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Genetic diversity and heterotic grouping of the core set of southern African and temperate maize (*Zea mays* L) Inbred lines using SNP markers

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Abstract

The establishment of heterotic groups and heterotic patterns is crucial to a successful maize hybrid breeding programme. Molecular markers can be used for differentiating maize into heterotic groups which can be used for maximum exploitation of heterosis. A core set of 45 maize inbred lines was selected from 96 maize inbred lines that were obtained from major breeding programmes in Zambia, Zimbabwe, CIMMYT, IITA, and USA. The 45 inbred lines were assessed for their genetic diversity and assigned to different heterotic groups using 129SNPs. The genetic distance ranged from 0.03 to 0.99, with the highest distance observed between inbred lines B73 and Mo17 and the least between L3233 and N3. The inbred lines were clustered into four groups which corresponded to the N, SC, BSS, and Lancaster heterotic groups. The genetic divergence among temperate inbred lines was larger than that among tropical inbred lines. Temperate inbred lines with potential to improve the heterotic response of the N and SC heterotic groups were identified. The study has shown that including temperate inbred pairs from established and well-known heterotic groups is recommended for effective molecular characterisation of Southern African maize inbred lines. It is recommended that the genetic distance based grouping should be verified by combining ability studies.

Keywords: heterotic groups, SNPs, genetic distance, cluster analysis, principal coordinate analysis

Introduction

The development of maize inbred lines and the search of their best hybrid combinations is the focus of maize hybrid breeding programme in Zambia (Ristanovic et al, 1987). This involves the development and improvement of open pollinated varieties as sources of inbred lines, followed by identifying the best inbred line combination determined by evaluating testcrosses in more than six locations for more than 3 years (Ristanovic et al, 1987). The procedure is thus time consuming and expensive especially when resources are limiting (Mienie and Fourie, 2013). As a consequence, the Zambian maize breeding programme has been crossing exotic elite lines to local elite lines followed by inbreeding and selection. In developing hybrids, modified single crosses were often used compared to single crosses. This type of inbred line development may result in having a high percentage of inbred lines that are very similar (genetic distance less than 0.05), a sign of having duplicates, if unchecked.

Classification of maize inbreds into known heterotic groups is one of the methods that can be used to reduce the number of duplicates while preserving diversity. Heterotic grouping results in maximising combining ability (Barata and Carena, 2006) and helps the breeder to make informed decisions on suitable hybrid combinations (Reid et al, 2011). This reduces the chance of evaluating a high number of undesirable crosses. The concept of heterotic groups and heterotic patterns in maize is meant for the systematic exploitation of grain yield heterosis (Melchinger and Gumber, 1998). Recently, the concept has been seen to be important for the development of «climate-change resilient maize cultivars» (Prasanna, 2012). Therefore, breeders have been identifying multiple heterotic groups and patterns to improve maize hybrid breeding or monitor changes in heterotic patterns after prolonged breeding (Choukan et al, 2006; Teng and Li, 2004; Zhang et al, 2000).

There are many methods of classifying maize inbred lines into heterotic groups (Aguiar et al, 2008a; Barata and Carena, 2006; Fan et al, 2003; Reid et al, 2011; Reif et al, 2003a; Senior et al, 1998; Suwarno et al, 2014; Xia et al, 2004). Molecular markers are among the methods that allow a greater number of inbred lines to be characterised and established into distinct cluster of genotypes based on genetic distance, thereby increasing the efficiency of breeding (Melchinger and Gumber, 1998; Reif et al, 2003a; Reif et al, 2003b). Studies have shown that molecular markers are effective in assigning maize inbred lines

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into heterotic groups (Aguiar et al, 2008b; Akinwale et al, 2014; Reid et al, 2011; Reif et al, 2003a; Suwarno et al, 2014). Several studies have demonstrated that molecular marker based heterotic grouping of maize inbred lines is positively correlated to F_1 grain yield and specific combining ability (Akinwale et al, 2014; Badu-Apraku et al, 2015; Drinic et al, 2002; George et al, 2011; Laude and Carena, 2015; Phumichai et al, 2008; Pinto et al, 2003; Reid et al, 2011; Zheng et al, 2008). Recently, SNP based heterotic grouping has been shown to be the most efficient compared to SCA and GCA (HSGCA) heterotic grouping even over heterotic grouping based on GCA of multiple traits (HGCAMT) (Badu-Apraku et al, 2015). Furthermore, it has been shown that including reference inbredlines (inbred lines with known genetic background and heterotic relationship) increases the integrity of molecular marker based heterotic grouping (Negrini et al, 2009). Since molecular characterisations of diverse maize inbred lines and populations from eastern and southern Africa has been inconclusive (Semagn et al, 2014; Semagn et al, 2012; Warburton et al, 2008; Warburton et al, 2005; Warburton et al, 2002), the inclusion of temperate maize inbred lines with known genetic background and heterotic relationships would help to properly classify the tropical lines. Therefore, this study was undertaken to determine the genetic inter-relationship and heterotic grouping of a core set of Southern African maize inbred lines. The maize inbreds with expired United States Plant Variety Protection (ex-PVPA), selected from well-known heterotic groups were included in the study, as reference materials (Negrini et al, 2009). In Zambia, despite having a well-developed maize breeding programme, there is no study that has been undertaken to classify the inbred lines into well-defined heterotic groups and patterns. Considering the large number of inbred lines in the Zambian breeding programme (Author, 2015), the use of molecular markers to characterisation the inbred lines will increase the efficiency of hybrid breeding and the development of genetically enhanced hybrids.

Table 1 - Name, origin and pedigree of the mini-core set of 45 maize inbred lines.

ID	Inbred	Source*	Pedigree
1	212-758	Zambia	[[SW1SR/COMPE1-W]-61-2-1-B/89[32/DRSTEW]#-107-2-3-X-1]-B-14-1-B-1-#-B X L12-2-2-4-2-B-B
2	213-813	Zambia	[TEWDSR-DrtTolSynS1#-8-X-X-1-B*4/CML390]-B-28-1-B-3-#-B X L1214-3-7-2-3-3-B-2
3	214-823	Zambia	[TIWD-EarlySelSynS1#-2-XX-2-B/[SW1SR/COMPE1-W]-126-2-1-B]-B-27-4-B-2-#-B X L1214-2-2-2-3-2-2
4	214-845	Zambia	[Ent67:92SEW1-17/[DMRESR-W]EarlySel-#I-3-3-B/CML391-2]-B-31-B-4-#-B X L1214-2-11-1-2-B-3
5	A632	USA	[(Mt42 X B14) B14(3)].
6	B73	USA	Iowa Stiff Stalk Synthetic C5
7	CML395	C-Z	90323(B)-1-B-1-B*4
8	CML444	C-Z	P43C9-1-1-1-1-1-BBBB
9	CML536	C-Z	[CML442/CML197//[TUXPSEQ]C1F2/P49-SR]F2-45-7-3-2-BBB]-2-1-1-1-1-B
10	CML539	C-Z	MAS[MSR/312]-117-2-2-1-B
11	CR1Ht	USA	(WI17Ht x Mo17Ht)
12	GVL1025	Zambia	K64R-7-3 X L5522-1-5-1-2-2-B-B
13	GVL1083	Zambia	L913xL1216-4-2-3 X ZEWA-4-2-4-1-2-4
14	GVL1282	Zambia	(CML386-5 X L710) X L710-3-2-6-5-B-B
15	GVL1292	Zambia	([[MSR123XI137TN-9-2-4-X-3/LZ956441]-B-1-5-5-BB-2-2 X L917) X L917-2-1-B-1
16	GVL506	Zambia	[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-BB-6-1-B-B
17	GVL522	Zambia	ZUCA 2000/1-2-2-1-1-4-2-B-B
18	GVL556	Zambia	90323(B)-1-X-5-BB-2-1-BBB-9-B-1
19	GVL721	Zambia	x (discard) 1 X L917-1-5-2-1-4
20	GVL82	Zambia	L12 M1 (220Gy)-150-3-2-1-1-3-2-1-1-B-4
21	GVL916	Zambia	SW89300-IP5S2-5-##1-1-3-B X L1214-2-3-1-1-1-2-2-B-2
22	H99	USA	Illinois synthetic
23	Houbai	China	Huojia Baimaya (Landrace)
24	IITA1	IITA	[TZM1501/KU1414/501]-1-4-3-1-B*6
25	K64-r	Zimbabwe	K64
26	L12	Zambia	Yugoslav germplasm
27	L1212	Zambia	L12 version
28	L152	Zambia	V01/87923-x-7575-3-3-1-2-3-2
29	L211	Zambia	L2 version
30	L3233	Zambia	L3233 version
31	L3234	Zambia	Unknown
32	L5522	Zambia	Contaminated SC selection
33	L917	Zambia	Yugoslav germplasm L9 version
34	Mo17	USA	C.I.187-2 x C103
35	N3	Zimbabwe	Salisbury White germplasm
36	ND405	USA	ND203 x OH51A
37	ND474	USA	[(WD x Wf9)WD(2)]
38	NK778	USA	(WI17XB37Ht). WI17
39	Oh43	USA	Oh40B X W8
40	PHG50	USA	(PH848 x PH207)
41	PHR36	USA	((((203 X 549) X 549) X 848) X 848) X 848
42	SC	Zimbabwe	Salisbury white
43	Suwan1	Thailand	Unknown
44	Tzi9	IITA	Sids 7734 x TZSR
45	*Zea diploperennis*	Mexico	maize progenitor

*C-Z = CIMMYT-Zimbabwe, USA = United States of America, IITA = International Institute of Tropical Agriculture.

Materials and Methods

Plant Materials

A mini core set of 45 inbred lines was developed from 96 maize inbred lines obtained from Southern Africa and the United States of America (USA), screened with 96SNPs. The 45 inbred lines consisted of 22 inbred lines from Zambia, three from Zimbabwe, four from CIMMYT, two from IITA and 21 inbreds from USA. The 21 inbred lines from USA included seven ex-PVPA (Nelson et al, 2008) and one maize progenitor, *Zea diploperennis*. Among the seven ex-PVPA, inbred lines PHG50 (PH207), B73, Mo17, A632, and Oh43 represented the three predominant heterotic groups in USA (Nelson et al, 2008; Reid et al, 2011). The other two inbred lines, namely ND474 and ND405, are from North Dakota, representing the Wf9 and Minnesota13 heterotic groups (Barata and Carena, 2006).

DNA extraction and genotyping with SNPs

The DNA for the 96 genotypes was extracted using the modified CTAB method (Saghai-Maroof et al, 1984). The DNA for the 76 genotypes from Southern Africa were extracted at CIMMYT/ILRI, BeCa in Nairobi (Kenya), while the DNA for the 20 inbreds from USA were extracted at the Department of Agronomy, Iowa State University, Ames in USA. The DNA samples of all the 96 genotypes were standardised and then sent to the Genomics Technology Facility (http://www.plantgenomics.iastate.edu/) at Iowa State University for genotyping with 96 SNPs using Sequenom technology (http://www.plantgenomics.iastate.edu/instrumentation.php). Thereafter, a mini core set of 45 inbred lines were selected form the 96 inbred lines. The names and pedigree of the selected inbred lines is shown in Table 1.

Data analyses

The Minor Allele Frequency (MAF), gene diversity, polymorphic information content (PIC) and the Rodgers' (1972) genetic distance were calculated using the genetic analysis software, the PowerMarker version3.25 (Liu and Muse, 2005). Minor allele frequency (MAF) were taken to be alleles with a frequency of <0.20 (Jones et al, 2007). The Rodgers' (1972) genetic distance was calculated based on shared allele frequency (Reif et al, 2005):

$$RGD = \frac{1}{m}\sum_{i=1}^{m}\sqrt{\frac{1}{2}\sum_{j=1}^{nj}(p_{ij}-q_{ij})^2}$$

where p_{ij} and q_{ij} are allele frequencies of the j^{th} allele at the i^{th} locus, n_j is the number of alleles at the j^{th} locus and m refers to the number of loci. The Rodgers (1972) distance matrix was then subjected to Darwin software, version 5.0.157 (Perrier and Jacquemoud-Collet, 2006) for Principal Coordinate Analysis (PCoA), cluster analysis and visualization of the dendrogram. Polymorphic information content (PIC) was calculated as: PIC = 1 - Σf_i^2, where f_i^2 is the frequency of the i^{th} allele, averaged across the loci.

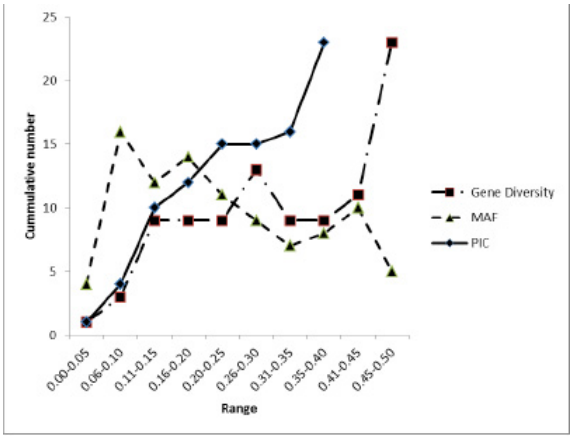

Figure 1 - Properties of 129 SNP markers used on 45 inbred lines.

Results

Characterisation of SNP diversity

Forty five inbred lines were surveyed with 96 SNPs of which 59 SNPs were polymorphic and their results for MAF, PIC, and gene diversity are shown in Figure 1. In the core set, 47.9% markers had minor allele frequency (MAF) of < 0.20 while 71.9% of the SNPs had high polymorphism content (PIC > 0.20). The MAF ranged from 0.02 to 0.49 with majority of the markers having MAF ranging from 0.06 to 0.10. Only 4 markers had MAF less than 5% and therefore, majority (95.8%) of the markers were of good quality. About 42 SNPs (43.8%) were relatively infrequent (MAF = 0.05 to 0.20). The average heterozygosity was 0.02 and gene diversity ranged from 0.04 to 0.50. The PIC ranged from 0.04 to 0.37 with majority of the SNPs having a PIC of 0.35 to 0.40. Only 12SNPs were non polymorphic among the tropical maize inbreds but were highly informative (PIC > 0.25), with high gene diversity among the temperate inbreds (Table 2). These markers can be used for discriminating between temperate and tropical inbred lines and for detection of rare alleles in tropical inbred lines.

Genetic similarity and clustering

The genetic distance between pairwise comparisons of the 45 mini core set, including *Zea diploperennis*, ranged from 0.03 to 0.99 (Supplementary Table 1). The highest distance of 0.99 was observed between inbreds B73 and Mo17, followed by that between inbreds B73 and CR1Ht (0.80). The lowest distance was between L3233 and N3 (0.03) followed by that between GVL1282 and GVL1292 (0.06). The genetic distance among the tropical inbreds ranged from 0.03 to 0.44, with a mean of 0.28 having a range of 0.41 (Supplementary Table 1). The highest GD was between N3 and Tzi9. On the other hand, the genetic distance (GD) among the temperate inbred lines ranged from 0.27 to 0.99, with a mean of 0.39 and range of 0.72. Considering the tropical/temperate inbred line pairs, the largest genetic distances were observed between B73 and TZi9 (0.60). The lowest

Table 2 - Properties of 12 SNPs that were missing (rare alleles) in tropical inbred lines.

SNo	Marker	Gene Diversity	Heterozygosity	PIC*	MAF*
1	11005W46	0.3200	0.0000	0.2688	0.2000
2	16676W3	0.2778	0.0667	0.2392	0.1667
3	20399W4	0.4898	0.0000	0.3698	0.4286
4	30618W25	0.3200	0.0000	0.2688	0.2000
5	32875W26	0.4978	0.0000	0.3739	0.4667
6	33362W31	0.1244	0.0000	0.1167	0.0667
7	39571W27	0.2311	0.0000	0.2044	0.1333
8	46177W5	0.2311	0.0000	0.2044	0.1333
9	72893W32	0.3200	0.0000	0.2688	0.2000
10	77095W27	0.4082	0.0000	0.3249	0.2857
11	91724W37	0.3200	0.0000	0.2688	0.2000
12	94591W13	0.3200	0.0000	0.2688	0.2000
	Maximum	0.4978	0.0667	0.3739	0.4667
	Minimum	0.1244	0.0000	0.1167	0.0667

*PIC = polymorphic information content, MAF = minor allele frequency.

genetic distances were observed between inbred lines GVL1282 and Huobai (0.21). Among all the key ex-PVPA temperate inbred lines (Mo17, B73, A632, PHR36, H99 and CR1Ht), only Mo17, B73 and CR1Ht had genetic distances greater than 0.44 with most of the tropical inbred lines (Supplementary Table 1). The temperate inbred lines, namely NK778, Oh43, PHG50, Suwani1, Huobai, and ND405 failed to have genetic distances greater than 0.44 with tropical lines.

When the inbreds were clustered using *Zea diploperennis* as the root (outgroup), four groups were observed (Figure 3), clearly separating temperate inbreds, Mo17 and B73 into groups 1 and 3 respectively, and separating tropical inbreds N3 or L3233 and SC or L5522 into groups 2 and 4 respectively. Inbred line, K64r was alone. Group 1 had five temperate inbred lines and 10 tropical inbreds and the notable line in this group was B73. Group 2 had mostly tropical inbred lines with only Houbai as a temperate. Mo17 and Oh43 were clustered together with other 7 temperate inbred lines in group 3, with TZi9 and CML536 as the only tropical lines in this group. Group 4 had tropical inbred lines only which included the southern African N3 and SC heterotic patterns; N3 and L3233. The highest gene diversity (0.32) was observed in group 3 followed by those in group 1 (0.30) (Table 3). The gene diversity for group 2 and 4 were 0.22 and 0.23 respectively (Table 3). Group 1 had a high proportion of tropical inbreds while group 3 had a high proportion of temperate inbred lines.

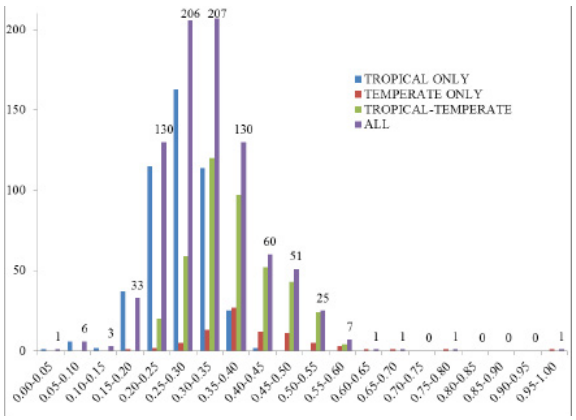

Figure 2 - Genetic distances of 45 inbred lines genotyped with 129 SNPs.

The Principal Coordinate analysis shows that the temperate inbred lines are scattered on the graph (Figure 4). The tropical inbred lines, however, are not as dispersed as temperate inbreds but are mostly clustered around the centre. The inbreds A632, Mo17, B73 and N3/L3233 are located on the extremes (or vertex) of the scatter plot. The temperate inbred lines, A632, Mo17, and B73 were dispersed on the PCoA plot indicating that they are divergent.

Discussion

Genetic divergence and clustering of maize inbred lines

The study revealed that the temperate inbreds were more divergent, having high mean genetic distance (0.38) and range (0.72) compared to the tropical inbreds with low mean genetic distance (0.28) and low range (0.41). The observed large divergence among temperate lines has been reported before (Wen et al, 2012; Zheng et al, 2008). Mo17 and B73 were identified as the most divergent, which is consistent to their known heterotic grouping and response. The large genetic distance between Mo17 and B73 observed in this study has been reported before (Choukan et al, 2006). To the contrary, the genetic distances between N or L3233 and SC or L5522 were low (0.32 and 0.34, respectively), yet according to Mickelson et al (2001), the pairs are key heterotic groups of maize hybrid breeding in Eastern and southern Africa. The GD observed between the N and SC heterotic groups is three times less than the distance of their counterpart Mo17 and B73 heterotic groups. The observed heterotic response to grain yield between the N and SC groups, despite low genetic distances, has been largely attributed to the large dominance gene effects (80%) and 20% accounted for additive and additive x additive mode of gene action (Derera and Musimwa,

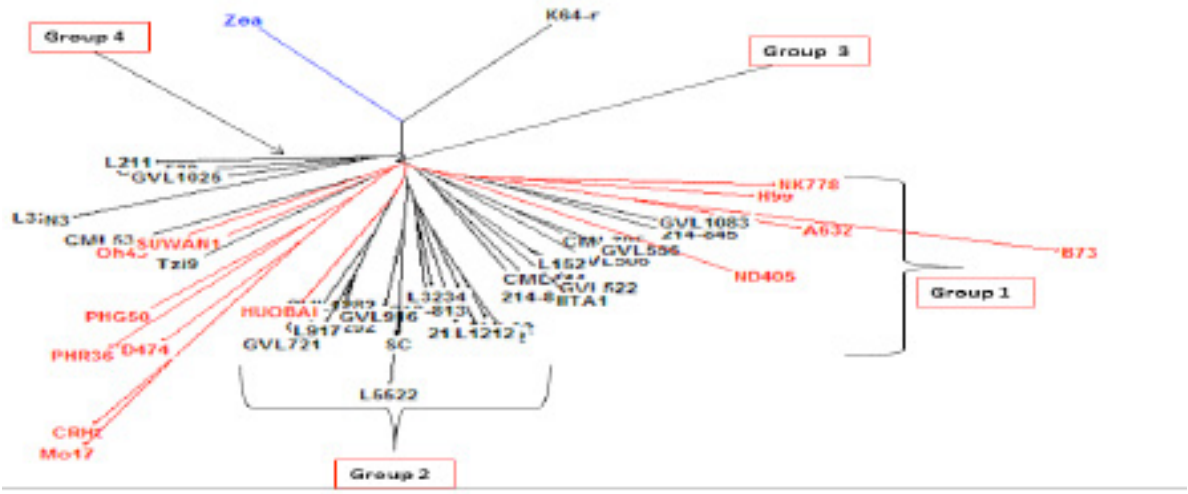

Figure 3 - Phylogenetic relationship of the mini core set of 45 maize inbred lines (Red= Temperate, Black= Tropical and Blue= *Zea diploperennis*).

2015). Thus, the genetic basis of heterosis between the N and SC heterotic groups needs further investigation.

The number of clusters formed in molecular marker based classification is reflective of heterotic groups, which depends on the degree of genetic divergence. This is because there is a relationship between genetic distance and heterosis (Moll et al, 1965), which is characterised by significantly high positive correlations (Reif et al, 2003a; Reif et al, 2003b). Furthermore, genetic diversity is highly related to transcriptional variation, which accounts for 80% of heterosis observed in maize hybrids (Stupar et al, 2008). SNP based genetic distances of between 0.44 and 0.77 have been reported to be highly correlated to grain yield and specific combining ability (George et al, 2011; Reid et al, 2011). Thus cluster analysis separated the tropical inbred lines having the topical inbred lines into the N and SC heterotic groups, while the temperate lines being grouped into the BSSS and Lancaster heterotic groups. The separation of inbred lines into temperate and tropical groups is commonly reported in molecular diversity studies (Fan et al, 2003; Zheng et al, 2008). We also expected that the seven prominent USA inbred lines, namely Mo17, PHG50, B73, A632, Oh43, ND474, and ND405 would form individual clusters. However, they were clustered into Iowa Stalk Synthetic (BSSS) and Lancaster sure crop (LSC) heterotic groups (Preciado–Ortiz and Johnson, 2004). The two north Dakota inbred lines, ND405 and ND474 with Minnesota 13 and Wf9 background respectively (Barata and Carena, 2006), were also grouped into the BSSS and LSC respectively (Figure 3). The grouping of ND405 with the BSSS is expected as most North Dakota lines have BSSS background (Barata and Carena, 2006). Since the temperate inbred lines were grouped according to the expected heterotic groups, we can therefore confidently say that the clusters represent the true reflection of the heterotic groups. Therefore, the main heterotic groups were identified as: B73 heterotic group (group 1), SC heterotic group (Group 2), Oh43/Mo17 heterotic group (group 3), N heterotic group (group 4) and the K heterotic group (K64r). Subgroups were also observed in some of the main heterotic groups. The clustering results indicates that the K group has not been extensively used or utilized in breeding as there was only one member in the group, K64r. The inbred lines from CIMMYT-Zimbabwe are classified as heterotic group A or B, with inbred lines CML395, CML444 belonging to heterotic group B while CML536 and CML539 belonging to heterotic group A (Semagn et al, 2012). In this study, inbreds CML536 and CML539 were clustered separately, indicating that they are far apart to be clustered together. CML536 was put in the Mo17 group and CML539 in the N group. The clustering of CML539 (CIMMYT-A group) together with N group indicates that much of the N genome was recovered from N3 during the conversion of the Mexican Tuxpeno germplasm to a MSV tolerant line, using N3 as a donor (Vivek et al, 2009). However, it seems less genome of SC was recovered from Mexican ETO Blanco germplasm during conversion of CML536 to MSV resistant inbred line using SC as a donor (Vivek et al, 2009). The observed disparity in classification of CML536 and CML539 have also been reported in West African Maize inbred lines (Adetimirin et al, 2008).

The non-divergence of key historical tropical maize inbreds compared to the temperate inbreds explains, in part, the non-heterotic responses observed among tropical lines (Xia et al, 2005). The non-existence of alternative heterotic groups apart from the N and SC indicates that no breeding efforts were made to improve the divergence among the two groups. In Zambia, this can be attributed to the change in focus of the breeding programmes due to the influence of the donors (Ristanovic et al, 1985; Ristanovic et al,

Table 3 - Genetic properties of the clusters.

Cluster*	MAF	Sample Size	NFO	Allele Number	Gene Diversity	Het	PIC
1	0.2162	15	14.3646	1.9167	0.2999	0.0239	0.2432
2	0.1567	14	13.3438	1.7188	0.2188	0.0172	0.1783
3	0.2458	9	8.6771	1.8542	0.3211	0.0301	0.2550
4	0.1763	7	6.7083	1.6354	0.2342	0.0252	0.1872

*MAF = minor allele frequency, NFO = number of observations, Het = Heterozygosity and PIC = polymorphic information content.

1987). Another probable reason for failure to distinctly separate the lines is caused by developing lines from crosses between different groups (Mienie and Fourie, 2013), leading to having lines with mixed origin. However, successes has been scored by deriving the lines from crosses between sub-groups within the same heterotic group, which is an efficient way of developing hybrids between groups (Zhang et al, 2002). Therefore, there is need to expand the genetic base of the tropical inbred lines in relation to the observed heterotic groups.

Inbred line development

Most commercial breeding companies intermate inbred lines within a heterotic pattern or family (Lu and Bernardo, 2001; Mikel, 2008), also called «inbred recycling». In Zambia, pedigree breeding is used for inbred lines development. However, looking at the pedigree information elite exotic lines were crossed to elite local lines for extracting inbred lines. Therefore, it is possible for the Zambian inbred lines to be diverse and/or have a high number of duplicates. Therefore, it is recommended that inbred lines be extracted from well-established divergent populations. Since there are no populations based on the N and SC grouping, marker based clustering can be used to form synthetics which can be used as base population for inbred line extraction. Studies have shown that synthetics formed by crossing inbred lines with low genetic similarity (GS = 0.32 and 0.34) performs better than synthetics formed based on high genetic similarity (GS = 0.44 and 0.77) or based on combining ability (Narro et al, 2012). Once the synthetics are formed, their divergence can be increased by recurrent selection (Hinze et al, 2005), before being used for inbred line extraction.

Enhancement of heterotic response of tropical inbreds by CIMMYT, IITA and temperate inbred lines

Wen et al (2011) observed that incorporating exotic lines with unique alleles and clear heterotic patterns enhances heterosis and grain yield of local

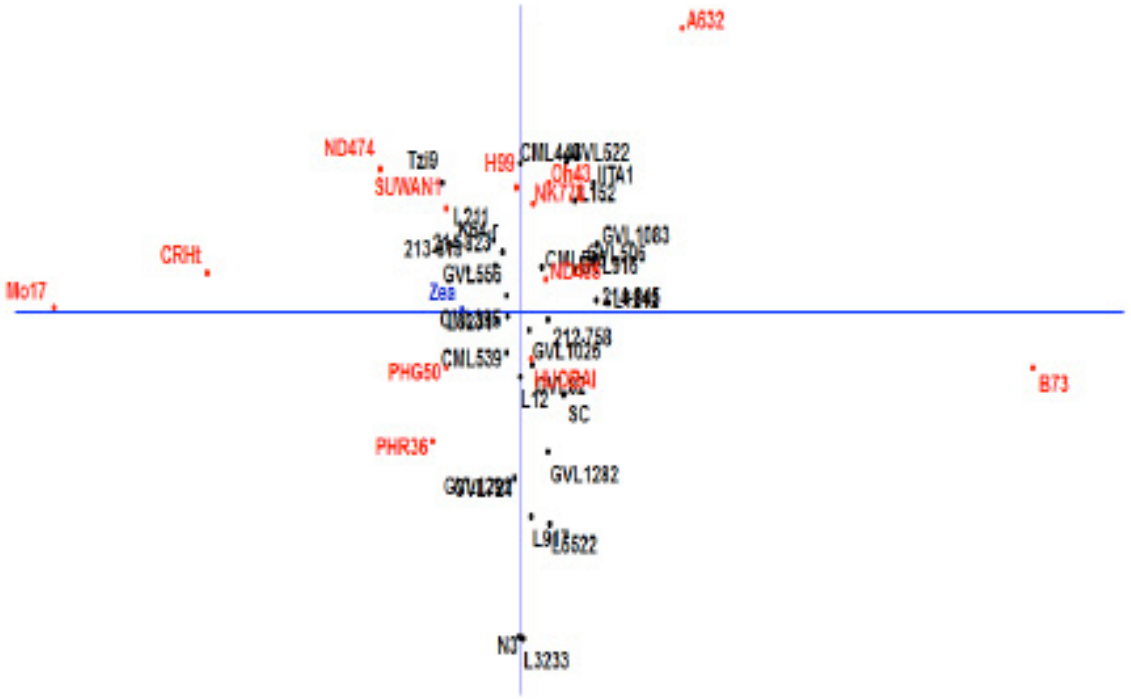

Figure 4 - Principal Coordinate Analysis of 45 inbred lines genotyped with 96 SNPs.
Red = Temperate, Black = Tropical, Blue = *Zea diploperennis*.

germplasm. In this case the mini core set forms an initial working panel for increasing the heterotic response of the N and SC groups of tropical maize. Studies have shown that hybrid performance is correlated to SNP based genetic distance of between 0.44 and 0.77 of parental pairs (Badu-Apraku et al, 2015; George et al, 2011; Laude and Carena, 2015). Therefore, temperate-tropical pairs with genetic distance between 0.44 and 0.77 have potential to be used in tropical breeding. All the CIMMYT and IITA inbred lines had genetic distances less than 0.44 with the N and SC groups, except for Tzi9, which had a genetic distance of 0.44 with N3. The temperate inbred lines: Mo17, B73, A632, H99, CRHt, ND474, and PHR36 had genetic distances greater than 0.44 with the N and SC heterotic groups (Supplementary Table 1). The K group (K64r) had genetic distances greater than 0.44 with B73, CRHt, and H99. Since the inbred lines; B73, Mo17, PHG50, A632, and Oh43 represents the three predominant heterotic groups (Nelson et al, 2008a), they would be potentially useful in improving the N and SC groups. Similarly, the inbred lines ND405 and ND474 has Minnesota13 and W9f background, respectively (Barata and Carena, 2006), but only ND474 would be useful, as it had genetic distances of 0.44 with both L3233 and N3. Although CIMMYT inbred lines are sources of favourable alleles, Xia et al (2005) and Semagn et al (2012) observed that their usage requires a systematic approach. This entails that the Zambian germplasm should first be well characterised into heterotic groups before IITA and CIMMYT inbred lines can be incorporated based on their combining ability with local germplasm.

Conclusion

The development of germplasm that belongs to different heterotic groups and/or patterns is vital for breeding high yielding and stress tolerant maize hybrids. Therefore, the ability to efficiently determine the heterotic grouping and patterns as well as identify new alternative heterotic groups for introduced germplasm is critical to the success of maize hybrid breeding programme in Zambia and other sub-Saharan African (SSA) countries. The inclusion of temperate inbred lines from well-known heterotic groups can aid in identifying potential heterotic groups of germplasm that has not been characterised. Based on the grouping of temperate inbred lines one would be able to infer the classification of tropical lines. The study showed that tropical inbred lines had 47% rare alleles (frequency < 0.20) and temperate lines had 33% rare alleles. This indicates that more temperate lines should be screened with more markers for identifying useful inbred lines. The study identified B73, Mo17, A632, ND474, H99, PHR36, and CR1Ht for improving the N and SC heterotic groups. The study shows that including inbred lines from established heterotic groups in a molecular characterisation program, is essential for the proper identification of potential heterotic groups of Southern African maize inbred lines.

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