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Analysis of Genetic Diversity in Thirteen Turfgrass Cultivars Cultivated at Golf Courses Using RAPD Markers

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ABSTRACT. This study was carried our to examine the genetic relationship of 13 commercial turfgrass cultivars using Random Amplified Polymorphic DNA to provide genetic informations more efficient golf course management. Analysis of 56 random hexamer primers generated 13 to 54 polymorphic bands among the 13 cultivars with an average of 30.7 bands per primer. The results of cluster analysis based on RAPDs revealed that three major variety groups: Group I - 'Shadow II', 'Aurora Gold', 'Little Bighorn Blue', 'PennA-1', and 'PennA-4'; Group II - 'Midnight II', 'Prosperity', 'Moon light SLT', 'Bright star SLT', and 'Silver dollar'; and Group III - 'Olympic Gold', 'Silver Star', and 'Tar Heel II'. The genetic similarity coefficients among 13 turfgrass cultivars ranged from 0.039 to 1.0 with highest coefficient in Group III. Studies on morphological characters and the effective molecular markers such as sequence characterized amplified regions are further needed to identify relationships and genetic diversities within species and among species.

Key words: Cultivar, Genetic similarity, Random Amplified Polymorphic DNA, Turfgrass

Introduction

Turfgrasses have low growing habit, prostrating tendency, high shoot density, and coarse-to-fine leaf texture (Beard, 1973; Alderson and Sharp, 1994). Most turfgrasses have been established, adapted, and persisted in areas distant from their initial origin (Beard, 1973). All cultivated turfgrass have been improved by selection and breeding from collected germpla and distributed mainly during the first half of 20th century (Hitchcock, 1950).

Warm-season turfgrasses have been used extensively as lawn grass or on athletic fields in subtropical and transitional zones because of their high stress tolerance such as drought and shade and excellent disease resistance (Beard, 1973; Cockerham et al., 1994; Kim et al., 1987). Morphological classification of turfgrass cultivars has mostly been conducted with multi-genic and quantitative traits, leaf blade color, texture, shape, length, blade angle, and growth habit (Rafalski and Tingery, 1993). The application of DNA technology in agricultural research has progressed rapidly over the last twenty years, especially in cultivar identification (Nyborm, 1990).

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Among the turfgrass genera, common bermudagrass (*Cynodon dactylon* L. Pers.), tall fescue (*Festuca arundinacea* Schreb.), and perennial ryegrass (*Lolium perenne* L.) are perennial species wildly used in landscaping (Thorogood, 2003). Common bermudagrass is a member of the *Poaceae* family, *Eragrostoideae* sub-family, and *Chlorideae tribe*. It is a native of eastern Africa (Beard, 1973).

Recently, a wide range of molecular markers have been available. The techniques applied to turfgrass study include those that are based in nucleic acid hybridization, such as RFLP analysis (Botstein et al., 1980), those that selectively amplify highly informative genomic regions, such as SSR analysis (Litt and Luty, 1989; Weber and May, 1989), and those that scan nucleic acids by amplification, such as RAPD (Williams et al., 1990), DAF (Caetano-Anolles et al., 1991), and derived methods such as ASAP (Caetano-Anolles and Gresshoff, 1996), and RAFLP analysis (Sweeney and Danneberger, 1996).

PCR based methods require lower amounts of genomic DNA, are relatively low cost, and can be developed rapidly. RAPD marker can be used in the systematic study of wild plants and new crops. A major advantage of RAPD markers over some other DNA based markers is that they require no prior sequence information, and no prior knowledge about any particular gene in a target taxon (Golembiewski et al., 1997; Palumbi, 1996; Al-Humaid and Motawei, 2004)

Continuous expansion will be hampered by turfgrass

Table 1. List of turfgrass used in the study.

Cultivar	Common name	Scientific name
Shadow II	Chewing's fescue	*Festuca rubra* L. spp. *Commutata* Gaud.
PennA-1	Creeping bentgrass	*Agrostis palustris* Huds.
PennA-4	Creeping bentgrass	*A. palustris* Huds.
Aurora Gold	Hard fescue	*F. longifolia* Thuill.
Little Big Horn Blue	Hard fescue	*F. longifolia* Thuill.
Midnight II	Kentucky bluegrass	*Poa pratensis* L.
Moonlight SLT	Kentucky bluegrass	*P. pratensis* L.
Prosperity	Kentucky bluegrass	*P. pratensis* L.
Bright Star SLT	Perennial ryegrass	*Lolium perenne L.*
Silver Dollar	Perennial ryegrass	*L. perenne* L.
Olympic Gold Turf-Type	Tall fescue	*F. arundinacea Schrub*.(syn. *F. elatior*, *L. arundinaceum)*
Silver Star Turf-Type	Tall fescue	*F. arundinacea Schrub*.(syn. *F. elatior, L. arundinaceum)*
Tar Heel II Turf-Type	Tall fescue	*F. arundinacea Schrub*.(syn. *F. elatior, L. arundinaceum)*

susceptibility to disease and pest and its ability to adapt to the environment because of low genetic diversity of turfgrass species in Korea (Warnke et al., 1997; Choi and Yang, 2006; Kubik et al., 2009). Golf course managers are now searching for alternative control of the disease (Im and Kim, 1999; Chang et al., 2009; Chang et al., 2010).

This study analyzed the genetic diversity of the commercial turfgrass cultivars used as mono and hybridization components on golf courses in Korea to provide basic information on developing turfgrass cultivars.

Materials and Methods

Plant materials

A total of 13 commercial turfgrass cultivars from six species were used in the study: one chewing's fescue (*Festuca rubra* L. spp. *commutata* Gaud., 'Shadow II'); two creeping bentgrass (*Agrostis palustris* Huds., 'PennA-1' and 'PennA-4'); two hard fescue (*F. longifolia* Thuill., 'Aurora Gold' and 'Little Big Horn Blue'); three Kentucky bluegrass (*Poa pratensis* L., *F. longifolia* Thuill., 'Midnight II', 'Moonlight SLT', and 'Prosperity'); two perennial ryegrass (*Lolium perenne* L., 'Bright Star SLT' and 'Silver Dollar'); and three tall fescue (*F. arundinacea* Schreb., 'Olympic Gold', 'Silver Star', and 'Tar Heel II') (Table 1). These cultivars were planted and maintained in the greenhouses for two months.

DNA extraction

DNA was extracted using modified Cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). Genomic DNA was extracted from about 0.2 g of individual leaf tissue sample of each of the 13 cultivars. Each leaf sample was powdered finely with liquid nitrogen. Genomic DNA was extracted in 750 μL of pre-warmed (65°C) 1% CTAB buffer consisting of 1.5M NaCl, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, and 1% CTAB. The homogenate was incubated for 30min at 60°C and saturated with equal volume of chloroform/isoamyl alcohol (24:1). After being vortexed gently for three minutes the mixtures were centrifuged at 12,000 rpm for 10min at 4°C. The supernatant was transferred to new tubes and cold isopropanol was added to 2/3 volume of supernatant. After 30 min on ice, genomic DNA was precipitated by centrifugation at 12,000 rpm for 10min at 4°C. The pellets were washed with 70% ethanol and dissolved in TE buffer (pH 7.5). The quality of DNA was examined on a 1% agarose gel stained with ethidium bromide. The DNA concentration was quantified by Nano Drop system (Thermo, Wilmington, U.S.A.), diluted to working concentration of 10 ng/μL, and stored at 4oC.

PCR conditions and genotyping of the RAPD markers

The PCR analysis followed the procedures by Bioneer PCR premix kit (Bioneer Co. Ltd., Korea). A total of 56 random hexamer primers were selected for assessing RAPD analysis of 13 turfgrass cultivars (Table 2). Polymorphic DNA bands were amplified with Bioneer premix kit (Bioneer Co. Ltd., Korea) in a reaction volume of 20 uL containing 16 uL of deionized distilled water, 2.0 uL of random primer, and 2 uL of template DNA (20 ng).

Amplifications were carried out in a MyGenie96 Thermal cycler (Bioneer Co. Ltd., Korea). The programmed DNA template was initially denatured at 94°C for 5min, followed by 30 cycles of PCR amplifications with a 30-sec denaturation at 94°C, 30-sec primer annealing from 42 to 60°C, and a

Table 2. List and information of random primers in this study.

Primer	Sequence(5'→3)	Primer	Sequence(5'→3)	Primer	Sequence(5'→3)	Primer	Sequence(5'→3)
P127	ATCTGGCAGC	P153	GAGTCACGAG	P214	CATGTGCTTG	P247	TACCGACGGA
P128	GCATATTCCG	P154	TCCATGCCGT	P217	ACAGGTAGAC	P250	CGACAGTCCC
P129	GCGGTATAGT	P156	GCCTGGTTGC	P218	CTCAGCCCAG	P251	CTTGACGGGG
P130	GGTTATCCTC	P159	GAGCCCGTAG	P219	GTGACCTCAG	P254	CGCCCCCATT
P131	GAAACAGCGT	P160	CGATTCAGAG	P225	CGACTCACAG	P256	TGCAGTCGAA
P133	GGAAACCTCT	P167	CCAATTCACG	P229	CCACCCAGAG	P260	TCTCAGCTAC
P135	AAGCTGCGAG	P171	TGACCCCTCC	P231	AGGGAGTTCC	P265	CAGCTGTTCA
P139	CCCAATCTTC	P179	TCACTGTACG	P232	CGGTGACATC	P266	CCACTCACCG
P141	ATCCTGTTCG	P182	GTTCTCGTGT	P237	CGACCAGAGC	P268	AGGCCGCTTA
P144	AGAGGGTTCT	P183	CGTGATTGCT	P239	CTGAAGCGGA	P269	CCAGTTCGCC
P146	ATGTGTTGCG	P185	GTGTCTTCAC	P240	ATGTTCCAGG	P273	AATGTCGCCA
P147	GTGCGTCCTC	P189	TGCTAGCCTC	P242	CACTCTTTGC	P274	GTTCCCGAGT
P150	GAAGGCTCTG	P197	TCCCCGTTCC	P244	CAGCCAACCG		
P151	GCTGTAGTGT	P211	GAAGCGCGAT	P246	TATGGTCCGG		

1-min primer extension at 72°C allowing for completion of primer extension, with final extension at 72°C for 10 min. Initially, 5 μL of the amplified product was electrophoretically resolved in a 1.5% agarose gel in 0.5X Tris-acetate-EDTA (TAE) buffer (pH 8.0) and visualized under UV light after staining with 0.1 ug/ml of ethidium bromide (Et-Br). The polymorphic bands were scored "1" if present and "0" if absent. A similarity coefficient matrix was also formed. This matrix was analyzed by the "Power Marker" (http://statgen.ncsu.edu/powermarker/) software to construct a dendrogram and principal coordinate analysis. Using this similarity coefficient matrix, genetic distance was analyzed by a UPGMA method.

Data analysis

The clear bands from the gel images were scored manually, either as present, "1" or absent, "0" and a similarity coefficient matrix was formed. The results were confirmed with NTSYS-pc (Version 2.0, Numerical Taxonomy and Multivariate Analysis System) software. Similarity matrix was calculated using SIMQUAL. Sequential, agglomerative, hierarchical, and nested clustering was carried out with unweighted pair-group method and arithmetic average (UPGMA) method. A simple matching coefficient was used to calculate the similarity coefficient matrix in NTSYS-pc.

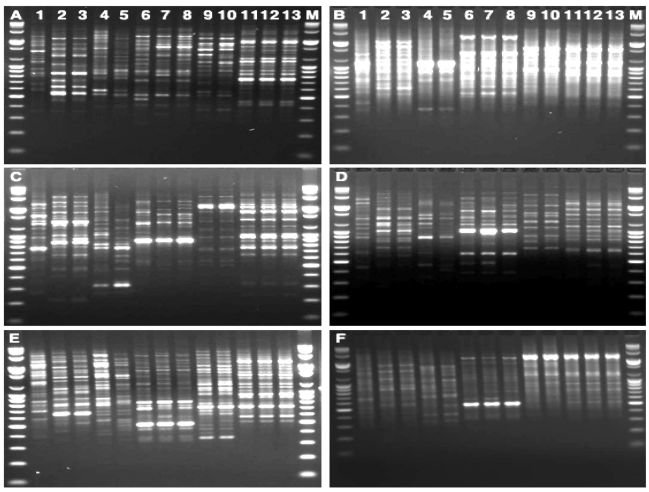

Fig. 1. Polymorphism patterns with random primers P127 (A), P128 (B), P129 (C), P130 (D), P131 (E), and P133 (F) to identify genetic differences for 13 turfgrass cultivars. M lane: Molecular marker (100 bp of ladder DNA plus); lane 1: Shadow II; lane 2: 'PennA-1'; lane 3: 'PennA-4'; lane 4: 'Aurora Gold'; lane 5: 'Little Big Horn Blue'; lane 6: 'Midnight II'; lane 7: 'Moonlight SLT'; lane 8: 'Prosperity'; lane 9: 'Bright Star SLT'; lane 10: 'Silver Dollar'; lane 11: 'Olympic Gold'; lane 12: 'Silver Star' and lane 13: 'Tar Heel II'.

Results and Discussion

Using RAPD analysis, 13 - 54 polymorphic bands were amplified and an average of 30.7 bands per primer generated. Only two primers, P128 and P130 which exhibited 100% polymorphic alleles, amplified the genetically varied bands, while 16 primers formed a single band (Fig. 1 and Table 3).

A dendrogram analysis showed that 13 turfgrass cultivars were divided into three major groups, Group I, Group II, and Group III; Group I included five cultivars, Shadow II, 'Aurora Gold', 'Little Bighorn Blue', 'Penn A-1', and 'Penn A-4'. Also, Group II included five cultivars, 'Midnight II',

Table 3. Number of polymorphic bands amplified from 13 turfgrass cultivars with each random primer.

Primer No.	GC content (%)	No. of total bands	No. of single bands	Primer No.	GC content (%)	No. of total bands	No. of single bands
P127	60	34	2	P211	60	32	3
P128	50	26	0	P214	60	19	1
P129	50	44	5	P217	50	42	9
P130	50	36	2	P218	70	24	4
P131	50	52	8	P219	60	35	3
P133	50	20	0	P225	60	51	14
P135	60	23	2	P229	70	28	8
P139	50	32	4	P231	70	25	3
P141	50	44	6	P232	60	15	1
P144	50	26	4	P237	70	19	4
P146	50	25	4	P239	60	20	3
P147	70	26	1	P240	50	30	2
P150	60	26	6	P242	50	36	9
P151	50	43	10	P244	70	25	5
P153	60	13	2	P246	60	39	6
P154	60	18	1	P247	60	43	9
P156	70	20	2	P250	70	26	4
P159	70	54	13	P251	70	22	3
P160	50	23	5	P254	70	48	7
P167	50	44	7	P256	50	29	4
P171	70	32	6	P260	50	37	7
P179	50	40	9	P265	50	36	6
P182	50	45	5	P266	70	32	5
P183	50	41	8	P268	60	40	8
P185	50	24	5	P269	70	29	4
P189	60	32	4	P273	50	27	5
P197	70	30	3	P274	60	37	10

'Prosperity', 'Moon light SLT', 'Bright star SLT', and 'Silver dollar'. Group III included three cultivars, 'Olympic Gold', 'Silver Star', and 'Tar Heel II'. This is confirmed by their genetic distance (Fig. 2).

The turfgrass cultivar classification is difficult because it has mostly relied on morphological characteristics that are multigenic, quantitative, and easily modified by environmental factors. Individual performance of cultivars and varieties within each classification may be vary widely and require comparative observation (Rafalski and Tingery, 1993).

The genetic similarity of Group I, Group II, and Group III were 0.039~0.966, 0.827~0.977, and 1.0, respectively. Group III had the highest genetic similarity (Fig. 3 and Table 4).

Most of the cultivated pasture and turfgrasses originated from Eurasia (Hartley and Williams 1956). They were key elements in the migration of livestock agriculture throughout Eurasia. Numerous perennial grasses have developed tolerance to saline soils in coastal salt marshes and beaches to rocky, alpine environments (Wu, 1981; Acharya et al., 1992).

Characterization of each accession of these species is critically important to be able to use turfgrass genetic breeding materials. Molecular markers are a powerful, reliable, and cost-effective tool for evaluating genetic diversity and can provide important insights for breeding. It is also necessary to identify the relationships and the differences of genetic diversity within species. Morphological studies need to be carried and the effective markers such as SCARs need further study.

This study revealed the genetic diversity of 13 turfgrass cultivars commercially used on the golf courses in Korea. Turfgrass cultivars most widely used on the golf courses in Korea were effectively classified through morphological

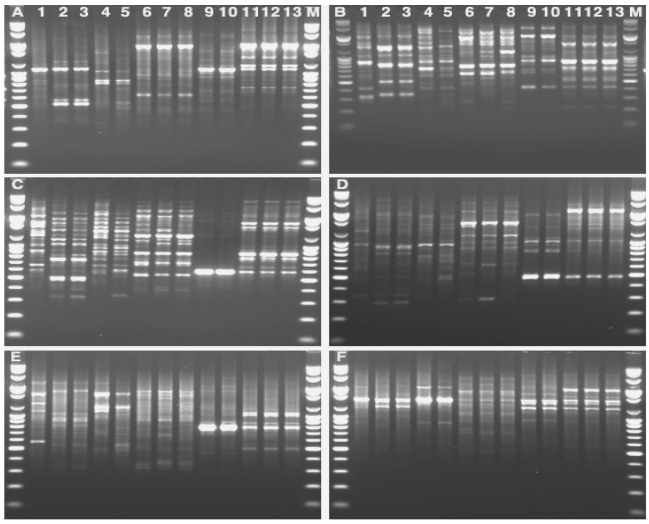

Fig. 2. PCR band patterns generated by random primers P135 (A), P139 (B), P141 (C),P144 (D), P146 (E), and P 147 (F) to identify genetic differences for 13 turfgrass cultivars. M lane: Molecular marker (100 bp of ladder DNA plus); lane 1: Shadow II; lane 2: 'PennA-1'; lane 3: 'PennA-4'; lane 4: 'Aurora Gold'; lane 5: 'Little Big Horn Blue'; lane 6: 'Midnight II'; lane 7: 'Moonlight SLT'; lane 8: 'Prosperity'; lane 9: 'Bright Star SLT'; lane 10: 'Silver Dollar'; lane 11: 'Olympic Gold'; lane 12: 'Silver Star'and lane 13: 'Tar Heel II.

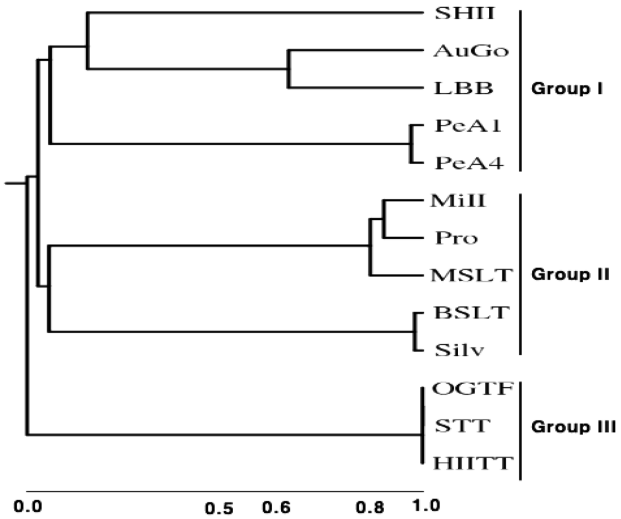

Fig. 3. The genetic dendrogram of 13 turfgrass cultivars was constructed from similarity coefficients and showing the clustering of the unweighted pair group average linkage of Pearson coefficients. SHII: 'Shadow II'; PeA1: 'PennA-1'; PeA4: 'PennA-4'; AuGo: 'Aurora Gold'; LBB: 'Little Big Horn Blue; MiII: 'Midnight II'; MSLT: 'Moonlight SLT'; Pro: 'Prosperity'; BSLT: 'Bright Star SLT'; Silv: 'Silver Dollar'; OGTF: 'Olympic Gold' ; STT: 'Silver Star' and HIITT: 'Tar Heel II'.

Table 4. The genetic similarity matrix among 13 turfgrass cultivars used on golf courses in Korea based on Pearson coefficient (r).

	SHII[x)]	PeA1	PeA4	AuGo	LBB	MiII	MSLT	Pro	BSLT	Silv	OGTF	STT	HIITT
SHII	1	-0.087	-0.087	0.039	-0.011	-0.048	-0.071	-0.077	-0.107	-0.102	-0.021	-0.021	-0.021
PeA1		1	0.966	-0.076	-0.134	-0.126	-0.126	-0.098	-0.188	-0.181	-0.236	-0.236	-0.236
PeA4			1	-0.076	-0.134	-0.126	-0.108	-0.098	-0.188	-0.181	-0.253	-0.253	-0.253
AuGo				1	0.606	-0.157	-0.157	-0.183	-0.154	-0.148	-0.195	-0.195	-0.195
LBB					1	-0.134	-0.113	-0.160	-0.14	-0.134	-0.151	-0.151	-0.151
MiII						1	0.827	0.886	-0.089	-0.102	-0.173	-0.173	-0.173
MSLT							1	0.867	-0.110	-0.124	-0.173	-0.173	-0.173
Pro								1	-0.054	-0.087	-0.161	-0.161	-0.161
BSLT									1	0.977	-0.147	-0.147	-0.147
Silv										1	-0.140	-0.140	-0.140
OGTF											1	1	1
STT												1	1
HIITT													1

[x]SHII: 'Shadow II'; PeA1: 'PennA-1'; PeA4: 'PennA-4'; AuGo: 'Aurora Gold'; LBB: 'Little Big Horn Blue; MiII: 'Midnight II'; MSLT: 'Moonlight SLT'; Pro: Prosperity'; BSLT: 'Bright Star SLT'; Silv: 'Silver Dollar'; OGTF: 'Olympic Gold' ; STT: 'Silver Star' and HIITT: 'Tar Heel II'.

characterization. Their genetic similarity was analyzed based on RAPD data. These results can be providing basic information on identifying exported t turfgrass cultivars in the markets.

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RAPD 마커를 이용한 국내골프장의 잔디 13 품종의 유전적 다양성 분석

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요 약: 본 연구는 무작위 분자마커(RAPD)를 이용한 우리나라 골프장에서 이용되고 있는 잔디 13개 잔디품종의 유전적 다형성을 조사하여 보다 효과적인 골프장 관리를 위한 유전적 정보를 제공하고자 조사하였다. 본 연구에서 사용한 54개의 random hexamer primer를 이용하여 RAPD분석을 실시한 결과 13~54개의 다형성 밴드를 형성하였으며 primer당 평균 30.7개의 다형성 밴드를 확인할 수 있었다. RAPD분석 결과 13개의 잔디품종은 크게 3개의 그룹으로 나눌 수 있었다. Group I은 Shadow II, Aurora Gold, Little Big Horn Blue, PennA-1, PennA-4, Group II는 Midnight II, Prosperity, Moonlight SLT, Bright Star SLT, Silver Dollar, Group III은 Olympic Gold Turf-Type, Silver Star Turf-Type, Tar Heel II Turf-Type을 포함하였다. 13개 잔디 품종의 유전적 근연 정도는 0.039~1.0으로 나타났으며, Group III이 유전적 근연 정도가 가장 높게 나타났다. 이상의 결과를 통해 향후, 잔디 종 또는 이종간의 유전적 다양성의 상호관계나 차이점을 규명하기 위해서는 형태적인 특성과 SCARs 마커와 같은 특이적인 분자마커에 대한 연구가 추가적으로 필요할 것으로 사료된다.

주요어: 잔디, 품종, 유전적 유연관계, RAPD