

Article

Diversity and Pre-Breeding Prospects for Local Adaptation in Oat Genetic Resources

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Abstract: Acreage of oat (*Avena sativa* L.) in Europe was steadily declining during the last century due to less breeding progress compared to other cereals. However, oat remains a valuable crop for food and feed, as well as for sustainable crop rotations. To unravel the genetic and phenotypic diversity in oat breeders' germplasm collections, a diversity panel including 260 accessions was investigated by molecular markers and in multi-environment field trials. Due to the large genetic variation in the present diversity panel, high heritabilities were observed for most agromorphological traits, even for complex traits such as grain yield. Population structure analyses identified three subpopulations which were not straightforwardly related to the geographic origin of the accessions. Accessions from France, Germany, and the Czech Republic in particular were present in approximately equal proportions among all three subpopulations. Breeders' selection after one year of field trials was mainly based on grain yield, grain weight, grading, plant height, and maturity and did not result in a loss of genetic diversity. However, the low number of polymorphic markers must be considered in this case. The present study provides basic knowledge for further oat improvement through the identification of valuable genetic resources which can be exploited in breeding programs as e.g., parental genotypes in crossings.

Keywords: *Avena sativa*; genetic resources; grain yield; heritability; population structure; regional adaptation; retrospective selection index; trait association

1. Introduction

Oat (*Avena sativa* L., $2n = 6x = 42$, AACCCDD) was domesticated a few millennia later than wheat and barley (i.e., about 4500 years ago), however, it was frequently present and thus under selection pressure as an agricultural weed in cultivated wheat and barley habitats since the origin of agriculture. Oat finally became a secondary domesticate in the regions of North-eastern Europe due to its superior performance in marginal climatic and edaphic conditions [1], where it played an important role in agriculture production systems over several centuries e.g., as an excellent horse feed and for food. Nevertheless, since the mechanization of agriculture and transportation in the 20th century, the cultivation of oats decreased gradually [2]. Markedly, a tremendous decrease in acreage occurred during the 1990s in European countries (Figure 1). This decrease in acreage was compensated for by increased grain yields [3] in some countries (e.g., Australia, Canada, and Finland) where the production is oriented towards export markets, whereas the global acreage and production

decreased significantly. Oat is thus only a minor cereal crop in some Northern countries today with Canada, Russia, and the Nordic countries being the most important producers, although in recent years several health-promoting nutraceutical compounds, e.g., tocots, avenanthramides, and beta-glucans, were identified in oat [4–6]. It is alarming that in some European countries, the grain yield of oat has been stagnating since the 1980s. Reduced input, cultivation on marginal land, but also the cultivation of older cultivars can be identified as reasons for this trend [3].

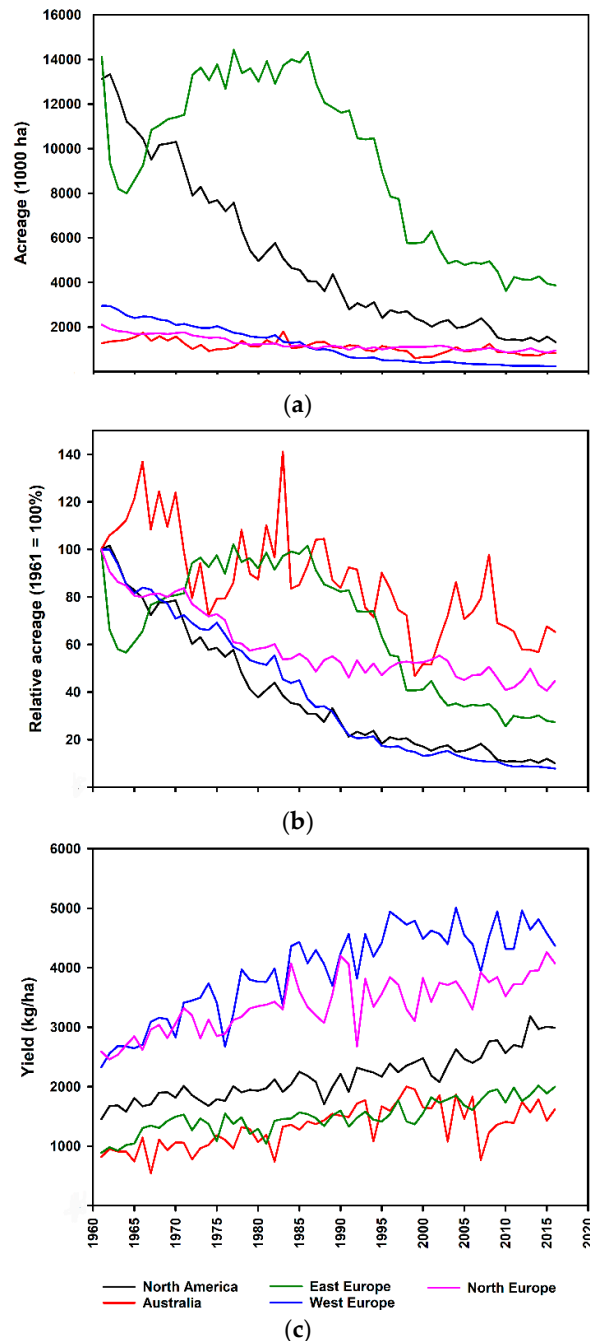


Figure 1. Development in oat acreage: (a) absolute values; (b) relative changes in relation to 1961 and (c) grain yield in five major production areas from 1961 to 2016. (Source of data: FAOSTAT Crops, Food and Agriculture Organization of the United Nations, Rome, Italy; <http://www.fao.org/faostat/en/#data/QC>).

Low acreages, however, lead to a vicious circle with abandoned breeding programs, reduced genetic diversity, reduced number of improved cultivars, small breeding gains, and finally again a decline in acreage and production. For example, in Austria and Germany the number of oat breeding programs decreased by more than 80% in the last 20 years [3,7]. To exploit the diversity in cultivated oat more efficiently, a better characterization of modern breeding material and genetic resources is needed, especially since the population structure in oat has been found to be weak, without any clear morphological source. Traits such as caryopsis type, lemma color, and panicle type have not been found to contribute significantly to genetic clustering patterns [7,8]. The genome of hexaploid oat is large (~13 Gb) [9] and genetic diversity in oat was described using different marker technologies [3,9–14].

The aim of the present study was thus to evaluate the genetic and phenotypic diversity of an oat diversity panel which was established within the FP7 HealthyMinorCereals project and evaluated over four years in two different European locations.

2. Materials and Methods

2.1. Plant material and phenotypic data

A world-wide collection of 260 oat accessions (*Avena sativa* L.) was assembled for this study and furthermore distinguished into husked, naked, and black oats. The entire population of 260 accessions was phenotyped in two locations (Czech Republic and Estonia) in 2014 in an unreplicated yield trial with 1 m² plots that included two replicated check varieties (i.e., Atego and Raven) to account for spatial field trends. The 87 most promising accessions were selected after the first year by the involved breeders for further testing in completely randomized designs in Pernarec (CZ; 49°50'43"N, 13°5'35"E) in 2015, 2016, and 2018, and randomized complete block designs in Jõgeva (EE; 58°45'44"N, 26°23'51"E) from 2015 to 2018, resulting in a total of nine environments for grain yield, 1000-grain weight, hectoliter weight, and plant height. Yield components such as number of panicles per unit area (panicles/m²), panicle length (cm), and grading (% grains >2 mm) were additionally assessed in 3–6 of the environments, while disease scorings for leaf rust (*Puccinia coronata* f. sp. *avenae*) and leaf blotch (*Septoria avenae* f. sp. *avenae*) were obtained in 3–4 environments with natural infections using a modified 1–9 scale (1=resistant; 9=susceptible) [15]. Morphological parameters like days to heading and maturity (days after sowing), ground coverage (1=shallow; 9=dense), frequency of plants with recurved flag leaves (1=all flag leaves are rectilinear; 5=50% of the plants with recurved flag leaves; 9=all flag leaves are recurved), second leaf width (mm), hairiness of the margin of the leaf below the flag leaf (1=absent; 5=medium; 9=very strong), hairiness of the uppermost node (3=weak; 5=medium; 7=strong), glume glaucosity (1=absent or weak; 7=very strong), lemma color (1=white; 2=yellow; 3=brown; 4=grey; 5=black), and panicle shape (1=erect; 5=horizontal; 9=strongly dropping) were finally scored in 3–5 environments. The detailed scoring schemes can be found in the respective technical protocol of the Community Plant Variety Office for testing of distinctness, uniformity, and stability (DUS) [16]. Traits and/or environments for which only low or no genetic variation was observed were not included in the statistical analysis.

2.2. Statistical analysis of phenotypic data

Phenotypic analysis for the population of 260 oat accessions was conducted separately for each trial by trait combination using a linear mixed model of the form:

$$y_{ik} = \mu + g_i + b_k + r_{ik} \quad (1)$$

where y_{ik} are the phenotypic records, μ is the grand mean, and the effect of the k^{th} block b_k was modeled as random, while r_{ik} designates the residual effect with $\mathbf{r} \sim N(\mathbf{0}, \mathbf{I}\sigma_r^2)$ in trials with a randomized complete block design. The effect of the k^{th} block b_k was accordingly omitted in the analysis of trials that were laid out as completely randomized designs. The effect g_i of the i^{th} line was modeled as random to estimate the genetic variance σ_g^2 in the first analysis, and afterwards fixed to derive best linear unbiased

estimates (BLUEs). The heritability of each trial by trait combination was determined according to Piepho and Möhring [17]:

$$h^2 = \frac{\sigma_g^2}{(\sigma_g^2 + \frac{1}{2}MVD)} \quad (2)$$

where σ_g^2 is the genetic variance and MVD the mean variance of a difference of the BLUEs. The derived BLUEs from the first stage of analysis were subsequently used for the analysis across trials:

$$y_{ij} = \mu + g_i + e_j + ge_{ij} \quad (3)$$

where y_{ij} is once again the phenotypic records for each trait of interest, μ the grand mean, the fixed effect of the j^{th} environment e_j , and g_i the effect of the i^{th} accession with $\mathbf{g} \sim N(\mathbf{0}, \mathbf{I}\sigma_g^2)$. The random genotype by environment interaction ge_{ij} was confounded with the residual effect in this model. The heritability was again estimated by formula (2), and BLUEs for each trait were obtained by modeling a fixed accession effect. Three subsets of data were created in this way for further analyses: (a) performance for all 260 accessions using the available data from all environments; (b) performance for all 260 accessions only for the initial year of selection (2014); (c) performance for the 87 selected accessions in the subsequent years (2015–2018).

All phenotypic analyses were conducted with the statistical package ASReml 3 (VSN International Ltd., Hemel Hempstead, UK) for the R programming environment (R Development Core Team, Vienna, Austria).

2.3. Genotypic data and population structure

DNA was extracted from bulks of approximately 30 plants grown in pots in the greenhouse using CTAB [18]. A part of one leave was taken per plant. Quality and concentration of DNA samples were verified electrophoretically and spectrophotometrically, while samples were diluted to a concentration of 100 ng μl^{-1} . A set of 42 SSR primer combinations (Supplementary Material Table S1), selected from previous publications [19–22], was used to genotype all 260 oat accessions. The products of amplifications were separated using the ABI PRISM 3130 capillary electrophoresis instrument (Applied Biosystems, Foster City, CA), and electrophoretograms were analyzed with GeneMapper Software v3.7 (Applied Biosystems). Monomorphic markers and markers with an allele count larger than 30 were excluded from further analysis resulting in a reduced set of 28 microsatellites. The gene diversity for each marker in this subset was calculated as:

$$H_{e_j} = 1 - \sum_{i=1}^n p_i^2 \quad (4)$$

where p_i is the frequency of the i^{th} allele at the j^{th} marker locus, and markers with $H_{e_j} > 0.2$, gave a final dataset of 15 SSR markers for population genetic analyses. Population structure was subsequently analyzed with ancestry estimates using a non-negative matrix factorization algorithm, which previously did provide highly accurate estimates in populations with fully inbred individuals [23]. The optimal number of subpopulations was thereby determined by minimizing a cross-entropy criterion, where marker genotypes of 25% of the lines were initially masked and subsequently predicted in a cross-validation manner with 100 replicates for a range of $K = 1$ to 10 subpopulations. The population structure analysis was thereby based on the R-package *LEA* [24]. The genetic diversity within the entire collection, each region of origin, and the found subpopulation was subsequently determined by the total and average number of alleles, and average gene diversity for the k^{th} group with:

$$H_{e_k} = \frac{1}{m} \sum_{j=1}^m H_{e_j} \quad (5)$$

where m is the number of markers. Estimates of Rogers' distance [25] among the accessions and a neighbour joining tree served additionally to differentiate between the sets of 87 selected and 173 non-selected accessions to assess the potential loss of genetic diversity by selection. Differentiation between the above-described groups was moreover assessed by Wright's F_{ST} statistic [26], where values larger than 0.05 suggested a moderate to high diversity between the investigated subpopulations [27]. The population genetic analyses were conducted with the package *adegenet* [28,29] for R (R Development Core Team).

2.4. Trait correlations and response to selection

The relationship between the assessed traits was investigated with the Fruchterman and Reingold [30] algorithm as implemented in the R package *sna* [31,32], where the according network analysis was based on trait characteristics of all 260 accessions across environments and the corresponding correlation matrix. A retrospective index was calculated after standardizing the phenotypic data in order to determine the relative importance that the breeders allocated to each trait:

$$b = P^{-1}s \quad (6)$$

where b are the index weights, P^{-1} is the inverse of the phenotypic variance-covariance matrix among traits, and s is a vector of selection differentials [33,34]. The index weights were determined using the phenotypic data from the initial selection year 2014, while the absolute and relative response to selection was estimated with all available data. The accuracy for predicting the accessions breeding values in the initial trials was finally estimated by the phenotypic correlation between the 2014 and 2015–2018 trials using the 87 accessions that were tested in all years.

3. Results and Discussion

3.1. Quantitative genetic parameters and trait correlations

High heritabilities were observed for most agro-morphological traits (Table 1). Similar high heritabilities were reported for a nursery of 120 oat varieties of worldwide origin [35], which had 44 varieties in common with our study, and a population of 101 F_6 recombinant inbred lines (RILs) developed from a cross between a naked and husked Canadian variety [36]. The high heritability estimates resulted from the large genetic variation and low genotype by environment (G×E) interaction present in the studied oat collection. Even for the complex trait grain yield, heritability was high due to the large genetic variation despite a substantial G×E (Table 1). Also, most DUS criteria like lemma color and stem hairiness were among the traits with the highest heritability (Table 1) which justifies their inclusion in the technical protocol for variety testing. On the other hand, leaf hairiness showed an only medium heritability due to an equal variation of genotype and G×E, the latter mainly due to the minor, and therefore difficult to score, differences between scores 3 (i.e., weak) and 5 (i.e., medium). Contrary to other studies [35,36], heritability for the percentage of plump kernels (grading >2.0 mm) was also only medium, and the correlation between test weight and kernel plumpness was negative ($r = -0.47$; $p < 0.0001$; Figure 2). Test weight and kernel plumpness are important physical quality traits for industrial milling [37] as they are easy and quick to determine. Usually the two traits are positively related [35]. In our study, the negative correlation is caused by the inclusion of naked oat varieties which have a low kernel plumpness but a high test weight [36] due to having a higher packing proportion. If the naked oat accessions are deleted from the data, then the correlation becomes significantly positive ($r = 0.34$; $p < 0.0001$), and further increases when only European oat accessions are considered ($r = 0.42$; $p < 0.0001$). Moreover, it must be considered that seed plumpness in the present study was more prone to G×E due to both a higher number of test environments and more heterogeneous environments compared to other studies [35,36]. The present oat panel consisted of 14, 21, and 225 accessions of black, naked, and husked oats, respectively. Naked oats were responsible for the large variation in seed plumpness. Husked and black oats had very similar average grading percentages with 86.6% and 87.7%, respectively, whereas the grading had

the range 43.5%–79.8% for the naked oats, reflecting their smaller size due to lacking a husk. However, the naked oat with the highest grading surpassed 11 accessions from the other two groups regarding this trait.

Table 1. Mean, range, variance components and heritability for grain yield-related, morphological, and disease resistance traits of all 260 oat accessions across the entire trial series 2014–2018.

Trait	Trials (n)	σ_G^2 ¹	σ_{GE}^2	h^2	Min	Mean	Max
Grain yield (dt ha ⁻¹)	9	112.9	82.4	0.81	7.5	46.9	69.3
1000 grain weight (g)	9	11.2	2.5	0.93	22.5	34.5	47.0
Test weight (kg hL ⁻¹)	9	10.8	3.9	0.89	41.2	50.4	63.2
Grading >2 mm (%)	8	50.1	54.1	0.59	43.5	86.0	97.8
Plant height (cm)	9	109.8	35.2	0.91	67.8	100.3	149.0
Heading (d after sowing)	5	9.0	0.8	0.94	51.7	61.1	74.7
Maturity (d after sowing)	5	2.8	1.0	0.81	97.2	103.0	114.2
Leaf rust (1–9)	3	1.1	1.6	0.51	1.0	3.4	6.5
Leaf blotch (1–9)	4	0.1	0.4	0.35	1.9	3.6	4.9
Panicle number (n m ⁻²)	4	1311	2175	0.44	336.8	489.9	768.0
Panicle length (cm)	3	5.3	1.2	0.86	13.1	17.5	32.2
Panicle shape (1–9)	3	0.8	0.5	0.68	1.3	5.9	9.3
Lemma color (1–9)	3	2.2	0.4	0.88	1.0	2.3	9.0
Glume glaucosity (1–9)	3	2.4	1.0	0.77	1.0	4.5	8.8
Recurved flag leaves (1–9)	3	2.4	0.6	0.84	1.0	3.7	8.1
Second leaf width (mm)	3	3.0	6.4	0.40	17.0	24.8	35.4
Ground coverage (1–9)	3	0.5	0.4	0.63	2.8	6.3	8.8
Stem hairiness (1–9)	3	3.3	0.2	0.95	1.0	1.8	9.0
Leaf hairiness (1–9)	3	0.2	0.2	0.48	1.0	1.2	7.2

¹ h^2 : heritability; σ_G^2 : genotypic variance; σ_{GE}^2 : genotype×environment interaction

High G×E and, therefore, low and medium heritabilities were observed for leaf blotch and leaf (crown) rust resistance, respectively. For both diseases, a natural infection of the germplasm was observed only in Estonia. For leaf blotch, genetic variation was limited, and no accession was identified with high or very high susceptibility. For leaf rust, the medium heritability of 0.51 is significantly higher compared to the estimates determined in F₇ offspring populations of two Canadian crosses [38], but lower than in two US crosses [39,40]. Differences in heritability measurements of disease resistances are not astonishing, especially with respect to race-specific diseases such as leaf rust, due to differences in the investigated germplasm or the disease evaluation method (e.g., natural field infection with or without the use of susceptible spreader rows vs. artificial inoculation with prevalent races of the pathogen).

Substantial G×E was also found for grain yield and other yield components like the panicle number per unit area. The according variance component was though comparably low for thousand grain weight that showed, thus, the highest heritability among all yield components. Nevertheless, a high data quality was obtained for most of these traits due to their assessment in several environments as reflected by medium to high heritabilities. A substantial variation was present among all accessions in the collection with regard to grain yield ranging from 7.5 to 69.3 dt ha⁻¹. This variation also reflected the issue that many accessions were not well adapted to the two test locations in Estonia and the Czech Republic. Nevertheless, 52 accessions in the diversity panel surpassed the checks in their grain yield performance that amounted 57.9 dt ha⁻¹ across the two test locations, highlighting the potential value of the genetic resources in the collection at hand. The grain yield of

oat is strongly influenced by environmental conditions, resulting in fluctuating yields from year to year [3,41,42]. Therefore, oat breeding for broad adaptation is hard to achieve. On the other hand, breeding for regional adaptation, e.g., drought tolerance in Southern and Central Europe, and adaptation to day length and fall sowing in Northern and Western Europe, respectively, make long-term financing less difficult despite decreasing acreages and decreasing income from royalties [3]. Recurrent selection was proposed and shown to be effective for breeding for broad adaptation [41,43,44], but had also drawbacks such as a decline in test weight [41]. Therefore, it was suggested to use superior lines/varieties developed for broad adaptation as breeding parents in crosses with locally adapted material for further improvement in overall performance [3,41].

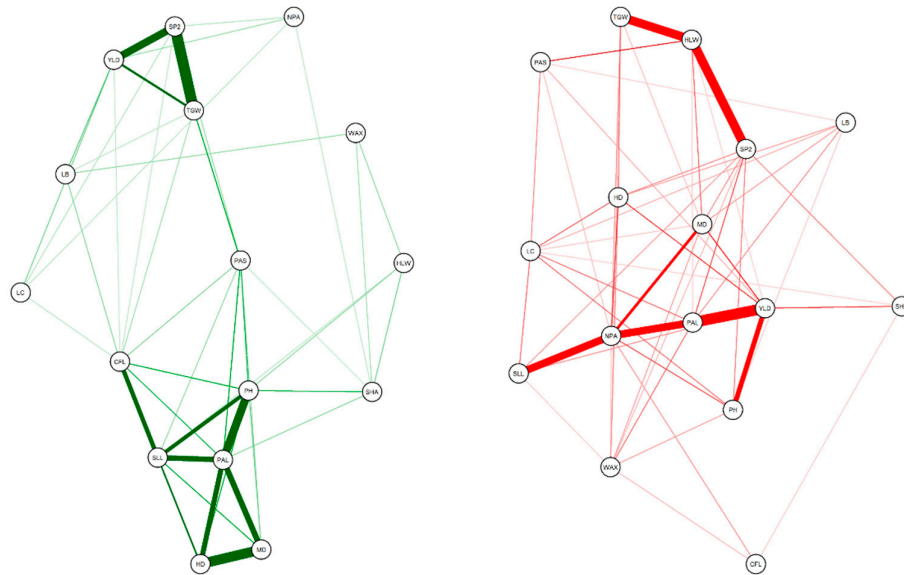


Figure 2. Network analysis of grain yield components, morphological and disease resistance traits, showing both positive (green) and negative (red) correlations as well as their magnitude (thickness) between the respective traits. Grain yield (YLD), 1000-grain weight (TGW), hectoliter weight (HLW), grading (SP2), heading date (HD), maturity date (MD), plant height (PH), LB (leaf blotch), number of panicles per unit area (NPA), lemma color (LC), glume glaucosity (WAX), panicle shape (PAS), panicle length (PAL), recurved flag leaf (CFL), second leaf length (SLL), stem hairiness (SHA).

For the plant height the check mean was 98 cm, which was surpassed by 130 out of the entire 260 accessions in the collection, and turned to be too tall for the target environments. The variation in plant height (i.e., 68 to 149 cm; Table 1) was similar to the values reported for a European hexaploid oat collection screened within the AVEQ project [45]. Plant height is associated with lodging, but many other aspects also affect lodging of oats [46]. Therefore, the correlation between plant height and lodging is often only moderate when a large collection of germplasm is screened [45]. In the present study, no correlation between plant height and lodging was observed as the breeders discarded too tall accessions after the first year and environmental conditions did not favor lodging thereafter. Similar to plant height, a high level of genetic variation was observed for heading date and maturity. Although numerous accessions were either too early or too late from the current breeders' point of view, this germplasm may have potential with respect to adopting to changing cropping systems caused by global warming [47,48].

Given that a combination of performance in several traits is decisive, a thorough investigation of the relationship between traits was conducted by using a network analysis (Figure 2). A strong correlation was found between kernel plumpness and grain weight, which mostly reflected the

difference between husked and naked oats. Grain weight showed also a positive association with grain yield ($r = 0.35$), which was however lower than the one between grading and yield ($r = 0.50$). The latter might lead to favoring husked oats, as the weight of the husk was also included into yield, thus introducing some bias when considering the actual harvested end-product. This trend was on the other hand inverted for the hectoliter weight, where naked oats were favored due to lacking a bulky husk, which resulted in a negative correlation between the hectoliter weight and grading as well as the grain weight ($r = -0.47$). However, as already outlined above, this negative correlation became positive if the naked oat genotypes were deleted from the analysis. Negative correlations were also observed between grain yield and morphological traits, foremost with plant height, but also for the panicle length, which was closely related to plant height. Surprisingly, only a very low correlation of $r = 0.10$ was present between panicle number per unit area and grain yield, which underlined the larger influence of grain weight and, probably, grain number per panicle on yield in this study. Heading and maturity were furthermore slightly negative correlated with grain yield because late accessions were most likely not well adapted, as mentioned beforehand. In addition, selection for earliness and grain yield was an important combination of traits and was successfully improved by oat breeders. Further traits like lemma color or leaf blotch were eventually relatively independent from the outlined trait complexes.

3.2. Phenotypic and genotypic population structure

Given the complex interaction between traits, a principal component analysis was carried out in order to further dissect these relationships and investigate the phenotypic population structure in the present oat collection. The loadings of the first two principal components corresponded to a large magnitude with previously observed correlations (Figure 3). Following the previous-mentioned relationships among grading, grain weight and grain yield, the naked and husked oats formed clearly two distinct groups, while the black and husked oats constituted a common group that was for the larger part merely structured by the lemma color. Nevertheless, these differentiations could not be verified by molecular markers where naked, black, and husked oats were rather mixed and showed a comprehensive pattern across all groups, which confirms the results of various previous studies on the phenotypic and genotypic diversity of oat collections of diverse origins [7,35,36]. Notwithstanding this, three large subpopulations with sizes of 69, 84, and 107 accessions, respectively, could be identified by a genetic population structure analysis (Figure 4). Accession origins' were diverse within each subpopulation, and accessions from France, Germany, and the Czech Republic were split into approximately equal proportions among all three subpopulations.

North American accessions from the United States and Canada were on the other hand more prevalent in subpopulation I, while accessions from Austria clustered mostly into subpopulation III. The genetic population structure analysis accordingly showed some admixture between subpopulations; hence germplasm grouping according to geographic origin was not straightforward, especially for smaller groups of accessions derived from Russia and Northern Europe. The genetic distance between accessions showed likewise a large range, with very close to distant relationships even within a given subpopulation, which resulted in a highly diverse kinship pattern. The genetic diversity was largest in subpopulation I, followed by subpopulations III and II giving the same pattern as the one observed for the average allele count within each subpopulation (Table 2). Furthermore, the F_{st} values and average Rogers' distance suggested that subpopulation III had a closer relationship to the two other subpopulations, while subpopulation I and II were more distinct from each other. Overall, population structure analyses reflected both the high genetic and phenotypic diversity within the identified subpopulations and across all accession in the investigated oats collection.

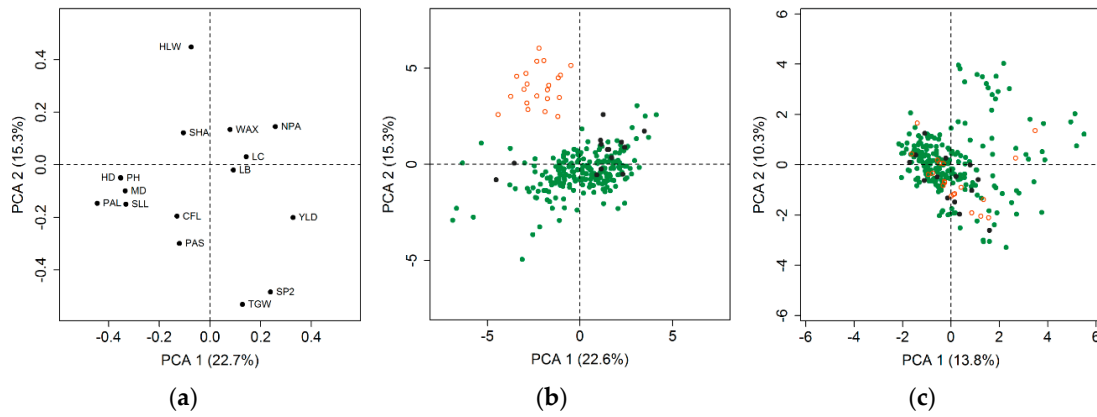


Figure 3. Loadings (a) and population structure inferred by a principal component analysis with the phenotypic (b) as well as the molecular data (c). Closed green and black symbols represent the husked and black oats, respectively, while the open red circles indicate the naked oats. For trait abbreviations, see Figure 1.

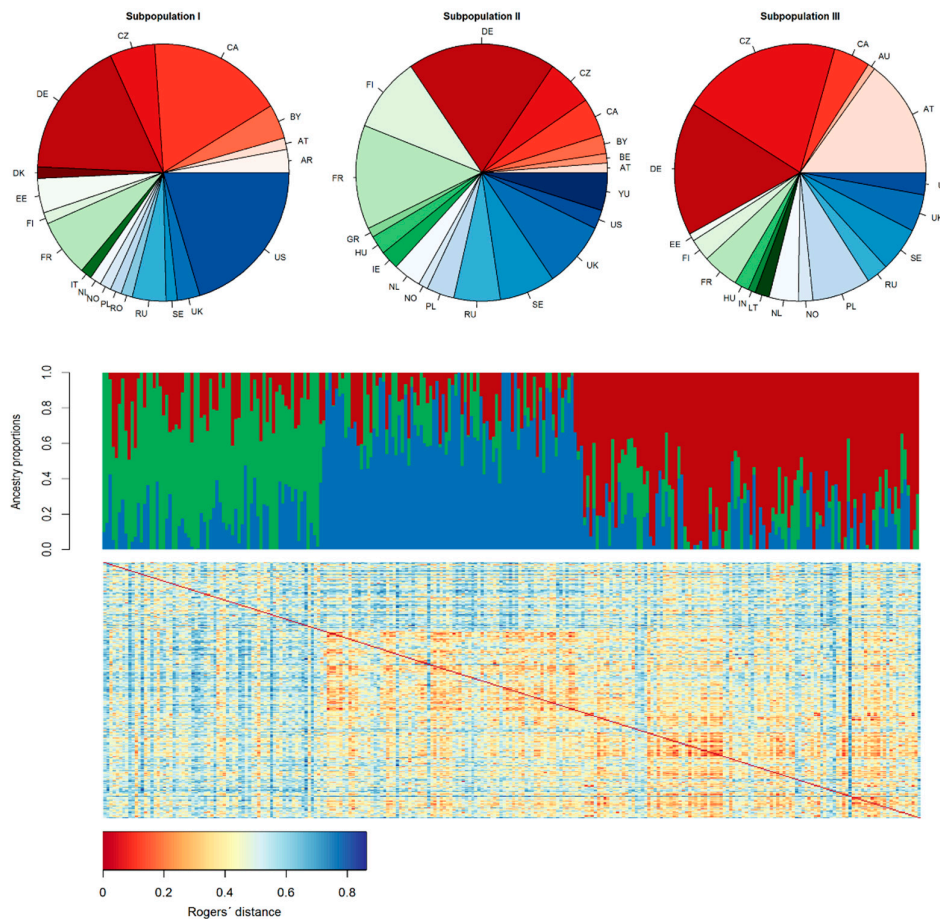


Figure 4. Percentage memberships of accessions to the subpopulations inferred by the population structure analysis (top), their estimated ancestry coefficients with the respective subpopulations (middle), and the genetic relationship between all 260 analyzed oat accessions (bottom).

Table 2. Gene diversity and allele count (diagonal), F_{st} values (above diagonal), and Rogers' distance (below diagonal) among the three subpopulations defined by the population structure analysis.

	Subpopulation I	Subpopulation II	Subpopulation III
Subpopulation I	0.612 (9.4)	0.056	0.029
Subpopulation II	0.230	0.488 (7.5)	0.033
Subpopulation III	0.169	0.150	0.505 (8.3)

With the emergence of molecular techniques, a few studies concerning the population structure of oat collections were published [7,8,10,13,14,45,49–53]. Although all studies are biased with respect to the geographic origin of the investigated germplasm, a main conclusion is that the genetic diversity of North American oat varieties is larger compared to European varieties, and that population structures are generally weak and are not associated with morphological traits such as lemma color, caryopsis type, or panicle shape [7,8,50–52]. Within North American material, a much larger genetic diversity was reported for US oat germplasm, whilst Canadian oat diversity was described to be narrow and a decrease in the average genetic diversity was observed for varieties released since 1950 [10,49–52]. A significant loss of genetic diversity during the transition from landraces and old varieties to modern varieties was also observed in Northern Europe [13]. A negative effect of modern plant breeding on genetic diversity was demonstrated for various crops and is heavily disputed because the allelic diversity of particular loci is sensitive to plant breeding practices [10], the impact of specific breeding methods was rarely considered, and some studies were experimentally inadequate and contained technical biases from the sampling of cultivars and genomes [54]. Breeding programs not only decrease but also increase genetic diversity. For example, the large genetic diversity in North American oat is astonishing considering that the foundation of oat breeding in the first half of the 20th century was based only on a few parental landraces introduced from Europe [55]. Studying more than 10,000 oat accessions by morphological characters it was concluded that the greatest richness of diversity was observed for countries with intensive oat breeding programs [56]. Similarly, the often-reported low diversity within European oat germplasm [7,8,50–52] was not observed in the present study. On the contrary, German, French, Swedish, and Czech varieties were present in almost equal shares in all three subpopulations. This reflects the present situation in European oat breeding programs very well. Due to decreasing acreages and, therefore, decreasing income from royalties, the remaining oat breeding programs are forced to breed for broader adaptation and/or regional adaptation beyond their traditional target area. Thus, nowadays the introgression of material from other gene pools, and testing and selection in multi environment trials is a more common situation [3].

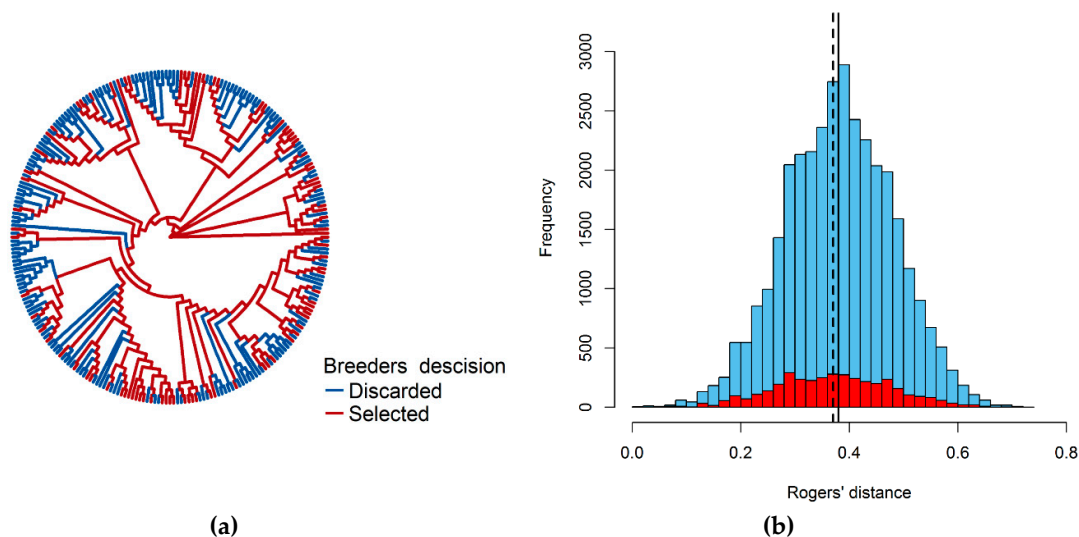
3.3. Breeders' decisions and selection for local adaptation

Considering the large diversity of oats, it was very feasible to select accessions with suitable trait combinations that were adapted to the target environments in the study at hand. The 87 most promising out of all 260 accessions were thus selected in 2014 and retested in several environments from 2015 to 2018. These accessions came from multiple origins and spanned the entire diversity of the collection, revealing no clear tendency for favoring certain genetic backgrounds when conducting selection decisions (Figure 5). Likewise, the difference in average Rogers' distance among the selected accession and the whole collection was negligible with $\Delta = 0.01$ and suggested hardly any loss in genetic diversity. However, it can be presumed that the low number of 15 polymorphic markers used in calculating Roger's distance affected this result. Notwithstanding this, a large selection gain was achieved for grain yield that amounted to an absolute value of 8.8 dt ha⁻¹ and a relative value of 19% when compared to the population average of all accessions (Table 3). It was evident from the relative importance in the retrospective selection index that grain yield was the main target when undertaking the selection decisions, followed by grain weight, grading, and lemma color. This reflects the importance of grain yield, yield stability, and primary grain characteristics for oat improvement in practical breeding and trade [3,57,58].

Table 3. Mean, range, variance components, and heritability for grain yield-related, morphological, and disease resistance traits of all 260 oat accessions across the entire trial series 2014–2018.

Trait	Retrospective selection index		Selection gain		Accuracy
	Index weight	Relative importance (%)	ΔG_{abs}^1	ΔG_{rel}	
Grain yield (dt ha ⁻¹)	+0.68	23.9	+8.84	119	0.64
1000 grain weight (g)	+0.60	21.2	+1.68	105	0.95
Test weight (kg hL ⁻¹)	-0.10	3.6	-0.69	99	0.69
Grading >2 mm (%)	-0.36	12.8	+3.74	104	0.28
Plant height (cm)	-0.15	5.5	-4.09	96	0.87
Heading (d after sowing)	+0.15	5.4	-0.69	99	0.89
Maturity (d after sowing)	-0.16	5.8	-0.72	99	0.69
Leaf blotch (1–9)	-0.05	1.6	+0.09	102	0.11
Panicle number (<i>n</i> m ⁻²)	-0.01	0.4	+8.68	102	0.55
Panicle length (cm)	-0.06	2.0	-1.01	94	0.70
Panicle shape (1–9)	-0.07	2.6	-0.04	99	0.50
Lemma color (1–9)	-0.21	7.4	-0.27	88	0.95
Glume glaucosity (1–9)	-0.01	0.3	+0.03	101	0.64
Recurved flag leaves (1–9)	+0.00	0.0	-0.10	97	0.78
Second leaf width (mm)	-0.12	4.2	-0.63	97	0.30
Stem hairiness (1–9)	+0.09	3.3	-0.15	91	0.92

¹ ΔG_{abs} : absolute selection gain expressed as observed selection differential; ΔG_{rel} : selection gain relative to the population average (100%)

**Figure 5.** Neighbor joining tree displaying the genetic relationship between the selected and discarded accessions (a) and the Rogers' distance between both sets (b). The vertical solid and dashed lines highlight the average Rogers' distance between the entire 260 (blue) and 87 selected (red) accessions.

The selection gain for grain weight could be attributed to the correlated selection response for grain yield, whereas grading appeared as an important selection criterion as most of the naked oats were discarded. For naked oats less breeding effort has been undertaken, resulting in a comparable low number of released varieties [59,60]. Therefore, this type has only regionally a niche market [57,59]. All black oats were discarded as well, as there is generally no market for this germplasm in Estonia or the Czech Republic, where the accessions were tested. Hence, merely some varieties with intermediate coloring were retained. Markedly, the average yield of black oats was 45.8 dt ha⁻¹, not considerably lower in comparison with husked oats that yielded 48.2 dt ha⁻¹ on average. Similar results were reported earlier [35].

Aside from yield components, the selection for morphological traits focused on the reduction of plant height to prevent lodging as well as stabilizing the selection for heading and maturity date to fitting into the seasonal climate conditions of the target region. The accuracy for predicting these traits was high across years and was of a similar magnitude to traits like hairiness and lemma color which are often used as DUS criteria. Prediction accuracy was medium to high for grain yield, grain weight, and hectoliter weight that allowed for achieving the high selection gain seen in this study. This selection gain was though mainly attributed to the presence of numerous low yielding accessions in the collection. The average grain yield performance of the selected accessions relative to check average was only 96.1% and with 100.2% slightly higher for grain weight, and selection decisions followed for the largest part the retrospective index (Figure 6). Nevertheless, a total of 32 accessions surpassed the check average in grain yield, while this number with 38 accessions was slightly higher for grain weight, and with 58 accessions was substantially higher for hectoliter weight. Finally, the 11 most promising accessions surpassed the checks for all three of these major agronomic traits. They were furthermore characterized by a plant height of 96.9 cm and 102 days to maturity, where the former was 1.3% lower than the check average and the latter corresponded to the check average and reflected the selection for local adaptation. Hence, several interesting accessions that represent valuable genetic resources for further oats breeding were identified in this study.

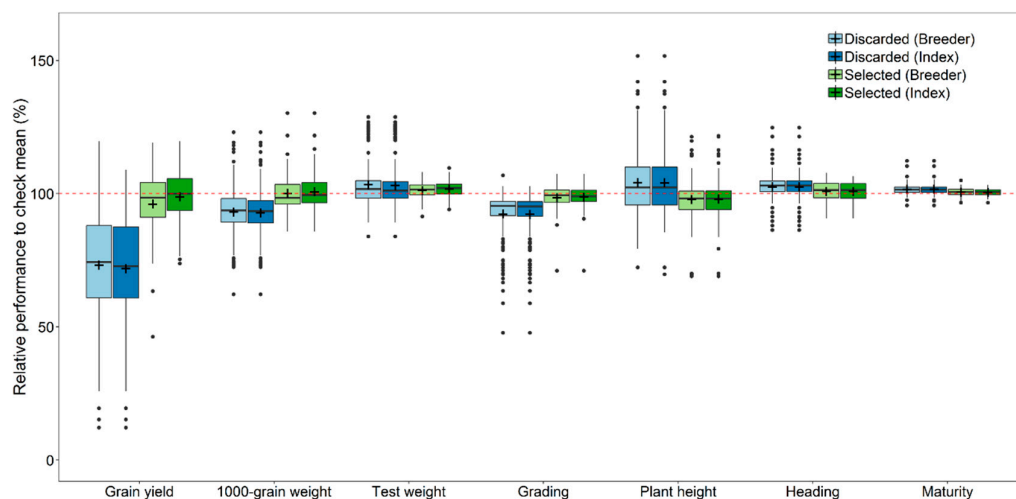


Figure 6. Relative performance relative to the check varieties of the selected and discarded accession according to the retrospective selection index and the actual selection decisions for major agronomic traits.

4. Conclusions

Oat is a valuable crop for sustainable production of cereal food and feed. To face the decreasing acreage, reduced number of breeding programs, and changing environmental conditions due to global warming, the more efficient description and exploitation of oat genetic resources is necessary to secure oat production and thereby retain agricultural diversity. Progress in yield and yield stability is of utmost importance, irrespective of the scientifically well described nutritional benefits. The present study describes the genotypic and phenotypic diversity of working collections of oat germplasm originating from 27 countries evaluated by European breeders. The presented results demonstrated a great genetic diversity in important agro-morphological traits, mainly high heritabilities and no loss of genetic diversity after one selection cycle. Compared to other cereals, the progress in grain yield from 1960 to 2005 on a global scale was smaller in oat [61], whereas in Western and Northern European countries such as Germany, grain yield improvement was comparable to other inbreeding cereals [3,57]. Information on the multiple trait performance of oat genetic resources in multi-environment trials, as presented in this study, are, together with new breeding techniques and strategies [57,62], essential for further breeding progress in oat.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Description of the employed microsatellite markers.

Data availability: The description of the investigated germplasm and BLUEs for the evaluated traits are available at <https://doi.org/10.5281/zenodo.3557367>.

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