

Article

Hidden Diversity of Crown Rust Resistance within Genebank Resources of *Avena sterilis* L.

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Abstract: The most widespread and damaging fungal disease of the oat plant is crown rust. Resistance to the crown rust pathogen, *Puccinia coronata* Cda. f. sp. *avenae* (*Pca*), at the seedling stage of *Avena sterilis* accessions from the Polish national genebank was characterised by five North American and Polish pathotypes of *Pca* of diverse pathogenicity. *Pca* pathogenicity was determined on a series of 34 differential lines carrying known seedling resistance genes. Seventy-five percent of studied accessions showed a heterogeneous infection pattern, 17% behaved as homogenous susceptibles, and 7% of tested genotypes could be unambiguously described as resistant. This study proved that *A. sterilis* accessions preserved in a genebank as complex populations could be a very valuable source of resistance to crown rust. The complexity of analysed populations was ascertained by a detailed variance analysis of transformed resistance/susceptibility data. We demonstrate here that hidden sources of resistance may be discovered in accessions with general susceptibility.

Keywords: accessions heterogeneity; complex populations; *Puccinia coronata* f.sp. *avenae*; resistance diversity



Citation: Paczos-Grzęda, E.; Boczkowska, M.; Sowa, S.; Koroluk, A.; Toporowska, J. Hidden Diversity of Crown Rust Resistance within Genebank Resources of *Avena sterilis* L. *Agronomy* **2021**, *11*, 315. <https://doi.org/10.3390/agronomy11020315>

Academic Editors: Caterina Morcia and Valentina Manstretta
Received: 30 December 2020
Accepted: 5 February 2021
Published: 10 February 2021

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1. Introduction

Puccinia coronata f. sp. *avenae*, which causes crown rust in oats, exhibits a high level of pathogenic variation and dynamic shifts in virulence as a consequence of both sexual and asexual reproduction [1–5]. The fungus displays a huge number of pathogenic variants and efficient adaptability [6]. Crown rust is considered the most harmful disease of the oat plant, and resistance conditioned by race-specific (*Pc*) genes has been the primary means of control [7]. Over 100 *Pc* genes have been discovered in different species belonging to the *Avena* genus [3,8], but the diversity of the pathogen rapidly leads to loss of effectiveness in resistance conferred by single dominant genes with different specificities towards *Pca* isolates, so that new resistance sources are needed urgently. Because of the large number of resistance genes identified in different wild species [7,9], it seems unlikely that these resources have been exhausted. Nazareno et al. [10] proposed that systematic evaluations of germplasm collections could uncover new genes and improve options for crown rust management.

The *Avena* collections maintained in gene banks worldwide consist of 131,000 accessions, stored by 125 holders in 63 countries, and are the eighth most numerous collections of crop germplasm after wheat, rice, barley, maize, beans, sorghum, and soybeans [11]. Twenty-four percent of *Avena* global resources are identified as wild species, of which the most plentiful is the hexaploid *Avena sterilis* L., with more than 23,000 accessions. Ninety-two percent of these are stored in Canada, the United States, and Israel. Moreover, 66% of *A. sterilis* accessions originate from Israel, a relatively small representative of the species' geographical range. *A. sterilis* genotypes collected from Israel and other Mediterranean

countries were the sources of forty-four crown rust resistance genes [7,12–15]. Some of these genes were introduced into North American, European, and Australian oat cultivars. Accessions originating from Israel provided *Pc39*, *Pc48*, *Pc58*, *Pc59*, *Pc60*, and *Pc61*; accessions from Algeria provided *Pc38* and *Pc68*; and the *Pc50* gene donor was from Tunisia [7,12–14].

Polish germplasm resources of genus *Avena* are the ninth most numerous collection worldwide overall, with the *A. sterilis* collection ranking seventh [11]. The studies presented here use a detached leaf method to screen individual seedling resistance to crown rust in accessions from this collection [16]. Our survey proved that *A. sterilis* accessions collected in Morocco and preserved in a genebank as a complex population could be a very valuable source of resistance to crown rust. In the present paper, as a continuation of previous efforts, the complexity of analysed populations has been characterized and precisely assessed by detailed variance analysis of transformed resistance/susceptibility data. The level of the internal variation of *Avena* species has never been analysed using the approach applied in our studies.

2. Materials and Methods

The research was carried out on 41 accessions of the *A. sterilis* (Table 1). The seeds were obtained from the National Centre for Plant Genetic Resources in Radzików, Poland. The accessions were acquired during field expeditions in Morocco (18 accessions), Ukraine (5 accessions), and Iran (2 accessions) from 1986 to 2006. The origin of 15 accessions could not be identified.

Table 1. Accession list containing basic information and statistical data.

No.	Genebank Number	Acquisition Date	Country of Origin	uHe	I	Post Hoc Group Comparison
1	PL 50280	1983	na	0.087	0.061	ABCDEFGHJIJ
2	PL 51557	1985	MAR	0.163	0.114	GHIJ
3	PL 51559	1985	MAR	0.162	0.110	GHIJ
4	PL 51565	1985	MAR	0.022	0.015	A
5	PL 51589	na	MAR	0.084	0.059	ABCDEFGH
6	PL 51818	1993	MAR	0.157	0.107	FGHIJ
7	PL 51823	1993	MAR	0.038	0.025	ABC
8	PL 51832	1993	MAR	0.127	0.087	CDEFGHIJ
9	PL 51836	1993	MAR	0.060	0.043	ABCDE
10	PL 51837	1993	MAR	0.096	0.066	ABCDEFGHJIJ
11	PL 51838	1993	MAR	0.119	0.083	BCDEFGHIJ
12	PL 51839	1993	MAR	0.138	0.090	DEFGHIJ
13	PL 51840	1993	MAR	0.097	0.066	ABCDEFGHJIJ
14	PL 51841	1993	MAR	0.180	0.116	J
15	PL 51851	1993	MAR	0.170	0.118	HIJ
16	PL 51856	1993	MAR	0.147	0.098	EFGHIJ
17	PL 51857	1993	MAR	0.050	0.035	ABCD
18	PL 51860	1993	MAR	0.146	0.098	EFGHIJ
19	PL 52105	1995	na	0.122	0.084	BCDEFGHIJ
20	PL 52106	1995	na	0.078	0.052	ABCDEFGH
21	PL 52108	1995	na	0.085	0.060	ABCDEFGHI
22	PL 52109	1995	na	0.098	0.067	ABCDEFGHJIJ
23	PL 52110	1995	MAR	0.071	0.046	ABCDEFG
24	PL 52111	1995	na	0.178	0.116	IJ
25	PL 52205	1997	na	0.061	0.042	ABCDE
26	PL 52209	1997	na	0.060	0.043	ABCDE
27	PL 52212	1997	na	0.107	0.070	ABCDEFGHJIJ
28	PL 52217	1997	na	0.102	0.068	ABCDEFGHJIJ
29	PL 52278	2000	na	0.100	0.070	ABCDEFGHJIJ
30	PL 52353	2001	UKR	0.136	0.094	DEFGHIJ

Table 1. Cont.

No.	Genebank Number	Acquisition Date	Country of Origin	uHe	I	Post Hoc Group Comparison
31	PL 52355	2001	UKR	0.045	0.030	ABCD
32	PL 52363	2001	UKR	0.088	0.062	ABCDEFGHJIJ
33	PL 52435	2004	UKR	0.065	0.047	ABCDEF
34	PL 52442	na	na	0.155	0.105	FGHIJ
35	PL 52459	2006	na	0.091	0.064	ABCDEFGHJIJ
36	PL 52460	2006	na	0.098	0.065	ABCDEFGHJIJ
37	PL 52461	2006	na	0.068	0.047	ABCDEF
38	PL 52462	2006	na	0.031	0.022	AB
39	PL 501552	2004	IRN	0.064	0.044	ABCDEF
40	PL 502692	2005	UKR	0.052	0.035	ABCD
41	PL 502695	2004	IRN	0.105	0.071	ABCDEFGHJIJ

I = Shannon index, uHe = Nei coefficient, IRN = Iran, MAR = Morocco, UKR = Ukraine, na = not available

Crown rust resistance of each of the accessions was assessed using five highly virulent *P. coronata* isolates (*Pca*). CR230, CR241, and CR257 were kindly supplied by Dr. J. Menzies from the Morden Research and Development Centre, AAFC, Canada, whereas 94(63) and 51(22) were selected from a wide collection of single-pustule pathotypes that originated from Polish fungus populations. The virulence of each isolate used in the study was previously characterized on the basis of the susceptibility/resistance reaction of 34 differential oat lines with single gene resistance (Table 2).

Table 2. Virulence profile of *Puccinia coronata* f.sp. *avenae* isolates used for testing resistance of *Avena sterilis* accessions.

Isolate Number	Phenotype Code ¹	Virulence to Differentials
51(22)	SBLP	<i>Pc14, Pc35, Pc40, Pc45, Pc46, Pc51, Pc54, Pc57, Pc62, Pc6, Pc96, Pc97, Pc98, Pc101, Pc104</i>
94(63)	NJBP	<i>Pc36, Pc39, Pc40, Pc46, Pc48, Pc54, Pc55, Pc57, Pc61, Pc62, Pc64, Pc70, Pc71, Pc94, Pc96, Pc97, Pc98, Pc103-1</i>
CR230	LQCB	<i>Pc14, Pc35, Pc36, Pc38, Pc39, Pc40, Pc55, Pc57, Pc59, Pc60, Pc61, Pc63, Pc70, Pc71, Pc91</i>
CR241	DSGB	<i>Pc14, Pc36, Pc38, Pc39, Pc46, Pc48, Pc52, Pc55, Pc61, Pc63, Pc70, Pc71, Pc103-1</i>
CR257	BRBG	<i>Pc36, Pc38, Pc39, Pc55, Pc56, Pc61, Pc63, Pc68, Pc70, Pc71, Pc94</i>

¹ Phenotype code based on the standard differentials set.

The host/pathogen test [17] according to Sowa et al. [18] was applied. To screen the resistance of the *A. sterilis* accessions, ten leaf fragments were used, each from a different seedling. Leaves were placed into Petri dishes or 12-well culture plates filled with agar (0.6%) and benzimidazole (3.4 mM) medium according to the methodology of Hsam et al. [17]. Inoculations were performed in a settling tower by applying 500–700 spores of *P. coronata* per square centimetre. The plates were incubated for 10 days in a phytotron at about 18 °C with 70% humidity and light intensity of approximately 4 kLx. Crown rust disease symptoms were evaluated 10 days after inoculation using the 0–4 infection type (IT) qualitative scale [10,19], which were transformed to S, MS, MR, R, and HR, where S = 4 = susceptible, large to moderately large pustules with little or no chlorosis; MS = 3 = moderately susceptible, moderately large pustules surrounded by extensive chlorosis; MR = 2, 2N, 12C, ;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 [10,16,18–20].

The raw data were transformed into a binary matrix. In order to ensure that the matrix reflects the accuracy of the initial results in the best possible way, each plant resistance response level to a particular pathotype of *Pca* (assessed in 0-4 IT scale) was treated as a single variable. The presence of each resistance response level was encoded as 1, while its absence was encoded as 0. The diversity parameters such as Shannon's [21] and Nei's [22] diversity indices were calculated, and the results were subjected to one-way analysis of variance (one-way ANOVA) with Tukey's test for post hoc comparison. In order to compare individuals and their relationships, the dissimilarity matrix was built using a Dice coefficient [23] according to the following formula:

$$D = 1 - \frac{2x_{ij}}{2x_{ij} + x_i + x_j}$$

where x_{ij} is the number of variables present both in i -th and j -th individuals, x_i is the number of variables unique for the i -th individual, and x_j is the number of variables unique for the j -th individual. Intra-accession differences were calculated according to Nei's unbiased distance formula:

$$uD = -\ln(I)$$

$$I = \frac{J_{xy}}{\sqrt{J_x J_y}}$$

$$J_{xy} = \sum_{i=1}^k p_{ix}^2 p_{iy}^2$$

$$J_x = \sum_{i=1}^k p_{ix}^2$$

$$J_y = \sum_{i=1}^k p_{iy}^2$$

where p_{ix} and p_{iy} are the frequency of i -th variable in accessions x and y [24]. For hierarchical clustering, dissimilarity matrices were used to construct dendrograms using Ward's method. Principal coordinate analysis (PCoA) was performed to visualize the relationships between individuals and accessions. Analysis of molecular variance (AMOVA) was used to partition the diversity [25]. The variance components were tested statistically using 9999 permutations. The binary data were also analysed for population structure using a model-based Bayesian clustering. The models were computed for $K = 1 \div 10$ (K —number of subpopulation). Each model was tested five 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. All of the above-mentioned analyses were performed using GenAlEx 6.5 [26], R environment for statistical computing, STRUCTURE v2.3.4 [27], and CLUMPAK [28] software.

3. Results

Resistance reactions of 41 *A. sterilis* accessions to *P. coronata* isolates in the host/pathogen tests ranged from susceptible (S) to highly resistant (HR). Thirty-one (76%) of the tested *A. sterilis* accessions displayed heterogeneous phenotypes, and the specific *Pca* inoculation response of single seedlings within accessions could vary from highly resistant to susceptible. Only seven accessions (17%) were completely susceptible on all tested races of *P. coronata*. The remaining three accessions (7%) displayed a consistently resistant phenotype. Two of these accessions were immune to races 51(22) and 94(63) (PL 51589, PL 52110), and the last one (PL 51836) was assessed as resistant or highly resistant to all *Pca* strains.

Variation within the accessions was determined on the basis of the level of resistance reaction of each plant inoculated with five isolates of *P. coronata*. The average values of both calculated coefficients were relatively low and were, respectively, 0.101 for the Shannon index (I) and 0.069 for the Nei coefficient (uHe). In the group with the lowest variation

we found both accessions, wherein all individuals were characterized by complete lack of resistance to all tested isolates (PL 51565, PL 51823, PL 51857), as well as accessions wherein all individuals were highly resistant to all *Pca* isolates (PL 51836) (Table 1). Low variability was also noted for accessions that did not show full resistance to all tested *Pca* isolates, but wherein all individuals displayed the same resistance level and pattern. The greatest diversity was observed in accessions PL 51841, PL 52111, and PL 51851. These were mixtures of individuals with different levels of resistance to all *Pca* isolates, which displayed the highest level of heterogeneity. The analysis of variance (ANOVA) revealed a statistically significant difference between the examined accessions with respect to the Shannon index ($p = 0.01$). Homogeneous groups were identified by a post hoc Tukey's test, and the results are given in Table 1. The analysis of molecular variance (AMOVA) showed that the distribution of variability among individuals and among accessions was comparable ($\phi_{PT} = 0.515$).

Due to the specificity of the data, the coefficient of dissimilarity of individuals reached its maximum range, i.e., 0-1. The distance among accessions ranged from 0.003 (PL 51837-PL 52109 and PL 51838-PL 52459) to 0.251 (PL 51565-PL 52462). Hierarchical clustering and principal coordinate analysis (PCoA) were performed on the basis of both dissimilarity matrices.

A dendrogram based on accession dissimilarity identified four main clusters, composed of 9, 11, 14, and 7 accessions, respectively (Figure 1). The second group consisted mainly of the Moroccan accessions, which were generally characterized by the highest level of resistance. The fourth cluster encompassed seven accessions originating mainly from Ukraine and Iran. They were characterized by susceptibility to all tested *Pca* isolates.



Figure 1. The Ward's clustering dendrogram of 41 accessions of *Avena sterilis* L. based on Nei's unbiased distance matrix [24]. The accessions were labelled with numbers in accordance with Table 1. Four main clusters were surrounded by contours and filled in.

A dendrogram based on individuals' data also showed the presence of four main clusters composed of 124, 109, 25, and 152 individuals, respectively (Figure S1). As a result of internal differentiation of accessions, individuals from the same source could be placed in different groups. The smallest and most interesting cluster, cluster 3, was composed of 25 individuals belonging to five accessions: PL 51836 (10 individuals), PL 51856 (9 individuals),

PL 51851 (4 individuals), and PL 51841 and PL 52111 (1 individual each). Most of them displayed the highest level of resistance. The remaining clusters were composed of a mixture of less resistant or totally susceptible individuals. They were grouped according to the resistance level and pattern in the subordinate units.

The first three axes of PCoA of individuals explained 33.12% of the total variance (12.63%, 10.62%, and 9.87% respectively) (Figures 2a and 3a). The plot of the first two PCoAs presented large variation; however, a clear identification of any grouping pattern was difficult (Figures 2b and 3b). Clearly, the individuals from the accessions gathered in Morocco covered all the detected variability, which was also reflected in the results of the coefficients of variation (Figure 4). The plot in Figure 3b also clearly indicated that four to five isolate-resistant individuals were concentrated in quarter IV. All of them were of Moroccan origin (Figure 2b). Analysis of resistance to individual *Pca* isolates presented in Figure 5b,d,f,h,j showed that individuals resistant to a particular isolate were present in different parts of the plot, which indicated that there were many potential sources of resistance.

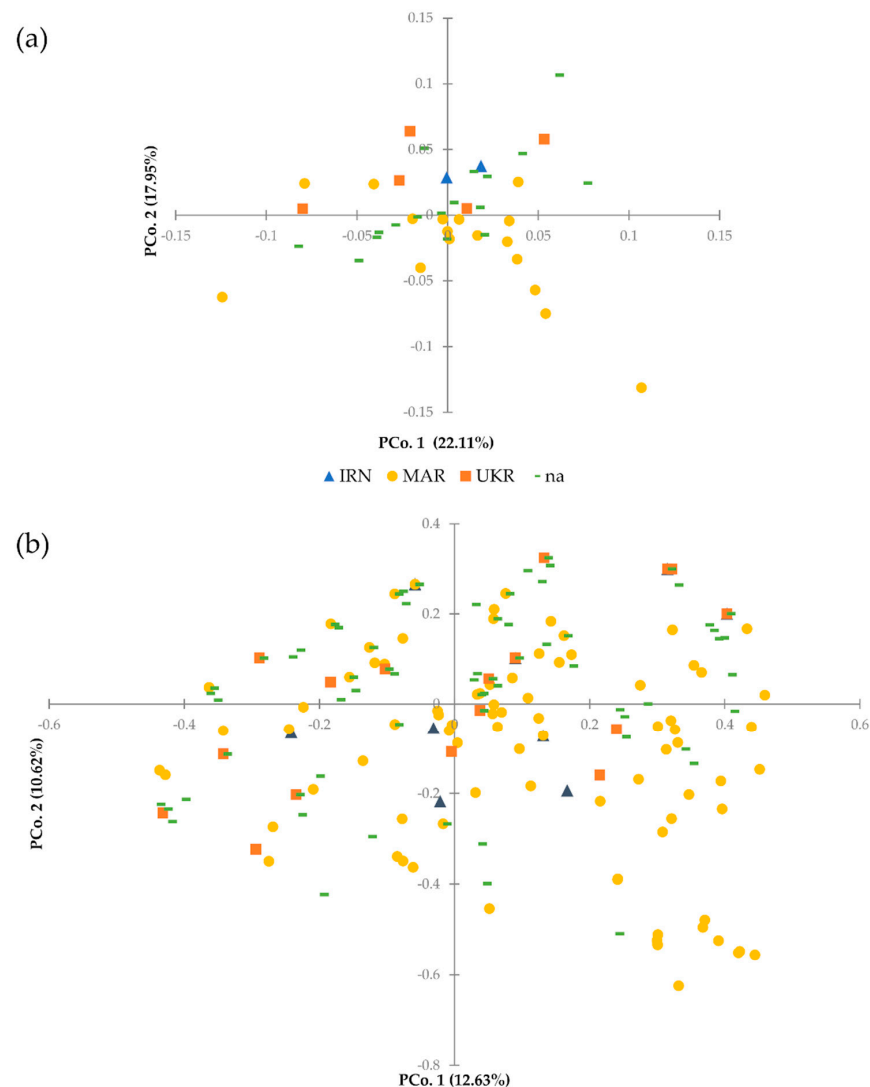


Figure 2. Analysis of principal coordinates (PCoA) with an accession origin marking; (a) for 41 accessions of *Avena sterilis* L. based on Nei's unbiased distance matrix [24]; (b) for 410 individuals of 41 accessions of *A. sterilis* based on Dice's dissimilarity matrix [23]. IRN = Iran, MAR = Morocco, UKR = Ukraine, *na* = not available

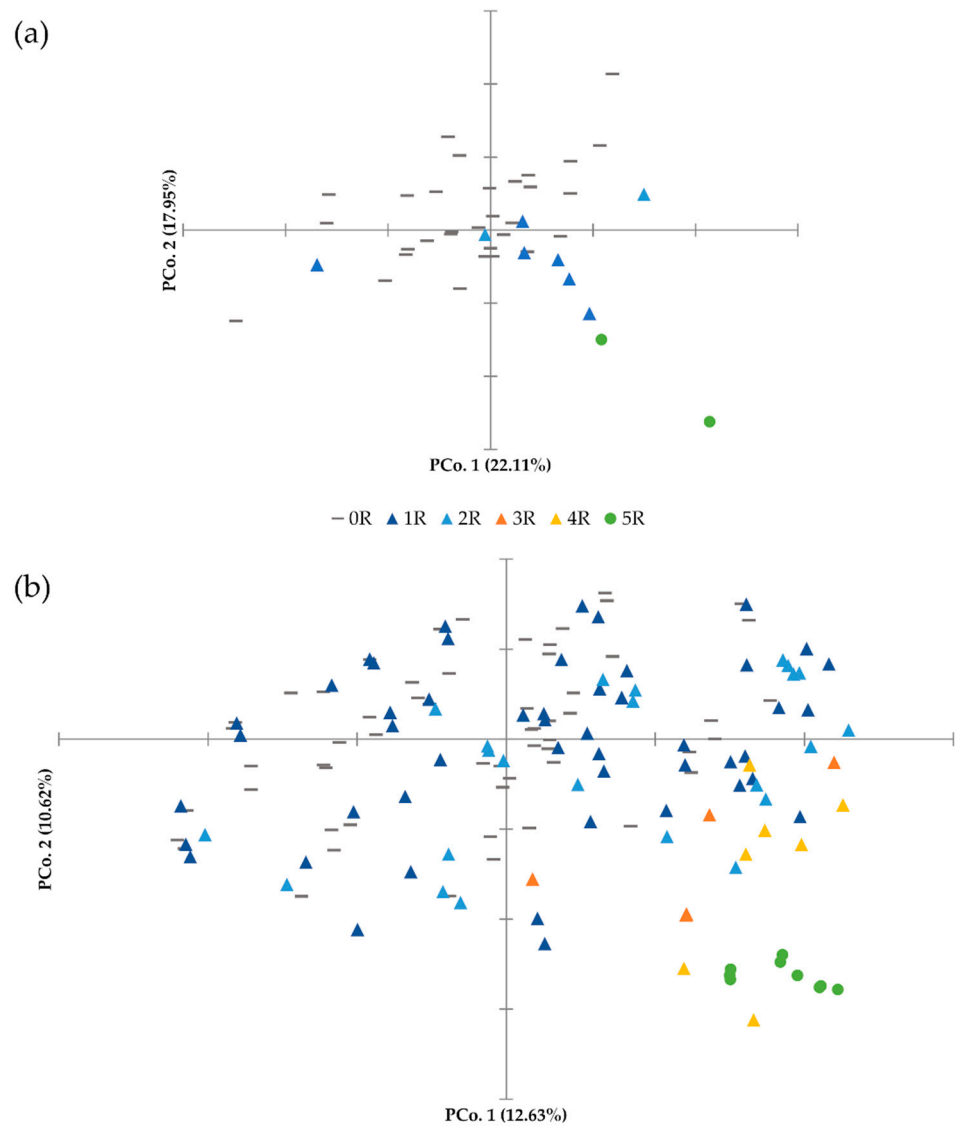


Figure 3. Analysis of principal coordinates (PCoA) with determination of resistance to tested isolates of *Puccinia coronata* f. sp. *avenae*, where 0R means susceptibility to all tested isolates, 1R–4R means resistance to 1–4 of the tested isolates, and 5R means resistance to all tested isolates; (a) for 41 accessions of *Avena sterilis* L. based on Nei’s unbiased distance matrix [24]; (b) for 410 individuals of 41 accessions of *A. sterilis* based on Dice’s dissimilarity matrix [23].

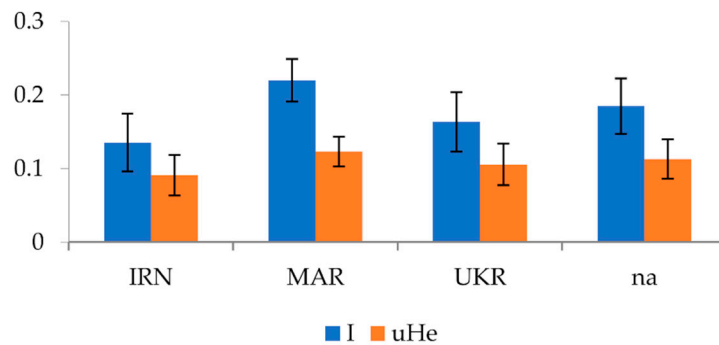


Figure 4. Levels of Shannon’s index [21] and Nei’s coefficient [22] indicating diversity for the groups determined by the collection site.

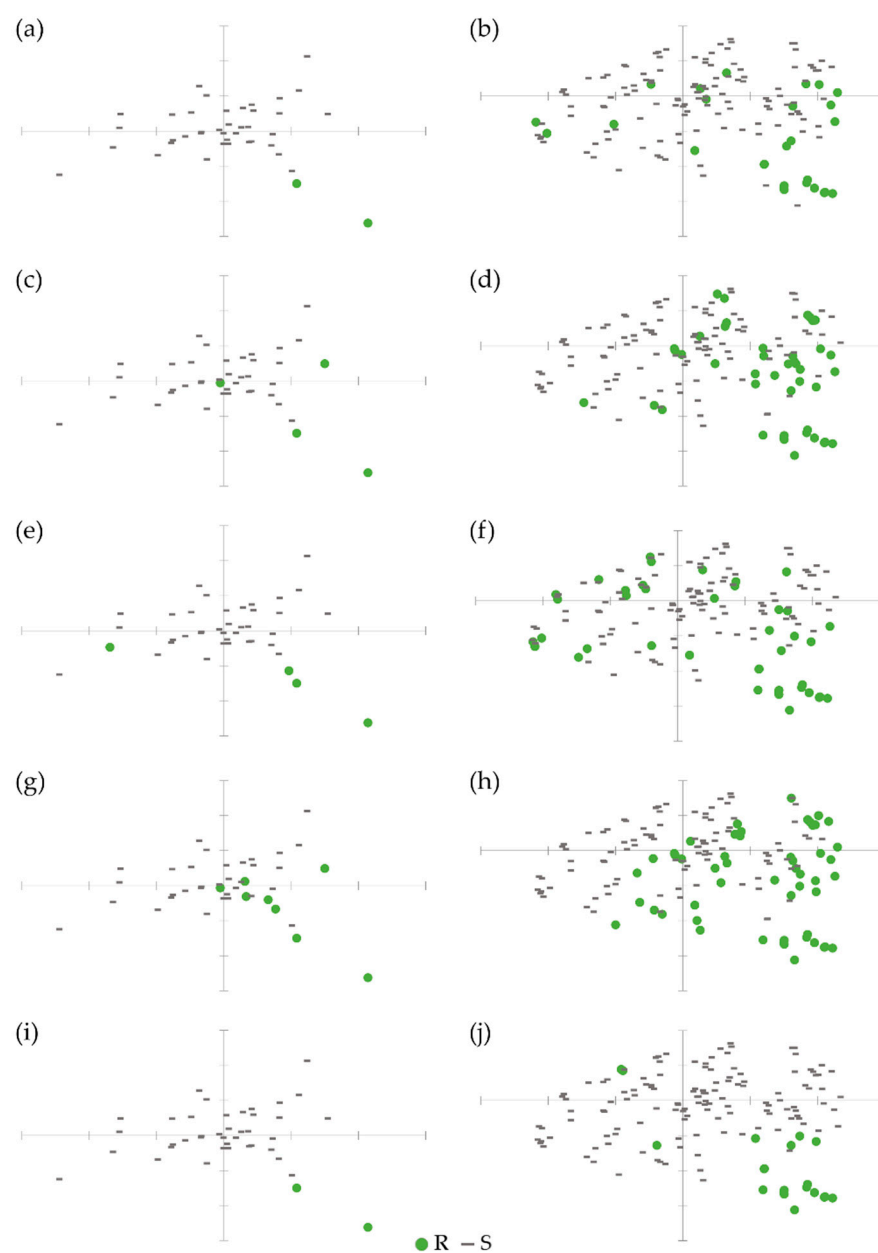


Figure 5. Plots of 1 vs 2 axis of principal coordinates analysis (PCoA) with the resistance (○R) and susceptibility (–S) of 41 accessions of *Avena sterilis* L. to specific isolates of *Puccinia coronata* f. sp. *avenae* (Pca) indicated; (a) generalized resistance of the accessions to the CR257 Pca isolate; (b) individuals' resistance to the CR257 Pca isolate; (c) generalized resistance of the accessions to the 51(22) Pca isolate; (d) individuals' resistance to the 51(22) Pca isolate; (e) generalized resistance of the accessions to the CR230 Pca isolate; (f) individuals' resistance to the CR230 Pca isolate; (g) generalized resistance of the accessions to the 94(63) Pca isolate; (h) individuals' resistance to the 94(63) Pca isolate; (i) generalized resistance of the accessions to the CR241 Pca isolate; (j) individuals' resistance to the CR241 Pca isolate.

Resistance to *Pca* isolate CR257 occurred in 46 individuals derived from 14 accessions (Figure 5b). Thirty-six individuals (eight accessions) came from Morocco, and the remainder came from six accessions of unknown origin. Only in one accession (PL 51836) were all individuals resistant to this isolate.

Seventy-four individuals from 16 accessions showed resistance to *Pca* isolate 51(22) (Figure 5d). A total of 63.5% of individuals (eight accessions) came from Morocco, 27% (five accessions) from unknown locations, and the rest from Ukraine (three accessions).

Among the Moroccan accessions, two (PL 51589 and PL 51836) demonstrated resistance of all individuals, while nine out of ten tested plants from the third (PL 51856) were resistant.

Seventy-one individuals were not susceptible to isolate CR230; they originated from a total of 17 accessions (Figure 5f). Forty-four plants were sourced from 10 accessions collected in Morocco, 1 was sourced from Ukraine, and the remaining 6 were sourced from accessions with an unknown collection site. All plants from three accessions (PL 51832, PL 51836, and PL 52209) were resistant to this *Pca* isolate.

Almost one in every four tested individuals showed resistance to isolate 94(63) (Figure 5h). Sixty-two plants came from ten Moroccan accessions, three came from one from Ukraine, and single plants came from each of the two Iranian accessions. The remaining 32 individuals came from 10 populations of unknown origin. All individuals in the three populations PL 51589, PL 51836, and PL 52110 were resistant. Ninety percent of plants of PL 51586 were also resistant.

Only 37 plants were characterized by resistance to isolate CR241 (Figure 5j). A total of 94.5% of these originated from seven Moroccan populations, and the remaining were from two accessions with unknown collection sites. Among them, in only one case (PL 51836) were all individuals resistant. Nine of the ten tested plants of PL 51856 also showed resistance.

The Bayesian model approach implemented in the STRUCTURE software was used for population structure analysis. The ΔK peak was the highest for $K = 5$, supporting the presence of five distinct populations. Forty-one accessions were divided into five populations (Figure 6a) containing 12, 7, 9, 9, and 4 accessions. Further, on the basis of the membership fraction, accessions were categorized as pure (probability ≥ 0.8) or admixed. P1 contained four pure accessions, or 76 (18.5%) pure individuals (Figure 6b). In P2, no pure accession was included; however, as many as 64 (15.6%) pure individuals were found in the whole set. P3 was composed of four pure accessions, or 64 (15.6%) individuals. P4 contained three pure accessions, or 32 (7.8%) individuals; and finally, in P5, two pure accessions were found, or 32 (7.8%) individuals. The remaining 28 accessions and 171 individuals were classified as admixed. In general, P3 corresponded to susceptibility to all tested *Pca* strains, whereas P5 corresponded to the resistance to these isolates. P2 was associated with a decreased susceptibility to isolate 51(22). The higher the proportion of these groups in the accession, the higher the proportions of individuals with the above-described resistance pattern. For individuals, the contribution of P5 was reversely proportional to the number of isolates capable of infecting the particular plant, while P3 contribution was directly proportional to the susceptibility to the tested isolates. The other two populations, P2 and P4, indicated intermediate susceptibility values to the tested isolates. The analysis of the population structure in a graphical way refers to the results of the level of variation within the accession obtained with the use of the coefficient of Nei's diversity and Shannon's index.

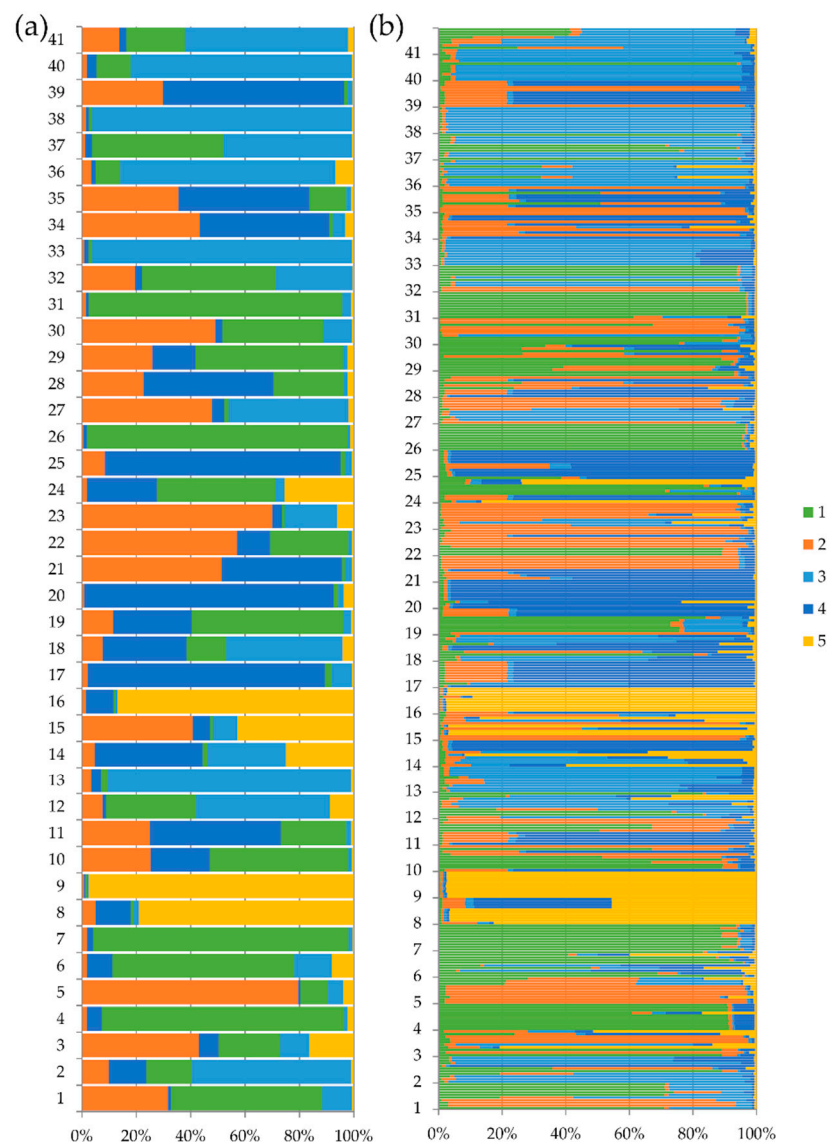


Figure 6. Bar graphs showing diversity structure of 41 accessions of *Avena sterilis* L. for resistance to five isolates of *Puccinia coronata* f. sp. *avenae* as assessed using STRUCTURE software [27]; (a) the graph for accessions; (b) the graph for 410 individuals. Each population is represented by a different colour.

4. Discussion

Currently oat breeding programmes, especially North American and Australian, are in urgent need of genetic sources from which new genes of *P. coronata* resistance can be introduced. The primary oat gene pool consists of hexaploid species that cross easily with cultivars and are widely used as donors of crown rust race-specific resistance genes. *A. sterilis* accessions collected in Israel and other Mediterranean countries in the 1960s and early 1970s were the main source of these genes [15,29].

The research presented here was carried out on 41 accessions from the Polish National Centre for Plant Genetic Resources, most of which were acquired during expeditions in Morocco, Ukraine, and Iran. Fifteen accessions of unconfirmed origin were also included. A set of highly virulent and diverse *P. coronata* isolates derived from populations collected in North America and Europe were used in this survey, enabling identification of potentially valuable resistance for use in current breeding programmes. Thirty-one (75%) of the accessions studied showed a heterogeneous infection pattern, seven (17%) were homogeneously susceptible, and three (7%) genotypes could be unambiguously described

as resistant. Other studies on wild oat resistance have also revealed variable response to the rust inoculation within single accessions [18,20,30]. This study proved that *A. sterilis* accessions collected in Morocco, in particular, could be a very valuable source of resistance to crown rust. Among 17 investigated populations from this country, 11 displayed high seedling resistance to the *P. coronata* races used. This percentage of hexaploid species accessions with resistance to crown rust was higher than observed in other studies [30,31], but it was comparable to our previous results obtained in a survey of the tetraploids *Avena magna* Murphy et Terrell, *Avena murphy* Ladiz, and *Avena insularis* Ladiz [18]. The highest level of resistance in that study was also observed in accessions originating from Morocco. Saidi et al. [32] evaluated crown rust resistance in natural infection conditions of 288 accessions, which represented 13 species of wild oat gathered in Morocco. One hundred genotypes of *A. sterilis* were characterised, but only four were assessed as resistant or moderately resistant. Among the other analysed samples, 23 representing *Avena longiglumis* Durieu, *Avena damascena* Rajhathy et Baum, *Avena wiestii* Steud, *Avena barbata* Pott ex Link, and *A. magna* proved to be resistant. Tan and Carson [30] also analysed crown rust resistance of 332 accessions originating from Morocco and representing 11 wild oat species from the USDA-NSGC (United States Department of Agriculture National Small Grains Collection) in Aberdeen. Most of them were diploids and tetraploids, and only two accessions represented hexaploid *A. sterilis*. After screening this collection with a highly diverse population of *P. coronata*, about 50% of the accessions were recorded as at least moderately resistant at the seedling and adult plant stages. However, one *A. sterilis* accession was susceptible, and the other only moderately resistant. The results obtained by Saidi et al. [32] and Tan and Carson [30] favour the wild tetraploids and diploids from Morocco as the richest source of crown rust resistance.

Morocco is considered to be the centre of genus *Avena* origin, and out of all *Avena* species only three (*Avena canariensis* Baum, *A. insularis*, and *Avena macrostachya* Bal. ex Coss. et Dur) have not been recorded there [11,33]. Morocco's crop diversity results from long-term adaptation to various local environmental conditions such as drought, cold, and salinity [34,35]. Genetic variation at the genomic and population levels in both pathogens and their host plants are the effect of antagonistic interaction between them and their environment [36]. The fungus evolves parallelly and convergently to its hosts and results in the accumulation of many resistance and virulence genes [37]. Oates et al. [38] claim that in environments where pathogens are widespread and variable, heterogeneous resistance arises within populations.

A further complexity is the natural heterogeneity of accessions representing wild species gathered in genebanks [18,30,39]. Here, we have compared resistance within as well as between accessions. On the basis of the agglomerative hierarchical clustering and PCoA analysis, the distinctiveness among *A. sterilis* individuals and among accessions was demonstrated. Information based on the average of resistance across individuals from a given accession, which is standardly available in the gene bank databases, may obscure the presence of individuals with significant resistance to pathogens. The generalized information on the susceptibility of the entire accession makes researchers less interested in looking for new sources of resistance within it. As we have shown in this paper, at a time when available sources of oat resilience have failed, it is worth looking for desirable traits in accessions with general susceptibility, which have proven to be a hidden, valuable source of effective resistance.

5. Conclusions

Detailed variance analysis of transformed resistance/susceptibility data revealed hidden sources of resistance in *A. sterilis* accessions with general susceptibility. The study proved that accessions preserved in a genebank as complex populations could be a very valuable source of resistance to crown rust.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4395/11/2/315/s1>, Figure S1: The Ward’s clustering dendrogram of 410 individuals of 41 accessions of *A. sterilis* based on Dice dissimilarity matrix [23]. The accessions were labelled with numbers in accordance with Table S1. Individuals from each accession were additionally labelled with a–j letters. Four main clusters were surrounded by contours and filled in, Table S1: Characteristics of *A. sterilis* accessions reactions to inoculation with 5 *P. coronata* isolates at the seedling stage. S = susceptible; MS = moderately susceptible; MR = moderately resistant; R = resistant; HR = highly resistant. Individuals from each accession were additionally labelled with a–j letters.

Author Contributions: Conceptualization and methodology, E.P.-G., M.B. and S.S.; investigation, J.T. and A.K.; resources, M.B.; writing—original draft preparation, E.P.-G. and M.B.; writing—review and editing, S.S.; visualization, M.B.; project administration, E.P.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Ministry of Agriculture and Rural Development of the Republic of Poland Grant 2014–2020: “Crown rust resistance genes pyramiding in oat genome and identification of DNA markers for these genes”. The calculations were made at the Interdisciplinary Centre for Mathematical and Computational Modelling of Warsaw University (ICM UW) as part of the computational grant no G72-19.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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