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Comparative single nucleotide polymorphism analysis of maize Iodent and BSSS germplasms

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Abstract

The analysis of single nucleotide polymorphisms of 107 maize inbreds was performed on 384 special single nucleotide polymorphism (SNP) markers to receive their unique certificates and determine their degrees of affinity and heterotic potential. All inbreds were selected in the steppe zone of Ukraine; among them, 39 inbreds belonged to the Iodent and 28 inbreds to the BSSS germplasms. 40 inbreds of the Iodent/BSSS breeding group developed after hybridization of Iodent and BSSS, were also analysed by the same SNP markers. The average homozygosity of lines amounted to 98.05%, and the genetic diversity was 0.1746. According to pairwise SNP distances, lines of the Iodent and BSSS pedigrees formed two separate clusters, while the Iodent/BSSS lines were distributed among them. The allelic patterns of SNP markers specific for Iodent and BSSS inbred lines in comparison with each other and with the original inbreds P165, B14, B37, and B73 were formulated and discussed. Inbreds within the Iodent germplasm were on average much more closely related (GD = 0.2494) than those within the BSSS germplasm (GD = 0.3900) and the Iodent/BSSS breeding group (GD = 0.3967). The potential of the heterotic model Iodent×BSSS was assessed as high (mean GD = 0.4509). Based on SNP distances, inbreds have been recommended for the development of single cross heterotic hybrids, sister hybrids, and initial populations for subsequent breeding cycles.

Keywords: genetic distances, heterosis, molecular markers, single nucleotide polymorphism, *Zea mays*.

Introduction

Changes of single nucleotides in DNA constitute a widespread type of gene mutations and lead to single-nucleotide polymorphisms (SNPs), the most common background of the genome polymorphism within species. In plant biotechnology single-nucleotide changes are often applied as SNP markers in genotype identification, certification, affinity determination, clustering and seed purity assessment (Syvänen 2001) or in marker-assisted selection for the improvement of economically valuable and other traits (Hasan *et al.* 2021). Modern panels of SNP markers differ in size and selection criteria, while technologies of SNP analysis are continually evolving (Xu *et al.* 2017, Chen *et al.* 2021).

Maize (*Zea mays* L.) is a very suitable and efficient model in genetic and biotechnological research. Intensive investigations on heterosis (Xiao *et al.* 2021), full plant

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Abbreviations: df - degrees of freedom; GD - pairwise genetic distance; MS - mean squares; PIC - polymorphic information content value; SNP - single nucleotide polymorphism; SS - sums of squares.
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genome sequencing (Schnable *et al.* 2009), genetic modification (Armstrong *et al.* 1995), haploid production (Cherchel *et al.* 2020), and molecular markers (Xu *et al.* 2017) were performed on maize as a model plant. The results allowed to determine the peculiarities and genetic control of a majority of life processes in plants, as well as to develop modern breeding technologies. *Z. mays* is also a valuable food, feed, and technical plant and one of the most common crops in the world (Ranum *et al.* 2014). In the 2021/2022 3.46% world maize production was in Ukraine (https://www.statista.com/statistics/1156213/global-corn-production/).

The variability of possible ways of maize evolution, the long period of "folk" selection in the 19th and early 20th centuries, and achievements of scientific selection in the 20th and 21st centuries have led to a significant intraspecific genotypic diversity and have given rise to a great number of currently existing forms (Buckler *et al.* 2006, Qu and Liu 2013).

Since the beginning of the 20th century, the model of the development of highly productive crop genotypes has been based on the phenomenon of heterosis and involves inbreeding to obtain homozygous lines and hybridization of selected inbred lines for a maximum heterotic effect. Heterosis value significantly depends on the degree of relatedness of parental components, which is directly connected with the similarity of their genomes (Rehman *et al.* 2021). Thus, the structurization of a multitude of maize genotypes according to their origin, inheritance, and interrelationships is highly important.

The concept of germplasm is widely used in the analysis of the intraspecific genetic diversity of cultivated species. A specific type of germplasm includes genotypes that have a common phylogenetic origin, *i.e.*, originate from one initial variety and most likely contain related genetic material. The main germplasm types of modern maize are Lancaster, Iodent, Reid, BSSS, European Flint, Lacaune, Mix, as well as different exotic germplasms (Troyer 2000). Representatives of these germplasm types are adapted to specific soil characteristics and climatic conditions. Crossed inbred lines from alternative germplasms often show a more pronounced heterotic effect than inbred lines from the same germplasm (Cherchel *et al.* 2020). Maize hybrid production is usually realized in particular heterotic models, such as the Iodent×BSSS model.

The abundance of single-nucleotide changes in the maize genome is quite high and estimated to be 1 at 44-75 bp (Gore *et al.* 2009, Ganal *et al.* 2011), one at 31 bp in noncoding regions and 124 bp in coding regions (Ching *et al.* 2002). Wide genomic studies have made it possible to establish inbred affiliation and determine genetic distances between genotypes within defined heterotic models (Alseekh *et al.* 2021). However, investigations on the specific allelic states of SNP markers in certain types of maize germplasm are limited. Specific single-nucleotide polymorphisms of Lancaster germplasm were partially analysed by Derkach *et al.* (2017a,b), while a broad comparative genomic analysis of other germplasms was not performed. At the same time, the expansion of certain heterotic models in actual maize breeding makes comparative genomic characteristics of germplasm types relevant and opportune. In this regard, the aim of our study was to compare maize inbreds of Iodent and BSSS germplasm types, as well as the Iodent/BSSS breeding group on single-nucleotide polymorphisms to determine the degrees of their affinity and heterotic potential.

Materials and methods

A set of 107 maize inbred lines was used for single-nucleotide polymorphism analysis (Table 1S). The majority of the inbreds were developed in the steppe zone of Ukraine by cumulative selection over the last twenty years. According to the inbreds' pedigrees, the set included 39 lines of the Iodent germplasm, 28 lines of the BSSS germplasm, and 40 lines of Mix germplasm. These 40 lines of Mix germplasm were classified in our research as the Iodent/BSSS breeding group, since they had been previously developed by crossing Iodent and BSSS inbreds. All lines (inbreds) were at the S_{10}-S_{12} stages of inbreeding. Grain for analyses was obtained under field conditions as a result of artificial controlled self-pollination.

Genotyping was performed by the analysis of single-nucleotide polymorphisms on SNP markers with the *GoldenGate* test and *Illumina VeraCode* (Fan *et al.* 2006) on the basis of *Eurofins BioDiagnostics* (River Falls, USA) under the financial and organizational support of *SPFE Company* "*Mais*" (Synelnykove, Ukraine).

For each line, 10 seeds as an average sample were selected and germinated on filter paper at 26°C in the dark for one week. DNA was isolated from shoots by the CTAB method (Murray and Thompson 1980). The concentration of isolated DNA was adjusted to 50 ng μL^{-1}, and 5 µL of DNA solution was taken for genotyping. The isolated DNA was applied to a *Sentrix* array matrix.

A *BDI-III* panels with 384 SNP markers (*BioDiagnostics*, River Falls, USA) on an *Illumina VeraCode Bead Plate* was used (Venkatramana *et al.* 2010). These SNP markers were biallelic, located on all 10 maize chromosomes, and had a designability rank score >0.6, which was efficient in *GoldenGate* testing. SNP markers of the *BDI-III* panel had PIC >0.25, as they were specifically selected for genotyping modern temporary maize breeding materials.

SNP genotyping was performed in fully automated mode on an *Illumina BeadStation 500 G* equipped with a *BeadReader* device (*Illumina*, San Diego, CA, USA). The intensity of fluorescence was analysed with *Illumina BeadStation* genome studio software. SNP genotyping and determination of the fluorescence signal for each inbred were performed 30 times for each marker. Only fluorescence signals with strengths greater than 0.25 were considered. In the studied set, the amplification of 16 SNP markers out of the total amount of 384 was not sufficient. Thus, the analysis of SNP genotyping information was performed on 368 SNP markers (Table 2S). The number of studied SNP loci for which it was possible to determine allelic states was 39 158, that was

approximately 366 loci per line.

The results of SNP genotyping of individual maize lines, and the sets of 39 Iodent lines, 28 BSSS lines and 40 Iodent/BSSS lines, were analysed for homozygosity [%], frequency of monomorphic markers in a set of lines [%], major allele frequency of a marker *i* in a set of lines, genetic diversity of an individual line, and genetic SNP distance between two lines (pairwise genetic distance, GD) according to Botstein *et al.* (1980) and Lu *et al.* (2009) with modifications (Table 3S). Heterozygous sites were excluded from all types of calculations.

To carry out the comparative statistical analysis, we conditionally considered Iodent as an original group, while BSSS and Iodent/BSSS as derivative groups. The difference between the frequency of a major allele of every SNP marker in the original group and the frequency of the same allele of the same marker in the derivative group was also calculated and noted as D. The genetic SNP distance between two lines was calculated for each line of the Iodent, BSSS, and Iodent/BSSS sets in relation to *1*) each line of the same set and *2*) each line of two other sets. Then mean and maximal GD values for lines in such comparisons were calculated and represented.

Allele frequencies, genetic distances and other characteristics were presented as the mean ± confidence interval. Confidence interval was calculated as SE × $t_{0.05}$, where SE - standard error, $t_{0.05}$ - Student's test at a level of significance 0.05. Student's test was chosen as a test for a small sample size analysis. In tables, the values with the same letter do not differ significantly at the level of significance 0.05. The differences between the major allele frequency in the Iodent germplasm and the frequency of the same allele in the BSSS germplasm were proved also by χ^2 test.

One-way analysis of variance of pairwise genetic SNP distances with *F*-statistics was provided by *ANOVA* in *Excel Version 2108*, *Microsoft Office LTSC Professional Plus 2021*. Qualitative clustering analysis of Iodent, BSSS and Iodent/BSSS lines was carried out by the construction of the dendrogram based on pairwise genetic SNP distance values by the method of UPGMA in *Tassel 3.0* software (Bradbury *et al.* 2007, http://genomes.urv.es/UPGMA/, https://sourceforge.net/projects/tassel/).

Results

SNP genotyping was performed for 107 maize lines, and the examples of SNP genetic passports were represented for DK7736SVZM (Iodent), DK3931MV (BSSS), and DK6469SVZM (Iodent/BSSS) inbreds (Tables 4S - 6S).

In the whole set of 107 lines, the average homozygosity was very high and reached 98.08 ± 2.70%, and the average value of genetic diversity was 0.1746 ± 0.0038. The average genetic distance in the set was 0.3853 ± 0.0091, while the maximum genetic distance reached 0.6146 ± 0.0091.

For the compared sets of the Iodent and BSSS germplasms and the Iodent/BSSS group, the average homozygosity of lines was in the range of 97.60 - 98.78% (Table 1). The average genetic diversity varied in the interval 0.1599 - 0.1745 being the smallest in the BSSS and higher in the Iodent and the Iodent/BSSS. Monomorphic markers were significantly more abundant in the Iodent germplasm and least spread in the Iodent/BSSS breeding group.

The estimation of relationships between lines on SNP distances (Fig. 1S) proved that lines of different germplasms belonged to different branches of maize genetic resources: the overwhelming majority of the Iodent lines fell into one cluster (*yellow*), except for line MSST64. The BSSS lines fell into another separate cluster (*sea-green*), except IM_131, MSST146 and I_M137 which localized at the beginning of the Iodent cluster. Lines of the Iodent/BSSS group (*purple*) were dispersed among the Iodent and BSSS lines in both clusters.

When Iodent and BSSS germplasms were compared with each other a significant disequilibrium in the major allele frequencies on 182 SNP markers (51.41%) was found. For further analysis, 14 SNP markers (top markers) with the largest differences (D) between the major allele frequencies in the original group (the Iodent germplasm) and the frequencies of the same allele in the derivative group (the BSSS germplasm) were selected (Table 2). For all the selected markers the significant differences between the major allele frequency in the Iodent germplasm and the frequency of the same allele in the BSSS germplasm were proved through the comparison of confidence intervals as well as by χ^2 test. The chosen top markers were localized on 5 out of 10 maize chromosomes, particularly on chromosomes 1, 3, 4, 7, and 9.

For the 14 top SNP markers, the range of major allele frequencies for inbreds of the Iodent germplasm was 0.72 - 0.97, while in BSSS germplasm, the frequencies of the same alleles dropped to 0.36 - 0.07. When comparing the mean values for the 14 top markers, a sharp decrease

Table 1. Main characteristics of the Iodent, BSSS germplasms, and the Iodent/BSSS breeding group based on SNP analysis. The means ± SEs × $t_{0.05}$, where SE - standard error, $t_{0.05}$ - Student's test at a level of significance 0.05; $n = 30$ for each individual line on every marker. The values of a characteristics with *the same letter* do not differ significantly at $P < 0.05$.

Characteristics	Iodent	BSSS	Iodent/BSSS
Numbers of inbreds analysed	39	28	40
Average homozygosity [%]	97.67 ± 0.25[a]	98.78 ± 0.21[b]	97.60 ± 0.26[a]
Average genetic diversity [fractions of a unit]	0.1744 ± 0.0064[a]	0.1599 ± 0.0071[b]	0.1745 ± 0.0063[a]
Frequency of monomorphic markers [%]	9.86 ± 3.10[a]	4.11 ± 2.07[b]	3.27 ± 1.85[b]

in the frequencies of alleles that were major in the original group was observed in BSSS: 0.21 ± 0.04 in BSSS *versus* 0.88 ± 0.03 in Iodent. Thus, the allelic patterns of SNP markers specific for both germplasms were demonstrated, and according to genotyping on the BDI-III panel, inbred lines of the Iodent germplasm compared to BSSS could be defined as 120A, 007G, 008C2G, 009C2G, 297G, 329A, 105A, 374C2G, 096G, 144A, 155C2G, 156C2G, 128G, 129G. Inbred lines of the BSSS germplasm compared to Iodent could be defined with alleles of SNP markers as 120G, 007A, 008C2A, 009C2A, 297A, 329G, 105G, 374C2A, 096A, 144T, 155C2A, 156C2A, 128A, 129A, 097A. The Iodent/BSSS inbreds on 4 of the 14 top SNP markers listed in Table 2 (096, 155C2, 156C2, and 097) were close to Iodent but differed significantly from BSSS. For the allelic state of 2 out of the 14 top SNP markers (374C2 and 144), inbreds of the Iodent/BSSS group did not differ significantly from BSSS, but were far from Iodent. For SNP marker 128 the lines of the Iodent/BSSS group did not differ from both basic germplasms. The Iodent/BSSS inbreds differed reliably from both the Iodent and the BSSS inbreds on 7 rest markers. Six of them, 120, 007, 008C2, 009C2, 297, and 329, showed a tendency to move towards the values of the Iodent germplasm, and only the marker 105 had a corresponding allele frequency approaching the value specific for BSSS. The mean value of the analysed trait in the Iodent/BSSS group (0.60 ± 0.04) was significantly different from the major allele frequency as in Iodent (0.88 ± 0.03) as well as in BSSS (0.21 ± 0.04) germplasms.

Analysis of variances for genetic SNP distances between lines inside germplasms and a breeding group (Table 3) demonstrates that the diversity within Iodent, BSSS, and Iodent/BSSS differs significantly in comparison with it in the whole set of 107 lines. Thus, intrapopulation diversity appeared specific in comparison with the interpopulation

Table 2. Changes in allele frequencies of top SNP markers in maize lines of the BSSS germplasm and the Iodent/BSSS breeding group in comparison with the Iodent germplasm (D - the difference between the frequency of a major allele of every SNP marker in the original group and the frequency of the same allele of the same marker in a derivative group). SNP markers of BDI-III panel are indicated according to Table 2S, possible alleles of biallelic markers are noticed as bases: A - adenine, T - thymine, G - guanine, C - cytosine. The number of inbreds studied for the Iodent germplasm is 38 - 39, for the BSSS germplasm 27 - 28, for the Iodent/BSSS group 38 - 40. The values are presented as the mean ± SE × $t_{0.05}$, where SE - standard error, $t_{0.05}$ - Student's test at a level of significance 0.05. The values of a characteristics with *the same letter* do not differ significantly at $P < 0.05$. For χ^2 test $P < 0.0001$ in all the pairwise comparisons except Iodent/BSSS - Iodent for markers 096 ($P = 0.11$), 155C2 ($P = 0.15$), 156C2 ($P = 0.15$), 097 ($P = 0.08$).

SNP marker	Original group	Derivative groups		
	Iodent germplasm major allele frequency	BSSS germplasm frequency of allele which is major in original group	D	Iodent/BSSS group frequency of allele which is major in original group
120AG	A = 0.87 ± 0.11^a	A = 0.07 ± 0.10^b	0.94	A = 0.59 ± 0.16^a
007AG	G = 0.97 ± 0.05^a	G = 0.19 ± 0.15^b	0.79	G = 0.63 ± 0.15^c
008C2AG	G = 0.97 ± 0.05^a	G = 0.19 ± 0.15^b	0.79	G = 0.63 ± 0.15^c
009C2AG	G = 0.97 ± 0.05^a	G = 0.19 ± 0.15^b	0.79	G = 0.62 ± 0.16^c
297AG	G = 0.97 ± 0.05^a	G = 0.21 ± 0.16^b	0.76	G = 0.62 ± 0.16^c
329AG	A = 0.97 ± 0.05^a	A = 0.26 ± 0.17^b	0.71	A = 0.62 ± 0.16^c
105AG	A = 0.73 ± 0.15^a	A = 0.07 ± 0.10^b	0.66	A = 0.39 ± 0.16^c
374C2AG	G = 0.72 ± 0.15^a	G = 0.07 ± 0.10^b	0.65	G = 0.15 ± 0.12^b
096AG	G = 0.79 ± 0.14^a	G = 0.14 ± 0.13^b	0.65	G = 0.58 ± 0.16^a
144AT	A = 0.95 ± 0.07^a	A = 0.32 ± 0.18^b	0.63	A = 0.62 ± 0.16^b
155C2AG	G = 0.87 ± 0.11^a	G = 0.29 ± 0.17^b	0.58	G = 0.79 ± 0.13^a
156C2AG	G = 0.87 ± 0.11^a	G = 0.29 ± 0.17^b	0.58	G = 0.79 ± 0.13^a
128AG	G = 0.84 ± 0.12^a	G = 0.31 ± 0.18^b	0.53	G = 0.62 ± 0.16^{ab}
097AT	T = 0.85 ± 0.12^a	T = 0.36 ± 0.18^b	0.49	T = 0.74 ± 0.14^a
Mean	0.88 ± 0.03^a	0.21 ± 0.04^b	0.67	0.60 ± 0.04^c

Table 3. Analysis of variance of genetic SNP distances within the Iodent and the BSSS germplasms and the Iodent/BSSS breeding group (df - degrees of freedom, F - value for an F-distribution, $F_{0.05}$ - 0.05 critical value for an F-distribution, MS - mean squares, SS - sums of squares).

Source of variation	df	SS	MS	F	P-value	$F_{0.05}$
Between groups	2	0.4831	0.2416	129.9	< 0.0001	3.09
Within groups	104	0.1934	0.0019			
Total	106	0.6766				

diversity and proves the independence of Iodent, BSSS, and Iodent/BSSS.

The mean SNP distances between the 39 Iodent inbreds themselves were substantially low (Fig. 1, Table 4). The smallest mean SNP distance within the Iodent set (0.0234) belonged to DK4498SVZM, while the largest distance (0.3776) belonged to the MSST64 line. The maximum GDs (Fig. 2, Table 4) in such a comparison varied from 0.3880 (between MSST64 and MSST0B176) to 0.5052 (between MSST64 and DK4464SVZM). The minimum GDs ranged from 0.0234 (between DK4498SVZM and MSST17) to 0.3776 (between MSST64 and MSST60).

The mean SNP distances of the Iodent lines with BSSS lines were within the interval 0.4008 - 0.5067, with the largest distance calculated for DK4464SVZM. The maximum GDs between the Iodent and BSSS lines were located in the range from 0.4688 to 0.5911. The highest value was recorded between Iodent line DK4464SVZM and BSSS line MSST67.

The mean SNP distances between Iodent germplasm lines and lines of the Iodent/BSSS group varied from 0.3314 to 0.4502, when the highest value was observed for MSST63. The maximum GDs ranged from 0.5104 to 0.5990. The largest maximum GD was recorded between Iodent line DK5401 and Iodent/BSSS line VIK71. The minimum GDs ranged from 0.0859 (between DK7436SVZM-Iodent and DK7440-Iodent/BSSS) to 0.3307 (between MSST0B17-Iodent and DK7440-Iodent/BSSS).

Similarly, pairwise genetic SNP distances were evaluated for the BSSS and Iodent/BSSS sets, with the exception of the information on the Iodent lines already analysed above. The analysis of pairwise genetic distances of BSSS lines themselves showed that line MSST45A was the line with the smallest mean value (0.3444) while line IM_131 had the largest mean GD (0.4374). Maximum GDs among BSSS lines ranged from 0.4479 (between MSST45A and MS44SV, DK1212 and MSSTV-50) to 0.5130 (between MSST33 and MSST261), whereas minimum GDs varied from 0.0130 (between DK3931MV and DK3129) to 0.3802 (between DK3301 and MSST37, IM_131 and MSST45A). Analysis of SNP distances between BSSS and Iodent/BSSS lines showed the smallest value for MSST146 (0.1953) and the highest for MSSTV20 (0.3958). The maximum GDs in this comparison were located in the range from 0.5078 to 0.6146, where the latter value was fixed between BSSS line MSST37 and Iodent/BSSS line VIK71. The minimum GDs ranged from 0.1953 (between BSSS line MSST146 and Iodent/BSSS line DKS41) to 0.3958 (between BSSS line MSSTV20 and Iodent/BSSS line DK978).

Analysis within the Iodent/BSSS group detected the smallest mean SNP distance for DK4118SVZM (0.3394) and the largest one for VIK71 (0.5349). Maximum GDs ranged from 0.4714 (between DKS46 and DK4401SVZM) to 0.6146 (between DK7270 and VIK71), and minimum GDs ranged from 0.0260 (between DK6469SVZM and

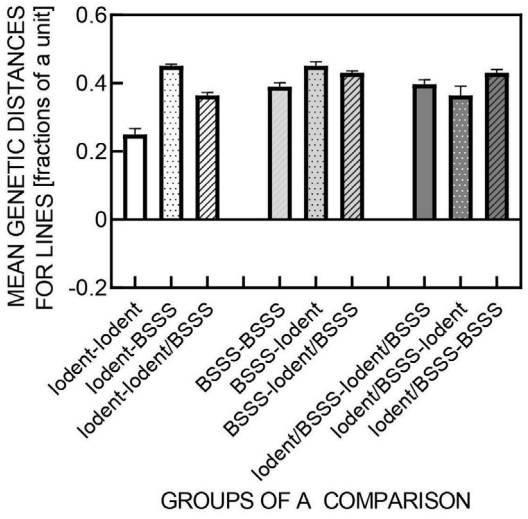

Fig. 1. Pairwise comparison of mean genetic SNP distances for lines between the Iodent, BSSS germplasms, and the Iodent/BSSS group. *Bars* show mean values with confidence interval on the 0.05 level of significance.

Table 4. Genetic SNP distances between maize lines of the Iodent, BSSS germplasms, and the Iodent/BSSS breeding group (*n* - number of lines compared). The values with *the same letter* do not differ significantly at $P < 0.05$.

Compared sets of inbreds	*n*	Mean SNP distances for lines [fractions of a unit]		Maximum SNP distances for lines [fractions of a unit]
		min - max	mean for a set	min - max
Iodent - Iodent	39	0.1718 - 0.4376	0.2494 ± 0.0174[a]	0.3880 - 0.5052
Iodent - BSSS	39	0.4008 - 0.5067	0.4509 ± 0.0055[b]	0.4688 - 0.5911
Iodent - Iodent/BSSS	39	0.3314 - 0.4502	0.3646 ± 0.0084[c]	0.5104 - 0.5990
BSSS - BSSS	28	0.3444 - 0.4374	0.3900 ± 0.0107[a]	0.4479 - 0.5130
BSSS - Iodent	28	0.3944 - 0.5188	0.4509 ± 0.0119[b]	0.4688 - 0.5911
BSSS - Iodent/BSSS	28	0.4034 - 0.4613	0.4303 ± 0.0059[c]	0.5078 - 0.6146
Iodent/BSSS - Iodent /BSSS	40	0.3394 - 0.5349	0.3967 ± 0.0134[a]	0.4714 - 0.6146
Iodent/BSSS - Iodent	40	0.2049 - 0.5506	0.3646 ± 0.0262[a]	0.4141 - 0.5990
Iodent/BSSS - BSSS	40	0.3907 - 0.5568	0.4303 ± 0.0097[b]	0.4557 - 0.6146
Average range	-	0.1637	-	0.1233

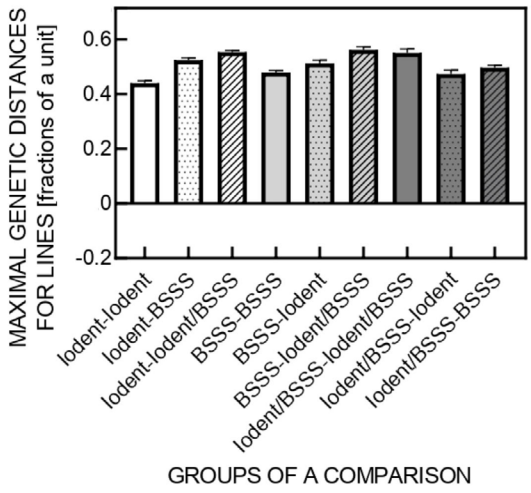

Fig. 2. Pairwise comparison of maximal genetic SNP distances for lines between the Iodent, BSSS germplasms, and the Iodent/BSSS group. Bars show mean values with confidence interval on the 0.05 level of significance.

DK6869SVZM) to 0.3724 (between DKS42 and DKS30).

In summary, a distribution of pairwise SNP distances between lines of three sets - the Iodent germplasm, the BSSS germplasm, and the Iodent/BSSS group - showed that the magnitude of their mean values varied from 0.0579 to 0.3458 with an average value of 0.1637 (Table 4). The size range of the maximum SNP GDs was slightly smaller than 0.1233. The size range of mean values of pairwise SNP distances was somewhat inversely correlated with the minimum mean SNP distances for the groups studied (r = -0.85, $r_{0.05}$ = 0.67) and directly correlated with the range size of maximum mean SNP distances (r = 0.71, $r_{0.05}$ = 0.67).

The final estimate of the distribution of genetic distances showed that the studied lines of the Iodent germplasm were significantly more closely related to each other than to the lines of the BSSS germplasm or the Iodent/BSSS breeding group. The lines of the latter group were related to each other as BSSS lines. Despite the different levels of the mean values of GDs, the GD ranges were much wider in the Iodent - Iodent and Iodent/BSSS - Iodent/BSSS comparisons than in the BSSS - BSSS comparison (0.2658, 0.1954, and 0.0930, respectively). The same observation applied to the range of maximum GDs, which were much larger in the Iodent - Iodent (0.1172) and Iodent/BSSS - Iodent/BSSS (0.1432) comparisons than in the BSSS - BSSS comparison (0.0651).

Furthermore, it was essential to compare the genetic SNP distances between germplasms and groups to establish a baseline for selecting parents of maize hybrids in the Iodent×BSSS model. The largest mean GD of lines was in the Iodent - BSSS comparison (0.4509), the smallest was in the Iodent - Iodent/BSSS comparison (0.3646), and an intermediate mean GD was noted in the BSSS - Iodent/BSSS comparison (0.4303). The same trend was observed for the ranges of mean values of pairwise GDs in the sets. Partially, it was due to the higher affinity of Iodent/BSSS lines to Iodent than to BSSS, as mentioned above.

Discussion

The essential assumption for hybrid seed production is to maintain parental lines in homozygosity and in accordance with reference patterns. For prospective inbreds, the development of molecular genetic passports is actual for reference control in their registration, certification, and the defence of intellectual property. The BDI-III platform with 384 SNP biallelic markers that was applied to maize has a theoretical resolution of 2^{384} and considers only SNP markers with a sufficient detection signal - 2^{368}. It has made it possible to have unique genetic passports for a number of lines denoted by 111 orders. This resolution was quite significant, and the SNP passports of 107 lines of the analysed set were reliable. The same approach to inbred certification with specific panels of SNP markers has been proven by other researchers (Chen *et al.* 2021, Josia *et al.* 2021). However, unifying SNP panels to compare the results of different research groups is still a major challenge. The problem of uniform marker platforms is better solved for SSR markers, while FAO has officially published a panel with 20 SSR markers, 2 markers per chromosome, primers, and the allelic status of these markers in classical maize inbreds (ISO/TR 17623:2015). In this approach, a balance between making genotyping results widely available and preserving copyrights is essential (Aubry 2019).

Certification of genetic resources using panels of not only markers of genetic polymorphism but also functional markers and subsequent systematization within germplasms and heterotic models would allow the promotion of cross-pollinated crops to the level of genomic selection. Such an approach has already been partially implemented in the classical inbreds of the main maize germplasms - the B73, H99, 207, Mo17, and BSSS populations (Pea *et al.* 2013, Gerke *et al.* 2015, Wu *et al.* 2016, Qiu *et al.* 2021). At the same time, extensive studies with SNP markers have revealed specific genomic characteristics of breeding materials and local populations that have emerged as the result of screening under specific soil and climatic conditions in different geographical zones. These include American and European landgrasses (Arca *et al.* 2021), early maturing tropical lines of West and Central Africa (Adu *et al.* 2019), local sub-Saharian populations (Badu-Apraku *et al.* 2021), tropical lines from public breeding programs in Brazil (de Faria *et al.* 2022), Chinese breeding materials (Zhang *et al.* 2016, Yan *et al.* 2022), *CIMMYT* and Indian inbreds (Kumar *et al.* 2022), and inbreds of Lancaster germplasm developed and cultivated in Ukraine (Cherchel *et al.* 2020). According to our data, the maize breeding pool of the Iodent and the BSSS germplasms, which was selected in the basic area of maize cultivation in Ukraine - the steppe zone - also possesses specific SNP characteristics.

The absence of significant differences in gene diversity between Iodent, BSSS, and Iodent/BSSS may be related to

the large number of BDI-III panel markers with alternative alleles A↔G. It reflects the general tendency that transitions of purine nucleotides are more abundant than transitions of pyrimidine nucleotides, while transversions are significantly rare (Lu *et al.* 2009).

Specific alleles of SNP markers of certain germplasm types can be regarded as a contribution to marker-assisted selection if the type of germplasm is considered a trait. From this point of view, a standard for a germplasm type should be chosen as the typical or original line. It is known that inbred P165 is considered to be an original line for the Iodent, while B14, B37, and B73 inbreds for the BSSS germplasm (Troyer 2000, Cherchel *et al.* 2020). In previous research, SNP analysis of these lines was performed properly on the modified panel BDI-III (Cherchel *et al.* 2020). The comparison of 39 Iodent inbreds studied in the given research and typical Iodent inbred P165 on the same ten top markers of the BDI-III panel (120, 007, 008C2, 297, 329, 105, 374C2, 096, 144, 156C2, 128, 097) showed that they coincided by 92.3%, and P165 had alternative allele A by marker 155C2. The typical inbred of the BSSS germplasm B14 coincided with the studied BSSS lines by 46.2% and had alternative alleles G by markers 297, 374C2, 096, 155C2, 156C2, A by marker 329 and T by marker 097. Another typical BSSS inbred B37 matched 92.3% with the studied BSSS lines and had an alternative allele A by marker 144. Typical BSSS inbred B73 is 69.2% similar to the studied BSSS lines and had alternative alleles G by marker 297, 096 and A by marker 329. A certain divergence of modern Iodent and BSSS lines from their ancestors can be explained by long-term selection in specific soil and climatic conditions of the steppe zone and the intensive improvement of these germplasms in breeding programmes. It was noteworthy that the highest concordance of the studied BSSS lines occurred in line B37, which was the most common standard of BSSS in the steppe zone. Thus, original and modern Iodent and BSSS inbreds are close enough. The SNP characteristics of modern Iodent and BSSS lines can be used to control germplasm type when establishing initial populations for subsequent selection cycles and assessing genetic relatedness.

Several publications have shown a relationship between heterosis, yield, and allelic state of molecular markers, although the type of markers was significant (Barata and Carena 2006, Tomkowiak *et al.* 2019, 2020; Auinger *et al.* 2021). Maize lines with pairwise SNP distances up to 0.3400 were shown to be more closely related and did not demonstrate high heterosis values. Otherwise, genotypes with pairwise SNP distances of 0.3500 and above are already considered genetically distant and have a high heterotic potential (Cherchel *et al.* 2020). In our study, the mean pairwise SNP distances and their limits showed that Iodent genotypes were more related to each other than BSSS ones. Significant differences in minimum SNP distances between BSSS lines again denoted the heterogeneity of the BSSS germplasm, especially since it had three main branches derived from B14, B37, and B73. The greater genetic variation of BSSS lines was also confirmed by their lower abundance of monomorphic markers compared to Iodent lines.

Pairs of lines studied within the Iodent or BSSS sets that had SNP distances of 0.3400 or less are valued for use in improvement programmes for these germplasms, as well as the development of sister lines. These observations should also be considered in the construction of new synthetic populations based on Iodent and BSSS germplasms. Their mixing for self-pollination and screening inbreds in subsequent cycles as well as in the selection of parental components of sister hybrids have also to take into account the sizes of SNP distances. As a result of Iodent and BSSS hybridization and subsequent selection, SNP distances within the Iodent/BSSS group were greater than those of the two initial germplasms, reaching a maximum of 0.5516 and indicating considerable genetic removal. The Iodent/BSSS breeding group seems to be very heterogeneous and closer to the Iodent based on SNP markers. This situation can be explained by the fact that the evolution of the Iodent/BSSS group occurred simultaneously with selection for a shorter duration of the growing season and increased both yield and stress tolerance, which were inherent to Iodent in the steppe zone. As demonstrated, many Iodent/BSSS genotypes showed affinity for Iodent, and some were genetically closer to BSSS. Hence, it is important to use pairwise SNP distances within this group and with Iodent and BSSS lines to determine the specific heterotic potential of each Iodent/BSSS line. For example, the initial populations for the next cycle of sister line development can be held at the crossing of DK6869SVZM-Iodent/BSSS with DK6469SVZM-Iodent/BSSS, DK7440-Iodent/BSSS with DK7436SVZM-Iodent or DKS41-Iodent/BSSS with MSST146-BSSS. Introduced comparisons are important in the selection of parental lines for high-heterosis hybrids.

The average SNP distances between the Iodent and BSSS lines examined were much greater than 0.3500, indicating a significant number of nucleotide substitutions. The mean and maximum GD values between the lines in the Iodent - BSSS comparison confirm the significant heterotic potential of the Iodent×BSSS model based on lines having been selected in the steppe zone. The largest SNP distance between DK4464SVZM-Iodent and MSST67-BSSS (0.5911), allows the prediction of the most highly heterotic combinations according to a given heterotic model. The SNP distances between DK5401-Iodent and VIK71-Iodent/BSSS (0.5990) and DK7270-BSSS and VIK71-Iodent/BSSS (0.6146) also imply a large heterotic ability. Such pair of genotypes can be recommended as parental components of highly heterotic F_1 crosses.

The classification of modern maize genetic resources into heterotic models and groups is generally an important task, as it allows the prediction of the heterotic potential of hybrid combinations. There are different points of view on the classification of maize resources into heterotic groups summarized by Beckett *et al.* (2017). According to our results, lines of alternative the Iodent and BSSS germplasms selected in Ukraine demonstrated large genetic differences on SNP markers, which proved

the effectiveness of the model under steppe conditions. In the crosses between these two germplasms, the hybrids realize their new potential, but it is necessary to verify pairwise SNP distances in specific hybrid combinations.

Conclusions

Maize inbreds of Iodent and BSSS germplasms possessed unique alleles of single-nucleotide polymorphism markers. Inbreds of the breeding group Iodent/BSSS obtained by crossing Iodent and BSSS lines demonstrated SNP alleles of both germplasms, but selection in the steppe zone resulted in a closer relationship with Iodent as a more adapted germplasm. The differences in allelic states of SNP markers verify the large distances between Iodent and BSSS lines and the high potential of the Iodent×BSSS heterotic model. The level of affinity of all the analysed lines estimated on allelic states of SNP markers allows to choose systematically parental forms of highly heterotic crosses and sister hybrids, as well as new initial populations for subsequent breeding cycles.

References

Adu G.B., Badu-Apraku B., Akromah R. *et al.*: Genetic diversity and population structure of early-maturing tropical maize inbred lines using SNP markers. - PLoS ONE **14**: e0214810, 2019.

Alseekh S., Kostova D., Bulut M., Fernie A.R.: Genome-wide association studies: assessing trait characteristics in model and crop plants. - Cell Mol. Life Sci. **78**: 5743-5754, 2021.

Arca M., Mary-Huard T., Gouesnard B. *et al.*: Deciphering the genetic diversity of landraces with high-throughput SNP genotyping of DNA bulks: methodology and application to the maize 50k array. - Front. Plant Sci. **11**: 568699, 2021.

Armstrong C.L., Parker G.B., Pershing J.C. *et al.*: Field evaluation of European corn borer control in progeny of 173 transgenic corn events expressing an insecticidal protein from *Bacillus thuringiensis*. - Crop Sci. **35**: 550-557, 1995.

Aubry S.: The future of digital sequence information for plant genetic resources for food and agriculture. - Front. Plant Sci. **10**: 1046, 2019.

Auinger H.-J., Lehermeier C., Gianola D. *et al.*: Calibration and validation of predicted genomic breeding values in an advanced cycle maize population. - Theor. Appl. Genet. **134**: 3069-3081, 2021.

Badu-Apraku B., Garcia-Oliveira A.L., Petroli C.D. *et al.*: Genetic diversity and population structure of early and extra-early maturing maize germplasm adapted to sub-Saharan Africa. - BMC Plant Biol. **21**: 96, 2021.

Barata C., Carena M.J.: Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. - Euphytica **151**: 339-349, 2006.

Beckett T.J., Morales A.J., Koehler K.L., Rocheford T.R.: Genetic relatedness of previously Plant-Variety-Protected commercial maize inbreds. - PLoS ONE **12**: e0189277, 2017.

Botstein D., White R.L., Skolnick M., Davis R.W.: Construction of genetic linkage map in man using restriction fragment length polymorphisms. - Am. J. Hum. Genet. **32**: 314-331, 1980.

Bradbury P.J., Zhang Z., Kroon D.E. *et al.*: TASSEL: software for association mapping of complex traits in diverse samples. - Bioinformatics **23**: 2633-2635, 2007.

Buckler E.S., Gaut B.S., McMullen M.D.: Molecular and functional diversity of maize. - Curr. Opin. Plant Biol. **9**: 172-176, 2006.

Chen Z., Tang D., Ni J. *et al.*: Development of genic KASP SNP markers from RNA-Seq data for map-based cloning and marker-assisted selection in maize. - BMC Plant Biol. **21**: 157, 2021.

Cherchel V.Yu., Dziubetskyi B.V., Satarova T.M. *et al.*: [Initial material of Lancaster germplasm in maize selection and biotechnology.] Pp. 352. Agrarna nauka, Kyiv 2020. [In Ukrainian]

Ching A., Caldwell K.S., Jung M. *et al.*: SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. - BMC Genet. **3**: 19, 2002.

de Faria S.V., Zuffo L.T., Rezende W.M. *et al.*: Phenotypic and molecular characterization of a set of tropical maize inbred lines from a public breeding program in Brazil. - BMC Genomics **23**: 54, 2022.

Derkach K.V., Satarova T.M., Borysova V.V. *et al.*: [Grouping and clustering of maize Lancaster germplasm inbreds according to the results of SNP-analysis.] – Regul. Mech. Biosyst. **8**: 343-348, 2017b. [In Ukrainian]

Derkach K.V., Satarova T.M., Borisova V.V., Cherchel V.Yu.: [The allelic state of SNP-markers specific for Lancaster germplasm maize inbreds.] - Bull. Ukr. Soc. Genet. Breed. **15**: 32-39, 2017a. [In Ukrainian]

Fan J.-B., Gunderson K.L., Bibikova M. *et al.*: Illumina universal bead arrays. - Method. Enzymol. **410**: 57-73, 2006.

Ganal M.W., Durstewitz G., Polley A. *et al.*: A large maize (*Zea mays* L.) SNP genotyping array: development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. - PLoS ONE **6**: e28334, 2011.

Gerke J.P., Edwards J.W., Guill K.E. *et al.*: The genomic impacts of drift and selection for hybrid performance in maize. - Genetics **201**: 1201-1211, 2015.

Gore M.A., Chia J.-M., Elshire R.J. *et al.*: A first-generation haplotype map of maize. - Science **326**: 1115-1117, 2009.

Hasan N., Choudhary S., Naaz N. *et al.*: Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. - J. Genet. Eng. Biotechnol. **19**: 128, 2021.

ISO/TR 17623:2015, Molecular biomarker analysis – SSR analysis of maize. Pp. 6. Geneva, 2015. *Available at*: https://www.iso.org/standard/60171.html.

Josia C., Mashingaidze K., Amelework A.B. *et al.*: SNP-based assessment of genetic purity and diversity in maize hybrid breeding. - PLoS ONE **16**: e0249505, 2021.

Kumar B., Rakshit S., Kumar S. *et al.*: Genetic diversity, population structure and linkage disequilibrium analyses in tropical maize using genotyping by sequencing. - Plants-Basel **11**: 799, 2022.

Lu Y., Yan J., Guimarães C.T. *et al.*: Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. - Theor. Appl. Genet. **120**: 93-115, 2009.

Murray M.G., Thompson W.F.: Rapid isolation of high molecular weight plant DNA. - Nucleic Acids Res. **8**: 4321-4326, 1980.

Pea G., Aung H.H., Frascaroli E. *et al.*: Extensive genomic characterization of a set of near-isogenic lines for heterotic QTL in maize (*Zea mays* L.). - BMC Genomics **14**: 61, 2013.

Qiu Y., O'Connor C.H., Della Coletta R. *et al.*: Whole-genome variation of transposable element insertions in a maize diversity panel. - G3-Genes Genom. Genet. **11**: jkab238, 2021.

Qu J., Liu J.: A genome-wide analysis of simple sequence repeats

in maize and the development of polymorphism markers from next-generation sequence data. - BMC Res. Notes **6**: 403, 2013.
Ranum P., Peña-Rosas J.P., Garcia-Casal M.N.: Global maize production, utilization, and consumption. - Ann. N. Y. Acad. Sci. **1312**: 105-112, 2014.
Rehman A.U., Dang T., Qamar S. *et al.*: Revisiting plant heterosis – from field scale to molecules. - Genes **12**: 1688, 2021.
Schnable P.S., Ware D., Fulton R.S. *et al.*: The B73 maize genome: complexity, diversity, and dynamics. - Science **326**: 1112-1115, 2009.
Syvänen A.-C.: Accessing genetic variation: genotyping single nucleotide polymorphisms. - Nat. Rev. Genet. **2**: 930-942, 2001.
Tomkowiak A., Bocianowski J., Kwiatek M., Kowalczewski P.Ł.: Dependence of the heterosis effect on genetic distance, determined using various molecular markers. - Open Life Sci. **15**: 1-11, 2020.
Tomkowiak A., Bocianowski J., Radzikowska, D., Kowalczewski P.Ł.: Selection of parental material to maximize heterosis using SNP and SilicoDarT markers in maize. – Plants-Basel **8**: 349, 2019.
Troyer F.: Temperate corn – background, behaviour and breeding. - In: Hallauer A.R. (ed.): Specialty Corn. Second Edition. Pp. 74. CRC Press, Boca Raton-London-New York-Washington 2000.
Venkatramana P., Carlson C., Blackstad M. *et al.*: Development and characterization of single nucleotide polymorphism (SNP) panel for marker assisted backcrossing in corn. - Seed Technol. **32**: 153, 2010.
Wu X., Li Y., Fu J. *et al.*: Exploring identity-by-descent segments and putative functions using different foundation parents in maize. - PLoS ONE **11**: e0168374, 2016.
Xiao Y., Jiang S., Cheng Q. *et al.*: The genetic mechanism of heterosis utilization in maize improvement. - Genome Biol. **22**: 148, 2021.
Xu C., Ren Y., Jian Y. *et al.*: Development of a maize 55 K SNP array with improved genome coverage for molecular breeding. - Mol. Breeding **37**: 20, 2017.
Yan Y., Sun S., Xing R. *et al.*: Identifying parameters for defining "essentially derived varieties" of maize inbred lines using high-throughput genome-wide SNP markers. – Plants-Basel **11**: 1909, 2022.
Zhang X., Zhang H., Li L. *et al.*: Characterizing the population structure and genetic diversity of maize breeding germplasm in Southwest China using genome-wide SNP markers. - BMC Genomics **17**: 697, 2016.