

An integrated linkage map of rice

O. Ideta, A. Yoshimura, and N. Iwata

To furnish significant information for genome analysis of rice, we have tried to integrate the morphological and restriction fragment length polymorphism (RFLP) linkage maps of all chromosomes. Thirty-nine morphological markers and 82 RFLP markers were mapped together based on segregation analysis of 19 F_2 populations derived from the crosses between indica variety IR24 and japonica lines with different morphological markers. Both linkage maps of rice were completely orientated. The gene *d2*, which had been thought to belong to the linkage group of chromosome 4, was linked to some RFLP markers on chromosome 1. Therefore, chromosomal location of *d2* was shifted to chromosome 1 from chromosome 4. Unmapped genes *gl1* on chromosome 5 and *I-Bf* on chromosome 9 were mapped on their respective linkage groups with some RFLP markers. The segregation distortion was detected on chromosomes 1, 2, 3, 6, 11, and 12. In these regions, the japonica/japonica allele frequencies were significantly less than the normal F_2 frequency of 25%. The RFLP linkage maps on chromosomes 2, 3, 6, 7, and 8 were considered not to be saturated. On these chromosomes, some marker genes were estimated to be located beyond the respective terminal ends of RFLP linkage maps.

Rice linkage studies have been conducted for the last 50 yr. Nagao and Takahashi (1963) proposed 12 possible linkage groups corresponding to the haploid chromosome complement of rice ($n=12$). Cytogenetic stocks such as trisomics and reciprocal translocations were used to establish relationship between these linkage groups and the 12 chromosomes of rice (Iwata 1986).

Recently, restriction fragment length polymorphism (RFLP) linkage maps of rice have been constructed (McCouch et al 1988, Saito et al 1991, Causse et al 1994, Kurata et al 1994). It is important to integrate the morphological linkage map with the RFLP linkage map for various genetic studies involving molecular tagging of genes

and marker-aided selection. Although some efforts to integrate the morphological linkage map and the RFLP linkage map have been reported (Kishimoto et al 1992, Causse et al 1994, Yu et al 1995), the integrated linkage map of all 12 chromosomes of rice had not been accomplished until now. In this study, we describe an integrated linkage map of the 12 chromosomes of rice constructed from the morphological linkage map (Iwata et al 1984, 1989a,b) and the RFLP linkage map (Saito et al 1991).

Materials and methods

Plant materials

Nineteen F_2 populations derived from the crosses between indica variety IR24 and japonica lines with from one to four genetic marker genes were used. Thirty-nine segregating marker genes of these F_2 populations are listed in Table 1.

RFLP analysis

Eighty-two RFLP markers mapped on the molecular map (Saito et al 1991) were used. DNA of F_2 populations were extracted from leaves and digested with six different restriction enzymes (*Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, and *Hind*III). Electrophoresis, Southern blotting, and hybridization were performed as described by Saito et al (1991).

Linkage analysis

Linkage analysis was performed on F_2 segregation data. Recombination values were estimated by the maximum likelihood equation (Allard 1956), and the recombination values were converted into genetic map distances (cM) using the Kosambi function (Kosambi 1944).

Table 1. Marker genes used in this study.

Chromosome	Marker genes ^a
1	<i>fs2, d18, d2, r12, spl6, eg</i>
2	<i>tri, bl1, spl2</i>
3	<i>chl1, fc1, dl, spl3</i>
4	<i>d11, Ph, lg</i>
5	<i>gl1, d1, spl7, nl1</i>
6	<i>dp1, spl4, Cl</i>
7	<i>g1, d6, spl5, v11</i>
8	<i>v8, sug</i>
9	<i>IBf, dp2, drp2, Dn1</i>
10	<i>spl10, pgl</i>
11	<i>z2, v9</i>
12	<i>spl1, r11</i>

^aSee Kinoshita (1993).

Results and discussion

Among the 172 RFLP markers randomly selected from the molecular map (Saito et al 1991), a total of 147 RFLP markers gave polymorphism in the genomic Southern hybridization patterns of indica variety IR24 and japonica variety Nipponbare. Among the 147 RFLP markers, 82 RFLP markers randomly selected were used for linkage analysis.

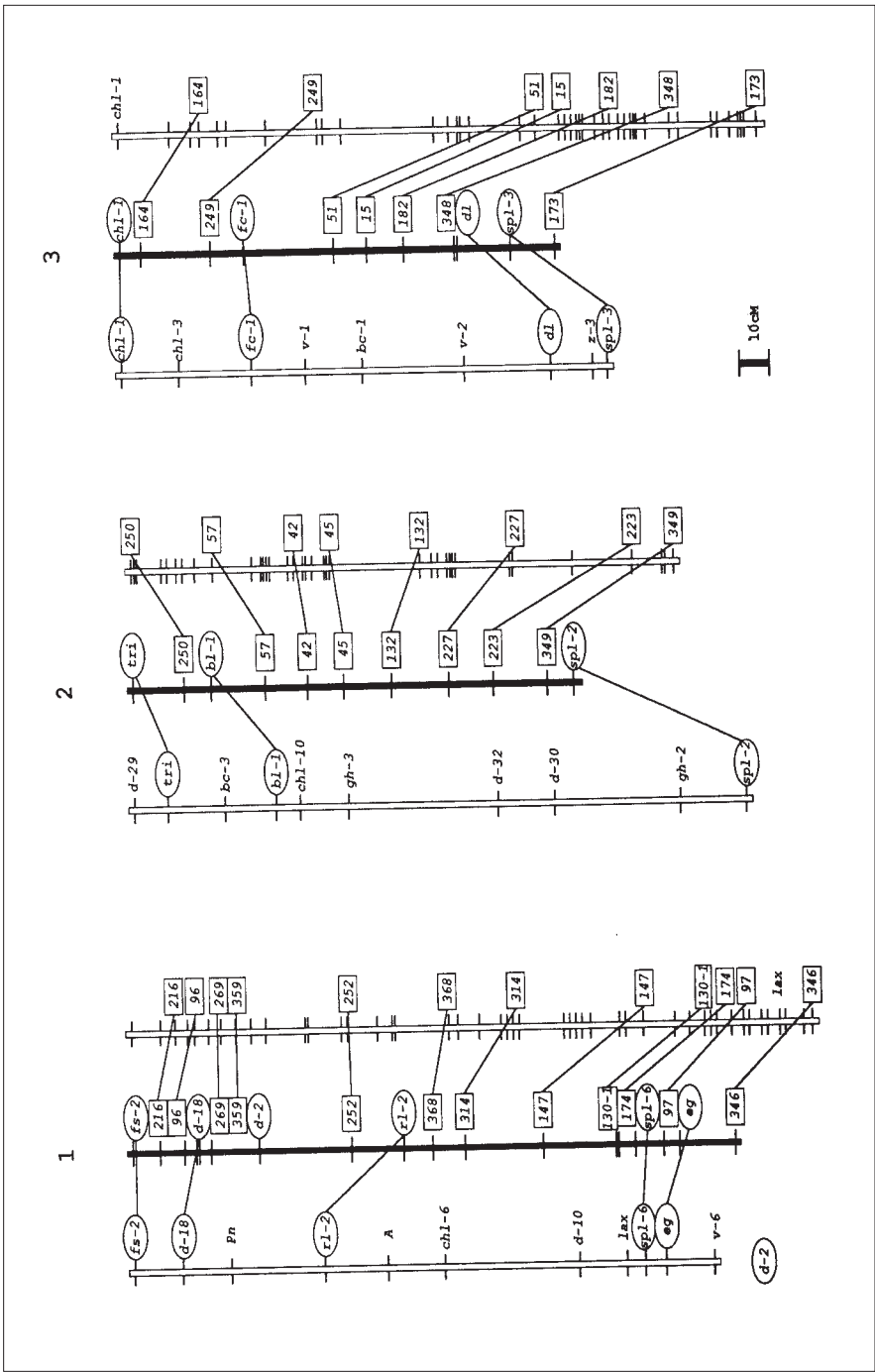
Japonica lines with different morphological markers were crossed to indica variety IR24. The segregation of morphological and RFLP markers was evaluated in 70-140 F₂ individuals. Based on the segregation analysis of these F₂ populations, an integrated linkage map of rice containing 39 morphological markers and 82 RFLP markers was constructed (Fig. 1). When the integrated linkage map was compared with both the morphological and RFLP marker maps, the following was observed.

Gene *d2*, which was previously assigned to chromosome 4, was found to be located on chromosome 1 through detection of linkages between *d2* and several RFLP markers mapped on chromosome 1. Previously, *d2* was estimated to be located on the terminal end of the morphological map of chromosome 4. In this case, gene *lg* was located on the middle of the morphological linkage map of chromosome 4. But Saito et al (1991) located *lg* on the terminal end of the molecular map of chromosome 4. Therefore, a long gap was estimated between *lg* and *d2* on chromosome 4 where there were no RFLP markers. In this study, this gap was closed by shifting the *d2* locus from chromosome 4 to chromosome 1.

Gene *gl1* was located on chromosome 5 and its relationship with other morphological marker genes located on linkage group 5 was established. For chromosome 6, a linear order of *dp1-spl4-XNpb209-XNpb165-1* was determined. As gene *wx* (glutinous endosperm) was located on the terminal end of the morphological linkage map, the arrangement of *wx-dp1-spl4-XNpb209-XNpb165-1* was suggested. On the other hand, Saito et al (1991) located a waxy gene from maize (*cmWX*) on the linear order of *XNpb209-cmWX-XNpb165-1*. Allelic tests between *wx* and *cmWX* need to be made.

For chromosome 7, *XNpb50* assigned to chromosome 7 by trisomic gene dosage analysis (Ideta et al 1990) was located at the terminal end of the integrated linkage map. The linear order of *XNpb50-gl-d6-spl5* on the integrated linkage map was different from that of *d6-gl-spl5* found on the morphological linkage map. More investigation is needed to determine the order of *d6* and *gl*. For chromosome 9, the locus of the previously unlocated gene *IBf* was determined and the linear order of classical marker genes *IBf-dp2-drp2-Dn1* was established.

The segregation distortion was detected in six regions. In the regions of *fs2-XNpb216-XNpb96-d18-XNpb269-XNpb359-d2* on chromosome 1, *bl1-XNpb57-XNpb42* on chromosome 2, *XNpb164-XNpb249-fc1-XNpb51-XNpb15-XNpb182* on chromosome 3, *XNpb165-1* on chromosome 6, *XNpb389-XNpb78-z2-gmZ410* on chromosome 11, and *r11-XNpb148-XNpb198* on chromosome 12, the japonica/japonica allele frequencies of both the morphological and RFLP markers showed significantly less than the normal frequency of 25%.



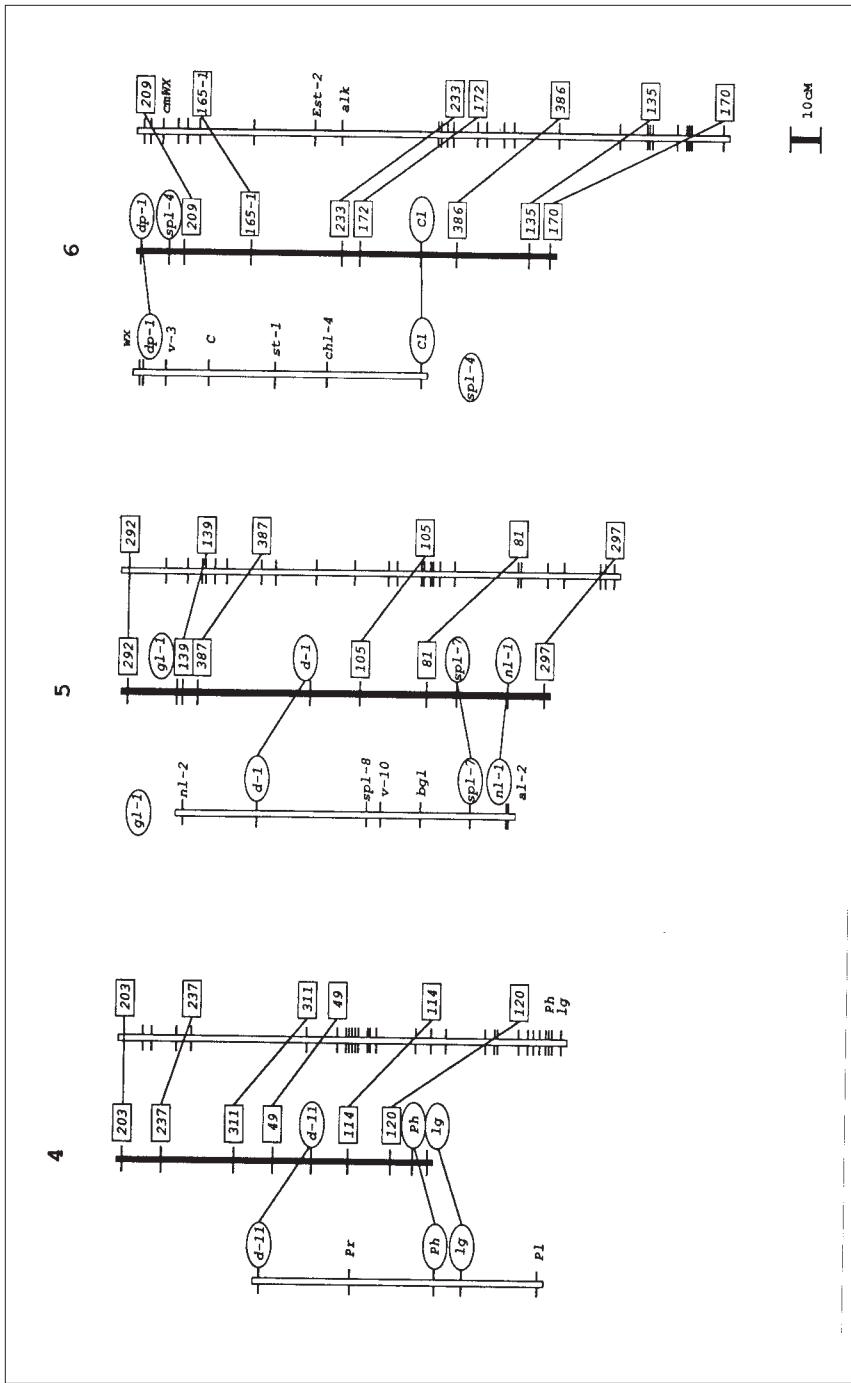
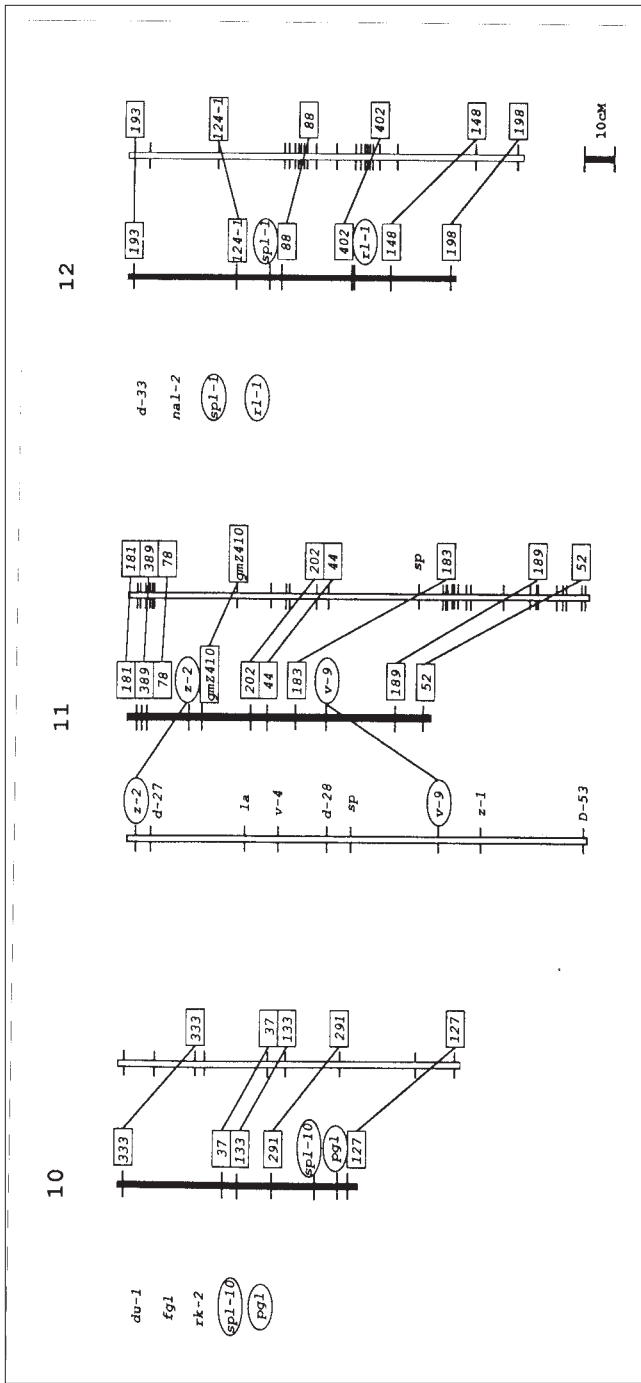


Fig. 1. An integrated linkage map of rice. The classical linkage map is shown on the left and the RFLP linkage map (Saito et al 1991) on the right. Numbers at the top indicate chromosomes. The prefixes of RFLP markers, XNpb, are omitted. The loci in ellipses are morphological markers used in this study, those in boxes are RFLP markers.



The origin of segregation distortion detected in this study was not examined. However, a gametophyte gene (*ga9*) tightly linked to *d18* on chromosome 1 (Maekawa and Kita 1985), *ga3* (Nakagahra 1972) tightly linked to *XNpb51* on chromosome 3 (Sobrizal 1995), *ga1* (Iwata et al 1964) and *ga4* (Mori et al 1973, Nakagahra et al 1974) located around the *wx*—*C* region on chromosome 6, and the gametic-lethal gene loosely linked to *la* on chromosome 11 (Tomita et al 1989) can cause segregation distortion. In addition, Nakagahra (1978) had reported segregation distortion of *r11* on chromosome 12 without revealing its cause. The new segregation distortion found on chromosome 2 needs to be further investigated.

Genes *tri*, *spl2* (chromosome 2), *chl1* (chromosome 3), *dp1*, *spl4* (chromosome 6), *d6*, *g1*, *spl5* (chromosome 7), *sug* and *v8* (chromosome 8) were estimated to be located beyond the terminal ends of the RFLP linkage map by the three-point linkage test. The RFLP linkage map (Saito et al 1991) was not saturated. Probably, the molecular map constructed by Kurata et al (1994) can cover these regions since it is saturated with more than 1,300 DNA markers.

The morphological linkage map and the RFLP linkage map were integrated for all 12 chromosomes of rice. The integrated linkage map would enhance tagging of genes governing agronomic traits with both the morphological and RFLP markers. Recently, Kurata et al (1994) constructed a high-resolution rice genetic map containing 1,383 DNA markers. The relationship between the RFLP linkage map of Saito et al (1991) and that of Kurata et al (1994) was determined using recombinant inbred lines (Yoshimura et al 1995). A well-saturated linkage map integrated with morphological, isozyme, and RFLP markers would enhance genetic and breeding research aimed at rice improvement.

Cited references

- Allard RW. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. *Hilgardia* 24:235-278.
- Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* 138:1251-1274.
- Ideta O, Yoshimura S, Yoshimura A, Saito A, Kawase M, Kishimoto N, Yano M, Nakano M, Ogawa T, Nakagahra M, Iwata N. 1990. Assignment of rice RFLP clones to their respective chromosomes by using gene dosage effect in hybrid primary trisomics [in Japanese]. *Jpn. J. Breed.* 40(1):466-467.
- Iwata N. 1986. The relationship between cytologically identified chromosomes and linkage groups in rice. In: *Rice genetics. Proceedings of the International Rice Genetic Symposium; 27-31 May 1985; Los Baños, Philippines. Manila (Philippines): International Rice Research Institute.* p 229-238.
- Iwata N, Nagamatsu T, Omura T. 1964. Abnormal segregation of waxy and apiculus coloration by a gametophyte gene belonging to the first linkage group in rice [in Japanese, English summary]. *Jpn. J. Breed.* 14:33-39.
- Iwata N, Satoh H, Omura T. 1984. The relationships between chromosomes identified cytologically and linkage groups. *Rice Genet. Newsl.* 1:128-132.

- Iwata N, Satoh H, Yoshimura A. 1989a. Linkage studies in rice (*Oryza sativa* L.). Linkage map of chromosome 3. Bull. Inst. Trop. Agric. Kyushu Univ. 12:1-9.
- Iwata N, Satoh H, Yoshimura, A. 1989b. Linkage map for Nishimura's chromosome 8. Rice Genet. Newsl. 6:106-108.
- Kinoshita T. 1993. Report of the Committee on Gene Symbolization, Nomenclature, and Linkage Groups. In: Rice genetics cooperative. Rice Genet. Newsl. 10:7-39.
- Kishimoto N, Shimosaka E, Matsuura S, Saito A. 1992. A current RFLP linkage map of rice: alignment of the molecular map with the classical map. Rice Genet. Newsl. 9:118-124.
- Kosambi DD. 1994. The estimation of map distances from recombination values. Ann. Eugen. 12:172-175.
- Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyoma T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y. 1994. A 300-kilobase interval genetic map of rice including 883 expressed sequences. Nat. Genet. 8:365-372.
- Maekawa M, Kita F. 1985. New gametophyte genes located in the third linkage group (chromosome 3) of rice. Jpn. J. Breed. 35:25-31.
- McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. 76:815-829.
- Mori K, Kinoshita T, Takahashi M. 1973. Segregation distortion and its causation of an endosperm character in crosses of distantly related rice varieties (Genetical studies on rice plant, LVIII) [in Japanese, English summary]. Mem. Fac. Agric. Hokkaido Univ. 53:72-130.
- Nagao S, Takahashi M. 1963. Trial construction of twelve linkage groups in Japanese rice (Genetical studies on rice plant, XXVII). J. Fac. Agric. Hokkaido Univ. 53:72-130.
- Nakagahra M. 1972. Genetic mechanism on the distorted segregation of marker genes belonging to the eleventh linkage group in cultivated rice. Jpn. J. Breed. 22:232-238.
- Nakagahra M. 1978. Newly found distorted segregations in wide crosses of Asian cultivated rice. Jpn. J. Breed. 28(1):102-103.
- Nakagahra M, Omura T, Iwata N. 1974. New certation gene on the first linkage group found by inter-subspecific hybridization of cultivated rice. J. Fac. Agric. Kyushu Univ. 18:157-167.
- Saito A, Yano M, Kishimoto N, Nakagahra M, Yoshimura A, Saito K, Kuhara 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. Jpn. J. Breed. 41:665-670.
- Sobrizal. 1995. Genetic studies on gametophyte genes on chromosome 3 of rice (*Oryza sativa* L.). Ph D thesis, Kyushu University, Japan.
- Tomita M, Tanisaka T, Okumoto Y, Yamagata H. 1989. Genetical analysis for semi-dwarfness of rice 9. Identification of the chromosomes carrying a semi-dwarfing gene of Hukuriku 100 and its complementary gametic lethal gene [in Japanese]. Jpn. J. Breed. 39(1):228-229.
- Yoshimura A, Tsunematsu H, Nagamura Y, Kurata N, Yano M, Sakaki T, Iawata N. 1995. Construction of rice framework map for analyzing linkage of DNA markers using recombinant inbred population. [in Japanese] Breed. Sci. 4(1):85.
- Yu ZH, McCouch SR, Kinoshita T, Sato S, Tanksley SD. 1995. Association of morphological and RFLP markers in rice (*Oryza sativa* L.). Genome 38:566-574.

Notes

Authors' address: Faculty of Agriculture, Kyushu University, Fukuoka 812-81, Japan.

Citation: [IRRI] International Rice Research Institute. 1996. Rice genetics III. Proceedings of the Third International Rice Genetics Symposium, 16-20 Oct 1995. Manila (Philippines): IRRI.