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Full Length Research Paper

Genetic diversity and relationship analysis among accessions of *Aegilops* ssp. in Turkey using amplified fragment length polymorphism (AFLP) markers

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Amplified fragment length polymorphism (AFLP) DNA markers were used to assess the genetic diversity and relationships between 55 accessions of genus *Aegilops*, including the species *Aegilops triuncialis* L. (UUCC), *Aegilops geniculata* Roth (MMUU), *Aegilops cylindrica* Host (CCDD) and *Aegilops umbellulata* Zhuk (UU). The samples were collected from Aegean region and East Anatolia, Turkey. 16 AFLP selective primer combinations generated a total of 3200 polymorphic amplification products. 50 *Aegilops* accessions were analyzed using the data analysis software, unweighted pair-group method arithmetic average (UPGMA) method and numerical taxonomy and multivariate analysis system (NTSYSpc-2.02k). The similarity index coefficients were calculated according to simple matching coefficient. Using 16 AFLP primer combinations, species from Aegean region and east Anatolia were clustered as four major groups. *Aegilops* species having U genome clustered together and *A. cylindrica* host was out grouped.

Keywords: *Aegilops cylindrica*, *Aegilops triuncialis*, *Aegilops umbellulata*, *Aegilops geniculata*, amplified fragment length polymorphism (AFLP), Li-COR, genetic relationship, unweighted pair-group method arithmetic average (UPGMA), principle coordinate analysis.

INTRODUCTION

The wild species of *Triticeae* family, especially the genus *Aegilops* L. are valuable sources of genetic variation for wheat improvement since they possess the genetic background of all the cultivated wheat having still un-identified important characters such as resistance to different biotic and abiotic stresses (Rekika et al., 1998; Zaharieva et al., 2004). The genus *Aegilops* L. has been the most intensively studied group of grasses, especially since it is closely related to the cultivated wheat (van Slageren, 1994). *Aegilops* ssp. is thought to be a genetic reserve for the improvement of the wheat cultivars (Damania, 1993). *Aegilops* spreads mainly in central Asia and Mediterranean region (van Slageren, 1994). Turkey is the center of diversity for this genus and it is rich in wild populations of tetraploid species: *Aegilops triuncialis* L. (UUCC), *Aegilops geniculata* Roth (MMUU), *Aegilops cylindrica* Host (CCDD) and diploid species: *Aegilops umbellulata* Zhuk. (UU). *A. cylindrica* and *A. triuncialis* are widely distributed in Turkey adding up to 15 species of *Aegilops* (Davis, 1985). Ecogeographic studies are necessary as they determine the genetic relationship and also guide conservation programs for the target plant species (Anikster and Noy-Meir, 1991). DNA-based molecular markers are particularly useful both for quantifying genetic diversity within plant species and for identifying and characterizing closely related genotypes (Jasieniuk and Maxwell, 2001). Molecular markers can

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provide information needed to select genetically diverse parents for developing breeding and mapping populations, among which the AFLP markers have been successfully used to determine genetic diversity in many plant species (Sharma et al., 1996; Pillay and Myers, 1999).

AFLP markers are generated by selective amplification of a subset of restriction fragments from total genomic DNA (Vos et al., 1995; Mueller and Wolfenbarger 1999). The reproducibility, heritability, effectiveness and reliability of these amplified DNA products have substantial advantages when compared with other marker systems (Russell et al., 1997). The PCR-based AFLP markers are amenable to automation for high-throughput genotyping and, being anonymous, do not require any sequence information (Rouf Mian et al., 2002). AFLP fingerprinting is considerably informative, allowing the survey of variation in more than 50 co-amplified restriction fragments in each AFLP reaction (Incirli and Akkaya, 2001; Sudupak et al., 2004; Yildirim and Akkaya, 2006). Li-COR IR^2 automated DNA sequencers and associated software have been demonstrated to efficiently generate and analyze complex AFLP patterns of various genomes (Qui et al., 1999; Remington et al., 1999). We applied AFLP markers to characterize the genetic diversity and relationships among different populations of *Aegilops* in Turkey using Li-COR instrument.

MATERIALS AND METHODS

The materials of the study consist of *A. cylindrica, A. geniculata, A.truncialis* and *A. umbellulata* gathered from the Aegean and the Eastern Anatolia Regions in 2005. 50 individuals from a total of 11 populations belonging to these plants were collected (Table 1).

DNA isolation

The seeds of *Aegilops* were germinated and DNA was isolated from the seedlings of two weeks old leaves starting with 200 mg young leaf tissue using a minor modified cetyl trimethyl ammonium bromide (CTAB) method.

AFLP analysis

AFLP analysis was carried out according to Vos et al. (1995) using fluorescently labeled primers and bands were detected using a Li-COR automated sequencer (model 4300). All the chemicals and enzymes apart from 10X reaction buffer and Taq DNA polymerase were present in the kit provided by Li-COR (IRDye Fluorescent AFLP Kit for Large Plant Genome Analysis). Genomic DNA (200 ng) was double digested with 1.5 units each of *Eco*RI and *Mse*I (MBI Fermentas) in a final volume of 20 µl and incubated at 37°C for 2 h. 7.5 and 75 pmols of adaptors *Eco*RI and *Mse*I, respectively, were ligated to the resulting fragments (20 µl of the digestion mix) using 1 unit of T4 DNA ligase (Roche diagnostics GmbH) in a final volume of 25 µl buffer ligase 1X and incubated for 2 h at 25°C. The ligation mix was diluted 1/10 and 2.5 µl were added to the preamplification reaction containing AFLP Pre-amp primer mix, 1X PCR reaction buffer, 2.5 U *Taq* DNA polymerase (Roche diagnostics GmbH) in a final volume of 25 µl. Preamplification was performed in a PTC-100 MJ Research Inc. thermocycler as in the following steps: 2 min 95°C, 20 cycles of 30 s at 94°C, 1 min at 56°C, 1 min at 72°C and 4 min at 72°C. The preamplification mix was diluted to 1/40 and 2 µl was added to the selective amplification reaction, containing 1 µM IRDye 700 labeled *Eco*RI primer A, 1 µM IRDye 800 labeled *Eco*RI primer B and 1 µM *Mse*I + 3 (Table 2), reaction buffer lX, 0.25 mM each dNTPs and 1 unit of *Taq* DNA polymerase in a final volume of 20 µl. Selective amplification was performed on a Stratagene Mx3005P Real Time Thermal Cycler as follows: 13 touchdown cycles (- 0.7°C per cycle) of 30 s at 94°C, 30 s at 65°C, l min at 72°C; 23 cycles of 30 s at 94°C, 30 s at 56°C, 1 min at 72°C and 10 min at 72°C. A total of 16 selective primer combinations were used.

The PCR products were separated by electrophoresis in a 6.5% polyacrylamide gel using the Li-COR 4300 DNA Analyzer and analyzed using the Saga Generation Software. Genetic similarity and diversity analysis among 55 *Aegilops* varieties were performed using the data analysis software, UPGMA method and NTSYSpc-2.02k (Rholf, 1997). The similarity index coefficients were calculated according to simple matching (SM) coefficient (Rholf, 1997).

RESULTS

Turkey is rich in tetraploid species: *A. triuncialis* L. (UUCC), *A. geniculata* Roth (MMUU), *A. cylindrica* Host (CCDD) and diploid species: *A. umbellulata* Zhuk. (UU). Thus, it is important to find the genetic diversity of these species in Turkey. 16 selective primer combinations resulted in 3200 polymorphic bands to measure the genetic diversity within 55 accessions of *Aegilops* genus. A dendrogram was generated from the data using the UPGMA and the program NTSYSpc 2.02k (Figure 1). Principle coordinate analysis of the data was also determined (Figures 2 and 3).

The genetic diversity within the 55 accessions of *A. triuncialis* L. (UUCC), *A. geniculata* Roth (MMUU), *A. cylindrica* Host (CCDD) and *A. umbellulata* Zhuk were calculated using 16 selective primer combinations which resulted in 3200 polymorphic bands (UU). In the tree, the species sharing the U genome formed a main cluster and *A. cylindrica* which is intensely associated with *A. squarrosa* (DD), was clearly different from the other species, due to the influence of the presence of distant D genome. This genome has undergone less divergence than other diploid genomes during evolution and therefore appears to be less modified and is well separated within the *Triticeae* (Damania, 1993; Badaeva et al., 1996).

AFLP based UPGMA dendrogram of *Aegilops* accessions is presented in Figure 1, in which there are four main clusters: *A. cylindrica* (Ac)*, A. triuncialis* (At)*, A. umbellulata* (Au) and *A. geniculata* (Ag). The closest genetic similarity between genotypes (0.980 simple matching coefficient) was determined between Ag9 and Ag10 genotypes. Other genetic similarity results are respectively as follows: the similarity between Au5 and Au6 (0.958 simple matching coefficient), the similarity between At9 and At10 (0.948 simple matching coefficient), the

Table 1. The species of *Aegilops* L. collected from Turkey: sample numbers, locations, species and genomes.

S/N	Location (city)	Coordinate	Species	Genome
1	Uşak	38° 40.507N, 029° 18.648E, 928 m	*A. cylindrica*	CCDD
2	Uşak	38°40.507N, 029° 18.648E, 928 m	*A. cylindrica*	CCDD
3	Uşak	38°40.507N, 029° 18.648E, 928 m	*A. cylindrica*	CCDD
4	Uşak	38° 40.507N, 029° 18.648E, 928 m	*A. cylindrica*	CCDD
5	Uşak	38° 40.507N, 029° 18.648E, 928 m	*A. cylindrica*	CCDD
6	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. cylindrica*	CCDD
7	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. cylindrica*	CCDD
8	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. cylindrica*	CCDD
9	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. cylindrica*	CCDD
10	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. cylindrica*	CCDD
11	Van	38° 31.868N, 043° 20.808E, 1671 m	*A. cylindrica*	CCDD
12	Van	38° 31.868N, 043° 20.808E, 1671 m	*A. cylindrica*	CCDD
13	Van	38° 31.868N, 043° 20.808E, 1671 m	*A. cylindrica*	CCDD
14	Van	38° 31.868N, 043° 20.808E, 1671 m	*A. cylindrica*	CCDD
15	Van	38° 31.868N, 043° 20.808E, 1671 m	*A. cylindrica*	CCDD
16	Uşak	38° 40.507N, 029° 18.648E, 928 m	*A. triuncialis*	UUCC
17	Uşak	38° 40.507N, 029° 18.648E, 928 m	*A. triuncialis*	UUCC
18	Uşak	38° 40.507N, 029° 18.648E, 928 m	*A. triuncialis*	UUCC
19	Uşak	38^{v} 40.507N, 029° 18.648E, 928 m	*A. triuncialis*	UUCC
20	Uşak	38° 40.507N, 029° 18.648E, 928 m	*A. triuncialis*	UUCC
21	Van	38^{v} 25.543N, 043° 15.695E, 1664 m	*A. triuncialis*	UUCC
22	Van	38° 25.543N, 043° 15.695E, 1664 m	*A. triuncialis*	UUCC
23	Van	38° 25.543N, 043° 15.695E, 1664 m	*A. triuncialis*	UUCC
24	Van	38° 25.543N, 043° 15.695E, 1664 m	*A. triuncialis*	UUCC
25	Van	38° 25.543N, 043° 15.695E, 1664 m	*A. triuncialis*	UUCC
26	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. triuncialis*	UUCC
27	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. triuncialis*	UUCC
28	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. triuncialis*	UUCC
29	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. triuncialis*	UUCC
30	Van	38° 33.794N, 043° 17.839E, 1672 m	*A. triuncialis*	UUCC
31	Van	38^{0} 25.544N, 043° 15.697E, 1664 m	*A. umbellulata*	UU
32	Van	38° 25.544N, 043° 15.697E, 1664m	*A. umbellulata*	UU
33	Van	38° 25.544N, 043° 15.697E, 1664m	*A. umbellulata*	UU
34	Van	38° 25.544N, 043° 15.697E, 1664m	*A. umbellulata*	UU
35	Van	38° 25.544N, 043° 15.697E, 1664m	*A. umbellulata*	UU
36	Van	38^{v} 31.868N, 043° 20.808E, 1671m	*A. umbellulata*	UU
37	Van	38° 31.868N, 043° 20.808E, 1671m	*A. umbellulata*	UU
38	Van	38^{v} 31.868N, 043^{0} 20.808E, 1671m	*A. umbellulata*	UU
39	Van	38° 31.868N, 043° 20.808E, 1671m	*A. umbellulata*	UU
40	Van	38° 31.868N, 043° 20.808E, 1671m	*A. umbellulata*	UU
41	Uşak	38° 40.476N, 029° 16.412E, 902 m	*A. umbellulata*	UU
42	Uşak	38° 40.476N, 029° 16.412E, 902 m	*A. umbellulata*	UU
43	Uşak	38° 40.476N, 029° 16.412E, 902 m	*A. umbellulata*	UU
44	Uşak	38° 40.476N, 029° 16.412E, 902 m	*A. umbellulata*	UU
45	Uşak	38° 40.476N, 029° 16.412E, 902 m	*A. umbellulata*	UU
46	Izmir	38° 31.229N, 026° 37.433E, 16 m	*A. geniculata*	MMUU
47	Izmir	38° 31.229N, 026° 37.433E, 16 m	*A. geniculata*	MMUU
48	Izmir	38° 31.229N, 026° 37.433E, 16 m	*A. geniculata*	MMUU
49	Izmir	38° 31.229N, 026° 37.433E, 16 m	*A. geniculata*	MMUU
50	Izmir	38° 31.229N, 026° 37.433E, 16 m	*A. geniculata*	MMUU
51	Uşak	38° 40.620N, 029° 22.638E, 904 m	*A. geniculata*	MMUU

Table 1. Contd

52	Uşak	38° 40.620N, 029° 22.638E, 904 m	*A. geniculata*	MMUU
53	Uşak	38° 40.620N, 029° 22.638E, 904 m	*A. geniculata*	MMUU
54	Uşak	38° 40.620N, 029° 22.638E, 904 m	*A. geniculata*	MMUU
55	Uşak	38° 40.620N, 029° 22.638E, 904 m	*A. geniculata*	MMUU

Table 2. *Mse*I and IRDye 700 labeled *Eco*RI primers used in selective amplification reaction.

Primer	Flourescent label	Sequence (5'-3')
M-CAA	-	GATGAGTCCTGAGTAACAA
M-CAC	-	GATGAGTCCTGAGTAACAC
M-CAG	-	GATGAGTCCTGAGTAACAG
M-CAT	-	GATGAGTCCTGAGTAACAT
M-CTA	-	GATGAGTCCTGAGTAACTA
M-CTC	-	GATGAGTCCTGAGTAACTC
M-CTG	-	GATGAGTCCTGAGTAACTG
M-CTT	-	GATGAGTCCTGAGTAACTT
E-AAC	IRDye 700	GACTGCGTACCAATTCAAC
E-AAG	IRDye 700	GACTGCGTACCAATTCAAG
E-ACA	IRDye 700	GACTGCGTACCAATTCACA
E-ACT	IRDye 700	GACTGCGTACCAATTCACT
E-ACC	IRDye 800	GACTGCGTACCAATTCACC
E-ACG	IRDye 800	GACTGCGTACCAATTCACG
E-AGC	IRDye 800	GACTGCGTACCAATTCAGC
E-AGG	IRDye 800	GACTGCGTACCAATTCAGG

similarities between Au3 and Au4, Ag7 and Ag9 (0.946 simple matching coefficient). The least genetic similarity between genotypes was found between Au8 and Au9 (0.800 simple matching coefficient). Christiansen et al. (2002) and Lage et al. (2003) showed similar results and generally followed the trend that increased geographical distance correlates with increased genetic distance.

In the analysis of the dendrogram, with 2 and 3 dimensional scaling; it was detected that the genotypes of Ag2, Ag5, Ag6, Au8 and Au9 had a different branching pattern from the one expected. Ac3, Ac13, At1, At2 and At15 had different branching patterns and positions from other genotypes. It was found that genotypes with high similarity (Ag6, Ag7, Ag9 and Ag10) and genotypes gathered from similar populations (Au5, Au6 and At9, At10) had the same branching pattern and positions in general (Figures 2 and 3). Since the genus *Aegilops* L. is a valuable source of genetic variation, the data presented here might have advantage in various studies in future.

DISCUSSION

Periodical genetic diversity assessments of all kinds of wild type plant species are very important for many reasons, but it is more crucial for the wild types of wheat since they are genome donors of cultivated wheat. Since the cultivated wheat has a very narrow genetic diversity, for crop improvement, we always need to investigate the traits hidden in the wild types or the ancestors of the wheat, so that we can maintain the sustainable agri-culture to feed ever increasing human population. Thus, by continual diversity assessment in nature, we can maintain the most diverse species in gene banks for conservation and crop improvement purposes. The effects of environmental changes or climate fluctuations on the natural diversity can also be traced by continual analyses. This is especially the case for *Aegilops* species in Turkey since the land is the center of origin for these *Aegilops* species.

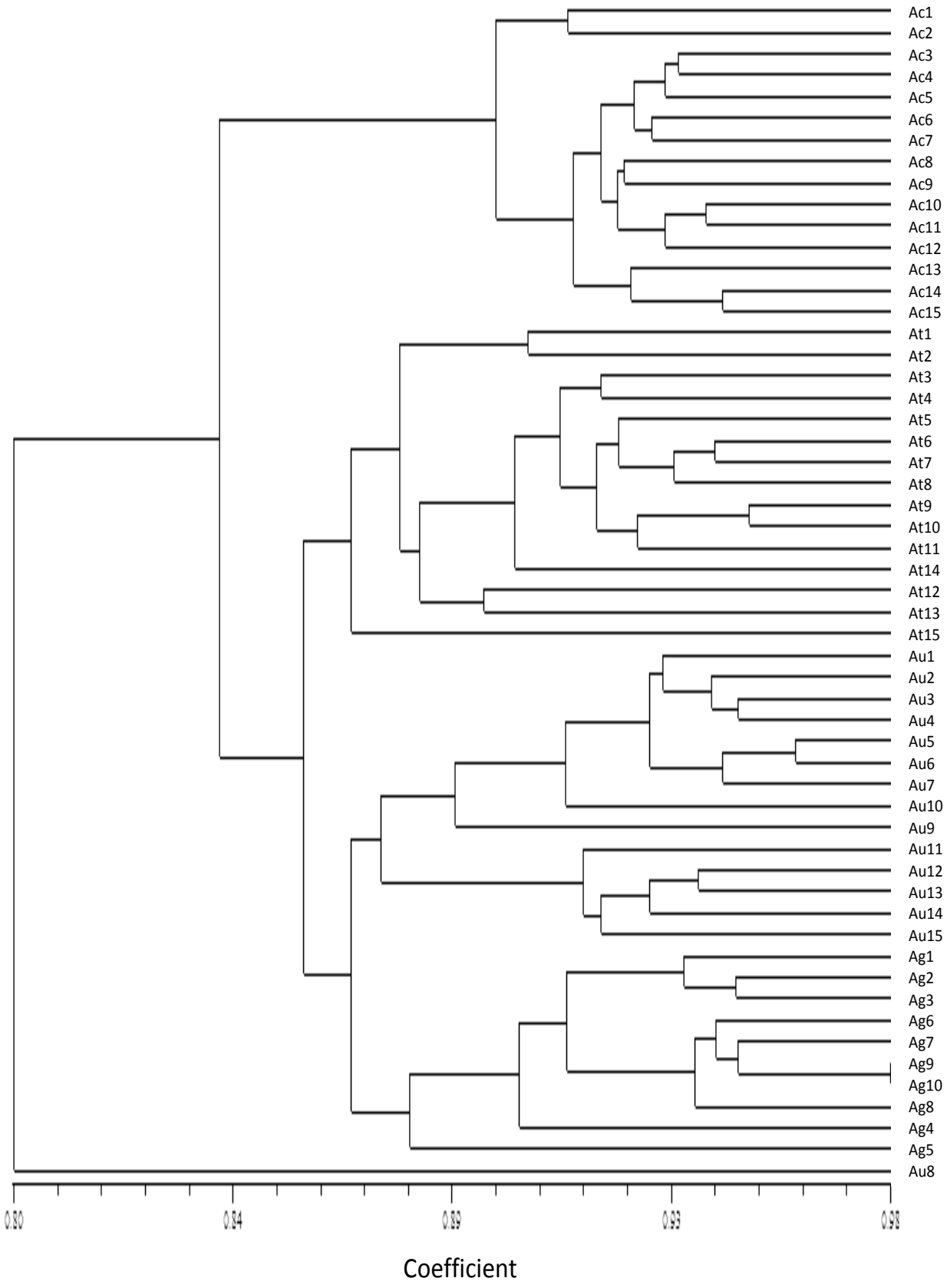

Figure 1. AFLP-based UPGMA dendrogram of *Aegilops* accessions.

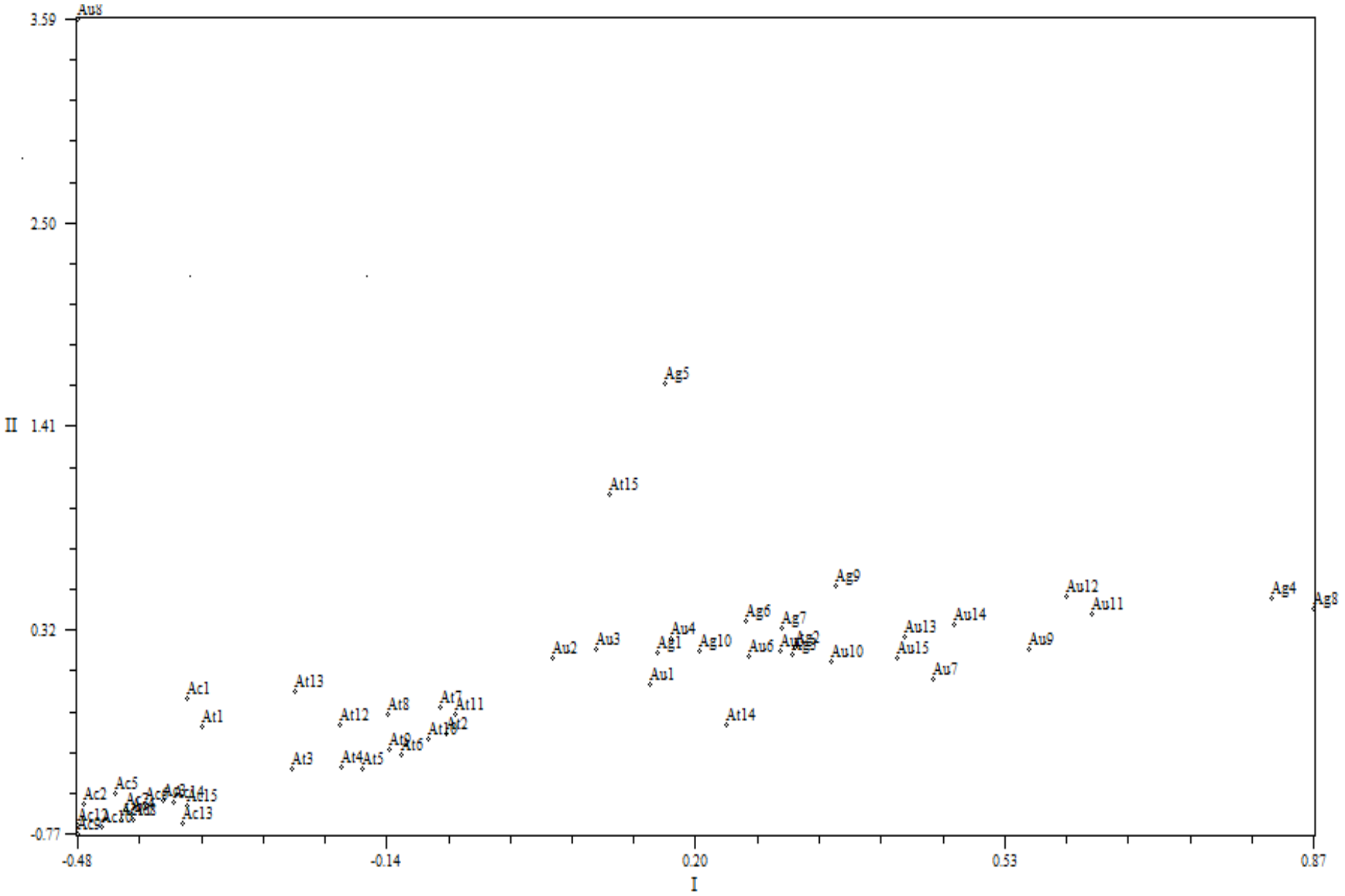

Figure 2. 2D plot generated by principle coordinate analysis.

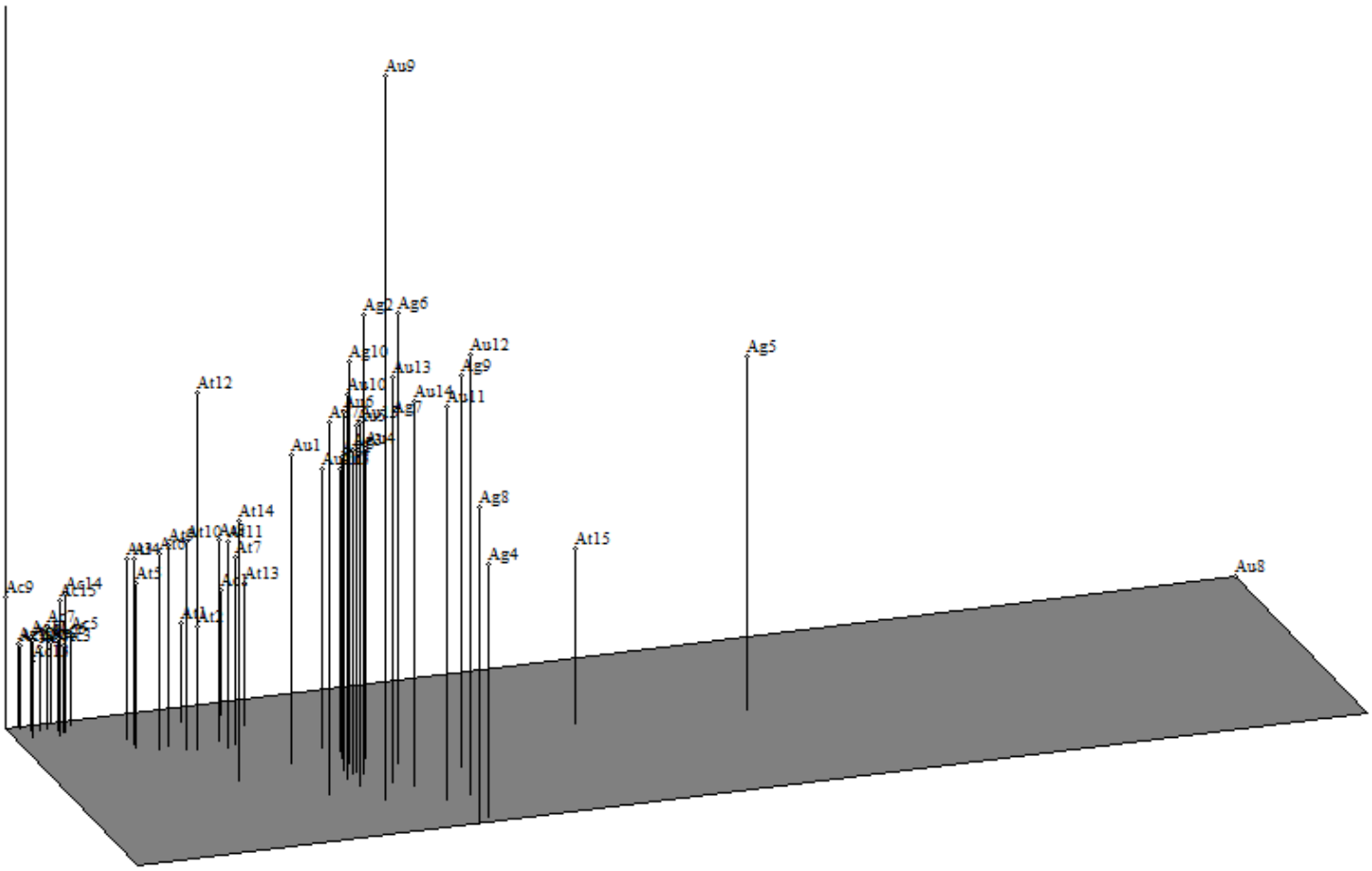

Figure 3. 3D plot of the principal coordinate analysis of the AFLP data generated by NTSYS.

AFLP marker system to test the genetic diversity is one of mostly used marker system since, it is multi-locus, thus highly polymorphic, reproducible and high through-put instrumentation is available, such as Li-Cor. Thus, in this study, we preferred to perform analysis using AFLP highly polymorphic marker system. We believe the data presented here will be a tool for other wheat researchers.

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