the ever-evolving genome editing technology offers an opportunity for the easier insertion of the desired genes utilizing targeted genome engineering techniques [27]; so, it is worth characterizing possible donors of valuable breeding traits. Previous research focused on the screening of *A. strigosa* accessions gave the first insight into the resistance potential of this species [28–30]. The current work complements the existing data and reveals the spectrum of putative new resistance genes or alleles present in the diploid sand oat gathered by the Polish Genebank (National Centre For Plant Genetic Resources, The Plant Breeding and Acclimatization Institute, NRI, Radzików, Poland). Considering the presumable heterogeneity of the analyzed populations, testing many plants of one accession was conducted with the use of various *P. coronata* races. Such an approach enabled us to reveal the complexity of the studied wild species populations gathered in the genebanks and to identify the most resistant individuals.

## 2. Materials and Methods

### *2.1. Plant Material and Virulence Assessment*

The study was carried out on 39 accessions of *A. strigosa* (Table 1) obtained from the National Centre for Plant Genetic Resources in Radzików, Poland. The accessions were landraces from Poland (22 accessions), the United Kingdom (5 accessions), Spain (2 accessions), Chile (2 accessions), Russia (2 accessions), France (1 accession), Uruguay (1 accession), the Netherlands (1 accession), Bulgaria (1 accession), and Brazil (1 accession). The origin of one accession was unknown.

The reactions of the seedlings to crown rust were recorded using five *P. coronata* race isolates with the virulence profile characterized based on the susceptibility/resistance reaction of 34 differential oat lines with single *Pc* genes [31,32], described by Paczos-Grzęda et al. [31,33]. CR230, CR241, and CR257 were obtained from the Morden Research and

Development Centre, AAFC, Canada, whereas 94(63) and 51(22) were selected from a collection of races' isolates originating from populations collected in Poland [34].

Ten seedlings from each *A. strigosa* accession were tested with all five *P. coronata* races using a detached leaf assay [35], according to the host–pathogen test methodology of Hsam et al. [36] with modifications [34]. The leaves were placed into Petri dishes or 12-well culture plates filled with agar medium (0.6%) with benzimidazole (3.4 mM) using susceptible cv. Kasztan as the infection control in each well [37]. The inoculations were performed in a settling tower, and the plates were incubated for 10 days and assessed using an infection type (IT) qualitative scale [38,39]. The infection results were transformed to HS, S, MS, MR, and R as described by Sowa and Paczos-Grzęda [34,37,40].

### *2.2. Data Mining and Analysis*

The reactions to the isolate infections were grouped into two classes: the phenotypes described as HS, S, and MS were considered susceptible, and the remainder were considered resistant. The infection profiles (IP) of differential lines were compared with the infection profiles assigned to the analyzed *A. strigosa* seedlings to select the genotypes with crown rust resistance conditioned by genes not described so far. The infection scores were transformed into a binary matrix. Each plant's resistance response level to a particular *P. coronata* race was treated as a single variable. The resistance or susceptibility was considered as 1 or 0, respectively. The phenotypic diversity of the accessions was described by the Normalized Shannon diversity index (Sh) [41] and Nei's diversity (Hs) [42,43], calculated by the Virulence Analysis Tool (VAT) software [44,45]. For hierarchical clustering, dissimilarity matrices were used to construct a dendrogram using Ward's method. Principal component analysis (PCA) was performed to visualize the relationships between the accessions. The groups and subgroups were determined with 1000 bootstrap analyses performed in PAST 4.11 software [46]. The analysis of molecular variance (AMOVA), performed by GenAlex 6.502 [47], was used to partition the diversity [48]. The variance components were tested statistically using 9999 permutations. The binary data were also evaluated for population structure using a model-based Bayesian clustering in STRUCTURE v2.3.4 [49]. The models were computed for K = 1 ÷ 10 (K—number of subpopulations). Each model was tested ten times with 10,000 burn-in cycles and 100,000 iterations. The results were tested to find the best model with the highest ΔK value using the web-based software StructureSelector [50] integrating the Clumpak program [51].

**Table 1.** Crown rust resistance phenotypes of *Avena strigosa* L. genotypes. Accessions, within which fully resistant plants were present, are highlighted in grey.

| No. | Plant ID | Origin | *Puccinia coronata* Race [1] | | | | | Infection Profile (Number of Resistant Seedlings) [2] | Diversity [3] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 51(22) | 94(63) | CR230 | CR241 | CR257 | | Sh | Hs |
| 1 | 51022 | Brazil | HS, S | MS, R | S, MS, R | S, R | HS, R | 0 (2), 1.2 (6), 2.5 (1), 4.1 (1) | 0.473 | 0.200 |
| 2 | 51199 | Bulgaria | HS, S | HS, S, MS | S, MS, R | S, HS | HS, S, MS | 0 (9), 1.3 (1) | 0.141 | 0.036 |
| 3 | 51326 | unknown | S | S, MS | MS, MR, R | HS | HS | 0 (7), 1.3 (3) | 0.265 | 0.084 |
| 4 | 51518 | Poland | HS, S, MS, R | HS, MS | HS | HS, S, MS | HS | 0 (7), 1.1 (3) | 0.265 | 0.084 |
| 5 | 51520 | Poland | HS, S, R | HS, MS, R | HS | HS, S, MS, MR | HS | 0 (4), 1.1 (2), 1.2 (2), 1.4 (1), 2.1 (1) | 0.639 | 0.204 |
| 6 | 51523 | Poland | HS, S, MS, R | HS, MS, R | HS | HS, MS, R | HS, S, MR, R | 1 (3), 1.2 (1), 1.5 (2), 2.4 (2), 2.8 (1), 3.7 (1) | 0.736 | 0.280 |
| 7 | 51524 | Poland | HS, S, MS, R | HS, MS, R | HS | HS, MS, MR, R | HS, S, MS | 0 (3), 1.1 (3), 1.2 (1), 1.4 (1), 2.3 (1), 2.4 (1) | 0.714 | 0.236 |
| 8 | 51575 | Holland | S | MR, R | MR | HS, MS, R | HS, R | 2.5 (2), 3.2 (5), 4.1 (3) | 0.447 | 0.148 |
| 9 | 51578 | Uruguay | HR | HR | R | R | HS, S, MS, MR | 4.4 (9), 5 (1) | 0.141 | 0.036 |
| 10 | 51579 | Russia | HS, S, R | HS, S, MS | HS | HS, S, MS, R | HS | 0 (4), 1.1 (5), 1.4 (1) | 0.410 | 0.136 |
| 11 | 51581 | Russia | HS, S, MS, R | HS, S, MS, R | HS | HS, S, MS | HS | 0 (9), 2.1 (1) | 0.141 | 0.072 |
| 12 | 51582 | Spain | HS | R | MS, MR | S | MS | 2 (1), 2.5 (9) | 0.141 | 0.036 |
| 13 | 51583 | Spain | R | MR, R | HS, R | R | HS, R | 3.8 (1), 4.2 (1), 5 (8) | 0.278 | 0.100 |
| 14 | 51584 | France | HS, R | HS | HS | S, MS, R | HS, S | 0 (8), 1.1 (1), 2.3 (1) | 0.278 | 0.100 |
| 15 | 51585 | Poland | HS, S, MS, R | HS, S | MR, R | S, MS, R | MS, R | 3 (1), 2.7 (6), 3.1 (2), 3.6 (1) | 0.473 | 0.136 |
| 16 | 51586 | Poland | HS, S, MS, MR, R | HS, S, MR, R | HS, S, MR, R | HS, S, MS, R | HS, R | 0 (3), 1.1 (2), 1.2 (2), 2.1 (1), 2.3 (1), 5 (1) | 0.736 | 0.332 |
| 17 | 51596 | Chile | HR | R | R | R | HS, HR | 4.4 (9), 5 (1) | 0.141 | 0.036 |
| 18 | 51597 | Chile | HS, S, MS, MR, R | R | R | R | S | 3.5 (3), 4.4 (7) | 0.265 | 0.084 |
| 19 | 51598 | Poland | S, MS | MS, MR, R | HS, S | S | HS, S, MS, R | 0 (1), 1.2 (8), 1.5 (1) | 0.278 | 0.100 |
| 20 | 51613 | Poland | HS, R | S, MS, R | HS | HS, S | HS, S | 0 (9), 2.1 (1) | 0.141 | 0.072 |
| 21 | 51630 | Poland | S, MR | MS, MR | HS | HS | HS | 0 (8), 1.2 (1), 2.1 (1) | 0.278 | 0.100 |
| 22 | 51732 | Poland | S, MS, MR | MR, R | MR, R | MS, MR, R | S, MS, MR, R | 2.5 (5), 3.2 (1), 3.5 (2), 4.1 (1), 4.4 (1) | 0.590 | 0.196 |
| 23 | 51750 | Poland | S, MS, MR, R | MR, R | MS, MR, R | S, MS, R | HS, MS, MR, R | 2.9 (1), 2.8 (2), 3.2 (1), 4.1 (2), 4.4 (3), 5 (1) | 0.736 | 0.360 |

**Table 1.** *Cont.*

| No. | Plant ID | Origin | *Puccinia coronata* Race [1] | | | | | Infection Profile (Number of Resistant Seedlings) [2] | Diversity [3] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 51(22) | 94(63) | CR230 | CR241 | CR257 | | Sh | Hs |
| 24 | 51751 | Poland | HS, S, MS, R | HS | HS | HS, MS | HS, R | 0 (5), 1.1 (4), 1.5 (1) | 0.410 | 0.132 |
| 25 | 51753 | Poland | S, MS, MR | MR, R | MS, R | MS | MS, MR, R | 2 (1), 2.5 (1), 2.9 (5), 3.2 (1), 4.3 (2) | 0.590 | 0.224 |
| 26 | 51754 | Poland | S, MS, MR | S, MS, MR | MR, R | S | HS | 3 (7), 2.5 (2), 3.4 (1) | 0.348 | 0.120 |
| 27 | 51755 | Poland | MS, R | MS, MR, R | MS, R | S, MS | HS | 0 (1), 1.2 (7), 2.1 (1), 3.4 (1) | 0.408 | 0.136 |
| 28 | 51887 | Poland | MR, R | MR, R | R | MR, R | MR, R | 5 (10) | 0.000 | 0.000 |
| 29 | 51987 | Poland | S, R | MS, MR, R | S, MS, R | S, MS, R | HS, S, MS, R | 0 (1), 1.2 (1), 2.9 (2), 4.4 (2), 5 (4) | 0.639 | 0.420 |
| 30 | 52339 | Poland | S, MS | S, MS, MR, R | S, MS | S, MS, MR, R | HS | 0 (3), 1.2 (2), 1.4 (3), 2.6 (2) | 0.593 | 0.196 |
| 31 | 52340 | Poland | S | MR, R | S, MS | S, R | HS, S, MS, R | 2 (6), 2.6 (2), 2.9 (1), 3.3 (1) | 0.473 | 0.148 |
| 32 | 52341 | Poland | MR, R | S, MS | MS, MR, R | MS | S, MS, MR, R | 7 (2), 2.4 (3), 3.1 (5) | 0.447 | 0.148 |
| 33 | 52342 | Poland | MS, MR, HR | MS, MR, HR | MR, HR | MS, MR, R | MS, R | 3.1 (1), 3.5 (1), 4.3 (2), 5 (6) | 0.473 | 0.192 |
| 34 | 501048 | Poland | HS, S, MR, R | HS | HS, MS | HS | HS | 0 (8), 1.1 (2) | 0.217 | 0.064 |
| 35 | 502855 | United Kingdom | MS, MR, HR | S | S, MS, MR, R | S | HS | 1 (4), 1.3 (1), 2.2 (5) | 0.410 | 0.132 |
| 36 | 502856 | United Kingdom | MR, R | MS, R | MS, MR, R | MS, MR | HS, MS, MR, R | 1 (5), 2.3 (1), 2.4 (1), 2.8 (1), 3.1 (1), 5 (1) | 0.651 | 0.288 |
| 37 | 502857 | United Kingdom | MS, MR | HS, R | MS | S, MS | HS, R | 0 (8), 1.5 (1), 2.8 (1) | 0.278 | 0.136 |
| 38 | 502858 | United Kingdom | S, MS, MR | HS | S | MS | HS | 0 (9), 1.1 (1) | 0.141 | 0.036 |
| 39 | 502859 | United Kingdom | S, MS | HS | S, MS, MR | S | HS | 0 (9), 1.3 (1) | 0.141 | 0.036 |

[1] Resistance phenotype: HS = 4 = highly susceptible—large pustules with little or no chlorosis; S = 3 = susceptible—moderately large pustules with little or no chlorosis; MS = 2 = moderately susceptible—moderately large pustules surrounded by extensive chlorosis; MR = 2N, 2C, ;1C = moderately resistant—small pustule surrounded by chlorosis or necrosis; R = ;-N, ;C, ;+C, ;1N = resistant—chlorotic or necrotic flecking; and 0 = HR = highly resistant—no visible reaction. [2] IP—infection profile—infection pattern determined for the five tested isolates characterized in Table 2; the number of resistant seedlings in brackets. [3] Sh—Normalized Shannon diversity index; Hs—Nei's diversity.

**Table 2.** The infection profiles (IP) of the tested *A. strigosa* accessions based on the reaction to *P. coronata* race infection.

| IP | *Puccinia coronata* Race | | | | | Oat Differential Line with a Corresponding Phenotype |
|---|---|---|---|---|---|---|
| | 51(22) | 94(63) | CR230 | CR241 | CR257 | |
| 0 | H [1] | H | H | H | H | - |
| 1.1 | L | H | H | H | H | Pc36, Pc39, Pc55, Pc61, Pc70, Pc71 |
| 1.2 | H | L | H | H | H | - |
| 1.3 | H | H | L | H | H | - |
| 1.4 | H | H | H | L | H | - |
| 1.5 | H | H | H | H | L | - |
| 2.1 | L | L | H | H | H | Pc38, Pc63 |
| 2.2 | L | H | L | H | H | - |
| 2.3 | L | H | H | L | H | - |
| 2.4 | L | H | H | H | L | - |
| 2.5 | H | L | L | H | H | - |
| 2.6 | H | L | H | L | H | - |
| 2.7 | H | H | L | H | L | - |
| 2.8 | L | L | H | H | L | - |
| 2.9 | H | L | H | H | L | Pc14 |
| 3.1 | L | H | L | H | L | Pc48, Pc103-1 |
| 3.2 | H | L | L | H | L | - |
| 3.3 | H | L | H | L | L | Pc35 |
| 3.4 | L | L | L | H | H | - |
| 3.5 | H | L | L | L | H | - |
| 3.6 | H | H | L | L | L | Pc54, Pc62, Pc64, Pc96, Pc97, Pc98 |
| 3.7 | L | H | H | L | L | - |
| 3.8 | L | L | H | L | H | - |
| 4.1 | H | L | L | L | L | Pc45, Pc51, Pc101, Pc104 |
| 4.2 | L | L | H | L | L | Pc59, Pc60, Pc91 |
| 4.3 | L | L | L | H | L | Pc52 |
| 4.4 | L | L | L | L | H | Pc56, Pc68 |
| 5 | L | L | L | L | L | - |

[1] H = high infection (virulent reaction); L = low infection (avirulent reaction).

## 3. Results

Five *P. coronata* isolates were used to perform the host–pathogen tests on ten plants of each of the 39 *A. strigosa* accessions. The host reactions ranged from highly susceptible (HS) to highly resistant (HR) (Table 1). All of the tested *A. strigosa* accessions displayed heterogeneous phenotypes. None of the accessions was completely susceptible to all *P. coronata* races; however, 118 of all 390 tested plants were susceptible to all *P. coronata* pathotypes. The largest number of resistant plants (177) was found for the most aggressive race 94(63), which was virulent to 18 of 34 evaluated *Pc* genes. Resistance to the 51(22) race was shown by 146 plants, and 148 plants were resistant to CR230. The lowest number of resistant plants, 96 and 97 were obtained for race CR241 (virulent to 13 *Pc* genes) and CR257 (virulent to 11 *Pc* genes), respectively. For nine accessions (51199, 502858, 502859, 501048, 51326, 51518, 51751, 51579, and 51598), only single seedlings were rated as resistant or moderately resistant to one crown rust race. Thirty-three plants within nine accessions (Table 1) were fully resistant to all the crown rust races used. Within accessions 51578 from Uruguay and 51596 from Chile, one plant each was resistant to all races, and nine plants were immune only to CR257. The exceptional accession was 51887 originating from Poland, which was resistant or moderately resistant to all *P. coronata* races.

Based on the seedling reactions to the five *P. coronata* races, 28 infection profiles (IPs) were determined for the 390 *A. strigosa* plants (Table 2). IP 0 corresponded to virulent reactions to all of the rust races. Resistance to one of five races was assigned as IP 1.1–IP 1.5, while resistance to two races was described as IP 2.1–IP 2.8. IP 3.1–3.8 indicated a

combination of resistance to three crown rust races. Resistance to four races was assigned as IP 4.1–4.3, and resistance to all five races was assigned as IP 5.

The IPs of the *A. strigosa* accessions were compared with the IPs of the differential Pc lines. The infection profile of the reference lines containing genes *Pc36, Pc39, Pc55, Pc61, Pc70*, and *Pc71* corresponded to IP 1.1 (Table 2) and was present within nine accessions (Table 1). The Pc38 and Pc63 lines were identical with IP 2.1, as observed within six accessions. The resistance to 94(63) and CR257 was characteristic of the differential line for *Pc14*. This pattern was assigned as IP 2.9 and was observed for four accessions. IP 3.1 corresponded to Pc48 and Pc103-1 and was found in two accessions. IP 3.3 (Pc35), IP 3.6 (Pc54, Pc62, Pc64, Pc96, Pc97, and Pc98) and IP 4.2 (Pc59, Pc60, and Pc91) were observed in one accession each. IP 4.1 with a virulent reaction to 51(22) was identical with Pc45, Pc51, Pc101, and Pc104 and was present within four accessions. IP 4.3 corresponding to Pc52 was present within two accessions. IP 4.4 (Pc56, Pc68) was observed within six accessions.

The variation within the accessions was assessed based on the level of the resistance reaction of each plant inoculated with five isolates of *P. coronata*. The average values of both calculated coefficients were 0.383 for the Normalized Shannon index (Sh) and 0.143 for Nei's diversity index (Hs). In the group with the lowest variation, we found accessions wherein all individuals were characterized by a low level of resistance to the tested isolates (51199, 51581, 51582, 51613, 502858, and 502859), as well as accessions wherein all individuals were highly resistant to all (51887) or almost all (51578 and 51596) isolates (Table 1). The greatest diversity was observed in accessions 51586, 51750, 51523, and 51987. These were mixtures of individuals with different levels of resistance to all *P. coronata* isolates, displaying the highest level of heterogeneity.

The analysis of molecular variance (AMOVA) determined that the majority of the observed variability was due to variation among accessions (64%, $p = 0.001$). The variation within the accessions accounted for 36% of the total variation.

The first two axes of the principal component analysis (PCA) of the accessions explained 51.3% of the total variance (41% and 11.3%, respectively) (Figure 1). The plot presented a large variation; however, a clear identification of groups composed of resistant or moderately resistant accessions was possible. The most resistant accession 51887 was localized on the opposite side of the plot along with 51583 and 52342. The middle part of the plot was occupied by genotypes with large variations in the immune response, e.g., 52341 (Sh = 0.447; Hs = 0.148), 502856 (Sh = 0.651; Hs = 0.288), and 52340 (Sh = 0.473; Hs = 0.148).

A dendrogram based on the accessions' dissimilarity identified two main clusters, composed of 25 and 14 accessions, respectively (Figure 2). The second group consisted of eight accessions from Poland, two from Chile, two from Russia, one from the Netherlands, and one from Uruguay. These were generally the most resistant accessions largely corresponding to the PCA group of resistant genotypes. Some genotypes from the same countries with identical or very similar profiles could be seen, e.g., 502855 and 502856 as well as 502858 and 502859 from the United Kingdom or 51518 and 51630 from Poland.

For population structure analysis, the Bayesian model approach implemented in STRUCTURE software was used (Figure 3). The $\Delta K$ peak was the highest for $K = 2$, supporting the presence of two distinct populations. On the basis of the membership fraction, the accessions were categorized as homogeneous (probability $\geq 0.8$) or admixed. P1 contained seven homogeneous accessions. In P2, fifteen homogeneous accessions were included. The remaining 17 accessions were classified as admixed. In general, P1 corresponded to resistance to most of the tested *P. coronata* race isolates, whereas P2 corresponded to susceptibility to these isolates. The analysis of the population structure in a graphical way refers to the results of the variation level within the accession obtained with the use of Shannon's index and the coefficient of Nei's diversity.

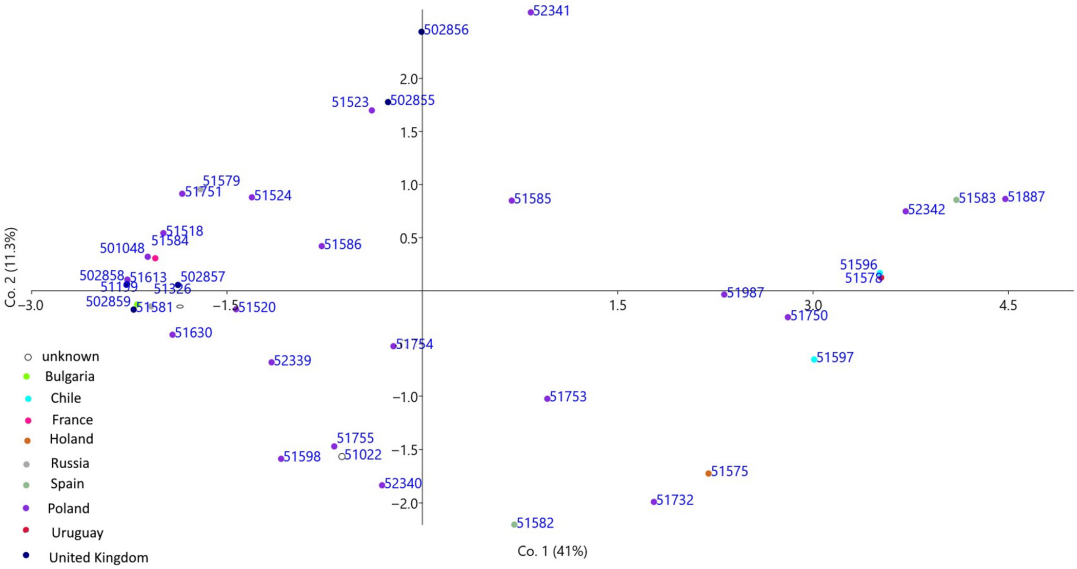


**Figure 1.** Principal component analysis of the *Avena strigosa* L. accessions. A scatter plot of PC1 (explaining 41% of the variance) versus PC2 (explaining 11.3% of the variance). Label colors indicate the origin countries of the samples.

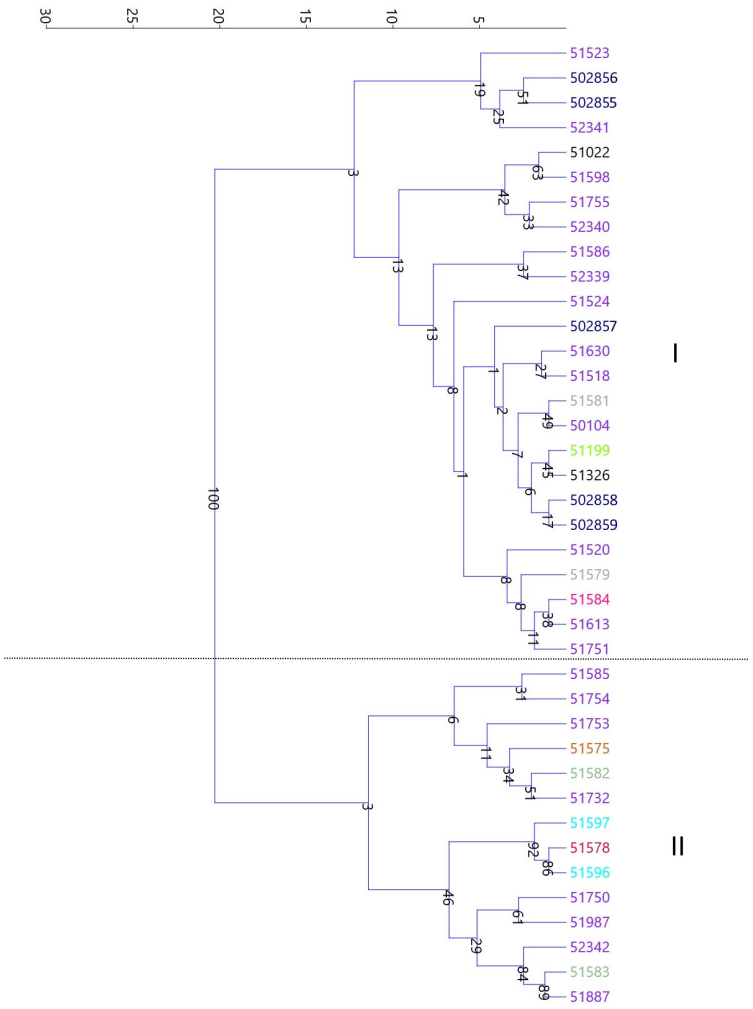


**Figure 2.** Ward's dendrogram of 39 accessions of *Avena strigosa* L. The accessions were assigned with the numbers used in Table 1 and the colors indicating the origin countries of the samples used in Figure 1.

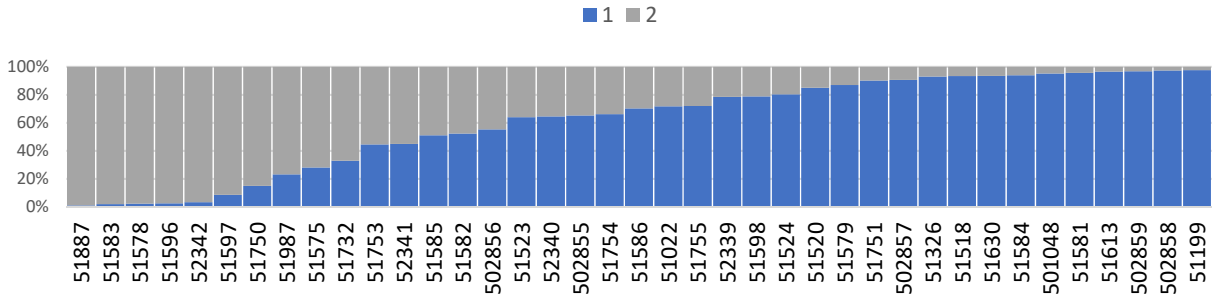


**Figure 3.** Bar graphs showing the population structure of 39 accessions of *Avena strigosa* L. based on the resistance to five isolates of *Puccinia coronata* f. sp. *avenae* as assessed using STRUCTURE. Each population is represented by a different color.

## 4. Discussion

The constant evolution of the *P. coronata* pathogen has caused the rapid loss of the effectiveness of the currently used crown rust resistance genes; therefore, the search for new ones is necessary. Wild oat progenitors have been proven to be a rich source of useful genes [32,33,35,52]; however, *A. strigosa* still remains unexploited. This study was carried out on 39 mostly European accessions from the Polish National Centre for Plant Genetic Resources with the majority originating from Poland (22 genotypes). A set of five *P. coronata* isolates was used to postulate the presence of potentially novel resistance genes.

Thirty-eight (97%) of the accessions studied showed a heterogeneous infection pattern; none of the accessions displayed homogeneous susceptibility, and only one, 51887, was homogeneously resistant to all *P. coronata* races. The heterogeneity of response to the rust inoculation within a single accession was already reported in the studies of wild oat species [32,33,53,54]; however, the level of phenotype variability within the analyzed *A. strigosa* was significantly higher, similar to the results obtained for *A. fatua*, where 85% of tested genotypes showed a heterogeneous infection pattern [31].

More than half of the *A. strigosa* genotypes screened in this research were characterized in terms of genetic (ISSR and SRAP), isoenzymatic, and morphological diversity by Podyma et al. [55,56]. According to Rodionova et al. [57], 17 botanical *A. strigosa* varieties can be distinguished based on clearly recognizable morphological traits. The results of the abovementioned authors revealed both genetic, botanical, and isoenzymatic variations in the analyzed objects. In terms of the morphology, within each accession, one to four varieties were recorded. Interestingly, within the most phenotypically variable *A. strigosa* accessions identified in our study (51586, 51520, 51523, and 51524), according to previous research, only one or two botanical varieties could be distinguished.

In this study, a detached leaf assay was used for resistance evaluation, allowing for the simultaneous testing of multiple rust isolates on a single plant. This is an important consideration when genotypes may be mixed as for many genebank accessions of landrace or previously uncharacterized material [35,58]. Additionally, the use of *P. coronata* races for the establishment of infection profiles (IP) allows postulating known and new *Pc* genes/alleles by matching the IPs of the tested plants with the profiles identified in differential lines. Twenty-eight infection profiles were defined within the 39 *A. strigosa* accessions, of which ten corresponded to the profile of known *Pc* genes. One hundred and sixty-eight out of 390 plants revealed an IP indicative of uncharacterized genes. Among the most resistant genotypes, 43 plants were immune to four of the five *P. coronata* races; each of these profiles corresponded to that of a highly efficient known *Pc* gene. However, this does not rule out that the identified profiles may correspond to new genes. To assess the genetic background of this resistance, it would be necessary to perform crosses with susceptible parents to determine the heredity model and conduct allelism tests to determine gene novelty.

Thirty-three plants within nine accessions were resistant to all crown rust races. A highly resistant response was shown by accessions 51578 from Uruguay and 51596 from Chile, with one plant in each resistant to all races and nine plants immune only to CR257.

The most homogeneous accession was 51887 from Poland, resistant or moderately resistant to all isolates. According to the Polish Genebank, 51578 was acquired by the USDA (United States Department of Agriculture, Agricultural Research Service Small Grains Collection in Aberdeen, ID) in 1951 from the Instituto Fitotecnico y Semillero Nacional, Montevideo, and the equivalent of this number is PI 194201. This accession obtained from the USDA was tested by Admassu-Yimer et al. [59] for seedling resistance against eight *P. coronata* races. PI 194201 along with PI 193040, PI 237090, and PI 247930 were resistant to all the races used; however, according to USDA, all of the genotypes are classified as *A. sativa.* In the study of Podyma et al. [56], 51578 obtained from Polish genebank was assessed in terms of a range of morphological traits, and two *A. strigosa* varieties within the analyzed plants were recorded, which confirms the correct species classification. Both PI 194201 and 51578 exhibited high crown rust resistance; moreover, PI 194201, according to the GRIN database, possesses resistance to other important diseases of oats, including stem rust, barley yellow dwarf virus, and smut. The classification of both accessions should be reexamined; regardless, both could prove valuable for oat breeding programs. The equivalent number of 51596 from Chile is PI 436103. Apart from the crown rust resistance exhibited by this accession in this study, it displayed a high level of seedling resistance to five of six *P. graminis* races used in the study of Gold Steinberg et al. [26], which further increases the agronomic value of the genotype. The highest level of resistance was observed in 51887 acquired by the IHAR genebank in 1994 and classified as a Polish landrace. No further data regarding the resistance of this accession to other oat diseases are available, so it is worth conducting additional analysis, as previous studies indicate that the *A. strigosa* species can exhibit a wide range of resistance to various pathogens.

## 5. Conclusions

The results obtained in this research confirmed the complexity and heterogeneity of the accessions gathered in genebanks. Here, we compared the resistance within as well as between the accessions based on PCA and agglomerative hierarchical clustering. The generalized average of resistance across individuals from a given accession, standardly available in the genebank databases, may obscure the presence of individuals with significant resistance to pathogens and reduce interest in looking for new resistance sources within it. Even accessions with poor overall resistance, such as 51586, may contain very resistant and often overlooked plants. At a time when many available sources of oat resistance have been overcome, it might be worth looking for desirable traits within susceptible accessions, which could be hidden donors of effective pathogen resistance.

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