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Full Length Research Paper

Genetic diversity in some Turkish pepper (*Capsicum annuum* L.) genotypes revealed by AFLP analyses

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The genetic relationships among 14 Turkish pepper (*Capsicum annuum* L) genotypes, 6 of them inbred lines, were determined by comparing their molecular traits. The taxonomic relationships and genetic variation among these genotypes were investigated with those of 5 foreign pepper genotypes. Fifty-six (26%) polymorphic AFLP markers out of total 215 DNA fragments from 4 primer pairs were used to define the genetic similarity among the pepper genotypes by dendrograms or two and three dimensional scaling. Two genotype-specific markers for the genotype PM-702 were among the polymorphic ones. The inbred lines of Alata Agricultural Research Institution were partitioned to similar clusters and constituted extremely low genetic variation. On the other hand, other local Turkish genotypes had comparatively higher genetic diversity.

Key words: *Capsicum annuum*, AFLP, Genetic diversity.

INTRODUCTION

Pepper (*Capsicum annuum* L; 2n = 2x = 24) are important vegetable species both worldwide and in Turkey with the 24.80 and 1.75 million t of production on 1.725 millions and 88 thousands ha area, respectively (Anonymous, 2005). It has been observed that local pepper genotypes in Turkey are rich in diversity and breeding studies have been underway. Turkish local pepper genotypes have been collected for breeding programs where breeders have attempted to evaluate these collections. A comparison of the plant phenotype is the simplest approach for the detection of genotypes and the assessment of genetic diversity; however, phenotypic evaluation is influenced by environment and might not distinguish between closely related genotypes (Rodriguez et al., 1999). Molecular DNA marker analyses which are not affected by environment have been suggested for the determination of genetic similarity among genotypes (Gilbert et al., 1999).

Morphological markers such as flower and fruit morphologies in pepper have been known for very long time and these visually observed markers are small in number and might have epistatic effects (Geleta et al., 2005; Rodriguez et al., 1999). However, DNA markers such as RFLP, RAPD, AFLP, and micro-satellites have been beneficial by being large in number and not affected by the environment, especially in fingerprinting, marker assisted selection and genome mapping. (Paran et al., 1998; Rodriguez et al., 1999; Minamiyama et al., 2006; Lanteri et al., 2003; Rao et al., 2003; Toquica et al., 2003; Portis et al., 2004; Geleta et al., 2005; Ben-Chaim et al., 2001; Oyama et al., 2006).

Amplified fragment length polymorphism (AFLP) is a powerful and reproducible method with the ability to generate a number of polymorphic loci (Vos et al., 1995). With many other crop species (Park et al., 2000), this method is being widely used in pepper studies (Paran et al., 1998; Minamiyama et al., 2006; Lanteri et al., 2003; Toquica et al., 2003; Portis et al., 2004; Geleta et al., 2005; Ben-Chaim et al., 2001). The retrospective analysis of the consequences of breeding and selection for the production of new lines could be succeeded by AFLP (Geleta et al., 2005).

Alata is a state research institute near the Mediterranean sea in Turkey. This institute is working on development new vegetables varieties; pepper is one of these

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Table 1. List of pepper genotypes used in the experiment.

Genotype	Type	Source	Classification of germplasm	County of Origin
HDA-160	Triangular	INRA*	Breeding line	France
Yolo Wonder	Blocky	INRA	Cultivar	France
Ilıca-256	Elongate	Local	Cultivar	Turkey
Kandil	Blocky	Local	Cultivar	Turkey
Alata-7	Elongate	Local	Breeding line	Turkey
Alata-17	Elongate	Local	Breeding line	Turkey
Alata-29	Elongate	Local	Breeding line	Turkey
Alata-38	Elongate	Local	Breeding line	Turkey
Alata-42	Elongate	Local	Breeding line	Turkey
Alata-43	Elongate	Local	Breeding line	Turkey
Kraska	Blocky	Local	Cultivar	Poland
Olenka	Blocky	Local	Cultivar	Poland
Islahiye	Elongate	Local	Cultivar	Turkey
K. Maras	Elongate	Local	Cultivar	Turkey
Pazarcik	Triangular	Local	Cultivar	Turkey
Urfa -246	Blocky	Local	Cultivar	Turkey
PM-702	Campanulate	INRA	Breeding line	France
Urfa local	Blocky	Local	Cultivar	Turkey
Elazig	Blocky	Local	Cultivar	Turkey

vegetables. It has a large number of pepper breeding lines. The institution tries to improve especially elongate fruit shape types pepper for Turkey. Its pepper types are generally elongate and have thin fruit walls. In this study, all of the Alata breeding lines have elongate fruit shape, green (immature)-red (mature) fruit colour, thin fruit wall (10-15 cm fruit length). Plant height ranges from 46 to 65 cm and plant canopy width varies from 90 to 150 cm. Plant branching habit is sparse and leaves have generally ovate shape. Flower position is determined as erect.

The aims of this study are to (1) describe the AFLP-based diversity present among the studied pepper genotypes, (2) identify diagnostic AFLPs which can be used for the identification of breeding lines and (3) compare the level of genetic variation among these genotypes.

MATERIALS AND METHODS

Plant materials

A total of nineteen pepper genotypes (*Capsicum annuum* L.) were used in this study, including eight breeding lines and 11 cultivars. Names, types, sources and origins of breeding lines or cultivars are given in Table 1.

DNA extraction and AFLP analyses

Leaf samples were taken from each accession, frozen in liquid nitrogen and stored at -70°C until use. Genomic DNA was extracted from leaf tissue by the CTAB method of Doyle and Doyle (1987) with minor modifications (Kafkas et al., 2005). The AFLP amplification was performed according to Vos et al. (1995) with minor modifications (Özkan et al., 2006; Kafkas et al., 2005), using four AFLP primer combinations (E_{ACG}/M_{AGG}, E_{AAG}/M_{AAT}, E_{AGC}/M_{AGG} and E_{ACG}/M_{AGT}). The adaptor sequences, pre-selective amplification primers and selective primers are listed in Table 2.

A total of 10 µl of the AFLP and SAMPL selective amplification product were mixed with 10 µl of loading buffer, then denatured at 94ºC for 5 min and placed immediately on ice. About 3 µl of mixture were loaded onto a 4.5% (w/v) polyacrylamide denaturing gel with 0.5 X TBE buffer, with a prerun electrophoresis at 60 W for 30 min and a run at 60 W until the loading dye reached the bottom of the gel. The gels were dried at 80°C for 3 h; an autoradiographic Hyperfilm-MP (Amersham, England) was exposed to the gels for 2 days.

Band scoring and data analysis

The AFLP bands were scored manually as present (1) or absent (0). Only the clearest and strongest bands were recorded and used for the analysis. Genetic distances, based on the proportion of different bands between all pair-wise combinations of genotypes, were calculated by the PAUP 4.0b program (Swofford, 1998). These distances were used to construct an unweighted pair-group method with arithmetic means (UPGMA) tree.

The unweighted pair-group method using arithmetic average (UPGMA) cluster analysis, the resulting dendrograms and multidimensional scaling (MDS) were performed on the genetic distance matrices using the computer program NTSYpc version 2.02k (Rohlf, 1997).

The computer program POPGENE (Yeh et al., 1997) was used to calculate the statistical measures of genetic variation (that is, Nei's gene diversity (Nei, 1973)), Shannon's information index (Shannon and Weaver, 1949) and percentage of polymorphic loci as measured by AFLP markers for Turkish (Alata breeding lines and other local genotypes) and foreign pepper genotypes.

Table 2. Sequences of oligonucleotide adaptors and primers used in the characterization of 9 breeding lines and 10 cultivars of pepper by AFLP marker.

Adaptor / primer	Code	Sequence
Adaptors		
*Eco*RI adaptors		5'- CTC GTA GAC TGC GTA CC -3'
		3'- CAT CTG ACG CAT GGT TAA -5'
*Mse*I adaptors		5'- GAC GAT GAG TCC TGA G -3'
		3'- TA CTC AGG ACT CAT -5'
Preselective amplification primers		
*Eco*RI primer + A	E_A	5'-GACTGCGTACCAATTC+A-3'
*Mse*I primer +C	M_A	5'-GATGAGTCCTGAGTAA+A-3'
Selective amplification primers		
*Eco*RI + 3-ACG	E_{ACG}	5'- GACTGCGTACCAATTC+ ACG -
*Eco*RI + 3-ACG	E_{ACG}	5'- GACTGCGTACCAATTC+ ACG -
*Eco*RI + 3-AGC	E_{AGC}	5'- GACTGCGTACCAATTC+ AGC -
*Mse*I + 3-AGG	M_{AGG}	5'- GATGAGTCCTGAGTAA+ AGG -
*Mse*I + 3-AGT	M_{AGT}	5'- GATGAGTCCTGAGTAA+ AGT -
*Mse*I + 3-AAT	M_{AAT}	5'- GATGAGTCCTGAGTAA+ AAT -3'

Table 3. AFLP primers combination, total number of bands, number and percentage of polymorphic bands detected in the 9 breeding lines and 10 cultivars of pepper.

Primer combination	Total number of bands	Polymorphic bands	
		No.	Percentages
E_{ACG} / M_{AGG}	54	15	278
E_{AAG} / M_{AAT}	29	5	17.2
E_{AGC} / M_{AGG}	56	22	39.3
E_{ACG} / M_{AGT}	60	14	23.3
Total	215	56	26.0
Average	49.75	12.50	

RESULTS

Genetic dis/similarities among pepper genotypes

Four AFLP primer combinations were used in the molecular characterization of 19 pepper genotypes, including eight breeding lines and eleven cultivars. Out of total of 215 DNA fragments, 56 (26%) were polymorphic, averaging 49.75 total bands and 12.50 polymorphic bands per primer combination (Table 3). The maximum number of bands (60) was found for primer combination E_{ACG}/ M_{AGT}, whereas the lowest number of bands (29) was obtained with primer pair E_{AAG}/ M_{AAT} (Table 3). The highest level of polymorphism (39.3%) found with primer pairs E_{AGC}/M_{AGG}, whereas the lowest one (17.2%) was generated with E_{AAG}/M_{AAT} primer combination.

Two genotype-specific markers for PM-702 were found among the 56 polymorphic bands analyzed. If these finding are confirmed using a broader range of accessions, these unique bands can be useful for the identification sequence-tagged site primers.

The primary aim of this study was to evaluate the exiting levels and patterns of genetic diversity among nineteen breeding lines and cultivars of pepper. The dendrogram, 2D and 3D scaling based on molecular (AFLP) data were formed (Table 3; Figures 1, 2 and 3). The dendrogram derived by unweighed pair group method with arithmetic mean algorithm (UPGMA) analyses clearly split 8 breeding lines and 11 cultivars of peppers into two clusters. Cluster I was divided into four subclusters:1A, containing 12 genotypes, 2A, containing only genotype Urfa local, 3A, containing only Olenka and 4A, containing PM-702. Cluster II was also divided into two sub-clusters: IB, with 3 genotypes and 2B with one genotype (Urfa- 246).

In this study, the dendrogram, 2D and 3D scaling clearly split genotypes based on their genetic relatedness (Figures 1, 2 and 3). According to the dendrograms, 2D and 3D scaling, Islahiye, Pazarcik, K.Maras, PM702 and Urfa-246 genotypes were the most distant ones than the others. Alata breeding lines clustered together and Kandil, Kraska and Ilica 256 were in closer position to them. The pair-wise genetic distance values in all genotypes range 0.013 (between Ilıca and Alata-29) to 0.208 (between and Islahiye and PM 702) (Table 4).

Genetic variation among pepper genotypes

The present study demonstrated that level of variation

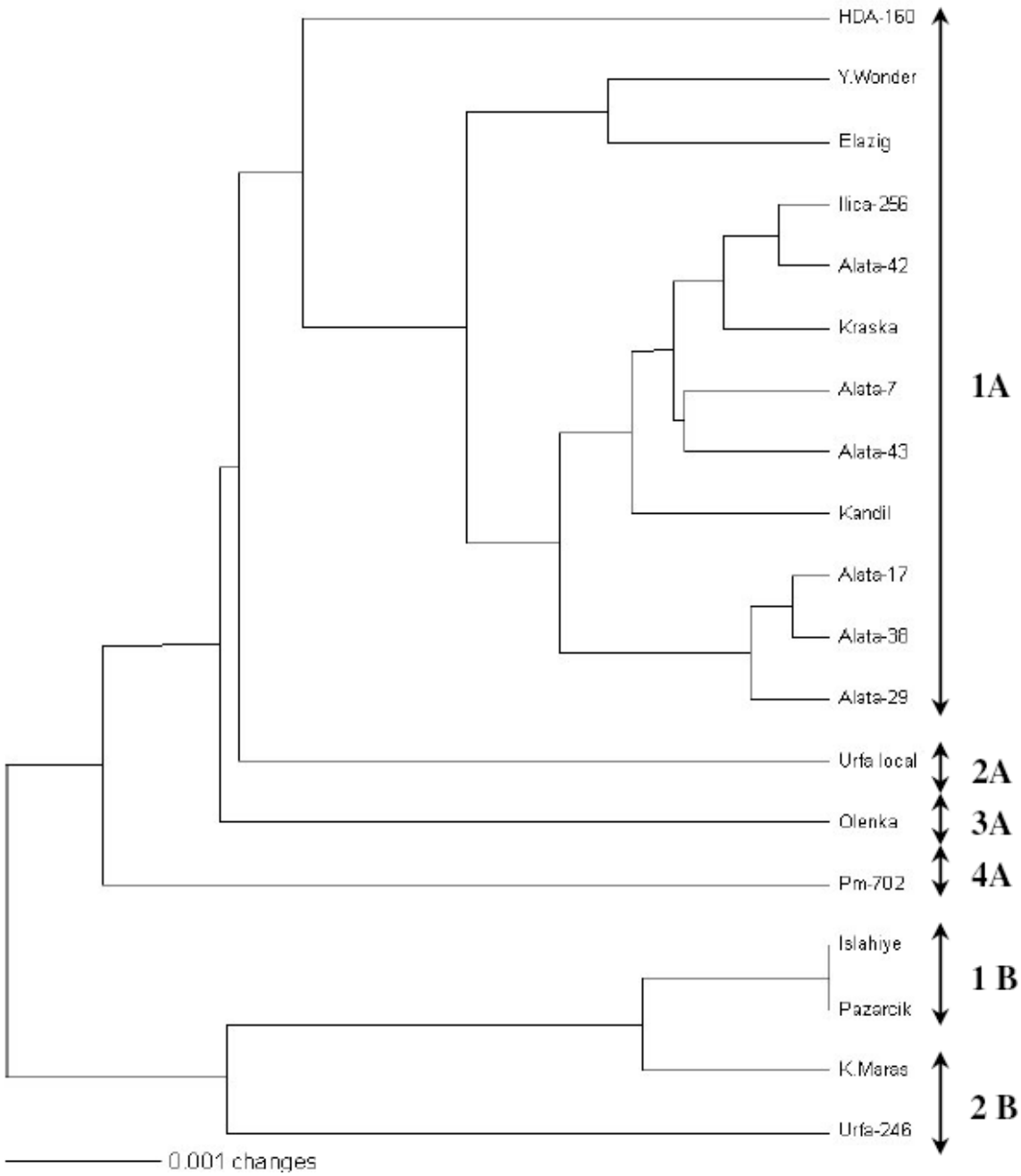

Figure 1. Dendrogram representing the phylogenetics relationship among 19 pepper accessions revealed UPGMA cluster analyses.

among the studied is rather low. However, the statistical variation measures showed that the genetic diversities among the populations were significantly different (Table 5). The genetic diversity among the Alata breeding lines was the smallest (H = 0.0170 and I = 0.0242) followed by the studied foreign genotypes (H = 0.0504 and I = 0.0759). The genetic diversity among the other local Turkish genotype was the highest (H = 0.1773 and I = 0.2551). Moreover, these Turkish pepper genotypes found to have large polymorphic loci (41.07%).

DISCUSSION

In the present study, AFLP method was used to assess the genetic relationship among some Turkish pepper genotypes because of its high efficiency (Vos et al., 1995; Paran et al., 1998; Rodriguez et al., 1999; Park et al., 2000; Lanteri et al., 2003; Rao et al., 2003; Toquica et al., 2003; Portis et al., 2004; Geleta et al., 2005; Ben-Chaim et al., 2001; Minamiyama et al., 2006; Oyama et al., 2006).

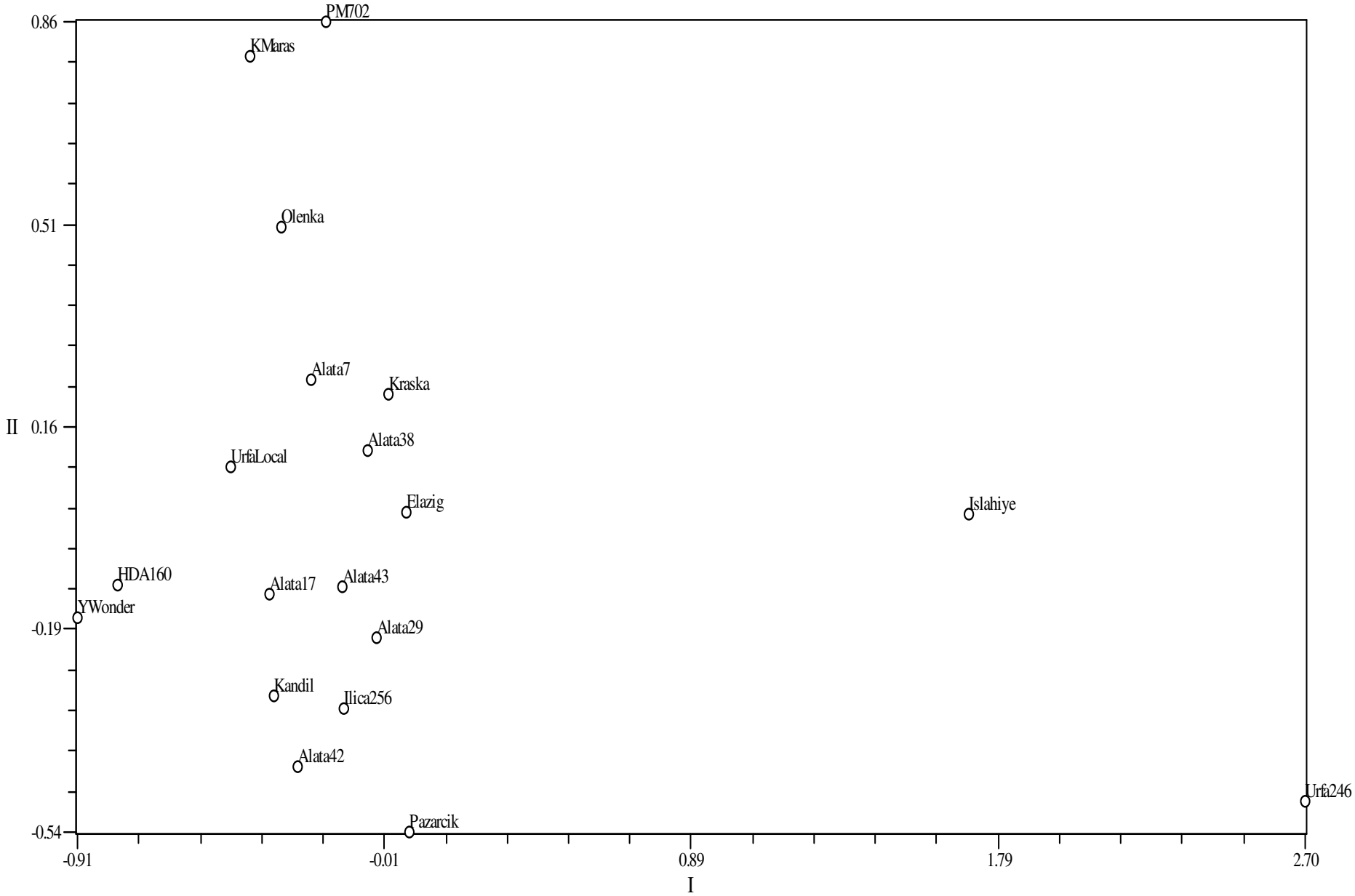

Figure 2. 2D-Multidimensional scaling plot of 19 pepper genotypes based on Euclide similarity coefficients from 56 AFLP markers.

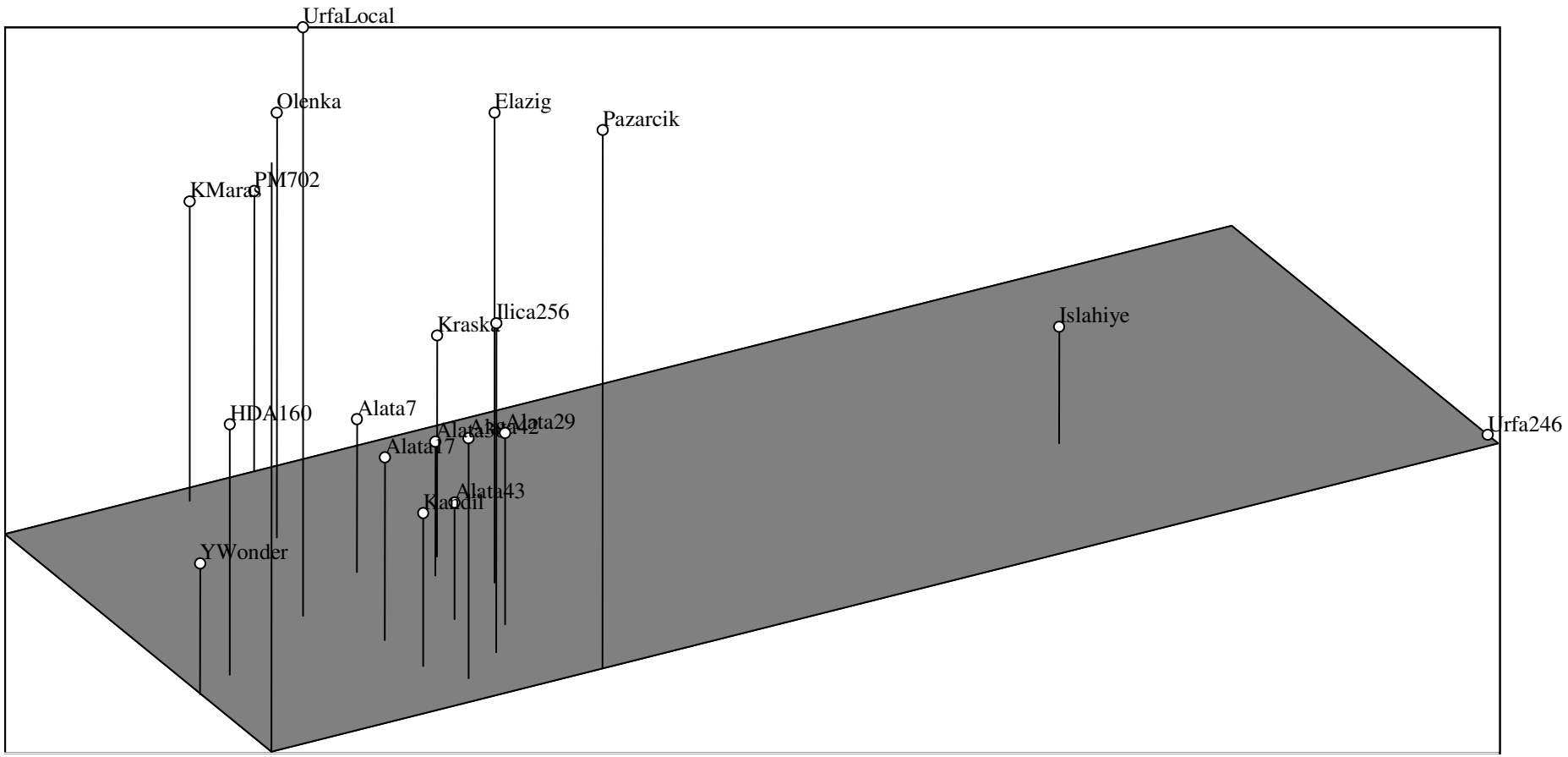

Figure 3. 3D-Multidimensional scaling plot of 19 pepper genotypes based on Euclide similarity coefficients from 56 AFLP markers.

Genetic dis/similarities among pepper genotypes

The polymorphic levels in this research are high (26%) compared to some published studies; for example Kochieva and Ryzhova (2003) found 16.5% polymorphism in 14 pepper genotypes using 9 primer pairs, Paran et al. (1998) detected 13% polymorphism in 34 pepper genotypes using ten primer pairs, and Tam et al. (2005) observed 8.03% polymorphism in 35 genotypes using 9 primer pairs. However, genotypes and primer pairs in the above mentioned researchers were different, making the results not easily comparable. On the other hand, Geleta et al. (2005) obtained 352 polymorphic markers in the analysis of 39 accessions using six AFLP primer pairs.

The efficiency of the different marker techniques for estimating DNA polymorphism in pepper is variable. For example, using 9 RAPD primers in 14 pepper cultivars, Kochieva and Ryzhova (2003) found 35% polymorphism; similarly Paran et al. (1998) detected 22% polymorphism, analyzing 34 pepper genotypes. Using 13 SSR in 35 pepper genotypes, and Tam et al. (2005) detected 2.38 alleles/locus. For RFLPs, the number of polymorphic bands per probe/enzyme combination was

Table 4. Genetic distances among 9 breeding lines and 10 cultivars of pepper.

Genotypes	HDA-160	Y. Wonder	Ilıca-256	Kandil	Alata-7	Alata-17	Alata-29	Alata-38	Alata-42	Alata-43	Kraska	Olenka	Islahiye	K. Maras	Pazarcik	Urfa -246	PM-702	Urfa local	Elazig
HDA-160	-	0.043	0.076	0.069	0.085	0.074	0.053	0.080	0.085	0.096	0.090	0.090	0.136	0.128	0.138	0.129	0.105	0.101	0.064
Y. Wonder		-	0.045	0.037	0.053	0.053	0.045	0.048	0.064	0.064	0.069	0.069	0.106	0.106	0.106	0.098	0.098	0.080	0.032
Ilıca-256			-	0.023	0.023	0.045	0.013	0.053	0.008	0.030	0.015	0.091	0.106	0.106	0.106	0.068	0.091	0.068	0.038
Kandil				-	0.027	0.048	0.038	0.043	0.037	0.027	0.032	0.085	0.098	0.112	0.112	0.091	0.105	0.074	0.037
Alata-7					-	0.053	0.030	0.048	0.032	0.021	0.016	0.090	0.083	0.106	0.096	0.076	0.098	0.090	0.053
Alata-17						-	0.008	0.005	0.043	0.043	0.048	0.080	0.136	0.128	0.138	0.098	0.090	0.090	0.053
Alata-29							-	0.015	0.015	0.023	0.023	0.075	0.130	0.128	0.135	0.091	0.083	0.090	0.045
Alata-38								-	0.048	0.037	0.053	0.074	0.129	0.133	0.133	0.106	0.098	0.085	0.048
Alata-42									-	0.021	0.016	0.101	0.098	0.128	0.117	0.076	0.098	0.090	0.064
Alata-43										-	0.016	0.090	0.076	0.117	0.096	0.083	0.105	0.080	0.064
Kraska											-	0.096	0.091	0.112	0.101	0.083	0.090	0.085	0.059
Olenka												-	0.106	0.101	0.101	0.099	0.113	0.096	0.069
Islahiye													-	0.030	0.000	0.083	0.208	0.098	0.114
K. Maras														-	0.021	0.083	0.188	0.122	0.117
Pazarcik															-	0.083	0.188	0.112	0.117
Urfa-246																-	0.143	0.121	0.106
PM-702																	-	0.143	0.098
Urfa local																		-	0.048
Elazig																			-

Mean genetic distance: 0.079.

1.46 in 14 pepper genotypes. Comparative studies on soybean and maize demonstrated that AFLP is the most efficient compared to RFLP, RAPD and SSR (Powell et al., 1996). In the study, using only four AFLP, we observed 215 bands, fifty-six (26%) of them were polymorphic, which shows that AFLP is one of the most efficient marker systems in the case of pepper.

Peppers are classified into different commercial varieties based on fruit characters landraces and improved cultivars of peppers are grouped according to their mutual resemblance for several characters (Geleta et al., 2005).

Our distance values indicated comparatively low genetic diversity among the breeding lines and cultivars of pepper. The low level of genetic diversity in this study was agreement with the results of Kochieva and Ryzhova (2003) and Paran et al. (1998) who studied samples from gene bank accessions, but not with the findings of Oyama et al. (2006) who studied the wild and domesticcated pepper (*Capsicum annuum* L.) populations of North-western Mexico where wild populations of *C. annuum* L. are widely distributed. Moreover, Geleta et al. (2005) found moderately high genetic variability among the 39 *C. annuum* L. genotypes with different geographical origins.

Genetic variation among pepper genotypes

The genetic diversity among the local Turkish line is consistent with the findings (H = 0.182) of

Table 5. Genetic diversity among the pepper genotypes.

Genotypes	N	H	I	Polymorphism (%)
All genotypes	19	0.1691	0.2523	46.43
Turkish pepper genotypes	14	0.1710	0.2504	42.86
a-Alata breeding lines	6	0.0170	0.0242	3.57
b- Local genotypes	8	0.1773	0.2551	41.07
Foreign genotypes	5	0.0504	0.0759	14.29

N = Genotype number; H = Nei's genetic diversity index; I = Shannon's genetic diversity index.

Toquica et al. (2003) in *C. frutescens* and *C. annuum* genotypes of Colombia. Lanteri et al. (2003) studied the genetic variation in a landrace of pepper (*Capsicum annuum* L.) grown in North-west Italy with Shannon's diversity index. Their results revealed that this studied pepper landrace showed rather little genetic variation. This was not surprising as the starting material for selecting the different cultivars of pepper at presently grown represented a rather limited gene pool. Portis et al. (2004) showed that seed harvested on the basis of farmer selection criteria had caused a progressive decrease in within genetic variation of a landrace population of pepper (*C. annuum* L.) grown in North-west Italy. In the present study, it was also noticed that the genetic variation in Alata breeding lines had little genetic variation due to narrow genetic back-ground and extensive selection procedures.

CONCLUSION

AFLP markers are powerful tools for describing genetic dis/similarities and diversity among the studied pepper genotypes. The observed genetic relationship and diversity among the pepper genotypes are helpful for current and future breeding programs in order to select genetically distinct parents. Artificial selection might decrease the genetic diversity within the populations as seen in the Alata's breeding lines. The potential responses from selection lie in genetic diversity (Rodriguez et al., 1999); therefore, necessary precautions should be taken to increase genetic diversity in any breeding program. Genetic diversity should be enlarged by combining desired traits from different local and wild populations of different geographical origins into the breeding lines.

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