Characteristics of RAPD markers in breeding of *Cucumis sativus L.*

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Abstract

The goal of this research was to assess genetic polymorphism of RAPD fragments that are relevant to their use in the genetic analysis of cucumber genotypes. It was discussed potential application of RAPD primers in genotype classification by cluster analysis. Besides, RAPD profiles were determined to be dominantly and co-dominantly inherited in first generation hybrids. It was possible to distinguish some of the genotypes due to the presence of several specific DNA products. Genetic distances between parental lines, estimated on the base of RAPD patterns, showed medium (-0,34 and 0,39) and strong (0,81 and 0,84) correlations with productivity of hybrids. As a result, it was shown the possibility of implementation in prognosis of heterosis effect in F_1 hybrids and in breeding programs of Cucumis sativus L.

Keywords: dominance, co-dominance, *Cucumis sativus* L., RAPD

Introduction

The commercial cucumber (*Cucumis sativus* L. var. *sativus*) has a narrow genetic base [3, 10], which hardens the identification of the parameters used to classify cultivars or hybrids of various plants. Several approaches have been developed for this purpose. Isozymes, restriction fragment length polymorphisms (RFLPs), morphological markers, and more recently PCR based methods have been used extensively for genome exploration in cucumber [1, 8, 9, 25, 26]. Each marker type has advantages and disadvantages incumbent for their use [23]. The application of molecular marker technologies has allowed for an increased understanding of its genome and reduces the errors that may arise from phenotypic plasticity. [8, 9, 13]. The potential value of molecular markers for plant variety protection and hybrid production in cucumber has also been discussed [3, 20, 21, 22].

The methodologies, as RAPDs, which amplify DNA fragments in regions containing moderate to high copy sequences can provide for markers in regions of the genome previously inaccessible to analysis by Southern hybridization [14]. RAPD, one of the commonly used and relatively cheap techniques, has shown to be useful for genetic diversity study [29]. Generally, 10-mer primers with 50-80% G+C content are preferred [17]. Thus, well-defined, repeatable RAPD markers can be used in conjunction with co-dominant markers to better describe genomes [24].

Recently, RAPD strategy was still weakly explored for cultivars due to its low reproducibility [5], because only 14% of arbitrary primers can distribute reproducible results regarding molecular marker polymorphism and their identification [28]. High values of RAPD polymorphism was assessed for sunflower - 83% [11], potato – 77,6% [2], sorghum - 55,3% [27].

During breeding program, the RAPD technique was introduced to determine if there is genetic diversity among cucumber cultivars preserved. Likewise, genome mapping of

cucumber has indicated that the frequency of polymorphism increases progressively as the genetic distance between the parents increases [8]. Where the genetic distance between mapping parents is relatively small (i.e., in narrow crossing), the use of RAPD technologies may provide for more critical elucidation of the genome.

The goal of this research was to assess genetic polymorphism of RAPD fragments and the possibility of their implementation in prognosis of heterosis effect in F_1 hybrids and in breeding programs of *Cucumis sativus* L.

Materials and methods

Plant material. As study material were six hybrids of cucumber (*Cucumis sativus* L.) and their parental forms, offered by the Institute of vegetable-growing and irrigation from Tiraspol (tab.1). ***Vzglead*** and ***Epilog*** are commercial hybrids, approved in Republic of Moldova. The rest were experimental hybrids which differed by their productivity: H274 and H273 were high productive, and H6 and H7 – low productive.

Table 1. Genotypic structure of cucumber hybrids

	Hybrid	Parental form	
		♀	♂
High productivity	**H 274**	L 203	L 216
	H 273	L 222	L 203
Standard productivity	**Vzglead**	L 371	Beregovoi
	Epilog	L 371	Favorit
Low productivity	**H 6**	L 222	Beregovoi
	H 7	L 222	Favorit

DNA isolation. Molecular analyses were done on etiolated plants grown for five days. DNA samples were isolated with DNAzol and quantified by spectrophotometer [17].

PCR-RAPD analysis. PCR mixes had volume of 25µl: 50 ng DNA, 200µM of each dNTP, 1 µM of primer, 1,25 U per reaction of GoTaq ADN-polymerase, 2,5 mM $MgCl_2$. The cycling profile was: 95ºC - 3 min, then 45 cycles: 95ºC – 1 min, 36ºC – 1 min, 72ºC – 2 min and 72ºC – 5 min. Amplification products were displayed through electrophoresis in 1,6% agarose gel with 0,5 µg/ml ethidium bromide.

There were screened eight RAPD decamere primers: P28 (GACCGCTTGT), P36 (CCGAATTCGC), P37 (CTGACCAGCC), P43 (AGTCAGCTGC), P44 (GGACCCCGCC), P46 (GGTTGGGGAG), P48 (GCGGTGCTCG), P49 (GACAGCCTA) with G-C base content of 60 – 80%.

The amplicons were notated as P^n, where ***n*** indicating amplicon size, bp.

Data analysis. Amplified fragments were scored as the presence (+) or absence (—) of a fragment [5]. In instances where two or more phenotypic classes were classified as fragment intensity differences, the fragment was counted only if one of the classes was its virtual absence, and the intensity variants were summed into a single 'presence' class.

Genetic similarity (GS) and genetic distance (GD) between the genotypes were estimated [7, 12, 16, 19].

The construction of dendrogrammes was realized on the base of distance matrixes using UPGMA (Unweighted Pairwise Group Method with Arithmetic mean) method [15].

Nonparametric correlations were calculated between GD of parental lines and hybrid productivity [30]

Results

Out of eight primers, four of them (P43, P46, P48, P49) showed no differences of DNA fragments profiles (data not shown), but the other four manifested polymorphism and yielded a total of 37 fragments, with the average of six DNA fragments per primer. About 30 % of amplified products (eleven amplified regions) were reproducible in all the genotypes. RAPD profiles analysis of parental lines revealed PCR products with fragment size of 100-1300 bp. There were shown the differences between the genotypes by the number of components and the intensity of identified bands.

The analysis RAPD amplicons inheritance. Genetic analyses, carried out at various cultivated plants, gave evidence that RAPD fragments were dominant markers [7], though co-dominant inheritance also was observed [4] The segregating fragments were either present or absent confirming the dominant character of RAPD variation in majority of cases.

Primer P28. The analysis of electrophoretic profiles of P28 primer's amplicons revealed their dominant inheritance from parental genotypes to hybrids in all cases (tab. 2). From 12 amplyfied regions, dominantly were transmitted amplicons of 270 bp (at hybrids H273 and H7 from their maternal form L222 and at hybrids H274 and Vzglead from their paternal forms L216 and, respectively, Beregovoi). In two cases it was shown dominant inheritance of 700 bp amplicon, at hybrid Epilog from paternal genotype Favorit and at H6 – from ♀L222. Only at hybrid H274 was observed dominant inheritance of 130 bp amplicons from paternal line L216.

Table 2. RAPD pattern inheritance at different cucumber genotypes

Genotype	P28			P36		P37			P44			
H273		P^{270}			P^{900}					P^{380}	P^{400}	P^{1000}
♀L222		P^{270}			P^{900}					P^{380}	P^{400}	
♂L203												P^{1000}
H274	P^{130}	P^{270}			P^{900}	P^{480}	P^{560}	P^{900}				P^{1000}
♀L203						P^{480}						
♂L216	P^{130}	P^{270}			P^{900}		P^{560}	P^{900}				P^{1000}
Vzglead		P^{270}				P^{480}	P^{560}		P^{300}			P^{1000}
♀L371						P^{480}			P^{300}			P^{1000}
♂Beregovoi		P^{270}					P^{560}					
Epilog			P^{700}	P^{450}	P^{900}			P^{900}				
♀L371				P^{450}								
♂Favorit			P^{700}		P^{900}			P^{900}				
H6			P^{700}		P^{900}			P^{900}				
♀L222			P^{700}		P^{900}			P^{900}				
♂Beregovoi												
H7		P^{270}				P^{480}	P^{560}					
♀L222		P^{270}					P^{560}					
♂Favorit						P^{480}						

Note: index indicate amplicons size, bp; dominant inheritance ☐ and co-dominant inheritance ■.

Primer P36. The fragments amplified by P36 primer were dominantly and co-dominantly transmitted (tab. 2). Hybrids H273 and H6 were characterized by the presence of 900 bp amplicon, similar to maternal form L222, as hybrids H274 and Epilog inherited au 900

bp band from ♂L216 and, respectively, ♂Favorit. For the last hybrid was shown dominant inheritance of 450 bp amplicon, but from maternal line L371. Thus, both of these amplicons of 900 bp and 450 bp specific to different parental genotypes determines co-dominant inheritance and allow to evidence hybrid genotype.

Primer P37. The inheritance of DNA fragments amplified by P37 primer was dominant in two cases and co-dominance in three cases (tab. 2). Altogether, dominantly were transmitted amplicons of 480 bp, 560 bp and 900 bp. Dominant inheritance was revealed at hybrids Epilog and H6, the fragment of 900 bp being passed from ♂Favorit and, respectively, ♀L222.

At hybrid H274 was highlighted co-dominant inheritance of amplicons of 480 bp (from maternal form L203) and of 560 and 900 bp (from ♂L216). Hybrid form Vzglead inherited 480 bp fragment from ♀L371 and 560 bp band from ♂Beregovoi. The same RAPD fragments were transmitted at hybrid H7 (480bp – from ♂Favorit, 560 bp – from ♀L222).

Primer P44. Dominant inheritance of obtained amplicons on the base of P44 primer was evidenced for 1000 bp band at hybrid H274 from paternal line L216 and at hybrid Vzglead from maternal genotype L371 (tab. 2). Also, this line dominantly transmitted in the first generation 330 bp amplicon.

Co-dominantly inheritance of 380 bp, 400 bp, and 1000 bp amplicons was identified at hybrid H273 (two first fragments were transmitted from maternal form L222, the last – from paternal line L203).

Genetic diversity between parental forms. Genetic distance (GD) of parental lines varied between GD=0,00-0,56, in dependency of used primer (tab. 3). Several tendencies were observed: parental forms ♀L222-♂L203 of the hybrid H273 and ♀L222-♂Favorit of hybrid H7 shared similar amplicon profile produced by P28 RAPD primer and, respectively, P36. Parental lines ♀L222-♂L203 of the hybrid H273 and ♀L203-♂L216 of the hybrid H274 exhibited higher variations of molecular pattern generated by RAPD primers.

Table 3. Genetic distance between parental forms of cucumber

	Genetic distance			
	P28	**P36**	**P37**	**P44**
♀L222-♂Beregovoi	0,29	0,15	0,15	0,18
♀L222-♂Favorit	0,41	0,00	0,29	0,18
♀L222-♂L203	0,00	0,15	0,56	0,34
♀L203-♂L216	0,41	0,29	0,56	0,18
♀L371-♂Beregovoi	0,15	0,15	0,41	0,18
♀L371-♂Favorit	0,29	0,15	0,15	0,18

RAPD variations in hybrids. It was determined that GD between parental and hybrid genotypes was between 0,00-0,77 (tab. 4). GD estimated on the base of DNA products of P44 primer showed three minimal values for pairs **H7 - ♂Favorit**, **Vzglead - ♀L371** and **Epilog - ♂Favorit**. Amplicons of P36 primer manifested a single case of total similarity between **H6 - ♀L222.** Maximal value GD (0,77) characterized the differences between **H274** and **♀L203** genotypes based on the DNA fragments produced by P28 primer.

Table 4. Genetic distance between parental and hybrid forms of cucumber

Hybrid	**Parental form**	**Genetic distance**			
		P28	**P36**	**P37**	**P44**
H6	♀L222	0,51	0,00	0,12	0,34
	♂Beregovoi	0,22	0,15	0,29	0,18

H7	♀L222	0,41	0,12	0,12	0,18
	♂Favorit	0,56	0,56	0,56	0,00
H273	♀L222	0,61	0,12	0,12	0,40
	♂L203	0,61	0,29	0,29	0,63
H274	♀L203	0,77	0,29	0,41	0,15
	♂L216	0,34	0,22	0,22	0,18
Vzglead	♀L371	0,41	0,29	0,29	0,00
	♂Beregovoi	0,22	0,15	0,29	0,18
Epilog	♀L371	0,41	0,12	0,29	0,18
	♂Favorit	0,32	0,22	0,12	0,00

Cluster analysis based on RAPD amplified regions. DNA polymorphism expressed by arbitrary primers served to differentiate the genotypes through their grouping (fig. 1).

Primer P28. Cluster analysis of genetic distance between cucumber genotypes demonstrated different capacity to differentiate the genotypes. Thus, on the base of amplicons produced by P28 primer were distinguished two major clusters genotypes:

- I –H274, H273, Vzglead, Epilog, H6 and H7 hybrids, and parental lines L216 and Beregovoi;
- II – maternal lines L222, L371, paternal line Favorit and L203 genotype.

It should be noticed that all hybrid genotypes are situated in the same cluster, but hybrids Vzglead and Epilog are placed on the same position. This showed that RAPD profile obtained on the base of P28 primer didn't ensure strict differentiation between these hybrids.

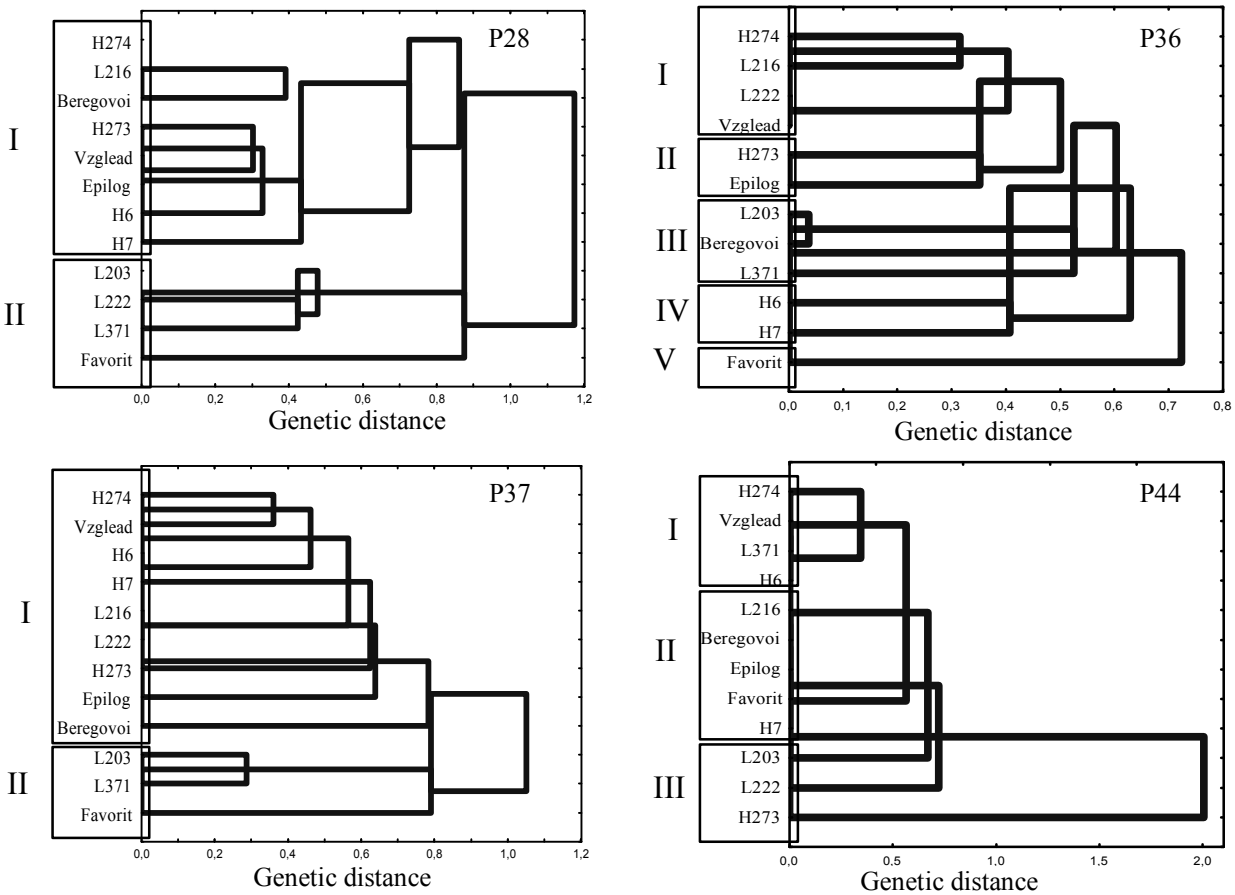

Figure 1. Cluster analysis of cucumber genotypes based on RAPD fragments polymorphism I-V – clusters numbering

Primer P36. Ranging the genotypes on the base of P36 primer amplicons showed a separation in more clusters:

- I –H274 and Vzglead hybrids, and ♂L216 and ♀L222 lines;
- II –H273 and Epilog hybrids;
- III – L203, ♂Beregovoi and ♀L371 lines;
- IV –H6 and H7 hybrids;
- V –♂Favorit line.

It was not possible to distinguish L222 genotype, as parental form, and hybrid Vzglead. Also, cluster analysis showed that H6 and H7 hybrids are situated together, but separately from other genotypes.

Primer P37. On the base of P37 primer were determined two major groups of genotypes:

- I – all hybrid genotypes and maternal line L222 and paternal forms L216 and Beregovoi;
- II – line L203, maternal form L371 and paternal form Favorit.

There was obtained a similar clustering of hybrid genotypes in the same group, as in case of P28 primer. Besides, hybrids H7 and H6 and, respectively, forms ♀L222 and ♂L216 showed a high similarity.

Primer P44. Classification of studied genotypes on the base of P44 primer revealed a lower capacity of differentiation in comparison to other primers, as the analysis places more genotypes in the same position.

Thus, as the result of cluster analysis, hybrids Vzglead and H6 and maternal line L216 occupy the same position, that was determined by lack of the differences for the amplified regions. A higher level of distinction from this group showed several genotypes of other cluster: paternal lines L216, Beregovoi and Favorit and first generation hybrids Epilog and H7. Moreover, all clusters are not well defined. The other genotypes are placed in solitary groups.

The study of correlation of the GD between parental forms and hybrid yield. To check the association of genetic distance to the productivity of cucumber hybrids, there was done correlation analysis according to amplicons profiles obtained by different primers. The correlation varied between -0,34 to 0,84 (tab. 5). It was noticed that genetic distances estimated on the base of P28 primers negatively correlates with the productivity.

Table 5. Correlation analysis between GD and hybrids' productivity

Hybrid	Hybrid productivity*, q/ha	Range of productivity	Genetic Distance			
			P28	P36	P37	P44
H274	***194***	***6***	0,41	0,29	0,56	0,18
H273	***189***	***5***	0,00	0,15	0,56	0,34
Vzglead	***185***	***4***	0,15	0,15	0,41	0,18
Epilog	***160***	***3***	0,29	0,15	0,15	0,18
H6	***150***	***2***	0,29	0,15	0,15	0,18
H7	***145***	***1***	0,41	0,00	0,29	0,18
Correlation coefficient*			**-0,34**	**0,84**	**0,81**	**0,39**

* *the productivity was estimated during field tests for three years by hybrids' authors.*

Similar value of correlation, but positively, was shown for distances calculated on the base of P44 primers. GD estimated on the base of P36 and P37 primers correlates highly positively with hybrid's productivity.

Discussions

Generally, it was disclosed that hybrids H6 and H7 owned high similarities to each other and same tendency of diversity to other genotypes, because they possessed the same maternal line. Moreover, they came from different paternal lines (Beregovoi and, respectively, Favorit cultivars), which in all cases were separated in the same group by all RAPD amplified regions, with exception for P44 primer.

Though the last one possessed low capacity of genotype differentiation, it revealed amplification of three specific fragments: 900 bp and 190 bp for H273 and 800 bp for H274. Also, it was observed co-dominant inheritance for hybrid H273, which showed its usage in evaluation of hybrid level for this genotype.

In comparison to primer P44, P28 was characterized by a high level of genotype differentiation and by dominant inheritance at all hybrids, manifesting two bands: one of 400 bp specific to hybrid H273 and of 730 bp specific to Favorit paternal genotype. As a result, it should be recommended for the cucumber genotypes classification.

Similar features possessed P36 primer, but it was observed also a case of co-dominant inheritance. So, it may be applied both for genotype classification and for estimation of hybrid level (Epilog hybrid).

P37 primer possesses an intermediate degree of differentiation of cucumber genotypes in comparison to P44 primer and P28 and P36 primers. The advantage of its application in hybrid level determination is the fact that it manifested three cases of co-dominant inheritance of its DNA fragments.

RAPD primers used in this research assessed various resolutions of the relationships between cucumber genotypes. Thus, most prospective showed to be primer P28 and P36 which allow revealing genetic relationships. Three of four primers manifested co-dominant inheritance, which allow to their implementation in hybrid level estimation. Moreover, genetic distances between parental genotypes estimated on the base of DNA profiles generated by primers P36 and P37 correlated positively strong with higher productivity at hybrid genotypes. All these, approve mentioned primers to be applied in cucumber breeding programs as good tools in assessing genotype classification and quality control in researching the hybrid vigor manifestation.

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