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GENETIC DIVERSITY OF WINTER WHEAT CULTIVARS AND STRAINS DETERMINED BY ELECTROPHOREGRAMS OF GLIADIN AND GLUTENIN PROTEINS

ABSTRACT

Based on the polymorphism of gliadin and glutenin proteins relationships of 45 cultivars and strains of winter wheat were evaluated. The cluster analysis showed a considerable variation of the investigated genotypes. The similarity indices were calculated using the Nei and Li formula(1979). The genetic distances between the cultivars ranged from 1.00 to 0.12. The highest similarity index - SI=1.00- being proof of the identical physicochemical composition of storage proteins, was found for the pair Farmer and Elena. The groups of similar and genetically distant cultivars have been presented in the form of a dendrogram. The possibility of using the results obtained from the cluster analysis in breeding programmes has been discussed.

Key words: cluster analysis, electrophoresis, gliadins, glutenins, polymorphism

INTRODUCTION

The analysis of polymorphism and the identification of new sources of genetic diversity make possible to prevent undesirable effects of narrowing the gene pool, resulting from selection and provide information on the value of various genotypes as parental components in breeding programmes (Ferguson and Robertson 1996, Loarce *et al.* 1996). The identification of genotypes, formerly based on the observation of morphological traits, is curently carried out with the use of biochemical and molecular markers (Kongkiatngam *et al.* 1996). When studying the present-day publications a new approach to the analysis of genetic diversity can be noticed. It is the efect of combining statistical methods with biochemical and molecular techniques that are dynamically entering the fields of genetics and plant breeding. A special role in the studies on genetic diversity is played by the cluster analysis, the results of which are presented in the form of a dendrogram dividing the investigated genotypes into groups of closely related individuals. In the foreign

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literature one can find a number of research works where the cluster analysis is used to estimate the degree of relationship among cultivars belonging to various plant species. In those works polymorphic fragments of DNA obtained from the polymerase chain reaction (PCR) or related techniques are usually used as molecular markers (Loarce *et al.* 1996, Kongkiatngam *et al.* 1996, Bohn *et al.* 1999, Stappen *et al.* 2000).

Storage proteins are another type of polymorphic markers. As regards wheat, the diversity of gliadins and glutenins has been a subject of intensive studies for years. It is due to the genetic linkage found between the diversity of allelic variants of these proteins (the so-called protein blocks) and the differentiation of various important traits connected mainly with the technological quality. The results of these studies are a source of valuable information helpful in the selection of parental components in breeding programmes. They also facilitate the selection of genotypes with desirable combinations of protein blocks, i.e. those showing definite traits. The information which the electrophoretic analysis of glutenins gives on the technological traits of the investigated genotypes is considered to be of special value.

The objective of the research was to evaluate the diversity of cultivars and strains from the winter wheat collection with regard to the allelic composition of storage protein coding loci as an important selection trait. The method applied was the cluster analysis, which until that time had not been used till nowfor studying the polymorphism of storage proteins.

MATERIAL AND METHODS

For the purpose of the research winter wheat cultivars and strains from the collection of the Plant Breeding and Acclimatization Institute, harvested in 1998, were used. From among 120 forms, 45 were chosen, those whose electrophoretic patterns of gliadins and glutenins made it possible to identify unmistakably the protein fraction. Each of those forms was a pure line, owing to which the pattern of storage proteins did not get falsified as a result of the overlapping of fractions from various biotypes. Besides, when selecting material for the research it was decided to choose genotypes the pattern of which - mainly as regards gliadins - was made of sharp, outstanding bands, distinctly different from other bands, facilitating the comparison of the individuals of a given population.

Gliadins and glutenins were analysed by polyacrylamide gel electrophoresis described many a time in former papers (Waga 1997).

For each genotype the total number of gliadin and glutenin fractions was determined, and then the number of common bands was calculated, pairing the genotypes in all the possible combinations, using the protein block catalogues. For

glutenins the Payne catalogue was employed, while for the interpretation of gliadins the catalogue worked out at the Plant Breeding and Acclimatization Institute, Department of Cereals was used (Payne and Lawrence 1983, Waga 2000). The number of common bands was determined according to the following scheme. Let us assume that three cultivars are characterized by the combination of protein blocks:

Cultivar A	*Glu A1* Null	*Glu B1* 7+9	*Glu D1* 2+12
Cultivar B	*Glu A1* Null	*Glu B1* 6+8	*Glu D1* 2+12
Cultivar C	*Glu A1* Null	*Glu B1* 6+8	*Glu D1* 5+10

The electrophoretic patterns of these protein fractions are presented in Fig. 1. In this case the sum of bands for the cultivars A and B is eight, two of them are common (2 and 12) and two pairs of bands are different (6 and 8, 7 and 9). Similar relations occur in the case of the cultivars B and C (common bands - 6 and 8, different bands - 2 and 12, 5 and 10), while the cultivars A and C show no common bands.

The similarity indices (SI) for the compared genotypes were calculated using the Nei and Li formula (Nei and Li 1979):

$$SI = 2 \times \frac{c}{a+b}$$

where:
a – is the number of bands for the cultivar A
b – is the number of bands for the cultivar B
c – is the number of bands common to the cultivars A and B.

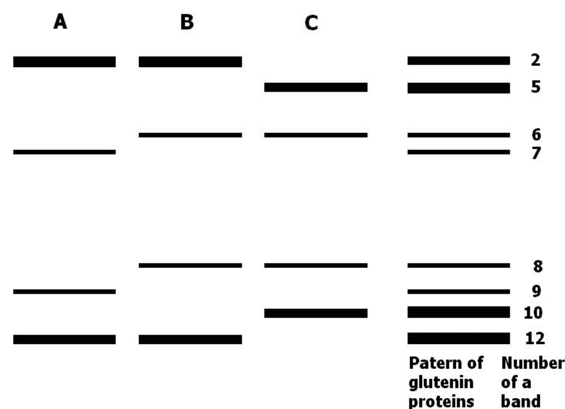

Fig. 1 A scheme of glutein protein electrophoretic patterns of wheat cultivars A, B and C

In the above theoretical example the double number of bands common to the pairs of the cultivars A and B, and B and C (four) in relation to the

sum of bands for both the pairs gives the value of SI equal to 0.5. However, when comparing the cultivars A and C, the value of SI equals zero.

The values of similarity indices can range from 0.00 to 1.00. The zero values and those nearing zero mean a lack or a small number of common electrophoretic bands, which should be interpreted as a lack of relationship,that is a significant differentiation of the analysed cultivars as regards the storage protein coding genes. On the contrary, the SI values equal to zero or those nearing one mean identity or a considerable similarity of the electrophoretic patterns, and thus they can be proof of a close relationship between the pair of the compared genotypes.

In order to join the investigated cultivars and strains into groups characterized by similar SI values, the cluster analysis was used. The SI values as well as the clusters of the cultivars and strains were determined using the computer program CLUSTER written at the Plant Breeding and Acclimatization Institute, Department of Cereals.

Table 1

Number of winter wheat cultivars and strains containing identified storage protein fractions: gliadins (*Gli*) and glutenins (*Glu*)

Proteins	Symbol of protein fraction	Storage proteins coding loci										
		Gli A1	*Gli B1*	*Gli D1*	*Gli A2*	*Gli 2 SI*	*Gli 2 SII*	*Gli 2 SIII*	*Gli 2 SIV*	*Glu A1*	*Glu B1*	*Glu D1*
Gliadins	1	25	16	37	25	38	30	23	40			
	2	7	17	8	6	4	15	14	5			
	3	4	8		6	3		8				
	4	2	3		8							
	5	4	1									
	6	2										
	7	1										
Glutenins	1									8		
	2									12		
	Null									25		
	7+9										34	
	6+8										6	
	7+8										4	
	7										1	
	5+10											21
	2+12											24

RESULTS

Polymorphism of storage proteins, found in 45 cultivars and strains from the winter wheat collection of the Plant Breeding and Acclimatization Institute, is presented in Table 1. From these data it can be seen that the selected genotypes vary considerably in respect of the allelic variants of genes coding for that group of proteins.

Table 2

Electrophoretic formulas and total number of bands for the analyzed cultivars

Number	Cultivars	Storage proteins coding loci											Number of protein fractions
		Gli A1	*Gli B1*	*Gli D1*	*Gli A2*	*Gli 2 SI*	*Gli 2 SII*	*Gli 2 SIII*	*Gli 2 SIV*	*Glu A1*	*Glu B1*	*Glu D1*	
1	STH 235	1	2	2	1	1	1	3	1	N	7+9	5+10	28
2	Mikon	5	4	1	3	1	2	1	1	1	7+9	5+10	19
3	Farmer	3	1	1	1	2	2	1	1	N	7+9	2+12	19
4	BOA 426	3	2	1	4	1	2	2	1	N	7+9	2+12	25
5	Elena	3	1	1	1	2	2	1	1	N	7+9	2+12	19
6	STH 1096/1	4	1	1	1	1	1	1	1	2	7+9	5+10	23
7	CHD 165/89	1	2	1	1	1	1	1	1	N	7+9	5+10	26
8	SMH 3749	1	1	1	3	1	1	2	1	N	7+9	2+12	22
9	GRH 216	7	2	1	4	1	1	4	1	2	6+8	2+12	31
10	STH 391	2	1	1	1	1	1	1	1	2	7+9	5+10	21
11	Mir 808	5	4	2	3	1	1	1	1	1	7+9	5+10	21
12	SMH 2843	1	5	1	1	1	2	3	2	N	7+9	2+12	23
13	MOB 2860	5	3	1	1	1	1	4	1	N	6+8	2+12	22
14	DED 528/84	2	2	1	1	1	1	2	1	N	7+9	2+12	25
15	SMH EGRO	1	2	2	1	1	2	1	2	2	7+9	5+10	28
16	STH 373	2	1	2	4	1	1	1	1	1	7+9	2+12	23
17	LAD 226/89	1	2	1	4	1	1	1	1	2	7+9	2+12	28
18	MIB 3338/89	1	2	1	1	1	1	2	1	2	7+8	5+10	27
19	Glockner	1	1	2	2	1	1	2	1	2	7+9	5+10	24
20	LAD 181/91	1	2	1	4	1	1	2	1	1	7+9	5+10	28
21	OZH 3061	6	3	1	1	3	2	1	2	N	7+9	5+10	23
22	Wilga	1	2	1	3	1	1	2	1	N	7+9	2+12	27

Table 2

Continued

Number	Cultivars	Storage proteins coding loci											Number of protein fractions
		Gli A1	*Gli B1*	*Gli D1*	*Gli A2*	*Gli 2 SI*	*Gli 2 SII*	*Gli 2 SIII*	*Gli 2 SIV*	*Glu A1*	*Glu B1*	*Glu D1*	
23	LAD 350/93	2	3	1	1	1	1	2	1	N	7	5+10	20
24	SMH 3391	1	3	1	1	1	1	2	1	N	7+8	2+12	22
25	STH 1096/2	1	1	2	1	1	1	1	1	2	7+9	5+10	23
26	BOA 333	1	2	1	4	1	2	3	1	N	7+9	2+12	27
27	LAD 420	1	2	1	3	3	1	2	1	N	7+9	2+12	28
28	STH 9055	1	3	1	2	1	1	1	1	N	7+9	5+10	23
29	SMH 4320	5	4	1	4	1	1	1	1	1	6+8	2+12	21
30	NAD 649/92	1	2	1	2	1	2	2	1	2	6+8	2+12	23
31	Begra	4	1	1	1	1	1	4	1	N	7+9	5+10	23
32	TAW 119452/82	1	2	2	3	1	1	3	1	2	7+9	5+10	30
33	MIB 2939/89	1	3	1	1	2	1	1	1	N	7+8	2+12	22
34	KOC 2730/90	1	2	1	1	3	2	2	1	N	7+9	2+12	26
35	CHD 236/90	1	2	1	1	1	1	1	1	N	7+9	2+12	26
36	SMH 3340	3	3	1	1	1	2	1	2	N	7+9	2+12	21
37	Jawa	2	1	1	1	1	1	1	1	N	7+9	2+12	20
38	STH 60	1	1	1	1	1	1	1	1	2*	7+9	2+12	22
39	OLH 1389	1	1	1	1	1	1	2	1	1	7+9	5+10	22
40	OLH 3521	1	1	1	1	2	2	1	1	2	7+8	5+10	21
41	Bussard	2	1	1	4	1	2	1	2	1	7+9	5+10	22
42	Tarso	1	2	1	2	1	1	2	1	N	7+9	5+10	27
43	HE 509/93	6	1	1	1	1	2	3	1	1	7+9	2+12	22
44	SMH 4043	2	1	1	2	1	1	1	1	N	6+8	5+10	21
45	Almari	1	3	2	2	1	2	1	1	N	6+8	2+12	23

Twenty-seven different groups of gliadin bands and ten glutenin blocks were found. Some of them, within the individual groups of chromosomes, were in the majority, for example the gliadin fractions: *Gli A1-1* (from the chromosome *1A* group), *Gli D1-1* (from the chromosome *1D* group), *Gli A2-1* (from the chromosome *6A* group), *Gli SI-1* and *Gli SIV-1* (from the chromosome *6B* group) or the glutenin blocks: *Glu A1 Null* (from the chromosome *1A* group) and *Gli B1* 7+9 (from the chromosome *1B* group). The others, such as the gliadins *Gli B1-1* and *2* (from the chromosome *1B* group) or the glutenins *Glu D1 5+10* and *2+12* (from the chromosome *1D* group) were equal in number to their allelic variants.Finally, some protein fractions, such as the gliadins *Gli A1-7* and *Gli B1-5* or the glutenin subunit *Glu B1-7* were observed in few cultivars and strains.

The cultivars and strains selected for the similarity index analysis, their complete electrophoretic formulas for gliadins and glutenins and the total number of polymorphic electrophoretic bands are presented in Table 2. The number of bands for individual genotypes ranged from 19 to 31. Taking as a starting point their electrophoretic characterization (the description of storage protein fractions and the total number of electrophoretic bands), the number of bands common to all the nine hundred and ninety combinations of paired cultivars and strains was calculated, using the method of mutual comparisons of the investigated objects.

The SI values obtained for the investigated cultivars ranged from 1.00 to 0.12, the value 1.00 being noted only for one pair of cultivars - Farmer and Elena.This results logically from the electrophoretic formulas of both the cultivars (Table 2). Such a wide range of SI values is the additional confirmation of the strong polymorphism of storage proteins in the investigated population.

The results of the cluster analysis are presented graphically in the form of a dendrogram (Fig.2). The horizontal axis distributes cultivars, and the vertical axis represents SI values. The groups of cultivars similar in respect of storage protein coding genes are bracketed. The height of the brackets corresponds to the calculated SI value and defines the similarity of the joined genotypes. The cluster analysis was done according to the highest SI values and that is why the cultivars showing the greatest similarity (as regards the physicochemical composition of storage proteins), Farmer and Elena, are directly joined - forming the simplest cluster - with the brackets reaching the point corresponding to 1.00 on the Y-axis. A form lying at some distance from them is the strain SMH 3340, so the next brackets join it to the pair Farmer - Elena. The greatest similarity to that group, formed already by three genotypes, is shown by another group of two forms - the cultivar Jawa and the strain STH 60. The cluster of five genotypes formed that way is equally similar to the strains STH 391 and LAD 350. The above-mentioned seven genotypes are joined to other seven genotypes (STH 1096, Begra, OZH 3061, SMH 3391, MIB 2939, OLH 3521 and HE 509) to form one common cluster at the 0.86 SI level. Then this cluster is joined to the

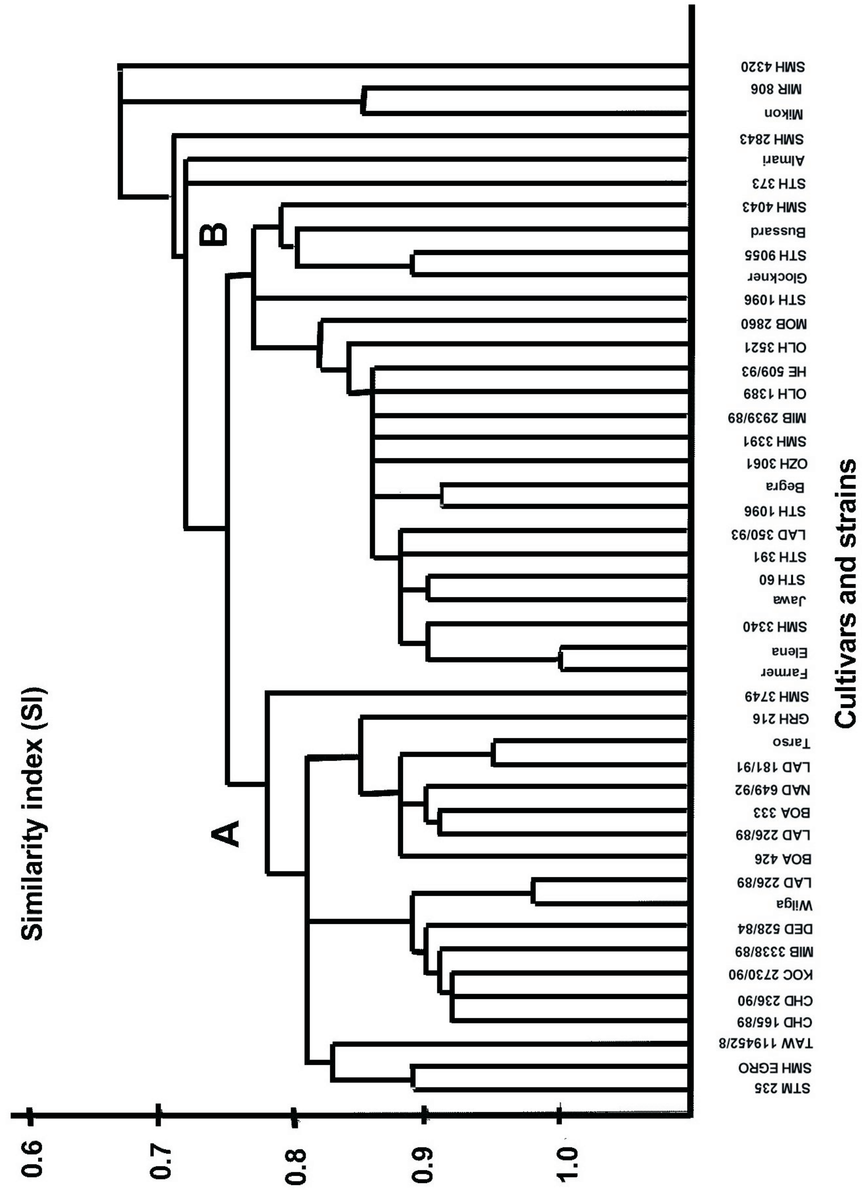

Fig. 2 Dendrogram based on similarity indices of 45 winter wheat cultivars

strain OLH 3521 at 0.84 SI level, and later on to the strain MOB 2860 at the 0.82 SI level. In turn, the pair of genotypes - the cultivar Glockner and the strain STH 9055, forming a simple cluster at the 0.89 SI level, is joined to the cultivar Bussard at the 0.80 SI level, and then to the strain STH 373 at the 0.79 SI level. These four genotypes are bracketed with the above-mentioned group of sixteen cultivars and strains, and separately - at the 0.77 SI level - with the strain STH 1096/2. The group of twenty-one forms made that way is marked with the letter B. Another group of eighteen hierarchically joined - at the 0.78 SI level - cultivars and strains located on the left-hand side of the dendrogram is marked with the letter A.

When comparing the electrophoretic formulas of the genotypes in the groups A and B special attention should be given to the specific distribution of gliadin blocks coded by the chromosome *1B*. From among eighteen genotypes in the group A as many as seventeen contained the block marked with the symbol *Gli B1-2*. Only one strain in that group (SMH 3749) contained the block *Gli B1-1* (Table 2). When studying the arrangement of clusters in the dendrogram we can see that this strain forms a cluster with the whole group of the remaining seventeen genotypes containing the block *Gli B1-2*. In other words, the strain SMH 3749, being a component of the group A, is at the same time most distant from the other genotypes in the group. In turn, the cultivars constituting the group B contain the chromosome *1B* coded gliadin subunits different from those found for the group A. They are *Gli B1-1* and *Gli B1-3*, the former being over twice as numerous as the latter.

The groups A and B are bracketed together to form one cluster at the 0.75 SI level. Above it there are only three cluster levels joining them to separate and - considering storage proteins - fairly distant cultivars. They include genotypes containing rarely observed blocks *Gli B1-4* and *Gli B1-5*, which have not been classed among either of the two groups. The highest cluster reaching the 0.67 SI level groups two directly joined varieties - Mironowska 808 and Mikon, and then the strain SMH 2843, joining them to all the other analysed forms of winter wheat to make one group.

DISCUSSION

The objective of the research was to evaluate the diversity of winter wheat cultivars and strains, from the collection of the Plant Breeding and Acclimatization Institute, in respect of the composition of storage protein subunits - high-molecular-weight glutenins and gliadins.The cluster analysis was used, which made it possible to estimate the genetic similarity of wheat forms comprising the investigated population, based on the similarity indices calculated according to the Nei and Li formula (1979). The results of the cluster analysis

presented in the form of a dendrogram show that the group of selected winter wheat cultivars and strains was considerably differentiated in respect of the storage protein coding genes. The presented research work is connected with the studies on the genetic relationship between various agronomically important traits and the diversity of storage proteins, carried out at the Plant Breeding and Acclimatization Institute, Department of Cereals. One of the aspects of these studies is the evaluation of breeding materials in respect of the possibility of using them as parental components in crossing (Waga 1997). The authors are of the opinion that the cluster analysis can facilitate the selection of parental components by informing which combinations of genotypes will give genetically differentiated progeny, and which combinations will give uniform or only slightly differentiated progeny. Two groups of cultivars and strains, marked in the dendrogram with the letters A and B, differing in the gliadin blocks coded by the *Gli B1* loci, confirm that hypothesis. Almost all the wheat forms comprising the group A contain the *Gli B1-2* block, which is the marker for rye 1B/1R translocation. The group B includes genotypes which contain the remaining chromosome *1B* coded protein fractions. Gliadins from the chromosome *1B* group are related to the variation of a number of important traits, mainly those connected with the technological quality. Thus the selection of parental components guarantees the genetic diversity of cross progeny. In addition, the information obtained from the cluster analysis can be helpful in working out breeding strategies by showing the genotypes which should be joined so as to obtain individuals with highly favourable traits and inconsiderably unfavourable traits.

The characterization of the polymorphism of storage proteins in winter wheat cultivars and strains has been the subject of many publications. However the cluster analysis was not used as a method for estimating the similarity of the investigated genotypes. In this respect the present work is a new approach to the problem of diversity of gliadins and glutenins as related to the technological quality and other important traits.

CONCLUSIONS

The investigated group of winter wheat cultivars and strains was considerably differentiated in respect of the genes coding for storage proteins - gliadins and glutenins.

From among all the blocks of storage proteins, gliadins coded by the chromosome *1B* diversify the selected forms of winter wheat to the greatest degree.

The relationship between the chromosome *1B* coded gliadins and the technological quality, presented in earlier works, enables us to conclude that the analysis of polymorphism using the cluster method divides the investigated cultivars and strains into groups differing in that trait.

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