Gliadin genotypes worldwide for spring wheats (*Triticum aestivum* L.) 1. Genetic diversity and grain-quality gliadin alleles during the 20th century

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ARTICLE INFO

Keywords:

Dough quality
Genetic diversity
Genetic erosion
History

ABSTRACT

High genetic diversity ($H = 0.85 \pm 0.03$) of spring common wheats (290 cultivars from nine countries, four continents) was discovered through the identification of alleles at the gliadin-encoding (*Gli*) loci. There were no indications of the existence of genetic erosion (narrowing of polymorphism with time) either for wheat germplasm studied throughout the 20th century, or in the group of cultivars bred in any country. At the same time, there was a significant change of the frequency of occurrence, in wheat germplasm of Australia, Canada, Mexico, Nordic countries, Spain, and the former USSR, of certain gliadin alleles related (as shown earlier) to end-use wheat quality, mainly to dough strength. The increase in frequency, in genotypes of groups of more recent cultivars, of gliadin alleles positively influencing dough strength (*Gli-B1b*, *Gli-B1d*, and *Gli-B2c*) caused a loss of genetic diversity at the corresponding *Gli* loci. It is suggested that these alleles may serve as an indirect targets of breeders' selection for dough quality. Mechanisms of the involvement of some allelic variants of the *Gli* loci in the manipulation of dough quality are discussed.

1. Introduction

During the 20th century, the common way for wheat improvement was based on crosses between different genotypes. However, the repeated use in breeding of a restricted number of parental genotypes, successful in producing new cultivars, may cause a decrease of the level of genetic diversity of wheat germplasm and thereby restrict the potential of wheat improvement (Porceddu et al., 1988). One of the main factors which may influence negatively on wheat genetic diversity is the selection, common for many countries during the second part of the 20th century, for improved end-use (dough) quality. There is a set of reliable genetic markers which may be used for the evaluation not only of the diversity of wheat germplasm in general, but, at the same time, to reveal its possible temporal changes which might be caused by the selection for quality. These markers are provided by the wheat seed storage protein complex, gliadin.

The gliadins possess a unique assortment of merits: immense polymorphism, well-studied genetics, and complex structure of the gliadin-coding loci (*Gli*) which include different alleles. The synthesis of gliadins is encoded at the six main *Gli* loci (*Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2*, *Gli-D2*), and a vast multiple allelism has been described for each

of these loci. Each allele at each *Gli* locus encodes several polypeptides (“a block”) inherited as a Mendelian co-dominant trait. Recombination frequency between gliadin-coding genes of a *Gli* locus does not exceed 1% (reviewed in Metakovsky, 2015).

Alleles at the *Gli* loci might be identified through the electrophoretic analysis of the set of different gliadin polypeptides produced by a given wheat genotype. It is established that gliadin electrophoregrams do not depend on the growing conditions of the plant (eco-climatic conditions, the level of fertilization and/or watering), nor on grain protein content or on the maturity of the grain. Alleles at the same locus differ in number and in the electrophoretic mobility of the respective gliadin polypeptides composing a block (reviewed in Metakovsky, 2015). A recent catalogue includes more than 180 alleles, in sum, at the six *Gli* loci, and there was no instance of the identity of gliadin genotypes for two unrelated cultivars among more than 1000 cultivars studied (Metakovsky et al., 2018b). Obviously, in their resolving power, the extensive family of gliadin alleles may successfully compete with other genetic markers, including any DNA markers, for wheat genotype identification and distinction and, therefore, for evaluation of genetic diversity in a group of cultivars.

In addition, an important advantage of gliadins as genetic markers

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would be their relation to dough quality. It has been demonstrated that the contribution of the gliadin fraction of gluten is to dough extensibility and loaf volume of bread rather than to dough strength, the latter balancing characteristic being attributed especially to the glutenin fraction, which (unlike the gliadins) have cysteine residues to form intermolecular bonds. Thus, it was shown that a higher proportion of gliadin in flour reduces dough strength (Gupta and MacRitchie, 1994).

An influence of individual gliadin alleles on dough quality has been documented in many publications (reviewed in Metakovsky, 2015) and explained (Payne et al., 1984) through the linkage of the *Gli-1* and *Glu-3* loci. It is known that synthesis of the wheat-seed storage protein, low-molecular-weight (LMW) glutenin, is encoded at the *Glu-A3*, *Glu-B3*, and *Glu-D3* loci, tightly linked to the *Gli-A1*, *Gli-B1*, and *Gli-D1* loci, respectively (Payne et al., 1984; Singh and Shepherd, 1988). The recombination frequency between *Gli-1* and *Glu-3* loci does not exceed 3% in different studies (reviewed in Metakovsky, 2015). Moreover, analysis of some extended DNA sequences shows that there is no clear border between *Gli-1* and *Glu-3* loci at the DNA level (Gao et al., 2007; Anderson et al., 2012).

Also, it was discovered that, in some variants of the complex *Gli* loci, there are genes encoding polypeptides similar to LMW glutenin subunits (Kasarda et al., 1987; Lew et al., 1992; Anderson et al., 2001; Masci et al., 2002; reviewed in Metakovsky, 2015).

In general, there is no doubt now that allelic variation at the DNA regions of common wheat chromosomes marked by each of the six *Gli* loci make significant contributions to end-use wheat quality via the casual effects of *Gli-1* and/or *Glu-3* alleles. Therefore, the gliadin alleles are likely to be involved in breeders' selection (positive or negative) as indirect targets for modifying grain quality. To provide new insights into relationships between common breeding for dough properties and wheat genetic diversity, it would be valuable to evaluate the possible effects of breeders' selection on the frequency of some gliadin alleles known to relate to end-use wheat quality.

In this work, we studied, using gliadin alleles, the diversity of spring common wheat germplasm worldwide with respect to time scale throughout the 20th century. Also, we intended to find gliadin alleles and *Gli* loci which might be indirectly involved in breeders' activity and that thereby might influence changes in genetic diversity for wheat in the future.

2. Materials and methods

2.1. Grain materials

Nearly all grain samples of spring common wheat (*T. aestivum* L.) were obtained from genetic and/or breeding laboratories from the countries of origin of the cultivars studied. The internet site (wheatpedigree.net) was used to establish the pedigree of a cultivar, its winter/spring habit, year of release, and the country where it was bred. Also, we followed the spelling of names of cultivars used at this site.

In total, *Gli* alleles in 290 cultivars bred in nine countries situated in four continents were identified.

2.2. Methods

The gliadin allele compositions for 275 spring cultivars used in our analysis have been published earlier (Metakovsky et al., 2018b). Gliadin alleles for a further 15 varieties were identified in this work (Table S1, Supplementary materials) using the procedure described earlier (Metakovsky et al., 2018b). The full list of cultivars involved in this work is presented in the List S1 (Supplementary materials).

An allele occurring with the frequency of > 40% at a given *Gli* locus in a given group of cultivars was assumed to be the most frequent gliadin allele (MFGA) (Table 1). Frequency of the most frequent allele at each *Gli* locus in each group of cultivars studied is also shown (Table

S2, Supplementary materials).

The Nei's genetic diversity index (H) at a *Gli* locus in a group of cultivars was computed as $H = 1 - \sum p^2$, where p is the frequency of an allele at this locus. The overall diversity in a group of cultivars was assumed to be an average of H obtained for each of the six *Gli* loci.

The significance of the difference of occurrence of an allele in two temporal groups of cultivars was analyzed by the χ^2 test with one degree of freedom (Rana and Singhal, 2015).

3. Results

Genetic diversity of the 290 spring common wheat cultivars studied was as high as $H = 0.85 \pm 0.03$. To provide insight into possible changes of genetic diversity of cultivars bred during the 20th century, 280 cultivars with known year of registration were divided into eight temporal groups with similar numbers of cultivars in each: 1901–30 (29 cultivars); 1931–50 (38); 1951–62 (35); 1963–69 (40); 1970–75 (35); 1976–80 (35); 1981–85 (33); and 1986–93 (35).

The level of genetic diversity at a germplasm-studied scale varied during the 20th century between $H = 0.80 \pm 0.04$ (1986–93) and $H = 0.85 \pm 0.02$ (1963–69) with an insignificant decrease with time not exceeding 0.04 units of H during the whole century (Fig. 1). Therefore, we did not find any reliable indication of decrease of genetic diversity of spring common wheat, at the world germplasm level, during the 20th century. It should be noted, however, that results of analysis of genetic variation in global wheat germplasm performed by joining together cultivars from many countries may not reflect the temporal processes occurring in wheat germplasm of each country. Therefore, genotypes in groups of cultivars bred at different times during the 20th century in each country were studied. To compare genotypes of cultivars released in a time course, all cultivars bred in one country were divided into temporal groups, with similar numbers of cultivars in each group (Table 1).

Judging from our results (Table 1), the wheat germplasm of Australia has undergone two dramatic changes in its set of the MFGA (after 1950 and after 1973): only allele *Gli-B1b* (Fig. 2) occurred as the MFGA throughout of the 20th century. It was found that three alleles, *Gli-A1a* (Fig. 2b), *Gli-B2c* (Fig. 2a–d), and *Gli-D2q* spread over Australian wheats so that frequencies of these alleles in the last temporal group of cultivars studied (1974–85) were significantly higher than in cultivars bred in the first part of the 20th century. The increase of the frequency of these three alleles was accompanied by an elimination from Australian wheat germplasm of the alleles *Gli-A1m*, *Gli-B2an*, and *Gli-D2w*, respectively (see Table 2), which are present in the ancient cultivars Gluyas Early, Nabawa and Ranee (see Table S1 in Metakovsky et al., 2018b).

In spite of these changes in allelic frequencies, the overall diversity index H and the number of different gliadin alleles in a group were identical, comparing four temporal groups of Australian cultivars (Table 1). Through application of DNA markers, it was also established earlier that the genetic diversity of Australian wheat was relatively high throughout the 20th century (White et al., 2008).

In Spain, a considerable difference of genotypes, judging from sets of MFGA, between two temporal groups of cultivars studied was observed, but there were no changes either in the genetic diversity or in the number of *Gli* alleles identified (Table 1). However, there was a significant increase, as in Australia, in the group of more recent cultivars, of the frequency of the allele *Gli-A1a* accompanied by the complete elimination of the allele *Gli-A1m* (Tables 1 and 2).

A fall of the genetic diversity accompanied by a decrease of the number of *Gli* alleles was observed in Canadian cultivars released in 1953–85. However, in the group of the most recent cultivars studied (1986–88), both these parameters have fully recovered. A few differences in the MFGA between temporal groups of cultivars studied were observed (Table 1), but there were at least five statistically significant differences between genotypes in the group of eight cultivars bred in

Table 1

Sets of the most frequent gliadin alleles (MFGA), the value of genetic diversity H , and number of different alleles present in groups of common wheat cultivars bred in different countries during the 20th century.

N ^a	Country ^b	Years	The most frequent allele at the <i>Gli</i> loci						H ^c	Number of alleles
			-A1	-B1	-D1	-A2	-B2	-D2		
16	Australia	1901–49	<i>m</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>an</i>	<i>w</i>	0.66	31
16	Australia	1951–66	– ^d	<i>b</i>	–	<i>c</i> + <i>a</i> ^e	–	–	0.68	31
16	Australia	1967–73	–	<i>b</i>	–	<i>c</i>	<i>c</i>	–	0.69	30
15	Australia	1974–85	<i>a</i> ^f	<i>b</i>	<i>b</i>	<i>f</i> ^f	<i>c</i>	<i>q</i>	0.65	31
8	Canada	1907–52	<i>m</i>	<i>b</i>	<i>a</i>	<i>m</i>	<i>c</i>	<i>m</i>	0.55	19
8	Canada	1953–72	<i>m</i>	<i>d</i>	<i>j</i>	<i>m</i>	<i>c</i>	<i>h</i>	0.34	15
9	Canada	1974–85	<i>m</i>	<i>d</i>	<i>j</i>	<i>m</i>	<i>c</i>	<i>h</i>	0.39	18
6	Canada	1986–88	–	<i>d</i>	<i>j</i>	<i>f</i>	<i>c</i>	–	0.54	19
10	Italy	1913–55	<i>a</i>	–	–	–	–	–	0.73	31
8	Italy	1966–87	<i>a</i>	–	<i>k</i>	–	–	–	0.73	32
7	Mexico	1962–66	<i>a</i>	<i>b</i>	<i>b</i>	–	<i>c</i>	–	0.57	21
6	Mexico	1967–76	<i>o</i>	<i>d</i>	–	<i>f</i>	<i>c</i>	<i>j</i>	0.56	20
7	NC	1921–68	<i>m</i>	<i>k</i>	<i>a</i>	<i>m</i>	<i>g</i>	<i>a</i>	0.52	18
8	NC	1972–81	<i>o</i>	<i>b</i>	<i>a</i>	<i>u</i>	–	–	0.59	22
19	former USSR	1923–45	–	<i>e</i>	<i>a</i>	<i>q</i>	–	–	0.68	38
20	former USSR	1946–59	–	<i>e</i>	<i>a</i>	<i>q</i>	–	–	0.67	40
19	former USSR	1961–75	–	<i>e</i>	<i>a</i>	<i>q</i>	–	<i>e</i>	0.67	42
19	former USSR	1976–80	<i>f</i>	<i>e</i>	<i>a</i>	<i>q</i>	–	<i>e</i>	0.65	45
16	former USSR	1981–86	–	<i>e</i>	<i>a</i>	–	–	–	0.67	45
17	former USSR	1987–93	–	<i>b</i> + <i>e</i> ^e	<i>a</i>	–	–	–	0.71	40
6	Spain	1921–66	<i>m</i>	–	<i>i</i>	–	–	<i>a</i>	0.70	26
9	Spain	1975–92	<i>a</i>	–	<i>b</i>	–	<i>c</i>	–	0.73	28

^a Number of cultivars in the group.

^b Canada without Quebec; NC: Finland (6 cultivars) + Norway (5) + Sweden (5).

^c For Nordic countries, H was calculated using data for five *Gli* loci (without *Gli-B2*).

^d No single allele reached 40% for a given locus in a given group.

^e Two different alleles at the same *Gli* locus occurred each with a frequency exceeding 40%.

^f Frequency of this allele at this locus in this group = 40%.

the first part of the 20th century and nine cultivars released in 1985–88. It was found that the alleles *Gli-B1d* (Fig. 2c) and *Gli-A2f* increased their frequencies while *Gli-D1a* decreased, and the allele *Gli-B2w* was substituted by *Gli-B2c* (Table 2). In addition, there was a substitution, identical to that observed in Australia and Spain, of the allele *Gli-A1m* for *Gli-A1a*: the decrease of the frequency of the *Gli-A1m* and the increase of the *Gli-A1a* were both close to being statistically significant (each, $p < 0.1$).

Temporal changes of genotypes of Canadian Hard Red Spring cultivars were documented earlier using different sets of microsatellites as genetic markers although genetic diversity among cultivars released from different breeding periods, breeding programs, and ancestral families did not change significantly during the 20th century (Fu et al., 2006). At the same time, a decrease in the level of genetic diversity tested through the loss of some identified and unique alleles was observed (Fu and Somers, 2011).

There were some differences of genotypes between two temporal groups of Mexican cultivars studied without change neither in the genetic diversity nor in the number of *Gli* alleles identified in each of these

two groups (Table 1). The only significant difference of genotypes between the two temporal groups was an increase of the frequency of the allele *Gli-B1d* (as it was in Canada) in the more recent group (Table 2).

No significant differences were discovered between six temporal groups of the former USSR cultivars either in their sets of the MFGA, or in the level of genetic diversity H , or in the number of different alleles present in one group (Table 1). The only significant substitution was an increase, with time, of the frequency of the allele *Gli-B1b* (Fig. 2a) accompanied by the loss of the *Gli-B1m*. The local allele *Gli-D2s* disappeared nearly completely from cultivars bred in 1987–93 (Table 2).

The changes in genotypes of cultivars bred in Italy and Nordic countries (NC) were accompanied by a slight increase in genetic diversity and in the number of alleles found in NC cultivars, and was not reflected in this parameter for Italian ones (Table 1). There were no statistically significant temporal changes of the frequency of any allele in these two germplasms studied, although the frequency of the allele *Gli-B1b* increased and of the *Gli-A1m* and *Gli-B1k* decreased with time in NC (each, $p < 0.1$).

Therefore, it was discovered that the genetic diversity H at the

Fig. 1. Levels of genetic diversity (H) and their standard deviations in time-based groups of spring common wheat germplasm during the 20th century.

Fig. 2. Gliadin electrophoregrams of spring common wheat cultivars: a, Gabo (Australia); b, Siete-Cerros-66 (Mexico); c, Laura (Canada); d, Farneto (Italy). Blocks of jointly inherited electrophoretic components encoded by alleles, *Gli-A1a*, *Gli-B1b*, *Gli-B1d* and *Gli-B2c* are shown schematically. B - a universal standard cultivar Bezostaya-1.

corresponding individual *Gli* locus decreased only when the frequency of alleles *Gli-B2c* (Australia, Canada), or *Gli-B1b* (the former USSR and, probably, NC), or *Gli-B1d* (Canada, Mexico) increased with time. Statistically significant temporal increases in the frequency of other gliadin alleles (*Gli-A1a*, *Gli-D1a*, *Gli-A2f*, *Gli-D1q*) did not reduce the genetic diversity at the corresponding *Gli* locus (Table 2).

4. Discussion

There was no considerable loss of polymorphism of common wheat during the 20th century, as shown using different types of genetic markers (reviewed in Van de Wouw et al., 2010). Our results obtained by using highly-polymorphic gliadin markers for the description of genotypes of spring *T. aestivum* cultivars bred during the 20th century fully confirmed this conclusion: we did not find indications of narrowing genetic polymorphism with time throughout the 20th century in spring *T. aestivum* neither at a country level, nor at the world-wide germplasm level: there was neither a permanent temporal decrease of the index *H* nor of the number of registered gliadin alleles.

Analysis of polymorphism of the storage protein gliadin which influences dough quality (Gupta and MacRitchie, 1994) opens a possibility to reveal effects of breeders' selection for higher end-use wheat quality on individual gliadin alleles and *Gli* loci. In this work, a significant variation of the frequency of certain gliadin alleles, in wheat

germplasm of each (except Italy) country studied, was discovered.

Earlier, a few of the many gliadin alleles studied were shown to relate to improved dough quality. For example, only three (*Gli-B1b*, *Gli-A2b* and *Gli-B2c*), out of 28 gliadin alleles studied, related statistically to dough strength (W) in Italian cultivars (Metakovsky et al., 1997a).

The changes discovered in genotypes of Australian wheat (Table 1) might be caused by the transition from using local cultivars to Mexican (CIMMYT's) wheats (O'Brien et al., 2001). Indeed, the alleles *Gli-A1a* and *Gli-B2c*, which increased their frequencies with time in Australian cultivars, are characteristic of Mexican wheats (Table 1). However, the allele *Gli-D2q* (its frequency also increased with time in Australian germplasm) was not found in Mexican wheats studied. Therefore, changes occurred in Australian germplasm in the 20th century could not be explained only through the appearance of Mexican cultivars in Australian breeding programs.

The presence of the allele *Gli-B2c* in genotypes from Italy (Metakovsky et al., 1997a) and France (Metakovsky et al., 1997b) (in both cases, mainly winter wheats were studied) correlated statistically with higher dough strength. It is shown now that the frequency of this allele significantly increased with time in Australian and Canadian wheat germplasm. In both these countries, an increase of the occurrence of the allele *Gli-B2c* was accompanied by a selective loss of genetic diversity at the *Gli-B2* locus (Table 2). The allele *Gli-B2c* is present in each Canadian (without Quebec) cultivar bred in 1985–88 and also

Table 2Temporal changes of the genetic frequency of individual alleles and genetic diversity at individual *Gli* loci.

N ^a	Country	Allele ^b	Years ^c	Frequency (%) ^d	P ^e	H ^f
16	Australia	↓ <i>Gli-A1m</i> ^g	1901–49	43.8	**	0.703
15			1974–85	6.7		0.702
16		↑ <i>Gli-A1a</i>	1901–49	0.0	***	0.703
15			1974–85	40.0		0.702
16		↓ <i>Gli-D1a</i>	1901–49	40.6	*	0.736
14			1974–85	7.1		0.663
16		↑ <i>Gli-D1b</i>	1901–49	6.3	**	0.736
14			1974–85	42.9		0.663
16		↑ <i>Gli-D1f</i>	1901–49	6.3	*	0.736
14			1974–85	35.7		0.663
16		↓ <i>Gli-D1o</i>	1901–49	25.0	*	0.736
14			1974–85	0.0		0.663
16		↑ <i>Gli-B2c</i> ^h	1901–49	12.5	***	0.602
15			1974–85	66.7		0.524
16		↓ <i>Gli-B2an</i>	1901–49	56.3	***	0.602
15			1974–85	0.0		0.524
16		↑ <i>Gli-D2q</i>	1901–49	10.0	*	0.655
15			1974–85	43.8		0.694
16		↓ <i>Gli-D2w</i>	1901–49	50.0	*	0.655
15			1974–85	13.3		0.694
8	Canada	↑ <i>Gli-B1d</i> ^h	1907–52	12.5	*	0.656
9			1985–88	66.7		0.519
8		↓ <i>Gli-D1a</i>	1907–52	68.8	***	0.430
9			1985–88	0.0		0.537
8		↑ <i>Gli-A2f</i>	1907–52	0.0	**	0.680
9			1985–88	50.0		0.654
8		↑ <i>Gli-B2c</i> ^h	1907–52	62.5	*	0.469
9			1985–88	100.0		0.000
8		↓ <i>Gli-B2w</i>	1907–52	37.5	*	0.469
9			1985–88	0.0		0.000
7	Mexico	↑ <i>Gli-B1d</i> ^h	1962–66	0.0	***	0.653
6			1967–77	66.7		0.514
6	Spain	↑ <i>Gli-A1a</i>	1921–66	0.0	*	0.500
9			1975–92	55.6		0.617
6		↓ <i>Gli-A1m</i> ^g	1921–66	66.7	***	0.500
9	former USSR		1975–92	0.0		0.617
6		↓ <i>Gli-D2a</i>	1921–66	50.0	**	0.667
9			1975–92	0.0		0.790
19	former USSR	↑ <i>Gli-B1b</i> ^h	1923–45	6.8	***	0.732
17			1987–93	50.0		0.528
19		↓ <i>Gli-B1m</i> ^g	1923–45	21.1	*	
17			1987–93	0.0		
19		↑ <i>Gli-B2b</i>	1923–45	0.0	*	0.758
17			1987–93	20.6		0.848
19	former USSR	↓ <i>Gli-D2s</i>	1923–45	28.9	*	0.666
17			1987–93	2.9		0.763

^a Number of cultivars studied in a given group for a given locus.^b Alleles whose frequency significantly changed with time: ↓, decrease; ↑, increase.^c Years of registration of cultivars composed a given group.^d Frequency of the allele under study in a given group.^e Statistical significance of the alteration of frequency of a given allele in a given group of cultivars (*p < 0.05; **p < 0.02; ***p < 0.01).^f Genetic diversity at a given locus in the group of cultivars studied.^g It was shown earlier that the presence of this allele in common wheat genotype influences negatively on dough quality (dough strength).^h It was shown earlier that the presence of this allele in common wheat genotype influences positively on dough quality (dough strength).

in each of eight Portugal cultivars studied (see Table S1 in Metakovsky et al., 2018b). All these facts together may indicate indirect breeders' selection, in different countries, in favour of one particular allele (related to end-use wheat quality) at a particular locus.

The fastest polypeptide of the block controlled by the *Gli-B2c* and located in the β-zone of an electrophoregram of gliadin (Fig. 2) is a characteristic of this block: it did not occur in other blocks controlled by other known alleles at the *Gli-B2* locus. Obviously, it is a mutant form of a polypeptide common to most of the other blocks. The polypeptide encoded by the *Gli-B2c* has higher electrophoretic mobility and it is

shorter than similar ones presented in other blocks (Metakovsky et al., 2018a) probably due to mutational reduction of polyglutamine stretches. Mutational changes in the length of the polyglutamine stretches are usual for the α-type gliadin polypeptides (Anderson and Green, 1997; Noma et al., 2016). The allele *Gli-B2c* has a proven positive influence on dough strength, while it is generally assumed that mutant glutenin-like gliadin polypeptides, encoded at the *Gli-2* loci, must have a negative effect on dough quality (Lew et al., 1992; Anderson et al., 2001; Masci et al., 2002).

It was shown earlier, that the allelic variant of the *Gli-B1* locus, present in the cultivar Nuri-70, was better in one of the quality parameters studied, namely, dough breakdown value (Lagudah et al., 1988). Our analysis of the data presented in the works of Lagudah et al. (1988) and Masci et al. (2000) led us to the conclusions that, first, the cultivar Nuri-70 possessed, without doubt, the allele *Gli-B1d*, and second, that exclusively cultivars with this particular allele produced a specific polypeptide of the LMW glutenin type with a molecular mass of 42 kD which might relate to better dough quality (discussed in Metakovsky, 2015). In this work, it was found that the frequency of the allele *Gli-B1d* significantly increased with time causing a decrease of genetic diversity at the *Gli-B2* locus in Canadian and Mexican germplasms. Therefore, it is probable that the allele *Gli-B1d* may be also (as the *Gli-B2c*) a target of non-direct breeders' selection because it relates, due to linkage with a glutenin-coding gene, positively to dough quality.

Allele *Gli-B1b* was recognized earlier as one of the best alleles at the *Gli-B1* locus with a positive effect on dough quality, namely, on sedimentation value (several publications of Ukrainian scientists [comparing lines obtained from crosses of different wheat genotypes] mainly of Poperelya and Sozinov (1974–80), reviewed in Metakovsky (2015)). In our work, we revealed that this allele occurred as the most frequent at the *Gli-B1* locus throughout the 20th century in Australia (Table 1), its frequency increased significantly with time in the former USSR (Table 2) and, probably, in NC wheat germplasms. It was shown that this allele was nearly always accompanied by the allele *Glu-B3b* (Gupta et al., 1994) which was one of the strongest alleles at the *Glu-3* loci in its positive influence on end-use wheat quality (Gupta et al., 1991). Quite possibly, the positive effects of the *Gli-B1b* allele were due to its neighbour allele *Glu-B3b*.

In the former USSR germplasm, an increase of the frequency of the *Gli-B1b* with time reduced strongly (Table 2) the genetic diversity at the *Gli-B1* locus. However, the allele *Gli-B1e*, characteristic of Russian wheat spring germplasm, maintained its frequency with time, probably because this allele encoded so-called ω-gliadin d4, which also related positively to dough quality (Branlard et al., 2003).

It was also shown in Ukrainian publications of Poperelya, Sozinov et al. (1974–80) (reviewed in Metakovsky, 2015) that the allele *Gli-A1m* had a statistically significant negative influence, in the background of winter wheat, on flour quality (tested as sedimentation value). It was shown later that this allele was always accompanied by the *Glu-A3e* (Jackson et al., 1996) which is one of the worst alleles at the *Glu-A3* in relation to dough quality (Gupta et al., 1991). In our work, it was shown that this allele disappeared from the groups of more recent Australian, Spanish, and, probably, Canadian and NC cultivars. Many high-quality Canadian cultivars possess the allele *Gli-A1m* (Table 1), but its frequency in Canadian germplasm dropped from 75.0% in 1907–52 to 33.3% in 1985–88.

5. Conclusion

Alleles at the *Gli* loci were used as genetic markers of *T. aestivum* genotypes. No indications of any narrowing of genetic polymorphism throughout the 20th century were found neither at the spring world-wide wheat germplasm level, nor at the country level. At the same time, the frequency of occurrence of some *Gli* alleles, including those known to relate to dough strength (in particular, *Gli-B1b*, *Gli-B1d* and *Gli-B2c*), significantly increased with time in groups of cultivars bred in the

different countries studied. It was assumed that alleles related (as shown earlier) to end-use wheat quality, were indirect targets of breeders' selection for this wheat character. Changes in the frequency of occurrence of these alleles in wheat germplasm reduced genetic diversity at the corresponding *Gli* loci, but did not influence overall wheat genetic diversity.

Conflicts of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcs.2019.03.008>.

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