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Use of Random Amplified Polymorphic DNA Markers to Estimate Heterosis and Combiming Ability in Tomato Hybrids

[1]A. Mirshamsi, [2]M. Farsi, [2]F. Shahriari and [3]H. Nemati
[1]Research Center for Plant Science, Ferdowsi University of Mashhad, Iran
[2]Department of Biotechnology and Plant Breeding, College of Agriculture, Ferdowsi University of Mashhad, Iran
[3]Department of Horticulture, College of Agriculture, Ferdowsi University of Mashhad, Iran

Abstract: Random Amplified Polymorphic DNAs (RAPD) were used to estimate genetic distances and determine the correlation between genetic distance and hybrid performance of 29 tomato lines that were the parents in a diallel mating design. Among 97 observed bands, 69 showed polymorphism and were used for establishing genetic distances based on the Nei coefficient between parents. A UPGMA dendrogram and Multi-Dimensional Scaling (MDS) analysis based on Nei genetic distances clearly clustered each group, confirming the variation at a molecular level. Correlations between genetic distances of the parents and performances of hybrids were established for various quantitative traits. Significant correlations were found between RAPD markers estimated genetic distances and MPH, HPH, SCA for some traits. The low correlation between parental genetic distances and hybrid performances for some quantitative traits suggested that RAPD markers have low linkage to Quantitative Trait Loci (QTLs) or have inadequate genome coverage for these traits. The results indicated that RAPD markers can be used as a tool for determining the extent of genetic diversity among tomato lines, for allocating genotypes into different groups and also to aid in the choice of the superior crosses to be made among tomato lines, so reducing the number of crosses required under field evaluation.

Key words: RAPD markers, Nei genetic distance, UPGMA, QTLs, genetic diversity

INTRODUCTION

Nearly one hundred years after Shull`s proposal of hybrid breeding, recognition of lines with superior cross performance is still the most costly and time-consuming phase in hybrid development projects. The general procedure is to assess the performance of crosses between lines from different heterotic groups through extensive field tests (such as open-pollinated progeny test, topcross test, polycross test, single cross test, diallel cross mating and line x tester analysis). If lines could be screened and superior crosses predicted before field evaluation, this would greatly enhance the efficiency of hybrid breeding programs (Melchinger *et al*., 1990).

In tomato, prediction of hybrid performance and heterosis is important and has attracted large interest over the past decades. Recently, advances in genome research have generated new tools in predicting heterosis and hybrid performance using molecular markers in hybridization projects (Zhang *et al*., 1996). Genetic diversity between lines for molecular markers has been considered as a possible way for predicting heterosis and combining ability. The impetus for this approach stems from the positive association between heterosis and indirect measures of genetic diversity reported for crosses among lines of maize. Furthermore, quantitative genetic theory shows that for any degree of dominance greater than zero, heterosis expressed in a cross is a function of the allele frequency differences in parents (Melchinger *et al*., 1990; Zhang *et al*., 1996). The use of markers to assess the genetic divergence among pairs of lines has been suggested as a mean to overcome the drawbacks referred to above, allowing the prediction of hybrid performance. Various investigators have used markers to assess directly the genetic diversity of parental genotypes. Isozymes, Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) have been used to estimate genetic diversity of parental genotypes in several experiments.

The use of isozymes (Heidrich-Sobrino and Corderio, 1975) and RFLP (Goldshalk *et al*., 1990; Lee *et al*., 1984, 1989; Bernardo, 1992, 1993; Duddley, 1991, Ajmone-Marsan *et al*., 1998) has been proposed to predict hybrid performance from the genetic divergence of lines. However, the correlations of the genetic distance

Corresponding Author: A. Mirshamsi, Research Center for Plant Science, Ferdowsi University of Mashhad, Iran

based on isozymes and the grain yield of the hybrids are too low to be useful to predict hybrid performance. In maize (*Zea mays* L.), results indicated that isozyme allelic differences between lines are not predictive of hybrid performance (Lamkey *et al*., 1987; Price *et al*., 1986). In rice (*Oryza sativa* L.). Peng *et al.* (1988) did not find any association between the magnitude of heterosis in F_1 and isozyme variation among parents. However, he suggested that esterase and peroxidase patterns in the parents may be of value for predicting F_1 yield heterosis.

RFLP markers have proved useful for assigning maize to heterotic groups and for detecting relationships among them (Smith *et al*., 1990; Dudley *et al*., 1991; Melchinger *et al*., 1991; Bernardo, 1993). The association of RFLP-based genetic distance with F_1 performance and heterosis has been tested in several studies (Lee *et al*., 1984, 1989; Goldshalk *et al*., 1990; Melchinger *et al*., 1990, 1991, 1992; Boppenmaier *et al*., 1992; Bernardo, 1993; Zhang *et al*., 1994, 1995, 1996; Cerna *et al*., 1997; Zhao *et al*., 1999; Benchimol *et al*., 2000) with the results appearing to be highly dependent on the origin of parental inbreds. A study reported significant correlations between RFLP-based genetic distances and heterosis for yield in Maize, suggesting that measures of similarity calculated from RFLP data could allow maize breeders to predict combination of lines resulting in high-yielding single-cross hybrids (Smith *et al*., 1990). However some studies conducted in maize indicated that there is no relationship between RFLP markers and heterosis expression (Dudley *et al*., 1991; Goldshalk *et al*., 1990; Lee *et al*., 1989; Melchinger *et al*., 1990).

In rice (Zhang *et al*., 1994, 1995) showed the relationship between marker heterozygosity and hybrid performance and heterosis in a number of characters, including yield and yield component traits and found mostly low correlation between general heterozygosity and F_1 performance and heterosis. In contrast, very high correlations were detected between midparent heterosis and specific heterozygosity for a number of traits other than yield and yield components.

A significant improvement in the correlations between genetic distance and hybrid performance was noted in maize and alfalfa by using AFLP markers in comparison with RFLP markers (Ray and Lerma, 1999; Wu, 1999).

RAPD markers have also been used to determine the extent of diversity among lines, for allocating genotypes into different groups and to aid in the choice of superior crosses to be made (Arcade *et al*., 1996; Lanza and Souza, 1997; Wu, 1999; Parentoni *et al*., 2001).

The aim of the present study was to investigate the relationships between genetic dissimilarity of the parental lines and hybrid performance in tomato. RAPD markers were selected because of the high number of markers that can be generated in a short time and technically they are easy to use. The different steps of the study are to assessment of genetic variability among lines and parental lines involved in a diallel mating design, to study of the relationships between genetic distance and hybrid performance for various quantitative traits and to the evaluation of the potential of marker-based genetic distance in predicting the performance of tomato hybrids.

MATERIALS AND METHODS

Parental lines and crosses: Plant materials used in this study are shown in Table 1. Seeds were obtained from breeding programs in Florida, Russia, Italy and the collection of the Tomato Genetic Resource Center (TGRC). Out of 29 lines, fifteen lines selected in early field evaluation (Data not shown) and were inter-mated in all possible pairs excluding reciprocals to form a half diallel crosses, during the cropping season 2000, in Ferdowsi University of Mashhad and produced 105 hybrids.

Greenhouse experiment: Out of 105 F_1 hybrids, twenty-one hybrids and their seven parental lines were randomly selected and examined for agronomic performance in the

Table 1: List of tomato lines used in this study

No.	Accession	Source	Background genotype
1	LA2443	TGRC[1]	Unknown
2	LA3004	TGRC	Rutgers
3	LA3035	TGRC	Gardner
4	LA3168	TGRC	Alisa Craige
5	LA3728A	TGRC	Alisa Craige
6	LA3899	TGRC	Ohio8245
7	LA3247	TGRC	Alisa Craige
8	LA3000	TGRC	Rutgers
9	LA3898	TGRC	FM6203
10	LA2374	TGRC	Caro Red
11	LA0588	TGRC	Condine Red
12	LA0611	TGRC	Condine Red
13	LA0643	TGRC	Long Red
14	LA1793	TGRC	Italian cultivar
15	IL2-377	TGRC	XL-pearson
16	LA3006	TGRC	San marzano
17	LA3723	TGRC	Alisa Craige
18	I L-345	TGRC	Money Maker
19	KalGN3	Falat	Italian cultivar
20	Super H	Falat	Italian cultivar
21	Viva	Falat	Italian cultivar
22	Kingston	Falat	Italian cultivar
23	Fla7771	IFAS[2]	Unknown
24	BoIII	VIR[3]	Russian cultivar
25	R2	VIR	Russian cultivar
26	R22	VIR	Russian cultivar
27	Re1	VIR	Russian cultivar
28	C17	VIR	Russian cultivar
29	B3	VIR	Russian cultivar

[1]: Tomato Genetic Resource Center; [2]: Institute of Food and Agricultural Sciences; [3]: Vavilov Research Center of Plant Production

greenhouse, in a randomized complete block design with two replications, during the year 2001. The field management included: irrigation, weed control, fertilizer and pesticide applications, essentially the same as under normal conditions of tomato production. Three plants of each hybrids were examined for eight quantitative characters namely: Plant Height (PLH), Yield per plant (Y), Fruit Weight (FW), Fruit Number (FN), Days to Flowering (DFL), Days to Ripening (DRP), Leaf numbers to first inflorescence (LI), Days from Flowering to Ripening (DF-DR). Based on the analysis of hybrids, by means of analysis of variance, the sums of squares were portioned into general and specific combining abilities using diallel analysis method (2) proposed by Griffing (1956).

DNA extraction: For each parental line, genomic DNA was extracted from 0.5 g of young leaves, harvested in bulk from four to five weeks old plants per genotype. DNA was extracted using a modified Dellaporta procedure (Dellaporta *et al*., 1983). DNA concentration was determined by spectrophotometer, following procedures supplied by the manufacturer. Agarose gel electrophoresis also was carried out for DNA quantity and quality analysis. For use in PCR, the DNA was diluted with TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) to 50 ng μL^{-1} and stored at 4°C until used for RAPD analysis.

DNA amplification: A series of optimization experiments were conducted by changing the concentrations of template DNA, primers and $MgCl_2$ to determine which condition gave the strongest and the most reproducible patterns. RAPD amplification was performed in a reaction volume of 25 μL, containing 50 ng template DNA, 0.2 μM dNTPs, 1.5 mM $MgCl_2$, 17.5 pmol primer (Cinagen Inc.), One micro Taq polymerase and 10X PCR buffer (10 mM Tris-HCl, 50 mM KCl). Controls run with each amplification, included at least one sample of the reaction mix with no template DNA. PCR cycles consisted of initial denaturation at 94°C for 4 min, followed by 35 cycles of amplification, each having denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min. A final extension step at 72°C for 10 min was followed by termination of the cycle at 4°C. Following amplification, PCR products (10 μL) were loaded in 1.2% agarose gels and separated by electrophoresis at 75 v for about 3 h. RAPD fragments were stained with ethidium bromide and photographed on UV photo documentation system. The size of amplification products was determined by comparison with 1 kb DNA ladder (MBI Fermentas) and using labwork software.

Data scoring and statistical analysis: As RAPD markers are dominant markers, presence and absence represent the two allelic forms at a locus. The presence and absence of

Table 2: Genetic distance matrix in percentage for 29 genotypes estimated on the basis of RAPD markers

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
R2	0																												
R22	24	0																											
Re1	26	52	0																										
IL2-377	27	41	36	0																									
LA0611	39	58	33	34	0																								
LA3899	19	18	36	26	32	0																							
LA3035	58	67	44	52	25	56	0																						
LA1793	56	50	42	54	44	58	39	0																					
C17	69	63	50	59	33	59	26	27	0																				
Kingston	54	52	44	49	33	45	23	25	21	0																			
LA0588	61	52	44	63	52	56	41	25	34	32	0																		
LA3168	59	58	42	50	23	47	42	34	30	42	27	0																	
LA0643	67	44	42	69	44	61	36	32	30	33	33	41	0																
Super H	52	41	45	58	54	54	39	29	36	27	25	41	34	0															
B3	59	54	49	69	58	61	42	26	33	36	12	37	34	21	0														
LA3898	83	63	65	76	49	67	41	33	29	37	32	36	45	36	27	0													
Fla.7771	63	50	59	54	44	54	39	44	30	42	39	37	44	29	34	30	0												
KalGN3	59	58	42	61	50	54	45	32	36	39	27	44	44	34	23	25	41	0											
Viva	61	56	54	59	45	52	44	39	37	44	44	39	49	36	42	37	33	36	0										
LA3000	94	80	83	90	76	91	61	56	61	65	58	71	56	67	67	65	59	80	78	0									
LA3728A	96	95	78	80	90	91	61	49	61	61	54	90	56	67	59	58	67	56	69	34	0								
LA3247	98	88	90	96	88	96	59	47	59	63	42	65	58	61	47	52	58	50	76	22	19	0							
LA2374	83	85	65	63	59	76	44	56	47	58	61	76	49	71	59	65	59	59	74	34	37	33	0						
LA3006	80	74	90	80	78	88	63	54	76	63	52	88	69	65	58	63	69	58	63	39	27	32	45	0					
LA3723	98	80	74	90	89	95	65	56	69	78	61	85	49	76	63	69	76	63	69	37	23	30	29	39	0				
IL-345	76	96	56	60	47	78	56	61	63	85	71	44	78	83	69	63	58	65	63	52	52	47	33	65	49	0			
BOIII	78	90	50	50	59	76	65	52	61	74	65	42	80	71	67	61	67	63	61	54	47	42	41	63	58	15	0		
LA2443	80	88	59	60	61	83	56	54	52	67	52	61	61	69	58	63	58	58	67	36	39	32	19	54	30	26	22	0	
LA3004	83	85	61	60	56	80	58	63	74	65	65	80	52	71	71	78	71	63	65	34	29	39	29	39	26	52	50	36	0

bands were recorded for each parent. The genetic similarity index (GS_{NL}) was determined from the RAPD pattern of individual plants in each population according to the Nei coefficient (Nei and Li, 1979).

$$GS_{NL} = 2N_{11}/(2N_{11}+N_{01}+N_{10})$$

Assuming that:
N_{11}: No. of bands shared by both parents P_1 and P_2.
N_{01}: Total No. of bands presented by parents P_1.
N_{10}: Total No. of bands presented by parents P_2.

For clarity, only strong and reproducible bands were scored as present (1) or absent (0) for calculating Nei coefficient of similarity. Ambiguities were scored as missing data. The genetic distance D was deduced from genetic similarity as D = 1-S. The dissimilarity matrix (Table 2) was used to construct the dendrogram using UPGMA and to do Multi-Dimensional Scaling (MDS) analysis employing the STATISTICA ver.5.5.

Pair-wise genetic distances between the seven parental lines, based on the RAPD analysis, were examined to find any possible association with yield performance of the 21-hybrid. The relationship between Nie genetic distances and General Combining Abilities (GCA), Specific Combining Abilities (SCA), High Parent Heterosis (HPH), mid Parent Heterosis (MPH) and Mean were evaluated by Pearson correlations.

RESULTS

Marker polymorphisms: Twenty 10-mer primers were used for the ability to detect polymorphism in 4 randomly chosen tomato lines (LA1793, LA3006, BOIII and KalGN3). The primers that presented the highest degree of polymorphism were selected for this study. These primers produced a total of 97 reproducible bands, which 71.1% of them were polymorphic. An average of 24.2 bands per primer was obtained, ranging from 290 to 3500 bp (Fig. 1), mostly concentrated from 400 to 2100 bp. The UPMGA dendrogram based on Nei`s genetic distances (Fig. 2A) showed three clusters: one comprising the Russian lines and two others clusters included TGRC lines and majority of the Italian cultivars. The frequencies of the intra-group polymorphic markers were 62.5, 77.08 and 78.04%, respectively. Also, maximum average of polymorphic markers was 0.299 and the minimum was 0.179. The MDS analysis of 29 tomato lines (Fig. 2B) indicated very little difference in clustering among sub-groups; however, in general this MDS analysis confirmed the cluster results.

Hybrid performance and heterosis: There were significant differences in the performance of the crosses in all studied traits. The amounts of heterosis differed drastically among the crosses and also varied widely from one trait to another (Table 3). The results of diallel analysis indicated that variation among crosses was attributed primarily to GCA effects, however, SCA effects were also significant except for LI and FN (data not shown). This behavior would be expected if additive effects were of major importance.

Relation of hybrid performance and heterosis with genetic distance: In general our results showed positive correlations between genetic distances based on RAPD markers and genetic parameters such as SCA, GCA, MPH, HPH and mean of hybrids (Table 4). Significant

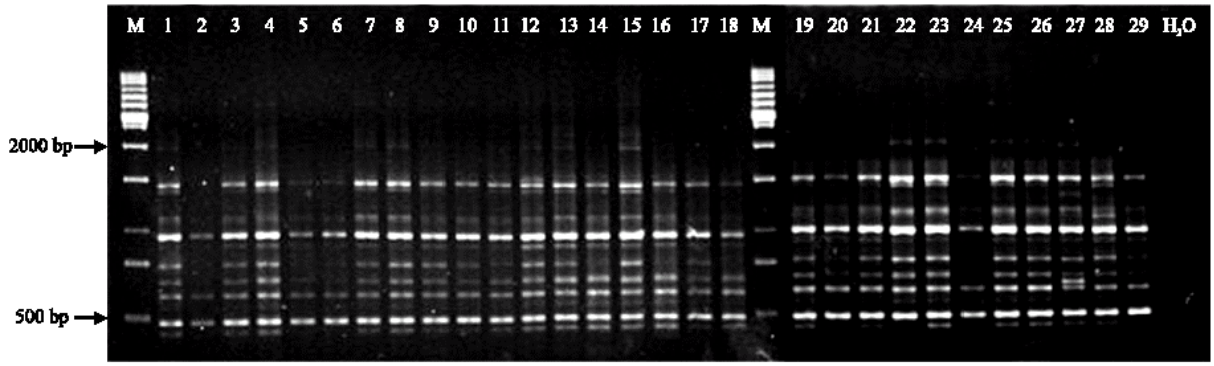

Fig. 1: RAPD profiles for 29 tomato lines using primer OPJ-10 (5'-AAGCCCGAGG-3'). Lanes are as follows from left to right: 1 kb DNA ladder (MBI Fermentas), LA3035, R2, LA1793, C17, Kingston, LA0611, LA0588, LA3168, LA0643, R22, Super H, B3, LA3898, Fla7771, KalGN3, Re1, Viva, LA3899, 1 kb DNA ladder, LA3000, IL2-377, LA3728A, LA3247, LA2374, LA3006, LA3723, IL-345, BoIII, LA2443, LA3004, (H_2O) Negative Control

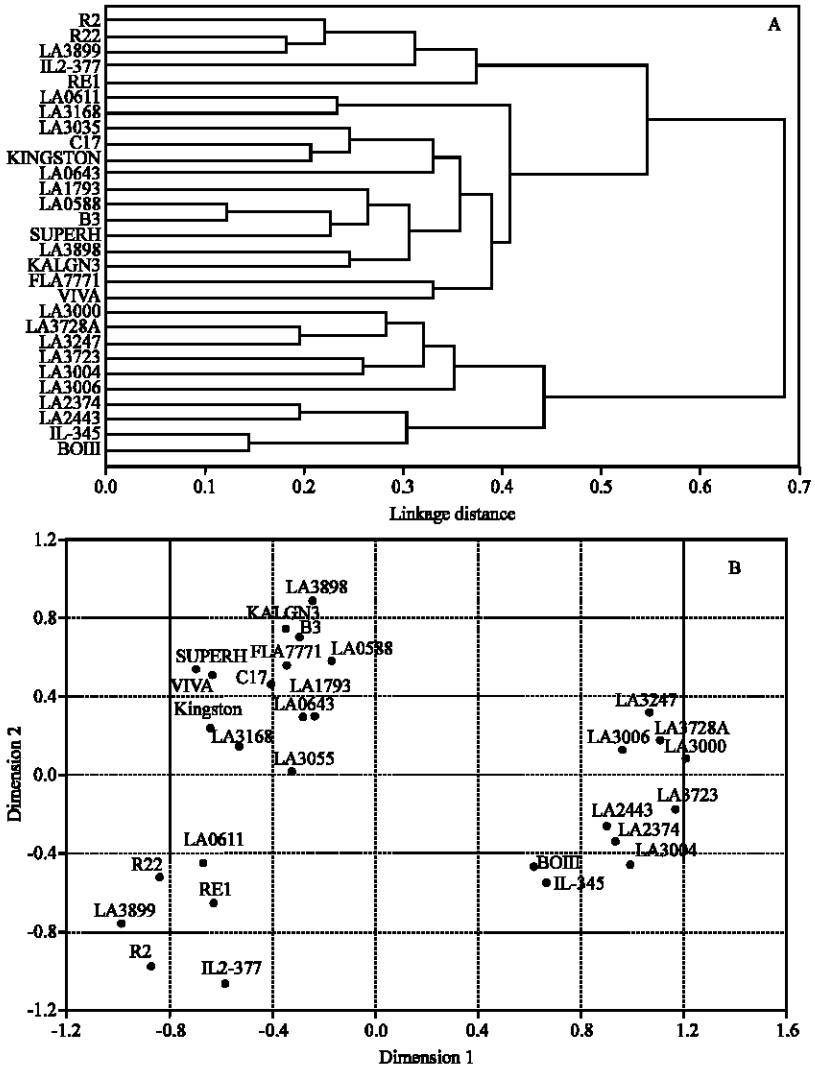

Fig. 2: (A) Dendrogram among 29 tomato lines resulting from UPGMA cluster analysis based on Nei coefficient. (B) Two-dimensional plot from multidimensional analysis of 29 tomato lines based on RAPD data

Table 3: Measurements of SCA, MPH and HPH value for the 21 hybrids of diallel cross

	Y			PLH			DFL			DF-DR			DRP			FN			FW			LI		
Cross	SCA	MPH	HPH	SCA	MPH	HPH	SCA	MPH	HPH	SCA	MPH	HPH	SCA	MPH	HPH	SCA	MPH	HPH	SCA	MPH	HPH	SCA	MPH	HPH
6×7	-18.0	-127.0	-302.00	-34.30	64.90	38.80	0.09	-0.40	-1.0	-0.10	-2.70	-5.30	0.08	-3.10	-5.0	0.48	-0.20	-0.60	-3.90	-11.00	-290.00	0.00	0.09	-0.30
5×7	-87.0	-265.0	-265.00	-0.40	16.00	10.40	-1.60	3.00	-4.0	2.90	12.00	-33.00	5.50	-18.00	40.0	0.20	-1.10	2.80	6.10	-17.00	-24.00	0.48	0.60	0.00
5×6	-41.0	-102.0	-277.00	6.63	22.60	-9.10	-0.40	-1.40	-1.8	-5.10	-15.00	-33.00	-6.50	-19.00	-39.0	0.45	1.17	-0.10	1.00	-0.50	-10.00	0.39	0.59	0.38
4×7	-44.0	-182.0	-224.00	21.30	39.20	35.20	-1.70	-3.10	-3.2	0.44	0.76	-0.80	-1.10	-2.30	-4.0	-0.90	-2.20	-2.70	-5.10	-13.00	-17.00	0.85	1.48	0.42
4×6	69.0	48.5	-85.00	-13.00	4.11	-26.00	0.10	-1.10	-1.9	-0.60	-1.10	-2.10	0.60	-2.30	-2.5	1.39	1.72	0.79	14.60	15.50	1.75	0.59	1.27	-0.20
4×5	-50.0	-139.0	-181.00	-5.00	-1.80	-3.40	1.09	-0.80	-2.0	-8.80	-16.00	-35.00	-8.70	-20.00	-40.0	-0.70	-0.60	-2.80	2.62	0.50	-3.30	0.09	0.81	-0.90
3×7	-38.0	-201.0	-248.00	-21.00	-4.90	-25.00	1.41	-0.10	-1.5	5.30	-10.00	-16.00	-3.50	-9.80	-17.0	-1.10	-2.00	-3.20	-3.00	-16.00	-18.00	-0.80	-1.30	-2.40
3×6	-11.0	-56.0	-184.00	15.20	31.30	-15.00	-0.90	-2.10	-4.0	0.11	-5.90	-9.40	-0.40	-7.30	-12.0	0.77	1.47	0.67	-5.00	-9.40	-29.00	0.27	-0.20	-0.90
3×5	-77.0	-191.0	-238.00	0.64	2.54	-12.00	-0.50	-2.60	-4.9	-9.90	-23.00	-37.00	-11.00	-27.00	-42.0	-0.10	0.44	-0.10	-0.70	-8.20	-18.00	0.00	-0.40	-0.90
3×4	-53.0	-127.0	-132.00	18.30	21.40	5.45	-1.60	-3.70	-4.9	3.56	0.50	-4.00	2.36	-2.50	-7.5	-1.50	-1.40	-3.10	-4.70	-9.80	-16.00	0.60	0.68	-1.50
2×7	-100.0	-198.0	-281.00	12.10	24.20	16.50	-0.30	-2.10	-4.3	3.66	4.80	-2.90	3.49	2.68	-7.2	-0.70	-0.80	-1.70	-10.00	-20.00	-25.00	-0.50	-1.20	-2.50
2×6	77.5	96.3	4.17	1.51	16.20	-21.00	0.64	-0.80	-2.0	2.23	2.50	-7.80	3.00	1.75	-10.0	-1.50	-0.10	0.60	10.70	9.60	-3.30	-1.10	-1.70	-2.70
2×5	-48.0	-98.0	-180.00	4.11	4.63	-0.50	-3.00	-5.40	-6.5	-4.60	-11.00	-39.00	-8.60	-19.00	-51.0	1.35	2.61	1.87	-1.30	-5.40	-8.30	-0.40	-1.00	-1.70
2×4	31.5	22.2	-19.00	-20.00	-18.00	-25.00	-0.40	-2.80	-5.0	5.06	8.25	-1.00	4.81	5.50	-6.0	2.47	3.34	1.89	-3.40	-5.00	-5.90	0.00	-0.10	-2.50
2×3	-39.0	-73.0	-109.00	12.20	12.80	3.38	-3.40	-5.90	-9.4	-1.00	-3.30	-17.00	-4.00	-8.50	-25.0	1.49	2.73	2.45	-12.00	-19.00	-26.00	-1.10	-2.30	-2.50
1×7	-167.0	-301.0	-321.00	16.30	33.30	10.00	0.22	0.29	-1.2	0.73	-0.70	-2.40	1.09	-0.50	-0.7	-2.40	-3.90	-5.20	-4.90	-17.00	-32.00	0.07	0.00	-0.60
1×6	-62.0	-77.0	-273.00	16.10	32.70	-17.00	0.74	1.25	-0.9	-3.50	-5.90	-10.00	-2.70	-4.60	-6.8	0.31	0.50	-0.40	-14.00	-17.00	-50.00	0.22	0.21	0.00
1×5	42.1	-42.0	-63.00	-2.40	0.05	-18.00	0.68	0.25	-2.3	-3.00	-12.00	-34.00	-3.20	-14.00	-36.0	0.18	0.16	-0.20	-4.60	-10.00	-33.00	-0.20	-0.10	-0.10
1×4	-50.0	-94.0	-156.00	8.29	12.20	-7.10	-1.20	-1.70	-3.0	5.09	5.67	-2.42	3.91	3.88	2.0	-1.30	-1.80	-3.60	-3.50	-6.70	-26.00	0.30	0.81	-0.90
1×3	21.8	-47.0	-115.00	-21.00	-18.00	-22.00	0.65	0.06	-0.1	-4.50	-9.40	-17.00	-3.40	-8.60	-16.0	1.27	1.24	1.14	4.91	-3.70	-17.00	-1.10	-1.80	-2.30
1×2	138.0	133.0	29.60	-11.00	-10.00	-23.00	0.99	0.13	-3.5	2.73	4.13	-1.90	3.52	3.88	-5.8	0.89	1.62	1.24	8.93	3.75	-16.00	0.34	-0.50	-1.20

Plant height (PLH), Days to flowering (DFL), Days from flowering to ripening (DF-DR), Days to ripening (DRP), Fruit number (FN), Fruit weight (FW), Leaf numbers to 1st inflorescence (LI), Yield per plant (Y), Mid-Parent Heterosis (MPH), High Parent Heterosis (HPH), Specific Combining Ability (SCA)

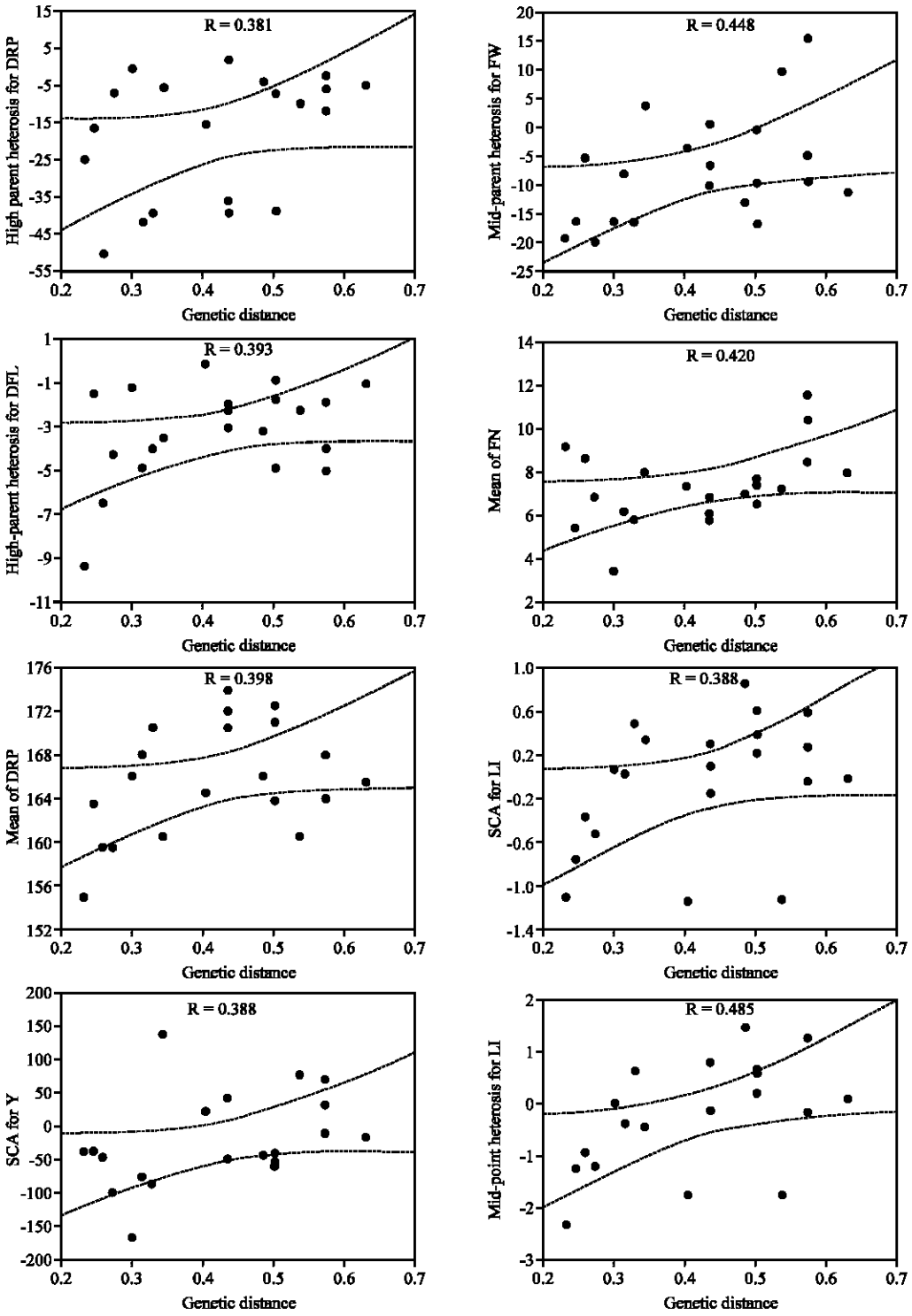

Fig. 3: Correlation between RAPD's estimated genetic distance and Specific Combining Ability (SCA), Mid Parent Heterosis (MPH), High Parent Heterosis (HPH) and mean of some study traits. Plant height (PLH), Yield per plant (Y), Fruit Weight (FW), Fruit Number (FN), Days to Flowering (DFL), Days to Ripening (DRP), Leaf numbers to 1st inflorescence (PLH), Days from flowering to ripening (DF-DR), High Parent Heterosis (HPH), Mid-Parent Heterosis (MPH), Specific Combining Ability (SCA)

Table 4: Correlation coefficient of hybrid performance, heterosis and SCA with genetic distance

Parameters	Mean	MPH	HPH	SCA
PLH	-0.3525	0.3035	-0.0276	0.1970
p-value	0.1170	0.1810	0.9060	0.3920
DFL	0.1808	0.2801	0.3931	0.2179
p-value	0.4330	0.2190	0.0780	0.3430
DF-DR	0.3115	0.2530	0.3489	0.2785
p-value	0.1690	0.2690	0.1210	0.2220
DRP	0.3988	0.3032	0.3814	0.3408
p-value	0.0730	0.1820	0.0880	0.1310
FN	0.4210	0.1533	0.1152	0.1602
p-value	0.0570	0.5070	0.6190	0.4880
FW	-0.1257	0.4487	0.1289	0.2585
p-value	0.5870	0.0410	0.5780	0.2580
LI	0.0789	0.4855	0.3356	0.3889
p-value	0.7340	0.0260	0.1370	0.0810
Y	-0.0112	0.4251	0.1890	0.3884
p-value	0.9610	0.0550	0.4120	0.0820

Plant height (PLH), Days to flowering (DFL), Days from flowering to ripening (DF-DR), Days to ripening (DRP), Fruit number (FN), Fruit weight (FW), Leaf numbers to 1st inflorescence (LI), Yield per plant (Y), Mid-Parent Heterosis (MPH), High Parent Heterosis (HPH), Specific Combining Ability (SCA)

correlations ($p<0.10\%$) were found between D of the parents and performances of the hybrids for some quantitative traits. MPH for FW (R = 0.45), Y (R = 0.42) and LI (R = 0.48) were positively correlated with the genetic distances of parents. Also, our results indicated that significant correlations were found between genetic distances and HPH for DFL (R = 0.39), DRP (R = 0.34). Significant correlations were found between SCA in absolute values for Y (R = 0.39), LI (R = 0.39) and genetic distances. However, in other traits these correlations were not significant ($p<0.10\%$). Finally, a positive significant correlation ($p<0.10\%$) between genetic distance and mean of FN (R = 0.42), DRP (R = 0.40), was observed (Fig. 3).

DISCUSSION

Since present results showed a positive correlation between RAPD-based genetic distances and SCA, MPH, HPH and mean of most traits, it can be expected that some of the RAPD markers are linked to QTLs; although a lower significant correlation ($p<0.10$) were observed in some cases, in absolute values, these estimates were generally small. One possible reason could be the fact that the calculation of marker genetic distance includes many markers not linked to yield or yield components. Bernardo (1992) identified the following conditions for the effective prediction of hybrid performance using molecular markers: strong dominance effects, the allele frequencies at individual loci in parental lines should be negatively correlated, high trait heritability, the narrow range variation of average parental allele frequencies, 30-50% of QTL have to be linked to molecular markers and not more than 20-30% of molecular markers have to be randomly dispersed or unlinked to QTLs (Bernardo, 1992). Estimation of gene action involved in the expression of traits, the level of additive effects and the degree of dominance also are very important in developing a breeding method for the trait of interest. Alleles with dominance or additive phenotypic effects influence heritability differently, depending on whether they are in homozygous or heterozygous conditions (Mohammadi *et al*., 2002). In tomato, some of these conditions may not be met and current knowledge may not be sufficient to establish the effectiveness of molecular markers as predictors for heterosis expression of yield. For instance, most gene actions reported in tomato for economically important traits are additive and heritability estimates are low (Burdick, 1954; Mittal and Singh, 1977; Singh and Singh, 1984). In other instances, even though relationships between QTLs and molecular markers have been reported (Doganlar and Tanksley, 2000), no relationships have yet been determined between RAPD markers and QTLs for yield. Low or negative correlation between RAPD-based genetic distance and mean of traits, in the results may be indicated that these traits have complex inheritance and low heritability.

Since we have found a positive correlation between RAPD-based genetic distance and FN, DRP, it can be expected that some of the RAPD markers are linked to QTLs. Also, fruit number is a trait with high heritability and dominance effects, which is in agreement with Bernardo`s predictions.

The assessment of the effectiveness of RAPD markers in breeding tomato for yield and economically important traits may need further consideration. The evaluation of the association between RAPD marker diversity and the expression of heterosis would require more genotypes in hybrid combinations and also that the RAPD marker used to estimate genetic divergence should be linked to QTLs of the traits. Another reason for the poor association between genetic distance and MPH, HPH in crosses could be due to using the arbitrarily selected primers to estimate the Quantitative Trait Loci (QTLs) affecting yield. Different loci affecting yield expression in different crosses or loci with multiple alleles and epistatic effects may also reduce the correlation. The type of markers used may be decisive in determining the relationships between genotypes. Indeed, the RAPD markers allow the amplification of any site of the genome, especially in non-coding regions that are more likely to accumulate mutations and to generate greater polymorphism between individuals or between species than coding regions such as isozyme loci (Arcade *et al*., 1996).

The identification of individual loci that code for quantitative traits would be a more suitable approach to an understanding of their way of expression and

predicting hybrid performance. The influence of genetic distance would not have resulted from cumulative effects of single-locus heterozygosity but mainly from the accumulation of different and favorable alleles provided by parents (Arcade *et al*., 1996).

In conclusion, the analysis of relationship between genetic distances of the parents and hybrid performances using RAPD markers has provided important results. Firstly other techniques would not have been likely to produce as many markers in the same period of time. This observation is in agreement with other researches (Jain *et al*., 1994; Vaillancourt *et al*., 1995). However, it should be kept in mind that bands of similar size are not necessarily homologous and that their sequence homology should be checked either by hybridization or sequencing. RAPD markers are, however, dominant markers and an imprecision always remains regarding the genotype of the parents with respect to homozygosity or heterozygosity for the marker alleles.

Secondly, there is a significant and positive correlation between genetic distance of the parents and performance of the hybrids. This result represents a potential selection criterion in breeding program if some yield component are the desired characters. Crosses should then be carried out in order to ensure a maximal genetic distance between parents.

Concerning other traits such as plant height, investigations should focus on the identification of marker linked to QTLs involved in expression of the character and could lead to a marker-assisted selection scheme in the tomato breeding.

According to present results, RAPD-based genetic distances could be used to help in the choice of the crosses to be made among tomato lines and in this way reducing the number of hybrids to be evaluated.

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