Isolation and Characterization of New Polymorphic Simple Sequence Repeat Loci in *Chrysopogon aciculatus* (Retz.) Trin.

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Abstract. *Chrysopogon aciculatus* (Retz.) Trin. is a perennial turfgrass for its low management and resistance. To develop simple sequence repeat (SSR) markers for *C. aciculatus*, we used four Roche 454 pyrosequencing, combined with the magnetic bead enrichment method FIASCO (fast isolation by amplified fragment length polymorphism of sequences containing repeats) to isolate from the *C. aciculatus*. A total of 66,198 raw sequencing reads were obtained with 4289 sequences (6.48%) were fit for primer pair design. One hundred microsatellite loci were selected to test the primer amplification efficiency in 20 accessions, and out of these, 11 loci were polymorphic. The amount of observed alleles ranged from three to six, with an average of 3.64. Nei's genetic diversity values ranged from 0.085 to 0.493, with an average of 0.293. Shannon's information index values ranged from 0.141 to 0.686, with an average of 0.428. Twenty accessions were clustered into three groups by unweighted pair-group method with arithmetic means (UPGMA). These SSR markers will provide an ideal marker system to assist with gene targeting, cultivar variety or species identification, and marker-assisted selection in *C. aciculatus* species.

Chrysopogon aciculatus (Retz.) Trin. is a perennial turfgrass with many features of drought resistance, shade resistance, leanness resistance, and low management. It is usually found in sunny, dry, exposed areas such as roadsides, lawns, pasture, bank of rivers, and water courses (Sun, 1995). All *C. aciculatus* individuals are polyploidy, with $2n = 20$ (Liao et al., 2011). In China, the wild germplasm resources of *C. aciculatus* are mainly distributed in the tropical and subtropical climate regions, such as Hainan, Guangdong, Guangxi, Yunnan, and Guizhou (Liu et al., 1998; Zheng et al., 2005). It is an excellent kind of grass for water and soil conservation often used for highway slope conservation, and also for common green land and shade turf in tropical and subtropical region of China. Previous studies of *C. aciculatus* have mainly focused on its morphological characteristics (Liao et al., 2011; Wagner et al.,

1999). Liu et al. (1997) had investigated its distribution in the range of China. Bai et al. (2002) evaluated about the ecological adaptation. Reddi et al. (2010) studied pollination and seed of *C. aciculatus*. Ambasta and Rana (2013) studied the taxonomy of *C. aciculatus*. Up to date, the genetic diversity of its wild germplasms is little known. Therefore, developing a set of reliable and informative DNA markers to aid in assessing the genetic diversity of its wild germplasm resources would provide theoretical basis for the future study of breeding excellent cultivars. Microsatellite or SSR is a novel molecular genetic markers. Microsatellite has the characteristics of being a total of codominant modes, following the Mendel's law, large number, wide and uniform distribution, the high polymorphism information content, and easy to be amplified by polymerase chain reaction (PCR) (Nichols et al., 2003). It has been widely used in population genetic analysis, paternity testing, genome mapping, and marker assisted selection. FIASCO first proposed in 2002 (Zane et al., 2002) has been maturely used in many field (Chen et al., 2007; Hou et al., 2011; Wang et al., 2014; Xu et al., 2006; Yang et al., 2009; Zhang et al., 2008). However, this method is complicated to operate, and cannot obtain a large number of SSRs in one experiment. Nowadays next-generation sequencing has

been the most popular approach in the research of transcription group (Li et al., 2013), such as the launch of Roche 454 pyrosequencing, has got good application for the structural variation in the genome (Alagna et al., 2009; Barakat et al., 2009; Dassanayake et al., 2009; Guo et al., 2010; Kumar et al., 2014; Swarbreck et al., 2011; Wang and Wang, 2015). We used Roche 454 pyrosequencing, combined with the magnetic bead enrichment method FIASCO, to isolate SSR markers from the *C. aciculatus* genome. The objectives of this study were 1) using Roche 454 pyrosequencing to isolate SSR markers from the *C. aciculatus* genome and 2) to study the genetic relationships among 20 accessions of *C. aciculatus* from China and describe the genetic variation.

Materials and Methods

Genomic DNA extraction and SSR mining. Genomic DNA was extracted using a plant genomic DNA kit (Tiangen Biotech, Beijing, China) from silica gel-dried leaves. A shotgun library was constructed following Roche protocol with about 1 µg of genomic DNA. The shotgun library was further enriched by seven 5'-biotinylated probes: (AC)₁₂, (AG)₁₂, (AAT)₈, (AGG)₈, (AGC)₈, (AGAT)₆, (ACAG)₆, according to the protocol of Toth and Gaspari (2000). Roche 454 GS-FLX+ System (Shanghai, China) was used to sequence the enriched products on a picotiter plate to search for microsatellite sequences by MISA (MicroSatellite identification tool) (<http://pgrc.ipk-gatersleben.de/misa/>). Search parameters were set as 10 repeat units for mononucleotides, six repeat units for dinucleotides, five repeat units for trinucleotides, tetranucleotides, pentanucleotides, and hexanucleotides. The maximum difference between two SSRs was set at 100 nucleotides. Primer design was conducted with Primer 3 (Rozen and Skaletsky, 2000). The PCR product size range was set at 100–400 bp and the remaining parameters were set at default values.

PCR amplification and genotyping. A total of 20 individuals of *C. aciculatus* from five provinces in China (Table 1) were used to analyze the polymorphisms of the microsatellite primers. PCRs were performed in a total volume of 20 µL, which contained 10 ng of genomic DNA, 0.18 mM/L of each dNTP, 0.2 µM/L of each primer, 2.0 µL of 10× polymerization buffer, and 1.0 unit of Taq polymerase (Tiangen Biotech, Beijing, China), using a PTC-200 Thermal Cycler (MJ Research, Watertown, MA). The PCR was conducted as follows: an initial denaturation for 5 minutes at 95 °C, followed by 40 cycles of denaturation for 30 seconds at 94 °C, annealing for 30 seconds at 48–60 °C (optimized for each locus, Table 2), extension for 45 seconds at 72 °C, and a final extension step of 10 minutes at 72 °C.

The PCR products were electrophoresed on an 8% polyacrylamide gel and visualized by silver staining. A 100-bp DNA ladder (Takara, Dalian, China) was used as reference for estimating allele sizes.

Data analysis. Polymorphism statistics, including allele number, Nei's measure of

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gene diversity (Nei, 1973), were recorded using POPGENE v.1.3.2, software for genetic similarity analysis (Yeh and Boyle, 1996). Genetic relationships between 20 individuals of *C. aciculatus*, based on Nei's genetic distance (Nei,

1978), were examined using the UPGMA in NTSYS-pc 2.10e (Rohlf, 2000). Shannon's information index (I) were used to construct a UPGMA dendrogram within the sequential agglomerative hierarchical nested cluster analysis

module of NTSYS (taxonomy and multivariate analysis system) (Sneath and Sokal, 1973). Finally, a Mantel test between the geographic and genetic distances have been carried out using COPH (cophenetic values) and MXCOP (matrix comparison plot) modules in NTSYS-pc software (Mantel, 1967).

Table 1. Details of locations of 86 *Chrysopogon aciculatus* accessions.

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	Changtai, Fujian
4	CA04	Qionghai, Hainan	47	CA49	Pinghe, Fujian
5	CA05	Qionghai, Hainan	48	CA50	Wengyuan, Guangxi
6	CA06	Qionghai, Hainan	49	CA51	Lingao, Hainan
7	CA07	Danzhou, Hainan	50	CA52	Guiping, Guangxi
8	CA08	Qionghai, Hainan	51	CA53	Qinzhou, Guangxi
9	CA09	Wanning, Hainan	52	CA54	Hepu, Guangxi
10	CA10	Wanning, Hainan	53	CA55	Qiongzong, Hainan
11	CA11	Wanning, 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	Dingan, Hainan	58	CA60	Wuzhishan, Hainan
16	CA18-1	Danzhou, Hainan	59	CA61	Wuzhishan, Hainan
17	CA18-2	Danzhou, Hainan	60	CA62	Wuzhishan, Hainan
18	CA19	Qionghai, Hainan	61	CA63	Wuzhou, Guangxi
19	CA20	Haikou, Hainan	62	CA64	Qiongzong, Hainan
20	CA21	Dongfang, Hainan	63	CA65	Wuzhou, Guangxi
21	CA22	Changjiang, Hainan	64	CA66	Qiongzong, Hainan
22	CA23	Sanya, Hainan	65	CA67	Shanghang, Fujian
23	CA25	Cenxi, Guangxi	66	CA68	Wuzhou, Guangxi
24	CA26	Yingde, Guangdong	67	CA69	Hepu, Guangxi
25	CA27	Guigang, Guangxi	68	CA70	Xuwen, Guangdong
26	CA28	Beihai, Guangxi	69	CA71	Maoming, Guangdong
27	CA29	Sanya, Hainan	70	CA72	Bobai, Guangxi
28	CA31	Chengmai, Hainan	71	CA73	Suixian, Guangdong
29	CA31	Chengmai, 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
43	CA45	Daxin, Guangxi	86	CA89	Baisha, Hainan

Results and Discussion

A total of 66,198 raw sequencing reads were obtained. 17,161 sequences (25.92%) contained SSRs and only 4289 sequences (6.48%) were fit for primer pair design. The properties of mono-, di-, tri-, tetra-, penta-, and hexaploid were 6.35%, 47%, 41.42%, 4.85%, 0.05%, and 0.35%, respectively. We randomly selected 100 microsatellite sequences to design primer pairs and tested. These sequences were deposited in GenBank (KT946824-KT946834). Twenty-six primer pairs, which could successfully amplify target fragments, were tested for polymorphisms in 20 accessions collected from five provinces in China (Table 1); 11 loci were polymorphic and 15 displayed monomorphic. The genetic diversity parameters of each material were represented in Table 2. The amount of observed alleles ranged from 3 (IJ3Q0KF02FQL5F, IJ3Q0KF02GCT8C, IJ3Q0KF02GKSXP, IJ3Q0KF02IC1FO, IJ3Q0KF02IWEAL, IJ3Q0KF02FH3GM) to 6 (IJ3Q0KF02IILLR), with an average of 3.64. Nei's genetic diversity values ranged from 0.085 (IJ3Q0KF02FQL5F) to 0.493 (IJ3Q0KF02H9WIF), with an average of 0.293. Shannon's information index values ranged from 0.141 (IJ3Q0KF02FQL5F) to 0.686 (IJ3Q0KF02H9WIF), with an average of 0.428. This result suggested that 20

Table 2. Primer sequences and characterization of 11 microsatellite loci isolated from *Chrysopogon aciculatus*.

Primer	Primer sequence (5'-3')	Repeat motif	Ta ^a (°C)	Allele size (bp)	A ^b	H _E ^c	I ^w	GenBank accession no.
IJ3Q0KF02HEEVY	F:AGGAGGAAGAGGACGAG R:CCATGAGGAGGAAGAGCAAG	(GGA)12	59.9	166-364	4	0.383	0.567	KT946824
IJ3Q0KF02JB94X	F:ATCCCAGCATATCCAAATCG R:CACCACCAATCCACCATGTA	(AAG)8acgacgacgacc(AAG)6	60.1	86-315	4	0.158	0.246	KT946825
IJ3Q0KF02IILLR	F:CGCAAGAAGCTCTGGAGGAAC R:TCGTAATAGCTGCACCATGC	(AG)24	59.9	117-318	6	0.306	0.459	KT946826
IJ3Q0KF02H9WIF	F:CCCCTCCATCACCATAGCTT R:CTGCAAGCCAACAGAAACAA	(TTG)12	61.2	178-395	4	0.493	0.686	KT946827
IJ3Q0KF02FQL5F	F:CATCGCAGATTGCTCTCTCA R:GGAGCATTGAAGAGTGAGGC	(AAG)8gacaagagagagaagaagaaca agaaggagaagagaagaaggttgagtac (AAG)5	59.9	164-433	3	0.085	0.141	KT946828
IJ3Q0KF02FOQLC	F:GCCAATGACGTTGAACACC R:TCAGCAGATGAGCAGAGTCG	(TCT)19	59.9	111-295	4	0.204	0.297	KT946829
IJ3Q0KF02GCT8C	F:CGGATTTCATGTGACCTGTGT R:TTGGTGATTCTTCGTCCC	(CA)16(TA)6	59.4	213-409	3	0.460	0.652	KT946830
IJ3Q0KF02GKSXP	F:GCCGACAGGAATAGGACAGA R:ACACATCCTGGAACCCATA	(AC)27	60.2	104-372	3	0.165	0.229	KT946831
IJ3Q0KF02IC1FO	F:CACGTGATCTTGACAATGG R:TCACACTATAAAGAGCGAGCG	(TGAGA)5(GAT)9*	59.7	98-348	3	0.297	0.465	KT946832
IJ3Q0KF02IWEAL	F:CCTGATCGATCTCGACACAA R:TTTTCTGTGTCTCTGTGTGTG	(AG)6ggagaagaaacacacacattc (AG)6	59.8	41-300	3	0.472	0.665	KT946833
IJ3Q0KF02IRPRC	F:TCACTTCATCGCAATTGTC R:TGTAGCATGCCAATGAGGTC	(AGA)5aggagaagaaagaaaaagtt tgagtac(AAG)5	59.7	158-541	3	0.198	0.297	KT946834

^aPolymerase chain reaction annealing temperature.

^bNumber of alleles.

^cNei's measure of gene diversity.

^wShannon's information index.

Fig. 1. Unweighted pair-group method with arithmetic means tree illustrating the genetic relationships between 20 accessions of *Chrysopogon aciculatus* based on Nei's genetic distance.

accessions possessed a great amount of variation and represented the accessions of *C. aciculatus* possessed great genetic diversity.

We could know from the UPGMA tree, individuals CA06, CA08, CA09, CA11, and CA14 had closed phylogenetic relationships in group I. All of these materials come from Hainan Province. Group II included all individuals coming from Guangxi Province (CA25, CA27, CA36, CA53, CA63, CA64, and CA69), Guangdong Province (CA71 and CA73), Fujian Province (CA49 and CA48), Yunnan Province (CA77), and one individual from Hainan Province (CA42). CA46 and CA87 in group III (Fig. 1) were both coming from Qiongzong, Hainan. CA49 (Pinghe, Fujian) and CA69 (Hepu, Guangxi) had the closest phylogenetic relationship. UPGMA showed the materials from the same area were more easily clustered together. This phenomenon also mentioned in other studies (Budak et al., 2004; Tani et al., 1998; Wang and Wang, 2015; Wang et al., 2010, 2013). *C. aciculatus* has been characterized as a species with strong vegetative reproduction, and through human activities it is easily to be taken to different place, and it would have a high level of genetic diversity with extension, and reproduction. Introgression may lead to cross clustering among materials from different regions. In this study, eight accessions, which came from Hainan (CA06, CA08, CA09, CA11, CA14, CA42, CA46, and CA87), were clustered into different groups. Actually, they exhibited great difference on turf characteristics. CA46 and CA87 were clustered in group III, both two accessions have higher length of leaves both stolons and upright stems, length of internode, and relatively larger dense inflorescence. The five accessions in group I (CA06, CA08, CA09, CA11, and CA14) demonstrated opposing characteristics to those of

group III. CA42 from Haikou, Hainan were clustered in group II which included 13 accessions from 5 provinces. Characteristics of CA42 and other materials in group II were generally in-between those of group I and group III. The relationship between their genetic distance and geographical origins were complex. We should make full use of excellent *C. aciculatus* germplasm gathered in different types or subtypes during the selection of crossbreeding parents, without being limited to geographical factors.

We also used the COPH and MXCOP module calculate goodness-of-fit generated between the geographic and genetic distances, the result of Mantel test was $r = 0.7372$. This turned out that the geographic and genetic distances correlated positively.

In conclusion, this study was the first to isolate and use microsatellite markers in *C. aciculatus*. The studies indicated that there was great genetic diversity among *C. aciculatus* germplasms. This first set of microsatellite markers developed for *C. aciculatus* was promised to be useful in the studies of cultivars identification, genetic relationship, and assessment of wild germplasm resources.

Literature Cited

- Alagna, F., N. D'Agostino, L. Torchia, M. Servili, R. Rao, M. Pietrella, G. Giuliano, M.L. Chiusano, L. Baldoni, and G. Perrotta. 2009. Comparative 454 pyrosequencing of transcripts from two olive genotypes during fruit development. *BMC Genomics* 10:399.
- Ambasta, N. and N.K. Rana. 2013. Taxonomical study of *Chrysopogon aciculatus* (Retz.) Trin. a significant grass of Chauparan, Hazaribag (Jharkhand). *Sci. Res. Rptr.* 3:27.
- Bai, C.J., G.D. Liu, and J.S. Wei. 2002. The selection and utilization of *Zoysia matrella* cv. Huanan. *Acta Agrestia Sinica* 10:112-117.
- Barakat, A., D.S. DiLoreto, Y. Zhang, C. Smith, K. Baier, W.A. Powell, N. Wheeler, R. Sederoff, and J.E. Carlson. 2009. Comparison of the transcriptomes of American chestnut (*Castanea dentata*) and Chinese chestnut (*Castanea mollissima*) in response to the chestnut blight infection. *BMC Plant Biol.* 9:51.
- Budak, H., R.C. Shearm, I. Parmaksiz, and I. Dweikat. 2004. Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. *Theor. Appl. Genet.* 109: 280-288.
- Chen, G.Q., L.F. Li, G. Hao, S.H. Shi, and X.J. Ge. 2007. Characterization of seven genomic and one dbEST-derived microsatellite loci in the river mangrove *Aegiceras corniculatum* (Myrsinaceae). *Conserv. Genet.* 9:449-451.
- Dassanayake, M., J.S. Haas, H.J. Bohnert, and J.M. Cheeseman. 2009. Shedding light on an extremophile lifestyle through transcriptomics. *New Phytol.* 183:764-775.
- Guo, S., Y. Zheng, J.G. Joung, S. Liu, Z. Zhang, O.R. Crasta, W.S. Bruno, X. Yong, H. Sanwen, and J.F. Zhang. 2010. Transcriptome sequencing and comparative analysis of cucumber flowers with different sex types. *BMC Genomics* 11:384.
- Hou, X.G., D.L. Guo, S.P. Cheng, and J.Y. Zhang. 2011. Development of thirty new polymorphic microsatellite primers for *Paeonia suffruticosa*. *Biol. Plant.* 55:708-710.
- Kumar, S., N. Shah, V. Garg, and S. Bhatia. 2014. Large scale in-silico identification and characterization of simple sequence repeats (SSRs) from denovo assembled transcriptome of *Catharanthus roseus* (L.). *G. Don. Plant Cell Rpt.* 33:905-918.
- Li, X.B., L. Xiang, J. Luo, B.L. Hu, S.P. Tian, M. Xie, and C. Sun. 2013. The strategy of RNA-seq, application and development of molecular marker derived from RNA-seq. *Chinese J. Cell Biol.* 35:1-7.
- Liao, L., C.J. Bai, X.L. Guo, and Z.Y. Wang. 2011. Morphological diversity of *Chrysopogon aciculatus*. *Chinese J. Trop. Crops* 32:2042-2047.
- Liu, J.X., S.A. He, and Y.D. Liu. 1997. Characteristics and economic value of warm season turfgrasses in east China. *Grassland China* 4:62-66.
- Liu, J.X., Y.D. Liu, S.A. He, and S.L. Chen. 1998. Species diversity of Chinese warm season

- turfgrass and the characteristic of geography distribution. *Acta Agrestia Sinica* 6:45–52.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27:209–220.
- Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA* 70:3321–3323.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 76:379–390.
- Nichols, K.M., W.P. Young, B.D. Robison, C. Rexroad, M. Noakes, R.B. Phillips, P. Bentzen, I. Spies, K. Knudsen, F.W. Allendorf, B.M. Cunningham, J. Brunelli, H. Zhang, S. Ristow, R. Drew, K.H. Brown, P.A. Wheeler, and G.H. Thorgaard. 2003. A consolidated linkage map for rainbow trout (*Oncorhynchus mykiss*). *Anim. Genet.* 34:102–115.
- Reddi, C.S., N.S.N. Raju, and M.V.S. Rao. 2010. Pollination and seed set in tropical wetland grasses. *Nord. J. Bot.* 28:354–365.
- Rohlf, F.J. 2000. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 2.1. Exeter Publ., Setauket, NY.
- Rozen, S. and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers, p. 365–386. In: S. Krawetz and S. Misener (eds). *Bioinformatics methods and protocols: Methods in molecular biology*. Humana Press, Totowa, NJ.
- Sneath, P.H.A. and R.R. Sokal. 1973. Numerical taxonomy. Freeman, San Francisco, CA.
- Sun, J.X. 1995. Turf science. China Agriculture Press, Beijing, China.
- Swarbreck, S.M., E.A. Lindquist, D.D. Ackerly, and G.L. Andersen. 2011. Analysis of leaf and root transcriptomes of soil-grown *Avena barbata* plants. *Plant Cell Physiol.* 52:317–332.
- Tani, N., N. Tomaru, Y. Tsumura, A. Masayuki, and O. Kihachiro. 1998. Genetic structure within a Japanese stone pine (*Pinus pumila* Regal) population on Mt. Aino-dake in Central Honshu. *Plant Res.* 111:7–15.
- Toth, G. and Z.J. Gaspari. 2000. Microsatellites in different eukaryotic genomes: survey and analysis. *Genome Research* 10:967–981.
- Wang, Z., K.E. Kenworthy, and Y.Q. Wu. 2010. Genetic diversity of common carpetgrass revealed by amplified fragment length polymorphism markers. *Crop Sci.* 50:1366–1374.
- Wang, Z.Y., L. Liao, X.J. Yuan, H.L. Guo, A.G. Guo, and J.X. Liu. 2013. Genetic diversity analysis of *Cynodon dactylon* (bermudagrass) accessions and cultivars from different countries based on ISSR and SSR markers. *Biochem. Syst. Ecol.* 46:108–115.
- Wang, X.L. and Z.Y. Wang. 2015. Genetic diversity of carpetgrass germplasm based on simple sequence repeat markers. *HortScience* 50:797–800.
- Wang, M., F. Xue, P. Yang, X.Y. Duan, Y.L. Zhou, C.Y. Shen, G.Z. Zhang, and B.T. Wang. 2014. Development of SSR markers for a phytopathogenic fungus, *Blumeria graminis* f.sp. *tritici*, using a FIASCO protocol. *Integr. Agr.* 13:100–104.
- Wagner, W.L., D.R. Herbst, and S.H. Sohmer. 1999. Manual of the flowering plants of Hawaii, p. 1516–1517. Revised edition. University of Hawaii Press, Honolulu, HI.
- Xu, Y.Q., Y. Wang, Z.Z. Li, Z.X. Bao, J.F. Zhou, and H.W. Huang. 2006. Characterization of polymorphic microsatellite loci in a traditional Chinese medicinal plant, *Gastrodia elata*. *Mol. Ecol. Notes* 6:316–318.
- Yang, J.B., J. Yang, H.T. Li, Y. Zhao, and S.X. Yang. 2009. Isolation and characterization of 15 microsatellite markers from wild tea plant (*Camellia taliensis*) using FIASCO method. *Conserv. Genet.* 10:1621–1623.
- Yeh, F.C. and T.J.B. Boyle. 1996. Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belg. J. Bot.* 129:157.
- Zane, L., L. Bargelloni, and T. Patarnello. 2002. Strategies for microsatellite isolation: A review. *Mol. Ecol.* 11:1–16.
- Zhang, J., X. Yao, X. Wei, L. Chen, and M. Jiang. 2008. Development and characterization of 14 polymorphic microsatellite loci in the endangered tree *Euptelea pleiospermum* (Eupteleaceae). *Mol. Ecol. Resour.* 8:314–316.
- Zheng, Y.Z., J.B. Xi, and Z.Y. Yang. 2005. Studies on distribution and morphological variation of wild grass *Chrysopogon aciculatus* collected from the tropics and subtropics of China. *Acta Agrestia Sin.* 3:117–122.