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MORPHOLOGICAL DIVERSITY AND DNA POLYMORPHISM OF COMMON OAT (*AVENA SATIVA* L.) LANDRACES CULTIVATED IN POLAND

ABSTRACT

The aim of the work was characterization of morphological diversity and DNA polymorphism of common oat landraces. 25 Morphological traits and DNA polymorphism have been examined using AFLP methods. It has been found, that identification of oat landraces is possible based on the examined morphological traits. The examined accessions were differentiated by traits important for intraspecific taxonomy of oat: type of panicle, presence of awns, colour of grain, as well as other morphological traits, such as shape of panicle, rigidity of stem leaves, and type of awns. Relationships of morphological traits of leaves, grains, and stem with some DNA fragments suggesting presence of molecular markers of these morphological traits have been found. Morphological similarity of landraces doesn't correspond to affinity complied with DNA similarity of these objects.

distinguishing unique combination of morphological traits as well as different DNA polymorphism have been identified. They could be used in process of new varieties breeding. Complex characterization of examined objects of oat collection, collected in National Centre for Plant Genetic Resources, will make possible its effective use in breeding and research works and will facilitate management of this collection.

Key words: common oat, DNA polymorphism, landraces, morphological traits,

INTRODUCTION

The assurance of food for permanently growing human population on Earth is becoming a priority aim of the XXI century. In the face of progressing genetic erosion and parallel increase of demand for healthy and valuable food, landraces of many crop plants, included common oat, collected in a gene bank, could prove a rich source of genetic variability for new varieties breeding, responding to increasing consumers needs.

The aim of this work was characterization of morphological diversity and DNA polymorphism of common oat landraces. Complex characterization of oat collection, located at the National Centre for Plant Genetic Resources, will

make possible effective use of examined landraces in breeding and research works and facilitate management of this collection.

MATERIAL AND METHODS

Research material constituted 68 of oat landraces, collected in the four regions of the country (Northern Poland, Eastern Poland, southeastern Poland, and mountain areas) and stored in the National Centre for Plant Genetic Resources of Plant Breeding and Acclimatization Institute.

Methodology and research analysis of morphological traits

Research was conducted on 20 randomly chosen plants for each object. Twenty five morphological traits – 23 qualitative and two quantitative have been examined. Selection of the traits for morphological research was made on the basis of Słaboński (1949) monograph and oat descriptors, published by Scientific-Technical Board of the COMECON (CMEA) Countries for Collecting Wild and Crop Plant Species (1984), International Board for Plant Genetic Resources IBPGR (1985), Research Institute of Plant Production Praha-Ruzyne (1986) and PBAI and RCCT (Traczyk and Nawaracała 1988).

Estimation of the differences, relevance among the objects with respect to investigated morphological traits was done by non-parametric Kruskal – Wallis test.

To separate groups of objects similar to each other in respect of morphological traits cluster analyses were made. For calculation of Euclidean distance square $d(x, y) = \sum_i (x_i - y_i)^2$ (Sieczko 2003) mean values of morphological traits were used. Grouping was made by UPGMA method. In order to identify the traits the best differentiating of the investigated objects and to indicate eigenvectors, Principal components analysis (PCA) concerning the mean values of morphological traits was performed.

With the aim of linkage analysis of morphological traits and DNA polymorphism of the investigated objects, Spearman's rank correlation coefficient and canonical correlation coefficient were used.

Molecular research

Genome DNA analyses of 68 oat landraces were carried out by AFLP method (Vos et. al 1995). Genetic differentiation analyses of the investigated objects were conducted with application of four combinations of primers on AbiPrism 377XL apparatus. Obtained results were used to binary matrix elaboration. On the basis of this matrix Jaccard similarity coefficient was calculated for pairs of investigated landraces and phenogram of similarity of the studied objects by UPGMA method was constructed.

Canonical correlation analyses were performed with the aim of affirmation of relationship among polymorphic DNA fragments groups and morphological traits of the studied oat landraces.

Shannon-Weaver Index of diversity was calculated for morphological traits and genetic features of groups of landraces collected in the seventies, the eighties, and the nineties, and for landraces originated from four regions of Poland.

Research with application of GIS – Geographical Information System

With the aim of ascertaining of potential relationships among appurtenances of landraces to geobotanical departments of Poland, represented potential flora, (Matuszkiewicz *et al.* 1995), and morphological traits or DNA traits of studied landraces, cluster analysis has been conducted.

With the aim of studying of relationships between diversity of morphological traits of the examined landraces and environmental conditions, and among DNA polymorphism of those landraces and environmental conditions, the following variables were used: longitude, latitude, altitude, annual sum of rainfall, potential evapotranspiration, annual water balance, average year temperature, weighted index and valorization index of agricultural space of production (VIASP) (Witko 1981).

Relationships between variables characterizing collection places of landraces and morphological traits, and DNA polymorphism of those landraces, were estimated by Spearman Rank Correlation Test.

RESULTS

Botanical and morphological diversity of *Avena sativa* landraces

Among the studied landraces 25 objects were homogeneous botanical varieties; 15 of them belonged to *aurea* varieties. Seven of them belonged to *flava* varieties. Three landraces belonged to *mutica* varieties. Remaining 43 landraces were compounds of different botanical varieties.

Among the studied landraces, plants characterized by all types of growth habit were observed: erect, semi-erect, intermediate, semi-prostrate, and prostrate. Uniform landraces with respect to this trait were not found: Each object was characterised by two, three, sometimes even four types of growth habit. The most uniform in this respect was landrace PL51604 with 95% individuals with prostrate type and landrace PL51610 with 95% individuals with semi-prostrate type. Different classes of hairiness of sheaths were observed among individuals of nine landraces.

Two landraces (PL51610 and PL52265 –in 95%) were left-handed. 14 landraces were not uniform with respect to this trait; they were left and right-handed. Remaining 52 landraces were right-handed. Among the studied objects strong differentiation with respect to intensity of leaves rotation was found. Presence of plants with weak rotation of leaves (about 1/2 of turn), medium (about 1/3 of turn), strong (about 1/4 of turn) and very strong (the whole turn) was observed. The most frequent were plants with strong rotation of leaves.

Five types of blade leaves growth were distinguished: erected, bent, poorly pendulous, pendulous and strongly pendulous. The most of objects had at least

two type of leaves growth: strongly pendulous and pendulous leaves. Individuals with strongly pendulous leaves were found in all landraces excluding PL51521. Homogenous with respect of this feature was originated from Lublin district landrace PL50438. In the studied landraces, three types of angle to culm of leaves (acute, intermediate, and obtuse) were noted, with decided prevalence of intermediate angle. 50 Landraces had all leaves pendulous. Remaining objects were characterized by different types of angle of flag leaf to culm. Hairiness of leaf margin was present in 14 landraces.

Among the studied objects three colours of leaves were affirmed: light green in PL52351 landraces, green in six landraces and bottle green in remaining landraces. As opposed to leaf colour, most of all populations (62) had green colour of panicle. Light green colour of panicle was present in six populations.

Seven landraces had all the highest nodes glabrous. Among remaining objects different degrees of hairiness were observed.

In the studied material three types of panicle shape were distinguished. Unilateral type of panicle characterized seven landraces. Six objects were heterogeneous in respect of the type of panicle: PL51603, where 80% individuals had equilateral panicle and the rest had intermediate panicle, three populations with dominant equilateral panicle and remaining individuals with unilateral panicle, and two landraces with dominant unilateral panicle and remaining individuals with equilateral panicle. Remaining 55 landraces were characterised by equilateral type of panicle.

Depending on attitude of branches of panicle, 9 landraces with erect panicle were distinguished. Besides, four objects with bushy panicle, six populations with pendulous type of panicle, and landrace PL52191 with strongly pendulous panicle were observed. All studied oat landraces were characterized by drooping erection of spikelets.

The most frequent (45 objects) were yellow grains oats. White grains were present in 13 objects. Landrace PL50613 had grey colour of grains. Within the limits of two populations, white and grey grains were observed. Six landraces had grains with white and yellow colour of grain. Landrace PL51616 contained white, yellow and grey kernels. Most objects had plants with two grains in spikelet, but only in 11 landraces in 100%. Less numerous were plants with three grains in spikelets. In 21 populations occurred single grains in spikelets.

Number of storeys of branches in the panicle has fluctuated from four to nine. The most frequent were panicles with seven and six stages. Among the studied objects 33 landraces were awnless. Rest of the objects were to slight extent awned. Among them a big differentiation of awn types was observed. The most numerous were landraces with weak awns on seeds. The strong awns were present only in nine landraces.

Objects were characterized by big variability of hairiness at basal part of the primary grain. 22 landraces had glabrous basal part of the grain. The most frequent were weak hairiness of grain base. Medium hairiness of grain base was

observed in 13 landraces. Strong hairiness of grain base had 5 landraces and very strong – were presence in 4 landraces. All landraces had glabrous lemmas.

Hairiness of a lemma was absent in 56 landraces. Medium hairiness was observed in 5% individuals of PL50556, and weak- in 12 landraces. Hairiness of a rachilla was absent in most of objects. Medium hairiness was observed in six landraces, and weak – in 12 objects. Besides, the studied objects weren't homogenous with respect to length of rachilla. 66 Landraces had grains with medium – long rachilla. Short rachilla, with a different frequency, were observed in 57 landraces. Long rachilla were in 11 landraces. Very short rachilla were present only in six landraces.

Three shapes of grain base were distinguishing: convex, intermediate and flat. Grains with flat grain base were observed in 12 landraces. Other objects had shape of grain base convex and intermediate. Four shapes of grain were distinguished. The convex grains were present only in PL50554 landrace. In the remaining objects intermediate, slender or pointed shapes of grain were observed.

Some morphological traits are found only among landraces: hairiness of sheaths, prostrate and half- prostrate type of growing of young plants and hairiness of leaf margin.

Estimation of the significance of differences between objects in relation to the studied morphological traits was conducted by the Kruskal-Wallis nonparametric test. All differences were statistically significant at the 99%confidencelevel.

Numerical and statistical analysis of morphological traits.

Cluster analysis of 68 oat landraces has proved, that mutual similarity of the studied objects is compatible to a large degree with their botanical classification. Yellow – grain, white – grain and yellow – grain objects with unilateral panicle were grouped in separated clusters. (Fig. 1). The obtained dendrogram confirmed uniqueness of phenotypes of PL51604, PL52351 and PL51610 landraces. Similar image of relationship between landraces were obtained conducting principal component analysis (Fig. 2). Landraces with rare and unique combination of morphological traits formed groups the most remote from the others ones.

DNA polymorphism of oat landraces

For oat landraces four combination of AFLP primers which differentiated the studied objects were obtained. Landraces were characterised by 68 polymorphic DNA fragments.

Cluster analysis of 68 oat landraces performed on the basis of DNA polymorphism has proved grouping of objects only partly according to their botanical affinity. Symptomatic is often a close similarity of objects belonging to the different botanical varieties. Landrace PL52351 -POLNAR99-45, originated from the Narew Valley, differs the most from the all landraces with regards to

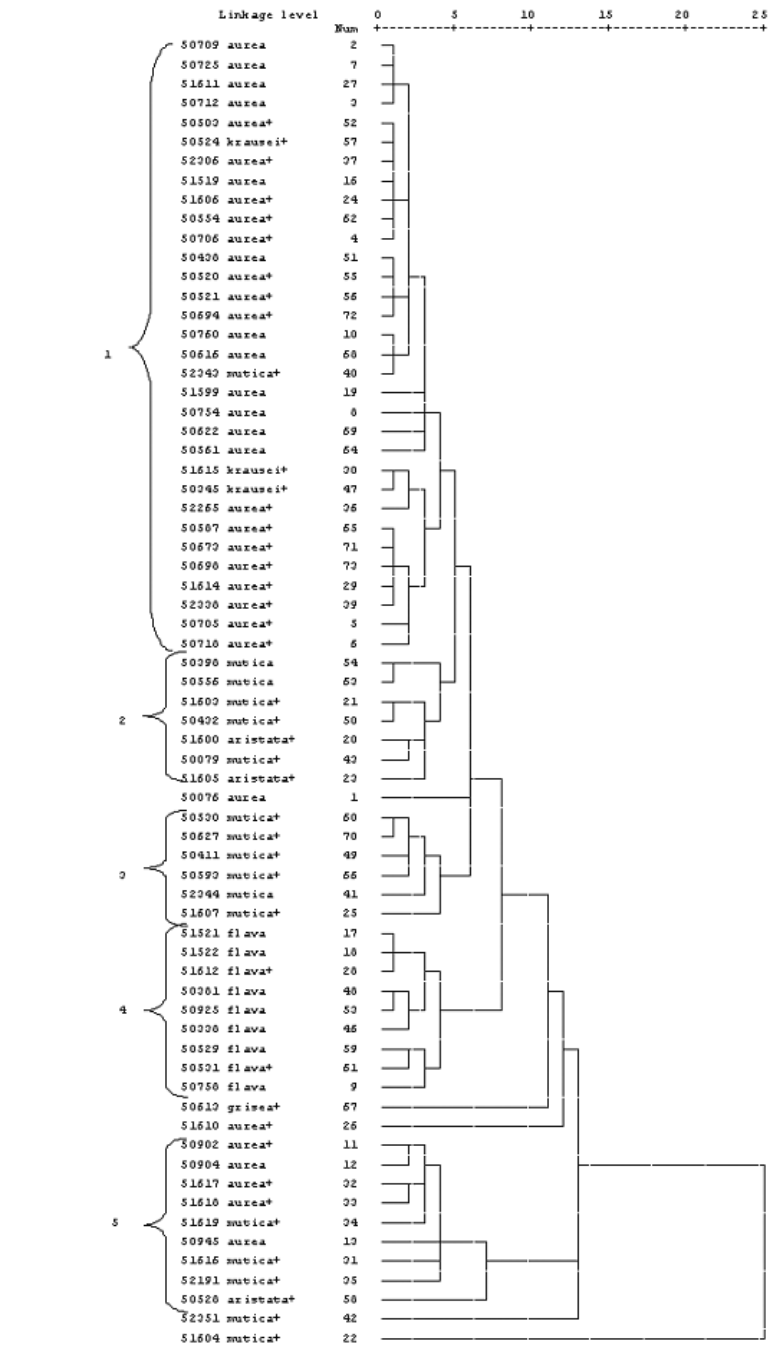


Fig. 1. Phenogram of morphological similarity of 68 oat landraces.
Square of the Euclidean distance / UPGMA method

the morphological traits, was also the most distinct with respect to DNA polymorphism.

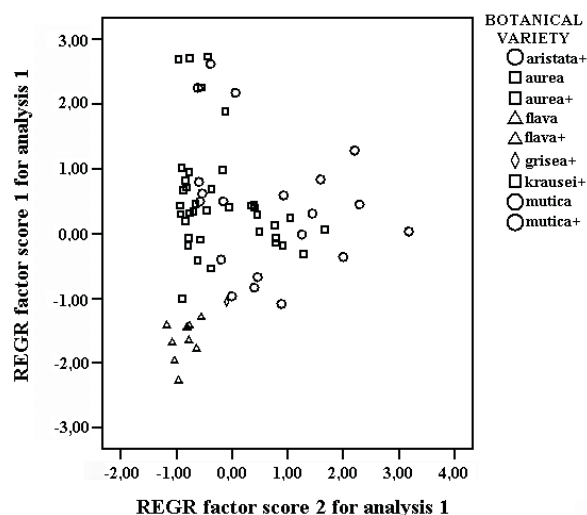


Fig. 2. The distribution of oat landraces on the level of the first two principal components

Relationship between variability of morphological traits and variability at the DNA level

Correlation analysis of morphological and genetic traits of the studied objects was performed by Spearman nonparametric test and revealed significant correlations among 13 studied morphological traits and 99 polymorphic DNA fragments. These correlations were statistically significant at the confidence level $P = 0.01$ or 0.05 .

Canonical correlation analysis revealed relationship among groups of polymorphic DNA fragments and morphological traits of the studied oat landraces. Correlation of DNA fragments with canonical variable was weak. Correlation coefficients reached values from 0.5 to 0.7 only for the seven canonical variables with seven morphological traits: hairiness of leaf margin, leaf colour, shape of panicle, type of panicle, colour of panicle, colour of grain and angle of flag leaf to culm. 12 to 45 DNA fragments have influenced the values of the described canonical variables.

Relationship between variability of morphological traits and environmental factors

Analyses of Spearman correlation coefficient values for morphological traits and variables characterizing collecting places of the studied landraces have proved relationships among the studied traits. These correlations were statistically significant at the confidence level $P = 0.01$ or 0.05 for 21 morphological traits and all the environmental variables.

Relationship between DNA polymorphism and environmental conditions

Analyses of Spearman correlation coefficient values of DNA polymorphism and variables characterizing collecting places of the studied landraces have proved weak relationships among the studied traits. From all the 118 polymorphic DNA fragments, only 25 revealed significant correlations with environmental conditions. The highest values of the correlation coefficient occurred for DNA fragment indicated by number 14, with annual water balance and annual sum of rainfall on the 0.32 level and with potential evapotranspiration on the 0.39 level.

Morphological and molecular variability of oat landraces in relationship with region of origin against the background of geobotanical regions of Poland

Collecting places of oat landraces were located in six, from nine existing geobotanical regions of Poland. Cluster analysis, grouping landraces according to similarity of morphological traits, revealed weak relationships of characteristic combinations of morphological traits with origin of landraces from particular geobotanical region of Poland.

Similar result was obtained on the basis of DNA cluster analysis.

Diversity Indexes

Values of Shannon - Weaver diversity index for morphological traits of landraces collected in three decades, and average coefficient, including all traits for each decade and coefficient for DNA fragments, did not reveal significant differences. Values of Shannon - Weaver diversity index for morphological traits of landraces collected in four regions of Poland were also similar. Values of diversity index for DNA fragments of the studied objects were the lowest for landraces originated from eastern Poland and the highest for mountain landraces.

DISCUSSION

Botanical differentiation of *Avena sativa* landraces and factors determining them

In the studied material seven botanical varieties were found. These were botanical varieties belonging to *convarietas sativa* and *orientalis*. Varieties belonging to subspecies *nudisativa* were not found. Oat varieties (*A. sativa* ssp. *sativa*) cultivated in Poland differ from each other mainly by grain colour. The prevailing colour is white and yellow.

Yellow grain varieties were always more willingly cultivated by farmers than white grain ones (Arseniuk *et al.* 2003). They are mature mostly earlier, so they are less attacked by blight. According to Lewicki and Mazurek (1967) yellow oat varieties are more appropriate for a medium and poor soil. They are characterized by higher resistance to drought than white grain varieties. However, the last ones, more fertile and better exploiting rich soils, were preferred by breeders (Saloni 1956).

Frequency of occurrence of botanical varieties within the studied landraces is related to agricultural values which these varieties present. According to Sawicki (1975), the highest agricultural value characterized yellow and white grain oat varieties. These varieties were also the most frequently collected during expeditions. Varieties with unilateral panicle were rarer found and the rarest were primitive oat forms with brown and grey colour of grain.

The observed botanical variability of oats landraces was also similar to botanical variation of varieties, historically cultivated on the Polish territory. Botanical compositions of the studied oat populations confirmed Mordvinkina observations (Rodionova *et al.* 1994), that the botanical varieties characteristic for this region are *mutica*, *aristata*, *aurea*, *flava*, more rare are *cinerea* or *obtusata*.

Variability of morphological traits of *Avena sativa* landraces

The most determining reciprocal similarity of landraces are taxonomically important traits (Słaboński 1949). Only objects with traits rarely found among studied individuals, as strong hairiness of sheaths of the lowest leaves, untypical colour of panicle and leaves, do not belong to clusters of the same or close botanical varieties (Fig. 1).

The results obtaining from principal component analysis (Fig. 2) are confirming the results of cluster analysis, reveal the most differentiating traits of the studied objects – type of panicle, awn presence, colour of grain – that are taxonomically important features (Mordvinkina 1936), and also attitude of branches, type of growing of blade leaves and type of awns – traits the most correlated with principal components. 40% of variation explained by principal components showed high significance of morphological traits, with them these components are connected, in differentiation of oat landraces.

Correlations of the morphological traits of landraces with variables characterizing of environment in the places of collecting, in spite of their significance, had low or medium values. The highest values of the correlation coefficient were obtained for the trait of “glabrous basal part of the primary grain” with annual rainfall sum and annual water balance (negative correlations) and latitude (positive correlations). It can mean, that in regions with low water availability oat forms with glabrous basal part of the primary grain had selective advantage. Negative correlations of white colour of grains with potential evapotranspiration confirmed opinion of Lewicki and Mazurek (1967), that white grains varieties are characterized by lower drought resistance than oat varieties with yellow colour of grain. These results can suggest, that factors commonly regarded as influencing the growth, development and oat yield, such as water availability, influence also indirectly presence of some morphological traits.

DNA Diversity of *Avena sativa* and factors determining them

Among studied landraces high polymorphism of DNA has been observed. Values of Shannon - Weaver diversity index for DNA traits of landraces were high, what confirm their big genetic diversity. It is to be supposed, that besides of adaptations to local conditions, in which landraces have evolved, their polymorphism was also influenced by random genetic drift.

Relationship between DNA variability and environmental features in the places of landraces collecting was low. It was noticeable only for the landraces collected in mountain region. The high values of correlation coefficients between DNA fragments and environmental parameters characterizing water accessibility for plants indicate existence of molecular markers connected with water plant management.

Relationship between morphological diversity and DNA polymorphism of *Avena sativa* landraces

Relationship between morphological diversity and DNA polymorphism of *Avena sativa* landraces is very poor. As resulted from the conducted research landraces originated from the same region (for example Łapszanka village) were characterized by high morphological similarity. However, analysis of DNA polymorphism showed considerable differences between these landraces.

On the basis of Spearman correlation analysis of morphological traits with polymorphic DNA fragments of the studied landraces, we can presume, that describing DNA fragments can play role of markers of some morphological traits.

Analysis of the canonical correlations confirmed relationship between some DNA fragments with morphological traits. Such features as hairiness of leaf margin, colour of leaves, colour of grain, have been correlated with canonical variables. Other traits, as type of growth, intensity of rotation, and hairiness of lemma, significant in Spearman correlation analysis, did not prove connection with canonical variables. However, there were not such traits correlated with canonical variables which were not significant in Spearman correlation analysis. This fact confirms usefulness of Spearman method, as a basic tool in studying relationship of morphological traits with DNA profiles.

Morphological and DNA differentiation of *Avena sativa* on the background of geobotanical regions of Poland

Oat landraces originated from the same or neighbouring geobotanical regions of Poland were morphologically similar. The obtained results have confirmed existence of the ecological groups of crop plants (Mordvinkina 1936), related to particular soil-climatic conditions. However, only 40% of landraces originated from the same or neighbouring geobotanical departments of Poland were similar in respect to DNA. Thus, as opposed to morphological diversity, DNA variability doesn't reveal connection with features of natural habitats lo-

cated in geobotanical regions described by Matuszkiewicz and co- authors (1995).

Importance of the work results for gene bank and breeding

Topic of the work is concentrated on basic problems concerning plant genetic resources conservation, such as: studying of affinity, identification of objects and rationalizing of collection by duplicates analysis.

Results of the present work could be particularly useful during setting up of core collection of oat. Creation of such collections belongs to the gene banks priority (Bhattacharjee 2002). In studied materials a number of landraces characterized by unique combination of morphological and genetic traits was found (landraces PL51604 (E 1490), PL51610 (E1485), PL52351 (POLNAR99-45), PL 50076 (Stopka) and PL50613 (E0179)). Landraces collected in mountain region, as objects characterized by the most morphological diversity and the greatest DNA polymorphism deserve for particular attention. These objects can constitute a valuable initial material in breeding works. AFLP method of DNA analysis has proved useful as a tool improving precision of variability description in oat collection, and particularly enabling to prove uniqueness of some landraces.

CONCLUSIONS

- identification of oat landraces is possible based on examined morphological traits,
- examined landraces were differentiated as well by traits approved as important for intraspecific taxonomy of oat: type of panicle, presence of awns, color of grain, as well as other morphological traits,
- correlation of morphological traits with selected DNA fragments suggests presence the of molecular markers of this morphological traits
- DNA analyses by AFLP method allow to distinguish oat landraces, being a precious tool for their identification,
- morphological similarity of oat landraces is not related with DNA similarity of these objects,
- landraces distinguishing unique combination of morphological traits as well as different DNA polymorphism have been identified.

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