植 物 学 报 2000，42(9)：988－990
Acta Botanica Sinica

·快讯 Short Communication·

双探针原位杂交揭示稻属 BB、CC 和 EE 基因组之间的分化

李常宝[1] 张大明[1*] 葛 颂[1] 卢宝荣[2] 洪德元[1]

(1. 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，北京 100093；
2. *Genetic Resources Center*，*International Rice Research Institute*，MCPO Box 3127，1271 Makati City，Philippines)

摘要： 利用双探针原位杂交技术，揭示了稻属 *Oryza officinalis* 复合体中 BB、CC 和 EE 基因组之间的分化。以标记的 BB 基因组(来自二倍体的 *O. punctata* Kotechy ex Steud.)的总 DNA 为探针，同 BBCC 基因组(*O. minuta* J. S. Presl. et C. B. Presel)的中期染色体杂交。结果表明，BB 基因组的 DNA 探针同与四倍体 *O. minuta* 中的 BB 基因组的染色体之间有强烈的杂交信号，而与 CC 基因组的染色体之间的杂交信号很弱。而 EE 基因组(来自 *O. australiensis* Domin)的总 DNA 标记的探针同 *O. minuta* 基因组的所有染色体均有不同程度的杂交，且杂交信号的大小和强弱在 BB 基因组的染色体和 CC 基因组的染色体之间无显著的差异。由此证明：(1)CC 和 BB 基因组之间的分化程度很大；(2)EE 基因组同 CC 和 BB 基因组具有一定的亲缘关系；(3)CC 基因组与 EE 基因组之间的关系较近而同 BB 基因组关系较远。

关键词： 基因组原位杂交(GISH)；稻属；基因组分化；BBCC 基因组；BB 基因组；EE 基因组

中图分类号：Q941 **文献标识码：**A **文章编号：**0577-7496(2000)09-0988-03

Detection of Differentiation Among BB, CC and EE Genomes in the Genus *Oryza* by Two-probe Genomic *in situ* Hybridization (GISH)

LI Chang-Bao[1], ZHANG Da-Ming[1*], GE Song[1], LU Bao-Rong[2], HONG De-Yuan[1]

(1. *Laboratory of Systematic and Evolutionary Botany*, *Institute of Botany*, *The Chinese Academy of Sciences*, Beijing 100093, China;
2. *Genetic Resources Center*, *International Rice Research Institute*, MCPO Box 3127, 1271 Makati City, Philippines)

Key words: genomic *in situ* hybridization (GISH); *Oryza*; genomic differentiation; genome **BBCC**; genome **BB**; genome **EE**

The genus *Oryza* consists of two cultivated species (*O. sativa* L. and *O. glaberrima* Steud.) and approximately 20 wild relative species widely distributed in the pan-tropics. These species have been classified into four complexes following the Vaughan's taxonomic system[1]. The *O. officinalis* complex is the largest complex in the genus, which includes ten species, having **BB**, **CC**, **DD**, and **EE** genomes in the diploids as well as **BBCC** and **CC**DD genomes in the tetraploids. The relationships among the **BB**, **CC**, and **EE** genomes still remain unclear, although previous studies have indicated certain affinities of these genomes[2-4]. Genomic *in situ* hybridization (GISH) is a powerful technique to detect the relationships among the related genomes at chromosome and DNA levels. The objective of the present study was to investigate the relationships among the **BB**, **CC** and **EE** genomes in the genus *Oryza* by the two-probe GISH.

1 Materials and Methods

Three wild *Oryza* species were used in this study and described in Table 1. Seeds of the three *Oryza* species were obtained from the International Rice Research Institute (IRRI), Los Baños, the Philippines, and cultivated in a greenhouse in the Institute of Botany, The Chinese Academy of Sciences, Beijing. Two diploid wild rice species, *O. punctata* Kotechy ex Steud. (2n = 2x = 24, **BB**) and *O. australiensis* Domin (2n = 2x = 24, **EE**), were used as genomic probes. The tetraploid species, *O. minuta* J. S. Presl. et C. B. Presel (2n = 4x = 48, **BBCC**) was used as the target, from which chromosome spreads were prepared.

Received：2000-02-25 Accepted：2000-05-10
Foundation item：The Key Project B"Studies on Evolutionary Biology of Some Important Endangered Plant Species"(KZ951-B1-102) of the Chinese Academy of Science.
* Author for correspondence. E-mail：<zhangdm@ns.ibcas.ac.cn>.

Table 1 The genomes and origins of the wild *Oryza* species used in the study

Species	Accession No.	2n	Genome	Country of origin
O. punctata	IRGC 103896	24	**BB**	Tanzania
O. australiensis	IRGC 105263	24	**EE**	Australia
O. minuta	IRGC 101082	48	**BBCC**	Philippines

The method of chromosome preparation was modified from Fukui and Iijima[5], and Fukui *et al*[6]. The total genomic DNA of *O. punctata* (**BB**) was labeled with DIG-11-dUTP (Boehringer Mannheim, Cat. No. 1093088) and that of *O. australiensis* (**EE**) was labeled with bio-14-dATP (GIBCO BRL Cat. No. 19524-016) by nick translation. The labeled probes were purified with GFX™ PCR DNA and Gel Band Purification Kit (Pharmacia, 27-9602-01). The probe length was 200 – 600 bp estimated by gel electrophoresis. The procedures of *in situ* hybridization and probe detection followed those described by Leitch *et al*[7], with some modifications. The hybridization mixture consisted of 50% deionized formamide, 2 × SSC, 10% (W/V) dextran sulfate, 0.1% (W/V) SDS, 0.25 μg/μL sheared salmon sperm DNA (about 100 bp), and 3 – 5 ng/μL probe DNA. Detection of the biotin-labeled probe was achieved using avidin-FITC (Boehringer Mannheim) and the digoxigenin-labeled probe using anti-digoxigenin rhodamine conjugate (Boehringer Mannheim). The chromosomes were observed with a fluorescence microscope (Leica, DMRBE).

Fig. 1 a. Metaphase chromosome of *Oryza minuta* counterstained with DAPI. b. The same chromosome spread hybridized by digoxigenin labeled with DNA of the **BB** genome (*O. punctata*) and detected with anti-dig-rhodamine conjugate, showing strong hybridization signals of the **BB** genome. c. The same chromosomes were probed with biotinylated **EE** genome and detected with avidin-FITC, showing even hybridization signals on the **B**- and **C**-genome chromosomes. d. The same chromosomes hybridized with DNA of both **EE** and **BB** genomes, showing co-hybridization of the **BB** and **EE** genomes (yellow) and the **EE** genome (green). bar = 5 μm.

Photographs were taken with Kodak Ektachrome 400 slide film.

2 Results and Discussion

Oryza minuta is a tetraploid species and its genomic constitution was designated as **BBCC**[8]. Fig. 1a shows a metaphase spread of *O. minuta* stained with DAPI, confirming its tetraploid nature with 48 chromosomes and its small chromosome size. Fig. 1b shows the chromosomes of *O. minuta* probed by digoxigenin-labeled total DNA from diploid *O. punctata* (**BB** genome). The 24 chromosomes showing bright-red fluorescence signals belonged to the **BB** genome, while the remaining 24 with weak hybridization signals were chromosomes of the **CC** genome. The result confirmed the existence of the **BB** genome in *O. minuta*. The differentiation between the **BB** genome and the **CC** genome is significantly large, considering chromosomes of the **CC** genome had weak hybridization signals with the **B**-genome probe. The same chromosome spread was probed with biotinylated total genomic DNA of *O. australiensis* (**EE** genome) and the green hybridization signals presented on all chromosomes in *O. minuta* (Fig. 1c). This result indicates that the **EE** genome has certain relationship with both the **BB** and **CC** genomes. However, compared with the result of hybridization with digoxigenin-labeled total DNA from the **BB** genome, we believed that the differentiation between the **CC** and **BB** genomes is more significant than that between the **CC** and **EE** genomes. Fig. 1d shows the hybridization result of two-probe GISH with both **B**- and **E**-genome probes. The chromosomes of the **BB** genome were painted as yellow signals and the chromosomes of the **CC** genome as green signals. Yellow fluorescence located on the **B**-genome chromosomes indicated a mixture of red and green colors by co-hybridization of the **B**-genome (red) and **E**-genome (green) probes. The green signals indicated the hybridization of the **E**-genome probe alone on the **C**-genome chromosomes. The image clearly shows that the **EE** genome can hybridize with both of the **BB** genomes, and that the hybridization signals in general have no distinct difference in domain and intensity between the **C**- and **B**-genome chromosomes. Based on these results, we concluded that the **EE** genome has certain affinities with the **CC** and **BB** genomes, and the **CC** genome has a closer relationship with the **EE** than with the **BB** genome.

References:

[1] Vaughan D A. The genus *Oryza* L. Current status of taxonomy. Manila: *IRRI Research Paper Series*, 1989, **138**: 1 – 21.

[2] Ge S, Sang T, Lu B R, Hong D Y. Phylogeny of rice genomes with emphasis on origins of allotetraploid species. *Proc Natl Acad Sci USA*, 1999, **96**: 14400 – 14405.

[3] Wang Z Y, Second G, Tanksley S D. Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. *Theor Appl Genet*, 1992, **83**: 565 – 581.

[4] Aggarwal R K, Brar D S, Nandi S, Huang N, Khush G S. Phylogenetic relationships among *Oryza* species revealed by AFLP markers. *Theor Appl Genet*, 1999, **98**: 1320 – 1328.

[5] Fukui K, Iijima K. Somatic chromosome map of rice by imaging methods. *Theor Appl Genet*, 1991, **81**: 589 – 596.

[6] Fukui K, Ohmido N, Khush G S. Variability in rDNA loci in genus *Oryza* detected through fluorescence *in situ* hybridization. *Theor Appl Genet*, 1994, **87**: 893 – 899.

[7] Leitch A R, Schwarzacher T, Jackson D, Leitch I J. *In Situ* Hybridization—A Practical Guide. London: BIOs Scientific Publishers, 1994. 33 – 108.

[8] Morinaga T. Cytogenetical studies on *Oryza sativa* L. Ⅵ. The cytogenetics of hybrid of *O. minuta* Presl. and *O. latifolia* Desv. *Jpn J Bot*, 1943, **12**: 347 – 357.

(路安民编委推荐)(责任编辑:李长复)