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

ABSTRACT

The aim of the work was characterization of morphological diversity and DNA polymorphism of common oat breeding varieties. 25 morphological traits and DNA polymorphism have been examined using AFLP and RAPD methods. It has been found, that identification of oat breeding varieties 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, 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 breeding varieties doesn't correspond to affinity complied with DNA similarity of these objects.

Breeding varieties 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 the examined objects of oat collection, maintained in the National Centre for Plant Genetic Resources of Plant Breeding and Acclimatisation Institute, will make possible its effective use in breeding and research works and will facilitate management of this collection.

Key words: breeding varieties, common oat, DNA polymorphism, morphological traits

INTRODUCTION

The old varieties of crops, stored in gene banks in form of seeds, still could play an important role in breeding programs. The old oat varieties, cultivated many decades ago, are still valuable for organic farmers. They are also attractive for breeders, who claim for the lack of divers materials suitable for crossing aims.

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

Resources, will make possible effective use of examined varieties in breeding and research works and facilitate management of this collection.

MATERIAL AND METHODS

Research material constituted 64 of oat breeding varieties, cultivated in Poland in XX century and stored in the National Centre for Plant Genetic Resources of Plant Breeding and Acclimatization Institute. Among them were varieties bred in a period before the I World War (I), interwar period (II), and after the II World War to the 60. (III) and 70. - 80. (IV).

Research was conducted on 20 randomly chosen plants for each object. Twenty five morphological traits – 23 qualitative and two quantitative have been examined.

Genome DNA analyses of 64 oat breeding varieties, were carried out by RAPD (Williams *et al.* 1990) and AFLP method (Vos *et al.* 1995).

Details of methodology of analysis of morphological traits and molecular research has been described by Nowosielska and Nowosielski (2008).

Shannon-Weaver diversity index was calculated for morphological traits and genetic features of breeding varieties originated from four periods of breeding.

RESULTS

Botanical and morphological diversity of *Avena sativa* breeding varieties

Among the studied breeding varieties 38 objects were homogeneous botanical varieties. Most of them belonged to *aurea* variety. Seven of them belonged to *mutica* variety, four to *flava* variety. Two objects belonged to *grisea* variety and Tatarski variety belonged to *sativa* one. Pszenicznik, having a brown lemma, appertain to *sativa* and *montana* varieties. Zielony variety, characterised by white lemma and unilateral panicle, belonged to *obtusata* and *tartarica* varieties. Remaining 26 breeding varieties were compounds of different botanical varieties.

Among the studied objects, plants characterized by all types of growth habit were observed: erect, semi-erect, intermediate, semi-prostrate, and prostrate. Uniform varieties 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 Proporczyk variety, where 95% of plants were represented by an intermediate growth habit. Every studied breeding varieties has naked spathe of leaves.

Three objects: Borek, Pszenicznik and Podkowa Dłużewski varieties, were left-handed. Twelve varieties were not uniform with respect to this trait: they were left and right-handed. Remaining 49 varieties were right-handed. Among the studied objects strong differentiation with respect to intensity of leaves turn was found. Presence of plants with weak rotation

of leaves (about $\frac{1}{4}$ of a turn), medium (about $\frac{1}{2}$ of a turn), strong (about $\frac{3}{4}$ of a turn) and very strong (the whole turn) was certified. The most frequent was state of strong turn of leaves, which prevailed in 70 – 95% in Tatarski, Kościelecki, Ułan, Płatek, Leniak, Kanarek Mikulicki, Lach, Dupkowski, Ligowo Sielecki, Puławski Wczesny, Ozimowy and Pomorski Żółty varieties. Five types of blade leaf growth were distinguished: erected, bent, poorly pendulous, pendulous and strongly pendulous. Most of the objects were characterized by at least two types of leaf growth: strongly pendulous and pendulous. Individuals with strongly pendulous leaves were found in all varieties. The highest frequency of erected blade leaves (45%) and bent ones (35%) was present in Podkowa Dłużewski variety. In the studied varieties, three types of angle to culm of leaves (acute, intermediate, and obtuse) were noted, with decided prevalence of an intermediate angle, which was noticed in 90% individuals of Tatarski, Borek and Lubelski varieties. Obtuse angle of leaves were present in 75 to 95% range in Zielony, Pomorski Późny and Przebój I varieties. 85% individuals of Leniak variety were characterized by acute angle of leaves.

Setting of flag leaf to culm is a characteristic feature of variety after heading. 39 breeding varieties had all leaves pendulous. Remaining objects were characterized by different types of angle of flag leaf to culm. Hairiness of oat leaf margin most of all is present on lower and middle leaves. In the studied objects hairiness of leaf margin were present in five breeding varieties. The most “hairy” object was Markus variety.

Among the studied objects two colours of leaves were affirmed: green in 12 varieties and bottle green in the remaining ones. As opposed to leaf colour, most of all varieties (59) had green colour of panicle. Light green colour of panicle was present in four varieties: Leniak, Grodkowicki Biały, Dupkowski i Biały Mazur. Bottle green colour of panicle was present in Podkowa Dłużewski variety.

Seventeen breeding varieties had all the highest nodes glabrous. Among the remaining objects different degrees of hairiness were observed. Strong hairiness of nodes (from 5 to 25% individuals) was characteristic for Rychlik Kozarowski variety. Very strong hairiness was observed only on one plant of Jeżewski variety and on 8% individuals of Hrywak one.

In the studied material three types of panicle were distinguished. Unilateral type of panicle characterized five breeding varieties: Huzar Oryginalny, Grzywacz Późny Wołyński, Hrywak, Saski and Zielony. Intermediate panicle was present in four varieties: Sołacki Wczesny, Puławski 292, Rychlik Oberek and Rychlik Trybański. One object was heterogeneous in respect of the type of panicle: It was Leniak variety, where 95% of individuals had an unilateral type of panicle and rest of them an equilateral. Remaining 53 breeding varieties were characterised by equilateral type of panicle.

Depending on attitude of branches of panicle, seven breeding varieties with erected panicle were distinguished (Huzar Oryginalny, Grzywacz Późny

Wołyński, Hrywak, Saski, Udycz 100, Boruta and Zielony), as well as four objects with bushy panicle (Jeżewski, Piaskowy, Niemierczański and Grodkowicki Biały). Most of objects (49) had a branchy shape of panicle. All the studied oat breeding varieties were characterized by drooping erection of spikelets.

The colour of grains was not an uniform feature. The most frequent (31 objects) were yellow grains oats and white grains (24 objects). Two varieties had grey colour of grains (Udycz Biały, Rychlik Kozarowski) and two had brown colour of kernels (Tatarski and Pszenicznik). Among individuals of Kolumb variety, plants with white or grey grains were observed. Four varieties (Leniak, Perona, Kanarek Mikulicki and Dupowski) had grains of white and yellow colour. Most of objects had plants with two grains in spikelet, but only in 8 varieties in 100%. Three varieties (Puławski Najwcześniejszy, Lach and Markus) were always characterized by plants with three grains in spikelets. In 21 varieties single grains occurred in spikelets, in range from 5 to 30%.

A 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 a different types of awns were observed. 40 varieties were awnless. Rest of the objects were to a slight extent awned. The most numerous were breeding varieties with weak awns on lemma, for example Podkowa Dłużewski variety. Plants with seeds with medium awns were considerably the rarest. They were present in 26 varieties. Most of them were noticed in Sobieszyński (85%), Ozimowy (63%) and Sołacki Wczesny (40%) varieties. The strong awns were present only in eight varieties, most of them were observed in Sołacki Wczesny (45%) variety.

Objects were characterized by a big variability of hairiness at basal part of the primary grain. Ten breeding varieties had glabrous basal part of the grain. The most frequent were weak hairiness of grain base, but only Piaskowy variety was uniform with respect of this feature. Medium hairiness of grain base was observed in 21 varieties. Strong hairiness of grain base had 8 varieties (The highest frequency 50% in Tatarski and Jeżewski variety) and very strong – were present in two varieties - Rychlik Trybański – 17.6% and Tatarski – 10% of individuals).

Thirty-nine varieties were without hairs on lemma. Medium hairiness was observed in Tatarski variety (5% of individuals), and weak in remaining 25 breeding varieties, with a frequency from 5% to 94% in Ozimowy variety. Hairiness of a rachilla was present only in 8 varieties. Medium hairiness was present in 5 objects and weak hairiness in 17 varieties, the most often in Jagiełło and Piaskowy varieties. Besides, the studied objects weren't homogenous with respect to length of rachilla. 61 breeding varieties had grains with medium – long rachilla. Short rachilla, with a different frequency, were observed in 49 varieties. Modzurowski variety was only object, homogenous with respect to this feature. Long rachilla occurred in

14 varieties, with frequency from 55% in Rychlik Trybański to 85% in Piaskowy. Very short rachilla were present only in 11 varieties, the most frequent in Lach (65%) and Boruta (45%).

Three types of shapes of grain base were distinguished: convex, intermediate and flat. Grains with a flat grain base were observed in five varieties (46% of Puławski Wczesny individuals). Other objects had shape of grain base convex and intermediate. Three shapes of grain were distinguished: intermediate, slender or pointed shapes. Slender shape was present in individuals of 63 varieties, but only Udycz Biały and Markus were homogenous with respect to this features. Pointed shape was noticed in 47 varieties, with frequency from 5% to 95%. Intermediate shape of grain has occurred in 43 varieties; the most frequently in Podkowa Dłużewski variety.

Estimation of 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% level of confidence.

Numerical and statistical analysis of morphological traits.

Cluster analysis of 64 oat varieties has proved, that mutual similarity of the studied objects was 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 Pszenicznik, Podkowa Dłużewski and Leniak varieties. Similar image of relationship between varieties were obtained conducting principal component analysis (Fig. 2). Varieties with rare and unique combination of morphological traits formed groups the most remote from the others ones.

DNA polymorphism of oat varieties

Four combination of AFLP primers which differentiated the studied objects were obtained. Varieties were characterised by 66 polymorphic DNA fragments.

Cluster analysis of 64 oat breeding varieties 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. DNA analysis have revealed for example, uniqueness of Pszenicznik and Modzurowski variety.

Analysis of 64 varieties similarity depending on breeding period have proved, that breeding varieties from inter-war period and varieties bred directly after second world war are similar to each other with respect to DNA profiles (Fig. 3). However varieties selected before the I World War and developed in period 70. to 80. are definitely different from other varieties and from each other.

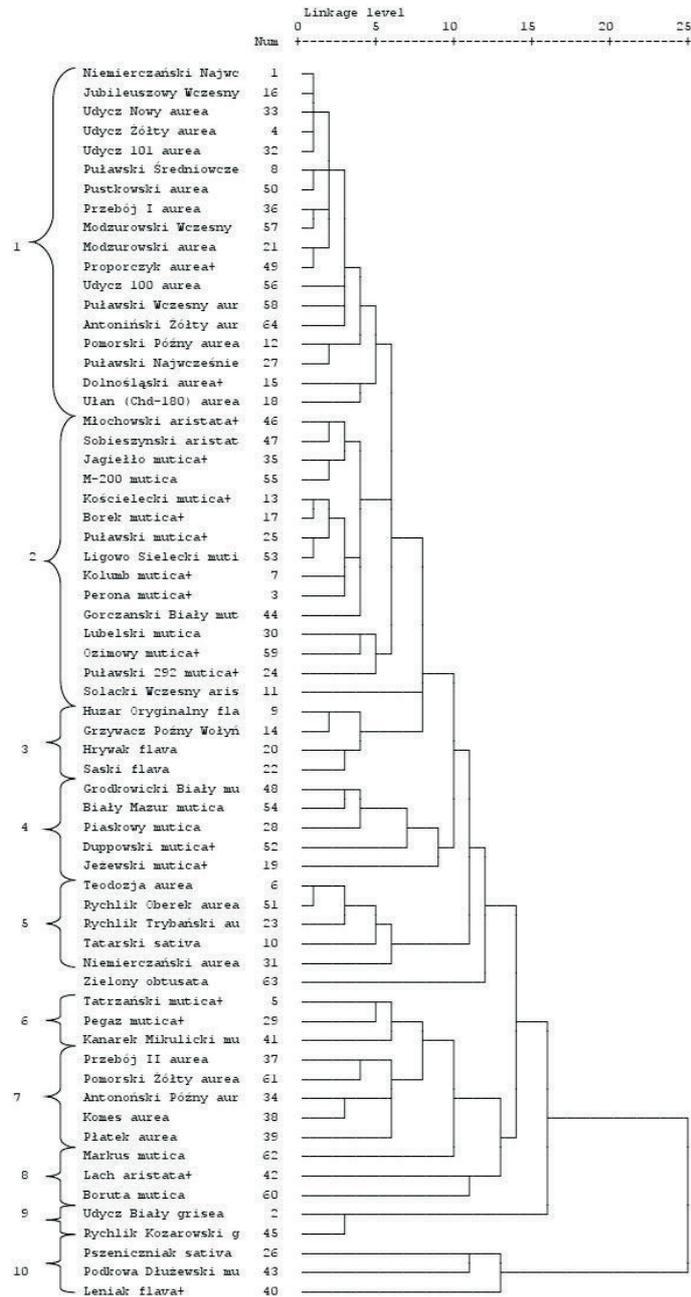


Fig. 1. Phenogram of morphological similarity of 64 oat breeding varieties. Square of the Euclidean distance / UPGMA method. (* + means presence of additional botanical varieties within prevalent variety)

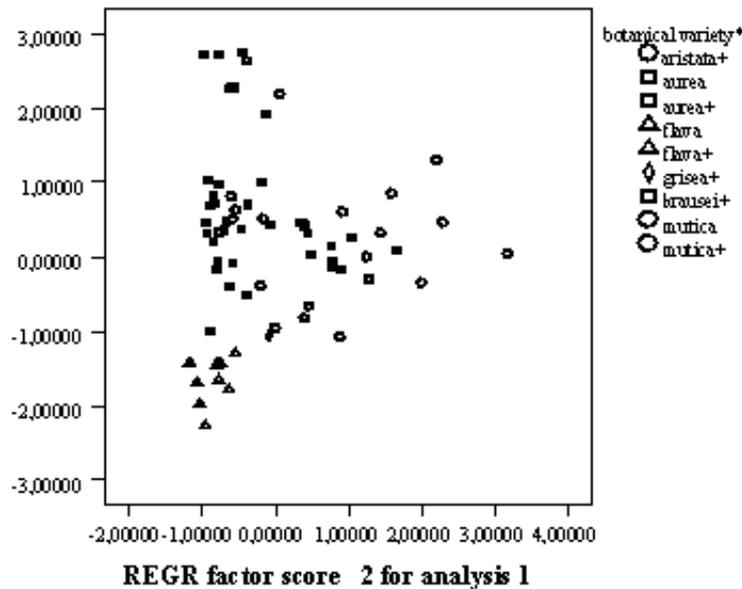


Fig. 2. The distribution of oat breeding varieties on the level of the first two principal components.
* + means presence of additional botanical varieties within prevalent variety

From 33 used RAPD primers, only 3 have generated polymorphic stripes, which differentiated the studied objects. Varieties were characterised by 32 polymorphic DNA fragments. Cluster analysis of 64 oat breeding varieties performed on the basis of DNA polymorphism has proved grouping of objects only partly according to their botanical affinity (Fig.4). Besides, the similarity of objects recognized by RAPD method was completely different from similarity obtained by AFLP techniques.

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 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. From 12 to 45 DNA fragments have influenced on the values of the described canonical variables.

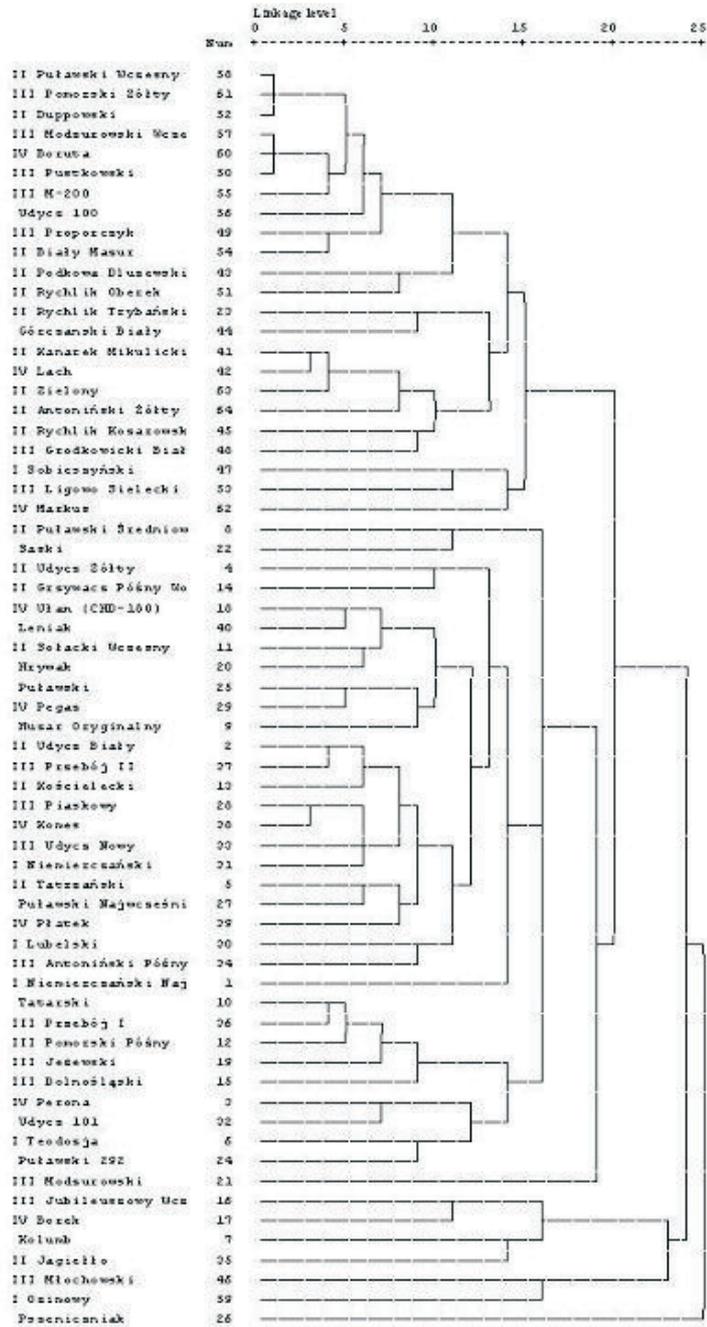


Fig. 3. Phenogram of DNA similarity of 64 oat breeding varieties depending on breeding period. Jaccard coefficient/UPGMA method.

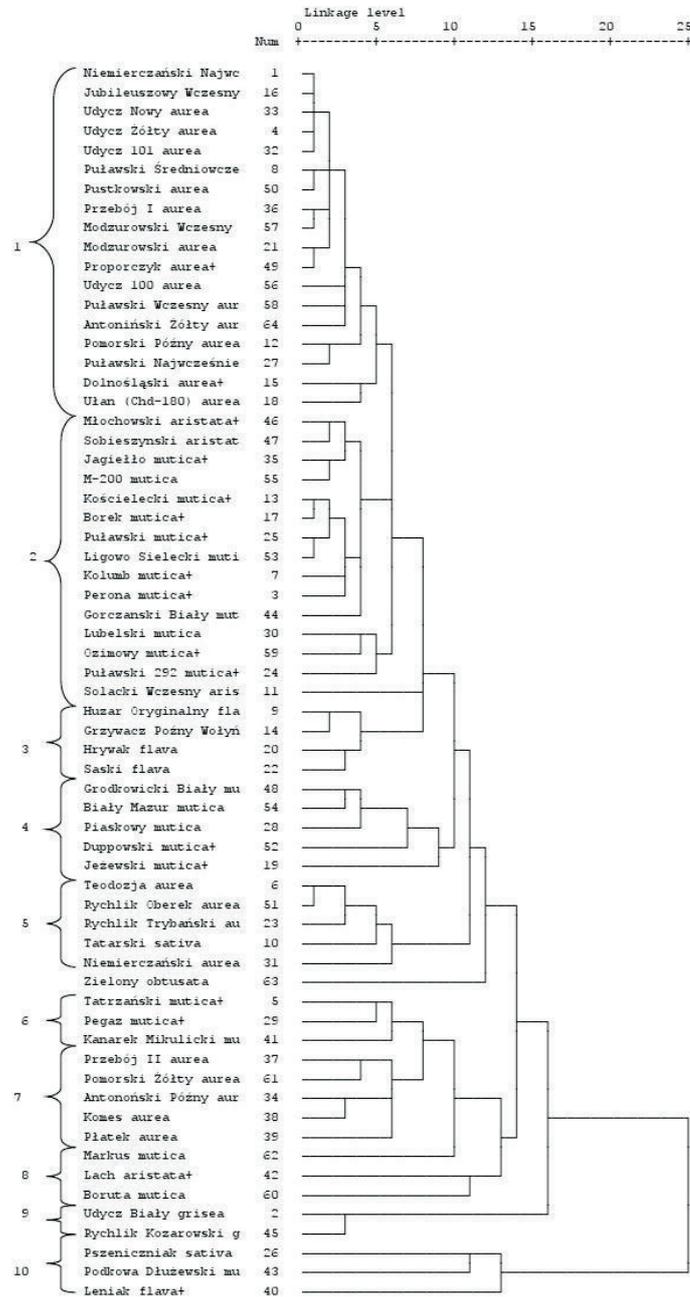


Fig. 4. Phenogram of DNA similarity of 64 oat breeding varieties. Jaccard coefficient/ WARD method.

DISCUSSION

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

In the studied material ten 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. Varieties characterised by those colours were preferred by farmers and breeders, according to their good yields and a higher resistance to diseases and environmental stress ((Saloni 1956), Lewicki and Mazurek (1967), Sawicki (1975), Arseniuk *et al.* 2003, Nowosielska and Nowosielski 2008). Botanical compositions of the studied breeding varieties of oat confirmed Mordvinkina observations (Rodionova *et al.* 1994), that the botanical varieties characteristic for Middle-East of Europe, where Poland is located, are *mutica*, *aristata*, *aurea*, *flava*, more rare *cinerea* or *obtusata*.

Variability of morphological traits of *Avena sativa* varieties

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

The results obtained from principal component analysis (Fig. 2) are confirming the results of cluster analysis, revealing the most differentiating traits of the studied objects – type of panicle, awn presence, colour of grain – that are taxonomically important features (Mordvinkina 1936), and shape of panicle, 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 big significance of morphological traits, with them these components are connected, in differentiation of oat varieties. Principal component analysis indicated also a big influence of traits connected with plant habit for differentiation of breeding varieties. The traits describing shape of leaves were the most correlated with the first principal component.

Values of Shannon - Weaver diversity index for morphological traits of varieties bred during four periods, and average coefficient, including all traits for each period and coefficient for DNA fragments, did not reveal significant differences. Average coefficient for morphological traits was the lowest for the varieties bred before the I World War and the highest for varieties from inter-war period. The highest values of Shannon - Weaver diversity index was obtained for feature “intensity of leaves turn” (1.027).

Values of Shannon - Weaver diversity index for DNA fragments of the studied objects from four periods of breeding has been systematically falling (excluding varieties from the last period).

Analysis of the morphological diversity of varieties depending on period of breeding has proved lack of morphological similarity of varieties descending before the I World War (period I). On this basis and on the basis of their pedigrees we can suppose, that they were bred from genetically distant initial varieties. 70% varieties bred between the two World Wars and after II World Wars to the 60. are morphologically similar. This fact can suggest some restriction of the gene pool of initial materials, took to breeding in this two periods. Lower morphological similarity among varieties was found in the group of the varieties, bred in the 70. and the 80. Higher values of Shannona – Weaver index also confirm the extension of varieties variability, bred in the last period, in comparison with varieties bred in the previous period. Doubtless we can see here influence of foreign varieties, which have been taken to crossing. Nevertheless, the most distant are old varieties bred before I World War, with uniquely combinations of morphological traits.

DNA Diversity of *Avena sativa* varieties and factors determining them

As a result of DNA variability has been proved, that description of diversity obtained by RAPD method differs from description obtained by AFLP one. Result of cluster analysis made for DNA polymorphism of breeding varieties confirms conclusions obtained from morphological research. The oldest varieties, bred before the I World War (I period), are not similar to each other in the respect of DNA polymorphism. Although almost 80% varieties bred during inter-war period (II period) and during after-war period to the 60., are similar to each other in the respect of DNA variability. After war to the 60. breeding is a continuation of the works on the same initial materials (Spiss 2003). On the other hand, varieties originated from the 70. and the 80., are very differentiated in the respect of DNA polymorphism.

Relationship between DNA polymorphism of *Avena sativa* varieties generated by ALFP and RAPD methods

View of polymorphism of 64 oat varieties, obtained based on RAPD method (Fig.3) differed in significant way from pictures of polymorphism revealed by AFLP technique. Only for very few varieties genetic similarity by RAPD has been proved and then this result has been confirmed by AFLP method. Despite that most of molecular methods are regard as credible and repeatable, in literature we can find information, that results obtained by differ methods are do not correspond with each other (Li at all. 2000). Demissie et all. (1998) having studied barley landraces from Ethiopia, ascertained also lack of meaningful correlation between results obtained by molecular method RFLP, isoenzymes method and morphological method. According to those authors, these methods apply to study distinct areas of

genome, with the very different steps of polymorphism, thus giving different results. In common opinion, AFLP is much more distinct and reliable method of studying polymorphism DNA (Bednarek *et al.* 1999, Łojkowska 2004).

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

Relationship between morphological diversity and DNA polymorphism of *Avena sativa* varieties is very poor. On the basis of Spearman correlation analysis of morphological traits with polymorphic DNA fragments of the studied varieties, we can presume, that description of DNA fragments can fulfill role of marker of some morphological traits.

Analysis of canonical correlations confirmed relationship between some DNA fragments and 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 growing, 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.

Importance of the work results for gene bank and breeding

Topic of the work is concentrated on basic problems concerning conservation of plant genetic resources, such as: studying of affinity, identification of objects and rationalizing of collection by duplicate 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 the studied materials a number of varieties, characterizing both unique combination of morphological and genetic traits was found (varieties Pszenicznik, Podkowa Dłużewski and Leniak). These objects could be valuable initial materials 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 proving uniqueness of some breeding varieties.

Conducted consideration of literature data (Słaboński 1949, Arseniuk *et al.* 2003) with the state of oat collection in the National Centre for Plant Genetic Resources at Radzików has indicated, that studied oat varieties composed 57% of all varieties cultivated in Poland from times before the I World War to the end of the 80. of XX century. It can be presumed, that the rest of varieties were in the same degree diverse in the respect of molecular and morphological traits and contained many precious properties and adaptations. The founding of the

work encourages to quest for 48 lacking varieties of Polish origin in foreign gene banks and to complete the research.

CONCLUSIONS

- identification of oat breeding varieties is possible based on the examined morphological traits,
- examined varieties were differentiated as well by traits approved as 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, type of stalk leaves growing and type of awns,
- correlation of morphological traits of leaves, grains, and stalk with selected DNA fragments suggest presence of the molecular markers of these morphological traits,
- DNA analyses by AFLP method enable distinguishing of oat breeding varieties, being a useful tool for their identification,
- morphological similarity of breeding oat varieties is not related with DNA similarity of these objects,
- RAPD method of DNA analyze shows another view of DNA polymorphism of oat breeding varieties than AFLP method,
- breeding varieties distinguishing unique combination of morphological traits as well as different DNA polymorphism has been identified.

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