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DELLA MUTATIONS IN PLANTS WITH SPECIAL EMPHASIS ON WHEAT

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DELLA proteins are a subfamily of transcriptional regulators from the GRAS protein family. They act as growth repressors. Gibberellins (GA) promote growth by overcoming the DELLA-mediated growth restraint. A number of dominant mutations in highly conserved orthologous DELLA genes are known in various plant species. These mutations reduce plant height owing to production of more active forms of growth repressors. The aim of the current study is to estimate the genetic similarity of DELLA nucleotide and amino acid sequences stored in GenBank and to identify mutant and wild type alleles that encode DELLAs in modern Ukrainian winter bread wheat varieties. We have performed nucleotide and amino acid sequence search and alignment of the *Rht-D1* wheat dwarfing gene and DELLA-encoding genes of other plant species with BLAST and MUSCLE software. A dendrogram illustrating phylogenetic relations among DELLA sequences of 35 plant species has been constructed using MEGA v5. In addition, PCR-based genotyping for the major wheat dwarfing genes *Rht-B1* and *Rht-D1* has been performed in wheat cultivars from different regions of Ukraine. It is shown that the mutant *Rht-D1b* allele is the commonest dwarfing allele among those tested in Ukrainian wheat cultivars (68 %), but its distribution over Ukraine is not random.

Key words: DELLA, wheat, dwarfing genes, phylogenetic relations, PCR genotyping.

Introduction

The improvement of plants by introduction of new agriculturally important genes facilitates the enhancement of productive potential. The introduction of the dwarfing genes *Rht-B1b* and *Rht-D1b* into the wheat genome reduced the plant height and resulted in 2-3 fold increase of yield in grain crops, becoming a basis for the so called «green revolution» (Borojevich, Borojevich, 2005; Bonnet *et al*., 2010; Pearce *et al*., 2011). The importance of the dwarfing genes displays the necessity of detailed understanding of the processes followed by changes in the habitus of plants after introduction of dwarfing genes.

The *Rht-B1* and *Rht-D1* genes in wheat encode DELLA-proteins (Hirsh, Oldroyd, 2009). In Figure 1, we summarize the information about structure of DELLAs from the literature. DELLA proteins consist of functional GRAS and regulatory DELLA-domain at the less conservative N-terminus of the protein (Fig. 1), that may function as a receptor for upstream GA signals (Ashikari *et al*., 2003). DELLAs N-termini show high homology to each other between 34 and 84 % similarity (Bolle, 2004). Mutations in these genes produce amino acid substitutions, deletions or insertions in the N-terminal region of the translated DELLA protein (in wheat, *Rht-B1b* and *Rht-D1b* alleles encode proteins that have deletion between DELLA and TVHYNP motifs in LExLE region). These mutations affect binding to GA-receptor and GA allowing accumulation of mutant DELLAs and repress growth.

In the nucleus, DELLA of wild type binds to GA-receptor (e.g. GID1 known for Arabidopsis), GA and SCF E3 ubiquitin ligase complex. Such a large complex is recognized by 26S proteasome and destroyed. The disappearance of DELLA proteins stimulates GA responsive processes such as seed germination, stem and root elongation, and fertility (Hirsh, Oldroyd, 2009).

In the absence of GA, or in the case of mutation in nucleotide sequence of DELLA domen, the ubiquitination becomes impossible. Accumulation of the mutant DELLA proteins cause continuous

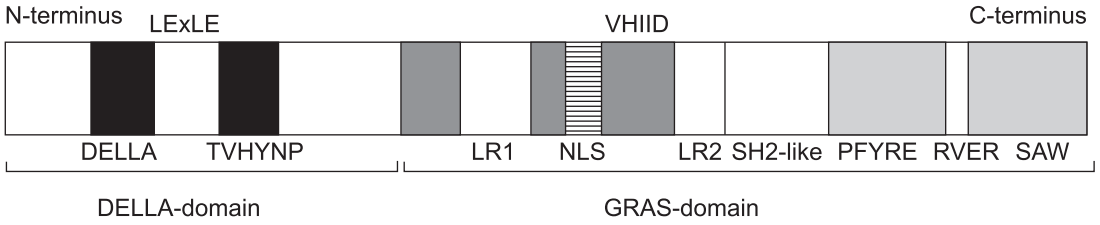

Fig. 1. Summarized scheme of DELLA protein structure according to Peng *et al*. (1997), Peng *et al*. (1999), Olszewski *et al*. (2002), Bolle (2004), and Pearce *et al*. (2011). Conserved N-terminal regulatory region consists of DELLA, LexLe and TVHYNP amino acid motifs. In the C-terminus, functional domains are indicated (LR1, LR2 – leucine rich regions; NLS – nuclear localization signals; SH2 – (Src Homology2)-like domains; VHIID, PFYRE, RVER and SAW – amino acid motifs).

growth inhibition and, accordingly, leads to agronomically advantageous dwarfed plant height and improved straw strength by inhibition of stem cell elongation (Dalrymple, 1986; Flintham *et al*., 1997; Peng *et al*., 1999).

As has been shown for barley embrio (Gubler *et al*., 2002; http://plantcellbiology.masters.grkraj.org), DELLAs repress transcription and processing of *GAMYB* gene (GA induced Amylase-beta), that is transcriptional regulator of α-amylase gene regulatory elements called *GARE* (GA response elements) and induce amylase gene expression (Fig. 2).

DELLAs inhibit growth by interfering with the activity of growth-promoting transcription factors (Harberd *et al*., 2009). Mutants of wheat, barley, and rice, that are affected in GA signaling, display an altered aleurone α-amylase response. For example, dominant mutations at the homeoallelic wheat *Rht-B1a* and *Rht-D1a* loci confer dwarfism and a reduced growth response to GA (Börner *et al*., 1996; Peng *et al*., 1999). Severely dwarfing alleles, such as *Rht-B1c*, abolish the GA response of mutant aleurone cells (Gale, Marshall, 1975; Ho *et al*., 1981; Börner *et al*., 1996).

DELLAs are conservative due to the essential role in plant cell. They help to establish GA homeostasis by direct feedback regulation on the expression of GA biosynthetic and GA receptor

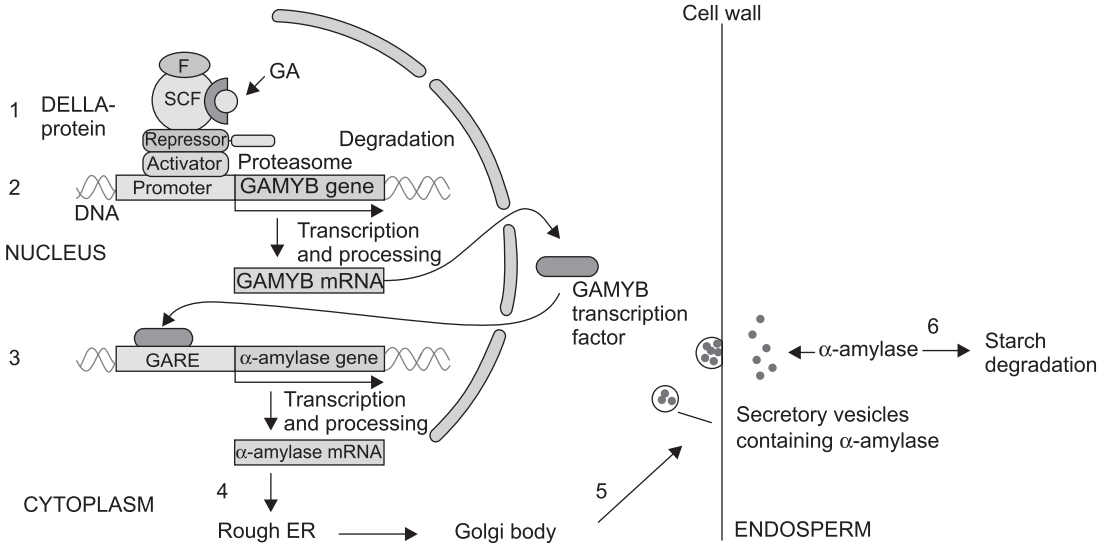

Fig. 2. Model of regulation of amylase biosynthesis by DELLA-protein (http://n0b3l1a.blogspot.com/2010/03/gibberellins-cytokinins.html) with modifications.
1 – DELLA-SCF E3 ubiquitin ligase complex degradation starts after binding with GA; 2 – the promoter of *GAMYB* gene becomes active and GAMYB transcription factor is synthesized; 3 – GAMYB transcription factor activates α-amylase gene; 4 – α-amylase and other hydrolytic enzymes are synthesized in rough endoplasmic reticulum and secreted by the Golgi body (5). Secretory vesicles go through the cell wall, and α-amylase starts the starch degradation in endosperm.

genes, and promote the expression of downstream negative components that are putative transcription factors/regulators or ubiquitin E2/E3 enzymes (Zentella *et al*., 2007). In addition, one of the putative DELLA targets, *XERICO*, promotes accumulation of abscisic acid (ABA) that antagonizes GA effects. Therefore, DELLA may restrict GA-promoted processes by modulating both GA and ABA pathways (Zentella *et al*., 2007).

DELLA-related proteins were identified in a lycophyte (*Selaginella kraussiana*) and a bryophyte (*Physcomitrella patens*) species – ancient plant groups first appeared around 400 and 430 million years ago, respectively (Yasumura, Harberd, 2006).

The aim of the current study was to estimate the genetic similarity of the DELLAs nucleotide and amino acid sequences lodged in GenBank and to detect mutant and wild type alleles that encode DELLA-proteins in Ukrainian modern winter bread wheat varieties.

Materials and Methods

Amino-acid and nucleotide sequences data of DELLAs deposited in GenBank (NCBI database available at http://www.ncbi.nlm.nih.gov/) were used in genetic similarity analysis. In molecular analysis, winter bread wheat varieties created after 1990th in different breeding centers of Ukraine were exploited. We divided all varieties that have been involved in our investigation into three groups according to geographical position of the breeding centers. Thus, 79 varieties from Plant Breeding and Genetics Institute (Odessa), and Institute of Agriculture of Southern Region (Kherson) were assigned to the southern part; 21 varieties from Poltava State Agrarian Academy, Institute of Plant Production, Donetsk Institute of Agroindustrial production, Lugansk Institute of Agroindustrial production and Dnepropetrovsk State Agrarian University – to the eastern part; 14 varieties from the V.N. Remeslo Institute of Wheat in Mironovka, Institute of Plant Physiology and Genetics, Institute of sugar beet NAAS, and Belaya Tserkov experiment breeding station – to the central part of Ukraine.

The BLAST tool (Altschul *et al*., 1997) was used for finding homologous sequences. Multiple protein sequence alignment was done with MUSCLE software (MUSCLE tool online at ttp://www.ebi.ac.uk). Phylogenetic and molecular evolutionary analyses of protein sequences were performed using MEGA v5 program (Tamura *et al*., 2011) the UPGMA algorithm.

Total genomic DNA was extracted from seedlings using the modified CTAB method (Verbitskaya, 1998). The *Rht-B1b* and *Rht-D1b* alleles were detected as recommended by Ellis *et al*. (2002). The PCR-products were separated in 7 % PAAG. Visualization of amplification fragment was done according to Promega Technical Manual (1999).

Results and Discussion

Results of Blast and phylogenetic analysis

Based on the data that all DELLAs have similar protein structure features we chose the sequence of wheat DELLA-protein encoded by the *Rht-D1* dwarfing allele and BLASTed it against the NCBI database to compare genetic similarity level of DELLA sequences among different species. We found highly identical sequences among 35 species from different divisions: Bryophyta, Lycopodiophyta and Magnoliophyta (or Angiospermae). Among angiosperm, DELLAs were presented in monocots and dicots such as pea, wheat, rice, maize, cultural soybeans, lettuce, snapdragons, tarragon, cabbage etc. and several species of apple, *Arabidopsis*, and cotton families. These data suggest that the function of GA-signalling repression, in which DELLA takes a special role, is highly conserved within monocots and dicots (Peng *et al*., 1999; Boss, Thomas, 2002; Sun, Gubler, 2004; Muangprom *et al*., 2005). We found several sequences of grape, sorghum, rice and *Arabidopsis* proteins, predicted DELLAs, highly identical (93–100 %) to wheat DELLA-proteins.

We used MUSCLE tools to align these sequences and then MEGA v5 to estimate phylogenetic and molecular evolutionary relationship among DELLAs of different species (Fig. 3). On the dendrogram, DELLAs are divided into two clusters, one of which is formed by bryophytes and lycophytes, the most ancient species, and the second one contains flowering plants (Fig. 3). These data also have been confirmed by analysis of nucleotide sequences that encode DELLA pro-

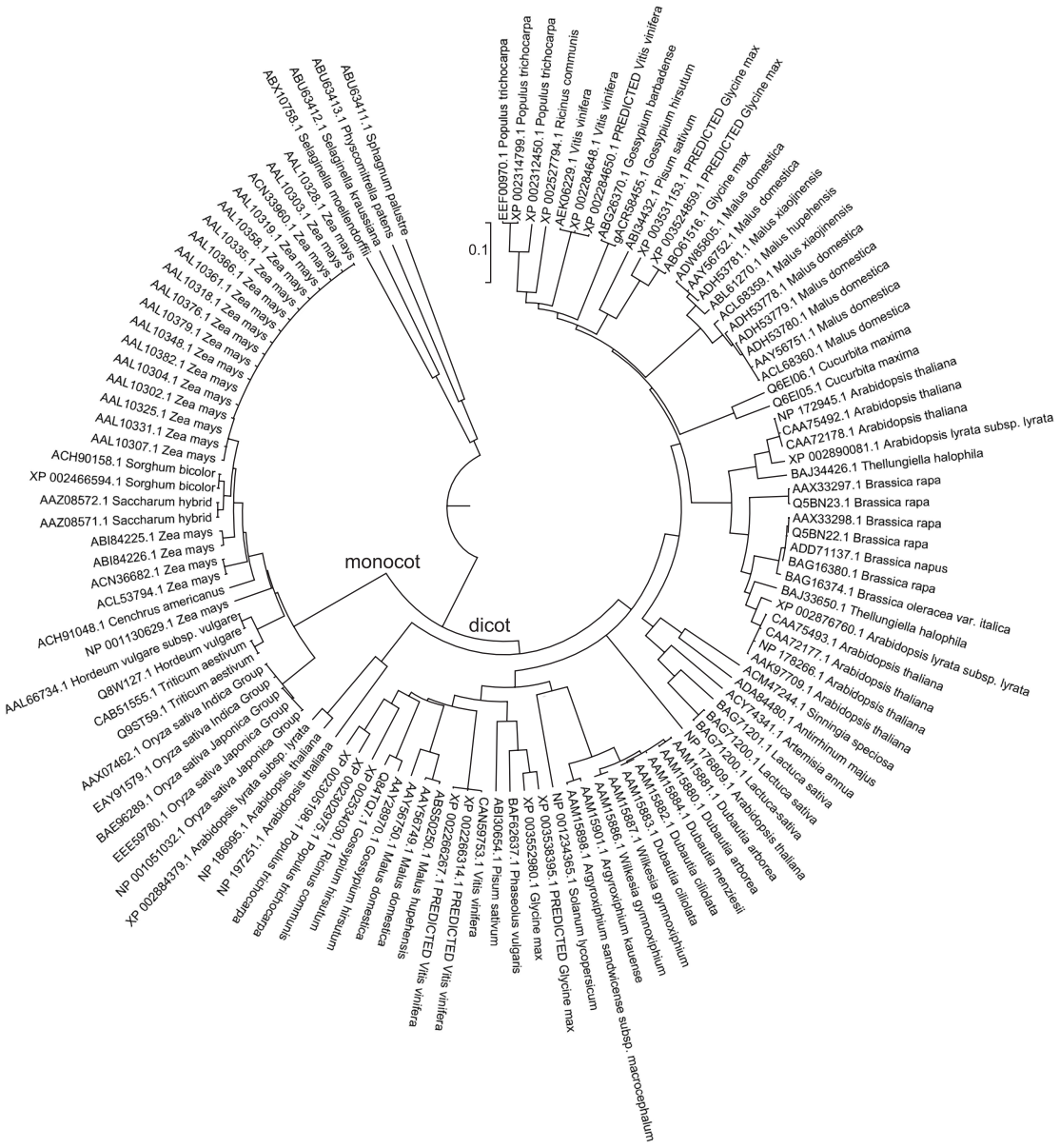

Fig. 3. Dendrogram of sequences of DELLA-proteins in plant species according to the Mega v5.

teins of these species. Angiosperm cluster consists of monocot and dicot subclusters. The cluster of monocots includes a total of 37 sequences and is divided into several subclasses (Fig. 3). The first subclass was formed by DELLAs from two accessions of *Orysa sativa indica* and three ones of *O. sativa japonica*, the second subclass is represented by *Hordeum vulgare* SLN1 protein and *Triticum aestivum* DELLA-protein Rht1, the third subclass consists of *Zea mays* d8 (dwarf 8) protein and proteins of *Saccharum hybrid*, *Sorghum bicolor*, *Cenchrus americanus*.

Among the DELLAs sequences of dicot, proteins of one species can be found in different clusters on the dendrogram, e.g. apple, grape, cotton, *Arabidopsis*, castor bean, pea and poplar. We can speculate that proteins from one species in different clusters are paralogues.

The number of gene copies that encode DELLAs in genome vary between species. In wheat, three genes *Rht-A1*, *Rht-B1* and *Rht-D1* on chromosomes 4A, 4B and 4D, respectively, are known (1 per diploid genome), having high level of sequence similarity to each other. *Rht-B1* and

Rht-D1 have many alleles that differ from the wild type alleles by single nucleotide substitutions (*Rht-B1b* and *Rht-D1b*), insertions (*Rht-B1c*), increased number of gene copies (*Rht-D1c*); introduction of premature stop codon (*Rht-B1d* and *Rht-B1e*) (Pearce *et al*., 2011). For the homoeologous locus on chromosome 4A only one allele *Rht-A1a* (wild type monomorph) is known (Voss, 2010; Pearce *et al*., 2011). There are five members of DELLA gene family in *Arabidopsis*, whereas rice (*SLN1*), maize (*d8*), barley (*SLR1*), *Brassica rapa* (*dwf2*) and grape (*Vvgai1*) contain only one known DELLA protein orthologue. These numbers of orthologs could reflect specific adaptive processes and/or differences between monocots and dicots (Bolle *et al*., 2004).

Detection of alleles of genes that encode DELLAs in wheat

Rht-B1 and *Rht-D1* encode transcription factors which belong to the DELLA proteins (Bolle, 2004; Pearce *et al*., 2011). The alleles *Rht-B1b* and *Rht-D1b* were transferred to European winter wheat from Japanese variety 'Norin 10' and increased significantly wheat production in the last century (Borojevich, Borojevich, 2005; Bonnet *et al*., 2010). With the help of molecular markers (Ellis *et al*., 2002) we have detected *Rht-B1b* and *Rht-D1b* alleles in genotypes of modern winter wheat varieties from different parts of Ukraine (Fig. 4).

The distribution of the mutations in sequences that encode DELLA proteins, analyzed among winter wheat varieties from different breeding centers of Ukraine, is shown in Fig. 5. These mutations are often used in breeding programs in wheat varieties created after 1990th in Ukraine, and the mutant allele *Rht-D1b* has higher frequency than the *Rht-B1b* allele. In the previous investigations, wheat varieties created before 1960th have been shown to have wild type alleles of the *Rht-B1a* and *Rht-D1a* genes. During 1980th, breeders started to introduce dwarfing GA-insensitive alleles in varieties from the southern part of Ukraine, on the other hand varieties from the central part of Ukraine were predominantly GA-sensitive (Chebotar, 2008).

By analysis of allele frequency we revealed, that dwarfing allele *Rht-B1b* is used in breeding programs of Ukraine rarer than its wild type variant – *Rht-B1a*. The allele *Rht-D1b* has higher frequency among tested varieties from eastern and southern parts of Ukraine than wild variant of this locus – *Rht-D1a*. For wheat varieties from the central part of Ukraine, the wild type alleles of the *Rht-B1* and *Rht-D1* genes are more common. This could be due to the priority of breeding programs and/or using special ancestral genotypes in crossing.

Conclusions

In this study, we estimated genetic similarity of the DELLAs nucleotide and amino acid sequences that are involved in gibberellin signaling pathways in plants. We summarized the information about protein sequence of DELLAs for 35 species from the NCBI database. Comparative analysis of DELLA protein sequences for all accessions in NCBI database showed subdivision on two clusters, the first one is formed by non-vascular (bryophytes) and vascular (lycophytes) land plants and the second one by seed plants. In the seed plants cluster, we have observed two quite separate subclusters – monocot and dicot.

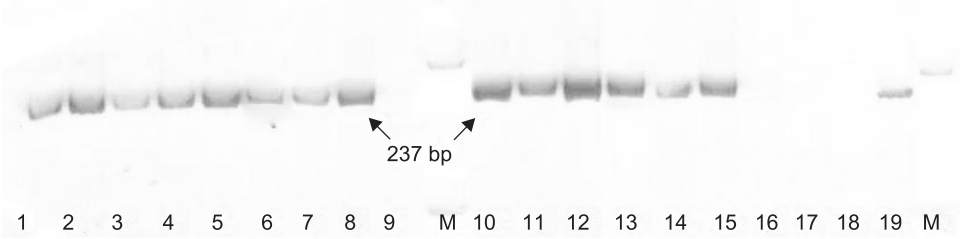

Fig. 4. Electrophoresis of allele-specific PCR-fragments derived from *Rht-D1b* allele in 7 % PAAG of wheat varieties.

1, 2 – 'Jayvir'; 3–5 – 'Ujinok'; 6–8 – 'Barviy', 9 – 'Stepnyak 1' (control line with *Rht-D1a* allele); 10–12 – 'Nebokray'; 13–15 – 'Juravka'; 16–18 – 'Turunchuk'; 19 – 'Stepnyak 2K' (control line with *Rht-D1b* allele); M – molecular weight marker pUC19/Msp I.

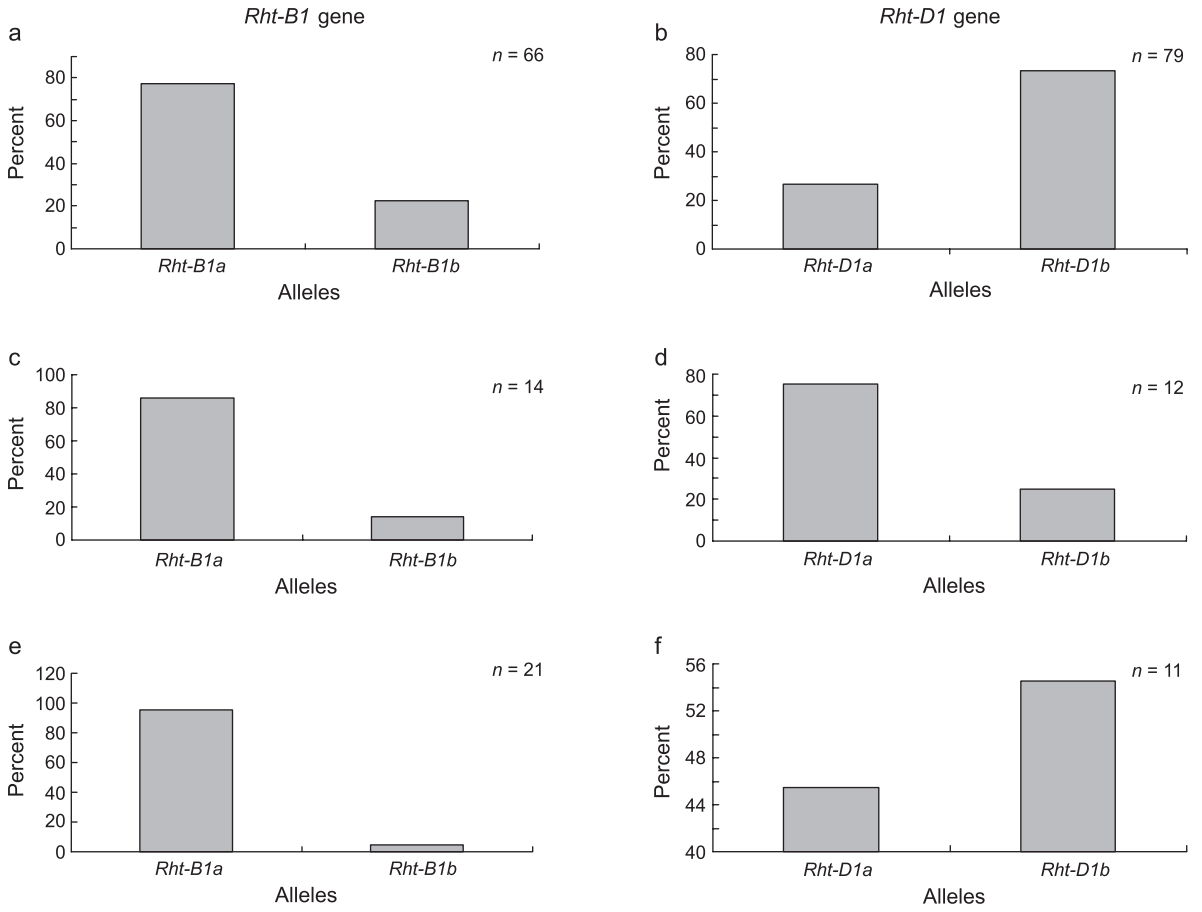

Fig. 5. Diagram of the allele frequency of *Rht-B1* and *Rht-D1* genes in winter wheat varieties of different breeding centers of Ukraine.

a, b – Southern part of Ukraine; c, d – Central part of Ukraine; e, f – Eastern part of Ukraine.

We detected mutant (*Rht-B1b*, *Rht-D1b*) and wild type (*Rht-B1a*, *Rht-D1a*) alleles that encode DELLA-proteins in Ukrainian winter bread wheat varieties created after 1990th. The allele *Rht-D1b* is the most frequently used dwarfing gene in Ukrainian varieties among tested alleles, it is presented in 68 % of wheat varieties that have been analyzed, but its distribution is not random on the territory of Ukraine.

Acknowledgement

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DELLA-МУТАЦИИ РАСТЕНИЙ НА ПРИМЕРЕ ПШЕНИЦЫ

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Репрессоры роста растений DELLA относятся к семейству белков-регуляторов транскрипции GRAS. Гиббереллины способствуют преодолению ограничения роста растений, обусловленного влиянием DELLA. Мутации в высококонсервативных генах, кодирующих DELLA, были описаны у разных видов растений. Эти мутации приводят к снижению высоты растений за счет образования более активных форм репрессоров роста. Цель настоящего исследования состояла в оценке генетического сходства нуклеотидных и аминокислотных последовательностей DELLA, представленных в базах данных, и определении аллелей, кодирующих DELLA у современных украинских сортов озимой мягкой пшеницы. С помощью программного обеспечения BLAST и MUSCLE, были проведены поиск и сравнительный анализ последовательностей гена *Rht-D1* и генов других растений, кодирующих DELLA. При использовании программы MEGA v5 была построена дендрограмма, иллюстрирующая сходство аминокислотных последовательностей DELLA у 35 видов растений. ПЦР-идентификация аллелей *Rht-B1* и *Rht-D1* генов короткостебельности пшеницы в сортах из разных регионов Украины показала, что наиболее часто в изученной коллекции встречается мутантный аллель *Rht-D1b* (68 %), причем распределение частот его встречаемости неоднородно на территории Украины.

Ключевые слова: DELLA-протеины, пшеница, гены короткостебельности, ПЦР-генотипирование.