Relationships among Creeping Bentgrass Cultivars Based on Isozyme Polymorphisms

S. E. Warnke,* D. S. Douches, and B. E. Branham

ABSTRACT
An understanding of the genetic variability within a crop species is essential to its improvement. The objectives of this research were to study the utility of isozyme patterns for creeping bentgrass (Agrostis palustris Huds.) cultivar identification and to evaluate the relationships between creeping bentgrass cultivars based on isozyme patterns. Seventy-three plants from each of 38 creeping bentgrass cultivars and 25 plants from one plant introduction were scored for 24 isozyme polymorphisms representing six loci. All cultivars except a small group containing the cultivars Pennlinks, ProCap, Southshore, and Lopez were uniquely characterized based on a 20% or greater band frequency in one cultivar versus absence of the band in the most closely clustered cultivar. The isozyme patterns from each plant were used to calculate the genetic distance within a cultivar, and the average band frequency within a cultivar was used to calculate genetic distances between cultivars. The cultivars Pennlinks, ProCap, Southshore, and Lopez had the highest average within-cultivar genetic distances indicating that additional marker loci will be needed to distinguish these cultivars. The unwighted pair group method with arithmetic average (UPGMA) cluster analysis generated from the between-cultivar genetic distance matrices divided the cultivars into two groups. One group contains 39 cultivars including the variety Seaadie which may have provided initial germplasm for this group. The second group contains the cultivars Pennlinks, Southshore, ProCap, Lopez, and four cultivars with unique allozymes. The plant introduction PI215499 was distinctly related to the cultivar U.S. germplasm indicating that European material could be a source of genetic diversity to broaden U.S. bentgrass germplasm. The Bentgrasses are native to Western Europe (Harlan, 1992) with the genus Agrostis consisting of approximately 200 species (Hitchcock, 1951). The four species commonly used as turfgrasses are A. palustris Huds.—creeping bentgrass (2n = 4x = 28), A. canina L.—velvet bentgrass (2n = 2x = 14), A. tenella Sibth.—colonial bentgrass (2n = 4x = 28), and A. gigantea Roth.—redtop bentgrass (2n = 6x = 42). Creeping bentgrass is the most widely utilized of the turf-type bentgrasses because it has excellent tolerance of low mowing heights and a strong stoloniferous growth habit making it ideally suited for the establishment of golf course greens and fairways in temperate and subarctic climate zones.

An understanding of the genetic diversity present in the cultivated germplasm of a species as well as the location of new sources of genetic variability is important for the optimal utilization of genetic resources. Plant breeders must have an understanding of the genetic variability of elite germplasm because continued rescission within this germplasm can narrow the genetic base of elite material and ultimately increase the potential vulnerability to pests and abiotic stresses. Information about the location of new sources of genetic variability can help broaden the genetic base of elite material and maintain long-term improvement. Information about the genetic relationships of creeping bentgrass cultivars is limited because it is a cross polinated species, and in many cases the parental clones of synthetic cultivars are of unknown origin. However, in other species, genetic similarity between cultivars has been estimated based on molecular markers such as isozymes in Glycine max L.—soybean (Cox et al., 1985) and Solanum tuberosum L.—potato (Douches and Ludlam, 1991); RFLPs in Hordeum vulgare L.—barley (Melchinger et al., 1994), Zea mays L.—corn (Messier et al., 1993), and Festuca arundinacea Schreb.—tall fescue (Xu et al., 1994); and RAPD markers in [Bambusa] dicrcyleides (Nutt.) Engelm.—bamboo (Huff et al., 1997; Wu and Lin, 1994). Isozyme markers are not as numerous as RFLPs or RAPD markers; however, they are polymorphic in creeping bentgrass populations (Warnke et al., 1997) and technically simpler to apply with large population sizes. The objectives of this research were to (i) assess isozyme patterns for creeping bentgrass cultivar identification, (ii) determine the optimum sample size for estimating allozyme frequencies of creeping bentgrass populations, and (iii) estimate the genetic relationship between creeping bentgrass cultivars based on allozyme frequencies.

MATERIALS AND METHODS
Eighteen major creeping bentgrass cultivars commercially available in the USA and one plant introduction were assayed for isozyme polymorphisms. Seed was obtained from the 1993 National Turfgrass Evaluation Programs (NTEP) bentgrass cultivar trial and directly from the proprietary seed companies. In a few cases seed from the 1989 NTEP trial was used (Table 1). Individual plants at least 60 d old were used for isozyme analysis. Plants were maintained in the greenhouse and fertilized every 2 wk with a (20:8:7:16 N-P-K) water soluble fertilizer solution and trimmed regularly to promote tiller production. Healthy plants free from disease and insect pressure were found to provide much higher enzyme activity. Furthermore, electrophoretic consistency was improved by utilizing eliased tissue from plants maintained at low light levels and high temperature.

*Corresponding author.

Abbriviations: UPGMA, unwighted pair group method with arithmetic average; AWGD, average within-cultivar genetic distance; PGM, phosphoglucomutase; PPDF, phosphofructokinase isozyme; TPI, triosephosphate isomerase; GST, glutathione S-transferase transmembrane; NTEP, National Turfgrass Evaluation Programs.

constant temperature for 1 wk prior to analysis. A four- to five-leaf sample of newly expanded leaves was collected from each plant and crushed with a pestle and mortar, and then the extract was collected and stored at -80°C for later analysis. The isolated RNA was treated with DNase I and subsequently used for RT-PCR to amplify the cDNA corresponding to the target gene.

RESULTS AND DISCUSSION

A total of 24 alloys were sequenced for 70 plants in each of the 18 cultivars. Segregation data is available for 14 of the 24 alloys sequenced (Worsele et al., 1996). The assignment of additional alloys was based on mobility differences compared with the control plants run with each gel. The Pm-2 locus in creeping bentgrass is highly variable with seven scorable alleles present; however, the dimeric structure of this enzyme and close mobilities of many alleles does not allow for the accurate classification of all alleles present in some genotypes without progeny testing. Therefore, only the fastest and slowest migrating alleles for each plant, i.e., those that were easily detected, were scored. Average allele frequency ranged from 0.01 for Pm-1 and Pm-2 to 1.00 for Pm-3 and Cm-2 (Table 2). Genetic distances between cultivars ranged from 0.007 for Southshore and Penlink to 0.277 for Cato and PI251945 (Table 3). The UPGMA cluster analysis separates the 18 U.S. cultivars into two main groups (Fig. 1). The first group includes 10 cultivars (Penncroft, Putter, Penncross, Timelin, Viper, Emerald, 18th Green, Cobra, Crenshaw, and Seaside). With the exception of Crenshaw these are strongly creeping cultivars having a prostrate to semi-erect growth habit. The cultivar Seaside is the oldest variety in this group and may have provided germplasm used in the development of some of the creeping bentgrasses in this group. Seaside originated as a naturalized population growing in tidal flats near Coos Bay, OR, and was the only widely available seedbed bentgrass in the U.S. from the 1920s until 1955 when Penncross was released (Duch, 1985). The second cluster contains eight cultivars that can be divided into two groups. Four of them (Southshore,
<table>
<thead>
<tr>
<th>Allele</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025</td>
<td>0.032</td>
<td>0.034</td>
<td>0.043</td>
<td>0.043</td>
<td>0.063</td>
<td>0.062</td>
<td>0.069</td>
<td>0.067</td>
<td>0.064</td>
<td>0.069</td>
<td>0.052</td>
<td>0.068</td>
<td>0.026</td>
<td>0.098</td>
<td>0.014</td>
<td>0.012</td>
<td>0.012</td>
<td>0.015</td>
<td>0.017</td>
<td>0.013</td>
</tr>
<tr>
<td>0.063</td>
<td>0.062</td>
<td>0.069</td>
<td>0.067</td>
<td>0.064</td>
<td>0.069</td>
<td>0.052</td>
<td>0.068</td>
<td>0.026</td>
<td>0.098</td>
<td>0.014</td>
<td>0.012</td>
<td>0.012</td>
<td>0.015</td>
<td>0.017</td>
<td>0.013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.012</td>
<td>0.008</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

1 Culture code described in Table 1.2 Allele number specified in Warren et al. (1996).
Allozyme differences between the cultivars could also arise due to genetic drift because of the small population sizes or to differences in the strength and direction of selection imposed by breeders.

Creeping bentgrass cultivar identification via isoenzymes is complicated by the fact that cultivars are synthetics and considerable within-cultivar variation exists. The establishment of isozymes as a reliable characteristic for fingerprinting requires that allozyme frequencies in a given cultivar be stable in different environments and generations. In this study, all cultivars except Southshore, Pennlinks, Pro/Cup, and Lopez were distinguished by the presence of an allozyme in one cultivar at a frequency greater than 20% and its absence from the most closely-related cultivar. The cultivar Lopez is close to meeting this requirement at the Gpi-2 allelic.

However, further research needs to be done on seed lot variability and post-establishment effects on allozyme frequencies to determine the overall utility of isozymes for creeping bentgrass cultivar discrimination. Additionally, more enzyme systems will be needed to be evaluated to reliably distinguish Pennlinks, Pro/Cup, and Southshore.

Yamamoto and Duich (1994) examined the utility of isozyme analysis with bulk plant leaf sampling and were able to distinguish 12 creeping bentgrass cultivars using only the Pgi-2 locus. Oosterhoudt and Nielsen (1994) investigated the effectiveness of the Pgi-2 locus for cultivar identification in tetraploid ryegrass and found that allelic frequencies did not differ among seed lots. Additionally, seed size differences and low germination did not lead to significant within-cultivar allozyme frequency differences. Hayward et al. (1978) demonstrated Pgi-2
isoenzyme profiles in perennial ryegrass did not differ significantly between seed stocks and samples from 3-yr old stands. Similar studies must be conducted for creeping bentgrass to establish the utility of isozyme analysis for creeping bentgrass cultivar discrimination in the turf industry.

Genetic diversity studies of cross-pollinated species require adequate sampling to ensure the accuracy of allele frequency estimates. One method for determining optimum sample sizes was described by Xu et al. (1994) in an RFLP analysis of tall fescue cultivars. Average genetic distances were calculated for different sample sizes and the point at which there was no longer a significant difference between the means of two sample sizes was considered the most efficient sample size. In their study, the largest sample size examined was 20 and there was no significant difference between sample sizes of 16 and 20 plants. In our study, average genetic distances between cultivars were calculated using data from 4, 8, 12, 16, 20, 25, and 70 plants. The mean distance between cultivars decreased and became consistent as sample size increased (Fig. 2). The difference between means was not significant (p = 0.05) for sample sizes of 16, 20, or 16 and 25; however, there was a significant difference between sample sizes of 16 and 70, 20 and 70, and 20 but not between 25 and 70. The results suggested that a random sample of 25 plants is the minimum number of plants needed to estimate accurately the isozyme variation within these creeping bentgrass cultivars.

ACKNOWLEDGMENTS

The authors wish to thank the Michigan Turfgrass Foundation for providing financial assistance for this research.

REFERENCES


