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利用 RAPD 分子标记评价仲彬草属的种间关系

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Relationships among *Kengyilia* species assessed by RAPD markers

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Abstract To assess the interspecific relationships in *Kengyilia*, fourteen species of *Kengyilia* were used for RAPD assay. Of the 34 random primers tested, twenty (58.8%) produced polymorphic products. A total of 112 products amplified from 14 primers were selected for RAPD analysis. Eighty-three (74.1%) amplified products were found to be polymorphic among 14 species. Data were used to generate Jaccard's similarity coefficients and to construct a dendrogram using UPGMA in the NTSYS computer programs. It is concluded from this study that: (1) There was great genetic diversity among the 14 species of *Kengyilia*; (2) Distinct RAPD variations existed between the species from the Qinghai-Xizang Plateau and those from Xinjiang; (3) The species with similar morphological characters and the species from the same areas or neighboring geographical regions were clustered together; (4) RAPD results are comparable with those obtained from studies on morphology and cytology. RAPD assay is a useful additional method for assessing the relationships among the species in *Kengyilia*.

Key words *Kengyilia*; RAPD assay; Genetic variation; Relationships; Morphology

Kengyilia Yen et Yang (Poaceae: Triticeae) was described with *K. gobicola* Yen et J. L. Yang as the type species in 1990 (Yen, Yang, 1990a). Morphologically, it is a genus intermediate between *Agropyron* and *Roegneria*. *Kengyilia* differs from *Roegneria* by having an erect spike with dense spikelets, lemma densely pilose or hirsute, short-awned, and differs from *Agropyron* by the flat glume and lemma with rounded back, not keeled from tip to bottom, a terminal spikelet more often presented. These characters are obviously similar to those of section *Paragropyron* of the genus *Roegneria* (Baum *et al*., 1995; Keng, Chen, 1963). Cytologically, *Kengyilia* contains StYP genomes, and StY comes from *Roegneria*, and P from *Agropyron* (Yen, Yang, 1990b). Genome analysis and karyotype studies were carried out on most of the species in sect. *Paragropyron* of *Roegneria* and in *Kengyilia*. The results indicated that they contained StYP genomes (Zhang *et al*., 1998; Jensen, 1996, 1990; Zhou, 1994; Sun *et al*., 1993). Yang *et al*. (1992) included the species of sect. *Paragropyron* of *Roegneria* in the genus *Kengyilia*. Up to now, there are about

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23 species and 9 varieties, mainly distributed in western and south-western regions of the former USSR, western China and neighboring regions with the altitude about 1100 ~ 5100 m. They thrive in subalpine meadows, alpine and montane steppes on lush green slopes of glade and fringes of forests and also in semidesert or extremely dry stony desert (Yang *et al.*, 1992).

Since 1990, the random amplified polymorphic DNA (RAPD) technique has been used to construct genetic maps, to identify and localize molecular markers linked to important genes, to identify cultivars, accessions and species, to measure genetic variation for establishing genetic and evolutionary relationships, and to generate phylogenetic trees for genomes, species, subspecies and populations (Zhou *et al.*, 1999; Wei *et al.*, 1997; Jain *et al.*, 1994). The objectives of the present study were to analyze RAPD marker variations among the species of *Kengyilia* and to evaluate the usefulness of RAPD markers in the studies of interspecific relationships.

1 Materials and methods

1.1 Plant materials

One hundred and sixty-six individual plants representing 14 *Kengyilia* species were analyzed in this study (Table 1). Nine to 16 plants of each species were examined.

Table 1 Species and their locality of experimental materials in *Kengyilia*

No.	Species	Accession No.	Chromosome No.	Genome Constitutions	Geographic Origin	No. Of Plants
1	*K. gobicola*	Y9503	42	StYP	Xinjiang, China	15
2	*K. thoroldiana*	Y2884	42	StYP	Qinghai, China	15
3	*K. hirsuta*	Y2919	42	StYP	Gansu, China	10
4	*K. melanthera*	Y9509	42	StYP	Sichuan, China	10
5	*K. kokonorica*	Y2880	42	StYP	Qinghai, China	10
6	*K. grandiglumis*	Y2857	42	StYP	Qinghai, China	10
7	*K. rigidula*	Y9510	42	StYP	Gansu, China	12
8	*K. stenachyra*	Y2922	42	—	Gansu, China	12
9	*K. laxiflora*	Y2893	42	StYP	Sichuan, China	11
10	*K. longiglumis*	Y9514	42	—	Xinjiang, China	10
11	*K. mutica*	Y9307	42	—	Qinghai, China	16
12	*K. pamirica*	Y9501	42	—	Xinjiang, China	16
13	*K. laxistachys*	Y9508	42	—	Xinjiang, China	10
14	*K. tahelacana*	Y0573	42	—	Xinjiang, China	9
Total	14					166

1.2 Template DNA preparation

Three grams of fresh leaf tissue from three-month-old plants were frozen in liquid nitrogen, ground and used for DNA extraction. The extraction buffer and procedure were based on the phenol/chloroform protocols of Sharp *et al.* (1988). The DNA concentration was determined by using LKB ultroscpec Ⅲ DNA fluorometer (Phamacia Company) and comparing band intensities with known standards of lambda DNA on an agarose gel. The genomic DNA samples was diluted in sterilized water to a concentration of 20 ng/μl.

1.3 RAPD assay

Decamer oligonucleotides used in this study were obtained from Opron Technologies (California, U.S.A.). The Taq DNA polymerase, 10× buffer and 30 mM $MgCl_2$ were obtained from Hua-

Mei (Beijing, China). All reagents except template DNA were premixed and aliquoted. The optimized amplification reaction mixture (20 μl) contained 1 × buffer, 1.8 mM $MgCl_2$, 0.1 mM dNTP, 0.2 μM primer, 20 ng template DNA and 1 unit of Taq DNA polymerase. About 25 μl of mineral oil overlaid on each reaction mixture. DNA amplification was performed using a MJ Research Inc. PTC-200 PCR programmed for 50 cycles of 45 sec at 94℃, 45 sec at 36℃ and 2 min at 72℃ using the fastest available transitions followed by storage at 4℃. Appropriate loading buffer was added. The RAPD products were separated by agarose gel electrophoresis in 1 × TBE on 1.5% agarose containing 0.5 μg/ml ethidium bromide. DNA fragments were visualized and photographed under UV light.

1.4 Data analysis

Photographs were used to score the RAPD data. DNA fragment sizes were determined by comparing with DNA size markers. The DNA bands were scored for their presence (1) or absence (0) in each accession. These data matrices were entered into NTSYS-PC program (Rohlf, 1993). Data were analyzed using Simqual (Similarity for Qualitative Data) routine to generate Jaccard's similarity coefficients. Similarity coefficients were used to construct dendrograms using the unweighted pair group method with arithmetic average (UPGMA) and the SHAN (sequential, hierarchical, agglomerative, and nested clustering) routine in the NTSYS programs.

2 Results and discussion

2.1 Primer selection

A total of 34 primers were tested to select those that produced polymorphic DNA bands. A polymorphic band was defined as an amplified DNA fragment that was present in at least one accession and was absent in at least one accession. Of the 34 primers tested, eight (23.5%) had no detectable product or produced ambiguous products. Six (17.7%) produced uniform fragments among accessions, and 20 (58.8%) produced some polymorphic fragments. Fourteen primers were selected for RAPD assay (Table 2). These primers produced 112 bands, ranging from 3 ~ 12 bands per primer. The RAPD results in 14 *Kengyilia* taxa produced by primer OPA-04 was showed in Fig. 1.

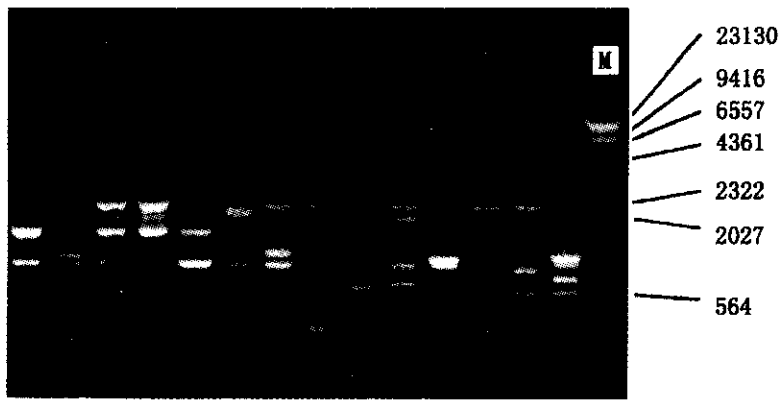

Fig.1 RAPD results in 14 *Kengyilia* taxa produced by primer OPA-04
The material order from the right to the left was described in the same order as in the Table 1. Lambda DNA digested with Hind Ⅲ was used as molecular weight marker(M), and the size of the fragments was indicated in the right.

2.2 RAPD marker variation among the taxa

Of the 112 bands, twenty-nine (25.9%) fragments amplified were present in all the 14 species. Eighty-three (74.1%) unambiguous polymorphic bands were used to analyze the genetic diversity among the 14 species studied. The Jaccard's genetic similarity coefficients ranged from 0.463 ~ 0.857 (Table 3). The similarity coefficients were used to generate a dendrogram with UPGMA (Fig. 2).

From the dendrogram, the 14 species was divided into three groups.

Table 2 List of primers, their sequences, and amplification results

Primer	Sequences	Total bands	Scorable polymorphic bands
OPA-01	5' CAGGCCCTTC 3'	3	2
OPA-02	5' TGCCGAGCTG 3'	6	3
OPA-04	5' AATCGGGCTG 3'	13	9
OPA-05	5' AGGGGTCTTG 3'	9	9
OPB-07	5' GGTGACGCAG 3'	7	5
OPB-08	5' GTCCACACGG 3'	9	8
OPB-10	5' CTGCTGGGAC 3'	7	5
OPB-11	5' GTAGACCCGT 3'	8	8
OPC-12	5' TGTCATCCCC 3'	5	4
OPC-13	5' AAGCCTCGTC 3'	9	8
OPD-20	5' ACCCGGTCAC 3'	9	4
OPR-13	5' GGAGGACAAG 3'	11	6
OPR-16	5' CTCTGCGCGT 3'	12	10
OPX-02	5' TTCCGCCACC 3'	4	2
Total	14	112	83

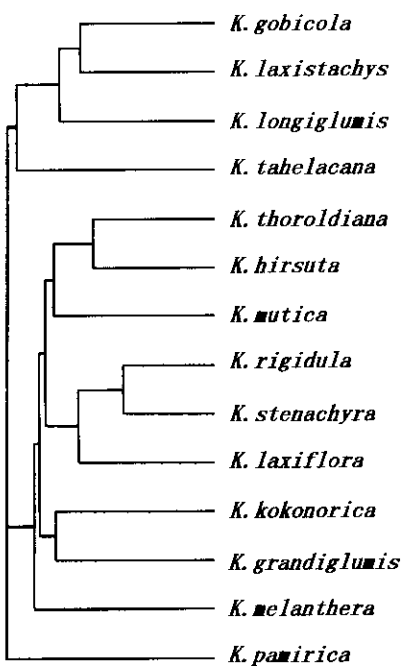

Fig. 2 A dendrogram generated from RAPD markers for 14 *Kengyilia* species. Scale is Jaccard's coefficients similarity.

In group Ⅰ, there is one species, i. e. *K. pamirica*. *K. pamirica* with densely arranged spikelets was distributed in the Pamir Plateau, south Xinjiang, China.

Table 3 Matrix of Jaccard's coefficients of genetic similarity based on RAPD data*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.000													
2	0.676	1.000												
3	0.688	0.798	1.000											
4	0.545	0.731	0.658	1.000										
5	0.591	0.672	0.671	0.612	1.000									
6	0.573	0.691	0.646	0.676	0.698	1.000								
7	0.618	0.671	0.688	0.610	0.682	0.662	1.000							
8	0.644	0.694	0.671	0.636	0.697	0.645	0.857	1.000						
9	0.593	0.693	0.679	0.646	0.648	0.613	0.763	0.744	1.000					
10	0.675	0.575	0.584	0.512	0.575	0.554	0.654	0.659	0.667	1.000				
11	0.595	0.746	0.646	0.608	0.651	0.639	0.663	0.667	0.654	0.554	1.000			
12	0.568	0.554	0.580	0.539	0.554	0.611	0.571	0.557	0.549	0.588	0.634	1.000		
13	0.757	0.608	0.627	0.494	0.565	0.557	0.580	0.585	0.558	0.740	0.577	0.613	1.000	
14	0.634	0.542	0.506	0.463	0.522	0.506	0.532	0.538	0.494	0.588	0.547	0.500	0.592	1.000

* The material order from 1, 2, 3,……, to 14 is described in the same order as in Table 1.

In group Ⅱ, there are 9 taxa. They are *K. thoroldiana*, *K. hirsuta*, *K. mutica*, *K. rigidula*, *K. stenachyra*, *K. laxiflora*, *K. kokonorica*, *K. grandiglumis* and *K. melanthera*. *K. rigidula*, *K. stenachyra* and *K. laxiflora* were clustered together. They were distributed in the border of Sichuan, Qinghai and Gansu provinces in China, with lax spikes. *K. rigidula* was closely related to *K. stenachyra*. Morphologically, *K. rigidula* differs from *K. stenachyra* only by length and width of glume, glume vein and length of lemma. *K. laxiflora* was treated as *Roegneria laxiflora* in the sect. *Clinelymus* of *Roegneria* by Yang (1987). Cytological study indicated that *K. laxiflora* contained StYP genomes (Zhang *et al*., 1998). Therefore, *K. laxiflora* was a valid taxon in *Kengyilia*. Although *K. thoroldiana*, *K. hirsuta* and *K. mutica* were clustered together, *K. thoroldiana* and *K. hirsuta* were closer to each other than either to *K. mutica*. These three species were distributed in the Qinghai-Xizang Plateau, with dense spikes. *K. kokonorica* and *K. grandiglumis* with dense spikes were distributed in Qinghai, China. Although the genetic similarity between them was much less than that between *K. thoroldiana* and *K. hirsuta*, *K. kokonorica* and *K. grandiglumis* were clustered together. *K. melanthera* was a species with dense spikes, distributed in western Sichuan, China. It was distant from *K. kokonorica*, *K. grandiglumis* and *K. thoroldiana*.

In group Ⅲ, *K. gobicola*, *K. laxistachys*, *K. longiglumis* and *K. tahelacana* were grouped together. They are taxa with lax spikes, commonly distributed in the Karakorum Mountain Range, southern Xinjiang, China. *K. longiglumis* was also found in southwestern Gansu. *K. longiglumis* was closely related to *K. gobicola* and *K. laxistachys*.

3 Conclusions

Although the number of RAPD markers in this study was small, the results from RAPD data provided some supports for those from morphological and cytological studies. The present study indicated that the taxa from the Qinghai-Xizang Plateau had greater genetic differences than those from the species in Xinjiang. This suggests that the original species of *Kengyilia* species in Qinghai-Xi-

zang and in Xinjiang were different. It was inferred that the StY genomes of *Kengyilia* species in Xinjiang might have originated from *Roegneria* in the Middle Asia and the StY of *Kengyilia* in the Qinghai-Xizang Plateau came from *Roegneria* in the Himalayas. This is in our further pursuit.

The RAPD technique is simpler, less costly, and less labor-intensive than other DNA marker methodologies when studying genetic relationships of different groups of plant species (Caetano-Anolles *et al.*, 1991). It also has some limitations. One problem with RAPDs is the degree of homology between fragments of similar size on which the similarity measures are based. At least in closely related individuals these are likely to be allelic, so the technique is recommended for comparison of very closely related taxa, i.e., at the intraspecific level or among very closely related species (Oppen *et al.*, 1994). The most limiting factor is its sensibility, which affects the reproducibility of the results. But it may be overcome by eliminating variation in DNA concentration, and taking care to ensure consistent reaction conditions and thermal profile during amplification (Wei *et al.*, 1997; Rafalski *et al.*, 1995). In this study, when the concentrations of all reagents in RAPD reactions were optimized, and after two times each primer was tested for each individual analysis, the reproducibility was quite good. Therefore, we concluded that the RAPD technique was a highly useful additional method for assessing the relationships among the species of *Kengyilia*.

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摘要　利用随机扩增多态性DNA(RAPD)技术分析了14种仲彬草属 *Kengyilia* 植物的种间关系。对34个OPRON公司十聚体随机引物进行多态性筛选，20个(58.8%)能产生多态性。14个引物产生的112个DNA片断，用于计算种间Jaccard遗传相似性系数分析，在NTSYS程序中利用UPGMA构建系统发育树状图。分析结果表明：(1)14个 *Kengyilia* 物种存在较大的遗传多样性；(2) 青藏高原的物种与新疆的物种的RAPD变异极大；(3)形态相似、地理分布一致的物种有一定的亲缘关系，聚类在一起；(4)RAPD结果与形态学和细胞学等分析结果一致。RAPD分析方法将为 *Kengyilia* 系统分类提供DNA水平上丰富的资料。

关键词　仲彬草属；RAPD分析；遗传变异；亲缘关系；形态学

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新书《系统与进化植物学中的分子标记》出版

由中国科学院植物研究所系统与进化植物学开放研究实验室邹喻苹、葛颂两位研究员和王晓东博士编写的新书《系统与进化植物学中的分子标记》2000年10月由科学出版社出版，各地新华书店发行。

全书共分12章，约30万字。本书深入浅出地阐述了RAPD，PCR-RFLP，SSR以及AFLP等常用分子标记技术的原理与方法及介绍有关分子生物学背景知识；通过大量典型实例举一反三地展示了上述分子标记技术在植物居群生物学、保护生物学、遗传资源保护和利用、品种鉴定、野生种亲缘关系分析以及植物系统发育等研究中的广泛应用；同时也详细介绍了分子标记数据处理的基本原理和方法以及一些常用的数据分析软件。本书内容反映了国际最新动态，反映了我国和本开放室的研究成果，且通俗易懂，可读性较强，是从事植物分子系统学、分子生态学、居群生物学、保护生物学和生物多样性等方面研究的科研人员和管理人员了解本领域的入门指南。鉴于分子标记技术在诸多领域应用广泛，本书对于从事遗传育种、植物生理与病理、园艺、环境保护、遗传病与流行病的检测与诊断、亲子鉴定以及法医诊断的工作者也颇具参考价值。

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