Rapid publication

Conservation of Duplicated Segments between Rice Chromosomes 11 and 12

Yoshiaki Nagamura, Takakazu Inoue, Baltazar A. Antonio, Tomotoshi Shimano, Hiromi Kajiya, Ayahiko Shomura, Shao Yang Lin, Yoshihide Kuboki, Yoshiaki Harushima, Nori Kurata, Yuzo Minobe, Masahiro Yano and Takuji Sasaki

Rice Genome Research Program, National Institute of Agrobiological Resources (NIAR)/Institute of Society for Techno-innovation of Agriculture, Forestry and Fisheries (STAFF), Tsukuba, Ibaraki 305, Japan

Summary

In the process of constructing a detailed genetic map of rice using two cultivars, Nipponbare and Kasalath, 79 DNA probes (approximately 6.1 % of total DNA markers) showed two or more RFLP bands in Southern hybridization and were mapped at duplicate or triplicate loci over the 12 chromosomes. Thirty-three DNA probes were mapped in the distal regions of rice chromosomes 11 and 12. Thirteen among 33 DNA probes were detected at duplicate loci. Linkage alignment of these 13 markers on both chromosomes agreed perfectly within a distance of ca. 12 cM from the distal ends of chromosomes 11 and 12. However, the other 20 DNA probes were mapped only on either chromosome 11 or chromosome 12, because the other bands were monomorphic between Nipponbare and Kasalath. These results indicate that the regions on rice chromosomes 11 and 12 are highly conserved as a duplicated chromosomal segment. Such highly conserved segments were not found anywhere else in the other chromosomes in our map.

Key Words : *Oryza sativa*, RFLP analysis, duplicate loci, conserved chromosomal segment.

Introduction

Through the development of molecular analysis, especially Restriction Fragment Length Polymorphism (RFLP), genetic maps with many DNA markers have been constructed for various plants (McCouch *et al.* 1988, Saito *et al.* 1991, Tanksley *et al.* 1992, Burr *et al.* 1993, Kleinhofs *et al.* 1993, Causse *et al.* 1994, Kurata *et al.* 1994b). RFLP analysis using DNA markers is also an effective method to reveal chromosomal rearrangements, such as translocations, insertions and duplications, and to analyse the degree of conservation in gene content and order in a particular chromosome during long periods of evolution (Wendel *et al.* 1986, Helentjaris *et al.* 1988, Kishimoto *et al.* 1994). On the basis of these genetic maps, several comparative genetic studies have also been successfully carried out by many researchers to examine the ancestral and evolutionary

relationship within and between species (Ahn and Tanksley 1993, Jena *et al.* 1994, Kurata *et al.* 1994a).

In a recent paper (Kurata *et al.* 1994b), our group has described a high resolution genetic map of rice with 1383 DNA markers mapped by RFLP analysis. In the course of the construction of the detailed RFLP linkage map, we noticed that several DNA markers were mapped at duplicate or triplicate loci. In this paper, we describe a highly conserved duplicated segment on chromosomes 11 and 12 in rice.

Materials and Methods

In this study two rice cultivars, Nipponbare (a *japonica* variety) and Kasalath (an *indica* variety) were used as well as their F₂ progenies consisting of 186 plants. As the source of DNA markers for RFLP analysis, we used mainly cDNA clones (Sasaki *et al.* 1994) and genomic clones which were prepared from Nipponbare (Kurata *et al.* 1994b). To detect RFLP between both parents and to analyze the segregation of 186 F₂ plants, total DNA was individually prepared from green leaves by the CTAB method (Murray and Thompson 1980).

Each extracted DNA was digested with the following 8 restriction enzymes; *Bam* HI, *Bgl* II, *Hin* dIII, *Eco* RI, *Eco* RV, *Apa* I, *Dra* I and *Kpn* I. Electrophoresis of digested DNA (2 µg DNA per lane) was carried out during 12 hours. After electrophoresis, the DNA was blotted onto a positively charged nylon membrane filter (Boehringer Mannheim) in 0.4N NaOH by capillary transfer and the membrane filter was washed in 2xSSC, air-dried and baked at 120°C for 20 min. Most of the DNA probes for Southern hybridization were amplified by PCR (Model 480, Perkin Elmer). The preparation of labelled probes with HRP (horseradish peroxidase), Southern hybridization and the detection of chemiluminescence on X-Ray film were carried out according to the protocol of ECL direct nucleic acid labeling and detection systems (Amersham International plc). The segregation pattern in 186 F₂ plants was determined and linkage analysis was performed using MAPMAKER Version 3.0 (Lander *et al.* 1987). Map distance (cM) was estimated using the Kosambi function (Kosambi 1994).

Received March 31, 1995. Accepted July 3, 1995.

Results and Discussion

In the course of the construction of a high resolution RFLP map (Kurata *et al.* 1994b), we observed that while most of the DNA clones used as probes showed a single-copy band in genomic Southern hybridization, some DNA probes had more than one band and were located at duplicate or triplicate loci. A total of 79 probes (approximately 6.1 % of total mapped DNA probes) were mapped at duplicate or triplicate loci over the twelve chromosomes. The location of these multiple loci suggested that a conserved chromosomal segment might be present in rice chromosomes 11 and 12. Thirteen of the 33 mapped DNAs were mapped in this region as duplicate loci, namely, G189, R642, R769, C1116, C104, R318, W120, C362, S790, C372, R2253, G24 and TEL2, which were distributed and aligned in the same order on both chromosomes 11 and 12 (Fig. 1).

The genetic distances of these conserved segments were 10 centimorgan and 11.8 centimorgan on chromosomes 11 and 12, respectively. The fact that the 13 DNA markers were located at a distance of about 12 centimorgan in exactly the same order indicates that this region is highly conserved as a duplicated segment between chromosomes 11 and 12 and that these conserved sequences in both chromosomes have not diverged appreciably.

RFLPs of 13 DNA probes mapped in this region are shown in Fig. 2. Most of the DNA probes had duplicate

loci, as shown by the two codominant polymorphic bands with almost the same intensity in genomic Southern hybridization. The TEL2 probe, one of the TAS (telomere-associated sequence) clones mapped on our map, was located at duplicate loci at the distal end of chromosomes 11 and 12 by two polymorphic bands, each present only in one parent (Ashikawa *et al.* 1994). On the other hand, all the other 20 DNA probes including C562 were mapped only on either chromosome 11 or chromosome 12 in this region, because one of two pairs of hybridizing bands was monomorphic. Typical Southern hybridization pattern of the probe with duplicate loci and probe with a single locus located on the duplicated segment in chromosomes 11 and 12 is shown in Fig. 3. This Southern hybridization pattern indicates that the C562 DNA probe has two copies in the genomic DNA of rice based on the minimum number of hybridizing bands per genome with eight kinds of restriction enzymes.

We are also constructing other rice maps in 4 *japonica/indica* crosses different from the Nipponbare/Kasalath cross. Similarly, the conserved segments in chromosomes 11 and 12 were also observed in these linkage maps (unpublished data). In addition to the duplicate loci of 13 DNA markers in the Nipponbare/Kasalath cross, the results obtained corroborate the existence of the duplication of chromosomal segments between chromosomes 11 and 12 in rice. Therefore, it is likely that these conserved segments on chromosomes 11 and 12 are commonly observed in all or most of the

Fig. 1. Highly conserved linkage segments with 13 DNA markers on chromosomes 11 and 12 in rice. The genetic distances of these conserved regions were 10 cM and 11.8 cM on chromosomes 11 and 12, respectively. The genetic distance (cM) is shown on the left side. Horizontal bars crossed by vertical lines correspond to loci of DNA markers. Locus names are listed to the right and left of the chromosomes. First letter of locus names indicates the following; C, callus cDNA cloners; R, root cDNA clones; G, random genomic clones; S, green shoot clones; TEL, telomere-associated clone and W, wheat clone. The markers XNpb24 and XNpb189 of Saito *et al.* (1991) are denoted in this map as marker G24 and G189, respectively.

Fig. 2. Polymorphism of 13 DNA probes in Nipponbare (N) and Kasalath (K) showing fragments (A, B and C) mapped on duplicated segment in chromosomes 11 and 12. The genomic DNA from both parents was digested with eight restriction enzymes, *Bam* HI, *Bgl* II, *Hin* dIII, *Eco* RI, *Eco* RV, *Apa* I, *Dra* I and *Kpn* I, but only the enzymes polymorphic in the two parents and used for the segregation analysis of F₂ population are shown here.

Fig. 3. Typical Southern hybridization patterns of probe with duplicate loci (A) and probe with single locus (B) between Nipponbare (a *japonica* variety) and Kasalath (an *indica* variety). A: Two loci of probe C1116 were located on chromosome 11 (N: 3kb, K: 6kb, on *Hin* dIII-digested DNA lanes) and chromosome 12 (N: 3.3 kb, K: 4 kb, on *Hin* dIII-digested DNA lanes). B: Probe C562 was mapped only on chromosome 11 based on the presence of a polymorphic band on *Hin* dIII-digested DNA lanes (N: 5 kb, K: 2.7, 3.5 kb).

varieties of *Oryza sativa*. Kishimoto *et al.* (1994) reported that there were duplicated segments in the same order on chromosomes 1 and 5 which were detected by using three kinds of cDNA clones. In the Nipponbare/Kasalath cross, highly and extensively conserved regions, as represented by duplicated segments between chromosomes 11 and 12, were not observed in other chromosomes.

With respect to RFLP linkage map of rice, Causse *et al.* (1994) constructed a high density molecular map based on a backcross derived from the interspecific cross, *O. sativa*/*O. longistaminata*//*O. sativa* (a wild species from Africa, AA genome), using genomic or cDNA clones from rice, oats, barley and maize. Recently, com-

parative RFLP mapping of a wild rice species, *Oryza officinalis* (CC genome), and cultivated rice species, *O. sativa* (AA genome) has also been reported (Jena *et al.* 1994). In both papers, we noticed two interesting facts. First, the locus names CDO127B and CDO127A derived from oats cDNA library were located in the distal region of both chromosomes 11 and 12, respectively (Causse *et al.* 1994). Second, RG235 clone had been mapped on chromosome 11 of *O. sativa* and chromosome 12 of *O. officinalis* (Jena *et al.* 1994). These facts suggest that highly conserved segments on chromosomes 11 and 12 may be present not only in *O. sativa* but in other species of the genus *Oryza*, as well. According to all the results, the duplication of these segments seems to have

occurred early in the evolution of the genus *Oryza*.

Kurata *et al.* (1994b) have also reported that an extensive conservation of gene order between rice and wheat was revealed by reciprocal mapping of DNA probes. However, there was no distinct synteny relationship between chromosomes 11 and 12 of rice and wheat chromosome groups. In the conserved region which we identified in this study, it is noteworthy that two loci of the W120 clone derived from wheat were mapped on both chromosomes, while this clone showed a single-copy locus on group 5 of wheat genome. A more detailed analysis of rice chromosomes 11 and 12 will be required to resolve the organization of these chromosomes in relation to the wheat chromosome groups. In addition, in the comparative mapping of the rice and maize genomes, Ahn and Tanksley (1993) observed that the majority (72 %) of the single-copy loci in rice were duplicated in modern maize. Unfortunately, no DNA markers on conserved segments of chromosomes 11 and 12 have been mapped on maize chromosomes. However, further detailed studies in this region will help to determine the evolutionary relationships between rice and maize. The degree of extensively conserved chromosomal segments on chromosomes 11 and 12 at the sequence level will be elucidated further with the construction of a physical map of YAC or cosmid clones in the near future.

Acknowledgements

We thank Dr. Ilkka Havukkala for helpful comments about the manuscript. We also thank Dr. Kaetsu Kobayashi for his advice and encouragement.

Literature Cited

- Ahn, S. and S. D. Tanksley (1993) Comparative linkage maps of the rice and maize genomes. *Proc. Natl. Acad. Sci.* 90 : 7980-7984.
- Ashikawa, I., N. Kurata, Y. Nagamura and Y. Minobe (1994) Cloning and mapping of telomere-associated sequences from rice. *DNA Research* 1 : 67-76.
- Burr, B., F. A. Burr and E. C. Matz (1993) Maize molecular map (*Zea mays* L.) (2N=20) In "Genetic maps, Book 6 Plants" O'Brien, S. J. (ed), Cold Spring Harbor Laboratory Press, New York, 6.39-6.60.
- Causse, M. A., T. M. Fulton, Y. G. Cho, S. N. Ahn, J. Chunwongse, K. Wu, J. Xiao, Z. Yu, P. C. Ronald, S. E. Harrington, G. Second, S. R. McCouch and S. D. Tanksley (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* 138 : 1251-1274.
- Helentjaris, T., D. Weber and S. Wright (1988) Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. *Genetics* 118 : 353-363.
- Jena, K. K., G. S. Khush and G. Kochter (1994) Comparative RFLP mapping of a wild rice, *Oryza officinalis*, and cultivated rice, *O. sativa*. *Genome* 37 : 382-389.
- Kishimoto, N., H. Higo, K. Abe, S. Arai, A. Saito and K. Higo (1994) Identification of the duplicated segments in rice chromosomes 1 and 5 by linkage analysis of cDNA markers of known functions. *Theor. Appl. Genet.* 88 : 722-726.
- Kleinhofs, A., A. Kilian, M. A. Saghai Maroof, R. M. Biyashev, P. Hayes, F. Q. Chen, N. Lapitan, A. Fenwick, T. K. Blake, V. Kanazin, E. Ananiev, L. Dahleen, D. Kudrna, J. Bollinger, S. J. Knapp, B. Liu, M. Sorrells, M. Heun, J. D. Franckowiak, D. Hoffman, R. Skadsen and B. J. Steffenson (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. *Theor. Appl. Genet.* 86 : 705-712.
- Kosambi, D. D. (1944) The estimation of map distances from recombination values. *Ann. Eugenet.* 12 : 172-175.
- Kurata, N., G. Moore, Y. Nagamura, T. Foote, M. Yano, Y. Minobe and M. Gale (1994a) Conservation of genome structure between rice and wheat. *Bio/Technology* 12 : 276-278.
- , Y. Nagamura, K. Yamamoto, Y. Harushima, N. Sue, J. Wu, B. A. Antonio, A. Shomura, T. Shimizu, S.-Y. Lin, T. Inoue, A. Fukuda, T. Shimano, Y. Kuboki, T. Toyama, Y. Miyamoto, T. Kirihaara, K. Hayasaka, A. Miyao, L. Monna, H. S. Zhong, Y. Tamura, Z.-X. Wang, T. Momma, Y. Umehara, M. Yano, T. Sasaki and Y. Minobe (1994b) A 300 kilobase interval genetic map of rice including 883 expressed sequences. *Nature Genetics* 8 : 365-372.
- Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln and L. Newburg (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1 : 174-181.
- Murray, M. G. and W. F. Thompson (1980) Rapid isolation of high molecular weight plant DNA. *Nucl. Acids. Res.* 8 : 4321-4325.
- Saito, A., M. Yano, N. Kishimoto, M. Nakagahra, A. Yoshimura, K. Saito, S. Kuhara, Y. Ukai, M. Kawase, T. Nagamine, S. Yoshimura, O. Ideta, R. Ohsawa, Y. Hayano, N. Iwata and M. Sugiura (1991) Linkage map of restriction fragment length polymorphism loci in rice. *Japan. J. Breed.* 41 : 665-670.
- Sasaki, T., J. Song, Y. Koga-Ban, E. Matsui, F. Fang, H. Higo, H. Nagasaki, M. Hori, M. Miya, E. Murayama-Kayano, T. Takiguchi, A. Takasuga, T. Niki, K. Ishimaru, H. Ikeda, Y. Yamamoto, Y. Mukai, I. Ohta, N. Miyadera, I. Havukkala and Y. Minobe (1994) Toward cataloguing all rice genes: large-scale sequencing of randomly chosen rice cDNAs from a callus cDNA library. *The Plant Journal* 6 (4) : 615-624.
- McCouch, S. R., G. Kochert, Z. H. Yu, Z. Y. Wang, G. S. Khush, W. R. Coffman and S. D. Tanksley (1988) Molecular mapping of rice chromosomes. *Theor. Appl. Genet.* 76 : 815-829.
- Tanksley, S. D., N. W. Ganai, J. P. Prince, M. C. de Vicente, M. W. Bonierbale, P. Broun, T. M. Fulton, J. J. Giovannoni, S. Grandillo, G. B. Martin, R. Messeguer, J. C. Miller, L. Miller, A. H. Paterson, O. Pineda, M. S. Röder, R. A. Wing, W. Wu and N. D. Young (1992) High density molecular linkage maps of the tomato and potato genomes. *Genetics* 132 : 1141-1160.
- Wendel, J. F., C. W. Stuber, M. D. Edwards and M. M. Goodman (1986) Duplicated chromosomal segments in maize (*Zea mays* L.): Further evidence from hexokinase isozymes. *Theor. Appl. Genet.* 72 : 178-185.