Isolation and Characterization of 88 Polymorphic Microsatellite Markers in Kentucky Bluegrass (Poa pratensis L.)

Josh A. Honig¹, Stacy A. Bonos, and William A. Meyer
Department of Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers University, 59 Dudley Road, Foran Hall, New Brunswick, NJ 08901-8520

Abstract. Kentucky bluegrass (Poa pratensis L.) is an important facultative apomictic temperate perennial grass species used for both forage and cultivated turf. Through apomixis, this species is able to propagate diverse and odd ploidy levels, resulting in many genetically distinct phenotypes. A wide range of diverse cultivars and accessions of kentucky bluegrass have been previously characterized based on common turf performance or morphological characteristics as well as by random amplified polymorphic DNA (RAPD) markers. Although previous characterization efforts have provided valuable information, the use of both morphological characteristics and RAPD markers for genetic diversity analysis has limitations. In the current report, we developed and characterized 88 novel microsatellite markers for kentucky bluegrass. Polymorphism for each marker was assessed in 265 kentucky bluegrass cultivars, experimental selections, collections, and hybrids. The number of alleles for individual microsatellites ranged from four to 81 with an average of 38.3 alleles per simple sequence repeat. These polymorphic microsatellite markers would be useful tools for investigating genetic diversity, creation of genetic linkage maps, assessment of levels of apomixis in cultivars and experimental varieties, and identification of aberrant progeny in apomorphic kentucky bluegrass breeding programs.

The bluegrasses, also commonly referred to as meadowgrasses, are one of the most economically important genera of the Poaceae (Huff, 2010; Soreng and Barrie, 1999). Kentucky bluegrass (Poa pratensis L.) is the botanical-type species for the genus Poa (Soreng and Barrie, 1999) and is recognized as one of the most widely used temperate perennial grass species for both forage and amenity turf in the northern United States and Canada (Huff, 2003, 2010). Kentucky bluegrass is a facultative apomictic species, which has a highly variable chromosome number, creating a series of polyploidy and aneuploidy ranging from 2n = 28 to 154 (Akerberg, 1939; Graziani, 1939; Grazi et al., 1961; Huff, 2003; Love and Love, 1975; Meyer and Funk, 1989; Munzing, 1933; Nielsen, 1946; Tinney, 1940). Although this complex polyploidy may sometimes present a challenge to breeding efforts in this species, the ability of kentucky bluegrass to propagate diverse and odd ploidy levels through apomixis results in many genetically distinct phenotypes within the species (Huff, 2010). This wide range of diversity of cultivars and accessions of kentucky bluegrass has been previously characterized based on common turf performance or morphological characteristics (Bara et al., 1993; Bonos et al., 2000; Murphy et al., 1997; Shortell et al., 2009) as well as by random amplified polymorphic DNA markers (Curley and Jung, 2004; Huff, 2001; Johnson et al., 2002).

Although currently used in many plant variety protection (PVP) schemes, the use of morphological characteristics to determine genetic diversity and distinctness, uniformity, and stability (DUS) of cultivars has numerous drawbacks, including the maintenance of increasingly large reference collections for comparative analyses, a limited number of descriptors available to distinguish varieties, time-consuming field-based measurement of large numbers of samples and replicates, and the potential for the expression of morphological traits to be influenced by the environment (Giancola et al., 2002; Ibanez et al., 2009; Kwon et al., 2005; Lombard et al., 2000; Roldan-Ruiz et al., 2001). As a result of these drawbacks of using morphological characters for determining genetic diversity or for DUS testing, numerous researchers have proposed using molecular markers for PVP (Bonow et al., 2009; Borchert et al., 2008; Cooke and Reeves, 2003; Giancola et al., 2002; Gunjaca et al., 2008; Heckenberger et al., 2002, 2003, 2005a; Ibanez et al., 2009; Kwon et al., 2005; Roldan-Ruiz et al., 2001; Smith et al., 2009; Smykal et al., 2008; Tommassini et al., 2003; van Eenwijk and Law, 2004; Vossman et al., 2004). In contrast to morphological characters, molecular markers offer a number of advantages, including a nearly unlimited number of characters, high degree of polymorphism, ease of scoring, and they are unaffected by the environment (Lombard et al., 2000; Tommasini et al., 2008; Vossman et al., 2004). In this report, we describe the development of the first polymorphic microsatellite markers for ongoing molecular genetic research in kentucky bluegrass.

Total genomic DNA was extracted from a single plant of the kentucky bluegrass cultivar Cabernet (Bonos et al., 2004) using the Sigma GenElute Plant Genomic DNA Miniprep Kit (St. Louis, MO) according to the manufacturer’s instructions. DNA was sent to Genetic Identification Services Inc. (GIS, Chatsworth, CA) for construction of simple sequence repeat (SSR) libraries enriched for CA, GA, AAT, and CAG SSRs. Methods for DNA library construction and enrichment were developed following Jones et al. (2002). Briefly, genomic DNA was partially digested with a cocktail of seven blunt-end restriction enzymes (Rsal, HaeIII, Bsu36I, Pvull, Stul, Scal, EcoRV). Fragments in the size range of 300 to 750 bp were cloned into oligonucleotides, which contained a HindIII site at the 5’ end and subjected to magnetic bead capture (CIPG, Inc., Lincoln Park, NJ) using 5’-biotinylated CA15, GA15, AAT12, and CAG12, as capture molecules according to the manufacturer’s instructions. Captured molecules were amplified using amplimers to the adaptor, digested with HindIII to remove the adaptor sequences, and ligated into the HindIII site of pUC19. Recombinant plasmids were then electrotransformed into Escherichia coli DH5α. GIS delivered DNA libraries 50% enriched for CA and GA repeats and libraries 15% enriched for the trinucleotides AAT and CAG.

Several 50-µL aliquots of each SSR library were plated out onto LB agar plates containing ampicillin, IPTG, and Bluo-Gal. The plates were incubated at 37 °C overnight. Three thousand individual colonies were chosen from the CA- and GA-enriched libraries and grown in 6 ml LB broth containing ampicillin. Five hundred colonies were chosen from the AAT- and CAG-enriched libraries. DNA was isolated from the cultures using the Qiagen QIAprep Spin Miniprep kit (Valencia, CA). Samples were sequenced using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). A total of 2523 clones were sequenced. Sequence data from clones in the AAT and CAG libraries proved to be complex (long sequences of highly repetitive DNA), and the percentage appropriate for primer design was less than 1%. As a result, only the dinucleotide libraries were used for further analyses. Six hundred seventy-four dinucleotide clones contained no repeat, whereas 546 clones had other problems that precluded primer design (poor sequence, repeat too close to cloning site, etc.). One thousand seventy-one clones contained either CA or GA repeat motifs suitable for primer design.

Sequence data from the clones containing dinucleotide SSRs were analyzed for primer
Table 1. Primer sequences and characteristics of 88 *Poa pratensis* (L.) microsatellite markers obtained from the cultivar Cabernet tested on 265 *Poa pratensis* cultivars, experimental selections, collections, and hybrids.

| Marker ID | GenBank accession no | Primers (5'–3') | Repeat motif | N<sub>0</sub> | Original cloned allele (bp) | Allele size range (bp) | Range of PIC values for N<sub>0</sub> alleles |
|----------|----------------------|-----------------|--------------|---------|-----------------------------|----------------------|---------------------------------|
| NJPpGA1  | HM136087             | F: AAAGCCTGCTGTTGTAATCTCACG | (CT)<sub>17</sub>(GT)<sub>10</sub> | 40      | 252                         | 199–305              | 0.01–0.48                      |
| NJPpGA6  | HM136088             | F: GGTGTTGCTGCTTCAATAGTGG | (GA)<sub>2</sub>(GA)<sub>3</sub> | 22      | 279                         | 181–314              | 0.01–0.45                      |
| NJPpGA9  | HM136089             | F: GCCGAATAGTGGAGGAAGAC | (CT)<sub>21</sub> | 40      | 208                         | 142–275              | 0.01–0.50                      |
| NJPpGA107| HM136090            | F: TTTGGCTCACTAGTATTTATCC | (GA)<sub>22</sub> | 39      | 238                         | 187–268              | 0.01–0.50                      |
| NJPpGA108| HM136091            | F: GAGTGGAGATCACAACACATG | (CT)<sub>23</sub> | 81      | 213                         | 161–292              | 0.01–0.44                      |
| NJPpGA111| HM136092            | F: CGACCAGACTTGTAACGAC | (GA)<sub>30</sub> | 32      | 229                         | 179–268              | 0.01–0.43                      |
| NJPpGA115| HM136093            | F: CCACCCGACATTTGACTG | (GA)<sub>31</sub> | 24      | 277                         | 281–328              | 0.01–0.50                      |
| NJPpGA124| HM136094            | F: GTGCCCTCTTCCAGAAAGTC | (CT)<sub>25</sub> | 33      | 114                         | 117–173              | 0.01–0.50                      |
| NJPpGA125| HM136095            | F: GCAGAAACAAAAAGTCTACTG | (GA)<sub>2</sub>(GA)<sub>18</sub> | 64      | 231                         | 158–439              | 0.01–0.50                      |
| NJPpGA128| HM136096            | F: GACCGCAAGGACCTACCTCTC | (CT)<sub>37</sub> | 30      | 156                         | 131–199              | 0.01–0.50                      |
| NJPpGA129| HM136097            | F: CCAGCACTACCTAGCAGC | (CT)<sub>32</sub> | 27      | 296                         | 272–322              | 0.01–0.48                      |
| NJPpGA132| HM136098            | F: TTCCGAAGAACCTGATTTGG | (CT)<sub>3</sub>(TT)<sub>2</sub> | 4       | 300                         | 311–321              | 0.01–0.50                      |
| NJPpGA134| HM136099            | F: ACCCCCTCTTGGATATCG | (CT)<sub>33</sub> | 15      | 150                         | 129–172              | 0.02–0.48                      |
| NJPpGA274| HM136700            | F: GACGACAAAACTCGACACT | (CT)<sub>35</sub> | 35      | 298                         | 191–444              | 0.01–0.50                      |
| NJPpGA329| HM136701            | F: TGTGCTTATCTGGAAAGCA | (CT)<sub>33</sub> | 35      | 292                         | 282–382              | 0.01–0.48                      |
| NJPpGA379| HM136702            | F: AATGTGTTATGGCATCACC | (GA)<sub>32</sub> | 42      | 295                         | 286–370              | 0.01–0.50                      |
| NJPpGA393| HM136703            | F: CATGAGAGACAGCAAGACG | (CT)<sub>33</sub> | 26      | 248                         | 239–335              | 0.01–0.50                      |
| NJPpGA405| HM136704            | F: TGTGCTTATCTGGAAAGCA | (CT)<sub>33</sub> | 18      | 262                         | 231–297              | 0.01–0.49                      |
| NJPpGA412| HM136706            | F: GCAACCTGGAACAAATTTAT | (CT)<sub>34</sub> | 38      | 300                         | 293–379              | 0.01–0.48                      |
| NJPpGA422| HM136707            | F: TGCTGTCCTTCAACATACAT | (CT)<sub>34</sub> | 64      | 260                         | 244–335              | 0.01–0.48                      |
| NJPpGA446| HM136710            | F: CTCCGTGAGTAGACCGTGTT | (CT)<sub>34</sub> | 37      | 299                         | 267–419              | 0.01–0.50                      |
| NJPpGA468| HM136711            | F: TGCCCAAGGAAAAATTATGA | (GA)<sub>2</sub>(GA)<sub>2</sub> | 41      | 284                         | 233–366              | 0.01–0.33                      |
| NJPpGA470| HM136712            | F: GCTTCTATGTGATCGAGAAA | (GA)<sub>2</sub>(GA)<sub>2</sub> | 32      | 294                         | 242–313              | 0.01–0.50                      |
| NJPpGA480| HM136713            | F: CTCCGTGAGTAGACCGTGTT | (CT)<sub>32</sub> | 41      | 284                         | 233–366              | 0.01–0.33                      |
| NJPpGA498| HM136714            | F: TGGTTTGATGAAAAGAC | (CT)<sub>34</sub> | 48      | 290                         | 269–494              | 0.01–0.50                      |
| NJPpGA508| HM136715            | F: ACAGGAGATCCTAGAGTCTCG | (CT)<sub>34</sub> | 23      | 187                         | 161–242              | 0.01–0.50                      |
| NJPpGA748| HM136716            | F: TTTGGCTCACGGCGAGAAAA | (GA)<sub>2</sub>(GA)<sub>2</sub> | 27      | 294                         | 255–357              | 0.01–0.50                      |
| NJPpGA771| HM136717            | F: TCGGAGAGATGAGAGAACAAAT | (GA)<sub>2</sub>(GA)<sub>17</sub> | 35      | 279                         | 253–337              | 0.01–0.49                      |
| NJPpGA783| HM136718            | F: AAAGTAAAGATCGCTGAGC | (CT)<sub>34</sub> | 49      | 274                         | 255–357              | 0.01–0.50                      |
| NJPpGA799| HM136719            | F: TTTGGAGAAAGAGTAATGGA | (GA)<sub>2</sub>(GA)<sub>2</sub> | 36      | 250                         | 251–317              | 0.01–0.50                      |
| NJPpGA806| HM136720            | F: TCCCTCAAGTGAATTCTGGA | (CT)<sub>32</sub> | 38      | 298                         | 249–352              | 0.01–0.49                      |

(Continued on next page)
Table 1. (Continued)

| Marker ID   | GenBank accession no. | Primers (5'-3') | Repeat motif | N<sub>a</sub> | Original cloned allele (bp) | Allele size range (bp) | Range of PIC values for N<sub>a</sub> alleles |
|-------------|-----------------------|-----------------|--------------|-------------|-----------------------------|-----------------------|--------------------------------------------|
| NJpGA815    | HM136723              | F: CACTAAAAGCCAACAACCAAGA (GA)<sub>13</sub>A(AGA)<sub>3</sub> | 41           | 286         | 179–365                     | 0.01–0.50             |                                            |
| NJpGA832    | HM136724              | F: TCCAGAGCATACAGGAGAGTTAACA (CT)<sub>21</sub>G(CGT)<sub>3</sub> | 45           | 250         | 236–338                     | 0.01–0.50             |                                            |
| NJpGA836    | HM136725              | F: AACATAAAGCACAACAACAGA (GA)<sub>13</sub>A(GA)<sub>5</sub> | 50           | 286         | 257–365                     | 0.01–0.50             |                                            |
| NJpGA892    | HM136726              | F: TGTCGCTAGAGCTCTCTTTGCACTCTG | 20           | 250         | 224–291                     | 0.01–0.49             |                                            |
| NJpGA897    | HM136727              | F: ACTCTCACTACATCGCTCTCCAAGATGAG (CT)<sub>22</sub> | 40           | 300         | 267–362                     | 0.01–0.50             |                                            |
| NJpGA900    | HM136728              | F: ATTCGGAATGCTTAGGTCCTAGT GAAGGTGTCGAGATGAG (CT)<sub>22</sub>G | 42           | 299         | 152–374                     | 0.01–0.49             |                                            |
| NJpGA914    | HM136729              | F: CCCCACATCTCTTCTCTACAAATGAG (GA)<sub>19</sub> | 60           | 278         | 274–353                     | 0.01–0.50             |                                            |
| NJpGA931    | HM136730              | F: CTTCGTTTGGAGAGGTTGTG (CT)<sub>14</sub>G | 22           | 245         | 183–290                     | 0.01–0.49             |                                            |
| NJpGA954    | HM136733              | F: TACCCAGTACCATGGTCTCTCCTTGCAAAGAG (CT)<sub>16</sub>A(GA)<sub>13</sub> | 12           | 286         | 286–419                     | 0.01–0.36             |                                            |
| NJpGA957    | HM136734              | F: TCCAAGGCTACTCACCATCAGTG (CT)<sub>17</sub>G | 25           | 286         | 284–3550                    | 0.01–0.47             |                                            |
| NJpGA963    | HM136735              | F: ATACATTGGGGTGCATGGT (CT)<sub>23</sub> | 23           | 299         | 289–342                     | 0.01–0.50             |                                            |
| NJpGA964    | HM136736              | F: AACCCGGTTTTGCCTTATACTG | 28           | 291         | 243–305                     | 0.01–0.48             |                                            |
| NJpGA973    | HM136737              | F: ATCTTCAGATCCTGGGTAGC (CT)<sub>19</sub>G | 35           | 281         | 236–328                     | 0.01–0.50             |                                            |
| NJpGA986    | HM136738              | F: CATGAGAAGAGACACAGAGGAG (CT)<sub>19</sub>G | 54           | 298         | 283–351                     | 0.01–0.48             |                                            |
| NJpGA993    | HM136739              | F: GAGACCCAAAAATCGTCCTC (CT)<sub>18</sub>G | 28           | 291         | 289–350                     | 0.01–0.48             |                                            |
| NJpGA1003   | HM136740              | F: GGGGQATACAAACAATTCCGATAC (CT)<sub>23</sub> | 23           | 293         | 270–316                     | 0.01–0.48             |                                            |
| NJpGA1054   | HM136741              | F: ATACATTGGGGTGCATGGT (CT)<sub>23</sub> | 13           | 286         | 243–305                     | 0.01–0.50             |                                            |
| NJpGA1071   | HM136742              | F: CACTCTCGTGGATTGATTGCTCCTG | 61           | 334         | 240–357                     | 0.01–0.50             |                                            |
| NJpGA1092   | HM136743              | F: GACATCAAGACCTGCCAGCAAAG (CT)<sub>12</sub>G | 25           | 299         | 292–346                     | 0.01–0.49             |                                            |
| NJpGA1095   | HM136744              | F: ATTTAAAGCAGGGGAGCAAG (CT)<sub>17</sub>G | 29           | 292         | 274–338                     | 0.01–0.50             |                                            |
| NJpGA1100   | HM136745              | F: CTTAGGCTGATTTTTCCCACTTC (CT)<sub>14</sub>G | 47           | 250         | 249–336                     | 0.01–0.50             |                                            |
| NJpGA1101   | HM136746              | F: CACTCCCCGATTGGATGTGCGCT (CT)<sub>18</sub>G | 35           | 298         | 285–342                     | 0.01–0.50             |                                            |
| NJpGA1102   | HM136747              | F: TCTCTCTCTCTCCCCCTTCTTTG (CT)<sub>20</sub>G | 38           | 360         | 247–329                     | 0.01–0.50             |                                            |
| NJpGA1110   | HM136748              | F: TGGAGAGTTCGCTGTATGGAG (TA)<sub>10</sub>G | 39           | 291         | 240–365                     | 0.01–0.42             |                                            |
| NJpGA1112   | HM136749              | F: AAAAACTGATGGCTCGACAAT (TA)<sub>14</sub>G | 55           | 281         | 236–328                     | 0.01–0.50             |                                            |
| NJpGA1117   | HM136750              | F: CTCCAGAGTACAGGGTTGCTG | 25           | 299         | 253–360                     | 0.01–0.49             |                                            |
| NJpGA1119   | HM136751              | F: TCCAGCAGCTGTTATTTTCTTCTC (TA)<sub>14</sub>G | 40           | 285         | 257–344                     | 0.01–0.50             |                                            |
| NJpGA1148   | HM136752              | F: GCAGTATCCCAAGGTTATTACAAAAGAAG (TA)<sub>14</sub>G | 40           | 250         | 234–389                     | 0.01–0.47             |                                            |
| NJpGA1152   | HM136753              | F: AACAGTGATGCTCGACAAT (TA)<sub>13</sub>G | 58           | 281         | 216–328                     | 0.01–0.50             |                                            |
| NJpGA1156   | HM136754              | F: CTCCAGGATAGCGGAAG (TA)<sub>14</sub>G | 41           | 291         | 276–360                     | 0.01–0.50             |                                            |
| NJpGA9307   | HM136755              | F: ACGCAGAGAGCACAAACAGAAG (GA)<sub>20</sub>G | 67           | 283         | 258–394                     | 0.01–0.44             |                                            |
| NJpGA9314   | HM136756              | F: GACAAGCGTGATGCTCTCGACTG | 48           | 290         | 271–340                     | 0.01–0.41             |                                            |
| NJpGA9317   | HM136757              | F: GTGCAGCGTGATGCTCTCGGAGG (GA)<sub>13</sub>G | 53           | 294         | 240–357                     | 0.01–0.45             |                                            |

(Continued on next page)
selection, and polymerase chain reaction (PCR) primers were designed to flank regions surrounding the SSR motif using Primer3 software (Rozen and Skaletsky, 2000). The forward primer in the pair was elongated at the 5′ end using the M13(-21) 18-bp sequence (5′-TGTAAAACGACGGCCAGT-3′) for economic fluorescent labeling (Schuelke, 2000) and synthesized by Integrated DNA Technologies (Coralville, IA). Of the 1071 clones containing dinucleotide repeats, PCR primer pairs were designed for 500 of these sequences, of which 88 (17.6%) produced distinct, repeatable polymorphic bands in a panel of 265 kentucky bluegrass cultivars, experimental selections, collections, and hybrids with texas bluegrass (Poa arachnifera L.) as the 5′ end of the SSR motif. The individual SSR allele was calculated according to the formula $P_i = 1 - \sum P_i^2$, where $P_i$ is the frequency of the $i$th allele in the genotypes examined. For dominant markers, this formula can be simplified to $PIC = 2P_iQ_i$, where $P_i$ is the frequency of presence and $Q_i$ is the frequency of absence of a particular band (Tehrani et al., 2000). The high level of polymorphism observed for the described microsatellite markers supports their application in genetic studies of kentucky bluegrass. The potential usefulness of these markers includes assessment of the combination of high genetic diversity, creation of genetic linkage maps, assessment of levels of apomixis and high ploidy levels in the genotypes under investigation. The PIC value ranges for the majority of the SSR markers approached the maximum PIC value for dominant markers, indicating that each SSR marker produced alleles that were highly polymorphic (Table 1).

Table 1. Characteristics of the SSR markers and their primer sequences are shown in Table 1. All SSR markers produced well-defined discrete alleles. The total number of alleles produced by the 88 SSR markers in the 265 cultivars and accessions was 3373. The number of alleles for individual SSR markers ranged from four to 81 with an average of 38.3 alleles per SSR. The high average number of alleles per SSR is likely the result of the combination of high genetic diversity and high ploidy levels in the genotypes under study. A range of PIC values for all alleles generated by a particular SSR marker (Table 1).

Characteristics of the SSR markers and their primer sequences are shown in Table 1. All SSR markers produced well-defined discrete alleles. The total number of alleles produced by the 88 SSR markers in the 265 cultivars and accessions was 3373. The number of alleles for individual SSR markers ranged from four to 81 with an average of 38.3 alleles per SSR. The high average number of alleles per SSR is likely the result of the combination of high genetic diversity and high ploidy levels in the genotypes under investigation. The PIC value ranges for the majority of the SSR markers approached the maximum PIC value for dominant markers, indicating that each SSR marker produced alleles that were highly polymorphic (Table 1).

Table 1. Characteristics of the SSR markers and their primer sequences are shown in Table 1. All SSR markers produced well-defined discrete alleles. The total number of alleles produced by the 88 SSR markers in the 265 cultivars and accessions was 3373. The number of alleles for individual SSR markers ranged from four to 81 with an average of 38.3 alleles per SSR. The high average number of alleles per SSR is likely the result of the combination of high genetic diversity and high ploidy levels in the genotypes under investigation. The PIC value ranges for the majority of the SSR markers approached the maximum PIC value for dominant markers, indicating that each SSR marker produced alleles that were highly polymorphic (Table 1).
appropriate for PVP analyses in other crops (Heckenberger et al., 2005b; Smith et al., 2009).

Literature Cited

Heckenberger, E. 1939. Apomictic and sexual seed formation in Poa pratensis L. Hereditas 25:359–370.

Bara, R.F., W.K. Dickson, J.A. Murphy, D.A. Smith, and C.R. Funk. 1993. Performance of Kentucky bluegrass cultivars and selections in New Jersey turf trials. Rutgers Turfgrass Proc. 25:49–94.

Becher, S.A., K. Steinmetz, K. Weising, S. Boury, D. Bara, R.F., W.K. Dickson, J.A. Murphy, D.A. Akerberg, E. 1939. Apomictic and sexual seed formation in Pol- ergonium. Theor. Appl. Genet. 101:643–651.

Bonos, S.A., T.M. Ford, R.B. Meyer, and C.R. Funk. 2004. Registration of ‘Caber- net’ Kentucky bluegrass. Crop Sci. 44:1480.

Bonos, S.A., W.A. Meyer, and J.A. Murphy. 2000. Classification of Kentucky bluegrass genotypes grown as spaced-plants. HortScience 35:910–913.

Bonow, S., E.V.R. Von Pinho, M.G.C. Vieira, and Bonos, S.A., T.M. Ford, R.F. Bara, W.A. Meyer, and S.A. Bonos. 2009. Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties: II. Genetic and technical sources of variation in AFLP data and comparison with SSR data. Mol. Breed. 12:97–106.

Huff, D.R. 2001. Characterization of Kentucky bluegrass cultivars using RAPD markers. Int. Turfgrass Sci. Res. J. 9:169–175.

Huff, D.R. 2003. Kentucky bluegrass, p. 27–38. In: Casler, M.D. and R.R. Duncan (eds.). Turfgrass biology, genetics, and breeding. Wiley, Hoboken, N.J.

Huff, D.R. 2010. Bluegrasses, p. 345–379. In: Boller, U.K. Posselt, and F. Veronesi (eds.). Fodder crops and amenity grasses, handbook of plant breeding 5. Springer Science+Business Media, LLC, New York, NY.

Ibanez, J., M.D. Velez, M.T. de Andres, and J. Borrego. 2009. Molecular markers for establishing distinctness in vegetatively propagated crops: A case study in grapevine. Theor. Appl. Genet. 119:1213–1222.

Johnson, R.C., W.J. Johnston, C.T. Golob, M.C. Nelson, and R.J. Soreng. 2002. Characterization of the USDA Poa pratensis collection using RAPD markers and agronomic descriptors. Genet. Resources Crop Evol. 49:349–361.

Jones, K.C., K.F. Levine, and J.D. Banks. 2002. Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (Cervus canadensis). Mol. Ecol. Notes 2:425–427.

Kwon, Y.S., J.M. Lee, G.B. Yi, S.I. Yi, K.M. Kim, E.H. Soh, K.M. Bae, E.K. Park, J.H. Song, and B.D. Kim. 2005. Use of SSR markers to complement tests of distinctiveness, uniformity, and stability (DUS) of pepper (Capsicum annum L.) varieties. Mol. Cells 19:428–435.

Liao, W.J., B.R. Zhu, Y.F. Zeng, and D.Y. Zhang. 2008. TETRA: An improved program for classification and inheritance of morphological and agronomic characteristics in Kentucky bluegrass (Poa pratensis L.). HortScience 44:274–279.

Smith, J.S.C., E. Hoot, G. Cole, H. Lu, E.S. Jones, S.J. Wall, and D.A. Berry. 2009. Genetic diversity among US sunflower inbreds and hybrids: Assessing probability of ancestry and potential for use in plant variety protection. Crop Sci. 49:1295–1303.

Heyl, P.J., J. Horacek, R. Dostalova, and M. Hybl. 2008. Species discrimination in pea (Pisum sativum L.) by molecular, biochemical and morphological markers. J. Appl. Genet. 49:155–166.

Soreng, R.J. and F.R. Barrie. 1999. Proposal to conserve the name Poa pratensis (Gramineae) with a conserved type. Taxon 48:157–159.

Tehrani, M.S., M. Mardi, H. Saeidi, B. Gharehyazi, and M. Assadi. 2008. Transferability of genomic and EST-microsatellites from Festuca arundinacea Schreb. to Lolium persicoides Boiss. and Hohen, ex Boiss. Int. J. of Bot. 4:476–480.

Timney, F.W. 1940. Cytology of parthenogenesis in Poa pratensis. J. Agr. Res. 60:351–360.

Tommasini, L., J. Batley, G. Arnold, R. Cooke, P. Donini, D. Lee, J. Law, C. Lowe, C. Moule, M. Trick, and K. Edwards. 2003. The development of multiplex simple sequence repeat (SSR) markers to complement distinctness, uniformity and stability testing of rape (Brassica napus L.) varieties. Theor. Appl. Genet. 106:1091–1101.

van Eeuwijk, F.A. and J.R. Law. 2004. Statistical analysis of DNA fingerprint data in crop improvement. In: Asay, and J.F. Pedersen (eds.). Contributions for breeding forage and turf grasses. Crop Sci. 44:1132–1140.

Weir, B.S. 1996. Genetic data analysis: Methods for discrete population genetic data. Sinauer Associates, Inc., Sunderland, MA.