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Molecular Characterization of Selected Maize (*Zea mays* L.) Inbred Lines

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Abstract Genetic knowledge of germplasm diversity among parental inbred lines has significant impact in the development of improved maize hybrids in the breeding program. The aim of our study was to assess maize inbred lines for variability in molecular traits and to estimate genetic distance among different parental lines. Maize inbred lines were genotyped through 200 SNPs markers. The results revealed low levels of variability across the lines. Two major clusters of lines were observed where the first major group was made of 16 sub-groups of 28 lines. The genetic distance between the studied lines was low. Therefore, the prediction of the heterosis effect of the crosses between the maize parental lines would have been in the negative way.

Keywords Inbred line; SNP marker; Variability; Genetic distance

Introduction

Genetic diversity is the average sequence divergence between any two individuals for a given loci (Ahmad et al., 2010). The strategies used in maize breeding programs are frequently characterized by a decrease of genetic diversity in the pool of germplasms and an increase in the genetic evenness in cereal production (Lee, 1998; Morales et al., 2010). This might cause important problems, particularly sensitivity to new diseases and/or a decreased tolerance to high temperatures or drought (Duvick, 1989).

Different methodologies have been used to characterize genetic diversity in the maize germplasm including morphological characters, pedigree analysis, heterosis and the detection of variation at the DNA level using markers (Udaykumar et al., 2013). The advent of molecular genetics has enhanced selection accuracy for quantitative traits by incorporating molecular information into genetic improvement programs (Tang and LI, 2006). Analysis of genetic diversity and relationships among the elite breeding materials can significantly aid in crop improvement. In maize, this information is useful in planning for hybrid and line development, assigning lines to heterotic groups and in plant variety protection (Yuan et al., 2002; Yadav and Singh, 2010).

Morphological and molecular studies of inbred lines have not yet been undertaken under acid soils of the Humid Forest Zone of country. For an effective and efficient national maize breeding program in the Cameroon, there is an urgent need to gather useful information in this regard.

The objectives of the present study were to: Assess maize inbred lines for variability in molecular traits; Estimate genetic distance among different parental lines.

1 Results

1.1 Grouping of inbred lines based on SNP markers

Clusters were generated through DARwin by a simple matching dissimilarity index, a threshold equality of 0%, with 15 nodes (degree: minimum = 2, maximum = 3) (Figure 1). The edge length sum of the graph was 0.46. Different colors were applied to discriminate introduced inbred lines from the local (lines from IRAD were in black color). Two major clusters of lines were observed: group one include 28 inbred lines and the second group two lines (ATP S6 31Y-2 and ATP S6 20Y-1). The first group was divided into 16 sub-clusters: the first 10 sub-clusters each contained one line. The sub-cluster k, l and m had 2 lines each, n had 5 lines, o had 4 lines and p had 4 lines (Table 1). The introduced inbred lines were colored in blue while

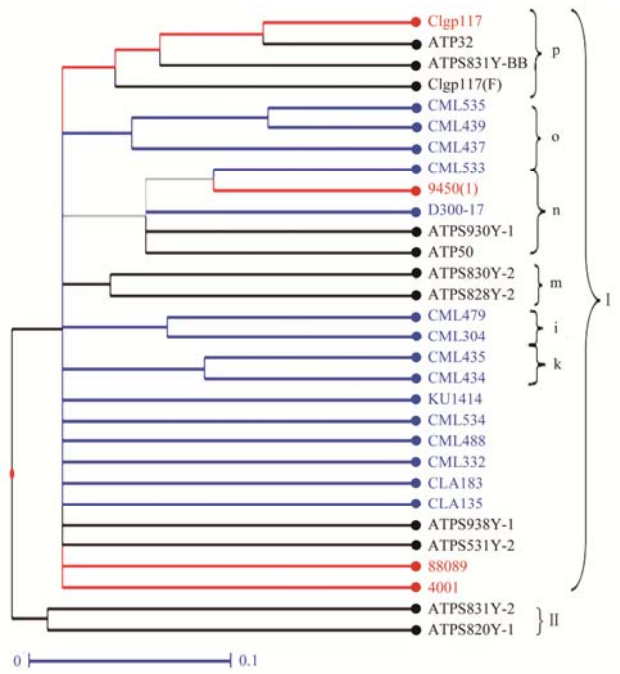

Figure 1 Dendrogram of maize inbred lines generated from the genotyping result of 200 SNP markers

Table 1 Clustering of maize inbred lines based on diversity in SNP markers

Cluster			Line
Cluster I	16 sub-clusters	a	4001
		b	88069
		c	ATP S9 36Y-1
		d	ATP S5 31Y-2
		e	CLA 135
		f	CLA 183
		g	CML 332
		h	CML 486
		i	CML 534
		j	Ku 1414
		k	CML 435, CML 434
		l	CML 304, CML 479
		m	ATP S8 30Y-2, ATP S8 26Y-2
		n	ATP 50, ATP S9 30Y-1, D300-17, 9450, CML 533
		o	CML 535, CML 439, CML 437, CML 533
		p	Cam Inb gp1 17, ATP 32, ATP S6 31Y-BB, Cam Inb gp1 17 (F)
Cluster II			ATP S6 31Y-1
			ATP S6 20Y-1

the inbred lines used as heterotic testers in the next chapter were colored in red (Figure 2).

1.2 Genetic distance among inbred lines

The genetic distance among the inbred lines varied from 0.1 observed between CML 535 and CML 439 to 0.4 (Table 2). The genetic distance between inbred 88069 and most of the ATP lines (from number 4 to 13) was 0.4. The genetic distance between 4001, 88069, 9450 and Cam Inb gp1 17 varied from 0.3 to 0.4.

2 Discussion

The description of maize inbred lines based on molecular analysis is the one way of identify the little difference between each of them. The molecular markers were used to get more information on the inbred lines of the study. The IRAD lines were interspersed between the introduced inbred lines on the dendogram. Two major groups were identified. ATP S9 36Y-1 and ATP S5 31Y-2 were the only lines in the main cluster II. They are local inbred lines from IRAD. The main group I was subdivided into 16 sub-clusters. The genotypes in red are the lines used in the next chapter as testers (Cam Inb gp1 17, 9450, 4001 and 88069). These were found to be in different sub-clusters in group I. The genetic distance among these testers varied from 0.3 to 0.4 indicating that they were closely related. Genetic distance varied from 0.1 to 0.4 for the 30 inbred lines. The minimum distance (0.1), indicating closely related inbred lines, was between Cml 439 and Cml 535. Sserumaga et al., (2014) found that gene diversity ranged from 0. 18 to 0.92 between maize inbred lines with under adapted regimes of water.

Tester 9450 was genetically similar to several CIMMYT Cml lines and was closely related to Cam Inb gp1-17 but the hybrid between these two testers was the highest yielding in stressed plots in (Tandzi et al., 2015). Testers 88069 and 4001 were in adjacent sub-clusters suggesting that they are genetically very close but their hybrid was the highest yielding under control conditions (Tandzi et al., 2015) indicating that they are in different heterotic groups. The results here again demonstrate that genetic distance as measured by molecular markers is not associated with heterosis. The prediction of the heterosis effect of the crosses between these inbred lines would have been in a negative way. According to Warburton et al. (2002)

Table 2 Genetic distance among inbred lines using line codes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	0.3																													
3	0.4	0.4																												
4	0.3	0.4	0.3																											
5	0.4	0.4	0.2	0.3																										
6	0.3	0.4	0.4	0.3	0.3																									
7	0.4	0.4	0.4	0.4	0.4	0.4																								
8	0.4	0.4	0.4	0.4	0.4	0.4	0.4																							
9	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.4																						
10	0.3	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.4																					
11	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.3																				
12	0.3	0.3	0.2	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4																			
13	0.3	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.4	0.4																		
14	0.3	0.4	0.3	0.2	0.3	0.3	0.4	0.4	0.2	0.3	0.3	0.3	0.3																	
15	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.2																
16	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4															
17	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3														
18	0.3	0.4	0.4	0.3	0.4	0.3	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3													
19	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.3												
20	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3											
21	0.3	0.4	0.4	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.2										
22	0.3	0.4	0.4	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.3									
23	0.3	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.2	0.3								
24	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.2	0.3	0.4	0.4	0.3	0.3							
25	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.4	0.4	0.3						
26	0.4	0.4	0.2	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4					
27	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.4				
28	0.3	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.3	**0.1**	0.3	0.3	0.4	0.3			
29	0.3	0.3	0.2	0.3	0.3	0.3	0.4	0.4	0.3	0.4	0.4	0.3	0.4	0.3	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.4		
30	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	

and Xia et al. (2004), heterosis has been predicted on the basis of genetic distance based on molecular markers. Goff (2011) found that generally the greater the genetic diversity of the parents, the higher the level of heterosis achieved. This suggested that since the genetic distance among lines was not wide, it would have been impossible to get any good hybrid combination. The current results differed from the assumption used to establish the heterotic groups based on molecular maker data as stated by Reif et al. (2005). All these findings were different from the results got from the present study. Also Sserumaga et al. (2014) realized that high overall genetic diversity (0.65) among the inbred line combinations indicates an opportunity to exploit the inbred lines for the development of varieties and start point of pedigree breeding population used to produce promising inbred lines (Sserumaga et al., 2014). For a crop like maize, the strategy of developing good hybrids depends on genetic diversity present in the available inbred lines. In the present study, the molecular markers identified genetic diversity. Analysis of genetic diversity and of relationship among the elite breeding materials could significantly aid in crop improvement. Moreover, the lines find themselves close to clusters due to a decrease in variation between them.

3 Conclusions

The variability among the inbred lines in this study was not very high. Also, the genetic distance between the studied lines was low. The prediction of the heterosis effect of the crosses between them would have been in the negative way. Cluster I contained all the introduced inbred lines and most of the locally adapted lines. It was subdivided into 16 sub-clusters. All the four inbred lines used as heterotic testers in the next chapter were found to be in different sub-clusters based on molecular characterization but were all in the same main cluster I.

4 Materials and methods

4.1 Plant material and data collection

Thirty inbred lines were collected from CIMMYT, IITA and IRAD. They were planted in the breeding nursery during the 2013. Fourteen of these lines were from CIMMYT, three from IITA and thirteen from the Institute of Agricultural Research for Development (IRAD). The origin of these lines and their respective characteristics are presented in Table 3. Fresh leaf samples of the 30 maize genotypes were collected, packed and sent in double wells per genotype to Laboratory of the Government Chemist (LGC) Genomics for genotyping. Young leaves were harvested from 14 day old seedlings from two plants per inbred line. Four samples of six millimeter leaf discs were taken from each inbred line and placed in a 96 well plate. The 96 well plates containing the leaf samples were sealed with a perforated heat seal and sent to LGC Genomic for genotyping. The sampling was carried

Table 3 Maize inbred lines used in the study

Lines	Code	Origin	Characteristics
4001	1	IITA	Tolerant to low N
88069	2	IRAD	Good root volume
9450 (1)	3	IITA	Temperate adapted
ATP 32	4	IRAD	Acid soil tolerant
ATP 50	5	IRAD	Acid soil tolerant
ATP S5 31Y – 2	6	IRAD	Acid soil tolerant
ATP S6 20Y – 1	7	IRAD	Acid soil tolerant
ATP S6 31Y-2	8	IRAD	Acid soil tolerant
ATP S6 31Y-BB	9	IRAD	Acid soil tolerant
ATP S8 26Y – 2	10	IRAD	Acid soil tolerant
ATP S8 30Y – 2	11	IRAD	Acid soil tolerant
ATP S9 30Y – 1	12	IRAD	Acid soil tolerant
ATP S9 36Y – 1	13	IRAD	Acid soil tolerant
CI gp1 17	14	IRAD	Tolerant and P efficient
CI gp1 17 (F)	15	IRAD	/
CLA 135	16	CIMMYT	Susceptible
CLA 183	17	CIMMYT	Acid soil tolerant
CML 304	18	CIMMYT	Susceptible
CML 332	19	CIMMYT	Susceptible
CML 434	20	CIMMYT	Acid soil tolerant
CML 435	21	CIMMYT	Acid soil tolerant
CML 437	22	CIMMYT	Acid soil tolerant
CML 439	23	CIMMYT	Acid soil tolerant
CML 479	24	CIMMYT	Acid soil tolerant
CML 486	25	CIMMYT	Acid soil tolerant
CML 533	26	CIMMYT	Acid soil tolerant
CML 534	27	CIMMYT	Acid soil tolerant
CML 535	28	CIMMYT	Acid soil tolerant
D300-17	29	CIMMYT	Acid soil tolerant
KU 1414	30	IITA	Tolerant to low N

out following the protocol of the Kbioscience leaf sampling kit. The genotyping was conducted using the KAPS method with 200 SNP markers. The details on the principle and procedure of the DNA assay are available at http://www.kbioscience.co.uk/reagents/KASP.

Data analysis

DARwin5 software was used for the tree construction from the molecular markers (http://darwin.cirad.fr/darwin).

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