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Global durum wheat diversity: structure and origin revealed by means of the gliadin markers

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Abstract. Genetic diversity for the alleles of gliadin-coding loci was studied within 563 durum wheat cultivars from 42 countries and in 98 Bulgarian durum wheat landraces. In total 116 alleles for 4 gliadin-coding loci were identified. The highest genetic diversity was revealed for durum wheat cultivars having origin from Middle East, Trans Caucasia, the Pyrenean Peninsula, and the Balkans countries. Ward's clustering analysis based on gliadin-calculated Euclidian distances among cultivars from different countries divided the collection on three separate groups. The groups significantly differed in the gliadin alleles frequencies. Two groups were proposed be formed by ancient genetic branches of durum wheat. A "Southern" branch included mostly durum wheats from the Mediterranean region, the Middle East, and Transcaucasia. A "Northern" branch included Russian and Ukrainian cultivars as well as cultivars bred on their basis in North America, China and some other countries. An additional group included durum wheat cultivars that had been bred in several past decades on the basis of the material of the International Agricultural Research Institutes, viz.,CIMMYT and ICARDA. This group displayed low genetic diversity.
The results made it possible to emphasize the factors forming present global durum wheat genetic diversity: climatic conditions and historical factors in the areas of cultivation on one hand and an international breeding trend on the other hand.

Keywords. Gliadin loci – Genetic diversity – Geno-geography – Durum wheat.

Diversité du blé dur à l'échelle mondiale : structure et origine révélées à l'aide des marqueurs de la gliadine

Résumé. *La diversité génétique des allèles des loci codant pour la gliadine a été étudiée chez 563 cultivars de blé dur provenant de 42 pays et 98 variétés locales bulgares de blé dur. Au total, 116 allèles pour 4 loci codant pour la gliadine ont été identifiés. Les cultivars de blé dur originaires du Moyen-Orient, de la Transcaucasie, de la péninsule des Pyrénées et des Balkans ont montré la diversité génétique la plus importante. L'analyse de regroupement selon la méthode de Ward, basée sur les distances euclidiennes calculées pour les gliadines au niveau des cultivars de différents pays, a permis de répartir la collection en trois groupes distincts. Les groupes différaient significativement pour la fréquence des allèles de la gliadine. On a avancé l'hypothèse que deux groupes étaient formés par les anciennes branches génétiques du blé dur. Une branche du "Sud" incluait la plupart des blés durs de la région méditerranéenne, du Moyen-Orient, et de la Transcaucasie. Une branche du "Nord" incluait des cultivaras russes et ukrainiens ainsi que des cultivars sélectionnés sur leur base en Amérique du Nord, en Chine et dans d'autres pays. Un groupe supplémentaire incluait des cultivars de blé dur qui ont été sélectionnés au cours de plusieurs décennies, en s'appuyant sur le matériel des Instituts internationaux de recherche agricole CIMMYT et ICARDA. Ce dernier groupe se caractérisait par une faible diversité génétique.*
Les résultats ont permis de faire ressortir les déterminants de la diversité génétique actuelle du blé dur à l'échelle mondiale : des conditions climatiques et des facteurs historiques dans les zones de culture, d'une part, et une tendance à la sélection internationale, d'autre part.

Mots-clés. *Loci de la gliadine – Diversité génétique – Géno-géographie – Blé dur.*

I – Introduction

The application of polymorphic DNA markers in biodiversity and phylogenetic studies in plants has become a routine research procedure now (Agarwal *et al*., 2008). However, in addition to

DNA based methods some polymorphic proteins could be successfully used. Such markers are probably less modern and advanced genetic tools than DNA, but also very informative and useful. For more than 30 years the Plant Genetics Department of the Vavilov Institute of General Genetics Russian Of the Academy Of Sciences has been studying polymorphism of gliadins – the wheat seed storage proteins. These proteins are very readily available and cheap to extract as markers, and allow the exploration of genetic differentiation among wheat cultivars as well as internal genetic structure of each cultivar involved in the analyses (Novoselskaya-Dragovich *et al.*, 2011). The genetics of gliadins is well studied and documented. Being separated by acid polyacrylamide gel (PAG) electrophoresis, gliadins form an electrophoretic spectrum containing about 40 distinct bands All these protein bands are coded by individual gliadin-coding genes in clusters and located on short arms of the chromosomes of the 1st and 6th homeological groups (Wrigley and Shepherd, 1973). Tetraploid durum wheat has 4 loci of gliadin-coding genes – located on the chromosomes 1A, 1B (loci *Gli-1*) and 6A, 6B (loci *Gli-2*) (Joppa *et al*., 1983). Each locus usually contains more than one gliadin-coding gene and controls more than one band in the typical electrophoretic spectrum. The genes gathered in one locus are closely linked genetically and are even often separated by retrotranspason elements (Gu *et al*., 2004). There is no recombination in the gliadin-coding locus. Consequently, the gliadin proteins controlled by such gene clusters are inherited together as a single Mendelian trait (Metakovsky *et al*., 1984). Such group of proteins were named as blocks of gliadin components and it is possible to discriminate all four blocks which form total electrophoretic spectrum of durum wheat gliadins (Kudryavtsev, 1994). Due to the polyallelism in gliadin-coding loci, allelic variants of blocks of gliadin components differ in mobility, staining intensity, and amount of their components (Metakovsky *et al*., 1984). That is the reason why different wheat cultivars display distinct gliadin spectra – almost each cultivar has its own, unique electrophoretic spectrum of gliadin.

In this study we applied gliadin markers to study a global collection of modern breeding cultivars of durum wheat.

II – Material and methods

To estimate the global diversity of durum wheat *Triticum durum* Desf., we examined 563 cultivars, which were developed mostly from the 1940s to the 1990s, and 28 landraces from 45 countries. Grains were obtained from the collection of the N.I. Vavilov All Russian Institute of Plant Industry and from our colleagues from different countries and organizations. Eight to one hundred grains were examined for each accession. Gliadin was extracted with 70% ethanol; polyacrylamide gel electrophoresis was carried out by a standard method (Metakovsky and Novoselskaya, 1991).

The allelic variants of gliadin component blocks were identified and designated according to available catalogs and an accepted system of allele designation (Kudryavtsev, 1994; Kudryavtsev *et al*, 1996; Melnikova *et al*., 2012). Genetic diversity for the loci of gliadin-coding genes was estimated according to Nei (Nei, 1973) as $H = 1 - \Sigma pi$ where H is Nei’s index of genetic diversity (per locus) and pi is the allele frequency for the locus. Statistical analysis was performed using the Statistica (StatSoft) software package. To estimate the genetic similarity for groups of accessions from different regions and countries, we computed the Euclidean distances on the basis of allele frequencies and performed a clustering according to Ward (Ward, 1963).

III – Results and Discussion

In total 119 alleles for 4 gliadin-coding loci of durum wheat were identified. Most of these alleles have low occurrence and only 15 ones were relatively frequent (more than 5%). The most common alleles for four loci were: *c* (frequency 0.42), *g* (0.17), and *b* of the *Gli-A1*^{d} locus; *c* (0.49), *a* (0.30), and *b* (0.12) of the *Gli-B1*^{d} locus; *a* (0.21), *g* (0.21), *b* (0.18), and *o* (0.14) of the *Gli-A2*^{d}

locus; and *h* (0.45)and *a* (0.25) of the *Gli-B2*^{d} locus. At the same time, it was found that allele frequencies vary significantly in material from different countries. For example the allele *Gli-A1g* has frequency 0.17 in whole world collection. In Italian durum wheats it is rather rare allele with the frequency 0,07 but in Russian germplasm it is predominant allele having frequency 0,6 in old breeding varieties and 1.0 in modern ones (Melnikova and Kudryavtsev, 2009).

The genetic distances between cultivars from different countries were calculated using routine statistical procedure: On the basis of the allele frequencies we computed the Euclidean distances between the sets of national cultivars and performed clustering according to Ward. Three distinct clusters of durum wheat cultivars were isolated (fig.1).The first cluster join durum wheat accessions from Australia, Spain, Tunisia, Azerbaijan, Portugal, Turkey, the Republic of South Africa, Israel, Moldova, Bulgaria, India, Uzbekistan, Egypt, Cyprus, Syria, Bolivia, Peru, Armenia, Iraq, Iran, Hungary, Romania, and Georgia. The second cluster included the accessions of Austria, the United States, Canada, Kazakhstan, China, Russia, Ukraine, Germany, Poland and Yugoslavia. The third cluster included the accessions of Algeria, Morocco, Italy, Argentina, Chili, San Marino, Ethiopia, Greece, Jordan, Mexico, France, and Kenya.

The clusters (or groups) significantly differed in allele frequencies. In the case of the *Gli-A1* locus, the most common alleles were *b* (frequency 0.25) and *c* (0.25) in group I, *g* (0.45) in group II, and *c* (0.85) in group III. In the case of the *Gli-B1* locus, higher frequencies were observed for allele *c* (0.45) in group I, *a* (0.68) in group II, and *c* (0.75) in group III. In the case of the *Gli-A2* locus, the most common was allele *g* (0.31) in group I, allele *a* (0.45) in group II, and allele *b* (0.32) in group III. In the case of the *Gli-B2* locus, higher frequencies were characteristic of allele *h* in groups I and III (0.44 and 0.65, respectively) and allele *a* (0.58) in group II.

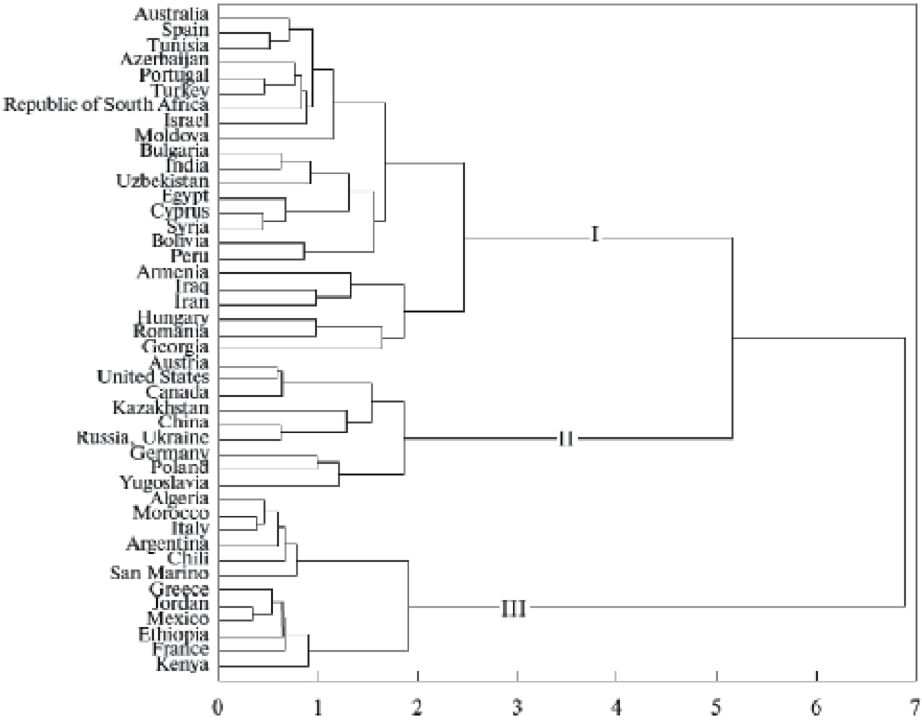

Figure 1. Clustering of the samples of durum wheat accessions from different countries by alleles of the gliadin-coding loci.

The Nei heterogeneity indexes (*H)* were calculated for each country and for each group (fig.2) On this diagram the countries are arranged according to the diversity level and shaded according to their group attribution. In general, genetic diversity was higher (0.64 on average) in the accessions of group I and lower (0.42 on average) in the accessions of group III. The genetic diversity indexes greatly varied in the cultivars of group II, averaging 0.5.

It is well known that the genetic diversity of cultivated plants usually is higher in the historical centers of origin or secondary diversification (Vavilov and Dorofeev, 1992). So looking on this graph we can suppose that there were at least two historical centers of durum wheat diversification: the first one is in the Mediterranean region, and the second one is in Russian and Ukrainian steppes. In a separate study, we demonstrated that this steppe ecotype of durum wheat could be brought from South Russia or Volga river region into the territory of ex-Yugoslavia by ethnic Bulgarians who migrated into Balkans after the Great Bulgaria disintegration about one thousand years ago and then evolved there independently (Melnikova *et al*., 2010). We know also that durum wheat of Canada, China and USA were bred not more than one hundred years ago based on Russian durum wheat germplasm. This is an explanation why the genetic diversity is wide in Russia, Ukraine and ex-Yugoslavia and relatively narrow in other countries of the second group. As to the third group of cultivars, it seems that we are dealing with the most advanced breeding cultivars which substituted completely the local durum wheat germplasm of these countries (if such landraces existed before). At least, this can be surely affirmed concerning Ethiopian and Italian durum wheats. We have studied old landraces of these countries and found cardinal genetic differences between new and old cultivars (un published results). Probably the genetic diversity in this group was formed also as a result of breeding activity of the International Centers like CYMMIT and ICARDA.

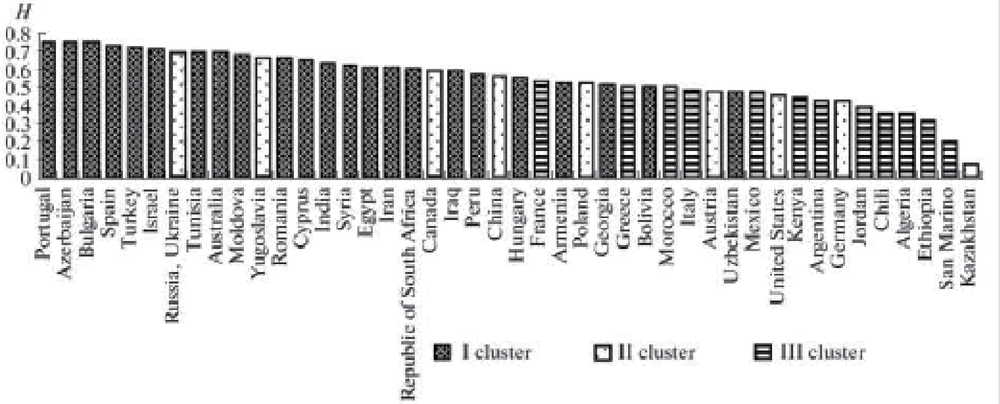

Figure 2. Genetic diversity *H* for the accessions from different countries. The clusters are indicated by column filling.

All these results and our observations bring up again the question of genetic erosion in modern durum wheats. In general, the genetic erosion has two aspects of manifestation. The first one is the loss of genetic heterogeneity and the second one is the loss of specific, local alleles due to their replacement by foreign ones. For the countries of the third cluster it is clear that here we deal with both aspects of the erosion - with complete change of local alleles on new ones and with the decreasing of the heterogeneity level. For the countries of first cluster it seems that genetic heterogeneity has not decreased during breeding process, however in this case we can observe certain signs of genetic erosion defined by the allele loss or exchange. The same situation is for Russian durum wheat (second group). The most clear erosion was shown earlier for *Gli-A1* locus. The Russian landraces displayed wide allele diversity for this locus, but now we have only one allele *g* (Melnikova and Kudryavtsev, 2009).

At least one important practical conclusion could be deduced from these results: Evidently durum wheat cultivars form two evolutionary old groups (the first and the second ones) that are adapted to different climatic conditions and represent two different ecotypes of the species. Therefore, the national agricultural research centers should consider this fact and develop breeding strategies based on use of proper donor genotypes which genetically belong to the group appropriate for local climatic conditions. As to the third group, cultivars it could be supposed that they belong to the same agroecological type as the first group of cultivars but differ from them with some technological characteristics. In this case we deal with clear trend of globalization in durum wheat breeding which is stimulated by practical needs but result in genetic erosion.

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