RESEARCH ARTICLE

Genetic variability and relationship between MT-1 elephant grass and closely related cultivars assessed by SRAP markers

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Abstract

Genetic variability and relationships among elephant grass cultivars were estimated by the SRAP (sequence-related amplified polymorphism) assay. A total of 60 individuals collected from five cultivars in China were analysed. Sixty-two selected primer combinations generated 1395 bands, with an average of 22.5 per primer combination. The average value of percentage of polymorphic bands (PPB) was 72.8% at species level. The PPB was from 15.2% to 75%, with an average of 39.6% at cultivar level. H_{POP}, within-cultivar Shannon's index was 1.738 at cultivar level; at species level, the Shannon's index (H_{SP}) was 3.880. An assessment of diversity between cultivars $[(H_{SP} - H_{POP})/H_{SP}]$ indicated that most of the diversity (55.2%) was detected among cultivars, and only 44.8% was within cultivars in total genetic variation. According to UPGMA dendrogram, the five cultivars were clustered into three main groups. One group included MT-1 and Mott with a bootstrap support of 100%, another consisted of Huanan and N51 with a bootstrap support of 81%, and last one was only Guimu-1. The results indicate that the MT-1 and Mott have a closest genetic relationship; Huanan and N51 possess a relatively close relationship, and Guimu-1 is the most distinct from the other four cultivars.

[Xie X-M., Zhou F., Zhang X-Q. and Zhang J-M. 2009 Genetic variability and relationship between MT-1 elephant grass and closely related cultivars assessed by SRAP markers. *J. Genet.* **88**, 281–290]

Introduction

Elephant grass (*Pennisetum purpureum* Schum.) is a tropical C4 bunch grass with high rate of growth and biomass production, native throughout humid, tropical mainland Africa and the island of Bioko (Holm *et al*. 1977; Burkill 1994). It has been planted for forage and has been naturalized in many tropical areas in Asia, the Americas, Oceania, the Middle East, Australia and the Pacific islands, and much effort has been devoted to determine its palatability and nutritional values as an alternative forage crop (Wang *et al*. 2002). This species is also cut for hay and fermented for silage. A number of forage samples of different ages of grass from several countries varied from 4 to 15 per cent in crude protein, 28 to 40 per cent in crude fiber, 10 to 16 per cent in ash, 0.9 to 3.8 per cent in fat, and 39 to 49 per cent in nitrogen-free extract (Skerman and Riveros 1990). This species can be planted as hedgerows for erosion protection and forage production in the alley cropping system of agroforestry (Magcale-Macandog *et al*. 1998). It is also effective as a windbreak for agricultural crops (Karschon and Heth 1958). In Africa, this plant is used for thatch, and the thick culms are made into fences, screens, and reinforcement for mud huts. The young leaves and shoots are eaten in soups and stews (Burkill 1994). Elephant grass is used for mulch in East Africa where a 25-cm depth of mulch is needed for good weed control (Nishimoto 1994). Extracts of the plant are strongly diuretic and are used in Africa for this purpose. It is also used in a number of other herbal remedies (Burkill 1994). In China, the cultivars of *Pennisetum* are mainly used as forage and raw materials for paper, sometimes as culture substrate for mushroom (Zhou *et al*. 2007). As of 2003, there were nine cultivars registered and a few local cultivars unregistered in the Chinese Herbage Cultivar Registration Board in China (Wu 1999; CHCRB 2001, 2002, 2003).

DNA markers revealing polymorphism at DNA level have been shown to be a powerful tool for characterization and genetic diversity estimation. Tinker *et al*. (1993) sug-

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Keywords. genetic diversity; cluster analysis; DNA markers; Shannon information index; *Pennisetum purpureum*.

gested that the diversity estimates based on molecular markers are better suited than pedigree data for parental selection. The use of molecular markers for diversity analysis can also serve as a tool to discriminate between closely related individuals from different breeding sources (Lombard *et al*. 2000) and may help to eliminate redundancy in phenotype base germplasm collections. To probe into the genetic relationships among elephant grass cultivars, including some new lines, the RAPD molecular marker technique was employed (Xie and Lu 2005; Chen *et al*. 2007). Recently, a new marker system, sequence-related amplified polymorphism (SRAP) was developed by Li and Quiros (2001), which aimed at the amplification of open reading frames (ORFs). It is a PCR-based marker system with two primers, a forward primer of 17 bases and a reverse primer of 18 bases. The forward primers consist of a core sequence of 14 bases. The first 10 bases starting at the 5′ end are 'filler' sequences of no specific constitution, followed by the sequence CCGG and then by three selective nucleotides at the 3′ end. Variation in these three selective nucleotides generates a set of primers sharing the same core sequence. The reverse primers consist of the same components as the forward primers with the following variations: the filler is followed by AATT instead of the CCGG sequence. Following the AATT sequence, three selective bases are added to the 3′ end of the primer. SRAP marker system is a simple and efficient marker system that can be adapted for a variety of purposes in different crops, including map construction, gene tagging, genomic and cDNA fingerprinting, and map based cloning. It has several advantages over other systems: simplicity, reasonable throughput rate, discloses numerous co-dominant markers, allows easy isolation of bands for sequencing and, most importantly, it targets ORFs (Li and Quiros 2001). This marker system has been demonstrated to be more powerful for revealing genetic diversity among closely related cultivars than SSR, ISSR and RAPD markers on buffalo grass (Budak *et al*. 2004).

In this study, SRAP markers were used to examine genetic variation in elephant grass cultivars. The objectives were: (i) to investigate if SRAP data can reveal the genetic relationships and diversity among different elephant grass cultivars and among individuals within cultivar; especially the relationships between MT-1 (a new line of elephant grass) and others, and (ii) to compare the difference and similarity of results assessed by SRAP markers and by RAPD markers. The results from this study will be beneficial for the breeding of elephant grass, and to provide a rapid and effective way for the germplasm identification and other molecular biological research of elephant grass.

Materials and methods

Plant material and DNA extraction

Five elephant grass genotypes, including a hybrid between pearl millet (*P. americanum* (L.) Leeke) and elephant grass, were obtained from the Forage Introduction Garden of South China Agricultural University, PR China. These genotypes were introduced from USA and Indonesia, and some are new cultivars and lines bred in China (table 1).

Fresh leaf tissue of 12 individuals of each genotype was used for DNA extraction. The procedure for total genomic DNA extraction was based on that of Doyle and Doyle (1987) with some modifications. DNA quality and quantity were determined visually from the band intensities following standard horizontal electrophoresis on 1.0% agarose gels and ethidium bromide staining.

SRAP procedure

SRAP analysis was conducted according to previously established protocols (Li and Quiros 2001). In this assay, 62 different primer combinations were employed using 12 forward primers and 17 reverse primers combined randomly (table 2). Polymerase chain reaction was performed in 25-μL reaction mixture with a final composition of 2.5-μL 10x buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTP (dATP, dCTP, dGTP and dTTP), 1 U *Taq* DNA polymerase, 0.5-μM primer, 50-ng DNA template. The amplifications were carried out in a PTC-100TM Programmable Thermal Controller (MJ Research, Watertown, USA) with a heated lid

Table 1. Elephant grass genotypes evaluated in this study, together with a description of their origin.

Cultivar	Species	Individual number	Germplasm origin	Citation
MT-1	*P. purpureum* Schum. cv. MT-1	12	A new line selected from Mott, 1999	Unregistered
Huanan	*P. purpureum* Schum. cv. Huana	12	Indonesia, 1960	Registered in 1990 in China (Wu 1999)
Mott	*P. purpureum* Schum. cv. Mott	12	USA, 1987	Registered in 1994 in China (Wu 1999)
N51	*P. purpureum* Schum. cv. N51	12	USA, 1985	Unregistered in China (Chen et al. 1991)
Guimu-1	(*P. americanum* × *P. purpureum*) × *P. purpureum* cv. Guimu No.1 or (*P. americanum* cv. Tift23A × N51) × Mott	12	Improved cultivar from hybrid between Hybrid and Mott, 2000, Guangxi, PR China	Registered in 2000 in China (CHCRB 2001)

Table 2. Information of the 29 primers used for SRAP analysis.

Forward primer (name: sequence)	Reverse primer (name: sequence)
ME1: 5-TGAGTCCAAACCGGATA-3′	EM1: 5′-GACTGCGTACGAATTAAT-3′
ME2: 5′-TGAGTCCAAACCGGAGC-3′	EM2: 5′-GACTGCGTACGAATTTGC-3′
ME3: 5′-TGAGTCCAAACCGGAAT-3′	EM3: 5′-GACTGCGTACGAATTGAC-3′
ME4: 5′-TGAGTCCAAACCGGACC-3′	EM4: 5′-GACTGCGTACGAATTTGA-3′
ME5: 5′-TGAGTCCAAACCGGAAG-3′	EM5: 5′-GACTGCGTACGAATTAAC-3′
ME6: 5′-TGAGTCCAAACCGGTAA-3′	EM6: 5′-GACTGCGTACGAATTGCA-3′
ME7: 5′-TGAGTCCAAACCGGTCC-3′	EM7: 5′-GACTGCGTACGAATTCAA-3′
ME8: 5′-TGAGTCCAAACCGGTGC-3′	EM8: 5′-GACTGCGTACGAATTCTG-3′
ME9: 5′-TGAGTCCAAACCGGTAG-3′	EM9: 5′-GACTGCGTACGAATTCGA-3′
ME10: 5′-TGAGTCCAAACCGGTCT-3′	EM10: 5′-GACTGCGTACGAATTCAG-3′
ME11: 5′-TGAGTCCAAACCGGTGT-3′	EM11: 5′-GACTGCGTACGAATTCCA-3′
ME12: 5′-TGAGTCCAAACCGGCAT-3′	EM12: 5′-GACTGCGTACGAATTATG-3′
	EM13: 5′-GACTGCGTACGAATTAGC-3′
	EM14: 5′-GACTGCGTACGAATTACG-3′
	EM15: 5′-GACTGCGTACGAATTTAG-3′
	EM16: 5′-GACTGCGTACGAATTTCG-3′
	EM17: 5′-GACTGCGTACGAATTGTC-3′

under the flowing block temperature cycle. After an initial heat denaturation at 94°C for 5 min, the first five cycles were run at 94°C for 1 min, 35°C for 1 min and 72°C for 1 min, for denaturing, annealing and extension, respectively. Then the annealing temperature was raised to 48°C for another 35 cycles. The separation of amplified fragments was accomplished by 6% denatured polyacrylamide gel electrophoresis (PAGE) at 300 V for 2 h at room temperature. The amplified products were visualized by simplified silver staining method (Xu *et al*. 2002).

Data analysis

SRAP fragments were treated as a unit character coded as 1 (present) or 0 (absent) in each sample. Genetic diversity was estimated by the Shannon's information index (Gauer and Cavalli-Molina 2000):

$$H = -\sum_{i=1}^{K} p_i \log_e p_i,$$

where K is the number of SRAP bands, and p_i is the frequency of the ith band in a given cultivar. H is the SRAP diversity for each primer combination: the higher the H value, the greater the genetic diversity. These data were averaged to obtain estimates of within-cultivar SRAP diversity (H_{POP}), and SRAP diversity for the species (H_{SP}), which is also referred to as total genetic diversity of species, was calculated using band frequencies of all individuals. Using the data, Nei's (1973) coefficient of genetic differentiation among cultivars is given by $(H_{SP} - H_{POP})/H_{SP}$, and the percentage of polymorphic bands (PPB) were calculated. Meanwhile, the software of molecular evolutionary genetics analysis (MEGA 3.1) (Kumar *et al*. 1993) was applied to analyse the data. According to the working requirements of the software, the amplified products of SRAP was recorded as 'a' (if absent) or 't' (if present) in each individual. A matrix of genetic distance coefficients based on p-distance model in MEGA 3.1 was subjected to cluster analysis by UPGMA (un-weighted pair group method with arithmetic average) to develop a dendrogram. Statistical support of the clusters was assessed by means of 1000-bootstrap replicates.

Results

The SRAP polymorphism profile

The 62 selected primer combinations generated 1395 bands ranging in size from 100 to 2000 bp. The total number of bands scored per primer combination ranged from 10 (primer pair of ME4–EM3) to 39 (ME6–EM9, ME7–EM6 and ME10–EM16), with an average of 22.5 bands per primer combination. Of these bands, the polymorphic bands were from 33.3% (ME7–EM6) to 100% (ME4–EM2, ME5–EM16, ME6–EM9 and ME10–EM6) among 60 individuals, and the average of percentage of polymorphic bands (PPB) was 72.8% at species level. Among five cultivars, the polymorphic bands were from 15.2% (ME8–EM9) to 75% (ME8–EM8), and the average of PPB was 39.6% at cultivar level (table 3).

Within-cultivar Shannon's index H_{POP} ranged from 0.316 (ME8–EM11) to 4.708 (ME5–EM16), with an average of 1.738 at the cultivar level. At the species level, the Shannon's index (H_{sp}) ranged from 1.753 (ME4–EM3) to 9.790 (ME6–EM9), and the average was 3.880 (table 4).

We obtained 91 cultivar-specific bands, where five of them were for MT-1, eight for Mott, 18 for Huanan, 15 for N51 and 45 for Guimu-1. Figure 1 shows examples of SRAP profiles illustrating this variability.

Table 3. Primer combinations used for generating SRAP amplification, number of bands per primer combination and percentage of polymorphic bands (PPB) among cultivars and individuals.

Primer combinations	Total no. of bands	PPB among cultivars (%)	PPB among individuals (%)	Primer combinations	Total no. of bands	PPB among cultivars (%)	PPB among individuals (%)
ME1–EM2	24	25	58.3	ME8–EM8	12	75.0	91.7
ME1–EM7	24	37.5	45.8	ME8–EM9	33	15.2	60.6
ME1–EM10	35	57.1	65.6	ME8–EM11	16	43.8	56.3
ME1–EM12	27	40.7	55.6	ME8–EM12	20	45.0	75.0
ME2–EM1	18	38.9	83.3	ME8–EM15	26	26.9	53.9
ME2–EM2	17	52.9	76.5	ME8–EM17	19	15.8	73.7
ME2–EM6	27	29.6	66.7	ME9–EM1	18	55.6	83.3
ME2–EM9	16	68.8	75.0	ME9–EM2	21	42.9	95.2
ME2–EM12	25	52	68.0	ME9–EM4	27	18.5	40.7
ME2–EM17	19	36.8	42.1	ME9–EM5	15	53.3	73.3
ME3–EM13	12	50	75.0	ME9–EM6	21	33.3	57.1
ME3–EM15	20	40	60.0	ME9–EM7	22	27.3	77.3
ME4–EM2	22	36.4	100	ME9–EM9	25	48.0	80.0
ME4–EM3	10	40.0	90.0	ME9–EM11	30	36.7	66.7
ME4–EM5	14	50.0	78.6	ME9–EM12	20	25.0	65.0
ME4–EM9	30	43.3	96.7	ME9–EM14	21	33.3	57.1
ME4–EM17	17	41.2	94.1	ME9–EM16	19	36.8	79.0
ME5–EM1	15	40.0	73.3	ME9–EM17	17	47.1	82.4
ME5–EM13	12	50.0	66.7	ME10–EM1	17	29.4	82.4
ME5–EM15	22	22.7	40.9	ME10–EM5	27	40.7	66.7
ME5–EM16	24	54.2	100	ME10–EM6	33	42.4	100
ME6–EM1	29	48.3	96.6	ME10–EM7	28	35.7	71.4
ME6–EM2	19	31.6	57.9	ME10–M16	39	35.9	74.4
ME6–EM9	39	61.5	100	ME11–EM1	30	46.7	63.3
ME6–EM10	35	17.1	51.4	ME11–EM8	28	28.6	46.4
ME6–EM16	17	23.5	64.7	ME11–EM10	33	39.4	84.9
ME6–EM17	19	31.6	79.0	ME12–EM5	17	52.9	76.5
ME7–EM1	25	44	96.0	ME12–EM11	19	36.8	63.2
ME7–EM6	39	15.4	33.3	ME12EM14	17	29.4	88.2
ME8–EM1	20	35	80.0	ME12–EM15	19	47.4	89.5
ME8–EM3	13	53.9	84.6	ME12–EM17	21	42.9	81.0
				Mean	22.5	39.6	72.8

Table 4. Genetic diversity of five cultivars and partitioning of the genetic diversity within and between cultivars (Shannon's index) for the 62 primer combinations analysed.

Primer combinations	MT-1	Mott	Huanan	N51	Guimu-1	H_{POP}	H_{SP}	$(H_{SP} - H_{POP})/H_{SP}(\%)$
ME1–EM2	1.260	1.570	0.486	3.149	0.730	1.439	3.005	52.1
ME1–EM7	0.000	0.530	0.693	0.960	0.000	0.437	3.026	85.6
ME1–EM10	0.582	1.701	0.749	1.010	0.000	0.808	6.413	87.4
ME1–EM12	0.638	1.179	1.385	1.161	0.947	1.062	4.134	74.3
ME2–EM1	1.599	1.619	1.209	1.903	2.185	1.703	3.468	50.9
ME2–EM2	0.296	2.084	0.486	1.274	1.624	1.153	3.462	66.7
ME2–EM6	1.471	0.661	0.347	1.269	3.091	1.368	3.519	61.1
ME2–EM9	0.679	1.462	0.000	1.047	0.613	0.760	3.705	79.5
ME2–EM12	1.099	1.661	1.160	0.943	0.957	1.164	4.614	74.8
ME2–EM17	0.645	0.693	0.270	0.347	0.000	0.391	2.292	82.9
ME3–EM13	1.076	1.627	1.226	1.243	0.432	1.121	2.764	59.5
ME3–EM15	1.079	0.833	0.975	1.442	1.027	1.071	2.967	63.9
ME4–EM2	3.150	1.685	0.944	1.000	2.306	1.817	4.552	60.1
ME4–EM3	0.878	1.181	1.234	1.017	0.152	0.892	1.753	49.1
ME4–EM5	1.708	1.966	0.905	2.247	1.755	1.716	3.087	44.4

Table 4 *(contd)*

Primer combinations	MT-1	Mott	Huanan	N51	Guimu-1	H_{POP}	H_{SP}	$(H_{SP} - H_{POP})/H_{SP}(\%)$
ME4–EM9	2.620	2.776	5.110	4.794	3.794	3.819	7.661	50.2
ME4–EM17	2.027	1.789	1.079	1.687	1.771	1.671	3.554	53.0
ME5–EM1	1.549	0.080	1.596	0.810	1.425	1.092	2.540	57.0
ME5–EM13	0.833	0.299	1.045	2.150	0.661	0.998	2.362	57.8
ME5–EM15	0.585	0.909	0.661	0.000	1.327	0.696	2.197	68.3
ME5–EM16	3.113	2.901	7.006	5.204	5.316	4.708	6.988	32.6
ME6–EM1	2.501	4.323	5.383	4.556	4.072	4.167	7.852	46.9
ME6–EM2	0.757	0.314	1.291	1.106	1.624	1.018	2.286	55.4
ME6–EM9	5.223	2.540	1.808	3.640	4.244	3.491	9.790	67.7
ME6–EM10	1.676	0.873	1.437	0.232	1.575	1.159	2.631	56.0
ME6–EM16	1.418	1.328	1.597	1.582	0.879	1.361	2.195	38.0
ME6–EM17	1.697	1.932	2.938	3.392	1.613	2.314	4.252	45.6
ME7–EM1	4.194	3.050	2.150	1.764	2.872	2.806	6.484	56.7
ME7–EM6	0.679	1.646	1.989	2.692	0.714	1.544	3.123	50.6
ME8E–M1	2.041	1.474	1.557	1.194	1.710	1.595	2.929	45.5
ME8–EM3	1.286	1.073	1.591	2.616	1.579	1.562	3.150	50.4
ME8–EM8	1.286	0.693	1.906	0.347	3.265	1.499	2.807	46.6
ME8–EM9	2.857	2.830	1.954	0.962	2.168	2.154	2.807	23.3
ME8–EM11	0.299	0.299	0.365	0.270	0.347	0.316	2.510	87.4
ME8–EM12	2.024	1.235	1.203	0.574	1.274	1.262	3.438	63.3
ME8–EM15	2.434	1.365	1.751	1.171	0.893	1.523	3.337	54.4
ME8–EM17	3.105	0.850	2.053	1.503	1.696	1.842	2.613	29.5
ME9–EM1	3.633	1.629	1.990	1.152	1.533	1.988	3.988	50.2
ME9–EM2	2.021	0.964	1.668	2.023	1.316	1.598	3.318	51.8
ME9–EM4	2.010	2.223	1.211	1.253	1.343	1.608	2.957	45.6
ME9–EM5	1.500	1.330	1.999	0.845	1.111	1.357	3.284	58.7
ME9–EM6	1.141	1.439	0.671	1.777	0.448	1.095	1.993	45.1
ME9–EM7	1.833	1.786	0.868	1.751	1.331	1.514	3.535	57.2
ME9–EM9	2.041	3.769	0.347	2.156	3.726	2.408	5.380	55.3
ME9–EM11	2.682	1.967	2.143	2.927	2.556	2.455	5.029	51.2
ME9–EM12	2.744	1.724	1.213	2.540	1.546	1.953	2.860	31.7
ME9–EM14	2.203	1.446	1.542	1.413	1.093	1.539	3.118	50.6
ME9–EM16	2.387	2.083	1.291	1.751	2.366	1.976	3.688	46.4
ME9–EM17	2.260	1.788	1.263	2.633	2.682	2.125	3.597	40.9
ME10–EM1	2.679	3.506	1.894	1.221	1.691	2.198	3.599	38.9
ME10–EM5	1.786	2.211	2.428	2.332	1.742	2.100	4.465	53.0
ME10–EM6	3.453	3.661	3.355	3.527	2.688	3.337	7.753	57.0
ME10–EM7	3.017	2.890	3.539	3.801	4.157	3.481	5.313	34.5
ME10–EM16	3.452	2.342	3.058	2.808	3.503	3.032	5.915	48.7
ME11–EM1	1.176	1.551	1.100	1.892	1.138	1.371	4.668	70.6
ME11–EM8	0.674	1.197	1.826	1.442	2.637	1.555	3.686	57.8
ME11–EM10	2.442	3.828	2.663	1.567	2.472	2.594	5.414	52.1
ME12–EM5	0.566	0.986	1.096	0.530	0.937	0.823	3.230	74.5
ME12–EM11	2.321	1.215	1.720	0.499	0.831	1.317	3.303	60.1
ME12–EM14	1.934	1.772	2.098	2.348	2.371	2.105	3.156	33.3
ME12–EM15	1.147	1.083	0.994	1.701	1.530	1.291	3.638	64.5
ME12–EM17	0.893	1.751	1.291	1.908	1.402	1.449	3.425	57.7
Mean	1.812	1.696	1.658	1.775	1.755	1.738	3.880	55.2

H_{POP}, mean within-cultivar genetic diversity for all cultivars; H_{SP}, genetic diversity of species; $(H_{SP} - H_{POP})/H_{SP}$, coefficient of genetic differentiation among cultivars.

Within-cultivar variability

Many different SRAP patterns were detected within each cultivar of elephant grass. The number of polymorphic bands per primer combination ranged from 0 (primer pair of ME1–EM7) to 19 (ME6–EM9), with an average of 7.16 per primer combination for MT-1, while the

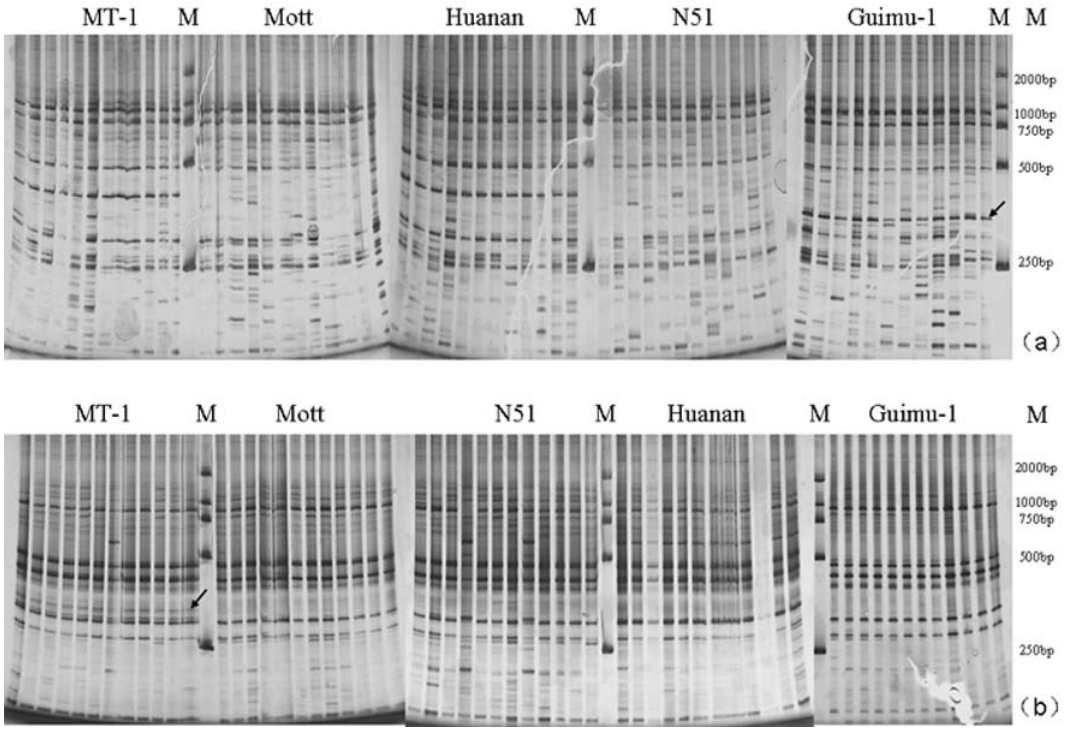

Figure 1. SRAP profiles for 60 accessions of five elephant grass cultivars. (a) primer combination of ME9–EM7, (b) primer combination of ME9–EM11. The bands arrowed are cultivar-specific bands for (a) 'Guimu-1', and (b) 'MT-1'.

PPB ranged from 0 (ME1–EM7) to 86.7% (ME5–EM16), with an average PPB of 40%. As for Mott, the number of polymorphic bands produced by each primer pair ranged from 1 (ME5–EM1, ME5–EM13, ME6–EM2 and ME8–EM11) to 16 (ME11–EM10), with an average of 6.45, while the PPB varied from 7.7% (ME6–EM2) to 70% (ME6–EM1), with an average PPB of 36.4%.The number of polymorphic bands per primer pair ranged from 0 (ME2–EM9) to 21 (ME5–EM16), with an average of 6.4 in Huanan, while the PPB varied from 0 (ME2–EM9) to 87.5% (ME5–EM16), and the average PPB was 34.5%. As regards N51, the PPB ranged from 0 (ME5–EM15) to 82.6% (ME4–EM9), with an average PPB of 38.7%, and the average number of polymorphic bands per primer pair was 7.10. The PPB of Guimu-1 ranged from 0 (ME1–EM7, ME1–EM10 and ME2–EM17) to 84.2% (ME6–EM1), with an average of 37.1%. The average number of polymorphic bands per primer pair was 6.8. The order of PPB from higher to lower was MT-1 (40%) > N51 (38.6%) > Guimu-1 (37.1%) > Mott (36.4%) > Huanan (34.5%).

The average cultivar diversity using the Shannon's information index was 1.812, and ranged from 0 (ME1–EM7) to 5.223 (ME6–EM9) for MT-1, 1.696 (ranged from 0.080 (ME5–EM1) to 4.323 (ME11–EM10)) for Mott, 1.658 (ranged from 0 (ME2–EM9) to 7.006 (ME5–EM16)) for Huanan, 1.775 (ranged from 0 (ME5–EM15) to 5.204 (ME5–EM16)) for N51 and 1.755 (ranged from 0 (ME1–EM7, ME1–EM10 and ME2–EM17) to 5.316 (ME5–EM16)) for Guimu-1 (table 4). The order of Shannon's index from higher to lower is same as that of PPB among different cultivars. According to the ANOVA, however, the within-cultivar variability between cultivars was statistically not significant (P = 0.94 for Shannon information index; P = 0.543 for PPB).

Figure 2 shows a dendrogram for the individuals from the five cultivars. Grouping of plants from each cultivar was observed on the dendrogram. The individuals from the five cultivars were clustered into five groups, and supported by bootstrap value of 100%, respectively. This result may indicate a greater similarity within cultivars than between cultivars for every cultivar.

Between-cultivar diversity

From what was mentioned above, the PPB was 40% for MT-1, 36.4% for Mott, 34.5% for Huanan, 38.7% for N51 and 37.1% for Guimu-1, and the average within cultivars was 37.4%, while the PPB between cultivars was 39.6% (table 3), indicating the genetic diversity between cultivars was bigger than that within cultivars. Shannon's index of phenotypic diversity was used to partition SRAP diversity into within-cultivar and between-cultivar components (table 4). The average diversity of SRAP markers for elephant grass (H_{SP}) was 3.88, and an assessment of diversity present between cultivars [$(H_{SP}-H_{POP})/H_{SP}$] indicated that most of the diversity

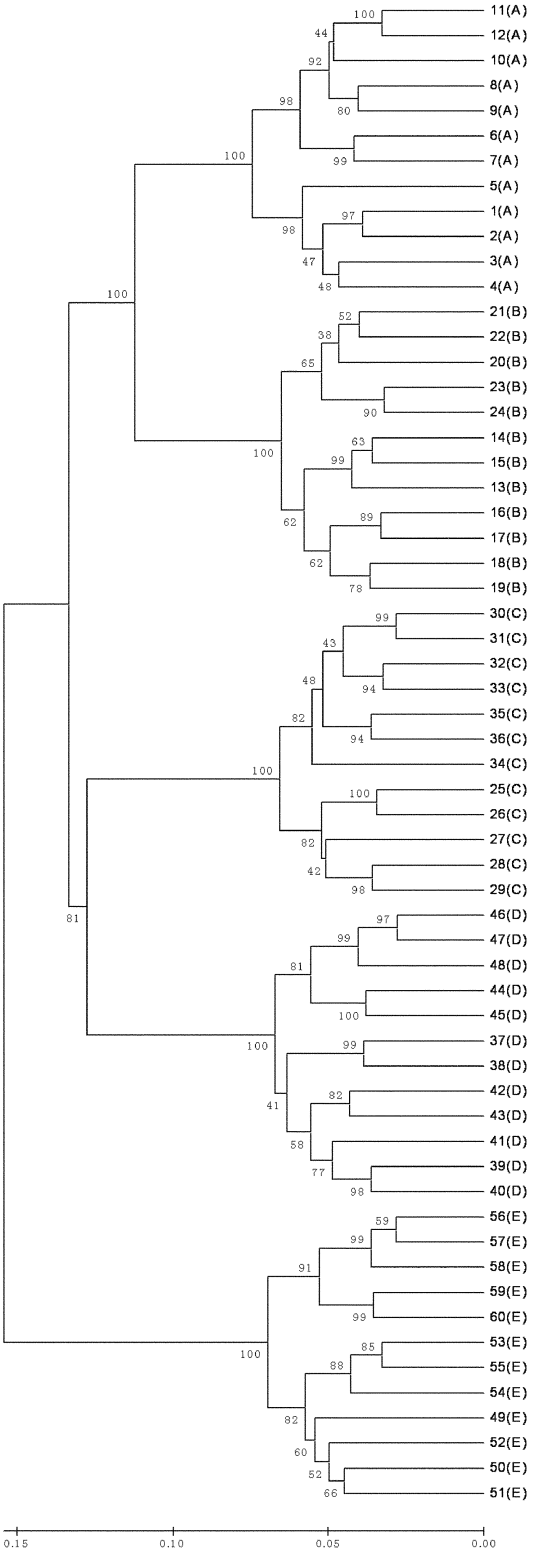

Figure 2. UPGMA dendrogram based on p-distance model of MEGA 3.1 for elephant grass cultivars. Numbers at nodes are percentage over 1000-bootstrap replicates. A, MT-1; B, Mott; C, Huanan; D, N51; E, Guimu-1.

(55.2%) was detected between cultivars, and only 44.8% was within cultivars in total genetic variation.

UPGMA method of clustering was carried out to estimate genetic relationships among five cultivars (figure 2). The five cultivars were clustered into three main groups. One group included MT-1and Mott with a bootstrap support of 100%, another consisted of Huanan and N51 with a bootstrap support of 81%, and the last one was only Guimu-1. The results indicate that MT-1 and Mott have a closest genetic relationship; Huanan and N51 possess a relatively close relationship, and Guimu-1 is the most distinct from the other four cultivars which clustered into one group again.

Discussion

Genetic variability within and between cultivars

Several studies demonstrated considerable differences among elephant grass cultivars at DNA level (Daher *et al*. 2002; Xie and Lu 2005; Chen *et al*. 2007), but no study has been conducted on the genetic variation within cultivars. In this study, the SRAP markers were used for the first time to study both within-cultivar and between-cultivar variations. The results showed that the PPB was 40.04% for MT-1, 36.42% for Mott, 34.52% for Huanan, 38.65% for N51 and 37.13% for Guimu-1, and the Shannon's index was 1.812 for MT-1, 1.696 for Mott, 1.658 for Huanan, 1.775 for N51, and 1.755 for Guimu-1 (table 4), indicating that there are the genetic variations within cultivars, but the degree of variation is different for different cultivars. MT-1 is a new line selected from Mott population in 1999, and it displayed a biggest genetic variation in five cultivars. It is clear that MT-1 still has a potential of genetic differentiation further, and other new lines may be bred from it.

The PPB was 72.8% at species level in this study, showing a high genetic diversity, similar to that obtained by RAPD markers (Xie and Lu 2005; Chen *et al*. 2007). In this total genetic variation, however, the proportion of within cultivars was 44.8%, leaving 55.2% of the diversity between cultivars. Population genetic structures are usually affected by a number of factors including breeding system, genetic drift, population age and size, environmental heterogeneity, seed dispersal, gene flow, genetic drift, evolutionary history, life history, as well as natural selection (Loveless and Hamrick 1984; Hamrick and Godt 1990). In general, outcrossing species commonly have higher levels of genetic diversity and lower differentiation among populations than selfing and clonal plants (Rossetto *et al*. 1995). Elephant grass cultivars belong to the clonal plants with low seed setting and seed germination rate such that they are spread by means of asexual reproduction of stem in field. Therefore, gene flow among cultivars is low, and the most genetic variation resides between cultivars rather than within cultivars. It is also the pressure of artificial selection that brings about the higher levels of genetic differentiation among the cultivars.

Pedigree relationships among cultivars

In China, there are nine registered and several unregistered cultivars for the genus *Pennisetum*. Some are from elephant grass (*P. purpureum* Schum.), some are from pearl millet (*P. americanum* (L.) Leeke), some are from the hybrids of elephant grass and pearl millet, and one is from kikuyu grass (*P. dandestinum* Höchst. Ex. Chiov.). Of these, only Mott dwarf elephant grass, Huana elephant grass, N51 elephant grass are derived from the species *P. purpureum* Schum. In the experimental cultivars, although Guimu-1 hybrid elephant grass is bred from the hybrids of elephant grass and pearl millet, it is a triple hybrid ((*P. americanum* cv.Tift23A × N51) × Mott) (Wu 1999; CHCRB 2001), which has more genetic material of elephant grass than the single cross hybrid of elephant grass × pearl millet.

MT-1 elephant grass is a new line gained occasionally from Mott population in 1999. It is the major research material, and we plan to register it as a cultivar in China, because it has similar characteristics on the texture and indumentum of leaf, either metaphylla or spire, but the height and the biomass yield of MT-1 are much higher than that of Mott (You *et al*. 2004). To reveal the genetic variability and relationships between MT-1 and other cultivars in *Pennisetum*, these four closely related genotypes, such as Mott, Huanan, N51 and Guimu-1, were treated as control materials, owing to the unambiguous pedigree relationships amongst the five genotypes.

Genetic relationships among cultivars

The result of cluster analysis indicates that MT-1 and Mott are the closest cultivars in genetic relationship, and that Guimu-1 is the most distinct one (figure 2). This finding is consistent with the result from RAPD markers (Chen *et al*. 2007) and similar to the result obtained by Xie and Lu (2005). At the same time, the closest genetic relationship between MT-1and Mott is also in agreement with their pedigree relationships. It was reported that open-pollinated progenies of Mott and 7262 (new strains of tall elephant grass) were used to investigate the effects of dwarfing genes on forage yield and chemical composition (Cheng *et al*. 1995). Progenies from open-pollinated Mott segregated into dwarf and tall plants, and most of the progenies from open-pollinated 7262 were tall plants. Hybrids from Tift 85DB (dwarf pearl millet (*Pennisetum glaucum*)) × Mott segregated into dwarf and extreme dwarf plants, but most of the hybrids from Tift 85DB ×7262 were tall. These results showed that Mott was heterozygous and has dwarf genes. Intergenomic dominance and/or epistasis might be involved in the inheritance of tallness in *Pennisetum* interspecific hybrids. In the yield trial, strain 7728 selected from Mott open-pollinated progenies was better than Mott for quality, and also better than Mott for yield by 8%. There were positive correlations between forage yield and plant height, leaves and stem diameter. In quality, there was a positive correlation between leaf/stem ratio and crude protein, and a negative correlation between leaf/stem ratio and plant height (Cheng *et al*. 1995). The MT-1 selected by us is similar to the strain 7728 in yield and plant height, so we speculate that MT-1 may be the open-pollinated progenies of Mott, but needed more evidences.

'Guimu-1' is the most distinct cultivar from others (figure 2), because it is a triple hybrid ((*P. americanum* × *P. purpureum*) × *P. purpureum*). Its genome consists of genes from both elephant grass and pearl millet, while other four cultivars contain only the genetic materials of elephant grass. Hence Guimu-1, with 45 cultivar-specific SRAP bands, has very different genetic component from other four cultivars. The cluster analysis result just reflects the genetic divergence between cultivars, and the divergence will increase during the long term cultivation and selection.

Research value of genetic diversity

In striving to slow or halt the loss of biodiversity, the conservation of diversity within species has been recognized as fundamentally important. The value of such intraspecific genetic diversity is evident from the often deleterious impacts of its loss on populations through effects such as increased inbreeding and genetic drift (Oostermeijer *et al*. 2003; Frankham 2005). Likewise, genetic diversity has a fundamental role in both the evolutionary history and future evolutionary trajectory of a species (Crozier 1997; Mace *et al*. 2003; Jump *et al*. 2009). Genetic diversity is the raw material for evolution, and all species have arisen via an evolutionary walk where each step depends on the variation present at the last. Although not every genetic variant is potentially adaptive, a proportion will be, even if most of the genetic variation within a population remains effectively neutral throughout its lifetime. When subjected to environmental change, genetic diversity therefore has a value that is likely to be proportional to its amount. Increasing evidence shows that maintaining genetic diversity within natural populations can maximize their potential to withstand and adapt to biotic and abiotic environmental changes (Jump *et al*. 2009).

Effects of genetic diversity on plant productivity can arise as a consequence of the combined effects of genotypic replacement (selection) or complementarity. Assuming that different genotypes of a species differ in their productivity, as the number of genotypes within a population increases, so does the probability that the population will include a genotype that is unusually productive. If highly productive genotypes are better competitors within a mixture, then increased genetic diversity can lead to increased productivity through this sampling effect and subsequent selection for increased abundance of the most productive genotype (Jump *et al*. 2009). In many ways, the effects described at the intra-population level are mirrored by those at the intra-individual level. Essentially, this is the notion of heterozygote advantage (Ding and Goudet 2005), where, in its simplest definition, the fitness of an individual having two different alleles at a given locus (heterozygote) is higher than the fitnesses of

individuals with two copies of either allele (homozygotes). The MT-1 elephant grass is a heterozygous cultivar with high genetic diversity, and has great potential of adapting in a new environment. Therefore, it is worth being conserved and extended.

As the important forage resources, elephant grass is extensively planted around the world, and generates many new cultivars and lines, resulting in increasingly genetic differentiations under the condition of artificial selection. In this case, it is very difficult to identify so many different germplasm resources relying solely on the traditional classification. The DNA marker technique is useful for correct identification of cultivars. Correct identification of a cultivar is an important step in a breeding programme, to ensure the right line for breeding purposes is chosen and not the same line under different names. Fingerprinting keys can also be used for protection of new cultivars. Similarly, a dendogram is a practical way to show relationships among cultivars tested. When using new or distinct materials for a breeding programme, the dendogram will show the distance between new or distinct materials and existing cultivars. This will assist plant breeders in choosing which cultivars will be used in their breeding programme. In this study, SRAP showed more clear, reproducible, stable and abundant bands, as well as a high level of polymorphism. For example, the average amplified bands per primer was 4.5 (Daher *et al*. 2002), 8.9 (Xie and Lu 2005) or 11.7 (Chen *et al*. 2007) using RAPD markers to asses genetic diversity of elephant grass, but the average of 22.5 bands per primer combination for SRAP markers. Therefore, the SRAP markers can be used for detecting genetic variations between elephant grass cultivars and such information could be useful to determine optimal breeding strategies to allow continued progress in elephant grass breeding.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (no. 30671489) and Guangdong Project Technologies Program (no. 2006A10703003).

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Received 5 February 2009, in revised form 10 May 2009; accepted 26 May 2009
Published on the Web: 21 October 2009