UDC 631.522:58.083.5

SNP-GENOTYPING AND ALLELE STATUS IN EARLY MAIZE INBREDS

[1,2] ***T. M. Satarova,*** *doctor of biological sciences;*
V. Yu. Cherchel, *candidate of agricultural sciences;*
O. E. Abraimova, *candidate of biological sciences*
V. V. Borisova, Yu. O. Goncharov
[1]SE Agricultural Steppe Zone Institute of NAAS
O. M. Starostina
[2]Ukrainian State University of Chemistry and Technology

The results of the analysis of single nucleotide polymorphism (SNP) on 384 DNA-markers for Ukrainian maize inbreds of different maturity groups are represented. Alleles of top-SNP-markers which are specific for early inbreds in comparisons with early-middle, middle and late-middle ones are determined.

Key words: *maize, single nucleotide polymorphism, SNP-marker, early maturing, early-middle inbreds, middle inbreds, late-middle inbreds.*

Analysis of DNA single polymorphism (SNP-analysis) is a modern molecular method of crop marker-assisted selection [4]. The investigations of the ways of potential application of SNP-analysis have confirmed its high efficiency for genotype certification and heterosis prediction in maize [1]. The actual task is the elaboration of methodological principles of application of SNP-genotyping for marker-associated selection of valuable traits. The attraction of SNP-alleles for maize phenotypical markering has been researched for several parameters of abiotic and biotic tolerance [6; 7] and grain quality [3]. SNP-markering in traits connected with the duration of period of vegetation has not been provided yet. The aim of the given investigation is the SNP-genotyping of maize lines differed in maturity groups and the comparative analysis of their allele status.

As the material for SNP-genotyping maize inbred lines of Ukrainian selection were used: early, FAO 150–200 (48 inbreds), early-middle, FAO 201–300 (25 inbreds), middle, FAO 301–400 (123 inbreds) and late-middle, FAO 401–450 (19 inbreds). SNP-genotyping had been fulfilled with GoldenGate-testing, reading system Illumina VeraCode and Sentrix array matrice (SAM) [2]. SNP-analysis has been provided on 384 SNP-markers arranged in panel BDI-III. This SNP-panel had been elaborated on the base of Illumina VeraCode Bead Plate and kindly presented for the given investigation by BioDiagnostics, Inc. (USA). 384 SNP-markers had been selected as the most suitable for the estimation of polymorphism in modern maize breeding germplasm [5]. SNP-markers of BDI-III-panel cover all ten maize chromosomes, have score rank >0,6, are biallelic.

DNA was isolated from 7-day maize seedlings. SNP-genotyping was done in automatic regime with equipment Illumina BeadStation 500G (Illumina, Inc. San Diego, CA, USA) incomeple-ted with BedReader for the detection of the results of single nucleotide polymorphism identifica-tion. After genotyping of each group of inbreds, for each marker the frequencies of major and minor alleles were calculated.

For the identification of alleles connected with the demonstration of alternative groups of maturity the pair comparisons of frequencies of major and minor SNP-alleles of early inbreds and other maturity groups were made. For each SNP-marker the probabilities of disequilibrium of the ratio of frequencies of major and minor alleles in groups were estimated according to χ^2-method at the significance level 0,05. For further analyses markers with $\chi^2_{fact} \geq \chi^2_{0,05}$ were selected. For each marker with reliable disequilibrium of the given ratio the differences between the frequencies of major alleles in groups of early-middle, middle and late-middle lines and the frequencies of the same-named alleles in early group were determined. These differences were marked as “D” and are represented in tables as absolute values. After ranging markers according to value of “D” the first ten SNP-markers (*top*-markers) were chosen for further analysis. Then the formula of allele status of ten *top*-markers (haplotype formula) was determined.

Maize inbred genotyping has shown that 367 out of 384 SNP-markers of BDI-III-panel are representative for breeding samples of Ukrainian selection. The total number of SNP-markers with significant disequilibrium of allele ratio between early and other maturity groups is presented in table 1.

1. Comparison of maize maturity groups on SNP-markers

Compared groups of maize inbreds	SNP-markers with significant disequilibrium of allele ratio	
	number	%
early – early-middle	178	48,5 ± 5,8
early – middle	213	58,0 ± 7,5
early – late-middle	238	64,9 ± 11,7

In pair comparisons of maturity groups 48,8–64,9 % of SNP-markers had disequilibrium of allele ratio proved by χ^2-method. In tables 2–4 the frequencies of major alleles for ten *top*-markers with the biggest estimates of index D are shown. They have the most rank alteration of major allele frequencies in transfer to the group of early maize inbreds.

2. Frequencies of major alleles of top-SNP-markers in pair comparison of early and early-middle maize inbreds

SNP-marker	Major allele frequency in the group of early-middle inbreds	Frequency of the same-named allele in the group of early inbreds	D	$\chi^2_{fact.}$
219AG	A = 0,71 ± 0,07	A = 0,28 ± 0,14	0,43	91,1
223AT	T = 0,50 ± 0,08	T = 0,09 ± 0,09	0,41	68,7
328AT	T = 0,69 ± 0,07	T = 0,32 ± 0,14	0,38	66,3
175AG	A = 0,81 ± 0,06	A = 0,47 ± 0,15	0,34	77,0
174AG	A = 0,81 ± 0,06	A = 0,47 ± 0,15	0,34	77,0
261AG	G = 0,64 ± 0,11	G = 0,31 ± 0,18	0,33	47,6
292AG	A = 0,77 ± 0,07	A = 0,45 ± 0,16	0,32	59,1
218AG	G = 0,80 ± 0,06	G = 0,49 ± 0,15	0,32	63,0
20AG	G = 0,53 ± 0,08	G = 0,83 ± 0,13	0,30	35,7
16AG	A = 0,53 ± 0,08	A = 0,82 ± 0,11	0,30	35,1
Average	0,68 ± 0,07	0,29 ± 0,14	0,35	-
$\chi^2_{0,05}$				3,8

In tables 2–5 the denomination of SNP-marker consists of its number in BDI-III-panel and potential allele status. SNP-alleles are marked according to the nitrogenous bases of DNA: A-adenine, T-thymine, G-guanine, C-cytosine.

3. Frequencies of major alleles of top-SNP-markers in pair comparison of early and middle maize inbreds

SNP-marker	Major allele frequency in the group of middle inbreds	Frequency of the same-named allele in the group of early inbreds	D	$\chi^2_{fact.}$
309AG	A = 0,92 ± 0,05	A = 0,40 ± 0,15	0,52	356,6
223AT	T = 0,60 ± 0,09	T = 0,09 ± 0,09	0,51	109,7
332AG	A = 0,74 ± 0,08	A = 0,26 ± 0,13	0,48	122,4
219AG	A = 0,75 ± 0,08	A = 0,28 ± 0,14	0,47	121,7
261AG	G = 0,77 ± 0,12	G = 0,31 ± 0,18	0,46	121,4
16AG	G = 0,62 ± 0,09	G = 0,18 ± 0,11	0,44	80,9
310AG	G = 0,72 ± 0,08	G = 0,32 ± 0,14	0,40	79,1
20AG	A = 0,56 ± 0,09	A = 0,17 ± 0,13	0,39	60,4
112AG	G = 0,58 ± 0,09	G = 0,20 ± 0,12	0,38	59,4
297AG	G = 0,55 ± 0,09	G = 0,17 ± 0,11	0,37	55,9
Average	0,68 ± 0,09	0,24 ± 0,13	0,44	-
$\chi^2_{0,05}$				3,8

4. Frequencies of major alleles of top-SNP-markers in pair comparison of early and late-middle maize inbreds

SNP-marker	Major allele frequency in the group of late-middle inbreds	Frequency of the same-named allele in the group of early inbreds	D	$\chi^2_{fact.}$
309AG	A = 0,95 ± 0,10	A = 0,40 ± 0,15	0,54	600,9
310AG	G = 0,84 ± 0,17	G = 0,32 ± 0,14	0,52	205,7
295AG	A = 0,56 ± 0,23	A = 0,11 ± 0,09	0,51	143,6
274AG	G = 0,84 ± 0,17	G = 0,34 ± 0,14	0,50	186,3
329AG	G = 0,58 ± 0,23	G = 0,11 ± 0,10	0,47	91,6
215AC	A = 0,56 ± 0,23	A = 0,11 ± 0,10	0,44	80,0
216AC	A = 0,56 ± 0,23	A = 0,11 ± 0,10	0,44	80,0
147AG	A = 0,84 ± 0,17	A = 0,10 ± 0,14	0,44	144,2
148AG	A = 0,84 ± 0,17	A = 0,40 ± 0,14	0,44	144,2
136AC	C = 0,84 ± 0,17	C = 0,41 ± 0,15	0,43	141,0
Average	0,74 ± 0,19	0,27 ± 0,14	0,47	-
$\chi^2_{0,05}$				3,84

5. The summarized results of SNP-markering early maturity in maize

Number of SNP-marker and major allele in early inbreds	Frequency of major allele in the group of early lines	Frequency of the same-named allele in the group of		
		early-middle inbreds	middle inbreds	late-middle inbreds
SNP-markers for early lines in comparison with early-middle and middle ones simultaneously				
219G	0,72	0,29	0,25	0,68
223A	0,91	0,49	0,40	0,53
261A	0,69	0,36	0,23	0,20
20G	0,83	0,53	0,44	0,53
16A	0,82	0,53	0,38	0,58
SNP-markers for early lines in comparison with middle and late-middle ones simultaneously				
309G	0,60	0,32	0,28	0,05
310A	0,68	0,56	0,28	0,16
SNP-markers for early lines in comparison with early-middle ones				
328A	0,68	0,31	0,32	0,42
175G	0,53	0,19	0,20	0,26
174G	0,53	0,19	0,21	0,26
292G	0,55	0,23	0,25	0,44
218A	0,51	0,20	0,17	0,21
SNP-markers for early lines in comparison with middle ones				
332G	0,74	0,48	0,26	0,47
112A	0,80	0,57	0,42	0,37
297A	0,83	0,55	0,45	0,79
SNP-markers for early lines in comparison with late-middle ones				
274A	0,66	0,49	0,54	0,16
295A	0,74	0,62	0,56	0,24
329A	0,89	0,84	0,76	0,42
215C	0,89	0,85	0,78	0,44
216C	0,89	0,85	0,78	0,44
147G	0,60	0,37	0,32	0,16
136A	0,59	0,39	0,23	0,16
148G	0,60	0,37	0,32	0,16

The range of major allele frequencies in the group of early-middle maize lines for ten *top*-SNP-markers was 0,50–0,81, but in the group of early lines for the same alleles was changed into 0,09–0,83. The raising of the top limit of frequencies in the group of early lines is connected with those facts that for markers 20AG and 16AG not the decreasing, but the increasing of frequencies of the same-named alleles had happened. The allele status of *top*-SNP-markers typical for the early group on major allele frequencies identifying in pair comparison with the early-middle group is BDI-III-219G, BDI-III-223A, BDI-III-328A, BDI-III-175G, BDI-III-174G, BDI-III-261A, BDI-III-292G, BDI-III-218A, BDI-III-20G, BDI-III-16A.

The range of major allele frequencies of ten *top*-SNP-markers in the group of middle lines varied from 0,55 to 0,92. In the group of early lines in comparison with middle ones the frequencies of the same alleles decreased to 0,09–0,40. The markers of early maize on the frequencies of major alleles in the pair comparison with middle lines are: BDI-III-309G, BDI-III-223A, BDI-III-332G, BDI-III-219G, BDI-III-261A, BDI-III-16, BDI-III-310A, BDI-III-20G, BDI-III-112A, BDI-III-279A.

The range of the frequencies of major SNP-alleles in the group of late-middle lines composed 0,56–0,95. The frequencies of the same alleles in early lines in comparison with late-middle decreased significantly and accounted 0,11–0,41. Early maize lines in pair comparison with late-middle ones can be described with the next formula: BDI-III-309G, BDI-III-310A, BDI-III-295G, BDI-III-274A, BDI-III-329A, BDI-III-215C, BDI-III-216C, BDI-III-147G, BDI-III-148G, BDI-III-136A.

In table 5 the summarized results of SNP-marking the trait of early maturity in maize in comparison with groups of early-middle, middle and late-middle lines are shown.

Among identifying *top*-SNP-markers the common markers in comparison of the group of early lines with together early-middle and middle ones have not been found. The markers which would characterize early lines simultaneously in comparison with early-middle, middle and late-middle were absent too.

The summarized table 5 shows that identifying markers describe major allele frequencies in early group in range 0,51–0,89. These estimates differ greatly from the frequencies of the same-named alleles in other analyzed groups of maturity. This fact is remarkable in pair comparisons with early-middle and middle lines. Not very big difference in allele frequencies between early and late-middle lines could be explained through the limited size of the last group.

Conclusions. The summarizing haplotype of early group of maize inbreds is determined as BDI-III-219G, BDI-III-223A, BDI-III-261A, BDI-III-309G, BDI-III-310A. This formula is recommended for application in maize breeding to prognose the early maturity of initial material. For the detalization of the status of early maturity the characteristics of allele status of SNP-markers identifying in pair comparisons with other groups of maturity have to be also taken into account.

Bibliography

1. Оцінка різних типів гібридів кукурудзи за генетичними дистанціями та ступенем гетерозису / [*В. Ю. Черчель, Б. В. Дзюбецький, В. В. Борисова, Т. Н. Сатарова*] // Вісн. аграр. науки. – 2013. – № 8. – С. 33–37.
2. Illumina universal bead arrays / *J. B. Fan, K. L. Gundetsson, M. Bibikova* [et al.] // Methods Enzymol. – 2006. – Vol. 410. – P. 57–73.
3. Natural variation in the sequence of PSY1 and frequency of favorable polymorphisms among tropical and temperate maize germplasm / *Z. Fu, Y. Chai, Y. Zhou* [et al.] // Theor. Appl. Genet. – 2013. – Vol. 126. – № 4. – P. 923–935.
4. *Syvänen A.-Ch.* Accessing genetic variation: genotyping single nucleotide polymorphism / *A.-Ch. Syvänen* // Nature reviews / Genetics. – 2001. – Vol. 2. – P. 930–942.
5. Development and characterization of single nucleotide polymorphism (SNP) panel for markers-assisted backcrossing in corn [Електронний ресурс] / *P. Venkatramana, C. Carison, M. Blackstad* [et al.] – Режим доступу: http://www.biodiagnostics.net/wp-content/up-loads/2012/07/singlenuc leotide.pdf. – Назва з екрану.
6. Molecular mapping of the major resistance quantitative trait locus qHS2.09 with simple sequence repeat and single nucleotide polymorphism markers in maize / *J. Weng, X. Liu, Z. Wang* [et al.] // Phytopathology. – 2012. – Vol. 102, № 7. – P. 692–699.

7. Genome-wide association analysis for nine agronomic traits in maize under well-watered and water-stressed conditions [Електронний ресурс] / *Y. Xue, M. I. Warburton, M. Sawkins* [et al.].// Theor. Appl. Genet. – 2013. – Режим доступу: PMID: 23884600. – Назва з екрану.