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Genetic Diversity of Seashore Paspalum Revealed with Simple Sequence Repeat Markers

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Abstract. Seashore paspalum (*Paspalum vaginatum*) is an important warm-season turfgrass distributed in tropical and coastal areas. It has excellent resistance to abiotic stresses, such as salinity, drought, and low temperature. However, the research on genetic diversity of local *P. vaginatum* collections from China is limited. In this study, the genetic diversity among 58 *P. vaginatum* accessions from four different provinces in China and four cultivars were assessed using simple sequence repeat (SSR) markers. The results indicated that a total of 45 alleles were detected by 19 polymorphic markers, with a range of 2 to 4 and an average of 2.4 alleles per marker. The genetic similarity coefficients between each pair of the 58 *P. vaginatum* accessions and four cultivars ranged from 0.51 to 1.00, with an average of 0.77. The range of variation of Shannon diversity index of each SSR marker was 0.047 to 1.075, with an average of 0.486. The polymorphic information content of each SSR marker varies from 0.016 to 0.577, with an average of 0.249. The results of cluster analysis and principal component analysis (PCA) showed that 58 *P. vaginatum* accessions and four cultivars were divided into four groups. These results provide the theoretical basis for the genetic diversity assessments and molecular marker–assisted breeding of *P. vaginatum* species.

Paspalum vaginatum is a warm-season perennial grass of the Gramineae family that is distributed in the coastal areas of the tropical and subtropical regions of the world (Chen et al., 2005). It mainly grows in coastal areas, mudflats, and other habitats with high salinity. It has excellent resistance to abiotic stresses, such as salinity, drought, and low temperature (Jia et al., 2015a; Shahba et al., 2014; Wu et al., 2018; Ye et al., 2010). As turfgrass, *P. vaginatum* has been widely used on golf courses, sport fields, and landscapes (Berndt, 2007). In terms of water and soil conservation, *P. vaginatum* plays a very important role for preventing soil erosion because of its ecologically aggressive and fast growing characteristics under extremely warm and humid climates (Xie et al., 2004). It also has been used for the bioremediation of contaminated soil and to improve the productivity of soil [e.g., heavy metals and organic chemicals (Duan et al., 2016; Wang et al., 2010)].

As an important warm-season turfgrass, there have been many studies on *P. vaginatum*, such as salt tolerance, drought resistance, and cadmium tolerance (Chen et al., 2012; Wu et al., 2015; Xie and Lu, 2004; Xu et al., 2018). However, there were few reports on genetic diversity of *P. vaginatum* in recent years. Liu et al. (1994) used random amplified polymorphic DNA (RAPD) to detect the genetic diversity of 46 *P. vaginatum* ecotypes. The genetic diversity, size, and distribution pattern of three wild accessions and one cultivar of *P. vaginatum* from Guangdong Province were analyzed by RAPD molecular marker technique (Xie et al., 2004). Four introduced cultivars and one thick-stem mutant of *P. vaginatum* were assessed using morphological and amplified fragment length polymorphism (AFLP) analysis; the results showed that the genetic distance of morphology was consistent with the clustering result of similarity coefficient of AFLP markers (He et al., 2011). The optimization of sequence-related amplified polymorphism (SRAP) molecular marker reaction system and the selection of primers of *P. vaginatum* were carried out based on four typical *P. vaginatum* germplasms (Liu et al., 2016). Molecular markers have been proven to be powerful tools to assess the

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genetic diversity of *P. vaginatum* for germplasm collection and plant breeding programs. These studies can promote the development of genetic diversity of *P. vaginatum.*

Compared with other molecular markers, SSR markers have been widely used in many fields of plant research due to their high frequency, high polymorphism, repeatability, codominant inheritance, and simple methodology (Sun et al., 2011). Recently, SSR markers have been applied to detect the genetic diversity in turfgrasses such as *Cynodon dactylon* (Long et al., 2016) and *Zoysia japonica* (Guo et al., 2008); however, limited SSR sequence information is available for *P. vaginatum*, which restricts the development of molecular markers and analysis of genetic diversity. Therefore, more SSR markers need to be developed for characterization and evaluation of *P. vaginatum*. Wang et al. (2006) selected and used 40 SSR markers from wheat (*Triticum aestivum*), maize (*Zea mays*), and sorghum (*Sorghum bicolor*) to genetically distinguish 73 *P. vaginatum* accessions. Harris-Shultz et al. (2013) used 80 SSR markers to assess genetic relationships among 18 *P. vaginatum* accessions. Li et al. (2016) developed 50 pairs of expressed sequence tag (EST)-SSR primers and selected 10 turfgrass cultivars to test the validity and polymorphism of these primers.

In addition to the introduction and cultivation of *P. vaginatum* in China, the research, development, and utilization of local resources are extremely rare. To accelerate the efficient development and utilization of *P. vaginatum*, SSR molecular marker technology was used to analyze the genetic diversity of local *P. vaginatum* germplasm in China in this study. These results provide the theoretical basis for the genetic diversity and molecular marker–assisted breeding of *P. vaginatum* species.

Materials and Methods

Plant materials. The experimental materials were 62 *P. vaginatum* germplasm resources, including 58 *P. vaginatum* accessions collected from four different provinces in China, of which 36 accessions were collected from Hainan province, 14 accessions were collected from Guangxi province, seven were collected from Guangdong province, and one was collected from Jiangxi province, plus an additional four cultivars from the University of Georgia (Table 1).

All materials were propagated from healthy stolons gathered from their native habitat and planted in the experimental field of Danzhou campus of Hainan University. Materials were collected according to geographical distribution, the distance between the materials was 2 km. Materials from the same or adjacent regions are collected according to morphological differences. The experimental materials were planted according to the serial number sequence, the stolon segment of each accession was planted in separate 1 × 1-m plots. The distance between the individual plots was 50 cm. The plot edges were trimmed twice weekly to prevent cross contamination between adjacent plots. Routine irrigation and fertilization were carried out to maintain healthy growth.

Extraction, amplification, and electrophoretic detection of DNA. The DNA was extracted from fresh leaves of *P. vaginatum* by the cetyl trimethylammonium bromide method (Doyle and Doyle, 1987). The quality and concentration of DNA were measured by 1.0% agarose gel electrophoresis. Samples were diluted with sterile water to a final concentration of 100 ng·μL^{-1} and stored at –20 °C.

SSR amplification. In this study, 58 SSR markers previously reported by Jia et al. (2015b) and Li et al. (2016) were evaluated in *P. vaginatum*. Four *P. vaginatum* accessions (USA17-4, USA17-29, HN17-22, and HN7-54) were randomly selected to screen the 58 SSR markers. The markers with clear and distinct bands were used for polymerase chain reaction (PCR) amplification of 58 *P. vaginatum* accessions and four cultivars (Table 2). The PCR amplification system and procedures were performed according to the method by Jiang (2017). For PCR amplification, a 20-μL reaction with 1.0 μL DNA (100 ng·μL^{-1}), 1.0 μL primers (5 μmol·L^{-1}), 0.3 μL Taq DNA polymerase (5 U·μL^{-1}), 1.4 μL deoxynucleoside triphosphates (2.5 mmol·L^{-1}), 2.0 μL 10 × PCR buffer, and 14.3 μL double-distilled H_2O was used. The amplification program was as follows: 4 min at 94 °C; followed by 38 cycles of 94 °C for 40 s, 55 °C for 45 s, and 72 °C for 45 s; with a final extension at 72 °C for 5 min; and then the reaction was cooled to 4 °C. The amplification products were separated on an 8% nondenaturing polyacrylamide gel, which was electrophoresed at a voltage of 140 V for 3 h. Then the gel was silver stained (Li et al., 2004), developed with formaldehyde solution, and finally photographed and recorded.

Data processing and analysis. The SSRs generated by PCR were manually scored using a 50–base pair DNA ladder marker as a reference. Spreadsheet software (Excel; Microsoft, Redmond, WA) was used to conduct the "0, 1" system statistics on each band, with band presence marked as "1" and absence marked as "0", to generate the original data matrix. Following the method proposed by Nei and Li (1979), the distance matrix and system tree diagram of the tested material were calculated using NTSYS-pc software [version 2.10e (Rohlf, 2000)]. Genetic similarity cluster analysis was conducted according to unweighted pair group method using arithmetic average [UPGMA (Sneath and Sokal, 1973)]. POPGENE 1.32 software (Yeh et al., 1999) was used to calculate the genetic diversity parameters of each marker, package the number of alleles (Na), Shannon polymorphism index (I), observed heterozygosity (Ho), and excepted heterozygosity (He) (Nei, 1973). Polymorphic information content (PIC) was calculated by using the frequency of each allele of SSR marker according to the method of Botstein et al. (1980). The formula is as follows:

$$PIC = 1 - \sum_{i=l}^{n} P_i^2 - \sum_{i=l}^{n-l} \sum_{j=i+l}^{n} 2P_i^2 P_j^2$$

In the formula, n represents the number of alleles detected by each marker, and P_i, P_j represent the frequency of the presence of the i, j alleles in the tested materials.

Results

Genetic diversity. In this study, 27 markers with clear and distinct bands were selected from the 58 SSR markers. Using PCR to amplify the 27 markers, we found that 19 SSR markers were polymorphic (Table 2) in our 62 *P. vaginatum* samples. A total of 45 alleles were detected, with a range of 2 to 4 and an average of 2.4 alleles per marker (Fig. 1, Table 3). The markers with the most alleles were SP-18 and SP-37. The range of Ho of 19 SSR markers was 0.000 to 0.694, with an average of 0.241. The He ranged from 0.016 to 0.657, with an average of 0.295 (Table 3). The Ho and the He of primer ESSR3 were the highest. The range of variation of I of each SSR marker was 0.047 to 1.075, with an average of 0.486. The PIC of each SSR

Table 1. The origins of 58 *Paspalum vaginatum* accessions and four cultivars used in genetic diversity analysis revealed with simple sequence repeat markers.

No.	Accession	Origin	Latitude	Longitude	No.	Accession	Origin	Latitude	Longitude
1	HN17-1	Wanning, Hainan	18°53′07″N	110°36′55″E	32	HN17-101	Wenchang, Hainan	19°37′32″N	110°47′32″E
2	HN17-2	Wenchang, Hainan	20°01′42″N	110°36′42″E	33	HN17-102	Wenchang, Hainan	19°37′32″N	110°47′32″E
3	HN17-3	Wanning, Hainan	18°53′07″N	110°30′55″E	34	HN17-103	Wenchang, Hainan	19°37′32″N	110°47′32″E
4	HN17-8	Wanning, Hainan	18°53′30″N	110°30′06″E	35	HN17-104	Wenchang, Hainan	19°37′32″N	110°47′32″E
5	HN17-11	Wanning, Hainan	18°33′36″N	110°30′06″E	36	HN17-105	Haikou, Hainan	20°04′39″N	110°20′32″E
6	HN17-13	Wanning, Hainan	18°55′45″N	110°30′07″E	37	GX17-7	Dongxing, Guangxi	21°35′03″N	108°09′55″E
7	HN17-15	Wanning, Hainan	18°53′50″N	110°29′45″E	38	GX17-12	Fangchenggang, Guangxi	21°31′42″N	108°09′00″E
8	HN17-16	Wenchang, Hainan	20°01′37″N	110°36′34″E	39	GX17-13	Fangchenggang, Guangxi	21°37′41″N	108°31′41″E
9	HN17-18	Wenchang, Hainan	20°01′38″N	110°36′37″E	40	GX17-18	Fangchenggang, Guangxi	21°41′40″N	108°34′14″E
10	HN17-21	Wanning, Hainan	18°56′18″N	110°28′12″E	41	GX17-21	Fangchenggang, Guangxi	21°41′40″N	108°34′14″E
11	HN17-22	Wanning, Hainan	18°53′03″N	110°31′05″E	42	GX17-24	Qinzhou, Guangxi	21°44′41″N	108°32′12″E
12	HN17-23	Wanning, Hainan	18°53′02″N	110°31′02″E	43	GX17-43	Qinzhou, Guangxi	21°43′09″N	108°35′39″E
13	HN17-25	Wanning, Hainan	18°53′03″N	110°31′06″E	44	GX17-48	Qinzhou, Guangxi	21°43′05″N	108°42′23″E
14	HN17-26	Wanning, Hainan	19°41′21″N	109°12′54″E	45	GX17-53	Beihai, Guangxi	21°38′09″N	108°57′28″E
15	HN17-29	Dongfang, Hainan	19°05′43″N	108°36′55″E	46	GX17-58	Beihai, Guangxi	21°35′18″N	109°08′03″E
16	HN17-30	Wanning, Hainan	19°44′23″N	109°12′46″E	47	GX17-62	Beihai, Guangxi	21°24′24″N	109°08′58″E
17	HN17-36	Dongfang, Hainan	19°05′54″N	108°37′37″E	48	GX17-67	Beihai, Guangxi	21°38′44″N	109°31′55″E
18	HN17-37	Sanya, Hainan	18°16′25″N	109°40′26″E	49	GX17-72	Beihai, Guangxi	21°28′40″N	109°45′26″E
19	HN17-38	Sanya, Hainan	18°16′25″N	109°40′27″E	50	GX17-75	Beihai, Guangxi	21°29′12″N	109°45′13″E
20	HN17-39	Sanya, Hainan	18°16′33″N	109°40′34″E	51	GD17-76	Zhanjiang, Guangdong	21°23′22″N	109°54′54″E
21	HN17-40	Sanya, Hainan	18°16′33″N	109°40′34″E	52	GD17-84	Zhanjiang, Guangdong	21°25′20″N	110°56′47″E
22	HN17-41	Sanya, Hainan	18°16′33″N	109°40′34″E	53	GD17-85	Zhanjiang, Guangdong	21°25′20″N	110°56′47″E
23	HN17-52	Lingao, Hainan	20°00′19″N	109°42′57″E	54	GD17-88	Zhanjiang, Guangdong	21°17′10″N	110°37′11″E
24	HN17-55	Lingao, Hainan	20°00′19″N	109°42′57″E	55	GD17-90	Zhanjiang, Guangdong	20°51′42″N	110°09′47″E
25	HN17-56	Lingao, Hainan	20°00′19″N	109°42′57″E	56	GD17-92	Zhanjiang, Guangdong	20°50′51″N	110°18′48″E
26	HN17-62	Wenchang, Hainan	20°01′43″N	110°35′07″E	57	GD17-93	Zhanjiang, Guangdong	20°17′13″N	110°03′49″E
27	HN17-63	Qionghai, Hainan	19°08′45″N	110°34′41″E	58	JX17-97	Leping, Jiangxi	28°47′22″N	117°16′04″E
28	HN17-65	Danzhou, Hainan	19°44′40″N	109°13′24″E	59	Sealsle 2000	University of Georgia	Introduced cultivar	
29	HN17-69	Lingao, Hainan	20°00′19″N	109°42′57″E	60	Platinum	University of Georgia	Introduced cultivar	
30	HN17-94	Qiongzhong, Hainan	19°04′58″N	109°38′57″E	61	Adalayd	University of Georgia	Introduced cultivar	
31	HN17-95	Qiongzhong, Hainan	19°04′58″N	109°38′57″E	62	Seaspray	University of Georgia	Introduced cultivar	

Table 2. Sequence of primers used for simple sequence repeat analysis in 58 *Paspalum vaginatum* accessions and four cultivars.

No.	Primer code[z]	Forward primer (5′- 3′)	Reverse primer (5′- 3′)	Repeat sequence	Polymorphic?	Reference
1	SP-1	GGGGCTCCATAGCAACTTT	GGTCGACGAAGAAGTGGTG	$(CGG)_6$	No	Jia et al. (2015b)
2	SP-3	AGGAAGCACACAAAAACCG	GATCCAGGATTCGTTCCAA	$(TCA)_6$	No	Jia et al. (2015b)
3	SP-4	AATGCCAGGAAGATCCACA	GGTGATTATTGGTGCCTCG	$(GCAGCC)_4$	No	Jia et al. (2015b)
4	SP-8	GGAAGCTCACTGTGCGAAT	AAGAATGGCATGGACGTGT	$(GCGAAC)_4$	No	Jia et al. (2015b)
5	SP-10	GGTCGAATGGTCCATCATC	AACCACTCCTCCTTCTGGG	$(TGA)_6$	Yes	Jia et al. (2015b)
6	SP-12	TCGAATTGAAGCAGCACAG	GGCTCGCACTTGTTCAGAT	$(TGC)_7$	No	Jia et al. (2015b)
7	SP-13	GCAACGAGTAGTGCCCAAA	GCTCTCTACCTGCAACCCA	$(CACAA)_3$	Yes	Jia et al. (2015b)
8	SP-14	CATGGAAGGAAGGACGATG	CATTGGGCTTGATCTTTGC	$(CAG)_5$	No	Jia et al. (2015b)
9	SP-15	GTCCATATCCACCCATCCA	TCTGGGACTGGGTCAAGAA	(GGC)5	Yes	Jia et al. (2015b)
10	SP-17	AAGGTCGATCCTTTCTCCG	TCCATCTGTATGCGTGTGTG	$(TC)_9$	Yes	Jia et al. (2015b)
11	SP-18	ATTGCTTTCTTCCTCGGCT	GGCGACCACACCTATTTTG	$(AAC)_5$	Yes	Jia et al. (2015b)
12	SP-19	TTGGCAAAGTAGTCCTCGC	TAAATCGGGTTTGCCCTTC	$(AGA)_6$	Yes	Jia et al. (2015b)
13	SP-25	AGCAGAGCTGACGGATCTC	CACAGACAGCAGGTGATCG	$(CTG)_5$	No	Jia et al. (2015b)
14	SP-26	TGTGCACCAATTCTGGTTG	ACATACCTCGCCCTCTTCC	$(CTGA)_6$	Yes	Jia et al. (2015b)
15	SP-34	GCAAGGGCTCTATCGACAA	GTGTGAACGAGACCGATCC	$(TCCTC)_4$	Yes	Jia et al. (2015b)
16	SP-37	CTGAAATGCATGCAAGGTG	GACTGCGCACATACTGACG	$(TCC)_6$	Yes	Jia et al. (2015b)
17	SP-40	AAGGTGGAACAGGATGCAA	GCTGCCATCCTTCTCGTTA	$(AGA)_5$	Yes	Jia et al. (2015b)
18	SP-41	TTGTAATTGCTCAGGATGGC	GTACCGAATTCCCCGAAAG	$(CGG)_5$	Yes	Jia et al. (2015b)
19	SP-42	CGCAAGCTGATTCCAAGTT	AACGCATGCCCAAGAGTAG	$(TTTG)_5$	Yes	Jia et al. (2015b)
20	SP-43	GACCACGACACTGCCTTCT	TCCGGCTCCTCTTAGGATT	$(GTG)_8$	No	Jia et al. (2015b)
21	SP-45	AGGAGGACGAGAAGAGCGT	GAATTGAATCGATCAGCGG	$(ACG)_5$	Yes	Jia et al. (2015b)
22	SP-46	GCAACGAGTAGTGCCCAAA	GCTCTCTACCTGCAACCCA	$(CACAA)_3$	Yes	Jia et al. (2015b)
23	ESSR3	AATCGAGAATGCTGGCTTG	GACGGAGGGGAACTAGGAA	$(AG)_{11}$	Yes	Li et al. (2016)
24	ESSR5	ATTGCTTTCTTCCTCGGCT	GGCGACCACACCTATTTTG	$(AAC)_5$	Yes	Li et al. (2016)
25	ESSR8	TTGTAATTGCTCAGGATGGC	GTACCGAATTCCCCGAAAG	$(CGG)_9$	Yes	Li et al. (2016)
26	ESSR28	GCAACGAGTAGTGCCCAAA	GCTCTCTACCTGCAACCCA	$(CACAA)_4$	Yes	Li et al. (2016)
27	ESSR33	GCAAGGGCTCTATCGACAA	GTGTGAACGAGACCGATCC	$(TCCTC)_4$	Yes	Li et al. (2016)

[z]The primer codes are the same as those in the reference articles.

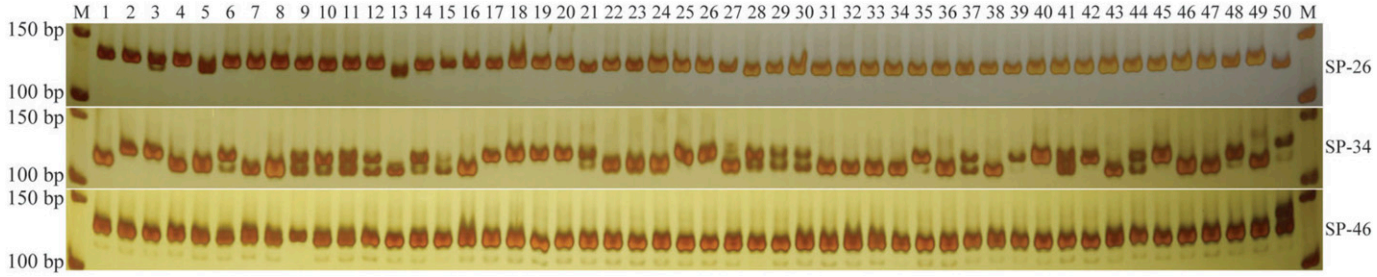

Fig. 1. Amplification profiles of part of simple sequence repeat (SSR) primers in the 50 *Paspalum vaginatum* accessions [lane M = 50–base pair (bp) DNA ladder marker]. SSR profiles of 50 *P. vaginatum* accessions with primers SP-26, SP-34, and SP-46 (lanes 1–50).

marker varies from 0.016 to 0.577, with an average of 0.249 (Table 3). The I and the PIC were the highest in primer ESSR3 and the lowest in primer SP-15, which indicated that ESSR3 had higher detection efficiency of polymorphism.

Cluster analysis. Based on the data of 19 polymorphic SSR markers, the genetic similarity coefficients (GSCs) among different materials were calculated. The GSCs between each pair of the 58 *P. vaginatum* accessions and four cultivars ranged from 0.51 to 1.00, with an average of 0.77. The lowest GSC (0.51) existed between HN17-2 and HN17-26 populations, indicating that they were least related. Cluster analysis based on GSCs showed that the 58 *P. vaginatum* accessions and four cultivars were classified into four distinct major groups: A, B, C, and D (Fig. 2) using a similarity index of 0.75. Most of the accessions or cultivars collected from the same region or nearby regions had high genetic similarity and tended to cluster within the same group or neighboring groups; however, there was no significant relationship between genetic diversity and geographic location. Cluster A contained six *P. vaginatum* accessions: one from Guangxi (GX17-62) and five from Hainan province (Fig. 2). Cluster B included 29 *P. vaginatum* accessions and four cultivars, which was divided into two subgroups [B1 and B2 (Fig. 2)]. The subgroup B1 contained 25 accessions, including 14 Hainan accessions, 7 Guangxi accessions, 3 Guangdong accessions, 1 Jiangxi accession (JX17-97), and two cultivars (Seaspray and Adalayd) (Fig. 2). Subgroup B2 included four Hainan accessions and two cultivars (Platinum and Sealsle 2000). Cluster C consisted of 21 *P. vaginatum* accessions, and could be classified further into three relatively distinct subgroups of C1, C2, and C3 (Fig. 2). The subgroup C1 included 1 Hainan accession and 2 Guangxi accessions; the subgroup C2 comprised 15 accessions, which were 11 Hainan accessions, 2 Guangxi, and 2 Guangdong accessions; and the subgroup C3 contained 1 Hainan accession and 2 Guangdong accessions (Fig. 2). Cluster D included GX17-13 and GX17-48, both from Guangxi. According to the clustering results, the materials from the same area were not completely clustered in one group, so there were large genetic differences among the tested materials.

Principal component analysis. Based on the genetic resemblance matrix, the PCA was conducted on 58 *P. vaginatum* accessions and four cultivars to further understand the ecological distribution of different accessions. Figure 3 presents the distribution of the different accessions according to the three principal axes of variation using PCA. The percentages of variance revealed by principal component 1 (PC1), principal

Table 3. Polymorphic analysis of simple sequence repeat markers within 58 *Paspalum vaginatum* accessions and four cultivars.

No.	Primer code	Alleles (no.)	Observed heterozygosity	Expected heterozygosity	Shannon index	Polymorphic information content
1	SP-10	2	0.161	0.150	0.280	0.137
2	SP-13	2	0.258	0.227	0.385	0.199
3	SP-15	2	0.016	0.016	0.047	0.016
4	SP-17	2	0.113	0.475	0.664	0.360
5	SP-18	4	0.177	0.564	0.941	0.468
6	SP-19	2	0.177	0.163	0.300	0.149
7	SP-26	2	0.000	0.462	0.650	0.353
8	SP-34	2	0.419	0.452	0.640	0.348
9	SP-37	4	0.436	0.416	0.808	0.384
10	SP-40	2	0.307	0.262	0.428	0.226
11	SP-41	2	0.048	0.048	0.114	0.046
12	SP-42	2	0.032	0.032	0.083	0.031
13	SP-45	3	0.355	0.370	0.652	0.326
14	SP-46	2	0.355	0.294	0.468	0.249
15	ESSR3	3	0.694	0.657	1.075	0.577
16	ESSR5	3	0.258	0.235	0.474	0.220
17	ESSR8	2	0.048	0.048	0.114	0.046
18	ESSR28	2	0.355	0.294	0.468	0.249
19	ESSR33	2	0.371	0.446	0.635	0.345
	Mean	2.4	0.241	0.295	0.486	0.249

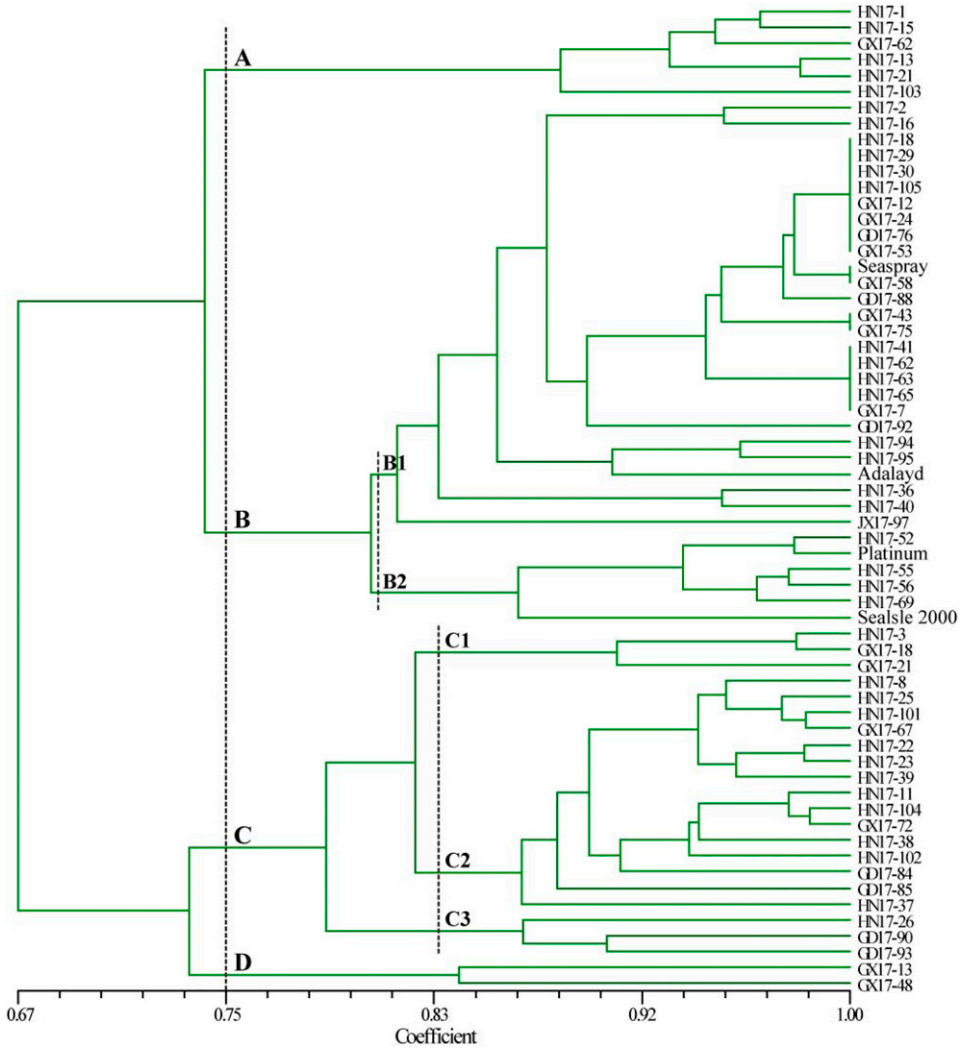

Fig. 2. Unweighted pair group method using arithmetic average clustering for 58 *Paspalum vaginatum* accessions and four cultivars revealed with simple sequence repeat (SSR) markers. The 58 *P. vaginatum* accessions and four cultivars were divided into four groups (A, B, C, and D) with the cluster analysis. Group B was divided into two subgroups of B1 and B2. Group C was classified into three subgroups of C1, C2, and C3.

component 2 (PC2), and principal component 3 (PC3) were 76.50%, 9.24%, and 3.30%, respectively. According to the first three principal components of 62 materials, the PCA three-dimensional scattered point distribution map (Fig. 3) was consistent with the results of the UPGMA cluster analysis. Fifty-eight *P. vaginatum* accessions and four cultivars were also divided into four groups, of which group A was more concentrated, whereas groups B and C were relatively scattered. In contrast, PCA (Fig. 3) is more intuitive than cluster analysis (Fig. 2) in revealing the genetic relationships among different materials.

Discussion and Conclusions

Molecular markers are important tools for plant genetic research and molecular marker–assisted breeding (Ding et al., 2015). Compared with the other molecular markers based on PCR, SSR markers are abundant in quantity, highly polymorphic, codominant, and user friendly (Bian et al., 2019). SSR makers have been applied in many fields (Ding et al., 2015). SSR markers have previously been used successfully in genetic diversity studies of *P. vaginatum.* At present, 220 SSR markers have been developed and applied in *P. vaginatum* (Harris-Shultz et al., 2013; Jia et al., 2015b; Li et al., 2016; Liu et al., 1995; Wang et al., 2006). In this study, a total of 45 alleles were detected by 19 polymorphic SSR markers, with a range of 2 to 4 and an average of 2.4 alleles per marker. There were fewer alleles detected in our experiment compared with the studies of Wang et al. (2006), in which they found 209 alleles using 40 SSR markers, with an average of five alleles per marker. The higher the PIC value, the more effective the primer is in evaluating genetic diversity (Smith et al., 2000). The PIC was the highest in primer ESSR3, indicating that ESSR3 has high detection efficiency of genetic diversity. The PIC for 58 *P. vaginatum* accessions and four cultivars was lower than that reported by Harris-Shultz et al. (2013), which ranged from 0.00 to 0.78 and with an average value of 0.31 for all markers. The differences observed might be due to the different SSR markers used in the two studies, or the fact that the accessions used in their studies were collected from much more diverse areas, even different continents (Budak et al., 2004). Therefore, SSR technology is an effective method for genetic diversity analysis of *P. vaginatum.*

Cluster analysis and PCA, based on these SSR markers, separated all 58 *P. vaginatum* accessions and four cultivars into four groups in this study. These results were inconsistent with the findings of Harris-Shultz et al. (2013) and Wang et al. (2006), which were clustered into two and three major groups, respectively. The different results may be due to the different sources of experimental materials. The experimental materials of this study were mainly concentrated in Hainan, Guangdong, and Guangxi provinces, which were very close to each other. Most of the accessions or cultivars, which were collected from the same region or nearby regions, had higher GSCs and tended to cluster within the same group or neighboring groups (Wang et al., 2013). The four cultivars had a high level of genetic similarity in the study. However, the accessions from the same area were not completely grouped together. For example, Guangxi accessions were distributed in all four groups, and Hainan accessions were also distributed in three groups (groups A, B, and C). Similar results were also reported by Wang et al. (2011, 2013). In addition, there were accessions from different regions clustered in the same group. For instance, Hainan, Guangxi, and Guangdong accessions were clustered in group B or C. This also appeared in other species (Liao et al., 2016;

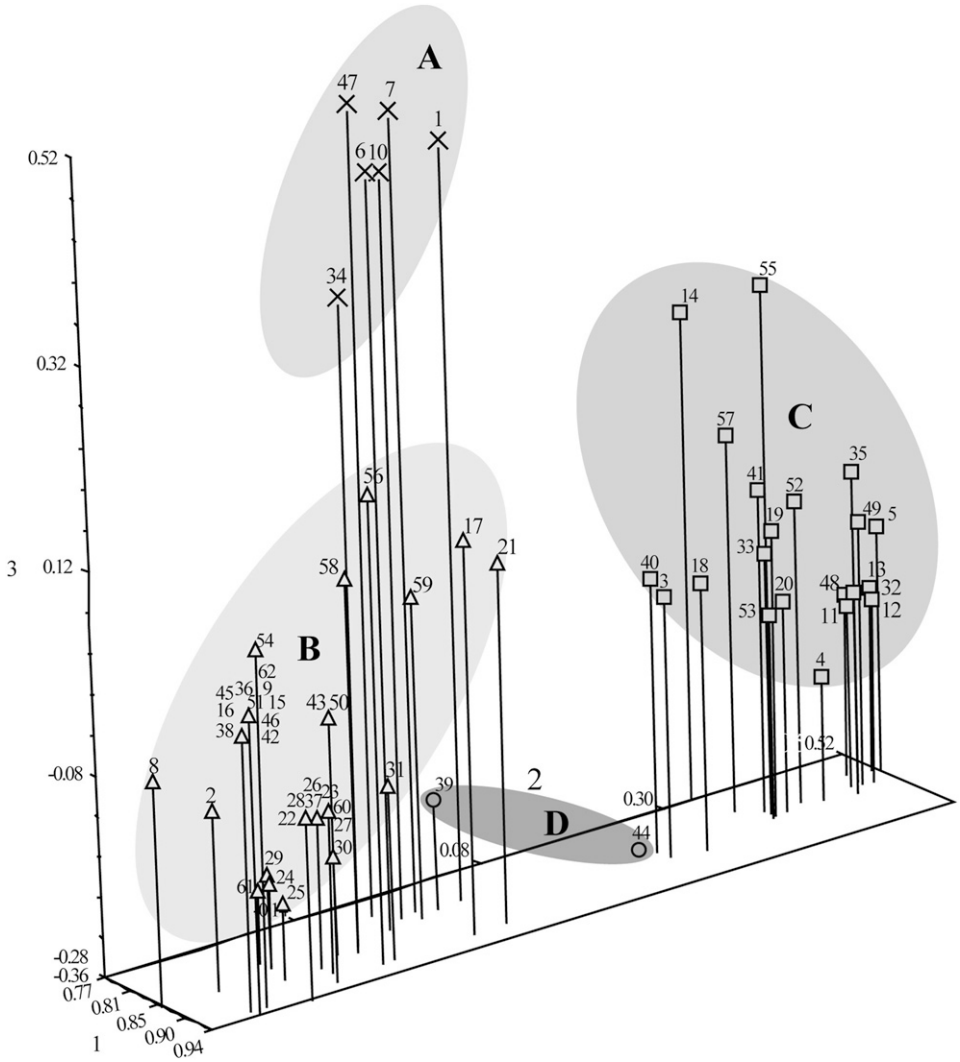

Fig. 3. Three-dimensional scatter plot with the principal component analysis (PCA) for 58 *Paspalum vaginatum* accessions and four cultivars revealed with simple sequence repeat markers. The numbers for materials in the figure are corresponding to the numbers of accessions given in Table 1. The 58 *P. vaginatum* accessions and four cultivars were divided into four groups (A, B, C, and D) with the PCA. The groups A, B, C, and D in the figure are consistent with those in Fig. 2.

Wang et al., 2015). The clustering results of most materials were not strictly consistent with the geographical sources, which were largely due to the limitations of the sampling. These results showed that most of the accessions or cultivars from similar or adjacent regions were clustered in one group but some of the accessions or cultivars from the similar or adjacent regions were not clustered together. There were many reasons to explain these phenomena. First, *P. vaginatum* accessions or cultivars were propagated by a single asexual reproduction in the world (Evers and Burson, 2004). *P. vaginatum* asexual reproduction was usually spread by stolons. Second, different habitats and environments were clustered together by consciously artificial or unconscious introduction, such as the material collected by the roadside might be a cultivar that has been widely used. The materials were collected according to the geographic location, so it was possible to accidentally collect similar material during sampling. Third, there was a lack of sufficient materials and primers in this study. A large number of materials will be collected to accurately evaluate the polymorphism of *P. vaginatum* by enough SSR markers in the future. In conclusion, SSR markers were successfully used to study the genetic diversity of *P. vaginatum*, which provided an important theoretical basis for the collection of turfgrass germplasm and genetic breeding.

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