CHAPTER II

Genetic Differentiation of Tetraploid Creeping Bentgrass and Hexaploid Redtop Bentgrass Genotypes by AFLP and Their Use in Turfgrass Breeding

ABSTRACT

The turf industry in the last decade has seen doubling in number of new creeping bentgrass (*Agrostis stolonifera* var. *palustris* Huds. and *A. stolonifera var. stolonifera* Huds.) cultivars, many with unknown variability and lineage. Understanding the genetic diversity of putative parental and wild stocks would be useful in breeding programs. AFLP analysis was conducted to investigate genetic variability among old and new cultivars of creeping bentgrasses, redtop bentgrasses (*Agrostis gigantea* Roth.), plant introductions and selected genotypes with resistance to gray snow mold (*Typhula incarnata* Lasch). Seven chosen primer combinations resulting in 355 polymorphic markers were used to differentiate the bentgrasses. Three groups were extracted using principal component analysis. Using UPGMA analysis, mean similarity coefficients of creeping bentgrass genotypes found in the first group was 0.78. Creeping bentgrasses in the USA were clustered as a subgroup and separated from European plant introductions, indicating that most selection and genetic exchanges in the last fifty years have evolved locally. Redtop bentgrasses were the most diverse and were found in different groups. Selected lines from northern Michigan, MI 20104, MI 20215 and MI 203164 were differentiated from the other cultivars and would be advantageous to use as sources of disease resistant traits and for development of populations for future gene mapping.

Predictive estimates of genetic variation and molecular identification of new cultivars and interrelationships would be important for advanced breeding of bentgrass species.

Key words: AFLP analysis, turfgrass breeding, *Agrostis stolonifera* var. *palustris* Huds., *Agrostis stolonifera* var. *stolonifera* Huds., *Agrostis gigantea* Roth.

INTRODUCTION

Creeping bentgrasses (2n=4x=28) are the premier and most widely used cool-season turfgrasses for golf course putting greens, tees and fairways in the USA (Funk, 1998). Redtop bentgrasses (2n=6x=42) are widely used in seed mixtures for rapid vegetation cover. These two species of bentgrasses are differentiated from other species by their profuse creeping stolons forming dense mats, vigorous shallow roots and their attractive range of bluish-green to grayish appearance. From 1927 to 1994, less than twenty cultivars have been released by different institutions and private seed companies in the USA but within the past eight years, the number of cultivars that has been bred have doubled. Creeping bentgrasses are synthetic products created from crossing of three or more clones and recurrent selection. Improved turf quality and appearance, tolerance to close mowing, regenerative and seed yield potential, and tolerance to diseases are some important parameters for selection. Some of these new cultivars may be closely related but their interrelationships are unknown. Diversity within creeping bentgrasses appears limited as their genetic variability may be narrow (.007 to .08%) as revealed by isozyme polymorphism (Yamamoto and Duich 1994; Warnke et al., 1997). Plant introductions from germplasm collections could be used to widen the genetic base.

Morphologically, creeping bentgrasses are difficult to distinguish and taxonomic confusion has resulted in many synonymous names for the tetraploid species. *Agrostis stolonifera* L. was listed as being synonymous with *A. alba* var. *palustris* Huds.; *A. alba* var. *stolonifera* (L.) Sm; *A. stolonifera* var. *compacta* Hart.; A. *stolonifera* var. *palustris* (Huds.) Farw. and *A. maritima* Lam. (Texas A&M, BONAP Poaceae Listing 2003; Plant Gene Resources of Canada, GRIN-CA Taxonomy, 2003). Hexaploid bentgrasses were referred to as *A. stolonifera* sp. *gigantea* (Huds.), *A. gigantea* L. or *A. alba* sp. *gigantea* Huds. Adding to the taxonomic complexity are other factors; i.e. outcrossing nature of bentgrasses, ability to form interspecific hybrids and environmental fluctuations that may result in differences in phenotypic expressions and inconsistencies in classification. A more stable tool like DNA molecular marker technology may help resolve identification and offer insights to the degree of genetic variability and relationships among cultivars and to help define future breeding strategies.

Molecular marker studies using amplified fragment length polymorphism (AFLP) analyses have been used to differentiate bermudagrass (*Cynodon* spp.) genotypes (Zhang et al., 1999) and to construct a genomic map of zoysiagrass (*Zoysia* spp.) (Ebina et al., 1999). Some bentgrass cultivars and species have been differentiated using random amplified polymorphic DNA (RAPD) (Golembiewski et al., 1997, Scheef et al., 2001) and restriction fragment length polymorphism (RFLP) (Caceres et al., 2000). Our recent work in the study of genetic diversity in the *Agrostis* species using AFLPs has shown the advantage of this technique for biodiversity studies, distinguishing fourteen species of bentgrasses into seven major groups (Vergara and Bughrara, 2003). Stoloniferous bentgrasses from several countries formed a single cluster comprised of *A. stolonifera* L.

or *A. palustris* Huds., *A. gigantea* L., *A. mongolica* Roshev., and *A. transcaspica* Litv. The percentage of genetic dissimilarity (5 to 18%) within plant introductions of creeping bentgrasses indicated considerable potential for the improvement of turf.

Turfgrass breeders are currently interested in developing superior cultivars of *A. stolonifera* var. *palustris* with resistance to major diseases such as dollar spot (caused by *Rutstroemia floccosum*, teleomorph of *Sclerotinia homeocarpa* F.T. Bennett) (Powell, 1998), brown patch (*Rhizoctonia solani* Kuhn) and snow molds (*Typhula* spp. and *Microdochium nivale* Fr.) (Vargas, 1994; Hsiang et al., 1999). In 2000 and 2001, the Michigan State University (MSU) turf breeding program isolated and selected experimental lines, MI 20104, MI 20215 and MI 203164, with resistance to gray snow mold from replicated controlled screening trials. Our objective was to use AFLPs to investigate genetic variability among old and new cultivars of creeping bentgrasses, redtop bentgrasses, plant introductions and differentiate MSU experimental lines of creeping bentgrasses. Predictive estimates of genetic variation will be useful in planning turfgrass breeding programs and developing heterotic populations. Marker data and identified AFLP primer combinations will be useful in future mapping studies.

MATERIALS AND METHODS

Plant materials and DNA extraction

Bulk leaf samples from 25 plants each of cultivars 'Seaside', 'Penncross', 'Providence', 'L-93', 'Penn G2', 'Penn A4', 'S. Redtop', 'Emerald' and redtop bentgrass cultivar 'S. Redtop' were used. Widely used cultivars developed during different periods of time were chosen. Leaf samples from identical selected clones of MI 20104, MI 20215

and MI 203164 selected from Northern Michigan golf courses in 2000 were included for comparison. In addition, Plant Introductions (PI) of eight creeping bentgrass accessions and two redtop bentgrass accessions (source: USDA Plant Introduction Station, Pullman, WA) from different countries were included (Table 2.1). Fresh leaf tissues were ground in liquid nitrogen and an equal volume of extraction buffer was added. The extraction buffer consisted of Tris-EDTA-HCl, SDS, NaCl with 0.38 g/100 ml buffer of sodium bisulfite. The mixture was incubated at 65 °C for 20 min followed by the addition of 10 ml of 5M potassium acetate. After 20 min incubation with ice in a gyratory shaker, the suspension was centrifuged at 3000 *g* RCF (Sorvall RT7 model) for 20 min at 5 °C. The supernatant was collected to which 2/3 volume of cold isopropanol was added to precipitate the DNA. All DNA samples were treated with RNAse dissolved in TE buffer and twice reprecipitated by using 1/10 volume of 3M sodium acetate followed by two volumes of chilled absolute ethanol. Extracted DNA was stored in 1% TE buffer. DNA quality was checked by running 5 µl of the undigested samples in 1% agarose gel containing TBE buffer and compared to *EcoR*I digested samples. DNA quantification was done using a DyNA Quant 200 Fluorometer (Pharmacia Biotech, CA).

AFLP analysis

Approximately 150 to 200 ng of DNA was used for AFLP analysis of each genotype. Digestion was conducted using two restriction enzymes, *Eco*RI, a six base pair cutter and *Mse*-I, a four base pair cutter. The AFLP procedure used in this study was as described by Vos et al. (1995) with modifications. Pre-amplification was done on a PTC-100 thermal cycler (MJ Research, Inc., MA) using 72°C for 2 min, 30 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 1 min, followed by elongation at 72°C for 5 min and 4°C

Table 2.1. Cultivars, MSU experimental lines and plant introductions (PI) lines of creeping and redtop bentgrasses (*Agrostis* spp.) examined, year released or collected and their sources.

Bentgrass	Scientific name	Year[a]	Source/Origin
Cultivars			
Seaside	*A. stolonifera var. palustris*	1928	Tee-2 Green Corp., US
Penncross	*A. stolonifera var. palustris*	1955	Pure Seed Testing, Inc., US
Providence	*A. stolonifera var. palustris*	1987	Seed Research of OR, Inc., US
L-93	*A. stolonifera var. palustris*	1995	Agri-Biotech Inc., US
Penn G2	*A. stolonifera var. palustris*	1995	Tee-2 Green Corp., US
Penn A4	*A. stolonifera var. palustris*	1995	Tee-2 Green Corp., US
Emerald	*A. stolonifera var. palustris*	1973	Cebeco Intl. Seeds, Inc.,US
S. Redtop	*A. stolonifera var. palustris*	1989	Pickseed West, Inc., US
Experimental Lines			
MI 20104	*A. stolonifera var. palustris*	2000	Michigan State University, US
MI 20215	*A. stolonifera var. palustris*	2000	Michigan State University, US
MI 203164	*A. stolonifera var. palustris*	2000	Michigan State University, US
Plant Introductions			
PI 251 945	*A. stolonifera var. palustris*	1958	USDA PI from Austria (AT)
PI 235 440	*A. stolonifera var. palustris*	1956	USDA PI from Switzerland (CH)
PI 235 541	*A. stolonifera var. palustris*	1956	USDA PI from Sweden (SE)
PI 204 390	*A. stolonifera var. palustris*	1953	USDA PI from Turkey (TR)
PI 269 838	*A. stolonifera var.stolonifera*	1960	USDA PI from Germany (DE)
PI 494 119	*A. stolonifera var.stolonifera*	1984	USDA PI from Netherlands (NL)
PI 230 235	*A. stolonifera var.stolonifera*	1955	USDA PI from Iran (IR)
PI 439 027	*A. stolonifera var.stolonifera*	1978	USDA PI from Russia (RU)
PI 383 584	*A. gigantea*	1972	USDA PI from Turkey (TR)
PI 443 051	*A. gigante*	1980	USDA PI from US

[a] Year of release for cultivars and year collected for experimental lines and PIs.

hold. PCR products from initial ligation of adapters were checked on 1.5% TBE agarose gel. Combinations of fluorescent (*) dye labeled E and M primers each with 3 selective nucleotides at the 3' ends were used. The following E* primers were used: E*-ACA and E*-AGC. The following M primers were tested: M-CAT, M-CAG, M-CGG, M-CGA, M-CTT, M-CCT. The 15 µl selective amplification mixture consisted of 15 pmol E*-primer, 75 pmol M-primer , 2 mM dNTP, 1X PCR buffer, 37.5 mM $MgCl_2$, 1.0 unit Taq polymerase in deionized distilled water. The PCR cycling sequence used for selective amplification was as recommended by Vos et al. (1995). Products from selective amplification were checked initially on 1.5% agarose and diluted four times with 0.1 TE buffer. For separation on acrylamide gels, samples consisted of 0.5 µl of the amplified product and 0.5 µl loading dye. The samples were denatured at 95°C for 5 min prior to loading and run on a 6% Long Ranger polyacrylamide gel with 0.7X TBE buffer using a LI-COR DNA Analyzer 4200 (Lincoln, NE) at a constant 800 v for 6 hours at 50°C.

Data analyses

Gels were visualized using the software Gene ImagIR 4.0 (Scanalytics, Inc.,VA). Each informative polymorphic band was scored manually as 1 for presence and 0 for absence. Analyses were done using Numerical Taxonomy and the Multivariate Analysis System, NTSYS v.2.1 (Rohlf, 2000). Genetic similarities based on Jaccard's coefficients (Jaccard, 1908) were calculated among all possible pairs using the SIMQUAL option and ordered in a similarity matrix. The similarity matrix was run on Sequential, Agglomerative, Hierarchical and Nested clustering, SAHN (Sneath and Sokal, 1973) using the Unweighted Pair Group Method with the Arithmetic Mean (UPGMA) as an option (Sokal and Michener, 1958). Cophenetic correlation was calculated to measure

goodness of fit. Principal component analysis (PCA) was run using CPCA option (NTSYS) to identify the number of groups based on eigenvectors and verified using SYSTAT. The three dimensional PCA plot was generated from the NTSYS program using AFLP markers as observations and bentgrass genotypes as the operational taxonomic units (OTUs). The TREE module of NTSYS v.2.1 was used to produce the dendrogram (Rohlf, 2000).

RESULTS and DISCUSSION

From the initial twelve primer combinations, seven combinations were chosen for clarity and repetitiveness in duplicated gel runs (Table 2.2). A total of 380 polymorphic markers were initially scored for the 21 genotypes from the seven combinations. Cultivar Seaside had data from only four of the seven primer combinations. Two UPGMA analyses were performed, first using 248 markers for 21 genotypes which included Seaside and secondly, 355 markers for 20 genotypes, excluding 'Seaside'. The number of bands varied with each primer combination ranging from 100 to 150 bands and the number of polymorphic markers ranged from 25 to 100 per individual lane. Primer combination E-ACA/M-CAT gave the most robust polymorphism. Pairwise similarity coefficients (**sc**) were computed based on shared and unique amplification products using UPGMA (Table 2.3). The genetic similarity coefficients ranged from 0.56 to 0.93. The most similar bentgrass genotypes were the plant introductions (PI) from Switzerland and Sweden (**sc**=0.93) and the most dissimilar would be 'Penncross' and PI 443051 (**sc**=0.56).

Table 2.2. Number of polymorphic bands obtained from different primer combinations.

Primer Pair	No. of Polymorphic Bands	
	20 genotypes	21 genotypes
E-ACA/M-CAT	98	94
E-ACA/M-CAG	25	22
E-ACA/M-CGG	61	60
E-ACA/M-CGA		31
E-AGC/M-CAT	64	62
E-AGC/M-CGG		60
E-AGC/M-CGA		26
Total markers	248	355

Adapter and primer sequences were adapted from Vos et al. (1995).
E=5'-GACTGCGTACCAATTCA-3', M=5'-GATGAGTCCTGAGTAAC-3'.

Table 2.3. Genetic similarity coefficients* for 21 genotypes of creeping and redtop bentgrasses using fluorescence-labeled AFLP technique.

Bentgrass Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 Penncross	1.00																			
2 Providence	0.72	1.00																		
3 L-93	0.71	0.83	1.00																	
4 Penn G2	0.76	0.81	0.79	1.00																
5 Penn A4	0.74	0.76	0.78	0.79	1.00															
6 Emerald	0.73	0.76	0.79	0.81	0.81	1.00														
7 MI 20104	0.72	0.78	0.79	0.77	0.78	0.88	1.00													
8 MI 20215	0.75	0.79	0.77	0.80	0.76	0.78	0.80	1.00												
9 MI 203164	0.78	0.83	0.81	0.80	0.77	0.78	0.79	0.86	1.00											
10 S. Redtop	0.60	0.67	0.63	0.63	0.63	0.65	0.63	0.65	0.66	1.00										
11 PI 251 945_AT	0.69	0.73	0.74	0.74	0.75	0.75	0.74	0.77	0.76	0.65	1.00									
12 PI 235 440_CH	0.72	0.78	0.78	0.79	0.79	0.81	0.79	0.77	0.82	0.66	0.82	1.00								
13 PI 235 541_SE	0.74	0.82	0.79	0.80	0.78	0.80	0.79	0.79	0.81	0.68	0.81	0.93	1.00							
14 PI 204 390_TR	0.68	0.73	0.73	0.74	0.73	0.74	0.73	0.74	0.73	0.68	0.74	0.80	0.80	1.00						
15 PI 269 838_DE	0.72	0.76	0.74	0.78	0.75	0.78	0.77	0.80	0.77	0.67	0.80	0.84	0.84	0.78	1.00					
16 PI 494 119_NL	0.73	0.79	0.77	0.81	0.79	0.80	0.80	0.79	0.79	0.67	0.80	0.83	0.84	0.78	0.87	1.00				
17 PI 230 235_IR	0.60	0.59	0.60	0.60	0.63	0.61	0.62	0.63	0.61	0.64	0.62	0.63	0.64	0.68	0.62	0.65	1.00			
18 PI 439 027_RU	0.58	0.61	0.64	0.60	0.63	0.61	0.61	0.63	0.63	0.62	0.64	0.63	0.63	0.62	0.61	0.64	0.72	1.00		
19 PI 383 584_TR	0.66	0.65	0.67	0.68	0.70	0.66	0.67	0.69	0.68	0.64	0.72	0.71	0.72	0.77	0.70	0.73	0.65	0.60	1.00	
20 PI 443 051_US	0.55	0.55	0.56	0.55	0.57	0.56	0.55	0.56	0.55	0.59	0.54	0.59	0.59	0.56	0.58	0.59	0.67	0.59	0.63	1.00
21 Seaside	0.81	0.66	0.60	0.64	0.62	0.62	0.58	0.64	0.66	0.54	0.60	0.61	0.64	0.57	0.62	0.63	0.54	0.50	0.58	0.46

*Data from bentgrass genotypes 1 to 20 came from 355 AFLP markers and data from 'Seaside' came from 248 AFLP markers.

Using the subroutine programs of NTSYS, a rotated PCA with the markers as observations was used to determine the number of groups based on Eigen values greater than one. Three groups were extracted which explained 58% of the total variance. The first component accounted for 44% of the total variance with cultivar 'S.Redtop' bearing the lowest component loading (Figure 2.1). Seventeen genotypes were found distinguished by the first component. The second component, which accounted for 8% of the variation, separated Groups 1 and 2. The third component accounted for 5% of the variation and distinguished PI 443051.

From the two UPGMA analyses, dendrograms were generated. The two dendrograms did not differ in terms of genotypes within a cluster but only in the estimates of similarity coefficients. These slight differences (0.01 to 0.02 **sc**) could be due to the deletion or addition of allelic markers considered for an additional genotype. The higher number of markers used would predict a more accurate dendrogram presented in Figure 2.2. The dendrogram also indicated where 'Seaside' would cluster. The cophenetic correlation was calculated (r = 0.95) as a measure of goodness of fit of the similarity indices. The cophenetic values were plotted against the distances derived from SAHN's cluster analysis and the phenogram was plotted in Figure 2.3. Mean similarity coefficient for all creeping bentgrasses in the first group was 0.78. Similarity coefficient was lower between the redtop bentgrasses (**sc**=0.64). Group 2 consisted of *A. palustris* (Iran and Russia) and Group 3 consisted only of PI 443051, *A. gigantea* (US). The dendrogram showed a similarity coefficient of 0.64 for the three groups.

'Seaside' was found to be most similar to 'Penncross' with **sc**= 0.81 based on UPGMA analysis using data from 248 AFLP markers (Table 2.3). The dendrogram

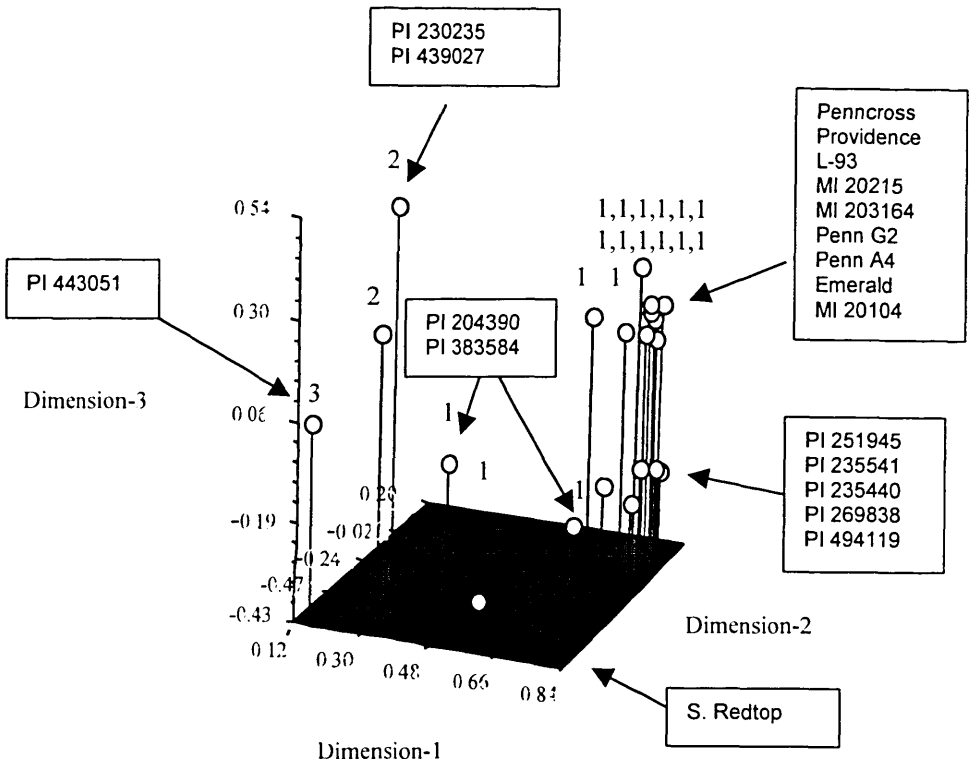

Figure 2.1. Three dimensional plot of principal component analysis (PCA) using 355 AFLP markers (observations) and bentgrass genotypes defining three groups marked as 1, 2 and 3 from the plot options of NTSYS v.2.1 (Rohlf, 2000).

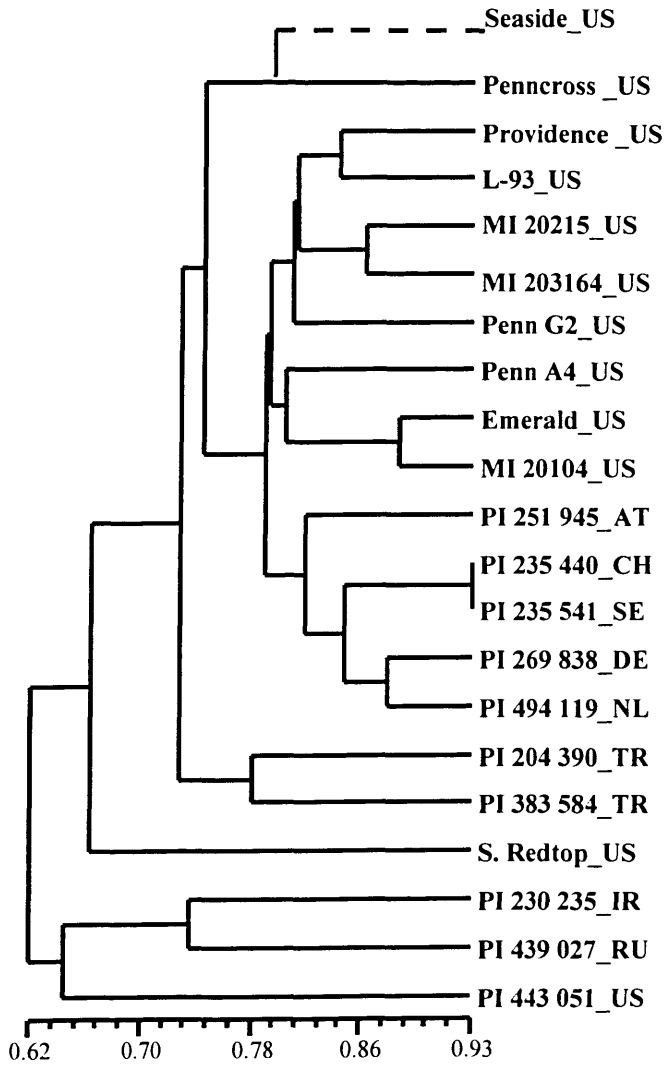

Figure 2.2. UPGMA dendrogram of creeping and redtop bentgrasses using data from 355 AFLP markers (solid lines) and genetic similarity of 'Seaside' using data from 248 AFLP markers (dashed lines).

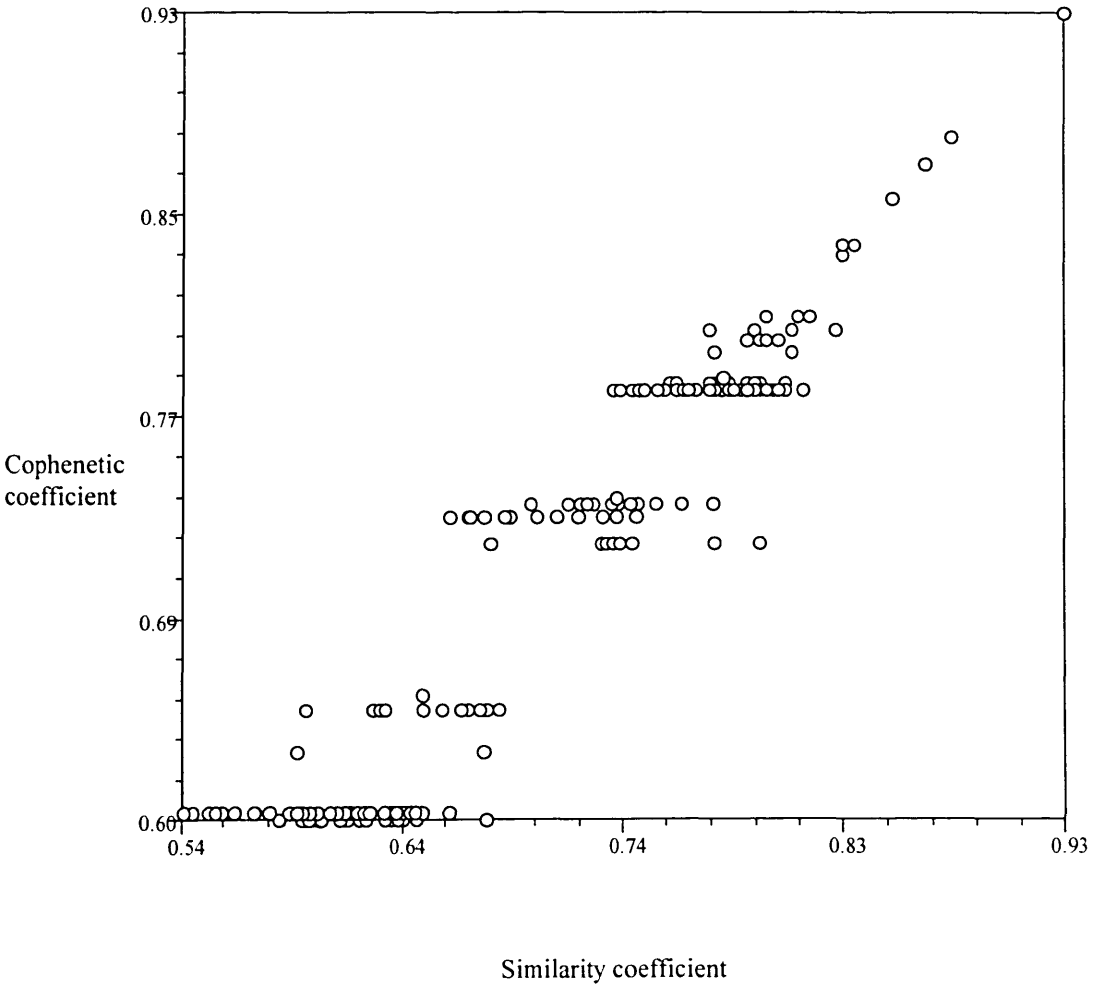

Figure 2.3. Plot analysis of cophenetic and similarity coefficients as a measure of goodness of fit of the similarity indices. r = 0.94808 = normalized Mantel statistics Z; Approximate Mantel t-test: t= 5.1548; P(Z<obs. Z: p = 1.0000).

using data from 355 markers showed that 14 of the 17 genotypes in Group 1, namely 'Penncross', 'Providence', 'L-93', 'Penn-G2', 'Penn A4', 'Emerald', 'MI 20104', 'MI 20215', 'MI 203164', PI 251945 (Austria), PI 235440 (Switzerland), PI 235541 (Sweden), PI 269838 (Denmark) and PI 494119 (Netherlands), were clustered close together at **sc**=0.78. Cultivar 'Penncross' may share genetic similarities with modern cultivars by a mean **sc**=0.75. The fourteen genotypes were slightly differentiated from PI 204390 and PI 383584 (Turkey) and 'S. Redtop'. Cultivar 'S. Redtop' was the most genetically distant in the first group. 'MI 20104' was most similar with 'Emerald' with **sc**=0.88 while 'MI 20215' and 'MI 203164' were more similar to 'Providence' with **sc**=0.79 and 0.83 respectively. 'Providence' and 'L-93' had a high **sc**=0.83 and shared close identity with 'MI 20215' and 'MI 203164' as well. Dendrogram results showed the three genotypes of *A. gigantea* did not group together. PI 443051, *A. gigantea* (US) separated from cultivar 'S. Redtop' (US) and from PI 383584 (Turkey).

Differentiating old and new cultivars of creeping bentgrasses

Most developed cultivars of creeping bentgrass, *A. stolonifera* var. *palustris*, in the USA such as 'Penncross', 'Penn A4', 'L-93' and others came from phenotypic recurrent selection of three or more clones descended from selection of adapted lines which were thought to be from earlier European origin. Warnke et al. (1997) used isozyme polymorphisms to distinguish between several creeping bentgrasses. Eighteen bentgrasses were divided into two groups based on cluster analyses. The first group included 10 cultivars ('Penncross', 'Emerald', 'Cobra', 'Crenshaw', 'Seaside', 'Penneagle', 'Putter', 'Trueline', 'Viper' and '18th Green'). With the exception of 'Crenshaw', they were all strongly aligned with creeping cultivars. 'Seaside' is thought to

be the oldest within the group and may have provided some of the germplasm used in the development of some bentgrasses in the grouping. Their genetic distance calculated from Nei's distance formula ranged from 0.007 to .08. The second group contained the cultivars 'Pennlinks', 'Southshore', 'ProCup', 'Lopez', 'Providence', 'SR1020', 'National' and 'Cato'. Both groups were differentiated from PI 251945, which is of European origin by genetic distance of 0.27. They found genetic differences to be small within cultivars and suggested that the PI line could be a source to broaden genetic diversity of bentgrasses in the USA.

In this study, UPGMA similarity coefficients from AFLP showed that 'Seaside' (1928), the oldest seeded cultivar used, and 'Penncross' (1955) were clustered closely but evidently showed genetic dissimilarity at 19%. Genetic dissimilarity was computed from 1-**sc** x 100 (Zhang et al., 1999). 'Seaside' creeping bentgrass, indigenous to coastal regions of Washington and Oregon, was known to be an extremely variable grass that develops into patches of individual strains with different colors, textures and densities. Most seed supplies came from natural stands. Hence to date after more than seventy-five years, 'Seaside' remains to be a highly heterogenous population.

In Figure 2.2, more recent cultivars, 'Providence' (1987) and 'L-93' (1995) were also clustered together with **sc**=0.84. The genetic dissimilarity between 'Providence' and 'L-93' is 16% and both are differentiated from 'Seaside' as much as 34%. 'Providence' was a consistently top rated (best five) bentgrass in the National Turfgrass Evaluation Program (NTEP 98-8) Putting Green and Fairway Test (Morris, 1997), and in trials throughout the world. The progenitors of 'Providence' were five clones that Dr. Richard Skogley of the University of Rhode Island selected as being unique and superior to

'Penncross' since 1965 (Seed Research Reports of Oregon, 2003). The newer cultivars, 'Penn G2' and 'Penn A4' (1995) were clustered together at **sc**=0.80 and differentiated from its early predecessor 'Penncross'. Pennsylvania State University developed cultivars with the epithet 'Penn'. 'Providence', 'Penn G2' and 'Penn A4' were reported as having better resistance to dollar spot in NTEP trials (Moris, 2001a) and would be useful as genetic stocks. Cultivars 'Seaside', 'Penncross' and 'Emerald' were in the same cluster using AFLP and supported similar findings by Warnke et al.(1997). Another similar observation to their findings from our results was that PI251945 (Austria) has a range of **sc**=0.65 to 0.77 to USA cultivars. PI251945 and other PIs from Switzerland, Sweden, Denmark and Netherlands would be useful to expand genetic variability in creeping bentgrass. In contrast to the results of Warnke et al. (1997), AFLP analysis showed that the genetic differences among USA cultivars were higher than the previous estimate using isozyme. Variability is important because selection becomes more effective when diversity is high.

Creeping bentgrass from Turkey (Central Asia) was the most distant in the first group and would be highly useful and informative for future mapping studies. Redtop bentgrass cultivar 'S. Redtop' in group 1 cluster was shown to share some genetic similarity with the tetraploid creeping bentgrasses. In contrast to creeping bentgrasses, redtop bentgrass is not used as fine turf but would be an important source of other important traits like drought and heavy metal tolerance (Winterhalder, 1990). Creeping bentgrasses (USA) clustered separately as a subgroup from the PIs from Europe at **sc** = 0.78, an indication that most development, selection and genetic exchanges in the last fifty years occurred locally. Support for European lineage for USA creeping bentgrasses

was also shown by AFLP results in Group 1 in this study. Previous findings comparing genetic similarities of plant introductions of *Agrostis* species using AFLPs showed that bentgrasses from the USA were closer to European PIs than accessions coming from other parts of the world (Vergara and Bughrara, 2003).

Differentiating MSU experimental lines with other creeping bentgrasses

MSU experimental lines were collected from old (>50 years) Northern Michigan golf courses which have not been overseeded for the last 10 years. Materials from these golf courses have been through natural selection pressures for abiotic and biotic stresses making them excellent genetic stocks for turf breeding programs. Data from AFLPs showed that the selected experimental lines were differentiated from other creeping bentgrasses and showed the closest genetic similarity to 'Providence', 'L-93' and 'Emerald'. The two cultivars, 'L-93' and 'Emerald' are currently internationally known for their dark, dense and aggressive growth habit. 'L-93' was rated premier out of 25 cultivars in the National Bentgrass Test – 1998 (NTEP 02-3, Morris, 2001b) on fairways and tees and also performed better in heat stress than 'Penncross' (Huang and Xu, 2001). Cultivar 'Emerald', is a descendant of a single synthetic clone originating from Sweden and is widely used in blends of two or more creeping bentgrasses in the USA. 'Emerald' is important as it also has moderate tolerance to heat like 'Providence', as compared to most cool-season bentgrasses. The high similarity coefficients (0.80 to 0.88) to top rated modern cultivars provided predictive estimates by which MI 20104, MI 20215 and MI 203164 with resistance to snow mold disease may be used to improve performance of newer cultivars without radically altering their genetic components. Correspondingly, cultivars to which the **sc** values were less gave indication of the high genetic diversity

favorable for studies on combining abilities. Populations derived from crosses with large differences in polymorphic markers may be used to map the snow mold disease resistance trait.

Results from dendrogram analysis showed that germplasm materials or plant introductions from Europe may also be used to widen the genetic base of modern USA creeping bentgrasses. The estimated mean genetic dissimilarity among all creeping bentgrass genotypes was 0.78 and suggested considerable diversity from which selection for improved cultivars may be generated. Creeping bentgrass from other subgroups (Central Asia) which are more differentiated by AFLPs would correspondingly further increase genetic variability. Diverse parental combinations would create segregating populations of various heterotic groups from which superior clones may be selected.

Genetic dissimilarity of tetraploid and hexaploid creeping bentgrasses

AFLP analysis indicated that most tetraploid creeping bentgrasses were grouped together and separated from hexaploid stoloniferous bentgrasses (Figure 2.2). PI 204390 (*A. palustris*, Turkey) was the most genetically distant among the tetraploid creeping bentgrasses in this study and may share closer genomic constitution with PI 383584 (*A. gigantea*, Turkey). Having similar geographic origins, possible genetic introductions between the progenitors of the two species may have occurred and allowed them to evolve sympatrically. Interspecific hybridizations between tetraploid and hexaploid bentgrasses like *A. stolonifera* x *A. gigantea* were known to occur naturally and are easy to produce (Davies, 1953; Jones,1955). The ability of cross-speciation enhances opportunities to transfer other important traits, e.g. tolerance to heavy metals and poor

soils, drought tolerance, and vigorous growth habit found in *A. gigantea* to creeping bentgrass.

Hexaploid bentgrasses, *A. gigantea* have been found to be genetically diverse within species and differentiated from the tetraploid *A. stolonifera var. palustris* using AFLPs (Table 2.3). The three *A. gigantea* genotypes were found to group separately from each other. S. Redtop (US) has only a **sc** = 0.59 with PI 443051, *A. gigantea* (US). This indicated that their genomic constitutions ($A_1A_1A_2A_2A_3A_3$) may be dissimilar and may have descended from different diploid and tetraploid progenitors. Much of the extensive variation in *A. gigantea* could have been generated by multiple or repeated cycles of hybridization of multiple origins. Our findings in the AFLP analysis of a number of *Agrostis* species were similar and indicated that only the A_1A_1 genome may be shared by the different accessions of *A. gigantea* (Vergara and Bughrara, 2003). Future cytogenetic and hybridization studies may be done to explain their differences.

Applications to Turfgrass Breeding

Turf breeders may gain advantage in selection when breeding materials are highly heterogenous and populations could be efficiently differentiated. Difficulty in differentiating outcrossing allopolyploid turfgrass species morphologically may be overcome with AFLP analyses. This study has shown considerable genetic dissimilarities between old and new cultivars, germplasm and MSU experimental lines. Dendrogram analysis revealed that USA creeping bentgrass cultivars have locally evolved and differentiated from European germplasm. Genetic similarity coefficients may predict which cultivars are more similar or distant and help define strategies for breeding. The AFLP data suggested that *A. stolonifera* var. *palustris* cultivars in the USA are highly

heterogenous but may be further diversified with materials from Europe and Turkey. Experimental materials from MSU with disease resistance to snow mold could be used to improve cultivars. Other important traits found in plant introductions of *A. gigantea,* may be used to improve 'S. Redtop' or transferred to creeping bentgrass cultivars by interspecific hybridization. In the future, the identified primer combinations and polymorphic marker data identified herein may be used to monitor introgression, map important traits, and used for protection of indigenous materials and developed cultivars.

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