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# Diversity of Ukrainian Winter Common Wheat Varieties with Respect to Storage Protein Loci and Molecular Markers for Disease Resistance Genes[1]

**N. A. Kozub[a, b, *], I. A. Sozinov[a, **], A. V. Karelov[a, b], Ya. B. Blume[b], and A. A. Sozinov[b]**

[a] *Institute of Plant Protection, NAAS of Ukraine, Kyiv, Ukraine*
[b] *Institute of Food Biotechnology and Genomics, NAS of Ukraine, Kyiv, Ukraine*
**e-mail: natalkozub@gmail.com*
***e-mail: sia1@i.com.ua*

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**Abstract**—Diversity of Ukrainian winter common wheat varieties was studied with respect to the storage protein loci *Gli-A1*, *Gli-B1*, *Gli-D1*, *Glu-A1*, *Glu-B1*, *Glu-D1*, *Gli-A3*, *Gli-B5*, and *Gli-A6* (362 varieties) and markers for the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene conferring moderate resistance to a number of biotrophic pathogens, the *Tsn1* gene for sensitivity to the toxins A of the necrotrophic fungi *Pyrenophora tritici-repentis* and *Stagonospora nodorum*, the *Tsc2* gene for sensitivity to the toxin B of *P. tritici-repentis*, and the *TDF_076_2D* gene for moderate resistance to *Fusarium* head blight (181 varieties). Significant differences in frequencies of alleles at these marker loci between groups of varieties developed in different soil and climatic zones were revealed. The retention of a set of predominant alleles in groups of varieties of a certain zone in different periods of breeding was confirmed. At the same time, the appearance of new allele associations in the groups of varieties of the Steppe (in particular *Gli-A1g* and *Glu-B1al*) and the Central Forest-Steppe (1AL/1RS and *Glu-B1d*) in the last two decades has been noted. Nonrandom associations between alleles of disease resistance genes as well as alleles of disease resistance genes and storage protein alleles were revealed.

*Keywords: Triticum aestivum*, storage proteins, resistance genes, alleles, gene associations, diversity
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## INTRODUCTION

Common wheat *Triticum aestivum* L. (the genomic formula AABBDD) is an important crop, which probably originated about 8000 years ago as a result of hybridization of the cultivated form of tetraploid wheat *Triticum turgidum* L. (AABB) with the wild wheat species *Aegilops tauschii* Coss. (DD) in the area of modern southeastern Turkey [1]. *T. aestivum* is the most widespread cultivated wheat, which accounts for 95% of the wheat grown in the world, it remains an important source of protein for human and livestock nutrition due to high yields of the crop, adaptation to a wide range of environments, and, most important, unique properties of dough, which are mainly determined by the complex of seed storage proteins [2].

Due to their high polymorphism, storage proteins, gliadins and high molecular weight (HMW) subunits of glutenin, were the first genetic markers for studying diversity of world wheat collections [3–11]. The HMW glutenin subunit loci, *Glu-A1, Glu-B1,* and *Glu-D1,* are located on the long arms of the homoeologous group 1 chromosomes and contain two genes encoding *x*- and *y*-subunits; in the case of the *Gli-A1* locus, only the x-subunit or none is expressed [12]. Based on the composition of HMW glutenin subunits it is possible to at least partially predict dough quality [13, 14]. Gliadins (ethanol-soluble proteins) are encoded by clusters of genes located in distal parts of the homoeologous group 1 (*Gli-A1, Gli-B1,* and *Gli-D1*) and 6 (*Gli-A2, Gli-B2,* and *Gli-D2*) chromosomes [12]. The loci containing genes for low molecular weight subunits of glutenin (*Glu-3*) are closely linked to the *Gli-1* loci [12]. Besides the major gliadin loci, there are a number of minor loci on the short arms of the group 1 chromosomes, in particular, *Gli-A3* and *Gli-B3* located at a distance of about 20 cM from *Gli-A1* and *Gli-B1,* and as well as more closely linked loci *Gli-A5, Gli-B5,* and *Gli-A6* (up to 5 cM from the respective major loci) [15–17].

It has been demonstrated that the major gliadin loci *Gli-1* are located in gene-rich parts of the group 1 chromosomes [18, 19]. Certain alleles at these loci in wheat and its relatives proved to be closely linked to disease resistance genes, in particular, this is the case of introgressed alleles. For instance, the leaf rust resis-

[1] The article was translated by the authors.

tance genes *Lr21* and *Lr42* and the stem rust resistance gene *Sr33* from *Ae. tauschii* are linked to the *Gli-D1* locus with the recombination frequency of about 5% [20–22]. The yellow rust resistance gene *Yr10* was mapped at a distance of 5% recombination from the *Gli-B1* locus [23]. The powdery mildew resistance gene *Pm3* is linked to the *Gli-A1* locus and the loci that are closely associated to it (*Gli-A5* and *Glu-A3*), in particular, the allele *Pm3g* was localized at a distance of 5 cM from *Gli-A5* [24]. The leaf rust resistance gene *Lr10* was mapped at a distance of 2.9 cM from the *Gli-A1* locus [25]. A prominent example of the use of storage proteins as markers is identification of the wheat-rye 1BL/1RS and 1AL/1RS translocations associated with manifestation of a number of important traits. The 1BL/1RS translocation from the rye Petkus (2x), which is the most widespread introgression among common wheat varieties [26], carries the disease resistance genes *Pm8, Sr31, Lr26,* and *Yr9* [27]. The *Dn2414* gene conferring resistance to biotype 2 of the Russian wheat aphid *Diuraphis noxia* (Kurdjumov) was also mapped on 1BL/1RS [28]. The 1AL/1RS translocation was first introduced into the wheat cultivar Amigo from the Argentinian rye (*Secale cereale* L.) cultivar Insave and contains the *Gb2* gene conferring resistance to biotypes B and C of the greenbug *Schizaphis graminum* (Rondani), the *Cm3* gene conferring resistance to the wheat curl mite *Aceria tosicheilla* (Keifer), as well as the disease resistance genes *Pm17* and $Sr1RS^{Amigo}$ [27], the latter being effective against stem rust race Ug99 biotypes [29].

The employment of DNA markers based on PCR techniques allowed studying diversity of wheats with respect to an unlimited number of either neutral or functional loci, among them, markers for pathogen resistance genes. In particular, in a number of studies mapping and sequencing of the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene conferring moderate resistance to biotrophic pathogens on chromosome 7D [30–32], the *Tsn1* gene for sensitivity to the toxins A of the necrotrophic fungi *Pyrenophora tritici-repentis* (Died.) Drechs. and *Stagonospora nodorum* (Berk.) E. Castell. and Germano on chromosome 5B [33], and the *TDF_076_2D* gene for moderate resistance to *Fusarium* head blight on chromosome 2D [34] were performed. The *Tsc2* gene conferring sensitivity to the toxin B of *P. tritici-repentis* was mapped on chromosome 2B [35].

Diversity of collections of Ukrainian common wheat varieties has been studied with the use of storage proteins as molecular-genetic markers, primarily with consideration for their effect on bread-making quality [5, 8, 36–39], as well as with SSR and other DNA markers [40–48]. Based on analysis of composition of storage protein alleles in varieties from different breeding centers of Ukraine developed in the periods of 1910–1960 and 1960–1995, the concept of formation of stable gene associations has been offered [36]. According to it, the most valuable gene associations derived from landraces or developed via hybridization are retained for a long time in commercial varieties of a given region, and a new breeding stage is characterized by involvement of new genes or gene complexes into such associations. Genotypes carrying valuable gene associations (leader varieties) not only provide progress in the practical use of the crop but also serve as progenitors for many new varieties with such associations [36].

The aim of this research was to characterize diversity of winter common wheat varieties developed in different agroecological zones of Ukraine in different periods of time, in particular, after 1995, with respect to storage protein loci on the homoeologous group 1 chromosomes and a number of important disease resistance genes, as well as to reveal possible stable gene associations.

## MATERIALS AND METHODS

A total of 362 Ukrainian winter common wheat varieties were analyzed (Table S1, http://cytgen.com/articles/512053s.pdf). Among them, there were 128 varieties of the breeding institutions of the Central Forest-Steppe of Ukraine: the Myronivka Remeslo Institute of Wheat of the National Academy of Agrarian Sciences of Ukraine (NAAS) (MIW), the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (IPPG), and the Bila Tserkva Experimental Station of the Institute of Bioenergy Crops and Sugar Beet of NAAS (BTsES). The varieties of the Steppe of Ukraine (149) were represented by 146 varieties developed in the Plant Breeding and Genetics Institute (PBGI), Odessa, and 3 varieties (Titona, Tronka, and Shestopalivka) developed by the Private Agricultural Research Breeding Company "BOR" (Dachne, Odessa region). Besides, 23 varieties of the Plant Production Institute nd. a. V.Ya. Yuryev (PPI) of NAAS, 30 varieties of the National Science Centre "Institute of Agriculture of NAAS" (IA), and a group of varieties developed in other breeding institutions of the Steppe zone from the South and the East of Ukraine (ES, 35 varieties) were analyzed. The varieties of the Central Forest-Steppe and the Steppe were assigned to two groups according to the year of development: varieties, developed before 1996 (period 1) and those developed since 1996 (period 2). The sample of Central Forest-Steppe varieties included 35 varieties developed before 1996 and 93 varieties of the second period. Among the Steppe varieties there were 62 of period 1 and 87 of period 2. The seed material was kindly provided by the National Center for Plant Genetic Resources of Ukraine of NAAS (Kharkiv), MIW, PBGI, and breeders.

For statistical analysis of data on markers for disease resistance genes, the results reported previously for 91 winter common wheat varieties of the Steppe zone of Ukraine (PBGI), 65 varieties developed in MIW and IPPG, as well as 25 IA varieties were used [44, 45, 47, 48]. For this research, a number of variet-

ies were studied additionally using the marker *INDEL1* of the *TDF_076_2D* gene by the procedure described in [34, 47]. The resistance-associated allele of the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene [32] was marked as *Lr34+*, the allele associated with absence of resistance as *Lr34–*; for the *Tsn1* gene, the allele for insensitivity to the toxin A [33] was designated as *tr*, the allele for sensitivity as *Ts*; for the *Tsc2* gene, the allele for insensitivity to the toxin B [35] was marked as *tsr*, the allele for sensitivity as *Tss*; for the *TDF_076_2D* gene, the allele conferring *Fusarium* head blight resistance [34] was designated as *TDF-1*, the allele for the absence of such resistance as *TDF-2*.

Acid polyacrylamide gel (APAG) electrophoresis of gliadins of 10–20 individual seeds of each variety was performed on 10% gels by the procedure [38]. HMW subunits of glutenin were analyzed by SDS-electrophoresis according to Laemmli [49] on 10% separating gel. Alleles at the HMW glutenin subunit loci *Glu-A1, Glu-B1,* and *Glu-D1* were identified according to the catalogue of Payne and Lawrence [4], alleles at the gliadin-coding loci *Gli-A1, Gli-B1,* and *Gli-D1* were identified using the catalogue of Metakovsky [50] with additions. Genotypes of a portion of varieties of the Central Forest-Steppe and IA at the *Gli-1* and *Glu-1* loci had been studied previously [38, 39], however genotypes of some of these varieties were corrected. Alleles at the *Gli-D1* locus were identified without taking into consideration the minor γ-gliadin with a high mobility [50], therefore the alleles *a* and *f* in this research were not differentiated and marked as *f*. The marker of the Amigo-type 1AL/1RS translocation is the presence of the secalin block designated as *Gli-A1w* (*Gld 1A17* [5]) on the APAG electrophoregram [38]. Alleles at the *Gli-A3* locus were designated in accordance with [27]; alleles at the *Gli-B5* locus were denoted according to [17], except for varieties with the 1BL/1RS translocation, for which the designation "*nnn*" was used. At the minor *Gli-A6* locus, the allele *c* was identified by the presence on APAG electrophoregrams of ω-components specific, in particular, for genotypes with the allele *Gli-A1f*, their absence was marked as the allele *a* [15], except for varieties with the 1AL/1RS translocation, for which the designation "*nnn*" was used. The allele *Gli-A1x* was identified by the presence of the gliadin component (GLD 1A9 [5]) as described in [38]. The allele controlling the block GLD 1D10 [5] was designated as *Gli-D1x*. Other rare gliadin alleles identified for the first time were marked with asterisks.

Allele frequencies in groups of Ukrainian varieties were calculated taking into consideration heterogeneity (the frequency of each of the two alleles in heterogeneous varieties was taken as 0.5). To analyze differences in allele frequencies between different groups of varieties, the $\chi^2$ test was used. Associations between alleles of resistance genes as well as at storage protein loci were estimated with use of the phi coefficient (φ) [51], for this, the genotypes were presented with the use of the binary system (0, 1). Nei's genetic diversity index [52] was calculated by the formula $H = 1 - \Sigma p_i^2$, the effective number of alleles per locus was determined as $n_e = 1/\Sigma p_i^2$, where $p_i$ is a frequency of a certain allele [53]. Frequencies of alleles at the *Gli-1* and *Glu-1* loci of common wheat varieties of Australia, France, United Kingdom (UK), the pooled sample of German and Dutch varieties (for *Gli-1*) or only German varieties (for *Glu-1*) were calculated on the basis of genotypes presented in [11] without considering heterogeneous varieties at respective loci. Based on allele frequencies, pairwise Euclidean distances between groups of varieties were determined and dendrograms were constructed by the neighbor-joining (NJ) method with 1000 bootstraps using the DARwin6 software [54].

## RESULTS AND DISCUSSION

Genetic diversity was assessed in the collection of 362 Ukrainian winter common wheat varieties at the storage protein loci *Gli-A1, Gli-B1, Gli-D1, Glu-A1, Glu-B1, Glu-D1, Gli-A3, Gli-B5,* and *Gli-A6* as well as 181 varieties with respect to markers for the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene conferring moderate resistance to a number of biotrophic pathogens [32], the genes *Tsn1* for sensitivity to the toxins A of the necrotrophic fungi *P. tritici-repentis* and *S. nodorum* [33], *Tsc2* for sensitivity to the toxin B of *P. tritici-repentis* [35], and the *TDF_076_2D* gene for moderate resistance to *Fusarium* head blight [34].

### *Genetic Diversity at Storage Protein Loci in the Total Sample of Ukrainian Winter Common Wheat Varieties*

Information about genotypes at the loci *Gli-A1, Gli-B1, Gli-D1, Glu-A1, Glu-B1, Glu-D1, Gli-A3, Gli-B5,* and *Gli-A6* is given in Table S1. Genotypes of some previously published [38] Central Forest-Steppe varieties were corrected. In particular, the genotypes of the varieties Vdiachna, Mironovskaya 25, and Mironovskaya 29 are presented with consideration for the genotype at the minor locus *Gli-A6* as *Gli-A1b Gli-A6c* instead of the previous designation *Gli-A1y* [38] (*Gld-1A-12* [5]), and the genotype of the variety Estet is given as *Gli-A1x Gli-A6c*. Some new rare alleles at the locus *Gli-B1*, which are absent in the available catalogues, have been identified. The allele provisionally denoted as *b** was detected in a biotype of the variety Odesskaya 267, most likely being of mutant origin. The APAG electrophoretic pattern of gliadins encoded by *Gli-B1b** differs from that encoded by *Gli-B1b* by the higher mobility of the lower ω-gliadin. The *Gli-B1* allele of the variety Hnom was temporarily designated as *gn**; it codes for the upper ω-gliadin and the γ-gliadin as in the allele *Gli-B1f* and its lower ω-gliadin has the mobility like that in the product of expression of the allele *Gli-B1b* on APAG electrophoregrams, which indicates the recombinant origin of *gn**.

**Table 1.** Indices of diversity of Ukrainian winter common wheat varieties at storage protein loci (362 varieties)

| Locus | Heterogeneous varieties, % | Number of alleles | Effective number of alleles | Nei's genetic diversity index |
|---|---|---|---|---|
| *Gli-A1* | 11 | 11 | 3.6 | 0.719 |
| *Gli-B1* | 6 | 10 | 2.1 | 0.516 |
| *Gli-D1* | 15 | 7 | 3.2 | 0.683 |
| *Glu-A1* | 15 | 3 | 2.4 | 0.586 |
| *Glu-B1* | 10 | 8 | 2.3 | 0.575 |
| *Glu-D1* | 1 | 4 | 1.3 | 0.199 |
| *Gli-A3* | 11 | 4 (5)* | 2.4 | 0.586 |

* The number of alleles taking into account the presence of the 1AL/1RS translocation is given in parentheses.

Many Ukrainian varieties (41%) are heterogeneous with respect to one or more storage protein loci: from 27% in the group of Central Forest-Steppe varieties to 51% for the Steppe varieties. The largest number of alleles was revealed at the *Gli-A1* and *Gli-B1* loci, and the highest index of genetic diversity and, respectively, the effective number of alleles are observed at the loci *Gli-A1* and *Gli-D1* (Table 1). Nei's genetic diversity index on average for seven loci (without consideration for the minor loci *Gli-B5* and *Gli-A6,* which are closely linked to the respective major loci) is 0.552.

In general, in the gene pool of Ukrainian varieties, the predominant storage protein alleles are the following: *Gli-A1b* (49%), *Gli-B1b* (68%), *Gli-D1b* (43%), *Gli-D1g* (33%), *Glu-A1a* (42%), *Glu-A1b* (47%), *Glu-B1c* (57%), *Glu-B1b* (32%), *Glu-D1d* (89%), *Gli-A3a* (41%), *Gli-A3b* (49%), *Gli-B5a* (82%), and *Gli-A6a* (84%) (Table 2). The alleles *Gli-A1f* (11%), *Gli-A1o* (14%), *Gli-B1l* (the translocation 1BL/1RS) (14%), and *Gli-D1j* (14%) are also rather frequent. Virtually all predominant alleles at the HMW glutenin subunit loci are alleles with the positive effect on bread-making quality, according the scale of Payne et al. [13], this is especially the case of alleles at the loci *Glu-A1* and *Glu-D1*, which is a characteristic feature of Ukrainian varieties. The data on predominant alleles at the major storage protein loci are in agreement with those obtained for previously analyzed samples of Ukrainian varieties [8, 37, 38].

The collection of Ukrainian varieties significantly differs in frequencies of predominant alleles at the *Gli-1* loci from those of other countries although there are many common predominant alleles (Table S2, http://cytgen.com/articles/512053s.pdf, frequencies calculated based on [11]). In particular, the alleles *Gli-A1f* (33%), *Gli-A1o* (36%), *Gli-B1b* (24%), *Gli-B1f* (54%), and *Gli-D1b* (74%) are frequent among French varieties, of which only *Gli-B1f* is rare among Ukrainian varieties. Among the UK varieties, the alleles *Gli-A1b* (24%), *Gli-A1f* (41%), *Gli-A1o* (22%), *Gli-B1f* (45%), *Gli-B1g* (28%), *Gli-B1l* (13%), *Gli-D1b* (69%), *Gli-D1f* (13%), and *Gli-D1m* (13%) are encountered with frequencies exceeding 10%. The alleles *Gli-A1f* (57%), *Gli-A1o* (29%), *Gli-B1f* (41%), *Gli-B1h* (18%), *Gli-B1l* (27%), *Gli-D1b* (68%), and *Gli-D1l* (23%) predominate in the pooled sample of German and Dutch varieties. In the Australian sample, the most frequent are *Gli-A1a* (13%), *Gli-A1f* (34%), *Gli-A1o* (24%), *Gli-B1b* (61%), *Gli-B1l* (23%), *Gli-D1a* (20%), *Gli-D1b* (19%), *Gli-D1f* (28%), and *Gli-D1l* (20%). Thus, it can be seen that the alleles *Gli-A1f, Gli-A1o,* and *Gli-D1b* are common for the groups of varieties of Ukraine and all the above-mentioned countries, as well as the alleles *Gli-B1b* and *Gli-B1l* predominate in the majority of these samples. The comparison of allele frequencies at the HMW glutenin subunit loci *Glu-1* of the collection of Ukrainian varieties and those of other countries, the USA [55], Argentina [56], Australia, France, the UK, and Germany [11], (Table S3, http://cytgen.com/articles/512053s.pdf) has demonstrated the high similarity of the group of Ukrainian varieties to those of the USA and Argentina (Fig. 1). This evidently is due to similar stringent requirements on bread-making quality of grain in the breeding process.

It can be seen that among the most common alleles in the sample of Ukrainian varieties there are all the storage protein alleles characteristic of the outstanding variety Bezostaya 1 (*Gli-A1b, Gli-B1b, Gli-D1b, Glu-A1b, Glu-B1c, Glu-D1d, Gli-A3b, Gli-B5a,* and *Gli-A6a*), which has played a significant part in breeding of Ukrainian varieties [36]. The proportion of varieties with the combination of these alleles (taking into account heterogeneous varieties) is 0.055, which significantly exceeds the expected frequency of such genotypes based on frequencies of these alleles in the total sample, 0.007 ($\chi^2 = 77.7$, $P < 0.01$).

### *Analysis of Allele Frequencies at Storage Protein Loci in Groups of Ukrainian Varieties Developed in Different Zones*

At the *Gli-A1* locus in all groups of varieties, the frequency of the allele *b* is 50% and higher, except for the Central Forest-Steppe varieties, where its frequency is much lower, 25%. The Steppe, Central Forest-Steppe, and PPI varieties also show a rather high frequency of the allele *o*. In the group of IA varieties, the alleles *x* and *f* are frequent, like in the Central For-

**Table 2.** Frequencies of alleles at storage protein loci among winter common wheat varieties of Ukrainian breeding

| Locus, allele | S (149) | CFS (129) | *P* | IA (30) | PPI (23) | ES (32) | Total sample (362) | Locus, allele | S (149) | CFS (129) | *P* | IA (30) | PPI (23) | ES (32) | Total sample (362) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Gli-A1* | | | | | | | | *Glu-A1* | | | |
| *b* | 0.644 | 0.246 | ** | 0.567 | 0.500 | 0.625 | 0.486 | *a* | 0.346 | 0.453 | | 0.233 | 0.870 | 0.531 | 0.424 |
| *c* | 0.037 | 0.113 | * | | 0.109 | 0.109 | 0.072 | *b* | 0.631 | 0.406 | ** | 0.367 | 0.087 | 0.375 | 0.472 |
| *f* | 0.007 | 0.246 | ** | 0.117 | 0.043 | 0.031 | 0.105 | *c* | 0.023 | 0.141 | ** | 0.400 | 0.043 | 0.094 | 0.104 |
| *g* | 0.141 | | ** | | | | 0.058 | | | | | *Glu-B1* | | | |
| *i* | | | | | 0.043 | | 0.003 | *al* | 0.111 | | ** | 0.033 | | 0.031 | 0.051 |
| *l* | | | | | | 0.094 | 0.008 | *a* | | 0.031 | * | 0.033 | | | 0.014 |
| *m* | 0.027 | | | | 0.130 | 0.031 | 0.022 | *b* | 0.497 | 0.125 | ** | 0.083 | 0.304 | 0.453 | 0.315 |
| *o* | 0.128 | 0.176 | | 0.067 | 0.174 | 0.078 | 0.138 | *c* | 0.386 | 0.723 | ** | 0.783 | 0.652 | 0.516 | 0.566 |
| $w^A$ | 0.013 | 0.117 | ** | | | | 0.047 | *d* | 0.007 | 0.109 | ** | 0.033 | 0.043 | | 0.047 |
| *x* | | 0.102 | ** | 0.250 | | 0.031 | 0.059 | *f* | | 0.008 | | | | | 0.003 |
| *null?* | 0.003 | | | | | | 0.001 | *i* | | 0.004 | | | | | 0.001 |
| | | | | *Gli-B1* | | | | *aa* | | | | 0.033 | | | 0.003 |
| *b* | 0.782 | 0.504 | ** | 0.383 | 0.870 | 1.000 | 0.675 | | | | | *Glu-D1* | | | |
| *c* | 0.037 | | * | 0.033 | | | 0.018 | *a* | 0.020 | 0.125 | ** | 0.483 | 0.043 | 0.016 | 0.097 |
| *d* | 0.077 | 0.039 | * | 0.133 | 0.022 | | 0.058 | *b* | | | | 0.083 | 0.043 | | 0.010 |
| *e* | 0.084 | 0.016 | * | 0.267 | 0.065 | | 0.066 | *d* | 0.980 | 0.863 | ** | 0.433 | 0.913 | 0.984 | 0.890 |
| *f* | 0.003 | 0.039 | | | | | 0.015 | *e* | | 0.012 | | | | | 0.004 |
| *h* | | 0.047 | ** | | 0.043 | | 0.019 | | | | | *Gli-A3* | | | |
| $l^B$ | 0.013 | 0.344 | ** | 0.150 | | | 0.140 | *a* | 0.483 | 0.396 | * | 0.150 | 0.196 | 0.516 | 0.409 |
| *x* | | 0.012 | | | | | 0.004 | *b* | 0.493 | 0.374 | | 0.717 | 0.804 | 0.484 | 0.493 |
| *gn** | | | | 0.033 | | | 0.003 | *c* | | 0.078 | ** | | | | 0.026 |
| *b** | 0.003 | | | | | | 0.001 | *d* | 0.010 | 0.022 | | 0.133 | | | 0.023 |
| | | | | *Gli-D1* | | | | $nnn^A$ | 0.014 | 0.130 | ** | | | | 0.049 |
| *b* | 0.221 | 0.664 | ** | 0.667 | 0.326 | 0.328 | 0.431 | | | | | *Gli-B5* | | | |
| *f* | 0.081 | 0.082 | | 0.083 | 0.065 | | 0.073 | *a* | 0.950 | 0.602 | | 0.817 | 0.957 | 1.000 | 0.820 |
| *g* | 0.379 | 0.203 | ** | 0.233 | 0.435 | 0.578 | 0.326 | *b* | 0.037 | 0.047 | | 0.033 | 0.043 | | 0.037 |
| *i* | 0.007 | 0.008 | | | | | 0.006 | $nnn^B$ | 0.013 | 0.352 | | 0.150 | | | 0.142 |
| *j* | 0.265 | 0.027 | ** | 0.017 | 0.174 | 0.094 | 0.140 | | | | | *Gli-A6* | | | |
| *l* | | 0.016 | | | | | 0.006 | *a* | 0.980 | 0.625 | ** | 0.883 | 0.957 | 0.969 | 0.844 |
| *x* | 0.047 | | * | | | | 0.019 | *c* | 0.007 | 0.258 | ** | 0.117 | 0.043 | 0.031 | 0.109 |
| | | | | | | | | $nnn^A$ | 0.013 | 0.117 | ** | | | | 0.047 |

Here and in Table 4: S—Steppe varieties (all of PBGI except for Titona, Tronka, and Shestopalivka); CFS—Central Forest-Steppe varieties (MIW, IPPG, and BTsES); IA—IA varieties, PPI—PPI varieties; ES—varieties of other breeding institutions of the South and the East of Ukraine. The number of varieties analyzed is given in parentheses. Differences in allele frequencies between the S and CFS groups are significant at * $P < 0.05$; ** $P < 0.01$. [A]—1AL/1RS; [B]—1BL/1RS.

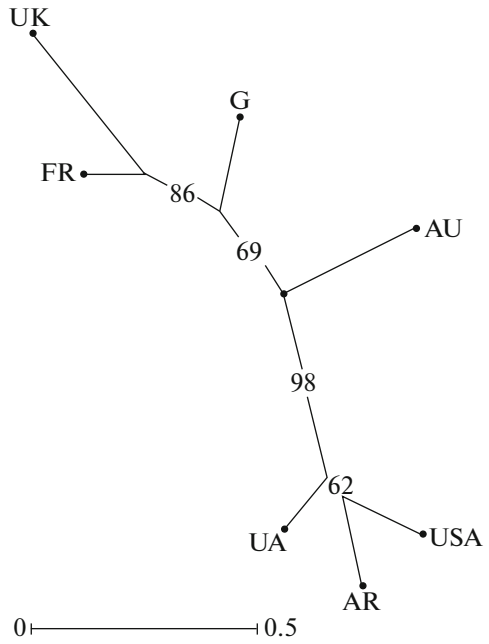


**Fig. 1.** Dendrogam of genetic similarity between collections of common wheat varieties of different countries on the basis of genotypes at *Glu-1* loci constructed using the NJ method (AR—Argentina, AU—Australia, FR—France, G—Germany, UA—Ukraine, UK—the UK, and USA—the USA) according to [11, 55, 56].

est-Steppe group. The group of Central Forest-Steppe varieties shows the highest diversity at the *Gli-A1* locus: Nei's genetic diversity index is 0.811, there are six alleles with frequencies higher than 10%, *b*, *c*, *f*, *o*, *x*, and *w*, a marker for the wheat-rye translocation 1AL/1RS (12%). The allele *g* is unique for the PBGI varieties (the Steppe group), and it is encountered in 14% of Steppe varieties. The group of Steppe varieties significantly differs from the group of Central Forest-Steppe varieties in frequencies of the majority of alleles at the *Gli-A1* locus (Table 2). A special feature of the Central Forest-Steppe and IA varieties is a rather high frequency of the allele *Gli-A6c* (26 and 12%, respectively), which encodes characteristic ω-gliadins. It is well known that this allele is closely linked to the allele *Gli-A1f* [15]. In the case of the varieties Vdiachna, Mironovskaya 25, and Mironovskaya 29, it is associated with the allele *Gli-A1b*, and in the variety Estet it is found in combination with the allele *Gli-A1x*. There are also some differences among the groups of varieties with respect to the minor locus *Gli-A3*. The alleles *a* and *b* are predominant ones with similar frequencies in the Steppe, Central Forest-Steppe, and ES groups, whereas the allele *b* prevails in the IA and PPI groups. A peculiarity of the IA group is a relatively high frequency of the allele *d* (13%), whereas the allele *c* was identified only among the Central Forest-Steppe varieties.

As for the locus *Gli-B1,* the allele *b* is predominant in all the groups of varieties. Its frequency is the lowest among the IA varieties, where other frequent alleles are *e* (27%) and *l,* a marker for the wheat-rye 1BL/1RS translocation (15%). In the Central Forest-Steppe group, the frequency of the allele *Gli-B1l* is 34%. The Steppe and Central Forest-Steppe samples significantly differ in frequencies of most of the alleles at the *Gli-B1* locus (Table 2). Among the Central Forest-Steppe varieties there is a group of genotypes in which the allele *Gli-B1h* is associated with the allele *Gli-B5b* (two specific ω-gliadins are expressed), but these varieties do not have red glumes: these are Tsyhanka and Poveliia, in addition to the previously identified varieties Monotyp, Estet, Harant, and Modus [38]. The old Kharkiv red-glumed variety Ferrugineum 1239 also carries the same combination of alleles *Gli-B1h* and *Gli-B5b* (Table S1).

At the *Gli-D1* locus, the group of Steppe varieties shows the highest value of Nei's genetic diversity index (0.728). The frequency of the allele *b* in the Central Forest-Steppe and IA varieties is twice as high as that in the other groups. The Steppe and Central Forest-Steppe samples significantly differ in the frequencies of the alleles *b, g,* and *j*. The allele provisionally designated *Gli-D1x* (GLD 1D10 [5]) is encountered only among IPBG varieties.

As for the locus *Glu-A1,* two alleles with the positive effect on bread-making quality, *a* and *b,* are found with high frequencies in the majority of groups of varieties. In the PPI group, the allele *a* predominates, whereas the group of IA varieties shows the high frequency of the allele *c*. The groups of Steppe and Central Forest-Steppe varieties significantly differ in the frequencies of the alleles *b* and *c* ($P < 0.01$).

The allele *c* is the most common allele at the *Glu-B1* locus in the group of Central Forest-Steppe varieties, whereas in the other groups of varieties another predominant allele is *b*. In 11% of Central Forest-Steppe varieties, the allele *d* was identified*:* these are chiefly the varieties with the 1AL/1RS translocation. A special feature of the Steppe group (IPBG varieties) is a relatively high frequency (11%) of the allele *al*, which is associated with extra-strong dough characteristics [57]. The samples of Steppe and Central Forest-Steppe varieties significantly ($P < 0.01$) differ in frequencies of the alleles *al, b, c,* and *d*.

The majority of groups of varieties show a high frequency (more than 90%) of the allele *Glu-D1d* associated with good bread-making quality. The group of IA varieties has the highest frequency of the allele *a* (48%) and shows the highest genetic diversity (0.572). The groups of Steppe and Central Forest-Steppe varieties significantly ($P < 0.01$) differ in frequencies of the alleles *a* and *d* (Table 2).

The value of Nei's genetic diversity index with respect to seven storage protein loci (*Gli-1, Glu-1,* and *Gli-A3*) is moderate for the groups of Steppe (0.469), Central Forest-Steppe (0.560), and IA (0.554) variet-

ies and somewhat lower for the PPI and ES groups (0.398 and 0.394, respectively).

### *Analysis of Frequencies of Alleles at Storage Protein Loci in Groups of Varieties Developed Within Two Periods of Time*

The frequencies of alleles at the storage protein loci were compared in groups of varieties developed prior to 1996 and since 1996 for two large samples, the Steppe and Central Forest-Steppe varieties (Table 3). In the Steppe group (IPBG varieties) developed after 1995, the frequencies of the alleles *Gli-A1g, Gli-D1g, Glu-B1al, Gli-A3a* ($P < 0.01$), and *Glu-A1b* ($P < 0.05$) significantly increased and the frequencies of the alleles *Gli-D1b, Glu-B1c,* and *Gli-A3b* decreased ($P < 0.01$). A special feature of the Steppe varieties of the second period is the formation of the nonrandom combination of the allele *Gli-A1g* and the extra-high quality allele *Glu-B1al*: it is encountered in 15 varieties developed after 1995. The frequency of genotypes with this two-locus combination is 0.172, which significantly differs from the expected one, 0,040 ($\chi^2 = 30.5$, $P < 0.01$). Such association is characteristic of the variety Odesskaya krasnokolosaya, which derived it from Lerma Roho-64 (http://wheatpedigree.net/sort/show/46264). On the basis of Odesskaya krasnokolosaya the first extra-strong Ukrainian varieties Panna and Leleka have been developed [57]. In addition, in the mentioned group of varieties, the combination *Gli-A1g* and *Glu-B1al* proved to be associated with the allele *Gli-D1g* in 80% of varieties, *Glu-A1b* in 80%, *Gli-B1b* in 87% and completely with *Gli-A3a* and *Glu-D1d*. Thus, this suggests the formation of a stable allele association above all due to breeding for high bread-making quality in the last 20 years.

A peculiarity of the group of Central Forest-Steppe varieties of the second period is the emergence of a number of varieties with the 1AL/1RS translocation of the Amigo type (16%) and the allele *Glu-B1d*. In the second period, the frequencies of the alleles *Gli-A1c* and *Glu-B1c* somewhat decreased ($P < 0.05$). Among varieties developed after 1995, a group of 12 varieties with the association *Gli-A1w* (1AL/1RS), *Glu-B1d,* and *Glu-D1a* stands out. The actual frequency of varieties with such a combination (0.129) significantly differs from the expected one, 0.004 ($\chi^2 = 364.9$, $P < 0.01$). It should be noted that the alleles *Glu-B1d* and *Glu-D1a* are associated with poor bread-making quality [13]. Evidently the main cause of formation of such stable association is the common origin of these varieties, which derived from the hybrid population involving TAM-107 via mutagenesis [58]. After 1995 the proportion of Central Forest-Steppe varieties with the 1BL/1RS translocation has not changed significantly and comprises 38%. In 94% of these 47 varieties, this translocation is linked to the allele *Glu-B1c*. On the whole, 87% of varieties with the 1BL/1RS translocation have HMW glutenin subunit alleles with the positive effect on bread-making quality (*Glu-A1a, Glu-A1b, Glu-D1d,* and *Glu-B1b*). Evidently, such combinations could compensate the negative effect of 1BL/1RS on this trait. Similarly, 36% of Argentinian common wheat varieties carry the 1BL/1RS translocation in combination with high-quality alleles at the *Glu-1* loci, moreover in nearly 20% of them the translocation is linked to the extra-high quality allele *Glu-B1al* [56].

Analysis of genetic distances between groups of varieties on the basis of frequencies at the storage protein loci revealed the high similarity of the ES group of varieties to the Steppe group (IPBG), in particular, somewhat closer to the group of Steppe varieties developed before 1996 (Table 4, Fig. 2). On the dendrogram, the group of varieties developed in IA, the breeding institution for the Polissya and in part for the Forest-Steppe zone of Ukraine, is positioned in the same cluster with the Central Forest-Steppe varieties (MIW, IPPG, and BTsES), and the PPI group is closer to the Steppe varieties developed before 1996. Thus, the use of storage protein alleles permitted differentiating groups of varieties in accordance to the zone of development (and consequently soil and climate conditions).

### *Analysis of Groups of Varieties with Respect to Markers of Resistance Genes and Their Associations*

Genotypes of Ukrainian winter common wheat varieties with respect to the markers for the moderate resistance *Lr34/Yr18/Pm38/Sr57/Bdv1* and *TDF_076_2D* genes as well as the *Tsn1* and *Tsc2* genes conferring toxin (in)sensitivity are given in Table S4 (http://cytgen.com/articles/512053s.pdf). Based on the results of studying varieties developed in different institutions and different climatic zones, frequencies of alleles of genes for resistance against necrotrophic and biotrophic fungal pathogens were determined (Table 5).

The frequencies of alleles at the marker loci significantly differ among the samples from different climatic zones (the Steppe and the Central Forest-Steppe, $P < 0.01$). For the sample of Central Forest Steppe varieties, the medium-effect association between alleles of *Tsn1* and *Tsc2* is observed ($\varphi = 0.37$, $P < 0.01$). The positive value of $\varphi$ indicates associations between alleles of the similar type (sensitivity or insensitivity to both the toxins A and B). The frequency of varieties with the insensitivity alleles of both the genes makes up 0.323, the frequency of those with the sensitivity alleles is 0.354. The substantial number of varieties with both the insensitivity alleles may testify to the selective pressure from the toxin A and B producing races of *P. tritici-repentis* during the breeding process. However, the approximately equal number of varieties with both the sensitivity alleles may be the evidence of some unidentified properties of functional (associated with sensitivity to the toxins) products of *Tsn1* and *Tsc2*. In particular, the product of the

**Table 3.** Frequencies of alleles at storage protein loci among winter common wheat varieties of the Steppe and the Central Forest-Steppe of Ukraine developed in different periods of time (1 – before 1996, 2 – since 1996)

| Locus, allele | Steppe | | Central Forest-Steppe | | Locus, allele | Steppe | | Central Forest-Steppe | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 (62) | 2 (87) | 1 (35) | 2 (93) | | 1 (62) | 2 (87) | 1 (35) | 2 (93) |
| | *Gli-A1* | | | | | *Glu-A1* | | | |
| *b* | 0.669 | 0.626 | 0.214 | 0.258 | *a* | 0.427 | 0.287 | 0.457 | 0.452 |
| *c* | 0.073 | 0.011 | 0.214 | 0.075* | *b* | 0.540 | 0.695* | 0.314 | 0.441 |
| *f* | 0.008 | 0.006 | 0.343 | 0.204 | *c* | 0.032 | 0.017 | 0.229 | 0.108 |
| *g* | 0.024 | 0.224** | | | | *Glu-B1* | | | |
| *m* | 0.056 | 0.006 | | | *al* | 0.016 | 0.178** | | |
| *o* | 0.169 | 0.098 | 0.171 | 0.177 | *a* | | | 0.029 | 0.032 |
| $w^A$ | | 0.023 | | 0.161* | *b* | 0.435 | 0.540 | 0.043 | 0.156 |
| *x* | | | 0.057 | 0.124 | *c* | 0.540 | 0.276** | 0.929 | 0.645** |
| *null?* | | 0.006 | | | *d* | 0.008 | 0.006 | | 0.151* |
| | *Gli-B1* | | | | *f* | | | | 0.011 |
| *b* | 0.855 | 0.730 | 0.586 | 0.473 | *i* | | | | 0.005 |
| *c* | 0.040 | 0.034 | | | | *Glu-D1* | | | |
| *d* | 0.048 | 0.098 | 0.086 | 0.022 | *a* | 0.032 | 0.011 | 0.043 | 0.156 |
| *e* | 0.032 | 0.121 | | 0.022 | *d* | 0.968 | 0.989 | 0.957 | 0.828 |
| *f* | | 0.006 | 0.057 | 0.032 | *e* | | | | 0.016 |
| *h* | | | | 0.065 | | *Gli-A3* | | | |
| $j^B$ | 0.016 | 0.011 | 0.243 | 0.382 | *a* | 0.328 | 0.592** | 0.500 | 0.359 |
| *x* | | | 0.029 | 0.005 | *b* | 0.672 | 0.368** | 0.383 | 0.371 |
| *b** | 0.008 | | | | *c* | | | 0.100 | 0.071 |
| | *Gli-D1* | | | | *d* | 0.000 | 0.017 | 0.017 | 0.024 |
| *b* | 0.355 | 0.126** | 0.543 | 0.710 | $nnn^A$ | 0.000 | 0.023 | | 0.176 |
| *f* | 0.097 | 0.069 | 0.129 | 0.065 | | *Gli-B5* | | | |
| *g* | 0.282 | 0.448** | 0.300 | 0.167 | *a* | 0.944 | 0.954 | 0.757 | 0.543* |
| *i* | | 0.011 | | 0.011 | *b* | 0.040 | 0.034 | | 0.0651 |
| *j* | 0.226 | 0.293 | 0.029 | 0.027 | $nnn^B$ | 0.016 | 0.011 | 0.243 | 0.392 |
| *l* | | | | 0.022 | | *Gli-A6* | | | |
| *x* | 0.040 | 0.052 | | | *a* | 0.992 | 0.971 | 0.600 | 0.634* |
| | | | | | *c* | 0.008 | 0.006 | 0.400 | 0.204* |
| | | | | | $nnn^A$ | | 0.023 | | 0.161 |

Differences in frequencies of alleles between groups of varieties developed in periods 1 and 2 are significant at * $P < 0.05$; ** $P < 0.01$. $^A$—1AL/1RS; $^B$—1BL/1RS. The number of varieties analyzed is given in parentheses.

**Table 4.** Pairwise genetic (Euclidean) distances between groups of Ukrainian varieties based on frequencies of alleles at storage protein loci

| Group of varieties | S (1) | S (2) | CFS (1) | CFS (2) | IA | PPI | ES |
|---|---|---|---|---|---|---|---|
| S (1) | 0.000 | 0.694 | 1.213 | 1.221 | 1.244 | 0.741 | 0.535 |
| S (2) | | 0.000 | 1.443 | 1.430 | 1.545 | 1.220 | 0.745 |
| CFS (1) | | | 0.000 | 0.722 | 1.198 | 1.221 | 1.209 |
| CFS (2) | | | | 0.000 | 1.084 | 1.327 | 1.324 |
| IA | | | | | 0.000 | 1.341 | 1.417 |
| PPI | | | | | | 0.000 | 0.748 |
| ES | | | | | | | 0.000 |

In parentheses: 1—before 1996; 2—since 1996.

*Tsn1* gene for the toxin A sensitivity is a protein similar to the family of R-proteins associated with the hypersensitive type of resistance against biotrophic pathogens [59]. The product of the *Tsc2* gene is arginase [35], which is also associated with resistance against biotrophic pathogens [60]. In the total sample of common wheat varieties developed in the Forest-Steppe Zone (the varieties of MIW, IPPG, and IA), the association between the alleles of *Tsn1* and *Tsc2* was also retained ($\varphi = 0.24$, $P < 0.05$). The frequency of the varieties with insensitivity alleles of both the genes makes up 0.289, the frequency of those with both alleles of sensitivity is 0.333. For the Forest-Steppe varieties, we also revealed the association between alleles of the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene (the allele *Lr34+*) and the *Tsn2* gene ($\varphi = -0.22$, $P < 0.05$): association between the absence of the allele *Lr34+* and presence of the allele *tsr* and vice versa. The frequency of varieties with the alleles *Lr34+* and *Tss* makes up 0.244, the proportion of varieties without the allele *Lr34+* and with *tsr* is 0.356. Such associations may testify to a negative role of the allele *Lr34+* conferring resistance to the biotrophic pathogens in breeding of varieties for resistance (insensitivity) to the necrotrophic pathogens in the Forest-Steppe climatic zone.

No association between alleles of the resistance genes was found for the Steppe varieties. For the total sample of Ukrainian varieties, the association between the alleles of the *Lr34/Yr18/Pm38/Sr57/Bdv1* (the allele *Lr34+*) and *TDF_076_2D* genes was revealed ($\varphi = -0.22$, $P < 0.01$): the association between the presence of the resistance allele *Lr34+* and the susceptibility allele *TDF-2* and vice versa. The frequency of varieties with the allele *Lr34+* and without the allele *TDF-1* (taking into consideration heterogeneous varieties) made up 0.260, the frequency of those without the allele *Lr34+* and with *TDF-1* was 0.350. These results suggest that the absence of the allele *Lr34+* was favorable to the selection of genotypes with the *TDF-1* allele irrespective of the climatic zone of development. Such correlations might be explained by peculiarities of expression of the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene. In particular according to [61], the expression of the allele *Lr34+* causes physiological changes in the cell similar to those during the hypersensitive resistance, but the cell death does not take place. The product of the *TDF_076_2D* gene possibly plays a key role in the regulation of cell defensive ways [34]. Therefore, it is possible that in the case of the presence of at least moderate natural infection background physiological changes happening in the cell as a result of the expression of the allele *Lr34+* negatively influence such regulation. And vice versa the regulatory features of the *TDF_076_2D* gene may adversely affect resistance conferred by the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene.

For the total sample of Ukrainian varieties, the association between the *Tsn1* and *Tsc2* genes was also revealed ($\varphi = 0.24$, $P < 0.01$). The frequency of varieties with the insensitivity alleles of both genes made up 0.483, the frequency of those with both alleles for sensitivity was 0.167. The data obtained may be the evidence of significant preference of selection of genotypes with resistance (insensitivity) to some races of *P. tritici-repentis* compared to those in which possible properties of the functional products of the *Tsn1* and *Tsc2* genes are displayed. We also observed a weak association between the *Lr34/Yr18/Pm38/Sr57/Bdv1* (the allele *Lr34–*) and *Tsn1* genes ($\varphi = -0.17$, $P < 0.01$). In particular, the presence of the alleles *Lr34–* and *Ts* was found out in 0.306 of the varieties and the absence of *Lr34–* and the presence of *tr* was detected in 0.244 of the varieties analyzed. That may testify to possible deeper mechanisms of regulation of interaction of plants with necrotrophic and biotrophic pathogens.

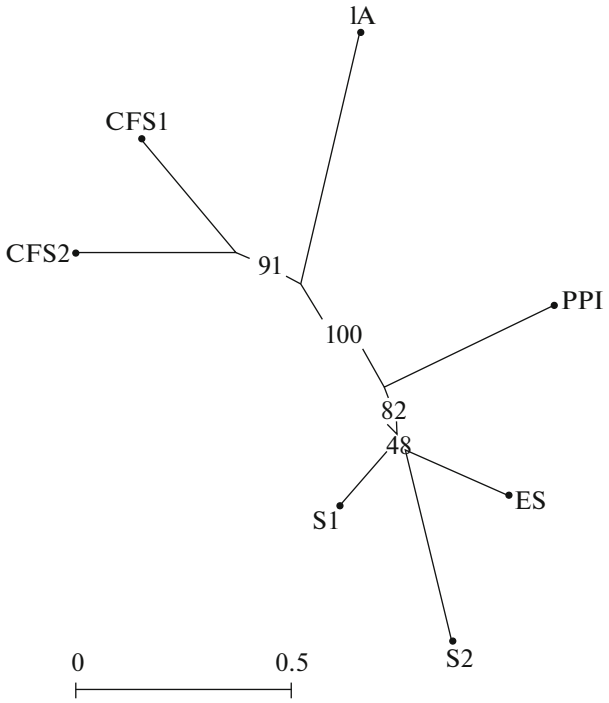


**Fig. 2.** Dendrogam of genetic similarity between groups of Ukrainian winter common wheat varieties on the basis of genotypes at storage protein loci constructed using the NJ method. S—Steppe (IPBG) varieties; CFS—Central Forest-Steppe varieties (MIW, IPPG, and BTsES); IA—IA varieties, PPI—PPI varieties; ES—varieties of other breeding institutions of the South and the East of Ukraine; 1—varieties developed before 1996, 2—since 1996.

### *Analysis of Associations of Alleles at Storage Protein Loci and Disease Resistance Genes*

In the group of Central Forest-Steppe varieties, we revealed a tendency to closer association of the allele *Gli-B1l* (the 1BL/1RS translocation) with the resistance gene *Lr34/Yr18/Pm38/Sr57/Bdv1* (the allele *Lr34+*) ($P < 0.05$) and the allele *Gli-B1b* with *Lr34–* ($P < 0.05$) (Table 6), taking into account heteroge-

**Table 5.** Frequencies of alleles of some disease resistance genes in groups* of Ukrainian winter common wheat varieties

| Locus, allele | S (91) | CFS (65) | IA (25) | Total sample (181) |
|---|---|---|---|---|
| *Lr34/Yr18/Pm38/Sr57/Bdv1* | | | | |
| *Lr34+* | 0.578 | 0.200 | 0.480 | 0.428 |
| *Lr34–* | 0.422 | 0.800 | 0.520 | 0.572 |
| *Tsn1* | | | | |
| *tr* | 0.711 | 0.431 | 0.600 | 0.594 |
| *Ts* | 0.289 | 0.569 | 0.400 | 0.406 |
| *Tsc2* | | | | |
| *tsr* | 0.956 | 0.538 | 0.320 | 0.717 |
| *Tss* | 0.044 | 0.462 | 0.680 | 0.283 |
| *TDF_076_2D* | | | | |
| *TDF-1* | 0.478 | 0.862 | 0.520 | 0.622 |
| *TDF-2* | 0.522 | 0.138 | 0.480 | 0.378 |

The number of varieties analyzed is given in parentheses. S—varieties of the Steppe zone (PBGI); CFS—varieties of the Central Forest-Steppe (MIW, IPPG, and BTsES); IA—varieties of IA.

**Table 6.** Some statistically significant associations between alleles of resistance genes and alleles at storage protein loci in Ukrainian winter common wheat varieties

| Group of varieties | Allele combination | φ |
|---|---|---|
| CFS | *Lr34+/Gli-B1l* | 0.28* |
| CFS | *Lr34-/Gli-B1b* | 0.27* |
| CFS | *tr/Gli-A3a* | –0.27* |
| CFS | *tr/Gli-A3b* | 0.29* |
| CFS | *TDF-1/Gli-A3a* | –0.3* |
| CFS | *TDF-1/Gli-A3b* | 0.27* |
| S | *TDF-1/Gli-A3a* | –0.27* |
| S | *TDF-1/Gli-A3b* | 0.28** |
| S | *TDF-1/Gli-D1j* | –0.24* |
| S | *TDF-1/Gli-D1b* | 0.21* |
| S + CFS + IA | *TDF-1/Gli-A3a* | –0.27*** |
| S + CFS + IA | *TDF-1/Gli-A3b* | 0.23** |
| S + CFS + IA | *TDF-1/Gli-D1j* | –0.26*** |
| S + CFS + IA | *TDF-1/Gli-D1b* | 0.23** |

S—Steppe varieties (PBGI); CFS—Central Forest-Steppe varieties (MIW, IPPG, and BTsES); IA—IA varieties. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

neous varieties. In this group of varieties, the allele for insensitivity to the toxin A (*tr*) occurs more frequently in combination with the allele *Gli-A3b* than with *Gli-A3a* ($P < 0.05$). In the groups of Steppe and Central Forest-Steppe varieties, the presence of the allele *Gli-A3b* proved to be associated with the presence of the allele *TDF-1,* which confers moderate resistance to *Fusarium* head blight, in contrast to the alternative allele, *Gli-A3a*. The allele *TDF-1* is also encountered more frequently in combination with the allele *Gli-D1b* and more rarely with *Gli-D1j* ($P < 0.05$). Such significantly increased frequencies of combinations of genes located on different chromosomes may be explained by their selective advantage. In the case of the *Gli-A3* and *TDF_076_2D* loci, the combinations of *Gli-A3b* with *TDF-1,* as well as *Gli-A3a* with *TDF-2*, turned out to be favorable in both of the soil-climatic zones. Statistically significant associations of the allele *TDF-1* with the above-mentioned alleles at the *Gli-D1* and *Gli-A3* are also retained in the total sample of Steppe, Central Forest-Steppe, and IA varieties ($P < 0.01$, $P < 0.001$, Table 6), which may indicate that they are nonrandom. It is interesting to note that two QTLs associated with *Fusarium* head blight resistance have been mapped on chromosome arm 1AS, one of which is marked by microsatellite locus *gwm1097* [62]. Judging from its position on the map, it is possible that *gwm1097* is located between the centromere and *Gli-A1* in the same region as the locus *Gli-A3*. Conceivably *Gli-A3* might be linked to this QTL, which calls for further investigation. Thus, in the sample of Ukrainian varieties, the allele *TDF-1* for moderate resistance to *Fusarium* head blight proved to be associated with the alleles *Gli-A3b* and *Gli-D1b*. The frequency of this association (0.249) significantly exceeds the expected frequency (0.138) ($\chi^2 = 18.8$, $P < 0.01$).

In summary, as a result of investigation of the collection of Ukrainian winter common wheat varieties, we revealed differences in frequencies of alleles at the storage protein loci *Gli-A1, Gli-B1, Gli-D1, Glu-A1, Glu-B1, Glu-D1, Gli-A3,* and *Gli-A6* as well as some important disease resistance genes, *Lr34/Yr18/Pm38/Sr57/Bdv1, Tsn1, Tsc2,* and *TDF_076_2D,* between groups of varieties developed in different breeding centers. The comparison of groups of varieties developed in different periods of time demonstrates that basically the same predominant storage protein alleles detected in previous studies [8, 37, 38] have been retained. At the same time, formation of new associations of storage protein alleles has been revealed. Among the Steppe varieties of the last two decades, this is the combination *Gli-A1g* and *Glu-B1al*, whereas the most important peculiarity of the Central Forest-Steppe group of this period is the development of a number of varieties with the wheat-rye 1AL/1RS translocation in combination with the allele *Glu-B1d*, which virtually was not encountered previously among Ukrainian varieties. Some nonrandom associations of disease resistance genes and storage protein alleles have also been revealed. Of interest is the association of the allele *TDF-1* for moderate resistance to *Fusarium* head blight and the gliadin alleles *Gli-A3b* and *Gli-D1b*, which may be of adaptive value. This is especially the case with the allele *Gli-A3b* since this association was detected in groups of varieties from two zones with different soil and climatic conditions.

The studying of the history of breeding, from our viewpoint, permits investigating adaptation of genotypes selected by breeders to several factors. The investigation of varieties grouped by place of development most probably enables studying adaptation of genotypes to soil and climatic conditions, whereas differences in allele frequencies between groups of varieties developed in different periods of time are influenced by climate changes as well as changes in growing technology, crop area structure, and procedures of variety field trials. Hence the revealed regularities may be of importance for accelerating the breeding process as, based on the most frequent combinations of alleles at marker loci, one could select genotypes that are the most adapted to a certain cultivation zone.

## CONCLUSIONS

The investigation of collections of Ukrainian winter common wheat varieties with respect to storage protein loci and a number of disease resistance genes provides support for the concept of formation of stable gene associations in the breeding process [36]: despite the involvement of a large amount of initial material from different countries, genotypes with alleles peculiar to a certain soil and climatic zone are selected in the breeding process and these sets of alleles are retained in varieties of a certain zone over a long period of time. In particular, the storage protein alleles characteristic of the variety Bezostaya 1 remain among predominant alleles of Ukrainian winter common wheat varieties. At the same time, the study confirmed the role of leader varieties and lines on the level of initial breeding material, which are involved in development of a group of varieties with new characteristics. Odesskaya krasnokolosaya turned out to be an initial leader form for the Steppe zone. It transmitted its association of high bread-making quality genes from Lerma Rojo-64 to the group of PBGI varieties of the last two decades. The material from crosses with TAM-107 carrying the 1AL/1RS translocation has played a similar part as initial leader material for Central Forest-Steppe group, in which the proportion of varieties with this translocation has increased from 0 to 16% in the last 20 years. In certain groups of Ukrainian varieties, nonrandom associations between disease resistance genes as well as disease resistance genes and storage protein alleles have been revealed.

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