苏铁属植物 RAPD 反应体系的优化及部分种类亲缘关系的探索

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摘 要：通过优化苏铁属植物的 RAPD 反应体系，进而探讨苏铁属部分种类的亲缘关系。结果表明，Mg^{2+}、dNTP、*Taq* 酶及随机引物浓度在 RAPD 反应中有重要影响，而模板 DNA 浓度有一个很大的适应范围。适合苏铁属植物 RAPD 分析的反应体系：20 μL 反应体系中，含有 10 mmol/L Tris-HCl(pH8.3)、50 mmol/L KCl、2.0 mmol/L $MgCl_2$、200 μmol/L dNTP、0.3 μmol/L 随机引物、模板 DNA 50ng、*Taq* 酶 1.0 U。聚类分析结果基本反应了苏铁属各个种间的亲缘关系，证明了 RAPD 适用于苏铁属种间亲缘关系分析。RAPD 聚类分析结合形态学研究表明：海南苏铁、台湾苏铁、广东苏铁、滇南苏铁和仙湖苏铁之间的亲缘关系较远，支持各自成为独立的种。

关键词：苏铁属；RAPD；亲缘关系

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Improving of RAPD reaction system in *Cycas* and studies on the genetic relationships of some species

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Abstract: In this paper, the reaction system of RAPD in *Cycas* had been established and applied to discuss the relationships among *C. hainanensis*, *C. taiwaniana*, *C. guangdongensis C. diannanensis and C. fairylakea*. The results showed that RAPD was affected by the concentrations of four components i. e. Mg^{2+}, dNTP, *Taq* DNA polymerase and primers. The optimal reaction system was as follows: 20 μL reaction volume, including 10 mmol/L Tris-HCl(pH 8.3), 50 mmol/L KCl, 2.0 mmol/L $MgCl_2$, 200 μmol/L dNTP, 0.3 μmol/L primers, 50 ng template DNA, 1.0 U *Taq* DNA polymerase. The reflection of RAPD cluster to the genetic relationship of all the species showed that RAPD was suitable for analysis of interspecific genetic relationship in *Cycas*. Genetic relationship analysis by RAPD markers showed that five defined species had far genetic relationships each other, combining with the analysis of morphological traits, it's concluded that they were five independent genetic units at species level.

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Key words: *Cycas*; RAPD; genetic relationships

Lots of studies of cycads which is known as "living fossil" for human being, have been carried out to discuss the evolutionary relationships among different cycads species, origin and immigration of existing cycads, and its mechanism adapted to various environments (Huang, 2001). However, the classification of some species is still obscure, and there is a dispute on whether the taxa of some species were credible or not (Huang, 2001), especially on the *Cycas hainanensis*, *C. taiwaniana*, *C. guangdongensis* or "Guangdong cycad" (local name), *C. diannanensis*, *C. fairylakea* and so on. It has become a barrier for the utilization and conservation of *Cycas*. Studies had showed that RAPD was suitable for the analysis of interspecific genetic relationship (Lanner *et al.*, 1996; Zhou, 1999; Wang *et al.*, 2003; Ding *et al.*, 2005; Xiao, 2006; Chen *et al.*, 2007; Wang & Ding, 2007; Wang *et al.*, 2008; Zhang & Huang, 2008). However, no study on *Cycas* by RAPD marker was reported in former researches. In this research, we try to establish an optimized reaction system of RAPD in *Cycas* and to clear some problems of species at molecular level, thereby, provide reference for setting up the molecular biology basis in evolvement investigation, and provide theories basis for further study on the taxonomy, propagation, utilization and conservation of cycads.

1 Materials and methods

1.1 Experimental materials

Six species of *Cycas* had been collected (Table 1), 7—15 samples for each. The leaves of *Cycas* were dried with silica gel right after being collected from the plant (Chase & Hills, 1991). The samples which DNA couldn't be extracted immediately were stored at -70 ℃ refrigerator.

In order to ascertain optimum reaction system, *Cycas balansae* O. Warburg was used as the experimental material to carry out the comparative study. Other five defined species were carried out for the molecular evolutionary relationships research.

Table 1 Colony and its origin of six species

No.	Species	Source site	Collection site	Time	Collector & voucher specimens No.	^Lodged
1	*C. balansae* O. Warburg	Yunnan Province of China	Cycad Garden of Qingxiushan Garden, Nanning of China	May 24, 2004	Huang Y. Y, Nong B. X 356	GXAC
2	*C. hainanensis* C. J. Chen	Hainan Province of China	South China Botanical Garden, Guangzhou of China	May 31, 2004	Huang Y. Y, Nong B. X 363	GXAC
3	*C. taiwaniana* Carruthers	Hainan Province of China	Shenzhen Fairy Lake Botanical Garden, Shenzhen of China	Jun 1, 2004	Huang Y. Y, Nong B. X 364	GXAC
4	*C. guangdongensis*	Guangdong Province of China	Shenzhen Fairy Lake Botanical Garden, Shenzhen of China	Jun 1, 2004	Huang Y. Y, Nong B. X 368	GXAC
5	*C. diannanensis* Z. T. Guan et G. D. Tao (♀)	Yunnan Province of China	Shenzhen Fairy Lake Botanical Garden, Shenzhen of China	Jun 1, 2004	Huang Y. Y, Nong B. X 370	GXAC
6	*C. diannanensis* Z. T. Guan et G. D. Tao (♂)	Yunnan Province of China	Shenzhen Fairy Lake Botanical Garden, Shenzhen of China	Jun 1, 2004	Huang Y. Y, Nong B. X 365	GXAC
7	*C. Fairylakea* Wang DY	Guangdong Province of China	Shenzhen Fairy Lake Botanical Garden, Shenzhen of China	Jun 1, 2004	Huang Y. Y, Nong B. X 358	GXAC

GXAC: Herbarium of the Agricultural College of Guangxi University.

1.2 DNA extraction

Every individual of species was mixed in equal weight respectively, then the DNA of these samples were extracted by using CTAB method (Doyle JJ & Doyle JL, 1987).

1.3 PCR reaction

121 pieces of primers were purchased from Shanghai Sangon Biological Engineering Technology & Services Company Ltd., China. And dNTP, Taq DNase were bought from TaKaRa Biotechnology (Dalian) Company Ltd., Dalian of China.

A series of concentrations and volumes of each factor were designed as follows: DNA (10, 25, 50, 75, 100 ng DNA in every tube, respectively); Mg^{2+} (0.5, 1.0, 1.5,

2.0,2.5,3.0 mmol/L);dNTP(50,100,150,200,250,300 μmol/L);Taq DNase(0.2,0.6,0.8,1.0,1.4 U). The primer concentrations were 0.1,0.2,0.3,0.4,0.5,1.0, 2.0 μmol/L. The reaction was conducted in Biometra Tgradient PCR apparatus. The optimal PCR amplification procedure was as follows: Pre-denaturation at 94 ℃ for 5 min and 35 cycles of denaturation at 94 ℃ for 30 s, annealing at 37 ℃ for 40 s, lengthening at 72 ℃ for 110 s; then lengthening at 72 ℃ for 7 min again. The final product was kept at 4 ℃. The production of PCR was isolated by electrophoresis on an agarose gel (1%) and dyed with ethidium bromide (EB), then were observed and photographed through the Imagine Master VDS.

1.4 Data analysis

Every reaction was repeated for three times. It was labeled with 1 or 0, respectively, according to whether it had RAPD band or not. The data were used to generate Jaccard similarity coefficient and to construct a dendrogram according to unweighted pair-group arithmetic average clustering (UPGMA) in NTSYS programs.

2 Results and analysis

2.1 RAPD reaction system of *Cycas*

2.1.1 The effect of template DNA concentrations The bands showed clearly when the template DNA ranged from 25 —75 ng, but obscurely when DNA less than 10 ng or more than 75 ng (Fig. 1). It's indicated that the range of DNA concentrations was not severely affected in the RAPD. Given the suitable range of DNA concentrations, it could obtain stable amplified production. But to avoid the influence of excessive DNA, every tube(20 μL reaction solution) contained 50 ng DNA was better.

2.1.2 The effect of Mg^{2+} concentrations Mg^{2+} plays an important role in the PCR reaction, Fig. 2 showed the results of treatments with different Mg^{2+} concentrations. When the Mg^{2+} concentration was 0.5 mmol/L, only one band was produced; when it was 3.0 mmol/L, the darken background was serious; when it was in the range from 1.0—2.5 mmol/L, similar bands could be seen, and when the concentration was 2.0 mmol/L, the bands were clearest. So we selected the 2.0 mmol/L of Mg^{2+} as the best concentration.

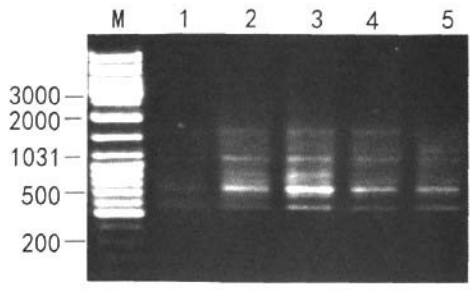

Fig. 1 Comparison of amplification effects of template DNA with different concentrations.
From left to right(Primer S404): 10,25,50,75,100 ng; M: Gene RulerTMDNA Ladder MIX

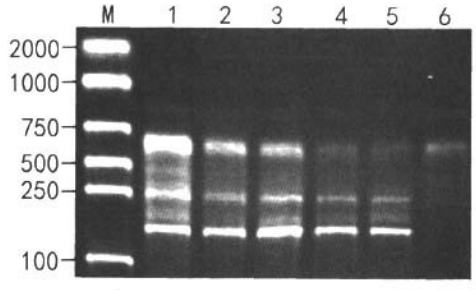

Fig. 2 Effects of Mg^{2+} concentrations on amplified results
From left to right(Primer S216): 3.0,2.5,2.0,1.5, 1.0,0.5 mmol/L; M: DNA Marker DL2000

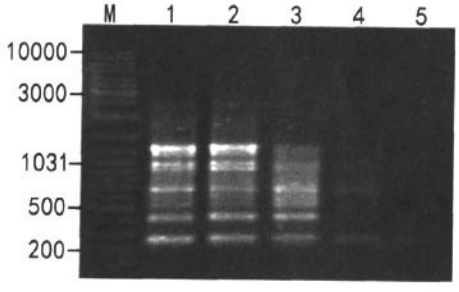

Fig. 3 Effects of dNTP concentrations on amplified results
From left to righ(Primer S347): 50,100,150,200, 250,300 μmol/L; M: Lambda DNA/HindⅢ.

2.1.3 The effect of dNTP concentrations The amplified production were basically similar when the concentrations of dNTP ranged from 50—200 μmol/L(Fig. 3), but it was clearer when the concentrations was 200 μmol/L; when it was 250 μmol/L, the bands couldn't be

seen clearly; when it was 300 μmol/L, there wasn't any band. So we selected 200 μmol/L dNTP as the best content for RAPD reaction.

2.1.4 The effect of *Taq* DNA polymerase Fig. 4 indicated that when the *Taq* DNA polymerase ranged from 0.8—1.4 U/μL, the amplified bands were almost consistent; when it was less than 0.6 U/μL, the bands were getting more and more obscure. Considering the stability and cost, we selected 1.0 U/μL Taq DNase for RAPD reaction.

2.1.5 The effect of primers concentrations The research results showed that when the primer concentrations ranged from 0.2—0.5 μmol/L, it could produce basical similar bands; when it was 0.3 μmol/L(Fig. 5), the bands were particularly clear; the primer concentrations was less than 0.2 μmol/L or more than 0.5 μmol/L, the amplified bands increased and got obscure. So we selected 0.3 μmol/L primer for RAPD reaction.

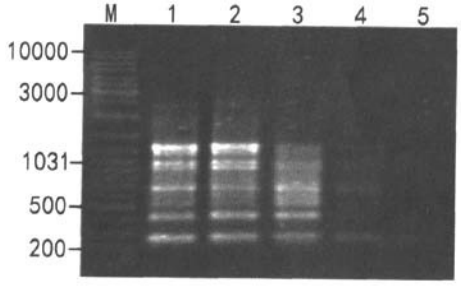

Fig. 4 Effects of *Taq* DNA polymerase concentrations on amplified results

From left to right(Primer S24): 1.4, 1.0, 0.8, 0.6, 0.2 U/μL; M: GeneRulerTMDNA Ladder MIX.

2.2 Primers selection, DNA amplified and clustering analysis

2.2.1 Primers selection 3 significant different species in morphologic were amplified with 121 primers to screen polymorphic markers for clustering analysis. 21 pieces of primer produced stably, clearly and polymorphic bands were selected(Table 2) and applied to the further analysis of five species. Total 202 DNA bands had been amplified from 21 primers with an average of 9.6 bands per primer, while there were 170 bands of the polymorphic DNA occupying 84.16 % of total, showing there were great diversity in *Cycas* interspecies, the band ranged from 200 — 3 000 bp. Fig. 6 showed the amplified bands of primer S 380.

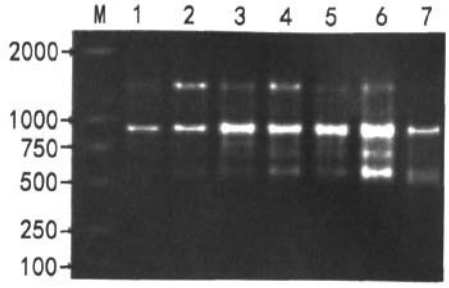

Fig. 5 Effects of primer concentrations on amplified results

From left to right(Primer S216): 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0 μmol/L; M: DNA Marker DL2000.

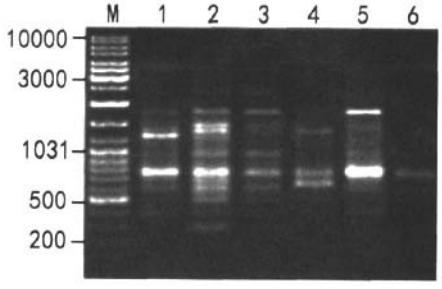

Fig. 6 Amplified bands of primer S380 for five species

1. *C. fairylakea*; **2.** *C. hainanensis*; **3.** *C. taiwaniana*; **4.** *C. guangdongenses*; **5.** *C. diannanensis*(♀); **6.** *C. diannanensis*(♂); M: GeneRulerTMDNA Ladder Mix.

Table 2 21 primers used in analysis

No.	Sequence (5′-3′)	Amplified loci	Polymorphic loci	Percentage of polymorphic loci (%)
S8	GTCCACACGG	11	11	100.0
S22	TGCCGAGCTG	10	10	100.0
S29	GGGTAACGCC	12	9	75.0
S31	CAATCGCCGT	11	9	81.8
S32	TCGGCGATAG	14	13	92.9
S36	AGCCAGCGAA	8	7	87.5
S38	AGGTGACCGT	11	7	63.6
S40	GTTGCGATCC	9	7	77.8
S41	ACCGCGAAGG	6	4	66.7
S102	TCGGACGTGA	7	6	85.7
S107	CTGCATCGTG	5	3	60.0
S132	ACGGTACCAG	10	9	90.0
S151	GAGTCTCAGG	10	9	90.0
S156	GGTGACTGTG	11	9	81.8
S198	CTGGCGAACT	7	7	100.0
S216	GGTGAACGCT	9	8	88.9
S380	GTGTCGCGAG	13	12	92.3
S404	GGCGGTTGT	9	8	88.9
S410	TCTGGCGCAC	7	4	57.1
S464	GTGTCTCAGG	11	10	90.9
S1427	GTGGCCGATG	11	9	81.8

It was labeled 1 or 0, respectively, according to whether there was RAPD band or not. The data were analyzed by NTSYS software, we adopted Jaccard coefficient and drew the clustering map using UPGMA(Fig. 7).

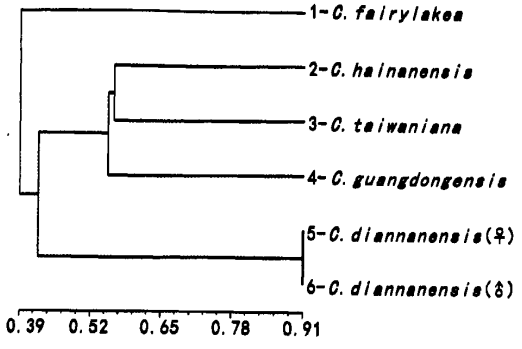

Fig. 7 Dendrogram generated by Jaccard coefficient of similarity

Table 3 Similarity coefficient and genetic distance among 6 taxa

Sequence number	1 C. fairylakea	2 C. hainanensis	3 C. taiwaniana	4 C. guangdongensis	5 C. diannanensis (female)	6 C. diannanensis (male)
1	1.0000					
2	0.3878	1.0000				
3	0.4121	0.5597	1.0000			
4	0.3636	0.5476	0.5504	1.0000		
5	0.3987	0.4351	0.4395	0.4133	1.0000	
6	0.3671	0.4118	0.4167	0.4082	0.9098	1.0000

2.2.2 Clustering analysis　The similarity coefficient between every two species ranged from 0.3636—0.5597 (Table 3), showed that they had a far genetic relationship each other. The biggest similarity coefficient was 0.5597, which located in *C. hainanensis* and *C. taiwaniana*, indicating that they had closest genetic relationship. The similarity coefficient of *C. fairylakea* and *C. guangdongenses* was the smallest among all species, it showed that there were farthest genetic relationship between them. The similarity coefficient of different sex colony in a same species: *C. diannanensis* was 0.9098, which was the biggest datum of the all materials; they clustered first of all, showing the closest genetic relationship in all materials. The results of RAPD cluster that reflected the genetic relationship of all the species showed that RAPD was very suitable for interspecies genetic relationship analysis of cycad.

3 Discussions

3.1 Several factors affecting the RAPD reation and RAPD essentiality to plant taxonomy

Template DNA, Mg^{2+}, dNTP, *Taq* DNA polymerase and the primers were the main factors affecting the RAPD reaction, if one of them was changed, the stability of whole reaction system could be influenced greatly. So it is important to ascertain the reaction parameters for RAPD analysis.

To summarize above results and analysis, the optimal reaction system of RAPD was as follows: 20 μL reaction volume including 10 mmol/L Tris-HCl (pH8.3), 50 mmol/L KCl, 2.0 mmol/L $MgCl_2$, 200 μmol/L dNTP, 0.3 μmol/L primers, 50 ng template DNA, 1 U *Taq* DNA polymerase.

Many results of comparative studies on RAPD with ISSR methods to many families of plants showed that the results were very high coherence. Such as correlation analysis among genetic similarities based on RAPD, ISSR markers and phenotypic data showed the correlation coefficient of RAPD and ISSR marks were 0.932, indicating they were significantly correlated. The number of fragments per primer, the percentage of polymorphic loci was in turn 9.09%, 83.73 % for RAPD markers, and 5.88%, 79.79 % for ISSR markers. It's showed that these indexes of RAPD were higher than ISSR. Although ISSR had higher percentage of polymorphic loci in C. annuum than RAPD, and effective number of alleles(Ne), Shannon's Information index(I), heterozygosity(Ht), the genetic differentiation coefficient (Gst) based on ISSR markers were bigger than those based on RAPD as well. But these indexes had very small difference, the number commonly was the scope in 0.01—0.04, whereas some numbers were RAPD higher than ISSR(Chen *et al.*, 2007).

In another study, for RAPD analysis, 13 random primers were identified with polymorphism among the entries; in total, 167 bands were produced, of which 50 bands were polymorphic; percentage of polymorphic bands was 29.9%. For ISSR analysis, 7 primers were

identified with polymorphism among the entries; in total, 96 bands were produced, of which 44 bands were polymorphic; percentage of polymorphic bands was 45.8%(Xiao,2006).

Study results from Zhang *et al.* (2007) showed that 225 and 196 bands were amplified by 12 RAPD primers and 12 ISSR primers respectively. There were 215 and 196 polymorphic bands amplified by RAPD and ISSR primers respectively, and the percentages of polymorphic bands were 95.56% and 100.00% respectively. The gene diversity among species(Ht) and within species(Hs) were 0.3689, 0.3575 and 0.1077, 0.1380, respectively.

Hereinbefore these results indicated that polymorphic bands amplified, percentages of polymorphic bands general were higher RAPD than ISSR, in the gene diversity among species(Ht) and within species(Hs) Ne, I, Ht and Gst, RAPD and ISSR were very appropinquity, and some species had higher ISSR than RAPD, some species had higher RAPD than ISSR, but their number difference was very small.

These studies fully proved that RAPD method was a very trusty and stabilization to analysis plant relationship between species, subspecies, variation and form, even variety(Wang *et al.*, 2003; Qi *et al.*, 2003, 2004; Ding *et al.*, 2005; Xiao, 2006; Chen *et al.*, 2007; Wang & Ding, 2007; Xiao *et al.*, 2007; Zhang *et al.*, 2007; Jia *et al.*, 2008; Pan et al., 2008; Wang *et al.*, 2008; Zhang *et al.*, 2008). After some scholars had completed the comparative studies RAPD and ISSR, proved RAPD a very and exact method to research the relationships between species, subspecies, variation and form, they continue to use RAPD for the farther studies on some species in same genera, such as Zhang *et al.* had made the studies which comparative studies RAPD and ISSR on *Iris* L. Plants and published(2007), then they continued to carry out RAPD studies on other species of Iris L. plants(2008).

The improving of RAPD reaction system for *Cycas* in this study was significant to afford right and effective method on analysed the relationship between species, subspecies, variation and form in Cycadopsida.

3.2 Evolutionary relationships of five species

Researchers have been disputed on the taxon of *C. fairylakea*, *C. hainanensis*, *C. taiwaniana*, *C. guangdongensis*, *C. diannanensis* for a long time. Both Huang (2001) and Wang *et al.* (1996) considered that *Cycas guangdongensis* (the brand hang in the sample tree located in Shenzhen Fairy Lake Botanical Garden) and *C. taiwaniana* were different names of a same species. Chen pointed that *C. hainanensis* distinguished from *C. taiwaniana* by megasporophyll with less lateral segments, and so on(Hill, 1994). Wang *et al.* (1996) made wide investigation in Hainan Province, finding that Megasporophyll terminal segments shape of type form of *C. taiwaniana* and *C. hainanensis* were broad deltoid, furthermore, it had a series of variation from deltoid to subulate-lanceolate, numbers of later segments also had a certain range of variation, so *C. hainanensis* was a modified form, and should be merged into the latter. Huang (2001) considered that characteristics of *C. hainanensis* had been mistakenly included in the morphologic illustrating of *C. taiwaniana* by Wang, Wang (1996) thought *C. taiwaniana* had many varieties, because he had illustrated characteristics of *C. taiwaniana* including all the megasporophyll shapes of *C. hainanensis*. Chen (1999) merged *C. diannanensis* and *C. fairylakea* into *C. taiwaniana*, then renamed *C. taiwaniana*'s while Chinese name as *C. guangdongensis*. Anatomical research showed that micro-shapes were apparently different between *C. taiwaniana* and *C. hainanensis*, they were two independence species(Huang, 2001).

In this research, different sexes of *C. diannanensis* were clustered in the first group, their similarity coefficient was 0.9098. and it was the biggest one of all the species, *C. taiwaniana*, *C. hainanensis* and *C. guangdongensis* were clustered together, the similarity coefficient between each other of them ranged from 0.5476 — 0.5597. It indicated that *C. hainanensis* was closer genetic relationship to *C. taiwaniana*, and *C. hainanensis* and *C. guangdongensis*, *C. taiwaniana* and *C. guangdongensis* also had closer genetic relationship. The similarity coefficient among *C. fairylakea*, *C. diannanensis* and *C. taiwaniana* ranged from 0.3671—0.4395, and it showed that they had a far genetic relationship each other.

According to the common view(Lane, 1993), those

species which their similarity coefficients each other was more than 0. 90 could be merge to the same species, those which similarity coefficients ranged from 0. 67—0. 90 were different subspecies and variations, the similarity coefficients being less than 0. 67 was at the species level. In present study, the similarity coefficients of 5 defined species of *Cycas* were all less than 0. 67, therefore, the authors supported that they were established as five independent species.

Analyzing the results of molecular mark, the similarity coefficient was very high(more than 0. 90)between male and female of *C. diannanensis*, other similarity coefficient of defined species each other was lower and very low(all were less than 0. 687), it was approximately coincident with the classical taxon by other research methods. It indicated that RAPD was a propitious way to study *Cycas* taxonomy. Present research is useful for further research on the evolutionary relationships of every taxon of *Cycas* and afforded effectively using molecular marker method.

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References:

Chase MW, Hills HH. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies[J]. *Taxon*, **40**: 215—220

Chen JR, Stevenson DW. 1999. Cycadaceae[M]// Wu ZY, Peter HR(eds). Flora of China(Vol. 4)[M]. Science Press: Beijing & Missouri Botanical Garden Press, 1—7

Chen XJ, Cheng ZF, Chen JF, *et al*. 2007, Genetic diversity of pepper germplasm with RAPD, ISSR and phenotypic data[J]. *Act Bot Boreal-Occident Sin*, **27**(4): 662—670

Ding CB, Zhou YH, Yang RW, *et al*. 2005. Relationships among *Pseudoroegneria* species based on random amplified polymorphic DNA(RAPD)[J]. *Acta Pratacul Turae Sin*, **14**(1): 38—43

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue[J]. *Phytochemical Bull*, **9**(1): 11—15

Hill KD, Chen JR. 1994. On *Cycas taiwaniana* Carruthers(Cycadaceae)and the cycads of southeastern China[J]. *Acta Phytotaxon Sin*, **32**(6): 538—548

Huang YY. 2001. Studies On Systematics and Evolution of Cycadaceae of China [M]. Beijing, Meteorology Press

Jia JB, Li WL, Wu WL, *et al*. 2008. RAPD analysis of *Rubus* spp. [J]. *J Plant Res Environ*, **17**(3): 18—22

Lane MA, Wang IR. 1993. *Rhododendron albiflorum* Hook(Ericaceae): one taxon or two[J]. *Rhodora*, **95**: 11—20.

Lanner C, Bryngelsson T, Gustafsson M. 1996, Genetic validity of RAPD markers at the intra- specific and inter-specific in wild *Brassica* species with n=9[J]. *Theor Appl Genet*, **93**: 9—14.

Pan YZ, Pang B, Sun ZY, *et al*. 2008, Phylogenetic relationship analysis among 13 species of *Primula* L. in south-west Sichuan using RAPD marker[J]. *Fore Res*, **21**(3): 391—396

Qi JM, Zhou DX, Wu WR, *et al*. 2003. The application of RAPD technology in genetic diversity detection of jute[J]. *Acta Genetica Sin*, **30**(10): 926—932

Qi JM, Zhou DX, Wu WR, *et al*. 2004, A comparison between RAPD and ISSR technology in detection of genetic diversity of jute[J]. *Sci Agric Sin*, **37**(12): 2 006—2 011

Wang AH, Ding Y. 2007, Genetic diversity of wild close relatives of barley(*Hordeum vulgare*)in Tibet of China by using RAPD and ISSR markers[J]. *J Wuhan Univ*(*Nat Sci Edi*), **53**(6): 723—730

Wang FX, Liang HB, Chen TQ. 1996. Chinese Cycads[M]. Guangzhou, Guangdong Science and Technology Press

Wang JG, Che DD, Liu SK, *et al*. 2008. RAPD analysis of twenty-six general species in *Gladiolus hybridus*[J]. *Bull Bot Res*, **28**(3): 321—324

Wang SQ, Lin YQ, Tang H, *et al*. 2003. RAPD and ISSR analysis of germplasmic resources of Chewing Cane[J]. *Acta Agric Univ Jiangxi*, **25**(3): 412—417

Xiao BG. 2006, Assessment of genetic relationships between the flue-cured Tobacco varieties by RAPD and ISSR markers[J]. *J Wuhan Bot Res*, **24**(5): 392—396

Xiao HJ, Xu Z, Li LH, *et al*. 2007. netic diversity of *Roegneria* genera studied by ISSR markers[J]. *Acta Agric Boreali-Sin*, **22**(4): 146—150

Zhang M, Huang SZ, Qiu S, *et al*. 2007. Analysis of RAPD and ISSR on genetic diversity of *Iris* plants[J]. *J Plant Res Environ*, **16** (2): 6—11

Zhang M, Huang SZ. 2008, RAPD Analysis on swome germplasm of *Iris* plants[J]. *Acta Bot Boreal-Occident Sin*, **28**(5): 933—939

Zhou YH. 1999. Research the relationships among the species of *Roegneria* koch by RAPD[J]. *Plant Journal*, **41**(10): 1 076—1 081

苏铁属植物RAPD反应体系的优化及部分种类亲缘关系的探索

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参考文献(22条)

1. Chase MW;Hills HH Silica gel:An ideal material for field preservation of leaf samples for DNA studies 1991

2. Chen JR;Stevenson DW Cycadaceae 1999

3. 陈学军,程志芳,陈劲枫,娄群峰,耿红 辣椒种质遗传多样性的RAPD和ISSR及其表型数据分析[期刊论文]-西北植物学报 2007(4)

4. 丁春邦,周永红,杨瑞武,张利,郑有良 应用RAPD分子标记探讨拟鹅观草属的种间关系[期刊论文]-草业学报 2005(1)

5. Doyle JJ;Doyle JL A rapid DNA isolation procedure for small quantities of fresh leaf tissue 1987(01)

6. Hill KD;Chen JR On Cycas taiwaniana Carruthers(Cycadaceae)and the cycads of southeastern China 1994(06)

7. Huang YY Studies On Systematics and Evolution of Cycadaceae of China 2001

8. 贾静波,李维林,吴文龙,闾连飞,郭巧生 悬钩子属植物的RAPD分析[期刊论文]-植物资源与环境学报 2008(3)

9. Lane MA;Wang IR Rhododendron albiflorum Hook(Ericaceae):one taxon or two 1993

10. Lanner C;Bryngelsson T;Gustafsson M Genetic validity of RAPD markers at the intra-specific and inter-specific in wild Brassica species with n=9 1996

11. 潘远智,庞博,孙振元,陈强 川西南13种报春花属植物亲缘关系的RAPD分析[期刊论文]-林业科学研究 2008(3)

12. 祁建民,周东新,吴为人,林荔辉,方平平,吴建梅 应用RAPD指纹探讨黄麻属种间遗传多样性及其亲缘关系[期刊论文]-遗传学报 2003(10)

13. 祁建民,周东新,吴为人,林荔辉,方平平,吴建梅 RAPD和ISSR标记检测黄麻属遗传多样性的比较研究[期刊论文]-中国农业科学 2004(12)

14. 汪爱华,丁毅 西藏近缘野生大麦RAPD和ISSR分子标记的遗传多样性[期刊论文]-武汉大学学报（理学版） 2007(6)

15. Wang FX;Liang HB;Chen TQ Chinese Cycads 1996

16. 王金刚,车代弟,柳参奎,杨传平 26个唐菖蒲品种RAPD分析[期刊论文]-植物研究 2008(3)

17. 王水琦,林彦铨,汤浩,高三基,潘世明 果蔗种质资源的RAPD和ISSR分析[期刊论文]-江西农业大学学报(自然科学版) 2003(3)

18. 肖炳光 利用RAPD和ISSR标记分析烤烟品种间遗传关系[期刊论文]-武汉植物学研究 2006(5)

19. 肖海峻,徐柱,李临航,马玉宝,曹授俊 利用ISSR标记研究鹅观草属种质资源的遗传多样性[期刊论文]-华北农学报 2007(4)

20. 张敏,黄苏珍,仇硕,孙延东 鸢尾属植物遗传多样性的 RAPD和ISSR分析[期刊论文]-植物资源与环境学报 2007(2)

21. 张敏,黄苏珍 鸢尾属部分植物种质资源的RAPD分析[期刊论文]-西北植物学报 2008(5)

22. Zhou YH Research the relationships among the species of Roegneria koch by RAPD 1999(10)

本文读者也读过(10条)

1. 王朝晖. 李劲平. 王培训. WANG Zhao-hui. LI Jing-song. WANG Pei-xun 不同产地穿心莲药材的RAPD鉴别初步研究[期刊论文]-中医药导报2006,12(5)

2. 杨泉光. 李楠. 李志刚. 林平义. 罗斌. YANG Quan-Guang. LI Nan. LI Zhi-Gang. LIN Ping-Yi. LUO Bin 苏铁属花粉萌发及保存条件研究[期刊论文]-广西植物2009,29(5)

3. 张一平. 王进欣. 刘玉洪. 马友鑫. 李佑荣. ZHANG Yi-ping. WANG Jing-xin. LIU Yu-hong. MA You-xin. LI You-rong 热带次生林林窗干热季光照特征初步分析[期刊论文]-广西植物2001,21(1)

4. 朱华. 赵见明. 蔡敏. 刘世龙. 李黎 云南德宏州种子植物区系研究(Ⅰ)--科和属的地理成分分析[期刊论文]-广西植物2004,24(3)

5. 张宏达. 丘华兴 值得注意的中国植物(续)[期刊论文]-广西植物2002,22(1)

6. 金振洲. Jin Zhenzhou 滇川干热河谷种子植物区系成分研究[期刊论文]-广西植物1999,19(1)

7. 王晓鹏 安徽皇甫山琅琊山野生太子参生态环境比较研究[期刊论文]-广西植物2003,23(5)

8. 王文采. WANG Wen-sai 四川毛茛科翠雀属一新种[期刊论文]-广西植物2008,28(5)

9. 李锋. 盘名德. LI Feng. PAN Ming-de 突出科技与经济结合这个核心创造改革与建设的新辉煌--广西植物研究所65周年回顾与展望[期刊论文]-广西植物2000,20(3)

10. 张巧玲. 王少平. 胡启明. 郝刚. ZHANG Qiao-Ling. WANG Shao-Ping. HU Qi-Ming. HAO Gang 中国紫金牛属圆齿组花粉形态研究及其分类学意义[期刊论文]-广西植物2007,27(3)

引证文献(4条)

1. 周明昆,李正文,李志刚,李素丽,李楠,刘鹏 模拟酸雨对德保苏铁叶片光合作用及根系分泌有机酸的影响[期刊论文]-南方农业学报 2012(05)

2. 王小艳,张恩慧,许忠民,程永安,许念芳,马勇斌 甘蓝Ogura胞质雄性不育基因的RAPD标记[期刊论文]-西北农业学报 2010(06)

3. 郑道君,谢良商,张文,张治礼 海南龙血树RAPD-PCR反应体系的优化[期刊论文]-广西植物 2011(01)

4. 农保选,黄玉源,刘驰 基于RAPD分析的中国苏铁属部分种类亲缘关系探讨[期刊论文]-广西植物 2011(02)

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