

# Genetic Diversity and Heterotic Orientation of South Africa Maize Inbred Lines Towards Tropical and Temperate Testers

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## Abstract

An efficient hybrid breeding program defines and utilizes few heterotic groups. The objectives of this study were to determine genetic diversity and alignment of South Africa maize inbred lines collection towards tropical and temperate testers. Forty-two maize inbred lines were genotyped with 56110 single nucleotide polymorphism (SNP) DNA markers, using the Illumina MaizeSNP50 Bead chip. The 42 lines were crossed to two tropical and two temperate inbred line testers. The testcrosses were evaluated across seven environments, in South Africa, during 2014-2016. Genotypes and specific combining ability (SCA) effects of hybrids were significantly different ( $P < 0.05$ ) for grain yield. There was a weak correlation between molecular genetic distances and both grain yield mean and specific combining ability effects of hybrids, indicating that productivity of maize inbred lines could not be reliably determined based on molecular genetic distances. The SCA data was capable of classifying these maize inbred lines into three heterotic groups with respect to both tropical and temperate testers. Only a few lines could not be grouped on the basis of SCA data. The study also indicated high level of diversity among the maize inbred lines, which was shown by both the dendrogram and molecular genetic distances. The SNP marker data classified the inbred lines into 11 clusters that could be simplified into three major groups of normal maize endosperm and two groups of quality protein maize (QPM) endosperm types. However, the SNP data indicated that maize lines were more aligned towards tropical than temperate inbred testers. This information would be useful for simplifying heterotic classification of the lines with profound implications for breeding progress.

## Introduction

Advances in maize breeding have an important role in continually developing and deploying new maize cultivars, exhibiting improved grain yield and desirable agronomic characters. The strength and success of any breeding program depends on a well-established and properly arranged germplasm (Rajendran et al., 2014), which provides potential exploitation of maximum heterosis in crosses (Bidhendi et al., 2012; Librando and Magulama, 2008). Therefore, identification of heterotic groups (HGs) and heterotic patterns is the principal step towards developing a well-established germplasm (Barata and Carena, 2006; Delucchi et al., 2012) for a hybrid oriented breeding programme. Melchinger and Gumber (1998) defined a heterotic group as a group of related or unrelated genotypes from the same or different populations, which show similar combining ability and heterotic response when crossed with genotypes from other genetically different groups, whereas heterotic pattern is observed when a cross between a specific pair of two heterotic groups expresses high level of heterosis.

Classification of germplasm into heterotic groups offers several advantages in most breeding programs. When inbred lines from different known and unknown origins are used, a large number of hybrid combinations are made which are later evaluated in multi-location environments (Bidhendi et al., 2012). However, this is labour-intensive, lengthy and costly (Aguar et al., 2008). However, when heterotic groups are formed, undesirable combinations are avoided by crossing inbred lines from the existing and divergent groups (Aguar et al., 2008). This allows the exploitation of maximum heterosis (Melchinger and Gumber, 1998) among selected lines in hybrid combination, reduces the chance of missing superior hybrids and subsequently improves breeding efficiency. According to Fan et al. (2009) breeding efficiency is the percentage of superior hybrids, which are obtained relative to the total number of crosses made between lines from distinct heterotic groups in a breeding program. The employment of simple heterotic groups would simplify germplasm management (Fan et al., 2003). Selecting parental lines from defined heterotic groups is rapid and efficient (Kanyamasoro et al., 2012). The information on simplified heterotic orientation is crucial for setting up an efficient maize breeding program.

Knowledge of combining ability among maize inbred lines in the program is paramount for establishing heterotic orientations. General combining ability (GCA) and specific combining ability (SCA) as defined by Sprague and Tatum (1942) data are used to determine utility of maize inbred lines. They reveal the ability of inbred lines to combine amongst each other during hybridization and transmit the desirable genes to the  $F_1$  crosses (Fasahat et al., 2016). A survey of the literature indicates that SCA data is an important statistical tool for classifying maize germplasm into heterotic groups. The SCA data has a high predictive value for  $F_1$  grain yield than heterosis data (Betran et al., 2003). However, the SCA data can be used together with other methods that are cheaper and even more precise, because they are not affected by genotype x environment interaction effects. This includes molecular markers such as SNP genotyping.

Genotyping maize inbred lines with molecular markers and pedigree analyses are some of the methods which can be used to establish levels of diversity and classifying germplasm into potential heterotic groups. These methods have been reported in the literature (Parentoni et al., 2001; de Pinto et al., 2003; Fan et al., 2003; Fan et al., 2004; Menkir et al., 2004; Barata and Carena, 2006; Aguiar et al., 2008; Badu-Apraku et al., 2013; Rajendran et al., 2014). Effectiveness of these methods in classifying germplasm is mainly validated by estimating SCA of the hybrids using the line by tester method. The line x tester mating scheme utilizes a few divergent testers of known heterotic groups to determine heterotic orientation of inbred lines. Among other tools, the line x tester mating scheme has been widely used and reported in the literature (Vasal et al., 1992; Menkir et al., 2003; Li et al., 2007; Aguiar et al., 2008; Fan et al., 2010; Rajendran et al., 2014; Fasahat et al., 2016).

The public maize germplasm lines in South Africa have been maintained in at least seven heterotic groups since the beginning of professional maize breeding in the country. These heterotic groups have been utilised extensively in different combinations to develop commercial maize hybrids, since the Saunders and Gevers eras (Fourie and Gevers, 1987; Gevers and Whyte, 1987; Gevers and Lake, 1998). The use of a large number of heterotic groups from different populations complicates the breeding process and decision making during selection of parental lines. Employment of many heterotic groups is associated with several major drawbacks, including high costs of making and evaluating hybrids, and extended timeline to obtain results (de Pinto et al., 2003; Aguiar et al., 2008; Bidhendi et al., 2012) and subsequent reduction in breeding efficiency (Fan et al., 2014). This prompts the need to simplify the heterotic classification of the lines in South Africa for use in breeding.

Due to climate change, there is need to continuously introgress and incorporate germplasm from other countries, such as tropical Africa, tropical South America and USA temperate maize inbred lines. This would enhance genetic diversity and adaptation of the lines to climate change effects, such as increasing frequency and severity of drought, and global warming among other challenges.

Given the foregoing, the objectives of the current study were to determine diversity of South Africa maize inbred lines, and investigate their orientation towards the widely utilized tropical and temperate testers. The use of tropical and temperate testers was prudent because South Africa maize production falls within subtropical to warm temperate conditions. In this regard, both tropical and temperate maize germplasm could be exploited to develop productive hybrids, using a simplified heterotic classification system.

## Materials And Methods

### Germplasm

Forty-two maize inbred lines were selected from the Agricultural Research Council of South Africa maize germplasm database. Six representative maize inbred lines were randomly selected from each of the seven traditional heterotic groups. The seven heterotic groups were described by fore-bearers of the public maize breeding programs in the region, such as Saunders, Gevers, Olver, Fourie and others (Gevers and Whyte, 1987; Gevers and Lake, 1998; Olver, 1998) and Fourie (2017, personal communication). According to the literature, the inbred lines used were developed from germplasm which was introduced from the USA, Australia and also mainly from the local open-pollinated varieties which mostly originated from the USA corn-belt (Saunders, 1942; Gevers, 1997). There is a possibility of infusion of temperate and tropical genetic backgrounds through both incorporation and introgression of USA's temperate germplasm, in South Africa. Therefore, it was prudent that the 42 lines were crossed with four testers, representing the heterotic Groups A and B of both tropical and temperate origin. The maize inbred line testers were represented by the International Maize and Wheat Improvement Center's (CIMMYT) tropical testers from Group A (CML312) and Group B (CML444). The temperate testers were represented by the Group A (B73) and Group B (MO17) inbred lines of the USA. These testers and their derivatives are widely used worldwide. The B73 and MO17 maize testers are temperate inbred lines representing the US Reid (heterotic Group A) and Lancaster heterotic groups (heterotic group B), respectively. These testers were developed by the University of Missouri and Iowa State University, respectively. The CML312 and CML444 are CIMMYT tropical maize inbred lines that were developed by the CIMMYT breeding program in Mexico and Harare in Zimbabwe, respectively. The CML312 is an intermediate maturing and subtropical line that is drought tolerant. The CML444 is a late maturing and mid-altitude or subtropical adapted inbred line. According to CIMMYT (2001), the heterotic Group A to which CML312 belongs consists of the pro-Tuxpeno (Mexico), Kitale (Kenya), BSSS and N3 (Zimbabwe) germplasm types. Most of the germplasm which is aligned to this group is of more dent grain texture. The CML444 is the counterpart of the CML312 in CIMMYT's heterotic Group B. It corresponds to the ETO, Ecuador 573, Lancaster and the SC (Zimbabwe) germplasm (CIMMYT, 2001). The germplasm lines in this group tend to be of a flint grain texture type in the CIMMYT heterotic system. CML444 is drought and low nitrogen tolerant (CIMMYT, 2001). The N3 and SC heterotic pattern has been reviewed and discussed in detail (Derera and Musimwa, 2015; Musimwa and Derera, 2015).

### Experimental Design and Management

Field experiments were conducted at the Agricultural Research Council's Summer Grain Crops Institute station, in Potchefstroom (26.740S; 27.08°E; altitude 1349 m) and the Cedara Research Station (29.54°S; 30.26°E; altitude 1068 m), in South Africa, during the 2014/15 and 2015/16 summer season. Rainfall amount was below average of the long-term weather data of the previous 29 growing seasons (29 years). During the 2015/16 season, the Potchefstroom Station received 67% and Cedara got 78% of the long term average total rainfall of 541 mm and 662 mm, respectively; while both stations received 93–95% of the long term total rainfall in the 2014/15 season. The 2015/16 season was characterised by the *El Nino* associated drought, which affected the whole Southern Africa region. Maximum daily average temperatures were at least 2°C above the long term average of 29°C and 25°C at Potchefstroom and Cedara stations, respectively. The average night or minimum temperatures were at least 1°C below the long-term average of 15°C and 14°C at Potchefstroom and Cedara, respectively.

All trials were laid out as  $\alpha$ -lattice design with two replicates within the sites. A compound fertiliser (NPK, 3:2:1) was applied as a basal fertiliser prior to sowing, at a rate of 25 kg N ha<sup>-1</sup>, 17 kg P ha<sup>-1</sup> and 8 kg K ha<sup>-1</sup>. Each hybrid entry was planted to a two-row plot of 4 m length, and the intra-and inter-row spacing were 0.25 m and 0.75 m, respectively. Thirty-four seeds (two per hill) were initially sown and later thinned to 17 vigorous plants per row to achieve 53 333 plants ha<sup>-1</sup>. Lime ammonium nitrate (LAN, 33% N) was applied as a top dressing at a rate of 150 kg ha<sup>-1</sup>, at four weeks after crop emergence. Weeds were controlled using pre-emergence herbicide, Bateleur Gold 650EC (Flumetsulam (sulfonanilide), s – metalachlor), at 1.3 L ha<sup>-1</sup> and post-emergence herbicide, Basagran (480 g/L bendioxide (thiadiazine)) at 2.5 L ha<sup>-1</sup>. This was augmented by occasional manual weeding when needed. Insecticides were applied to control stalk-borer, using Karate (50 g/L lambda-cyhalothrin) at 70 ml ha<sup>-1</sup>. Due to the below normal total rainfall, which was experienced at both stations, supplementary irrigation water was applied to augment rainfall amount until crops reached the physiological maturity, especially during the 2015/16 season. Grain yield per plot was estimated and converted to tonnes ha<sup>-1</sup> at 12.5 % grain moisture content in line with the standard practice for maize production, in South Africa.

### Field Data Analysis and Classification of Lines

Analysis of variance (ANOVA) was performed on plot means for grain yield data across sites for each season. Lines that did not cross successfully with all testers were not included in the analysis, hence 31 lines were used to perform the line x tester analysis. Specific combining ability effects for grain yield were estimated using the line by tester procedure, which is embedded in the Analyses of Genetic Designs computer software with 'R' (AGD-R, version 3.0) (Rodriguez et al., 2015). Lines and testers were treated as fixed effects. The statistical model used for the combined analysis was as follows:

$$Y_{ijkl} = \mu + L_i + T_j + (L \times T)_{ij} + E_l + (L \times E)_{il} + (T \times E)_{jl} + R_k(E_l) + (L \times T \times E)_{ijl} + e_{ijkl},$$

where  $Y_{ijkl}$  is the measured grain yield data of the testcross of the  $i^{\text{th}}$  line crossed to  $j^{\text{th}}$  tester in the  $l^{\text{th}}$  site and  $k^{\text{th}}$  replicate;  $\mu$  is the grand mean;  $L_i$  is the effect of the  $i^{\text{th}}$  line;  $T_j$  is the effect of the  $j^{\text{th}}$  tester;  $E_l$  is the effect of the  $l^{\text{th}}$  site;  $(L \times T)_{ij}$  is the interaction effect of the cross between the  $i^{\text{th}}$  line and the  $j^{\text{th}}$  tester;

$(L \times E)_{ij}$  is the interaction effect of the  $i^{\text{th}}$  line and the  $l^{\text{th}}$  site;  $(T \times E)_{jl}$  is the interaction effect of the  $j^{\text{th}}$  tester and the  $l^{\text{th}}$  site;  $R_k(E_l)$  is the effect of the  $k^{\text{th}}$  replicate nested in the  $l^{\text{th}}$  site;  $(L \times T \times E)_{ijl}$  is the interaction effect of the  $i^{\text{th}}$  line,  $j^{\text{th}}$  tester and the  $l^{\text{th}}$  site and  $E_{ijkl}$  is the random error term.

Classification of lines into heterotic groups was based on the specific combining ability (SCA) value of a line and a tester for grain yield as proposed by Vasal et al. (1992). Lines that showed negative SCA effects for grain yield when crossed to a particular tester were deemed to belong to the same heterotic group with the tester; whereas lines that exhibited positive SCA effects with one tester indicate that those lines belong to the opposite heterotic group. The lines displaying positive SCA with both testers (A and B) were allocated to the AB heterotic group and those displaying negative SCA with both testers could not be classified on the basis of SCA data.

## SNP Genotyping and Diversity Analysis

The 42 maize inbred lines were planted in the glasshouse for DNA sampling. Leaf samples bulked from four plants of each inbred were taken at two weeks after planting. Genomic DNA was extracted at the Agricultural Research Council-Grain Crops laboratory, following a modified CTAB procedure (Doyle, 1987). DNA samples were sent to the Agricultural Research Council-Biotechnology platform in South Africa for genotyping using the single nucleotide polymorphism (SNP) markers. Genotyping was carried out using the Illumina MaizeSNP50 Bead chip, which was derived from the B73 reference genome ([www.illumina.com](http://www.illumina.com)). The protocol provided 56110 SNP markers, which were densely covering all the ten chromosomes of the maize genome. The genetic distance (dissimilarity) estimates among inbred lines were calculated based on the Gower's distance (Gower, 1971). Genotypes were subsequently grouped by the Unweighted Pair Group Method using Arithmetic average (UPGMA) algorithm, using the R studio Software version 3.3 (RStudio Team, 2015).

## Results

### Diversity Analysis based on SNP markers

The UPGMA dendrogram of 40 maize inbred lines and four testers is shown in Fig. 1. The SNP markers divided the South African germplasm into 11 clusters (Table 1). The principal clusters were the USA Corn-belt temperate containing the two testers B73 and MO17, and the other mega cluster consisted of South Africa maize inbred lines. The South Africa maize inbred lines were on the same side with the tropical testers, CML444 and CML312, indicating that they were more inclined towards the tropical CIMMYT testers than the USA temperate testers. The South Africa maize inbred lines had shorter genetic distance from the tropical lines than the temperate testers (Table 1).

Table 1  
Summary of the dendrogram clusters and mean genetic distance between the clusters and testers

Cluster	Inbred lines within a cluster	General description of the cluster	Average genetic distance with testers			
			B73	MO17	CML444	CML312
2	RO421W (M)	A QPM line derivative of M37W, singleton cluster	0.81	0.83	0.77	0.76
3	K64R.22 (K), R2565Y (K), J80W (M), P28 (R), P612MSV (R), P614MSV (R), U71Y (R)	Mainly B73 introgressed into South African lines with BC to South Africa lines	0.91	0.98	0.70	0.71
4	K64 (K)	Kansas inbred line, singleton cluster	0.81	0.87	0.61	0.61
5	P590MSV (L), P594MSV (L), P598MSV (L)	Derivatives of Lancaster Sure crop through introgression of MO17 into South Africa lines via a BC to the South Africa lines	0.74	0.86	0.68	0.67
6	B1138T (F), P588MSV(L)	Purely South Africa germplasm, with no known temperate alignment	0.83	0.92	0.55	0.55
7	I-16 (I), I-34 (I), I-39 (I), I-41 (I), I-42 (I), NC258 (L)	South Africa inbred lines, of mainly the I-heterotic group	0.97	1.07	0.66	0.64
8	U127Y (K), CML312 (A), CML444 (B)	Tropical lines from CIMMYT and one South African line (U127Y)	0.83	0.92	0.52	0.52
9	U2540W (K)	K64R/M162W derivative, singleton cluster	0.83	0.91	0.55	0.57
10a	BO394Y (F), E30Y (F), RO544W (F), UO705Y (F), VO430Y (F), FO215W (P), SO713W (P)	QPM lines, which were derived from the F2834T and Natal Potchefstroom Pearl (NPP)	0.91	1.01	0.60	0.61
10b	FO215W (P), SO713W (P), SO607W (P) VO495Y (P), VO500Y (P), VO501Y (P)	QPM lines, which are derivatives of the Natal Potchefstroom Pearl (NPP)	0.88	0.97	0.59	0.59
11	MO17HtHtN (L), RO452W (M), SO503W (M), VO617Y (M), SO181Y (M), S198Y (R), SO1224Y (R)	Reid derivatives and QPM inbred lines from predominantly the South African M-group (M37W)	0.97	1.07	0.70	0.70

The average genetic distances between lines and different testers are shown in Table 2. The widest (0.95) average genetic distance was between the South Africa lines and the USA Corn-belt testers. The distance ranged from 0.68 and 1.19, with the minimum distance between P598MSV and B73 and the maximum between J80W and MO17. The mean genetic distance with the tropical testers was 0.80 and ranging from 0.52 to 0.80. The lowest genetic distance was

estimated between U127Y and both CIMMYT testers, CML312 and CML444, while the largest genetic distance was found between J80W with both tropical testers, and between RO452W and CML444.

Table 2  
Genetic distances between the South Africa maize inbred lines and tropical (CML312 and CML444) and temperate USA testers (B73 and MO17)

Inbred line Code	Current RSA HG	Abbreviated Pedigree	Genetic distance with testers			
			B73	MO17	CML444	CML312
B1138T	F	TEKO YELLOW	0.822	0.908	0.545	0.547
BO394Y	F	F2834T.402	0.976	1.085	0.640	0.647
E30Y	F	B390YxM136Y	0.932	1.038	0.588	0.595
RO544W	F	BO160W.3J400W	0.905	1.005	0.609	0.609
UO705Y	F	YOFE1(S4)	0.890	0.983	0.546	0.553
V0430Y	F	(HO466Y.1B0310Y)	0.885	0.990	0.575	0.577
I-16	I	I-16	0.880	0.975	0.577	0.555
I-34	I	I-34	0.909	1.007	0.594	0.575
I-39	I	I-39	1.033	1.139	0.733	0.712
I-41	I	I-41	1.011	1.109	0.708	0.688
I-42	I	I-42	1.029	1.134	0.729	0.709
K64	K	Pride of Saline	0.814	0.874	0.614	0.613
K64R.22	K	K64R-22	0.849	0.935	0.569	0.572
R2565Y	K	K64R2(I137TN1.K64R)	0.790	0.823	0.779	0.794
U127Y	K	M162W.1K0326Y	0.832	0.923	0.516	0.521
U2540W	K	M162W1.DO940Y-J34	0.834	0.908	0.554	0.565
MO17HtHtN	L	MO17HtHtN	0.826	0.922	0.564	0.569
NC258	L	NC258	0.943	1.048	0.614	0.604
P588MSV	L	MRSxVHMO17	0.838	0.925	0.555	0.558
P590MSV	L	MRSxVHMO17	0.774	0.889	0.713	0.701
P594MSV	L	MLSxVHMO17	0.778	0.893	0.709	0.698
P598MSV	L	21A-6xVHMO17	0.678	0.799	0.621	0.613
J80W	M	D800W2.HtN	1.083	1.191	0.796	0.798
RO421W	M	DO940Y-11.02(W)	0.811	0.831	0.768	0.764
RO452W	M	DO940Y-13.NHK	1.044	1.147	0.798	0.797
SO503W	M	KO315Y2.NPPES1	1.036	1.136	0.790	0.788
VO617Y	M	(1)"M37W.TE/TEO"	1.013	1.125	0.724	0.723
S0181Y	M	KO326Y2.NPPES1	0.919	1.020	0.619	0.620
F0215W	P	NPPES14.02S14	0.956	1.061	0.617	0.625
SO607W	P	POWS1(S4)	0.874	0.978	0.593	0.587
SO713W	P	POWS1(S4)	0.852	0.927	0.633	0.634
V0495Y	P	POWS12.Y	0.812	0.893	0.584	0.587
VO500Y	P	POWS12.Y	0.910	1.012	0.603	0.600
V0501Y	P	POWS12.Y	0.904	1.012	0.590	0.586
P28	R	P28	0.912	0.967	0.714	0.742
P612MSV	R	B73xVHKG/C1	0.878	0.962	0.613	0.631
P614MSV	R	B73xVHKG/C1	0.949	1.008	0.730	0.758
S198Y	R	M28Y1.DO620Y	0.902	1.000	0.658	0.654
SO1224Y	R	M28Y1.KO288Y	1.036	1.142	0.745	0.751
U71Y	R	M28Y2.NP	0.930	1.005	0.671	0.698

Inbred line Code	Current RSA HG	Abbreviated Pedigree	Genetic distance with testers			
			B73	MO17	CML444	CML312
Average			0.901	0.993	0.647	0.648
Min			0.678	0.799	0.516	0.521
Max			1.083	1.191	0.798	0.798

There were generally large average genetic distances between lines within clusters, ranging from 0.31 to 0.58 (Table 3). The minimum distance was between the temperate inbred lines in cluster 1, whereas the maximum was in clusters 3 and 6. The distance between lines within cluster 11 showed the widest range (0.17 to 0.70). The average genetic distance between clusters ranged from 0.70 to 0.86. The minimum genetic distance was recorded between clusters 6 and 8 and clusters 8 and 9; while the maximum genetic distance was between clusters 1 and 12 (Table 3).

Table 3  
Average genetic distances (GD) between maize germplasm clusters and within clusters

Cluster	1	2	3	4	5	6	7	8	9	10a	10b	11	Average GD between lines within clusters	GD Range within a cluster
1	-												0.31	
2	0.83	-											Singleton	
3	0.84	0.81	-										0.54	0.45–1.06
4	0.79	0.76	0.78	-									Singleton	
5	0.80	0.77	0.78	0.73	-								0.40	0.07–0.51
6	0.79	0.75	0.77	0.72	0.73	-							0.54	
7	0.85	0.81	0.83	0.78	0.79	0.77	-						0.42	0.05–0.59
8	0.78	0.74	0.76	0.71	0.72	0.70	0.76	-					0.51	0.49–0.52
9	0.79	0.75	0.77	0.72	0.73	0.71	0.77	0.70	-				Singleton	
10a	0.82	0.79	0.80	0.76	0.76	0.75	0.81	0.74	0.75	-			0.45	0.31–0.58
10b	0.81	0.78	0.79	0.74	0.75	0.74	0.80	0.73	0.74	0.77	-		0.48	0.38–0.54
11	0.86	0.83	0.84	0.79	0.80	0.79	0.85	0.78	0.79	0.82	0.81		0.48	0.17–0.70

The average distances among the current South African heterotic groups and among tester lines are shown in Table 4. The average genetic distance between the South Africa heterotic groups ranged from 0.74 (L and K) to 0.86 (I and M as well as M and R). The average genetic distance between the South Africa heterotic groups and the tester maize inbred lines was the highest (0.97) between group R and temperate testers, while the minimum genetic distance (0.59) was between heterotic group F and the tropical testers. The distance between testers was generally large, ranging from 0.31 for B73 and MO17 to 0.91 for MO17 and CML444 (Table 4).

Table 4  
Average genetic distances between current South African heterotic groups and between testers

HG	F	I	K	L	M	P	R	B73	MO17	CML444	CML312
F	-										
I	0.80	-									
K	0.75	0.79	-								
L	0.76	0.79	0.74	-							
M	0.83	0.86	0.81	0.82	-						
P	0.77	0.80	0.75	0.76	0.83	-					
R	0.80	0.84	0.78	0.79	0.86	0.80	-				
B73	0.90	0.97	0.82	0.81	0.98	0.88	0.93	-			
MO17	1.00	1.07	0.89	0.91	1.08	0.98	1.01	0.31	-		
CML444	0.58	0.67	0.61	0.63	0.75	0.60	0.69	0.83	0.91	-	
CML312	0.59	0.65	0.61	0.62	0.75	0.60	0.71	0.81	0.90	0.49	-

The analysis of variance results of yield in 2014/15 and 2015/16 are presented in Table 5. During the 2014/15 season, the mean squares associated with the sites main effects was highly significant ( $P < 0.001$ ) for grain yield. The results also showed significant differences due to lines main effects ( $P < 0.05$ ) and testers main effects ( $P < 0.001$ ). Although the genotype x site interaction effects were not significant ( $P > 0.05$ ), the site main effects and tester interaction

effects were highly significant ( $P < 0.001$ ) for grain yield data. The mean squares associated with the line x tester interaction effects was not significant ( $P > 0.05$ ) for grain yield, and site x line and site x line x tester interaction effects were not significant ( $P > 0.05$ ). There were no significant differences between sites main effects, testers main effects and tester x sites interaction effects, during the 2015/16 season (Table 5). However, the mean squares associated with genotypes, lines main effects and the interaction between sites x lines were highly significant ( $P < 0.001$ ) for grain yield, during the 2015/16 season. The interactions between lines x testers, sites x genotypes were also significant ( $P < 0.01$ ) during the 2015/16 season. The mean squares associated with site x line x tester interaction effects was also significant ( $P < 0.05$ ). The results indicated the presence of sufficient variability among the lines to be classified into heterotic groups.

Table 5  
Analysis of variance of maize hybrids for grain yield at two sites in each of the 2014/15 and 2015/16 summer season, in South Africa

Source	DF	MS	DF	MS
	2014/15		2015/16	
Site	1	35.81***	1	0.09
Rep(site)	2	5.03	2	10.24
Genotypes	41	4.90**	71	5.71***
Line	13	5.22*	17	7.61***
Tester	2 <sup>!</sup>	26.85***	3	0.32
Line x tester	26	3.05	51	5.40**
Site x genotypes	41	3.28	71	5.20**
Site x line	13	3.15	17	7.93***
Site x tester	2	29.06***	3	2.81
Site x line x tester	26	1.36	51	4.44*
Residuals	82	2.38	142	3.09

\*\*\*, \*\*, \* Data significant at  $P < 0.001$ , 0.01 and 0.05, respectively; DF - degree of freedom;! Testers excluding MO17.

## Heterotic grouping of lines based on SCA effects

Classification of 26 South African maize inbred lines in accordance with their specific combining ability effects with tropical testers is shown in Table 6. Heterotic classification of South Africa maize inbred lines based on their SCA with the tropical CIMMYT maize inbred testers indicated that 10 maize inbred lines were oriented towards CML312 and were fitted in heterotic Group A, 12 inbred lines with negative SCA with CML444 were placed in Group B; while two inbred lines which exhibited positive SCA with both tropical testers were classified as heterotic Group AB. Only two lines, which displayed negative SCA effects with both tropical testers, were not classified on the basis of SCA data. The results indicated a weak correlation ( $r < 0.30$ ) between genetic distance with both grain yield mean and specific combining ability data of the testcrosses of South Africa lines with tropical inbred testers (Table 6).

Table 6  
Heterotic orientation of 26 South Africa maize inbred lines towards tropical maize inbred testers based on SCA data

No.	Line	HG	SCA (t ha <sup>-1</sup> )		Orientation	Genetic distance		Testcross mean yield (t ha <sup>-1</sup> )	
			CML312 (A)	CML444 (B)		CML312	CML444	CML312	CML444
1	B1138T	F	0.88	-1.47*	B	0.547	0.545	6.65	4.26
2	E30Y	F	0.90	-0.82	B	0.595	0.588	6.91	5.34
3	RO544W	F	2.05*	-1.3	B	0.609	0.609	7.72	4.51
4	I-16	I	-0.10	1.58	A	0.555	0.577	5.98	7.82
5	I-42	I	-0.20	0.75	A	0.709	0.729	4.96	6.05
6	I-39	I	-1.04	1.12	A	0.712	0.733	5.84	7.96
7	K64	K	-0.98	0.87	A	0.613	0.614	5.52	7.32
8	K64R-22	K	-0.15	0.89	A	0.572	0.569	5.42	6.41
9	M162W	K	-2.32*	0.72	A			3.39	6.57
10	U127Y	K	0.18	-0.68	B	0.521	0.516	4.86	4.14
11	U2540W	K	0.02	-0.15	B	0.565	0.554	5.92	5.90
12	MO17HtHtN	L	0.69	-1.16	B	0.569	0.564	6.06	4.35
13	P594MSV	L	-0.75	0.01	A	0.698	0.709	5.82	6.53
14	J80W	M	-0.55	0.96	A	0.798	0.796	4.58	6.23
15	RO421W	M	-1.90*	-0.76	-	0.764	0.768	5.76	7.05
16	RO452W	M	0.22	-0.17	B	0.797	0.798	5.92	5.67
17	SO181Y	M	0.80	-0.77	B	0.620	0.619	6.76	5.34
18	FO215W	P	-0.78	3.01**	A	0.625	0.617	6.19	10.13
19	VO495Y	P	1.40	-0.29	B	0.587	0.584	7.58	6.04
20	SO713W	P	0.23	-1.14	B	0.634	0.633	7.26	5.83
21	VO500Y	P	0.20	0.05	AB	0.600	0.603	6.56	6.37
22	P614MSV	R	1.40	-0.83	B	0.758	0.73	7.16	5.08
23	S198Y	R	-0.29	-0.47	-	0.654	0.658	5.92	5.89
24	U71Y	R	-0.20	0.83	A	0.698	0.671	6.20	7.38
25	SO1224Y	R	0.44	-0.14	B	0.751	0.745	6.21	5.59
26	P612MSV	R	1.11	0.21	AB	0.631	0.613	7.35	6.41
<b>Correlation with GD</b>			<b>-0.26</b>	<b>0.10</b>	<b>-</b>			<b>-0.13</b>	<b>0.16</b>
*Significant at P < 0.05; SCA, Specific combining ability; GD, Genetic distance; SCA <sub>SE</sub> (P < 0.05) = 0.98									

Heterotic orientation of 18 South Africa maize inbred lines on the basis of their SCA effects with temperate USA corn-belt testers is shown in Table 7. Five inbred lines which showed negative SCA with B73 were fitted into heterotic group A, while six inbred lines, which showed negative SCA with MO17, were put in Group B. Three inbred lines, which exhibited positive SCA with both temperate testers, were allocated to the Group AB. Four maize inbred lines, which showed negative SCA with both temperate testers, could not be fitted into any of the heterotic groups on the basis of SCA data.

Table 7

Heterotic orientation, SCA effects, genetic distance and mean yield of 18 South Africa maize inbred lines with temperate inbred testers across environments

No.	Inbred Line	Current South African Heterotic Group	SCA effects		Orientation towards testers	Genetic distances with testers		Testcross Mean yield	
			(t ha <sup>-1</sup> )			(t ha <sup>-1</sup> )		(t ha <sup>-1</sup> )	
			B73(A)	MO17(B)		B73	MO17	B73	MO17
1	E30Y	F	-0.88	0.80	A	0.932	1.038	5.27	6.92
2	RO544W	F	0.66	-1.41	B	0.905	1.005	6.47	4.36
3	I-16	I	-0.90	-0.59	-	0.880	0.975	5.32	5.60
4	I-42	I	-0.33	-0.22	-	1.029	1.134	4.97	5.04
5	K64	K	0.83	0.93	AB	0.814	0.874	6.33	6.39
6	M162W	K	0.72	0.89	AB	-	-	6.57	6.70
7	U127Y	K	1.20	-0.70	B	0.832	0.923	6.02	4.08
8	U2540W	K	1.13	-0.99	B	0.834	0.908	7.17	5.01
9	MO17HtHtN	L	-0.57	1.05	A	0.826	0.922	4.94	6.52
10	J80W	M	-0.45	0.05	A	1.083	1.191	4.8	5.27
11	RO421W	M	1.79	0.86	AB	0.811	0.831	9.58	8.63
12	RO452W	M	0.08	-0.13	B	1.044	1.147	5.91	5.67
13	SO181Y	M	0.36	-0.38	B	-	-	6.46	5.68
14	FO215W	P	-1.98*	-0.25	-	0.956	1.061	5.12	6.82
15	VO495Y	P	-0.70	-0.41	-	0.812	0.893	5.62	5.87
16	P614MSV	R	0.31	-0.88	B	0.949	1.008	6.2	4.98
17	S198Y	R	-0.09	0.84	A	0.902	1.000	6.26	7.16
18	U71Y	R	-1.19	0.55	A	0.930	1.005	5.34	7.05
	Correlation with GD		-0.40	-0.15				-0.48	-0.28
	Grand mean (t ha <sup>-1</sup> )		5.98						
	Standard error (SCA)		0.98						

\*Significant at P < 0.05; SCA, specific combining ability; GD, genetic distance

## Discussion

### Classification of maize inbred lines by SNP markers

The SNP markers genotyping was highly effective in confirming classification of the germplasm collection in South Africa. As expected, the SNP markers divided the germplasm lines into two principal clusters of USA Corn Belt temperate testers and the South African maize lines clustering with the tropical testers from CIMMYT. This indicated that South Africa public lines were more inclined towards the tropical CIMMYT than the USA temperate testers. However, there were 11 sub-clusters within the South African germplasm collections indicating tremendous diversity. Only the lines from the current "I" heterotic group were placed in the closest cluster with both CIMMYT's tropical testers, CML444 and CML312, indicating that these lines have never been introgressed with temperate germplasm, and were more pro-tropical than the rest of the 40 lines in the sample. The findings from the study were consistent with pedigree data. Based on the pedigree data, lines with a history of temperate germplasm introgression, such as P614MSV and P612MSV with B73 introgression and P590MSV and P594MSV with MO17 introgression were placed in clusters 3 and 5, which is near the temperate cluster (Cluster 1) on the dendrogram, indicating their orientation towards the temperate material. Clusters 10 and 11, which are furthest away from the temperate cluster on the dendrogram are formed by a group of quality protein maize lines, which is a pro-tropical germplasm collection. These lines resulted from the opaque-2 breeding program, which led to the release of yellow and white opaque-2 modified inbred lines (Gevers, 1972). These lines were bred from tropical germplasm. However, within the QPM group, the inbred line MO17HtHtN from the current L heterotic group was non-QPM by origin. Clustering of this line within the QPM collection is an implication of more QPM alleles than normal maize in its genetic background, which could have been introduced along with Northern corn leaf blight (Ht) resistance.

Although genotypic classification by SNP markers was closely linked with the origin of inbred lines and pedigree records, there were deviations that were observed. The lines NC258 and U127Y which are currently classified as L and K heterotic groups, respectively, in South Africa, were actually placed in the same

sub-cluster with the I and CIMMYT's tropical testers, respectively. This indicates that the classification of South African lines in the whole collection might require confirmation with SNP genotyping. In the same vein, lines RO421W (M), K64 (K) and U2540W (K) which were expected to cluster with the current M and K heterotic groups of South Africa, formed singleton clusters, and were not associated with any of the South African lines. They have also displayed a lack of association with the USA temperate and tropical CIMMYT lines, qualifying them as representing distinct germplasm groups within the South African inbred lines collection.

## Classification based on Specific combining ability

Based on specific combining ability effects for yield data, inbred lines were grouped into three heterotic groups by both temperate and tropical testers, which was consistent with the system that was described by previous researchers. The SCA value reveals the genetic relationship between two parents or between a line and a tester (Vasal et al., 1992). High SCA value (positive) between two parents indicates a distant relationship and low (negative SCA) value is an indication of close relationship (Fan et al., 2003). The tropical testers were capable of discriminating the lines, such that six lines were allocated into tropical group A, indicating that they were oriented towards CML312, whereas nine lines placed into tropical group B indicating their orientation towards CML444. Only three lines which showed negative SCA with both tropical testers could not be classified on the basis of SCA data. The classification of the South Africa lines using their interaction (SCA effects) with the temperate testers allocated five lines into temperate group A, indicating that they were B73-orientated; while six lines were placed in temperate group B indicating that they were orientated towards MO17. Three lines, which exhibited positive SCA with both B73 and MO17, were classified as temperate AB group on the basis of SCA data; while four lines, which displayed negative SCA effects with both temperate testers, could not be classified.

## Relationship between SNP and SCA-based classification

Heterotic orientation of inbred lines under non-stress environments corresponded with the SNP marker classification. It was noted that lines that did not belong to any heterotic group when tropical testers were used could be classified clearly using temperate testers and vice versa. For example, in SNP cluster analysis, the I-group representatives were more aligned to both tropical testers. However, heterotic orientation by temperate testers did not classify them into any heterotic group, as revealed by negative SCA effects with both B73 and MO17. The grouping of the I-group lines with only tropical testers was an indication of a distant relationship between the I heterotic group and the temperate lines. This was further supported by the maximum average genetic distance between the I-group and both temperate testers, B73 (0.97) and MO17 (1.07). Therefore, delineation of inbred lines by either temperate or tropical lines is influenced by the predominance of either temperate or tropical genome in their genetic background, which was clearly confirmed by the SNPs marker data. Findings from this study are consistent with previous reports. Adetimirin et al. (2008) also observed that markers clearly discriminated temperate from tropical germplasm. They further observed that some lines that were known to be temperate x tropical were aligned with the tropical lines, indicating that they were more closely related to the tropical than temperate material. In this study, some lines were consistent in their heterotic orientation by both USA temperate and CIMMYT tropical lines. However, the strongest relationship towards either temperate or tropical lines was observed from the magnitude of SCA effects and their placement in the dendrogram.

There are inbred lines including RO421W, K64 and U2540W that formed distinct singleton clusters. Use of the SCA heterotic orientation approach did not clearly classify these lines. For example, RO421W did not belong to any heterotic group under tropical classification, while classification based on temperate testers allocated this line into heterotic the temperate group AB. The inbred line K64 was assigned into temperate AB group by temperate testers and into heterotic tropical group A by tropical testers.

Grouping lines by SCA effects therefore revealed that when inbred lines are classified into heterotic groups using two sets of testers, failure of one set of testers to classify lines could be an indication of distant relationship between a line and a particular set of testers. Alternatively, classification of some lines might not be clear with both sets, such lines could represent distinct germplasm groups within the germplasm collection. Therefore, when the molecular data is not available for validation, there should be no preference for any set of testers over another. However, inclusion of two sets of testers could be a more accurate approach, especially in distinguishing germplasm like the South African collection with a history of both tropical and temperate introgression.

The inbred lines used in the study were pooled from seven known South African heterotic groups to minimise the number of heterotic groups for improved breeding efficiency. The 11 clusters obtained could be simplified into a broader group of normal endosperm maize and quality protein maize lines. Normal maize endosperm lines may be grouped into three groups, with one group forming temperate germplasm (clusters 1 and 3) with pro-MO17 and B73. The second group is a group of South African lines in clusters five and six, with no temperate or tropical alignment, while the I-group lines and those lines with tropical alignment formed the third group. The QPM lines on the far right of the dendrogram could be split into two groups (clusters 10 and 11). Intra-group combinations among the normal maize groups and between the QPM groups are expected to give high heterosis in crosses. Cross combinations between normal maize and some lines in the QPM group, particularly the white versions are also recommended to maximise heterosis. The heterotic potential of these groups was shown by a cross between FO215W from the QPM cluster and the tropical tester, CML444 (data not presented). Lines that represented distinct germplasm should also be considered for utilisation in crosses; for example in the current study, high heterosis was revealed by crosses RO421W x B73 and RO421W x MO17. Previous investigations revealed high heterosis in crosses including F and I, M and K, K and P, M and P and crosses between M and F and R and L group combinations (Gevers and Whyte, 1987). Based on the new clusters formed, these combinations also included temperate x tropical lines. The identified groups will likely result in superior tropical x temperate combinations with new favourable alleles, wider genetic base, and consequently improvement in agronomic performance and adaptation of local varieties.

From the current seven heterotic groups of South Africa studied, some groups clearly clustered into the identified clusters. The study therefore indicated that the established patterns would be beneficial in inferring the classification of lines that were not included in the study, but represented in the seven heterotic groups. The F, P and M group lines and the rest of the Opaque-2 modified lines from other South African heterotic groups belong to the QPM group. Lines from the L-group form a distinct group of normal maize endosperm lines; the I-group lines form a distinct group closest to the CIMMYT lines, while the temperate group include the R lines and K-group lines with a normal maize endosperm type. However, classification of some South African heterotic groups, particularly

the K-group lines may not be straightforward as lines from this group were distributed in different clusters. Such deviations may be an indication of the presence of diversity within the K-group; this also suggests that inbred lines extracted from the same population do not always cluster together. This was also observed in many previous investigations (Vasal et al., 1992; Yu et al., 2001; Fan et al., 2003; Barata and Carena, 2006) that reported that maize inbred lines which were derived from the same population classified differently.

## Genetic distances

Genetic distances summarise the extent of genetic differences between populations or species (Dogan and Dogan, 2016). Genetic distances were estimated to determine the relatedness among inbred lines studied. The average genetic distances computed between clusters, South African heterotic groups, inbred lines within clusters and among tester inbred lines indicated the presence of sufficient variability between the identified clusters and therefore the possibility of obtaining superior hybrid combinations from inter-cluster crosses. The average genetic distances between lines within clusters clearly indicated that lines that formed the same cluster were genetically related by distance, as the average genetic distance ranged from 0.31 to 0.58 between clusters. However, for some clusters, the genetic distance range of inbred lines showed a considerable diversity within a cluster. For example, the genetic distance in cluster 11 ranged from 0.17 to 0.70. This therefore showed that the collection of QPM lines that formed cluster 11 were derived from distinct genetic backgrounds. The maximum average genetic distance between clusters 1 and 11, further demonstrated the reliability of SNP markers in distinguishing between inbred lines. Genetic distances between testers clearly revealed that both temperate USA Corn-belt testers, MO17 and B73, are closely related which reflected their temperate adaptation domain. The closest relationship was further confirmed by the grouping together of these inbred testers on the dendrogram. Similarly, the close genetic relationship between tropical CIMMYT testers, CML312 and CML444, was confirmed by the minimum genetic distance between them, and their placement within the same cluster, indicating their tropical adaptation domain.

The genetic differences among the current South African heterotic groups were generally large, indicating the presence of diversity within the South African germplasm collection. The largest genetic distance was between group R and M, and I and M, indicating genetic dissimilarity between these South African heterotic groups. The R group lines were grouped in cluster 3, with temperate alignment, whereas the M group lines were mainly grouped along with the QPM lines in cluster 11; the M group also showed the highest genetic dissimilarity with the I group. Inbred lines from heterotic Group I formed a distinct group in cluster 7, that was closest to the tropical testers, and were thus not closely associated with the M-group lines. The highest average genetic distance between these heterotic crops thus indicated that they are the most distinct heterotic groups within the seven South African heterotic groups. The minimum distance between the South African heterotic groups K and L was an indication of genetic similarity. Although these groups did not cluster together, their placement by the SNP markers in closest clusters, for example allocation of the K lines in clusters 3 and 4 and the L-group lines in cluster 5, could be an explanation of the minimum genetic distance estimates between them. Overall, the average genetic distance was generally large between South Africa inbred lines and temperate testers than with the tropical testers, indicating that although some lines may be aligned towards temperate than tropical testers, the lines are of South Africa origin and were closer to the tropical testers. This is expected because South Africa falls within the subtropical to a warm temperate environment, which places its germplasm between tropical and temperate although with a bias placement towards tropical orientation.

## Correlation of genetic distance with grain yield and specific combining ability

There were low correlations detected between genetic distance with grain yield mean and specific combining ability data, indicating that hybrid performance could not be efficiently predicted using molecular marker-based genetic distances. The low predictive value of genetic distance was also confirmed by the degree of heterosis observed in crosses (data not shown). For example, a pair of lines that showed the widest range of genetic distances did not necessarily show maximum heterosis in their testcrosses. These observations are in agreement with previous studies where poor (Makumbi, 2005; Parentoni et al., 2001; Xu *et al.*, 2004) to no (Rajendran et al., 2014) correlation was demonstrated between genetic distance with grain yield and SCA. The low correlation observed in several studies has been associated with the markers used to compute genetic distances. The markers used are generally many, evenly distributed on the genome, and may not be closely linked to grain yield and associated quantitative characters (Melchinger, 1999). This therefore reduces the association between genetic distances and heterosis. Furthermore, the genetic differences between parents are due to many traits and loci which may not all be correlated with yield. Zhang et al. (2010) thus suggested the use of only yield-related markers to analyse genetic differences, and subsequently to reliably predict hybrid performance.

In sharp contrast to findings in the current study, Schrag et al. (2010) and Gichuru et al. (2017) reported high predictive value of molecular marker genetic distances in heterosis; the observed degree of correlation was associated with the genetic differences and complementary traits of lines used. The ability of genetic distance to predict heterosis in crosses therefore varies with germplasm used (Betran et al., 2003) and probably the set of molecular markers used. In the current study, the observed levels of correlations therefore underlines the need for conducting field trials to evaluate hybrid performance, irrespective of known genetic distances between inbred lines. However, despite the inability of genetic distances to reliably predict hybrid performance, their usefulness as a guide during selection of inbred lines for hybrid make-ups is underscored.

## Conclusion

The study confirmed existence of high level of genetic diversity among the South Africa line collection. These lines have shown alignment towards both tropical and temperate testers. However, they were more inclined towards the tropical than temperate testers. The SNP markers genotyping was highly effective in confirming classification of the germplasm collection in South Africa. On the basis of SNP genotyping data the germplasm could be simplified into two broader groups, which are consistent with biochemical composition of the grain endosperm type. The first group comprised quality protein maize (QPM) lines and the second group is normal endosperm type of maize inbred lines. However, there are further sub-divisions within these broad germplasm groups indicating that the program in South Africa has maintained the diversity in both quality protein and normal endosperm maize germplasm. The SNP data was complemented by SCA data which grouped the germplasm into three heterotic groups (A, B and AB), indicating that South Africa germplasm lines have

orientation towards both tropical and temperate testers, and that some lines showed heterosis with both testers within the two broad adaptation groups. These findings have profound implications for optimising breeding programmes that target the warm temperate production environments in South Africa.

## Declarations

## CONFLICT OF INTEREST

The authors have no conflict of interest.

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## Figures

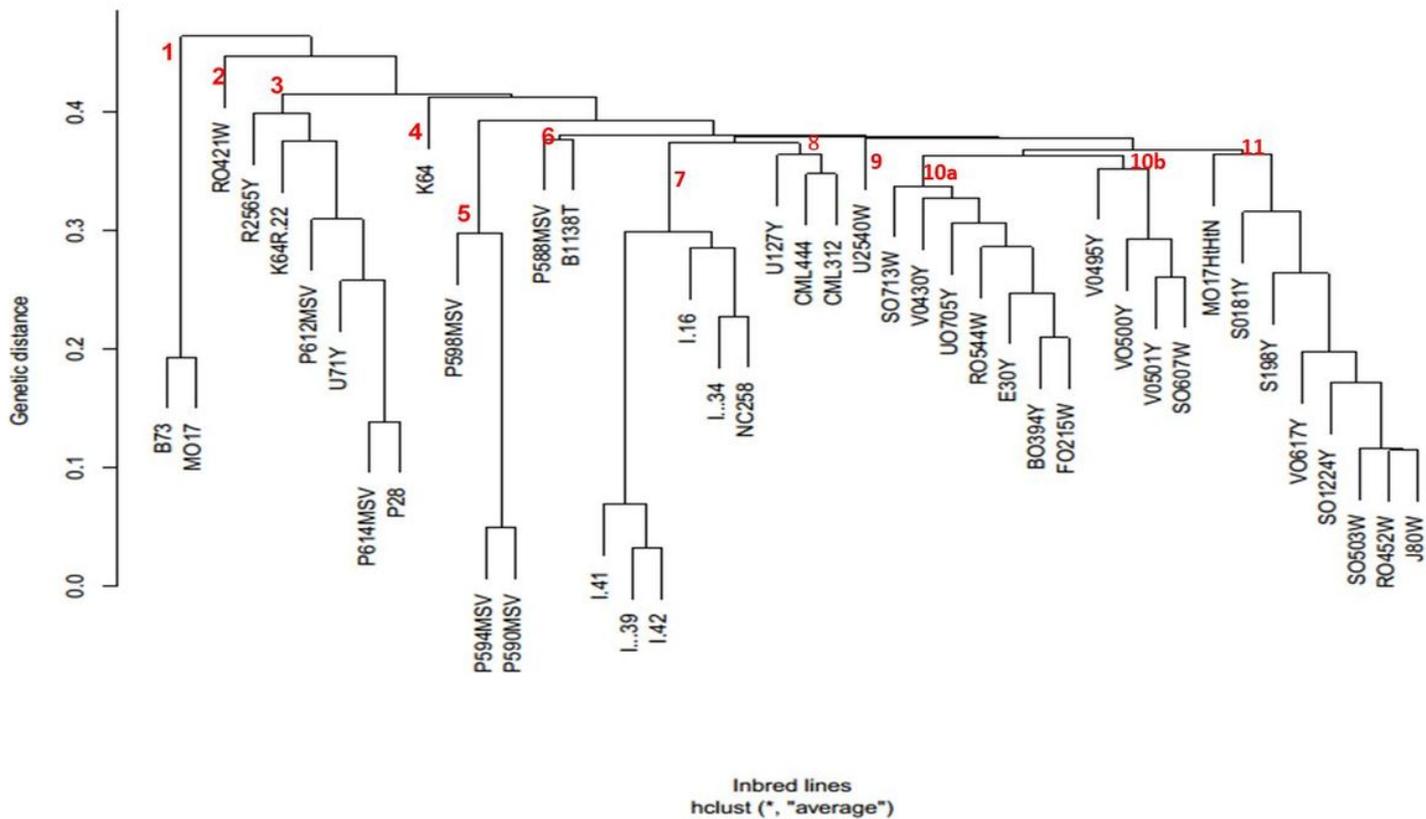


Figure 1

UPGMA dendrogram based on the analysis of 40 maize inbred lines and four testers using the Gower's distance (Gower, 1971)