Research on genetic diversity and phylogeny of *Saccharum spontaneum* L. in China

Hui Chen[1], Yuanhong Fan[2], Qing Cai[3] and Ya-ping Zhang[4]

[1] Sugarcane Research Institute, Yunnan Academy of Agricultural Sciences, Kaiyuan, Yunnan , China, 661600
Email: chenhuiysri@ yahoo.com
[2] Sugarcane Research Institute ,Yunnan Academy of Agricultural Sciences, Kaiyuan, Yunnan ,China, 661600
Email fyhysri@sohu.com.
[3] Sugarcane Research Institute ,Yunnan Academy of Agricultural Sciences, Kaiyuan, Yunnan ,China, 661600
Email caiqingysri@ sohu.com.
[4] Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology ,the Chinese Academy of Sciences,
Kunming ,Yunnan, China 650223 Email yapingzhang@mail.kiz.ac.cn

Abstract

In this study, 195 accessions of *Saccharum Spontaneum* L. from different geographical populations in China were studied using random amplified polymorphic DNA (RAPD) analysis to estimate their genetic diversity. Phylogenetic analysis was done to assess relationships among haplotypes. A total of 266 bands were scored of which 145 were reproducibly polymorphic. The extent of genetic variability was between 62.95% and 37.05% within populations. The topology of the phylogenetic tree of *S. spontaneum* corresponds to the population geographical distribution. The genetic variation and diversity among different populations was consistent with existing knowledge on the geographical distribution of *S. spontaneum* in China. We tentatively propose that Yunnan province was the center of origin for *S. spontaneum* in China, based on previous collections and archeological information when combined with these results.

Media summary

Based on our research on genetic diversity and phylogeny combined with previous collections and archeological information, Yunnan province is the center of origin for *S. spontaneum* in China. and

Key Words

Saccharum Spontaneum L., RAPD, genetic diversity, phylogeny

Introduction

China's vast territory, complicated geological formation and various climate types create favorable conditions for the evolution of wild sugarcane. Diverse sugarcane genetic resources evolved in these variable environments. Germplasm collections are an important component of sugarcane improvement programs as they provide breeders with sources of useful traits. Basic germplasm has often been used in sugarcane breeding. Most modern commercial sugarcane clones represent a complex interspecific combination between the genomes of *S. officinarum* and *S. spontaneum* (Besse et al. 1997). At present, sugarcane breeding in China is progressing towards the introduction of valuable genes, with positive effects on agronomically important traits, from new basic germplasm into modern sugarcane clones. This is especially true for genes from the species *S. spontaneum*, which has played a very important role in sugarcane breeding in the development of interspecific hybrids. *S. spontaneum* has the widest geographical distribution of the six species of the genus *Saccharum* (*S. officinarum* L., *S. spontaneum* L., *S. barberi* Jeswiet., *S. sinense* Roxb., *S. robustum* Brandes & Jeswiet ex Grassl. and *S. edule* Hassk.). *S. spontaneum* has many desirable characters including disease resistance, vigour, tillering ability, drought tolerance, water tolerance, frost tolerance, and general adaptability. Because of these traits, sugarcane

breeders all over the world have considerable interest in the collection, maintenance, evaluation, and exploitation of its genetic potential (Tai et al. 1999). *S. spontaneum* clones are distributed throughout 8 provinces in China. Clones have been collected on a number of occasions, and maintained in the National Nursery for Sugarcane Germplasm Resources, Yunnan Sugarcane Research Institute, Kaiyuan, Yunnan province, China. In this paper, we examine the use of RAPDs for estimating the level of genetic diversity among 195 *S. spontaneum* accessions from different geographical populations in China, and discuss the implications of our results for the origin and diffusion of the wild sugarcane.

Materials and Methods

Materials

195 accessions from eight geographic populations were considered in this study (Table 1). All samples were obtained from the National Nursery for Sugarcane Germplasm Resources in Yunnan province, China.

Table 1. The geographical distribution and climatic characteristic in 8 geographical colonies of *Saccharum spontaneum* L in China.

Colony	Number of samples	Geographical distribution and climatic characteristic samples
Yunnan (YN)	86	locate in the southwest of China, attrib to semi-tropic, plateau, moisture and monsoon climate
Sichuan(SC)	36	locate in the southwest of China, attrib to semi-tropic, plateau, moisture and monsoon climate
Guizhou(GZ)	10	locate in the southwest of China, attrib to semi-tropic, plateau, moisture and monsoon climate
Guangxi (GX)	13	locate in the south of China, attrib to tropic, moisture and monsoon climate
Guangdong(GD)	18	locate in the south of China, attrib to tropic, moisture and monsoon climate
Hainan(HN)	14	locate in the south of China, attrib to tropic, moisture and monsoon climate
Fujian(FJ)	14	locate in the southeast of China, attrib to semi-tropic, moisture and monsoon climate
Jiangxi(JX)	4	locate in the southeast of China, attrib to semi-tropic, moisture and monsoon climate

Methods

DNA was isolated from apical meristem and young leaf tissues using a modified method (Fan et al. 1999). PCR mixtures (10μL total) contained: 25ng DNA, 2.5mM dNTP, 0.2μM primers (products of Operon Technology Company),10 mM Tris-HCl (pH8.9), 50 mM KCl, 0.2mM BSA, 2.5mM $MgCl_2$, and 1.0 unit of Taq polymerase (products of Takara Biotechnology Company). Reactions were overlaid with 20μL of mineral oil to prevent evaporation. Samples for enzymatic amplification were subjected to initial denaturation at 95?C for 3 min follow by 40 cycles of 94?C for 1min (denaturation), 36?C for 1min (annealing), and 72?C for 2min (extending) with a final extension at 72?C for 5min. Fragments generated by amplification were separated according to size electrophoresis on 1.5% agarose gel in 1?TAE, stained with ethidium bromide, and photographed by an EAGLE EYE imager (Williams et al. 1990; Wachiro et al. 1995; Chen et al. 2001).

Results

RAPD Analysis

Out of a total of 25 primers used, 20 (80%) generated polymorphic loci. A total of 266 bands were scored of which 145 (54.5%) were reproducibly polymorphic. The number of products generated by each primer varied from 7 to 15 with an average of 13. The size of the amplified fragments that were scored ranged from 0.2-2kb.

Genetic Diversity

The phenotypic frequencies detected with the 20 primers were calculated and used in estimating diversity (*Ho*) within population types (Table 2). The Jiangxi population exhibited the lowest within population variability (0.6937), but had the smallest population size, which affects *Ho*. The Yunnan population exhibited highest within population variability (1.7126).

Shannon's index of phenotypic diversity was used to partition the diversity into within and between population components (Table 3). Primer OPI-08 detected the most within population variability, while primer OPA-19 detected the least.. An assessment of the proportion of diversity present within population Hpop/Hsp, compared with that between populations, *(Hsp-Hpop)/Hsp*, indicates that, on average, most of the diversity (62.95%) is detected between populations (38.15%-71.86%). 37.05% of the variation was detected within populations. However, the distribution of variability did vary slightly between and within populations with different primers.

Table 2. The genetic diversity index of 8 geographical colonies of *Saccharum Spontaneum* L. in China

Primer	YN	SC	GZ	GX	GD	HN	FJ	JX
OPA-07	2.0387	1.7673	1.4536	1.3849	1.2767	1.0885	1.1456	0.9764
OPA-19	2.0909	1.8421	1.7654	1.4308	1.5692	1.2048	1.0923	0.8732
OPB-14	1.4758	1.8122	0.9091	0.7422	0.7288	0.7059	0.4378	0.6496
OPD-01	2.1492	1.9678	1.6216	1.4358	1.2697	0.9739	0.7262	0.6519
OPF-01	1.3591	1.2943	1.0249	0.9739	0.7618	0.7618	0.8732	0.6348

OPF-05	2.2241	1.9769	2.0349	1.3851	1.0624	1.0348	0.9792	0.7567
OPF-12	1.8526	1.9769	1.7743	1.3155	1.2349	0.9849	0.8492	0.6599
OPH-01	1.5101	1.3844	1.0989	0.9432	1.1143	0.8938	0.7607	0.8349
Primer	YN	SC	GZ	GX	GD	HN	FJ	JX
OPH-19	1.8122	1.4278	1.3342	1.1893	0.9866	0.8654	0.7348	0.6549
OPI-08	1.4637	1.3859	1.2248	1.1049	1.0876	0.9703	0.8785	0.7786
OPJ-07	1.2917	1.0977	0.9771	0.8718	0.7185	0.6507	0.5873	0.4336
OPJ-09	1.7298	1.6348	1.3285	1.2853	1.0928	0.9385	0.6418	0.5186
OPJ-14	1.2875	1.4637	1.1524	1.0335	0.8785	0.8324	0.6545	0.5448
OPJ-18	1.8819	1.6405	1.4969	1.1721	0.9898	0.8671	0.7703	0.6479
OPK-18	1.9416	1.8803	1.6581	1.3272	1.2717	1.0068	0.8781	0.7589
OPL-17	1.9769	1.7734	1.5266	1.6534	1.2548	0.9849	0.8834	0.6599
OPM-04	1.6309	1.3154	1.4403	1.1191	1.0348	0.8779	0.5946	0.6343
OPM-07	1.7898	1.9416	1.6034	1.4432	1.0781	1.2249	0.9789	0.8744
OPN-02	1.1143	1.5533	0.9073	0.6471	0.8411	0.5489	0.5302	0.4812
OPN-11	1.6309	1.2951	1.4038	1.1438	0.9779	1.0433	0.9342	0.8495
Average	1.7126	1.6216	1.3868	1.1801	1.0615	0.9230	0.7965	0.6937

Table 3. Partitioning of the genetic diversity into within and between populations for 20 primers

Primer	Hsp	Hpop	Hpop/Hsp	(Hsp-Hpop)/Hsp

OPA-07	3.9898	1.3915	0.3487	0.6513
OPA-19	5.2715	1.4836	0.2814	0.7186
OPB-14	2.3744	0.9327	0.3928	0.6072
OPD-01	4.0458	1.3495	0.3335	0.6665
OPF-01	1.6233	0.9605	0.5917	0.4083
OPF-05	4.8902	1.4318	0.2928	0.7072
OPF-12	3.9773	1.3311	0.3347	0.6653
OPH-01	1.9903	1.0675	0.5364	0.4636
OPH-19	2.8188	1.1256	0.3993	0.6007
OPI-08	1.7977	1.1118	0.6184	0.3815
OPJ-07	1.7671	0.8285	0.4688	0.5312
OPJ-09	3.2252	1.1463	0.3554	0.6446
OPJ-14	2.2187	0.9809	0.4421	0.5579
OPJ-18	3.4079	1.1833	0.3472	0.6528
OPK-18	4.3257	1.3403	0.3098	0.6902
OPL-17	4.5241	1.3392	0.2961	0.7039
OPM-04	2.5573	1.0809	0.4227	0.5773
OPM-07	4.0844	1.3668	0.3346	0.6654
OPN-02	2.3378	0.8279	0.3541	0.6459

OPN-11	2.0307	1.1598	0.5711	0.4289
Average	3.1619	1.1719	0.3705	0.6295

Genetic Differentiation and Genetic Relationship

To examine the genetic differentiation and genetic relationship between the different populations, a genetic distance matrix based on the proportion of shared fragments (Nei 1978) was used to establish the level of relatedness between the different populations of *S. spontaneum* studied. Figure 1 shows a dendrogram generated by UPGMA cluster analysis, based on the estimates of genetic distances between populations.

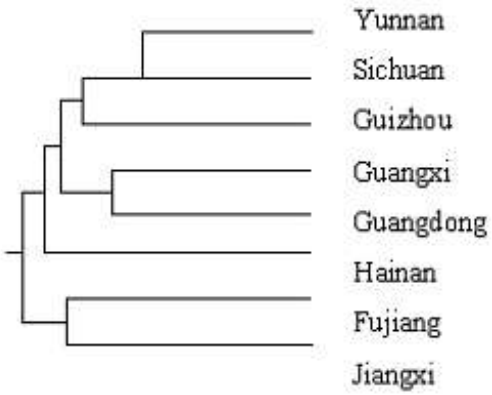
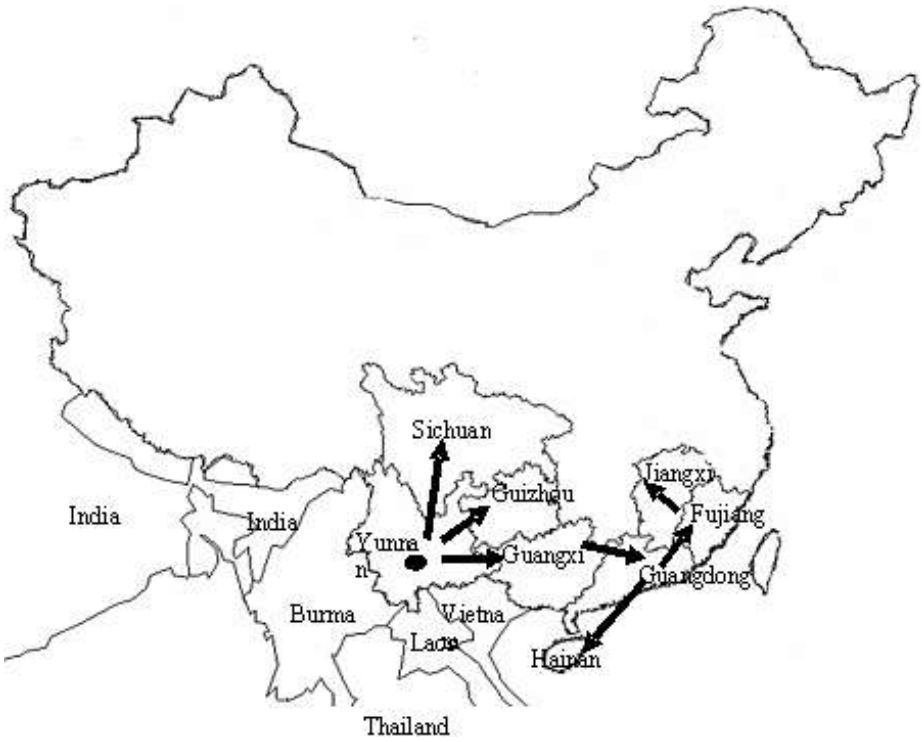


Figure 1. A dendrogram generated by UPGMA cluster analysis, based on the estimates of genetic distances between populations.

Conclusion

This study demonstrated that *S. spontaneum* clones within the Yunnan population exhibited more extensive genetic variability, as well as more abundant genetic diversity and many original types of wild sugarcane. Yunnan province has the widest geographical distribution of *S. spontaneum* of all provinces in China. Moreover, according to our results, studies on sugarcane collection, maintenance, evaluation and exploitation of its genetic potential over many years, and related archeological information, we tentatively proposed that Yunnan province was the possible origin center of *S. spontaneum* in China, which then spread into Sichuan, Guizhou and Guangxi provinces. *S. spontaneum* in Guangxi further spread into Guangdong province, followed by spread into Fujiang and Jiangxi provinces northward and Hainan province southward.(Figure 2)

Figure 2. The possible origin center and diffusion paths of *Saccharum Spontaneum* L. in China were demonstrated by the dot and the arrows respectively.



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