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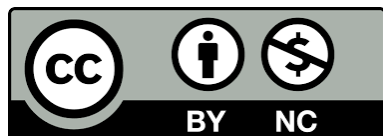
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Microsatellite-based heterotic grouping of temperate maize (*Zea mays* L.) inbred lines

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Abstract: Maize (*Zea mays* L.) is an essential cereal crop globally, with breeding efforts aiming to develop high-yielding hybrids through heterotic patterns. This study assessed the feasibility of classifying 51 maize inbred lines into the heterotic groups using 30 Simple Sequence Repeat (SSR) markers. Out of the 30 marker pairs tested, 28 displayed polymorphism, producing a total of 68 alleles, ranging from 2 to 4 alleles per locus, with an average of 2.43 alleles per locus. The primers umc2152 and Bnlg1194 exhibited the highest number of alleles, while the marker mmc0481 had the highest allele frequency. Polymorphic Information Content (PIC) values ranged from 0.08 to 0.93, with an average value of 0.56. The highest ΔK value resulted in the classification of the inbred lines into five distinct heterotic groups. The findings suggest that SSR markers effectively reveal significant genetic diversity, making them valuable tools for the classification of maize inbred lines. This categorization can assist in identifying heterotic patterns and predicting heterosis for future hybrid production.

Keywords: Maize, heterotic group, genetic diversity, simple sequence repeats.

Introduction

Maize (*Z. mays* L.), a diploid ($2n=2x=20$) monoecious crop belonging to the family of Gramineae, is currently the second most widely grown cereal after wheat in terms of grain and forage production [1]. Its grain is generally used for food, feed, and industrial products including ethanol, biodegradable foams, plastics, and adhesives. Its forage, on the other hand, is generally utilized to feed ruminant livestock, biofuel production, and chemical production (Scott and Emery, 2016).

Development of hybrid maize varieties, by crossing two different parental inbred lines, has played a crucial role in improving the performance and productivity of maize. This breeding technique has been instrumental in supporting farmers' livelihoods and reducing risks. By effectively harnessing and utilizing heterosis, the superior performance of hybrids compared to their parents, the yield of F_1 maize cultivars has witnessed a significant increase of 50-100% compared to the traditional open-pollinated varieties [3]. Evaluating and adopting the proper parental lines, which combine well together, is the initial step towards exploiting heterosis. However, the process of testing inbred lines for their combining ability, which involves a large number of line-to-line tests to determine hybrid performance, is considered the most challenging and time-consuming aspect of F_1 hybrid breeding (Rudolf-Pilih et al., 2019). To overcome this obstacle and maximize the potential of heterosis from the available gene pools, plant breeders suggest identifying heterotic groups and patterns. This approach helps in efficiently identifying combinations of parental lines that exhibit strong hybrid vigor.

Heterotic groups are referred to as "groups of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups" (Melchinger and Gumber, 1998). By comparison, the term "heterotic pattern" refers to a specific pair of two heterotic groups expressing the highest heterosis and consequently the highest hybrid performance in their crosses (Melchinger and Gumber, 1998). In addition to improving the accuracy of hybrid

prediction, determining heterotic groups also aids in the incorporation of cytoplasmic male sterility (CMS) in the female pool and restorer gene(s) exclusively in the male pool for commercial hybrid production (Hussain et al., 2022). This approach not only enhances the efficiency of hybrid breeding but also allows for the controlled production of hybrids by manipulating the reproductive traits of the parent lines. Despite numerous studies, the precise physiological, biochemical, and molecular mechanisms underlying this phenomenon remain largely elusive (Virmani et al., 2004), and they have not been able to accurately predict heterotic combinations.

Duly assignment of inbred lines into heterotic groups is a prerequisite for attaining functional heterotic patterns. This assignment can be carried out *via* different approaches such as pedigree analysis, combining ability analysis, phenotypic and molecular marker-assisted clustering. Acquiring information on the origin of inbred lines necessary for pedigree analysis can be challenging and sometimes unattainable. Furthermore, the classification of breeding materials based on mating designs and phenotypic methods can be misleading due to unknown genetic mechanisms and environmental cues (Oyetunde et al., 2020). Providing a more stable and consistent approach for trait identification across different growing conditions, molecular markers are widely employed to assess the genetic distance between different maize genetic materials and assign inbred lines to specific heterotic groups. These markers can be incorporated into selected parental lines to develop heterotic hybrids that benefit from mechanisms not typically observed in the individual inbred lines (Virmani et al., 2004; Suwarno, 2014). Among the various molecular approaches, Simple Sequence Repeat (SSR, or microsatellites) and Single-Nucleotide Polymorphism (SNP) markers are commonly used to predict heterotic groups and patterns. The current research aims to investigate genetic diversity and determine heterotic groups in 51 maize inbred lines to maximize the exploitation of heterosis and select testers within the heterotic group for future breeding programs.

Materials and Methods

Plant materials

In this study, 51 maize inbred lines (42 early and 9 late maturing) were used for determining heterotic groups at Seed and Plant Improvement Institute (SPII), Karaj, Iran (Table 1).

DNA extraction

Seeds were sown in trays containing peat and perlite (1:1; v:v) with 8 replications in the laboratory of Maize and Forage Crops Research at the Seed and Plant Improvement Institute. Leaf sampling was conducted after the seedlings reached 2-3 leaf stage. The genomic DNA of young fresh leaves was extracted using the CTAB (cetyl trimethyl ammonium bromide) method. The concentration of the extracted DNA was measured by the NanoDrop 2000 spectrophotometer. The quality of the DNA was verified by running electrophoresis on a 1% agarose gel. In this study, a total of thirty SSR markers were selected from the entire maize genome. The information regarding these SSR markers was obtained from the Maize Genetics and Genomic Database (www.maizegdb.org) (Table 2).

Polymerase chain reaction (PCR)

The PCR was performed according to the method previously described by Shahata [10]. Each 15 µl PCR reaction consisted of 7.5 µl PCR master mix from DNA Biotech, 3.5 µl deionized H₂O, 1 µl reverse and forward primer, and 1 µl DNA (50 ng/µl). The PCR reaction involved an initial denaturation at 94°C for 3 minutes, followed by denaturation at 94°C for 30 seconds, primer annealing at 54-62°C (as specified in Table 2) for 30 seconds, and extension at 72°C for 30 seconds. This cycle was repeated 35 times, followed by a final extension at 72°C for 5 minutes, and the reaction was then stopped at 10°C. The PCR products were separated into 8% polyacrylamide: bisacrylamide (29:1) gels using vertical electrophoresis. The gels were subsequently stained with 50 µl of safe stain for one hour. After staining with a safe stain, the DNA was observed under UV light and photographed using the Gel-doc device from the American BIO-RAD company.

Statistical analysis

The Popgen32 software was used to calculate genetic diversity indicators such as the observed number of alleles (Na), the effective number of alleles (Ne) (Kimura and Crow, 1964), polymorphic information content (PIC), Shannon's information index (I) (Lewontin, 2014), and Nei's index (Nei, 1973). PIC was calculated based on the number of identified alleles and their frequency in the population according to the following formula:

$$PIC = 1 - \sum p_i^2$$

(where P_i is the frequency of allele i among all alleles produced by the genotypes used (Anderson et al., 1992). The marker index (MI) is derived from the number of polymorphic alleles for each marker. The MI value for each marker is calculated using the formula: $MI = PIC \times EMR$. The Effective Multiplex Ratio (EMR) is obtained by calculating the ratio of polymorphic markers to the total number of markers. The population structure analysis was carried out using the Bayesian Markov Chain Monte Carlo model (MCMC) implemented in STRUCTURE v2 software (Pritchard et al., 2000). The Bayesian-based model included in the Structure program was utilized for the genetic structure analysis of molecular data in the maize-inbred lines investigated.

The precise genotypic classification (K values) into suitable subpopulations based on genetic structure was confirmed using the Bayesian model-based method and Structure software. The optimal value of K, representing the number of subpopulations, was determined considering indices such as $\ln P(D)$ and ΔK (Zhou et al., 2018). The parameters used in this model included K (ranging from 4 to 9 for microsatellite data), and 10 repetitions for each K. The number of runs and the MCMC repetitions were set to 10000 to achieve the maximum likelihood curve (Anderson et al., 1992).

Results and Discussion

Genetic diversity of maize inbred lines

The genetic diversity analysis of maize inbred lines in this study was conducted using divergent reproducible amplification produced by 30 SSR primers tested (Figure 1; Table 3).

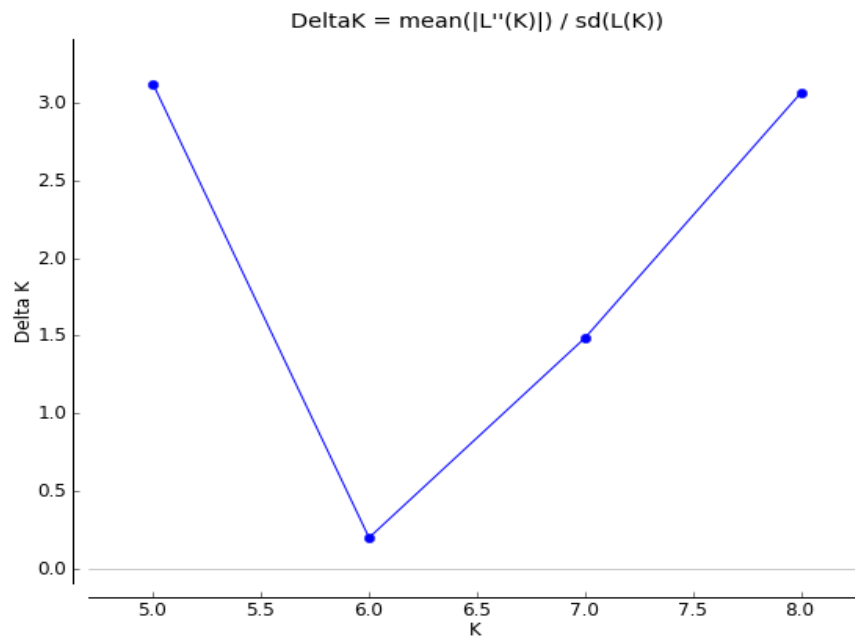


Figure 1. ΔK curve for determining the optimal value of K.

Results revealed 68 alleles distributed across 28 marker loci, with an average of 2.43 alleles per locus. Previous studies indicated varying allele numbers ranging from 1 to 8, depending on the number of SSR primers used (Nikhou *et al.*, 2013; Nikolić *et al.*, 2015; Synrem *et al.*, 2017; Aung *et al.*, 2023). Expectedly, small variations in the number of alleles between studies may primarily be attributed to the size of samples investigated, their pedigrees, homogeneity, and most importantly the repetition type of SSR primers employed. Genetic diversity levels were found to vary from 0.04 (umc1648) to 0.67 (bnlg1194), with an average value of 0.4. The SSR primers in this investigation displayed significant values of gene diversity together with allelic variations.

The PIC value is considered a critical metric used to assess the effectiveness of a marker in estimating genetic variation among genotypes. In this study, the PIC value ranged from 0.11 (umc1648) to 0.93 (umc1525) with an average value of 0.57 (Table 3). Based on the highest PIC values, the primers phi2513 (0.93), phi041 (0.78), bnlg1194 (0.74), bmc1979 (0.72), umc1795 (0.71), and bnlg244 (0.7) were found to be the most potent markers for

assessing genetic diversity among the inbred lines. The average PIC value observed in this research was greater than the values documented by Shiri *et al.* (2014) (ranging from 0.23 to 0.79 with an average of 0.53) and Aung *et al.* (2023) ranging from 0.01 to 0.76 with an average of 0.48). The mean PIC value in our study, however, turned out to be inferior to the PIC value observed by Vathana *et al.* (2019) which ranged from 0.311 to 0.903 with a mean value of 0.743.

Strong molecular markers have the potential to be a useful tool for classifying genotypes into different heterotic groupings (Xia *et al.*, 2004). Information on the degree of polymorphisms among genotypes is greatly aided by research on genetic variation and PIC values in plant breeding programs (Eltaher *et al.*, 2018). Low polymorphism is indicated by a PIC value of less than 0.25, moderate polymorphism is shown by a value between 0.25 and 0.5, and a highly polymorphic locus is indicated by a value more than 0.5 [16]. With the use of 28 SSR markers, the current study offers insightful data on the genetic makeup of 51 genotypes of maize inbred lines. PIC values ranged from 0.11 to 0.28 for 10.7% of markers had low polymorphism, 0.28 to 0.47 for 14.3% of the

markers had average polymorphism, and 0.53 to 0.93 for 75% of the markers had high polymorphism.

Population structure analysis and genetic relationship

The Bayesian-based model included in the Structure program was utilized for the genetic structure analysis of molecular data in the maize inbred lines investigated. First presented by Pritchard *et al.* (2000), the Structure program is a clustering technique based on an independent marker system and a genetic model. Before experimentation or observation, Bayesian approaches assign probabilities or distributions to the data based on optimal assumptions; then, they repeatedly modify these assumptions following the experiment (Kumar *et al.*, 2017).

In the present study, the ideal number of K was found to range from 4 to 9 groups with the largest ΔK value (Figure 1) at K=5, signifying the division of the lines into 5 groups. The matrix of membership proportions of individuals in each cluster was calculated, and K=5 was shown to be the ideal K for predicting the structure of these inbred lines (Figure 1 and Table 3). The vertical color column identified each line and its matching number in a biplot (Figure 2) based on the findings of the molecular

data analysis performed with the Structure software. Each line's presence of color diversity reflects the genetic mixing of that line and shows how genetically similar the lines are to one another. To put it more clearly, an individual 'n' might have inherited some DNA from its population K ancestors. In this instance, the person is a member of the group in that cluster with the broadest color range.

The 51 inbred lines investigated in this study were divided into five groups based on the aforementioned information (Figure 2). There are 12 genotypes in the red group (23.53%), 9 genotypes in the green group (17.65%), 11 genotypes in the blue group (21.57%), 10 genotypes in the yellow group (19.61%), and 9 genotypes in the pink group (17.675%). The red group includes inbred lines 4, 5, 7, 13, 14, 15, 25, 26, 28, 30, 31, and 40, all of which belonged to the early maturing corn lines. The green group included inbred lines 1, 2, 20, 23, 24, 38, 39, 42, and 50 all, except for numbers 42 and 50 were early corns. Likely, one of the parents of inbred lines 42 and 50 had already been selected from the early maturing types. In the blue group, the most of inbred lines, except for lines numbers 6, 22, and 41, were considered to be late-maturing types.

Table 1. Names and codes of the maize inbred lines investigated.

No.	Early inbred lines	No.	Early inbred lines	No.	Early inbred lines	No.	Early inbred lines	No.	Late inbred lines
1	K1263/17	12	KE78011/6112	23	KE80001/72111	33	KE81027/3-1-1	42	K3653/2
2	K1263/2-1	13	KE78015/111	24	KE81015/211	34	KE81027/2-3-1-1	43	MO17
3	R319	14	KE78005/511	25	KE81015/521	35	KE81027/4-2-1	44	B73
4	R59	15	KE78027/1113	26	KE83008/2211	36	KE83001/4111	45	K3547/4
5	KE72012/12	16	KE78008/212	27	KE81009/311	37	K2331	46	K3640/3
6	KE75016/232	17	KE78011/6121	28	KE81009/511	38	K615/1	47	K47/2-2-1-3-3-1-1-1
7	KE75006/212	18	KE78010/421	29	KE81010/521	39	K722	48	K74/1
8	KE77008/1	19	KE78011/61231	30	KE81015/1-1-1	40	OH43/1-42	49	K18
9	KE77005/3	20	KE78011/61232	31	KE81012/3-1-1	41	K1264/1	50	K222
10	KE77008/2	21	KE79017/3211	32	KE81027/4-4-3			51	KE81018/611
11	KE78016/212	22	KE80001/5212	32	KE81027/4-4-3				

Table 2. Characteristics of the applied SSR markers.

Marker code	Forward and reverse sequence	Annealing Tm(°C)	Bin	Marker code	Forward and reverse sequence	Annealing Tm(°C)	Bin
phi056	ACTTGCTGCGCTGCCGTTAC(F) CGCACACCACTCCCAGAA(R)	57	1.00	umc1406	AGAGGAGACAGGAGGTCGGTAGTT(F) TGTGGTGTGGTCTTCTCTCTCTG(R)	62	7.05
umc2124	ATGCGGAGGGTCTACTACACATA(F) CTGTGTCTCACTGGAAATGACGAT(R)	61	1.00	bnlg1194	GCGTTATTAAGCAAGCTGC (F) ACGTGAAGCAGAGGATCCAT(R)	54	8.02
phi002	CATGCAATCAATAACGATGGCGAGT(F) TTAGCGTAAACCCTTCTCCAGTCAGC(R)	62	1/08	umc1858	GTTGTCTCCTTGCTGACCAGTTT(F) ATCAGCAAATTAAGCAAAGGCAG(R)	57	8.04
umc1542	TAAAGCTATGATGGCACTTGCAGA(F) CATATTTGCCTTTGCCCTTTTGTA(R)	60	2/02	umc1957	CATGATCGCCGGGATTAATACTAC (F) GTCCAAGGACGACGATTACGAC (R)	61	9
umc1326	GACCAAAGAATCCCTCCCCTA(F) TACCTAGTACTCGGCCAGTTCCTC(R)	61	2/04	umc1982	TTCATCTTCTAGTCTCGTCTCCG(F) AATCGTACTTGGAGGAGGCGTT(R)	59	9.08
phi2513	CCAGTCCAATGGAGAGGG(F) GAGATCCCCTGCAGGACT(R)	57	2.07	uhi041	TTGGCTCCCAGCGCCGAAA(F) GATCCAGAGCGATTTGACGGCA(R)	61	10
umc1394	CCCGAGTCAGAAAAACATTCACTT(F) CCTAACCTGAAGAAGGGAGGTCAT(R)	55	3.01	bnlg1360	TTGGCTCCCAGCGCCGAAA(F) GATCCAGAGCGATTTGACGGCA(R)	55	10
umc1135	TTTTTAACCTCACGAGCATCGTCT(F) CGCTAGCTTAGCTCCATCGTTTA(R)	60	3.07	bnlg244	GATGCTACTACTGGTCTAGTCCAGA(F) CTCTCCACTCATCAGCCTTGA(R)	60	9.02
umc2152	TAGCTTACCTGATGATCTTGCAC(F) CCTTTGTCTCCGCTATCTTCTT(R)	62	3.09	umc1648	CTGCAGTACGTGAGCCTGTACG(F) GCTTGAGCTGTGAGGAAGTTTGA(R)	61	10.04
umc1008	TCTAGCTTGTGGTGGTGGTTGA(F) ACATGAGCACAAAGACTGACGC(R)	59	4	umc1792	CATGGGACAGCAAGAGACACAG(F) ACCTTCATCACCTGCAACTACGAC(R)		5.08
umc2285	GAAGAAGAGGGAAAGGAAGGGAG(F) AAGTAGCTGGGCTTGGAGGG(R)	60	4.08	umc1979	AATTCGGGAAACAGGCCAT(F) GAGTCCCCGAAACTGAACACC(R)	54	6.04
phi006	AGGCGCGTGCTGAACACCT(F) CGCTTCATCTCCCGTGACAATG(R)	61	4.11	umc1883	GAATAATCAATCCATCGATCTCGC(F) AACTGCTGTGGATGAAAGAGGAAG(R)	58	6
mmc0481	TGTTTGAGCCGTTCTAGACT(F) GCACCTGCGAGACTAGG(R)	52	5.06	umc1795	CCCTCTTCTCTAGGTTATCGTT(F) CAGCGCGTCTTGAAGAGTAG(R)	60	6.05
mmc0483	CTTCTCTCTGGAGCGTGTATTG(F) ATATGTTGCAGAACCATCCAGGTC(R)	60	6.02	umc1301	CATCCATAAGCTGAAGGAGTGAGG(F) AACAGTCAAGCTCACTTCCCGC(R)	60	7.03
mmc1270	ACAAGGCAGGCAGACTACTTCTTG(F) CCTAAGAAGTGCGCAACCC(C)	59	7.01	umc1260	CTTAAGCAGAGCTCAAAAAGTCC(F) TAAATTGTCAAGCGAGGTTGGAT(R)	58	5.00

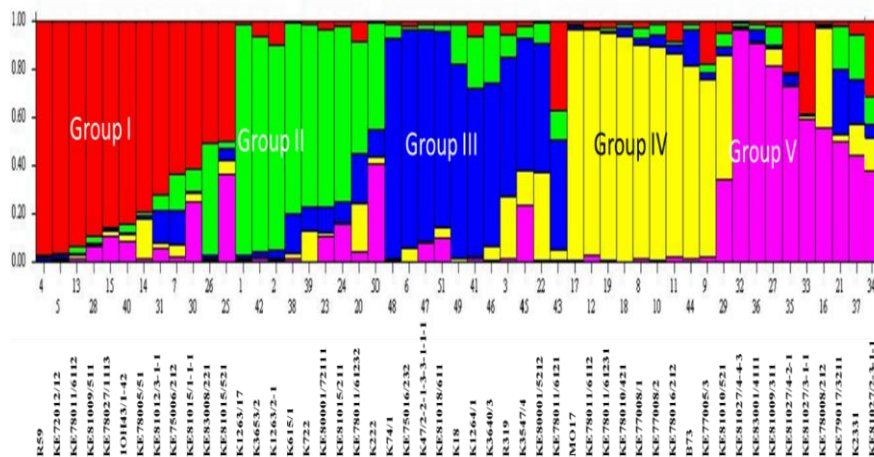
**Figure 2.** Population structure analysis of 51 maize lines using a model-based approach with SSR markers.

Table 3. Information of 28 SSRs, including number of alleles, Na, PIC, Ne, Nei, I, MI and RP across 51 maize inbred lines.

Number	Marker code	Na	A. frequency	PIC	Ne	Nei	I	MI	RP
1	phi056	2	0.76	0.62	1.8	0.44	0.63	1.24	1.18
2	umc2124	3	0.88	0.68	2.59	0.62	1.02	2.05	1.76
3	phi002	2	0.88	0.47	1.47	0.32	0.5	0.94	0.94
4	umc1542	3	0.88	0.63	2.08	0.52	0.86	1.88	1.57
5	umc1326	2	0.7	0.58	1.18	0.15	0.28	1.15	0.82
6	phi2513	2	0.27	0.93	1.15	0.13	0.26	1.87	0.55
7	umc1394	2	0.94	0.28	1.18	0.15	0.28	0.56	0.51
8	umc1135	2	0.88	0.53	1.64	0.39	0.58	1.05	1.18
9	umc2152	4	0.76	0.79	2.8	0.64	1.14	3.16	1.53
10	umc1008	3	0.9	0.35	1.3	0.23	0.48	1.04	0.59
11	uhi006	2	0.82	0.61	1.74	0.42	0.62	1.22	1.37
12	mmc0481	3	1	0.65	2.87	0.65	1.07	1.96	2.00
13	umc0483	2	0.96	0.18	1.13	0.11	0.23	0.37	0.31
14	umc1406	2	0.9	0.37	1.29	0.59	0.38	0.74	0.67
15	bnlg1194	4	0.92	0.74	3.08	0.67	1.2	2.94	1.84
16	umc1858	3	0.96	0.56	2.1	0.61	0.87	1.68	1.57
17	umc1957	2	0.67	0.62	1.25	0.2	0.35	1.25	0.94
18	umc1982	3	0.74	0.64	1.54	0.35	0.66	1.92	1.14
19	phi041	2	0.5	0.78	1.16	0.14	0.27	1.55	1.02
20	bnlg1360	3	0.96	0.64	2.55	0.61	1.02	1.91	1.88
21	bnlg244	3	0.9	0.7	2.76	0.64	1.05	2.09	1.80
22	umc1648	2	0.96	0.11	1.04	0.04	0.1	0.23	0.16
23	umc1792	2	0.7	0.66	1.45	0.32	0.49	1.31	1.14
24	umc1979	2	0.74	0.72	1.97	0.5	0.69	1.44	1.49
25	umc1883	2	0.94	0.54	1.94	0.49	0.68	1.09	1.69
26	umc1795	2	0.74	0.71	1.9	0.47	0.67	1.42	1.49
27	umc1301	2	0.94	0.54	1.94	0.49	0.68	1.09	1.69
28	umc1260	2	0.96	0.47	1.74	0.42	0.62	0.94	1.25
total		68	23.16	16.1	50.64	11.31	17.68	40.09	34.08
mean		2.423	0.83	0.575	1.81	0.4	0.63	1.43	1.22

By crossing the inbred line K722 (paternal parent from the green group) and the inbred line 1264/1 (paternal parent from the blue group) with the inbred line B73 (from the yellow group) the single crosses KSC-604 and KSC-647 were developed, respectively (Botstein *et al.*, 1980). Likewise, by crossing the inbred lines K3640/3 and K3547/4 (both as the maternal parent from the blue group) with MO17 (paternal parent from the yellow group), the single crosses KSC-705 (Dehghanpour *et al.*, 2018) and KSC-706 (Dehghanpour *et al.*, 2018) were developed, respectively. When the inbred line K615/1 (maternal parent from the green group) was crossed with K1264/1 (paternal parent from the yellow group), the single cross K260 was developed.

Dehghanpour *et al.* (2018) and Choukan *et al.* (2013) collectively pointing out to the validity of the present clustering.

Conclusion

In the current study, 28 out of 30 SSR primers proved to be polymorphic. In total, 68 alleles with a range of two to four alleles (and an average of 2.43 alleles per locus) were amplified. Out of the 30 SSR markers examined, markers phi2513, umc2152, bnlg1194, phi041, bnlg244, umc1979, and umc1795 exhibited the highest power for assessing genetic diversity among the inbred lines based on their PIC values. These robust molecular markers can be effectively utilized for genotype identification,

allowing for the classification of genotypes into distinct heterotic groups. Population structure analysis categorized 51 inbred lines into five distinct clusters. The highest and lowest MI values were found in the markers umc2152 (3.16) and umc1648 (0.23), respectively. The highest value of the Nei's index was observed in the bnlgl1194 marker (3.08), and the lowest Nei's index was obtained in the umc1648 marker (1.04). Moreover, the highest Shannon's index value was found in the bnlgl1194 marker (2.10). In conclusion, the use of microsatellite markers provides valuable insights into the genetic diversity of maize inbred lines, offering a robust tool for various applications in genetic studies and breeding programs.

Supplementary Materials

There is no supplementary material available for this article.

Author Contributions

Conceptualization, B.A. and A.M.; methodology, B.A. and M.S.; software, S.D.; validation, B.A. and S.D.; formal analysis, S.D.; investigation, B.A. and

A.M.; resources, B.A., M.S.; data curation, B.A., and M.S.; writing—original draft preparation, M.O. and B.A.; writing—review and editing, B.A.; visualization, M.O. and B.A.; supervision, B.A.; project administration, B.A.; funding acquisition, B.A., M.S., and A.M. All authors have read and agreed to the published version of the manuscript."

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Conflict of Interest Statement

The authors have no relevant financial or non-financial interests to disclose.

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گروه‌بندی هتروتیکی با استفاده از نشانگرهای ریزماهواره در اینبرد لاین‌های ذرت (*Zea mays* L.) مناطق معتدله

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چکیده: ذرت (*Zea mays* L.) یکی از غلات مهم در جهان محسوب می‌شود که اصلاح آن به منظور توسعه هیبریدهای با عملکرد بالا از طریق الگوهای هتروتیک انجام می‌گردد. در این تحقیق، امکان گروه‌بندی ۵۱ لاین خالص ذرت به گروه‌های هتروتیک را با استفاده از ۳۰ نشانگر توالی تکراری ساده (SSR) مورد ارزیابی قرار گرفت. از ۳۰ جفت نشانگر آزمایش شده، ۲۸ نشانگر چند شکلی را نشان دادند و مجموعاً ۶۸ آلل تولید کردند که از ۲ تا ۴ آلل، با میانگین ۲.۴۳ آلل در هر لوکوس متغیر بود. آغازگرهای umc2152 و Bnlg1194 بیشترین تعداد آلل‌ها را نشان دادند، در حالی که نشانگر mmc0481 بالاترین فراوانی آللی را دارا بود. مقادیر محتوای اطلاعات پلی‌مرفیسم (PIC) از ۰.۰۸ تا ۰.۹۳ متغیر و میانگین آن ۰.۵۶ بود. بالاترین مقدار ΔK نیز منجر به طبقه‌بندی لاین‌های خالص به پنج گروه هتروتیک متمایز گردید. یافته‌های این تحقیق نشان می‌دهد که نشانگرهای SSR می‌توانند به طور مؤثری تنوع ژنتیکی را آشکار نمایند، و این موضوع آن‌ها را به ابزاری با ارزش برای گروه‌بندی لاین‌های خالص ذرت تبدیل می‌کند. این گروه‌بندی می‌تواند در شناسایی الگوهای هتروتیک و پیش‌بینی هتروزیس برای تولید هیبریدها در برنامه‌های آینده کمک نماید.

کلمات کلیدی: ذرت، گروه هتروتیک، تنوع ژنتیکی، توالی تکراری ساده.