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Full Length Research Paper

Assessing genetic diversity of perennial ryegrass (*Lolium perenne* L.) from four continents by inter-simple sequence repeat (ISSR) markers

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In this study, inter-simple sequence repeat (ISSR) markers were used to compare genetic diversity between commercial cultivars and natural germplasm which were obtained from Europe, Africa, Asia, and North America. There was a relatively high genetic variation in the whole collection judged by the polymorphism rate (97.16%), Nei's gene diversity (0.28), and Shannon's information index (0.44). Results indicate lower genetic diversity in commercial cultivars than natural germplasm. The European group showed the highest genetic diversity. The genetic distance (GD) between cultivars 'Exacta' and 'ABT-99-4.560' was the closest (0.19), while largest GD occurred between 'PI 632472' and 'PI 547390' (0.85). Based on Jaccard's similarity coefficient, 12 groups were distinguished with a cut-off point at 0.44. Using the concept of core collection, we suggested 'Headstast 2', 'PI 598909', 'Catalina II', 'PI 538976', 'PI 598440', 'PI 610925', 'PI 598877', 'PI 516605', and 'PI 619554' be included in a core collection of germplasm to accommodate maximum genetic diversity.

Key words: Genetic distance, genetic erosion, unweighted pair group method with arithmetic mean (UPGMA), cluster analysis, germplasm.

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.), native to Eurasia, is one of the most important forage and turfgrasses used in temperate region due to its rapid establishment, adaptability, and nutrition values (Thorogood, 2003). Because of the high economic value, breeders throughout the world have made a great deal of effort to develop elite cultivars. However, most of the breeding programs in the world have been relying heavily on very narrow genetic resources (Thorogood, 2003). As perennial ryegrass in old pastures and grasslands is being replaced by new cultivars without increasing the genetic diversity at the same time, there is a threat of genetic erosion (Adebooye and Opabode, 2004) despite its cross-pollination nature (Golembiewski et al., 1997). Although, this trend is difficult to quantify, comparison of variability of traits over geological distance has provided useful informaiton of genetic diversity of ecological systems (Monestiez et al., 1994). Also, understanding the genetic diversity before, during, and after the release of cultivars is of vital importance to maintain broad genetic background (Cresswell et al., 2001; Günther et al., 1996).

It is well known that genetic diversity in natural and culture populations are increasingly declining because of over-exploration, changing environments and habitat fragmentation (Tang, 2007; Yang et al, 2011). Faced with the problem of preserving species diversity of perennial rye-grass, some biologists are now paying their concerns on genetic diversity in natural and culture populations (Balfourier and Charmet, 1991; Kolliker et al., 1999; Roldan et al., 2000; Kubik et al., 2001; Ghariani et al., 2003; Bolaric et al., 2005). Roldan et al. (2000) revealed the high degree of genetic diversity within commercial

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Abbreviations: ISSR, Inter-simple sequence repeat; **GD,** genetic distance.

ryegrass using amplified fragment length polymorphism (AFLP). Ghariani et al. (2003) examined genetic diversity of 16 wild perennial ryegrass accessions from Tunisian using ISSR and found large genetic diversity. Bolaric et al. (2005) assessed the genetic diversity within and among perennial ryegrass ecotypes from Germany using RAPD and found that genetic variation within cultivars (67%) was much larger than between them (33%). Although, great efforts have been focusing on its cultivation and natural germplasm, there is hardly any information on genetic diversity in the commercial and natural populations over larger geographical regions including Asia.

Inter-simple sequence repeats (ISSR) marker works by amplifying DNA segment between two SSR sequences based on *polymerase chain reaction* (PCR) method (Zietkiewicz et al., 1994). Compared to morphological, allozyme markers, random amplified polymorphic DNA (RAPD) technique, ISSR technique is simple, economical and reliable to assess the phylogenetic relationships and identify cultivars of various plants, which has beeen tested in both dicotyledon and monocotyledon species (Bornet and Branchard, 2001; Girma et al., 2010; Godwin et al., 1997; Singh et al., 2007). The objective of this study was to confirm that ISSR could provide sufficient polymorphism in perennial ryegrass collected from Europe, Africa, Asia and America. A second objective was to compare genetic diversity between commercial cultivars with natural germplasm to understand the current status of genetic erosion. And finally, to establish a core collection list that could facilitate germplasm collection for breeding.

MATERIALS AND METHODS

Plant materials

75 accessions of perennial ryegrass were obtained from 21 countries and four continents. These accessions included 47 commercial cultivars and 28 natural germplasm and were coded according to their origins (Table 1). The plant materials were established and maintained in a hydroponic system using half strength Hoagland solution (Hoagland and Arnon, 1950). Each accession was planted in a plastic tube 10 cm in diameter and 15 cm deep, which was filled with ceramsite to 12 cm depth and covered with a 0.5 cm layer of sand. A nylon screen was secured to the bottom of the tube to allow free passage of roots into the cultural solution. The tubes were inserted 8.5 cm below the surface of hydroponic solution through a supporting rack placed on the top of 45.2 L containers. The Hoagland solution was replenished weekly. Each accession had four replicates and a total of 12 containers were maintained in a greenhouse with temperature of 22/18°C (day/night). The plants were fully established 30 days after seeding and were cut to 6 cm height every other day.

DNA preparation and ISSR genotyping

At the 6-leaf stage, fresh newly developed leaves were cut with scissors and frozen immediately with liquid nitrogen before storing in a freezer under -80ºC for further analysis. Total genomic DNA was extracted using a modified CTAB protocol described by Wang., (2009). Amplification reactions of ISSR analysis were carried out in a total volume of 25 µL per sample, which contained 1.0 U Taq DNA polymerase (BestBio, China), 1×polymerase buffer (BestBio, China), 1.5 µ*M* $MgCl_2$, 150 µ*M* dTNP (Pharmacia, America), 0.2 µ*M* primers, and 40 ng DNA template. PCRs were performed in a Biometra Uno II thermal cycler programmed for one cycle of 94°C for 5 min, followed by 38 cycles of 94°C for 45 s, 45 s annealing for different primer at 53 to 58°C, and 72°C for 90 s, with a final elongation at 72°C for 7 min. The amplified products were separated electrophoretically on a 1.6% agarose gel, stained in ethidium bromide (0.5 µg/ml) and digitally photographed under UV light using Gel Doc XR system (Bio-rad, America). The size of amplification products was estimated with a D2000 molecular marker (BestBio, China).

Sixty ISSR primers were initially synthesized based on the results from previous research (Fan et al., 2007; Haijun et al., 2007; Wei et al., 2007; Zeng et al., 2006), and twenty eight were chosen because of their stability, polymorphism, and reproducibility. The optimized annealing temperatures for the 28 primers were confirmed using Thermocycler T-gradient. All tests were repeated twice.

Data analysis

The distinct and reproducible bands of each ISSR were scored as either present (1) or absent (0) to represent the genetic identity of each individual sample. Genetic diversity parameters were calculated using the version 1.32 of PopGene software (Yeh et al., 2000), which included group size (GS), number of polymorphic loci (NPL), polymorphism rate (PR), observed number of alleles (N_a), and the following.

Effective number of alleles (*Ne*) was estimated from:

$$Ne = 1 + 4\bar{N}\upsilon$$

Where, $\bar{N}$ is the effective size of a haploid population and υ is the average mutation rate (Maruyama and Kimura, 1980).

Shannon's information index (*I*) was estimated for each locus using the following equation:

$$I = -\sum \mathrm{p_i} \ln \mathrm{p_i} (i = 1 - S),$$

Where, P_i is the frequency of the i^{th} allele and S is the sum total of alleles in the locus (Shannon and Weaver, 1949).

Average Nei's (1973) gene diversity (*He*) was estimated from:

$$He = 1 - \sum p_i^2,$$

Where, P_i is the frequency of the j^{th} allele (Nei, 1987).

A pairwise genetic similarity matrix was analyzed using Jaccard's coefficient between each and everyaccession (Jaccard, 1912). A dendrogram was constructed based on the Unweighted-Pair Group Method arithmetic Average (UPGMA) using the version 2.01 of numerical taxonomy multivariate analysis system (NTSYS) (Rohlf, 2000). Genetic distances (GD) and principal coordinate analysis (PCA) were also performed using NTSYS.

Polymorphism information content (PIC) values were calculated using the algorithm:

$$\mathrm{PIC} = 1 - \sum_{\mathbf{i=1,}}^{n} f_i^2$$

Where, f_i^2 is the frequency of the ith allele (Smith et al., 1997).

The partition, within- and among-group, of all parameters was analyzed using the version 6.1 of analysis of molecular variance (AMOVA) software in GenALEx (Peakall and Smouse, 2006).

Table 1. A list of 75 perennial ryegrass (*Lolium perenne* L.) accessions (germplasm and cultivars) used for genetic diversity analysis using inter-simple sequence repeats markers.

Accession code[a]	Accession name	Origin	Accession code	Accession name	Origin	Accession code	Accession name	Origin
a1-1	PI 619033	Romania	c2	PI 502413	Uzbekistan	D2-23	BAR LP 4420	United States
a1-2	PI 610804	Romania	c3	PI 547390	Iran	D2-24	Panther GLS	United States
a1-3	PI 598453	Romania	d1	PI 403838	Canada	D2-25	Silver Dollar	United States
a2-1	PI 610795	France	D2-1	DP1	United States	D2-26	Pinnacle	United States
a2-2	PI 598439	France	D2-2	E-99	United States	D2-27	PST-217	United States
a2-3	PI628693	France	D2-3	Linn	United States	D2-28	Premier	United States
a3-1	PI 632472	Italy	D2-4	Pizzazz	United States	D2-29	Friesta	United States
a3-2	PI 598928	Italy	D2-5	AF	United States	D2-30	Overdrive	United States
a4-1	PI 577254	Luxembourg	D2-6	Prosport	United States	D2-31	Nexus XD	United States
a4-2	PI 418722	Luxembourg	D2-7	PST-2L96	United States	D2-32	Sunshine 2	United States
a5	PI619554	United Kingdom	D2-8	CAS LP84	United States	D2-33	Inspire	United States
a6	PI 632510	Hungary	D2-9	APR 1232	United States	D2-34	Quickstart II	United States
a7	PI628717	Bulgaria	D2-10	Phantom	United States	D2-35	Charger II	United States
a8	PI 610802	Norway	D2-11	MP103	United States	D2-36	Citation Fore	United States
a9	PI 577272	Turkey	D2-12	Koos R-71	United States	D2-37	Quick Trans	United States
a10	PI 598440	Switzerland	D2-13	Yatsugeen	United States	D2-38	Salinas	United States
a11	PI 422478	Germeny	D2-14	Barlennium	United States	D2-39	Gray Star	United States
a12	PI 423136	Spain	D2-15	Exacta	United States	D2-40	Catalina II	United States
a13	PI 538976	Russian federation	D2-16	BAR LP 4317	United States	D2-41	Showtime	United States
b1-1	PI 598877	Morocco	D2-17	ABT-99-4.560	United States	D2-42	Chaparral II	United States
b1-2	PI 516605	Morocco	D2-18	Headstast 2	United States	D2-43	Majesty II	United States
b2-1	PI 598909	Tunisia	D2-19	DCM	United States	D2-44	Transformer	United States
b2-2	PI 610925	Tunisia	D2-20	PST-2LAN	United States	D2-45	Brightstar SLT	United States
b3	PI 410155	South Africa	D2-21	Quicksilver	United States	D2-46	Uno(DO411T)	United States
c1	PI 420124	Japan	D2-22	APR 1648	United States	D2-47	Fiestoc	United States

[a] Numbers immediately following letters represent populations of a country. Lower-case letter represents natural germplasm. Upper-case letter refers to commercial cultivars. The numbers after the hyphen represent population codes within the same country.

RESULTS

Genetic diversity

A total of 176 bands were generated from the 28 primers, 171 (97.16%) of which were polymorphic ranging from 100 to 2000 bp in size (Table 2). Each primer produced 2 to 9 polymorphic bands with an average of 6.1. The PIC values ranged from 0.13 for UBC842 to 0.31 for P7, with a mean of 0.23 for the 28 primers (Table 2).

Statistics with AMOVA revealed 12.09 and 87.91% variance among and within geographical population, respectively. It also showed 11.23 and 88.77% variance explained among and within population of both groups (cultivars and natural accessions). Variance differentiation was significant ($P < 0.001$) for all components (Table 3). This result suggests that genetic variance was high within groups and low among groups.

The European materials showed highest diversity judged from the means of *Ne*, *He*, and *I*, while Asia accessions showed the lowest diversity

Table 2. Characteristics of the 28 ISSR primers used for the detection of polymorphism in 75 perennial ryegrass (*Lolium perenne* L.) genotypes.

Primer	Sequence (5′~3′)	Annealing temperature (℃)	Total loci	Polymorphic loci	Polymorphism rate (%)	Size range of fragments(bp)	PIC[a]
UBC807	$(AG)_8T$	55	9	9	100	350-1700	0.19
UBC817	$(CA)_8A$	53	8	8	100	300-1900	0.23
UBC821	$(GT)_8T$	55	7	7	100	400-1000	0.21
UBC823	$(TC)_8C$	53	7	7	100	100-1800	0.20
UBC835	$(AG)_8GCC$	55	4	4	100	450-1700	0.20
UBC836	$(AG)_8YA$	55	9	9	100	270-1800	0.28
UBC840	$(GA)_8YT$	58	8	8	100	260-1800	0.19
UBC842	$(GA)_8YG$	55	8	8	100	250-1600	0.24
UBC849	$(GT)_8YA$	55	9	9	100	400-2000	0.27
UBC855	$(AC)_8YT$	55	7	7	100	300-1400	0.30
UBC856	$(AC)_8YA$	55	7	7	100	370-1500	0.20
UBC857	$(AC)_8YG$	55	3	2	66.7	600-1800	0.16
UBC873	$(GACA)_4$	56	7	7	100	300-2000	0.30
UBC880	$(GGAGA)_3$	55	6	6	100	450-1500	0.17
P 1	$(GA)_8YA$	55	6	6	100	600-2000	0.22
P 2	$(GA)_8RC$	55	5	5	100	250-1900	0.31
P 3	$(GGGGT)_3$	55	4	4	100	500-1000	0.21
P 4	$(AC)_8GCT$	55	8	8	100	270-1800	0.27
P 5	$(AC)_8TG$	55	6	6	100	400-2000	0.27
P 6	$(TCC)_5TG$	55	6	6	100	400-1400	0.28
P 7	$(AC)_8GT$	55	8	7	87.5	270-1300	0.15
P 8	$(AG)_8TC$	55	7	6	85.7	400-1600	0.19
P 9	$(GA)_8GCC$	55	8	8	100	300-1600	0.31
P 10	ACT ACG ACT $(TG)_7$	55	6	5	83.3	500-2000	0.24
P 11	ACT CGT ACT $(AG)_7$	55	3	3	100	400-1700	0.23
P 12	CGT AGT CGT $(CA)_7$	55	3	2	66.7	500-1000	0.13
P 13	AGT CGT AGT $(AC)_7$	55	4	4	100	400-1500	0.22
P 14	$(AC)_8CG$	55	3	3	100	350-750	0.20

[a].PIC, Polymorphism information content (Smith et al., 1997).

(Table 4). Although, the means were not necessarily statistically significant and comparable due to small sizes from Asia and Africa, the trend was consistent for all four parameters. Genetic erosion was shown from the comparison between commercial cultivars and natural germplasm, with the former had lower *Ne*, *He*, and *I* than the later (Table 5). The genetic diversity evaluated from NPL, PR, and *Na* supported the results from *Ne*, *He*, and *I* (Table 6). Again, European materials demonstrated higher variation than other regions. Cultivated varieties showed less genetic variation than wild germplasm.

Genetic distance

The genetic distance between accessions ranged

Table 3. Analysis of molecular variance (AMOVA) of profiles developed from inter-simple sequence repeats markers in 75 perennial ryegrass (*Lolium perenne* L.).

Source of variation	df	Sum of squares	Variance component	Percentage of variation	*P*-value[a]
Analysis for four geographical groups					
Among groups	3	197.67	3.25	12.09	<0.001
Within groups	71	1678.15	23.64	87.91	<0.001
Total	74	1875.81	26.89		
Analysis for cultivars and natural accessions groups					
Among groups	1	130.09	3.03	11.23	<0.001
Within groups	73	1745.73	23.91	88.77	<0.001
Total	74	1875.81	26.94		

[a]Levels of significance were obtained through nonparametric procedures using 999 permutations.

Table 4. Variation of genetic parameters developed from inter-simple sequence repeats markers for different geographical groups of perennial ryegrass (*Lolium perenne* L.).

Statistic	America	Europe	Africa	Asia
Effective number of alleles, *Ne*				
Mean	1.39	1.49	1.48	1.35
Standard deviation	0.33	0.33	0.37	0.40
Minimum	1	1	1	1
Maximum	2	2	1.92	1.80
Nei's gene diversity, *He*				
Mean	0.24	0.29	0.27	0.19
Standard deviation	0.17	0.16	0.19	0.22
Minimum	0	0	0	0
Maximum	5	0.50	0.48	0.44
Shannon's information index, *I*				
Mean	0.38	0.44	0.40	0.27
Standard deviation	0.23	0.22	0.27	0.32
Minimum	0	0	0	0
Maximum	0.69	0.69	0.67	0.64

Table 5. Comparison of genetic variation between commercial cultivars and natural germpasm using parameters developed from inter-simple sequence repeats markers.

Statistic	Cultivated varieties	Natural germplasm	Overall
	Effective number of alleles, *Ne*		
Mean	1.39	1.51	1.46
Standard deviation	0.33	0.32	0.32
Minimum	1	1	1
Maximum	2	2	2
	Nei's gene diversity, *He*		
Mean	0.24	0.31	0.28
Standard deviation	0.17	0.15	0.15
Minimum	0	0	0

Table 5. Continue

Maximum	0.5	0.5	0.5
	Shannon's information index, *I*		
Mean	0.37	0.47	0.44
Standard deviation	0.23	0.20	0.20
Minimum	0	0	0
Maximum	0.69	0.69	0.69

Table 6. Differences of genetic diversity parameters among continents and between collections based on different classifications of perennial ryegrass (*Lolium perenne* L.) developed from inter-simple sequence repeats markers.

Comparison	Category	Statistic			
		GS[a]	NPL[b]	PR[c]	*Na*[d]
Continent vs continent	America	-	153	86.93	1.87
	Europe	-	156	88.64	1.89
	Africa	-	122	69.32	1.69
	Asia	-	76	43.18	1.43
Cultivated vs natural	Cultivars	47	152	83.36	1.86
	Natural germplasm	28	165	93.75	1.94
Recommended core vs whole	Recommended core	9	142	80.68	1.81
	Whole collection	75	171	97.16	1.97

[a]GS, Group size; [b]NPL, number of polymorphic loci; [c]PR, polymorphism rate; [d]N_a, observed number of alleles.

from 0.18 to 0.94. The commercial cultivars 'Panthers GLS' and 'Nexus XD' from United States had the closest GD (0.18). The largest GD (0.94) occurred between 'premier' from United States and 'PI 619554' from United Kingdom. Natural perennial ryegrass generally had a greater GD than commercial cultivars. However, greater GD also observed between a few natural accessions and commercial accessions. For example, GD between 'Headstast 2' and 'PI 516605' reached 0.80. The genetic distance between 'PI 628693' and 'PI 538976' was 0.88. An average GD of 0.73 was observed between 'PI 619554' and the rest accessions.

Phylogenetic analysis

The Jaccard's similarity coefficient ranged from 0.32 to 0.72 (Figure 1). Based on the polymorphic bands, 75 perennial ryegrass accessions were clustered into 12 groups (I–XII) with a cut-off point at 0.44. The accessions from same geological regions were likely to be clustered into the same group. Natural germplasm and commercial varieties were generally clustered into different groups. Group I included 56 accessions (74.7%), which consisted of 45 commercial cultivars and 11 natural accessions. Group I was further divided into 5 subgroups at Jaccard's similarity coefficient of 0.48. Group I covered materials from United States (45) and Europe (11). The subgroup I-1 had 46 perennial ryegrass accessions, 43 of which were commercial cultivars from the United States, and the other three were natural accessions from three different European countries. The subgroup I-2 was composed of 6 natural accessions from Europe. The subgroup I-3 included 2 natural accessions from two European countries. The subgroup I-4 and I-5 each had only one accession, 'Quick Trans' from United States and 'PI 619033' from Romania, respectively. Group III contained 3 natural accessions from Europe. Group VI included 7 natural accessions, 3 of which came from Europe, 3 others were from Asia and the last one from Africa. Group II, IV, V, VII, VIII, IX, X, XI and XII each had only one accession and collectively accounted for 83% of the total variation based on genetic diversity parameters (NPL, PR, *Na*, *Ne*, *He*, and *I*) (Table 6). They were 'Headstast 2', 'PI 598909', 'Catalina II', 'PI 538976', 'PI 598440', 'PI 610925', 'PI 598877', 'PI 516605' and 'PI 619554', respectively. This suggested that a core germplasm list can be potentially constructed, which enrich its diversity.

Two-dimensional plot based on PCA of ISSR data revealed a similar grouping result as from UPGMA. The first and second principle coordinates accounted for 11.28% of the total variation. 'Headstast 2', 'PI 598909', 'PI

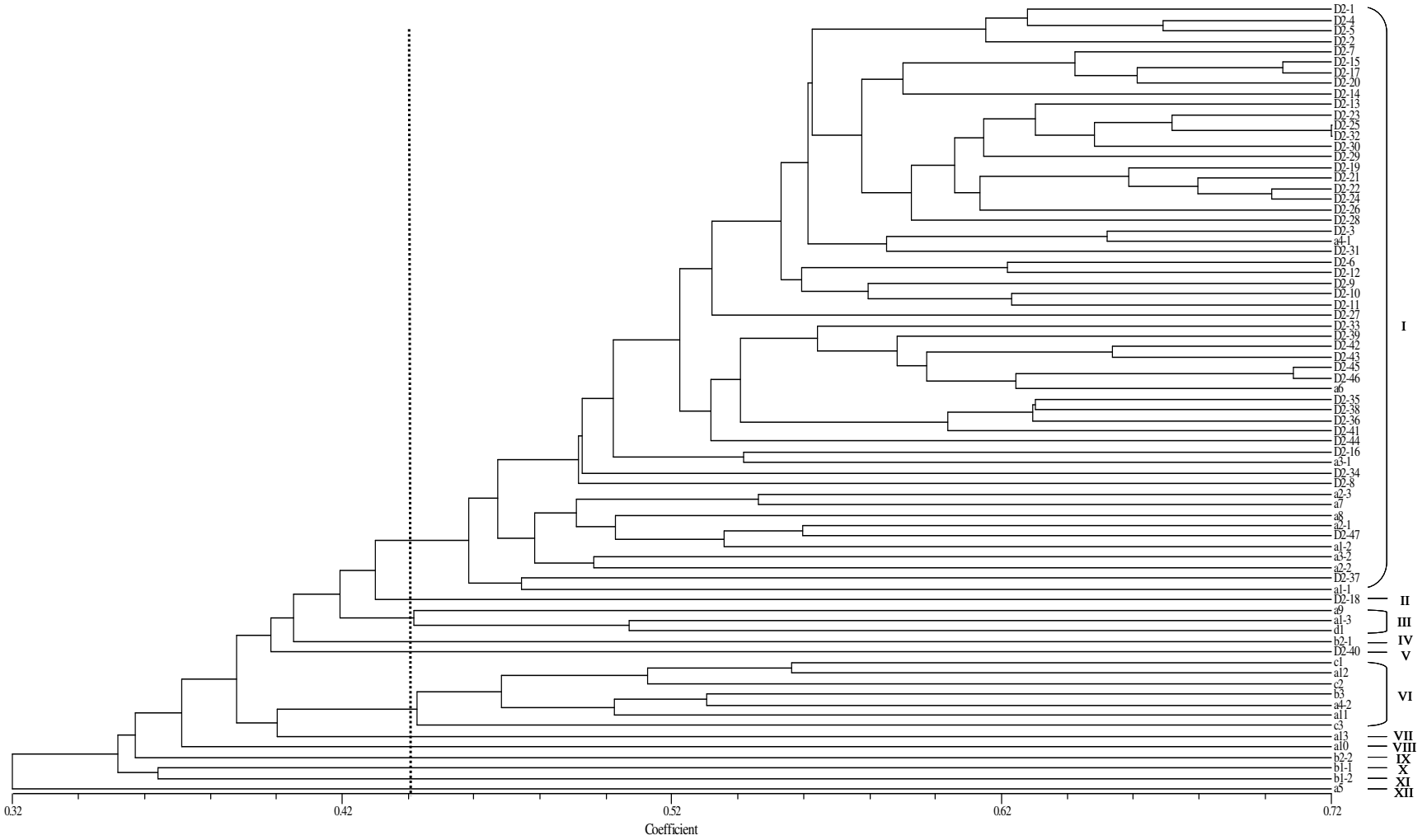

Figure 1. Jaccard's similarity coefficient of 75 perennial ryegrass (*Lolium perenne* L.) based on inter-simple sequence repeats markers.

538976' and 'PI 598877' were all distinctly differentiated from the other accessions by the two principle coordinates, and were clustered into groups having one accession each (Figure 2).

DISCUSSION

Although, not necessarily comparable among geological regions, the present study showed polymorphism rates as high as 97.16% for the whole population. The interpretation was evidently affected by the population size, as only 43.18% was found in 3 Asian accessions. Nevertheless,

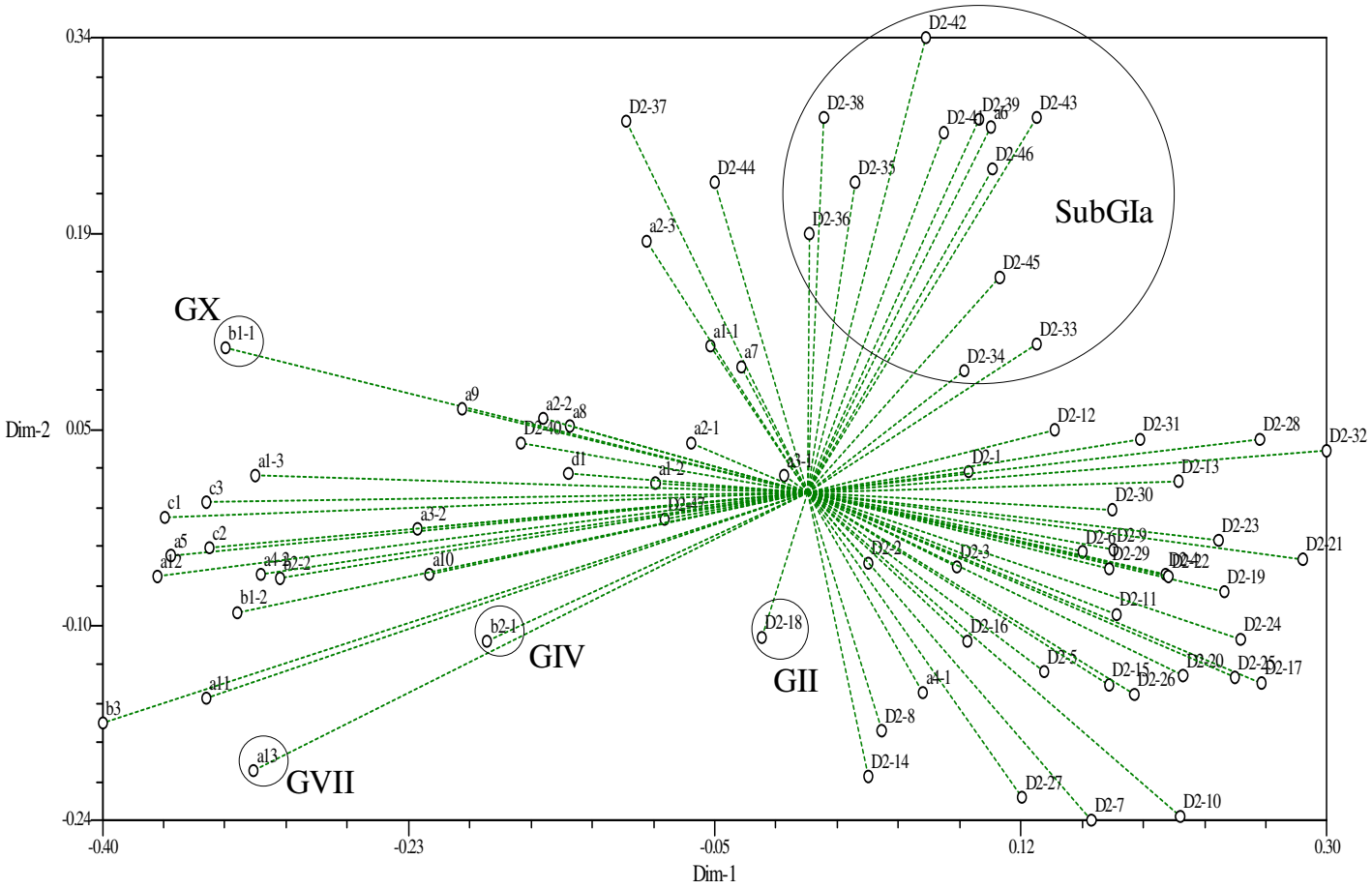

Figure 2. Two-dimensional plot based on principle component analysis using inter-simple sequence repeats markers for 75 perennial ryegrass (*Lolium perenne* L.) accessions, where Dim-1 is the first component and Dim-2 is the second component.

the polymorphism was comparable or higher than previously reported in perennial ryegrass with comparable population size by several PCR-based molecular markers techniques (AFLP, RAPD, and SSR). Jones et al. (2001) detected 67% polymorphism in diverse genotypes with 2 to 7 alleles per locus based on SSR. Guthridge et al. (2001) reported a polymorphism of 89.6% in two perennial ryegrass populations based on AFLP. The present study also corroborated the ISSR results by Ghariani et al. (2003) and suggested that ISSR markers technique is one of the best in detecting genetic diversity in perennial ryegrass.

The present study indicated that the whole population of 75 perennial ryegrass accessions had a relatively high level of genetic diversity (PR=97.16%, *He* =0.28 and *I*=0.44). The present study indicated that GD ranged from 0.18 to 0.94 with an average of 0.48, which was in line with Ghariani et al. (2003), who found that natural perennial ryegrass population had a GD of 0.28 to 0.78. These results further suggested that there was a greater level of genetic diversity among the 75 perennial ryegrass accessions. The genetic diversity was contributed to the growing environment in different regions and the far geographical distance engendered gene isolation. The similar results were reported in some previous research (Galván et al., 2003; Hou et al., 2006; Song et al., 2006). This high level of genetic diversity in perennial ryegrass might imply complicated and independent evolutionary processes of this species.

In this study, a high degree of divergence was found between cultivated varieties and natural germplasm or among these accessions from different geographical regions by the analysis of phylogenetic relationships. This was in part due to the fact that an independent evolutionary history for these accessions themselves with little or no gene flow for a long time (Yang et al, 2011). Results from the present study indicated lower genetic diversity in commercial group than natural perennial ryegrass despite the open-pollination which enhances genetic hybridization and introgression. The results support the findings by Warpeha et al. (1998) and differ from that by Casler (1995). For example, the GD between two commercial cultivars ‘Exacta’ and ‘ABT-99-4.560’ was the closest (0.19). The greater GD occurred between ‘PI 632472’ and ‘PI 547390’ (0.85). Although reduction of genetic diversity may seem unavoidable due to the requirement of uniformity

in new cultivars, maintenance of diversity to a certain extent is desirable for adaptability to both biotic and abiotic stresses.

The present study reveals larger genetic variation within geographical groups (87.91%) than among geographical groups (12.09%). The European group showed the higher genetic diversity than the American group which supported the suggestion by Thorogood (2003) that the American breeding program has been based on a narrow germplasm mostly from Europe. The results also reflected the degree of genetic erosion in different continents and may be used as a benchmark to monitor the change of genetic diversity as new cultivars are released, which requires sampling and analyzing of plant materials in those regions over time.

We found that a random sample consisted of 9 accessions that accounted 12% of the initial collection maintains 83% of the diversity for perennial ryegrass species. Based on the concept of core collection, a minimum representative samples of the initial collection with maximum genetic diversity of a plant species and its relatives (Frankel, 1984), we suggested 9 germplasm ('Headstast 2', 'PI 598909', 'Catalina II', 'PI 538976', 'PI 598440', 'PI 610925', 'PI 598877', 'PI 516605' and 'PI 619554') be included in a core collection of germplasm. Further collections should be made to enrich the collection especially in those categories where only one accession was included.

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