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Original Article

Analysis of Genetic Diversity in 73 Kentucky Bluegrass Materials by SSR and SRAP Markers

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Abstract

Kentucky bluegrass (*Poa pratensis* L.) (KBG) is a commonly used grass that possesses excellent quality, as well as a complex genetic background and reproductive patterns. In this study, a total of 73 KBG germplasms were collected, of which 49 were imported varieties, 5 were Chinese breeding varieties, and 19 were wild materials. A total of 70 simple sequence repeat (SSR) and 75 sequence-related amplification polymorphism (SRAP) markers were selected to use for genetic diversity analysis. From these studies, high levels of polymorphisms were observed in SRAPs (91.8%) and SSRs (94.5%), respectively. Three dendrograms that were generated from SRAP, SSR, and SRAP+SSR combined data revealed a general similarity for the positioning of the majority of materials. However, certain materials, including Z65, Z25, and Z27, were found to be located in diverse clusters among different dendrograms. Further analysis demonstrated no significant association between geographical origin and molecular marker clusters in the wild materials. Combined with the seedling phenotype identification carried out in our prior study, it seems as though there is no significant relationship between agronomic characterization and marker-based clustering in these materials, except for in the case of leaf color. These studies provided an increased understanding of genetic diversity among KBG materials, which will be beneficial for genetic improvement and germplasm conservation in the future.

Keywords: cluster analysis; genetic resources; molecular markers; *Poa pratensis* L.; turfgrass

Introduction

Kentucky bluegrass (*Poa pratensis* L.) (KBG) is a forage crop native to Asia and Europe, and is one of the most widely used cool-season turfgrass in both temperate and cooler regions (Gan *et al.*, 2016). KBG is able to reproduce both sexually (seeds) and asexually (apomixes), and it has been demonstrated that a complex series of polyploidy and aneuploidy exists among the different strains (Muntzing, 1940; Löve, 1952; Huff, 2003). Both the variable ploidy and the versatile reproduction mode are likely results of its advanced adaptiveness (Raggi *et al.*, 2015). Over a long-term natural selection period in a wide area that is subject to great climatic variation, wild KBG genotypes have been shown to have an increased inherent diversity (Burt and Christians, 1990). With ecotype selection and intraspecific hybridization breeding, an increasing number of KBG cultivars have begun to appear on the commercial turf market (Curley and Jung, 2004).

Typically, morphological traits, disease resistance, and stress tolerance are used to distinguish KBG varieties. These KBG varieties include those with different plant height, panicle height, tillers, flag leaf dimensions, rhizome spread, length (Nittler and Kenny, 1976; Bonos *et al.*, 2000; Shortell *et al.*, 2009), winter color and dormancy, tolerance to and recovery from summer stress, resistance to leaf spot diseases, resistance to stripe smut disease, and other properties (Bara *et al.*, 1993; Murphy *et al.*, 1997). In addition, identification of protein polymorphisms was also used in cultivar classification, including differences in leaf protein, seed protein, isoenzyme, and other properties (Wilkinson and Beard, 1972; Wehner *et al.*, 1976; Wu *et al.*, 1984; Tamkoc and Arslan, 2010). However, with an increased number of KBG varieties, relying only on phenotypic traits and protein markers has made the discrimination of different materials increasingly difficult.

Developments in PCR technology and molecular markers have enabled the generation of a more efficient instrument for the assessment of genetic diversity in numerous plants. Compared with phenotypic traits and

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protein characterization methods, molecular markers possess clear advantages (Lombard *et al.*, 2000), including a short test time, easy operation, an environmentally independent method, and sufficient (infinite) numbers, in theory. Currently, different types of molecular markers have been used widely in turfgrass genetic diversity studies. In the case of KBG, random amplification of polymorphic DNA (RAPD) (Johnson *et al.*, 2002; Curley and Jung, 2004; Liang *et al.*, 2009; Fard *et al.*, 2012; Szenejko and Rogalski, 2015; Yuan *et al.*, 2015; Szenejko *et al.*, 2016), amplified fragment length polymorphism (AFLP) (Treuren, 2008), simple sequence repeat (SSR) (Honig *et al.*, 2010; Honig *et al.*, 2012; Raggi *et al.*, 2015), inter-simple sequence repeat (ISSR) (Goldman, 2008; Szenejko *et al.*, 2016), and sequence-related amplified polymorphism (SRAP) (Yuan *et al.*, 2015) were all utilized to assess the genetic variability present and to identify germplasms.

Microsatellite loci are defined as short tandem repeats of 2 to 6 bp core sequences that are widely distributed throughout eukaryotic genomes in both protein-coding and non-coding regions (Morgante *et al.*, 2002). SSR markers are advantageous in that they are multi-allelic, co-dominant, reliable, abundant in genomes, and contain high numbers of polymorphisms. While SSR marker development requires time and cost, up until now, numerous SSR markers have been reported to be present in forage and turfgrass, including perennial ryegrass (*Lolium perenne* L.) (Jones *et al.*, 2001; Gill *et al.*, 2006), Italian ryegrass (*Lolium multiflorum* Lam.) (Hirata *et al.*, 2000; Hirata *et al.*, 2006), white clover (*Trifolium repens* L.) (Kölliker *et al.*, 2001; Barrett *et al.*, 2004), tall fescue (*Festuca arundinacea* Schreb.) (Saha *et al.*, 2004; Saha *et al.*, 2006), timothy (*Phleum pratense* L.) (Cai *et al.*, 2003), zoysiagrass (*Zoysia* spp.) (Cai *et al.*, 2005), bentgrass (*Agrostis*) (Kubik *et al.*, 2011; Honig *et al.*, 2016), switchgrass (*Panicum virgatum* L.) (Tobias *et al.*, 2006; Wang *et al.*, 2011), seashore paspalum (*Paspalum vaginatum* Swartz) (Harris-Shultz *et al.*, 2013), and KBG (Honig *et al.*, 2010).

The SRAP marker system was first reported by Li and Quiros in *Brassica oleracea*, and was aimed to be used for the amplification of open reading frames (ORFs) (Li and Quiros, 2001). The CCGG sequence is used in the forward primers to preferentially amplify exons to ORF regions. However, because exonic sequences are typically conserved, the number of polymorphisms amplified with these primers is low across different individuals (Quiros *et al.*, 2001). Reverse primers are designed with the AATT sequence to target AT-rich regions, which are more frequently present in promoters, introns, and spacers (Lin *et al.*, 1999). Because spacers, promoters, and introns are variable, polymorphic bands could be generated across different individuals using different primer combinations. This marker technique is advantageous due to the simplicity, reliability, highly polymorphic, and more informative properties of the technique.

SSRs and SRAPs are powerful techniques that have been used widely for linkage map construction (Jones *et al.*, 2002; Cai *et al.*, 2005; Saha *et al.*, 2005; Xie *et al.*, 2010) and gene tagging (Zhou *et al.*, 2011; Lou *et al.*, 2015; Sun *et al.*, 2015) in turfgrass. These techniques have also been applied for numerous types of genetic polymorphism analyses in turfgrass. Budak *et al.* (2004b) used 34 SRAP markers to study the genetic diversity and phenetic relationships in 53 buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.] germplasms. In addition, they also analyzed the relationships of 21 cool and warm-season turfgrasses, as well as other Gramineae species, using 19 SRAP markers (Budak *et al.*, 2004a). For the analysis of orchardgrass (*Dactylis glomerata* L.), a total of 60 accessions from four continents were studied using 21 SRAP primer pairs (Zeng *et al.*, 2008). Using 11 SRAP primer combinations, the levels of genetic diversity in 56 centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] accessions were compared (Milla-Lewis *et al.*, 2012). In the case of KBG, a total of 247 cultivars, experimental selections, and collections were genotyped using 25 SSR markers (Honig *et al.*, 2012), and a total of 11 nuclear and 2 chloroplast SSR markers were used to study the genetic diversity of 33 *P. pratensis* accessions from 23 different countries (Raggi *et al.*, 2015). A total of 80 SSR markers were used to assess genetic relationships between 18 *Paspalum* accessions (Harris-Shultz *et al.*, 2013). Recently, an assessment of genetic relationships among 74 *Agrostis* cultivars and accessions were carried out using 23 nuSSR markers (Honig *et al.*, 2016). An overview of the diversity and genetic relationships among 59 accessions of 32 species of the *Festuca-Lolium* complex were achieved using 22 SRAP primer combinations (Cheng *et al.*, 2016).

In China, the import of turfgrass varieties has recently grown exponentially with every year, with the majority of these varieties introduced from Europe and the United States. In addition, there are several new varieties that were bred domestically, including 'Daqignhsan', 'Huhe' (He *et al.*, 2009), 'Qinghai' (Wang *et al.*, 2010), 'KBG03', and 'KBG04' (Yuan *et al.*, 2014). In this study, we have used SSR and SRAP markers to investigate the genetic diversity of 73 KBG materials from both China and abroad. These 73 materials include different varieties, breeding lines, and wild materials. The objectives of this study were to (i) investigate whether different types of molecular markers generate similar clustering results, and (ii) assess the correlation between marker-based grouping and geographical origin in wild materials.

Materials and Methods

Plant materials

For this study, a total of 73 KBG materials (Z1-Z73) were collected (Table 1), which included 49 imported varieties, 10 wild materials obtained abroad, 5 domestic varieties/lines, and 9 domestic wild materials (from Jilin, Gansu, Sichuan, and Guizhou provinces in China). Among these, a total of 45 materials (Z27, Z43-Z71) were provided by the State Medium-term Repository for Grass Germplasms in China. In October 2015, 100 grains of each material were sown in 10×10×10 cm plastic pots. Following one month, a total of 36 seedlings of each type of material were randomly selected and transplanted to potted trays. Plants were watered every other day and were treated with full-strength Hoagland nutrient solution once a month (Hoagland and Arnon, 1950). The entire cultivation process was carried out in a greenhouse at the School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China under natural conditions (15-30 °C temperature, 40-80% humidity, and natural sunlight).

Table 1. Names, types and sources of 73 Kentucky bluegrass materials

Code	Commercial name or place of origin	Types, Source*	Code	Commercial name or place of origin	Types, Source*
Z01	Odyssey	Imported varieties, Bright	Z38	Moonnight	Imported varieties, Clover
Z02	3 Freedom III	Imported varieties, Bright	Z39	Right	Imported varieties, Clover
Z03	Everest	Imported varieties, Bright	Z40	4-season	Imported varieties, Clover
Z04	Arcadia	Imported varieties, Bright	Z41	bright	Imported varieties, Bright
Z05	Nuglade	Imported varieties, Bright	Z42	Zahe	Domestic varieties, SABS SJTU
Z06	Award	Imported varieties, Bright	Z43	Nublue	Imported varieties, Bright
Z07	Nassau	Imported varieties, Bright	Z44	Tianzhu of Gansu Province1	Domestic wild materials, SMTRGG
Z08	Classic	Imported varieties, Bright	Z45	Tianzhu of Gansu Province 2	Domestic wild materials, SMTRGG
Z09	Blue Chip	Imported varieties, Bright	Z46	Qibian of Qinghai Province	Domestic wild materials, SMTRGG
Z10	Kingdom	Imported varieties, Bright	Z47	Tianzhu of Gansu Province 3	Domestic wild materials, SMTRGG
Z11	Barrister	Imported varieties, Barenbrug, China	Z48	Midnight 2	Imported varieties, Clover
Z12	Baron	Imported varieties, Barenbrug, China	Z49	Brilliant	Imported varieties, Clover
Z13	Merit	Imported varieties, Barenbrug, China	Z50	Merit	Imported varieties, Clover
Z14	Barvictor	Imported varieties, Barenbrug, China	Z51	Kenblue	Imported varieties, Clover
Z15	Midnight	Imported varieties, Clover	Z52	Miracle	Imported varieties, Clover
Z16	Rugby2	Imported varieties, Clover	Z53	Numerit	Imported varieties, Clover
Z17	Abbey	Imported varieties, Clover	Z54	CPP811 (Danmark)	Imported varieties, Clover
Z18	Sapphire	Imported varieties, Clover	Z55	CPP812 (Danmark)	Imported varieties, Clover
Z19	Blue fox	Imported varieties, Clover	Z56	Allure	Imported varieties, Clover
Z20	Park	Imported varieties, Clover	Z57	Harmony	Imported varieties, Clover
Z21	Kentucky	Imported varieties, TopGreen	Z58	Dushan of Guizhou Province	Domestic wild materials, SMTRGG
Z22	Brooklawn	Imported varieties, TopGreen	Z59	Daqingshan	Domestic varieties, SMTRGG
Z23	Bedazzled	Imported varieties, TopGreen	Z60	Qinghai Province	Domestic wild materials, SMTRGG
Z24	Nublue	Imported varieties, TopGreen	Z61	Changling of Jilin Province	Domestic wild materials, SMTRGG
Z25	Avalanche	Imported varieties, Bright	Z62	Russia	Wild materials abroad, SMTRGG
Z26	Huhe 2	Domestic varieties, SJTU	Z63	Russia	Wild materials abroad, SMTRGG
Z27	Jilin City of Liaoning Province	Domestic wild materials, SMTRGG	Z64	Kazakhstan	Wild materials abroad, SMTRGG
Z28	KBG04	Domestic varieties, SABS SJTU	Z65	Kazakhstan	Wild materials abroad, SMTRGG
Z29	KBG03	Domestic varieties, SABS SJTU	Z66	Kazakhstan	Wild materials abroad, SMTRGG
Z30	Evergreen	Imported varieties, TopGreen	Z67	Russia	Wild materials abroad, SMTRGGSMTRGG
Z31	Nu destiny	Imported varieties, TopGreen	Z68	Kazakhstan	Wild materials abroad, SMTRGG
Z32	Superglade	Imported varieties, Bright	Z69	Russia	Wild materials abroad, SMTRGG
Z33	Blueberry	Imported varieties, TopGreen	Z70	the United States	Wild materials abroad, SMTRGG
Z34	Gourment Midnight	Imported varieties, TopGreen	Z71	Russia	Wild materials abroad, SMTRGG
Z35	B6	Imported varieties, Barenbrug, China	Z72	Ninglan	Domestic wild materials, NXU
Z36	Jumpstart	Imported varieties, Barenbrug, China	Z73	Langra	Imported varieties, Bright
Z37	Liberator	Imported varieties, Clover			

*Imported varieties were distributed by: Bright, Beijing Bright Turf & Forage Co., Ltd.; Barenbrug China, Beijing Barenbrug International Co., Ltd.; Clover, Beijing Clover Turf & Forage Co., Ltd. and TopGreen, Beijing TopGreen Seed Co., Ltd. Domestic cultivars were distributed by: SABS SJTU, School of Agriculture and Biology Science of Shanghai Jiaotong University; SMTRGG, State Medium-term Repository for Grass Germplasms and NXU, Ningxia University.

DNA extraction and molecular marker polymorphism screening

Identical amounts of fresh leaves were collected from 10 individual plants per germplasm. Leaves were frozen for the subsequent genomic DNA extraction using the CTAB method (Clark, 1997). Extracted DNA samples were visualized on 1.0% agarose gels via electrophoresis. Extracted DNA was diluted to 40 ng μL^{-1} for PCR reactions. A total of 70 SRAP and 75 SSR primer pairs were used to screen polymorphisms, which were selected from a total of 100 SRAP primer pairs (forward: me1-me10, reverse: em1-em10) (Yuan *et al.*, 2015) and 88 SSR primer pairs (Honig *et al.*, 2010), respectively. Primer pairs were prescreened on six genotypes (Z15, Z25, Z28, Z29, Z30 and Z73) in order to identify primers that generated clear bands for further amplification reactions for the total 73 materials. All primers were synthesized by Sangon Biological Engineering Technology and Service Co. Ltd, Shanghai.

SSR and SRAP PCR reactions were carried out in a 10-μL mixture: 0.3 unit of Taq DNA polymerase (TaKaRa, Talien, China), 1×Taq Buffer, 1.5 mmol L^{-1} $MgCl_2$, 100 μmol L^{-1} dNTP mixture, 10 pmol of each primer, and 50ng genomic DNA. The SSR PCR reaction was carried out as follows: 94 °C for 3 min for the initial denaturing step, followed by 30 cycles at 94 °C for 10 s, 55 °C for 30 s, and 72 °C for 45 s for denaturing, annealing, and extension, respectively. The annealing temperature was then reduced to 53 °C for another 15 cycles, followed by a final extension step at 72 °C for 8 min. The SRAP PCR reaction was carried out as follows: 94 °C for 3 min for the initial denaturing step, followed by 8 cycles at 94 °C for 10 s, 37 °C for 30 s and 72 °C for 45 s for denaturing, annealing, and extension, respectively. The annealing temperature was then increased to 53 °C for another 35 cycles, followed by a final extension step at 72 °C for 6 min. The SSR and SRAP products were separated on 6% and 4% denatured polyacrylamide gels, respectively. DNA bands were visualized using $AgNO_3$ solution (Bassam *et al.*, 1991). All PCR reactions were confirmed at least twice.

Statistical analysis

Genetic relationships between the 73 KBG genotypes were studied using the bands amplified by SSR and SRAP primer pairs. Steady and clear polymorphic loci in each material were used for analysis, and the banding phenotypes were scored as band presence (1) or absence (0). Data were analyzed using the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc) version 2.10 (Exeter Software, Setauket, NY). Genetic similarities between genotypes were calculated using the simple matching coefficient, and a dendrogram was generated based on the unweighted pair group method with the arithmetic average (UPGMA).

Results

Amplification of SSRs and SRAPs

Among the 73 different KBG materials, 70 SSR and 75 SRAP primer pairs were tested for the polymorphic bands (Fig. 1, Fig. 2). A total of 1,137 bands were amplified by SSRs, in which 1,075 were found to be polymorphic (94.5%). Each primer pair generated between 10 and 36 clear bands, with an average number of 18.6 bands. A total of 1,526 bands were detected by SRAPs, of which 1,401 were found to be polymorphic, accounting for 91.8%. The number of fragments amplified by each SRAP primer combination ranged between 4 and 42 (20.3 bands per primer pair). Among these, a total of 595 reproducible and unambiguous bands (233 SSRs and 362 SRAPs) were selected for further genetic diversity analysis.

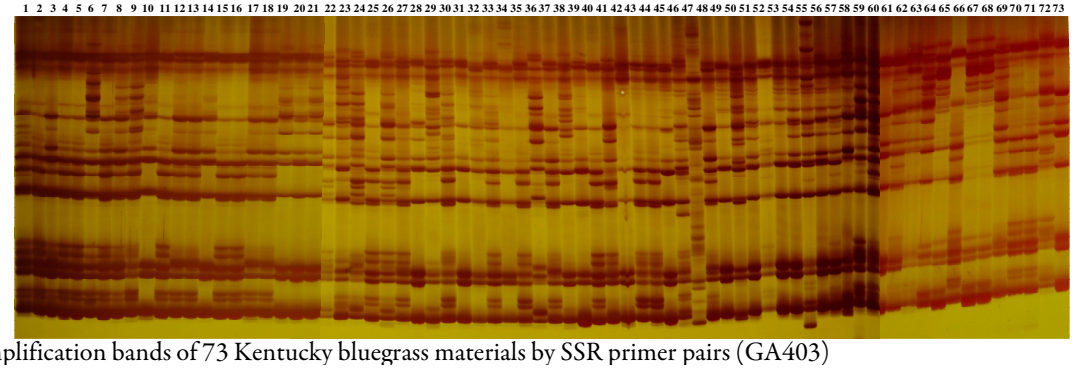

Fig. 1. Amplification bands of 73 Kentucky bluegrass materials by SSR primer pairs (GA403)

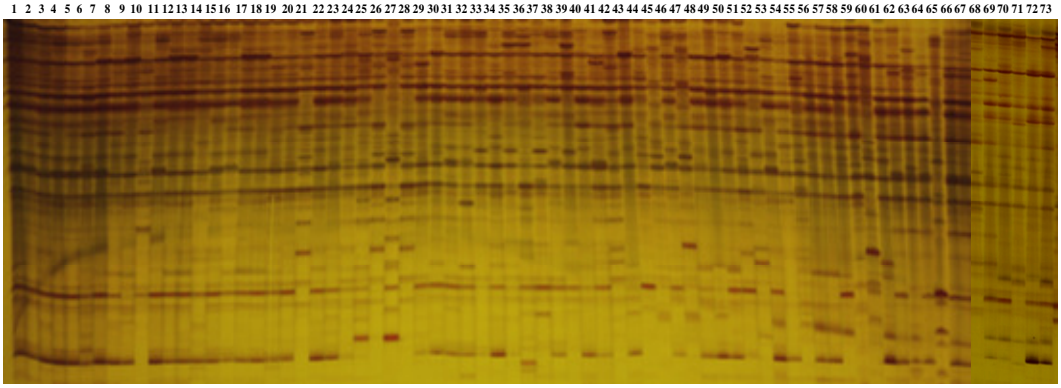

Fig. 2. Amplification bands of 73 Kentucky bluegrass materials by SRAP primer pairs (me10×em9)

SSR cluster analysis

Dendrogram analysis of the SSR data is depicted in Fig. 3. At the genetic similarity coefficient of 0.6, the 73 materials could be divided into three main clusters, denoted as Cluster I (SSR-I), Cluster II (SSR-II) and Cluster III (SSR-III). SSR-I comprised 46 materials, which accounted for 63% of the total material. With the exception of four domestic wild materials (Z27, Z46, Z61, and Z72), all others were found to belong to imported commercial cultivars. SSR-II was found to contain nine materials, including three domestic wild materials and one wild material from abroad, covering all domestic varieties/lines (Z26, Z28, Z29, Z42, and Z59). These results demonstrated that these five Chinese breeding lines were of close genetic relationship. SSR-III covered 18 materials, including 7 imported varieties, 2 domestic wild materials, and nearly all of the foreign wild materials.

SRAP cluster analysis

A dendrogram that was based on SRAPs divided all of the materials into two main clusters at a similarity level of 0.53 (Fig. 4). The first cluster included a total of 63 materials grouped together, which was comprised of all of the imported varieties (49) and the majority of the domestic and abroad wild materials (14). All domestic varieties/lines, three domestic wild materials, and two foreign wild materials were compiled into the second cluster (SRAP-II). Materials within the first cluster were grouped into two further subclusters at the similarity level of 0.62. The SRAP-III subcluster contained only one domestic wild material (Z27), while the SRAP-I subcluster comprised the remaining materials. SRAP-I, SRAP-II, and SRAP-III represented Cluster I, Cluster II, and Cluster III, respectively, in the SRAP dendrogram.

Cluster analysis of combined SSR and SRAP data

In addition, SSR and SRAP data were combined for UPGMA cluster analysis (Fig. 5). With this analysis, two major clusters were observed in the dendrogram at the genetic similarity coefficient of 0.54. The second major cluster was found to consist of 5 domestic varieties/lines, 2 foreign wild materials, and 3 domestic wild materials. The first major cluster was found to be comprised of the remaining 63 materials. With a higher similarity coefficient (0.63), two subclusters [(SSR+SRAP)-I and (SSR+SRAP)-III] were distinguished from the first major cluster. (SSR+SRAP)-I was found to be comprised of 47 materials: 43 of which were imported varieties and 4 of which were domestic wild materials (Z46, Z61, Z72, and Z27). (SSR+SRAP)-III was found to be comprised of a total of 16 materials, which included 2 domestic wild materials, 8 wild materials from abroad, and 6 imported varieties. At a similarity level of 0.63, the foreign wild material Z65 [(SSR+SRAP)-IV] was separated from the other 9 materials [(SSR+SRAP)-II] in the second major cluster. (SSR+SRAP)-I, (SSR+SRAP)-II, (SSR+SRAP)-III and (SSR+SRAP)-IV made up Cluster I to IV in the SSR+SRAP dendrogram.

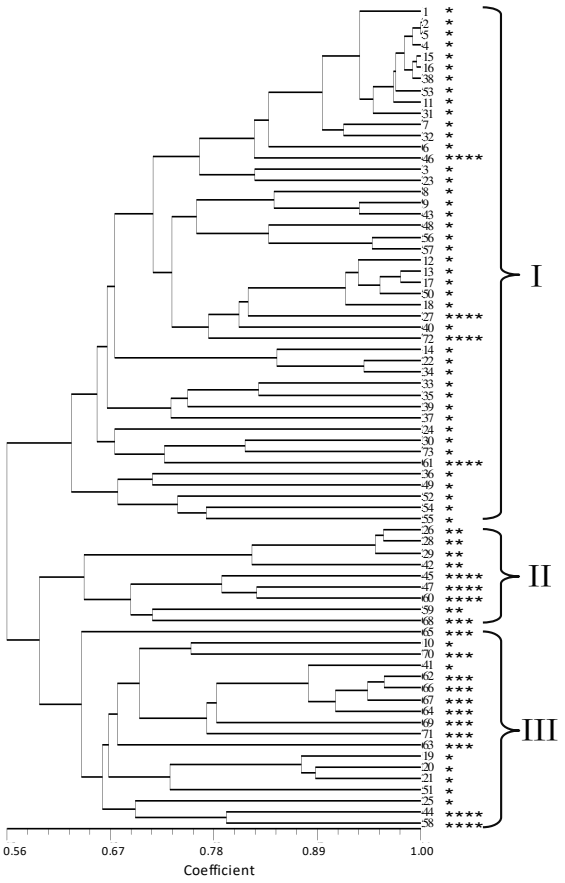

Fig. 3. UPGMA cluster analysis based on 233 SSRs of 73 Kentucky bluegrass materials; *: Imported varieties; **: Domestic varieties; ***: Wild materials abroad; ****: Domestic wild materials

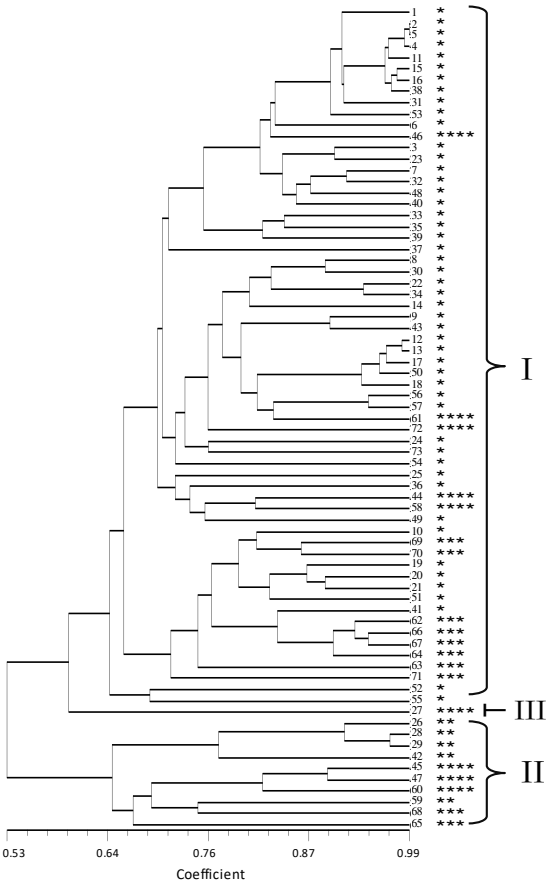

Fig. 4. UPGMA cluster analysis based on 362 SRAPs of 73 Kentucky bluegrass materials; *: Imported varieties; **: Domestic varieties; ***: Wild materials abroad; ****: Domestic wild materials

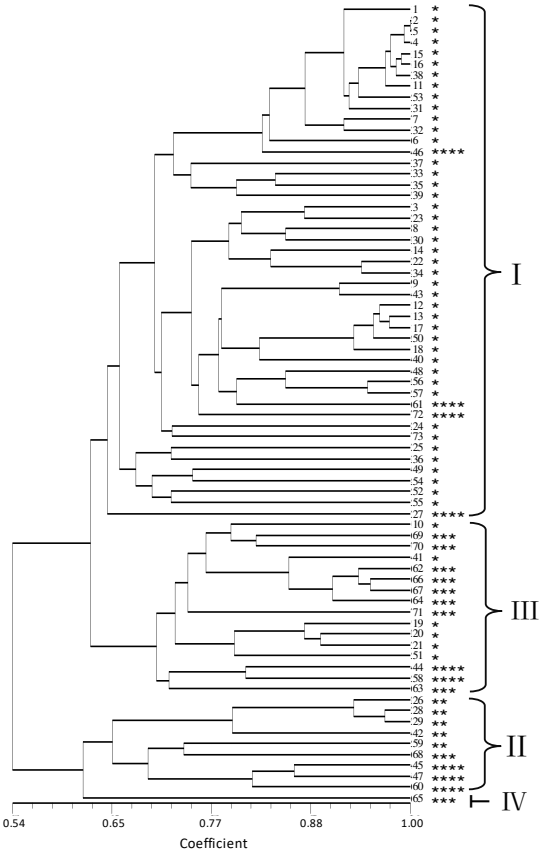

Fig. 5. UPGMA cluster analysis based on 233 SSRs and 362 SRAPs of 73 Kentucky bluegrass materials; *: Imported varieties: **: Domestic varieties; ***: Wild materials abroad; ****: Domestic wild materials

Discussion

SSRs and SRAPs have been applied to cultivar identification and genetic variation assessment for numerous turfgrass species. In the current study, we used SSR and SRAP markers to analyze relationships among various KBG material sources. Both SRAPs and SSRs were found to have high polymorphism (> 91%) among the 73 materials. This high level of polymorphisms was similar to that found in other grass studies: 67% in 8 perennial ryegrass genotypes (101 SSRs) (Jones *et al.*, 2001), 93% in 8 *Cynodon transvaalensis* genotypes (544 SSRs) (Tan *et al.*, 2014), 84% in 60 orchardgrass accessions (21 SRAPs) (Zeng *et al.*, 2008), 95% in 53 buffalograss germplasms (34 SRAPs) (Budak *et al.*, 2004b), and 100% in 59 *Festuca-Lolium* complex accessions (22 SRAPs) (Cheng *et al.*, 2016). The high number of polymorphisms identified in SSR markers was also observed in two other KBG studies (Honig *et al.*, 2012; Raggi *et al.*, 2015). All of these results demonstrate that SSRs and SRAPs represent ideal molecular markers that can be used for the efficient assessment of genetic diversity and relationships in turfgrass.

While the placement of some materials was different, three dendrograms that were generated from SSR, SRAP, and their combined data demonstrated generally similar results. The majority of materials in (SSR+SRAP)-II were found to correspond to those in SSR-II and SRAP-II. This included 9-10 of the same domestic and foreign wild materials and domestic breeding varieties/lines. In addition, the SSR-I germplasms were also found to be very similar to those in (SSR+SRAP)-I. SRAP-I included both Cluster I and Cluster III of SSR or SSR+SRAP dendrograms. In addition, three domestic wild materials obtained from Jilin (Z61), Qinghai (Z46), and Ningxia (Z72) were always found to be grouped with the majority of the commercial varieties obtained from abroad.

Some materials were located in different clusters within the three dendrograms. For example, the wild material 'Kazakhstan' (Z65) was found to be separated from all other materials in the SSR+SRAP dendrogram. However, it was gathered in SSR-III and SRAP-II, respectively. In addition, the imported variety 'Avalanche' (Z25) was not found in the corresponding clusters between SSR and SSR+SRAP dendrograms. The domestic wild material Z27 (obtained from Jilin Province in China) was grouped in Cluster I in both SSR and SSR+SRAP dendrograms. However, it was separated from all of the others in the SRAP cluster analysis.

In our previous study, we demonstrated that a dendrogram of the same materials (except for Z73) carried out with 20 RAPD primers was generally similar to the results obtained in this study (Tu *et al.*, 2017). Clusters I and III in the RAPD dendrogram were found to approximately correspond to that in the SSR and SSR+SRAP dendrograms. In this study, Cluster II materials were found to be equal to the sum of the materials in Clusters II and IV in the RAPD cluster analysis.

In the case of other species, genetic diversity analyses were also carried out using different types of molecular markers. In melon (*Cucumis melo* L.), studies have used RAPD and SSR markers to study genetic relationships between different accessions (Staub *et al.*, 2000). In the case of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], studies have used SRAP, SSR, and SRAP-SSR combined data to estimate genetic diversity (Wang *et al.*, 2015). The dendrograms that were generated from the use of different molecular markers all revealed a high similarity. However, among some Iraqi walnut genotypes (*Juglans regia* L.), the cluster analysis carried out by RAPD and SSR markers demonstrated a distinct diversity (Salieh *et al.*, 2013). In the case of *Monascus*, cluster results generated using SRAP and ISSR data were found to share immense similarity, with only minor differences identified in the subgroups (Shao *et al.*, 2011). Differences observed between cluster analyses based on diverse marker types could be caused by the use of different marker systems, different marker amounts, and/or different testing populations. Different molecular markers are developed using different principles, and therefore the position of the amplified fragment on the whole genome as well as the amount of genome coverage is expected to also differ. Within the same species, sampling variation could vary between populations. Therefore, a dendrogram that was constructed by a greater number of types and a greater number of molecular markers would be more realistic, such as the SSR+SRAP dendrogram used in this study.

In Napier grass (*Pennisetum purpureum* Schum) and bermudagrass (*Cynodon dactylon*) studies (Babu *et al.*, 2009; Farsani *et al.*, 2011; Li *et al.*, 2011), a correlation was found

between molecular marker clustering and geographical origin, to some degree. The majority of ecotypes were clustered according to their geographical distribution, and were also reported for KBG (Szenejko *et al*., 2016). In this study, based on the cluster analysis of SSR+SRAP data, a total of 80% of wild materials obtained from abroad (8 materials), including from Russia, Kazakhstan, and the United States, were grouped together in Cluster III, while a total of 9 domestic wild materials were distributed among three different clusters. In Cluster I, a total of 4 materials were collected from Qinghai, Jilin, Ningxia, and Liaoning provinces. A total of 2 germplasms in Cluster III were generated separately from Gansu and Guizhou Provinces, and a total of 3 materials in Cluster II were obtained from Gansu and Qinghai Provinces. We observed no obvious relationship between the geographical origin and the molecular marker clusters in the wild KBG materials studied here. This same result was observed in another clustering study in *Poa pratensis* accessions, representing 26 countries using RAPD markers (Johnson *et al*., 2002). The reasons for discrepancies could be due to the diffusion of species introduced, introgression through the hybridization of geographically diverse genetic stocks, and a potential inherent genetic overlap that could exist in these accessions.

Numerous studies have demonstrated a significant or partial correlation between RAPD-based grouping and morphological classification in KBG (Huff, 2001; Johnson *et al*., 2002; Curley and Jung, 2004; Szenejko and Rogalski, 2015). Combined with the seedling phenotype identification carried out in previous work (Tu *et al*., 2017), we found no significant association between agronomic characterization (plant height, tillers per plant, and leaf width) and marker-based clustering in these materials, with the exception of leaf color. This could be due to differences in experimental materials and morphological characterizations that were used in the different studies. In addition, certain critical agronomic traits typically received greater attention in the breeding program and were also selected in the morphological identification. Thus, some materials with similar phenotypes could possibly vary from their genetic source.

Conclusions

Both SRAPs and SSRs were found to possess high polymorphisms among the 73 KBG materials used in this study. Three dendrograms generated from SRAP, SSR, and SRAP+SSR combined data demonstrated a general similarity, and some materials studied were found to be placed in different clusters. Further analysis also demonstrated no significant association between the geographical origin and the molecular marker clusters in the wild materials studied. In addition, we observed no significant relationship between the majority of agronomic characterization and marker-based clustering in the materials studied, with the exception of leaf color. These results enable a better understanding of genetic diversity in KBG materials, and could function to facilitate further studies of genetic improvement and germplasm conservation.

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