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RELATIONSHIPS BETWEEN HYBRID PERFORMANCE AND GENETIC DISTANCE REVEALED BY MORPHOLOGICAL AND AFLP MARKER IN CUCUMBER

ABSTRACT

This study investigated the relationship of morphological and molecular genetic distance with hybrid performance and heterosis in cucumber in an attempt to make use of genetic distance in predicting hybrid performance. The results of this study showed that GD, in general, correlated poorly with heterosis and SCA. Results showed that the mean values of the hybrids were significantly larger or smaller for many traits when compared with the mean of parental lines, indicating that heterosis was present for these traits. In next step we compare inter group hybrids versus intra group hybrids. This test showed that intra group hybridization although increased the yield and yield component but decrease some fruit quality such as fruit color and shape.

Key words: cluster analysis, diallel, heterosis, orthogonal comparison, similarity matrix

INTRODUCTION

Hybrid vigor or heterosis is one of the most important phenomena in genetics, evolutionary biology and applied breeding. In general, heterosis refers to the higher performance of an F_1 hybrid over the mean of the two parents or better parent. In agricultural sense, the F1 should outperform the better parent to be useful. Heterosis has also been applied to adaptive traits like increased yield, viability and resistance to biotic and abiotic stress (Dobzhansky, 1950). Although breeders and farmers have long used hybrid varieties to produce high-yield and quality agricultural products, nonetheless the genetic basis for hetero-

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sis still remains unclear (Coors and Pandey, 1999). For example, the association of genetic distance with heterosis in elite inbred lines of corn may be very strong (Lee et al, 1989; Smith et al, 1990) or weak (Godshalk et al, 1990; Dudley et al, 1991), because the correlation between marker distance and F1 performance depends on the origin of lines studied (Melchinger *et al.* 1990; Boppenmaier *et al*. 1993). Genetic background plays an important role in heterosis.

Identification of parental combinations that produce hybrids of superior yield is the most important step in developing hybrids. However, this is one of the most costly and a time-consuming step in any hybrid breeding program, as it is necessary to cross the available inbred lines and evaluate the hybrids in extensive yield trials. Thus, because of space limitations only a limited number of hybrids generated from a relatively small number of inbred parents can be evaluated (Bernardo 1992). Some researcher believe that parents with a higher general combining ability and a large genetic distance produce a hybrid with better yield performance (Cox and Murphy 1990, Diers *et al.*1996).

By this background morphological and molecular marker genetic diversity studies in relation to hybrid performance have been undertaken in several crops. Positive associations between morphological genetic distance and heterosis were reported among crosses in wheat (Cox and Murphy 1990) and alfalfa (Riday *et al.* 2003). Investigations in maize, rice and oilseed rape have shown that the molecular genetic diversity of parents was significantly correlated with hybrid performance and that yield heterosis could be predicted using molecular markers (Diers *et al.* 1996, Riaz *et al.* 2001, Betran *et al.* 2003). In addition to the identification of potentially high-yielding hybrids, genetic distance measurements help to assign new pure lines to heterotic groups.

A prerequisite in using markers for predicting hybrid performance is a strong correlation between genetic distance and F1 performance (heterosis). Studies conducted in maize to investigate the relationship between marker distance and hybrid performance have produced mixed results (Lee et al 1989, Smith et al 1990, Godshalk et al 1990, Melchinger et al 1990, Dudley et al 1991, Boppenmaier *et al.* 1993). In rice, Zhang *et al.* (1994, 1995) studied the relationship of molecular marker heterozygosity with hybrid performance and heterosis in a number of characters using eight elite parental lines of hybrid rice in China. Their analyses showed correlations to be mostly low between general heterozygosity, which was based on all the markers included in the study, and F1 performance and heterosis. In contrast, very high correlations were detected between mid-parent heterosis and specific heterozygosity, which was based only on the markers that detected significant effects on each trait, for most of the traits. They suggested that such a high level of correlation might have a practical utility in predicting heterosis.

Ohara *et al.* (2005) showed that genetic distances based on AFLPs correlated significantly with heterosis over the mid-parent for each bunching onion seedling trait but the genetic distances were not correlated with heterosis over the

better parents. Liu et al (2002) showed that the genetic diversity among the parental lines was certainly related to F1 heterosis. In other hands Geleta et al (2004) showed that the correlations of AFLP measured genetic distances with mid and high parent heterosis were non-significant for all characters, except for pepper fruit diameter, and proved to be of no predictive value.

Thus, the objectives of this study were to measure morphological traits and amplified fragment length polymorphism (AFLP) marker-based genetic diversity among sex parental lines and assess the relationship between genetic distance and the performance of hybrids derived from them.

MATERIALS AND METHODS

Germplasm

Sample seeds of 6 cucumber lines were received from Europe (BH 502, BH 504, BH 604, BH 605) and two (115 and 118) from the World Vegetable Center in Asia. All lines were crossed with a partial diallel test in which reciprocal crosses are not used because previous research indicated that direct (Parent A as a female and parent B as a male) and reciprocal crosses (Parent B as a female and parent A as a male) do not affect many traits in cucumber (Kanobdee *et al.*, 1990; Wadid *et al.,* 2003).

Design

The experiment was a randomized complete block design with 15 F_1 hybrid and 6 parents and three replications. Forty seeds were planted in plots 3.1 m long as recommended by Swallow and Wehner (1986) on raised shaped beds. Plots were planted 8 July 2009. All research was conducted at the Agricultural research field in University of Guilan, Rasht, North of Iran I.R (37^o 16' N) using standard cultural procedures for growing pickling cucumbers. Plots were thinned to 30 plants (64,500 plants/ha) on 22 July 2009. Plots were harvested when almost of the plots contained oversized fruit (> 51 mm in diameter) as recommended by Miller and Hughes (1969) for optimum fruit yield in once-over harvest of pickling cucumbers.

Data collection

Number of fruits per plant was counted to obtain early (EY), marketable (MY) and total yield (TY). Early fruit were the number of oversized fruit at harvest (>51 mm in diameter). The number of marketable fruit was calculated as total fruit minus culls. Cull fruits (CF) were misshapen (crooked or nubbin).

Other characteristics including days to harvest (DH), number of node per plant (NN), plant length (PL), number of branch per plant (BN), day to first

male flower appearance (DM), day to first female flower appearance (DF), 1st midrib length (L1), 2nd midrib length (L2), 3rd midrib length (L3), first node length (FNL), marketable yield percent (MY%), simple weight index (SWI), plant length to first fruit (PLF) and number of fruit in main branch (NFMB) were recorded in filed.

In addition, fruit shape (FS), color (FC), seedcell size (FSC) and overall performance (OP) were rated on a scale of 1 to 9, where 1–3 = poor, 4–6 = intermediate, 7–9 = excellent (Strefeler & Wehner, 1986). SWI was calculated in following to Wehner and Cramer formula (Wehner and Cramer. 1996).

Evaluation of AFLP marker

DNA was extracted from approximately 1 g of young fresh leaves collected on ice following the protocol of Rogers and Bendich (1985). The AFLP protocol employed was similar to that of Vos *et al.* (1995). DNA was restricted with *Mse* I and *EcoR* I . A total of 21 primer pairs were used to detect the polymorphisms between parents and hybrids. The 21 primer combinations used in this study were:

M + AAC/E + AAG, M + AAC/E + AAT, M + AAC/E + ACG , M + AAC/E + ACT, M + AAC/E + AGT, M + AAC/E + ATC, M + AAC/E + ATT, M + AGA/E + AAG, M + AGA/E + AAT, M + AGA/E + ACG , M + AGA/E + ACT, M + AGA/E + AGT, M + AGA/E + ATC, M + AGA/E + ATT, M + AGT/E + AAG, M + AGT/E + AAT, M + AGT/E + ACG , M + AGT/E + ACT, M + AGT/E + AGT, M + AGT/E + ATC, M + AGT/E + ATT.

The number of polymorphic and monomorphic fragments was determined from the amplified fragments for each primer pair. Only polymorphic bands were used in the construction of a binary matrix. Clear and unambiguous bands were coded to a data matrix as present (1) or absent (0) for all the genotypes.

Data analysis

Griffing's method 2 (parents and one set of F_1 crosses) and 4 (One set of F_1 crosses) model 1 (fixed effect) diallel analysis was used to estimate specific combining ability (SCA) for hybrids (Griffing 1956). Heterosis was determined as follows:

$$MPH_{(\%)} = \frac{F_1 - MP}{MP} \times 100$$

$$HPH_{(\%)} = \frac{F_1 - HP}{HP} \times 100$$

where, $MPH_{(\%)}$ means Mid-parent heterosis, HPH(%) - High-parent heterosis F_1 is the F_1 performance, *MP=(P1 + P2)/2* where P1 and P2 are the performances of inbred parents, respectively, and *HP* is the high parent value.

SCA, MPH and HPH estimated by diallel analysis and simulation (version 1/1).

Genetic distances were determined and a dendrogram compiled via the unweighted pair group method (UPGMA) using NTSYSpc (version 2). Genetic distances among parent and hybrids were estimated according to Jaccard for molecular marker and Karl-Pearson for morphological marker. Values of genetic distance as measured by morphological traits and AFLP markers were correlated with MPH, HPH, and SCA effects to estimate their relationships.

RESULTS AND DISCUSSION

The genetic similarity estimates for all combinations ranged from 0.05 (504 vs. 115) to 0.77 (604 vs. 605) for AFLP and from 0.09 (502 vs. 115) to 0.25 (604 vs. 605) for morphology (Table 1). When the two distance measurements were compared, the mean morphological distance was greater than the AFLP distance, indicating the AFLP has better discriminating power (Geleta *et al.*, 2004).

Table 1:

Estimates of genetic similarity based on amplified fragment length polymorphism (upper diagonal) and morphological (lower diagonal) data for all pairwise combinations of sex parental lines

Parental lines	604	605	504	118	502	115
604	1	0.7692	0.3548	0.5667	0.5667	0.2432
605	0.2481	1	0.3750	0.6333	0.5312	0.2308
504	0.1660	0.1575	1	0.3235	0.4062	0.0476
118	0.1709	0.1720	0.1405	1	0.4706	0.3611
502	0.1355	0.1368	0.2054	0.1159	1	0.2250
115	0.1162	0.1065	0.1035	0.1119	0.0939	1

Cluster analyses based on morphological and AFLP measured distances provided good divisions of the parental genotypes into their heterotic groups (Fig. 1a, b).

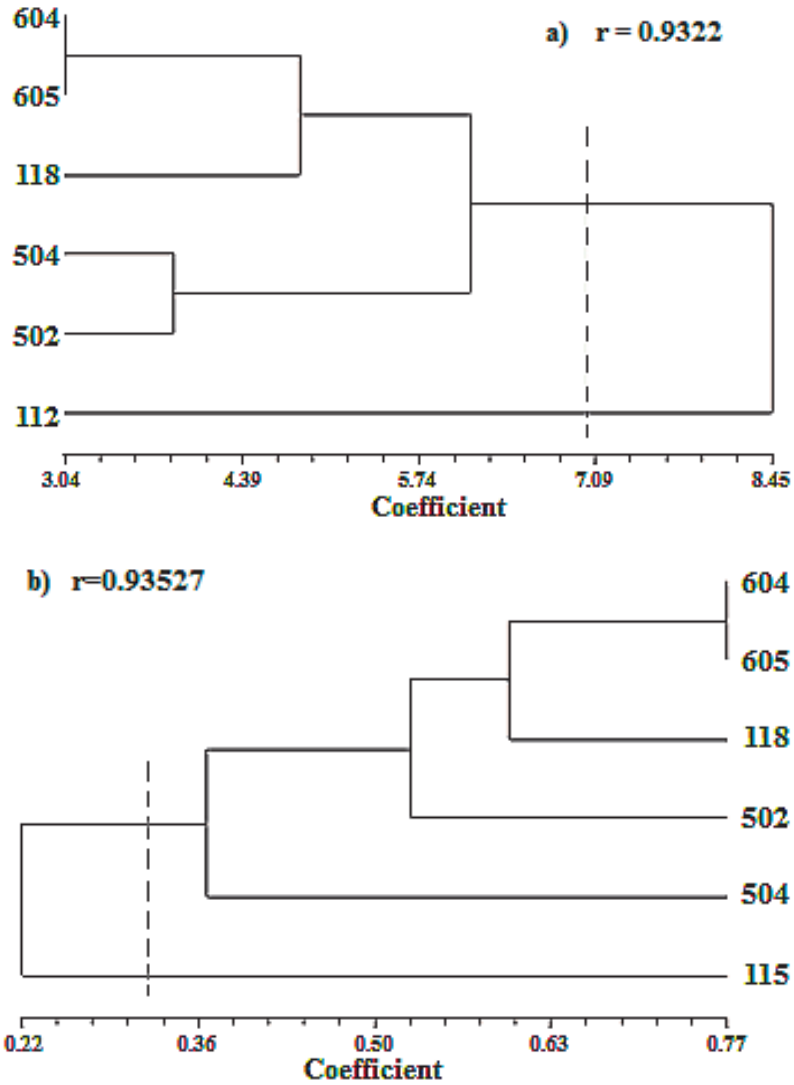

Fig. 1: Dendrogram of the sex parental lines clustered on the basis of morphological data (a) and AFLP marker (b)-based genetic distance estimates

Also, the two measures of distance did group the sex parents differently, but in both methods parental lines were clustered in two major groups. In cases, 502, 504, 118, 604 and 605 generally grouped together compared with 115. MANOVA analysis indicated this division (data not showed). Intra-group variation, as evidenced by GD, was lower than inter-group variation in both diversity measurements. However, the two diversity measures showed significant correlation (r=0.66) with each other.

Table 2

Correlation coefficients between genetic similarity (GS) and SCA estimated follow to Griffing's method 2 model 1 for measured characters

Genetic similarity (GS)	EY	MY	CF	TY	SWI	MY%	BN	PL
Morphological GS	0.495ns	-0.39ns	-0.12ns	-0.38ns	-0.49ns	-0.15ns	0.07ns	-0.18ns
Molecural GS	0.605*	-0.36ns	-0.32ns	-0.37ns	-0.41ns	-0.13ns	-0.04ns	-0.28ns
	NN	L1	L2	L3	FNL	PLF	NFMB	
Morphological GS	-0.08ns	-0.14ns	0.14ns	0.36ns	0.22ns	-0.05ns	-0.03ns	
Molecural GS	-0.08ns	-0.39ns	0.01ns	0.13ns	0.13ns	-0.08ns	-0.28ns	
	DM	DF	DH	FS	OP	FSC	FC	
Morphological GS	0.14ns	-0.05ns	-0.06ns	-0.45ns	0.11ns	-0.03ns	0.12ns	
Molecural GS	0.44ns	0.10ns	-0.06ns	-0.33ns	-0.18ns	-0.16ns	-0.22ns	

Table 3

Correlation coefficients between genetic similarity (GS) and SCA estimated follow to Griffing's method 4 model 1 for measured characters

Genetic similarity (GS)	EY	MY	CF	TY	SWI	MY%	BN	PL
Morphological GS	-0.062ns	-0.28ns	-0.13ns	-0.30ns	-0.21ns	-0.002ns	-0.14ns	0.15ns
Molecural GS	0.3782ns	-0.22ns	-0.22ns	-0.25ns	-0.15ns	0.028ns	-0.28ns	0.05ns
	NN	L1	L2	L3	FNL	PLF	NFMB	
Morphological GS	0.03ns	-0.004ns	0.23ns	0.35ns	-0.17ns	0.06ns	-0.14ns	
Molecural GS	0.03ns	-0.06ns	0.04ns	-0.10ns	-0.4ns	0.29ns	-0.17ns	
	DM	DF	DH	FS	OP	FSC	FC	
Morphological GS	0.12ns	-0.06ns	-0.07ns	-0.1ns	0.22ns	0.05ns	0.16ns-	
Molecural GS	0.24ns	-0.06ns	-0.05ns	-0.07ns	-0.05ns	-0.09ns	-0.62*	

The correlation between GS and SCA don't show any significant model and it is not useful for SCA predicting (Table 2-3) but we found significant correlation between GS and MPH for yield and yield component (Table 4) although these correlations were weaker for HPH (Table 5). However the significant correlations that found between HPH and GS for MY, TY and EY traits are hopeful for future research.

Table 4

Genetic similarity (GS)	EY	MY	CF	TY	SWI	MY%	BN	PL
Morphological GS	0.58*	-0.47ns	-0.11ns	-0.44ns	-0.61*	-0.26ns	0.24ns	-0.34ns
Molecural GS	0.60*	-0.57*	-0.34ns	-0.54*	-0.65**	-0.26ns	0.14ns	-0.42ns
	NN	L1	L2	L3	FNL	PLF	NFMB	
Morphological GS	-0.15ns	-0.1ns	0.05ns	0.53*	0.38ns	-0.13ns	0.24ns	
Molecural GS	-0.18ns	-0.34ns	-0.02ns	0.42ns	0.27ns	-0.08ns	0.12ns	
	DM	DF	DH	FS	OP	FSC	FC	
Morphological GS	0.13ns	0.04ns	-0.04ns	-0.55*	0.1ns	-0.08ns	0.33ns	
Molecural GS	0.53*	0.29ns	-0.06ns	-0.49ns	-0.12ns	-0.19ns	-0.07ns	

Correlation coefficients between genetic similarity (GS) and MPH for measured characters

Genetic similarity (GS)	EY	MY	CF	TY	SWI	MY%	BN	PL
Morphological GS	0.66**	-0.44ns	0.48ns	-0.38ns	-0.23ns	0.34ns	0.42ns	-0.07ns
Molecural GS	0.67**	-0.60*	0.37ns	-0.57*	-0.21ns	0.27ns	0.3ns	-0.25ns
	NN	L1	L2	L3	FNL	PLF	NFMB	
Morphological GS	0.23ns	-0.01ns	0.09ns	0.57*	0.47ns	-0.07ns	0.32ns	
Molecural GS	0.005ns	-0.29ns	-0.08ns	0.37ns	0.33ns	-0.11ns	0.11ns	
	DM	DF	DH	FS	OP	FSC	FC	
Morphological GS	0.26ns	0.08ns	0.21ns	0.1ns	0.54*	-0.06ns	0.31ns	
Molecural GS	0.32*	-0.05ns	0.56*	0.2ns	0.43ns	-0.22ns	0.07ns	

Table 5

Correlation coefficients between genetic similarity (GS) and HPH for measured characters

The orthogonal test of parent versus hybrids showed that hybridization increased positively yield, yield component and fruit quality (data not shown). In fact this test indicated that hybridization is a very important method in cucumber breeding program. Quantitative genetic theory states that heterosis is a function of genetic diversity between parents (Falconer 1989). In the present investigation, the mean values of the hybrids were significantly larger or smaller for many traits when compared with the mean of parental lines, indicat-

ing that heterosis was present for these traits. In next step we compare inter group hybrids versus intra group hybrids (Table 6). This test showed that intra group hybridization although increased yield and yield component but decreased some fruit quality such as fruit color and shape.

Table 6

Entry	EY [kg·plant^{-1}]	MY [kg·plant^{-1}]	CF [kg·plant^{-1}]	TY [kg·plant^{-1}]	SWI	MY [%]	BN	PL [cm]
Intra group hybrids	1.18	2.66	0.63	2.91	4.241	82.07	0.65	129.51
Inter group hybrids	1.1	2.01	0.55	2.18	4.237	83.78	0.59	124.68
Intra group hybrids vs.	ns	**	ns	**	**	ns	**	**
	NN	L1 [mm]	L2 [mm]	L3 [mm]	FNL [mm]	PLF [cm]	NFMB	
Intra group hybrids	23.67	49.34	80.91	90.19	35.29	5.54	1.32	
Inter group hybrids	25.45	49.63	82.38	93.96	40.65	5.49	1.49	
Intra group hybrids vs. Inter group hybrids	**	**	**	**	**	**	**	
	DM	DF	DH	FS	OP	FSC	FC	
Intra group hybrids	38.93	42.00	69.93	5.6	4	4.07	6.07	
Inter group hybrids	42.47	49.02	82.37	6.77	5.63	5.2	6.00	
Intra group hybrids vs. Inter group hybrids	**	**	**	**	**	**	**	

Mean values for fruit yield, yield components and other agronomic characters for parents and F1 hybrids from a sex-parent halfdiallel mating set in cucumber

The results of this study showed that GD, in general, correlated poorly with heterosis. Previous studies in various crop species such as maize (Ajmone-Marsan *et al.* 1998, Benchimol *et al.* 2000), rice (Kwon *et al.* 2002), wheat (Martin *et al.* 1995), alfalfa (Riday *et al.* 2003) chickpea (Sant *et al.* 1999) and pepper (Geleta *et al.*, 2004) also showed low correlations of GD with heterosis. Geleta *et al.*, believed that although poor correlations between morphological and AFLP distances with the heterosis of most of the measured characters were observed, the majority of the progeny expressed appreciable levels of heterosis in the desired directions for these characters. Heterosis probably also exists due to different allelic combinations at particular loci in each parent which when brought together in hybrid combination, complement each other, resulting in heterosis expression (Bingham *et al.* 1994). Riday *et al.* (2003) indicated that

such loci may not be directly related to observable morphological differences but could have an effect on the physiology of the plant.

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