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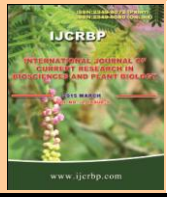

Original Research Article

Selection of Homozygosity and Genetic Diversity of Maize Inbred using Simple Sequence Repeats (SSRs) Marker

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A b s t r a c t

The level of homozygosity and genetic diversity are accurately detected by codominant SSRs markers. The research aimed to select homozygosity and analyze genetic diversity of 51 maize inbreds using 36 SSRs markers. The research was aimed to select among 51 maize inbreds with high homozygosity and to investigate the genetic diversity using 36 SSRs markers. The result shows that there were 30 inbreds indicating homozygosity level of more than 80%. The diversity of those inbreds was moderately high, with genetic similarity of between 0.22 and 0.87 distributed within six heterotic groups. The farthest genetic distance of 0.87 was detected on inbred pair 1044_3 vs Nei9008. Meanwhile the closest genetic distance of 0.22 was showed by inbred pair G20133077 vs G2013627. Inbred pairs with genetic distance of more than 0.7 were potentially generating high heterotic parental combinations.

K e y w o r d s

Genetic diversity
Homozygosity
Inbred maize

Introduction

Inbred lines are extracted from population or varieties through self cross (selfing) for 5 to 6 selfing generations which generates homozygous plants. Through self crossing, heterozygous loci are segregating to which increases the frequency of homozygous loci and decreases heterozygous loci (Singh et al., 1987). High frequency of homozygosity affects plant performance by decreasing plant vigor and productivity due to inbreeding depression.

Extraction of maize inbreds as parental lines supports the development of hybrid and synthetic maize. Breeding program to develop hybrid and synthetic maize requires high level of homozygosity and genetic diversity. Liu et al. (2003) stated that high genetic diversity of inbred lines distributed equally among heterotic group is useful in guiding breeders to select parental candidates for crossing program. Further, the diversity information enable breeders to select parental

lines to develop hybrid and synthetic maize (Legesse et al., 2007; Pabendon et al., 2008).

Detection of homozygosity level and genetic diversity can be performed conventionally based on the uniformity of morphological characters or plant color. Morphological data which refer to Union Pour la Protection des Obtention Vegetales (UPOV) has long been used as descriptive parameter to identify and distinguish varieties or inbreds (Gunjaca et al., 2007). However, morphological character poorly describes the genetic relationship due to the existence of genetic by environment interaction and also unknown genetic control. Therefore, selection of homozygosity level and genetic diversity based on the plant morphology is less accurate.

The advent of molecular marker as selection tool has extensively been utilized on plants and produces more accurate result compared to morphological data. Molecular marker selection is merely based on the genetic character of the plant and thus not affected by environment condition. Simple Sequence Repeats (SSRs) is one of molecular markers which have been used comprehensively on maize. Detection of this marker is based on polymerase chain reaction (PCR) using nucleotide sequence as primer (Gupta et al., 1996). The feasibility of SSRs markers for breeding program is proven, because it is abundantly and equally distributed throughout the genome with high variability (Powell et al., 1996), highly reproducible(Smith et al., 1997; Mingsheng et al., 2010), feasible for genetic diversity study (Li et al., 2002; Legesse et al., 2007; Shehata et al., 2009; Yang et al., 2011), and accurately detect the level of homozygosity and genetic purity of inbred lines (Mingsheng et al., 2010; Daniel et al., 2012; Hipi et al., 2012; Semagn et al., 2012; Mulsanti et al., 2013). The level of homozygosity and genetic purity is important key in developing novel hybrid and synthetic maize varieties (Gunjaca et al., 2008; Heckenberger et al., 2002). The research was aimed to select among 51 maize inbreds with high homozygosity and to investigate the genetic diversity.

Materials and methods

Selection of 51 maize inbred lines using SSRs markers was conducted at Molecular Biology Laboratory of Indonesian Cereal Research Institute (ICERI), Maros from April to June 2013. The research was aimed to select 51 maize inbred lines with homozygosity level of more than 80% and to investigate their genetic diversity. Genetic material used in this research consisted of 20 inbred lines tolerance to low N fertilizer and was introduced from CIMMYT; and 31 inbred lines collection of ICERI for high yielding and drought tolerance breeding program.

Fifteen seeds of each line were sown on a plant pot with diameter of 17 cm and height of 14 cm. The plot was filled with mix of soil and manure with ratio of 1:1 (v/v). Leave sample was collected at10 days after planting from 10 plants for each line, by cutting fully expanded leaves into small cutting. Leaves of 20 plants were mixed and sample was taken from the mixture as much as 0.4 gram /sample.

DNA extraction was performed based on George et al. (2004). DNA pellet was extracted through centrifugation, rinsing, drying and dilution of the DNA using TE buffer, and finally incubation at 60°C for 15 min.

DNA was amplified by Polymerase Chain Reaction (PCR) based on the markers used for the analysis. There were 36 markers equally distributed throughout maize genom were used. Those markers were seleceted based on their equal distribution on ten maize chromosoms and their elaborate utilization on maize (Warburton et al., 2002; George et al., 2004; Pabendon et al., 2007). PCR cocktail of 88 µl consisted of 1 µl DNA, 6,25 µl TaqDNA polymerase, 0.5 µl primer, 2.25 µl ddH2O. PCR process was consist of denaturation (1 min at 94°C), followed by touch down of 2 cycles for 1 min at 94°C, 1 min at 65°C and 2 min at 72°C. Annealing temperature was lowered down from 1°C every two cycles and finished as annealing temperature was reached. The cycle was repeated for 29 times.

PCR product was separated using vertical electrophoretic Triple Mini-vertical Electrophoresis System, and acrylamid gel with composition of 100 ml 8% acylamid, 100 µl Temed, and 1000 µl 10%APS. Electrophoretic was performed in 1×TBE buffer at 100 volt for 45–60 min. Silver staining was used to visualize DNA band according to Promega Silver Sequence protocol. The gel was soaked for 5 min in silver solution (1 g silver/l), then rinsed with aquades for 30 seconds, soaked in mixture solution of NaOH (20 g/l) and formaldehyde 3000 µl until DNA bands

were visualized. DNA bands were then labeled based on relative position of the base pair to marker fragments, which was φX174/Hin f I. The DNA band was scored based on the binary data with criteria of score 1: present of DNA band, score 0: absent of DNA band and 9: missing DNA band.

Level of polymorphism was analyzed based on the value of polymorphic information content (PIC) which shows level of genetic diversity (Weir, 1996). A locus with high number of alleles indicates high PIC value (Smith et al., 1997). PIC value was calculated using formula,

$$PIC = 1 - \sum_1^n f_i^2 \quad i = 1, 2, 3, \ldots\ldots\ldots\ldots n$$

where f_i^2 was i^{th} allele frequency. Cophenetic correlation coefficient was calculated using NTSYS program.

Level of hetezygosity was analized based on the number of DNA band that present as more than one bands per locus. This analysis helped to eliminate genotypes with high heterozygosity which was not detected on phenotypic selection. Formula to calculate level of heterozygosity was

$$\text{Heterozygosity} = \frac{\text{number of heterozygous locus}}{\text{total number of SSRs loci used}} \times 100\%$$

In order to generate an accurate data analysis, inbred lines with heterozygosity of more than 20% were eliminated at inital stage, and thus produced only parental lines with homozygosity of more than 80%.Level of genetic similarity was estimated by Jaccard coefficient (Rohlf, 2000) with formula,

$$S = \frac{m}{n + u}$$

where m was number of DNA band at the same position, n was total number of DNA (alleles) and u was number of DNA band at different position. Genetic similarity analysis was performed using NTSYS version 2.1based on Unweighted Pair Group Method Using Arithmetic Averages (UPGMA). Genetic distance matrix was calculated from genetic similarity analysis using formula S = 1 – GS, in which S was genetic distance and GS was genetic similarity. Boot Strapping analysis was performed to reveal the goodness of fit of clustering using Winboot program.

Results and discussion

Characteristic of SSRs markers on 51 maize inbred lines

Based on DNA band visualization of each marker, score 0 and 9 represent absent of the band and missing data respectively, while score 1 represents the present of DNA band. In order to produce valid data, markers with missing data of more than 15% were excluded from the analysis (CIMMYT, 2005). The total markers initially used for the analysis were 36 SSRs markers. However, among these markers were phi041 with missing data of 25.49% and phi45269 with missing data of 31.37%. Therefore, further heterozygosity and genetic diversity analysis was performed using 34 SSRs markers (Table 1).

The number of identified alleles from 30 inbred lines using 34 SSRs markers was 152. Meanwhile, the variation of allele number/inbred/marker was range from 2 to 8, with average of 4.28 alleles. The level of polymorphism was detected from 0.11 to 0.73, with the lowest level showed by marker phi448880 and the highest by marker bnlg1614. According to Buckler et al. (2006), the phenotypic variation in particular plant species is controlled by polymorphism of several genes. The high average of polymorphism level indicates high genetic variation among inbred lines.

Homozygosity selection

Level of homozygosity of certain genotype is identified using SSRs marker which based on the number of DNA band. Heterozygous locus will appear as more than one DNA bands /allele per marker per genotype, while homozygous locus is represented by one DNA band. The tolerable level of heterozygosity of maize inbred is 20%, with the assumption of homozygosity of more than 80% (CIMMYT, 2005).With the help of SSRs marker, genotypes with more than 20% of heterozygosity can be eliminated in the early stage of breeding program, which is difficult to be detected via phenotypic observation due to environmental effect.

The level of heterozygosity of 51 maize inbreds was ranged from 5.41% to 59.64% (Table 2). Among those inbreds, 30 genotypes were indicating level of heterozygosity of more than 20%, with the assumption of homozygosity percentage of more than 80%.

Table 1. List of SSRs markers for homozygosity selection and genetic diversity analysis.

No.	Marker	Bin No.	Repeat type	Base	Annealing temperature (°C)
1	phi109275	1.00	AGCT	CGGTTCATGCTAGCTCTGC // GTTGTGGCTGTGGTGGTG	54
2	bnlg1614	1.02	AG(15)	CCAACCCACCCAGAGGAGA // AGCGGGCGAGATCTTCAT	58
3	bnlg439	1.03	-	TTGACATCGCCATCTTGGTGACCA//CTTAATGCGATCGTACGAAGTTGTGGAA	54
4	umc1196	1.07	CACACG	CGTGCTACTACTGCTACAAAGCGA // AGTCGTTCGTGTCTTCCGAAACT	54
5	phi227562	1.12	ACC	TGATAAAGCTCAGCCACAAGG // ATCTCGGCTACGGCCAGA	54
6	phi083	2.04	AGATG	AGGAGGACCCCAACTCCTG // TTGCACGAGCCATCGTAT	54
7	bnl1621	2.07	AG(18)	CAAGTGCTCCGAGATCTTCCA // CGCGAACATATTCAGAAGTTTG	54
8	phi101049	2.09	AGCT	CCGGGAACTTGTTCATCG // CCACGTCCATGATCACACC	52
9	umc1504	3.00	AGATG	CCGGGAACTTGTTCATCG // CCACGTCCATGATCACACC	54
10	phi374118	3.03	ACC	TACCCGGACATGGTTGAGC // TGAAGGGTGTCCTTCCGAT	56
11	phi102228	3.04	ACC	TACCCGGACATGGTTGAGC // TGAAGGGTGTCCTTCCGAT	54
12	phi053	3.05	AAGC	ATTCCGACGCAATCAACA // TTCATCTCCTCCAGGAGCCTT	54
13	phi072	4.01	ATAC	ACCGTGCATGATTAATTTCTCCAGCCTT // GACAGCGCGCAAATGGATTGAACT	56
14	phi079	4.05	AAAC	TGGTGCTCGTTGCCAAATCTACGA // GCAGTGGTGGTTTCGAACAGACAA	52
15	phi093	4.08	AGATG	TGGTGCTCGTTGCCAAATCTACGA // GCAGTGGTGGTTTCGAACAGACAA	60
16	phi109188	5.00	AGCT	AGTGCGTCAGCTTCATCGCCTACAAG // AGGCCATGCATGCTTGCAACAATGGATACA	60
17	phi331888	5.04	AAAG	AAGCTCAGAAGCCGGAGC // GGTCATCAAGCTCTCTGATCG	54
18	phi048	5.07	ATCG	GCAAACCTTGCATGAACCCGATTGT // CAAGCGTCCAGCTCGATGATTTC	56
19	umc1153	5.09	AAGC	TTGCGCAAGTTTGTAGCTG//ACTGAACCGCATGCCAAC	58
20	phi423796	6.02	(TCA)4	CAGCATCTATAGCTTGCTTGCATT // TGGGTTTTGTTTGTTTGTTTGTTG	54
21	phi081	6.05	AGATG	CACTACTCGATCTGAACCACCA // CGCTCTGTGAATTTGCTAGCTC	54
22	phi452693	6.06	GAT-TAC	CAAGTGCTCCGAGATCTTCCA // CGCGAACATATTCAGAAGTTTG	56
23	phi299852	6.08	AGCC	CAAGTGCTCCGAGATCTTCCA//CGCGAACATATTCAGAAGTTTG	52
24	umc2059	6.09	(CAG)8	GGAAAAGGAGGAACAGTGTAAGCA // AGCGTGATCAGACGTACAATGCTA	56
25	umc1545	7.00	AGC	GATGTGGGTGCTACGAGCC // AGATCTCGGAGCTCGGCTA	58
26	phi034	7.02	CCT	TAGCGACAGGATGGCCTCTTCT // GGGGAGCACGCCTTCGTTCT	54
27	phi328175	7.04	CCT	TAGCGACAGGATGGCCTCTTCT//GGGGAGCACGCCTTCGTTCT	56
28	umc1304	8.02	AGG	GGGAAGTGCTCCTTGCAG//CGGTAGGTGAACGCGGTA	54
29	phi233376	8.03	CCG	GGGAAGTGCTCCTTGCAG // CGGTAGGTGAACGCGGTA	58
30	umc1858	8.04	(TA)8	GTTGTTCTCCTTGCTGACCAGTTT // ATCAGCAAATTAAAGCAAAGGCAG	56
31	umc1279	9.00	(TCGA)4	GATGAGCTTGACGACGCCTG // CAATCCAATCCGTTGCAGGTC	54
32	umc1506	9.01	AGCC	TTGGCTCCCAGCGCCGCAAA//GATCCAGAGCGATTTGACGGCA	56
33	phi032	9.04	CCG	CCGGCAGTCGATTACTCC // CGAGACCAAGAGAACCCTCA	54
34	phi448880	9.05	(CCT)6	GATGAGCTTGACGACGCCTG//CAATCCAATCCGTTGCAGGTC	54
35	phi041	10.00	AAAG	CTCCAGCAAGTGATGCGTGAC//GACACCCGGATCAATGATGGAAC	56
36	phi96342	10.02	AAGC	CGATCCGGAGGAGTTCCTTA // CCATGAACATGCCAATGC	54

Source: http://www.maizegdb.org/ssr.php

They were G2013631, G20133036, 1044-30, CLRCY017, CLYN261, DTPYC9-F13-2-3-1-2-B, 1042-69, AMB07, CLYN253, CY12, Nei9008, CLRCY034, CLRCY039, CLYN257, CLYN260, DTPYC9-F46-3-9-1-1-B, G20133077, AMB20, CY11, CY14, CY6, G2013645, MR14, G2013649, CML161/NEI9008, CY15, CLYN249, DTPYC9-F46-1-2-1-2-B, DTPYC9-F65-2-2-1-1-B, and G2013627 (Table 3). The other 21 inbreds were eliminated from the analysis because the heterozygosity level was more than 20%.

Based on the pedigree and breeder information, the inbreds evaluated were 5th selfing generation (S5) with

moderately high homozygosity level. It was predicted that during crossing period and seed processing, the inbreds were contaminated with other genotype.

Genetic similarity and heterotic group analysis

High genetic diversity can be identified based on the level of genetic similarity and relationship. Based on the genetic similarity coefficient which was analyzed using UPGMA, a graph of genetic relationship, named dendrogram, was clustered the inbreds into six heterotic groups (Fig. 1). Meanwhile, bootstrapping analysis produced cophenetic coefficient (r) value of 0.78, which indicated the stability of grouping was categorized as good fit. Cophenetic coefficient showed the accuracy of genotypic grouping, performed based on the genetic similarity among the inbreds evaluated using particular number of markers. High number of polymorphic markers used to perform the analysis will produce higher cophenetic coefficient value. According to Wu et al. (2010) to improve the stability of heterotic grouping of 30 inbreds, it is important to increase the number of markers into 350 alleles. In this current research, the number of alleles used was 152 alleles, and thus it is necessary to increase the number of alleles in order to improve the stability of the grouping.

Table 2. Percentage of missing data, number of allele, level of polymorphism and relative size of loci detected on 51 maize inbred lines.

No.	Marker	Chromosome no.	Missing data (%)	Number of allele	Level of Polymorphism	Relative size of SSRs loci (bp)
1	phi109275	1.00	7.84	5	0.71	104.73 - 124.11
2	bnlg1614	1.02	0.00	6	0.73	168.15 - 294.09
3	bnlg439	1.03	3.92	4	0.52	195.54 - 306.23
4	phi227562	1.12	3.92	4	0.67	276.55 -297.22
5	phi083	2.04	0.00	5	0.57	122.4 - 179.58
6	bnlg1621	2.07	1.96	5	0.71	91.03 - 140.05
7	phi101049	2.09	0.00	4	0.51	261.4 - 377.28
8	umc1504	3.00	0.00	4	0.49	145.5 - 206.53
9	phi374118	3.02	0.00	4	0.53	204.45 - 246.77
10	phi102228	3.04	0.00	4	0.40	121.14 - 149.16
11	phi053	3.05	5.88	6	0.70	155.92 - 185.31
12	phi072	4.01	0.00	4	0.42	130.57 - 166.31
13	phi079	4.05	9.80	3	0.59	179.95 - 195.54
14	phi093	4.08	1.96	3	0.33	276.55 - 304.11
15	phi109188	5.00	3.92	5	0.60	156.65 - 210.88
16	phi331888	5.04	0.00	5	0.51	127.77 - 169.14
17	phi048	5.07	0.00	4	0.37	153.45 - 311.09
18	umc1153	5.09	1.96	5	0.71	100.12 - 111.36
19	phi423796	6.01	1.96	4	0.50	107.99 - 119.22
20	phi299852	6.08	0.00	8	0.72	106.75 - 200.00
21	umc2059	6.09	0.00	5	0.65	125.33 - 170.60
22	umc1545	7.00	0.00	3	0.56	68.91 - 83.89
23	phi034	7.02	0.00	5	0.66	118.00 - 135.61
24	phi328175	7.04	0.00	4	0.55	98.21 - 129.02
25	umc1304	8.02	7.84	4	0.59	122.41 - 130.11
26	phi233376	8.03	0.00	3	0.54	140.01 - 181.15
27	umc1858	8.04	0.00	4	0.68	111.99 - 380.6
28	umc1279	9.00	9.80	3	0.43	91.02 - 110.49
29	umc1506	9.01	5.88	4	0.62	104.73 - 124.11
30	phi032	9.04	0.00	6	0.66	96.72 - 149.16
31	phi96342	10.02	0.00	4	0.31	233.92 - 271.54
32	umc1196	10.07	7.84	5	0.65	137.25 - 155.90
33	phi448880	9.05	0.00	2	0.11	151.00 - 231.70
34	phi081	6.05	0.00	3	0.26	138.53 - 156.16
*	phi452693	6.06	25.49	-	-	-
*	phi041	10	31.37	-	-	-
	Total			152		
	Average			4	0.57	
*Markers excluded for further analysis						

The genetic similarity coefficient of 30 genotypes was ranged from 0.28 to 0.79. Genetic similarity indicates the closeness relationship among genotypes. The higher the genetic similarity coefficient, and thus the bigger the chance of relationship between genotypes. On the contrary, the smaller the genetic similarity coefficient, thus the smaller the chance of relationship between genotypes. The range of genetic similarity coefficient (0.28 – 0.79) illustrated the high genetic diversity among 30 inbred lines evaluated.

Based on the genetic similarity coefficient of 0.35, there were six groups of inbreds. They were group A to group F. Group A was consist of eight inbred lines, namely CML161_NEI9008, CY12, CY14, AMB20, CY11, CLYN261, CY15, and CLYN249. Group B was comprised of nine inbred lines, namely CY6, CLRCY034, CLRCY039, G2013645, MR14, CLRCY017, CLYN235, CLYN257, and CLYN260. Group C was consisting of five inbreds, namely DTPYC9_F65_2_2_1, DTPYC9_F13_2_3_1, DTPYC9_F46_1_2_1, DTPYC9_F46_3_9_1, and Nei9008. Group D was comprised of six inbred lines, namely G2013627, G20133077, G20133036, G2013649, 104269, and AMB07. Meanwhile group E and F were each consists only one inbred line, namely G2013631 and 104430 respectively (Fig. 1).

Fig. 1: Dendrogram of 30 maize inbred lines performed by cluster analysis of UPGMA based on of Jaccard genetic similarity coefficient using 34 SSRs markers.

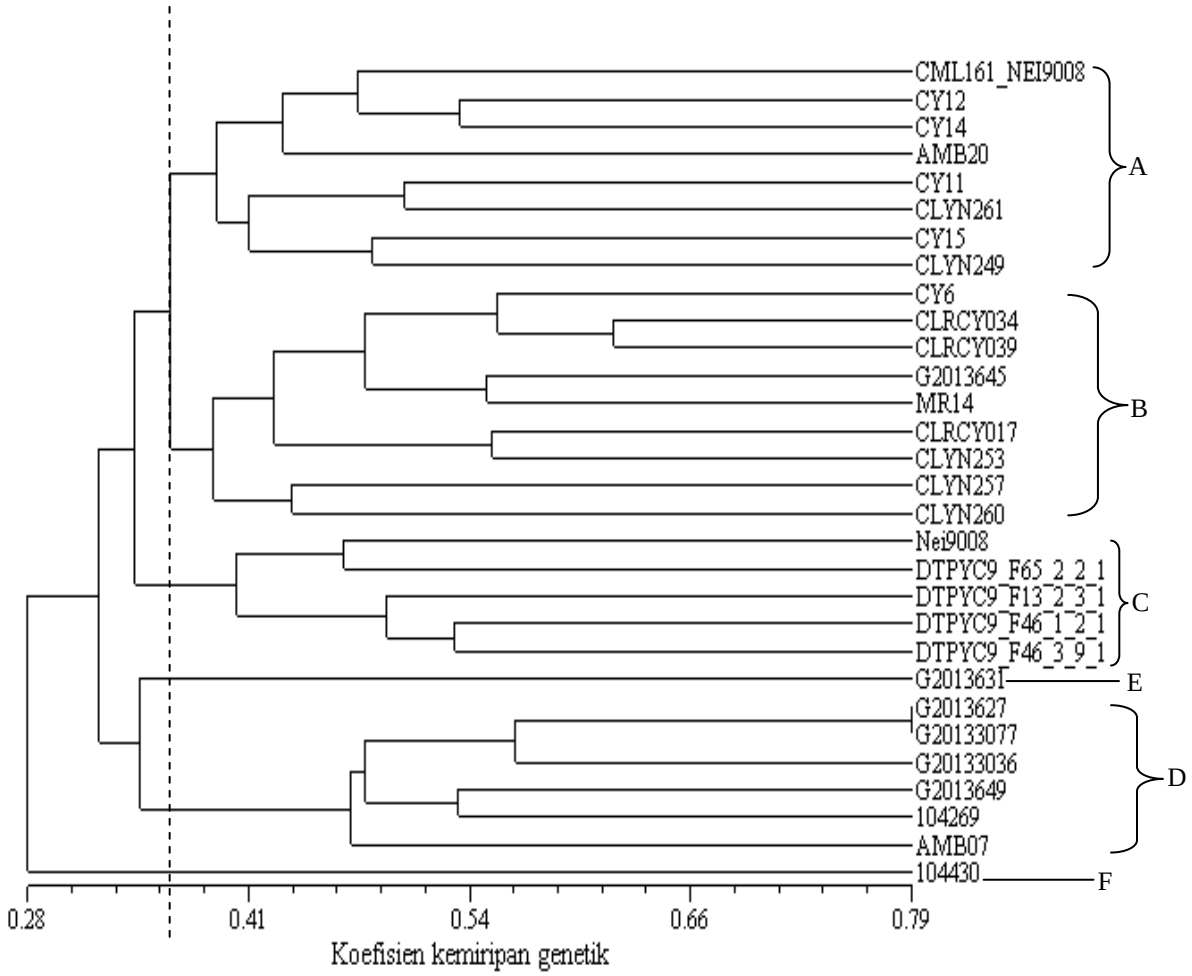

Table 3. The number and percentage of heterozygosity of 51 maize inbreds.

No.	Inbred name	Number of Heterozygous loci	Hetero-zygosity (%)	No.	Inbred name	Number of Heterozygous loci	Hetero-zygosity (%)
1	G2013631	2	5.41	27	CLYN249	7	18.92
2	G20133036	2	5.41	28	DTPYC9-F46-1-2-1-2-B	7	18.92
3	1044-30	2	5.41	29	DTPYC9-F65-2-2-1-1-B	7	18.92
4	CLRCY017	3	8.11	30	G 2013627	7	18.92
5	CLYN261	3	8.11	31	CY 4	8	21.62
6	DTPYC9-F13-2-3-1-2-B	3	8.11	32	CY 7	8	21.62
7	1042-69	3	8.11	33	DTPY C9-F47-1-7-1-B	8	21.62
8	AMB07	3	8.11	34	G180	8	21.62
9	CLYN253	4	10.81	35	G2013634	8	21.62
10	CY 12	5	13.51	36	CLYN248	8	21.62
11	Nei9008	5	13.51	37	DTPYC9-F114-2-4-1-1-B	8	21.62
12	CLRCY034	5	13.51	38	CY 10	9	24.32
13	CLRCY039	5	13.51	39	CLRCY031	9	24.32
14	CLYN257	5	13.51	40	CLYN262	9	24.32
15	CLYN260	5	13.51	41	DTPYC9-F143-5-4-1-2-B	9	24.32
16	DTPYC9-F46-3-9-1-1-B	5	13.51	42	AMB-36	9	24.32
17	G20133077	5	13.51	43	G2013640	10	27.03
18	AMB 20	5	13.51	44	G2013619	12	32.43
19	CY 11	6	16.22	45	G2013632	12	32.43
20	CY 14	6	16.22	46	G2013621	13	35.14
21	CY 6	6	16.22	47	CLYN231	14	37.84
22	G2013645	6	16.22	48	G2013620	17	45.95
23	MR 14	6	16.22	49	CY 16	18	48.65
24	G2013649	6	16.22	50	CLYN226	18	48.65
25	CML 161/NEI 9008	7	18.92	51	G2013623	22	59.46
26	CY 15	7	18.92				

The grouping of 30 inbred lines by SSRs markers was relatively valid. Therefore, SSRs marker allowed the grouping of inbred lines with the same initial pedigree into the same group, such as DTPY (drought tolerant population yellow) into one group of group C, which consist of DTPYC9_F65_2_2_1, DTPYC9_F13_2_3_1, DTPYC9_F46_1_2_1, and DTPYC9_F46_3_9_1. Inbred lines with initial pedigree G2013 was grouped into one group D; they were G2013631, G20133036, G2013649, G2013627, and G20133077 (Table 4).

Genetic distance analysis

Genetic distance of each 30 inbred lines is presented in Table 3. The genetic distance matrix of the inbreds was ranged from 0.22 to 0.87. The highest genetic distance 0.87 was indicated by inbred pair 1044_30 vs Nei9008. The inbreds were clustered in different groups, in which inbred 1044_30 was member of group F, while inbred Nei9008 was in group C. Inbred pair G20133077 vs G2013627 showed the lowest genetic distance of 0.22, to which both inbreds were belong to the same group D. Further, low genetic distance was also indicated by inbred pair CLRCY034 vs CLRCY039 which clustered in group B. Research conducted by Pabendon et al. (2008) showed that inbreds crossing from different heterotic groups provide opportunity to produce higher grain yield compare to inbreds crossing of one heterotic group. The higher the genetic distance between inbreds, the bigger the chance to explore high heterosis effect. As the consequence, clustering maize inbreds using SSRs markers is an important tool for breeder to select parental candidates to support the development of novel hybrid and syntehtic maize.

Genetic distance value is an accurate initial prediction tool to select or screen numbers of inbred lines as parental candidates to gain high heterosis effect. In addition, it helps to reduce the number of genetic materials to be crossed as selected inbreds with high genetic distance are the lines to be included in the crossing program. Several researches showed significant correlation between genetic distance and heterosis for grain yield, yet high genetic distance does not necessarily contribute to high heterosis (Drink et al., 2002; Su-Xia et al., 2004; Phumichai et al., 2008; Daniel et al., 2012; Akinwale et al., 2014).

Table 4. Matrix of genetic distance of 30 maize inbred lines.

Inbred	CML161NEI9008	CY11	CY12	CY14	CY15	CY6	G2013631	G2013645	MR14	Nei9008	CLRCY017	CLRCY034	CLRCY039	CLYN249	CLYN253	CLYN257	CLYN260	CLYN261	DTPYC9_F13_2_3_1¥	DTPYC9_F46_1_2_1¥	DTPYC9_F46_3_9_1¥	DTPYC9_F65_2_2_1¥	G2013649	G2013627	G20133077	G20133036	AMB20	1044_30	1042_69	AMB07
CML161NEI9008	0.00																													
CY11	0.54	0.00																												
CY12	0.49	0.51	0.00																											
CY14	0.52	0.53	0.45	0.00																										
CY15	0.66	0.51	0.57	0.60	0.00																									
CY6	0.58	0.68	0.60	0.62	0.66	0.00																								
G2013631	0.73	0.69	0.58	0.55	0.62	0.66	0.00																							
G2013645	0.49	0.55	0.50	0.54	0.61	0.45	0.68	0.00																						
MR14	0.52	0.61	0.60	0.58	0.66	0.55	0.69	0.44	0.00																					
Nei9008	0.73	0.69	0.74	0.66	0.71	0.71	0.68	0.64	0.48	0.00																				
CLRCY017	0.52	0.62	0.64	0.59	0.66	0.63	0.64	0.55	0.49	0.60	0.00																			
CLRCY034	0.62	0.64	0.66	0.61	0.65	0.40	0.68	0.47	0.49	0.57	0.53	0.00																		
CLRCY039	0.63	0.60	0.62	0.64	0.61	0.46	0.72	0.48	0.57	0.69	0.61	**0.37**	0.00																	
CLYN249	0.60	0.55	0.50	0.56	0.50	0.60	0.61	0.52	0.51	0.54	0.60	0.57	0.58	0.00																
CLYN253	0.61	0.65	0.55	0.60	0.59	0.57	0.67	0.54	0.58	0.60	0.45	0.56	0.57	0.47	0.00															
CLYN257	0.72	0.70	0.75	0.70	0.65	0.69	0.66	0.65	0.63	0.64	0.63	0.62	0.67	0.64	0.63	0.00														
CLYN260	0.74	0.71	0.72	0.72	0.75	0.69	0.71	0.66	0.60	0.63	0.60	0.52	0.65	0.61	0.65	0.57	0.00													
CLYN261	0.65	0.53	0.71	0.67	0.66	0.68	0.76	0.55	0.68	0.73	0.56	0.63	0.61	0.64	0.66	0.63	0.60	0.00												
DTPYC9_F13_2_3_1¥	0.61	0.68	0.69	0.66	0.76	0.69	0.74	0.71	0.68	0.71	0.65	0.64	0.65	0.66	0.70	0.57	0.68	0.60	0.00											
DTPYC9_F46_1_2_1¥	0.58	0.59	0.61	0.63	0.65	0.58	0.69	0.64	0.58	0.59	0.49	0.54	0.58	0.58	0.55	0.68	0.74	0.64	0.52	0.00										
DTPYC9_F46_3_9_1¥	0.73	0.58	0.62	0.62	0.70	0.66	0.62	0.70	0.76	0.67	0.64	0.56	0.66	0.67	0.64	0.68	0.71	0.61	0.55	0.47	0.00									
DTPYC9_F65_2_2_1¥	0.57	0.71	0.66	0.64	0.76	0.71	0.70	0.71	0.66	0.53	0.58	0.60	0.71	0.55	0.70	0.74	0.68	0.77	0.55	0.55	0.52	0.00								
G2013649	0.62	0.71	0.66	0.61	0.71	0.67	0.58	0.62	0.69	0.68	0.60	0.59	0.65	0.66	0.71	0.72	0.66	0.71	0.73	0.68	0.67	0.62	0.00							
G2013627	0.58	0.69	0.72	0.69	0.72	0.66	0.67	0.68	0.71	0.65	0.59	0.68	0.78	0.65	0.64	0.66	0.71	0.66	0.60	0.65	0.62	0.57	0.55	0.00						
G20133077	0.54	0.72	0.73	0.68	0.77	0.71	0.68	0.69	0.70	0.66	0.60	0.71	0.79	0.66	0.68	0.60	0.66	0.67	0.59	0.68	0.70	0.58	0.54	0.22	0.00					
G20133036	0.66	0.73	0.75	0.77	0.71	0.75	0.71	0.66	0.71	0.70	0.67	0.70	0.75	0.69	0.69	0.64	0.66	0.62	0.65	0.74	0.72	0.64	0.56	0.46	0.40	0.00				
AMB20	0.63	0.55	0.58	0.48	0.64	0.70	0.68	0.65	0.67	0.66	0.61	0.72	0.75	0.62	0.62	0.78	0.74	0.68	0.79	0.65	0.69	0.70	0.56	0.54	0.58	0.62	0.00			
1044_30	0.68	0.72	0.63	0.74	0.70	0.65	0.70	0.64	0.77	**0.87**	0.73	0.69	0.70	0.73	0.73	0.76	0.73	0.70	0.75	0.80	0.70	0.73	0.73	0.69	0.70	0.61	0.63	0.00		
1042_69	0.72	0.73	0.72	0.65	0.73	0.68	0.62	0.70	0.78	0.76	0.71	0.68	0.70	0.73	0.75	0.58	0.69	0.71	0.70	0.68	0.67	0.71	0.49	0.53	0.49	0.48	0.64	0.71	0.00	
AMB07	0.64	0.62	0.65	0.71	0.62	0.75	0.70	0.70	0.75	0.65	0.67	0.73	0.80	0.65	0.75	0.71	0.70	0.64	0.75	0.64	0.67	0.62	0.51	0.55	0.57	0.49	0.52	0.68	0.57	0.00

Pabendon et al. (2010) stated that parental pair with maximum genetic distance does not necessarily to be crossing pair with the highest heterosis effect; nevertheless it can be generated from parental pair with moderate difference of genetic distance value of more than 0.7. Xu et al. (2004) explained the reason of inconsistence prediction of hybrid heterosis using SSRs markers were (a) hybrid heterosis performance is strongly affected by environment. Productivity of maize will vary when it is cultivated in different agro-ecological condition, such as climate, fertility and pathogenesis present in that particular environment. On the contrary, SSRs markers are not influenced by environmental condition. (b) SSRs loci are equally distributed throughout the whole genome and thus SSRs data (SSRs allele) might not necessarily related to hybrid heterosis.

Conclusion

Homozygosity selection of 51 maize inbreds using SSRs markers has selected 30 maize inbred lines with homozygosity level of more than 80%. Genetic diversity of 30 inbred lines was relatively high with genetic similarity coefficient ranged from 0.22-0.87 and distributed in six heterotic groups. The highest genetic distance of 0.87 were indicated by inbred pair 1044_30 vs Nei9008, while the lowest genetic distance of 0.22 was displayed by inbred pair G20133077 vs G2013627.Inbred pairs with genetic distance of more than 0.7 are potential to generate high heterosis.

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