Kasetsart J. (Nat. Sci.) 44 : 70 - 78 (2010)

Early Hybrid Testing in Tropical Maize: Are Molecular Markers Useful for Selecting the Parental Component?

Pongsai Chuanchai[1]*, Tan Xuelin[2], Anek Silapapun[3], Pradit Suthipong[4], Luan Wei[2] and Rainer Messmer[5]

ABSTRACT

The combination of parental lines determines hybrid performance. Two methods for choosing parental components in early-generation hybrid testing were compared in the present study. The S_1 lines of two tropical maize populations from Yunnan and Guangxi were selected either based on the yield performance of reciprocal half-sib progeny (conventional method) or the maximization of the genetic distance (GD) between S_1 lines, calculated from the allelic information of fifty SSR markers. The GD between the two original populations was low, probably because of the narrow genetic base and only two generations of development. However, the weak positive correlations between the grain yield of F_1 hybrids and the GD as well as the specific combining ability (SCA), indicated that selecting the parents of testcrosses based on their genetic distance could help identify optimal genotype combinations. High-yielding F_1 hybrids could be undoubtedly produced by conventionally selecting the parents and without costly field testing. As some crosses of S_1 lines resulted in high-yielding progeny, the populations from Yunnan and Guangxi could provide additional new heterotic patterns for tropical maize breeding.

Key words: maize**,** heterosis, specific combining ability, simple sequence repeats (SSR), genetic distance

INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal crop of the world in terms of grain yield (FAOSTAT, 2008). In China, maize is grown on about 25.4 Gha and produces about 130 Gt yield, with an average yield of 5.12 t ha^{-1} (Zhang, 2005). The majority of global (Chang, 2005) as well as Chinese (Zhang, 2005) maize yields are produced with single cross hybrids. They have a higher yield potential in input-intensive agriculture than other hybrid types and open-pollinated varieties (Lee *et al*., 2005). The hybrid parents are selected usually based on their pedigree and the performance of test-cross progenies. However, conducting and evaluating these testcrosses are time-consuming and expensive. Therefore, more conveniently molecular marker-based methods to pre-select suitable parental components are highly desirable. Melchinger (1999) described the utility of molecular markers to delimit heterotic groups and to assign inbred lines into existing heterotic groups. Betrán *et al*. (2003) estimated the genetic diversity for restriction fragment length

1 Faculty of Agriculture and Biotechnology, Yunnan Agricultural University, Kunming 650201, China.
2 Rice Research Institute, Yunnan Agricultural University, Kunming 650201, China.
3 Bangkok Seed Industry, Soi Yenchit, Chand Road, Bangkok 10120, Thailand.
4 Xiang Fan Chia Tai Agriculture Development, Chengdu 610000, China.
5 ETH Zurich, Institute of Plant Sciences, Universitaetstrasse 2, 8092 Zurich, Switzerland.
* Corresponding author, e-mail: chuanchaip@yahoo.com

Received date : 12/05/09 Accepted date : 07/09/09

polymorphisms (RFLPs) within a set of tropical maize lines and classified the lines according to their genetic distance (GD). The results indicated that DNA markers represent a suitable tool to classify tropical maize inbreds developed from genetically diverse germplasm groups (Betrán *et al*., 2003). Moreover, a positive correlation between GD and hybrid performance was observed. However, this correlation largely depended on a few particular hybrids, and was considerably lower for other hybrids. It had previously been reported for temperate maize that GD correlated with hybrid performance for relatively closely related parental components (from the same heterotic group), but with less correlation for more distant material (different heterotic groups). Reif *et al*. (2003a) showed that panmictic mean parent heterosis (PMPH) increased with increasing genetic distance (among the 20 gene pools and populations of tropical, subtropical and temperate origins studied), but that adaptation problems could disturb this association. These authors also concluded that molecular markers were useful to complement field trials in order to identify heterotic groups and to introgress exotic germplasm systematically (Reif *et al*. 2003b).

Given this background, the present study aimed to test the usefulness of molecular marker (SSR) data for selecting parental components with favorable combining abilities. The grain yield of F_1-progeny resulting from crosses between two Chinese breeding populations of tropical maize was compared with the genetic distance between the respective individuals in order to test whether the yield of hybrids was positively related with genetic dissimilarity of the parental plants.

MATERIALS AND METHODS

Plant material and pre-testing

Two populations of tropical maize, Yunnan population (YNP) and Guangxi population (GXP), were developed by crossing a broad set of inbred lines. The lines used to develop the YNP flint population originated from Thailand, India, Southern China, and Vietnam, whereas the lines used to develop the GXP dent population originated from Thailand, Northern China, Brazil, Turkey and the USA. All the experiments were conducted at Jinghong, Yunnan, China, on a sandy clay loam soil (pH 6.0), applying 275, 68 and 68 kg ha^{-1} of N, P and K, respectively, according to local practices. The fields were furrow-irrigated every ten days.

For both populations, twenty inbred lines were grown at Jinghong, Yunnan, China, in 2004 (April-August) in one-row plots, 3 m long and 0.75 m apart, at a plant density of 5.3 m^{-2}. They were pollinated with a mixture of pollen collected on pollen donor plants of the same inbred lines grown in adjacent plots. At physiological maturity, the ears of all families were harvested and their seeds were bulked.

The bulked seeds were then used to grow more than 3,500 plants of both populations on the same site in 2004/05 (September-February); the populations were spatially separated to avoid contamination during natural (open) pollination. In both populations, the grains of more than 1,000 ears were harvested and bulked.

In 2005 (April-August), for both populations, again more than 3,500 plants were grown in the field. A random selection of 200 plants (S_0) from each population were used as pollen donors with the objective to compare the yield potential of progeny resulting from testcrosses between S_0 plants of YNP and GXP. Leaf tissues were harvested from all 400 S_0 plants for DNA extraction. The 400 S_0 plants were arbitrarily divided into four groups of 100 plants each (GXP_C, GXP_M, YNP_C, YNP_M, where C is the conventional selection method and M is the molecular marker selection method). The GXP_M and YNP_M plants were self-pollinated to produce S_1 seeds. The genetic distances between individual

plants of GXP_C and YNP_C as well as between plants of GXP_M and YNP_M were calculated and the 2*10 pairs of plants with the largest genetic distances were selected for factorial cross evaluation. All GXP_C and YNP_C plants were also self-pollinated. Additionally, pollen from each of the 100 GXP_C plants was used to pollinate five female plants in the YNP source population and *vice versa*. This procedure resulted in two reciprocal sets of 100 half-sib hybrid progeny. Half of the hybrid progeny were grown on the same site and under the same management practices as mentioned above in 2005/06 (September to February) in a randomized complete block (RCB) experiment with two-row plots, 5 m long and 0.75 m apart, at a plant density of 5.3 m^{-2}. The ears were harvested at physiological maturity, shelled and the grain humidity was measured with a Dole moisture tester. The grain weight per plot was adjusted for shelling percentage and for grain humidity (to 15%). The pollen donors of the ten highest-yielding hybrids were identified in both GXP_C and YNP_C.

Factorial crosses and hybrid evaluation

The S_1 seeds of the ten self-pollinated plants, selected from GXP_M, YNP_M, GXP_C and YNP_C, either based on their genetic distance or the estimate of general combining ability, were grown on the same site and under the same field management practices as described above in 2006 (April-August). The lines to be crossed were grown in adjacent plots (3 m long, 0.75 m apart). Leaf tissue of each S_1 family was harvested for DNA extraction. Factorial crosses were made between the plants from GXP_M and YNP_M, as well as between plants from GXP_C and YNP_C. The female plants were de-tasseled before flowering and then were pollinated with a mixture of pollen from the male plants of the given family. This procedure resulted in two sets of 100 F_1 crosses between lines of GYP and YNP.

These 200 F_1 crosses were grown in 2006/07 (September to February) by the CP Company at Jinghong, Yunnan together with the check hybrid CP619, a popular tropical maize single cross hybrid. An RCB experiment was used with two-row plots and two replications according to the same field management practices as described above. The plants were harvested at physiological maturity. The grain yield (kg ha^{-1}) was determined by adjusting grain weight per plot for the shelling percentage, the grain humidity (see above) and the area harvested. The entry-mean data were analyzed in SAS (The SAS Institute, Cary, NC), according to the model in Equation 1:

$$Y_{ij} = \mu + T_i + fl_j + E_{ij}, \quad (1)$$

where Y_{ij} is the yield of genotype, μ is the grand mean, T_i is the (additive) effect of the i^{th} replication, fl_j is the (additive) effect of the j^{th} block and E_{ij} is the random error for each plot the i^{th} replication and the j^{th} block.

Specific combining ability (SCA) between parental components was calculated with the Agrobase program (Agronomix Software, Inc., Winnipeg, Canada).

Molecular marker genotyping and genetic distance

The DNAs of the 200 S_0 plants and the 40 S_1 lines used for the factorial crosses were extracted from freeze-dried leaf tissues with the Fast Prep 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 µl ddH_20, 1 µl DNA solution (10 ng/ul), 5 µl forward and reverse primer (10 µmol/l), 1 µl dNTP, 0.125 µl Taq E (5 ū/µl) (Takara Biotechnology Dalian) 10 × PCR Buffer (Mg^{2+}1.250 µl), with a total reaction volume of 12.5 µl.

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 cycler (Stratagene, La Jolla, CA). The cycling profile included 8 min at 94°C to activate the AmpliTaq Gold polymerase (Applied Biosystems Inc., Foster City, CA), 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 min 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 PCR products were cooled to 10°C. The products were then separated by electrophoresis using 5% (w/v) Metaphor agarose gels (BioWhitaker Molecular Applications, Rockland, ME) in a 13 TBE buffer at 115V. The fragment sizes and the allelic pattern were manually recorded.

Nei's standard genetic dissimilarity (GD) (Nei, 1972) were calculated between the sub-populations GXP_M and YNP_M as well as between GXP_C and YNP_C for both the S_0 and the S_1 plants with the Pop32 software (Yeh *et al*., 1999), according to the formula in Equation 2:

$$GD = -\ln((r^{-1} * \Sigma^{r}_{j}\Sigma_{i}^{mj} x_{ij}y_{ij}) / (r^{-2} * \Sigma^{r}_{j}\Sigma_{i}^{mj} x_{ij}^{2} * \Sigma^{r}_{j}\Sigma_{i}^{mj} y_{ij}^{2})) \quad (2)$$

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 (1908) similarity coefficient, the genetic distances between pairs of S_1 lines from GXP_M and YNP_M as well as from GXP_C and YNP_C were calculated using Equation 3:

$$GD = (1 - v_{ij} * (v_{ij} + w_{ij} + x_{ij})^{-1})^{0.5} \quad (3)$$

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.

RESULTS AND DISCUSSION

Genetic distances

The 50 SSR markers revealed 153 alleles, with an average of 3.06 alleles per locus. The genetic distance (GD) between the two S_0 sub-populations YNP_M and GXP_M was 0.029 and the GD between the S_0 sub-populations YNP_C and GXP_C was 0.022. Therefore, the two methods to select S_0 plants did not affect the genetic distance between sub-populations. When Comparing the GDs to 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 two cycles, allowing for only a limited extent of recombination.

The genetic distances between the groups of S_1 lines selected from GXP_M and YNP_M and between the groups of S_1 lines selected from GXP_C and YNP_C were 0.089 and 0.077, respectively. These values are somewhat higher than the GDs between the S_0 sub-populations, which might be a consequence of one generation of inbreeding. However, it is likely that the different sample sizes of S_0 and S_1 plants also influenced the GDs.

The average GD (0.444) among individual S_1 lines of both GXP_M and YNP_M (which were chosen for the factorial crosses because of high genetic distances), was higher than

the average GD (0.367) among individual S_1 lines of both GXP_C and YNP_C, which were randomly selected (Tables 1 and 2). The GDs between pairs of S_1 lines ranged from 0.140 to 0.938 in the case of GXP_M versus YNP_M, whereas in the case of GXP_C versus YNP_C the range of GDs was smaller, from 0.140 to 0.651. The lower average and maximum GD between plants of GXP_C and YNP_C suggest that selecting parental components based on the grain yield of their cross-progeny does not necessarily maximize the genetic dissimilarity between them.

Grain yield

The average grain yields of the two reciprocal sets of 100 half-sib hybrid progeny (YNP x GXP and GXP x YNP) differed significantly by about the same amount (Table 3). The range in grain yield per family from the two reciprocal sets showed different levels of significance within each set) (Table 3).

The average grain yield (8,865 kg ha^{-1}) of the factorial crosses between lines of GXP_M and YNP_M did not differ significantly from the average grain yield (8,998 kg ha^{-1}) of the factorial

Table 1 Genetic distances between 10 GXP_M and 10 YNP_M S_1 lines.

Lines	YNP_M 01	YNP_M 02	YNP_M 03	YNP_M 04	YNP_M 05	YNP_M 06	YNP_M 07	YNP_M 08	YNP_M 09	YNP_M 10	*Mean*
GXP_M 01	0.362	0.427	0.427	0.245	0.302	0.191	0.191	0.362	0.302	0.496	*0.330*
GXP_M 02	0.496	0.427	0.570	0.496	0.427	0.570	0.191	*0.140*	0.427	0.832	*0.458*
GXP_M 03	0.362	0.427	0.570	0.496	0.302	0.737	0.570	0.496	0.427	0.832	*0.522*
GXP_M 04	0.496	0.427	0.427	0.496	0.570	0.570	0.570	0.362	0.302	0.496	*0.472*
GXP_M 05	0.427	0.362	0.496	0.427	0.245	0.496	0.362	0.191	0.362	*0.938*	*0.431*
GXP_M 06	0.496	0.570	0.427	0.496	0.427	0.302	0.302	0.362	0.427	0.650	*0.446*
GXP_M 07	0.570	0.496	0.362	0.737	0.362	0.362	0.496	0.427	0.245	0.302	*0.436*
GXP_M 08	0.496	0.302	0.427	0.362	0.191	0.427	0.570	0.496	0.427	0.650	*0.435*
GXP_M 09	0.496	0.427	0.427	0.496	0.302	0.427	0.570	0.496	0.427	0.496	*0.456*
GXP_M 10	0.496	0.570	0.302	0.362	0.570	0.191	0.427	0.650	0.427	0.496	*0.449*
Mean	0.470	0.444	0.444	0.461	0.370	0.427	0.425	0.398	0.377	0.619	0.444

Table 2 Genetic distances between 10 GXP_C and 10 YNP_C S_1 lines.

Lines	YNP_C 01	YNP_C 02	YNP_C 03	YNP_C 04	YNP_C 05	YNP_C 06	YNP_C 07	YNP_C 08	YNP_C 09	YNP_C 10	*Mean*
GXP_C 01	0.245	0.462	0.427	0.427	0.362	0.427	0.427	0.427	0.427	*0.651*	*0.428*
GXP_C 02	0.362	0.245	0.302	0.302	0.362	0.302	0.302	0.191	0.302	0.496	*0.317*
GXP_C 03	0.302	0.391	0.245	0.245	0.302	0.362	0.362	0.345	0.245	0.570	*0.337*
GXP_C 04	0.245	0.245	0.427	0.302	*0.140*	0.491	0.427	0.302	0.427	0.362	*0.337*
GXP_C 05	0.302	0.302	0.496	0.392	0.291	0.245	0.496	0.362	0.496	0.427	*0.381*
GXP_C 06	0.245	0.362	0.570	0.427	0.245	0.402	0.570	0.427	0.427	0.362	*0.404*
GXP_C 07	0.362	0.402	0.570	0.427	0.245	0.402	0.570	0.427	0.570	0.496	*0.447*
GXP_C 08	0.362	0.245	0.427	0.302	0.362	0.427	0.427	0.302	0.427	0.350	*0.363*
GXP_C 09	0.405	*0.140*	0.302	0.191	0.245	0.302	0.302	0.191	0.302	0.496	*0.287*
GXP_C 10	0.395	0.245	0.570	0.302	0.245	0.291	0.570	0.302	0.427	0.345	*0.369*
Mean	0.322	0.304	0.434	0.332	0.280	0.365	0.445	0.327	0.405	0.455	0.367

crosses between the lines of GXP_C and YNP_C (Table 4). This suggests that the selection of parental components of testcrosses based on molecular marker data, on average, is as effective as the selection of parental components based on the actual grain yield of their cross progeny, within a limited genetic background.

Ten progenies of the factorial crosses yielded more than the commercial check hybrid CP619 (Table 5). Seven of them resulted from crosses between lines that were selected based on their molecular marker data (GXP_M and YNP_M, Table 5). This indicates that selecting parental components of hybrids based on their genetic distance may have positive effects on their specific combining ability.

Relationship between genetic diversity and yield

The GD between parental components was positively correlated with the grain yield of their F_1 hybrids, with similar correlation coefficients for those lines that were chosen at random from the source population and for those that were chosen according to their genetic distance (r = 0.19 and 0.21, respectively; Figure 1). GD and SCA were also positively associated, but again with a low value (0.17; Figure 2). Betr·n

Table 3 ANOVA and average, minimum and maximum values of grain yield (kg ha^{-1}) of 200 reciprocal half-sib progeny tests between YNP and GXP.

Source	DF	EMS	%CV	LSD0.01	YieldMean	Yield Min	YieldMax
GXP x YNP	99	726,007.78**	10.70	1,342	7,557	8,307	9,200
YNP x GXP	99	688,727.67**	12.50	1,691	7,549	8,328	9,158

** = significant at 99%.

Table 4 ANOVA of grain yield (kg ha^{-1}) of F_1 hybrids resulting from two sets of factorial crosses.

Source	EMS	%CV	LSD0.01	Grain yield
Factorial crosses GXP_C – YNP_C	1,205,056.21**	8.80	1,312	8,998
Factorial crosses GXP_M - YNP_M	1,774,709.80**	7.96	1,170	8,865
All factorial crosses	1,445,638.64**	8.47	1,250	8,932

** = significant at 99%.

Table 5 Grain yield (kg ha^{-1}) of the 10 highest-yielding F_1 hybrids resulting from factorial crosses, and of the check hybrid, CP619.

Order	Crosses	Yield(Kgha^{-1})	GD	SCA	%Check
1	GXP_C 07 x YNP_C 06	11,060	0.402	887.08	105
2	GXP_C 07 x YNP_C 02	11,001	0.402	1219.98	105
3	GXP_M 01 x YNP_M 02	10,798	0.427	1011.58	103
4	GXP_M 08 x YNP_M 01	10,720	0.496	735.38	102
5	GXP_M 08 x YNP_M 02	10,686	0.302	56.68	102
6	GXP_M 08 x YNP_M 06	10,678	0.427	461.58	102
7	GXP_C 03 x YNP_C 10	10,643	0.650	869.08	101
8	GXP_M 09 x YNP_M 02	10,600	0.427	595.18	101
9	GXP_M 10 x YNP_M 02	10,555	0.570	1220.88	101
10	GXP_M 08 x YNP_M 04	10,515	0.362	330.28	100
11	CP619	10,490	-	-	100

GD = genetic distance based on Jaccard's dissimilarity coefficient.

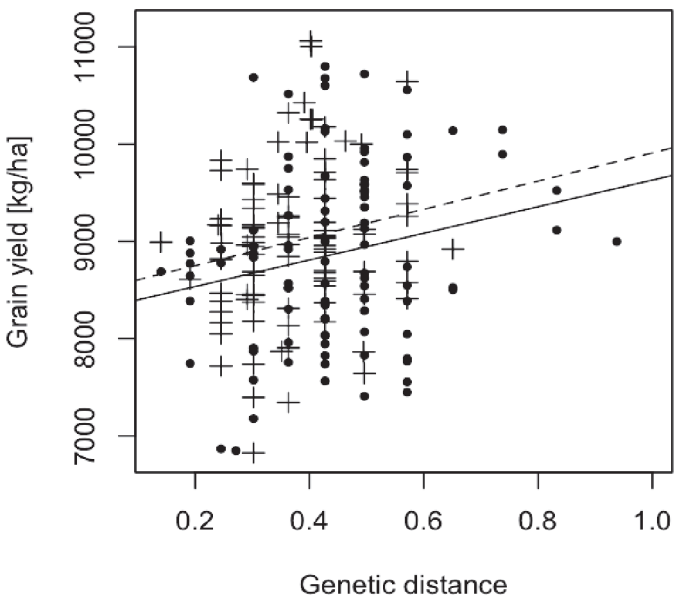

Figure 1 Relationship of the genetic distance (GD) between parental lines and the grain yield of the corresponding F_1 hybrids. The parents were selected either based on molecular marker data (GXP_M and YNP_M, filled circles, straight regression line), maximizing the genetic distance, or based on the grain yield of reciprocal testcrosses (GXP_C and YNP_C, crossed symbols, dashed regression line).

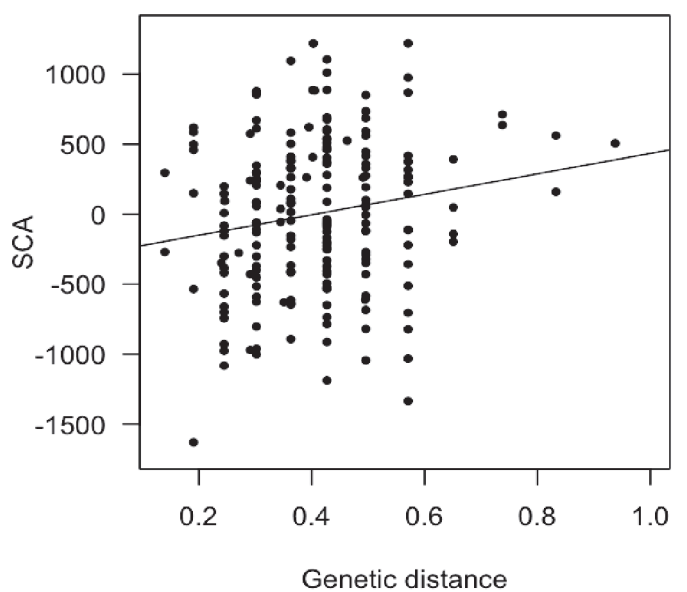

Figure 2 Relationship between genetic distance (GD) and specific combining ability (SCA) calculated for pairs of S_1 lines for which factorial crosses were performed.

et al. (2003), using RFLP markers in 17 tropical maize inbreds, showed that GD was positively correlated with grain yield and with SCA when the diversity was high; their F_1 hybrids mostly came from crosses between lines with high GD. The relationship between GD and F_1 hybrid grain yield and SCA was much lower in the present study, possibly because of the limited genetic diversity in the plant material and because of differences in linkage disequilibrium among markers used. Nevertheless, the results indicated that molecular markers are potentially useful to predict hybrid performance.

CONCLUSION

The results of the present study indicated that selecting parental components based on molecular marker data is advantageous for the performance of the hybrids. The use of molecular markers can reduce the costs associated with selecting suitable hybrid parents, as they offer a possibility to circumvent labor-intensive and time-consuming conventional testcrosses. Therefore, SSR markers could be used for increasing the effectiveness of hybrid breeding. Even though the genetic distance between the initial populations was low, some crosses of S_1 lines produced higher yields than the commercial hybrid variety used as a check. This was evidence of the potential contribution of the two populations, GXP and YNP, to future breeding efforts in tropical maize.

LITERATURE CITED

Betrán, F. J., J. M. Ribaut, D. Beck and D. Gonzalez de Leon 2003. Genetic diversity, specific combining ability and heterosis in tropical maize under stress and non-stress environment. **Crop Sci.** 43: 797-806.

Chang, M. T. 2005. Corn breeding achievement in United States, p.12. *In* **Proceedings of the Ninth Asia Regional Maize Workshop**, September 5-9, 2005, Beijing, China.

FAOSTAT. 2008. Food and agriculture organization of the United Stated nations for a world without hunger. Production cereals and share in world. http://www.fao.org/statistics/yearbook/vol_1_1/pdf

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. **Bull. Soc. Vaud. Sci. Nat.** 44: 223-270.

Lawrence, C. J., L. S. Mary, E. S. Trent, A. C. Darwin and C. H. Lisa. 2008. Maize GDB. http://journal.shouxi.net/html/qikan/jcyxyswyxgc/hsyjyxqk/20071351/wzjh/20080831043152634_351123.html

Lee, E. A., A. Ahmadzadeh and M. Tollenaar. 2005. Quantitative genetic analysis of the physiological processes underlying maize grain yield. **Crop Sci.** 45: 981-987.

Liu, X., M. S. Li, X. H. Li and S. H. Huang. 2005. Genetic diversity of Chinese maize OPVs determined by SSR analysis of bulk sample, p. 31. *In* **Proceedings of the Ninth Asia Regional Maize Workshop**, September 5-9, 2005, Beijing, China.

Melchinger, A. E. 1999. Genetic diversity and heterosis, pp. 99–118. *In* J. G. Coors and S. Pandey. (eds.). **The Genetics and Exploitation of Heterosis in Crops. ASA, CSSA, and SSSA**, Madison, WI.

Nei, M. 1972. Genetic distance between populations. **Amer. Nat.** 106: 283-291.

Prasanna, A., A. Singode, A. Garg, R. Kumar and B. B. Singh. 2005. Molecular characterization of maize genetic resources in India, p. 40. *In* **Proceedings of the Ninth Asia Regional Maize Workshop**, September 5-9, 2005, Beijing, China.

Reif, J. C., A. E. Melchinger, X. C. Xia, M. L Warburton, D. A. Hoisington, S. K. Vasal, G. Srinivasan, M. Bohn and M. Frisch. 2003a. Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. **Crop Sci.** 43: 1275-1282.

Reif, J. C., A. E. Melchinger, X. C. Xia, M. L. Warburton, D. A. Hoisington and S. K. Vasal 2003b. Use of SSRs for establishing heterotic groups in subtropical maize. **Theore. Appl. Genet.** 107(5): 947-957.

Yeh, F. C., R. C. Yang and T. Boyle. 1999. **Popgene program version 32**. A joint project development by university of Alberta and centre for international forestry research, 29 pp.

Zhang, S. H. 2005. Maize production and research in China: Advancement and challenges, p. 3. *In* **Proceedings of the Ninth Asia Regional Maize Workshop**, September 5-9, 2005, Beijing, China.