Theor Appl Genet (2001) 102:157–162

C.Q. Sun · X.K. Wang · Z.C. Li · A. Yoshimura
N. Iwata

Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers

Received: 19 May 1999 / Accepted: 26 April 2000

Abstract Forty fourth single-copy RFLP markers were used to evaluate the genetic diversity of 122 accessions of common wild rice (CWR, *Oryza rufipogon* Griff.) and 75 entries of cultivated rice (*Oryza sativa* L.) from more than ten Asian countries. A comparison of the parameters showing genetic diversity, including the percentage of polymorphic loci (*P*), the average number of alleles per locus (*A*), the number of genotypes (*Ng*), the average heterozygosity (*Ho*) and the average genetic multiplicity (*Hs*) of CWR and *indica* and *japonica* subspecies of cultivated rice from different countries and regions, indicated that CWR from China possesses the highest genetic diversity, followed by CWR from South Asia and Southeast Asia. The genetic diversity of CWR from India is the second highest. Although the average gene diversity (*Hs*)of the South Asian CWR is higher than that of the Southeast Asian CWR, its percentage of polymorphic loci (*P*), number of alleles (*Na*) and number of genotypes (*Ng*) are all smaller. It was also found that the genetic diversity of cultivated rice is obviously lower than that of CWR. At the 44 loci investigated, the number of polymorphic loci of cultivated rice is only 3/4 that of CWR, while the number of alleles, 60%, and the number of genotypes is about 1/2 that of CWR. Of the two subspecies studied, the genetic diversity of *indica* is higher than that of *japonica*. The average heterozygosity of the Chinese CWR is the highest among all the entries studied. The average heterozygosity of CWR is about two-times that of cultivated rice. It is suggested that during the course of evolution from wild rice to cultivated rice, many alleles were lost through natural and human selection, leading to the lower heterozygosity and genetic diversity of the cultivated rice.

Communicated by G. Wenzel

C.Q. Sun (✉) · X.K. Wang · Z.C. Li
Department of Plant Genetics and Breeding,
China Agricultural University, Beijing 100094, PR China
e-mail: rice@public.cau.edu.cn
Fax: +86-10-62891055

A. Yoshimura · N. Iwata
Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

Keywords Rice · Genetic diversity · DNA · Restriction fragment length polymorphism (RFLP)

Introduction

Rice is an important food crop in both China and the whole world. Owing to the narrow genetic basis of the parental materials, the progress of rice breeding in China has been rather slow during the last 10 years, limiting any further increase in yield. Therefore, for assessment of the genetic diversity of rice and its wild relatives, common wild rice (CWR, *Oryza rufipogon* Griff.), their preservation and utilization have become of ever-increasing importance.

Since RFLP analysis of nuclear DNA could detect a relatively large number of loci scattered throughout the genome, and the polymorphism revealed by RFLP markers is less influenced by time and space and environmental conditions, this technique has been widely used in studying genetic variation and phylogenetic relationships among populations, species and varieties (Wang and Tanksley 1989; Nakano et al. 1992; Nakano et al. 1992; Wang et al. 1992; Zhang et al. 1992; Doi et al. 1995). RFLP analysis aiso provides an useful tool for estimating the extent of the genetic diversity among and within populations and species. Cai et al. (1996) analyzed the genetic diversity of three natural populations of CWR from Dongxiong, Yuanjiang and Guigong of the Guangxi Province and some natural populations of CWR from Thailand, India and Indonesia using 15 probes. Wang et al. (1996) compared the genetic diversities of three populations of CWR from Guilin, Dongxiang and Fusui of the Guangxi Province using 14 RFLP probes. It was revealed that even though well isolated from cultivated rice, genetic diversity does exist within natural populations of CWR. Although isozyme analysis could detect a smaller number of loci, it was also used for the assessment of the genetic diversity of rice. Huang et al. (1996) compared 700 Chinese local cultivars from six geographical groups and indicated that the Yunnan varieties had the highest genetic diversity.

In the present study, 44 single-copy probes, 122 accessions of CWR and 75 entries of cultivated rice were used to compare the genetic diversity of CWR from different countries and regions so as to provide a theoretical basis for the preservation of the genetic diversity and the establishment of core collections.

Materials and methods

Plant materials

Materials used in this study contained 122 accessions of CWR and 75 entries of cultivated rice. Of the 122 accessions of CWR, 39 accessions came from China, 27 from India, 7 from Sri Lanka, and 6 from Bangladesh of South Asia; 17 from Thailand, 12 from Burma, 5 from Cambodia, 6 from Malaysia, 2 from Indonesia and 1 from the Philippines of Southeast Asia. The 39 Chinese CWR entries included 6 entries from Dongxiong of the Jiangxi Province, 1 from Hunan, 9 from Guangxi, 5 from Guangdong, 4 from Yuanjiang and 14 entries with unknown origins. The 75 entries of cultivated rice came from 11 Asian countries. According to our taxonomical studies (Sun et al. 1997), 37 entries belonged to *indica* subspecies, and 38 entries to *japonica* subspecies. One individual per entry was used for RFLP analysis.

DNA extraction

Fresh leaves of a single plant in each accessions were collected and ground in liquid nitrogen or dry ice. DNA was extracted from the ground-tissues by the CTAB (Cetyltrimethyl ammonium bromide) method (Murray and Thompson 1980).

RFLP markers

Forty four probes (Table 1) used in the study were from clones located on RFLP maps constructed by Saito, et al. (1991) (designated as *XNpb* numbers), Kurata et al. (1994) (designated as *C*, *G*. *R* numbers) and Tsunematsu et al. (1996) (designated as *Ky* numbers).

Southern hybridization

Three micrograms of genomic DNA digested with *Dra*I or *Hind*III was electrophoresed on a 0.8% agarose gel. The gels were blotted onto a positively charged Nylon membrane (Boehringer Mannheim) by capillary transfer in 0.4 N NaOH for 12 h and the membrane was washed in 2×SSC, dried and baked at 120°C for 20 min. All the probes were amplified by PCR and labeled with HRP (horseradish peroxidase) according to the protocol of the ECL direct nucleic acid labeling and detection system (Amersham). Hybridized filters were detected by enhanced chemiluminescence on Fuji X-ray film for 1–3 h.

Assessment of genetic diversity

Each of the 44 probes detected a locus where each polymorphic band could be represented an allele. The genetic diversity of the entries studied was estimated with parameters calculated according to the following formulae.

Percentage of polymorphic loci *P*:

$$P=(k/n)\times 100\%,$$

where k is the number of polymorphic loci, n is the total number of loci investigated.

Average number of alleles per locus *A*:

$$A=\Sigma\, Ai/n,$$

where Ai is the number of alleles at the ith locus, n is the total number of loci investigated.

Average number of alleles per polymorphic loci *Ap*:

$$Ap=\Sigma\, Api/n_p,$$

where Api is the number of alleles at a certain polymorphic locus, n_p is the total number of polymrphic loci investigated.

Average number of genotypes per locus *G*:

$$G=\Sigma\, g/n,$$

where g is the number of genotypes at a certain locus, n is the number of loci investigated.

Average heterozygosity per locus *Ho*:

$$Ho=\Sigma\, Hoi/n=(1-\Sigma\, q_{ij})/n,$$

where Hoi is the heterozygosity at a certain locus, n is the total number of loci investigated and q_{ij} is the frequency of homozygous genotypes of an allele at a certain locus.

Average gene diversity *Hs* (Sano and Morishima 1992):

$$Hs=1-1/n\,\Sigma\, q^2_{ij},$$

where q_{ij} is the frequency of the jth allele at the ith locus, n is the number of loci investigated.

Table 1 Probes used in RFLP analysis

Enzyme	Probe	Chromosome	Enzyme	Probe	Chromosome	Enzyme	Probe	Chromosome
*Dra*I	*XNpb*252	1	*Dra*I	*XNpb*296	4	*Dra*I	*XNpb*152	7
*Dra*I	*XNpb*343	1	*Dra*I	*Ky*4	4	DraII	*XNpb*33	7
*Dra*I	*C*970	1	*Dra*I	*C*859	5	*Dra*I	*XNpb*278	8
*Hin*dIII	*XNpb*113	1	*Dra*I	*XNpb*255	5	*Dra*I	*XNpb*103	9
*Dra*I	*C*955	1	*Dra*I	*XNpb*81	5	*Dra*I	*XNpb*108	9
*Dra*I	*C*1211	1	*Hin*dIII	*XNpb*292	5	*Dra*I	*XNpb*37	10
*Dra*I	*XNpb*227	2	*Hin*dIII	*C*1447	5	*Dra*I	*XNpb*291	10
*Dra*I	*XNpb*349	2	*Dra*I	*XNpb*27	6	*Dra*I	*C*794	11
*Dra*I	*XNpb*67	2	*Dra*I	*XNpb*342	6	*Dra*I	*XNpb*202	11
*Dra*I	*XNpb*132	2	*Dra*I	*Ky*11	6	*Dra*I	*C*1003	11
*Dra*I	*XNpb*395	2	*Dra*I	*XNpb*12	6	*Dra*I	*XNpb*280	11
*Dra*I	*XNpb*48	3	*Dra*I	*XNpb*135	6	HindIII	*G*1465	11
*Dra*I	*XNpb*238	3	*Dra*I	*XNpb*338	7	*Dra*I	*R*496	12
*Dra*I	*XNpb*249	3	*Dra*I	*XNpb*117	7	*Dra*I	*XNpb*239	12
*Hin*dIII	*XNpb*311	4	*Dra*I	*XNpb*20	7			

Result

Comparison of the genetic diversity of CWR originating from different countries

Number of polymorphic loci(Np) and percentage of polymorphic loci (P)

It can be seen from Table 2 that CWR from different countries have different *Np* and *P* values. The number and percentage of polymorphic loci of CWR from China are the largest; 40 of the 44 loci investigated are polymorphic. CWR from India ranks the next, and CWR from Bangladesh has the lowest *P* value, only 20 loci are polymorphic. The *P* value of CWR from Cambodia is also rather low.

The total number of alleles (Na) and the average number of alleles per locus(A)

The number of alleles reflects the abundance of genes in a population. Of the 39 Chinese CWR entries studied, there are 136 alleles at the 44 loci investigated, with an average of 3.09 alleles per locus. This is the highest *A* value among those of all the countries involved in our study, indicating the higher abundance of genes in the Chinese CWR. Among the three South Asian countries, CWR entries from India possess the largest number of alleles, whereas the Bangladesh entries have only 1.52 alleles.

Among the six countries in Southeast Asian, the CWR entries from Thailand have the largest total number of alleles. The *A* of CWR from the Philippines and Indonesia are smaller.

The average number of alleles per polymorphic locus (*Ap*)of CWR from China is also the largest, followed by that of CWR from India. Those of CWR from the Philippines, Indonesia and Bangladesh are smaller. With the exception of the Chinese CWR entries whose *Ap* are more than three, those of CWR from all the other countries are only 2–3, with small ranges of variation.

Comparison of the number of genotypes (Ng) of CWR from different countries

The number of genotypes at the 44 loci of CWR from different countries and regions are quite different from each other (Table 2). The Chinese CWR entries have the largest number of genotypes, with an average of 3.98 genotypes per locus, about two or more times of that of CWR from Sri Lanka, Bangladesh, Cambodia, Malaysia and the Philippines. The *Ng* of CWR from Indian is significantly higher than those of CWR from other South Asian countries. And in the Southeast Asian region, CWR entries from Thailand possess the largest number of genotypes.

Comparison of average heterozygosity per locus (Ho)

Heterozygosity is the percentage of heterozygous loci among the total number of gene loci. It can be seen from Table 2 that CWR from Bangladesh and Burma possess the lowest *Ho,* less than 0.01. The *Ho* of CWR from India and Sri Lanka of the South Asian region are quite similar. In the Southeast Asian region, the *Ho* of CWR from Cambodia is the highest, followed by those of CWR from the Philippines and Indonesia. *Ho* of CWR from China is the highest, about seven heterozygous loci per 100 loci. The high heterozygosity of CWR from China might be due to its low seedset percentage of selfed individuals. According to observations by Prof. Morishima (data not published), the seedset percentage of selfed CWR from South and Southeast Asia are obviously higher than that of CWR from China. The lower seedset percentage of selfed Chinese CWR makes it more liable to introgression by other genes (from cultivated rice), leading to higher heterozygosity.

Table 2 Parameter values showing genetic diversity of common wild rice from different countries and of cultivated rice. Note: *Np:* no polymorphic loci, *P*: proportion of polymorphic loci, *Na*: total no. of alleles; *A*: average no. alleles/locus, *Ap*: average no. alleles/polymorphic loci, *Ng*: no. genotype, *G*: average no. genotype/locus, *Ho*: degree of heterozygate/locus, *Hs*: average gene diversity

Species	Origin	*Np*	*P*	*Na*	*A*	*Ap*	*Ng*	*G*	*Ho*	*Hs*
O. rufipogon	India	37	84.1%	109	2.478	2.76	116	2.64	0.0135	0.2948
	Sri Lanka	25	56.8%	77	1.75	2.32	78	1.77	0.013	0.2389
	Bangladesh	20	45.5%	67	1.52	2.15	68	1.55	0.0076	0.2115
	Thailand	31	70.5%	97	2.2	2.71	101	2.29	0.0201	0.2467
	Cambodia	22	50.0%	78	1.77	2.55	77	1.75	0.0364	0.2232
	Malaysia	26	59.1%	84	1.9	2.54	83	1.88	0.0227	0.2771
	Burma	28	63.6%	83	1.89	2.39	84	1.91	0.0057	0.2409
	Philippines and Indonesia	27	61.4%	74	1.68	2.11	73	1.66	0.0303	0.2626
	China	40	90.9%	136	3.09	3.3	175	3.98	0.0696	0.3543
O. sativa	*indica*	28	63.6%	91	2.07	2.68	99	2.25	0.0141	0.1849
	japonica	29	65.9%	87	1.98	2.48	92	2.09	0.0144	0.1502
	Average	28.5	64.7%	89.4	2.03	2.54	95.1	2.16	0.0225	0.2441
	CV %	20.7	20.7	21.6	12.9	31.5	31.5	80.7	22.04	

Comparison of average gene diversity (Hs)

CWR from China shows the highest *Hs* value (Table 2). This is quite similar to the results of Wang et al. (1996) who found that the total *Hs* of three natural populations of Chinese CWR was 0.341. Among CWR from South Asia, CWR from India has the highest *Hs*, followed by the *Hs* of CWR from Sri Lanka. The *Hs* of CWR from Bangladesh is the lowest, not only among the CWR from the three South Asian countries, but also among all the countries involved in this study. Among CWR from Southeast Asia, the *Hs* of CWR from Malaysia is the highest, followed by those of CWR from the Philippines, Indonesia, Thailand, Burma and Cambodia.

The variability of the parameters of genetic diversity

It can be seen from Table 2 that the coefficients of variation (CV) of the average heterozygosity per locus (*Ho*) is the highest among all of the various genetic parameters. This might be due to the fact that common wild rice is a cross-pollinated plant. The CV of the number of genotypes (*Ng*) is the next highest. The CVs of the polymorphic loci (*Ng*), the number of alleles per locus (*A*) and the average gene diversity (*Hs*) are not very different from each other. The CV of *Ap* is the lowest, indicating that variation of the average number of alleles per polymorphic locus is rather low.

Comparison of the genetic diversity of CWR from different regions

The genetic diversity of CWR from different regions is compared in Table 3. It was found that the order of the values of several parameters, including the percentage of polymorphic loci (*P*), the number of alleles (*Na*) the number of genotypes (*Ng*) and heterozygosity (*Ho*), when arranged according to magnitudes usually show the following region-related tendency: China>Southeast Asia>South Asia. But the order of the average gene diversity (*Hs*) is: China>South Asia>Southeast Asia. Of all parameters of diversity, the CV of *Ho* is the highest, with the widest range of variation. The next is the CV of *Ng*. On average, CWR from China has one more genotype per locus than CWR from South, and 0.84 more genotypes than CWR from Southeast, Asia. The CVs of the remaining parameters are rather low, with small regional differences.

Comparison of the genetic diversity of the *indica* and *japonica* subspecies of cultivated rice

It can be seen from Table 2 that, in the 37 entries of *indica* and the 38 entries of *japonica*, the number of polymorphic loci (*Np*), the percentage of polymorphic loci (*P*), the number of alleles (*Na*)and the average number of alleles per locus (*A*), the number of alleles per polymorphic locus (*Ap*), the number of genotypes (*Ng*)and the average heterozygosity (*Ho*) of *indica* are always larger or higher than those of *japonica*, indicating a higher genetic diversity of the *indica* entries. It was also found that differences of *Np*, *Na* and *Ng* between the two subspecies are smaller than those among wild rice originating from different countries. The average gene diversity (*Hs*) of *indica* and *japonica* in our study were 0.1849 and 0.1502 respectively. This is quite similar to the results of isozyme studies (Sun et al. 1996) in which the *Hs* at ten isozyme loci of *indica* and *japonica* were 0.166 and 0.158 respectively.

It can also be seen that the *Ho* of *indica* and *japonica* are rather similar, about 0.014, indicating that among 100 loci of an entry, 1.4 loci might be heterozygous.

Comparison of the genetic diversity of CWR and cultivated rice

A comparison of the genetic diversity of wild rice and cultivated rice is given in Table 4. It was evident that the genetic diversity of wild rice is obviously higher than

Table 3 Parameter values, same as Table 2, showing genetic diversity of common wild rice from different regions in Asia

Regions	No. acc.	*Np*	*P*	*Na*	*A*	*Ap*	*Ng*	*G*	*Ho*	*Hs*
South Asia	40	37	84.1%	117	2.66	2.97	127	2.89	0.013	0.3131
Southeast Asia	43	38	86.4%	125	2.84	3.13	138	3.14	0.019	0.3036
China	39	40	90.9%	136	3.09	3.4	175	3.98	0.07	0.3452
Average		38.3	86.40%	126	2.86	3.15	146.7	3.34	0.034	0.3206
CV %		4.0	4.0	7.6	7.6	6.2	17.1	17.1	92.7	6.8

Table 4 Parameter values same as Table 2, showing genetic diversity of *O. rufipogon* and *O. sativa*

Species	No. acc.	*Np*	*P*	*Na*	*A*	*Ap*	*Ng*	*G*	*Ho*	*Hs*
O. rufipogon	122	43	97.7%	177	4.02	4.09	232	5.27	0.03285	0.3672
O. sativa	75	33	75.0%	103	2.34	2.84	121	2.65	0.0142	0.2939
O. sativa/*O. rufipogon*		0.767	0.767	0.582	0.582	0.694	0.521	0.503	0.432	0.8

that of cultivated rice. Of the 44 loci investigated, only one locus in wild rice does not show polymorphism,whereas 12 loci in cultivated rice do not show polymorphism. The ratio between the number of polymorphic loci (*Np*) of cultivated rice and that of wild rice is about 3:4. Of the 181 alleles detected from 44 loci, 177 appear in wild rice, while 103 appear in cultivated rice. Only four alleles existed exclusively in cultivated rice, whereas 78 alleles existed exclusively in wild rice, indicating that most of the genes of cultivated rice could be found in wild rice; by contrast, a great many genes of wild rice could not be found, or else were lost, in cultivated rice. This result was consistent with that from isozyme analysis (Shahi et al. 1967). The average number of alleles (*A*)of wild rice is 4.02, whereas that of cultivated rice is only 2.34, about 1/2 of that of wild rice. The average number of alleles per polymorphic locus (*Ap*) of wild rice is 4.09; that of cultivated rice is 2.84, about 3/4 of that of wild rice. At the 44 loci investigated, the total number of genotypes (*Ng*) of wild rice is about twice that of cultivated rice which is only 121. The average number of genotypes per locus (*G*) of wild rice is 5.26, whereas that of cultivated rice is only 2.65, about 1/2 of that of wild rice. The average heterozygosity (*Ho*) of wild rice is twice that of cultivated rice. The *Hs* of wild rice is about 3/4 of that of cultivated rice. No matter where it originated from, the average gene diversity of CWR is always higher than that of cultivated rice.

Discussion

A range of studies on the genetic diversity of natural populations of cultivated rice and CWR have been reported, including morphological, isozyme, and DNA levels. But the numbers of markers used and the entries examined were usually rather small. In the present study, 44 RFLP markers were used to analyze 122 accessions of CWR from more than ten Asian countries, including China, India, Thailand etc., and 75 entries of cultivated rice, including both improved varieties and landraces from different countries. Various parameters showing genetic diversity, including the percentage of polymorphic loci (*P*), the number of alleles (*Na*) and genotypes (*Ng*), the average heterozygosity (*Ho*) and the average gene diversity (*Hs*), were used to compare the genetic diversity of CWR, *indica* and *japonica* from different countries and regions, as well as to study the difference between the genetic diversity of wild rice and that of cultivated rice. The results of some parameters [such as the average gene diversity (*Hs*)] were in agreement with those of our previous isozyme studies.

With the rapid increase in the number of germplasm resources ever-increasing attention has been focused on studies of a core collection, where the minimum quantity of germplasm resources could represent the diversity of the entire resources collected to the maximum extent. Authentic assessment of the diversity of germplasm resources, therefore, is the basis for the establishment of the core collection. Although assessment by phenotypic variation is simpler and easier to handle, some characters, especially the yield-potential characters, are liable to influences of environmental conditions. The shortcoming of isozyme analysis is its fewer number of loci and its time-and space-conditioned gene expression. The RFLP marker method does not have these problems. It could not only calculate the frequency of different alleles in different populations, so as to estimate the genetic diversity of different populations, even of the entire species, but also determine the number of alleles and genotypes at the investigated loci of different populations and the entire species. Consequently, it could preserve, to the greatest extent, the total number of alleles and genotypes and the frequency and multiplicity of each gene.

In a previous taxonomic study (Sun et al. 1997) with the same set of materials, it was suggested that the Chinese CWR could be subdivided into three types, such as the original, *indica*-like and *japonica*-like types, based on the differentiation of DNA. It was also found that CWR from South Asia has only the original and *indica*-like types; it does not however have the *japonica*-like type, indicating that CWR from China is more affluent in types than CWR from South Asia. The result of the present study confirms once more that the genetic diversity of CWR from China is greater than that of CWR from the South Asia and Southeast Asia. Among CWR from South Asian and Southeast Asian countries, CWR from India has the largest genetic diversity. This confirms once more that China and South Asia (with India as the center) are two centers of the genetic diversity of wild rice. This also emphasizes the importance of preservation and further study of the Chinese wild rice resources.

Compared with its wild relatives, the CWR, the average gene diversity (*Hs*), the number of alleles (*Na*) and genotypes (*Ng*) and the heterozygosity (*Ho*) of cultivated rice are much reduced while the seedset of selfed individuals is much increased, indicating the course of the evolution of cultivated rice from wild rice, as a result of both natural and human selection and continuous selfing. The heterozygosity decreased while the homozygosity increased, the number of alleles became smaller and the polymorphism was lowered. Oka once pointed out that, from the aspect of isozyme studies, domestication is a process leading toward similarity rather than differentiation.

Narrowness of the genetic basis is one of the principal causes of the slow progress of rice breeding. Xiao et al. found two QTLs that could increase 18% in yield from low-yielding wild rice from Malaysia. The cloned gene *Xa21* (Khush et al. 1990; Song et al. 1995) and a *Xa22* gene, both highly resistant to rice bacterial wilt, were discovered from *O. 1ongistaminata* and a Yunnan landrace ”Zhachanlong” respectively. The result of our present study also reveals that a great many genes of wild rice were already lost in cultivated rice; the number of alleles at 44 loci of cultivated rice is only about 1/2 that of wild rice. All these facts indicate that there is great potential for the discovery and utilization of wild rice and landraces in rice breeding.

Acknowledgments This project was supported by a grant from the ”973” Project, the Ministry of Science and Technology of China, and a grant from the National Natural Science Foundation of China (NSFC).

Reference

Cai HW, Wang XK, Morishima H (1996) Genetic diversity of Chinese wild rice populations. In: Wang Xiangkun, Sun Chuanqing (eds) Monograph on the origin and differentiation of Chinese cultivated rice. China Agricultural University Press, Beijing, 1996, pp 154–156

Doi K, Yoshimura A, Nakano M., Iwata N, Vaughan DA (1995) Phylogenetic study of A-genome species of the genus *Oryza* using nuclear RFLP. Rice Genet Newslett 12:160–162

Huang YH, Sun XL, Wang XK (1996) Study on the center of genetic diversity of Chinese cultivated rice (In Chinese with English abstract). In: Wang Xiangkun, Sun Chuanqing (eds) Monograph on the origin and differentiation of Chinese cultivated rice. China Agricultural University Press Beijing, 1996, pp 85–91

Khush GS, Bacalangco E, Ogawa T (1990) A new gene for resistance to bacterial blight from *O. longistaminata*. Rice Genet Newslett 7:121–122

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antoio BA, Shomura A., Shimiu T, Lin S-Y, Inoue T, Fukuda A, Shimano T, Kuki Y, Toyama T, Miyamoto Y, Kirhara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang Z-X, Momma T, Umehara Y, Yano M, Sasaki T Minobe Y (1994) A 300-kilobase interval genetic map of rice including 883 expressed sequences. Nature Genet 8:365–372

Lin X H, Zhang DP, Xie YF (1995) Mapping a new gene for resistance to bacterial blight based on RFLP markers. Rice Genet Newslett 12:234–236

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucl. Acids Res. 4321–4325

Nakano M, Yoshimura A, Iwata N (1992) phylogenetic study of cultivated rice and its wild relatives by RFLP. Rice Genet Newslett 9:132–134

Sano R, Morishima H (1992) Indica-japonica differentiation of rice cultivars viewed from variations in key characters and isozymes with special reference to landraces from the Himalayan hilly areas. Theor Appl Genet 84:266–274

Shahi BB, Morishima H, Oka HI (1967) A survey of variations in peroxidase and phosphatase and esterase isozymes of wild and cultivated *Oryza* species. Jpn J Genet 44:303–319

Saito A, Yano M, Kishimoto N, Nakagahra M, Yoshimura A, Saito K, Kuhura S, Ukai Y, Kawase M, Nagamine T, Yoshimura S, Ideta O, Ohsawa R, Hayano Y, Iwata N, Sugiura M (1991) Linkage map of restriction fragment length polymorphism loci in rice. Japan J Breed 41:665–670

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995)The rice disease resistance gene, *Xa21*, encodes a receptor kinase-like protein. Science 270:1804–1806

Sun CQ, Wang XK, Yoshimura A, Iwata N (1997) RFLP analysis of nuclear DNA in common wild rice (*O. rufipogon* Griff.) and cultivated rice (*O. sativa* L.) (In Chinese with English abstract). Scientia Agric Sinica 30: 37–44

Sun XL, Cai HW, Wang XK (1996) Diversity and nonrandom association of rice isozyme genes (In Chinese with English abstract). Acta Genet Sinica 23:276–285

Tsunematsu H, Yoshimura A, Harushimu Y, Nagamura Y, Kurata N, Yano M. Sasaki T, Iwata N (1996) RFLP framework map using recombinant inbred lines in rice. Breed Sci 46:279–284

Xiao JH, Grandillo S, Ahn SN, McCouch SR, Tanksley SD, Li JM, Yuan LP (1996) Genes from wild rice improve yield. Nature 384:223–224

Wang ZS, Zhu LH, Liu ZY, Wang XK (1996) Gene diversity of natural wild rice populations detected by RFLP markers (In Chinese with English abstract). J Agric Biotechnol 4: 111–117

Wang ZY,Tanksley SD (1989) Restriction fragment length polymorphism in *Oryza sativa* L. Genome 32:1113–1118

Wang ZY, Second G, Tanksley SD (1992) Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor Appl Genet 83:565–581

Zhang QF, Saghai Maroof MA, Lu TY, Shen BZ (1992) Genetic diversity and differentiation of indica and japonica rice detected by RFLP analysis. Theor Appl Genet 83:495–499