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Research Article

Identification of Onion Varieties (*Allium Cepa* L.) of Ukrainian Breeding Using Microsatellite Markers

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ABSTRACT

It was provided an assessment of the genetic diversity in onion varieties of Ukrainian breeding based on the using of microsatellite markers and research findings of the allelic composition of the six microsatellite loci. The polymorphism ranged from 1–3 alleles per locus, indicating a low level of diversity among the cultivars. UPGMA cluster analysis based on genetic distance coefficients, distinctly separated all the varieties into two groups, and showed that the Ukrainian onion varieties are closely related. The formed clusters represent the significant stages in breeding headed by specific breeders. The dendrogram describing the stages of the onion variety genesis at the Institute of Vegetables and Melon Growing at National Academy of Agricultural Sciences (NAAS) from 1967 to 2006. Further research and experiments will be aimed at studying additional markers and genetic resources that are used in the breeding programs to create hybrids.

Keyword: Allele; electropherograms; locus; markers; dendrogram

INTRODUCTION

The onion (*Allium cepa* L.) is one of the most valuable and the most common vegetables in Ukraine. Every year from 45 to 80 hectares of land in Ukraine is cultivated to grow onions. And though in the recent past Ukraine used to import onions, starting from 2006 the country has positioned itself as a significant exporter of this agricultural produce.

The cultivars of onion, as the main type of cultivated onions in Ukraine are characterized with rich historical and research traditions. The Register of Ukrainian Plant Varieties contains 45 varieties of long-day bulb onion with 53% of them being the products of national breeding. The variety as an object of intellectual property rights requires protection of the breeders' copyrights for them to benefit from their use of up-to-date approaches and methods, including those based on molecular markers.

The onion diploid number (2n = 2x = 16) contains 32830 Mbp [1], which is, for comparison, 34 times as high as that of the genome of rice. 92-95% of the onion genome is represented by a non-coding DNA, including the fraction of microsatellite repeats. Molecular markers, developed on their basis, are a very

effective instrument for the assessment of the genetic variety of agricultural cultivars that is widely used for identification of varieties, including bulb onions [2].

The aim of our research is to analyze the genetic diversity of bulb onion varieties of Ukrainian breeding. The aim consisted of the following **tasks**: the variety diversification based on microsatellite markers, cluster analysis, development of identification formulae.

MATERIAL AND METHODS

Plant material

The source material for our research consisted of the 8 onion varieties (*Allium cepa* L.) developed by Institute of Vegetables and Melon Growing at NAAS (Table 1).

Genomic DNA isolation

DNA was extracted with the use of the DNA extraction set by "Agrogen" Ltd, Ukraine. Polymerase chain reaction (PCR) was conducted under the following temperature and time regime: first denaturation – 5 °C, 4 min; 28 cycles: 94 °C, 30 sec, 58 °C, 45 sec, 72 °C, 1 min; final elongation – 72 °C, 8 min. The reaction mixture contained: 1x DreamTaq Buffer with 2,0 mM $MgCl_2$ (Thermo Scientific, USA), 1 unit. DreamTaq-polymerase, 0,2 mM of each dNTP, 0,25 mM of each primer (MetaBion, Germany), 1 µl DNA. The sequence of primers to study the microsatellite loci – ACM004, ACM013, ACM018, ACM091, ACM115, ACM151 – according to J. Jakse [3] and K. Mitrova [4].

Table 1: The list of onion varieties analyzed in the present research

Variety	Breeders	Years when	
		the variety was registered	Planting regionalization was conducted
Amphora	Shabetia V.	2001	2004
Belyanochka	Shabetia V.	2001	2004
Veselka	Tymchuk V., Bilenka O.	1991	1994
Globus	Tymchuk V.	1995	1997
Zolotysta	Tkachenko F.	1967	1972
Lyubchik	Tymchuk V., Bilenka O.	2003	2006
Mavka	Tymchuk V.	2000	2003
Tkachenkivska	Tkachenko F.	1985	1991

Electrophoretic separation: of the extracted DNA – in 0.8% agarose gel, of the amplification products – in 2.0% agarose gel for primary identification of their presence and in 10.0% nondenaturating polyacrylamide gels in order to identify the allele sizes in the TBE-buffer. Visualization of the amplification products was ensured with the use of ethidium bromide as a fluorescent tag. The obtained electropherograms were recorded with a digital video camera.

Statistical analysis

The size of the amplification fragments was determined with the use of GeneAnalyzer 2010 software (free access). The genetic distances calculation as well as the cluster analysis and the graphic dendrogram development were made with the use of UPGMA method and MEGA6.0 as well as TREES 4.0 software (free access).

RESULTS AND DISCUSSION

Differentiation of onion varieties. In order to study molecular and genetic polymorphism of onion varieties we have selected from theoretical publications 6 microsatellite (MS) loci using the following criteria: the location on different chromosomes, the presence of trinucleotide repeats, PIC (Polymorphism information content) value above 0.25. Table 2 presents information on the selected MS loci of onion.

Table 2: Information on MS loci of bulb onion used in the present research

Microsatellite locus	The type of repeat	PIC	The size of the amplification fragment according to	
			Mitrova et al., 2015[4]	Jakse et al., 2005[3]
ACM004	$(CAA)_4$	0.36	201, 205, 212	203, 206, 213
ACM013	$(TCC)_9$	0.31	165, 168, 174	183, 186, 192
ACM018	$(CTT)_6$	0.28	275, 278	275, 278
ACM091	$(TCT)_{10}$	0.29	172, 174, 178	177, 183
ACM115	$(CAC)_6$	0.31	220, 223	239, 242
ACM151	$(ACA)_5$	0.37	243, 245, 247	264, 266

The use of the PCR-analysis of the six MS loci allowed studying the polymorphim of the 8 bulb onion varieties of Ukrainian breeding. PCR-analysis findings are presented in Table 3.

Table 3: Findings of the microsatellite analysis of loci in onion varieties

The variety name	The size of the microsatellite loci amplification fragment, bp					
	ACM004	ACM013	ACM018	ACM091	ACM115	ACM151
Amphora	212, 212	165, 165	270, 270	172, 183	237, 237	245, 264
Belyanochka	212, 212	165, 165	277, 277	172, 177, 183	237, 237	245, 264
Veselka	213, 213	165, 165	277, 277	172, 177, 183	237, 237	245, 245
Globus	213, 213	165, 165	277, 277	177, 183	237, 237	245, 264
Zolotysta	213, 213	165, 165	277, 277	183, 183	237, 237	245, 245
Lyubchik	212, 213	165, 165	277, 277	172, 177, 183	237, 237	245, 245
Mavka	213, 213	165, 165	277, 277	172, 177, 183	237, 237	245, 245
Tkachenkivska	213, 213	165, 174	277, 277	177, 183	237, 237	245, 245

* The presence of three alleles can be explained by the fact that DNA was extracted from the mixture of seeds.

12 alleles were detected – ranging from 1 to 3 per locus. So, in the present sample of varieties ACM115 locus turned out to be non-polymorphic: one allele with the size of 237 bp was identified. The rest of the loci had two alleles per locus - for ACM004, ACM013, ACM018, ACM151 loci, and three - for ACM091 locus.

ACM091 locus for four varieties contained three alleles per each. It can be explained by the fact that DNA was extracted from the mixture of seeds. This fact also indicates the heterogeneity of some varieties. There is a need to verify the variety purity of each individual variety sample with the use of PCR-method.

Cluster analysis of onion varieties. In order to provide graphic visualization of differences between the analyzed varieties the following dendrogram was constructed. The data on the alleles of each variety was used to identify the genetic distances between them and subsequently construct the dendrogram (Fig. 1). The dendrogram has two clusters. Associations between varieties revealed the reconstruction of the genesis stages for the breed varieties created by the Institute of Vegetables and Melon Growing from 1967 to 2006 (Table 1). The formed clusters represent the significant stages in breeding headed by specific breeders. Thus, such varieties as Amphora with its burgundy color of the skin and Belyanochka with its white skin in Cluster I were developed by the breeders headed by Shabetia V. the significant stages in breeding headed by specific breeders.

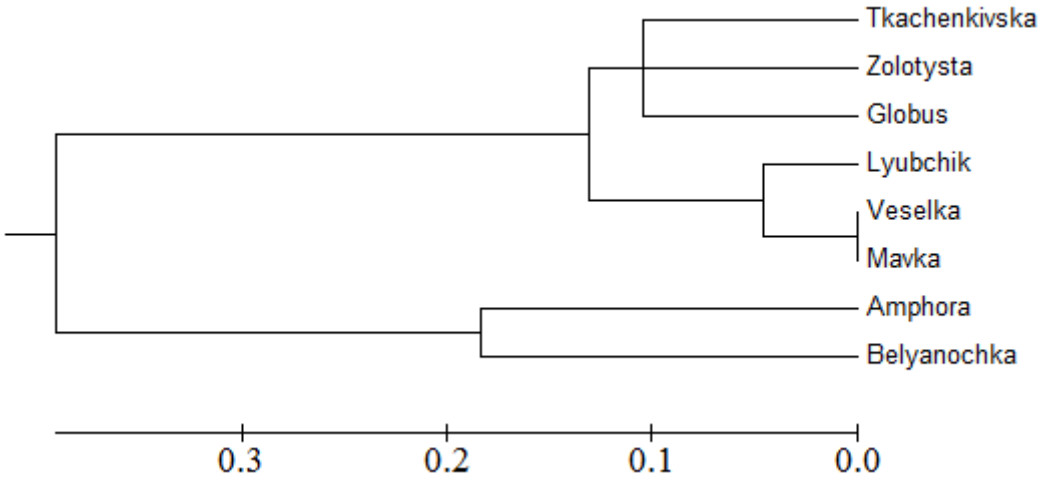

Fig.1. The onion varieties dendrogram based on the microsatellite analysis data. Genetic distances, standard units

Thus, such varieties as Amphora with its burgundy color of the skin and Belyanochka with its white skin in Cluster I were developed by the breeders headed by Shabetia V. while he was developing and studying the collection of genetic resources for the National Center for Genetic Resources of Plants in Ukraine (1990-2004). The other main cluster consists of samples developed by the school of Tkachenko F. Cluster II consists of two subclusters. The first subcluster consists of varieties developed by Tkachenko F. (1967 - 1997) with local forms – Tkachenkivska, Zolotysta and Globus; they were developed pursuant to his selection methods with the use of foreign forms from Southern Europe, theirs skins are of yellow-brown color and the shape is close to that of a ball. The second subcluster consists of varieties developed by his students: Bilenka O. and Tymchuk V. (1991-2006) with the use of the germplasm from Central Europe countries. At the same time the varieties with the violet-red color of the skin – Mavka and Veselka – are located separately from Lyubchik variety with its elliptical form and yellow color of the skin.

Comparison with the MS analysis findings from other samples. Here we present a number of examples of similar MS-marker-based research into onion samples from various geographic regions. So, the MS analysis of onion samples from India by 60 MS loci shows in clusterization a spinning off of a local short-day varieties group [2].

While studying the collection of 85 local samples and wild relatives of onions in Spain using 12 (out of 18) MS loci all the samples were differentiated and in three local forms the presence of specific alleles was identified [1]. Clusterization was not based on the geographical location.

Out of 21 MS loci 15 have allowed differentiating 16 commercial varieties of onion of Czech breeding [4]; 96 local samples, breeding lines and commercial varieties of onions from Turkey were differentiated by 46 MS loci [5].

The above mentioned research are characterized by a larger number of MS markers employed thus resulting in the differentiation of all the samples. It must be noted that the selected 6 MS markers in our research did not allow differentiating two varieties – Veselka and Mavka. So there is a need to widen the panel of markers and verify it on a certain selection of varieties.

One of the tasks of the present research was identified as the development of identification formulae. But we believe that the heterogenity of some varieties and the insufficient distribution capacity of the given panel of MS markers make it at the present stage not expedient to record the analyzed varieties in the form of identification formulae.

CONCLUSIONS

In the sample of the 8 onion varieties of Ukrainian breeds there have been identified from 1 to 3 alleles of the six MS loci. Despite the

fact that the genetic basis of commercial varieties is limited, the panel of markers employed in the research can be used for their differentiation but it must be enlarged. Associations between varieties in the MS analysis revealed the reconstruction of the genesis stages for the breed varieties created by the Institute of Vegetables and Melon Growing from 1967 to 2006. Further research and experiments will be aimed at studying additional markers and genetic resources that are used in the breeding programs to create hybrids.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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