Different Methods for Detecting Heterotic Groups in Maize

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Abstract. Comparing between different methods for classifying 12 maize inbred lines to heterotic groups was achieved using general and specific combining ability effects; agronomic traits to the maize inbreds per se; and ISSR-markers. The results of line-tester design showed that Giza656 lines had the highest yield for the cross with Gemmiza 1021 tester (7.20 ton per hectare) at Nubaria, while Giza655 lines had the highest yield for the cross with Gemmiza 1021 tester (8.24 ton per hectare) at Gemmiza. Regarding Heterotic specific and General Combining Abilities, the inbred lines were grouped into three groups as their testers were, but groups differed from Nubaria to Gemmiza locations.

The classification due to Inter Simple Sequence Repeats markers have agreement with pedigree data more than the HSGCA and agronomic characters, because the HSGCA method affected by testers, lines, environments, and the interactions between all of them, also, agronomic character method need to plant the inbreds and evaluate them at more locations and more seasons. Furthermore, all these cautions will not discard the environmental effects but they will reduce it to the minimum values. On the other hand, molecular marker will keep time and efforts needed to evaluate the inbred lines.

Keywords: heterotic groups, molecular markers, maize breeding.

Introduction

Heterosis is a major yield factor in all breeding categories except line breeding (Schnell, 1982). To exploit heterosis in hybrid breeding, the concept of heterotic groups and patterns was suggested, (Reif, *et al*, 2005).

The manifestation of heterosis usually depends on genetic divergence of the two parental varieties. Genetic divergence among varieties usually is unknown, and the only recourse is to determine level of genetic divergence empirically by means of variety crosses. Genetic divergence of the parental varieties is inferred from the heterotic patterns manifested in the series of variety crosses. If heterosis manifested from the cross of two

parental varieties is relatively large, it is concluded that the two parental varieties are more genetically diverse than two varieties that manifest little or no heterosis in their variety crosses. Establishment of heterotic patterns among varieties has important implications for selecting inbred lines as potential seed stocks in hybrids (Hallauer, *et al.*, 1988)

A heterotic group is a collection of closely related inbred lines which tend to result in vigorous hybrids when crossed with lines from a different heterotic group, but not when crossed to other lines of the same heterotic group (Lee, 2007). Depending on the objectives of the breeding program, breeders use different methods in selecting the best

parents for making crosses, and for assigning lines to a particular heterotic group, including (a) pedigree relations (b) phenotypic performance for specific traits, (c) adaptability and yield stability, (d) top crosses, (e) diallel crosses, and (f) genetic distances estimated from morphological and molecular markers, (Bertan *et al*, 2003).

The need of genetic diversity between inbred lines used in crosses is generally accepted. In the past, general experience usually but not always showed that crosses of unrelated genotypes contributed to greater yields. It usually required extensive evaluation trials to determine the unique combination of two parental inbred lines. Although the genetic basis of the importance of genetic diversity was not clear, general experience showed that the better hybrids involved inbred lines derived from two or more genetic backgrounds (Hayes, 1956).

Melchinger and Gumber, (1998) listed the following criteria which are useful for identifying heterotic groups and patterns 1. High mean performance and large genetic variance in the cross between heterotic groups. In other words the usefulness criterion in the population cross should be high. High performance and good adaptation of heterotic groups. Ideally inbred from both heterotic groups should be sufficient pollen and have high seed yields to provide adequate hybrid seeds. 2. Ability to maintain and propagate inbreds. Developing inbreds is difficult if inbreeding depression is too severe. 3. A stable cytoplasmic male sterility system, if needed.

The objectives of the present work were to: 1. Classifying 12 maize inbred lines into heterotic groups using: a. General and specific combining abilities derived from line-tester mating design (Heterotic General and Specific Combining Ability, (HSGCA method)), b. Cluster analysis for the 12 inbred lines agronomic characters, c. Cluster analysis for its DNA amplification with Inter Simple

Sequence Repeats (ISSR) markers. 2. Comparison between previous grouping methods with pedigree information, and determining breeding value for each method.

Materials and Methods

The present work was carried out at Maize research department, Field Crop Research Institute (FCRI), Agriculture Research Center (ARC), Egypt, during the period from 2010 to 2012. The present investigation consisted of three experiments; the first one was conducted to determine general and specific combining ability effects through Line x Tester mating design. The second experiment was conducted to determine some agronomic traits to these inbred lines under investigation. The third experiment was conducted to apply ISSR-analysis for detection of genetic polymorphism among 15 Maize inbred lines. These experiments were performed as follows:

A. Line x Tester experiment

Selected inbred lines for study: 15 Maize inbred lines and its genetic background presented in Table (1) were obtained from Maize research department. Three inbred lines Giza 639, Gemmiza 1021 and, Nubaria 552 were used as a tester for the other 12 inbred lines.

Experimental procedures:

In 2010 and 2011 seasons, at Nubaria Agriculture Research Station farm, 12 Maize inbred lines were used as female parents and crossed with three testers in a Line x Tester mating design yielded 36 yellow single crosses.

In 2012, 36 single crosses and check hybrid SC- 173 were evaluated at two locations which were Nubaria and Gemmiza. A randomized complete block design with three replications was used for each location. Each experimental unit was a single-row plot with a row spacing of 0.75 m and length of 6 m. Distance between two adjacent plants was 0.25 m and the population density was approximately 57000

plants ha^{-1} . Standard agronomic farming practices were followed at each experimental site. Grain yield (ton ha^{-1}): weight of grain yield detected in ton per hectare estimating by adjusting the ear weight according to its shelling percent and 15.5 % moisture content.

Statistical analysis:

Analysis of variance was done for each location alone, due to heterogeneity of error mean squares., 2.The procedure line x tester analysis according to Kempthorne (1957) was used for estimating general and specific combining ability effects were:

Lines: $g_i = (X_{i..} / tr) - (X_{...} / ltr)$, Testers: $g_j = (X_{.j} / lr) - (X_{...} / ltr)$, $S_{ij} = (X_{ij} / r) - (X_{i..} / tr) - (X_{.j} / lr) + (X_{...} / ltr)$.

Where: g_i = general combining ability for i th inbred line, $X_{i..}$ = total grain yield of testcrosses for i th inbred line, $X_{...}$ = overall total of grain yield for all crosses

l = number of inbred lines, t = number of testers, r = number of replications

S_{ij} = specific combining ability for i th inbred line and j th tester, $X_{.j}$ = total grain yield of testcrosses for j th tester.

HSGCA–method (Heterotic group's specific and general combining ability)

Heterotic group's specific and general combining ability (HSGCA) were made according to Fan *et al* (2009).

B. Agronomic traits for inbreds

Plant materials:

In 2012, at Nubaria griculture Research Station farm, a total of 15 maize inbred lines (shown in Table 1), were planted to determining agronomic traits for investigated inbred lines.

Experimental procedures:

Investigated inbred lines evaluated in a randomized complete block design with three replications. Each experimental unit was a three-row plot with a row spacing of 0.75 m and length of 6 m. Distance between two adjacent plants was 0.25 m and the population

density was approximately 57000 plant ha^{-1} . Standard agronomic farming practices were followed at experiment.

Molecular marker tools

Plant materials:

Polymerase chain reaction (PCR) was carried out using the genomic DNA from 15 Maize inbred lines as shown in Table 1. All inbred lines were grown in Nubaria greenhouse at 2012 season.

DNA extraction:

Bulk of leaf tissues of seven-days old plants from 10 plants from each inbred line (500 mg) were ground to a powder with pestle and mortar in liquid nitrogen N_2 . The powder was poured into tubes containing 9.0 ml of warm (65°C) CTAB extracting buffer.

ISSR analysis:

Sequences of 20 primers used for DNA polymorphism (Table 3) obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech., UK Limited, HP 79NA, England), were tested in this experiment, to amplify the templated DNA. The ISSR-PCR method was carried out according to Nagaoka and Ogiwara (1997). Amplification was carried out in 25 μl reaction volumes, containing 1 X Taq polymerase buffer (50 mM KCl, 10 mM Tris, pH 7.5, 1.5 mM MgCl_2) and 1 unit of Taq polymerase (Pharmacia Biotech, Germany), supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 50 pmol of ISSR primers, and 50 ng of total genomic DNA. Amplification was performed in a thermal cycler (Thermolyne AmpliTron). After completion of PCR, samples were cooled immediately to 10°C and stored at 4°C until gel separation. A gel-loading solution (5 μl) was added, and 10 μl of the total product volume was resolved in 1.5% agarose in 1X TAE buffer for 2 h aside with a 100-pb ladder (Pharmacia, Germany) as the size standard. Gels were stained in ethidium bromide and images were recorded and photographed on Polaroid films under U. V. light.

Data handling and cluster analysis:

Data were scored for computer analysis on the basis of the presence of the amplified products for each primer. If a product was present in an inbred line, it was designed as “1”, if absent, it was designed as “0”, after excluding the irreproducible bands. Pair-wise

comparisons of inbred lines, based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficient, according to Jaccard (1908).

Table 1. The 15 maize inbred lines, and its genetic background, used in the investigation.

Serial No.	Inbred line	Source
1	Gz 655	Cargill 922
2	Gz 656	Cargill 922
3	Gz 657	Cargill 922
4	Gz 658	Cargill 922
5	Gm1002	C-17-Tuxpino-1
6	Gm1004	DC-201
7	Nb 509	Population 80
8	Nb 517	Population (Nubaria100273)
9	Nb579	Population -40
10	Nb582	Population -40
11	Nb603	Population -40
12	Nb612	Population- 40
1	Gz 639*	Population of Sd6273
2	Gm 1021*	Gemmiza 7421 Population
3	Nb 552*	Nubaria 73 Ht population

*Tester inbred line

Table 2. Sequences of 20 ISSR primers used for polymorphism.

Serial No.	Primer name	Nucleotide sequence 5.....3
1	AD1	(AGC)6 G
2	AD2	(ACC)6 G
3	AD5	(CA)10 C
4	AD7	(AG)9 C
5	AD8	(AGC)6 G
6	AD9	(AC)9 G
7	ADM11	(CA)6 A
8	ADM2	(AC)8 CCT
9	Bra1	(GA)9 T
10	Bra2	(GGTA)4
11	Bra3	(GA)9 C
12	Bra4	(GACA)4
13	Bra5	(CCTA)4
14	HB13	(GAG)3 GC
15	HB15	(GTG)3 GC
16	MGDB1	(CA)6 AT
17	MGDB2	(GT)6 CA
18	MGDB3	(AGC)4 T
19	MGDB4	(GCT)4 T
20	TG1	(CAC)3 GC

Results

Line-tester experiment

Analysis of variance of line-tester mating design for grain yield (ton ha^{-1}) at the tested locations is presented in Table (3). Mean square of error for the two locations heterogeneous; therefore, the statistical analysis for each location was done separately. Highly significant differences were found among lines, testers, and line-tester interaction for grain yield within Nubaria and Gemmiza locations. The interaction line $\times$ tester for grain yield, at tested location indicated that the inbred lines performed differently in their respective test crosses depending on the type of tester used for these traits.

Grain yield as mean performance of top-crosses, for the 12 inbred lines at Nubaria and Gemmiza locations are presented in Tables (4, and 5). The results at Nubaria showed that Nb552 tester had the highest grain yield with the Gz656 and Nb517 inbred lines (5.77 and 5.59 ton ha^{-1} respectively), while Gz658 and Gm1004 inbred lines have the lowest grain yield with the same tester (3.49 and 3.37 ton ha^{-1} respectively, Table 4). Gz639 tester had the highest grain yield with the Gz656, Gz657, Gz658 and Gm1002 inbred lines (6.05, 6.01, 5.93 and 6.27 ton ha^{-1} respectively), while Nb612 inbred line had the lowest grain yield with the same tester (3.51 ton ha^{-1} , Table 4). Also, the results showed that Gm1021 tester had the highest grain yield with the Gz656 inbred line (7.20 ton ha^{-1}), while Nb517 inbred line had the lowest grain yield with the same tester (2.16 ton ha^{-1} , Table 4). Out of 36 single crosses evaluated at Nubaria location, 15 hybrids surpassed grain yield was significantly higher than the check SC173 mean (3.92 ton ha^{-1}),

and from those, there the three local commercial hybrids Gz639 $\times$ Gz656 (SC166), Gz639 $\times$ Gz657 (SC167), and Gz639 $\times$ Gz658 (SC168), had mean performance for grain yield 6.05, 6.01, and 5.93 ton ha^{-1} respectively. Out of the superior 15 hybrids, the new hybrid (Gz656 $\times$ Gm1021) yielded 7.20 ton ha^{-1} topping up all the hybrids under test.

Summarizing the rank of the 36 testcrosses resulting from the 12 lines with the three testers, the top yielding testcross with tester Nb552 were lines Gz656 and Nb517. While with the tester Gz639 the four lines Gz656, Gz657, Gz658, and Gm1002 were the top ranking hybrid. With tester Gm1021, Gz656 gave the best yielding hybrid. It be noticed that Gz656 inbred line had a significant heighest grain yield with the three testers at Nubaria location. Also, the Gz639 tester had the highest grain yield at Nubaria location comparing with Nb552, and Gm1021, (4.88, 4.55, and 4.57 ton/ha , respectevially).

At Gemmiza location, The results showed that Gm1002 and Nb509 inbred lines had the highest grain yield with the Nb552 tester (6.21 and 6.10 ton ha^{-1} respectively), while Gm1004 inbred line had the lowest grain yield with the same tester (4.00 ton ha^{-1} , Table 5). Gz639 tester had the highest grain yield with the Gz655 and

Gz657 inbred lines (7.74 and 7.83 ton ha^{-1} respectively), while Nb582 and Nb603 inbred lines have the lowest grain yield with the same tester (4.32 and 4.03 ton ha^{-1} respectively, Table 5). Gz655 inbred line had the highest grain yield with the Gm1021 tester (8.24 ton ha^{-1}), while Nb517, Nb579 and Nb612 inbred lines had the lowest grain yield with the same tester (4.52, 5.11 and 5.11 ton ha^{-1} respectively, Table 5).

Table 3. Analysis of variance for grain yield (ton/ha) from line x tester experiment at Gemmiza and Nubaria locations.

S.O.V	D.F	Grain yield (ton ha ⁻¹)	
		Nubaria	Gemmiza
Replications	2	1.244	8.860
Lines	11	6.347**	4.580**
Testers	2	1.220**	21.392**
Lines x testers	22	2.589**	2.959**
Error	70	0.227 ^a	0.933 ^b

** Significant and highly significant differences at 0.01 level of probability.

a, and b indicate heterogeneous error variance for the two locations.

Also, the Gm1021 tester had the highest grain yield mean at Gemmiza comparing with the Nb552, and Gz639 testers (6.49, 4.97, and 5.92 ton/ha, respectively).

Comparing the results of the topcrosses of the 12 inbred lines with the three testers indicated that the ranking of the testcross

differed from one tester to another due to the effect of general or specific combining ability or both.

For Nb552, both inbred line Gm1002 and Nb509 were the top, while for Gz639 Gz655 and Gz657 were the highest, and only Gz655 was the top hybrid with tester Gm1021.

Table 4. Grain yield means (ton ha⁻¹), for 12 lines topcrossed to three testers at Nubaria location.

Inbred line	Tester (ton ha ⁻¹) ⁺		
	Nb552	Gz639	Gm1021
Gz655	4.08 def	5.77 ab	6.22 b
Gz656	5.77 a	6.05 a	7.20 a
Gz657	4.61 bcd	6.01 a	6.18 b
Gz658	3.49 f	5.93 a	4.94 c
Gm1002	4.51 cde	6.27 a	4.86 c
Gm1004	3.37 f	4.28 cd	3.75 de
Nb509	4.16 def	5.02 bc	4.37 cd
Nb517	5.59 a	3.56 de	2.16 f
Nb579	4.71 bcd	4.07 de	3.90 de
Nb582	3.70 ef	3.74 de	3.15 e
Nb603	5.33 ab	4.32 cd	4.30 cd
Nb612	5.27 abc	3.51 e	3.77 de
Mean	4.55 c	4.88 a	4.57 b
SC173	3.92		

+ Means followed by the same letter are not differed according to FLSD_{0.05}.

Table 5. Grain yield means (ton ha⁻¹), for 12 lines topcrossed to three testers at Gemmiza location.

Inbred line	Tester (ton ha ⁻¹) ⁺		
	Nb552 ⁺	Gz639 ⁺	Gm1021 ⁺
Gz655	4.10 bc	7.74 a	8.24 a
Gz656	5.40 ab	6.19 bc	7.76 ab
Gz657	4.27 bc	7.83 a	7.30 abc
Gz658	4.32 bc	6.13 bc	7.38 abc
Gm1002	6.21 a	5.58 bcd	7.06 abc
Gm1004	4.00 c	6.11 bc	5.93 bcd
Nb509	6.10 a	6.57 b	7.68 ab
Nb517	5.17 abc	6.55 b	4.52 d
Nb579	4.93 abc	5.17 cde	5.11 d
Nb582	4.90 abc	4.32 e	5.63 cd
Nb603	5.32 abc	4.03 e	6.22 bcd
Nb612	4.91 abc	4.80 de	5.11 d
Mean	4.97 c	5.92 b	6.49 a
SC173	4.03		

+ Means followed by the same letter are not differed according to FLSD_{0.05}.

HSGCA (Heterotic group's from line-tester experiment)

Nubaria:

The inbred lines were divided into groups depending on its general combining ability plus its specific combining ability with every tester used in the line x tester experiment at the tested location according to the following steps.

Step1: Place all inbred lines (the 12 inbred lines) into the same heterotic group as their tester, in this step all the 12 inbred lines placed into each tester heterotic group, (Table 6).

Step 2: Kept the inbred line with the heterotic group where its HSGCA effects (Heterotic relying on Specific and General combining Ability effects) had the smallest value or largest negative value (Table 6).

Step 3: If the inbred line had positive HSGCA effect with all representative testers, it will be cautious to assign that line to any heterotic group, to get final groups.

Group 1 (Nb552): Gz655, Gz658, Gm1002, Gm1004, and Nb509. Group 2 (Gz639): Nb579, Nb603, and Nb612, Group 3 (Gm1021): Nb517, and Nb582.

Gemmiza:

The inbred lines were divided into groups depending on its general combining ability plus its specific combining ability with every tester used in the line x tester experiment at the tested location according to the following steps.

Step1: Place all inbred lines (12 inbred lines) into the same heterotic group as their tester, in this step all the 12 inbred lines placed into each tester heterotic group, (Table 7). Step 2: Kept the inbred line with the heterotic group where its HSGCA effects (Heterotic relying on Specific and General combining Ability effects) had the smallest value or largest negative value. Step 3: If the inbred line had positive HSGCA effect with all representative testers, it will be cautious to assign that line to any heterotic group, to get final groups. Group 1 (Nb552): Gz655, Gz657, Gz658, and Gm1004. Group 2 (Gz639): Gm1002, Nb582, and Nb603. Group 3 (Gm1021): Nb517, Nb579, and Nb612.

Table 6. HSGCA values depending on grain yield from line-tester experiment at Nubaria location for 12 inbred lines with three testers.

Inbred line	HSGCA (Nb.552)	HSGCA (G639)	HSGCA (Gm1021)
Gz655	-0.47	0.89	1.65
Gz656	1.22	1.17	2.63
Gz657	0.06	1.13	1.61
Gz658	-1.06	1.05	0.37
Gm1002	-0.04	1.39	0.29
Gm1004	-1.18	-0.6	-0.82
Nb509	-0.39	0.14	-0.2
Nb517	1.04	-1.32	-2.41
Nb579	0.16	-0.81	-0.67
Nb582	-0.85	-1.14	-1.42
Nb603	0.78	-0.56	-0.27
Nb612	0.72	-1.37	-0.8

Table 7. HSGCA values depending on grain yield from line-tester experiment at Gemmiza location for 12 inbred lines with three testers.

Inbred line	HSGCA (Nb552)	HSGCA (Gz639)	HSGCA (Gm1021)
Gz655	-0.87	1.82	1.74
Gz656	0.43	0.27	1.26
Gz657	-0.7	1.91	0.8
Gz658	-0.65	0.21	0.88
Gm1002	1.24	-0.34	0.56
Gm1004	-0.97	0.19	-0.57
Nb509	1.13	0.65	1.18
Nb517	0.2	0.63	-1.98
Nb579	-0.04	-0.75	-1.39
Nb582	-0.07	-1.6	-0.87
Nb603	0.35	-1.89	-0.28
Nb612	-0.06	-1.12	-1.39

Agronomic characters for inbred lines

Grouping inbred lines using agronomic characters

Mean performance of the 12 inbred lines per se in agronomic and morphological characters is presented in Tables (8, 9, and 10).

Grouping of tested inbred lines by cluster analysis for its agronomic characters using Wards minimum variance method (Ward, 1963), and using NCSS10 software are presented in figure 3.

Results showed that only seven inbred lines were divided into two heterotic groups as follow:

Table 8. Mean performance of number of days to mid-silking, plant height, ear height, grain yield, ear length, and weight of 100 grains for 15 inbred lines grown at Nubaria in 2012 season.

Inbred line	Number of days to mid-silking	Plant height (cm)	Ear height (cm)	Grain yield (g.plant ⁻¹)	Ear length (cm)	100 grains weight (gm)
Gz655	70.3 b	103.3 fg	61.6 def	49.01 abc	13.9 abc	22.84 gh
Gz656	71.6 a	118.0 de	68.3 bcd	55.45 abc	14.5 ab	25.10 defg
Gz657	70.0 b	114.0 defg	70.6 bc	40.03 bcd	12.8 cde	25.63 cdef
Gz658	70.6 b	120.6 bcde	74.0 ab	42.72 bcd	12.3 def	22.27 h
Gm1002	61.6 e	127.0 abcd	63.3 cde	20.98 d	10.9 g	27.17 bcd
Gm1004	66.6 d	102.0 g	60.3 def	60.33 ab	11.9 efg	17.65 i
Nb509	58.6 fg	108.3 efg	53.0 fg	65.33 ab	12.0 efg	23.47 fgh
Nb517	59.3 f	133.6 ab	55.6 efg	65.89 ab	13.6 bcd	25.61 def
Nb579	61.3 e	120.0 cde	50.3 g	57.74 ab	13.9 abc	25.86 cdef
Nb582	61.3 e	137.3 a	80.6 a	31.57 cd	11.0 fg	29.11 b
Nb603	61.0 e	124.0 abcd	66.0 bcd	64.31 ab	13.1 cde	26.58 cde
Nb612	58.6 fg	116.6 def	56.6 efg	63.61 ab	12.0 efg	37.76 a
Gz639	68.6 c	131.6 abc	74.0 ab	58.53 ab	15.2 a	24.12 efgh
Gm1021	66.0 d	125.3 abcd	64.0 cde	49.82 abc	12.4 def	28.12 bc
Nb552	58.0 g	132.6 abc	66.3 bcd	68.44 a	14.5 ab	22.07 h

Means had the same letters are significantly equals.

Group 1: Gz656, Gz657, and Gz658. Group 2: Nb579, Nb603, and Nb612.

But the Gm1002, Gm1004, Nb509, Nb517, and Nb582 inbred lines were not grouped because morphological characters were not sufficient for grouping them.

Disagreement between grouping based on pedigree and that based on morphological character was found in this study. Heckenberger, *et al* (2005) reported that morphological distance have disadvantages first, assessment of morphological traits requires extensive field trails across several years and locations to minimize environmental

effect. These measurements are therefore more expensive and time consuming. Second, the scoring of morphological traits is subjective to certain extent; therefore number of check inbred lines must be included in the study to warrant a high quality of morphological traits across different years and scoring persons.

Grouping inbred lines using ISSR-markers

Grouping 12 inbred lines using 10 Inter-Simple Sequence Repeats (ISSR) markers are presented in figure 4. Results showed that the 12 inbred lines are grouped into five groups as follows: Group 1: Gm1002, and Nb517.Group 2: Nb509, Nb579, and Nb582.Group 3: Gz655, Gz656, Gz657, and Gz658 Group 4: Gm1004, Nb603, and Nb612.

Fig. 3. Clustering inbred lines using 19 agronomic characters.

Fig. 4. Cluster analysis of the 12 maize inbred lines using ISSR markers.

Discussion

Different relatedness inbred lines were used in this investigation, inbred lines were sister lines in F3 stage and differed after yet (these released from Cargill 922), inbred lines from the same population but were not sister lines at any stage (Pop.40 released inbred lines), and other inbred lines are released from totally different sources (Gemmiza inbred lines), and that was to have a real vision how far could each used method detect these degrees of relatedness. The classification due to molecular markers have agreement with pedigree data more than the HSGCA and agronomic characters methods, because that the HSGCA method affected by testers, lines, and environments, also, agronomic character method need to plant the inbred lines and evaluate them at more location and more season and may need more planting date in the season, furthermore, all these cautions will not discard the environmental effects but they will reduce it to the minimum values. On the other hand, molecular marker will keep time and efforts needed to evaluate the inbred lines, also, molecular markers will discard the environmental effects on genetic distances determination.

The marker data is more accurate comparing with other classifying methods. Aguiar *et al* (2008) reported that, the formation of heterotic groups using information from test crosses is tester dependent. The discriminate of heterotic groups based on estimates of genetic diversity obtained with molecular marker data, on the other hand, is not influenced by environment interaction. Besides requiring less time, since no crosses has to be formed. Heckenberger *et al* (2005) reported that morphological distance and mid-parent agronomic heterosis have several disadvantages compared with molecular markers, first, assessment of morphological traits and mid-parent agronomic heterosis requires extensive field

trials across several years and locations to minimize environmental effect. These measurements are therefore more expensive and time consuming than molecular marker analyses. Second, agronomic heterosis estimates requires production and testing of hybrids. In addition, reciprocal crosses should be evaluated to minimize risk of maternal effects. Third, the scoring of morphological traits is subjective to certain extent; therefore number of check inbred lines must be included in the study to warrant a high quality of morphological traits across different years and scoring persons. Xiang *et al.*, (2010) found disagreement between heterotic groups based on specific combining ability and that based on molecular markers. For assessment of genetic diversity, molecular markers have been generally superior to morphological, pedigree, heterosis, and biochemical data (isozymes and chromatography), (Melchinger *et al.* 1991; Melchinger *et al.* 1992). Reif *et al.* (2003) reported that field trials for identification of promising heterotic patterns can be planned more efficiently based on prior information obtained from SSR molecular markers. Bruel, *et al.* (2006) concluded that the genetic distance revealed by RAPD markers can be used to establish consistent heterotic patterns between maize inbred lines. In contrast, Semagn *et al.* (2012) reported that there was no correlation between heterotic groupings based on Phenotypic and single nucleotide polymorphic markers. Estimated of relatedness based on pedigree data can differ from those based on molecular marker data (Bernardo and Kahler, 2001).

In Conclusion: Maize breeder should use ISSR molecular marker similarity data side by side with the pedigree information before crosses made to assist heterotic groups from maize inbred lines in breeding field reducing to efforts and money towards catching super crosses, also, data for grouping inbred lines according to HSGCA method, or

morphological characters for inbred lines per se are not sufficient for grouping Maize inbred lines, and grouping will be affected by the environment so far.

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طرق مختلفة لعمل مجموعات متباعدة وراثيًا في الذرة الشامية

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المستخلص. أجريت مقارنة بعض طرق تقسيم ١٢ سلالة من الذرة الشامية إلى مجموعات متباعدة وراثيًا، باستخدام تأثيرات القدرتين العامة والخاصة على الائتلاف وبعض الصفات المورفولوجية على السلالات ومن خلال المعلمات الجزيئية من النوع ISSR. أظهرت نتائج السلالة - الكشاف في النوبارية أن السلالات جيزة ٦٥٦ أعطت أعلى محصول حبوب مع الكشاف جميزة ١٠٢١ (٧,٢ طن للهكتار) بينما في الجميزة أعطت السلالة جيزة ٦٥٥ أعلى محصول حبوب مع الكشاف جميزة ١٠٢١ (٨,٢٤ طن للهكتار). وبناءً على قيم تأثيرات القدرتين العامة والخاصة على الائتلاف أمكن تقسيم السلالات إلى ثلاث مجموعات مختلفة في كل منطقة حسب الكشاف المستخدم ولكن اختلف التقسيم من النوبارية إلى الجميزة حسب الكشاف والسلالة والتفاعل مع البيئة. أظهرت نتائج الدراسة كذلك أن استخدام المعلمات الجزيئية من النوع ISSR كان أكثر توافقاً مع سجل نسب السلالات المستخدمة في الدراسة وأفضل من التقسيم باستخدام طريقة HSGCA من خلال الكشافات، وأقل كلفة وكذلك موفراً للوقت والمجهود، ولم تكن نتائج الصفات المورفولوجية جيدة في التقسيم بل قد تحتاج إلى أن تكرر في أكثر من موقع وأكثر من موسم نمو.