Advances in Bioresearch
Adv. Biores., Vol 16 (3) May 2025: 05-09

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.3.59

Advances in Bioresearch

ORIGINAL ARTICLE

Seed Identification of Commercial Wheat Varieties by Gliadin Proteins

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ABSTRACT
Evaluation of breeding material on the basis of protein markers makes it possible to carry out selection rather quickly and qualitatively and to control the transfer of desired traits from parental forms into hybrid populations. Electrophoretic spectra of gliadin proteins in wheat grain are specific for each genotype of a variety and have proven to be stable genetic markers. In this study, initial seeds of released wheat varieties from different regions of Uzbekistan were analyzed and identified by polyacrylamide gel electrophoresis (PAAG) based on gliadin protein spectra. The results of the study showed the specificity of gliadin spectra for the studied varieties. It was found that gliadin protein spectra of Andijan-4, Krasnodar-99 and Asr varieties obtained from the Research Institute of Grain and Legume Crops and its Namangan Experimental Station are monomorphic. However, differences in gliadin spectra were observed in samples of Andijan-2 and Tanya varieties from different regions, indicating the presence of polymorphism in these varieties, which is important for assessing their genetic stability and variability.
Keywords: *wheat, variety, genotype, electrophoresis, gliadin.*

Received 19.03.2025 Revised 21.04.2025 Accepted 05.05.2025

How to cite this article:
Ismoilova Gulyora M G, Baboyeva Sevara S Q, Qulmamatova Dilafruz E, Usmanov Rustam M, Buzurukov Sandjar S, Baboev Saidmurot K. Seed Identification of Commercial Wheat Varieties by Gliadin Proteins. Adv. Biores. Vol 16 [3] May 2025.05-09

INTRODUCTION

Common wheat (*Triticum aestivum* L.), also known as bread wheat, is one of the main agricultural crops in Uzbekistan and occupies a significant share in the diet of the population [1]. Scientific breeding continues to develop new varieties characterized by improved, economically valuable traits. In the State Register there are about 70 varieties of soft wheat authorized for wide sowing in all regions of Uzbekistan, which complicates the preservation of originality in seed production. Modern varieties of cereal crops are complex varieties-populations, which have high plasticity and adaptability, but it is difficult to preserve them in the process of seed production, because most of their constituent biotypes, differing in biological properties, are identical in morphological traits. The composition and ratio of biotypes of a variety can change significantly in 3- 4 years, so the issue of evaluation of the original genetic structure of a variety is of fundamental importance [2].

The abundance of existing cultivars highlights the need for their identification and certification, which would make it possible to distinguish between varieties, examine their authenticity, qualify samples, and assess their purity. The main method of establishing the cultivar assignment of seeds and determining cultivar purity is ground testing, which requires considerable material, resources, and time expenditures [3].

Gliadins are seed storage proteins in wheat (*Triticum aestivum* L.) and the key determinants of seed quality. They are genotype-specific, genetically determined proteins that are reproducible experimentally, independent of environmental variation or stage of plant ontogenesis [4]. The electrophoregram of gliadin composition is specific for the genotype of a wheat cultivar and does not depend on the growing conditions of the plant that produced that seed [5]. The spectra of gliadins formed in electrophoresis are

highly diverse and richly endowed with numerous components, making them highly suited to the detection of genetic polymorphism, phylogeny and pedigree relationships among wheat samples. Gliadin and glutenins make up 80% of the protein in wheat grain endosperm, and their electrophoretic spectrum does not change under the influence of external environmental factors [1]. Gliadin proteins soluble in 70% alcohol in one grain of wheat are divided into 20-25 components by one dimensional electrophoresis.

The use of protein markers (electrophoretic analysis of seed storage proteins) allows in laboratory conditions to carry out varietal identification, to evaluate varietal purity, to control the composition and ratio of biotypes.

Gliadins are monomeric proteins and are categorized into four groups known as α-, β-, γ-, and ω-gliadins based on their reduced mobility during electrophoresis in acidic pH media [6]. Genes controlling proteins belonging to the γ- and ω-groups are located at the Gli-1 (Gli-A1, Gli-B1, and Gli-D1) loci on the short arm of chromosome 1, whereas α- and β-type proteins are controlled by the Gli-2 (Gli-A2, Gli-B2, and Gli-D2) genes located on the short arm of chromosome 6 [7]. Each variety is characterized by a set of individual alleles of gliadin-coding loci, which makes it possible to use them to identify almost any variety (Kudryavtsev et al. 2014). Based on A-PAGE (the most efficiently electrophoretic method of gliadin separation) gliadins are divided into five groups: α, β, γ, ω-5 and ω-1.2, where α are the fastest and ω-1.2 the slowest moving protein fractions during separation on polyacrylamide gel [8].

MATERIAL AND METHODS

Seed materials. During the research, 50 samples consisting of the best seeds of soft wheat varieties released in the country, received from the nursery and submitted to the State Seed Control Enterprise for certification were selected. Since the seeds were obtained from different seed enterprises, some varieties were returned several times. These are varieties Zamin 1 (2), Andijan-4 (2), Andijan-2 (2), Vassa (5) and variety Tanya, returned 2 times. These varieties were planted in the experimental field of the "Durmon" Institute in two repetitions on plots of 1 m2 and analyzed morphological and economic traits, as well as electrophoretic analysis of spare gliadin proteins.

Field experiment. Seeds of 50 samples of commercial soft bread wheat varieties were sown in the experimental plot. The experimental design was a randomized complete block with three repetitions. The row spacing was 0.3 m. The plots were 1.0 m long and 1.2 m wide. The seeding rate was 400 seeds per 1 m. Seeds were sown in the second decade of October, before sowing seeds were treated with fungicides against fungal diseases.

Electrophoretic analysis. For electrophoretic analysis, 20 grains of each variety were milled separately and extracted in 70% ethanol for 30 min in a thermostat at 24 0C. The extract was centrifuged at 4000 rpm for 3 minutes. The supernatant was filtered and 80% sucrose solution was added dropwise. One-dimensional electrophoresis was performed according to the method of Bushuk and Zilman (1978), improved by Metakowskiy [8]. It was carried out in a unit for vertical electrophoresis in aluminum lactate buffer with pH=3.1 on 6.5% PAGE (polyacrylamide gel). The electrophorogram was stained with 1% Kummasi dye for 12 hours, then washed in plain water and varieties were determined based on the obtained electrophorogram spectra.

A nomenclature of components has been developed based on the electrophoretic spectra of prolamins. The reference spectrum is BP 654321 α-1234567, β-12345, γ-12345, ω-12345678910. The possible locations of the components in each zone are numbered from the start. In some positions, two or more subcomponents may occur. For example, ω -4, -6.-8. These are encoded by alleles of one gene and are, in turn, ω -4_1, ω- 4_2, ω -6_1, ω -6_2, etc. Here, the index 1 indicates that the component is biased towards the fast-moving component, 2 is in the middle, and the index 3 is biased towards the slow-moving component.

To record the results of electrophoretic analysis of gliadin, two recording methods are used depending on the nomenclature of spectrum components: biochemical, based on electrophoretic mobility, and genetic, based on genetic control of components.

RESULTS AND DISCUSSION

Most varieties of soft wheat are cultivated in Uzbekistan, which occupies more than 70% of the total area under irrigated conditions of grain crops of Russian breeding varieties. When analyzing the electrophoretic spectrum of the Zimnitsa cultivar belonging to the Krasnodar selection and cultivated in the main grain fields of Uzbekistan, it was observed that despite the fact that this variety has an almost monomorphic spectrum, it consists of two closely related biotypes.

Identification of Russian-bred varieties cultivated in Uzbekistan. Sixteen varieties widely cultivated in the region selected for analysis. According to the electrophoretic spectra of gliadin, the varieties Bezostaya-100, Aleksevich, Krasnodarskaya-99, Mars, Pervitsa, Tanya, Vekha, Vassa, Yuka and Zvezda were monomorphic. These varieties assessed as homogeneous. Of the homogeneous varieties, the electrophoretic spectrum of the Tanya variety obtained from two regions differed sharply from each other. These two samples were two different varieties under the same name. In addition, two different varieties found under the name Antonina, one of them was monomorphic, and the other turned out to be polymorphic. In addition to the main spectrum, this polymorphic variety had single seeds that differed in one or two components.

The first 4 spectra are the main biotypes of this variety, accounting for almost 90% of the variety. The remaining two biotypes occupy 5% of the analyzed spectrum. The first biotype of the Antonina variety differs sharply from the ω - zone of the main spectrum, as it is possible to observe the absence of the ω1, ω2, ω4, ω7 components and a shift of the ω - 8191 components towards the fast-moving component. The second biotype differs from the main spectrum of the variety by the absence of the ω10 and γ3 components (table.1).

Table 1. Electrophoretic formulas of wheat varieties and biotypes of Russian selection

T/p	Wheat cultivars and biotypes	α-groups	β-groups	γ-groups	ω-groups	Number of protein bands
1	Alexevich	457	25	14	12346789	15
2	Vassa	1234567	12345	12345	12348910	24
3	Vexa	457	235	12334	123489	17
4	Grom	457	235	234	123478910	17
5	Drujba	13467	1235	1345	1234678910	22
6	Krasnodar-99	13467	1235	12345	457891011	21
7	Mars	2457	1245	245	45689	16
8	Pervisa	57	235	145	34789	13
9	Zvezda	457	35	12345	245678910	18
10	Bezostaya 100	457	235	1234	123489	16
11	Yuka	24567	1245	1234	123468910	21
12	Tanya (1)	457	235	1345	245689	16
13	Tanya (3)	1357	235	1245	24568910	18
14	Antonina (1)	13457	245	234	12348910	18
15	Antonina (2)	1357	235	2345	379910	16
16	Biotype-1	467	235	24	1234689	15
17	Biotype-2	13457	235	1234	12348910	19
18	Gurt	4567	1235	1234	123478910	20
19	Biotype-1	457	235	245	123456789	18
20	Esaul	457	2345	234	24589	15
21	Biotype-1	457	2345	24	12489	14
22	Biotype-2	457	2345	145	5679	14
23	Zimnisa	457	235	12345	23489	16
24	Biotype-1	3457	235	12345	2348910	18

The electrophoretic spectrum of the Zimnitsa variety was observed to consist of two closely related biotypes. The main difference was manifested in the ω-zone of the electrophoregram. The second biotype of the Zimnitsa variety differs from the main biotype in the presence of the ω10 component, which has the slowest electrophoretic conductivity in the ω-zone, and the α4 component. This biotype accounts for approximately 10% of the variety.

Identification of Uzbek selection varieties

24 soft wheat varieties of Uzbekistan were divided into monomorphic and polymorphic varieties according to the electrophoretic spectrum and were analyzed electrophoretically. According to the results of the analysis, the protein formula of 17 varieties was determined as monomorphic according to the electrophoretic spectrum and seven varieties were determined as polymorphic according to the electrophoretic spectra. Accordingly, it was determined that the varieties “Istiklol”, “Janub Gawhari”, “Khisorak”, “Andijan 2” and “Jasmina” have 2 biotypes each, the variety “Navbahor” has 3 biotypes and the variety “Aq Marvarid” has five biotypes, and these varieties are polymorphic according to the electrophoretic spectrum.

DISCUSSION

Currently, molecular markers based on DNA analysis are widely used for genotyping and genetic identification in various crops [7]. The application of molecular markers was successful in the study of wheat genes controlling such traits as 1,000-grain weight, protein and gluten content [9, 12]. Nevertheless, molecular markers are relatively expensive in the equipment and reagents required, in typically well-established molecular laboratories. In contrast, biochemical markers based on proteins such as enzymes and storage proteins offer an alternative method involving cheaper and simpler protocols for crop breeding including wheat [10,11]. Acid-polyacrylamide gel electrophoresis (A-PAGE) is used to phenotype different varieties of wheat based on their gliadin profiles.

In the seed production system, there are cases of the release of other varieties under the same name, or even complete replacement of varieties. In this regard, it is necessary to certify varieties according to "Protein formulas". One of such varieties is the Vassa variety, which is considered one of the most popular among farms of the republic due to its high yield. However, analysis of the electrophoretic spectra of gliadin of this variety from different sources showed that different varieties are sown under this name. In another case, a variety consisting of two different varieties in a ratio of approximately 2:1 was encountered. The obtained data show the usefulness of compiling a catalog of varieties based on the electrophretic spectra of gliadin in wheat seed production.

CONCLUSIONS

This study highlights the evaluation of breeding materials based on protein markers, enabling the rapid and qualitative selection and control of trait transfer from parental forms into hybrid populations. The electrophoretic spectra of gliadin proteins in wheat grain are specific to each genotype of a variety and have proven to be stable genetic markers. Initial seeds of released wheat varieties from different regions of Uzbekistan were analyzed using polyacrylamide gel electrophoresis (PAGE) based on gliadin protein spectra. The results demonstrated the specificity of gliadin spectra for the studied varieties. The gliadin protein spectra of the Andijan-4, Krasnodar-99, and Asr varieties were found to be monomorphic. However, differences in gliadin spectra were observed in samples of the Andijan-2 and Tanya varieties from different regions, indicating the presence of polymorphism in these varieties. This finding is important for assessing their genetic stability and variability.

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