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应用 RAMP 分子标记探讨拟鹅观草属的种间关系*

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摘要：采用 RAMP（random amplified microsatellite polymorphism）**标记技术，分析了拟鹅观草属** 9 **种** 1 **亚种和鹅观草属** 6 **种植物之间的遗传变异和亲缘关系。** 33 **个引物组合产生的** 310 **条** DNA **扩增片段中，** 286 **条（**92.25%**）具有多态性，每个引物组合产生** 5～13 **条多态性带，平均为** 8.67 **条。利用** 310 **个** RAMP **标记，在** NTSYS-pc **软件中，计算** Jaccard **遗传相似系数，建立** UPGMA **聚类图。结果表明：（**1**）物种间遗传差异明显，具有丰富的遗传多样性；（**2**）阿拉善鹅观草和大丛鹅观草与拟鹅观草属的物种聚类在一起，表明它们与拟鹅观草属的亲缘关系较近，而与本试验所分析的另外** 4 **个鹅观草属物种的亲缘关系较远；（**3**）** RAMP **分子标记可以将拟鹅观草属的物种分开，而且形态相似、地理分布相同或相近的物种聚类在一起；（**4**）** RAMP **结果与形态学和细胞学的分析结果一致，表明** RAMP **标记是评价拟鹅观草属种间关系十分有效的方法。**

关键词：拟鹅观草属；RAMP **分析；聚类分析；亲缘关系；遗传变异**
中图分类号：Q 943 **文献标识码：**A **文章编号：**0253－2700(2005)02－0163－08

Relationships among *Pseudoroegneria* Species Based on RAMP

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Abstract： Relationships and genetic variation among nine species and one subspecies of *Pseudoroegneria* and six species of *Roegneria* were analyzed by using RAMP（random amplified microsatellite polymorphism）markers. A total of 310 products were amplified by 33 primer combinations，among which 286（92.25%）products were found to be polymorphic. 5～13 polymorphic bands were produced by each primer combination，with an average of 8.67. The data of 310 RAMP bands were used to generate Jaccard's similarity coefficients and to construct a dendrogram by means of UPGMA in the NTSYS-pc program. It was concluded as follows：（1）Distinct genetic differences and extensive genetic diversity were present among the species；（2）2 species in *Roegneria*，i.e.，*R. alashanica* and *R. magnicaespes*，and 10 accessions in *Pseudoroegneria* were grouped together，indicating that the two *Roegneria* species were closer to the species of *Pseudoroegneria* than to the other four species of *Roegneria* analysed in this study；

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(3) *Pseudoroegneria* species were separated clearly from each other based on RAMP markers. Meanwhile, the species with similar morphological characters and the species from the same areas or neighboring geographical regions were clustered together; (4) RAMP results were basically comparable with those obtained from morphological and cytological studies. It is concluded that RAMP is a useful method to assess the genetic relationships among *Pseudoroegneria* species.

Key words: *Pseudoroegneria*; RAMP analysis; Cluster analysis; Relationship; Genetic variation

Pseudoroegneria A. Löve, a newly established genus (Löve, 1980), includes 15 – 20 species occurring on open rocky hillsides from Middle East and Transcaucasia across Central Asia and northern China to western North America. The *Pseudoroegneria* grasses are caespitose, long-anthered, and cross-pollinating perennials; they have narrow, linear spikes with single, distantly spaced spikelets, with or without awns (Dewey, 1984; Löve, 1984). These grasses are exceptionally drought tolerant and they are excellent forage.

According to Löve (1982, 1984) and Dewey (1984), this genus contains a basic St genome, with diploid ($2n = 2x = 14$, StSt) and auto- and allo-tetraploid ($2n = 4x = 28$, StStStSt or $St_1St_1St_2St_2$) taxa. The St genome is one of the most important genomic components, present in more than half of the perennial Triticeae species. In combination with other genomes, the St genome has formed the polyploid genera, namely, *Elymus* L., *Elytrigia* Desv., *Roegneria* C. Koch., *Kengyilia* Yen et Yang, *Hystrix* Moench, *Sitanion* Raf. and *Pascopyrum* A. Löve (Dewey, 1984).

No *Pseudoroegneria* species was reported in Flora Reipublicae Popularis Sinicae and local Floras in China (Kuo, 1987). In fact, there are three species and one subspecies of *Pseudoroegneria*, i.e., *P. strigosa* (M. Bieb.) A. Löve, *P. cognata* (Hackel) A. Löve, *P. elytrigioides* (C. Yen et J. L. Yang) B. R. Lu and *P. strigosa* ssp. *aegilopoides* (Drobov) A. Löve reported in China. *P. elytrigioides* was first recognized as *Roegneria elytrigioides* by C. Yen et J. L. Yang (Yan and yang, 1984). *R. elytrigioides* shared the same basic St genome as that of *Pseudoroegneria*, i.e., $St_1St_1St_2St_2$, and was strongly recommended to transfer into the genus *Pseudoroegneria* by Lu (1994). *R. alashanica* Keng and *R. magnicaespes* (D. F. Cui) L. B. Cai, distributed in China, are similar to *P. elytrigioides* morphologically (Yan and Yang, 1984; Cui, 1990; Cai, 1997). Zhou *et al* (1999) found that *R. alashanica* and *R. magnicaespes* had intensive genetic differences from the other *Roegneria* species analysed in their study based on RAPD markers. According to cytogenetic analysis, Zhang *et al* (1999) reported *R. alashanica* and *R. magnicaespes* contained at least one St genome. So, *Pseudoroegneria* is a natural genus in China. The evaluation of *Pseudoroegneria* species and its geographical regions systematically in China is of significance to the discussion of the origin and evolution of the polyploid genera in China, such as *Elymus* L., *Elytrigia* Desv., *Roegneria* C. Koch., *Kengyilia* Yen et Yang and *Hystrix* Moench.

Random amplified microsatellite polymorphism (RAMP), as defined by Wu *et al* (1994), combined the advantages of simple sequence repeat (SSR) and random amplified polymorphism DNA (RAPD). Following RAMPs, 5'-anchored oligonucleotides complementary to SSRs were used in

combination with decamers of arbitrary sequence to amplify genomic DNA fragments, in the meantime detecting and mapping co-dominant microsatellite polymorphisms without cloning and sequencing (Davila *et al*, 1998). It has been previously shown that RAMPs reflected the genealogies of cultivars more faithfully than RAPDs did and were particularly fit for plant species of ambiguous genetic background (Davila *et al*, 1999; Cheng *et al*, 2001). RAMPs have been employed in studies on genetic diversity and relationship of peach cultivars and barley cultivars (Cheng *et al*, 2001; Zvingila *et al*, 2001). Zhang *et al* (2003) assessed interspecific relationships among *Kengyilia* species.

The objectives of the present study were (1) to analyze variations among 2 *Roegneria* species, i.e., *R. alashanica* and *R. magnicaespes*, and *Pseudoroegneria* species by means of RAMP marker; (2) to assess the interspecific relationships in *Pseudoroegneria*; (3) to compare the results of molecular marker with those obtained from morphological and cytological studies; (4) to evaluate the use of RAMP marker in the systematic study of *Pseudoroegneria* germplasms.

1 Materials and methods

1.1 Materials

The materials used in this study were listed in Table 1. To analyze RAMP marker variations among 2 *Roegneria* species, i.e., *R. alashanica* and *R. magnicaespes*, and *Pseudoroegneria* species, other 4 *Roegneria* species, i.e., *R. caucasica* C. Koch, *R. grandis* Keng, *R. kamoji* Ohwi and *R. ciliaris* (Trin.) Nevski were selected for test. All plants were planted in a space-planted nursery. All vouchers were preserved in Herbarium, Triticeae Research Institute, Sichuan Agricultural University, Dujiangyan (SAUTI).

Table 1　Materials used in this study

Number	Taxon	Chromosome no.	Genome constitutions	Locality	Voucher
1	*P. spicata*	14	StSt	California, United States	PI232123
2	*P. strigosa*	14	StSt	Xinjiang, China	PI499638
3	*P. strigosa* ssp. *aegilopoides*	14	StSt	Xinjiang, China	PI595164
4	*P. libanotica*	14	StSt	Iran	PI330687
5	*P. stipifolia*	14	StSt	Former Soviet Union	PI314058
6	*P. geniculata*	28	$St_1St_1St_2St_2$	Russian Federation	PI565009
7	*P. gracillima*	28	—	Former Soviet Union	PI420842
8	*P. kosaninii*	28	—	Turkey	PI237636
9	*P. tauri*	28	StStPP	Iran	PI380652
10	*P. elytrigioides*	28	$St_1St_1St_2St_2$	Xizang, China	Z2005
11	*R. alashanica*	28	StSt—	Ninxia, China	Z2006
12	*R. magnicaespes*	28	StSt—	Xinjian, China	Y9512
13	*R. caucasica*	28	StStYY	Former Soviet Union	PI531572
14	*R. grandis*	28	StStYY	Shanxi, China	Y92001
15	*R. kamoji*	42	StStHHYY	Sichuan, China	Y1416
16	*R. ciliaris*	28	StStYY	Sichuan, China	Y83006

1.2 Methods

Genomic DNA was extracted from fresh young leaves following the procedure described by Sharp *et al* (1988). The isolated genomic DNA was stored at 4℃. Two 5'-anchored oligonucleotides, GC $(CA)_4$ and GT $(CA)_4$, were used in combination with oligonucleotides of arbitrary sequence from Operon Technologies (kits A, B, H and R) to amplify genomic DNA of the 16 accessions. PCR reactions were carried out in a MJ Research PTC-200 Peltier Thermal Cycler

following the protocol of Davila *et al* (1999) with modification. The amplification reaction mixture (25 μl) contained 20 ng template DNA, 1 unit Taq DNA polymerase, 100 nmol/L GC $(CA)_4$ or 100 nmol/L GT $(CA)_4$, 100 nmol/L decamer oligonucleotide random primer, 100 μmol/L each of dNTP, 1.5 mmol/L $MgCl_2$ and 1 × PCR buffer. About 25 μl of mineral oil was overlaid on each reaction mixture. DNA amplification was performed for 4 min at 94℃ for initial denaturation, followed by 45 cycles of 1 min at 94℃, 1 min at 45℃ and 2 min at 72℃, with a final extension reaction of 10 min at 72℃. 5 μl loading buffer was added. The PCR products were separated by agarose gel electrophoresis in 1 × TAE on 2.0% agarose containing 0.5 ng/ml ethidium bromide (EB). Images were photographed, captured by Gel Doc 2000™ (Bio-Rad). Molecular weights were estimated by using a 100 bp DNA ladder plus.

Photographs were used to score the RAMP data. The DNA bands were scored for their presence (1) or absence (0) in each accession. These data matrices were entered into the NTSYS-pc program (Rohlf, 1993). Data were analyzed using a Simqual (similarity for qualitative data) routine to generate Jaccard's similarity coefficients. Similarity coefficients were used to construct a dendrogram by means of the unweighted pair group method with arithmetic average (UPGMA) and the SHAN (sequential, hierarchical, agglomerative, and nested clustering) routine in the NTSYS-pc program.

2 Results and discussion

2.1 RAMP polymorphisms

A total of 160 primer combinations were tested to select those producing polymorphic DNA bands. Of the 160 primer combinations tested, 45 produced polymorphic fragments. 33 primer combinations which were able to produce clear amplified bands, were selected for formal amplification reaction. The results were used for RAMP assay. These primer combinations produced 310 bands, ranging from 6 to 14 bands per primer combination (Table 2), with an average of 9.39. Of 310 bands, 286 (92.25%) polymorphic bands were obtained. The polymorphic bands produced by each primer combination ranged from 5 to 13, with an average of 8.67. This indicated that there was considerable RAMP variation among the species. Fig. 1 shows the results of amplification from primer pair GT $(CA)_4$ + OPA – 13.

Table 2 Primer combination and amplification results

Primer combination	Total bands	Scorable Polymorphic bands	Primer combination	Total bands	Scorable Polymorphic bands
GC $(CA)_4$ + OPA – 01	11	10	GT $(CA)_4$ + OPB – 05	9	9
GC $(CA)_4$ + OPA – 02	7	6	GT $(CA)_4$ + OPB – 07	6	5
GC $(CA)_4$ + OPA – 13	12	10	GC $(CA)_4$ + OPH – 01	8	8
GT $(CA)_4$ + OPA – 02	10	9	GC $(CA)_4$ + OPH – 03	7	6
GT $(CA)_4$ + OPA – 04	10	10	GC $(CA)_4$ + OPH – 06	10	10
GT $(CA)_4$ + OPA – 10	12	11	GC $(CA)_4$ + OPH – 10	11	11
GT $(CA)_4$ + OPA – 13	9	7	GC $(CA)_4$ + OPH – 11	9	8
GT $(CA)_4$ + OPA – 18	8	8	GC $(CA)_4$ + OPH – 16	8	7
GT $(CA)_4$ + OPA – 19	11	10	GC $(CA)_4$ + OPH – 19	9	8
GT $(CA)_4$ + OPA – 20	7	7	GC $(CA)_4$ + OPH – 17	9	8
GC $(CA)_4$ + OPB – 03	10	10	GC $(CA)_4$ + OPR – 04	9	8
GC $(CA)_4$ + OPB – 05	9	8	GC $(CA)_4$ + OPR – 05	10	10
GC $(CA)_4$ + OPB – 07	9	9	GC $(CA)_4$ + OPR – 07	14	13
GC $(CA)_4$ + OPB – 08	8	7	GC $(CA)_4$ + OPR – 11	8	7
GC $(CA)_4$ + OPB – 09	10	10	GC $(CA)_4$ + OPR – 14	10	8
GC $(CA)_4$ + OPB – 10	9	9	GC $(CA)_4$ + OPR – 18	10	9
GC $(CA)_4$ + OPB – 11	11	10	Total 33	310	286

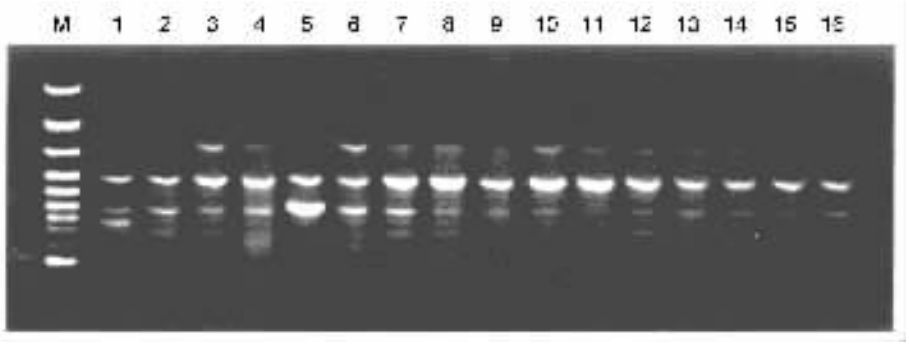

Fig. 1　RAMP polymorphism generated by primer combination GT $(CA)_4$ + OPA – 13

(The number of 1 – 16 refers to the species listed in Table 1. M: GeneRuler™ 100 bp DNA Ladder Plus marker)

2.2　Genetic similarities

All the 310 bands were used to calculate Jaccard's genetic Similarity (GS) coefficients among the 16 accessions (Table 3). The GS value ranged from 0.196 to 0.625, with the mean of 0.314. The highest GS value was found between *P. elytrigioides* and *R. alashanica*, while the lowest GS value was observed between *P. kosaninii* and *R. kamoji*.

Table 3　Genetic similarities (GS)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.000															
2	0.396	1.000														
3	0.341	0.453	1.000													
4	0.328	0.287	0.293	1.000												
5	0.358	0.340	0.328	0.364	1.000											
6	0.307	0.325	0.291	0.320	0.361	1.000										
7	0.322	0.302	0.289	0.345	0.303	0.401	1.000									
8	0.271	0.317	0.261	0.365	0.316	0.320	0.484	1.000								
9	0.255	0.278	0.234	0.408	0.425	0.330	0.354	0.323	1.000							
10	0.271	0.248	0.252	0.283	0.272	0.300	0.365	0.365	0.343	1.000						
11	0.276	0.272	0.276	0.308	0.331	0.346	0.359	0.317	0.328	0.625	1.000					
12	0.355	0.277	0.244	0.330	0.333	0.272	0.361	0.320	0.321	0.462	0.455	1.000				
13	0.268	0.264	0.237	0.291	0.325	0.352	0.260	0.212	0.322	0.300	0.285	0.242	1.000			
14	0.267	0.272	0.267	0.311	0.325	0.319	0.259	0.228	0.355	0.300	0.264	0.289	0.591	1.000		
15	0.283	0.290	0.254	0.338	0.292	0.265	0.274	0.196	0.220	0.246	0.301	0.295	0.412	0.388	1.000	
16	0.273	0.258	0.230	0.236	0.313	0.254	0.219	0.255	0.246	0.275	0.281	0.246	0.464	0.396	0.427	1.000

Note: The number of 1 – 16 refers to the species listed in Table 1.

2.3　Interspecific relationships

The Jaccard's Similarity coefficients were used to generate a dendrogram with UPGMA (Fig. 2). From the dendrogram, the 16 accessions were divided into two groups.

In group Ⅰ, there are four species of *Roegneria*. They are *R. caucasica*, *R. grandis*, *R. kamoji* and *R. ciliaris*. Among them, *R. caucasica*, *R. grandis*, and *R. ciliaris* contain StY genomes respectively, while *R. kamoji* contains StHY genomes. Cluster analysis based on RAMP markers indicates that *R. kamoji* was far from the other three *Roegneria* species.

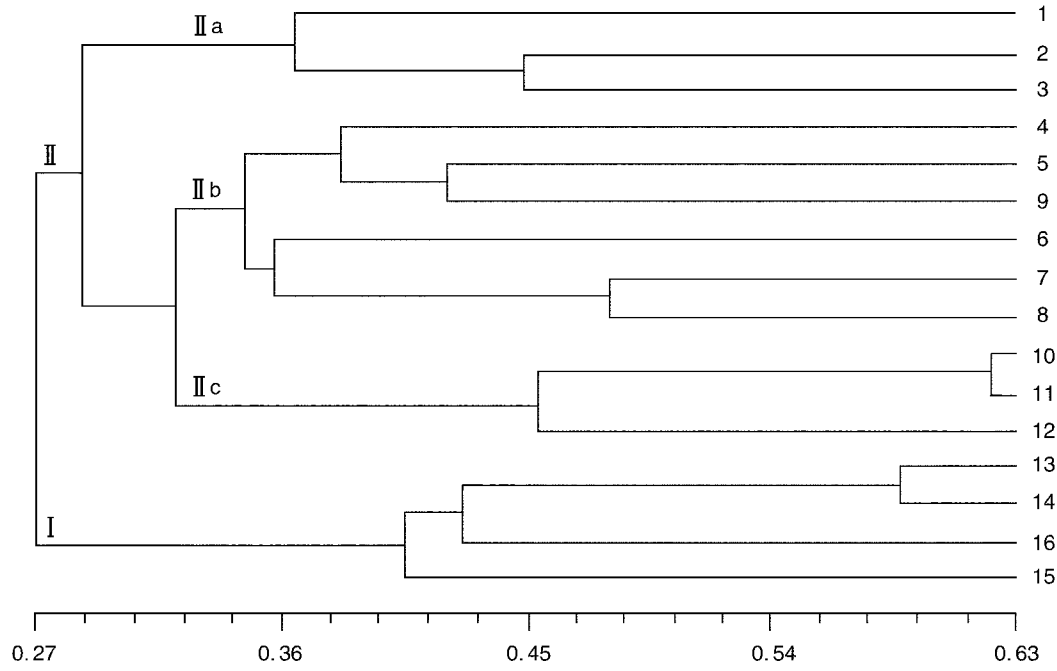

Fig. 2 A dendrogram generated by using Jaccard's coefficients of similarity
(The number of 1 – 16 refers to the species listed in Table 1)

In group Ⅱ, 2 species of *Roegneria*, i.e., *R. alashanica* and *R. magnicaespes*, and 10 taxa of *Pseudoroegneria* were clustered together. This indicates that the 2 *Roegneria* species were closer to the *Pseudoroegneria* species than those four *Roegneria* species in group Ⅰ. Results of this study and those of previous studies based on cytology (Lu, 1994; Zhang *et al*, 1999) are in considerable agreement. At the GS value of 0.32, group Ⅱ were divided into three subgroups. In subgroup Ⅱa, *P. strigosa* and *P. strigosa* ssp. *aegilopoides*, both from China, were clustered together first, then they grouped with *P. spicata* of America. In subgroup Ⅱb, *P. stipifolia*, *P. tauri* and *P. libanotica* were clustered together first, then clustered with three tetraploid species, i.e., *P. geniculata*, *P. gracillima* and *P. kosaninii*. Wang *et al* (1986) reported that *P. tauri* presented StStPP genomes, with P coming from *Agropyron* and St coming from *Pseudoroegneria* respectively. Jensen *et al* (1992) treated *P. tauri* as part of the genus *Pseudoroegneria* rather than *Agropyron* based on cluster analysis of morphological characters. In subgroup Ⅱc, *P. elytrigioides*, *R. alashanica* and *R. magnicaespes*, all distributing in China, were clustered together. Morphologically, they are similar to each other. *P. elytrigioides* differs from *R. alashanica* only in that it has longer rootstock and spike, and more spikelets (Yan and Yang, 1984). *P. elytrigioides* was treated as a variety of *R. alashanica* by Cai (1997). *R. magnicaespes* differs from *R. alashanica* only in that it forms bigger caespitose with dense pubescent rachilla (Cui, 1990). So, it is difficult to distinguish the three species based on morphological characters. But they were separated clearly from each other based on RAMP markers.

3 Conclusions

Jensen *et al* (1992) found within the genus *Pseudoroegneria* species separation was not clear based on cluster analysis of morphological characters. According to our results of RAMP markers, *Pseudoroegneria* species were separated clearly from each other. In the meantime, the species with similar morphological characters and the species from the same areas or neighboring geographical regions were clustered together, which indicates that RAMP loci were able to reveal more information than traditional morphological classification do.

According to the genomic system of classification, newly established by Löve (1982, 1984) and Dewey (1984), *Pseudoroegneria* contained St genome, and *Roegneria* presented StY or StHY genomes. Lu (1994) reported that *R. elytrigioides* contained $St_1St_1St_2St_2$ genomes and transferred it to *Pseudoroegneria* from *Roegneria*. Zhang *et al* (1999) reported that *R. alashanica* and *R. magnicaespes* had St genome but without Y genome. Ding *et al* (2004) found that *P. elytrigioides*, *R. alashanica* and *R. magnicaespes* had similar karyotypes. According to Oinuma 's (Oinuma, 1953) conclusion that there was an evolutionary parallelism between genome and karyotype, it was suggested that these three species had similar genomes, i.e., $St_1St_1St_2St_2$ genomes. Morphologically, the three species are similar to each other. In our RAMP analysis, they were grouped with *Pseudoroegneria* species. So, it was concluded that *R. alashanica* and *R. magnicaespes* may be the tetraploid species of *Pseudoroegneria* in China.

The RAMP technique could be considered a good multilocus markers system, for it is easy and reproducible to perform and able to detect high levels of polymorphism (Davila *et al*, 1998, 1999). In this study, the genetic diversity and interspecific relationships among the species of *Pseudoroegneria* were evaluated by using RAMP markers for the first time. The RAMP results were basically comparable with those obtained from studies on morphology and cytology. Therefore, it is concluded that RAMP is a useful method to assess the genetic relationships among *Pseudoroegneria* species.

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