

TURFGRASS SCIENCE

RAPD-Based Genetic Relationships in Kentucky Bluegrass: Comparison of Cultivars, Interspecific Hybrids, and Plant Introductions

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ABSTRACT

Kentucky bluegrass (*Poa pratensis* L.) is a widely used, facultatively apomictic, cool season turf and forage grass, with many known cultivars. Previously these have been classified on the basis of morphological traits in field tests. However, these determinations are lengthy, sensitive to environmental variations, and there are few traits that can be tested. Molecular markers provide an alternative way to classify these cultivars. Important advantages of molecular markers include lack of sensitivity to changes in environmental conditions, as well as a nearly unlimited potential number of markers and speed of the marker assays as compared with field tests. They can also provide additional information such as the amount of genetic divergence between Kentucky bluegrass cultivars and other genotypes, the amount of agreement between morphological and marker-based classification, and the amount of genetic variability between seedling replicates of genotypes. Random amplified polymorphic DNA (RAPD) marker data was collected from three replicates each of 123 Kentucky bluegrass cultivars, plant introductions (PIs), experimental breeding lines, and interspecific hybrids between Kentucky and Texas bluegrass (*Poa arachnifera* Torr.). From these data multidimensional scaling (MDS) plots were created, and ANOVA tests of significant differences between germplasm sources were performed. The PIs were the most genetically divergent from the cultivars, while the interspecific hybrids were not as distinct. Members of two morphological trait-based types were found to be genetically similar, and there was a wide range of genetic variability among germplasm sources. The genetic divergence of the PIs, combined with their potentially high turf utility reported in earlier studies, indicates their potential as a genetic resource. All but two of the morphological trait-based types contained genetically diverse individuals, so that cultivar blends of all but these two types would be morphologically similar yet genetically diverse. The rather high within-cultivar genetic variability could be due to a relatively high proportion of potentially sexually produced off-types. Thus, this study provides further evidence for the utility of RAPD markers for turfgrass genetics, as well as important genetic information for turf breeders and managers.

KENTUCKY BLUEGRASS is commonly used throughout the world for applications such as golf courses, athletic fields, lawns, and permanent pastures (Murphy et al., 1997). It is vigorous and attractive, easily established from sod because of its strong, dense rhizomes, and tolerant of a wide range of climate and soil conditions. These advantages combine to make it the “pre-

mier lawn grass” (Murphy et al., 1997) as well as the most popular and most widely used and propagated cool-season turfgrass in the USA (Burt and Christians, 1990; Huff, 2001).

The above advantages, along with improvement of intraspecific hybridization techniques (Pepin and Funk, 1971) have caused an increased interest in Kentucky bluegrass (Bonos et al., 2000a), resulting in a rapid increase in the number of registered Kentucky bluegrass cultivars. These new varieties are in addition to the many genotypes resulting from selection of promising, usually apomictic plants from old fields (Pepin and Funk, 1971) and other natural populations (Bonos et al., 2000b). Further, this species, and hence the genotypes within it, are inherently diverse because of adaptation to many climates and locations over many years (Burt and Christians, 1990).

The large, increasing number of Kentucky bluegrass cultivars can make registration of new cultivars difficult. In Europe, new *Brassica* cultivars must be tested for distinctness from previous cultivars, and uniformity and stability in their traits (Lombard et al., 2000); this is done to protect the breeder’s intellectual property and verify that new cultivars have sufficient difference from previously existing ones (Lombard et al., 2000). This testing is tedious, expensive, and the results are sensitive to environmental influences on trait expression.

In the USA, numerous parameters are listed in the USDA’s Plant Variety Protection Application Form for Kentucky bluegrass (Bonos et al., 2000b). These parameters include morphological traits such as plant height, panicle height, flag leaf dimensions, subtending leaf dimensions, and rhizome spread and length. These traits have also been used to classify Kentucky bluegrass cultivars into either seven (Murphy et al., 1997; Bonos et al., 2000b) or 12 types (Bonos et al., 2000a). The different morphological types are listed in Table 1, with the exception of the “Julia” type. The two main differences between the grouping systems are that (i) the “Compact” type in the seven group system has been divided into “Compact,” “Compact-America,” and “Compact-Midnight” in the revised 12 group system, and (ii) three new types have been added (“CELA,” “Julia,” and “Shamrock”). The “Other” type contains cultivars with characteristics intermediate between two or more types (Bonos et al., 2000b), while the “Unknown” listing contains cultivars for which the type name could not be determined in this study. The morphological data used

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Abbreviations: MDS, multidimensional scaling plot; RAPD, random amplified polymorphic DNA.

Table 1. List of the 85 Kentucky bluegrass cultivars, 21 plant introduction (PI) accessions, 13 experimental breeding lines†, and 4 Texas bluegrass × Kentucky bluegrass hybrids‡ used in this study (123 genotypes total), including their morphologically derived classification. Both the seven group and 12 group classifications are listed for the cultivars.

Name	Type (12-group)	Type (7-group)	Name	Type (12-group)	Type (7-group)	Name	Type (12-group)	Type (7-group)
Fairfax	Aggressive	Aggressive	Langara	Comp-America	Compact	Serene	Other	Other
Limousine	Aggressive	Aggressive	Boutique	Comp-America	Compact	Barcelona	Other	Other
Touchdown	Aggressive	Aggressive	Rugby2	Comp-Midnight	Compact	SR2100	Shamrock	Other
Northstar	Aggressive	Aggressive	Arcadia	Comp-Midnight	Compact	BA72-492 3-1705	Scott's Experimental Line	–
Banff	Bellevue	Bellevue	Award	Comp-Midnight	Compact	BA77-700 3-1707	Scott's Experimental Line	–
Freedom2	Bellevue	Bellevue	Award-B¶	Comp-Midnight	Compact	BA78-258 3-2836	Scott's Experimental Line	–
Classic	Bellevue	Bellevue	Quantumleap	Comp-Midnight	Compact	BA74-017 3-2954	Scott's Experimental Line	–
Suffolk	Bellevue	Bellevue	Midnight	Comp-Midnight	Compact	BA87-102 3-4261	Scott's Experimental Line	–
Parade	Bellevue	Bellevue	Nuglade	Comp-Midnight	Compact	BA76-372 3-5552	Scott's Experimental Line	–
Crest	BVMG‡	BVMG	Odyssey	Comp-Midnight	Compact	BA77-279 3-5569	Scott's Experimental Line	–
Merit	BVMG	BVMG	Liberator	Comp-Midnight	Compact	BA79-260 3-6510	Scott's Experimental Line	–
Cannon	BVMG	BVMG	Total Eclipse	Comp-Midnight	Compact	BA73-626 3-7401	Scott's Experimental Line	–
Gnome	BVMG	BVMG	Absolute	Comp-Midnight	Compact	BA74-114 2-8620	Scott's Experimental Line	–
Viva	BVMG	BVMG	ChicagoII	Comp-Midnight	Compact	BA70-242 2-8623	Scott's Experimental Line	–
Victa	BVMG	BVMG	Rugby	Comp-Midnight	Compact	BA72-500 2-8624	Scott's Experimental Line	–
Abbey	BVMG	BVMG	Explorer	Comp-Midnight	Compact	BA73-540 2-8684	Scott's Experimental Line	–
BlueChip	BVMG	BVMG	SR2000	Mid-Atlantic	Mid-Atlantic	TXHb337	Scott's TX-KY Hybrid	–
Baron	BVMG	BVMG	Monopoly	Mid-Atlantic	Mid-Atlantic	TXHb333	Scott's TX-KY Hybrid	–
Goldrush	BVMG	BVMG	Voyager	Mid-Atlantic	Mid-Atlantic	TXHb329	Scott's TX-KY Hybrid	–
Geronimo	BVMG	BVMG	Livingston	Mid-Atlantic	Mid-Atlantic	TXHb328	Scott's TX-KY Hybrid	–
Bluestar	BVMG	BVMG	Famous	Unknown	Unknown	PI371771	PI USPN626	–
Chache	BVMG	BVMG	Baritone	Unknown	Unknown	PI371775	PI USPN630	–
Envicta	BVMG	BVMG	Bluemoon	Unknown	Unknown	PI372738	PI USS103	–
Challenger	CELA§	Other	Bristol	Other	Other	PI372742	PI USS107	–
Denim	CELA	Other	Adelphi	Other	Other	PI349225	PI US2031	–
Alene	Common	Common	Nustar	Other	Other	PI368233	PI US67-126	–
Ginger	Common	Common	Cynthia	Other	Other	PI368241	PI US67-148	–
Kenblue	Common	Common	Chicago	Other	Other	PI371768	PI USPN623	–
Huntsville	Common	Common	Rita	Other	Other	PI303053	PI Sweden Primo	–
Rhonde	Common	Common	Washington	Other	Other	PI314734	PI Kazakhstan 415	–
Park	Common	Common	Sodnet	Other	Other	PI349160	PI US60-514	–
Indigo	Compact	Compact	Optigreen	Other	Other	PI349220	PI US2020	–
Glade	Compact	Compact	Nublu	Other	Other	PI505898	PI Sov. U. Morsanski 106	–
Alpine	Compact	Compact	Nassau	Other	Other	PI539057	PI Russian Fed AJC-520	–
Blacksburg	Compact	Compact	Ascot	Other	Other	PI574523	PI US Belturf	–
Blackstone	Compact	Compact	Coventry	Other	Other	PI227381	PI Iran	–
Moonlight	Compact	Compact	Buckingham	Other	Other	PI206725	PI Turkey	–
Nugget	Compact	Compact	Bartitia	Other	Other	PI380992	PI Iran 132	–
America	Comp-America	Compact	Chateau	Other	Other	PI229721	PI Iran	–
Brilliant	Comp-America	Compact	Cobalt	Other	Other	PI298098	PI Hungary G22	–
Unique	Comp-America	Compact	Sebring	Other	Other	PI237282	PI Denmark OTOFTE	–

† Experimental breeding lines and Texas bluegrass × Kentucky bluegrass hybrids were provided by The Scotts Co., Marysville, OH.

‡ Type named after the first cultivars released, Baron, Victa, Merit, and Gnome.

§ Type includes cultivars such as Challenger, Eclipse, and Liberty.

¶ Separately prepared duplicate of Award, from a different seed lot.

to classify cultivars into these types are supplemented by data on disease susceptibility, including stripe smut [caused by *Ustilago striiformis* (Westend.) Niessl.] and leaf spot [caused by *Drechslera poae* (Baudys.) Shoem.] (Murphy et al., 1997). The use of morphological and disease reaction traits to distinguish varieties is time-consuming, environmentally dependent, and is not always sufficient to reliably distinguish the new and existing varieties.

Molecular markers offer a powerful supplement to the morphological and disease resistance data currently used for variety protection, cultivar classification schemes, and estimation of the level of genetic diversity. Molecular markers have many advantages (Lombard et al., 2000) compared with morphological markers, such as robustness to environmental change, nearly unlimited number (unlike isozymes), and relative ease and rapidity of data collection. A commonly used DNA marker is RAPD, based on the polymerase chain reaction (PCR) and arbitrary sequence primers. Data from these markers, combined with appropriate statistical analysis, can be used to determine cultivar purity, rapidly verify apo-

mixis, show that a new cultivar is genetically distinct, and assist breeders in choosing genetically diverse parents for new cultivars, so as to broaden the genetic base and exploit hybrid vigor (Renganayaki et al., 2001).

Molecular markers, particularly RAPDs, have recently been applied to turfgrass research (Chai and Sticklen, 1998). In Kentucky bluegrass, markers have been successfully applied to characterization of the USDA germplasm collection (Johnson et al., 2002), identifying cultivar succession in swards (Lickfeldt et al., 2002), and determining the genetic origin of non-identical progeny from facultatively apomictic parents (Huff and Bara, 1993). Further, the utility of RAPD markers in discriminating among multiple Kentucky bluegrass cultivars has been demonstrated (Huff, 2001).

Therefore, this evidence suggests RAPD markers will provide an objective, rapid method of developing unique genetic profiles of each genotype. The use of RAPD marker data, combined with the known morphologically based classification system (Bonos et al., 2000b), can assist in developing cultivar blends that actually contain different genotypes, while providing the desired mix of

phenotypes. Because cultivars belonging to a given morphological trait-based type presumably have similar morphology, the morphological trait-based grouping system is useful for deciding which cultivars to include in blends. Therefore, it would be useful to know whether or not cultivars in a given morphological type are genetically related.

In addition, Kentucky bluegrass is facultatively apomictic (Pepin and Funk, 1971), meaning that some sexual seed production is possible and not all seeds produced will be genetically identical to the parent plant. The proportion of such apomictic seeds produced varies between cultivars (Porceddu et al., 2002). Thus, rapid molecular verification of apomixis (as opposed to progeny testing) will facilitate production of genetically stable turf material (seed or sod) not subject to changes in appearance over time due to changes in the genetic composition of the turf stand.

Furthermore, marker-derived genetic diversity analyses will facilitate the use of hybrid vigor in developing new cultivars. Applying molecular markers to determine genetic distinctiveness in new germplasm such as exotic plant introductions (PIs) or interspecific hybrids, especially if they are found to be genetically distant from currently available commercial cultivars, will be very useful in broadening the genetic base for breeders, providing opportunities for development of new, unique cultivars. Molecular markers applied in this way have significant economic potential.

This study used RAPD markers to study the genetic relationships of 123 Kentucky bluegrass genotypes. The specific objectives of this study were (i) to assess the amount of genetic divergence between Kentucky bluegrass commercial cultivars, plant introductions (PIs), experimental Kentucky bluegrass breeding lines, and hybrids between Kentucky bluegrass and Texas bluegrass, (ii) assess the correlation between trait-based classification and marker-based grouping, and (iii) determine the amount of intracultivar and seed source variability, which can help assess the degree of apomixis.

MATERIALS AND METHODS

Plant Material

A total of 123 Kentucky bluegrass genotypes were grown under greenhouse conditions. For each genotype, five seeds were planted, and three individual seedlings were retained for DNA extraction. These included 85 commercial cultivars from all seven and most of the 12 morphological types, 13 Kentucky bluegrass experimental breeding lines and four commercially produced Texas bluegrass \times Kentucky bluegrass hybrids (provided by The Scotts Co., Marysville, OH), and 21 PIs from around the world (Table 1). These PI accessions are part of the USDA *P. pratensis* collection at Pullman, WA, (Johnson et al., 2002) and were included to assess genetic distinction of these genotypes from the commercial cultivars.

RAPD

For each genotype, DNA was individually extracted from three different plants by grinding tissue in PEX (potassium ethyl xanthogenate) buffer with a Bio101 (Carlsbad, CA) Fast-prep machine, which pulverizes tissue by rapidly vibrating a

ceramic bead within a 2 mL screw-top microcentrifuge tube containing the tissue. The DNA was precipitated with 6:1 ethanol and 7.5 M ammonium acetate, treated with RNase, and finally precipitated with 10:1 ethanol and 3 M sodium acetate as described in Scheef et al. (2003).

The 12 RAPD primers used in this study (Operon primers A13, G19, M14, O15, P8, Y5, Y9, AE4, AE18, AF20, AG10, and AG14) consisted of 10 base-pair random sequences (Operon Technologies, Alameda, CA), chosen for ability to produce bright, polymorphic bands. Reaction mixtures, 10 μ L total, were 50 mM TrisHCl, pH 8.5, 10 mM KCl, 2 mM MgCl₂, 500 mg/mL bovine serum albumin (BSA), 0.01% (v/v) xylene cyanole, 1.5% (v/v) Ficoll 400, 20 ng plant DNA, and 0.6 U Taq polymerase (Scheef et al., 2003).

All PCR reactions were run in an MJ Research (Waltham, MA) PTC-100 Programmable Thermal Cycler. Reaction conditions consisted of one cycle of 91°C for 1 min, 42°C for 15 s, and 72°C for 1 min 10 s, followed by 38 cycles of 91°C for 15 s, 42°C for 15 s, and 72°C for 1 min 10 s, and then cooling to 4°C at the end of cycling.

Reaction products were electrophoresed on 1.5% (w/v) agarose gels stained with ethidium bromide and photographed with an instant camera under UV light. Eighty-five bright, reproducible bands were scored and confirmed by two different people for presence or absence.

Data Analysis

Genetic distances between genotypes were calculated as the complement to the simple matching coefficient (Gower, 1972), with the complement to the simple matching coefficient equaling 1 minus (simple matching coefficient). This is equal to the number of discordant bands between two genotypes divided by the sum of the number of discordant and concordant bands, as in Beebe et al. (1995). Genetic distances were calculated for all genotypes (each cultivar or seed source times three seedling replicates of each genotype). This was done to allow calculation of genetic distances between seedling replicates of cultivars or seed sources as well, by measuring genetic distance between the three replicates of each genotype.

These genetic distance matrices were then used to create a multidimensional scaling (MDS) plot using the Kruskal scaling option in Systat version 5.2 (Wilkinson et al., 1992). This type of plot graphically represents the matrices in the form of *x* and *y* coordinates (Wilkinson et al., 1992). The MDS coordinates are quantitative variables that contain weighted information from each of the 85 markers and, as such, hypotheses regarding among-group variability can be tested by analysis of variance techniques. The MDS procedure thus is very similar to principal components analysis (PCA), as each MDS coordinate explains a proportion of the total variance in the data. As more MDS coordinates are added, the proportion of variance explained decreases. An important difference from PCA is that MDS can often fit an appropriate model in fewer dimensions (Wilkinson et al., 1992). For example, Nienhuis et al. (1993) noted that MDS analysis provided a clearer separation of *Brassica* entries into two dimensions than did PCA.

Both MDS coordinates were analyzed by mixed models analysis, using the Statistical Analysis System, SAS (Littel et al., 1996). The repeated measures option of SAS was used to model heterogeneous variances among germplasm sources by a trial-and-error system and the Akaike's and Bayesian Information Criteria to choose the best model. Germplasm sources were considered to be a fixed effect and genotypes within sources were a random effect. Mixed models analyses were based on 15 germplasm sources: 12 morphological-trait-based cultivar types, plant introductions, experimental breeding

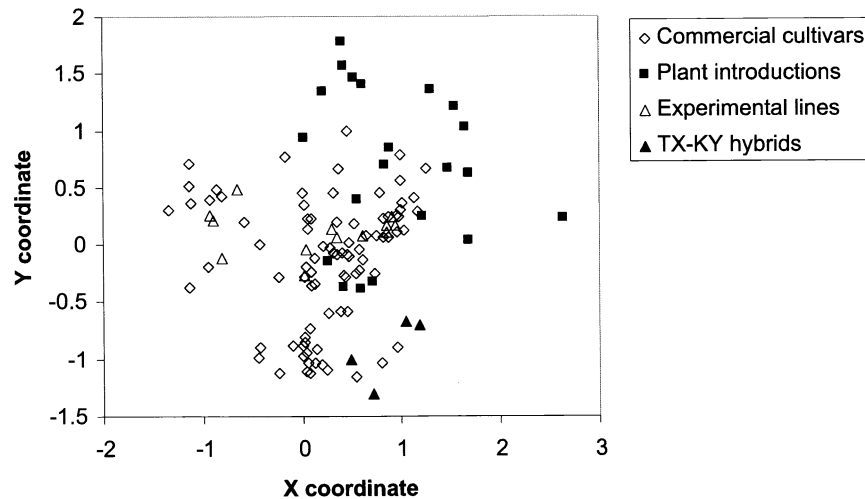


Fig. 1. Multidimensional scaling plot of the genetic distance matrix computed from RAPD data generated from 123 Kentucky bluegrass genotypes, classified into commercial cultivars, plant introductions, experimental breeding lines, and interspecific (*Poa pratensis* × *P. arachnifera*) hybrids, shown as (TX-KY).

lines, and interspecific hybrids. This was done to formalize the visual comparisons of the marker-based MDS groupings of the reported 12-group trait-based classification system (Bonos et al., 2000a), plant introductions, experimental breeding lines, and interspecific hybrids. Mixed models analysis based on means of MDS coordinates has been previously used by Casler et al. (2003) to detect sources of variation in MDS coordinate values among creeping bentgrass clones.

In addition, analysis of molecular variance (AMOVA, Schneider et al., 2000) was conducted by Arlequin version 2.000 software. This was done to determine the overall similarity of the genetic distance matrices obtained from the three seedling replicates, mainly as an error-checking measure.

RESULTS

Divergence between KBG Cultivars, PIs, Breeding Lines, and Hybrids

The molecular marker-derived genetic distances between the 123 Kentucky bluegrass genotypes is graphically presented with a multidimensional scaling (MDS) plot (Fig. 1). The stress of the plot was 0.228, which means that approximately 77% of the variance in the genetic distance data was explained by the two MDS coordinates. Further, the *x* coordinate of the MDS explained 39.8% of the total variance, while the *y* coordinate explained 37.2% of the total variance. The plot patterns were largely similar when calculated using the other two replicates of the 123 genotypes. Further, AMOVA treating the three replicates as separate populations indicated that only 2% of the genetic varia-

tion derives from among replicate differences ($p < 0.000001$). Thus the three genetic distance matrices are very similar overall, indicating that they are robust to the within cultivar or seed source variability found in this study, as well as to possible errors in the RAPD assay.

The most striking pattern is the general divergence of the PIs from the cultivars, lines, and interspecific hybrids, though there were eight PI entries which grouped together with the cultivars (Fig. 1). Second, the four interspecific hybrids were distinct from all but three of the cultivars. The breeding lines, however, were not generally distinct from the cultivars, though some of them appeared to differ from some cultivars. Lastly, the cultivars themselves, though they appear fairly heterogeneous as a group, do not appear to group uniformly. There are two areas of tight grouping of cultivars suggesting that some cultivars are more genetically related than others, which may be expected if multiple cultivars were derived from common gene pools.

When mixed models analysis using MDS-derived *x* and *y* coordinates of the 123 entries separated into each of the four above-mentioned germplasm sources was conducted, statistical significance was detected ($p < 0.0001$ for both coordinates). Looking at the group means separately, the mean of the PIs appears different by both coordinates from the mean of the cultivars (Table 2). An unequal variance *t* test using SAS shows that the means of both the *x* and *y* coordinates are significantly different between the cultivars and PIs ($p < 0.0001$ for both coordinates). Also, the mean of the interspecific hybrids was different from the mean of the cultivars by their *y* coordinates ($p < 0.0001$) but not by their *x* coordinates.

Table 2. Mean and standard error of the two multidimensional scaling coordinates (*x* and *y*) for Kentucky bluegrass cultivars, plant introductions (PIs), experimental lines, and Texas × Kentucky bluegrass hybrids.

Group name	Number in each group	Mean <i>x</i> coordinate	Mean <i>y</i> coordinate
Cultivars	85	0.226 ± 0.067†	-0.145 ± 0.064
Experimental lines	13	0.125 ± 0.171	0.115 ± 0.059
PIs	21	0.939 ± 0.135	0.692 ± 0.128
Hybrids	4	0.858 ± 0.308	-0.920 ± 0.107

† Mean of each coordinate plus or minus standard error.

Agreement between RAPD-Based MDS Grouping and Trait-Based Classification Systems

The MDS plots shows the genetic distance among entries in the 12-group system reported in Bonos et al. (2000a) (Fig. 2). Two areas of tight grouping in the MDS

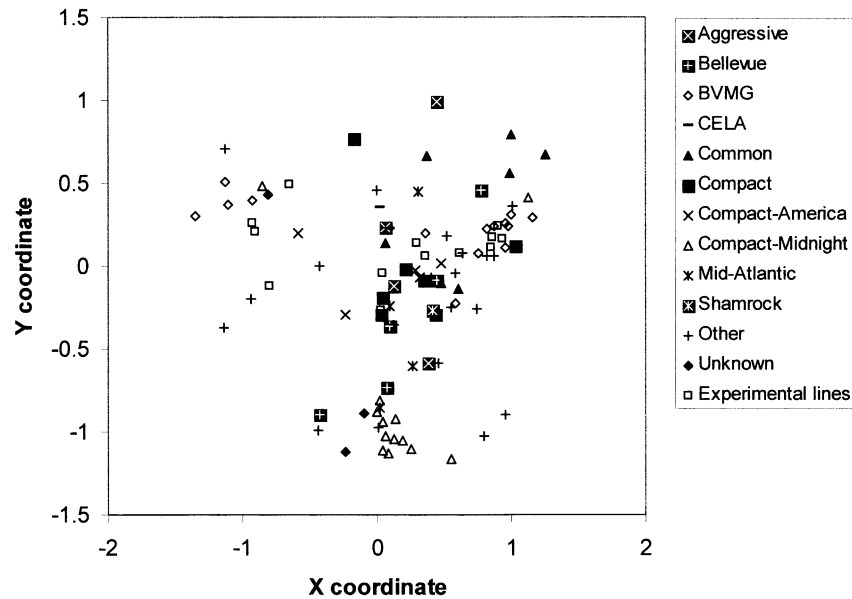


Fig. 2. Multidimensional scaling plot of the genetic distance matrix computed from RAPD data generated from all 85 Kentucky bluegrass cultivars, classified into 12 morphological trait-based groups, each represented by different symbols. Also included are the 13 experimental breeding lines.

plot were found to represent cultivars belonging to the same morphological trait-based type. Specifically, most of the entries in the BVMG type formed two distinct groups in the MDS plot (Fig. 2), while most of the Compact-Midnight type entries grouped together. Further patterns suggested in the plot were that seven of the thirteen experimental lines grouped near one or the other of the BVMG groups, and the Common type, although having only six entries, has four members that appear to group together, away from most of the other cultivars. No other obvious patterns emerged, suggesting that correlation between genetic relatedness and the morphological groupings exists only for the BVMG, Compact-Midnight, and possibly Common types.

When mixed models analysis using MDS coordinates of all 123 entries separated into 15 germplasm sources, i.e., the 12 morphological types, PIs, hybrids, and experimental lines was conducted, significant differences were detected ($p < 0.0001$ for both the x and y coordinates). The means and standard errors of the x and y coordinates for all fifteen germplasm sources are given in Table 3. On the basis of the unequal variance t test (SAS), the mean x coordinate of the PIs differed significantly from the mean x coordinates of these types: Aggressive, Bellevue, CELA, Compact, Compact-America, Compact-Midnight, Mid-Atlantic, Other, and the experimental lines. On the basis of the mean y coordinates, the Compact-Midnight type is significantly different from all these types: BVMG, CELA, Common, Compact, Compact-America, the hybrids, and the PIs. Also, the mean y coordinate of the PIs was significantly different from the mean y coordinate of these types: Bellevue, BVMG, Compact, Compact-America, Compact-Midnight, Other, the experimental lines, and the hybrids. The p -values ranged from <0.0001 to <0.002 for all the comparisons mentioned above.

Genetic Variability within Cultivars and Seed Sources

The within-cultivar and seed source variability was calculated by means of the difference in marker data between the three seedling replicates of each genotype; that is, the mean of the genetic distances between replicate 1 and replicate 2, replicate 1 and replicate 3, and replicate 2 and replicate 3. Even though only three replicates of each genotype were assayed, a wide range in the amount of variability was observed. This variability ranged from 0.05 (nearly all scored bands concordant over the three replicates) to nearly 0.5 (nearly half of the scored bands discordant over the three replicates). The mean value for this distance between seedling replicates for all 123 genotypes was 0.22, with a standard deviation of 0.097.

Table 3. Mean and standard error of the two multidimensional scaling coordinates (x and y) for all Kentucky bluegrass morphological trait-based types, plant introductions (PIs), experimental lines, and Texas \times Kentucky bluegrass hybrids, for a total of 15 groups.

Group name	Number in each group	Mean x coordinate	Mean y coordinate
Aggressive	4	0.255 \pm 0.313 [†]	0.128 \pm 0.300
Bellevue	5	0.194 \pm 0.280	-0.324 \pm 0.268
BVMG [‡]	14	0.280 \pm 0.167	0.236 \pm 0.051
CELA [‡]	2	0.060 \pm 0.443	0.285 \pm 0.135
Common	6	0.713 \pm 0.256	0.447 \pm 0.150
Compact	7	0.276 \pm 0.237	0.001 \pm 0.139
Comp-American	5	0.056 \pm 0.280	-0.034 \pm 0.085
Comp-Midnight	14	0.161 \pm 0.167	-0.741 \pm 0.160
Mid-Atlantic	4	0.175 \pm 0.313	-0.310 \pm 0.300
Shamrock	1	0.420 \pm 0.000	-0.270 \pm 0.000
Other	20	0.221 \pm 0.140	-0.206 \pm 0.134
Unknown	3	-0.380 \pm 0.362	-0.527 \pm 0.346
Experimental lines	13	0.125 \pm 0.174	0.115 \pm 0.053
PIs	21	0.939 \pm 0.137	0.692 \pm 0.131
Hybrids	4	0.858 \pm 0.313	-0.920 \pm 0.095

[†] Mean of each coordinate plus or minus standard error.

[‡] Group name abbreviations are identified in Table 1.

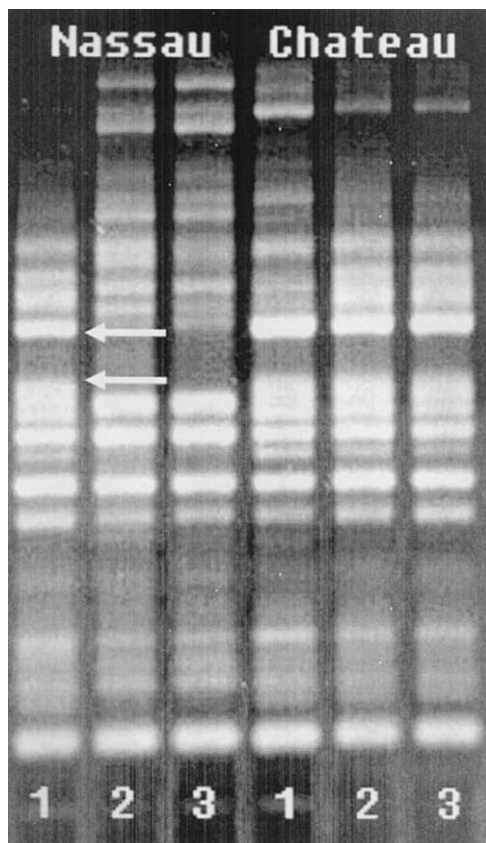


Fig. 3. RAPD-derived gel profile, using Operon primer AF20, of three seedling replicates, labeled '1', '2', and '3', of Kentucky bluegrass cultivars Nassau and Chateau. The arrows represent bands amplified in replicate 1 of cultivar Nassau but not in replicates 2 or 3.

As an example of this variability, Fig. 3 shows the RAPD profile based on Operon primer AF20 of the three replicate seedlings of cultivars Nassau and Chateau. Replicates 2 and 3 of cultivar Nassau had nearly identical banding patterns, while the two bands marked with arrows were amplified only in replicate 1. Thus seedling replicate 1 does not appear to be apomictically derived. On the other hand, all three replicates of cultivar Chateau have the same amplification pattern. Further, the amount of variability was not constant over the four major gene pools in this study (Table 4). The PIs had the highest level of variability, with the cultivars and interspecific hybrids intermediate, and the experimental breeding lines with the least within seed source variability.

Table 4. Results of ANOVA tests of differences of mean within cultivar or seed source genetic distance for the 4 Kentucky bluegrass germplasm sources: 85 commercial cultivars, 21 plant introductions, 13 experimental breeding lines, and 4 interspecific Texas \times Kentucky bluegrass hybrids. Means of these distances were compared over the four groups.

Group	Mean within-source distance
Plant introductions	0.292a [†]
Commercial cultivars	0.219b
TX-KY hybrids	0.195bc
Experimental lines	0.118c

[†] Values followed by the same letter are not significantly different at $\alpha = 0.05$ by Tukey's HSD test.

DISCUSSION

In this study, the RAPD marker-based genetic relationships of 123 Kentucky bluegrass genotypes yielded several important results. The first of these is that in the MDS plot, the 21 PIs appear to be generally genetically distinct from the cultivars, with only eight of the PI accessions grouping together with the cultivars. This grouping pattern was also well supported by the significant differences in the mean MDS coordinates detected by mixed models analysis. These differences suggest a potential of all of the PIs as a genetic resource. This is because, as a group, they not only can broaden the genetic base of Kentucky bluegrass germplasm, but many of these accessions were noted to have high turf potential by Johnson et al. (2002) and by our observations of these plants in the greenhouse. The interspecific hybrids and breeding lines, however, though not completely redundant with all cultivars, were not as genetically distinct from most of the cultivars as were the PIs.

Within the cultivars themselves, the distribution over the MDS plot was not uniform. Two groups of cultivars were noted, which upon comparison with the morphological trait-based classification systems were found to correspond with the BVMG type and the Compact-Midnight type. The BVMG type is interesting since it seems to form two groups in the MDS plot (Fig. 2), and as such little statistical significance was found between this type and others. The Compact-Midnight type, on the other hand, showed significant differences in the mean MDS y coordinate with several of the other morphological types. Since the y coordinate explains 37.2% of the genetic distance variance, this provides statistical evidence to support the grouping pattern seen in the MDS plot.

This correspondence suggests that most cultivars falling into these two groups are likely to be very similar genetically. No other correspondences between morphologically based cultivar groupings and molecular marker genetic distances were noted, except possibly for the Common type, which would suggest that the cultivars within these other groups are not necessarily genetically related. This way, they can be used for blended plantings with similar turf characteristics while still retaining genetic diversity within the planting.

These results generally agree with those of Huff (2001), in which the genetic relationship of 93 Kentucky bluegrass entries was studied using RAPDs. In that study, four out of five Compact-Midnight members were in the same dendrogram clade, while the fifth one, 'Explorer', clustered near the BVMG members as it did in this study. Further, all four BVMG cultivars clustered together, following the pattern of this study, and six of the eleven experimental lines tested in that study clustered in the same clade as BVMG, again similar to the results of this study, even though different experimental lines were used. Thus, evidence for this partial correspondence between RAPD-based grouping and morphological trait-based classification of cultivars is provided by two different studies. Further, since some of the cultivar groups have very few entries in this study,

it is possible more correspondence between the two grouping methods may be found if a larger sample size is tested in the future.

When the three seedling replicates of each genotype were compared against each other to calculate their genetic distance, a rather high amount of genetic variability within cultivar or seed source was found for some of them, with values as high as 0.5. The mean within-source distance for all the genotypes was 0.22, or an average of 22% of RAPD bands being different. This value appears high for a facultatively apomictic species, although the frequency of apomixis can vary from close to zero to nearly 100% (Porceddu et al., 2002). Further, occasional mitotic aberrations in meristems can change chromosome numbers within a plant (Porceddu et al., 2002), and presumably other genetic characters, and these changes can then be perpetuated by apomixis. So, it is possible that these differences reflect the known variation in the amount of reproduction by apomixis in this species.

Also, this estimate is likely to be biased upward slightly because of possible low frequency errors in scoring or misamplification of some of the RAPD bands. Skroch and Nienhuis (1995) reported a scoring error value of 2%, so a similar scoring error value can probably be expected in this study as well. Additionally, if fewer than 85 markers had been included in the analysis, it is possible that this genetic distance estimate would have been lower, because of possible undersampling of genetic differences between the three replicates (Skroch et al., 1992). However this seems unlikely since sampling efficiency, as measured by reduction in coefficient of variation, is near its maximum at a marker number of 85 (Skroch et al., 1992).

It should be noted that the sample size of three replicates for each genotype may be low. For example, Huff (2001) used 96 seedling replicates of two cultivars, Baron and Unique, to estimate the number of off-type plants in these two cultivars. However, the present study used three seedling replicates of all 123 cultivars and seed sources, so that comparisons of RAPD variability among replicates of different germplasm sources were possible.

Examination of Fig. 3, however, would suggest technical factors likely do not explain all of the observed variation. In this figure the second and third replicate of cultivar Nassau and all three replicates of cultivar Chateau appear identical as would be expected for apomictic reproduction. However, it appears that replicate 1 of cultivar Nassau is an off-type. It is also possible that this difference is caused by seed contamination, but these cultivars are well spaced from each other as well as from the rest of the cultivars in the MDS plot, which is based on all 85 RAPD markers. Another possibility is that it is the result of a sexually produced seed, either cross- or self-fertilized (Huff and Bara, 1993).

In contrast, there is also evidence for genetic uniformity of seeds of a given cultivar between seedlots. Specifically, a fourth seedling of cultivar Award, from a different seedlot than the other three (listed in Table 1 as Award-B), was included in this study. Compared with the three replicates of Award, the RAPD banding

patterns were 3.5, 8.2, and 8.2% different. Thus, even though some cultivars had variability between seedling replicates, this cultivar seemed fairly uniform even between seedlots.

Although further experimentation would be needed to determine the origins of the off-types, their frequency may increase as the age of the seed production field increases, due to a gradual buildup of an initially low proportion of sexually produced plants, which may or may not be derived from different cultivars. The age of the seed harvest fields from where the seeds originated in this study is not known. However, the experimental breeding lines had a lower value for within seed source variability than the cultivars, and since these genotypes are still in development rather than in commercial production, it is likely that the seed fields are kept more pure than for the cultivars. The PIs have the highest value for within seed source variability, suggesting a lower level of apomixis in these genotypes.

The information presented in this study provides support for the utility of RAPD marker-based genetic relationships for turfgrass genetics. Furthermore, the genetic information will be a valuable resource for turf breeders and managers, particularly in recommending cultivars to include in blends, choosing new, exotic genotypes to incorporate into breeding programs, and possibly providing a preliminary indication of variable levels of among-seedling genetic variability over different germplasm sources in Kentucky bluegrass.

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