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INHERITANCE OF SOME ω–GLIADIN PROTEIN SUBUNITS IN SPELT WHEAT AND THEIR LINKAGE WITH THE RED GLUME CODING *RG–1* LOCUS.

ABSTRACT

The Gli B1 locus controlling the synthesis of certain fractions of wheat storage proteins belonging to the group of ω–gliadins is tightly linked with the Rg1 gene responsible for red glume colour. The inheritance of selected ω–gliadin subunits and of glume colour was studied in order to identify markers of this trait among protein fractions specific to spelt wheat.

Electrophoretic analysis for 217 single plants of the F_2 generation in the cross *Tr. aestivum* (cultivar Elena) × *Tr. spelta* was done. Four out of five protein subunits specific for spelt wheat (O1, O3, O4 and O5) as well as gliadin block *Gli B1–1* of the Elena cultivar were inherited according to Mendelian segregation ratio 1:2:1 assumed for two allelic protein variants of one gene. The above mentioned fractions of spelt wheat formed a block, which has been denoted by the symbol *Gli B1–6*. The results of segregation analysis regarding glume colour indicate Mendelian, single–gene inheritance of this trait (3:1 ratio of red and white glumes), which differs from the results of other investigations suggesting the existence of two genes controlling the red colour. The genetic distance between loci *Gli B1* and *Rg1* has been estimated to be 1.9 cM.

Key words: A PAGE electrophoresis, gliadin subunits, glume colour, linkage analysis, protein blocks

INTRODUCTION

Gliadin proteins, when separated by acid–polyacrylamide gel electrophoresis (A PAGE) form a pattern composed of a certain number of subunits, depending on the genotype. As regards molecular weight, they can be divided into four groups: α, β, γ and ω with increasing molecular weight (Woychik *et al.* 1961). Their characteristic feature is marked polymorphism due to variability of allelic variants of six genes located on the short arms of chromosomes belonging to the first and the sixth homeological groups (1A, 1B, 1D, 6A, 6B, 6D) (Sozinov and Poperella 1980).

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The knowledge of polymorphism and inheritance of gliadin proteins identified among wheat cultivars and strains makes it possible to use them as genetical markers. The investigation of wild wheat species as well as of primitive forms broadens the spectrum of gliadin diversification by a number of subunits not observed in cultivated forms (Belitz *et al.* 1989). In a previous paper a few specific subunits from the group of ω–gliadins have been identified in one of the studied forms of spelt wheat (*Triticum aestivum* L. var. *spelta*) (Waga 2001).

Spelt wheat is one of six subspecies of hexaploid wheat and is commonly considered to be a form possessing better, compared with wheat cultivars (*Triticum aestivum* L. var. *vulgare*), nutritional qualities. On the other hand, primitive traits, such as brittle spike or poor threshing capacity, make it close to wild species hindering large–scale cultivation. Hence, attempts are being made to transfer advantageous traits of spelt wheat to cultivated varieties (Campbell 1997).

One of morphological traits differentiating the above mentioned form of spelt wheat from winter wheat varieties currently cultivated in Poland is the red glume colour. The gene controlling this trait (*Rg1*) is located on the *1B* chromosome and is linked with genes responsible for the synthesis of proteins belonging to the group of ω–gliadins (locus *Gli B1*) (Poperellya *et al.* 1980, Pogna *et al.* 1985). These investigations were conducted on hybrids of cultivated forms, both hexa– and tetraploidal, with different glume colour. As a result, a few subunits of gliadin proteins were identified which can serve as markers of *Rg* genes (Koval *et al.* 1986, Pogna *et al.* 1993).

In this work the results of research into inheritance of some ω–gliadin fractions and glume colour in *Tr. spelta* and *Tr. vulgare* hybrids are presented. The aim of these investigations was to identify biochemical markers of the *Rg* gene among spelt specific protein subunits. These markers can prove useful in the analysis of genetic linkage between glume colour and functional properties of spelt wheat. Their identification required the determination on which chromosomes are located genes coding particular fractions of ω–gliadin proteins and, further, finding out if, and to what degree, these genes are linked with the *Rg* gene controlling the red glume colour.

MATERIAL AND METHODS

The material for the studies consisted of 217 single plants of the F_2 generation obtained from the cross of a selected form of spelt wheat with the winter wheat variety Elena. The chosen genotypes originated from experiments conducted at the Plant Breeding Station in Strzelce.

The glume colour of selected hybrid crops was evaluated visually using parental forms as a reference. The glumes of Elena were yellow and differed markedly from the glumes of spelt wheat, which had a reddish–brown tinge. Among red forms various glume colour intensities

could be observed. However, this method made a precise assessment of differences impossible, hence all plants with a distinct red tinge were qualified to one class. In the case of yellow plants glume colour assessment was unequivocal – no diversification could be observed regarding colour intensity.

About a hundred kernels were ground from each single plant and the obtained flour was used for gliadin extraction. Proteins were extracted with 70% ethanol, using 400 ml of ethanol solution per ca. 50 mg flour. Analyses were conducted by the method of electrophoretic separation (A PAGE) according to the standard methodology developed by Bushuk and Zillmana (polyacrylamide gel 8%, lactate–aluminium buffer with pH=3.1, separation time about 4 hours at a constant voltage U=500V and current intensity I=85 mA) (Bushuk and Zillman 1978). Gels were stained with a solution of Coomassie Brillant Blue.

Within the group of selected single plants segregation was analysed regarding gliadin proteins fractions, glume colour, and then both these traits jointly. The results were assessed based on the χ^2 test. Recombination frequency between genes coding selected gliadin protein subunits and glume colour genes was calculated by the maximum–likelihood method according to Allard (Allard 1956). The genetic distance was calculated using the Kosambi function (Kosambi 1944).

RESULTS

All ω–gliadin proteins can be divided into two subgroups. The first one consists of fractions with higher molecular weight, coded mainly by the *1D* chromosome, while the lighter fractions – which are the subject matter of the present studies – are coded by chromosomes *1A* and *1B*. In the case of spelt wheat, their electrophoregram is made of four subunits de–

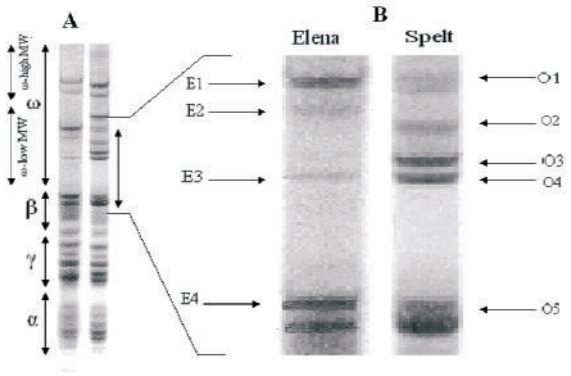

Fig. 1. Gliadin protein patterns of parental form: winter wheat cultivar Elena and *Tr. spelta*. A. The zones: α, β, γ and ω are marked. B. Enlargement of lower ω–gliadins zone. Gliadin protein subunits characteristic for Elena and spelt wheat are marked by the arrows.

noted by the symbols O1, O2, O3 and O4. For Elena, bands observed in this region were denoted by E1, E2, and E3 (Fig.1). The E1 and O1 gliadins occupy identical position, which indicates that these fractions have similar molecular weight. Their patterns are however different – E1 is a single, sharp and intensely stained band. In contrast, O1 is a weakly stained, extended spot. Owing to these characteristic features both protein fractions are easily distinguishable. Also the O4 and E3 bands occupy identical positions in the electrophoretic pattern, have sharp outlines, however the width of the former band is slightly larger than that of the latter. The remaining ω–gliadins: O2, O3 and E2 are lo–cated at different places and hence easily identifiable.

1. Inheritance of gliadin proteins

Specificity of the electrophoretic pattern for the studied form of spelt wheat is due to the components O3 and O4, which, owing to their close location as well as identical shape and staining intensity, form a pair of characteristic twin bands. One of them (O4) occupies – as mentioned above – the same position as band E4, which is an element of the protein block coded by the chromosome *1B* (locus *Gli B1*). In the catalogue, de–scribed in a previous paper, this block has been denoted by the symbol *Gli B1–1* (Waga 2000). In the Polish wheat varieties and strains this protein variant is the one most frequently observed. The analysis of spelt wheat ω–gliadin inheritance began with the identification of the

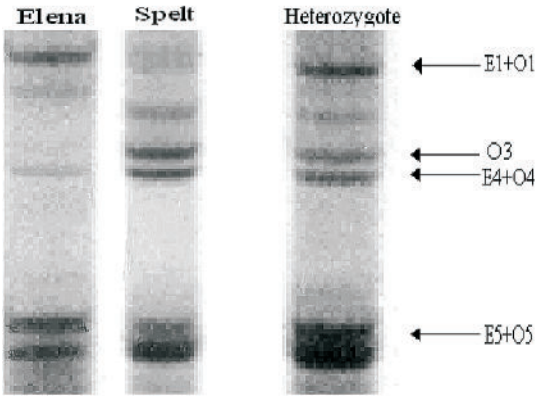

Fig.2. Electrophoretic patterns of ω–gliadins coded by the chromosome *1B* (locus *Gli B1*) ob–served in parental type homozygous genotypes and in the heterozygous hybrid genotypes

group of fractions coded by the protein variant allelic with respect to the *Gli B1–1* block.

Segregation analysis of O1+O3+ O4 and E1+ E3 gliadins

Assuming that O1, O3 and O4 gliadins form one protein block, which is an allelic variant of the group of subunits occurring in the Elena vari–ety (coded by the chromosome *1B* – block *Gli B1–1*), three types of hy–brid individuals were observed in the F_2 generation. Two of them

contained protein fractions characteristic of the parental forms, hence O1, O3 and O4 in the spelt wheat type hybrids and the *Gli B1–1* block in the Elena type hybrids. The third, heterozygotic type of hybrid, com–bined components characteristic of both spelt wheat and Elena (Fig. 2). Its electrophoretic pattern is composed of the following bands:

– E1 – formed as a superposition of the intensely stained and sharply outlined E1 fraction and the less intensely stained and blurred patch of the O1 fraction,
– O1 – characteristic of spelt wheat,
– O4 – formed as a result of superposition and mutual masking of the O4 and E3 fractions,

The frequency of the described genotypes should correspond to the theoretically predicted ratio 1:2:1 for two allelic gliadin blocks. Based on the χ^2 test it was found that the observed frequency of single plants agreed with that theoretically expected (Table 1). This indicates that bands O3 and O4 are coded by the *Gli B1* locus and they form a protein block, which has been denoted by the symbol *Gli B1–6* (the following se–rial number in the created catalogue of gliadin proteins). To this block also belongs the band O5, which appeared together with O1, O3 and O4 in the case of all hybrid individuals containing fractions of ω–gliadins which are typical for spelt wheat.

Table 1

χ^2 test for gliadin subunits O1+O3+O4, E1+E3, O2 and null O2.

Gliadin subunits	Sergregation	Expected	Observed	χ^2	P
O1+O3+O4	1	54.25	45		
O1+O3+O4+E1+E3	2	108.5	121	3.212	0.1–0.2
E1+E3	1	54.25	51		
O2	3	162.75	166	0.260	0.5–0.7
null O2	1	54.25	51		

Segregation analysis for O2 fraction

The location of the O2 and E2 bands in the electrophoretic pattern – with respect to each other as well as to blocks *Gli B1–6* and *Gli B1–1* – indicates that they are coded by two allelic variants of one gene. In such case one should expect the appearance of three hybrid types:

– containing the O2 fraction (parent type spelt wheat),
– containing both O2 and E2 fractions (heterozygotic type),
– containing fraction E2 (parent type Elena),

in the quantitative ratio 1:2:1. However, the observed numbers dif–fered significantly from those theoretically expected. Hence, a different model has been adopted to explain the inheritance of the O2 subunit. Two groups of individuals were formed – those which did and those, which did not contain the O2 band, respectively. All individuals in which the O2 band was well pronounced irrespective of its staining intensity

was counted in the first group. The second group consisted of all individuals, which did not contain the O2 band, hence those which contained the E2 band, whether in the form of a sharply outlined protein fraction (like in Elena), or a blurred patch without visible traces of the spelt wheat fraction (Fig. 3). In this interpretation it is assumed that the allele coding O2 is the "null" type variant coded by the so–called silent gene, whereas E2 is a product of a different gene and has no connection with O2 inheritance. The first group of individuals includes both homo– and heterozygotic genotypes regarding the gene coding the O2 band. In the second group there are homozygotic individuals regarding the gene for the "null O2" variant. In such case the theoretical segregation ratio of individuals belonging to the first and the second group would be close to 3:1. The χ^2 test confirms with high probability the agreement between

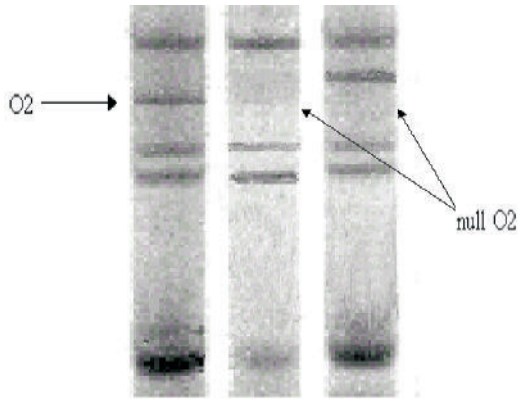

Fig.3. Spelt specific gliadin subunits O2 and their allelic form marked as „null O2"

the theoretically expected distribution and the observed one thus proving that the interpretation of O2 fraction inheritance is correct (Table 1).

Segregation analysis of gliadins: Gli B1–6, Gli B1–1, O2 and "null O2".

Fractions of ω–gliadin proteins, both parental components and hybrids of the parental component type, can be denoted by the following symbols:

– spelt wheat type – *Gli B1*–6/O2,
– Elena type – *Gli B1*–1/null O2,

The occurrence of two types of hybrids:

– combining the spelt wheat block *Gli B1*–6 with the "null O2" fraction of Elena – *Gli B1*–6/null O2,
– combining the Elena block *Gli B1*–1 with the O2 fraction of spelt wheat – *Gli B1–1*/O2,

demonstrates that O2 gliadin and its allelic variant of the type "null" are not components of the protein blocks coded by the *1B* chromosome. Therefore a hypothesis was adopted of an independent inheritance of genes responsible for synthesis of these gliadin fractions. It was ex–

pected that six phenotypic classes would occur in the ratio of 3:6:3:1:2:1 following from the combination of two other proportions: 1:2:1 for the *Gli*

Table 2

χ2 test for gliadin blocks *Gli B1–6*, *Gli B1–1* as well as gliadin subunits O2 and "null O2"

Gliadin subunits	*Gli B1* locus	Segregation	Expected	Observed	χ^2	P
O2	6	3	40.6875	33	4.814	0.3–0.5
O2	6+1	6	81.375	97		
O2	1	3	40.6875	36		
null O2	6	1	13.5625	12		
null O2	6+1	2	27.125	27		
null O2	1	1	13.5625	12		

Table 3

χ2 test for glume colour

Glume colour	Segregation	Expected	Observed	χ2	P
Red	3	162.75	168	0.68	03–0.5
White	1	54.25	49		

B1 locus and 3:1 for the O2 fraction. The result of the χ^2 test confirms the hypothesis of the independent inheritance of both loci (Table 2).

Taking into account that the O2 fraction is not an allelic variant of E2 (coded by the *1A* chromosome), neither is it coded by the *1B* chromo–some, the result obtained precludes the possibility of finding exactly which of the above two chromosomes codes the O2 band.

Glume colour inheritance

In the study of glume colour inheritance in F_2 hybrids of spelt wheat and Elena, two phenotypic classes were separated – plants of red and yellow colour. A significant outnumbering of the red forms over the yel–low ones confirms that the red colour is coded by dominant gene (*Rg*). It was assumed, therefore, that the group of red plants included both dom–inant homozygotes (*RgRg*) and heterozygotes (*Rgrg*), hence their ratio to the yellow plants (rgrg) should be close to 3:1. The result of the χ^2 test confirms the correctness of such assumption, which indicates a Mende–lian, single–gene inheritance of this trait (Table 3).

The inheritance of ω–gliadins and of the glume colour

Segregation analysis for the gene of O3 protein and of glume colour Rg

Assuming an independent model of inheritance of the gene coding the O3 gliadin fraction and of the gene controlling the glume colour *Rg*, four phenotypic classes were expected in a ratio 9:3:3:1, which follows from a combination of two 3:1 proportions assumed for each of these genes in–

Table 4

χ^2 test for gliadin subunits O2, null O2, *Gli B1* locus and spike colour

O2	Glume colour	Segregation	Expected	Observed	χ^2	P
O2	red	9	122.0625	130	1.306	0.5–0.9
null O2	red	3	40.6875	39		
O2	white	3	40.6875	36		
null O2	white	1	13.5625	12		
6	red	3	40.6875	45	179.868	<<0.001
6+1	red	6	81.375	120		
1	red	3	40.6875	3		
6	white	1	13.5625	0		
6+1	white	2	27.125	1		
1	white	1	13.5625	48		

dividually (Table 4). The result of the χ^2 test confirms the hypothesis of an independent inheritance of both these genes.

Segregation analysis for Gli B1 loci and glume colour Rg

Assuming independent inheritance of gliadin proteins coded by *1B* chromosome and glume colour – in total six phenotypic classes were expected in a ratio 3:6:3:1:2:1, which follows from combining two proportions: 1:2:1 for gliadins and 3:1 for glume colour. Analysis of frequencies in the six classes indicates a significant deviation from the assumed segregation ratio (Table 4). Only in the case of hybrids similar to spelt wheat (gliadins typical for spelt wheat, red glume colour) the observed frequency is consistent with that theoretically expected. In the other five classes significant deviations were observed. For example, no single plant was identified that contained gliadin fractions typical for spelt wheat and with yellow glume colour, whereas only three hybrids were identified which contained gliadins typical for Elena and with red glume colour. The hybrid form similar to Elena (gliadins typical for Elena, yellow glume colour) appeared in the group of individuals nearly four times larger than could be expected on the basis of theoretical segregation ratios. The result of the χ^2 test negates, therefore, the assumption of independent inheritance of the considered traits, which, in turn, indicates the existence of a linkage between the loci *Gli B1* and *Rg*. The genetic distance between them following from the recombination percentage calculated by the method of the maximum–likelihood was estimated to be 1.9±0.78 cM.

DISCUSSION

In this work the results of research into inheritance of glume colour and ω–gliadins coded by the *Gli B1* locus are presented. Hybrids of F_2 generation coming form the cross combination between spelt wheat with red glume colour and a winter wheat cultivar – Elena with yellow glume colour and differing in ω–gliadins composition were analysed. It was shown that protein fractions specific for spelt wheat, viz. O1, O3, O4, and O5, are coded by the *1B* chromosome and form a protein block, denoted by the symbol *Gli B1*–6, which has not been observed up to now in the studies of winter wheat cultivars and strains from plant breeding experiments conducted at the Cereals Department of the Plant Breeding and Acclimatization Institute in Kraków. The O2 fraction, located between bands O1 and O3, is inherited independently from the *Gli B1*–6 block. Although the result obtained does not allow for precise determination on which chromosome the gene coding O2 is located, it was nevertheless demonstrated that this band is not a component of the *Gli B1–6* block.

Segregation analysis of glume colour genes has confirmed that red colour is controlled by a dominant gene, whereas the intensively yellow colour occurs in the case of recessive homozygotes. However, certain discrepancies have been noted between the results obtained in the present work and those of other investigators regarding the number of genes controlling this trait (Rowland and Kerber 1974, Payne *et al.* 1986). Earlier works report two genes of the red glume colour located on chromosomes *1BS* (*Rg1*) and *1DS* (*Rg2)*. The results obtained in the present work suggest a Mendelian, single–gene inheritance of this trait (red to yellow ratio 3:1). The methodological simplification applied in this study, which consisted in qualifying all plants with various shades of red to one group does not account for the obtained discrepancies, since it did not affect the number of yellow plants, which could be estimated univocally. The essential difference between the investigations described in the present paper and those reported in the references concerned the plant material. In the works of cited authors the object of investigations was hybrids of yellow and red forms belonging to *Tr. vulgare* species, as well as hybrids from inter–specific cross–combinations of *Tr. vulgare* and *Ae. squarrosa*. A hypothesis can be formulated that in the case of spelt wheat (*Tr. spelta*), which differs evolutionally from *Tr. vulgare*, the inheritance mechanism of glume colour can be rather different.

The inheritance analysis of loci *Gli B1* and *Rg* jointly shows that genes coding these traits are mutually linked. The estimated recombination frequency (1.9%) is similar to those of other researchers: (Poperellya *et al.* 1980) – 1,6%, (Payne *et al.* 1986) – 1,8%, (Pogna *et al.* 1993) – 2,0%. This result indicates that lighter fractions of ω–gliadins peculiar to spelt wheat are glume colour markers. This marker has practical significance

in the selection of red–coloured genotypes. Red colour is coded by dominant gene and thus phenotypic selection will be rather ineffective, since it does not allow for precise distinction between dominant homozygote and heterozygote regarding glume colour. When choosing red plants, segregation must be expected with respect to this trait in next generations.

In contrast to phenotypic evaluation, the selection based on the use of protein markers will be effective. The electrophoretic pattern of ω–gliadins makes it possible to select individuals containing the protein block coded by locus *Gli B1*–6, which is linked to the dominant *Rg* gene. Thus, electrophoretic analysis of gliadins can be used to distinguish correctly dominant homozygotes (*RgRg)* from heterozygotes (*Rgrg*). Owing to correct genotype identification it will be possible to select such plants with red glume colour that will not segregate in next generations. Investigations of linkage between the *Gli B1–6* block and the variability of functional properties in hybrids of *Tr. spelta* and *Tr. aestivum* should verify practical value of the marker described in this work.

CONCLUSIONS

1. The glume colour of spelt wheat and Elena hybrids is simply inherited according to Mendelian laws. Gene controlling red glume colour is dominant (*Rg*).
2. The *Rg* gene is linked with the locus coding for the *Gli B1–6* protein block of spelt wheat. Thus, the electrophoretic pattern of *Gli B1–6* components can be a marker of the *Rg* gene.
3. Using lighter fractions of ω–gliadins as markers it is possible to distinguish dominant homozygotes (*RgRg*) from heterozygotes (*Rgrg*). Selection based on phenotypic evaluation does not give such a possibility.

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REFERENCES

Allard R.W. 1956. Formulas and tables to facilitatete calculation of recombination values in heredity. Hilgardia 24:235–278.

Belitz H.D., Seilmeier W, Wieser H. 1989. Die Proteine des Dinkels. Z. Lebensm. Forsch. 189:1–5.

Bushuk W., Zillman R.R. 1978. Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. Can J. Plant Sci. 58:505–515.

Campbell K.G. 1997. Spelt: agronomy, genetics, and breeding. Plant Breeding Revievs, Volume 15: 188–213.

Kosambi D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12:172–175.

Koval S.F., Metakovsky E.V., Kudryavtsev A.M., Sozinov A.A. 1986. On the linkage of genes controlling hairness and colour of glume with definite alleles of gliadin–coding loci in wheat. Selskhohoziaistvennaya Biologia 2:31–36. (In Russian).

Payne P.I., Holt L.M., Johnson R., Snape J.W. 1986. Linkage mapping of four gene loci, *Glu B1, Gli B1, Rg1* and *Yr10* on chromosome *1B* of wheat. Genet. Agrar.40:231–242.

Pogna N.E., Dal Belin Peruffo A., Mellini F. 1985. Genetic aspects of gliadin bands 40 and 43.5 associated with gluten strenght. Genet. Agrarie 39:101–108.

Pogna N.E., Metakovsky E.V., Radelli R., Raineri F., Dachkevitch T. 1993. Recombination mapping of *Gli–5*, a new gliadin–coding locus on chromosomes *1A* and *1B* in common wheat. Theor. Appl. Genet. 87:113–121.

Poperellya F.A., Bito M., Sozinov A.A. 1980. Associations between block of gliadin compo–nents and winter surviving of plants, productivity, colour of spikes, and flour quality of common wheat as studied in hybrids from the cross Bezostaya 1 × 'Crvena Zvezda'. Dokladi Vaskhnil 4:4–7 (in Russian).

Rowland G.G., Kerber E.R. 1974. Telocentric mapping in hexaploid wheat of genes for leaf rust resistance and other characters derived from *Aegilops squarrosa*. Can. J. Genet. Cytol.16:137–144.

Sozinov A.A., Poperella F.A., 1980, Genetic classification of prolamins and its use for plant breeding. Ann. Techn. Agric., 28: 229–245.

Waga J. 2000. Syntetyczna metoda klasyfikacji białek gliadynowych. Biul. IHAR. 215:35–60.

Waga J. 2001. Charakterystyka białek gliadynowych i glutenin u orkiszu. Biul. IHAR. 217:39–59.

Woychick J.H., Boundy J.A., Dimler R.J., 1961, Starch gel electrophoresis of wheat gluten proteins with concentrated urea. Arch. Biochem. Biophys, 94: 477.