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## ESTIMATION OF GENETIC DISTANCES AMONG GREEN PEPPER (*Capsicum annuum*. L.) LINES USING RAPD MARKERS AND ITS RELATIONSHIP WITH HETEROSIS

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**SUMMARY.** *Capsicum annuum* is widely cultivated around the world. The main producers are Hungary, India, Mexico, China, and Korea. The enormous genetic diversity available for pepper breeding has facilitated the development of new varieties and hybrids. Several pepper breeders coincide in that the level of heterosis exhibited by pepper hybrids is directly related with the genetic distance between their parental lines, therefore the importance of development of reliable techniques for the estimation of genetic distance. The search of superior hybrid parents in pepper breeding programs is commonly based on the estimation of the General Combining Ability (GCA) and Specific Combining Ability (SCA) of inbred lines. However, the application of this procedure is expensive and time consuming. The development of DNA based molecular markers represents an alternative procedure for the identification of promising parental lines for high performance hybrid production. The Random Amplified Polymorphic DNA (RAPD) markers have been widely used for the estimation of genetic distance among closely related individuals. Thus molecular markers, as RAPD, could be used for germplasm classification and clustering, producing so valuable information for heterosis prediction. The aim of this research was to study the relationship between the genetic distance, measured using RAPD markers, among parental lines, and the heterosis, observed as yield, of their F<sub>1</sub> hybrids. Estimations of GCA, SCA, and heterosis were performed using seven elite lines and their F<sub>1</sub> hybrids of "Serrano" pepper. The 28 treatments (7 lines and 21 hybrids) were distributed in the field following a complete block design with 4 four? replicates. A genetic analysis for the estimation of GCA and SCA was performed using the method 2 of Griffing (1956). Heterosis was estimated as the difference between the F<sub>1</sub> and the average of the two parents. The RAPD markers were generated using the set B of primers from OPERON (Alameda, California) following the protocol of Williams *et al.* (1990). The genotypes tested were statistically different for fruit yield. Among all the hybrids and parental lines, the F<sub>1</sub> (P05 x P01) produced the highest yield. Also, GCA and SCA were statistically significant, with P07 showing the highest GCA effect, and the F<sub>1</sub> (P05 x P01) the highest SCA. The F<sub>1</sub> (P06 x P01) showed the highest heterosis (178.5%). Genetic distances calculated on RAPD markers produced a dendrogram with seven nodes for the parental lines. However, the correlation between the matrix of genetic distances among parental lines and the matrix of heterosis was low ( $r = 0.3281$ ) and not significant.

**INTRODUCTION.** The "Serrano" or green pepper *Capsicum annuum* is widely cultivated around the world. The main producers are Hungary, India, Mexico, China, and Korea. The more probable ancestor of *C. annuum* is the "Piquín" pepper *C. annuum* var. *aviculare*. Piquín is extensively distributed from South America up to the south of The United States. However, probably the main area of domestication includes Central America and México (De Witt y Bosland, 1996).

The enormous genetic diversity available in Mexico has facilitated the development of new varieties and hybrids. The methods used for pepper breeding in Mexico include pedigree, single seed

descend, and a combination of both (Ramírez, 1996). Plant breeders working with several species have reported a direct relationship between the level of heterosis exhibited by the F<sub>1</sub>, and the divergence between their parents (Lee *et al.* 1989; Sekhon and Gupta, 1995). Therefore, estimation of genetic distance can be useful for prediction of high performance crossings (Smith *et al.* 1990). Actually, identification of parental lines is based on estimation of GCA and SCA (Ordás, 1991); these parameters are routinely estimated using diallelic designs (Griffing, 1956). Furthermore, diallelic designs can be used to estimate the genetic components of phenotypic variation (Vasal y Cordova, 1996). However, the application of these techniques is expensive and time consuming. The use of molecular markers has been proposed as an alternative procedure (Toby *et al.* 1999). Molecular markers based on polymorphisms of the DNA are specially useful for this enterprise, because they are not affected by the environment (Tatineni *et al.* 1996). Several examples of the application of molecular markers to calculate genetic distances have been produced in maize (Smith *et al.* 1990) and rice (Zhang *et al.* 1995).

The Random Amplified Polymorphic DNA (RAPD) technique has been widely used to quantify the genetic variation due to its simplicity and power to detect differences, even among closely related individuals, in species of *Brassica* (Jain *et al.*, 1994), *Lens* (Abo-elwafa *et al.* 1995), *Petunia* (Cerny *et al.* 1996) and *Pisum* (Hoey *et al.* 1996). Additionally, this type of markers are extremely useful to estimate genetic distances, mainly due to their productivity in terms of number of markers per essay, sensibility, quickness, and possibility of automation (Laucou *et al.* 1998).

The main aim of this research was to determine the relationship between genetic distance, estimated based on RAPD markers, and the heterosis for fruit yield in "Serrano" pepper. Additionally, GCA and SCA were quantified to study several parental lines and the heterosis shown by their F<sub>1</sub> hybrids.

**MATERIALS AND METHODS.** The field phase of this research was conducted at The Experimental Research Station "Sur de Tamaulipas" (CESTAM), of The Northeast Regional Research Center (CIRNE), National Forestry, Agricultural and Animal Science Institute (INIFAP) at Estación Cuauhtémoc, Tamaulipas, Mexico. The molecular markers work was developed at the Genetics Laboratory, Facultad de Agronomía, Universidad Autónoma de Nuevo León, at Marín, Nuevo Leon, Mexico.

Seven advanced lines of "Serrano", developed by the pepper breeding program of CESTAM, were crossed in all possible direct combinations to produce 21 F<sub>1</sub> hybrids. In this paper, only the results obtained for the accumulative fruit yield produced by the first three harvests is reported. The 28 genotypes (7 parental lines and 21 hybrids) were distributed in the field, following a complete block design with three replicates. The genetic design applied was the method 2 of Griffing (1956). Heterosis was estimated as the deviation of the F<sub>1</sub> from the average of its two parents (Vasal y Córdoba, 1996).

A modified version of the Doyle and Doyle (1990) protocol was used for DNA isolation. The DNA yield was estimated by flourimetry (Hoeffer Scientific, San Francisco, USA). The samples were obtained from plants growth two grams of leaves free of infections were collected.

RAPD markers were generated following the procedure proposed by Williams *et al.* (1990), using the set B (20 10mer oligonucleotides) of primers from Operon Technologies, (Alameda, CA. USA.). The PCR was performed in a Gene Cycler™ (BIORAD) thermocycler. All PCR components were buy from Promega



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(Madison, Wisconsin, USA). The PCR mixture consisted of dNTPs (0.1 mM), *Taq* DNA polymerase (1.25 U), *Taq* buffer (1X), MgCl<sub>2</sub> 3.5 mM primer 0.3 μM, genomic DNA (25 ng) in a total volume of 25 μl. The thermal program was: 94°C for 1 minute; 45 cycles (93°C for 1 minute, 40°C for 1 minute, 72°C for 2 minutes); and 72°C for 10 minutes. The PCR products were electrophoresed in a 2% agarose gels using a Sub-Cell GT apparatus (BIO-RAD). After tinsion in Ethidium Bromide, the gels were photographed on a UV transilluminator using a digital (Kodak model DS-120) and an instant camera (Polaroid).

Before extensive RAPD data were generated, the reaction conditions were optimized, mainly in terms of MgCl<sub>2</sub> concentration, to avoid spurious differences in PCR amplification. Each amplification product (or bands), of a specific molecular weight, was registered in each parent as a binary variable: absent (0), or present (1). The molecular weights of the RAPD bands were estimated using the Kodak Digital Science 1D™ softw are. A matrix of 7 columns (parental lines) by 53 rows (RAPD) was constructed with the molecular marker data. This matrix was used to estimate genetic distances by the formula of Nei (1972) which is implemented in the Tools for Population Genetic Analyses (TFPGA) software. Moreover, dendograms were produced by the same software using the UPGMA (Unweighted Pair Group Method with Arithmetical Averages) method. The Mantel test (Mantel, 1967) was applied to determine the statistical significance of the correlation between the matrix of genetic distances among parental lines and the matrix containing the heterosis values

**RESULTS AND DISCUSSION.** Highly significant differences among genotypes were detected for fruit yield (Table 1), indicating abundant genetic variability for this trait. The highest yielding parent was P03, even it was statistically similar to P02, P04, P05, and P07. Parental lines P06 and P01 presented the lowest fruit yield. Among all the hybrids and parental lines, the F<sub>1</sub> (P05 x P01) produced the highest yield (Table 2).

Table 1. Mean squares for fruit yield.

S.V.	M.S.	S.V.	M.S.
Treatments	431.084**	GCA	150.511 **
Replicates	3.625NS	SCA	540.322 **
Error	16.153	EE	16.784
C.V	9.54 %		9.77 %

NS. Non significant. \*Significant at 0.05. \*\*Significant at 0.01.

As can be seen in Table 1, the mean squares of GCA and SCA were highly significant. In accordance with Sprague y Tatum (1942), these results suggest the presence of additive and dominance effects in the genetic control of fruit yield.

The line P07 showed, by large, the highest general combining ability. On the other hand, the highest specific combining ability corresponded to P05 and P01; however, P04 x P02, P03 x P01, P07 x P04, and P06 x P02, produced hybrids of similar yield to P05 x P01.

As can be seen in Table 2, the F<sub>1</sub> (P06 x P01) showed the highest heterosis with 178.5%, and a relatively high SCA. In general, the heterosis values found in this study are well over others reported in pepper (Milerue and Nikornpun, 2000; Owen, 1992).

As mentioned before, P07 exhibited the highest GCA; however this line just participated in three out of ten of the best hybrids, in

terms of heterosis. On the other hand P01, with an intermediate GCA, produced five out of ten of the best hybrids in terms of heterosis, and in four out of ten of the highest values of SCA. These results suggest that the SCA effects were more important than the GCA effects in this population.

Table 2. Yield, GCA, SCA, and heterosis in genotypes of "Serrano" pepper

Genotype	Yield t ha <sup>-1</sup>	GCA	Heterosis %
P01 Chiser T-74	18.104	-0.2	-
P02 Chiser T-74-26	25.507	-2.1	-
P03 Chiser P8-60	31.685	-1.3	-
P04 Chiser 21-20-13	21.069	-2.4	-
P05 Chiser 16-34	27.467	0.6	-
P06 Chiser 28-102	19.162	0.8	-
P07 Chiser 29-119	30.086	4.6	-
-	-	SCA	-
P02 X P01	34.538	-5.1	58.4
P03 X P01	53.270	12.9	114.0
P04 X P01	40.670	1.4	107.6
P05 X P01	59.900	17.7	162.9
P06 X P01	51.893	9.5	178.5
P07 X P01	56.680	10.4	135.2
P03 X P02	36.997	-1.6	29.4
P04 X P02	50.507	13.0	116.9
P05 X P02	47.618	7.2	79.8
P06 X P02	51.947	11.3	132.6
P07 X P02	44.158	-0.3	58.9
P04 X P03	34.959	-3.3	32.5
P05 X P03	40.032	-1.2	35.4
P06 X P03	45.794	4.3	80.1
P07 X P03	49.577	4.3	60.5
P05 X P04	39.653	-0.5	63.4
P06 X P04	49.099	8.8	144.1
P07 X P04	56.880	12.8	122.4
P06 X P05	47.400	4.1	103.3
P07 X P05	50.943	3.9	77.0
P07 X P06	58.084	10.8	135.9
Tukey	12.187	-	-
D.M.S.	-	6.7	-

P= Parent.

The modification of the Doyle and Doyle (1990) protocol for the isolation of genomic DNA produced yields in the range of 508 to 773 ng μL<sup>-1</sup> (Table 3), and good quality.

After screening, seven out of twenty primers produced polymorphic bands considering the seven parental lines. The selected primers were: OPB-01 5'-GTTTCGCTCC-3', OPB-05 5'-TGCGCCCTTC-3', OPB-06 5'-TGCTCTGCC-3', OPB-07 5'-GGTGACGCAG-3', OPB-



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10 5'-CTGCTGGGAC-3', OPB-14 5'-TCCGCTCTGG-3', and OPB-17 5'-AGGGAACGAG-3'. These primers generated bands in the range of 500 and 2000 base pairs. Similar results were obtained by Williams *et al.* (1990) with random primers. Fifty three RAPD markers were scored and used to calculate genetic distances among the parental lines. In Figure 1, the band pattern produced by primer OPB-01 is shown.

Table 3. DNA yields

Parental line	DNA Concentration (ng $\mu\text{l}^{-1}$ )
P01	631.92
P02	686.14
P03	773.49
P04	631.92
P05	692.16
P06	508.43
P07	520.48

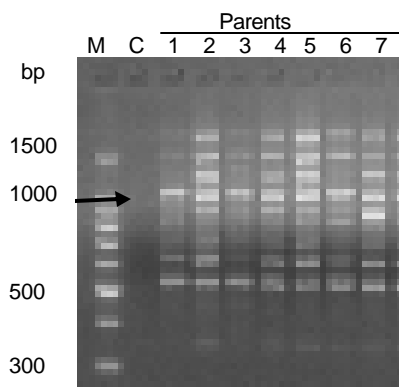


Figure 1. Amplification products generated with primer OPB- 01. M= Molecular weight marker. C= Negative control

As can be seen in Table 4, the longest genetic distance was between P01 and P08 with 0.879. It means that these two lines are 87.9% different in terms of the portion of the genome surveyed by the 53 RAPD markers. On the other hand, the closest genetic distance corresponded to P03 and P04 with 0.164.

Table 4. Genetic distances and heterosis values of seven advanced lines and their  $F_1$  hybrids.

GENETIC DISTANCES							
	P01	P02	P03	P04	P05	P06	P07
P01		0.387	0.505	0.569	0.879	0.281	0.603
P02	0.584		0.257	0.307	0.751	0.359	0.387
P03	1.139	0.294		0.164	0.474	0.474	0.233
P04	1.076	1.168	0.325		0.359	0.415	0.186
P05	1.628	0.798	0.353	0.633		0.536	0.333
P06	1.784	1.325	0.801	1.440	1.033		0.444
P07	1.352	0.588	0.605	1.223	0.770	1.358	
HETEROSIS VALUES							

A dendrogram containing the seven advanced pepper lines was constructed using the 53 RAPD markers (Figure 2). The dendrogram consisted of six nodes: 1) P03 and P04 were joined at a genetic distance of 0.1636, 2) P03, P04 y P07 at a distance of 0.2094, 3) P02, P03, P04 and P07 at 0.3167, 4) P01 and P06 at 0.2814, 5) P01, P02, P03, P04, P06 and P07 at 0.4694, and 6) P01, P02, P03, P04, P05, P06 and P07 at 0.5555. This indicates that the parental lines P03 and P04, and P01 and P06 are closely related among them and they differ only in 16 and 28 percent of the polymorphisms RAPD respectively.

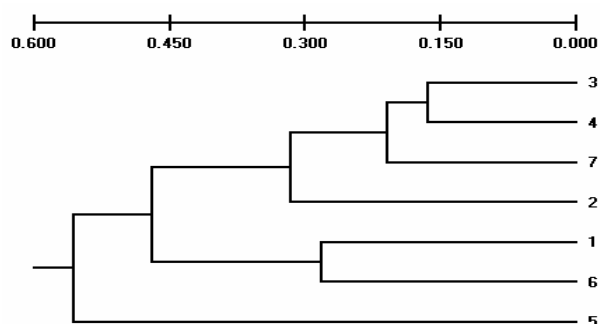


Figure 2. Dendrogram based on RAPD markers showing the genetic distances among parental lines.

The correlation between the genetic distances matrix and the heterosis matrix was low ( $r = 0.3281$ ) and non significant, or in other words, the genetic distances among parental lines is not related with the heterosis of their hybrids (Table 4). However, P05 and P01 were the more divergent parents and produced the highest fruit yield and the second highest heterosis, this shows an isolated tendency since, it was observed that parental lines with smaller divergence had low values of heterosis (Lee *et al.* 1989; Sekhon y Gupta, 1995). However, the limited genome coverage, or the possibility that the molecular markers used are not close enough to the genes controlling fruit yield in pepper, did not permitted to extract final conclusions about the relationship between genetic distance among parents and the heterosis of their  $F_1$ s. Similar results have been obtained in maize (Melchinger *et al.* 1990; Dudley *et al.* 1991). Also, Martín *et al.* (1995), Barbosa-Neto (1996) and Burkhamer *et al.* (1998) reported low correlations between genetic distances and heterosis in wheat. Charcosset *et al.* (1991) indicates that the detection of molecular markers which are not close to the genes responsible for the assayed trait may decrease the correlation between genetic distance and heterosis. Bernardo (1992), mentions that is essential to identify a specific marker related to the segments of the genome which determine the expression of the traits of interest to find a high correlation between genetic distance and heterosis. It may be expected that genetic distances, calculated using molecular markers, will become a useful way to predict heterosis until genes controlling important traits are placed on highly saturated genetic linkage maps and the adequate markers, those strongly linked, can be chosen to calculate the genetic distance.

**CONCLUSIONS.** The RAPD protocol worked efficiently in "Serrano" pepper producing 53 markers based on only seven primers. In general, the correlation between genetic distances among parents and the heterosis shown by their hybrids was no significant, maybe because the molecular markers used to calculate genetic distances are not linked to the genes controlling fruit yield.



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## BIBLIOGRAPHY

- Abo-elwafa, A., K. Murai, and T. Shimada. 1995. Intra and inter-specific variations in Lens revealed by RAPD markers. *Theor. Appl. Genet.* 90: 335-340.
- Barbosa-Neto, J.F., M.E. Sorrells, and G. Cisar, 1996. Prediction of heterosis in wheat using coefficient of parentage and RFLP based estimates of genetic relationship. *Genome* 39: 1142–1149.
- Bernardo, R. 1992. Relationship between single cross performance and molecular marker heterozygosity. *Theor. Appl. Genet.* 83: 628-634.
- Burkhamer, R.L., Lanning S.P., Martens R.J., Martin J.M., and Talbert L.E. 1998. Predicting progeny variance from parental divergence in hard red spring wheat. *Crop Sci.* 38: 243-248.
- Cerny, T.A., G. Caetano-Anollés., R.N. Trigiano, and T.W. Starman. 1996. Molecular phylogeny and DNA amplification Fingerprinting of *Petunia* taxa. *Theor. Appl. Genet.* 92: 1009-1016.
- Charcosset, A., M. Lefort-Buson, and A. Gallais, 1991. Relationship between heterosis and heterozygosity at marker loci: a theoretical computation. *Theor Appl Genet* 81: 571–575.
- De Witt, D., and P.W. Bosland. 1996. Peppers of the World. An Identification Guide. Ten Speed Press. Berkeley, California. 219 p.
- Doyle, J.J., and Doyle, L.H. 1990. Isolation of plant DNA from fresh tissue. *Focus.* 12: 13-15.
- Dudley, J.W., M.A. Saghai-Marooof, and G.K. Rufener, 1991. Molecular markers and the grouping of parents in maize breeding programs. *Crop Sci.* 31: 718–723.
- Griffing, B. 1956. Concept of general and specific ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* 9: 443-452.
- Hoey, B.K., K.R. Crowe., V.M. Jones., and N.O. Polans. 1996. Aphylogenetic analysis of *Pisum* based on morphological characters, and allozyme and RAPD markers. *Theor. Appl. Genet.* 92: 92-100.
- Jain, A., S. Bhatia., S.S. Banga., S. Prakash, and M. Lakshmikiran. 1994. Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationships to heterosis. *Theor. Appl. Genet.* 88: 116-122.
- Laucou, V., K. Haurogn., N. Ellis, and C. Rameau, 1998. Genetic mapping in pea. 1. RAPD-based genetic linkage map of *Pisum sativum*. *Theor. Appl. Genet.* 97: 905–915.
- Lee, M., E.B. Godshalk., K.R. Lambey, and W.L. Woodman. 1989. Association of restriction length polymorphism among maize inbreds whit agronomic performance of their crosses. *Crop Sci.* 29:1067-1071.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27: 209-220.
- Martin, J.M., L.E. Talbert., S.P. Lanning, and N.K. Blake. 1995. Hybrid performance in wheat as related to parental diversity. *Crop Sci.* 35: 104–108.
- Melchinger, A.E., M. Lee., K.R. Lamkey, and W.L. Woodman. 1990. Genetic diversity for restriction length polymorphisms: relation to estimated genetic effects in maize inbreds. *Crop Sci.* 30: 1033-1040.
- Milerue, N., and M. Nikompun. 2000. Studies on Heterosis of Chili (*Capsicum annum L.*). *Kasetsart J. Nat. Sci.* 34: 190-196.
- Nei, M. 1972. Genetic distance between population. *Am Nat.* 106: 283-292.
- Ordás, A. 1991. Heterosis in crosses between American and Spanish populations of maize. *Crop Sci.* 31: 931-935.
- Owens, K. 1992. Breeding pepper varieties for comercial processing. Proceedings of the conferences on chili pepper production in the tropics. Kualalumpur. 195-201p.
- Ramírez, M.,M. 1996. El chile. Biodiversitas. Boletín bimestral de la comisión nacional para el conocimiento y uso de la biodiversidad. 8: 8-14.
- Sekhon, M.S., and V.P. Gupta. 1995. Genetic distance and heterosis in Indian mustard: developmental isozymes as indicators of genetic relationships. *Theor. Appl. Genet.* 91: 1148-1152.
- Smith, O.S., J.S.C. Smith., S.L. Bowen., R.A. Tenborg, and S.R. Wall. 1990. Similarities among a group of elite maize inbreds as measured by pedigree, F 1 grain yield, grain yield, heterosis, and RFLPs. *Theor. Appl. Genet.* 80: 833–840.
- Sprague, G.F., and L.A. Tatum. 1942. General vs. specific combining ability in single crosses of corn. *J. Amer. Soc. Agron.* 34: 923-932.
- Tatineni, V., R.G. Cantrell, and D.D. Davis. 1996. Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. *Crop Sci.* 36: 186-192.
- Toby, A.J., T.M. Abberton., T.M. Michaelson-Yeates, and J.W. Foster. 1999. Relationships between genetic distance measured by RAPD-PCR and heterosis in inbred lines of white clover (*Trifolium repens L.*). *Euphytica* 107: 159–165
- Vasal, S.K. and H. Cordova. 1996. Heterosis en maíz: Acelerando la tecnología de híbridos de dos progenitores para el mundo en desarrollo. Memorias del Curso Internacional de Actualización en Fitomejoramiento y Agricultura Sustentable. México. pp. 32-54.
- Williams, J.G., A.R. Kubelik., K.J. Livak., J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.
- Zhang, Q., M.A. Saghai., G.P. Yang., K.D. Liu., Z.Q. Zhou., K.A. Gravois., C.G. Xu, and Y.J. Gao. 1995. Correlations between molecular marker polymorphism and hybrid performance in rice. *Plant Genome IV Conference. San Diego, CA.* p. 307.