RESEARCH ARTICLE

Nikolay Neykov [1]
Sonya Doneva [2]

Identification of reserve endosperm proteins in promising spring barley cultivars by SDS-PAGE electrophoresis

Authors' addresses:
[1] Institute of Plant Genetic Resources, Department of Plant Genetic Resources, 2 Drouzhba Blvd., Sadovo 4122, Bulgaria.
[2] Dobrudzha Agricultural Institute, General Toshevo 9521, Bulgaria.

Correspondence:
Nikolay Neykov
Institute of Plant Genetic Resources, Department of Plant Genetic Resources, 2 Drouzhba Blvd., Sadovo 4122, Bulgaria.
Tel.: +359 887522724
e-mail: neykov.nikolay@gmail.com

ABSTRACT
The aim of present study was to identified the reserve endosperm proteins in promising spring barley cultivars by SDS-PAGE electrophoresis. The material object of this study were seven cultivars of spring barley with different origin: Zernogradskii (Russia), Bodega, Fink, Scarlett, Barke (Germany), Josefin and Astoria (France). Cultivar Igri was used as a standard for biochemical analysis. Hordeins are divided into four groups based on their amino acid composition and electrophoretic mobility: A-, B-, C- and D-. The most significant allelic polymorphism was identified in B-hordein. The standard variety was characterized by six band with a different intensity. Two majors and four minor band were well expressed in the spectra of varieties Bodega, Fink and Barke. Electrophoretic profiles of Zernogradskii, Josefin and Astoria established a major, and between five and six minor subunits with a different intensity of color depending on the methodology used for fractionation of hordein.

Key words: spring barley, reserve proteins, hordein, SDS-PAGE electrophoresis

Introduction

The use of biochemical markers of genetic control of useful traits is increasingly used in the breeding of barley (Jones, 1982; Hauser et al., 1982; Stoyanova & Popova, 2002). As a result of long research found that electrophoretic spectra of spare proteins in barley - hordeins remain unchanged under the influence of environmental conditions (Konarev, 1983, 1983a). Studying the genetic nature of the traits in barley varieties on the basis of polymorphism of spare proteins is a guarantee for successful breeding program (Konarev al., 1986). The determination of reserve proteins are widely used in studies of plant populations, since variability in their characteristics is genetically determined. They are characterized by a high level of polymorphism and stability. External factors have little or no effect on their volume in the mature seeds. By using electrophoretic method spare proteins can be separated into individual fractions, each of which has a specific relationship with the economic valuable traits. Their expression is stable and independent of environmental conditions (Todorov et al., 2002).

Materials and Methods

The material objects of this study were seven cultivars of spring barley with different origin: Zernogradskii from Russia, Bodega, Fink, Scarlett, Barke from Germany, Josefin and Astoria from France. Cultivar Igri was used as a standard for biochemical analysis. In 2015, hordeins have been identified in the Laboratory of Biochemistry in Dobrudzha Agricultural Institute – General Toshevo by SDS-PAGE electrophoresis by two methods. In the method of Laemmli (1970) fractionation of hordein was performed on 12% separating gel. Is performed at a constant current of 20 mA per plate at room temperature for 18-20 hours. In barley is established significant polymorphism of B- and C-hordein. Many of the alleles of loci Hor 2 and Hor 3, respectively, encoding the expression of B- and C- hordein they correspond in several closely spaced band, which cannot be well separated by SDS-PAGE by the method of Laemmli, which subsequently hinders their exact identification. This requires the fractionation of hordein to be performed by one-dimensional SDS-PAGE electrophoresis by the method of Payne et al., 1980. The method is performed on a 35% separating gel for 3-4 hours at a constant current of 90 mA to the plate at room temperature. Hordein extraction is carried out using the method of Singh et al., 1991. The visualization of the protein components is accomplished by 0.03% solution of Coomassie Brilliant Blue-R250 in methanol and acetic acid.

Results and Discussion

As a result of research of many researchers has shown that the electrophoretic spectra of spare proteins in barley -

hordeins, are not altered under the influence of the environmental conditions. Studying the genetic nature of the trait in barley cultivars on the basis of polymorphism of spare proteins is a guarantee for successful breeding program. External factors have little or no effect on their volume in the mature seeds. By using electrophoretic method spare proteins can be separated into individual fractions, each of which has a specific relationship with the economic valuable traits.

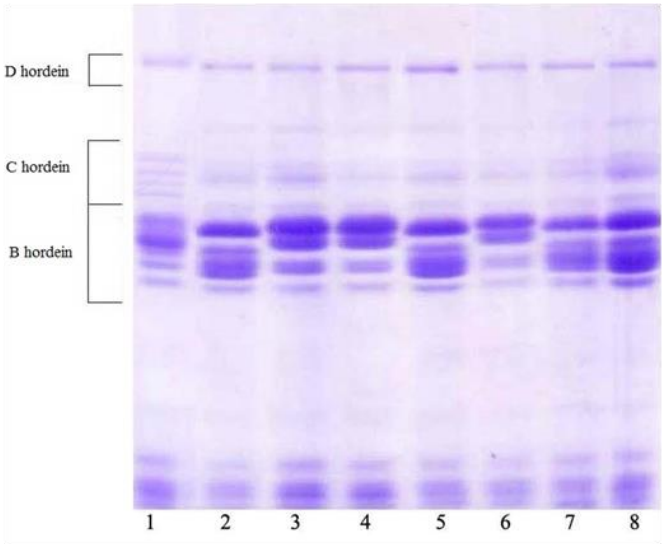

Figure 1. *SDS-PAGE according to Laemmli (1970).* ***Legend:*** *1 – Igri (standard); 2 – Zernogradskii; 3 – Bodega; 4 – Fink; 5 – Scarlett; 6 – Barke; 7 – Josefin; 8 – Astoria.*

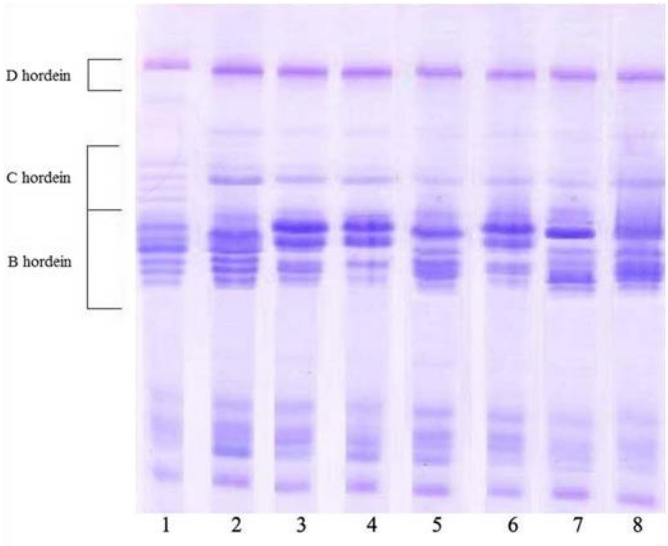

Figure 2. *SDS-PAGE according to Payne et al., 1980.* ***Legend:*** *1 – Igri (standard); 2 – Zernogradskii; 3 – Bodega; 4 – Fink; 5 – Scarlett; 6 – Barke; 7 – Josefin; 8 – Astoria.*

Hordeins are divided into four groups based on their amino acid composition and electrophoretic mobility: A-, B-, C- and D-. A-hordein is with lowest molecular weight. Usually they cannot be properly identified due to vague electrophoretic profiles and are not perceived as reserve proteins (Shewry et al., 1978).

D- hordein have the highest molecular weight, which is associated with a low electrophoretic mobility. Their synthesis is encoded by a locus Hor 3, localized in the long arm of chromosome 5 (Shewry & Tatham, 1990). They are represented by an intense subunit in the spectra of the standard variety Igri and analyzed accessions. These subunits are visually different relative electrophoretic mobility on the gel plate with each other and compared with the standard (Figure 1 and 2).

B- and C- hordein are encoded respectively by multi-alelic loci Hor 2 and Hor 1, localized in the short arm of chromosome 5. In the area of the C- hordein identified different number of subunits with a different intensity. In the spectrum of the standard Igri were established four well expressed minor band which are with different electrophoretic mobility as compared to the C- hordein in the analyzed spring varieties. One minor and one major subunits are established in electrophoretic profiles of the tested varieties of barley (Figure 1 and 2).

The most significant allelic polymorphism was identified in B- hordein. The standard variety is characterized by six bands with a different intensity. Two majors and four minor bands are well expressed in the spectra of varieties Bodega, Fink and Barke. Electrophoretic profiles of Zernogradskii, Josefin and Astoria established a major, and between five and six minor subunits with a different intensity of color depending on the methodology used for fractionation of hordein (Figure 1 and 2).

Observed subunits are probably different allelic variants of the D-, C- and B- hordein of barley varieties analyzed and the standard. This can be proven only by accurate calculation of the relative electrophoretic mobility of each subunit of the corresponding block in the process of White & Cook (1992) and the identification of hordein in the catalog of UPOV (2003) to the appropriate standards.

Conclusion

Hordein electrophoretic module of investigated barley cultivars are characterized by pronounced intercultivar polymorphism. Intercultivar allelic variation is the result of the presence or absence of the protein components and their different electrophoretic mobility.

References

Hauser K, Sasek A, Kubanek J, Krautova E, Cerny J. 1982. Hordein characteristics of spring barley varieties grown in Czechoslovakia. Symposium of Biochemical, Russia, 190-194.

Jones BL. 1982. Identifying United States malting barley varieties by electrophoresis of hordeins and esterase enzymes. Symposium of Biochemical, Russia, 195-199.

Konarev VG. 1983. Identification of domestic varieties of soft winter wheat byelectrophoretic gliadin spectra. Catalog of World Collections VIR, 386, 57.

Konarev VG. 1983a. Plant proteins as genetic markers, Moscow, Kolos, 320.

Konarev VG, Gavrilyuk PI, Gubarev KN, Peneva IT. 1986. Proteins as genetic markers in solving problems of applied botany, genetics and breeding. The Agricultural Science, 12: 45-50.

Laemmli UK. 1970 .Clavage of structural proteins during the assembly of the head of bacteriofage T4. Nature, 227(5259): 680-685.

Payne PI, Law CN, Mudd EE. 1980. Control by homeologous group chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor. Appl. Genet., 58: 113-120.

Shewry PR, Prat HM, Miflin BJ. 1978. Varietal identifications of single seeds of barley by analisis of hordein polypeptides. J. Sci. Food Agr., 29: 587-596.

Shewry PR, Tatham AS. 1990. The prolamine storage proteins of cereal seeds: structure andevalution. Biocemical Journal, 267: 1-12.

Singh NK, Shepherd KW, Cornish GB. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J. Cereal Sci, 14: 203-208.

Stoyanova S, Popova V. 2002. Identification of polymorphism in Bulgarian barley varieties by b - hordein spectrum. JubileeSci. Conference “Breeding and Cultivation of Field Crops”, 503-508 (Bg).

Todorov I, Ivanovand P, Ivanova I. 2002. Bio using polymorphism of grain storage proteins for identification of feed barley varieties 441 chemical markers in wheat and their use in breeding and seed production. In: JubileeSci. Conference “Breeding and Cultivation of Field Crops”, 1: 94-113 (Bg).

White J, Cook R. 1992. A standart classification system for the identification of barley Varieties by electrophoresis. Seed Science and Technology, 20: 663-676.

UPOV. 2003 .Protocol for distinctness uniformity and stability tests. *Hordeum vulgare* L. *sensu lato*. European Union.Community Plant Variety Office, 43.