Analysis of Genetic Diversity in *Chrysopogon aciculatus* Using Intersimple Sequence Repeat and Sequence-related Amplified Polymorphism Markers

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Abstract. Molecular genetic diversity and relationships among 86 *Chrysopogon aciculatus* (Retz.) Trin. accessions were assessed using intersimple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) markers. Twenty-five ISSR markers generated 283 amplification bands, of which 266 were polymorphic. In addition, 576 polymorphic bands were detected from 627 bands amplified using 30 SRAP primers. Both marker types revealed a high level of genetic diversity, with ISSR markers showing a higher proportion of polymorphic loci (PPL; 94%) than SRAP markers (91.87%). The ISSR and SRAP data were significantly correlated ($r = 0.8023$). Cluster analysis of the separate ISSR and SRAP data sets clustered the accessions into three groups, which generally were consistent with geographic provenance. Cluster analysis of the combined ISSR and SRAP data set revealed four major groups similar to those based solely on ISSR or SRAP markers. The findings demonstrate that ISSR and SRAP markers are reliable and effective tools for analysis of genetic diversity in *C. aciculatus*.

The perennial grass genus *Chrysopogon* is mainly distributed in tropical and subtropical regions of the world. Three species are indigenous to China: *Chrysopogon echinulatus* (Nees ex Steud.) Will. Watson, *Chrysopogon orientalis* A. Camus, and *Chrysopogon aciculatus*. *C. aciculatus* is the only member of the genus suitable for use as a turfgrass. The species is drought and shade tolerance, and shows low maintenance requirements. *C. aciculatus* is widely distributed in southern China and commonly used in lawns, sports fields, and shaded turf (Zheng et al., 2005).

The development and application of DNA-based molecular markers has revolutionized

the study of genetic diversity (Kalendar et al., 2011). DNA-based molecular markers are often simple to amplify and rapidly operable. Such markers have been widely used for research into interspecific and intraspecific genetic diversity among turfgrasses. Commonly used molecular marker technologies include random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs) (Kalia et al., 2011). Intersimple sequence repeat markers can be amplified simply and directly without knowledge of the flanking sequences, thus enabling easier development of specific primers compared with SSR markers. The development costs for ISSR markers are low, and ISSR markers show higher reproducibility and reliability compared with AFLP and RAPD markers (Barth et al., 2002; Huang et al., 2013; Zietkiewicz et al., 1994). ISSR technology has been widely used in genetic diversity analysis (Baliyan et al., 2014; Biswas et al., 2010; Djè et al., 2010; Tao et al., 2014; Wang et al., 2013) and DNA fingerprinting (Huang et al., 2010; Kalpana et al., 2012; Reunova et al., 2010; Smolik et al., 2010;

Zietkiewicz et al., 1994). The SRAP technique is based on polymerase chain reaction (PCR) amplification. SRAP primers are designed on the basis that exons are GC rich whereas promoters and introns are AT rich; the polymorphism reflects fundamental variation in the lengths of introns, promoters, and intergenic spacers, both among individuals and among species (Li and Quiros, 2001). The unique primer designs used in the SRAP technique allow direct amplification of open reading frames in the genome, regardless of flanking sequence (Budak et al., 2004a; Wang et al., 2009a). SRAP technology has been widely used in population genetic analysis, paternity testing, genome mapping, and marker-assisted selection. The technique has numerous advantages over other molecular marker technologies, such as abundant polymorphism, high reproducibility, easy amplification, and good stability (Babaei et al., 2014; Budak et al., 2004a; Cai et al., 2011; Li and Quiros, 2001; Uzun et al., 2009). Combination of ISSR and SRAP markers has been used for assessment of genetic diversity among diverse species, such as *Dianthus* L. species and *Salvia miltiorrhiza* Bunge (Budak et al., 2004a; Li et al., 2014; Liu et al., 2008; Song et al., 2010; Yildiz et al., 2011; Yilmaza et al., 2012).

Previous studies of variability within *C. aciculatus* have mainly focused on morphological characteristics (Clarkson, 1992; Liao et al., 2011; Zheng et al., 2005), with no information available on genetic diversity using molecular markers. In this study, we collected 86 *C. aciculatus* accessions from five provinces in China with the aim to evaluate the genetic diversity among the accessions and to assess the level of polymorphism detectable by the use of ISSR and SRAP markers.

Materials and Methods

Plant materials. Eighty-six *C. aciculatus* accessions were collected from the Hainan, Guangxi, Guangdong, Fujian, and Yunnan provinces of China (Table 1). The accessions were grown under field conditions at the Agronomy Garden, Hainan University, Haikou, Hainan, China (20°03' N, 110°19' E; 69.8 m above sea level). This area experiences a tropical monsoon climate. The average annual rainfall is 900–2200 mm and average annual sunlight hours exceed 2000 h. The temperature ranges from 9.4 to 38.7 °C, with an annual average of 23.8 °C. Detailed information for each collection provenance is provided in Table 1.

For each accession, 40 healthy stolons with two internodes of one accession were transplanted into separate 0.25-m² plot, with 50-cm spacing between each plot. Each plot was trimmed weekly to prevent interplot contamination. The plots were frequently mowed to a height of 50 mm. In accordance with the methods of Zheng et al. (2005), routine irrigation, fertilization, and fungicide application were performed to ensure active and healthy growth of the accessions.

DNA extraction. Genomic DNA of each accession was extracted from healthy, fresh,

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Table 1. Eighty-six *Chrysopogon aciculatus* samples collected from different locations in China and used in the study.

No.	Accession no.	Origin	No.	Accession no.	Origin
1	CA01	Haikou, Hainan	44	CA46	Qiongzong, Hainan
2	CA02	Qionghai, Hainan	45	CA47	Longzhou, Guangxi
3	CA03	Dongfang, Hainan	46	CA48	Chongzuo, Guangxi
4	CA04	Sanyai, Hainan	47	CA49	Pinghe, Fujian
5	CA05	Qionghai, Hainan	48	CA50	Wengyuan, Guangxi
6	CA06	Changjiang, Hainan	49	CA51	Lingao, Hainan
7	CA07	Guigang, Guangxi	50	CA52	Guiping, Guangxi
8	CA08	Qionghai, Hainan	51	CA53	Qinzhou, Guangxi
9	CA09	Wanning, Hainan	52	CA54	Beihai, Guangxi
10	CA10	Haikou, Hainan	53	CA55	Hepu, Guangxi
11	CA11	Baishau, Hainan	54	CA56	Hepu, Guangxi
12	CA12	Danzhou, Hainan	55	CA57	Hepu, Guangxi
13	CA14	Dingan, Hainan	56	CA58	Wuzhishan, Hainan
14	CA15	Danzhou, Hainan	57	CA59	Qiongzong, Hainan
15	CA16	Dongfang, Hainan	58	CA60	Wuzhishan, Hainan
16	CA18-1	Danzhou, Hainan	59	CA61	Wuzhishan, Hainan
17	CA18-2	Danzhou, Hainan	60	CA62	Qiongzong, Hainan
18	CA19	Qiongzong, Hainan	61	CA63	Wuzhou, Guangxi
19	CA20	Wanning, Hainan	62	CA64	Yingde, Guangdong
20	CA21	Dingan, Hainan	63	CA65	Wuzhou, Guangxi
21	CA22	Qionghai, Hainan	64	CA66	Qiongzong, Hainan
22	CA23	Qionghai, Hainan	65	CA67	Shanghang, Fujian
23	CA24	Sanya, Hainan	66	CA68	Wuzhou, Guangxi
24	CA25	Cenxi, Guangxi	67	CA69	Hepu, Guangxi
25	CA26	Yingde, Guangdong	68	CA70	Xuwen, Guangdong
26	CA27	Danzhou, Hainan	69	CA71	Maoming, Guangdong
27	CA28	Qiongzong, Hainan	70	CA72	Bobai, Guangxi
28	CA29	Chenxi, Guangxi	71	CA73	Suixian, Guangdong
29	CA31	Qiongzong, Hainan	72	CA74	Hepu, Guangxi
30	CA32	Laibin, Guangxi	73	CA75	Puer, Yunnan
31	CA33	Zhaoqing, Guangdong	74	CA76	Zhenyuan, Yunnan
32	CA34	Tunchang, Hainan	75	CA77	Ninger, Yunnan
33	CA35	Mangshi, Yunnan	76	CA78	Lianjiang, Guangdong
34	CA36	Changtai, Fujian	77	CA79	Xuwen, Guangdong
35	CA37	Wenchang, Hainan	78	CA80	Suixi, Guangxi
36	CA38	Lingshui, Hainan	79	CA81	Lingao, Hainan
37	CA39	Beihai, Guangxi	80	CA82	Lingao, Hainan
38	CA40	Tunchang, Hainan	81	CA84	Chengmai, Hainan
39	CA41	Haikou, Hainan	82	CA85	Haikou, Hainan
40	CA42	Haikou, Hainan	83	CA86	Haikou, Hainan
41	CA43	Qiongzong, Hainan	84	CA87	Sanya, Hainan
42	CA44	Longzhou, Guangxi	85	CA88	Qionghai, Hainan
43	CA45	Daxin, Guangxi	86	CA89	Wanning, Hainan

young leaf tissue using the cetyltrimethylammonium bromide method (Doyle and Doyle, 1987). DNA concentrations were determined by electrophoresis in 1.5% agarose gel by comparison with a sample of known concentration. Genomic DNA was diluted to a final concentration of 30 ng·μL⁻¹ and stored at -20 °C until required.

ISSR analysis. Twenty-five ISSR primers were chosen from 100 primers supplied by Invitrogen Biotech Co. (Shanghai, China) to amplify polymorphisms in the 86 *C. aciculatus* accessions. Detailed primer information is given in Table 2. The amplification procedure followed the protocol established by Wang et al. (2009a). Each PCR mixture (total volume 20 μL) consisted of 2 μL 10× buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl, and 15 mM MgCl₂], 2.5 mM dNTPs (Invitrogen), 0.2 μM primer (Invitrogen), 0.2 μL Taq DNA polymerase (Takara Biotechnology, Dalian, China), 14 μL ddH₂O, and ≈60 ng template DNA. Amplifications were carried out in a PTC-200™ Thermal Cycler (MJ Research, Watertown, MA) using the following PCR program: initial denaturation at 95 °C for

5 min; 45 cycles of 45 s degeneration at 94 °C, 1 min annealing at 52–61 °C, and 90 s elongation at 72 °C; and a final elongation step of 7 min at 72 °C. Following amplification, PCR fragments were electrophoresed in 1.5% agarose gel using a Hoefer vertical gel apparatus (JY-SCZ6; Beijing Liuyi Biotechnology Co., Ltd., Beijing, China) at 120 V for 2.5 h in 1× Tris/borate/EDTA buffer. A 100-bp DNA ladder (Dye Plus; Takara Biotechnology) was used as a reference for estimation of allele sizes. After electrophoresis, the gels were stained with ethidium bromide and photographed under ultraviolet light, as described by Wang et al. (2009a).

SRAP analysis. A total of 400 SRAP primer combinations were used for PCR amplification (Table 3). Thirty primer combinations were selected (Table 4) for further analysis. PCR amplification was based on the technique previously described for *Cynodon dactylon* (L.) Pers. (Wang et al., 2009b) with modifications. SRAP reactions were conducted in a total volume of 20 μL containing 2 μL 10× buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl, and 15 mM MgCl₂], 2.5 mM dNTPs, 0.2 μM

primer, 0.2 μL Taq DNA polymerase, 13.3 μL ddH₂O, and ≈30 ng template DNA. Amplifications were performed in a PTC-200™ Thermal Cycler using the following PCR program: 4 min denaturation at 94 °C, 1 min degeneration at 94 °C, 1 min annealing at 37 °C, and 10 s elongation at 72 °C. In the following 35 cycles, the annealing temperature was increased to 50 °C, with a final elongation step of 7 min at 72 °C. PCR amplicons were fractionated on 8.0% nondenatured polyacrylamide gels using a Hoefer vertical gel apparatus at 200 V for 2.5 h in 1× Tris/borate/EDTA buffer. A 100-bp DNA ladder (Dye Plus) was used as a reference for estimation of allele sizes. After electrophoresis, the gel was subjected to rapid silver staining for detection of DNA bands (Wang et al., 2009b).

Data analysis. The presence or absence of each SRAP or ISSR fragment was coded as 1 or 0, respectively. Popgene version 1.32 was used to calculate Nei's gene diversity (*He*) (Nei, 1973) and Shannon's information index (*I*) (Shannon and Weaver, 1949), which were used to compute Nei's standard genetic distance coefficients (Nei and Li, 1979) for the 86 accessions. The resulting distance matrix was used to construct a dendrogram with the unweighted pair-group method with arithmetic averages (UPGMA) (Sneath and Sokal, 1973) using NTSYS-pc version 2.10s software. The correlation between similarity matrices generated from the ISSR and SRAP data sets was estimated using the COPH and MXCOP modules in NTSYS-pc (Mantel, 1967). The COPH and MXCOMP programs were used to calculate the goodness of fit between the cluster analysis and the original distance matrix for three data sets (ISSR, SRAP, and ISSR + SRAP).

Results

Polymorphism analysis. Twenty-five ISSR primers amplified 283 scorable bands, with an average of 11.32 amplified fragments per primer pair (Table 2; Fig. 1). The number of bands ranged from 8 (ISSR827) to 15 (ISSR850 and ISSR858) bands per primer, with an average of 10.92 bands showing polymorphism. The percentage of PPL of the 25 primers tested ranged from 84.62% for primer ISSR895 to 100% for primers ISSR808, ISSR816, ISSR823, ISSR824, ISSR827, ISSR835, ISSR842, SSR859, ISSR866, and ISSR890 (Table 2). Of the 627 bands amplified by 30 SRAP primer pairs, 576 bands were polymorphic (91.87%). Polymorphisms of all loci ranged from a maximum of 100% (Me03-Em13, Me04-Em10, Me04-Em16, Me05-Em10, Me06-Em16, Me07-Em18, Me16-Em09, Me16-Em16, and Me19-Em09) to a minimum of 66.67% (Me08-Em19). An average of 20.9 bands was amplified per primer, and 19.2 bands were polymorphic (Table 3; Fig. 2).

Genetic diversity. Genetic variation accessed using ISSR and SRAP markers for all 86 *C. aciculatus* accessions are shown in Tables 2 and 4. The average values for PPL, *He*, and *I* obtained with the ISSR markers were 94%, 0.3, and 0.29, respectively. Genetic diversity

Table 2. Twenty-five primers sequences used for intersimple sequence repeat (ISSR) amplification and number of bands for each primer.

Primer	Primer sequence (5'-3')	Temp (°C)	Total no. of bands	Polymorphic no. of bands	Percentage of polymorphism
ISSR807	(AG) ₈ T	52	12	11	91.67
ISSR808	(AG) ₈ C	55	12	12	100
ISSR809	(AG) ₈ G	55	10	9	90
ISSR815	(CT) ₈ G	55	10	9	90
ISSR816	(CA) ₈ T	52	14	14	100
ISSR823	(TC) ₈ C	55	9	9	100
ISSR824	(TC) ₈ G	55	12	12	100
ISSR827	(AC) ₈ G	55	8	8	100
ISSR829	(TG) ₈ C	55	10	9	90
ISSR834	(AG) ₈ YT	54	10	9	90
ISSR835	(AG) ₈ YC	56	10	10	100
ISSR841	(GA) ₈ YC	56	13	12	92.31
ISSR842	(GA) ₈ YG	56	10	10	100
ISSR848	(CA) ₈ RG	56	10	9	90
ISSR849	(GT) ₈ YA	54	10	9	90
ISSR850	(GT) ₈ YC	56	15	14	93.33
ISSR855	(AC) ₈ YT	54	10	9	90
ISSR857	(AC) ₈ YG	56	10	9	90
ISSR858	(TG) ₈ RT	54	15	13	86.67
ISSR859	(TG) ₈ RC	56	10	10	100
ISSR866	(CTC) ₆	61	12	12	100
ISSR880	(GGAGA) ₃	60	13	12	92.31
ISSR890	VHV(GT) ₇	54	12	12	100
ISSR891	HVH(TG) ₇	53	13	12	92.31
ISSR895	(AG) ₂ TTGGT AG(CT) ₂ TGATC	56	13	11	84.62
Total			283	266	94
Average			11.32	10.64	

Table 3. Forward and reverse sequence-related amplified polymorphism primers used in this study.

Codes	Forward primers (5'-3')	Codes	Reverse primers (5'-3')
Me01	TGAGTCCAAACCGGATA	Em01	GACTGCGTACGAATTCAA
Me02	TGAGTCCAAACCGGAGC	Em02	GACTGCGTACGAATTCTG
Me03	TGAGTCCAAACCGGACC	Em03	GACTGCGTACGAATTGAC
Me04	TGAGTCCAAACCGGACA	Em04	GACTGCGTACGAATTGA
Me05	TGAGTCCAAACCGGTGC	Em05	GACTGCGTACGAATTAAC
Me06	TGAGTCCAAACCGGAGA	Em06	GACTGCGTACGAATTGCA
Me07	TGAGTCCAAACCGGACG	Em07	GACTGCGTACGAATTGAG
Me08	TGAGTCCAAACCGGAAA	Em08	GACTGCGTACGAATTGCC
Me09	TGAGTCCAAACCGGAAC	Em09	GACTGCGTACGAATTTC
Me10	TGAGTCCAAACCGGAAT	Em10	GACTGCGTACGAATTCAAT
Me11	TGAGTCCAAACCGGAAG	Em11	GACTGCGTACGAATTAAT
Me12	TGAGTCCAAACCGGTAG	Em12	GACTGCGTACGAATTGTC
Me13	TGAGTCCAAACCGGTTG	Em13	GACTGCGTACGAATTCTGA
Me14	TGAGTCCAAACCGGTGT	Em14	GACTGCGTACGAATTATG
Me15	TGAGTCCAAACCGGTCA	Em15	GACTGCGTACGAATTAGC
Me16	TGAGTCCAAACCGGGAC	Em16	GACTGCGTACGAATTACG
Me17	TGAGTCCAAACCGGGTA	Em17	GACTGCGTACGAATTAG
Me18	TGAGTCCAAACCGGGT	Em18	GACTGCGTACGAATTTCG
Me19	TGAGTCCAAACCGGCAG	Em19	GACTGCGTACGAATTGTC
Me20	TGAGTCCAAACCGGCAT	Em20	GACTGCGTACGAATTGGT

identified using the SRAP markers was similar to that of the ISSR markers, as indicated by the average values for PPL, *He*, and *I* of 91.87%, 0.29, and 0.26, respectively. The genetic similarity coefficients (GSCs) calculated using the UPGMA clustering method based on ISSR polymorphism ranged from 0.52 to 0.93. Accessions from the same geographic location (CA01 and CA10) in Haikou, Hainan Province, showed a GSC of 0.93. The GSC values derived from the SRAP data set ranged from 0.56 to 0.91. The largest GSC was between accessions CA21 (Dingan, Hainan Province) and CA24 (Sanya, Hainan Province). These results suggested that a high level of genetic diversity was exhibited among all samples.

Cluster analysis. A UPGMA dendrogram derived from the ISSR data were generated to

explore the genetic relationships among the 86 accessions. The accessions were divided into three main clusters (I, II, and III) (Fig. 3). Cluster I comprised of 19 samples from Hainan (18) and Guangxi (1) provinces. Cluster II included 54 accessions, which were further divided into two subclusters: subcluster II-1 contained eight accessions from Hainan (1), Guangxi (3), Guangdong (2), Fujian (1), and Yunnan (1) provinces; and subcluster II-2 contained 36 accessions from Guangxi (18), Hainan (12), Guangdong (4), and Fujian (2) provinces. Cluster III comprised 14 samples from Hainan (8), Yunnan (3), Guangdong (2), and Guangxi (1) provinces. Accessions from the same region were generally grouped within the same cluster.

The dendrogram (Fig. 4) derived from the SRAP data grouped the 86 accessions into

three main clusters (IV, V, and VI). Cluster V was divided into two subgroups, V-1 and V-2. Cluster IV was similar to cluster III. Cluster V was similar to cluster II, and cluster VI was similar to cluster I. CA04 was placed in cluster I, and CA22 and CA23 were grouped in cluster II in the ISSR analysis; in contrast, CA04, CA22, and CA23 were assigned to cluster IV in the SRAP analysis.

In addition, a dendrogram derived from a combined ISSR and SSR data set was constructed, for which the GSCs ranged from 0.56 to 0.88 (Fig. 5). In the combined data analysis, the 86 accessions were classified into four major clusters (VII, VIII, IX, and X). The groups were similar to those obtained in the ISSR analysis. Cluster I was similar to cluster X, except that CA01, CA04, CA06, CA07, and CA10 placed in cluster I based on the ISSR data (Fig. 3) were grouped in cluster VII (Fig. 5). CA20, CA22, CA23, and CA27 placed in subcluster II-1 (Fig. 3) were grouped in cluster X (Fig. 5). The accessions placed in subcluster II-2 and cluster III were identical to those grouped in clusters VIII and IX, respectively.

Discussion

Previous studies demonstrate that ISSR and SRAP markers are valuable for marker-assisted selection, analysis of genetic diversity, and population genetic analysis (Budak et al., 2004a, 2004b, 2004c; Carvalho et al., 2009; Gupta and Varshney, 2000). Both marker types have been successfully used for genetic analyses of warm-season turfgrasses, such as *Zoysia japonica* Steud. (Chen et al., 2009; Xie et al., 2012), *Cynodon dactylon* (Huang et al., 2010; Li et al., 2011; Ling et al., 2010; Wang et al., 2009b, 2013; Yi et al., 2008), *Cynodon arcuatus* J. Presl (Huang et al., 2013), and *Eremochloa ophiuroides* (Munro) Hack. (Zheng et al., 2009, 2013). The present study is the first assessment of the molecular genetic diversity among *C. aciculatus* accessions using ISSR and SRAP markers.

Evaluation of genetic diversity among a sample of accessions provides an important foundation for conservation of genetic resources and marker-assisted breeding. In the present study, ISSR and SRAP data revealed high genetic diversity among the 86 *C. aciculatus* accessions. The 25 ISSR markers yielded a higher PPL (94%) than the 30 SRAP markers (91.87%). The values of *He* and *I* generated by the ISSR markers (0.3 and 0.29, respectively) were slightly higher than those generated using SRAP markers (0.29 and 0.26, respectively), which indicated that ISSR markers provided more informative data than SRAP markers. Differences in the results obtained with the ISSR and SRAP markers probably reflect the different genomic regions amplified by the two marker types. Similar results were obtained for *Auricularia auricula-judae* Quel. (Tang et al., 2010), *Pogostemon cablin* Benth. (Wu et al., 2010), and *Prunella vulgaris* L. (Liao et al., 2012) using ISSR and SRAP molecular markers.

Table 4. Amplification results of 30 sequence-related amplified polymorphism primer combinations used in this study.

Primer combinations	Total no. of polymorphic	No. of polymorphic bands	Percentage of polymorphic bands
Me02-Em13	18	16	88.89
Me03-Em13	18	18	100
Me03-Em17	17	16	94.11
Me03-Em19	18	16	88.89
Me04-Em04	22	21	95.45
Me04-Em10	23	23	100
Me04-Em13	17	15	88.24
Me04-Em16	23	23	100
Me05-Em10	23	23	100
Me05-Em19	23	18	78.26
Me06-Em16	21	21	100
Me07-Em09	15	11	73.33
Me07-Em18	18	18	100
Me08-Em19	15	10	66.67
Me09-Em15	25	23	92
Me09-Em16	22	20	90.9
Me10-Em04	25	23	92
Me10-Em06	26	24	92.31
Me11-Em06	27	22	81.48
Me12-Em19	25	23	92
Me13-Em19	24	23	95.83
Me14-Em16	24	23	95.83
Me16-Em06	24	20	83.33
Me16-Em09	23	23	100
Me16-Em16	18	18	100
Me17-Em18	23	22	95.65
Me19-Em09	12	12	100
Me19-Em17	25	19	73.08
Me19-Em19	22	18	81.82
Me20-Em17	15	14	93.33
Total	627	576	91.87
Average	20.9	19.2	

The Mantel matrix correspondence test was used for an overall comparison of matrices of cophenetic values produced from dissimilarity matrices generated in the UPGMA cluster analysis (Mantel, 1967). The Mantel's test indicated that the ISSR and SRAP cophenetic values were significantly correlated ($r = 0.8023$), thus the ISSR and SRAP markers revealed similar patterns of genetic diversity in *C. aciculatus* accurately.

The dendrograms generated from the ISSR and SRAP data sets showed strong similarity in topology. Accessions with the same provenance were generally clustered in the same group. Accessions from the same geographical area that were clustered into different groups may reflect gene flow from introduced species or introgression (Li et al., 2011). Conversely, accessions from different regions that clustered together may be explained by the outcrossing breeding system of *C. aciculatus* or by exchange of germplasm resources between regions (Farsani et al., 2012). Genetic overlap occurs in neighboring regions (Li et al., 2011; Yi et al., 2008). In the present study, the 86 *C. aciculatus* accessions originated from five provinces, of which Fujian, Guangdong, Guangxi, and Yunnan provinces are adjacent to each other. *C. aciculatus* shows strong vegetative reproduction, and human activities may have aided dispersal, with subsequent vegetative spread and sexual reproduction leading to a high level of genetic diversity.

In this study, we combined the ISSR and SRAP data sets to examine the degree of

agreement between the individual marker types. Although combining data remains controversial (Mohammadi and Prasanna, 2003), combining the ISSR and SRAP data sets allowed a more comprehensive analysis of genetic diversity and provided greater information for future marker-assisted selection in the breeding of *C. aciculatus*. The 86 *C. aciculatus* accessions were clustered into four groups based on the combined data sets. The clusters generally reflected the geographical distribution of the accessions, similar to that based on separate analyses of the ISSR and SRAP data sets. In addition, we measured five indices of plant growth, namely chlorophyll content, maximum leaf length, withering rate, quality evaluation, and quality of dry matter, with the aim of assessing the shade tolerance of *C. aciculatus*. A clustering analysis diagram was generated based on the growth parameter data (X.Y. Zhang, Z.Y. Wang, and L. Liao, unpublished data). From these two cluster analyses, it was determined that cluster VII is characterized by excellent shade tolerance. The growth parameters of clusters VIII and IX are generally intermediate to those of clusters I and X, with the growth parameters of cluster IX slightly lower than those of cluster VIII. Accessions grouped in cluster X showed opposing characteristics to those of cluster VII. Accessions placed in clusters VII and VIII may prove useful turfgrasses for planting. Thus, the information provided by the combined marker analysis may prove useful for selection of parents in the breeding of improved shade-tolerant accessions of *C. aciculatus*.

In conclusion, this study assessed molecular genetic diversity and relationships among 86 *C. aciculatus* accessions using ISSR and SRAP markers. Both ISSR and SRAP markers are reliable and effective tools for analysis of genetic diversity in *C. aciculatus*, with high levels of genetic diversity detected among the 86 populations sampled. ISSR markers show a higher PPL (94%) than SRAP markers (91.87%). Cluster analysis of the ISSR and SRAP data independently yielded similar results. The *C. aciculatus* accessions were clustered into three groups. The clusters were generally consistent with the geographic provenances of the accessions. Cluster analysis of the combined ISSR and SRAP data sets generated similar groupings to those derived from separate analyses of the ISSR and SRAP data sets. Indices of shade tolerance differ among each group of accessions. Thus, ISSR and SRAP markers are informative for evaluation of genetic diversity in *C. aciculatus*, and may be useful in the conservation of genetic resources, development of biodiversity conservation strategies, and identification of wild germplasm resources. The markers may also contribute to future molecular marker-assisted breeding programs.

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Fig. 1. Intersimple sequence repeat amplified with primer ISSR842 in 86 *Chrysopogon aciculatus* DNA of *C. aciculatus* samples. The size standard is a 100-bp DNA ladder and the sample number is shown in Table 1.

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Fig. 3. Unweighted pair-group method with arithmetic averages clustering for 86 *Chrysopogon aciculatus* accessions based on intersimple sequence repeat markers.

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Fig. 4. Unweighted pair-group method with arithmetic averages dendrogram based on sequence-related amplified polymorphism data of 86 *Chrysopogon aciculatus* accessions.

Fig. 5. Unweighted pair-group method with arithmetic averages cluster for 86 *Chrysopogon aciculatus* accessions based on intersimple sequence repeat and sequence-related amplified polymorphism markers.

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