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POLYMORPHISM OF *GLI-A1* AND *GLI-D1* LOCI IN UKRAINIAN AND FOREIGN BREAD WHEAT CULTIVARS AND LINES

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Aim. The aim of this work was to analyse the polymorphism of *Gli-A1* and *Gli-D1* loci of bread wheat storage proteins within a larger (inter)national collection of bread wheat cultivars and lines, using acid polyacrylamide gel electrophoresis (A-PAGE) and classical PCR with allele-specific markers. Furthermore, to determine the correspondence between allelic variants of gliadins and alleles detected by PCR for these loci. **Methods.** In total 145 bread wheat cultivars and lines of Ukrainian and foreign selection were studied. Storage proteins electrophoresis was carried out in acid PAGE (A-PAGE) according to the method of Poperelya (1989), and the allelic variants of gliadins were marked according to the international nomenclature and catalogue (Metakovsky et al, 2018). DNA was isolated using an adapted CTAB method, and PCR was performed with allele-specific primers for the *Gli-A1* and *Gli-D1* loci (Zhang et al, 2003). PCR amplified products were separated in a 7% polyacrylamide gel and stained with silver nitrate. **Results.** In the PCR analysis the frequencies of the *Gli-A1.1* and *Gli-A1.2* alleles among 91 modern Ukrainian bread wheat cultivars and lines was 0.80 and 0.20, respectively. In a collection of 48 foreign wheat cultivars and lines, these frequencies were 0.69 and 0.31, respectively. The frequencies of the *Gli-D1.1* and *Gli-D1.2* alleles in the Ukrainian collection were 0.31 and 0.69, respectively. The *Gli-D1.1* allele prevailed in the foreign wheat cultivars tested, with a frequency of 0.64, the frequency of *Gli-D1.2* was 0.31. Using A-PAGE, seven allelic variants of gliadins were found among Ukrainian cultivars, the most common being *Gli-A1b* and 12 allelic variants in the foreign wheat cultivars, the most common being *Gli-A1a*. Five different allelic variants of gliadins encoded at the *Gli-D1* locus were found in the Ukrainian collection and eight in the foreign collection. Allelic variants of gliadins for six cultivars in the Ukrainian collection were undefined and marked as "possibly new". The most common allelic variant was *Gli-D1b*. It was determined that the *Gli-A1.1* allele corresponded to the allelic variants *Gli-A1a, Gli-A1ab, Gli-A1af, Gli-A1b, Gli-A1c, Gli-A1f,* and *Gli-A1l*, and the *Gli-A1.2* allele corresponded to *Gli-A1o, Gli-A1r* and *Gli-A1x* (*Gli-A1x* sensu Kozub et al, 2009). No correspondence was found between allelic variants of gliadins encoded at the *Gli-D1* locus and *Gli-D1.1* and *Gli-D1.2* alleles. **Conclusions.** The most common allele of the *Gli-A1* locus in a collection of 91 modern Ukrainian bread wheat cultivars and lines and that of 48 foreign cultivars was *Gli-A1.1*. For the *Gli-D1* locus the *Gli-D1.2* allele was most frequent in the Ukrainian cultivars, and *Gli-D1.1* in the foreign cultivars. For the *Gli-A1* locus, there was revealed correspondence between the allelic variants of gliadins encoded at the *Gli-A1* locus and the *Gli-A1.1/Gli-A1.2* alleles, but such a correspondence was not found for the *Gli-D1* locus.

Key words: *Triticum aestivum* L.; allelic variants of gliadins; molecular markers; allele-specific primers.

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INTRODUCTION

Gliadins and glutenins are the main storage proteins of the bread wheat endosperm, and critical components of gluten, on which the bread-baking quality of flour depends, namely the viscoelastic qualities of the dough extensibility with its elasticity and volume (Goutam et al, 2013; Urade et al, 2018). The properties of the gluten complex are primarily determined by polymorphism

and the ratio between the polymer high- and low-molecular weight (HMW and LMW) glutenin subunits, respectively, connected by disulfide bonds, and monomer gliadins, maintained with glutenins via intermolecular disulfide bonds and non-covalent interactions (Urade et al, 2018; Ma, 2019). The amount of accumulated gluten depends on the environmental conditions. Despite this fact, the impact of the genotype on the qualitative properties of gluten is decisive, and its amount does not measure the quality (Simic et al, 2006). Thus, the genes of glutenins and gliadins have been found relevant for marker-associated breeding and typing of wheat varieties (Kozub et al, 2009, 2020, 2024; Metakovsky et al, 2018a and b; 2024).

Glutenins contribute the most to bread-baking quality of flour; they are less polymorphic, and their genes are well-investigated and described; various DNA-markers have been proposed to detect most alleles of the genes (Dong et al, 2013; Wang et al, 2009 and 2010; Sui et al, 2010; Ravel et al, 2020). To the contrary gliadin genes are more numerous and polymorphic, they are responsible for up to 40 % of the total protein content in flour (Goesaert et al, 2005; Gras et al, 2001). Several studies have shown the reliable positive impact of some gliadins on the quality of gluten (Brandlard and Dardvet, 1985; Metakovsky, 1991).

Gliadins are divided into four types: α-, γ-, ω- and δ-gliadins, based on the electrophoretic motility and the biochemical composition of proteins (Anderson 2012; Shewry, 2023). The genes of γ-, δ- and ω-gliadins are localized at three loci: *Gli-A1, Gli-B1, Gli-D1,* located on short arms of chromosomes 1A, 1B, and 1D. The α-gliadins are encoded at *Gli-A2, Gli-B2,* and *Gli-D2* loci, located on short arms of chromosomes 6A, 6B, and 6D (Payne et al, 1982). A few minor gliadin loci is also distinguished, located on group I chromosome arms, viz. *Gli-A3, Gli-A5* and *Gli-A6* (on 1AS); *Gli-B3, Gli-B5* (on 1BS); *Gli-D3, Gli-D4, Gli-D6* (on 1DS) (Shewry et al, 2003; McIntosh et al, 2013).

Many researchers studied the polymorphism of gliadins using one- or two-dimensional electrophoresis in acid polyacrylamide electrophoresis (A-PAGE) (Metakovsky et al, 1991; Metakovsky et al, 2018; Kozub et al, 2009; Kozub et al, 2020; Utebayev et al, 2019; Utebayev et al, 2022). This method enables detection of so-called allelic variants of gliadins (blocks of gliadins). According to Hsia and Anderson (2001), the copy number of gliadin genes in a locus is different, from 15 to 150, depending on the type of gliadins and the cultivar. Based on sequence analysis and proteomic studies, the number of gliadin polypeptides/genes was described for such cultivars as Butte 86, Kembukang, Xiaoan 81, Pegaso and Chinese Spring (Dupont et al, 2011; Noma et al, 2016; Camerlengo et al, 2017; Wang et al, 2017; Huo et al, 2018a and b). At present, there are two known classifications (catalogues) of allelic variants of gliadins of bread wheat, other developed by Sozinov and Poperelya (1980) and one by Metakovsky et al (2018a). The latter is international and enlists 186 different allelic variants of gliadins, encoded at *Gli-1* and *Gli-2* loci and was applied in this study.

Current studies based on gliadin genes sequencing enable to determine the nucleotide sequences and an exact number of genes, encoding the polypeptides per allelic variant of gliadins as found in A-PAGE and are useful for determining coeliac disease epitopes (Qi et al, 2014; Shewry et al, 2016; Rybalka, 2017, Halstead-Nussloch et al, 2021). However, as of now, complete DNA sequences of *Gli-1* and *Gli-2* loci of 1–6.5 million bp were sequenced and composed only for one wheat cultivar, Chinese Spring (Huo et al, 2018a; Huo et al, 2018b).

Wang et al (2017) and Kawaura et al (2005) developed allele-specific, sequence tagged sites (STS) markers for 25 α-gliadin genes at *Gli-A2, Gli-B2* and *Gli-D2* loci, two γ-gliadin genes (at *Gli-A1, Gli-B1* loci) and one ω-gliadin gene (at the locus). Noma et al (2016) developed 11 primer pairs specific to α-gliadins of the cultivar Chinese Spring, used in real-time (RT) PCR. Li et al (2018) developed six DNA markers, for analysis of variation of the *Gli-D2* locus of *Aegilops tauschii,* and wheat cultivars Chinese Spring and Xiaoyan 81. Universal primers to α- and γ-gliadin genes were reported by Qi et al (2014) and Arentz-Hansen et al (2000). Six specific primer pairs for RT-PCR were described by Paris et al (2021) for durum wheat cultivar Svevo and a further eight pairs by Zhou et al (2022) for bread wheat. Based on the DNA sequences of two pseudogenes (on the 1A and 1B chromosomes) and the γ-gliadin gene (on 1D), three pairs of allele-specific primers, for singe nucleotide polymorphism (SNP) loci *Gli-A1, Gli-B1,* and *Gli-D1* were intended for detection of 1RS/1BL translocations and considered to be the potential markers for the *Glu-3* locus (Zhang et al, 2003). Regardless of such a large number of the elaborated molecular markers for some gliadin genes, there is still no system of DNA markers, which would identify all the allelic variants of gliadins, determined by the electrophoresis in A-PAGE to be used in the marker-assisted breeding.

However, while analysing the genotypes of 14 wheat cultivars and 6 near-isogenic lines, Polishchuk et al (2010) showed correspondence between molecular-genetic and storage protein electrophoretic data. Our previous studies of the polymorphism of the *Gli-B1* locus with a larger collection of Ukrainian cultivars and lines from different breeding centres and foreign bread wheat cultivars and lines using A-PAGE and PCR with allele-specific markers of Zhang et al (2003) for *Gli-B1* locus and primers for the microsatellite *Taglgap* of Devos et al (1995), also demonstrated correspondence between the two methods (Popovych et al, 2020, 2021a and b; Metakovsky et al, 2021).

Therefore, the aim of this work was to investigate the polymorphism of two other gliadin-encoding loci, *Gli-A1* and *Gli-D1*, using the above-mentioned PCR and primers of Zhang et al, (2003) with the same Ukrainian and foreign collection of bread wheat cultivars as for *Gli-B1*, mentioned above, with some extension in the range of cultivars and lines, and to determine the correspondence between allelic variants of the *Gli-A1* and *Gli-D1* loci, as determined by PCR and A-PAGE.

MATERIALS AND METHODS

The study of the polymorphism of *Gli-A1* and *Gli-D1* loci involved the collections of bread wheat, largely as previously studied for the *Gli-B1* locus (Popovych et al, 2020, 2021a; Metakovsky et al, 2021). The current Ukrainian collection consisted of 28 cultivars and lines from the Plant Breeding and Genetics Institute, the National Centre of Seed and Cultivar Investigation (PBGI-NCSCI), Odesa, Odessa region; 20 cultivars from the V.M. Remeslo Myronivka Institute of Wheat (MIW), Central, Myronivskyy area, Kiev region; 11 cultivars from the Bila Tserkva Experimental Selection Station (BTsESS), Mala Vilshanka, Bilotserkivsky area, Kiev region; ten cultivars from the Institute of Irrigated Agriculture (IIA), Naddniprianske, Kherson region; ten cultivars from the Poltava State Agrarian Academy (PSAA), Poltava, Poltava region; three cultivars and five lines from the Nosivka Breeding and Selection Station (NBES), Doslidne, Chernihiv region; two cultivars from Donetsk Institute of Agro-industrial Production of the Ukrainian Academy of Agrarian Sciences (DIAP), Pisky, Donetsk region; one cultivar from LLC Scientific and Production Company «Driada, Ltd», Kherson and one from Luhansk Institute of Breeding and Technologies (LIBT), Teplychne, Lugansk region. The collection of foreign cultivars consisted of 48 cultivars and lines with a diverse set of allelic variants of gliadins from 16 different countries (Australia, Belgium, Canada, China, France, Great Britain, Italy, Kazakhstan, Mexico, Netherlands, Portugal, Romania, Russia, Spain, Ukraine and USA). This collection was composed and provided for our study by Dr. E.V. Metakovsky (Unit of Genetics, Department of Biotechnology – Plant Biology, Universidad Politécnica de Madrid, Madrid, Spain). We also investigated six near-isogenic lines, created by Kopus (Kopus, 1994), based on the reference cultivar Bezostaya 1. A detailed list of all the investigated cultivars, the year of their registration and origin are presented in in the tables of the Results section.

Five kernels per cultivar/line were analysed separately. Each kernel was divided into two halves, one half of which was used for storage protein one-dimensional A-PAGE using the method of Poperelya (1989).

The allelic variants of gliadins were marked by the catalogue as published by Metakovsky et al (2018a). Only *Gli-A1x* was marked according to Kozub et al (2009) and not according to Metakovsky et al (2018a). Allelic variants of gliadins from the foreign collection of cultivars and lines were determined, using a similar methodology, which was shown in the earlier study of Metakovsky et al (2018a), so in this research, these data were integrally and unchanged included as such.

The other half of the kernel was used to for extraction DNA using CTAB, as described by Yu et al (2017) with the following modifications: the lysis buffer was prepared without β-mercaptoethanol, isopropanol was used instead of 99.9 % ethanol to precipitate DNA, and there was no stage of processing with RNAse. DNA concentrations were measured using a Nanodrop 2000 spectrophotometer («Thermo Fisher Scientific», USA) and samples of DNA were standardized to a final concentration of 100–150 ng/µl. PCR was performed with allele-specific primers for the *Gli-A1* and *Gli-D1* loci, according to Zhang et al (2003) (**Table 1**).

Two PCR reaction mixtures were used in the study. The first reaction mixture, in a total volume of 10 µL, contained 1 µL of 10xDreamTaq Buffer (ThermoScientific), 0.5 µL of 25 mM dNTPs (ThermoScientific), 0.5 µL of each primer (at a concentration of 10 pmol/µL), 0.05 µL of ThermoScientific™ DreamTaq™ DNA polymerase (Thermo Fisher Scientific, USA) with an activity of 5 units/µL, 100 ng of DNA (1 µL), and 6.45 µL of PCR-grade water. The second reaction mixture with a total volume of 10 µL was used to increase PCR specificity for some cultivars, due to detecting two alternative alleles in each grain studied, according to results obtained with the first reaction mixture

Table 1. Allele-specific primers, developed by Zhang et al (2003), used in this study

Locus	Marker (Allele specific primer)	F/R Primer	Primer sequence 5′–3′	Product length (bp)
Gli-A1	*Gli-A1.1*	F	CATAGCGTCGTGCATTCCAACG	168
		R	GCACATGTTTGGAAGGGATC	
	Gli-A1.2	F	CATAGCGTCGTGCATTCCAACA	168
		R	GCACATGTTTGGAAGGGATC	
Gli-D1	*Gli-D1.1*	F	AAGCGATTGCCAAGTGATGCG	264
		R	GTTTGCAACACCAATGACGTA	
	Gli-D1.2	F	AAGCGATTGCCAAGTGATGCG	270
		R	GCAAGAGTTTGCAACAGCG	

described in the results below. It consisted of 5 μL of Bio-Rad iProof HF Master Mix 2x («Bio-Rad», USA), 0.5 μL of each primer (at a concentration of 10 pmol/μL), 100 ng of DNA, and PCR-grade water. The annealing temperature of the primers and the amplification program were used according to Zhang et al (2003), namely 95 °C for 3 min followed by 38 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min. The PCR amplified products were separated in a 7% polyacrylamide gel (300V, 2 hours) and stained with silver nitrate (Silver sequence ™ DNA Sequencing System Technical Manual («Promega», «Madison», USA) (Promega, 1999). The allele frequencies were calculated, taking into account the heterogeneity of wheat cultivars according to Kozub et al (2020). Statistical significance of the difference between Ukrainian and foreign collections and between Ukrainian collection and six Ukrainian selection and breeding stations was assessed using the Chi-square method.

The cultivars Chinese Spring and Suneca, described by Zhang et al (2003), the Ukrainian cultivars Albatros odeskyi, Liubava and Strumok as well as six near-isogenic lines created by Kopus (1994), which were studied by Polischuk et al (2010), were used as reference.

RESULTS

The allele-specific primers, developed by Zhang et al (2003) allow detection of the following alleles of the *Gli-A1* locus, which are determined by SNPs: *Gli-A1.1* (based on the presence of guanine) and *Gli-A1.2* (based on the presence of adenine) with an expected size for both amplification fragments of 168 bp (Zang et al, 2003). When using the standard ThermoScientific DreamTaq DNA polymerase reagents in the PCR, insufficient specificity was observed with these primer pairs for nine cultivars. Contrary to cultivars, heterogeneous by the *Gli-A1* locus, such as cultivar Myronivska 61 (**Fig. 1**), these cultivars showed two alleles in all tested kernels, for instance cultivar Perlyna Lisostepu (Fig. 1). The probability of DNA contamination was ruled out by 3 repeats of DNA extractions from new samples, which gave the same result.

This problem was overcome by using Bio-Rad iProof HF Master Mix 2x, leading to the desired specificity: all nine cultivars showing two alleles, now were divided into two normal behaving groups (**Fig. 2**): the first one with *Gli-A1.1* allele (Lisova pisnia, Svitanok myronivskyi, Vilshana, Estafeta myronivska, lane KS1, Hovtva and the second with *Gli-A1.2* allele (Vodohrai bilotserkivskyi, Perlyna lisostepu, Charodiyka bilotserkivska), so this result was taken into account in the data analysis (Popovych, 2023).

In foreign cultivars and lines of bread wheat, the *Gli-A1.1* allele prevailed, which was found in 33 cultivars (frequency 0.69), and the *Gli-A1.2* allele was noted for 15 cultivars (frequency 0.31). Compared to the foreign collection, in the Ukrainian collection of cultivars and lines, the frequency of the *Gli-A1.1* allele was higher and amounted to 0.80 (*Gli-A1.1* was found in 71 cultivars). The *Gli-A1.2* allele was detected in only sixteen cultivars with a frequency of 0.20. Cultivars Antonivka, MIW Vyshyvanka, Myronivska 61 and Schedrist were heterogeneous according to PCR and A-PAGE results. Out of the six studied near isogenic lines, five were characterized by *Gli-A1.1*, and one line by *Gli-A1.2* (**Table 2**).

In A-PAGE, 12 cultivars of the foreign collection showed the *Gli-A1a* allelic variant, 11 foreign cultivars the *Gli-A1o* and 6 the *Gli-A1af* allelic variant. The

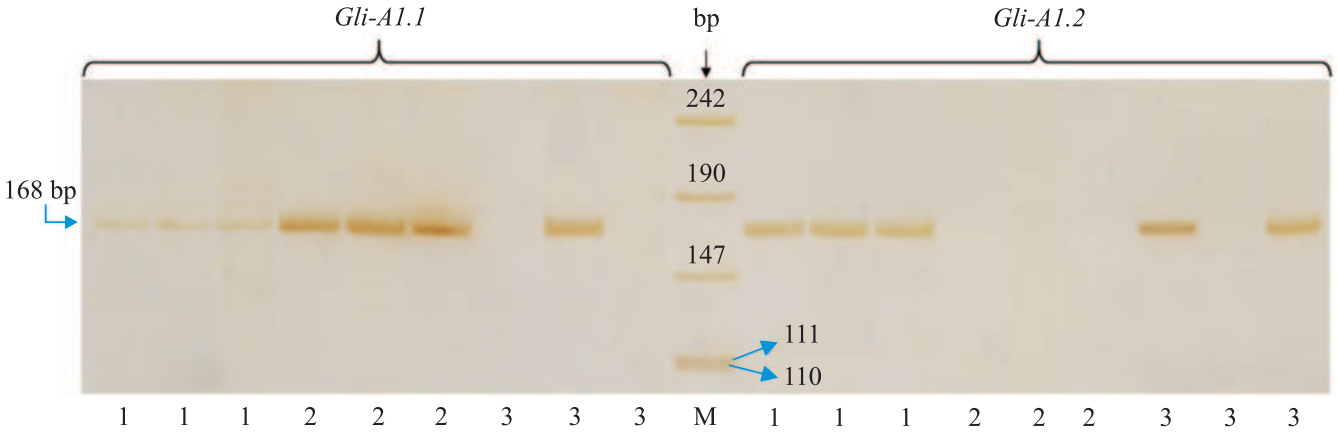

Fig. 1. Electropherogram of amplification products obtained in PCR with allele-specific primers for *Gli-A1.1/Gli-A1.2* alleles, using ThermoScientific DreamTaq DNA polymerase reagents. DNA: 1 – Perlyna lisostepu, 2 – Sahaidak, 3 – Myronivska 61, M – molecular weight marker *pUC19/Msp I*

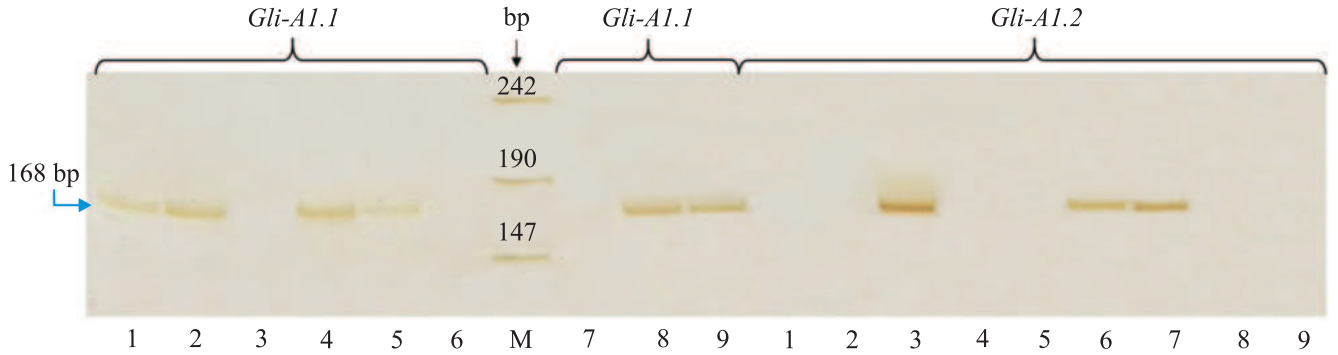

Fig. 2. Electropherogram of amplification products obtained in PCR with allele-specific primers for *Gli-A1.1/Gli-A1.2* alleles, using Bio-Rad iProof HF Master Mix 2x. DNA: 1 – Lisova pisnia, 2 – Svitanok myronivskyi, 3 – Vodohrai bilotserkivkyi, 4 – Vilshana, 5 – Estafeta myronivska, 6 – Perlyna lisostepu, 7 – Charodiyka bilotserkivska, 8 – lane KS1, 9 – Hovtva, M – molecular weight marker *pUC19/Msp I* according to Popovych (2023)

Gli-A1ab, *Gli-A1b*, *Gli-A1c*, *Gli-A1f*, *Gli-A1g*, *Gli-A1l*, *Gli-A1m, Gli-A1n* and *Gli-A1r* allelic variants were noted only in one up to three cultivars. Four cultivars of the foreign collection were found to be heterogeneous in A-PAGE with combinations of allelic variants *a+af* (Libero), *a+g* (Siete-Cerros-66), *b+f* (Donskaya polukarlikovaia) and *n+b* (Intensivnaya) (Table 2).

The results of A-PAGE demonstrated that the Ukrainian collection of bread wheat cultivars and lines was less polymorphic than the foreign collection. The following allelic variants of gliadins were found in it: *Gli-A1b* (37 cultivars), *Gli-A1c* (10 cultivars), *Gli-A1f* (7 cultivars), *Gli-A1g* (10 cultivars), *Gli-A1o* (8 cultivars). The *Gli-A1x* allelic variant described by Kozub (2009) occurred only in Ukrainian cultivars (8 cultivars). Eleven cultivars of the Ukrainian collection were found to be heterogeneous in A-PAGE with combinations of allelic variants *b+g* (seven cultivars), *b+o* (Antonivka), *f+o* (MIW Vyshyvanka), *f+x* (Myronivska 61), *m+b* (Schedrist) (Table 2). The *Gli-A1m* allelic variant of gliadins was detected only for one near-isogenic line Gli-A1-1, the other five cultivars had *Gli-A1b* (Table 2).

Ukrainian and foreign collections differed significantly ($p < 0.05$) by the allelic variant frequencies.

In total, seven allelic variants of gliadins encoded at the *Gli-A1* locus were found in the collection of modern Ukrainian bread wheat cultivars and lines, and 12 in the collection of foreign bread wheat cultivars and lines.

The cultivars sampled from different breeding centres of Ukraine, also differed in the results of PCR and A-PAGE. In the PCR analysis, *Gli-A1.1* prevailed in the cultivars of all breeding centres. In two of the six breeding centres (IIA and PSAA) the *Gli-A1.1* allele was detected in all studied cultivars (with a frequency of 1), the lowest frequency of this allele was observed in cultivars from MIW (0.50). Accordingly, the frequency of the al-

Table 2. Results of PCR with allele-specific primers and A-PAGE of storage proteins of Ukrainian and foreign bread wheat cultivars and near-isogenic lines

No.	Cultivar name	Allele * *Gli-A1*	Allelic variant ** *Gli-A1*	Origin of cultivar/line	Year
Ukrainian collection of bread wheat cultivars and lines					
1	Albatros odeskyi	*Gli-A1.1*	*b*	PBGI-NCSCI	–
2	Ariivka	*Gli-A1.1*	*b*	NBES	2017
3	Balada myronivska	*Gli-A1.1*	*b*	MIW	2018
4	Bilosnizhka	*Gli-A1.1*	*b*	DIAP	2006
5	Blaho	*Gli-A1.1*	*b*	IIA	2011
6	Borvii	*Gli-A1.1*	*b*	PBGI-NCSCI	2010
7	Burhunka	*Gli-A1.1*	*b*	IIA	2015
8	Estafeta myronivska	*Gli-A1.1*	*b*	MIW	2017
9	Hovtva	*Gli-A1.1*	*b*	PSAA	2013
10	Hurt	*Gli-A1.1*	*b*	PBGI-NCSCI	2013
11	Khyst	*Gli-A1.1*	*b*	PBGI-NCSCI	2013
12	Konka	*Gli-A1.1*	*b*	IIA	2014
13	Koshova	*Gli-A1.1*	*b*	IIA	2016
14	Levada	*Gli-A1.1*	*b*	PSAA	2005
15	Ledia	*Gli-A1.1*	*b*	IIA	2016
16	line KS1	*Gli-A1.1*	*b*	NBES	–
17	line KS14	*Gli-A1.1*	*b*	NBES	–
18	line L41/95	*Gli-A1.1*	*b*	NBES	–
19	Lira odeska	*Gli-A1.1*	*b*	PBGI-NCSCI	2013
20	Liubava odeska	*Gli-A1.1*	*b*	PBGI-NCSCI	–
21	Liutenka	*Gli-A1.1*	*b*	PSAA	–
22	Maria	*Gli-A1.1*	*b*	IIA	2013
23	Melodia odeska	*Gli-A1.1*	*b*	PBGI-NCSCI	–
24	Metalist	*Gli-A1.1*	*b*	LIBT	2014
25	Mudrist II	*Gli-A1.1*	*b*	PBGI-NCSCI	–
26	Mudrist III	*Gli-A1.1*	*b*	PBGI-NCSCI	–
27	Ovidii	*Gli-A1.1*	*b*	IIA	2009
28	Orzhytsia	*Gli-A1.1*	*b*	PSAA	2013
29	Podiaka	*Gli-A1.1*	*b*	PBGI-NCSCI	2008
30	Svitanok myronivskyi	*Gli-A1.1*	*b*	MIW	2014
31	Tsarychanka	*Gli-A1.1*	*b*	PSAA	2013
32	Shchedra nyva	*Gli-A1.1*	*b*	BTsESS	2011
33	Ukrainka poltavska	*Gli-A1.1*	*b*	PSAA	–
34	Vatazhok	*Gli-A1.1*	*b*	PBGI-NCSCI	2011
35	Vihen	*Gli-A1.1*	*b*	PBGI-NCSCI	2014
36	Vidrada	*Gli-A1.1*	*b*	BTsESS	2010
37	Vilshana	*Gli-A1.1*	*b*	PSAA	2010
38	Kokhana	*Gli-A1.1*	*b+g*	IIA	2009
39	line L59-95	*Gli-A1.1*	*b+g*	NBES	–
40	Nasnaha	*Gli-A1.1*	*b+g*	PBGI-NCSCI	2015
41	Optyma odeska	*Gli-A1.1*	*b+g*	PBGI-NCSCI	2018
42	Pamiati Remesla	*Gli-A1.1*	*b+g*	MIW	2009
43	Sonata	*Gli-A1.1*	*b+g*	PSAA	2018
44	Veteran	*Gli-A1.1*	*b+g*	PBGI-NCSCI	2014
45	Anatolia	*Gli-A1.1*	*c*	IIA	2015
46	Bilotserkivska napivkarlykova	*Gli-A1.1*	*c*	BTsESS	1999
47	Khersonska bezosta	*Gli-A1.1*	*c*	IIA	2002
48	Line KS22-04	*Gli-A1.1*	*c*	NBES	–

Continuation of table 2

No.	Cultivar name	Allele * *Gli-A1*	Allelic variant ** *Gli-A1*	Origin of cultivar/line	Year
49	Lisova Pisnia	*Gli-A1.1*	*c*	BTsESS	2008
50	Romantyka	*Gli-A1.1*	*c*	BTsESS	2009
51	Sydor Kovpak	*Gli-A1.1*	*c*	PSAA	–
52	Tsarivna	*Gli-A1.1*	*c*	BTsESS	2008
53	Yasochka	*Gli-A1.1*	*c*	BTsESS	2011
54	Zymoyarka	*Gli-A1.1*	*c*	MIW	2007
55	Lybid	*Gli-A1.1*	*f*	BTsESS	2006
56	Madiarka	*Gli-A1.1*	*f*	MIW	2008
57	Myronivska slava	*Gli-A1.1*	*f*	MIW	2017
58	MIW Dniprianka	*Gli-A1.1*	*f*	MIW	2018
59	Sahaidak	*Gli-A1.1*	*f*	PSAA	2010
60	Vezha myronivska	*Gli-A1.1*	*f*	MIW	2018
61	Zoriana nosivska	*Gli-A1.1*	*f*	NBES	–
62	Klarisa	*Gli-A1.1*	*g*	Driada Ltd.	2014
63	Kuialnyk AR	*Gli-A1.1*	*g*	PBGI-NCSCI	–
64	Mudrist IV	*Gli-A1.1*	*g*	PBGI-NCSCI	–
65	Panna	*Gli-A1.1*	*g*	PBGI-NCSCI	2013
66	Selianka	*Gli-A1.1*	*g*	PBGI-NCSCI	2003
67	Tradytsia I	*Gli-A1.1*	*g*	PBGI-NCSCI	–
68	Tradytsia II	*Gli-A1.1*	*g*	PBGI-NCSCI	–
69	Vidpovid odeska	*Gli-A1.1*	*g*	PBGI-NCSCI	2020
70	Zhaivir	*Gli-A1.1*	*g*	PBGI-NCSCI	2010
71	Zmina	*Gli-A1.1*	*g*	PBGI-NCSCI	2018
72	Antonivka	*Gli-A1.1/Gli-A1.2*	*b+o*	PBGI-NCSCI	2008
73	MIW Vyshyvanka	*Gli-A1.1/Gli-A1.2*	*f+o*	MIW	2017
74	Myronivska 61	*Gli-A1.1/Gli-A1.2*	*f+x*	MIW	1989
75	Shedrist	*Gli-A1.1/Gli-A1.2*	*m+b*	PBGI-NCSCI	2014
76	Gratsia myronivska	*Gli-A1.2*	*o*	MIW	2018
77	Donetska 48	*Gli-A1.2*	*o*	DIAP	1997
78	Myronivska zolotoverkha	*Gli-A1.2*	*o*	MIW	2012
79	Mudrist I	*Gli-A1.2*	*o*	PBGI-NCSCI	–
80	Oberih myronivskyi	*Gli-A1.2*	*o*	MIW	2014
81	Podolianka	*Gli-A1.2*	*o*	MIW	2003
82	Strumok	*Gli-A1.2*	*o*	PBGI-NCSCI	–
83	Yuvivata 60	*Gli-A1.2*	*o*	NBES	2013
84	Charodiika bilotserkivska	*Gli-A1.2*	*x**	BTsESS	2011
85	Kryzhynka	*Gli-A1.2*	*x**	MIW	2002
86	Myronivska 65	*Gli-A1.2*	*x**	MIW	2000
87	MIW Assol	*Gli-A1.2*	*x**	MIW	2018
88	Perlyna lisostepu	*Gli-A1.2*	*x**	BTsESS	2001
89	Trudivnytsia myronivska	*Gli-A1.2*	*x**	MIW	2017
90	Vodohrai bilotserkivskyi	*Gli-A1.2*	*x**	BTsESS	2014
91	Yuviliar myronivskyi	*Gli-A1.2*	*x**	MIW	2009
	Foreign collection of cultivars and lines, provided by E.V. Metakovsky				
1	Argelato	*Gli-A1.1*	*a*	Italy	1964
2	Caia	*Gli-A1.1*	*a*	Portugal	–
3	Cartaya	*Gli-A1.1*	*a*	Spain	1983
4	Chinese-Spring	*Gli-A1.1*	*a*	China	–
5	Diego	*Gli-A1.1*	*a*	Spain	1983

End of table 2

No.	Cultivar name	Allele * *Gli-A1*	Allelic variant ** *Gli-A1*	Origin of cultivar/line	Year
6	Escualo	*Gli-A1.1*	*a*	Spain	1981
7	Mentana	*Gli-A1.1*	*a*	Italy	1913
8	N10T1B (line)	*Gli-A1.1*	*a*	–	–
9	N1BT1D (line)	*Gli-A1.1*	*a*	–	–
10	1B-,1D- (line)	*Gli-A1.1*	*a*	–	–
11	Pane-247	*Gli-A1.1*	*a*	Spain	1960
12	Potam-70	*Gli-A1.1*	*a*	Mexico	1970
13	Libero	*Gli-A1.1*	*a+af*	Italy	1927
14	Siete-Cerros-66	*Gli-A1.1*	*a+g*	Mexico	1966
15	Galahad	*Gli-A1.1*	*ab*	Great Britain	1982
16	Arminda	*Gli-A1.1*	*af*	Netherlands	1976
17	Federation	*Gli-A1.1*	*af*	Australia	1901
18	Goelent	*Gli-A1.1*	*af*	France	1985
19	Insignia	*Gli-A1.1*	*af*	Australia	1946
20	Pavon-F-76 (Betres)	*Gli-A1.1*	*af*	Mexico	1976
21	Roblin	*Gli-A1.1*	*af*	Canada	1986
22	Bezostaya-1	*Gli-A1.1*	*b*	Russia	1959
23	Krasnodonka	*Gli-A1.1*	*b*	Ukraine	1969
24	Donskaya polukarlikovaia	*Gli-A1.1*	*b+f*	Russia	1984
25	Gazul	*Gli-A1.1*	*c*	Spain	1992
26	Ardec	*Gli-A1.1*	*f*	Belgium	1979
27	Mironovskaya -808	*Gli-A1.1*	*f*	Ukraine	1963
28	Prinqual	*Gli-A1.1*	*f*	France	1978
29	Gabo	*Gli-A1.1*	*g*	Australia	1942
30	Newcaster	*Gli-A1.1*	*l*	USA	1946
31	Salmone	*Gli-A1.1*	*l*	Italy	1980
32	Glenlea	*Gli-A1.1*	*m*	Canada	1972
33	Intensivnaya	*Gli-A1.1*	*n+b*	Kazakhstan	1978
34	Aragon-03	*Gli-A1.2*	*m*	Spain	1940
35	Katepwa	*Gli-A1.2*	*m*	Canada	1981
36	Marquis	*Gli-A1.2*	*m*	Canada	1907
37	Cajeme-71	*Gli-A1.2*	*o*	Mexico	1971
38	Capelle-Desprez	*Gli-A1.2*	*o*	France	1946
39	Cluij-650	*Gli-A1.2*	*o*	Romania	1954
40	Darius	*Gli-A1.2*	*o*	France	1974
41	Recital	*Gli-A1.2*	*o*	France	1986
42	Rinconada	*Gli-A1.2*	*o*	Spain	1981
43	Sideral	*Gli-A1.2*	*o*	France	1990
44	Splendeur	*Gli-A1.2*	*o*	France	1964
45	Suneca	*Gli-A1.2*	*o*	Australia	1981
46	Titien	*Gli-A1.2*	*o*	France	1985
47	Yecora-Rojo	*Gli-A1.2*	*o*	USA	1975
48	Laura	*Gli-A1.2*	*r*	Canada	1986
	Bread wheat near-isogenic lines, created by Kopus (Kopus, 1994)				
1	GLI-B1-3	*Gli-A1.1*	*b*	Ukraine	1994
2	GLI-B1-4	*Gli-A1.1*	*b*	Ukraine	1994
3	GLI-B1-12	*Gli-A1.1*	*b*	Ukraine	1994
4	GLI-D1-4	*Gli-A1.1*	*b*	Ukraine	1994
5	GLI-D1-5	*Gli-A1.1*	*b*	Ukraine	1994
6	GLI-A1-1	*Gli-A1.2*	*m*	Ukraine	1994

Note. *allele of the *Gli-A1* locus, determined by the PCR using allele-specific primers, ** allelic variant of gliadins, encoded at the *Gli-A1* locus, determined by the storage proteins electrophoresis in A-PAGE. PBGI-NCSCI – Plant Breeding and Genetics Institute, the National Centre of Seed and Cultivar Investigation, Odesa; MIW – V.M. Remeslo Myronivka Institute of Wheat, Central, Myronivskyy area, Kiev region; BTsESS – Bila Tserkva Experimental Selection Station, Mala Vilshanka, Bilotserkivsky area, Kiev region; IIA – Institute of Irrigated Agriculture (IIA), Naddniprianske, Kherson redion; PSAA – Poltava State Agrarian Academy, Poltava, Poltava region; NBES – Nosivka Breeding and Selection Station, v. Doslidne, Chernihiv region; DIAP – Donetsk Institute of Agroindustrial Production of the Ukrainian Academy of Agrarian Sciences, Pisky, Donetsk region, Driada, Ltd – LLC Scientific and Production Company "Driada, Ltd", Kherson; LIBT – Luhansk Institute of Breeding and Technologies, Teplychne, Lugansk region.

ternative allele *Gli-A1.2* in cultivars of different breeding centres ranged from 0 to 0.50 (Table 2).

In A-PAGE, the *Gli-A1b* allelic variant prevailed in cultivars of most breeding centres, namely in the ones from PBGI-NCSCI, PSAA, IIA and NBES with a frequency of 0.52 to 0.75. In the two remaining breeding centres MIW and BTsESS, *Gli-A1b* was present at a much lower frequency (0.18). Also, every group of cultivars from the different breeding centres, showed one or two more allelic variants of gliadins with a relatively high frequency of 0.10–0.41. For instance, for PBGI-NCSCI also frequently encountered and *Gli-A1g*, for BTsESS it is *Gli-A1c* and *Gli-A1x*, for MIW it is *Gli-A1x*, *Gli-A1o* and *Gli-A1f* (**Table 3**).

The analysis of the PCR and A-PAGE results using two collections of in total 145 bread wheat cultivars and near-isogenic lines, we found correspondence between allelic variants of gliadins (determined in A-PAGE) and alleles of the *Gli-A1* locus (determined in PCR) (**Table 4**). Thus, the *Gli-A1.1* allele corresponds to the allelic variants of gliadins *Gli-A1a, Gli-A1ab, Gli-A1af, Gli-A1b, Gli-A1c, Gli-A1f*, and *Gli-A1l*, while the *Gli-A1.2* allele corresponds to *Gli-A1o, Gli-A1r* and *Gli-A1x* (Table 3).

Table 3. Frequencies of alleles *Gli-A1.1/ Gli-A1.2* and allelic variants of gliadins, encoded at the *Gli-A1* locus

Breeding center								
AVG*	PBGI-NCSCI N = 28	MIW N = 20	BTsESS N = 11	IIA N = 10	PSAA N = 10	NBES N = 8	Ukrainian collection N = 91	Foreign collection N = 48
Frequency of AVG encoded at Gli-A1								
a	–	–	–	–	–	–	–	0.27
ab	–	–	–	–	–	–	–	0.02
af	–	–	–	–	–	–	–	0.14
b	0.52	0.18	0.18	0.75	0.75	0.56	0.46	0.07
c	–	0.05	0.45	0.20	0.10	0.13	0.11	0.02
f	–	0.25	0.09	–	0.10	0.13	0.10	0.08
g	0.37	0.02	–	0.05	0.05	0.06	0.16	0.03
l	–	–	–	–	–	–	–	0.04
m	0.02	–	–	–	–	–	0.01	0.08
n	–	–	–	–	–	–	–	0.01
o	0.09	0.23	–	–	–	0.13	0.10	0.11
r	–	–	–	–	–	–	–	0.02
*x**	–	0.28	0.27	–	–	–	0.09	–
Allele of Gli-A1 locus								
A1.1	0.89	0.50	0.73	1	1	0.87	0.80	0.69
A1.2	0.11	0.50	0.27	0	0	0.13	0.20	0.31

Note. * AVG – allelic variant of gliadins detected by A-PAGE.

Table 4. Correspondence of alleles of the *Gli-A1* locus, determined by using the primers of Zhang et al (2003), and allelic variants of gliadins determined by A-PAGE

Allelic variant of gliadins encoded at *Gli-A1* locus	Number of homogeneous cultivars/lines with *Gli-A1.1* or *Gli-A1.2* detected by PCR	
	Gli-A1.1	*Gli-A1.2*
Gli-A1a	12	–
Gli-A1ab	1	–
Gli-A1af	6	–
Gli-A1b	44	–
Gli-A1c	11	–
Gli-A1f	10	–
Gli-A1g	11	–
Gli-A1l	2	–
Gli-A1m	1	4
Gli-A1r	–	1
Gli-A1o	–	19
Gli-A1x	–	8

The only exception to this correspondence was the Canadian cultivar Glenlea, which had the *Gli-A1m* allelic variant and the *Gli-A1.1* allele, while other cultivars and lines with the same allelic variant were characterized by the *Gli-A1.2* allele (cvs Aragon-03, Katepwa, Marquis and line GLI-A1-1) (Table 3).

The polymorphism of the *Gli-D1* locus was studied using A-PAGE and PCR as well. The allele-specific primer pairs allowed to detect only two alleles with a single nucleotide polymorphism: *Gli-D1.1* (based on the presence of thymine) and *Gli-D1.2* (based on the presence of cytosine). Their amplification products were of the expected size, 264 bp and 270 bp, respectively (Zhang et al,2003). As was the case of PCR with primers to the *Gli-A1* locus, insufficient specificity was observed in PCR with primers to the *Gli-D1* locus using DreamTaq DNA polymerase. In this case, however, the use of HotStart DNA polymerase did not produce a more specific result for these cultivars, as was the case with the *Gli-A1* locus.

In the collection of foreign bread wheat cultivars and lines, the most common allele was *Gli-D1.1*, detected in 31 cultivars, with a frequency of 0.64. The *Gli-D1.2* allele was detected in 15 cultivars with a frequency of 0.31. The cultivar Darius and line 1B, 1D^{-} were characterized by a null allele (**Table 5**). The Ukrainian collection of cultivars differed from the foreign one by a higher incidence of the *Gli-D1.2* allele, detected in 57 cultivars with a fre-

Table 5. Results of PCR with allele-specific primers to the *Gli-D1* locus and A-PAGE of storage proteins of Ukrainian and foreign bread wheat cultivars and near-isogenic lines

No.	Cultivar name	Allele *Gli-D1**	Allelic variant ** *Gli-D1*	Origin of cultivar/line	Year
Ukrainian collection of bread wheat cultivars and lines					
1	Bilotserkivska napivkarlykova	*Gli-D1.1*	*b*	BTsESS	1999
2	Klarisa	*Gli-D1.1*	*b*	Ukraine	2014
3	Zymoyarka	*Gli-D1.1*	*b*	MIW	2007
4	Khyst	*Gli-D1.1*	*b+f*	PBGI-NCSCI	2013
5	Ledia	*Gli-D1.1*	*b+f*	IIA	2016
6	Hurt	*Gli-D1.1*	*f*	PBGI-NCSCI	2013
7	Vihen	*Gli-D1.1*	*f*	PBGI-NCSCI	2014
8	Mudrist II	*Gli-D1.1*	*g*	PBGI-NCSCI	–
9	Mudrist III	*Gli-D1.1*	*g*	PBGI-NCSCI	–
10	Panna	*Gli-D1.1*	*g*	PBGI-NCSCI	2013
11	Tradytsia I	*Gli-D1.1*	*g*	PBGI-NCSCI	–
12	Tradytsia II	*Gli-D1.1*	*g*	PBGI-NCSCI	–
13	Yuvivata 60	*Gli-D1.1*	*g*	NBES	2013
14	Vidrada	*Gli-D1.1*	*g*	BTsESS	2010
15	Bilosnizhka	*Gli-D1.1*	*j*	Ukraine	2006
16	Koshova	*Gli-D1.1*	*j*	IIA	2016
17	line L41/95	*Gli-D1.1*	*j*	NBES	–
18	Melodia odeska	*Gli-D1.1*	*j*	PBGI-NCSCI	–

Continuation of table 5

No.	Cultivar name	Allele *Gli-D1**	Allelic variant ** *Gli-D1*	Origin of cultivar/line	Year
19	Myronivska zolotoverkha	*Gli-D1.1*	*b*	MIW	2012
20	Mudrist IV	*Gli-D1.1*	*b*	PBGI-NCSCI	–
21	Vezha myronivska	*Gli-D1.1*	*j*	MIW	2018
22	Ukrainka poltavska	*Gli-D1.1*	*?*	PSAA	–
23	Liutenka	*Gli-D1.1/Gli-D1.2*	*?*	PSAA	–
24	Optyma odeska	*Gli-D1.1/Gli-D1.2*	*?*	PBGI-NCSCI	2018
25	Sydor Kovpak	*Gli-D1.1/Gli-D1.2*	*?*	PSAA	–
26	Tsarychanka	*Gli-D1.1/Gli-D1.2*	*?*	PSAA	2013
27	line L59-95	*Gli-D1.1/Gli-D1.2*	*b*	NBES	–
28	Myronivska slava	*Gli-D1.1/Gli-D1.2*	*b*	MIW	2017
29	Pamiati remesla	*Gli-D1.1/Gli-D1.2*	*b*	MIW	2009
30	Sonata	*Gli-D1.1/Gli-D1.2*	*b+?*	PSAA	2018
31	Nasnaha	*Gli-D1.1/Gli-D1.2*	*b+g*	PBGI-NCSCI	2015
32	Borvii	*Gli-D1.1/Gli-D1.2*	*g*	PBGI-NCSCI	2010
33	Sahaidak	*Gli-D1.1/Gli-D1.2*	*j*	PSAA	2010
34	Antonivka	*Gli-D1.1/Gli-D1.2*	*g+j*	PBGI-NCSCI	2008
35	Anatolia	*Gli-D1.2*	*?*	IIA	2015
36	Ariivka	*Gli-D1.2*	*b*	NBES	2017
37	Balada myronivska	*Gli-D1.2*	*b*	MIW	2018
38	Charodiika bilotserkivska	*Gli-D1.2*	*b*	BTsESS	2011
39	Donetska 48	*Gli-D1.2*	*b*	Ukraine	1997
40	Estafeta myronivska	*Gli-D1.2*	*b*	MIW	2017
41	Gratsia myronivska	*Gli-D1.2*	*b*	MIW	2018
42	Hovtva	*Gli-D1.2*	*b*	PSAA	2013
43	Khersonska bezosta	*Gli-D1.2*	*b*	IIA	2002
44	Kryzhynka	*Gli-D1.2*	*b*	MIW	2002
45	Lybid	*Gli-D1.2*	*b*	BTsESS	2006
46	line KS1	*Gli-D1.2*	*b*	NBES	–
47	line KS14	*Gli-D1.2*	*b*	NBES	–
48	Lisova Pisnia	*Gli-D1.2*	*b*	BTsESS	2008
49	Madiarka	*Gli-D1.2*	*b*	MIW	2008
50	Myronivska 61	*Gli-D1.2*	*b*	MIW	1989
51	Myronivska 65	*Gli-D1.2*	*b*	MIW	2000
52	MIW Assol	*Gli-D1.2*	*b*	MIW	2018
53	MIW Dniprianka	*Gli-D1.2*	*b*	MIW	2018
54	Oberih myronivskyi	*Gli-D1.2*	*b*	MIW	2014
55	Perlyna lisostepu	*Gli-D1.2*	*b*	BTsESS	2001
56	Podolianka	*Gli-D1.2*	*b*	MIW	2003
57	Romantyka	*Gli-D1.2*	*b*	BTsESS	2009
58	Shchedra nyva	*Gli-D1.2*	*b*	BTsESS	2011
59	Svitanok myronivskyi	*Gli-D1.2*	*b*	MIW	2014
60	Strumok	*Gli-D1.2*	*b*	PBGI-NCSCI	–
61	Trudivnytsia myronivska	*Gli-D1.2*	*b*	MIW	2017
62	Tsarivna	*Gli-D1.2*	*b*	BTsESS	2008
63	Vatazhok	*Gli-D1.2*	*b*	PBGI-NCSCI	2011
64	Vilshana	*Gli-D1.2*	*b*	PSAA	2010
65	Vodohrai bilotserkivskyi	*Gli-D1.2*	*b*	BTsESS	2014
66	Yuviliar myronivskyi asochka	*Gli-D1.2*	*b*	MIW	2009
67	Yasochka	*Gli-D1.2*	*b*	BTsESS	2011

Continuation of table 5

No.	Cultivar name	Allele *Gli-D1**	Allelic variant ** *Gli-D1*	Origin of cultivar/line	Year
68	Zoriana Nosivska	*Gli-D1.2*	*b*	NBES	–
69	MIW Vyshyvanka	*Gli-D1.2*	*b+g*	MIW	2017
70	Ovidii	*Gli-D1.2*	*b+g*	IIA	2009
71	Liubava odeska	*Gli-D1.2*	*b+j*	PBGI-NCSCI	–
72	Kuialnyk AR	*Gli-D1.2*	*d*	PBGI-NCSCI	–
73	Metalist	*Gli-D1.2*	*d*	Ukraine	2014
74	Levada	*Gli-D1.2*	*g*	PSAA	2005
75	Line KS22-04	*Gli-D1.2*	*g*	NBES	–
76	Mudrist I	*Gli-D1.2*	*g*	PBGI-NCSCI	–
77	Podiaka	*Gli-D1.2*	*g*	PBGI-NCSCI	2008
78	Selianka	*Gli-D1.2*	*g*	PBGI-NCSCI	2003
79	Veteran	*Gli-D1.2*	*g*	PBGI-NCSCI	2014
80	Zhaivir	*Gli-D1.2*	*g*	PBGI-NCSCI	2010
81	Zmina	*Gli-D1.2*	*g*	PBGI-NCSCI	2018
82	Albatros odeskyi	*Gli-D1.2*	*j*	PBGI-NCSCI	–
83	Blaho	*Gli-D1.2*	*j*	IIA	2011
84	Burhunka	*Gli-D1.2*	*j*	IIA	2015
85	Konka	*Gli-D1.2*	*j*	IIA	2014
86	Kokhana	*Gli-D1.2*	*j*	IIA	2009
87	Lira odeska	*Gli-D1.2*	*j*	PBGI-NCSCI	2013
88	Maria	*Gli-D1.2*	*j*	IIA	2013
89	Orzhytsia	*Gli-D1.2*	*j*	PSAA	2013
90	Shedrist	*Gli-D1.2*	*j*	PBGI-NCSCI	2014
91	Vidpovid odeska	*Gli-D1.2*	*j*	PBGI-NCSCI	2020
	Foreign collection of cultivars and lines, provided by Dr. E.V. Metakovsky				
1	Darius	–	*null*	France	1974
2	1B-,1D- (line)	–	*null*	–	–
3	N10T1B (line)	*Gli-D1.1*	*a*	–	–
4	N1BT1D (line)	*Gli-D1.1*	*a*	–	–
5	Cajeme-71	*Gli-D1.1*	*a*	Mexico	1971
6	Chinese-Spring	*Gli-D1.1*	*a*	China	–
7	Goelent	*Gli-D1.1*	*a*	France	1985
8	Marquis	*Gli-D1.1*	*a*	Canada	1907
9	Mentana	*Gli-D1.1*	*a*	Italy	1913
10	Newcaster	*Gli-D1.1*	*a*	USA	1946
11	Prinqual	*Gli-D1.1*	*a*	France	1978
12	Rinconada	*Gli-D1.1*	*a*	Spain	1981
13	Yecora-Rojo	*Gli-D1.1*	*a*	USA	1975
14	Intensivnaya	*Gli-D1.1*	*a+b*	Kazakhstan	1978
15	Potam-70	*Gli-D1.1*	*a+b*	Mexico	1970
16	Arminda	*Gli-D1.1*	*b*	Netherlands	1976
17	Cartaya	*Gli-D1.1*	*b*	Spain	1983
18	Diego	*Gli-D1.1*	*b*	Spain	1983
19	Escualo	*Gli-D1.1*	*b*	Spain	1981
20	Gazul	*Gli-D1.1*	*b*	Spain	1992
21	Laura	*Gli-D1.1*	*b*	Canada	1986
22	Pavon-F-76 (Betres)	*Gli-D1.1*	*b*	Mexico	1976

End of table 5

No.	Cultivar name	Allele *Gli-D1**	Allelic variant ** *Gli-D1*	Origin of cultivar/line	Year
23	Siete-Cerros-66	*Gli-D1.1*	*b*	Mexico	1966
24	Ardec	*Gli-D1.1*	*d*	Belgium	1979
25	Caia	*Gli-D1.1*	*f*	Portugal	–
26	Donskaya polukarlikovaia	*Gli-D1.1*	*f*	Russia	1984
27	Gabo	*Gli-D1.1*	*f*	Australia	1942
28	Roblin	*Gli-D1.1*	*f*	Canada	1986
29	Suneca	*Gli-D1.1*	*f*	Australia	1981
30	Mironovskaya-808	*Gli-D1.1*	*g*	Ukraine	1963
31	Katepwa	*Gli-D1.1*	*i*	Canada	1981
32	Insignia	*Gli-D1.1*	*i+j*	Australia	1946
33	Krasnodonka	*Gli-D1.1*	*q*	Ukraine	1969
34	Cluij-650	*Gli-D1.2*	*a*	Romania	1954
35	Argelato	*Gli-D1.2*	*b*	Italy	1964
36	Bezostaya-1	*Gli-D1.2*	*b*	Russia	1959
37	Capelle-Desprez	*Gli-D1.2*	*b*	France	1946
38	Galahad	*Gli-D1.2*	*b*	Great Britain	1982
39	Libero	*Gli-D1.2*	*b*	Italy	1927
40	Pane-247	*Gli-D1.2*	*b*	Spain	1960
41	Recital	*Gli-D1.2*	*b*	France	1986
42	Salmone	*Gli-D1.2*	*b*	Italy	1980
43	Sideral	*Gli-D1.2*	*b*	France	1990
44	Splendeur	*Gli-D1.2*	*b*	France	1964
45	Titien	*Gli-D1.2*	*b*	France	1985
46	Aragon-03	*Gli-D1.2*	*i*	Spain	1940
47	Federation	*Gli-D1.2*	*i*	Australia	1901
48	Glenlea	*Gli-D1.2*	*j*	Canada	1972
Bread wheat near-isogenic lines, created by Kopus (Kopus, 1994)					
1	GLI-D1-5	*Gli-D1.1*	*g*	Ukraine	1994
2	GLI-D1-4	*Gli-D1.2*	*j*	Ukraine	1994
3	GLI-A1-1	*Gli-D1.2*	*b*	Ukraine	1994
4	GLI-B1-3	*Gli-D1.2*	*b*	Ukraine	1994
5	GLI-B1-4	*Gli-D1.2*	*b*	Ukraine	1994
6	GLI-B1-12	*Gli-D1.2*	*b*	Ukraine	1994

Note. *allele of the *Gli-D1* locus, determined by the PCR using allele-specific primers, ** allelic variant of gliadins, encoded at the *Gli-D1* locus, determined by the storage proteins electrophoresis in A-PAGE. PBGI-NCSCI – Plant Breeding and Genetics Institute, the National Centre of Seed and Cultivar Investigation, Odesa; MIW – V.M. Remeslo Myronivka Institute of Wheat, Central, Myronivskyy area, Kiev region; BTsESS – Bila Tserkva Experimental Selection Station, Mala Vilshanka, Bilotserkivsky area, Kiev region; IIA – Institute of Irrigated Agriculture (IIA), Naddniprianske, Kherson redion; PSAA – Poltava State Agrarian Academy, Poltava, Poltava region; NBES – Nosivka Breeding and Selection Station, v. Doslidne, Chernihiv region; DIAP – Donetsk Institute of Agroindustrial Production of the Ukrainian Academy of Agrarian Sciences, Pisky, Donetsk region, Driada, Ltd – LLC Scientific and Production Company "Driada, Ltd", Kherson; LIBT – Luhansk Institute of Breeding and Technologies, Teplychne, Lugansk region.

quency of 0.69. *Gli-D1.1* was present in only 22 Ukrainian cultivars with a frequency of 0.31. A further 12 Ukrainian cultivars were either heterogeneous or positive for the two alleles *Gli-D1.1/Gli-D1.2* (Table 5).

Eight allelic variants of gliadins encoded at the *Gli-D1* locus were found in the foreign collection of bread wheat cultivars and lines by A-PAGE. Most frequent were *Gli-D1b* and *Gli-D1a* found in 19 and 12 cul-

Table 6. Frequencies of alleles *Gli-D1.1/ Gli-D1.2* and allelic variants of gliadins, encoded at the *Gli-D1* locus

AVG*	*Breeding center*							
	PBGI-NCSCI N = 28	MIW N = 20	BTsESS N = 11	IIA N = 10	PSAA N = 10	NBES N = 8	Ukrainian collection N = 91	Foreign collection N = 48
Frequency of AVG encoded at Gli-D1								
a	–	–	–	–	–	–	–	0.27
b	0.13	0.88	0.91	0.20	0.25	0.63	0.46	0.42
d	0.04	–	–	–	–	–	0.02	0.02
f	0.09	–	–	0.05	–	–	0.04	0.10
g	0.46	0.02	0.09	0.05	0.10	0.25	0.20	0.02
i	–	–	–	–	–	–	–	0.07
j	0.25	0.10	–	0.60	0.20	0.12	0.21	0.03
q	–	–	–	–	–	–	–	0.02
new	0.04	–	–	0.10	0.45	–	0.07	–
null	–	–	–	–	–	–	–	0.04
Allele of the Gli-D1 locus								
D1.1	0.43	0.20	0.18	0.20	0.35	0.31	0.31	0.64
D1.2	0.57	0.80	0.82	0.80	0.65	0.69	0.69	0.31

Note. * AVG – allelic variant of gliadins detected by A-PAGE.

tivars, respectively. In the remaining cultivars and lines *Gli-D1f, Gli-D1i, Gli-D1g, Gli-D1j* and *Gli-D1q* allelic variants were found, as well as the null allele for the Canadian cultivar Darius and line $1B^{-}$, $1D^{-}$ (**Table 5**).

Table 7. Correspondence of alleles of the *Gli-D1* locus, determined by using the primers of Zhang et al (2003), and allelic variants of gliadins determined by A-PAGE

Allelic variant of gliadins encoded et *Gli-D1* locus	Number of homogeneous cultivars/lines with *Gli-D1.1* or *Gli-D1.2* detected by PCR		
	Gli-D1.1	*Gli-D1.1* and *Gli-D1.2*	*Gli-D1.2*
Gli-D1a	11	–	1
Gli-D1b	11	3	48
Gli-D1d	1	–	2
Gli-D1f	7	–	–
Gli-D1g	8	1	8
Gli-D1i	1	–	2
Gli-D1j	7	1	12
Gli-D1q	1	–	–

Six allelic variants of gliadins encoded at the *Gli-D1* locus were detected in the Ukrainian collection in A-PAGE. Similar to the foreign collection, the Ukrainian collection *Gli-D1b* was also prevailed and found in 39 cultivars. A further 18 Ukrainian cultivars and lines contained *Gli-D1j* and 16 cultivars and lines *Gli-D1g*. *Gli-D1d* and *Gli-D1f* were found only in a small number of cultivars, and the allelic variant could not be identified in six cultivars, so they were designated as "possibly new". Eight cultivars were found to be heterogeneous. (Table 5). Ukrainian and foreign collections differed significantly ($p < 0.05$) by the frequencies of allelic variants of gliadins encoded at *Gli-D1*.

The *Gli-D1.2* allele dominated with a frequency of 0.57 to 0.82 in all groups of cultivars/lines from the different breeding centres in Ukraine. However, when using A-PAGE, groups of cultivars from different breeding centres differed in both the set of allelic variants of gliadins and their frequencies (Table 6). Although *Gli-D1b* was found in cultivars from all breeding centres, it was the most frequent only in samplings of cultivars from MIW, BTsESS, and NBES. *Gli-D1g* prevailed in the cultivars/lines from PBGI-NCSCI, and *Gli-D1j* in cultivars from IIA (**Table 6**).

The PCR results, obtained for the six near isogenic lines created based on the cultivar Bezostaya-1 by Ko-

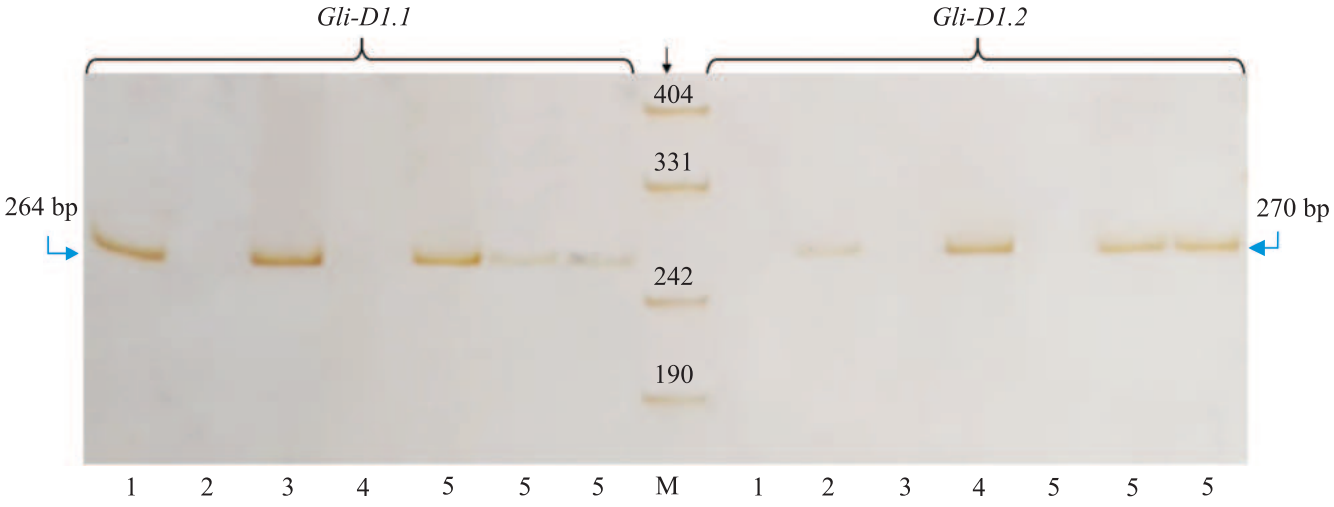

Fig. 3. Electropherogram of amplification products obtained in PCR with allele-specific primers of Zhang (2003) for *Gli-D1.1/Gli-D1.2* alleles. DNA: 1 – Klarisa, 2 – Lybid, 3 – Yuvivata 60, 4 – Myronivska 61, 5 – Sonata, M – molecular weight marker *pUC19/Msp I*

pus (Kopus, 1994), were used in our study to trace the correspondence between the allelic variants of gliadins and alleles of the *Gli-D1* locus. Four out of six lines were characterized by the same allelic variant of gliadin and allele by allele-specific PCR as recurrent parental cultivar Bezostaya-1: *Gli-D1b* and *Gli-D1.2*. Line GLI-D1-4 differed from parental cultivar Bezostaya-1 by the presence of the allelic variant *Gli-D1j* but also had *Gli-D1.2*. Presence of the *Gli-D1g* allelic variant in line *GLI-D1-5* led to a change of *Gli-D1.2* to *Gli-D1.1* (Table 5). The results, obtained for the near-isogenic lines demonstrate the correspondence between the allelic variants of gliadins and alleles of the *Gli-D1* locus: *Gli-D1.1* allele corresponds to *Gli-D1g* allelic variant and *Gli-D1.2* corresponds to *Gli-D1b* and *Gli-D1j* allelic variants. But the results for the *Gli-D1* locus, obtained by using PCR and A-PAGE, did not demonstrate the correspondence as observed for the *Gli-A1* locus (**Table 7**).

Most cultivars and lines (48, including near-isogenic lines) with the *Gli-D1b* allelic variant had the *Gli-D1.2* allele, and 11 cultivars had the *Gli-D1.1* allele, three cultivars had *Gli-D1.1* and *Gli-D1.2* alelles (Table 7). For instance, cultivar Klarisa has the amplification fragments that correspond to the *Gli-D1.1* allele, but Lybid and Myronivska 61 cultivars have the amplification fragments of the *Gli-D1.2* allele, but all these cultivars were found to contain the *Gli-D1b* allelic variant of gliadins (**Fig. 3**). Also grain of cultivar Sonata with the *Gli-B1b* allelic variant of gliadins (Fig. 3) have *Gli-D1.1* and *Gli-D1.2*.

A similar situation was observed for *Gli-D1g*: 8 cultivars with *Gli-D1.1* and 8 cultivars with *Gli-D1.2* allele), *Gli-D1j* (7 cultivars with *Gli-D1.1* and 12 cultivars with *Gli-D1.2* allele) (Table 7). Also, one cultivar, Ardec from the foreign collection with *Gli-D1d* allelic variant had the *Gli-D1.1* allele, while the *Gli-D1.2* allele was found in two Ukrainian cultivars (Kuialnyk AR and Metalist) with the same allelic variant.

DISCUSSION

Acid polyacrylamide gel electrophoresis (A-PAGE) has been found indispensable for the identification of allelic variants of gliadins, which are encoded by a single locus and inherited in a linked manner. This method allows for the simultaneous detection of allelic variants of gliadins encoded by all loci and is cheaper than PCR using specific primers for alleles of the genes encoding the gliadins. However, the difficulty of A-PAGE is that each *Gli* locus encodes several polypeptides, and one gel lane contains gene expression products (groups/blocks of gliadins) of the six gliadin-coding loci that may overlap, which greatly complicates the interpretation of electropherograms and the identification of allelic variants of gliadins (Metakovsky et al, 2018).

The availability of a DNA marker system enables 1) identification of allelic variants of gliadins from any part of the plant (not only from the endosperm), 2) analysis of each locus separately, 3) automation of the identification process, and 4) rejection of unnecessary alleles at early stages of breeding, which is very important for the use of gliadin alleles in marker-assisted breeding (Howitt et al, 2007; Landjeva et al, 2007; Goutam et al, 2013).

In our previous studies (Popovych et al, 2020, 2021a; Metakovsky, 2021), we found that amplification products obtained using allele-specific primers to the *Gli-B1* locus, developed by Zhang et al (2003), were polymorphic in length and could differ from the length specified by the developers due to microsatellite polymorphism within the amplified DNA sequence. Due to this, PCR simultaneously detected not only the SNP alleles for which the primers were designed, but also alleles formed by varying the number of microsatellite repeats. This allowed us in this case to detect a larger number of alleles that clearly corresponded to the allelic variants of gliadins of the *Gli-B1* locus detected in A-PAGE and to use definite DNA markers to identify allelic variants of gliadins encoded at the *Gli-B1* locus. PCR with allele-specific primers to the *Gli-A1* and *Gli-D1* loci in this study revealed only two alleles per locus, with no polymorphism in the length of amplification fragments, as described above for *Gli-B1* locus.

In our present study we found for a number of cultivars insufficient specificity of PCR with the primers to the *Gli-A1* and *Gli-D1* loci of Zhang et al (2003), which was not the case when using their primers for the *Gli-B1* locus. Although the use of a different polymerase increased specificity of PCR with primers to the *Gli-A1* locus, it remained unclear why this leads to higher specificity for certain cultivars/lines and not for others. Moreover, it did not explain why in some cultivars for the *Gli-D1* locus two alleles were detected in all samples (if these were heterozygotes, there should be homozygotes, but they were not detected). This apparent heterozygosity was reported by Ravel et al (2020) for 5 % of the wheat lines tested, using competitive allele-specific (KASP) markers to other, storage proteins (to high molecular weight glutenin subunits, HMWSs). In their case gene duplications that often occur in the bread wheat genome (Choulet et al, 2014; Glover et al, 2015) were suspected to be the reason.

Polymorphism of allelic variants of gliadins determined in A-PAGE was much higher than detected by PCR, using the specific primers of Zhang et al (2003). A number of common allelic variants encoded at *Gli-A1* and *Gli-D1* loci were found for a sampling of modern Ukrainian cultivars and a sampling of foreign bread wheat cultivars, namely *Gli-A1b, Gli-A1c, Gli-A1f, Gli-A1g, Gli-A1m* and *Gli-D1b Gli-D1g, Gli-D1j, Gli-D1f*, but their frequency in the two samplings differed significantly. *Gli-A1a, Gli-A1ab, Gli-A1af, Gli-A1l, Gli-A1n, GliA1r, Gli-D1a, Gli-D1i* and *Gli-D1q* were found only in foreign cultivars and lines, and *Gli-A1x* (sensu Kozub et al, 2009) only in the Ukrainian ones. It is noteworthy that the Ukrainian collection did not contain the *Gli-A1af* (*Gli-A1af* + *Gli-A6a*) allelic variant, which differs from the *Gli-A1f* (*Gli-A1af* + *Gli-A6b*) allelic variant, common in Ukrainian cultivars, by only one ώ-gliadin encoded by the minor locus *Gli-A6b* (Metakovsky et al, 2018a).

The study of allelic variants of gliadins with the aim to determine area or country specific valuable traits was carried out for bread wheat cultivars from Kazakhstan by Utebayev et al (2022), for different countries by Metakovsky et al. (2018a) and for Ukrainian cultivars by Kozub et al (2009, 2020, 2024).

In our study the most common allelic variant of gliadins encoded at the *Gli-A1* locus (*Gli-A1b*) in the Ukrainian sampling, was found in only two foreign cultivars, while the most common allele in the foreign sampling, *Gli-A1a*, was not found in Ukrainian cultivars and lines.

A difference in the set of allelic variants of gliadins was even observed between groups of cultivars from different breeding centres, which is consistent with the results of Kozub et al (2020), obtained for two of those breeding centres (MIW and PBGI-NCSCI) for cultivars developed between 1996 and 2010. In our study for the *Gli-A1* locus, the *Gli-A1b* and *Gli-A1g* allelic variants were most common among cultivars from PBGI-NCSCI. For MIW these were cultivars with *Gli-A1x*, *Gli-A1f*, *Gli-A1o* and *Gli-A1b*. If compare with investigations of genes *Pina-D1* and *Pinb-D1* affected hardness, there was not difference in allelic variants between group of cultivars from different breeding centers (Chebotar et al, 2012). Our present study showed that for the collection of Ukrainian bread wheat cultivars and lines developed from 1999–2020, the most frequent allelic variant of gliadins is again *Gli-A1b*, found earlier by Kozub et al (2009, 2020, 2024). As per the data of Metakovsky et al (2018a), *Gli-A1b* is most common for such countries as Bulgaria, Croatia, Hungary, Serbia and Ukraine, while *Gli-A1a* is most common among wheat cultivars in the foreign collection studied by the authors.

Metakovsky et al (2018a) determined for their catalogue using foreign and Ukrainian cultivars and lines that *Gli-D1b* was the most frequent allelic variant in foreign cultivars and lines and *Gli-D1j* for the Ukrainian ones. However, in the present study with differ-

ent Ukrainian samples we found *Gli-D1b* the most frequent, as for the foreign collection. The set and frequencies of allelic variants of gliadins for cultivars with PBGI-NCSCI are consistent with the study by Kozub et al (2020). However, for MIW we found a higher frequency of *Gli-D1b* (increased when compared to cultivars/lines developed before 2010), a second most frequent *Gli-D1j* and absence of *Gli-D1g*. This change in occurrence of the most common allelic variants may be induced by climate change (Kozub, 2009, 2021 and this study).The differences obtained between samplings of Ukrainian and foreign bread wheat cultivars can be explained by the fact that usually cultivars from each geographical region are by a certain specific set of alleles (Perrone et al, 2017; Utebayev et al, 2019.

Kozub et al (2009) described the new allelic gliadin variant *Gli-A1x* in some cultivars of MIW, which was not present in the catalogue of Metakovsky et al (1991). In his updated catalogue Metakovsky et al (2018a) describe a different *Gli-A1x,* detected mainly in cultivars from France. The latter should not be confused with the *Gli-A1x* as described by Kozub et al (2009) for the Ukrainian varieties. As Kozub et al (2009), we found that their *GliA1x* variant is closely similar in electrophoretic behaviour to *Gli-A1f*, but fortunately they are discriminated by PCR where the 'Metakovsky *Gli-A1f*' variant is linked to allele *Gli-A1.1* and the 'Kozub *Gli-A1x*' to the *Gli-A1.2* allele, as we determined in our study.

It once again shows that DNA markers can help in the recognition and isolation of new allelic variants of gliadins with very similar protein spectra.

The present study shows that cultivars with *Gli-A1a*, *Gli-A1ab*, *Gli-A1af, Gli-A1b, Gli-A1c, Gli-A1f, Gli-A1g* and *Gli-A1l* allelic gliadin variants are characte-rized by the *Gli-A1.1* allele, and cultivars with *Gli-A1r*, *Gli-A1o* and *Gli-A1x* are characterized by the *Gli-A1.2* allele. Therefore, the frequency of alleles detected in PCR for the *Gli-A1* locus correlated well with the frequencies of allelic variants of gliadins. In both foreign and Ukrainian samplings of wheat cultivars, the *Gli-A1.1* allele was the most common. However, for the *Gli-D1* locus, in the Ukrainian collection of cultivars, the most common was *Gli-D1.2*, and among the foreign cultivars this was *Gli-D1.1*, although according to the data of electrophoresis in A-PAGE for both Ukrainian and foreign cultivars, *Gli-D1b* was the most common. Therefore, correspondence between allelic variants of gliadins in A-PAGE and alleles of the *Gli-D1* locus determined by PCR primers of Zhang et al (2003) could not be established.

The correspondence between *Gli-A1.1* and *Gli-A1.2* alleles, and allelic variants of gliadins as determined by us in this study by using primers developed by Zhang et al (2003), complements the results obtained for the *Gli-B1* locus in our earlier studies using the same primers (Popovych et al, 2020, 2021; Metakovsky et al, 2021). This demonstrates the possibility of using the Zhang (2003) primers to reliably identify allelic variants of gliadins encoded at the *Gli-A1* locus. The negative side of using these primers is that they allow detecting only two alleles per locus determined by SNP polymorphism, while the polymorphism of proteins (allelic variants of gliadins) is much larger. Therefore, these DNA markers can only be auxiliary in the determination of allelic variants of gliadins or used in the analysis of recombinant-inbred lines or near-isogenic lines with different parental allelic variants of gliadins.

CONCLUSIONS

The most common allele of the *Gli-A1* locus in the collections of modern Ukrainian bread wheat cultivars and lines and that of foreign cultivars and lines (in total 145) was *Gli-A1.1*, and the most common gliadin variants as determined by A-PAGE were *Gli-A1b* for Ukrainian samples and *Gli-A1a* for the foreign ones. The most common allele of the *Gli-D1* locus in the collection of modern Ukrainian bread wheat cultivars and lines was the *Gli-D1.2* allele, and in the foreign collection of wheat cultivars it was the *Gli-D1.1* allele and in both cases the *Gli-A1b* allelic variant of gliadins. There is a correspondence between allelic variants of gliadins encoded at the *Gli-A1* locus and *Gli-A1.1/Gli-A1.2* alleles. *Gli-A1.1* is associated with *Gli-A1a, Gli-A1ab, Gli-A1af, Gli-A1b, Gli-A1c, Gli-A1f, Gli-A1g*, and *Gli-A1l*, and the *Gli-A1.2* allele with *Gli-A1r, Gli-A1o* and *Gli-A1x* sensu Kozub et al (2009). No correspondence was found between allelic variants of gliadins encoded at the *Gli-D1* locus and *Gli-D1.1/Gli-D1.2* alleles.

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Поліморфізм *GLI-A1* та *GLI-D1* локусів в українських та закордонних сортах та лініях пшениці м'якої

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Мета. Метою даної роботи було проаналізувати поліморфізм *Gli-A1* та *Gli-D1* локусів пшениці м'якої у межах доповненої української та закордонної колекцій сортів пшениці м'якої за допомогою методів електрофорезу запасних білків в кислому ПААГ і ПЛР з алель-специфічними маркерами, розробленими Zhang et al (2003) та з'ясувати відповідність між алельними варіантами гліадинів та алелями, виявленими в ПЛР для цих локусів. **Методи.** У роботі досліджували 145 сортів та ліній пшениці м'якої української та зарубіжної селекції. Електрофорез запасних білків проводили в кислому ПААГ за методикою Ф. О. Поперелі (1989), алельні варіанти гліадинів позначали за міжнародною номенклатурою (Metakovsky et al, 2018). ДНК виділяли СТАВ методом та проводили ПЛР з алель-специфічними праймерами до *Gli-A1* та *Gli-D1* локусів (Zhang et al, 2003). Продукти ПЛР фракціонували в 7% ПААГ та фарбували за допомогою аргентум нітрату. **Результати**. В ході ПЛР з алель-специфічними праймерами до *Gli-A1* у вибірці 91 сучасного українського сорту установлено, що частота *Gli-A1.1* алеля становила 0,80, *Gli-A1.2* алеля – 0,20, а у колекції 48 закордонних сортів пшениці м'якої – 0,69 та 0,31, відповідно. Частота *Gli-D1.1* та *Gli-D1.2* алелів в українській колекції сортів становила 0,31 та 0,69, відповідно. *Gli-D1.1* алель переважав у колекції закордонних сортів з частотою 0,64, частота *Gli-D1.2* становила 0,31. За результатами електрофорезу запасних білків у вибірці українських сортів знайдено сім алельних варіантів гліадинів, найбільш поширеним був *Gli-A1b*, у колекції закордонних сортів виявлено 12 алельних варіантів гліадинів, найбільш поширеним був *Gli-A1a*. П'ять різних алельних варіантів гліадинів, що кодуються *Gli-D1* локусом знайдено в українській колекції та вісім серед закордонних. Для шести сортів української колекції, алельні варіанти гліадинів не були визначені, тому вони позначені як «імовірно нові». Найбільш поширеним алельним варіантом гліадинів для двох вибірок був *Gli-D1b*. Установлено,що алель *Gli-A1.1* відповідає алельним варантами гліадинів *Gli-A1a*, *Gli-A1ab*, *Gli-A1af*, *Gli-A1b*, *Gli-A1c*, *Gli-A1f*, та *Gli-A1l*, а алель *Gli-A1.2* відповідає *Gli-A1o*, *Gli-A1r* та *Gli-A1x* алельним варіантам (*Gli-A1x* за Kozub et al, 2009). Відповідність між алельними варіантами гліадинів, що кодуються *Gli-D1* локусом та алелями *Gli-D1.1* і *Gli-D1.2* не виявлено. **Висновки.** Найбільш поширеним алелем *Gli-A1* локусу в колекції з 91 сучасного українського сорту та ліній пшениці м'якої та 48 закордонних сортів є *Gli-A1.1* алель, *Gli-D1.2* алель найчастіше зустрічається серед українських сортів пшениці м'якої та *Gli-D1.1* серед закордонних сортів. На відміну від *Gli-D1* локусу, в *Gli-A1* локусі існує відповідність між алельними варіантами гліадинів, що кодуються *Gli-A1* локусом та *Gli-A1.1/ Gli-A1.2* алелями.

Ключові слова: *Triticum aestivum L.*; алельні варіанти гліадинів; молекулярні маркери; алель-специфічні праймери.

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