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Allelic status of the microsatellite loci in modern grain sorghum varieties

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The results of analysis of 26 grain sorghum varieties selected in the Institute of Grain Crops of NAAS on four microsatellite loci (markers Sb4-32, Sb4-121, Sb6-57, Sb6-84) are represented. 33 alleles, in average 8.3 alleles per locus, were identified indicating a high level of genetic diversity of the studied varieties. Index of polymorphism information content of the given SSR-markers for the array of studied samples ranged from 0.42 to 0.73. Identified allelic status of the microsatellite loci is recommended for application in breeding process for certification, identification and registration of grain sorghum varieties of national selection.

Key words: *grain sorghum, SSR-markers, alleles, polymerase chain reaction, locus.*

Grain sorghum (*Sorghum bicolor* L.) is a promising food, forage and technical crop with a lot of advantages over the other grain crops due to its drought resistance and broad use for food and technical purposes. 100 kg of grain sorghum contain 118-130 feed units, its grain contains 71-82% carbohydrates, 12-15% protein, 3-5% fat, and 2.4-4.8%cellulose [1]. Effective and modern method of new crop varieties production is MAS-selection (**M**arker-**A**ssisted **S**election). MAS-selection with DNA-markers allows to conduct the selection for the improvement a trait directly through favorable genotype screening and to make comparative evaluation of the allelic status of DNA-markers for genotyping, certification, identification and registration of varieties[2]. Microsatellite markers are widespread type of DNA markers. Microsatellites (**S**ingle **S**equence **R**epeats, SSR) are short, from 1 to 6 nucleotides tandem repeats with a different number of copies. They represent high polymorphic regions of DNA with dozens of alleles at each locus and high rate of mutation. Alleles of microsatellite locus differ from each other in the lengths of amplified DNA fragments and in a number of repeats [2-4]. In plants short microsatellite repeats are abundant. They represent a substantial portion of noncoding DNA, however, a large number of microsatellites which contain GC-pairs are located in the coding regions of genome [3, 4]. Microsatellite markers (SSR-markers) are monolocus, polyallelic, codominant. These features permit their use as genetic markers for estimation of allelic status of loci in genomes of crops[2], among them in sorghum. Allelic status of sorghum microsatellite loci was previously investigated in the publications of Ukrainian [5, 6] and foreign [7-10] scientists, but the permanent production of new varieties of this crop requires molecular-genetic evaluation of initial and elite breeding materials. **The purpose of the research**. The purpose of the research was to characterize the allelic status of microsatellite loci in modern varieties of grain sorghum for application in breeding process. **Materials and methods.**26 varieties of grain sorghum, created in the steppe zone of Ukraine in Sinelnikovo breeding and experimental station (Dnipropetrovsk region) and Genichesk breeding station (Kherson region) of Institute of Grain Crops of NAAS of Ukraine served as the materials for the research. These varieties are pure lines and are propagated through self pollination. DNA was isolated from seedlings by CTAB-method according to plant DNA isolation protocol [11]. Clarification of the DNA concentrations was performed spectrophotometrically. The purity of DNA specimens was determined according to the ratio

of spectral absorption at wavelengths of 230, 260, 280 and 320 nm [12]. The DNA concentration in the samples was adjusted to 20 ng/ml.

Four SSR-markers used for the study of DNA polymorphism in grain sorghum are presented in Table 1.

Table 1. Characteristics of SSR-markers [5, 7, 13]

SSR-marker	Repeat (motif)	Sequences of primers to identify allelic status of SSR-marker (5'-3 ')
Sb4-32	AG	F: gaa aaa tct ccg tca atc cca aaa taa R: cgc tga aca acg aaa gga ata agt g
Sb4-121	AC	F: gaa aaa tct ccg tca atc cca aaa taa R: cgc tga aca acg aaa gga ata agt g
Sb6-57	AG	F: aca ggg ctt tag gga aat cg R: cca tca ccg tcg gca tct
Sb6-84	AG	F: cgc tct cgggat gaa tga R: taa cgg acc act aac aaa tga tt

For markers Sb6-57 and Sb6-84 duplex-PCR was conducted under the following conditions: initial denaturation at a temperature of 96°C for two minutes; then 35 cycles at 94°C for one minute, at 55°C for 30 seconds and at 72°C for one minute; final elongation at a temperature of 72°C for two minutes. Polymerase chain reaction for marker Sb4-121 and Sb4-32 was carried out under the following conditions: initial denaturation at a temperature of 94°C for two minutes; then 40 cycles at 94°C for 30 seconds; at 58°C for 30 seconds and at

72°C for one minute; final elongation at a temperature of 72°C for five minutes. Amplification products were separated by electrophoresis in a horizontal agarose gel (3%) on the device for electrophoresis Sub-cell GT (Bio-Rad) at a voltage of 5

V/cm and under the room temperature. For the analysis of the obtained results, in particular for the visualization of DNA fragments (amplicons), 5 μl/l of ethidium bromide was added to Tris-borate buffer (TBE), gels were analyzed on the device for visualization GelDocTM (Bio-Rad).

Index of polymorphism information content for marker *i* (PIC) was calculated by the formula [5]:

$$PIC = 1 - \sum_{i=1}^{n} fi^2$$ where *fi* - frequency of *i*-allele, *n* - number of alleles,

and was expressed in parts of a unit. PIC>0,25is considered high index of polymorphism information content. The frequency of allele occurrence was calculated as a ratio of the number of samples in which the given allele was registeredto the total number of samples analyzed and was expressed in parts of a unit.

The allele of the most common occurrence in a group of investigated samples was considered as a major allele. The frequency of a major allele for marker *i* was defined as a ratio of a number of breeding samples with major allele of marker *i* to the total amount of analyzed samples.

Results and discussion

All investigated samples of grain sorghum were homozygous in analysed microsatellite loci. Polymorphism of amplicon size in 26 varieties is presented in Table2.

Table 2. Characteristics of four microsatellite loci in 26 varieties of grain sorghum

SSR-marker	Number of investigated varieties	Number of alleles	Min-max lengths of alleles, bp	Length of major allele major,bp	The frequency of major allele	PIC
Sb4-32	26	7	156-190	173	0,23	0,42
Sb4-121	26	9	197-249	203	0,23	0,57
Sb6-57	25	8	250-294	282	0,32	0,73
Sb6-84	26	9	157-205	180	0,31	0,67

A number of alleles of four investigated microsatellite loci in selected 26 sorghum varieties ranged from 7 (Sb4-32) to 9 (Sb4-121 Sb6-84). 33 alleles were identified in total, in average 8.3 alleles per marker, which indicated a relatively high genetic diversity of studied samples. The diapason of allele lengths ranged of from 34 bp for Sb4-32 to 52 bp for Sb4-121. For two other markers this ratio was 44 bp for Sb6-57 and 48 bp for Sb6-84. Major alleles for markers Sb4-32 and Sb6-84 occupied the median position among the other alleles, while for the other two markers they were shifted towards shorter (Sb4-121) and longer (Sb6-57) alleles. An allele defined as a major one was met in 23% of the studied varieties for Sb4-

32 and Sb4-121 and in 31% and 32% of investigated varieties respectively for markers Sb6-84 and Sb6-57. Overall, a portion of varieties which demonstrate a major allele at the level of 23-32% indicates considerable genetic diversity in the studied collection of grain sorghum varieties on analyzed loci. Index of polymorphism information content for markers used was high enough, of 0.42 (Sb4-32) to 0.73 (Sb6-57). The average value of polymorphism information content index was 0.60, which confirms the high discrimination level of the chosen marker system.

Comparison of the obtained experimental data for grain sorghum varieties created in the steppe zone of Ukraine with the data on the allelic status of the same microsatellite loci Sb4-32, Sb4-121, Sb6-57and Sb6-84 in varieties of world collections to [5-10, 13, 14] showed that the number of alleles for each marker in the two groups was about the same level. The range of allele lengths for the national collection of varieties is slightly wider, but for Sb4-121 much broader than in results of other authors. Bounds of amplicons lengths in the national breeding material are shifted toward shorter alleles for markers Sb4-32, Sb6-57 and Sb6-

84,but PIC of all the markers is somewhat or substantially less than in the analysis of foreign varieties. The average number of alleles per locus in various studies [7,

8, 10] for 9-15 SSR-markers ranged between 4.4 and 18.3 proportionally increasing with the enlargement of investigated group from 27 to 380 samples. In our study of 26 varieties, having been selected in the steppe zone, the average number of alleles per marker SSR-locus was 8.3. Thus, the results indicate the similarity of grain sorghum varieties of Ukrainian selection and world collection in allele number of four investigated microsatellite loci and in average number of alleles per locus. However, in national varieties the diapason of allele lengths is wider, the borders of amplicon lengths are shifted toward shorter alleles, and PIC has smaller values.

The revealed characteristics of the allelic status of microsatellite markers Sb4-32, Sb4-121, Sb6-57 and Sb6-84 allowed designating the individual peculiarities of 26

Ukrainian varieties of grain sorghum Ukrainian selection, which can be used for their certification. A sufficiently large number of alleles at each locus provided the uniqueness of each sorghum variety in four microsatellite markers that is necessary for typing, identification and registration of grain sorghum varieties.

Conclusions

The allelic status of microsatellite markers Sb4-32, Sb4-121, Sb6-57 and Sb6-84 in 26 grain sorghum varieties was determined. 33 alleles were identified, in average of 8.3 alleles per locus, indicating a high level of genetic diversity of the varieties studied. Index of polymorphism information content for investigated markers and examined varieties ranged from 0.42 to 0.73. The allelic status of SSR-markers can be used to characterize the polymorphism of microsatellite loci, as well as for the certification, identification and registration of national grain sorghum varieties.

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