Development of Cotton and Wheat Variety Certification Using Isr-Pcr Markers

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Abstract. It is shown in this article, that electrophoretic spectrum of gliadin proteins consists of specific components in each variety. Among analyzed varieties there were homogeneous varieties by electrophoretic gliadin spectra as well as heterogeneous varieties. The wheat varieties Krasnodar-99, Kroshka, Zimnitsa and Tanya were homogeneous, while the varieties Vostorg, Grom, Zvezda, Andizhan-2, Asr, Durdona, Dustlik, Yaksart and Chillaki were heterogeneous with 2-3 phenotypes in their electrophoretic spectrum. In the varieties Zvezda and Sila, three phenotypes and in the variety Thunder two phenotypes were found. In each variety, in addition to the main spectrum, phenotypes up to 5% were found with an electrophoretic spectrum different from the main one. In the variety Andijan-2 four biotypes with percent ratio of 80:5:5:10 and this variety has the highest number of biotypes of heterogeneous varieties. In Asr variety 3 heterogeneous biotypes with 85:10:5 ratio, in Yenbosh variety 3 biotypes with 80:10:10 ratio, in Durdon variety 2 biotypes with 70:30 ratio and in Chillaki variety also 2 biotypes with 80:20 ratio were identified. Molecular marking and genotype passporting are used in basic genetics and in applied biological science. Some wheat varieties cultivated in Uzbekistan were found to be heterogeneous and have several phenotypes in their genotype according to the electrophoretic spectrum of gliadins. To accurately determine whether the identified phenotype belongs to a particular variety, it is necessary to screen all varieties from different seed growing farms, create a catalogue of EF-spectrum passports and evaluate the varietal purity of each variety. Keywords: electrophoretic spectrum, homogeneous varieties, heterogeneous varieties, phenotype, biotype, wheat and cotton varieties, passporting and molecular marking.

1 Introduction

Seed quality is a complex set of its genetic (varietal), physical, physiological and biochemical properties that determine the value of a seed lot for specific purposes [1, 2]. In agriculture, sowing seeds are used in accordance with the relevant normative and technical documentation. Seeds of any crop are the source material for cultivation and harvesting, the value and quality of which are determined by hereditary economic and biological properties

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of varieties under the influence of natural, biological, organizational and technological factors [3-7].

Seed quality has a genotype, epigenetic and modification variability arising as a response to the action of agrotechnical and environmental factors [8, 9], as well as responds to the conditions that develop in the period of seed formation, development and maturation [10].

Seeds of any crop are the starting material for cultivation and harvesting, the value and quality of which are determined by hereditary economic and biological properties of varieties under the influence of natural, biological, organizational and technological factors [3]. The role of genotype in increasing and stabilizing yields is constantly increasing, and the contribution of variety in zoning, according to Borisovets T.V. (2000), is estimated at 30...50%.

Maintaining the varietal purity of cultivated varieties is a high priority for seed production, since the possibility of cross-pollination contamination is not excluded when they are cultivated on pre-propagation farms, where several varieties are multiplied at the same time at close range [11-13]. Therefore, assessment of varietal purity is one of the first and necessary steps in seed multiplication.

Genetic markers play an extremely important role in the study of hereditary constitution of an organism and especially in the evaluation of starting and breeding material, because they facilitate the control of the inclusion of desired traits from parental forms in the varieties and hybrids being created [14-16].

The protein marker method is extremely important for improving the efficiency of wheat breeding. It can be used for identification and registration of varieties, in seed production and seed control, in the study of genetic diversity of a species or crop. In many CIS republics, protein markers are used to establish originality, uniformity and constancy of varieties of various crops [17-19]. The practical use of spare protein polymorphism for the above purposes is also stated in the ISTA decisions [20-21].

On the basis of analysis of the literature data, we can conclude that the use of molecular markers based on seed proteins is a promising direction in solving the problems of breeding and seed production.

2 Research methodology and materials

Researches were conducted in Scientific-Research Institute of selection, seed production and agrotechnology of cotton growing of Ministry of Agriculture of the Republic of Uzbekistan. Institute of Genetics and Experimental Biology of Plant Academy of Sciences of RUz and Center of Genomics and Bioinformatics.

Wheat varieties released in Uzbekistan - Krasnodar-99, Vostorg, Grom, Zvezda, Zimnitsa, Kroshka, Sila, Tanya, Andijan-2, Asr, Durdona, Dustlik, Enbosh, Yaksart, Chillaki were used as research material.

The cotton varieties "S-6565" and "Surhan-103" were used to create the passport.

3 Results

Electrophoretic analysis of gliadin spare proteins was carried out according to Bushuk and Zilman method modified by Metakovsky [22]. For this purpose 100 ears were selected from each variety and from each ear one grain was taken, extracted in 70% ethanol, then incubated for 30 minutes in an incubator at 240C, centrifuged for 3 minutes at 3000 rpm. An 80% sucrose stained with brilliant green was added to the supernatant. Electrophoresis was performed in 8% PAGE, pH 3.5. After electrophoresis, the gels were fixed for 30 min in 10% TCA, stained with 1% brilliant blue.

Electrophoregrams were compared with the reference spectrum of Bezostaya 1 and polymorphism by gliadin biotypes was described.

This work also aims to investigate the possibility of using PCR for molecular passportization of cotton varieties [23]. At the same time method was used: DNA genome was isolated and inserted by PCR in cotton varieties C-6565, G.hirsutum L. and Surhan-103 of barbadense species.

DNA genome by Dellaportaetal method [24] was isolated from plant leaves.

Specially designed SSR markers for DNA genome obtained for cotton plants were used. PCR reaction was performed using genomic DNA with a concentration of 50 ng, 2.5 µl 10XTaq buffer, 0.2 µl NTP (nucleic triphosphate DNA) 25 Mmol each, 5 pico mol/µl primer, 0.2 µl Taq polymerase (5 units/µl), water to 25 µl. The PCR was performed under the following conditions: 1 cycle - 940C, 3 min; 45 cycles - 940C 1 min; 550C, 1 min; 720C, 2 min; 1 cycle - 720C, 7 min.

The obtained PCR product was analysed against laser marker 25 DNA in a 3.5% agarose gel.

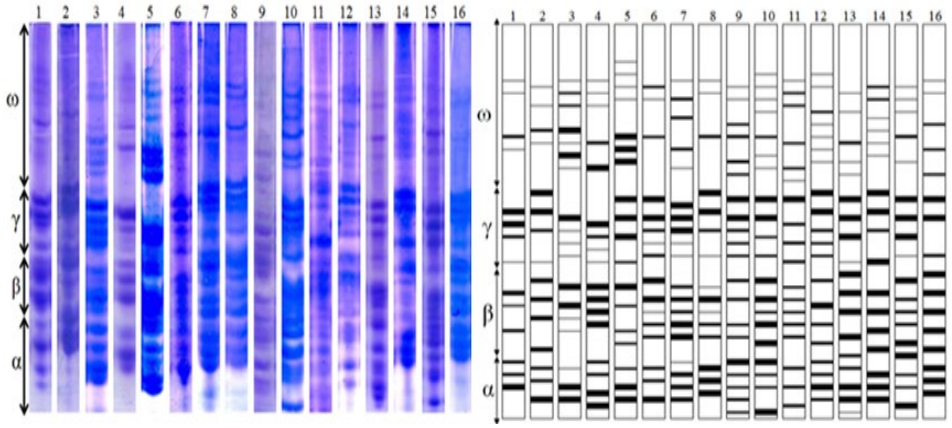

Fig. 1. Electrophoretic analysis of wheat varieties cultivated in Uzbekistan.

The electrophoretic spectrum of gliadin proteins in wheat is divided into α, β, γ and ω zones. In each variety the electrophoretic spectrum of gliadin proteins consists of certain components and is specific (Figure 1). Among the varieties analysed there were homogeneous varieties in terms of gliadin electrophoretic spectra as well as heterogeneous varieties. The wheat varieties Krasnodar-99, Kroshka, Zimnitsa, and Tanya showed homogeneity, while the varieties Vostorg, Grom, Zvezda, Andizhan-2, Asr, Durdona, Dustlik, Yaksart, and Chillaki were heterogeneous and had 2-3 phenotypes in their electrophoretic spectrum. In the varieties Zvezda and Sila, three phenotypes and in the variety Thunder two phenotypes were found. In each variety, in addition to the main spectrum, phenotypes up to 5% were found with an electrophoretic spectrum different from the main one.

In the variety Andijan-2 four biotypes with percent ratio of 80:5:5:10 and this variety has the highest number of biotypes of heterogeneous varieties were identified. In Asr variety 3 heterogeneous biotypes with 85:10:5 ratio, in Enbosh variety 3 biotypes with 80:10:10 ratio, in Durdon variety 2 biotypes with 70:30 ratio and in Chillaki variety also 2 biotypes with 80:20 ratio were found.

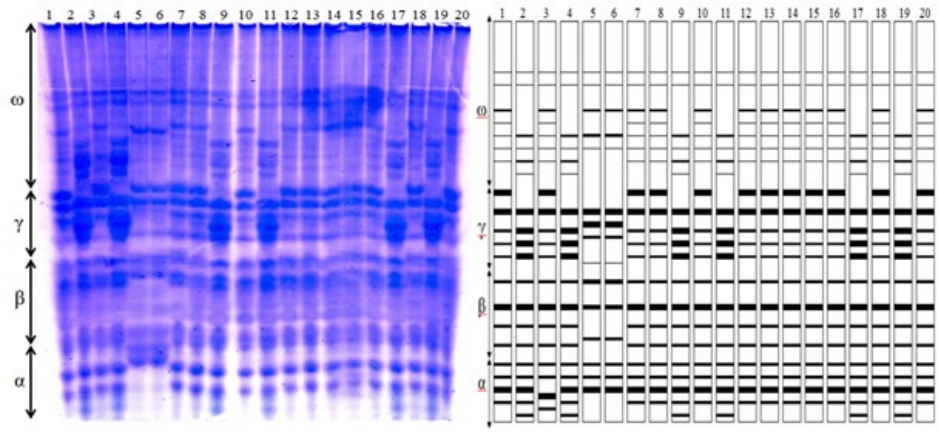

Fig. 2. Electrophoretic spectrum of cultivar Durdon. 5-6 ESP spectrum of Bezostaya 1 (control) and 1,3,7,8,10, 12-16, 18, 20 the first phenotype, 2, 4, 9,11, 17 and 19 the second phenotype.

Figure 2 describes the complete polymorphism in gliadin electrophoretic spectra of ancient Uzbek wheat varieties compared to commercial and newly released varieties. A total of 21 ancient, two new and one commercial varieties were analyzed and 40 phenotypes were identified using electrophoretic spectra. Of the analyzed varieties, 10 were monomorphic and 14 varieties were polymorphic, with many of the old varieties of wheat cultivated in remote regions, in private farms being monomorphic and those cultivars grown commercially or on large areas being polymorphic.

Molecular marking and genotype passporting are now used in basic genetics and in applied biological science.

Molecular markers are the most used in applied PCR, each type of marker has its own characteristics that need to be taken into account when choosing the most reliable and simple methods for genetic passportization of varieties of different cultivated plant species.

One of its applications is the passportisation of valuable genotypes. Molecular markers (MM) based on the application of PCR are the most widely used because of the simplicity and cheapness of the method [25].

A common one is RAPD (random amplified polymorphic DNA). The method does not require knowledge of the target DNA sequence. RAPD markers are generated by amplification of random DNA segments using single primers of any sequence [26].

The resulting PCR products are genotype-specific and can be easily separated in an agarose gel. In order to increase the number of PCR combinations, the primers can be combined in pairs rather than using individual primers. "Two-primer" RAPD yields more small fragments than the standard technique; more than half of the products synthesised are different from "single-primer" RAPD [25-28].

The medium-fibre cotton variety 'C-6565' was created from a hybrid combination (F17159-F x 02654), by multiple individual selection at the Research Institute of Breeding, Seed Production and Agrotechnology of Cotton Cultivation, by V. A. Avtonomov.

Thin-fibre cotton cultivar "Surhan-103" was developed from hybrid combination of F5 ML-120 x Giza-83 by multiple individual selection in Research Institute of Breeding, Seed Production and Agrotechnology of Cotton Cultivation. Authors O. Kimsanboev, V. Avtonomov. As a result of research the following was obtained:

Surxon-10311101010111101111101100110

C-6565 10010101111010110111001l011

10 SSR markers for molecular analysis on cotton were used: CIR_246, BNL-3034, BNL-3251, BNL-3638, BNL2634, BNL3599, BNL3280, JESPER220, BNL3442, BNL3923,

CIR_246_160, CIR_246_180, BNL-3034_175, BNL-3034_165, BNL-3251_200, BNL-3251_220, BNL-3638_240, BNL-3638_220, BNL-3638_260, BNL2634_200, BNL2634_250, BNL2634_280, BNL3599_200, BNL3599_215, BNL3599_240, BNL3280_190, BNL3280_260, BNL3280_280, BNL3280_420, JESPER220_130, JESPER220_140, JESPER220_155, JESPER220_170, BNL3442_130, BNL3442_150, BNL3442_175, BNL3923_100, BNL3923_120

Fig. 3. Surxon-103

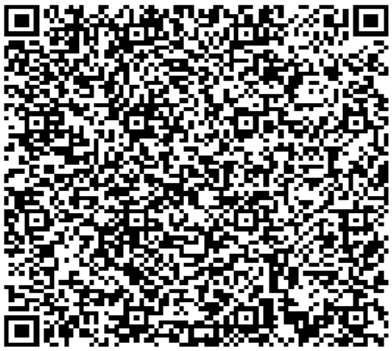

Fig. 4. C-6565

Along with cotton, wheat varieties were passported. Soft wheat varieties Bardosh, Pahlavon and Okmarvarid were used.

Soft wheat variety Bardos, developed by individual selection from CIMMYT collection, is resistant to yellow rust.

Bardoche 10101000100101011001010101010010100010101000

Fig. 5. Bardohe

The variety of soft wheat Pahlavon was created by individual selection based on electrophoretic spectra of gliadin of grain from the population of local variety Marjon.

Pahlavon 01011101001010011000101001011111000101000010

Fig. 6. Pahlavon

Ok Marvarid, a variety of soft wheat, was developed at the Institute of Genetics and Experimental Biology of the Academy of Sciences of the Republic of Uzbekistan by crossing the local variety Unumli Bugdoi with a sample from the local collection.

Ok marvarid 10101010010100110110010010100010011000010001

Fig. 7. Oc marvarid

14 SSR markers were used for molecular analysis on wheat: 513-WMS, 484-WMS, 374-WMS, 154-WMS, 522-WMC, 181-BARS, 614-WMS, 175-WMC, 177-WMC, 181-WMC, 257-WMC, 344-WMC, 397-WMC, 419-WMC, 513-WMS_220, 513-WMS_200, 484-WMS_230, 484-WMS_240, 374-WMS_100, 374-WMS_250, 374-WMS_275, 154-WMS_180, 154-WMS_200, 154-WMS_210, 154-WMS_220, 154-WMS_235, 522-WMC_250, 522-WMC_275, 522-WMC_300, 522-WMC_350, 181-BARS_240, 181-BARS_250, 181-BARS_275, 614-WMS_150, 614-WMS_175, 614-WMS_190, 614-WMS_210, 175-WMC_125, 175-WMC_140, 175-WMC_150, 177-WMC_230, 177-WMC_250, 181-WMC_220, 181-WMC_240, 181-WMC_300, 257-WMC_275, 257-WMC_300, 257-WMC_310, 344-WMC_110, 344-WMC_130, 344-WMC_145, 397-WMC_175, 397-WMC_185, 397-WMC_200, 419-WMC_160, 419-WMC_175, 419-WMC_190

4 Conclusions

As a result of research it was found that according to electrophoretic gliadin spectrum some wheat varieties cultivated in Uzbekistan are heterogeneous and have several phenotypes in their genotype. In order to accurately determine whether the identified phenotype belongs to a particular variety, it is necessary to screen all varieties from different seed production farms, create a catalogue of EF-spectrum passports and evaluate the varietal purity of each variety.

Electrophoretic analysis of stored grain proteins of some wheat varieties released in Uzbekistan Triticum aestivum L. showed slight polymorphism in ESP spectra of alcohol-soluble gliadin protein.

Requirements for certification of cotton varieties C-6565 and Surhon-103 as well as wheat varieties Bardosh, Pahlavon and Ok Marvarid have been developed.

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