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Biochemical Markers in Wheat Breeding

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ABSTRACT

Biochemical markers and specifically isozymes and seed storage proteins have been proved to be a very effective tool in wheat breeding and seed certification. Isozyme investigation contributed towards a better understanding of bread wheat (*T. aestivum*) genome structure (AABBDD). This investigation was also important in revealing the genome structure of related species and thus contributing to wheat evolution. Wheat storage proteins, i.e. gliadins and glutenins, due to their greater polymorphism, were found to be more informative in solving practical problems, especially in breeding for grain quality. Allelic variants of the high-molecular-weight and the low-molecular-weight glutenin subunits proved to be the most effective markers for grain quality breeding and were employed by most breeders in developing high-quality wheat cultivars. They were also effective markers in detecting lines possessing resistance genes to winter tolerance, to biotic, and to abiotic stress conditions. The revealed genes were recently employed in genetic engineering approaches for improving grain quality. Biochemical markers were informative in population-genetic studies and in analyses of the genetic diversity of the wild wheat species. Furthermore, their application enabled the identification of alien genetic material into the wheat genome. The close association between biochemical markers with productivity and adaptability was beneficial in producing advanced wheat germplasm. In conclusion, application of biochemical markers played an important role in investigating evolution, breeding and certification of cultivated wheats. Finally, they contributed to the increased employment of molecular markers in wheat breeding.

Keywords: gliadins, glutenins, electrophoresis, subunits, translocations, wheat improvement
Abbreviations: APAGE, acid polyacrylamide gel electrophoresis; **CIMMYT**, International Maize and Wheat Improvement Centre; **Gli**, gliadin; **Glu**, glutenin; **HMW**, high molecular weight; **LMW**, low molecular weight; **PCR**, polymerase chain reaction; **QTL**, quantitative trait loci; **RFLP**, restriction fragment length polymorphism; **SDS**, sodium dodecyl sulphate

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INTRODUCTION

The determination of effective criteria for evaluating new wheat germplasm is a very important task in breeding new varieties. These criteria were firstly based on the phenotype, which was influenced by the environment, and thus they were not reliable (Lookhart and Wrigley 1995; Gianibelli *et al*. 2002). However, the rapid development of molecular genetics resulted in new and more reliable criteria, based on the genotype, and for this they are minimizing the environmental effects.

The criteria applicable to breeding nowadays can be distinguished as morphological, biochemical markers (polymorphic proteins), and DNA markers (RFLP and various PCR markers). A marker could be efficiently employed in genetics and breeding if it meets the following requirements: 1) it has adequate polymorphism, 2) it is qualitatively independent of the growth conditions; and 3) it follows a co-dominant pattern of inheritance. There are three main areas in which genetic markers could be utilized in plant breeding and genetics. The first involves the employment of genetic markers for determining genetic relationships: i.e. varietal identification, protection of breeder's rights, and parentage determination. The second is referred to the use of genetic markers for identification and mapping of loci associated with quantitative and qualitative traits and for monitoring of these loci in breeding programs (marker-assisted selection). These two areas were proposed for DNA markers by Soller and Beckmann (1983) but biochemical markers could also be applied. The third area is the use of genetic markers in population-genetic studies.

The present review is focussed on the development, use and contribution of biochemical markers to wheat breeding.

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Invited Review

Table 1 Location of biochemical loci in wheat [based on the catalogue of McIntosh *et al*. 2003 and its version of 2005 (MacGene 2005)].

Chromosome arm	Loci
1AS	*Gpi-A1, Gpt-A1, Glu-A3**, Gli-A1**, Gli-A3**, Gli-A5*, Gli-A6*, Tri-A1*, Glo-A1*
1AL	*Mdh-A1, Glu-A1**, Pur-A1, Lec-A1*
1BS	*Gpi-B1, Hk-B1, Per-B1, Gpt-B1, Glu-B3**, Gli-B1**, Gli-B3*, Gli-B5*, Si-B2, Glo-B1*
1BL	*Mdh-B1, Glu-B1**, Pur-B1, Lec-B1*
1DS	*Gpi-D1*, Hk-D1, Per-D1*, Gpt-D1, Glu-D3**, Gli-D1**, Tri-D1*, Si-D2, Glo-D1*
1DL	*Mdh-D1, Glu-D1**, Pur-D1, Lec-D1, SbeI1*
2AS	*Est-A6*, Per-A2**
2AL	*Est-A7, Sod-A1, Isa-A1**
2BS	*Est-B6*, Per-B2**
2BL	*Est-B7, Sod-B1, Isa-B1**
2DS	*Est-D6*, Per-D2, Per-D5*
2DL	*Est-D7*, Sod-D1, Isa-D1*
3AS	*Est-A1, Est-A9, Hk-A2*, Pde-A1, Tpi-A1, Ndh-A4*
3AL	*Est-A5*, Est-A8, Got-A3, Per-A3*, Ndh-A3, Mal-A1*
3BS	*Est-B1, Est-B9, Hk-B2, Pde-B1, Tpi-B1, Ndh-B4, Iha-B1.1, Iha-B1.2**
3BL	*Est-B2, Est-B5**, Est-B8, Got-B3, Per-B3**, Ndh-B3*, Mal-B1*
3DS	*Est-D1, Est-D9, Hk-D2*, Pde-D1, Tpi-D1, Iha-D1*
3DL	*Est-D2, Est-D5*, Est-D8, Got-D3, Per-D3**, Ndh-D3, Mal-D1*
4AS	*Acph-A1**
4AL	*Adh-A1, Amp-A2*, Lpx-A1, Per-B4*, Pgm-A1, Ndh-A1**, Wx-B1***
4BS	*Adh-B1*, Amp-B2*, Lpx-B1*, Ndh-B1*
4BL	*Acph-B1, β-Amy-B1**, Cat-B1, Aco-B2***
4DS	*Adh-D1, Amp-D2*, Lpx-D1, Pgm-D1, Ndh-D1*
4DL	*Acph-D1, β-Amy-D1*, Aco-D2*
5AS	*Mdh-A3*, Skdh-A1*
5AL	*β-Amy-A1**, Lpx-A2, Tpi-A2, Aadh-A1*, Aco-A2, Ti-A2, Ibf-A1**, HstH1-A1, HstH1-A2**
5BS	*Mdh-B3*, Skdh-B1*
5BL	*Lpx-B2, Tpi-B2, Aadh-B1*, Ti-B2, Ibf-B1**, HstH1-B1, HstH1-B2**
5DS	*Mdh-D3*, Skdh-D1, Pina-D1**, Pinb-D1**, Gsp-D1***
5DL	*Lpx-D2, Tpi-D2, Aadh-D1, Ti-D2*, Ibf-D1**, HstH1-D1*, HstH1-D2*
6AS	*Amp-A1*, Got-A1, Gli-A2***
6AL	*α-Amy-A1*, Est-A4, Got-A2, Aco-A1*, Dip-A1*, Aadh-A2, AhasL-A1*
6BS	*Amp-B1*, Got-B1, Gli-B2**, Ep-B2*
6BL	*α-Amy-B1**, Est-B4, Got-B2, Aco-B1*, Dip-B1*, Aadh-B2, AhasL-B1*
6DS	*Amp-D1*, Got-D1, Gli-D2***
6DL	*α-Amy-D1*, Est-D4, Got-D2, Aco-D1, Dip-D1, Aadh-D2, AhasL-D1*
7AS	*Amp-A3*, Per-A4*, Wx-A1**, Sgp-A1*, Sgp-A3**
7AL	*α-Amy-A2, Ep-A1*, Adk-A1, Wsp-A1***
7BS	*Est-B3, Sgp-B1**, Sgp-B3**
7BL	*α-Amy-B2*, Ep-B1**, Adk-B1, Wsp-B1*, SbeI2*
7DS	*Est-D3, Per-D4*, Wx-D1**, Sgp-D1*, Sgp-D3, Ndh-D2*
7DL	*α-Amy-D2*, Ep-D1**, Adk-D1, Wsp-D1**

*2-3 alleles were reported, **more than 3 alleles were reported

BIOCHEMICAL MARKERS

Isozymes and seed storage proteins

Isozymes and storage proteins are the main biochemical markers in grasses. Their employment became possible after the development of methods of separation and identification of protein macromolecules (electrophoresis, isoelectric focusing). The use of nullisomic-tetrasomic and ditelosomic lines of the spring common wheat *Triticum aestivum* L. "Chinese Spring" produced by Sears (1954, 1966) enabled chromosome localisation of genes encoding different proteins in common wheat. Chromosome localisation of biochemical markers in wheat is presented in **Table 1**.

Isozymes

Isozymes (or isoenzymes) are different variants of the same enzyme, having identical or similar functions, and present in the same individual (Markert and Moller 1959). They are common enzymes expressed in plants. Although they differ in amino acid sequence, and consequently display different kinetic parameters, they catalyze the same chemical reaction. Biochemically, isozymes are isoforms of enzymes and in most cases are encoded by homologous genes that have diverged over time. They exhibit Mendelian inheritance, codominant expression, complete penetrance and absence of pleiotropic and epistatic interactions (Weeden and Wendel 1989). Their most important property is the simple genetic background of most polymorphisms. They most likely result from gene mutation, duplication, polyploidization or hybridization. Among widely used isozymes as genetic markers were esterases, alcohol dehydrogenase, α-, β-amylases etc. A list of isozymes with the relevant references was presented by Weeden and Wendel (1989).

Isozymes were a convenient tool in many genetic studies. They were effectively used as markers in breeding new wheat genotypes. Isozyme analysis is a quite economical and effective method for mutation and recombination differentiation in genes and chromosomes. According to Karcicio and Izbirak (2003), isozyme electrophoretic patterns could be used as markers in: a) linkage studies, b) establishing chromosome translocations, c) detecting gene localization on chromosomes, d) detecting gene expression changes observed at different developmental stages, e) providing qualitative and quantitative estimates of gene flow and divergence in gene manipulation, and f) determining spreading limits and species separation of natural plant populations. However, due to the fact that most cultivars are very similar, isozymes do not express adequate polymorphism. In addition, they are produced in small amounts and for this, changes in amino acid sequence resulted after changes in DNA sequence, cannot be stained and therefore differentiated well (Karcicio and Izbirak 2003).

Seed storage proteins

The second class of biochemical markers is the polymorphic group of seed storage protein loci. The main wheat seed storage proteins are gliadins and glutenins (Miflin *et al*. 1983; Payne 1987). Gliadins are alcohol-soluble proteins, which can be divided into four groups by acid polyacrylamide gel electrophoresis (APAGE): α-, β-, φ and ω-gliadins (Woychik *et al*. 1961). The S-rich prolamins, α-, β- and φ-gliadins (molecular weight of 36-44 kd) contain about 2% of cysteine, whereas ω-gliadins (molecular weight of 44-78 kd) are S-poor prolamins (Miflin *et al*. 1983). Glutenins are large aggregates of subunits joined by disulphide bonds. Glutenin subunits are divided into two classes: low-molecular-weight (LMW) glutenin subunits (31-51 kd) and high-molecular-weight (HMW) subunits (80-140 kd) (Payne and Corfield 1979).

In *T. aestivum* (2n = 6x = 42, AABBDD) gliadins are encoded by the six major loci *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2*, and *Gli-D2* located at the distal ends of the short arms of homoeologous group 1 and 6 chromosomes (Rybalka and Sozinov 1979; Dvorak and Chen 1984; Payne 1987; Singh and Shepherd 1988). The *Gli-A1*, *Gli-B1*, and *Gli-D1* loci contain clusters of genes for φ- and ω-gliadins (Payne 1987). They are closely linked to the loci *Glu-A3*, *Glu-B3*, and *Glu-D3* encoding most of the LMW glutenin subunits (B and C subunits) (Singh and Shepherd 1988). Clusters of genes encoding α- and β-gliadins occur at the *Gli-A2*, *Gli-B2*, and *Gli-D2* loci on the short arms of chromosomes 6A, 6B, and 6D, respectively (Payne 1987). Some β-gliadins are also encoded by the *Gli-A1* and *Gli-B1* loci and this can be seen from the corresponding patterns of certain gliadin alleles (Metakovsky 1991). HMW glutenin subunit loci *Glu-A1*, *Glu-B1*, and *Glu-D1* are located on the long arms of homoeologous group 1 chromosomes (Payne *et al*. 1982) and encode 0-1, 1-2, and 2 subunits respectively (Payne and Lawrence 1983).

Some minor gliadin loci [*Gli-A3*, *Gli-B3* (Sobko 1984; Payne *et al*. 1988), *Gli-A4*, *Gli-D4*, *Gli-B5*, and *Gli-D5* (Redaelli *et al*. 1992; Pogna *et al*. 1993; Rodriguez-Quijano and Carrillo 1996)] are also located on the short arms of homoeologous group 1 chromosomes, in addition to the major gliadin loci *Gli-1*. The *Gli-B5* and *Gli-D5* loci encoding ω-gliadins are located at a distance of, on the average, 1.4 and 3.7 cM from the *Gli-B1* and *Gli-D1* loci, respectively (Pogna *et al*. 1993; Rodriguez-Quijano and Carrillo 1996). The *Gli-D4* locus encoding φ-type gliadins is located between the centromere and the *Gli-D1* locus at a distance of 10.1 cM from it (Rodriguez-Quijano and Carrillo 1996). The loci *Gli-A3* and *Gli-B3* are located at a distance of 31 and 26% of recombination from the *Gli-A1* and *Gli-B1* loci, proximal to the centromere (Sobko 1984; Payne *et al*. 1988) and encode 0-2 components. Some of these proteins are ω-gliadins, whereas some others are D glutenin subunits. According to a hypothesis proposed by Masci *et al*. (1993), D glutenin subunits were formed as a consequence of a mutation of the ω-gliadin genes resulting in the formation of one or more cysteine codons. On chromosome 1D, genes for D glutenin subunits are closely linked to the *Gli-D1* locus (Payne 1987). Later studies demonstrated a further complexity of gliadin loci. A D glutenin subunit encoded by the gene at the *Glu-B3* locus was detected by Nieto-Taladriz *et al*. (1998). Metakovsky *et al*. (1997a) identified some proteins with properties of D glutenin subunits which were encoded at the *Gli-B1* locus. In the same study, it was also demonstrated that some proteins with the biochemical characteristics of C-type LMW glutenin subunits were encoded by the *Gli-B2* locus on chromosome 6B. Felix *et al*. (1996) referred two C-type LMW glutenin subunits encoded by genes on chromosome arm 6DS. Nieto-Taladriz and Carrillo (1996) reported that *Gli-A3* locus also encodes φ-gliadins. In addition, LMW glutenin subunits of a novel type (with amino acid sequence differing from that of known LMW glutenin subunit sequences) were identified (Sreeramulu and Singh 1997). These loci were designated as *Glu-D4* (on chromosome 1D) and *Glu-D5* (on chromosome 7D).

Multiple allelism characterises the major storage protein loci. Catalogues of alleles at gliadin loci were compiled by Sozinov and Poperelya (1980) and Metakovsky (1991), and they were supplemented with new alleles (Metakovsky and Branlard 1998; Metakovsky *et al*. 2000). Gliadin alleles involve 1-8 components inherited together, and for this they are called gliadin blocks. The main catalogues of alleles of HMW and LMW glutenin subunits are the respective catalogues of Payne and Lawrence (1983) and Gupta and Shepherd (1990). Patterns of LMW glutenin subunit alleles are also complex and involve 1-8 components.

Wheat storage proteins also include globulins – triplet proteins called triticins (Shepherd 1988). These proteins are tetramers consisting of subunits D (58 kd), δ (22 kd), A (52 kd), α (23 kd) linked together by disulphide bonds. Their structure is similar to the respective of the 11-12S globulins, which are storage proteins in legumes and some other cereals, including oats and rice (Shepherd 1988). Genes encoding triplet proteins (*Tri-1*) are located on the short arms of chromosomes 1A and 1D close to the centromere (Singh and Shepherd 1988).

Two more polymorphic seed proteins, although not storage proteins separated with the isoelectric focusing method, must be noted: water-soluble proteins (*Wsp-1*) and the iodine-binding factor (*Ibf-1*) (Liu *et al*. 1989; Liu and Gale 1989). The *Wsp-1* loci encoding water-soluble seed proteins are located on the long arms of the homoeologous group 7 chromosomes (Liu *et al*. 1989). In a sample of 44 wheat varieties, 5, 3, and 2 alleles at the *Wsp-A1*, *Wsp-B1*, and *Wsp-D1* loci were identified respectively. The loci for the iodine binding factor (a monomeric seed protein) are located on the long arms of the homoeologous group 5 chromosomes. At the *Ibf-A1*, *Ibf-B1*, and *Ibf-D1* loci, 5, 4, and 4 alleles, were identified, respectively (Liu and Gale 1989).

Application of biochemical markers to population-genetic studies and to the analysis of genetic diversity of wild wheat species

The discovery of isozyme polymorphism allowed the analysis of variation at many loci and the investigation of the genetic structure in natural populations of animals and plants as well as in cultivated species (Nevo 1983). The existence of non-random associations between allelic variants of genes and their selective value was demonstrated by Allard and his colleagues. The above mentioned scientists using esterases and other isozymes as biochemical markers investigated Composite Cross populations of barley (CCII, CCV) as well as natural populations of wild barley and oat, and collections of cultivated barley (Allard *et al*. 1972a, 1972b; Clegg *et al*. 1972; Kahler and Allard 1981; Muona 1982). Isozyme loci were included in extensive studies of genetic variation in wild emmer wheat [*Triticum turgidum* var. *dicoccoides* (Korn.) Thell] (2n = 4x = 28, AABB), a wild progenitor of cultivated wheat, which were conducted at macrogeographic (in the Near East Fertile Crescent) and microgeographic levels (four microsites north of the Sea of Galilee differing in their climatic and soil conditions) (Nevo *et al*. 2002). The genetic structure of 37 populations was studied at 42 isozyme loci and a substantial multilocus organisation was revealed. Isozyme diversity was significantly correlated with climatic and soil factors. This supported the hypothesis that allozyme polymorphisms are at least partly adaptive and determined by natural diversifying and balancing selection. Investigations on the microgeographic level also demonstrated that isozyme polymorphism is partly adaptive. For example, allozymic variation was analysed at 48 loci in 137 *T. dicoccoides* plants from a microsite in the location of Yehudiya in two climatic microniches: sunny between trees, and shady under the trees' canopies. Significant genetic differentiation at single and multilocus structures was found in neighbouring climatic niches, separated by only a few meters. This differentiation

suggested that isozyme polymorphisms are differentiated primarily at the multilocus level by the climatic factors related to temperature, water availability and light intensity stresses (Nevo *et al*. 2002). It should be noted that research with different DNA markers supported the main regularities detected with the use of biochemical markers (Nevo *et al*. 2002).

Seed storage proteins were also used as markers in population-genetic studies. Correlation of the genetic variation and the allele frequencies at HMW glutenin subunit loci in wild emmer wheat and their associations to climatic and natural factors were revealed (Nevo and Payne 1987; Levy and Feldman 1988). Ciaffi *et al*. (1993) demonstrated that seed storage protein differentiation in wild emmer was significantly correlated with the altitude of the collecting sites.

Isozymes and storage proteins were used for the analysis of genetic variation in other wild relatives of wheat (e.g. *Aegilops tauschii* Coss., *Ae. speltoides* Tausch, *T. monococcum* L., *T. urartu* Tum *ex* Gand, etc.). They were also used in wheat phylogeny studies, and in the evaluation of genetic resources for cultivated wheat improvement (Lagudah and Halloran 1988, 1989; Metakovsky and Baboev 1992; Jaaska 1995; Ciaffi *et al*. 1997; Hegde *et al*. 2000). Variation in HMW glutenin subunits and gliadin in *Ae. tauschii* Coss., the D genome donor of common wheat, was found to substantially exceed the existing diversity in common wheat (Lagudah and Halloran 1988).

The use of biochemical markers for varietal identification of cultivated wheat, and analysis of their diversity

Seed storage proteins are probably the most broadly used biochemical markers for identification, registration and purity analysis in wheat varieties. The HMW glutenin subunit composition is used as an additional tool in conducting tests of distinctness, uniformity and stability (UPOV 1996).

There is a vast number of wheat germplasm collections from different countries and breeding centres which have been analysed and certified with the use of seed storage proteins. Gliadin and glutenin allele composition was identified for common wheat cultivars developed in Russia (Chernakov and Metakovsky 1994; Novosel'skaya-Dragovich *et al*. 2003), Ukraine (Sobko and Sozinov 1999), North Kazakhstan (Metakovsky *et al*. 1988), USA (Lookhart et al. 1993), France (Metakovsky and Branlard 1998), Italy (Metakovsky *et al*. 1994), England (Chernakov and Metakovsky 1994), Portugal (Igrejas *et al*. 1999), Spain (Metakovsky *et al*. 2000), South Africa (Labuschagne *et al.* 2000), Argentina (Gianibelli *et al*. 2002), Japan (Tanala *et al*. 2003), India (Oak *et al*. 2004), Greece (Xynias *et al.* 2006) and in many other countries. The data with respect to the *Glu-1* loci of the world wheat collection are listed by Wrigley *et al*. (2006). In the same reference, information on LMW glutenin subunits and gliadin composition of the wheat germplasm is also included.

The investigation of wheat varieties developed in different periods of time allowed the elucidation of wheat breeding history. The analysis of the bread wheat varieties developed in the former USSR from the beginning of the 20th century till now revealed the existence of non-random associations of alleles at the storage protein loci and the role of leader varieties in their formation (Sozinov *et al*. 1999; Sozinov 2001). Based on the available information on HMW glutenin subunit composition of common wheats from different countries and their pedigrees, Rabinovich *et al*. (2000) were able to support evidence on the origin of the high-quality alleles of HMW glutenin subunits. These alleles, which exist in many cultivars in different countries of Europe, originated from the ancient Ukrainian wheat 'Red Fife', 'Ukrainka' and 'Krymka'.

Table 2 Ranking of alleles at the *Glu-1* (Payne *et al*. 1984), *Gli-1*, and *Gli-2* (Sozinov and Poperelya 1979, 1980) loci with respect to the effect on bread-making quality.

Locus	Alleles
Glu-A1	$a = b > c$
Glu-B1	$i;\ g;\ b > c > a > = d$
Glu-D1	$d > a = b > c$
Gli-A1	$7 > 4 > 2 > 5 > 3 > 1 \geq 6$
	$(- b > o > c > f > m \quad -)$*
Gli-B1	$1 > 2 \geq 7 > 5 > 4 > 3 > 6$
	$(b > d > - h > e > l > a)$*
Gli-D1	$4 > 5 > 1 > 2 > 3$
	$(j > g > b > f > a)$*
Gli-A2	$3 > 1$
Gli-B2	$2 > 1$
Gli-D2	$2 > 1 > 3$

* corresponding designations of alleles according to Metakovsky 1991.

Biochemical markers and the improvement of grain quality in wheat

Storage proteins are directly associated to bread-making quality. A detailed review on the role of storage proteins in the formation of dough properties and the relevant data on the association of biochemical markers based on storage proteins with quality indices are given by Skerritt (1998). Based on large-scale experiments gliadin alleles (Sozinov and Poperelya 1979, 1980) and HMW glutenin subunit alleles (Payne *et al*. 1984) were ranked with respect to their effect on bread-making quality (**Table 2**). In *T. durum* Desf., the presence of γ-gliadin 45 was considered as a marker for high pasta quality, whereas γ-gliadin 42 was associated with low quality (Damidaux *et al*. 1978). However, it was later demonstrated that the higher pasta quality directly depended on the presence of LMW glutenin subunits (pasta-making quality was strongly correlated with the presence of the LMW-2 type of the SDS-PAGE pattern, Masci *et al*. 2000), and γ-gliadins 45 and 42 were only the genetic markers of pasta firmness and elasticity (Feillet *et al*. 1989). These data suggested that effects of gliadin alleles on grain quality could be determined by their linkage to certain alleles at *Glu-3* loci. The effect of many LMW glutenin subunit alleles on dough quality was studied by Gupta and MacRitchie (1991) and the data were summarised in a review by Skerritt (1998). A close association between *Gli-1* and *Glu-3* loci hampers analysis of individual contributions of gliadins and LMW glutenin subunits to quality indices. However, in some other studies a direct effect of gliadins on gluten quality was detected. This was demonstrated by the association of allelic variants of gliadins encoded by the *Gli-2* loci with gluten strength, which was revealed in studies including biotypes, hybrid material and various collections of varieties (Sozinov and Poperelya 1980; Sozinov *et al*. 1993). Metakovsky *et al*. (1997b) reported a significant correlation of the alleles *Gli-A2b* and *Gli-B2c* to high gluten strength in Italian bread wheat varieties. Significant interaction effects between alleles of HMW glutenin subunits (Carrillo *et al*. 1990; Rousset *et al*. 1992), alleles at the *Glu-B1*,*Glu-A3/Gli-A1*, *Glu-D3*, and *Gli-B1* loci (Nieto-Taladriz *et al*. 1994), alleles at the *Glu-D1* locus and the 1BL/1RS translocation (Martin *et al*. 2001) on bread-making quality were also demonstrated. These results indicate that the whole complex of storage proteins should be taken into account in breeding for quality. The development of the super-strong common wheat varieties 'Panna' and 'Leleka', which produce high grain quality even when affected by *Eurygaster integriceps* Put. may serve as an example of the application of biochemical markers in breeding for improved quality (Poperelya and Blagodarova 1998). These varieties carry the "over-expressed" allele from 'Odesskaya Krasnokolosaya' at the *Glu-B1* locus, which can be differentiated from the common allele *Glu-B1b* by APAGE.

Electrophoretic screening was used for developing durum wheat lines carrying introgressions of the *Glu-D1* lo-

cus. This introduction of the *Glu-D1* locus improved bread-making quality in the range from 12 to 600% depending on the levels of the parameters tested in the original recipient lines and did not produce any significant effect on pasta quality (Lukaszewski 2003).

Use of biochemical markers for identification of alien material in common wheat genome

Before the development of DNA markers, biochemical markers, including monomorphic loci in wheat, were widely used for the identification of alien material in the wheat genome. One of the most glamorous examples was the identification of the wheat-rye 1BL/1RS and 1AL/1RS translocations (translocation of the short arm of rye chromosome 1R on the long arm of wheat chromosome 1B and 1A, respectively), which are widely-spread among modern common wheat varieties (Rabinovic 1998). The 1RS arm of these translocations carries several resistance genes to rusts, powdery mildew, greenbug (McIntosh *et al*. 1998) and to abiotic stress conditions and more precisely to drought and low temperature (Misic *et al*. 1998). It also increases yield in certain environments, although this performance is depended on the wheat background (Berzonsky and Franki 1999). Increased haploid production and enhanced green plant regeneration from anther culture were also attributed to the presence of the 1RS arm (Henry *et al*. 1993). For all those advantages, the1BL/1RS and 1AL/1RS translocations became widely spread within modern bread wheat varieties. According to Lukaszewski (1990) 11% of the wheat lines in 1989 USA wheat nurseries and 38% in the 21st International Winter Wheat Performance Nursery carried the 1RS arm. In a more recent study, Kazman *et al*. (1998) reported that 17% of the 454 European wheat cultivars studied also carried the 1RS arm. A similar situation was referred by Villareal *et al*. (1998) who stated that 50% of CIMMYT high yielding bread wheat advanced lines possessed the 1BL/1RS translocation. This widespread significance of the 1BL/1RS and 1AL/1RS translocations to wheat variety performance and wheat breeding programs resulted to a search for methods facilitating breeders and geneticists to identify the translocation.

The most convenient and simple way to identify the 1BL/1RS and 1AL/1RS translocations is acid gel electrophoresis of seed alcohol-soluble proteins. The presence of the specific block of components in the electrophoretic pattern of alcohol-soluble proteins of a seed marks the presence of the 1BL/1RS translocation. This block (allele) was designated as *Gli-B1-3* (*Gld1B3*, Sozinov and Poperelya 1977; Sasek and Bartos 1980) or as *Gli-B1l*, according to the international rules for wheat symbols (Metakovsky 1991). The second important, in terms of breeding, wheat rye translocation, 1AL/1RS, can be also identified from the presence of the specific secalin (secalin is the main seed storage protein in rye) block. The responsible allele in this case is *Gli-A1-17*, according to the nomenclature of Sobko and Poperelya (1986).

In many studies, biochemical markers were used for identification of alien chromosomes and translocations: e.g., identification of *Thinopyrum bessarabicum* chromosomes in disomic addition lines of wheat (William and Mujeeb-Kazi 1995), identification of *Aegilops* material in wheat lines developed after genome substitution using isozymes of α- and β-amylases (Antonyuk and Ternovskaya 1995) etc.

Application of biochemical markers for the identification of resistance genes in wheat

The above-mentioned secalin blocks (*Gli-B1-3* (*l*) and *Gli-A1-17*) may serve as biochemical markers for resistance genes associated with the wheat-rye 1BL/1RS and 1AL/1RS translocations. The 1RS arm in the 1BL/1RS translocation is originating from the diploid rye 'Petkus' (Villareal *et al*. 1998). This arm carries a number of resistance genes: i.e. *Pm8* (resistance to powdery mildew caused by *Erysiphe graminis*), *Sr31* (resistance to stem rust caused by *Puccinia graminis*), *Lr26* (resistance to leaf rust caused by *Puccinia recondita*), and *Yr9* (resistance to yellow rust caused by *Puccinia striiformis*) (McIntosh *et al*. 1998). The 1AL/1RS translocation was originally found in the wheat cv. 'Amigo' (Sebesta *et al*. 1995). The 1RS arm, which was transferred to the cv. 'Amigo' from 'Insave' rye (Sebesta *et al*. 1995), carries genes for resistance to greenbug (*Schizaphis graminum*) biotypes B and C (*Gb2*, Sebesta *et al*. 1995), to powdery mildew (*Pm17*, Heun *et al*. 1990), to wheat curl mite *Aceria tosicheilla* (Keifer) (Sebesta *et al*. 1994). The GRS 1201 bread wheat line (Porter *et al*. 1993) and a number of related lines (Porter *et al*. 1991) carry another 1AL/1RS translocation, which also derived from the rye cv. 'Insave'. The 1AL/1RS translocations in Amigo and in GRS 1201 bread wheat line can be distinguished with biochemical markers (secalins) (Graybosch *et al*. 1999). The 1AL/1RS translocation in the line GRS 1201 carries the gene *Gb6* conferring resistance to a wider range of greenbug biotypes, i. e. B, C, E, G, and I (Porter *et al*. 1993). A biochemical marker was found for the suppressor (*SuPm8*) of the powdery mildew resistance gene *Pm8* (located on the 1BL/1RS translocation). This marker turned out to be the gliadin allele *Gli-A1a* (Ren *et al*. 1996).

Relationships between endopeptidases and resistance genes were also detected. The *Ep-D1b* band of the endopeptidase enzyme is a biochemical marker for the effective gene *Pch1* offering resistance to *Pseudocercosporella herpotrichoides* causing eyespot (McMillin *et al*. 1986). This gene was introgressed from *Ae. ventricosa* into chromosome 7D of wheat. Another endopeptidase, *Ep-A1b*, is linked with a 15% recombination frequency to a second resistance gene, *Pch2* (de la Pena *et al*. 1996). The endopeptidase marker *Ep-D1b* was used in breeding programs, and in particular for the development of the cvs. 'Hyak' and 'Madsen' (Allan *et al*. 1989, 1990). However, no association between the endopeptidase pattern and resistance was found in a collection of *T. monococcum* (Cadle *et al*. 1997). Endopeptidase could also serve as a marker for the leaf rust resistance gene *Lr19* (McMillan *et al*. 1993; Winzeler *et al*. 1995), which was transferred from *Agropyron elongatum* to the 7DL arm of bread wheat.

Distal ends of the homoeologous group 1 chromosomes are gene-rich regions, as it was demonstrated by the identification of expressed sequence markers and expressed sequence tag analysis (Sandhu *et al*. 2002; Qi *et al*. 2004). Linkage of *Gli-1* loci to many resistance genes was demonstrated. The *Gli-D1* locus is linked to the leaf rust resistance gene *Lr21* (Jones *et al*. 1990) and to the stem rust resistance gene *Sr 33* (Jones *et al*. 1991; Czarnecki and Lukow 1992) with a 5.6% recombination frequency. The stripe rust resistance gene *Yr10* was mapped at a distance of 5% of recombination from the *Gli-B1* locus (Payne *et al*. 1986). The *Gli-A1* locus and the closely associated loci (*Gli-A5*, *Glu-A3*) on chromosome 1A are linked to powdery mildew resistance gene *Pm3* (*Pm3g* – 5cM from *Gli-A5*) (Sourdille *et al*. 1999) and to leaf rust resistance gene *Lr10* [Howes 1986 (2.9 cM from *Gli-A1*); Feuillet *et al*. 1997 (8 cM from *Glu-A3*)]. These data suggest that the corresponding alleles of gliadin loci may be used as biochemical markers for these resistance genes. For the above resistance genes, DNA markers were also elaborated (McIntosh *et al*. 2003). It should be mentioned that *Lr21* and *Lr10* were the first cloned resistance genes from the wheat genome (Feuillet *et al*. 2003; Huang *et al*. 2003). A major QTL associated with resistance to tan spot caused by *Pyrenophora tritici-repentis* (Died.) Drechs. *QTsc.ndsu-1A* was detected on the short arm of chromosome 1A (Faris *et al*. 1997; Effertz *et al*. 2001). The marker of this QTL was the RFLP marker *XGli1* with the allele from the line W-7984. *QTsc.ndsu-1A* accounted for 35 (Faris *et al*. 1997) to 64% (Effertz *et al*. 2001) of the variation in seedling resistance and 26% of the variation in adult plant resistance (Effertz *et al*. 2001). As a consequence, the *Gli-A1* locus allele of the synthetic hexa-

ploid W-7984 could be used as a biochemical marker for this QTL for resistance to chlorosis induction.

Association of biochemical markers with adaptation and productivity

It is well established that isozyme and storage protein polymorphism in populations of wild wheat relatives, is at least partly adaptive (Nevo *et al*. 2002). This was also confirmed by the formation of non-random associations of alleles at gliadin loci detected in wheat varieties from different breeding centres (Sozinov *et al*. 1999). Adaptation is closely related to the exploitation of allelic variant associations of marker loci with productivity traits and cold tolerance. The development of DNA markers based on PCR offers new possibilities for investigating these quantitative traits over the whole wheat genome (Gupta *et al*. 1999). However, the presence of gliadin loci in gene-rich regions in the distal parts of chromosomes (Qi *et al*. 2004), and more precisely *Gli-1* (Sandhu *et al*. 2001), suggests the possibility of associations between these loci with QTL responsible for adaptation and yield.

The study of hybrid genetic material revealed associations of some allelic variants of storage protein loci with yield and adaptation. Analysis of F_2 plants from the cross between the winter bread wheat varieties 'Bezostaya 1' and 'Crvena Zvezda' (Poperelya *et al*. 1980) lead to the conclusion that the *GLD 1B8* (*Gli-B1-8*) allele was associated with reduced frost resistance. Association of productivity with allelic variants at the *Gli-A1* locus was demonstrated in F_7–F_{10} progenies of individual plants derived from the winter wheat cross 'Bezostaya 1' × 'Dneprovskaya 521' (Sozinov and Poperelya 1985). Productivity was significantly higher in lines having the *GLD 1A1* allele (*Gli-A1m*) compared to those having the *GLD 1A4* allele (*Gli-A1b*).

Associations of the allelic variants of the storage protein loci *Gli-B1*, *Gli-D1*, *Glu-D1* with yield was demonstrated after studying the F_2 population of plants originating from the winter bread wheat cross 'B16' × 'Donskaya Polukarlikovaya' (Sozinov *et al*. 1993). Plants heterozygous at the *Gli-B1* locus (*Gli-B1-3.7* – heterozygous for the presence of the rye 1BL/1RS translocation) significantly surpassed the group of plants with the *Gli-B1-7.7* genotype in number of spikes, mass and number of seeds per plant. Plants with the genotype *Gli-D1-4.4* [*Gli-D1-j.j* according to the catalogue of Metakovsky (1991)] produced more mass and number of seeds per plant compared to plants homozygous at the *Gli-D1* locus having the allele *Gli-D1-3* (*Gli-D1a*). Plants heterozygous at the *Glu-D1* locus (*Gli-D1a.d*) produced less seeds per plant compared to plants homozygous at the *Gli-D1* locus (*Gli-D1a.a*). Sozinov and Khokhov (1987) after studying a F_2 population originating from the cross 'Obriy' × 'B16' demonstrated an association between heterosis in winter bread wheat hybrids and the allele state at the *Gli-B1* locus (heterozygosity for the 1BL/1RS translocation). Carrillo *et al*. (1990), using a set of F_8 recombinant inbred lines originating from the spring bread wheat cross 'Anza' × 'Cajeme 71', also detected associations between HMW glutenin loci and grain yield. The high-grain yield potential of cv. 'Anza' was strongly associated with the HMW glutenin alleles (*Glu-A1c*, *Glu-B1b*, and *Glu-D1a*) which were contributing to inferior bread-making quality.

The investigation of a large set of wheat collections and breeding germplasm (hybrids and lines) from breeding nurseries, suggested some relationships between certain allelic composition of gliadins and frost tolerance (Sozinov and Poperelya 1979). Winter wheat varieties with the best frost tolerance were those carrying the alleles *GLD 1A1* (*Gli-A1m*) *or GLD 1A2* (*Gli-A1o*), *GLD 1D5* (*Gli-D1g*), *GLD 6A3*, *GLD 6D2*. Metakovsky and Branlard (1998) investigated the diversity in gliadin allele composition in French bread wheat varieties. They detected that allelic variation at the *Gli-B1*, *Gli-A2* and *Gli-D2* loci was significantly correlated with earliness. Four alleles (i.e. *Gli-B1b*, *Gli-A2f*, *Gli-A2t*, and *Gli-D2m*) were the most frequent in the group of the earliest flowering cultivars. On the other side, the alleles *Gli-B1g*, *Gli-B1l* (the 1BL/1RS translocation) and *Gli-D2g* were characteristic of the latest flowering cultivars. It was also revealed that allelic variation at the *Gli-D2* locus was significantly correlated ($P < 0.05$) with the North/South habit. The *Gli-B1l* allele was only present in cultivars growing in Northern France. The authors suggested that certain gliadin alleles were associated with cold resistance: the frequency of the alleles *Gli-B1l*, *Gli-A2r* and *Gli-D2g* was significantly higher, and the alleles *Gli-A1a*, *Gli-B2c* and *Gli-D2m* significantly lower, in the group of the best cold resistant cultivars compared to the group of cultivars with the lowest resistance to cold.

Upelniek *et al*. (2003) suggested that the time and pattern of gliadin degradation during germination may be responsible for adaptability in grasses. Thus, the highest adaptability is characteristic of varieties showing early and rapid degree of proteolysis. Based on a sample of 16 spring common wheat varieties, the gliadin alleles *Gli-A1f*, *Gli-B1e*, *Gli-D1a*, *Gli-D1b* were identified as possible markers for adaptability in spring wheat.

The association between gliadin alleles and adaptation was referred by Metakovsky *et al*. (1986) in composite hybrid populations of spring bread wheat, by Kolyuchii and Sozinov (2000) in composite hybrid winter bread wheat populations, and by Kopus (1994) in populations of near-isogenic lines with respect to gliadin loci. The frequency of the *Gld 1D3* allele (*Gli-D1a*) in a complex hybrid spring common wheat population, after reproduction in the northern forest-steppe of Russia (near Novosibirsk), increased substantially with the presence of five alleles at this locus in the initial population (Metakovsky *et al*. 1986). Kolyuchii and Sozinov (2000) studied a complex hybrid population originating from crosses among five winter common wheat varieties. This population was reproduced for 10 years in Mironovka (the forest-steppe of Ukraine). The parents of the above population were varieties developed and released in the same ecological zone and, thus, well adapted to the conditions of the Forest-Steppe of Ukraine. It was reported that the gliadin allele frequencies of the parental associations (with one exception, cv. 'Illichevka') predominated over the newly-formed combinations. This indicated the adaptive value of such allele combinations.

CONCLUSIONS

Biochemical markers proved to be beneficial in solving a number of problems in wheat genetics, evolution and breeding. With their application, it was demonstrated that gene synteny is conserved among *Triticeae* genomes (Hart 1987). Biochemical markers were employed for studying world genetic diversity of wheat and its relatives. The association between allelic variants of certain loci and qualitative and quantitative traits, including grain quality, productivity, resistance to some diseases and pests was also detected. They became one of the most effective tools for seed production control and varietal identification. The discovery of co-adaptive gene association formation in the area of natural and artificial selection pointed out the importance of biochemical markers in wheat breeding. Finally, the use of biochemical markers could be considered as the initial source for the development of the theoretical basis for the use of DNA-markers in wheat genetics and breeding.

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