

CYTOGENETIC ANALYSIS AND POLYMORPHISM LEVEL ESTIMATION OF BREAD WHEAT CULTIVAR ZVEZDA AND ITS DERIVATIVES

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Abstract

The high level of polymorphism has been revealed in cv. Zvezda by C-banding and the wheat storage protein electrophoresis. Moreover, the cv. Zvezda derivatives have been found to be monomorphic. Genomic in situ hybridization and C-banding failed to reveal introgressions of E. intermedia into cv. Zvezda genome. The peculiarities found for Zvezda odnostebelnaya (single-stem) and Zvezda nizkostebelnaya (short-stem) lines are of special value for the further application in breeding programs.

Key words: wheat, cytogenetic analysis, GISH, polymorphism.

Introduction

Zvezda is known to be the winter bread wheat cultivar bred by intergeneric hybridization with successive step-by-step crossing F1 [*Triticum durum* (Harkovskaya 46) x *Elytrigia intermedia*] * *T. aestivum* F1 (Mironovskaya 808 x Lutescense 329) in RSAU-MTAA. Cv. Zvezda was proved to be a highly frost-resistant cultivar possessing superior bread-making characteristic, the main advantage of the cultivar being its high resistance to lodging. Due to the similarity to wheatgrass in growth and development rates cv. Zvezda differs sharply from the standard varieties in the mentioned characteristics. Moreover, the cultivar is distinguished from the others by rather high polymorphism of various characteristics. The cultivar productivity is maintained at the same level each year regardless of the weather conditions. Zvezda nizkostebelnaya (short-stem) and Zvezda odnostebelnaya (single-stem) lines were derived from the cultivar by the individual selection [10].

The main aim of the conducted research was to carry out the cytogenetic analysis as well as to estimate the polymorphism level of cv. Zvezda and the cv. Zvezda derivatives.

Protein is known to be the primary product of the genetic system, so, the pathway from the gene to the protein trait is much shorter than that to the morphological one. Therefore, the protein turns out to be independent from the ontogenesis stage and it is less subjected to the phenotype variability, which is of great importance for genetic labelling. Being the unique product of a particular genetic system, each protein molecule reflects its peculiarities perfectly. Protein polymorphism associated with the individual gene alleles is observed as the variation in the number, mobility and intensity of the components, which can be distinguished by SDS-PAGE analysis [15]. The high degree of the inherited polymorphism is typical of the wheat storage proteins within the species and particular populations. It appeared to be true for gliadins representing alcohol-soluble fraction of the wheat storage proteins called prolamins and accounting for 86% of the total seed protein.

The comparison of the C-band distribution in various polymorphic types of each of 21 wheat chromosomes provided the basis for the C-band classification into the following three groups: i) C-bands constant in their availability and size; ii) C-bands constant in

their availability but polymorphic in the size; *iii*) C-bands polymorphic in both patterns. The constant C-bands of the first two groups were shown to be useful for chromosome identification, while the polymorphic C-bands of the second and third groups confers the karyotype individuality of a given cultivar, line, sample etc. It is due to the polymorphism of the chromosome heterochromatin regions that enables to apply a detailed ideogram of the wheat cultivar karyotype as “the chromosome passport” [3]. The C-banding patterns were found to be a constant characteristic independent from the soil-climatic conditions and the reproduction level as well [2]. C-banding is widely used in the studies of various chromosome rearrangements, mainly for the Gramineae family.

Materials and Methods

The seeds of cv. Zvezda and cv. Zvezda-derivative lines were provided by the original breeder, A.A. Kondratyev; the seeds of *T. aestivum* \times *E. intermedia* derivative M3202 (2n=56) were obtained at the Department of Intergeneric Hybridization (The Main Botanical Garden of the Russian Academy of Sciences).

To evaluate the polymorphism of cv. Zvezda and its derivatives the spectra analysis of the storage proteins was carried out by means of SDS-electrophoresis. A random sample of 100 seeds of cv. Zvezda and its derivatives harvested in 2002 was analyzed. The total protein extraction from wheat seeds endosperm was conducted according to Yang et al. (2000) with slight modifications. The glutenins were separated in 12,5% SDS-PAGE at 30 mA, the gels were stained overnight with 12% (W/V) trichloroacetic acid solution containing 0,05% Commasie Brilliant Blue 250 in absolute ethanol (5% w/v) and destained subsequently with 25% ethanol and 10% acetic acid.

To reveal possible introgressions of *Elytrigia intermedia* into cv. Zvezda genome, the standard C-banding procedure [4] was conducted with slight modifications. The slides were air dried from 30 min to 24 h. Afterwards, the dried slides were treated with saturated barium hydroxide from 6 min at room temperature, rinsed in 1N hydrochloric acid for 10-15 sec and washed in running tap water. Then the slides were dried by hot air, incubated in 2^xSSC at 60°C for an hour, washed in running tap water for 15 min and dried. The staining was conducted in 4% Giemsa dye («Giemsa-Merck», cat. № 9204) diluted with 0,125M Tris-HCl buffer (pH 6,8) under the microscope control. After washing in running water the hot-air-dried slides were rinsed out in meta- or para-xylol. Then, entellan was dropped ("Merck", cat. № 7960) and the slides were covered with 22x22 mm or 24x24 mm coverslips with the following careful elimination of air bubbles and drying the slides on the flat horizontal surface for 24 h.

To reveal possible *Elytrigia intermedia* introgressions, genomic *in situ* hybridization was performed according to the standard procedure [8] with certain improvements. The total genomic DNA of bread wheat and the DNA of intermediate wheatgrass was extracted from five-day bleached-out seedlings via the standard procedure. The DNA was labelled with digoxigenin-11-dUTP by means of the standard nick-translation method. The DNA-block was obtained by autoclaving the total genomic wheat DNA for 7 minutes under pressure of 1 atm. The ratio label/block was 1:50. The signal was detected and visualized using FITC-antidigoxigenin system. The chromosomes were counter stained with 1 mg/ml propidium iodide. The Zeiss fluorescent microscope with a special light filter set was used for the signal visualization. The fluorescence signals were recorded on Fuji color 400 ASA film, scanned, digitized, and processed by means of the PhotoShop software.

Results

The ten biotypes of Zvezda have been revealed according to the result of the wheat storage protein electrophoresis, five of biotypes accounting for 89% of the analyzed plants and each of them showing more than 10% occurrence frequency (Fig. 1). The electrophoregrams obtained for the storage proteins of Zvezda odnostebel'naya and Zvezda nizkostebel'naya lines failed to reveal any polymorphism. Moreover, they appeared to be identical and to coincide with the spectrum of one of the isolated Zvezda biotypes which occurrence frequency was 21% (Fig. 2).

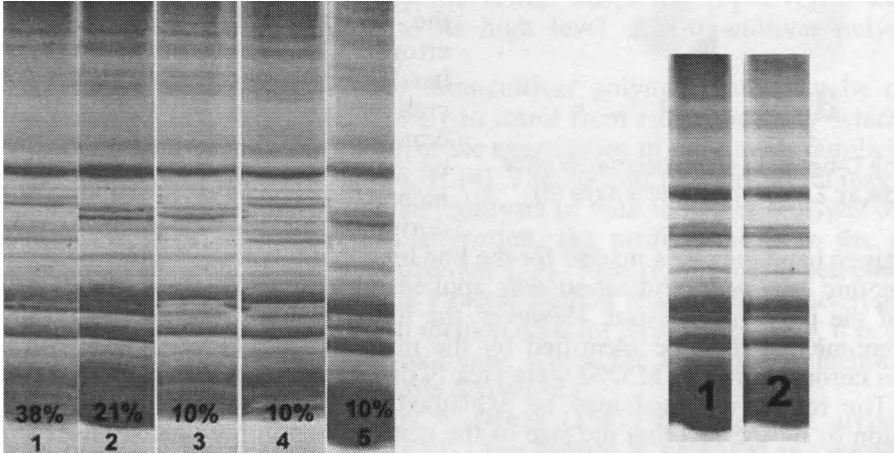


Fig. 1. The storage proteins electrophoresis spectra of the main cv. Zvezda biotypes

Fig. 2. The storage proteins electrophoresis spectra of Zvezda odnostebel'naya (1) and Zvezda nizkostebel'naya (2)

The analysis of the Zvezda C-banding karyotype enabled to classify it as a hexaploid wheat with the diploid chromosome set $2n=42$. The alien genome fragments have not been found by this method, though, the polymorphism of the bands (labelled with the arrowheads) have been observed in chromosomes 2A, 5A, 3B (Fig. 3) and 6B (Fig. 4). The obtained results agree with the data derived from the storage proteins electrophoresis and the morphological characteristics analysis.

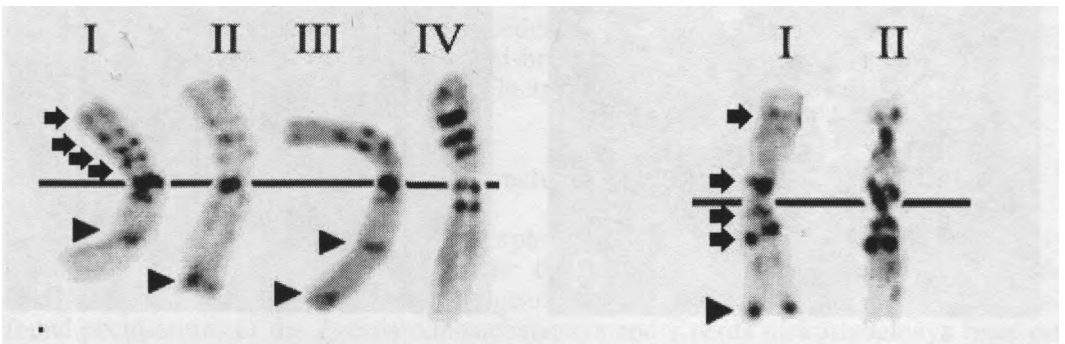


Fig.3. The C-band polymorphism of 3B chromosome of cv. Zvezda

Fig.4. The C-band polymorphism of 6B chromosome of cv. Zvezda

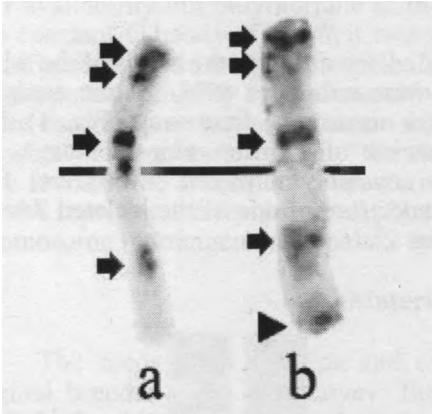


Fig. 5. The C-bands of chromosome 2B of cv. Zvezda(a); Zvezda odnostebel'naya (b)

time, the given band may be a marker for the line bred by individual selection.

Genomic *in situ* hybridization was applied both to Zvezda and M3202 for the purpose of the positive checkout. However, the introgression of *E. intermedia* into the Zvezda genome failed to be identified by the method used. Nevertheless, wheat and wheatgrass chromosomes of M3202 were clearly distinguished in the fluorescence colour (Fig. 6). The results obtained may be explained by either the lack of the discussed introgression or by the fact that the size of the genome region introduced into Zvezda is smaller than the least GISH resolving power, which is only 10 kbp.

The karyoanalysis of the Zvezda derivative lines has revealed the following specific bands: the interstitial band of 3BL polymorphic in Zvezda and the telomeric band of 6BL polymorphic for Zvezda. Thus, the Zvezda-derivative lines possess 3B chromosomes typical of the first type of 3B chromosome (Fig. 3, arrowheads) and 6B typical of the first type of 6B chromosome in the polymorphism row of the Zvezda chromosomes (Fig. 4, arrowheads). Besides, the little telomeric band of 2BL unrevealed in the original cultivar has been found in the Zvezda odnostebel'naya line (Fig. 5), which may be considered as the evidence for the incomplete coverage of all the biotypes during the cultivar screening. At the same

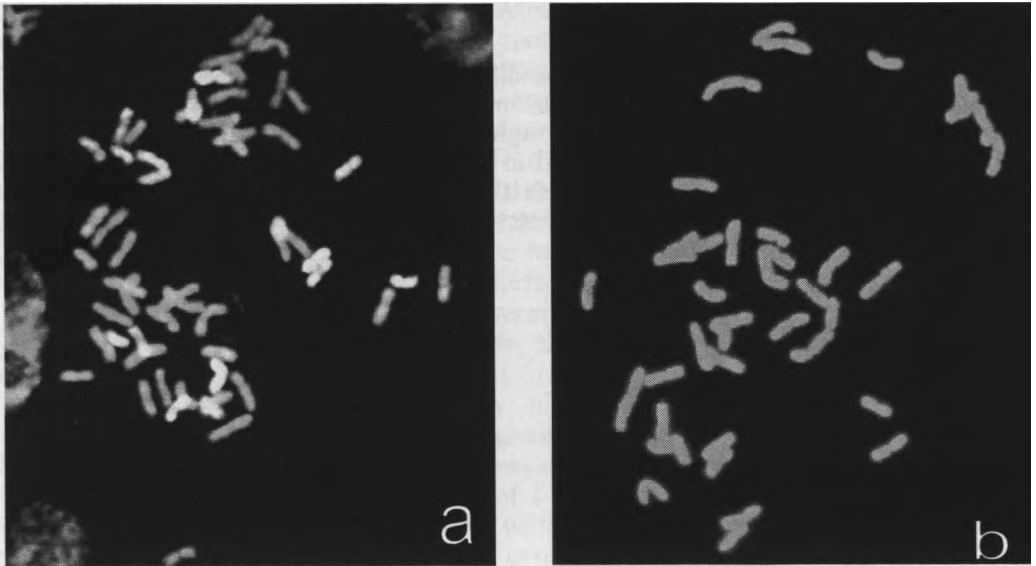


Fig. 6. Genomic *in situ* hybridization of M3202 (a) and cv. Zvezda (b)

Discussion

The intra-cultivar polymorphism is unlikely to be rare among wheat cultivars and turns out to correlate with their adaptive traits. All Russian wheat cultivars can be classified into three groups in line with the gliadin polymorphism estimation. The first group including very old and local polymorphic cultivars (Banatka, Krymka) is known for its high adaptivity. The second group developed mainly in the 1930-60-s (Mironovskaya 808, Bezostaya 1) is characterized by more or less high adaptive potential. The last group consisting of monomorphic cultivars developed in the 1970-80-s (Kavkaz, Avrora) is distinguished by the lowest adaptivity [9]. Zvezda seems to be referred to the second group due to its high level of intra-cultivar polymorphism, ecological plasticity and yield stability.

The most possible reasons for intra-cultivar polymorphism may be due to the cultivar heterozygosity. The latter is likely to result from either the early selection of the elite plants from the second generation or the aggregation of the similar families produced from the same hybrid combination [16, 7, 14]. The cultivar polymorphism may be caused by the inter-pollinating either with other cultivars or with the other biotypes of the same cultivar, as well as mechanical contamination, the artificial shift in the population structure or even spontaneous hybridization [13]. Finally, the mass selection of self-pollinating plants is also mentioned as a factor leading to the cultivar heterogeneity [7]. Taking into account the mass selection method used for Zvezda breeding, it is the method applied that causes the cultivar polymorphy, whilst the individual selection seems to result in the monomorphy of the Zvezda derivatives.

The classification suggested by N. Deeva (1987) proposed the division of the biotypes into “the frame” and “the shelf”. The former is supposed to contribute to the main structure of the cultivar while the latter provides the basis for the minor biotypes. However, both the structure and the content of the cultivar population changes from year to year [13, 1, 12]. In our research the five biotypes represented by the gliadin spectra (Fig. 1) seem to be referred to “the frame” of Zvezda accounting for 89% of the structure while the rest 11% can be designated as “the shelf”.

The polymorphism is believed to be interrelated with the introduction, that is changing the ecological and geographical cultivar niche. In this case the cultivar may demonstrate its inherited ecological plasticity [5, 6]. Such microevolution variations depending on the reproduction level and the region are likely to be due to the natural selection, the cultivar revealing itself as a polymorphic self-regulating system [5, 11]. The structure of cultivar population is affected by the cultivar breeding method and its primary seed breeding [7]. The breeder of the cultivar did not recommend any special techniques for Zvezda seed-breeding [10]. Therefore, the revealed polymorphism of gliadins (Fig. 1) reflects the structure of 2002 cultivar and may vary from year to year.

Conclusion

In conclusion, the revealed polymorphism of the C-bands and gliadin spectra may be interpreted as the genetic basis for the cv. Zvezda adaptivity. *E. intermedia* introgressions could not be identified by genomic *in situ* hybridization or C-banding. The found peculiarities of the Zvezda odnostebel'naya and Zvezda nizkostebel'naya lines can be of practical use in breeding programs.

Bibliography

1. Agafonov N.S., Pshenichnaya I.A., Turovskiy A.I., Velibekov M.D. The genetic identification of the wheat cultivars via gliadin formulae // The innovations in breeding and seed growing (in Russian) Kamennaya Step. 1987: 48-52.
2. Badaeva E.D. The evolution of wheat and its wild relatives genomes: molecular-cytogenetic analysis (PhD thesis - in Russian) / Engelgardt Institute of Molecular Biology. Moscow, 2000: 48.
3. Badaeva E.D., Badaev N.S., Sozinova L.F., Turbin N.V. C-banding method for the development of cereal «chromosome passport». // Selschohozyastvennaya Biologiya (in Russian), № 1, 1989.
4. Bolsheva N.L., Badaeva E.D., Kurochkina A.I., Badaev N.S. The comparison of the C-banded chromosomes of the two relative *Secale* species. Genetika (in Russian). 1984, 20: 2025-2030.
5. Deeva N.L. The intra-varietal polymorphism of gliadin and phenotypic diversity of winter bread wheat. The scientific research on application botanics, genetics and breeding. 1987, 114: 24-34.
6. Duktova N.A. The research of the intra-species variability in the quantitative traits of spring durum wheat // The condition and the ways of the development of the Belarus sugar beet production (in Russian). Minsk, 2003: 184-187.
7. Illychevskiy N.N., Kudryavtsev A.M., Upelnik V.P., Metakovskiy E.V. The analysis of the pedigrees of the cultivars of bread wheat on the basis of alpha-amylase polymorphism study. Genetika (in Russian). 1995, 31, 12: 1650-1654.
8. Karlov G.I., Khrustaleva L.I., Lim K.B., Van Tuyl J.M. Homologous recombination in 2n-gametes producing interspecific hybrids of lillium (*Liliaceae*) studied by genomic in situ hybridization (GISH). // Genome, vol 42, 1999, 681-686.
9. Konarev A.V. The application of molecular markers in terms of the solution of the genetic diversity problems in plant resources and breeding. Agrarian Russia (in Russian). 2006, 6: 4-22.
10. Kondratyeva N.N., Kondratyev A.A. Winter wheat Zvezda. // Selectsiya I Semenovodstvo (in Russian), №1, 1993, pp. 37-40.
11. Larionov Y.S., Larioinova L.M. The intra-varietal polymorphism and the yield of the cultivar of spring wheat. The theses of the All-Union conference “Agrophytocenoses and the ecological ways of increasing their stability and productivity” (in Russian). Izhevsk, 1988: 101-102.
12. Latipov A.Z., Snitko M.L. The intra-varietal genetic diversity of the cultivar samples of winter wheat of various origin. // The problems of crop plants production and the ways of their solution (in Russian). Gorki. 2000, 1: 123-129.
13. Olefirenko S.V., Blochin N.I. The intra-varietal variability in the grain quality of the mironovskaya-type cultivars of winter wheat and their gliadin polymorphism // The breeding, protection and agrotechnology of wheat, barley and triticale (in Russian). 1987: 63-68.
14. Pshenichnaya I. A. The intra-varietal polymorphism of gliadin in winter wheat and the perspectives for its application in breeding (PhD thesis) / Dokuchaev Agronomy Scientific-Research Institute of the Central Region of Chemozemye. Kamennaya Step, 1999: 22.
15. Shuaib M., Zeb A., Ali Z., Ali W., Ahmad T., Khan I. Characterization of wheat varieties by seed storage-protein electrophoresis. African Journal of Biotechnology. 2007, 6, 5: 497-500.
16. Surov V.Y. The application of the electrophoresis in the seed breeding of wheat. The theses of 6-th regional conference of the young scientists of Siberia and the Far East. Breeding and seed-breeding Section (in Russian). 1986: 40-42.
17. Yang Z.J., Li G.R., Ren Z.L. Identification of amphiploid between *Triticum durum* cv. Ailanmai native to Sichuan, China and *Secale africanum*. // Wheat Inf. Service, 91, 2000, pp. 20-24.

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