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Original Research

Evaluation of SRAP marker efficiency in identifying the relationship between genetic diversities of corn inbred lines with seed quantity and quality in derived hybrids

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Abstract: In order to estimate the efficiency of SRAP markers for identifying the performance of seed quantity and quality in maize single crosses, 13 inbred lines obtained from CIMMYT germplasm bank were crossed to A679, K166B, K18 and MO17 testers using the line×tester method. The inbred lines and derived hybrids were evaluated in two experiments separately in a randomized complete block design with three replications during two growing seasons in 2014 and 2015. In order to evaluate genetic variation in the inbred lines, 25 SRAP markers were also used. The results of variance analysis between inbred lines were showed a significant variation (P≤0.01) for seed quantity and quality. The analysis of variance among the hybrids derived from inbred lines was showed a significant variation (P≤0.01) for oil percent, starch content, protein content, seed yield and thousand seed weights and a significant variation for the dry matter (P≤0.05). The maximum Euclidian distance between the two lines was 24.5 times greater than the minimum distance between two lines. The PCR amplification for the 17 parentallines with the 25 combinations of SRAP primers generated a total of 205 clear and scorable bands, of which 135 were polymorphic (65.75%). The average distance between the studied lines was 0.324 on the bases of the Jaccard coefficient and maximum distance between two lines was 2.87 times greater than the minimum distance between two lines. The M1E1, M1E5, M5E3, M5E4, and M5E5 were superior to other primer combinations in expressing genetic diversity based on the primer information indices. The banding pattern of the studied primer combinations related to the genetic variation of the inbred lines based on the studied traits revealed that the M5E1 primer pair can predict the distance of inbred lines for dry matter better than other primers. Also, the primers combination of M4E4 for protein percentage, M4E4 for starch percentage, M2E3 for crude fiber, M4E3 for oil percentage, M2E5, M4E1 and M5E1 for thousand seed weight and M3E1 for seed yield, can be introduced as informative primer combination, to estimate genetic distance determination of inbred lines based on these traits. Due to the relationship between inbred lines variation based on primers combinations with the traits in hybrids progenies showed that the M2E1, M2E2, M4E1 and M5E3 for dry matter and M2E4 for starch percentage have the ability to detect hybrid performance for these traits. For traits, protein percentage, crude fiber and oil percent no suitable primers combination were found. Also, for the seed yield, three primer combinations of M1E5, M2E2 and M3E2 had the highest negative correlation. Therefore, the hybrids derived from the inbred lines with high genetic distance based on these primers combinations will have a low seed yield. The M1E2, M2E3 and M5E5 can be introduced to identify the prediction of higher thousand seed weight.

Key words: Corn; SRAP marker; Efficiency; Seed quality; Yield.

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops used for many years, as human and animals' food. One of the major objectives in maize breeding is higher yield (1-3). Considerable work has been carried out to develop varieties with higher seed yield and better qualitative characteristics (4). In this respect, the study of genetic diversity provides an opportunity for plant breeders to improve cultivars with favourable characteristics (5-8). The determination of this diversity is routinely performed using various techniques such as morphological, biochemical and molecular marker analysis (9-11). Selection based on morphological characteristics is not only affected by environmental conditions but also requires a lot of time and cost in breeding programs. Therefore, to reduce the impact of environmental effects in the analysis, researchers employed the biochemical techniques and then were used DNA-based molecular techniques. The molecular markers not only reduce the influence of the environment but also increases the resolution of measurements of genetic variation (12). The most important issue in examining molecular markers efficiency for revealing genetic diversity in breeding programs is their relationship with morphological characteristics that has been the subject of numerous researches (13-16).

However, the molecular markers are more efficient, precise and reliable for discriminating cultivars and therefore have great potential in marker-assisted breeding programs that is one of the most significant developments in the field of molecular genetics (17). Each of the molecular markers has advantages and disadvantages (18), but more attention should be given to the efficiency of them in genetic variation reports. The SRAP marker was introduced for the first time by Li and Quiros (19), for mapping and gene tagging in *Brassica oleracea* based on the amplification of open reading frames (ORFs) using two primers (forward and reverse). This marker system is a simple, efficient, cost-effective, and does not require any prior knowledge of the genome sequence that produces a number of co-do-

minant markers per amplification (17). The use of SRAP markers have been evaluated indetermination of relationships with morphological traits in a variety of plant species, and their potential for breeding has also been highlighted (20, 21). Limited information is available on the chromosomal locations of SRAP markers, their linkage with plant traits, and their potential for genetic diversity studies (14).

The phenomenon of heterosis describes the increased agronomic performance of heterozygous F1 plants compared to their homozygous parental inbred plants (22). On this base, identification of parental combinations that produce hybrids with superior yield is the most important step in developing hybrids. However, this is one of the costliest and time-consuming steps in any hybrid breeding programs, as it is necessary to cross the available inbred lines and evaluate the hybrids in extensive yield trials (13). Therefore, investigating the relationship between genetic distances of inbred lines based on molecular markers with morphological traits of hybrids can be an effective tool for heterosis evaluation. The purpose of this study was the evaluation of SRAP effectiveness in characterizing inbred parents considering the relationship between parental genetic distance with traits related to seed yield and quality in the derived hybrids.

Materials and Methods

Plant materials and experimental location

In this study, a set of 13 inbred lines from CYMMIT were selected and crossed through controlled pollination with four temperate maize testers using a line × tester matting design to produce 52 hybrid combinations. The origin and pedigree of the lines and testers are given in Table 1. Therefore, the plant materials consisted of 17 inbred lines and 52 single crosses from matting of the inbred lines.

The field experiments were conducted during two years (2014-2015) at the Research Farm of Agriculture and Natural Resources Research Center, Kermanshah, Iran (longitude of 47° 26' E, the latitude of 34° 8' N and altitude of 1346m) on a silty clay loam soil. The mean annual precipitation and temperature are 538 mm and 12.2 °C for the region, respectively.

Experimental design and traits measurements

Two separate but adjacent experiments were conducted to evaluate the genetic variation of inbred lines and hybrids based on a randomized complete block design (RCBD) with three replications. The first experiment consisted of 17 inbred lines and the second experiment consisted of 52 single crosses. Each plot included 2 rows of 4 m long with an inter-row spacing of 0.75 m and in row plant spacing of 18 cm.

The traits of grain yield and thousand seed weight per each plot were recorded. The seed quality characters of oil percentage, crude fiber, starch percentage, protein percentage per each plot were measured by NIR analyzer DA 7200.

SRAP analysis of inbred lines

Genetic characterization of all of the inbred lines was done using a set of 25 SRAP primer pairs. Genomic DNA was extracted from fresh leaves of each line according to the method of Murray and Thompson (23). The PCR reactions were performed in a 10µl reaction mixture and amplified products were resolved using 6% polyacrylamide gel followed by silver staining. The primers and primers combination are presented in Table 2.

Statistical analyses

Analysis of variance (ANOVA) for each experiment was conducted using the PROC GLM of SAS (SAS Institute 2008). The Euclidean distance was computed among inbred lines based on each morphological character and the traits related to seed quality separately and all studied characters together by $SPSS_{18}$.

The information of generated banding patterns for combined primers consisted of total bands, polymorphic bands, the percentage of polymorphism, polymorphism information content (PIC) and marker index (MI)

Table 1. Information on maize lines and testers used in the study.

Inbred lines	Name of lines and testers/pedigree	Origin
Line1	4-CHTSEY,2002/1389/9=1390/13=1391/10	derived from CIMMYT germplasm
Line2	4-CHTSEY,2002/1389/19=1390/21=1991/70	derived from CIMMYT germplasm
Line3	7-CHTSEY,2002/1389/33=1390/33=1391/61	derived from CIMMYT germplasm
Line4	7-CHTSEY,2002/1389/35=1390/37=1391/64	derived from CIMMYT germplasm
Line5	K18 ×2-CHTHIY, 2002/1389/59=1390/73=1391/43	derived from k18 × Cimmyt originated line
Line6	K18 × 2-CHTHIY, 2002/1389/61=1390/77=1391/46	derived from k18 × Cimmyt originated line
Line7	XT03	derived from unknown China -source
Line8	4-CHTSEY, 2002/1390/5=1391/6	derived from CIMMYT germplasm
Line9	4-CHTSEY, 2002/1390/9=1391/8	derived from CIMMYT germplasm
Line10	7-CHTSEY, 2002/1390/41=1391/22	derived from CIMMYT germplasm
Line11	20-CHTSEY,2002/1390/45=1391/25	derived from CIMMYT germplasm
Line12	20-CHTSEY,2002/1390/53=1391/31	derived from CIMMYT germplasm
Line13	MO17 × 6-CHTHEY, 2002/1390/69=1391/40	derived from MO17 × CIMMYT originated line
Line14 (Tester1)	K166B	CL. 187–2 × C103
Line15 (Tester2)	A679	derived from MO17 changes
Line16 (Tester3)	K18	A B73 back-cross derived line [(A662 × B73)(3)]
Line17 (Tester4)	MO17	derived from CIMMYT germplasm

Table 2. Forward and reverse primer names, sequence and annealing temperatures.

Primer name		Sequence	Temperature (°C)
Forward	Me1	5'-TGAGTCCAAACCGGATA-3'	50
	Me2	5'-TGAGTCCAAACCGGAGC-3'	55
	Me3	5'-TGAGTCCAAACCGGAAT-3'	50
	Me4	5'-TGAGTCCAAACCGGACC-3'	55
	Me5	5'-TGAGTCCAAACCGGAAG-3'	52
Reverse	Em1	5'-GACTGCGTACGAATTAAT-3'	49
	Em2	5'-GACTGCGTACGAATTTGC-3'	54
	Em3	5'-GACTGCGTACGAATTGAC-3'	54
	Em4	5'-GACTGCGTACGAATTTGA-3'	52
	Em5	5'-GACTGCGTACGAATTAAC-3'	52

were calculated by Microsoft Office$_{2013}$ in Excel environment. The genetic distances between 17 inbred lines were measured based on bands pattern of primer combinations and pattern of total bands of SRAP markers primers by Jaccard coefficient with NTSYS software. The pearson's correlation was measured between Euclidean distance of inbred lines based on studied traits with inbred lines distance based on Jaccard coefficients for pattern bands of primer combinations and pattern of total bands by SPSS18 and finally the Pearson's correlation was calculated among performance of studied traits in hybrids with inbred lines distance based on Jaccard coefficients for pattern bands of primer combinations and pattern of total bands.

Results

The results of the variance analysis between inbred lines for seed quantity and quality were showed a significant variation (P≤0.01) (Table 3). On the other hand, based on variance analysis among the hybrids derived from inbred lines, significant variation was observed for oil, starch, protein, seed yield and thousand seed weights (P≤0.01). Also, significant variation was observed for the dry matter (P≤0.05). The mean values of crude fiber did not show a significant variation among hybrids (Table. 4).

Euclidean distance between the inbred lines on the basis of the traits related to yield and seed quality is presented in Table 5. The average of Euclidean distance between the studied lines was 15.95, that L_4 with L_{15}had the most distance (46.00). On the other hand, the lowest Euclidian distance was observed between L_{11} and L_5 lines (1.87). Therefore, the maximum distance between the two lines was 24.5 times greater than the minimum distance between two lines. In additions, the lines of L_4 with L_{17} and L_{15} and the lines of L_{15} with L_7 and L_{16} had the most Euclidian distance based on seed quantity and quality. The lines of L_{13} with L_3 and L_6, the lines of L_8 with L_1 and the lines of L_{11} with L_5 had the lowest Euclidian distance.

The banding pattern for studied inbred lines based on M5M3 primer combination is presented in Figure 1. The PCR amplification for the 17 inbred lines with the

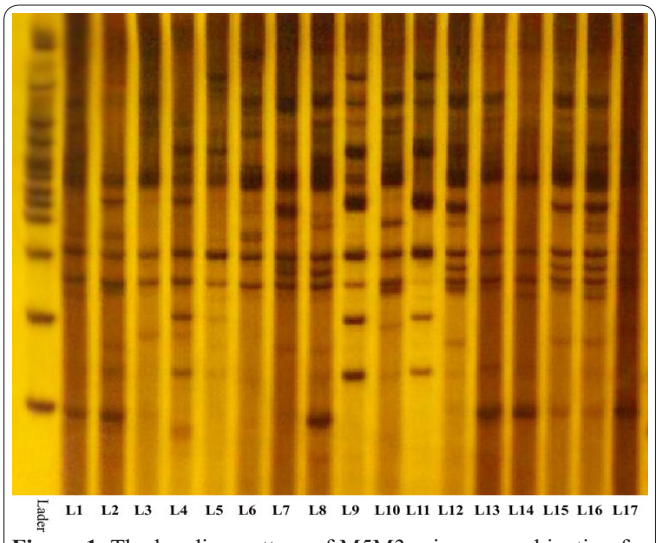

Figure 1. The banding pattern of M5M3 primers combination for the 17 inbred lines.

Table 3. Variance analysis for seed quantity and quality of inbred lines.

S.O.V	D. F.	Mean squares						
		Oil Percentage	Crude Fiber	Starch Percentage	Protein Percentage	Dry Matter	Seed Yield	Thousand seed weight
Replication	2	0.032^{ns}	0.005^{ns}	3.451^{ns}	1.276^{ns}	0.089^{ns}	0.482^{ns}	610.1^{ns}
Lines	16	0.848**	0.017**	10.322**	3.054**	0.850**	4.080**	505.0**
Error	32	0.169	0.005	4.371	1.229	0.079	0.301	374.0

Table 4. Variance analysis for seed quantity and quality of hybrids.

S.O.V	D. F.	Mean squares						
		Oil Percentage	Crude Fiber	Starch Percentage	Protein Percentage	Dry Matter	Seed Yield	Thousand seed weight
Replication	2	0.008^{ns}	0.003^{ns}	5.58^{ns}	0.069^{ns}	1.40^{ns}	30.64**	1042^{ns}
Hybrids	51	0.168**	0.008^{ns}	6.34**	1.50**	1.32*	7.41**	1688**
Errors	102	0.046	0.007	2.26	0.506	0.915	1.29	439.4

Table 5. Euclidean distance among studied inbred lines based on seed quantity and quality characters.

Inbred lines	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}	L_{11}	L_{12}	L_{13}	L_{14}	L_{15}	L_{16}
L_2	8.23															
L_3	18.70	11.03														
L_4	11.24	19.37	29.61													
L_5	15.60	7.83	5.27	26.63												
L_6	19.46	11.75	4.16	30.37	4.57											
L_7	6.83	14.57	25.33	6.24	22.04	26.02										
L_8	2.76	5.74	16.55	13.75	13.34	17.29	8.91									
L_9	9.55	4.81	10.00	19.90	8.69	11.74	16.20	7.88								
L_{10}	18.43	10.97	7.36	29.06	5.17	4.02	24.64	16.22	12.01							
L_{11}	14.91	7.36	5.40	25.85	1.87	4.63	21.44	12.78	7.88	4.90						
L_{12}	24.35	16.49	6.17	35.29	9.20	5.45	30.92	22.14	15.96	8.66	9.69					
L_{13}	19.89	12.12	2.00	30.82	5.54	3.06	26.47	17.72	11.49	6.46	5.71	4.66				
L_{14}	4.99	5.61	16.30	15.17	12.43	16.48	9.98	3.27	8.99	14.91	11.95	21.43	17.16			
L_{15}	35.06	27.05	17.27	46.01	19.65	15.83	41.42	32.75	27.02	17.48	20.26	11.25	15.64	31.60		
L_{16}	4.63	11.94	22.65	8.52	19.00	22.90	4.13	6.55	13.83	21.33	18.35	27.99	23.67	7.13	38.39	
L_{17}	31.19	23.26	12.99	42.15	15.88	11.89	37.72	28.94	22.84	14.26	16.41	6.97	11.51	28.11	4.92	34.73

Table 6. Parameters of genetic variation generated by combined primers of SRAP.

Combined Primers	Total bands	Polymorphic bands	Percentage of polymorphism	Polymorphism information content	Marker index
M1E1	16	9	56.20	0.47	4.20
M1E2	3	2	66.67	0.50	1.00
M1E3	15	6	40.00	0.44	2.65
M1E4	8	6	75.00	0.49	2.97
M1E5	14	9	64.29	0.50	4.50
M2E1	6	4	66.67	0.44	1.76
M2E2	8	4	50.00	0.43	1.74
M2E3	10	5	50.00	0.47	2.37
M2E4	6	5	83.33	0.49	2.47
M2E5	8	5	62.50	0.45	2.23
M3E1	12	9	75.00	0.50	4.48
M3E2	4	4	100.00	0.36	1.43
M3E3	0	0	----	----	-----
M3E4	2	2	100.00	0.49	0.98
M3E5	2	0	0.00	0.00	0.00
M4E1	6	5	83.33	0.49	2.45
M4E2	6	3	50.00	0.47	1.40
M4E3	9	6	66.67	0.47	2.80
M4E4	4	2	50.00	0.46	0.91
M4E5	6	4	66.67	0.49	1.98
M5E1	7	5	71.43	0.50	2.50
M5E2	10	7	70.00	0.49	3.45
M5E3	13	10	76.92	0.49	4.86
M5E4	15	13	86.67	0.47	6.06
M5E5	15	10	66.67	0.47	4.73
Average	9	6	65.75	0.43	2.66
Total	205	135	1578.02	10.83	63.90

25 combines of SRAP primers generated a total of 205 clear and scorable bands, of which 135 were polymorphic (65.75%). Among the 25 studied primer combinations used to study genetic variation of lines, M3E3 did not produce scorable bands and M3E5 produced only two monomorphic bands and therefore not included in the analysis. Information for primer combinations is presented in Table 6. As seen, M1E1, M1E3, M1E4 and M5E5 had the most produced bands, and M5E4, M5E5 and M5E3 had the most polymorphic bands. On the other hand, M3E2 and M3E4 produced 100% polymorphic bands. However, these primers along with

Table 7. Distance among studied inbred lines based on the banding pattern of SRAP markers by Jaccard coefficient.

Inbred lines	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}	L_{11}	L_{12}	L_{13}	L_{14}	L_{15}	L_{16}
L_2	0.273															
L_3	0.350	0.312														
L_4	0.314	0.340	0.327													
L_5	0.340	0.354	0.380	0.242												
L_6	0.336	0.283	0.248	0.333	0.295											
L_7	0.348	0.342	0.266	0.375	0.387	0.247										
L_8	0.286	0.333	0.269	0.367	0.379	0.306	0.245									
L_9	0.335	0.360	0.386	0.248	0.244	0.394	0.402	0.337								
L_{10}	0.309	0.325	0.245	0.360	0.383	0.260	0.299	0.291	0.360							
L_{11}	0.319	0.372	0.369	0.257	0.253	0.394	0.419	0.341	0.195	0.381						
L_{12}	0.325	0.359	0.277	0.371	0.383	0.292	0.233	0.247	0.371	0.299	0.337					
L_{13}	0.352	0.298	0.320	0.370	0.414	0.303	0.361	0.298	0.410	0.212	0.428	0.378				
L_{14}	0.327	0.353	0.276	0.395	0.388	0.291	0.240	0.232	0.422	0.342	0.376	0.220	0.329			
L_{15}	0.359	0.292	0.349	0.388	0.370	0.297	0.347	0.327	0.408	0.340	0.426	0.385	0.234	0.289		
L_{16}	0.308	0.290	0.301	0.350	0.333	0.283	0.331	0.290	0.390	0.336	0.372	0.298	0.310	0.298	0.292	
L_{17}	0.350	0.312	0.280	0.376	0.398	0.306	0.256	0.259	0.412	0.354	0.359	0.181	0.353	0.149	0.315	0.268

Table 8. Correlation coefficients between Euclidean distances of inbred lines based on studied traits with inbred lines distance based on Jaccard coefficients for pattern bands of primer combinations and pattern of total bands.

Primer combinations	Dry Matter	Protein Percentage	Starch Percentage	Crude Fiber	Oil Percentage	Thousand seed weight	Seed Yield
M1E1	0.016	0.016	-0.026	0.063	-0.105	-0.101	-0.133
M1E2	-0.013	0.033	0.000	0.066	-0.078	-0.048	0.134
M1E3	0.016	0.016	-0.026	0.063	-0.105	-0.101	-0.133
M1E4	-0.024	0.105	0.066	-0.044	-0.019	0.158	-0.112
M1E5	-0.291**	-0.078	-0.076	-0.113	-0.046	0.002	-0.158
M2E1	0.043	-0.084	-0.111	-0.166	-0.103	-0.096	0.042
M2E2	0.028	0.095	0.036	0.038	-0.103	0.149	-0.011
M2E3	0.159	-0.132	-0.123	0.247**	-0.100	0.363**	0.041
M2E4	-0.181*	0.093	-0.010	-0.071	0.025	-0.146	-0.090
M2E5	-0.121	-0.092	0.023	-0.192*	-0.167	0.202*	-0.180*
M3E1	0.150	0.003	0.061	0.075	0.040	0.096	0.269**
M3E2	-0.013	0.057	0.137	-0.143	0.007	-0.136	0.137
M3E3	Did not show any bands						
M3E4	-0.162	-0.051	-0.028	0.001	0.009	-0.255**	0.051
M3E5	Did not show any polymorphic bands						
M4E1	-0.191*	-0.014	-0.058	0.007	-0.026	0.177*	-0.031
M4E2	-0.016	0.221**	0.148	-0.039	-0.029	-0.020	0.135
M4E3	-0.150	0.136	0.046	-0.138	0.204*	-0.141	0.093
M4E4	-0.120	-0.087	0.198*	-0.160	-0.160	0.001	0.078
M4E5	-0.008	0.120	-0.024	-0.046	0.020	0.123	0.060
M5E1	0.182*	-0.002	0.019	0.097	-0.048	0.178*	0.123
M5E2	-0.014	0.000	0.063	-0.024	0.041	-0.130	0.119
M5E3	-0.124	0.068	0.115	-0.152	0.023	-0.126	0.096
M5E4	0.095	-0.133	-0.034	-0.021	-0.209**	-0.039	-0.023
M5E5	-0.109	0.074	0.060	0.085	0.003	-0.236**	-0.148
Total bands	0.016	0.016	-0.026	0.063	-0.105	-0.101	-0.133

M1E2, M3E5 and M4E4 generated the lowest numbers of bands. The parameter of polymorphism information content for primer combinations was showed that M1E1, M1E5, M3E1 and M5E1 had the most value and based on PIC were more informative. Finally, on the bases of marker index, primer combinations M1E1, M1E5, M3E1, M5E3, M5E4 and M5E5 had higher values and were considered superior in this respect.

Jaccard distance between the inbred lines on the basis of the SRAP marker banding pattern is presented in Table7. The average distance between the studied lines was 0.324 on the bases of the Jaccard coefficient and the L_{11} with L_{13} had the most distance (0.428). On the other hand, the lowest Jaccard distance was observed between L_{14} and L_{17} (0.149). Therefore, the maximum distance between the two lines was 2.87 times greater than the minimum distance between two lines. In additions, the lines of L_{11} with L_{13} and L_{15} and the lines of L_9 with L_{14} had the most distance based on SRAP markers. The lines of L_{17} with L_{12} and L_{14} and the lines of L_9 with L_{11} had the lowest distance.

The Pearson's correlation between Euclidean dis-

tance of inbred lines based on seed quantity and quality characters with inbred lines distance based on Jaccard coefficients for banding patterns of primer combinations and pattern of total bands is presented in Table 8. As seen, although the correlation between the distance of the inbred lines on the basis of dry matter with their distance based on all the bands obtained from SRAP primers was not significant (0.016), but a significant and positive correlation for the banding pattern of M5E1 at 5% level (0.182), a significant and negative correlation for the banding pattern of M1E5 at 1% level and a significant and negative correlation for the banding patterns of M2E4 and M4E1 at 5% level with dry matter were observed. Also, there was no significant correlation between protein percentage with total banding pattern, as well as, the correlation between genetic diversity of inbred lines based on the starch percentage, crude fiber, oil percentage, thousand seed weight and seed yield with their genetic variation based on total banding pattern were not significant. However, the banding pattern of M4E2 was showed a significant and positive correlation with protein percentage. The band pattern of M4E4 was showed a significant and positive correlation with the starch percentage at 5% level. M2E3 and M2E5 were showed a significant correlation with crude fiber at 1% and 5% levels respectively. For M4E3 a significant and positive correlation was observed with oil percentage at 5% level and M5E4 were showed a significant and negative correlation with oil percentage at 1% level. The weight of thousand seed was showed a significant and negative or positive correlation with M2E3, M2E5, M3E4, M4E1, M5E1 and M5E3 at 1% or 5% levels. For the seed yield a significant and positive correlation was observed with M3E1 at 1% level and a significant and negative correlation with M2E5 at 5% levels.

The Pearson's correlation between the performance of studied hybrids for seed quantity and quality characters with inbred lines distance based on Jaccard coefficients for the pattern of bands of primer combinations and pattern of total bands is shown in Table 9. As seen, although the correlation between the dry matter of hybrids with genetic distance of inbred lines based on all the bands obtained from SRAP primers was not significant (0.135), but a significant and positive correlation ($P \leq 0.01$) for the band pattern of M2E2 (0.359), and a significant and positive correlation ($P \leq 0.05$) for M2E1, M4E1 and M5E1 with dry matter of hybrids were observed. Also, there was no significant correlation between protein percentage with total banding pattern, as well as, lack of significant correlation between hybrids performance for starch percentage, crude fiber, oil percentage, thousand seed weight and seed yield with genetic variation of inbred lines based on total banding pattern.

The banding patterns of M2E4 and M4E4 were showed a significant and positive correlation with the starch percentage. M1E2, M2E3 and M5E5 combinations were showed a significant and positive correlation with thousand seed weight and M2E2 had a significant and negative correlation with thousand seed weight at 1% level. M1E5, M2E2 and M3E2 had a significant and negative correlation with seed yield.

Table 9. Correlation coefficients between studied traits in hybrids and the inbred lines. Distances are based on Jaccard coefficients for banding patterns of primer combinations and pattern of total bands.

Primer combinations	Dry Matter	Protein Percentage	Starch Percentage	Crude Fiber	Oil Percentage	Thousand seed weight	Seed Yield
M1E1	-0.002	0.119	0.016	0.112	-0.123	0.159	0.079
M1E2	0.083	-0.164	-0.058	-0.125	0.064	0.336*	0.228
M1E3	-0.120	0.163	-0.063	-0.041	0.088	0.243	0.263
M1E4	0.104	-0.109	0.265	0.206	0.212	-0.271	-0.271
M1E5	0.018	0.155	0.018	0.143	0.114	-0.160	-0.357**
M2E1	0.293*	0.028	-0.091	0.166	-0.085	-0.272	-0.036
M2E2	0.359**	-0.016	0.049	0.043	0.003	-0.360**	-0.302*
M2E3	-0.108	-0.094	0.080	-0.038	0.167	0.284*	0.108
M2E4	-0.152	-0.214	0.289*	0.234	0.138	-0.177	0.007
M2E5	0.016	-0.131	0.142	-0.019	-0.002	-0.128	-0.077
M3E1	-0.094	0.124	-0.032	-0.146	-0.155	0.139	-0.018
M3E2	-0.077	0.120	-0.118	-0.036	0.088	-0.046	-0.290*
M3E3	No bands showed						
M3E4	0.056	-0.102	-0.018	-0.099	-0.075	-0.103	0.068
M3E5	No polymorphic bands showed						
M4E1	0.286*	0.221	-0.209	0.029	0.140	-0.063	0.014
M4E2	0.071	0.016	-0.022	-0.107	0.101	0.211	0.039
M4E3	0.153	-0.138	0.096	-0.062	0.043	-0.028	0.117
M4E4	-0.100	0.205	0.025	0.100	0.064	0.008	0.018
M4E5	0.071	0.016	-0.022	-0.107	0.101	0.211	0.039
M5E1	0.019	0.097	-0.232	-0.007	-0.139	-0.013	0.014
M5E2	0.160	-0.054	-0.117	-0.095	-0.107	-0.022	-0.037
M5E3	0.288*	-0.158	0.041	0.074	0.116	-0.194	-0.166
M5E4	-0.080	-0.017	0.019	-0.089	-0.072	0.340	0.319
M5E5	0.068	-0.183	0.118	-0.192	-0.117	0.392**	0.214
Total bands	0.135	-0.056	0.058	-0.045	0.040	0.065	-0.025

Discussion

Understanding the genetic diversity of a crop is the basis of plant breeding programs (12, 24). The knowledge of its existence and how it is transmitted from parents to offspring, and also the application of it to select the superior cultivars, has been the subject of numerous researches (25, 26). In plants such as corn, that usually their single cross hybrids are introduced as a cultivar this information is more crucial. The relationship between the inbred lines and their hybrids, as well as the estimation of heterosis, has been evaluated in numerous studies (22, 27). Also, the knowledge of genes action controlling traits can play an important role in the understanding of genetic diversity structure and introducing appropriate breeding programs methods (28, 29). In the present study, there was a high genetic variation for traits related to seed quality, thousand seed weight and grain yield, among the inbred lines and between the hybrids derived from them.

It should be noted that in the present study the distance of the inbred lines was different based on genetic variation and the Euclidean distance ranged between 1.87 to 46 that was relatively a wide range. Therefore, the existing germplasm seemed to be very suitable for breeding programs to produce hybrid varieties. The existence of a high diversity among the hybrids derived from these lines also confirmed this point. Various studies have confirmed the necessity of genetic diversity and its use in the production of cultivars (11, 30). However, the use of molecular markers in the study of genetic variation provides more reliable information for plant breeding and genetic relationship among plant species populations (31). On the other hand, some studies have shown that the study of genetic diversity based on DNA markers cannot predict the appropriate hybrids derived from inbred lines (32, 33). Reports on the relationship between the genetic distance of genotypes based on DNA marker and genetic variation due to morphological traits are controversial. In some reports, these relationships were positively evaluated (15), but in others, the absence of this relation is reported (34, 35). The results of this study considering the genetic distance between inbred lines according to the SRAP marker was not considered high. The SRAP marker targets the ORFs and has been introduced in most reports as a reliable marker, which has a high ability to evaluate genetic variation and is highly efficient (17, 36).

The relationship between DNA markers with morphological characteristics is one of the main challenges in the study of genetic variation. In this study, a significant relationship was not observed between the morphological characters studied and the total banding patterns of SRAP primers. This absence of relation was observed for both inbred lines and the genetic diversity of hybrids derived from their crosses. However, in a few studies, the relationship between SRAP markers with morphological characteristics has been investigated. In some of these studies, positive relationships between the two markers were mentioned (15, 21) and in others, this relation was not existing (14). In additions, the efficiency of SRAP for expressing genetic variation, as already mentioned, has been expressed in many studies. Basically finding the positive relationship between the genetic distance of the inbred lines on the basis of morphological characteristics and heterozygosity obtained from these characteristics has been one of the important issues in corn research (27, 37).

Reaching the hybrids that have the appropriate performance for morphological characteristics, especially yield, based on the genetic distance due to DNA variation between inbred lines is a unique topic that has been mentioned in some research (38, 39). Our results showed that, although for the total banding patterns of the primer combinations, there was no significant relation with the traits studied in the inbred lines and the derived hybrids, for the banding pattern of the primer combinations separately, a significant relationship was found between the inbred lines and hybrids. These significant relationships were not always positive and in some cases they were negative. The existence of a positive relationship between genetic distances of inbred lines with the morphological characteristic indicated that the genetic variation revealed by the DNA marker has direct association with morphological characteristics studied. In other words, it may imply that the amplified regions are closely linked to the locations of genes that control the desired traits.

Nonetheless, the interpretation of the negative relation that existed between the genetic distance of the inbred lines on the basis of morphological characteristics and traits related to the seeds quality with their genetic distance based on the molecular markers is complicated and it poses the challenge that genetic diversity at the molecular level with genetic variation at the morphological level is not always in the same direction and in this relation the locations that have been amplified by SRAP markers, may be positioned near the location of characters that caused reduce the trait. Further research is needed for the explanation of this issue. On the other hand, a high relation was observed for correlation, between the genetic distance of inbred lines based on SRAP marker with the values obtained from derived hybrids on the bases of morphological characters and quality traits. The mean correlation coefficient between molecular markers with hybrids was 0.118, but the average correlation between the molecular markers with morphological characteristics of inbred lines was 0.089. Consequently, as the molecular genetics distance between inbred lines was higher, the derived hybrids had a better performance, which has been also reported in several other studies in corn with an emphasis on morphological traits (22, 40).

Although the M1E1, M1E5, M5E3, M5E4, and M5E5 were superior to the other primers combinations in the expression of genetic diversity based on the primers information indices, but the relation between the banding patterns of the studied primers combinations with the genetic variation of the inbred lines based on the studied traits were better explained by some other primers. For example, it was observed that the M5E-1banding pattern can predict the distance of inbred lines for dry matter production. Also, the primers combination of M4E4 for protein percentage, M4E4 for starch percentage, M2E3 for crude fiber, M4E3 for oil percentage, M2E5, M4E1 and M5E1 for thousand seed weight and M3E1 for seed yield.

Considering the relation between inbred lines varia-

tion based on primers combinations with the studied traits in hybrids, it was observed that M2E1, M2E2, M4E1 and M5E3 may have the ability to predict hybrids performance for dry matter and M2E4 for starch percentage respectively. For traits including protein percentage, crude fiber and percent oil a suitable primers combination were not found. Three primer combinations of M1E5, M2E2 and M3E2 had the highest negative correlation with seed yield. Thus, the hybrids derived from the inbred lines with high genetic distance based on these primers combinations will have a low seed yield. Finally M1E2, M2E3 and M5E5 can be introduced for prediction of thousand seed weight.

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