Euphytica **124:** 307–313, 2002.

Genetic diversity of Tainan-white maize inbred lines and prediction of single cross hybrid performance using RAPD markers

G.J. Shieh[1,2] & F.S. Thseng[1]
[1]*Department of Agronomy, National Chung Hsing University, Taichung, 40227 Taiwan;* [2]*Present Address: Department of Agronomy, Taiwan Agricultural Institute Wufeng, Taichung, 413 Taiwan*

Received 7 June 1999; accepted 16 June 2001

Key words: genetic diversity, heterosis, RAPD, *Zea mays*

Summary

To evaluate the genetic diversity of 13 maize inbred lines, and to determine the correlation between genetic distance and single cross hybrid performance, we employed the randomly amplified polymorphic DNA (RAPD)- a PCR-based technique. Six of these lines came from the Taichung population, and others derived from seven different sites. Forty different primers were used to give a total of 646 reproducible amplification products, 547 (84.7%) of them being polymorphic. Genetic divergence was determined using Jaccard's similarity coefficient, and a final dendrogram was constructed by UPGMA (unweighted pair-group method with arithmetical averages) cluster analysis in the CLUSTER procedure of the SAS system. The RAPD analysis was a useful tool in determining the extent of genetic diversity among Tainan-white maize inbred lines in the present case. Cluster analysis showed that the 13 inbred lines could be classified into distinct heterotic groups. There was no significant linear regression of grain dry weight heterosis value and mean performance of hybrids on genetic distance. And their coefficients of determination (R^2) are small, so that predictive value is limited. The present results showed that the Jaccard's similarity coefficients based on RAPD data cannot be used to precisely predict the F_1 hybrids yield performance and heterosis value.

Introduction

The amount of heterosis expressed in a F_1 hybrid is mainly affected by the genetic diversity (Griffing & Lindstrom, 1954; Moll et al., 1965). Previous studies have shown a positive relationship between genetic distance, as measured by geographical distance, F_1 grain yield and grain yield heterosis in maize (East, 1936; Hayes & Johnson, 1939). Moll et al. (1962) stated that heterosis in maize appeared to increase with genetic divergence of the parents. Many researchers have suggested that the parents with optimal genetic divergence can produce maximum expression of heterosis. But apparently, crosses between extremely divergent parents can result in a situation in which the harmonious functioning of alleles is disrupted. Consequently the physiological functions are not as efficient (Singh et al., 1981; Prasad & Singh, 1986; Gallais, 1987).

The phenomenon of heterosis has been exploited extensively in crop breeding, leading to significant increases in yield. Many maize breeding programmes are based on the development and selection of outstanding hybrids from inbred lines. Developing and selecting inbred lines for *per se* performance is quite easy but consuming time. It is not possible to predict hybrid performance from inbred parent performance because of the high dominance effect of the grain yield trait. In addition hybrid performance evaluation from extensive yield trials is costly and time consuming. Moreover, the large number of possible hybrid products from a relatively small number of inbred parents curtails the evaluation of all hybrids. To increase the breeding efficiency, parental genetic distance determined from assays of inbred lines for molecular markers (isozymes, storage proteins, and restriction fragment length polymorphisms) has been used as a potential tool to predict hybrid vigor of

single crosses. Some researchers have indicated that the genetic distance from RFLP cannot be used for predicting the yield performance and heterosis for F_1 hybrids (Godshalk et al., 1990; Melchinger et al., 1990a, 1992; Boppenmaier et al., 1992; Dhillon et al., 1993; Moser & Lee, 1994). And Chen et al. (1996) showed that there was no significant correlation between the RAPD-based genetic diversity and the performance of hybrids. However, Smith et al. (1990) and Bernardo (1994) believe that the degree of similarity calculated from RFLP data could allow maize breeders to predict the combination of lines that would result in high-yielding, single-cross hybrids. Lanza et al. (1997) and Liu et al. (1997) indicated that RAPD could be used as a tool for determining the extent of genetic diversity among maize inbred lines, for allocating genotypes into distinct heterotic groups.

Our objectives were (1) to investigate the genetic diversity for RAPDs within a set of inbred lines from the Tainan-white maize variety, (2) to assign them to heterotic groups, and (3) to examine the association of RAPD-based genetic distances of these inbred lines and mean performance of their single cross hybrids for grain dry weight.

Materials and methods

Field yield evaluation trial

Green corn 'Tainan-White' is a local variety and a open-pollinated population, which has been widely cultivated in Taiwan. Six S_5-selected inbred lines from the population cultivated at Taichung (A1, A2, A3, A5, A6, A9), and seven from Tainan (B), Yunlin (C),Taoyuan (D), Taitung (E), Changhua (F), Pingtung (G) and Hualien (H) (Shieh & Thseng, 1998) county in Taiwan were used in this study. The inbred lines were crossed following a diallel mating design producing 78 hybrids. 13 inbreds were added for a total of 92 entries. All were evaluated in the fall of 1996. The inbred lines and hybrids were separately planted in neighboring seed beds, using a randomized complete block design with four replications. Inbred lines were planted four rows, and F_1 hybrids were planted one row. One-row plot was 4.5m long with 0.80m between rows and 0.30 m between planted within rows. Fertilizers were applied at rate of 200 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1}. Grain dry weight was recorded at maturity.

DNA extraction

DNA was extracted using a modified version of Doyle et al. (1990). Leaf materials (0.2 g freeze dried leaf of chlorosis seedlings) were ground to fine powder in liquid nitrogen. The powdered leaf tissue was transferred to a breaker, and 5 ml of pre-heated extraction buffer (2% CTAB; 1.4 M NaCl, 0.2% 2-mercaptoethanol, 20 ml EDTA, 100 mM Tris-HCl, pH = 8.0) was added. After 30 minutes at 60 °C, 5 ml of Chloroform-isoamyl alcohol (24: 1 v/v) was added, and the upper clear part of the solution was collected by centrifugation at 7000 × g for 10 minutes at 4 °C. Then, 5 ml of Chloroform-isoamyl alcohol was again added, and the cell debris was removed by another 10 minutes of centrifugation at 4 °C. The DNA was precipitated by the addition of 3.3 ml of isopropanol and recovered by centrifugation for 5 minutes at 10000 × g after incubation in a freezer (–20 °C) for 3 hours. The pellet was dried and re-dissolved in 2 °Cml TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH = 7.4), 0.2 ml 2 M NaCl, and 5 ml alcohol (95%) 1 hour at –20 °C. Then, the precipitate was collected by centrifugation at 10000 × g for 5 minutes at 4 °C. The pellet was re-dissolved in 5 ml alcohol (95%) and re-collected by centrifugation at 12000 × g for 5 minutes at 4 °C. The final pellet was dried and dissolved in 0.5 ml TE buffer. The DNA concentration was determined using a fluorometer and following the procedures supplied by the manufacturer. The extracted DNA was stored at 4 °C in a cooler.

DNA amplification

40 random primers (Operon Technologies Inc. USA) were used in single-primed PCR reactions to generate polymorphisms. A 25 μl reaction was set ut as follows: 0.2 mM dNTPs, 0.2 μM, 3 ng/μgl Genomic DNA and 0.04U/μ 1 template DNA in 1X buffer. The reaction was overlaid with mineral oil and the reaction mix heated to 94 °C for 5 mins to denature the template DNA. The reaction mix was then held at 72 °C while the DNA polymerase (Dynazyme) was added to the reaction mix. Amplification proceeded on a DNA The rmalcycler (Perkin-Elmer) for 43 cycles of 1 min at 94 °C, 2 mins at 42 °C and 3mins at 72 °C and a final stage of 10 mins at 72 °C. The total volume of the reaction was loaded onto 1.5% agarose gel containing 0.3μg ml^{-1} ethidium bromide in 0.5X TBE buffer (pH = 8.3, with 45 mM Tris-base, 45 mM boric acid and 1 mM EDTA). The samples were separated

by electrophoresis at 110V for 3.5 hours, then the gels were photographed under UV.

Data analyses

Reproducible DNA bands were scored for presence (1) or absence (0) of amplification products. Pair-wise comparisons of sample were used to estimate Jaccard similarity coefficients (GS) = a/(a+b+c), where a = band number of positive coincidences in OTUi (operational taxonomic unit) and OTUj ($1 \neq j$, i, j were inbred lines cords), b = band number of positive incidence only in OTUi, c = band number of positive incidence only in OTUj (Jaccard, 1908). Genetic distances (GD) between pairs of lines were estimated as GD = 1-GS. The Genetic distances were used to construct a dendrogram by UPGMA (unweighted pair-group method with arithmetical averages) cluster analysis in the CLUSTER procedure of SAS (SAS Institute, 1993). Then calculated mid-parent heterosis = $(\bar{F}_1 - (\bar{p}_1 + \bar{p}_2)/2)/(\bar{p}_1 + \bar{p}_2)/2 \times 100\%$. A linear regression of grain dry weight heterosis value and mean performance of hybrids on genetic distance was performed, evaluating the coefficients of determination (R^2).

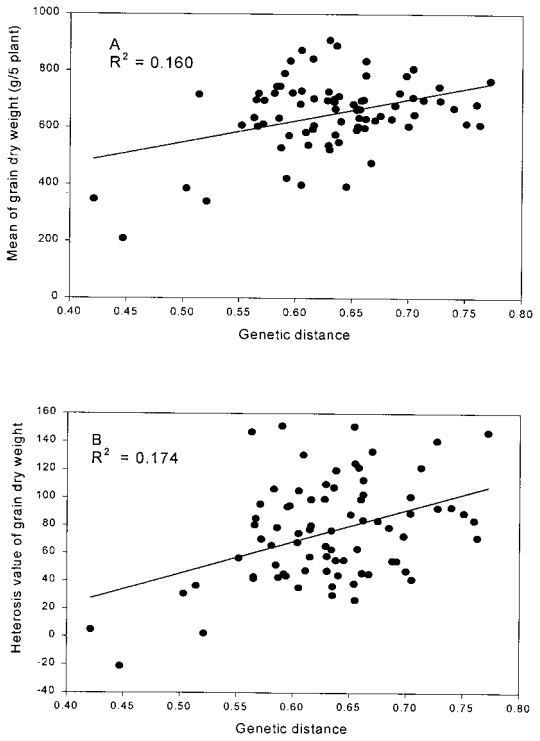

Figure 1. Relation between mean (A), heterosis value (B) of grain dry weight and genetic distance for F_1.

Results and discussion

The grain dry weights were quite different in inbred lines, which were derived from different populations of Tainan-white maize variety. The mean value was from 160(A2) to 521(A6) g/5 plant in Taichung population, and that of the all inbred lines were from 160 (A2) to 567 (E) g/5 plant in eight different populations (Table 1). The F_1 hybrids performance varied from 290 g/5plant (A × A6) to 909 g/5plant (E × A9), and difference was about 3.5 fold. The mid-parent heterosis varied from –21.2% (A1 × A6) to 151.2% (H × A3), and the difference was about 8 fold (Table 1). These data indicated that the 13 inbred lines expressed higher genetic diversity, and were sufficient to present the genetic structures of grain dry weight in Tainan-white maize variety.

Forty 10-mer primers were used for detecting polymorphism in the 13 maize inbred lines. Among the primers used for DNA amplification, 1 had no producer. The remaining 39 primers produced a total of 646 reproducible bands which 547 (84.7%) were polymorphic. The number of RAPD polymorphic band produced by each primer varied from 4 for OPB16 (operon primer) and OPB19 to as many as 29 for OPB-20. Two primers produced 1–5 reproducible polymorphic bands, eight primers produced 6–10 reproducible polymorphic bands, fourteen primers produced 11–15 polymorphic bands, eight primers produced 16–20 polymorphic bands, five primers produced 21–25 polymorphic reproducible polymorphic bands, and one primer produced 26–30 reproducible polymorphic bands.

The RAPD-based Jaccard's similarity coefficient analysis showed genetic distances between pair of inbred lines ranging from 0.421 to 0.772 (Table 2). Inbred lines A3 and A6 presented thesmaller genetic distance. The next closet pair was A6 and A1, which were derived from Taichung population. Inbred line A2 (Taichung population) and H (Hualien) produced the highest genetic distance. The data showed that these inbred lines have larger genetic variation in Tainan-white maize variety.

From the distribution of RAPD-genetic distance and mean of grain dry weight (Figure 1A), the linear regression produced small R^2 (R^2 = 0.16). For the distribution of RAPD-genetic distance and heterosis value of grain dry weight (Figure 1B), the linear re-

Table 1. Mean of genotypes and F_1 hybrid mid-parents heterosis (parenthesis) of grain dry weight (g/5 plant)

Inbreds	A1	A2	A3	A5	A6	A9	B	C	D	E	F	G	H
A1	457												
	–												
A2	421	160											
	(45.1)	–											
A3	398	392	407										
	(35.3)	(55.1)	–										
A5	385	584	635	376									
	(30.8)	(130.6)	(146.7)	–									
A6	209	476	348	340	521								
	(–21.2)	(45.1)	(5.0)	(2.4)	–								
A9	723	523	604	595	697	482							
	(94.3)	(58.0)	(80.3)	(77.2)	(70.1)	–							
B	703	697	631	549	592	623	465						
	(79.9)	(99.2)	(78.5)	(55.0)	(38.2)	(44.2)	–						
C	572	611	745	696	722	679	647	544					
	(43.7)	(71.2)	(106.2)	(92.6)	(65.5)	(54.6)	(41.3)	–					
D	643	745	613	696	608	691	599	634	391				
	(83.4)	(140.5)	(95.4)	(121.6)	(56.5)	(76.3)	(45.8)	(51.6)	–				
E	723	808	835	702	746	909	667	843	698	567			
	(54.4)	(89.0)	(93.5)	(62.7)	(78.4)	(47.4)	(26.4)	(57.5)	(43.0)	–			
F	608	664	626	604	632	727	539	684	538	576	313		
	(98.8)	(150.9)	(133.1)	(124.6)	(84.0)	(109.7)	(47.6)	(83.7)	(65.2)	(30.0)	–		
G	785	670	711	699	873	890	635	784	684	717	684	371	
	(102.5)	(93.2)	(119.6)	(99.0)	(105.0)	(107.4)	(41.8)	(72.4)	(67.8)	(36.6)	(88.8)	–	
H	707	766	791	698	721	835	606	730	529	667	616	666	303
	(100.9)	(146.5)	(151.2)	(121.6)	(85.0)	(112.6)	(47.4)	(74.4)	(42.5)	(36.3)	(88.9)	(63.1)	–

Table 2. Matrix of RAPD-genetic distance measured among 13 maize inbred lines estimated from amplified fragment data

Inbred lines	A1	A2	A3	A5	A6	A9	B	C	D	E	F	G
A2	0.592											
A3	0.605	0.645										
A5	0.503	0.609	0.563									
A6	0.447	0.667	0.421	0.521								
A9	0.597	0.630	0.566	0.615	0.572							
B	0.616	0.628	0.685	0.638	0.654	0.640						
C	0.594	0.763	0.583	0.728	0.581	0.688	0.705					
D	0.675	0.727	0.571	0.658	0.552	0.634	0.661	0.585				
E	0.692	0.704	0.595	0.634	0.586	0.630	0.655	0.615	0.565			
F	0.616	0.654	0.670	0.655	0.662	0.629	0.611	0.760	0.629	0.635		
G	0.662	0.740	0.638	0.660	0.605	0.636	0.656	0.698	0.604	0.514	0.651	
H	0.704	0.772	0.590	0.713	0.567	0.662	0.700	0.605	0.587	0.635	0.751	0.657

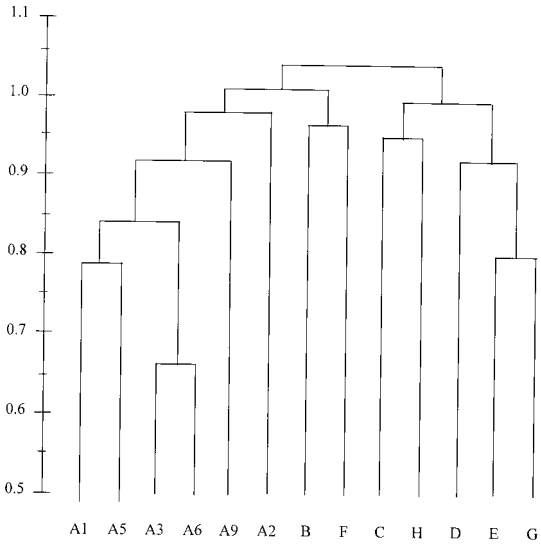

Figure 2. Association among 13 maize genotypes revealed by UPGMA cluster analysis of the Jaccard's similarity coefficients, calculated from 636 RAPD products generated by 38 primers.

Table 3. Means of grain dry weight and RAPD genetic distance among different heterotic group

Heterotic groups	I	II	III	IV
I	488.6[a]			
	56.9[b]			
	0.570[c]			
II	638.0	539		
	91.3	47.6		
	0.645	0.611		
III	711.9	638.2	730.0	
	95.9	65.3	74.4	
	0.662	0.692	0.605	
IV	741.5	616.5	687.0	699.6
	90.3	49.6	53.9	49.1
	0.644	0.648	0.630	0.561

a, b, c are mean, heterosis value and genetic distance, respectively.

gression produced small R^2 (R^2 = 0.174). This study demonstrated that RAPD analysis cannot be used to precisely predict the yield performance and heterosis value of F_1 hybrids according to this model.

Cluster analysis of the RAPD data, using Jaccard's genetic distance and the UPGMA cluster analysis to clustering. The results showed that 13 inbred lines could be classified into four groups (Figure 2). Group I contained A1, A2, A3, A5, A6 and A9; group II contained B and F; group III contained C and H; group IV contained D, E and G. The grain dry weight of F_1 hybrids in between group, I × IV, and III × IV expressed higher grain dry weight (Table 3). And these F_1 hybrids of A3 × E, A6 × g, A9 × E, A9 × g (I × IV) and C × E (III × IV) were in the top five orders. To obtain these information, and that can helped in establishing heterotic groups. In a traditional breeding program, thousand of crosses have to be done and F_1 grain yield has to be evaluated in experiment designs. According to our results, RAPD-based genetic distance could be used to help in the choice of the crosses to be made among maize lines derived from a broad genetic base population (Tainan-white variety). In crosses practice, belong to same group or near group of the inbred lines will not crosses. By so doing the number of single crosses hybrids to be evaluated is reduced.

Liu et al. (1997) employed RAPD analysis on fifteen self-crossing lines from different heterotic groups, and the results were the same as the heterosis of existing pedigrees. RAPD could be used as a tool for determining the extent of genetic diversity among tropical maize inbred lines and for allocating genotypes into district heterotic groups (Lanza et al., 1997). In this experiment, we used RAPD-marker to determine the extent of genetic diversity among Tainan-white populations. It was found that different self-crossing lines from the same variety could be allocated into distinct heterotic groups. The current study indicated that RAPD analysis was a useful tool in determining the genetic diversity, and could be classified into different heterotic groups using Jaccard's similarity coefficients.

The relationship of average grain dry weight of groups and genetic distance is shown in Figure 3A, and the linear regression produced a small R^2 level. And the relationship of average heterosis value of grain dry weight of groups and genetic distance showed in Figure 3B, and the linear equation models also produced a no significant level. The results showed a no significant linear correlation between the F_1 hybrid yield and the RAPD-based genetic diversity, which was different from Wang et al. (1994). The self-crossing lines used in this experiment were from eleven different sources with significant genetic diversity. As a result, the F_1 hybrid yield increased with the Euclidean genetic diversity in a range between 0.1 to 2.16. Beyond this range, the F_1 hybrid yield would decrease with the increase of genetic diversity (Singh et al., 1981; Prasad & Singh, 1986; Gallais, 1987). However, our results were different with Lanza et al. (1997)

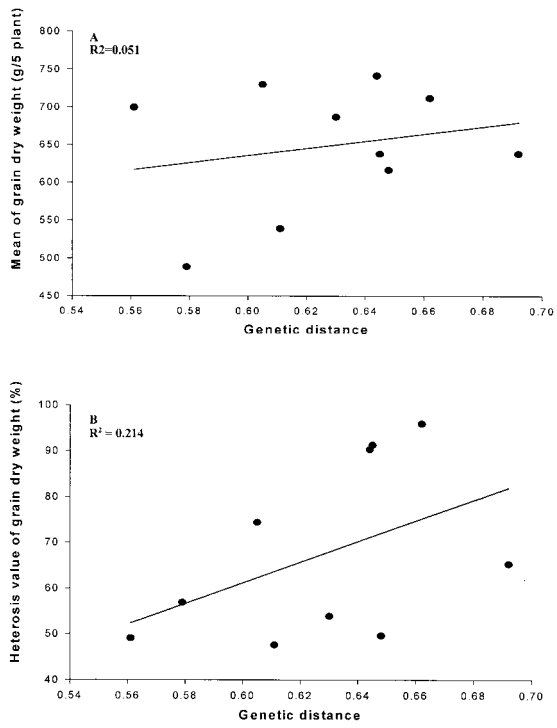

Figure 3. Relation between mean (A), heterosis value (B) of grain dry weight and genetic distance for F_1 after group.

that the F_1 hybrid yield was linearly correlated with the RAPD-based Jaccard's similarity coefficient. The self-crossing lines used in this experiment were from within one Tainan-white variety, and the genetic diversity was small. As a result, the F_1 hybrid yield was no significant linear relationship with the RAPD-based genetic diversity after group.

Currently, the association between hybrid performance and RAPD-based genetic diversity was not stronger for group among lines. Bernardo (1994) suggested that single-cross yield could only be predicted effectively based on parental RFLP data and yields of related inbred lines. Bernardo (1992) identified the following conditions for the effective prediction of hybrid performance using molecular heterozygosity: (1) strong dominance effects, (2) the allele frequencies at individual loci in parental inbreeds should be negatively correlated, (3) high heritability of trait, (4) the narrow range variation of average parental allele frequencies, (5) 30∼50% of QTLs (at least) have to be linked to molecular markers, and (6) not more than 20∼30% of molecular markers have to be randomly dispersed or unlinked to QTLs. According to our results, the grain yield correlated not consistently with RADP-based genetic distance. Regarding individual plants, grain yield is a trait with complex inheritance and low heritability; however, dealing with mean values, grain yield could be seen as a traits with high heritability and strong dominance effects, in agreement with Bernardo's prediction. But we have not found a positive correlation between RADP-based genetic distance and F_1 grain yield, suggesting that most of the RAPD marker weren't strongly linked to QTLs.

The results demonstrated non-correlation between RAPD-based genetic distance and maize single cross hybrid grain yield, using inbred lines from Tainan-white maize germplasm. This study showed that the RAPD-Jaccard similarity coefficients cannot be used to precisely predict the yield performance and heterosis value for F_1 hybrids. Due to the fact that their coefficients of determination for mean ($R^2 = 0.051$) and heterosis value ($R^2 = 0.214$) of grain dry weight are small, the predictive value is limited.

References

Bernardo, R., 1992. Relationship between single-cross performance and molecular marker heterozygosity. Theor Appl Genet 83: 628–634.

Bernardo, R., 1994. Prediction of maize single-cross performance using RFLPs and information from related hybrids. Crop Sci 34: 20–25.

Boppenmaier, J., A.E. Melchinger, E. Brunklaus-Jung, H.H. Geiger & R.G. Herrmann, 1992. Genetic diversity for RFLPs in European maize inbreds: Relation to performance of flint × crosses for forage traits. Crop Sci 32: 895–902.

Chen, S., K.K. Hwu & C. Chen, 1996. Prediction of heterosis with genetic diversity among maize (*Zea mays* L.) inbred lines revealed by random amplified polymorphic NDA. J Agric Res China 45(2): 147–155.

Dhillon, B.S., J. Boppenmaier,W.G. Pollmer, R.G. Herrmann & A.E. Melchinger, 1993. Relationship of restriction fragment length polymorphism among European maize inbreds with ear dry matter yield of their hybrids. Maydica 38: 245–248.

Doyle, J.D., J.L. Doyle & L.H. Bailey, 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

East, E.M., 1936. Heterosis. Genetics 21: 375–397.

Gallais, A., 1987. Heterosis: its prediction and utilization in plant breeding. Heredity 59: 312–313.

Godshalk, E.B., M. Lee & K.R. Lamkey, 1990. Relationship of restriction fragment length polymorphisms to single-cross hybrid performance of maize. Theor Appl Genet 80: 273–280.

Griffing, B. & E.W. Lindstrom, 1954. A study of the combining ability of corn inbreds having varying proportions of corn belt and non-corn belt germplasm. Agron J 46: 545–552.

Hayes, H.K. & Z.J. Johnson, 1939. The breeding of improved self lines of corn. J Amer Soc Agron 31: 710–724.

Jaccard, P., 1908. Nouvelles reserches sur la distribution florale. Bull Soc Vaud Sci Nat 44: 223–270.

Lanza, L.L.B., C.L. de Souza Jr., L.M.M. Ottoboni, M.L.C. Vieira & A.P. de Souza, 1997. Genetic distance of inbred lines and pre-

diction of maize single-cross performance using RAPD markers. Theor Appl Genet 94: 1023–1030.

Liu, X.Z., Z.B. Peng, J.H. Fu, L.C. Li & C.L. Huang, 1997. Heterotic grouping of 15 maize inbreds with RAPD markers. Scientia Agricultura Sinica 30(3): 44–51.

Melchinger, A.E., M. Lee, K.R. Lamkey, A.R. Hallauer & W.L. Woodman, 1990a. Genetic diversity for restriction fragment length polymorphisms and heterosis for two diallel sets of maize inbreds. Theor Appl Genet 80: 488–496.

Melchinger, A.E., M. Lee, K.R. Lamkey & W.L. Woodman, 1990b. Genetic diversity for restriction fragment length polymorphisms: Relation to estimated geneticeffects in maize inbreds. Crop Sci 30: 1033–1040.

Melchinger, A.E., J. Boppenmaier, B.S. Dhillon, W.G. Pollmer & R.G. Herrmann, 1992. Genetic diversity for RFLPs in European maize inbreds: II. Relation to performance of hybrids with versus between heterotic groups for forage traits. Theor Appl Genet 84: 672–681.

Moll, R.H., J.H. Lonnquist, J.V. Fortuna & E.C. Johnson, 1965. The relation of heterosis and genetic divergence in maize. Genetics 52: 139–144.

Moll, R.H., W.S. Salhuans & H.F. Robinson, 1962. Heterosis and genetic diversity in variety crossed of maize. Crop Sci 2: 197–198.

Moser, H. & M. Lee, 1994. RFLP variation and genealogical distance, multivarate distance, heterosis, and genetic variance in oats. Theor Appl Genet 87: 947–956.

Prasad, S.K. & T.P. Singh, 1986. Heterosis in relation to genetic divergence in maize (*Zea mays* L.). Euphytica 35: 919–924.

SAS Institute, Inc., 1993. SAS/STAT User's guide, Version 6, 4th ed., Cary, NC, USA.

Shieh, G.J. & F.S. Thseng, 1998. Development and evaluation of inbred lines derived from Tainan-white maize populations. J Agric Res China 47: 327–337.

Singh, Y.P., A. Kumar & B.P.S. Chaunan, 1984. Genetic divergence in pearl millet. Indian J Genet 41: 186–190.

Smith, O.S., J.S.C. Smith, S.L. Bowen, R.A. Tenborg & S.J. Wall, 1990. Similarities among a group of elite maize inbreds as measured by pedigree, F_1 grain yield, heterosis, and RFLPs. Theor Appl Genet 80: 833–840.

Wang, Y.L., C.G. Qiao, Q.Y. Wang & R.G. Zhao, 1994. Relation between genetic divergence and heterosis in popcorn. Acta Agronomica Sinica 20: 223–228.