THE USE OF SSR MARKERS TO IDENTIFY HETEROTIC PATTERN OF F_1 HYBRIDS IN TWO TROPICAL MAIZE POPULATIONS

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Abstract

The objectives of this study were to (i) select lines from two tropical maize populations using genetic distance (GD) with 50 simple sequence repeats (SSR) markers for F_1 hybrid production and (ii) study the relationship between GD and yield and GD and mid-parent heterosis (MPH), using SSR markers. The results showed that the GD of the S_1 selected lines from the two maize populations ranged from 0.14 to 0.94, with the average of 0.44, which manifested the high genetic diversity among the S_1 selected lines. The grain yield of the F_1 hybrids obtained from the crosses between the S_1 selected lines of both populations was evaluated. The mean grain yield of all F_1 hybrids was 8,865 kgha^{-1}. The F_1 hybrids from the crosses between G2001 × Y1008 and G2068 × Y1002 gave the highest grain yields of 10,799 kgha^{-1} and 10,721 kgha^{-1}, respectively. The GD showed a positive correlation with the grain yield of the F_1 hybrids and the MPH with the values of 0.21 and 0.07, respectively. However our research showed a low correlation between the GD and F_1 hybrid grain yield and the MPH, because there was a difference in the linkage disequilibrium among the markers used and the quantitative trait loci (QTLs) between the heterotic groups. This may be related to DNA markers being able to effectively predict hybrid performance with DNA heterozygosity. However the difference and number of markers studied may have also reflected the degree of correlation. The number of hybrids evaluated and the first generation of selfing in the lines studied may have also affected the value of the correlation.

Keywords: Maize, simple sequence repeats (SSR), population, heterosis, genetic distance (GD), marker assisted selection (MAS)

Introduction

Essentially all of the maize acreage grown in the world is planted to hybrid maize**,** with an increasing percentage of the acreage worldwide (65%) moving from open-pollinated populations,

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improved synthetics, and variety crosses to hybrids (Duvick, 1999). Tropical maize is grown on approximately 45 million ha in lowland tropical environments. Although hybrid development in tropical maize started in the 1940s, the sustainability and adoption has been variable (Vasal *et al*., 1999). The expanding utilization of hybrids and inbred-line-based synthetics in tropical areas has substantially increased the number of tropical inbred lines developed from landraces, populations, and synthetics by pedigree breeding (Hallauer, 1991). Several racial complexes have been preferentially used as a germplasm source for hybrid development including 'Tuxpeño', 'Cuban' and 'Coastal' tropical flints, 'Tuson' and 'ETO'. Promising heterotic patterns have been detected and developed as a result of increasing characterization of the maize germplasm and development of inbred lines for heterotic response (Goodman, 1985). Tropical maize, however, has a broad genetic base and shows a greater genetic diversity than temperate maize (Beck *et al.*, 1997). Therefore, the estimation and organization of the genetic diversity in tropical maize on the basis of DNA markers would assist in determining efficient breeding strategies.

The definition of heterosis groups and heterotic patterns is an empirical task in hybrid maize breeding that has, in temperate maize germplasm, contributed to a large increase in yield. Reciprocal recurrent selection programs (RRS) have proven to be effective in the improvement of heterotic groups for a systematic exploitation of heterosis, as they maximize selection gains within a heterotic group and differences between heterotic groups. Clear characterization of the genetic diversity of maize inbred lines derived from different origins will maximize the efficiency in hybrid combinations and the development of new inbreds. In temperate maize such as U.S. Corn Belt germplasm, a clear heterotic pattern (Reid Stiff Stalk vs. Lancaster) was established early on and inbred lines such as B73 and Mo17 from these two heterotic groups were chosen as testers for the selection of new maize inbreds. The use of representative testers allows the placement of a new inbred into the appropriate heterotic group using only a small number of field crosses (Xia *et al.*, 2005).

In the 1980s, DNA-based molecular markers were identified as having the potential to enhance maize breeding. Research has demonstrated the advantage of using molecular markers for selection of simply inherited traits, however only a few studies have evaluated the potentials to enhance genetic gain for quantitative traits (Sam *et al.*, 2007). Molecular genetic markers are powerful tools to delimit heterotic groups and to assign inbred lines into existing heterotic groups (Melchinger, 1999). The SSR markers offer advantages in reliability, reproducibility, discrimination, standardization, and cost effectiveness over other marker types (Smith *et al.*, 1997). The SSR markers show potential for large-scale DNA fingerprinting of maize genotypes due to the high level of polymorphism detected (Reif *et al*., 2003), their analyses by automated systems (Sharon *et al.*, 1997) and their high accuracy and repeatability (Heckenberger *et al*., 2002).

The objectives of this study were to (i) select lines from two tropical maize populations using genetic distance (GD) identified by 50 SSR markers for F_1 hybrids and (ii) study the relationship between GD and yield and GD and mid-parent heterosis (MPH), determined by SSR markers.

Materials and Methods

Genetic Materials

The Yunnan (YNP) and Guangxi (GXP) populations were used to initiate materials in this study. The YNP included germplasm 25% equally originated from Thailand, India, Southern China, and Vietnam with flint grain type. The GXP included germplasm 20% equally originated from Thailand, Northern China, Brazil, Turkey and USA, with dent grain type. These populations were formed during April 2005-February 2006 at Jinghong, Yunnan, China. Both populations were subjected to mass selection for 2 cycles of selection in each of which cycle of mass selection at least 1,000 ears

were used to form the population of the next cycle.

One hundred fifty plants were selected from both the YNP and GXP with the total of 300 plants. Leaf was cut from each plant before flowering for DNA extraction for molecular study, during flowering each plant was selfed for advanced S_1 lines, finally there were 300 S_1 lines for remnant seeds. S_1 lines were selected only based on GD.

Molecular Marker Genotyping

The DNA of the 300 S_0 plants and the 20 S_1 lines used for the factorial crosses was extracted from freeze-dried leaf tissue with the Fast Prep (10 lines from each population) System (Q-biogene, Carlsbad, CA). The DNAs were analyzed individually with 50 public SSR markers distributed over the whole genome, according to their position on the IBM2 Neighbors map available on MaizeGDB (Lawrence *et al*.,2008). PCR reactions consisted of 8.125 ul ddH_20, 1 ul DNA solution (10 ng/ul), 5 ul forward and reverse primer (10 umol/l),

1 ul dNTP, 0.125 ul *TakaRa Taq* (5 U/ul) (Takara Biotechnology Dalian Co.,Ltd., Dalian, Liaoning, China), 5 ul of 10 × PCR Buffer (Mg^{2+}plus), with a total reaction volume of 12.5 ul. The PCR reactions were performed in thin-walled 96-well microtiter plates (Diamed Inc., Mississauga, ON), topped with an equal volume of mineral oil (Sigma-Aldrich Canada Ltd., Oakville, ON) and covered with adhesive film (Diamed Inc., Mississauga, ON). The thermal cycling was conducted with a Robocycler 96-well temperature cycle (Stratagene, La Jolla, CA). The cycling profile included 8 minutes at 94°C, followed by 30 sec at 94°C and 55°C, and by 40 sec at 72°C. In the following 10 cycles, the annealing temperature was gradually decreased from 65°C to 55°C. After another 10 minutes at 94°C, the following steps were repeated 40 times: 30 sec at 94°C, 30 sec at 55°C and 40 sec at 72°C. Finally, the samples were cooled to 10°C. The PCR products were separated by electrophoresis using 5% (w/v) Metaphor agarose gels (BioWhitaker Molecular Applications, Rockland, ME) in a TBE buffer at 115V. The fragment sizes and the allelic pattern were manually recorded. Nei's genetic distance (GD) (Nei, 1972) was calculated between the sub-populations GXP_0 and YNP_0 as well as between GXP_1 and YNP_1 for both the S_0 and the S_1 plants with the popgene32 software (Yeh *et al*., 1999), according to the formula GD = -ln $((r^{-1} * \Sigma^{r}_{j}\Sigma^{mj}_{i} x_{ij}y_{ij}) / (r^{-2} * \Sigma^{r}_{j}\Sigma^{mj}_{i}\ x_{ij}^{2} * \Sigma^{r}_{j}\Sigma^{mj}_{i}\ y_{ij}^{2}))$ where x_{ij} and y_{ij} are the frequencies of the i^{th} allele at the j^{th} locus, m_j is the number of alleles at the j^{th} locus, and r is the number of loci considered.

Based on the Jaccard's (1908) similarity coefficient, the genetic distance between pairs of S_1 lines from the GXP and YNP were calculated as $(1 - v_{ij} * (v_{ij} + w_{ij} + x_{ij})^{-1})^{0.5}$, where v_{ij} corresponds to the number of bands in common between the two lines considered, w_{ij} is the number of bands present in the i^{th} line and absent in the j^{th} line, and x_{ij} is the number of bands absent in the i^{th} line and present in the j^{th} line.

Factorial Crosses and Hybrid Evaluation

The S_1 seeds of the 10 self-pollinated plants were selected from the GXP and YNP based on their genetic distance. Initially, parents' factorial crosses of 20 S_1 (10 × 10), were planted in a pair of 2 rows per cross with 3.0 m in length and a spacing of 0.75 m between rows and 0.25 m between plants during April-August 2006. The hybrids yield trial consisted of 100 hybrids with CP619, a tropical maize single cross hybrid of CP Company used as a check hybrid. This hybrid is popular among farmers in Guangxi and Yunnan provinces. The experiment used a randomized complete block (RCB) design with 2 replications at Jinghong, Yunnan, China. The experimental units were 2-row plots, 5.0 m in length, with spacing between rows of 0.75 m and between plants of 0.25 m, during September 2006-February 2007.

S_1 Lines Yield Trial

Twenty S_1 were planted separately from the hybrid evaluation with an RCB design, with 2 replications at Jinghong, Yunnan, China. The experimental units were 2-row plots, 3.0 m

in length with a spacing between rows of 0.75 m and between plants of 0.25 m, during September 2006-February 2007.

Statistical Analysis

Both S_1 lines and hybrid trials were analyzed as an RCB design by the SAS program (SAS, 1997) according to the following model:

$$Y_{ij} = \mu + T_i + \beta_j + Eij$$

where, Y_{ij} was the yield of genotype, μ was the grand mean, T_i was the (additive) effect of the i^{th} treatment (i = 1, 2, … , v), β_j was the (additive) effect of the j^{th} block (j = 1, 2, … , b), and *Eij* was the random error for the i^{th} treatment in the β^{th} block.

Mid-Parent Heterosis (MPH)

Mid-parent heterosis was calculated as following;

$$MPH = ((F_1\text{-}M_P)/ M_P)^*100,$$

where F_1 was the mean of the F_1 hybrid performance and $M_P = (P_1 + P_2)/2$ in which P_1 and P_2 were the means of the inbred parents, respectively.

Results and Discussions

Genetic Distance

The 50 SSR markers revealed a total of 153 alleles, with 3.06 alleles per locus on average. The genetic distance (GD) between the 2 S_0 sub-populations of the YNP_0 and GXP_0 showed a low difference value of 0.03 (Table 1). Compared with the results of other studies, these values were relatively low: Liu *et al*. (2005) reported genetic distances between 0.13 and 0.35 comparing the molecular data (70 SSRs) of 44 Chinese open-pollinating varieties (OPVs). Prasanna *et al*. (2005) observed even higher values of genetic distances (0.36 to 0.98) between 17 OPVs from India analyzed with 27 SSRs. The low genetic distances between S_0 sub-populations, according to which the sub-populations are closely related to each other, corresponds well to the fact that the genetic basis of both populations is relatively narrow. Furthermore, the experimental populations were developed in only 2 cycles, allowing for only a limited extent of recombination. However when studied among the YNP_1 and GXP_1, which came from the 10 S_1 lines of YNP_1 and the 10 S_1 lines of GXP_1, the GD was increased to 0.09, which showed the advance of selection for increase in the GD of population (Table 1).

The average GD of 0.44 from Table 2 showed high diversity among the S_1 lines selected. The range was 0.14 to 0.94, which were for the crosses between G2002 × Y1094 and G2038 × Y1137, respectively.

Grain Yield

The analysis of variation (ANOVA) showed highly a significant ($P<0.01$) difference among the grain yields of the F_1 hybrids and S_1 lines (Table 3). Grain yield of the hybrids averaged 8,865 $kgha^{-1}$, which ranged from 6,848 $kgha^{-1}$ to 10,799 $kgha^{-1}$ (Table 4). Grain yield of the S_1 lines averaged 2,590 $kgha^{-1}$, which varied from 2,244 $kgha^{-1}$ to 4,393 $kgha^{-1}$. The highest yielding hybrids were crosses between G2001 × Y1008, with gave a grain yield of 10,799 $kgha^{-1}$.

For the MPH grain yields of the F_1 hybrids, the data showed the average value of 205% and ranged from 107% to 300%, which were for the crosses between G2107 × Y1013 and G2051 × Y1028 and 2051 × Y1080, (Table 5).

Table 1. Total of alleles, allele no. and genetic distance (GD) of 2 tropical maize populations

Populations	Total of alleles	Allele No.	GD
GXP_0 x YNP_0	153	3.06	0.03
GXP_1 x YNP_1	153	3.06	0.09

Relationship Between Genetic Distance and Grain Yield of F_1 Hybrids

The GD was positively correlated with the hybrid grain yield, with the correlation coefficient of 0.21 (p = 0.12) (Figure 1) and correlated with the MPH of 0.07 (p = 0.59) (Figure 2). Correlation of the grain yield and MPH showed a highly positive correlation coefficient value of 0.56 (p = 0.01) (Figure 3); however, when ranked in the order of the top 10 high yield hybrids and low yield hybrids as in Table 6, we observed a highly positive correlation coefficient between GD and yield (r = 0.36; p = 0.28) (Figure 4). It showed that the high yielding F_1 hybrids came from crosses between high GD lines. Our result was similar to that of Betran *et al.* (2003) who used RFLP markers in 17 lowland white tropical maize inbreds which showed the GD was positively correlated with the grain yield, specific combining ability (SCA), MPH, and high parent heterosis (HPH) of F_1 hybrids in the whole environment.

However our research showed low correlationship between the GD and grain yield of the F_1 hybrid and MPH, because there was a difference in the linkage disequilibrium among the markers used and the QTLs between the heterotic groups. This may be related to DNA markers being able to effectively predict hybrid performances. However the difference and number of the markers studied may also reflect the degree of correlation (Reif *et al.,* 2003).

Bernardo (1992) concluded that at least 30% to 50% of the QTLs affected the traits, especially the grain yield of F_1 hybrids. However the number of hybrids evaluated and the first generation of selfing in lines studied may also affect the value of the correlation.

Comparing Grain Yield of F_1 Hybrids and Genetic Distance by SSR

The dendrogram of selected S_1 lines in Figure 5 can be classified into 4 groups: Group 1 including lines Y1002, Y1028, Y1080, G2001 and G2149; Group 2, lines Y1083, Y1094, G2002, G2038 and G2039; Group 3, lines

Table 2. Genetic distances between S_1 lines of 10 YNP (Y) and 10 GXP (G)

Lines	Y1002	Y1008	Y1013	Y1028	Y1064	Y1080	Y1083	Y1094	Y1105	Y1137	*mean*
G2001	0.36	0.43	0.43	0.25	0.30	0.19	0.19	0.36	0.30	0.50	*0.33*
G2002	0.50	0.43	0.57	0.50	0.43	0.57	0.19	*0.14*	0.43	0.83	*0.46*
G2028	0.36	0.43	0.57	0.50	0.30	0.74	0.57	0.50	0.43	0.83	*0.52*
G2033	0.50	0.43	0.43	0.50	0.57	0.57	0.57	0.36	0.30	0.50	*0.47*
G2038	0.43	0.36	0.50	0.43	0.25	0.50	0.36	0.19	0.36	*0.94*	*0.43*
G2039	0.50	0.57	0.43	0.50	0.43	0.30	0.30	0.36	0.43	0.65	*0.45*
G2051	0.57	0.50	0.36	0.74	0.36	0.36	0.50	0.43	0.25	0.30	*0.44*
G2068	0.50	0.30	0.43	0.36	0.19	0.43	0.57	0.50	0.43	0.65	*0.44*
G2107	0.50	0.43	0.43	0.50	0.30	0.43	0.57	0.50	0.43	0.50	*0.46*
G2149	0.50	0.57	0.30	0.36	0.57	0.19	0.43	0.65	0.43	0.50	*0.45*
mean	*0.47*	*0.44*	*0.44*	*0.46*	*0.34*	*0.43*	*0.43*	*0.40*	*0.38*	*0.62*	*0.44*

Table 3. ANOVA grain yield of F_1 hybrids and S_1 lines from 2 tropical maize populations

Source	DF	MSE	%CV	LSD 0.01
F_1 hybrid (GXP x YNP)	99	1,774,709.80**	7.96	1,170
S_1 Lines	19	646,654.41**	9.70	495

** = significant difference at 99%

Y1013, Y1137, Y1105, G2033 and G2051; and Group 4, lines Y1008, Y1064, G2068, G2107 and G2028. Data from Table 6 showed that the F_1 hybrids having a high grain yield mostly were from crosses between Group1 and Group 4. The F_1 hybrids were from G2001 × Y1008 and G2068 × Y1002, with the grain yield of 10,799 kgha^{-1} and 10,721 kgha^{-1}, respectively, and the GD values of 0.43 and 0.50, respectively, and the MPH values of 270% and 264%, respectively. These hybrids showed higher yields as compared with the check, CP619, of 107% and 106%, respectively (Table 6).

However we had observed that crosses within Group 4 showed high yields such as cross G2107 × Y1008 which showed a high yield of 10,601 kgha^{-1}, because this cross had a high GD value of 0.43. It was clear that when the crosses had a low GD, the F_1 hybrids showed a low grain yield. The F_1 hybrids crosses of G2038 × Y1064 showed a grain yield of 6,866 kgha^{-1}, with the GD values of 0.25 and the relative percentage

Table 4. Mean grain yield (kgha^{-1}) of 100 F_1 hybrids of crosses between YNP (Y) and GXP (G) and grain yield of 20 S_1 lines p*er se*

Lines	Y1002	Y1008	Y1013	Y1028	Y1064	Y1080	Y1083	Y1094	Y1105	Y1137	*mean*
G2001	9530	10799	8794	8777	7178	7745	8880	8921	8942	9351	2543
G2002	9131	9671	7796	9813	7564	9573	8772	8689	9313	9116	3171
G2028	8567	9198	8046	9505	7870	10146	8738	9526	8385	9524	3142
G2033	8623	8349	8209	8408	7555	10099	8386	9751	8954	8070	3951
G2038	8199	8960	8541	9442	6866	8674	7960	8646	8522	8999	2346
G2039	9591	9866	7738	8287	7945	8835	8877	8303	8567	8525	3519
G2051	8547	9582	8518	9895	7756	9872	7828	8037	8918	9113	2244
G2068	10721	10686	8030	10516	9005	10678	7768	9631	10163	10138	3071
G2107	9956	10601	7826	9921	7574	10130	7448	9455	9000	9190	4393
G2149	8966	10556	7904	9268	6848	8387	8348	8503	8206	7408	2417
Per se	2816	3296	3186	2707	2763	2693	2599	3380	2246	2491	2590

Mean grain yield of F_1 hybrids =8,865 kgha^{-1}

Table 5. Percentage of mid-parent heterosis (MPH) of 100 F_1 hybrids

Lines	Y1002	Y1008	Y1013	Y1028	Y1064	Y1080	Y1083	Y1094	Y1105	Y1137	*mean*
G2001	256	270	207	234	171	196	245	201	273	272	*233*
G2002	205	199	147	234	155	227	204	165	244	222	*200*
G2028	188	186	154	225	167	248	204	192	211	238	*201*
G2033	155	130	130	153	125	204	156	166	189	151	*156*
G2038	218	218	209	274	169	244	222	202	271	272	*230*
G2039	203	190	131	166	153	184	190	141	197	184	*174*
G2051	238	246	214	300	210	300	223	186	297	285	*250*
G2068	264	236	157	264	209	271	174	199	282	265	*232*
G2107	176	176	107	179	112	186	113	143	171	167	*153*
G2149	243	270	182	262	164	228	233	193	252	202	*223*
mean	*215*	*212*	*164*	*229*	*164*	*229*	*196*	*179*	*239*	*226*	*205*

to the check variety of only 68% (Table 6).

However we had observed some hybrids which showed highest GD (G2038 x Y1137: GD = 0.94) and lowest GD (G2002 x Y1094: GD = 0.14), that showed no difference in grain yields (8,999 $kgha^{-1}$ and 8,689 $kgha^{-1}$, respectively). It could be the problem of non adaptive lines. Samphantarak (2003) had reported that the 2 main factors affected high grain yield of hybrids were high GD and adaptability of both parental lines.

Conclusions

The GD showed a high difference among S_1 lines, which were selected from 2 populations by using SSR markers, and especially when the high grain yield of F_1 mostly came from crosses between high GD. It showed the potential of the population for long term improvement. As the selection cycle is further advanced in the future these populations will be an additional new heterotic pattern for maize breeding resources.

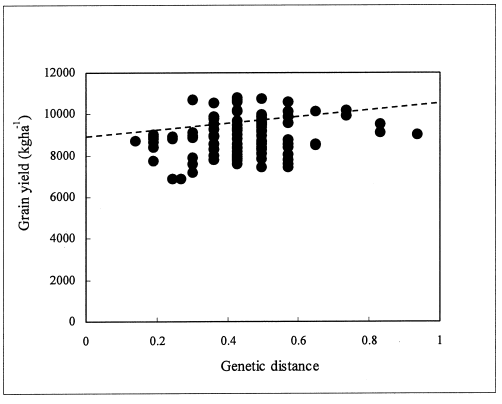

Figure 1. Relationship between genetic distance (GD) and grain yield ($kgha^{-1}$) of F_1 hybrids (r = 0.21, p = 0.12)

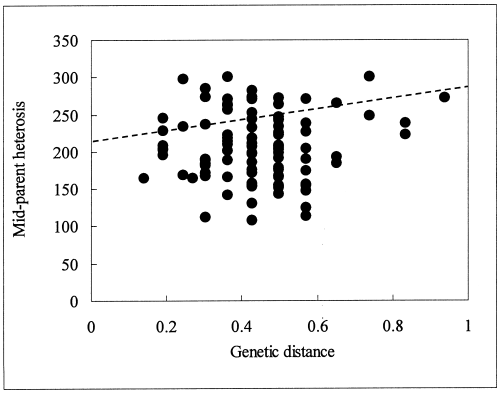

Figure 2. Relationship between genetic distance (GD) and mid-par ent heterosis (MPH) (r = 0.07, p = 0.59)

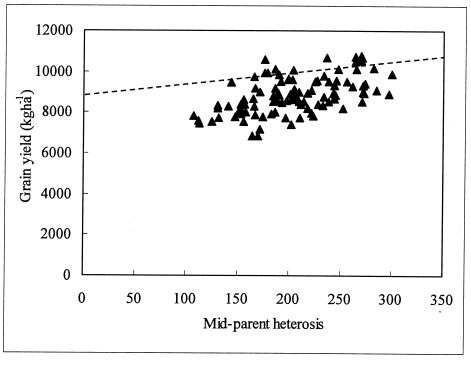

Figure 3. Relationship between grain yield ($kgha^{-1}$) of F_1 hybrids and percentage of mid-parent heterosis (MPH) (r = 0.56, p = 0.01)

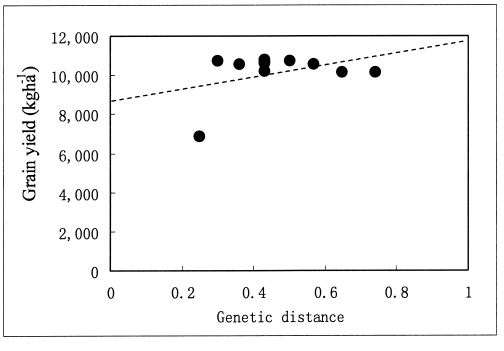

Figure 4. Relationship between genetic distance (GD) and grain yield ($kgha^{-1}$) F_1 hybrids of top10 high yielding hybrids and low yield hybrid (r = 0.36, p = 0.28)

The GD based on the SSR marker data as classified showed a positive correlation to yield of the F_1 hybrids and MPH, so that SSR markers could be a useful tool to increase the effectiveness in selection of new high yielding hybrids in the future as proved by the results of this study.

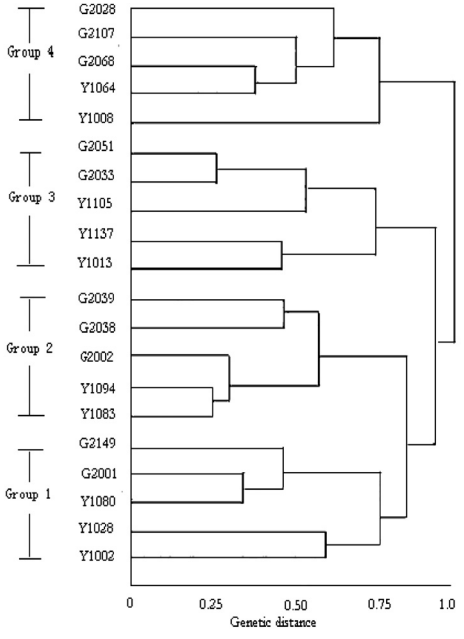

Figure 5. Dendrogram of 20 S_1 lines based on genetic distance (GD) identified by SSR

Table 6. Mean grain yield (kgha^{-1}) of top 10 high yielding and low yield, percentage relative to check variety, genetic diversity (GD) and percentage of mid-parent heterosis (MPH)

Crosses	Yield (kgha^{-1})	Relative to check (%)	GD	MPH (%)
G2001 x Y1008	10,799	107	0.43	270
G2068 x Y1002	10,721	106	0.50	264
G2068 x Y1008	10,686	106	0.30	236
G2068 x Y1080	10,678	106	0.43	271
G2107 x Y1008	10,601	105	0.43	176
G2149 x Y1008	10,556	104	0.57	270
G2068 x Y1028	10,516	104	0.36	264
G2068 x Y1105	10,163	101	0.43	282
G2028 x Y1080	10,146	100	0.74	248
G2068 x Y1137	10,138	100	0.65	265
G2038 x Y1064	6,866	68	0.25	169
Check CP619	10,109	100	-	-

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