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SSR ANALYSIS IN THE STUDY OF GENETIC DIVERSITY AND SIMILARITY OF BARLEY CULTIVARS

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The aim of research was to develop an evaluation system of the genetic polymorphism for barley cultivars of Ukrainian and foreign origin based on the analysis of simple sequence repeats and valuable agricultural trait loci as well as to compose the molecular genetic passports for those cultivars. PCRs with the following separation of amplification products by agarose and polyacrylamide electrophoresis were performed to find out genetic polymorphism. Unweighted Pair Group Method with Arithmetic Mean was used for phylogenetic relationship detection. The dandrogram of phylogenetic relationships of 55 barley cultivars was constructed and molecular genetic passports were developed. Molecular genetic passports can be involved in verification for the compliance with standards cultivars, stability and seed purity.

Key words: *Hordeum vulgare*, SSR markers, PCR analysis, molecular genetic passports.

Barley (*Hordeum vulgare* L.) is an important crop, which is used as fodder, raw material for malt production, and as human food [1, 2]. Barley is second only after wheat as the most important nutritional grain crop grown in low rainfall environments [2]. In addition, the cost of barley production is significantly lower than for other crops. Approximately 3.4 million hectares of spring and 400–500 thousand hectares of winter barley are sown in Ukraine annually [3, 4].

The study of genetic diversity of barley cultivars gives resource of potential genes donors in order to create and maintain modern crop cultivars and for direct farmer use [2, 5].

Molecular markers are of great value for characterization and evaluation of barley genetic diversity. Different types of molecular markers, used for characterizing germplasm, are based on different methods that differ fundamentally in application, type and number of detected polymorphism, cost and need for time [6].

Simple sequence repeats (SSR) markers are widely used in the study of barley genome. SSRs, also called microsatellite, are tandem repeats of 1–5 base pairs (bp), and are usually presented in the eukaryotes genomes [7]. The main benefit of all SSR loci is codominance, so they are useful for different breeding programs [8], e. g. genetic mapping [9] and the genetic diversity assessment [10]. SSRs are extremely unstable and this feature allows to distinguish similar plant cultivars [11]. Polymorphism of SSRs is easily analyzed with PCR. Finally, SSR markers are technically efficient, cost-effective and widely spread in barley genome [7, 9, 12–15]. These features make them effective molecular marker system for many types of genetic analyzes.

Besides the study of genetic diversity, results of SSR analysis should be used for composing molecular genetic passports of cultivars. Such a passport is a document that reflects the particular structure of cultivar's, line's, or hybrid's DNA and enables their

identification. Genotype passportization is performed by writing a formula that reflects characteristic of variable loci. DNA typing isn't a prerequisite for a cultivar registration in Ukraine yet. However, representation of a cultivar in the form of molecular genetic formula gives an idea of the cultivar structure, its compliance with the homogeneity and stability [16].

The process of the passport composing can take a month and a half instead of 2–3 years of field trials with UPOV DUS test [16, 17]. DUS test (test for distinctness, uniformity and stability [18]) does not provide registration of hybrids. Genetic formula allows us to determine varietal purity, carry out differentiation and identification of cultivars [16]. These passports can be complemented with data on valuable agricultural trait loci.

The objective of the work was to develop a system to evaluate genetic polymorphism of Ukrainian and foreign barley cultivars based on analysis of both SSR loci and valuable agricultural trait loci as well as to compose molecular genetic passports for barley cultivars.

Materials and Methods

The subject of the research were 55 barley cultivars of Ukrainian and foreign origing.

Total DNA was isolated with the modified CTAB method [19].

The reaction mixture of 20 µl included 0.5 µM of forward and reverse primers, 1×DreamTaq™ Green Buffer (Thermo Fisher Scientific), 0.2 mM of each deoxyribonucleotide-3-phosphate (Thermo Fisher Scientific), 1 unit of polymerase DreamTaq™ DNA Polymerase (Thermo Fisher Scientific), 30 ng of total plant DNA.

Primers for loci *EBmac0715*, *EBmac0874*, *MGB391*, *MGB402tt1*, *Bmag13*, *GMS1* and *MGB318* were used in the study [9, 20].

The PCR products were evaluated for polymorphisms on agarose (3%) and polyacrylamide (12%) gels using sodium borate and Tris-borate-EDTA buffers respectively [21–23]. Visualization of results was performed in UV-light with the photosystem Canon EOS 600D. Detection of the cultivars phylogenetic relationships was performed by the unweighed pair-group method using arithmetic averages cluster analysis (UPGMA) with the software DARwin 6.0.010 [24].

Results and Discussion

The subject of the study were 7 microsatellite loci (Table 1) to determine the level of genetic diversity and relationship of 55 barley cultivars of Ukrainian and foreign origin (Table 2). These selected microsatellite loci showed varying degree of polymorphism. To measure the informativeness of each SSR marker, the polymorphism information content (PIC) was calculated [25]. Using primers *EBmac0874* three fragments were amplified, among them, two were polymorphic and the alleles of these loci are found in various combinations. This fact enhances the value of the marker *EBmac0874* for the study of cultivars genotypes. The loci *MGB402tt1* and *Bmag13* also showed a high degree of polymorphism, and the loci *MGB391* and *MGB318* had a relatively low polymorphism (Fig. 1). The results demonstrated the effectiveness of using microsatellite markers in the genetic diversity study of barley cultivars, and detection the heterozygous forms.

Molecular genetic passports

Applying the results and the data on valuable agricultural trait loci (*Bmy1* determines the thermostability of β-amylase; *ITR1* — the synthesis of SE-protein, which causes the beer clouding; *Wax* — the synthesis of amylose) [26–28], molecular genetic passports were developed for 55 Ukrainian and foreign barley cultivars (Table 3).

The genotype passportization was carried out by writing a formula, which reflects the characteristic of variable loci. A locus was encoded with a letter of the Latin alphabet, and allele's molecular weight was pointed at subscript. The molecular weight of an allele was indicated in the case of the homozygous state of a locus (gene), and the molecular weight of several alleles — for heterozygous forms. Thus, SSR-loci were designated as A — *Ebmac0715*, B — *Ebmac0874*, C — *MGB391*, D — *MGB402tt1*, E — *Bmag13*, F — *MGB318*, G — *GMS1*; the valuable agricultural traits loci: H — *Bmy1*, I — *ITR1*, J — *Wax*.

Genetic diversity and similarity of barley cultivars

The results obtained during SSR analysis and the data on valuable agricultural trait loci (*Bmy1*, *ITR1* and *Wax*) were used in the research of the genetic diversity among 55 cultivars of Ukrainian and foreign origin [26–28]. The phylogenetic tree (Fig. 2) was

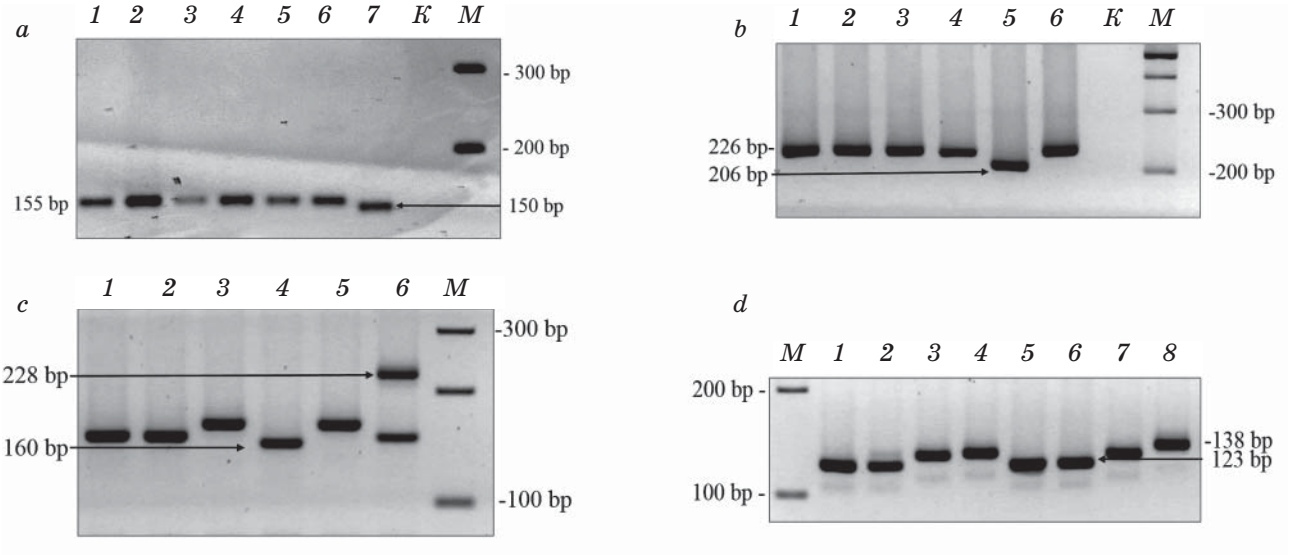

Fig. 1. **Agarose gels showing the allelic segregation of the SSR markers:**
A — EBmac0715; *B — MGB391*; *C — MGB318*; *D — GMS1*; Lane *1–8*, samples; *K* — negative control without DNA; *M* — marker of molecular weight GeneRuler™ DNA Ladder Mix

Table 1. **The characteristic of barley SSR markers**

Marker	Chromosome location	Fragment size, bp	Polymorphic amplified fragments	PIC
EBmac0715	2H (2)	150–155	2	0.401
EBmac0874	6H (6)	83–89 167–216	3 5	0.526 0.489
MGB391	2H (2)	206–226	2	0.370
MGB402tt1	5H (1)	215–260	7	0.792
Bmag13	3H (3)	146–156	5	0.651
MGB318	5H (7)	160–228	4	0.346
GMS1	5H (7)	123–131	3	0.410

performed by means of the method UPGMA using DARwin software.

The dendrogram consists of two main clusters. The first cluster group includes 54 cultivars, and only one Ukrainian cultivar Medikum 46 belongs to another cluster group. The first group divided into two subgroups. Two Canadian cultivars CDC Alamo and CDC Candle belong to one of them, the other Ukrainian and foreign cultivars — to another subgroup.

The dendrogram shows that cultivars of different breeding have significant genetic similarity. In addition, the similarity among a number of Ukrainian and foreign barley cultivars (e.g. Slavutych and Shakira, Ukrainian and German cultivars) is obvious. This fact can be an evidence of the possibility of the use of foreign elite barley to improve the genetic pool of national culture.

The various samples of a cultivar (e.g. Jennifer and Akhiles), that originated from different collections, are included in the various cluster groups, that is to say, they have different genotypes. The fact can indicate that barley cultivars from different growing areas don't comply with the standard. This confirms the necessity to control cultivars purity and cultivars to standards using DNA markers.

The detection of genetic diversity between a pair of Ukrainian cultivars Odeskyi 14 and Stepovyk failed using seven SSR loci and three valuable agricultural trait loci. Some additional loci should be included in further research to solve the problem.

Table 2. **Barley cultivars and their origin**

Cultivar	Country	Cultivar	Country	Cultivar	Country	Cultivar	Country
Akhiles	Ukraine	Ebson	Czech Republic	Kovzan	Ukraine	Odeskyi 69	Ukraine
Annabell	Germany	Enei	Ukraine	Kozatskyi	Ukraine	Odeskyi 70	Ukraine
Barke	Germany	Gladys	Netherlands	Xanadu		Palidum 32	Ukraine
Beatris	Germany	Golden promise	Ukraine	Luka	Ukraine	Quench	United Kingdom
Beatrix	Germany	Halychyn	Ukraine	Malz	Czech Republic	Rosalina	Denmark
Bojos	Czech Republic	Helios	Ukraine	Marthe	Germany	Scarlett	Czech Republic
CDC Alamo	Canada	Henrike	Germany	Medicum 46	Ukraine	Shakira	Germany
CDC Candle	Canada	JB Maltasia	Germany	Modern	Ukraine	Slavutych	Ukraine
CDC Gainer	Canada	Jennifer	Germany	Nutans 106	Ukraine	Stepovyk	Ukraine
Chorno-morets	Ukraine	Jersey	Netherlands	Nutans 244	Ukraine	Sviatohor	Ukraine
Claire	Germany	KBC Aliciana	Germany	Odeskyi 9	Ukraine	Yuzhnyi	Ukraine
Cristalia	United Kingdom	KBC Bambina	Germany	Odeskyi 14	Ukraine		
Danuta	Germany	Khadar	Ukraine	Odeskyi 18	Ukraine		
Datcha	France	Komandor	Ukraine	Odeskyi 36	Ukraine		

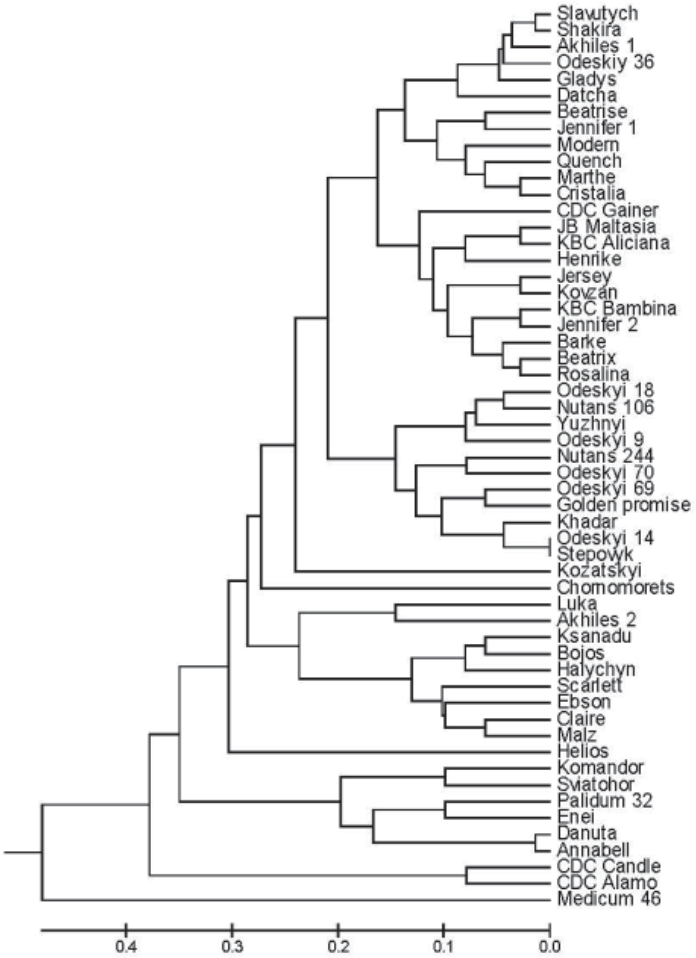

Fig. 2. **The dendrogram showing similarity and clustering of 55 barley genotypes**

Table 3. **Molecular genetic passports for barley cultivars**

Cultivar	Formula
Akhiles 1	$A_{155}\ B_{89,205}\ C_{226}\ D_{246}\ E_{149}\ F_{167}\ G_{123}\ H_{516}\ I_{455}\ J_{802}$
Akhiles 2	$A_{155}\ B_{89,167}\ C_{206,226}\ D_{225,235}\ E_{149}\ F_{172}\ G_{123}\ H_{643}\ I_{455}\ J_{802}$
Annabell	$A_{155}\ B_{83,170}\ C_{206}\ D_{215}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{1010}$
Barke	$A_{155}\ B_{83,170}\ C_{226}\ D_{225}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
Beatris	$A_{155}\ B_{83,170}\ C_{226}\ D_{215}\ E_{149}\ F_{172}\ G_{123}\ H_{516}\ I_{455}\ J_{802}$
Beatrix	$A_{155}\ B_{89,170}\ C_{226}\ D_{225}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
Bojos	$A_{155}\ B_{89,170}\ C_{206}\ D_{240}\ E_{149}\ F_{167}\ G_{123}\ H_{643}\ I_{455}\ J_{802}$
CDC Alamo	$A_{150}\ B_{89,170,205}\ C_{206,226}\ D_{230}\ E_{149,150}\ F_{167}\ G_{131,138}\ H_{643}\ I_{455}\ J_{592,802}$
CDC Candle	$A_{150}\ B_{89,205}\ C_{226}\ D_{230}\ E_{150}\ F_{167}\ G_{138}\ H_{643}\ I_{455}\ J_{592}$
CDC Gainer	$A_{155}\ B_{89,170}\ C_{226}\ D_{225}\ E_{149}\ F_{172}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
Chornomorets	$A_{155}\ B_{89,170,205}\ C_{226}\ D_{230}\ E_{146,150}\ F_{167,228}\ G_{123,131}\ H_{516,643}\ I_{0,455}\ J_{802,1010}$
Claire	$A_{155}\ B_{89,170}\ C_{206}\ D_{215}\ E_{149}\ F_{167}\ G_{123}\ H_{516}\ I_{455}\ J_{802}$
Cristalia	$A_{155}\ B_{89,170}\ C_{226}\ D_{215}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{455}\ J_{802}$
Danuta	$A_{155}\ B_{83,170}\ C_{206}\ D_{215}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{1010}$
Datcha	$A_{155}\ B_{89,170}\ C_{226}\ D_{246}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{455}\ J_{1010}$
Ebson	$A_{155}\ B_{93,170}\ C_{206}\ D_{230}\ E_{149}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
Enei	$A_{155}\ B_{83,170}\ C_{206}\ D_{225}\ E_{150}\ F_{172}\ G_{123}\ H_{643}\ I_{0}\ J_{1010}$
Gladys	$A_{155}\ B_{89,170}\ C_{226}\ D_{246}\ E_{149}\ F_{167}\ G_{131}\ H_{516}\ I_{455}\ J_{802}$
Golden promise	$A_{150}\ B_{83,170}\ C_{226}\ D_{230}\ E_{150}\ F_{172}\ G_{123}\ H_{643}\ I_{455}\ J_{802}$
Halychyn	$A_{155}\ B_{89,170}\ C_{206}\ D_{230}\ E_{149}\ F_{167}\ G_{131}\ H_{643}\ I_{455}\ J_{1010}$
Helios	$A_{155}\ B_{93,205}\ C_{226}\ D_{225}\ E_{150}\ F_{160}\ G_{131}\ H_{516}\ I_{455}\ J_{802}$
Henrike	$A_{150}\ B_{83,170}\ C_{226}\ D_{235}\ E_{149}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
JB Maltasia	$A_{155}\ B_{83,170}\ C_{226}\ D_{230}\ E_{149}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
Jennifer 1	$A_{155}\ B_{83,170}\ C_{226}\ D_{240}\ E_{148}\ F_{172}\ G_{123}\ H_{516}\ I_{455}\ J_{802}$
Jennifer 2	$A_{150}\ B_{89,170}\ C_{226}\ D_{235}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
Jersey	$A_{155}\ B_{89,205}\ C_{226}\ D_{230}\ E_{148}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
KBC Aliciana	$A_{155}\ B_{83,170}\ C_{226}\ D_{230}\ E_{148}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
KBC Bambina	$A_{150}\ B_{89,170}\ C_{226}\ D_{230}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
Khadar	$A_{150}\ B_{89,216}\ C_{226}\ D_{215,225}\ E_{150}\ F_{167}\ G_{123}\ H_{643}\ I_{455}\ J_{802}$
Komandor	$A_{155}\ B_{83,170}\ C_{206}\ D_{246}\ E_{148}\ F_{167}\ G_{131}\ H_{643}\ I_{0}\ J_{1010}$
Kovzan	$A_{155}\ B_{89,205}\ C_{226}\ D_{230}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{0}\ J_{802}$
Kozatskyi	$A_{155}\ B_{83,89,167,173}\ C_{226}\ D_{235}\ E_{148}\ F_{167}\ G_{123}\ H_{516,643}\ I_{455}\ J_{802}$
Ksanadu	$A_{155}\ B_{89,170}\ C_{206}\ D_{215}\ E_{149}\ F_{167}\ G_{131}\ H_{643}\ I_{455}\ J_{802}$
Luka	$A_{155}\ B_{89,216}\ C_{206}\ D_{225}\ E_{149}\ F_{172}\ G_{131}\ H_{516}\ I_{455}\ J_{802}$
Malz	$A_{155}\ B_{89,170}\ C_{206}\ D_{230}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{455}\ J_{802}$
Marthe	$A_{155}\ B_{83,170}\ C_{226}\ D_{215}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{455}\ J_{802}$
Medicum 46	$A_{155}\ B_{83,89,167,173}\ C_{226}\ D_{230,266}\ E_{146}\ F_{167,228}\ G_{131,138}\ H_{643}\ I_{0}\ J_{802}$
Modern	$A_{155}\ B_{89,170}\ C_{226}\ D_{215}\ E_{148}\ F_{167}\ G_{123}\ H_{643}\ I_{455}\ J_{802}$
Nutans 106	$A_{150}\ B_{89,216}\ C_{226}\ D_{230}\ E_{150}\ F_{167}\ G_{123}\ H_{516,643}\ I_{455}\ J_{802}$
Nutans 244	$A_{150}\ B_{83,170}\ C_{226}\ D_{225}\ E_{150}\ F_{167}\ G_{123}\ H_{516}\ I_{455}\ J_{802}$

Table 3. End

Cultivar	Formula
Odeskyi 9	A_{150} $B_{83,216}$ C_{226} D_{230} E_{150} F_{167} G_{123} H_{516} I_{455} J_{1010}
Odeskyi 14	A_{150} $B_{89,170}$ C_{226} D_{225} E_{150} F_{167} G_{123} H_{643} I_{455} J_{802}
Odeskyi 18	A_{150} $B_{83,89,170,216}$ C_{226} D_{230} E_{150} F_{167} G_{123} H_{516} I_{455} J_{802}
Odeskyi 36	A_{155} $B_{89,170}$ C_{226} D_{246} E_{149} F_{167} G_{123} H_{643} I_{455} J_{802}
Odeskyi 69	A_{150} $B_{83,170}$ C_{226} D_{225} E_{150} $F_{167,172}$ G_{123} H_{643} $I_{0,455}$ J_{802}
Odeskyi 70	$A_{150,155}$ $B_{83,89,170,205}$ C_{226} $D_{225,235}$ $E_{146,150}$ F_{167} G_{123} H_{516} I_{455} J_{802}
Palidum 32	A_{155} $B_{83,170}$ C_{206} D_{246} E_{156} F_{172} G_{138} H_{643} I_{0} J_{1010}
Quench	A_{155} $B_{83,170}$ C_{226} $D_{215,230}$ E_{148} F_{167} G_{123} H_{516} I_{455} J_{802}
Rosalina	A_{155} $B_{89,170}$ C_{226} D_{215} E_{150} F_{167} G_{123} H_{516} I_{0} J_{802}
Scarlett	A_{155} $B_{93,170}$ C_{206} D_{215} E_{149} F_{167} G_{131} H_{516} I_{455} J_{802}
Shakira	A_{155} $B_{89,170}$ C_{226} D_{246} E_{149} F_{167} G_{123} H_{516} I_{455} J_{802}
Slavutych	A_{155} $B_{89,170}$ C_{226} $D_{225,246}$ E_{149} F_{167} G_{123} H_{516} I_{455} J_{802}
Stepovyk	A_{150} $B_{89,170}$ C_{226} D_{225} E_{150} F_{167} G_{123} H_{643} I_{455} J_{802}
Sviatohor	A_{155} $B_{83,170}$ C_{226} D_{246} E_{149} F_{167} G_{131} H_{643} I_{0} J_{802}
Yuzhnyi	A_{150} $B_{89,170,216}$ C_{226} $D_{215,230}$ $E_{146,150}$ F_{167} G_{123} H_{516} I_{455} $J_{802,1010}$

The system of genetic diversity evaluation of barley cultivars with the SSR markers was developed. The results of SSR analysis and the data on valuable agricultural trait loci determined the genetic relationship or similarity between Ukrainian and foreign cultivars, which is of great value for breeders and farmers. In addition, the composed molecular genetic passports of barley cultivars should be involved in verification for compliance with standard cultivars, stability and seed purity.

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SSR-АНАЛІЗ У ДОСЛІДЖЕННІ ГЕНЕТИЧНОГО РІЗНОМАНІТТЯ ТА СПОРІДНЕНОСТІ СОРТІВ ЯЧМЕНЮ

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Метою роботи було провести оцінювання генетичного поліморфізму сортів ячменю української та зарубіжної селекції на основі SSR-аналізу та локусів цінних сільськогосподарських ознак, а також скласти молекулярно-генетичні паспорти сортів. Для встановлення генетичного поліморфізму здійснювали ПЛР з наступним розділенням продуктів ампліфікації методом електрофорезу в агарозних та поліакриламідних гелях. Для встановлення філогенетичних зв'язків було застосовано метод незваженого попарного середнього — UPGMA. Побудовано дендрограму філогенетичних зв'язків 55 сортів ячменю та складено їхні молекулярно-генетичні паспорти, які можуть бути використані для перевірки сортів на відповідність еталонам, стабільність та чистоту.

Ключові слова: *Hordeum vulgare*, SSR-маркери, ПЛР-аналіз, молекулярно-генетичні паспорти.

SSR-АНАЛИЗ В ИССЛЕДОВАНИИ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ И СРОДСТВА СОРТОВ ЯЧМЕНЯ

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Целью работы было провести оценку генетического полиморфизма сортов ячменя украинской и зарубежной селекции на основе SSR-анализа и локусов ценных сельскохозяйственных признаков, а также составить молекулярно-генетические паспорта сортов. Для установления генетического полиморфизма осуществляли ПЦР с последующим разделением продуктов амплификации методом электрофореза в агарозных и полиакриламидных гелях. Для установления филогенетических связей был использован метод невзвешенного попарного среднего — UPGMA. Построена дендрограмма филогенетических связей 55 сортов ячменя и составлены их молекулярно-генетические паспорта, которые могут быть использованы для проверки сортов на соответствие стандартам, стабильность и чистоту.

Ключевые слова: *Hordeum vulgare*, SSR-маркеры, ПЦР-анализ, молекулярно-генетические паспорта.