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Comparative genetic diversity analysis of oat (*Avena sativa* L.) by microsatellite markers and morphological rainfed expressions

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Equivalence was appraised between phenotypic and molecular markers (ISSR) to analyze the genetic diversity of 20 high yielding genotypes representing different geographical zones of the world. A moderate range of genetic similarity (0.84 to 0.20) was observed on the basis of 20 inter-simple sequence repeats (ISSR) markers, where it was found high (0.995 – 0.204) on the basis of 7 primary morphological rainfed expression. Genotypes in morphological character based dendogram were clustered into their respective geographic groups, while a random grouping was observed in dendogram based on the ISSR markers. A negative correlation (r = -0.186) was found among morphological and molecular marker systems, but the latter was found effective in distinguishing the genotypes using specific band positions for them. The genotypic classification agreed closely with the grouping observed in ISSR based 3D analysis.

Key words: *Avena sativa*, diversity, inter-simple sequence repeats (ISSR), morphology, oat, relationship.

INTRODUCTION

Oat (*Avena sativa* L.) is one of the most important forage and feed crops of the world. Oat is used as green fodder, straw, hay or silage. Oat grain makes a good balanced concentrate in the rations for poultry, cattle, sheep and other animals. Green fodder contains about 10 to 13% protein and 30 to 35% dry matter. Despite being high fed fodder crop, it is now gaining importance due to its unique and important quality characteristics, particularly the lipid and protein in grains. Hence, the first and foremost need is the identification or cataloguing of oats genotypes along with the assessment of genetic diversity prevalent in different geographical regions in the world. The genetic diversity analysis in oats has been done using either morphological characteristics or molecular markers such as random amplified polymorphic DNA (RAPD) (Loskutov, 2007; Perchuk, 2000), simple sequence repeats (SSR), restriction fragment length polymorphism (RFLP) (Pal, 2002), sequence-characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) (Orr et al, 2008) but, there are meager reports on the identification and characterization of oats germplasm using both the morphological and

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Abbreviations: RAPD, Random amplified polymorphic DNA; **SSR,** simple sequence repeats; **SCAR,** sequence-characterized amplified region; **CAPS,** cleaved amplified polymorphic sequence; **RFLP,** restriction fragment length polymorphism; **PCR,** polymerase chain reaction; **AFLP,** amplified fragment length polymorphism.

Table 1 Genetically pure seed material of 20 high yielding oat genotypes.

Genotype	Country of origin	Pedigree
D. Sel.-1	Pantnagar	Derivative of UPO 201/ UPO 211// UPO 212
D. Sel.-5	Pantnagar	Derivative of UPO 211 x UPO 212
D. Sel.-6	Pantnagar	Derivative of UPO 201 x UPO 210
Wright	U.S.A	Introduction from USA
HFO 114	Hisar	Selection line 37/ 14
OL 125	Ludhiana	Derivative of Appler × IPC-163
UPO 265	Pantnagar	Selection from UPO 201 x Kent
UPO 270	Pantnagar	Selection from UPO 228 x UPO 202
UPO 271	Pantnagar	Selection from UPO 212 x UPO 136
UPO 273	Pantnagar	Selection from UPO 202 x UPO 201
UPO 275	Pantnagar	Selection from Wright x UPO 233
Kent	Australia	Introduction fron USA
UPO 212	Pantnagar	US 1492 x Kent
No. 1	Pantnagar	Selection from local material
OS 6	Hisar	Derivative of HFO 10 x HFO 55
EC 605833	Exotic	Exotic material
EC 605836	Exotic	Exotic material
EC 605838	Exotic	Exotic material
UPO 260	Pantnagar	Derivative of UPO 204 x UPO 211
EC 246199	Exotic	Exotic material

Table 2. List of morphological characteristics and their stage of observation.

Characteristic	Stage of observation
Plant height at 50% heading	Maturity
Tiller diameter	100% Flowering
Number of productive tillers	50% Flowering
Days to maturity	Maturity
Biological yield per plant	100% Flowering
Grain yield per plant	100% Flowering
Straw yield per plant	100% Flowering

molecular markers to compare the two systems. However, the use of qualitative and quantitative morphological characteristics are often affected due to genotype x environment interaction, hence not much dependable but still it is easy to compare the phenotypic status of the plant with the genotypic level. Among a large category of molecular markers, microsatellite mar-kers (inter-simple sequence repeats, ISSR) can be effi-ciently applied to identify useful polymorphisms (Rafalski, 1993; Doldi et al., 1997). The resolving power of this tool is several folds higher than morphological or isozyme markers and is much simpler and technically less deman-ding than RFLP and other new generation markers. Mole-cular markers have proved their importance for diversity analysis in several crops and horticultural plants like neem (Deshwal et al., 2005) common bean (Marotti et al., 2007), strawberry (Kuras et al., 2004) and in oats (Wight et al., 2003). Since molecular marker technology has no effect on environment and genotype, it is an efficient tool to understand and explain between and within geographical variation and granting protection and crop improvement. Comparison between the morphological and molecular markers for estimating genetic relationship may provide critical assessment. Research information on such aspects are generally available, however more literature is documented on this topic in oats. The purpose of this study was to compare rainfed morphological expression and molecular markers for assessing genetic diversity and relationship in certain oat genotypes, to identify desirable parental combination and associate both morphological as well as molecular markers ISSR which could then be used in oat improvement programme.

MATERIALS AND METHODS

The seed material of 20 high yielding oat genotypes representing their origin from different geographical locations of the world were collected (Table 1).

Rainfed expression analysis

All the 20 varieties of oat for observation of phenotypic characteristics were planted in the experimental field at the Instructional Dairy Farm, Nagla, G.B. Pant University of Agriculture and Technology, Pantnagar in a plot size of 30.0 x 6.0 m (one meter long 5 rows spaced 20 cm apart for each variety), ans replicated thrice in randomized complete block design with no irrigation during the crop growing period for two consecutive years (2010 to 2012). Seven primary quantitative characteristics were recorded to analyze the genotypic variability among the treatments (Table 2) by using 20 ISSR primers (Table 3).

Data analysis

The mean of two years data on morphological observation was used to calculate the dissimilarity matrix to find out the genotypic relationship using NTSYpc, version 2.1. Dendrogram was constructed using Euclidean distance coefficients and the correlation between the tree and similarity matrices was estimated by means of the Mantel matrix correspondence test (Mantel, 1967).

ISSR analysis

Total genomic DNA was extracted using the method of Doyle and Doyle (1990) from ten days old seedlings. Amplifications were carried out in volumes of 25 µl reaction in a polymerase chain reaction (PCR) tube, containing in of 1X KCl buffer (Fermentas) containing 0.2 mM dNTPs, 30 ng of each primer, 1.5 mM MgCl2, 0.8U Taq DNA polymerase (Fermentas) and 100 ng of DNA. The reaction was performed in the form of master mix to minimize error. Amplification reaction were carried out in Thermal cycler PTC-100 (MJ Research Thermocycler) according to the following temperature profile: 4 min initial denaturation at 94°C; 37 cycles of 94°C for 1 min, 50°C for 45 s, 72°C for 1 min and final extension of 7 min at 72°C and final hold at 4°C. All amplifications were performed twice and independently to make sure that the results were correct. Electrophoresis was done at 50 V for 4 h in 1 X TBE electrophoresis buffer. The gels were stained using ethidium bromide solution and documented in Gel Doc system (Bio-Rad).

Scoring and data analysis

Amplified products were scored twice manually and independently for each primer (Figure 1). Only clear polymorphic ISSR bands of various molecular weight sizes were scored manually in binary formula of 1 or 0 for their presence or absence, respectively, mobility were considered as a single locus. The total numbers of bands, polymorphic bands, and average number of bands per primer with polymorphism percentage were calculated. Similarity matrix for (ISSR primers was constructed using the Jaccard's similarity coefficient values to find out genotypic relationship. These data were then subjected to unweighted pair-group method with arithmetic averages (UPGMA) analysis to generate dendrogram using NTSYSpc-version 2.11v. Principal coordinate (3D) analysis was performed in order to highlight the resolving power of the ordination.

RESULTS AND DISCUSSION

Genetic relationship as revealed by rainfed expression

On an average of 85%, genetic similarity was observed among 20 genotypes on the basis of Euclidian dissimilarity matrix. The dendrogram based on the Euclidean distance coefficient clustered 14 genotypes in the major cluster and six genotypes in the minor cluster (Figure 2). All genotypes from exotic collection included in the study viz., EC 246199, EC 605836, EC 605838 and EC 605833 fell into a single cluster breaking at 0.15 dissimilarity coefficient value. Four genotypes from Pantnagar viz. UPO 273, UPO 270, UPO 265 and D.Sel.-6 fell in same cluster breaking at 0.09 dissimilarity coefficient value. In agreement to the clustering pattern from molecular marker data, the clustering from rainfed plot data also placed the genotype HFO 114 and D.Sel.-5 close to each other showing more than 99% similarity between them (dissimilarity coefficient value 0.006). The maximum dissimilar pair identified from the rainfed dissimilarity coefficient was between UPO 260 and EC 246199 (>79%) (Table 6). In principal coordinate analysis (PCoA) analysis, a total of 8 components separated each genotype from each other in the 3D graph explaining for 100% variation (Figure 4).

Genetic relationship as revealed by ISSR marker

The Jaccard's similarity coefficient estimates between pairs of different genotypes included in the study (Table 7) indicated a range of genetic similarity values, which varied from 0.20 between UPO 270 and EC 605836 to 0.84 between HFO 114 and D. Sel.-5. Among the 20 genotypes, the three pairs with lowest GS value, that is, maximum diverse pairs were EC 605836 and UPO 270 (20% genetic similarity), KENT and UPO 212 (21 % genetic similarity), UPO 212 and EC 246199 and UPO 260 and EC 246199 (with GS value 24%, respectively). Genetic similarity between D. Sel. series genotypes varied from 69 to 75% showing high similarity between them. Similarly, three pairs with maximum GS value, that is, minimum diversity in the experimental material of the present study were HFO 114 and D. Sel. 5 (genetic similarity value 84%), D.Sel.-6 and HFO 114 (GS value 77%), between D.Sel.-1 and D.Sel.-5; KENT and D.Sel.-6 (genetic similarity value of 75%, respectively). The test genotypes were differentiable based on the absence or presence of amplified bands with different primers. For example, D.Sel.-1 was differentiated from Wright based on the amplification pattern with primer 2 at 2000 bp and with primer 14 at 1400 bp. Thus, combination of amplification pattern obtained with these two primers can be used effectively to distinguish different pairs of test genotypes. The dendrogram constructed from ISSR marker analysis in oats revealed that the first cluster broke at 0.31 Jaccard's coefficient of similarity, which separated UPO 212 from all other genotypes (Figure 3). Thus, the major gene cluster consisted of 19 oat genotypes leaving one genotype in the first minor gene cluster viz. UPO 212. Within the major gene cluster, D.Sel.-5 and HFO 114 were not further separated indicating the high level of genetic similarity (>84%) between the two, that is, some ancestral relationship between them. The secondary gene cluster was formed within the major gene cluster at 0.325 Jaccard's coefficient of similarity. The secondary gene cluster divided the major gene cluster into two sub-groups. In this cluster separation, the genotype EC 605836 came alone to the secondary minor cluster, the rest 18 genotypes fell in secondary major cluster. Thus, UPO 212 and EC 605836, separated distinctly from the rest of the genotypes while the tertiary cluster broke at 0.385 Jaccard's coefficient value where major gene cluster comprised of 14 genotypes while the four genotypes viz. UPO 260, EC 605833, OS 6 and Wright fell in the minor gene cluster. In the present study, 26 ISSR primers were used; 20 of them (Table 4) showed polymorphism (50 to 100%) and

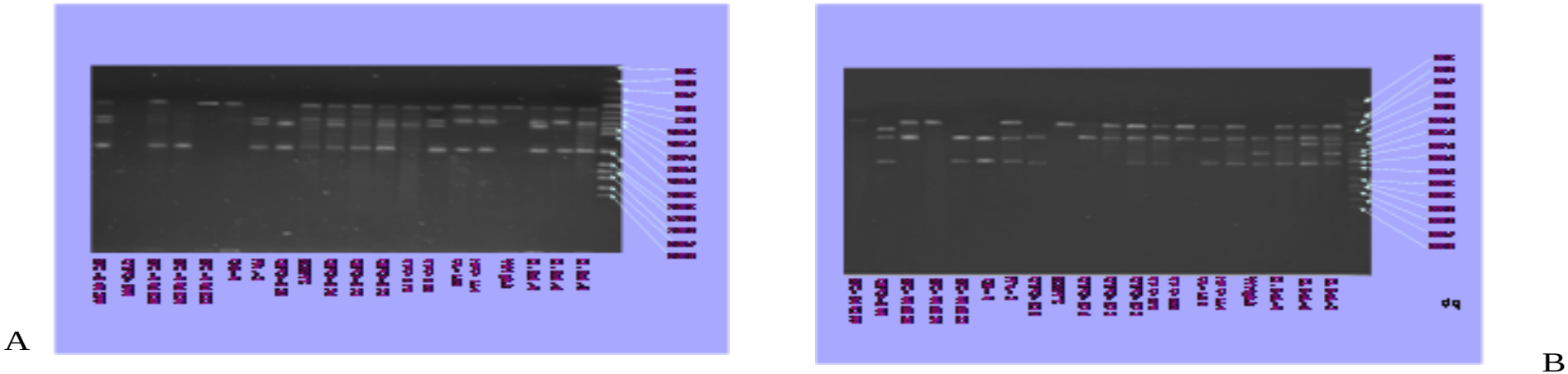

Figure 1. ISSR profile of oat genotypes on agarose gel. A, Amplification products obtained with primer 2; B, primer 14.

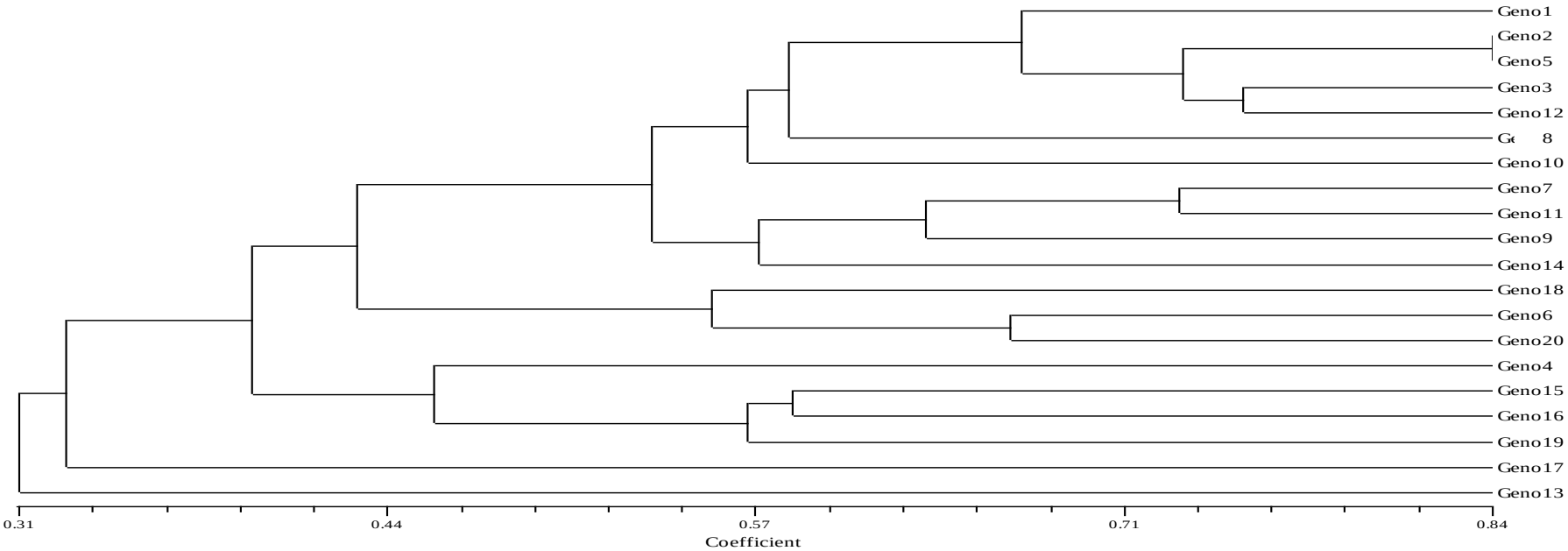

Figure 2. Dendrogram obtained from the pooled data of 20 ISSR profiles and 20 genotypes of oat. Geno, Genotype; 1, D. Sel.-1; 2, D. Sel.-5; 3, D. Sel.-6; 4, Wright; 5, HFO 114; 6, OL 125; 7, UPO 265; 8, UPO 270; 9, UPO 271; 10, UPO 273; 11, UPO 275; 12, Kent; 13, UPO 212; 14, No. 1; 15, OS 6; 16, EC 605833; 17, EC 605836; 18, EC 605838; 19, UPO 260; 20, EC 246199.

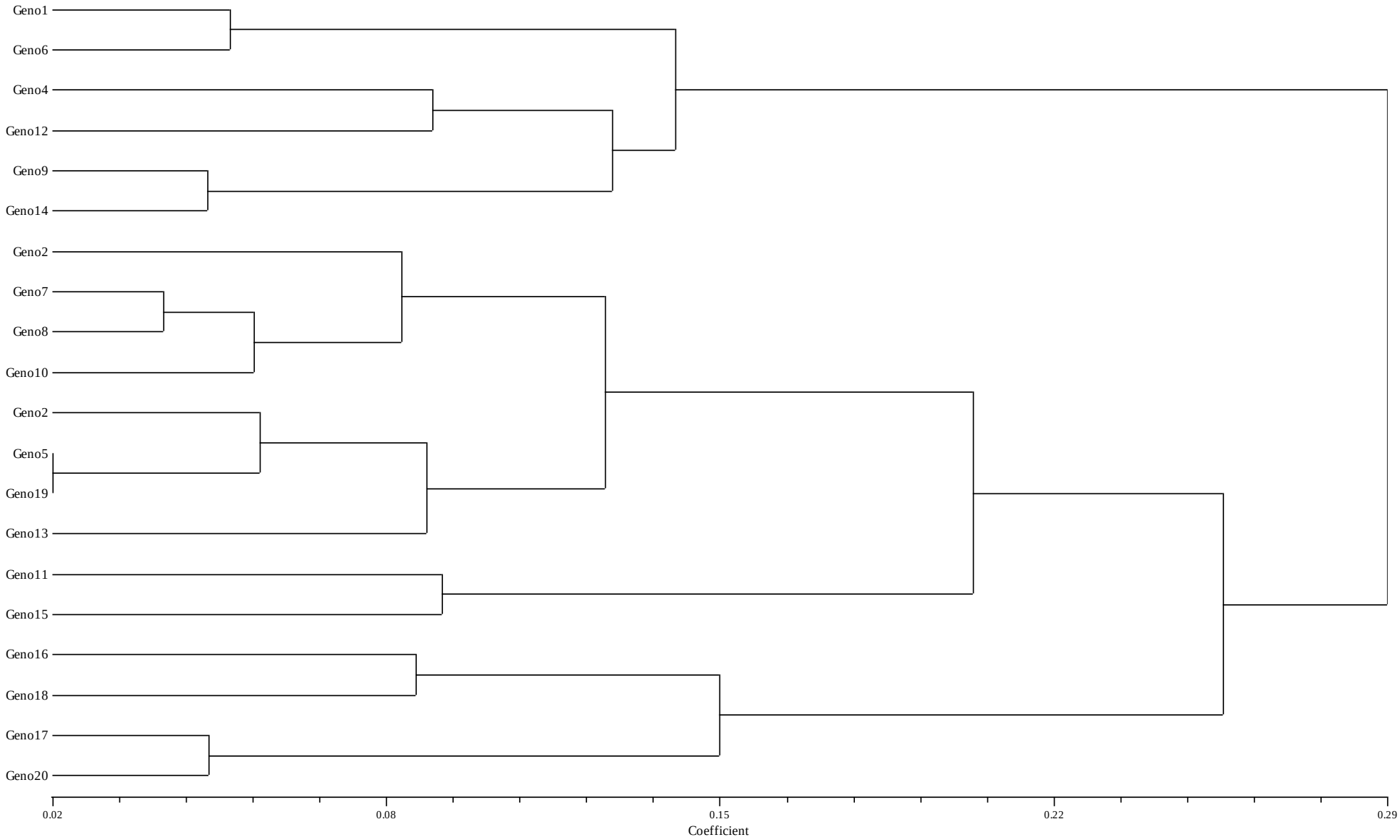

Figure 3. Dendrogram obtained from the pooled data of 8 quantitative variables from rainfed plots and 20 genotypes of oats. Geno, Genotype; 1, D. Sel.-1; 2, D. Sel.-5; 3, D. Sel.-6; 4, Wright; 5, HFO 114; 6, OL 125; 7, UPO 265; 8, UPO 270; 9, UPO 271; 10, UPO 273; 11, UPO 275; 12, Kent; 13, UPO 212; 14, No. 1; 15, OS 6; 16, EC 605833; 17, EC 605836; 18, EC 605838; 19, UPO 260; 20, EC 246199.

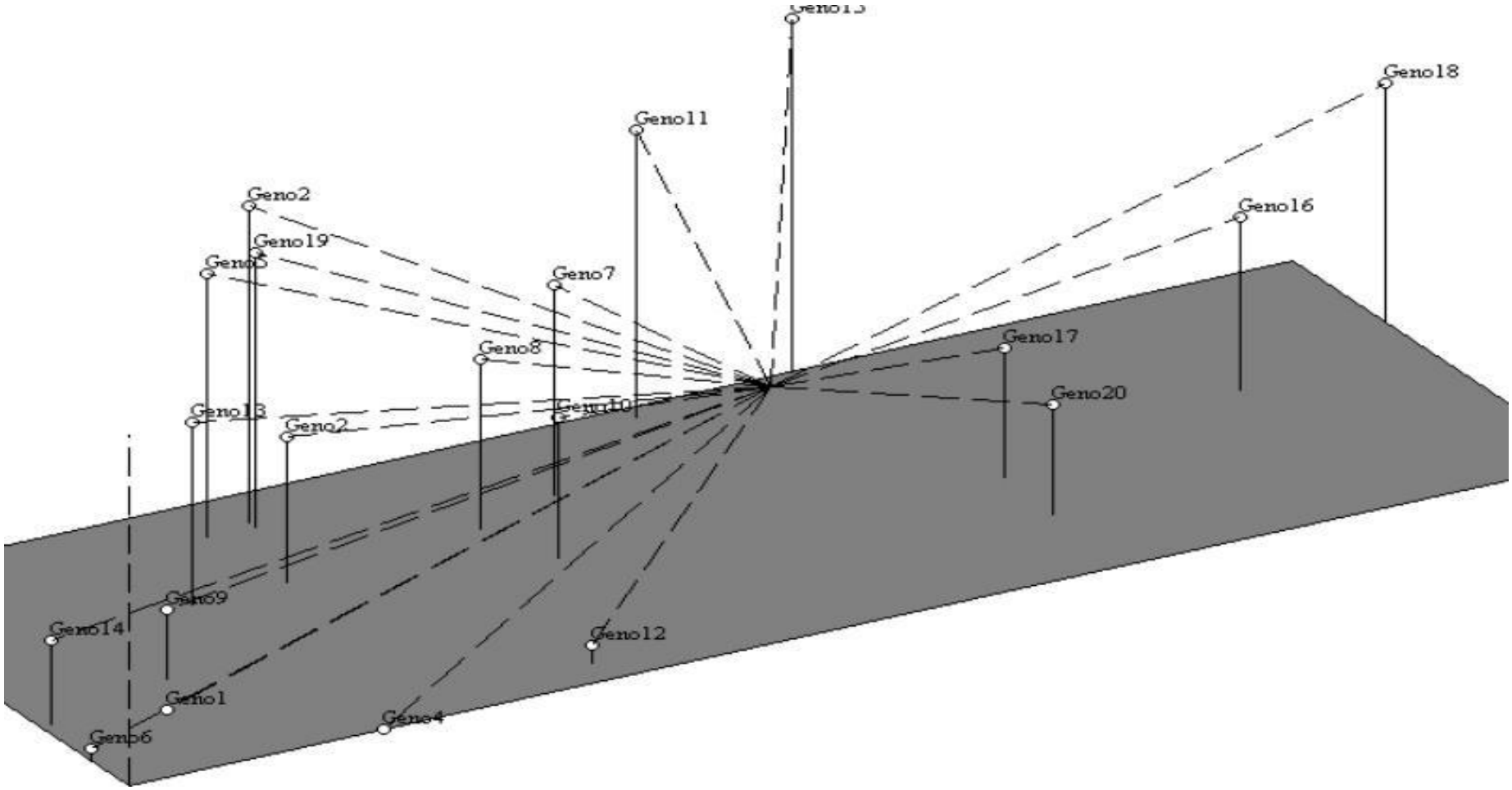

Figure 4. Relationships among 20 genotypes of oat visualized by principal component analysis (PCoA) of rainfed plot observations based genetic similarities. The numbers plotted represents individual genotypes. 1, D. Sel.-1; 2, D. Sel.-5; 3, D. Sel.-6; 4, Wright; 5, HFO 114; 6, OL 125; 7, UPO 265; 8, UPO 270; 9, UPO 271; 10, UPO 273; 11, UPO 275; 12, Kent; 13, UPO 212; 14, No. 1; 15, OS 6; 16, EC 605833; 17, EC 605836; 18, EC 605838; 19, UPO 260; 20, EC 246199.

clustered most genotypes according to their place of origin and amplification profile of these primers was revealed (Table 5). Similar kind of findings had also been reported by Loskutov et al. (2007) and Wight et al. (2003). The DNA based marker technology imparts a diagnostic tool that permits direct identification of genotypes or strains provided that the DNA marker is closely linked to the trait of interest. In this study, a unique band was identified with primer 14 in UPO 260 of 600 bp, which can be used for identification and characterization of this genotype. The number of loci for

Table 3. Characteristics of microsatellite ISSR primers.

Genetic code	Primer seq 5'- 3'	Mer	OD	%GC	Tm
4824-038	AGAGAGAGAGAGAGAGC	17	16.0	53%	46.8
4824-039	GAGAGAGAGAGAGAGAC	17	14.7	53%	43.3
4824-040	GAGAGAGAGAGAGAGAA	17	10.0	47%	44.3
4824-041	AGAGAGAGAGAGAGAGTT	18	11.6	44%	45.4
4824-042	AGAGAGAGAGAGAGAGCC	18	17.5	55%	52.2
4824-043	AGAGAGAGAGAGAGAGTA	18	9.8	44%	43.3
4824-044	GAGAGAGAGAGAGAGACC	18	11.6	55%	49.2
4824-045	GAGAGAGAGAGAGAGAA	17	5.4	47%	44.3
4824-046	ACACACACACACACACT	17	8.6	47%	49.2
4824-047	ACACACACACACACACC	17	11.0	53%	53.3
4824-048	GAGAGAGAGAGAGAGAT	17	7.7	47%	42.9
4824-049	GAGAGAGAGAGAGAGAC	17	18.4	53%	43.3
4824-050	GAGAGAGAGAGAGAGAA	17	8.0	47%	44.3
4824-051	GAGAGAGAGAGAGAGACT	18	9.1	50%	45.6
4824-052	AGAGAGAGAGAGAGAGTT	18	11.6	44%	45.4
4824-053	CTCTCTCTCTCTCTCTGC	18	13.4	55%	50.9
4824-054	CACACACACACACACAT	17	7.7	47%	51.1
4824-055	CACACACACACACACAGT	18	7.3	50%	53.7
4824-056	ACACACACACACACACGT	18	9.6	50%	56.3
4824-057	GTGTGTGTGTGTGTGTA	17	7.9	47%	47.6

Table 4. Summary of ISSR amplified products in 20 genotypes of oat.

Specification	Particular
Total number of primers tested	26
Number of polymorphic primers	20
Total number of monomorphic primers	6
Total number of unique bands identified	1
Total number of bands amplified	94
Size range of amplified products (in bp)	400 to 2000
Average number of bands per primer	4.7
Total number of polymorphic bands identified	75
Total number of monomorphic bands identified	19
Percentage of all bands that were polymorphic	79.78 %

each ISSR primer used in the study averaged around 4.7 loci per primer with average polymorphism content of 79.78%; similar detections have been reported by Wei-Tao et al. (2009) using ccSSR markers. Deletions, insertions, chromosomal inversion, to mention but a few, might be the main causes of differences at the DNA level which generate polymorphism or allelic diversity. Genotypes EC 605836 and UPO 270 had the lowest genetic similarity value (0.20) revealing that they were the most diverse pair of genotype used in the experimental material thus establishing the utility of microsatellite markers in identifying diverse pairs. In a similar attempt, LF dos Santos et al. (2011) studied diversity among cultivated oat varieties and validated the transferability of genome using microsatellite markers.

Association between molecular marker and rainfed classification

From the dissimilarity and similarity coefficient tables (Tables 6 and 7), it can be concluded that the genotype HFO114 and D.Sel.-5 were genetically very similar to each other since the genomic similarity value for them was high based on rainfed classification as well as molecular marker (ISSR) data analysis. This mark a possibility that the ISSR markers used in the study may be linked to the genomic region in these genotypes, which governs one or the other observation taken in the rainfed plot. Also, UPO 260 and EC 246199 were identified as the most dissimilar pair in both rainfed data (75%) and ISSR analysis (75%). Similar kind of association was established

Table 5. Amplified products and polymorphism obtained with ISSR primers.

Primer	Number of amplified loci	Polymorphic band(s)	Monomorphic band	Percent (%) polymorphism
1	4	3	1	75.00
2	5	5	0	100.00
3	3	3	0	100.00
4	2	1	1	50.00
5	6	5	1	83.33
6	7	5	2	71.42
7	5	4	1	80.00
8	5	4	1	80.00
9	3	3	0	100.00
10	3	3	0	100.00
11	4	2	2	50.00
12	4	3	1	75.00
13	6	4	2	66.66
14	5	5	0	100.00
15	7	6	1	85.71
16	5	4	1	80.00
17	6	5	1	83.33
18	6	4	2	66.66
19	3	3	0	100.00
20	5	3	2	60.00
Total	94	75	19	79.78

Table 6. Similarity coefficient between genotypes using 20 ISSR profiles.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.00																			
0.75	1.00																		
0.69	0.71	1.00																	
0.50	0.52	0.46	1.00																
0.68	0.84	0.77	0.44	1.00															
0.52	0.54	0.54	0.35	0.59	1.00														
0.54	0.56	0.62	0.45	0.60	0.48	1.00													
0.55	0.57	0.63	0.42	0.68	0.38	0.58	1.00												
0.64	0.61	0.67	0.52	0.59	0.48	0.70	0.58	1.00											
0.48	0.62	0.56	0.52	0.67	0.35	0.57	0.52	0.50	1.00										

Table 6. Contd.

0.41	0.52	0.57	0.42	0.50	0.38	0.73	0.43	0.58	0.52	1.00									
0.56	0.69	0.75	0.34	0.74	0.41	0.54	0.50	0.48	0.59	0.55	1.00								
0.26	0.22	0.38	0.28	0.29	0.29	0.35	0.32	0.30	0.35	0.32	0.41	1.00							
0.45	0.52	0.58	0.35	0.56	0.50	0.67	0.54	0.52	0.35	0.54	0.50	0.37	1.00						
0.44	0.46	0.46	0.56	0.38	0.42	0.52	0.42	0.46	0.39	0.55	0.39	0.35	0.48	1.00					
0.36	0.37	0.48	0.42	0.40	0.30	0.41	0.38	0.42	0.35	0.38	0.41	0.38	0.43	0.59	1.00				
0.35	0.36	0.36	0.26	0.28	0.35	0.27	0.20	0.24	0.33	0.25	0.35	0.27	0.29	0.33	0.28	1.00			
0.44	0.41	0.46	0.33	0.44	0.59	0.45	0.31	0.40	0.39	0.31	0.50	0.28	0.41	0.40	0.42	0.41	1.00		
0.39	0.36	0.41	0.40	0.33	0.29	0.33	0.31	0.30	0.33	0.31	0.34	0.44	0.35	0.56	0.59	0.50	0.40	1.00	
0.42	0.44	0.50	0.24	0.42	0.67	0.50	0.33	0.43	0.25	0.39	0.42	0.24	0.45	0.37	0.25	0.47	0.53	0.24	1.00

1= D. Sel.-1; 2= D. Sel.-5; 3 = D. Sel.-6; 4 = Wright; 5= HFO 114; 6= OL 125; 7 = UPO 265; 8 = UPO 270; 9 = UPO 271; 10 = UPO 273; 11 = UPO 275; 12 = Kent; 13 = UPO 212; 14 = No. 1; 15 = OS 6; 16 = EC 605833; 17 = EC 605836; 18 = EC 605838; 19 = UPO 260; 20 = EC 246199.

Table 7. Dissimilarity coefficient between genotypes using 8 quantitative variables rainfed plots.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0.000																			
0.175	0.000																		
0.339	0.175	0.000																	
0.136	0.180	0.018	0.000																
0.288	0.006	0.122	0.265	0.000															
0.005	0.222	0.022	0.155	0.330	0.000														
0.267	0.106	0.011	0.248	0.119	0.314	0.000													
0.235	0.007	0.010	0.212	0.108	0.283	0.004	0.000												
0.134	0.105	0.010	0.103	0.181	0.168	0.195	0.156	0.000											
0.219	0.085	0.085	0.178	0.141	0.264	0.007	0.004	0.142	0.000										
0.387	0.218	0.022	0.343	0.150	0.433	0.128	0.152	0.291	0.176	0.000									
0.133	0.163	0.016	0.094	0.267	0.165	0.209	0.182	0.143	0.146	0.319	0.000								
0.237	0.101	0.010	0.196	0.782	0.275	0.143	0.111	0.113	0.121	0.200	0.542	0.000							
0.116	0.138	0.014	0.114	0.214	0.139	0.236	0.198	0.005	0.186	0.334	0.163	0.149	0.000						
0.475	0.311	0.031	0.416	0.233	0.520	0.220	0.242	0.373	0.259	0.010	0.396	0.278	0.417	0.000					
0.410	0.299	0.030	0.361	0.312	0.453	0.210	0.233	0.361	0.224	0.206	0.300	0.327	0.406	0.225	0.000				
0.312	0.212	0.021	0.266	0.251	0.355	0.143	0.155	0.268	0.136	0.194	0.202	0.251	0.311	0.248	0.010	0.000			
0.497	0.373	0.037	0.441	0.362	0.540	0.275	0.302	0.437	0.299	0.229	0.386	0.386	0.482	0.209	0.009	0.187	0.000		
0.290	0.120	0.006	0.269	0.000	0.333	0.105	0.010	0.187	0.134	0.138	0.265	0.009	0.222	0.224	0.298	0.239	0.348	0.000	
0.310	0.228	0.023	0.247	0.271	0.349	0.173	0.176	0.265	0.147	0.222	0.188	0.258	0.307	0.268	0.120	0.005	0.203	0.796	0.000

1= D. Sel.-1; 2= D. Sel.-5; 3 = D. Sel.-6; 4 = Wright; 5= HFO 114; 6= OL 125; 7 = UPO 265; 8 = UPO 270; 9 = UPO 271; 10 = UPO 273; 11 = UPO 275; 12 = Kent; 13 = UPO 212; 14 = No. 1; 15 = OS 6; 16 = EC 605833; 17 = EC 605836; 18 = EC 605838; 19 = UPO 260; 20 = EC 246199.

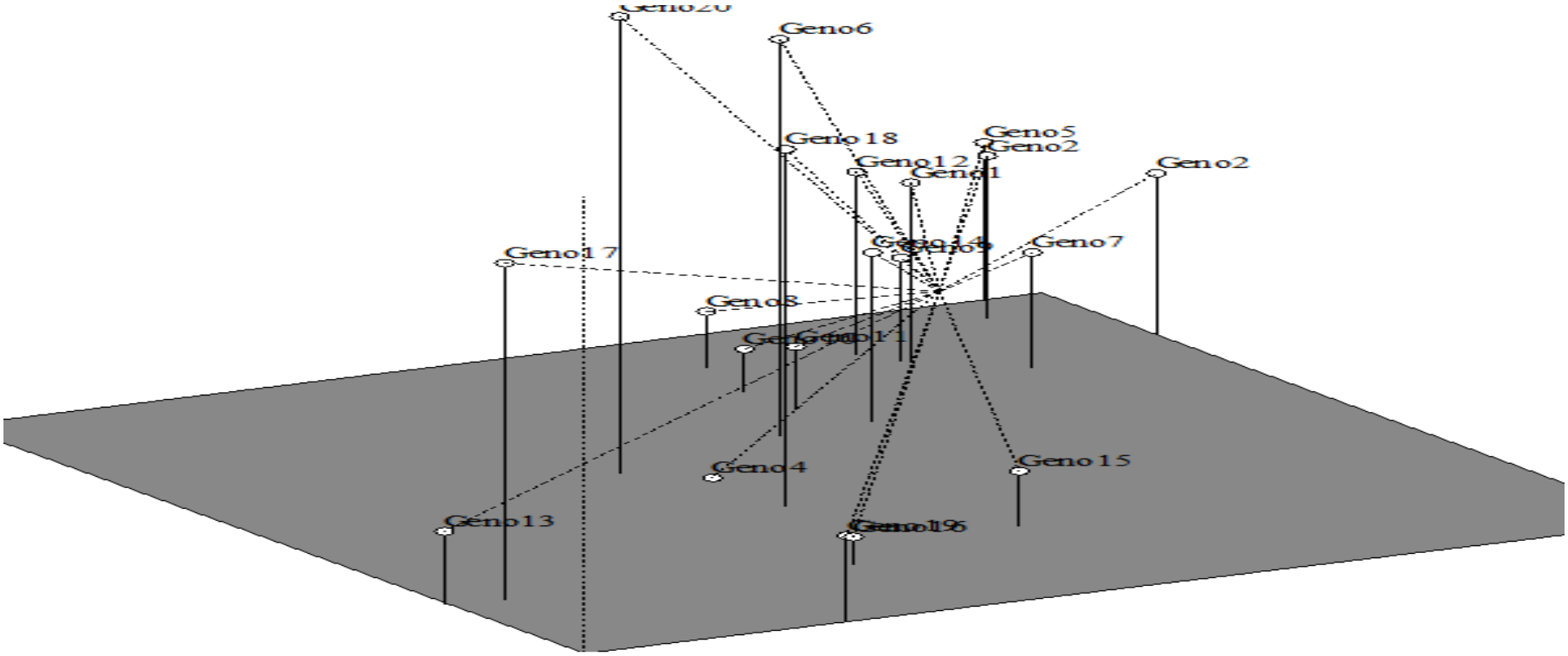

Figure 5. Relationships among 20 genotypes of oat visualized by PCoA of ISSR based genetic similarities. The numbers plotted represents individual genotypes.

by using amplified fragment length polymorphism (AFLP) markers for various traits including plant height and grain yield in oat (Achleitner et al., 2008). Thus, this probable linkage between the ISSR marker and observation taken in rainfed plots needs to be validated through precise investtigation for association either through bulk segregation analysis or near isogenic lines (Tanhuanpaa et al., 2007). ISSR markers have been used successfully to generate more repeatable microsatellite markers, thus can be used for characterization studies (Lian et al., 2001). In addition, the relationships between the Euclidean distance matrix based on rainfed plot observations and ISSR markers were analyzed using the matrix correlation approach developed (Mantel, 1967). A certain agreement of test for association between the two, that is, ISSR marker variability test and rainfed plot observations came but on the negative side, as evidenced by a low and non-significant correlation (r = -0.186) which is further con-firmed by a negative non-significant Mantel *t*-test value (*t* = -0.9753) between the morphological genetic distance matrix and the ISSR marker matrix. A weak correlation between Euclidean based genetic distance matrices indicated the discrepancy between the rainfed plot morphological data and ISSR markers based analysis which is also supported by the broad range of genetic similarities (0.204-0.995) based on morphological rainfed expressions as compared to the broad but less similar range based on ISSR (0.20-0.84) analysis. The greater similarity (Table 6) taken from rainfed plot might be due to similar environmental conditions and hence the near equivalent G x E interaction resulted in similar and narrow pattern of response of genotypes. The two methods were not found comparable in distinguishing all the genotypes individually which was evident by the dendrogram patterns (Figures 2 and 3) and 3D analysis (Figures 4 and 5). Several other comparisons between morphological and molecular marker based study

also indicated similar non-compatible results (Roldan-Ruiz, 2001) in different crops. The moderate association between genetic distances estimated using molecular and phenotypic markers can be explained by a range of factors. Molecular analysis provides a wider genome sampling than the morphological analysis, since a study comparing both the techniques rarely evaluates the same or even a similar, number of morphological and molecular markers. The association between estimates is also influenced by the fact that a large portion of the variation detected by molecular markers is non-adaptive and, therefore, not subject to either natural or artificial selection. On the other hand, the phenotypic characters are subject to both natural and artificial selection, aside from their high environmental dependence. Moreover, the observations taken from rainfed plots were quantitative in nature and so naturally covered many minor gene distributed non-uniformly within the genome; also it is not always the case that two identical phenotypes are determined by the same genes, that is, distinct genes may lead to similar phenotypes. Thus, it is clear that such estimates are closer when there is an association between the loci controlling the targeted morphological traits (quantitative trait loci, or QTLs) and the evaluated bands and when a large number of qualitative as well as quantitative traits are evaluated (Roy et al., 2004).

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