



Phenotypic Variation and Genetic Diversity in the Collections of *Erianthus Arundinaceus* (Retz.) Jesw.

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Abstract In order to provide information for further research and utilization of *Erianthus arundinaceus* (Retz.) Jesw., the phenotypic variation and genetic diversity in the collection of *E. arundinaceus* (Retz.) Jesw. germplasm in Guangxi, China were investigated. The coefficient of variation (CV) in four quantitative traits, plant height, stalk diameter, spikelet length and number of panicle nodes, of 50 *E. arundinaceus* (Retz.) Jesw. clones ranged from 12.71 to 16.14 %, stalk diameter had the highest coefficient of variation, followed by spikelet length, and number of panicle nodes showed the lowest. Fifteen start condon targeted polymorphism (SCoT) marker primers were used to assess the genetic diversity of the 50 collections of *E. arundinaceus* (Retz.) Jesw. germplasm. A total of 336 genotypes were investigated for SCoT polymorphism, which produced 284 amplicons with 83.97 % polymorphism, indicating that *E. arundinaceus* (Retz.) Jesw. germplasm had abundant diversity in Guangxi. The Jaccard's similarity coefficients among these accessions

varied from 0.61 to 0.88 with an average of 0.71. The UPGMA clustering analysis showed the 50 *E. arundinaceus* (Retz.) Jesw. accessions could be divided into four major groups at the similarity coefficient level 0.73. The UPGMA dendrogram reflected the geographic distribution patterns of these accessions.

Keywords *Erianthus arundinaceus* (Retz.) Jesw. · Phenotypic variation · Genetic diversity · SCoT

Introduction

Sugarcane is the most important sugar crop in tropical and subtropical regions of the world, and it has also become a very important bio-energy crop recently (Li 2010; Grivet and Arruda 2001). The cane sugar production accounts for over 90 % of total consumable sugar in China (Que et al. 2014). However, narrow genetic background is now the main restrictive factor in sugarcane breeding. Modern sugarcane cultivars have complex polyploidy genomes resulted from the crosses among several species of the *Saccharum* genus, namely *S. officinarum*, *S. spontaneum* and *S. barberi* (Li 2010). Since it is difficult to produce advantageous breakthrough through crossing between modern sugarcane varieties, and thus, widening the genetic basis of sugarcane with more wild germplasm is regarded as one of the key approaches for making a significant progress in sugarcane breeding. *Erianthus arundinaceus* (Retz.) Jesw. is the important wild germplasm of sugarcane with many useful characteristics for breeding, including strong vegetative vigor, good tillering ability and strong resistance to abiotic and biotic stresses such as drought and mosaic virus (Nair et al. 1993; Li 2010). However, research on the main quantitative traits variation and genetic

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diversity of *E. arundinaceus* (Retz.) Jesw. in China is limited.

In recent years, molecular markers have been widely used in crop genetic diversity analysis, offspring identification and gene mapping, etc. (Chen et al. 2010; Cai et al. 2012; Liu et al. 2012a, b; Su et al. 2012; Luo et al. 2013; Song et al. 2013; Zhang et al. 2013; Kalwade and Devarumath 2014; Sharma et al. 2014; Hu et al. 2015; Nerkar et al. 2015; Priya et al. 2005; Rajesh et al. 2015; You et al. 2015; Satya et al. 2015; Zan et al. 2015; Feng et al. 2016). Among the molecular markers reported, start codon targeted polymorphism (SCoT) has been proven to be effective for analyzing crop genetic diversity and clustering relationship (Collard and Mackill 2009; Xiong et al. 2010; Wu et al. 2010, 2013; Chen et al. 2012; Zhao et al. 2012; Wei et al. 2012). In the present study, we analyzed the phenotypic variation, genetic diversity and clustering relationship of fifty accessions of *E. arundinaceus* (Retz.) Jesw. collected in Guangxi, China, in order to provide a reference for further research and utilization of the wild germplasm in sugarcane improvement.

Materials and Methods

Plant Materials

The experiment was conducted at Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences/Sugarcane Research Center, Chinese Academy of Agricultural Sciences, Nanning, China in 2015. Fifty *E. arundinaceus* (Retz.) Jesw. accessions were collected from its originally distributed areas in Guangxi, China, and preserved at the nursery of Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences. All the 50 accessions could be distinguished morphologically. The detailed information of the studied accessions is summarized and presented in Table 1.

Traits Survey

The experiment was laid out in a randomized block design with three replications. Each accession was accommodated in a plot having three rows of 5 m in length with spacing 1.2 m between rows. The field management was done as for commercial sugarcane production. Observations were recorded for four quantitative traits viz. plant height, stalk diameter, spikelet length and number of panicle nodes.

DNA Extraction

Genomic DNA was extracted from approximately 0.5 g of fresh leaf samples using a modified SDS method as

described by Liu et al. (2012b). The quantity and quality of extracted DNA were verified using the spectrophotometer and agarose gel electrophoresis and then stored at $-20\text{ }^{\circ}\text{C}$ for PCR amplification.

SCoT Marker Analysis

SCoT reaction condition and protocol were adapted from the previous reports with minor modifications (Wu et al. 2010; You et al. 2015). All primers were synthesized by Shanghai Sangon Biotech (Shanghai, China) (Table 2). PCR amplification of these primers was carried out in a thermal cycle in 20 μL volume, with the reaction mixture containing 2 μL of $10\times$ PCR buffer, 0.4 μL dNTPs (10 mM), 2 μL primer (10 μM), 0.2 μL *Taq* DNA polymerase (5 U/ μL) and 0.5 μL template DNA (50 ng/ μL). Cycling conditions were: initial denaturation at $94\text{ }^{\circ}\text{C}$ for 4 min followed by 36 cycles of $94\text{ }^{\circ}\text{C}$ for 50 s, $50\text{ }^{\circ}\text{C}$ for 40 s, $72\text{ }^{\circ}\text{C}$ for 2 min and final extension at $72\text{ }^{\circ}\text{C}$ for 5 min. After amplification, PCR products were run on a polyacrylamide gel as described for the SCoT products.

Data Analysis

Only clearly amplified products from the SCoT primers were used for further analysis. The bands were scored as absent (0) or present (1). Software package NTSYS-pc version 2.0 was used for further analysis using the scored results. Genetic similarity analysis between the accessions was estimated using similarity matrix, generated by calculating Jaccard's similarity coefficient. These similarity coefficients were used for cluster analysis, and a dendrogram was constructed by the unweighted pair group method (UPGMA) and the sequential hierarchical and nested clustering (SHAN) routine in the NTSYS program. Four quantitative traits viz. plant height, stalk diameter, spikelet length and number of panicle nodes were analyzed with the coefficients of variation using software SAS.

Results

Phenotypic Variation Analysis

The variation range of four quantitative traits, plant height, stalk diameter, spikelet length and number of panicle nodes, of the 50 *E. arundinaceus* (Retz.) Jesw. clones was 12.71–16.14 % (Table 3). The range of plant height was 219–404 cm, and the average was 337 cm; the range of stalk diameter was 0.56–1.07 cm, and the average was 0.82 cm; the range of spikelet length was 40.2–80.4 cm, and the average was 59.7 cm; and the number of panicle nodes was 11–21, and the average was 17. There were

Table 1 Names and origins of tested *E. arundinaceus* (Retz.) Jews. clones in Guangxi, China

Name	Origin	Altitude (m)	Latitude	Longitude	Biotope
GUX01	Chuaitian, Lingchuan County, Guilin	197	25°57'	110°21'	Mid-level bushes
GUX02	Chuaitian, Lingchuan County, Guilin	201	25°36'	110°23'	Wilderness
GUX03	Chuaitian, Lingchuan County, Guilin	201	25°36'	110°23'	Wilderness
GUX04	Lingchuan County, Guilin	178	25°25'	110°20'	Wilderness
GUX05	Fushi, Rongan County, Hechi	107	25°60'	109°11'	Mid-level wilderness
GUX06	Fushi, Rongan County, Hechi, Guangxi	188	25°70'	109°22'	Mid-level wilderness
GUX07	Fushi, Rongan County, Hechi	188	25°70'	109°22'	Mid-level wilderness
GUX08	Fushi, Rongan County, Hechi	276	25°60'	109°23'	Mid-level wilderness
GUX09	Sanhe, Rongshui County, Liuzhou	140	25°30'	109°12'	Hilltop wilderness
GUX10	Sanhe, Rongshui County, Liuzhou	132	25°30'	109°12'	Low-level wilderness
GUX11	Sanhe, Rongshui County, Liuzhou	101	25°30'	109°13'	Low-level wilderness
GUX12	Lijian, Wuming County, Nanning	78	23°24'	108°07'	Hill wilderness
GUX13	Lijian, Wuming County, Nanning	78	23°24'	108°07'	Hill wilderness
GUX14	Lijian, Wuming County, Nanning, Guangxi	80	23°22'	108°22'	Hill wilderness
GUX15	Lijian, Wuming County, Nanning	81	23°22'	108°22'	Hill wilderness
GUX16	Lijian, Wuming County, Nanning	81	23°22'	108°22'	Hill wilderness
GUX17	Mendoutun, Luocheng County, Hechi	262	24°51'	109°10'	Mid-level bushes
GUX18	Mendoutun, Luocheng County, Hechi	262	24°51'	109°10'	Mid-level bushes
GUX19	Mendoutun, Luocheng County, Hechi	262	24°51'	109°10'	Mid-level bushes
GUX20	Mendoutun, Luocheng County, Hechi	262	24°51'	109°10'	Mid-level bushes
GUX21	Shuangqiao, Wuming County, Nanning	72	23°08'	108°15'	Hill wilderness
GUX22	Shuangqiao, Wuming County, Nanning	76	23°08'	108°07'	Hill wilderness
GUX23	Shuangqiao, Wuming County, Nanning	76	23°08'	108°07'	Hill wilderness
GUX24	Shuangqiao, Wuming County, Nanning	79	23°08'	108°07'	Hill wilderness
GUX25	Xiajianhe, Liusanjie Township, Yizhou City, Hechi	167	24°32'	108°40'	Low-level wilderness
GUX26	Xiajianhe, Liusanjie Township, Yizhou City, Hechi	170	24°32'	108°40'	Low-level wilderness
GUX27	Xiajianhe, Liusanjie Township, Yizhou City, Hechi	130	24°32'	108°40'	Low-level wilderness
GUX28	Xiajianhe, Liusanjie Township, Yizhou City, Hechi	130	24°32'	108°40'	Low-level wilderness
GUX29	Xiajianhe, Liusanjie Township, Yizhou City, Hechi	130	24°32'	108°40'	Low-level wilderness
GUX30	Xiajianhe, Liusanjie Township, Yizhou City, Hechi	147	24°31'	108°45'	Low-level wilderness
GUX31	Longteng, Luocheng County, Hechi	262	24°51'	109°12'	Mid-level bushes
GUX32	Longteng, Luocheng County, Hechi	262	24°51'	109°12'	Mid-level bushes
GUX33	Huojia Village, Rongshui County, Liuzhou	450	25°40'	109°27'	Mid-level bushes
GUX34	Huojia Village, Rongshui County, Liuzhou	404	25°60'	109°26'	Mid-level bushes
GUX35	Huojia Village, Rongshui County, Liuzhou	357	25°30'	109°27'	Mid-level bushes
GUX36	Yongfu County, Guilin	252	25°10'	104°01'	Mid-level bushes
GUX37	Yongfu County, Guilin	263	25°10'	104°01'	Mid-level bushes
GUX38	Yongfu County, Guilin	302	24°59'	109°40'	Mid-level bushes
GUX39	Yongfu County, Guilin	243	25°40'	109°43'	Mid-level bushes
GUX40	Tianqiao Village, Tongde Township, Yizhou City, Hechi	171	24°29'	108°27'	Hilltop wilderness
GUX41	Tianqiao Village, Tongde Township, Yizhou City, Hechi	171	24°29'	108°27'	Hilltop wilderness
GUX42	Bailin, Bama County, Hechi	652	23°88'	107°40'	Hilltop wilderness
GUX43	Bailin, Bama County, Hechi	631	23°88'	107°40'	Hilltop wilderness
GUX44	Pingxiang City, Chongzuo	226	22°10'	106°77'	Hilltop wilderness
GUX45	Pingxiang City, Chongzuo	205	22°10'	106°77'	Hilltop wilderness
GUX46	Xiashi Township, Pingxiang City, Chongzuo	289	22°12'	106°90'	Hilltop wilderness
GUX47	Xiashi Township, Pingxiang City, Chongzuo	250	22°12'	106°90'	Hilltop wilderness
GUX48	Xiazhangping, Rongshui County, Hechi	138	25°30'	109°20'	Wilderness
GUX49	Tianyang County, Baise	234	23°73'	106°95'	Wilderness
GUX50	Siding Township, Yongfu County, Guilin	266	25°30'	109°31'	Hill wilderness

Table 2 Sequences of primers for SCoT marker analyses

Code	Sequence	Code	Sequence
SCoT1	CAACAATGGCTACCACCA	SCoT30	CAACAATGGCTACCAGCC
SCoT2	CCATGGCTACCACCGCA	SCoT31	GCAACAATGGCTACCACC
SCoT24	ACCATGGCTACCACCGGG	SCoT35	ACCATGGCTACCACCGCC
SCoT25	CAACAATGGCTACCACCC	SCoT36	ACCATGGCTACCACCGTC
SCoT26	CAACAATGGCTACCAGCA	SCoT37	ACGACATGGCGACCACGT
SCoT27	ACGACATGGCGACCCACA	SCoT39	ACGACATGGCGACCGCGG
SCoT28	CAACAATGGCTACCACGT	SCoT42	CCATGGCTACCACCGCAC
SCoT29	ACCATGGCTACCACCGCA		

obvious morphological differences among the different materials. Stalk diameter showed the largest variation range and the variation coefficient reached 16.14 %, followed by spikelet length whose variation coefficient was 15.97 %, and the variation coefficients of plant height and number of panicle nodes were 13.12 % and 12.71 %, respectively.

Polymorphism Detected Using SCoT Markers

In the 50 *E. arundinaceus* (Retz.) Jesw. accessions, a total of 46 SCoT primers were screened, and 15 primers were successfully produced, which showed scorable, reproducible with clear high-intensity and highly polymorphic bands (Fig. 1). The total number of bands scored was 336, of which 284 were polymorphic (83.93 %) with an average of 19.0 per primer (Table 4). For each primer, the fragment sizes ranged from 250 to 1000 bp and the total number of bands varied from 17 (SCoT2) to 30 (SCoT27) with a mean 22, and the polymorphic rate ranged from 69.57 to 100 % and the total number of polymorphic bands varied from 12 (SCoT2) to 26 (SCoT27) with a mean 19.

Similarity Coefficient Analysis

The scored bands were used to calculate the genetic diversity among the 50 *E. arundinaceus* (Retz.) Jesw. accessions. The Jaccard's similarity coefficients varied from 0.61 to 0.88 with an average of 0.71. The accessions GUX09 and GUX10 had the highest genetic similarity coefficient of 0.88, while GUX10 and GUX30 had the lowest genetic similarity coefficient of 0.61. In terms of the genetic similarity coefficient of the accessions, the majority of the accession from the same area showed high similarity coefficient. For example, GUX01, GUX02, GUX03 and GUX04 coming from Lingchuan County, Guilin City, and their similarity coefficients were over 0.78; GUX12, GUX13, GUX14, GUX15 and GUX16 coming from

Wuming County, Nanning City, and their similarity coefficients were over 0.75; GUX25, GUX26, GUX27, GUX28 and GUX29 coming from Liusanjie Township, Yizhou City, and their similarity coefficients were over 0.76. These results indicated that the accessions of *E. arundinaceus* (Retz.) Jesw. clones from the same area have relatively lower genetic differences and closer genetic relationships compared with those from different areas.

Clustering Analysis

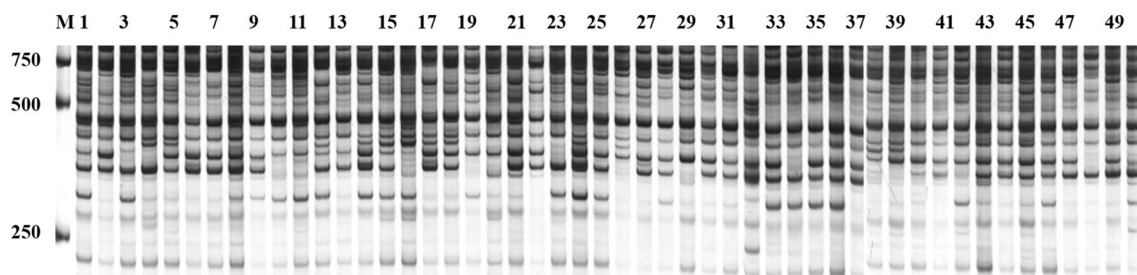
A UPGMA dendrogram based on the similarity coefficients was constructed to estimate the genetic relationships among the 50 *E. arundinaceus* (Retz.) Jesw. accessions (Fig. 2). As shown in Fig. 2, these accessions were distinctly clustered into four groups, which shared a common node at similarity coefficient of 0.73. The accessions GUX45, GUX49 and GUX50 belonged to cluster I, GUX48 belonged to cluster II, GUX38 belonged to cluster III, and the rest 45 clones belonged to cluster IV. The cluster IV was further subdivided into six subgroups, that is, eight were in subcluster A, 21 in subcluster B, six in subcluster C, six in subcluster D, two in subcluster E and two in subcluster F, which shared a common node at similarity coefficient of 0.76. The result shows that most of the accessions collected from the same area are gathered in the same class. For example, the accessions GUX01, GUX02, GUX03 and GUX04 from Lingchuan County, and GUX31, GUX32 and GUX33 from Hechi City were classified into subcluster A; GUX5, GUX6, GUX7, GUX8, GUX9 and GUX10 from Rongan County and neighbored Rongshui County, and GUX12, GUX13, GUX14, GUX15, GUX16, GUX21, GUX22, GUX23 and GUX24 from Wuming County were classified into subcluster B; GUX25, GUX26, GUX27, GUX28 and GUX29 from Liusanjie Village, Yizhou City were classified into subcluster C; and GUX40, GUX41 GUX42 and GUX43 from different areas of Hechi City are classified into subcluster D.

Table 3 Morphological diversity in agronomic traits of *E. arundinaceus* (Retz.) Jesw. clones from different areas of Guangxi, China

Code	Plant height (cm)	Stalk diameter (cm)	Spikelet length (cm)	Number of panicle nodes
GUX01	280	0.83	61.3	18
GUX02	272	0.58	50.2	18
GUX03	301	0.56	40.2	11
GUX04	285	0.64	49.8	15
GUX05	325	0.88	64.7	19
GUX06	279	0.78	54.2	18
GUX07	334	0.91	65.6	17
GUX08	313	0.83	62.1	18
GUX09	370	0.75	57.7	17
GUX10	373	0.73	59.1	15
GUX11	296	0.72	57.4	13
GUX12	378	0.88	55.8	16
GUX13	296	0.72	54.2	14
GUX14	372	0.76	65.5	16
GUX15	328	0.94	59.7	18
GUX16	219	0.61	60.6	17
GUX17	400	0.91	57.7	15
GUX18	299	0.76	66.9	18
GUX19	325	1.07	65.2	21
GUX20	395	0.82	78.2	21
GUX21	404	1.01	52.0	16
GUX22	277	0.70	42.2	14
GUX23	339	0.94	64.2	18
GUX24	391	0.96	60.3	17
GUX25	340	0.70	59.9	18
GUX26	356	0.87	80.4	19
GUX27	343	0.62	68.7	20
GUX28	320	0.96	64.0	20
GUX29	403	0.87	72.8	20
GUX30	361	0.79	66.9	19
GUX31	318	0.74	49.0	20
GUX32	363	0.92	61.1	17
GUX33	354	1.02	60.8	18
GUX34	360	0.89	52.8	17
GUX35	355	0.96	56.3	16
GUX36	271	0.63	44.4	13
GUX37	317	0.75	59.4	17
GUX38	358	0.86	56.5	16
GUX39	315	0.75	53.4	15
GUX40	401	1.01	75.1	19
GUX41	360	0.75	65.8	17
GUX42	329	0.82	53.8	16
GUX43	384	0.98	78.8	21
GUX44	353	0.87	60.6	19
GUX45	356	0.85	52.8	18
GUX46	375	0.85	54.4	20
GUX47	322	0.93	57.0	19

Table 3 continued

Code	Plant height (cm)	Stalk diameter (cm)	Spikelet length (cm)	Number of panicle nodes
GUX48	292	0.70	54.3	16
GUX49	354	0.87	63.4	18
GUX50	342	0.84	56.0	18
Mean	337	0.82	59.7	17
Minimum	219	0.56	40.2	11
Maximum	404	1.07	80.4	21
Sd	44	0.13	9.5	2
CV (%)	13.12	16.14	15.97	12.71

**Fig. 1** SCoT fingerprints amplified with primer SCoT29 for the *E.arundinaceus* (Retz.) Jesw. clones collected from different areas of Guangxi, China**Table 4** Genetic diversity of SCoT primers

Code	Total no. of bands	No. of polymorphic bands	Percentage of polymorphic bands (%)	Code	Total no. of bands	No. of polymorphic bands	Percentage of polymorphic bands (%)
SCoT1	21	17	80.95	SCoT30	19	18	94.74
SCoT2	17	12	70.59	SCoT31	28	21	75.00
SCoT24	19	19	100.00	SCoT35	23	16	69.57
SCoT25	20	17	85.00	SCoT36	24	17	70.83
SCoT26	28	25	89.29	SCoT37	18	16	88.89
SCoT27	30	26	86.67	SCoT39	22	18	81.82
SCoT28	25	23	92.00	SCoT42	23	20	86.96
SCoT29	19	17	89.47				
Mean	22	19	83.93				
Total	336	284	–				

Discussion

The coefficient of variance (CV) is an index of population variation. According to Wang et al. (2012), CV 0–10 % is on the lower variation level, 10–20 % is on the medium variation level and over 20 % is on the high variation level. In this study, the CVs of four quantitative traits, plant height, stalk diameter, spikelet length and number of panicle nodes,

in the 50 *E. arundinaceus* (Retz.) Jesw. clones collected from Guangxi ranged from 12.71 to 16.14 % which were on the medium variation level, lower than those reported by Liu et al. (2013) who found that the CVs for the quantitative traits of *E. arundinaceus* (Retz.) Jesw. accessions from Hainan Province were on the high level. In this study, the CV of stalk diameter was the highest; therefore, this trait has the greatest potential for genetic improvement.

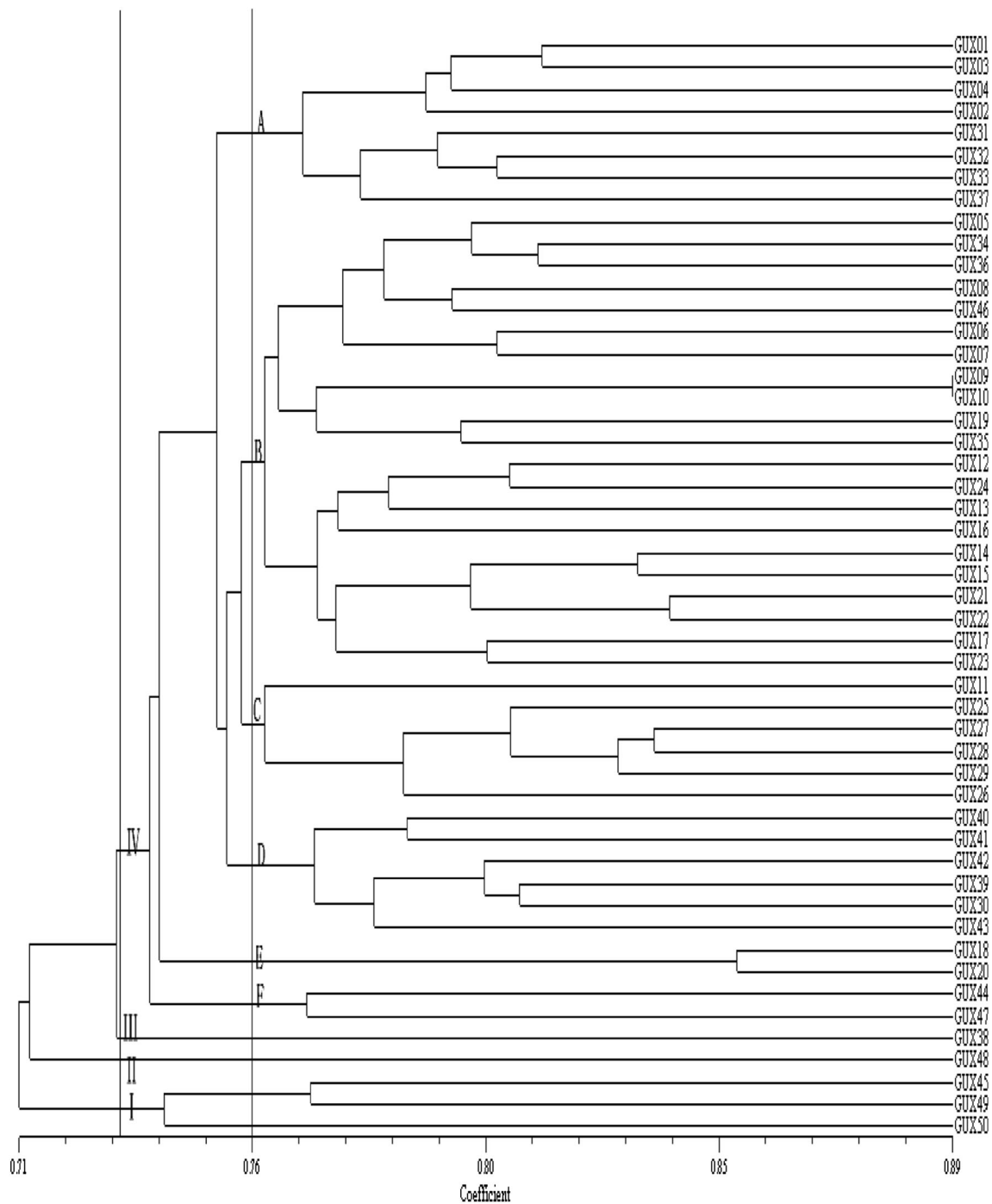


Fig. 2 Dendrogram of 50 accessions of *E. arundinaceus* (Retz.) Jesw. collected from different areas of Guangxi, China using UPGMA

The SCoT markers are expected to be linked to functional genes and corresponding traits; thus, the amplicons can be converted to gene targeted marker systems (Xiong et al. 2011). SCoT markers have been used for analyses of genetic diversity and clustering relation in several crops (Collard and Mackill 2009; Xiong et al. 2010; Wu et al. 2010; Chen et al. 2012; Zhao et al. 2012; Wei et al. 2012; Wu et al. 2013). Compared with RAPD, ISSR, AFLP and

SSR, SCoT marker analysis has the advantages of simplicity, low cost and good repeatability. Zhang et al. (2015) analyzed the genetic diversity of *E. arundinaceus* (Retz.) Jesw. collections from Sichuan using SRAP markers and the polymorphic bands ratio was 74.63 %. Rao et al. (2014) analyzed the genetic diversity of *E. arundinaceus* (Retz.) Jesw. collections using RAPD markers and the polymorphic bands ratio was 78.85 %. In

this study, the polymorphism of 50 *E. arundinaceus* (Retz.) Jesw. collections from Guangxi was analyzed using 15 SCoT primers and the total polymorphic bands ratio reached up to 83.93 %, which was higher than the previously reported results. The Jaccard's similarity coefficients ranged from 0.61 to 0.88, and the UPGMA dendrogram reflected the geographic distribution patterns of the 50 accessions of *E. arundinaceus* (Retz.) Jesw. collected from different areas of Guangxi, indicating that the collections of *E. arundinaceus* (Retz.) Jesw. from Guangxi have rich genetic diversity. Collection, identification, rational conservation and exploitation of the *E. arundinaceus* (Retz.) Jesw. germplasm in Guangxi might be potentially useful in sugarcane breeding.

Conclusion

The CVs of four quantitative traits, plant height, stalk diameter, spikelet length and number of panicle nodes, of 50 *E. arundinaceus* (Retz.) Jesw. collection from Guangxi ranged from 12.71 to 16.14 %. The polymorphism and similarity analyses based on 15 SCoT primers reflected the rich diversity and geographic distribution patterns of these accessions.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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