Theor Appl Genet (1996) 93: 257–261

H.-J. Koh · M.-H. Heu · S. R. McCouch

Molecular mapping of the *ge*^{s} gene controlling the super-giant embryo character in rice (*Oryza sativa* L.)

Received: 27 September 1995 / Accepted: 2 February 1996

Abstract The giant-embryo character is useful for quality improvement in rice. Three alleles controlling embryo size have been reported at the *ge* locus. Based on trisomic analysis, this locus is known to reside on chromosome 7. The objective of the present study was to identify linkage between molecular markers and the *ge*^{s} gene using an existing molecular map of rice and an F_2 population derived from Hwacheongbyeo-*ge*^{s} (super-giant embryo)/Milyang 23. The bulked-segregant method was used to screen 38 RFLPs and two microsatellite markers from rice chromosome 7. *RZ395* and *CDO497* flanked the *ge*^{s} gene, at 2.4 cM and 3.4 cM, respectively. The two microsatellite markers, *RM18* and *RM10*, were linked with *ge*^{s} at 7.7 cM and 9.6 cM, respectively. The availability of molecular markers will facilitate selection of this grain character in a breeding program and provide the foundation for map-based gene isolation.

Key words Rice (*Oryza sativa*, L.) · Super giant-embryo gene (*ge*^{s}) · RFLP · Microsatellite · Molecular markers

Introduction

During the past three decades, breeding efforts to improve the nutritional quality of rice have focused mainly on the quantity and quality of protein in the grain endosperm, but without any great advances (Coffman and Juliano 1987; Rutger 1981; Schaffer and Sharpe 1987; Kumamaru et al. 1988). Recently, mutants for grain quality have been developed by Satoh and Omura (1981) and Kim et al. (1991), and these offer promising sources for quality diversification in rice to meet the demands of rice markets (Heu and Park 1990). One such mutant, referred to as the giant-embryo mutant (Fig. 1), has an enlarged embryo that results from an increase of scutellar tissue without any accompanying change in the size of the shoot or root primordia (Koh et al. 1994). Satoh and Omura (1981) first reported a giant-embryo mutant from a Japanese *japonica* cultivar, Kinmaze, and this gene was located to chromosome 7 based on trisomic analysis (Satoh and Iwata 1990). Kim et al. (1992) developed a series of giant-embryo mutants consisting of three different embryo sizes from a Korean *japonica* cultivar, Hwachungbyeo, and demonstrated that they were the result of multi-alleles (*ge*^{m}, *ge*, *ge*^{s}) at the same locus as reported by Satoh and Iwata (1990). The giant-embryo mutant is a potentially useful character for quality improvement because the embryo has been reported to have an exceptionally high quality compared to other parts of the grain (Juliano 1985). In fact, super-giant embryo grains showed higher protein, oil and vitamin contents than did regular grains (Koh et al. 1994). Specifically, lysine, a-tocopherol, and vitamin B1 contents were much

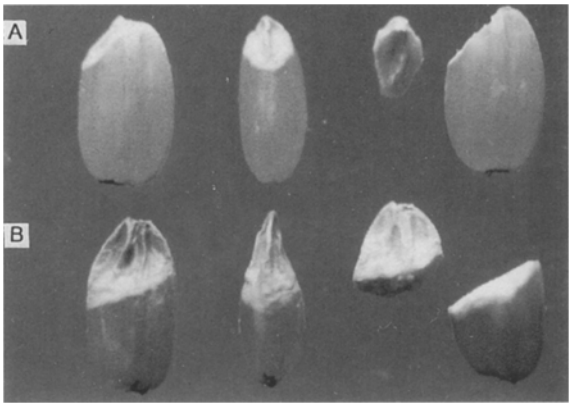

Fig. 1 Appearance of a regular (*A*) and super-giant-embryo grain (*B*) of Hwacheongbyeo

Communicated by G. E. Hart

H.-J. Koh · S. R. McCouch (✉)
Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853-1901, USA

H.-J. Koh · M.-H. Heu
Department of Agronomy, College of Agriculture and Life Science, Seoul National University, Suwon 441-744, Republic of Korea

higher. Therefore, the giant-embryo character may be useful for nutritional quality improvement in brown rice and rice bran, or for specific medicinal purposes utilizing embryos.

Over 50 embryogenesis-related (*emb*) mutants and 63 mutations affecting kernel development (*dek*) have been identified in maize (Clark and Sheridan 1991; Scanlon et al. 1993) and chromosomal locations were reported for 25 and 53 of them, respectively. Based on comparative mapping studies in maize and rice, there is a large region of conserved gene order on the long arm of maize chromosome 7 and rice chromosome 7 (Ahn and Tanksley 1993). None of the *emb* genes reported by Clark and Sheridan (1991) were located to maize chromosome 7, but two of the *dek* mutants were present on maize 7L. Both of these were associated with the development of small, inviable embryos, suggesting that ge^s in rice is uniquely valuable as breeding material. No useful genes conferring enlarged embryos have been reported in any other cereal crops to date.

The availability of DNA markers closely linked to the ge^s gene would facilitate breeding efforts to develop improved rice lines containing the giant-embryo character, and would provide the basis for map-based cloning of this gene. The present study aimed to locate the ge^s gene on the molecular map of rice and to identify easily selectable, tightly linked markers.

Materials and methods

Plant materials and genotype evaluation

Of three giant-embryo mutants induced via chemical mutagenesis using *N-methyl-N-nitroso-urea* from a Korean *japonica* cultivar, Hwacheongbyeo (Kim et al. 1991), the super-giant-embryo (ge^s) mutant was used in this study. The Hwacheongbyeo-ge^s line was crossed with Milyang 23, a genetically divergent *tongil* type rice [a high-yielding plant type derived from an *indica* × *japonica* cross in Korea (Suh and Heu 1978)], and though the F_1 plants were semi-sterile, an F_2 population was produced and used for mapping. F_1 seeds harvested from the Hwacheongbyeo-ge^s (seed parent) had grains with uniformly normal (small) embryos. One-hundred-and-forty six F_2 seeds and their parents were planted in pots (1 plant/pot) in the greenhouse at Cornell University. Leaves were collected from each plant at the maximum tillering stage and used immediately for DNA extraction. F_3 seeds were harvested from each F_2 plant and evaluated for embryo size after hulling (Kim et al. 1992). The difference in embryo size among grains was easily distinguishable (Fig. 1) and F_2 plants were readily classified into three phenotypic groups: those having seeds with uniformly normal embryos, those segregating for embryo size, and those having seeds with only super-giant embryos.

DNA extraction and construction of bulks

DNA was extracted from mature rice leaves of each F_2 plant and the parents at the maximum tillering stage according to Causse et al. (1994). Equal amounts of DNA from each of five plants showing uniformly large or small embryo size were used to construct bulks from F_2 segregants. Two bulks containing ge^sge^s segregants, and one containing *GeGe* segregants, were used in bulked segregant analysis (Michelmore et al. 1991).

Restriction digests, electrophoresis, clones, and Southern analysis

DNA from the two parents and three bulked samples was digested with six restriction enzymes, *Eco*RI, *Eco*RV, *Dra*I, *Hin*dIII, *Xba*I and *Bam*HI (BRL and NE Biolab), size separated on a 0.9% agarose gel and blotted onto Hybond N^+ (Amersham Corp.) according to the manufacturer's instructions. Thirty-six DNA clones previously mapped to chromosome 7 (Causse et al. 1994; Kurata et al. 1994; G. Kochert, University of Georgia, personal communication), including seven rice genomic (RG) clones, eight rice cDNAs (RZ), three oat cDNAs (CDO), two barley cDNAs (BCD), ten rice cDNA clones (UCH, from H. Uchimiya, Institute of Molecular and Cellular Biosciences, Tokyo, Japan), and six rice genomic and cDNA clones (G and C respectively, from T. Sasaki, Rice Genome Project, Tsukuba, Japan) were PCR-amplified as described in Causse et al. (1994), random hexamer labelled with ^{32}P (Feinberg and Vogelstein 1983) and used in Southern analysis to screen the parents and bulked DNA samples for polymorphism, following the basic protocols described in McCouch et al. (1988).

Analysis of microsatellite DNA

Two microsatellite markers, *RM10* and *RM18*, located on chromosome 7 (Panaud et al. 1996), were used in this study. PCR amplication of the F2 genomic DNAs, separation of PCR products on a DNA sequencing gel containing 6% polyacrylamide, staining with silver nitrate (Promega Co. 1993), and development of the X-ray film were all according to Panaud et al. (1996).

Linkage analysis

Linkage analysis was conducted using MAPMAKER computer software (Lander et al. 1987). Marker order was determined with a LOD score > 3.0, and map distances were estimated using the Kosambi function (Kosambi 1944).

Results

Identification of molecular markers putatively associated with the ge^s gene

Of 38 molecular markers surveyed for polymorphism, 26 RFLP markers and two microsatellites displayed variation between the parents, Hwacheong-ge^s and Milyang 23. Bulked segregant analysis (Michelmore et al. 1991) clearly discriminated between markers that were closely linked to *ge* and those that were separated by several recombination events. As shown in Fig. 2B, *CDO497* was tightly linked

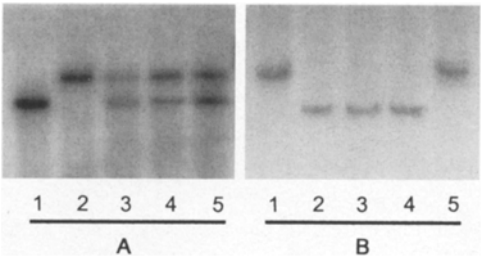

Fig. 2A, B Bulked-segregant analysis; *lane 1*: Milyang 23, *lane 2*: Hwacheongbyeo-ge^s, *lanes 3 and 4*: ge^sge^s bulk, *lane 5*: *GeGe* bulk. **A** Hybridized with *RZ509*; **B** hybridized with *CDO497*

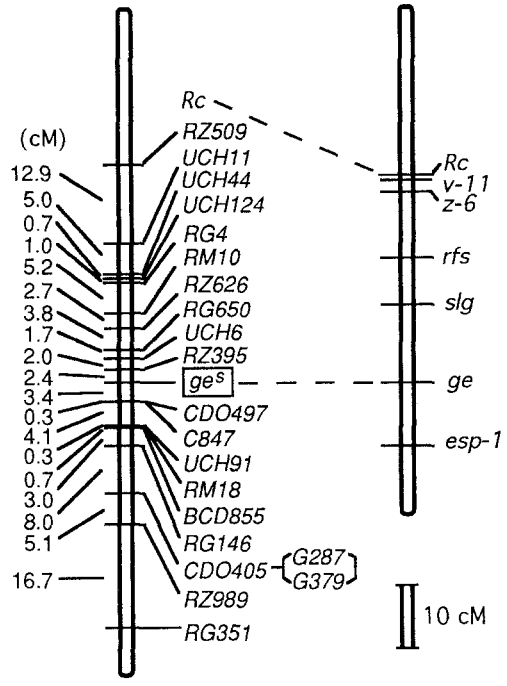

Fig. 3 Linkage map of the segment of rice chromosome 7 around the *ge* locus. Molecular map developed during this study on left; markers on the framework map are located with LOD > 3.0; genetic distances in Kosambi (1944) units. Classical linkage map on right (Kinoshita 1993)

to the ge^s locus, with both ge^sge^s bulks (lanes 3 and 4) showing a homozygous banding pattern corresponding to the Hwacheongbyeo-ge^s allele, and complete absence of this allele from the *GeGe* bulk (lane 5). In contrast, *RZ509* (Fig. 2A) was loosely linked to ge^s, and the presence of both Hwacheongbyeo-ge^s and Milyang 23-derived alleles could be detected in both bulks. Of the 28 polymorphic markers, 19 RFLP and 2 microsatellites were scored for segregation in the 146 F_2 plants.

Mapping of the ge^s gene

All 19 RFLP markers, both of the microsatellite markers, as well as the embryo character demonstrated the expected 1:2:1 segregation ratio (χ^2=5.99, n.s.). A saturated map of the lower half of chromosome 7 containing the ge^s locus is shown in Fig. 3. The marker loci closest to the ge^s gene were *RZ395* and *CDO497*, at distances of 2.3 cM and 3.4 cM, respectively. Microsatellite loci *RM18* and *RM10* were linked with ge^s at distances of 7.7 cM and 9.6 cM, respectively. The map of this region integrates loci detected with clones from a variety of sources: five UCH rice cDNA clones, three JRGP rice cDNA clones, four CU rice genomic, four CU rice cDNA clones, two CU oat cDNA clones, one CU barley cDNA clone, and two microsatellite markers. The two loci (*G287* and *G379*) shown in parentheses to the right of the chromosome-7 framework map showed a dominant/recessive (present/absent) banding pattern and could not be mapped with a LOD > 3.0. When segregation was scored using *G287* as a probe, a dominant band was inherited from Milyang 23, while for *G379* a dominant band was inherited from Hwacheongbyeo-ge^s.

The order of loci in this region of chromosome 7 is similar to that on the interspecific framework map described by Causse et al. (1994). A few of the marker loci such as *BCD855*, *CDO497* and *RG4* which appeared in parentheses as low-LOD markers (LOD < 2.5) in Causse et al., mostly due to missing data, have been located with greater resolution in this population. *RG634* is a multiple-copy clone with one locus previously located to this region of chromosome 7 (Causse et al. 1994), but in our study the polymorphic band did not map to chromosome 7. *RG146* is a multiple-copy clone with a complex hybridization pattern. One of the loci detected by this clone has previously been reported to be linked to whitebacked plant hopper resistance in the target region of chromosome 7 (McCouch and Tanksley 1991), while another locus has been reported on chromosome 1 (Causse et al. 1994). In the present study, *RG146* detected a 5.3-kb fragment that segregated with ge^s at a distance of 10.8 cM on *Eco*RI-digested DNA. The only polymorphic band in *Eco*RV-digested DNA segregated independently of ge^s, but its map location was not confirmed.

Figure 3 shows the classical linkage map of chromosome 7 (Kinoshita 1993) to the right of the molecular map developed during this study. Genes that have been mapped on both are indicated with dotted lines. The information provided by our study has clarified the orientation of the molecular and the classical linkage maps of chromosome 7. Previously only the *Rc* locus had been located on both maps (Causse et al. 1994). In keeping with new evidence about the location of rice centromeres (Singh et al. 1996), the orientation of the chromosome-7 map presented in Causse et al. (1994) has been reversed.

Figure 4 illustrates the observed co-segregation of *RZ395*, the ge^s locus, and *CDO497*. When Hwacheongbyeo and the derived mutant, Hwacheongbyeo-ge^s, were hybridized with both *RZ395* and *CDO497*, identical banding patterns were observed, despite the fact that these two varieties are phenotypically different. This suggested that the mutation in Hwacheongbyeo-ge^s affected only a very small region of the *ge* locus.

Discussion

Molecular mapping of the ge^s gene provides a clear indication of its position on chromosome 7 and also provides several markers that could be used in early plant selection for this grain character. The marker closest to the *ge* locus was *RZ395*, at a distance of 2.4 cM. If 100 F_2 plants were selected using *RZ395*, nearly all of them would be expected to have the ge^sge^s genotype, with a predicted error rate of

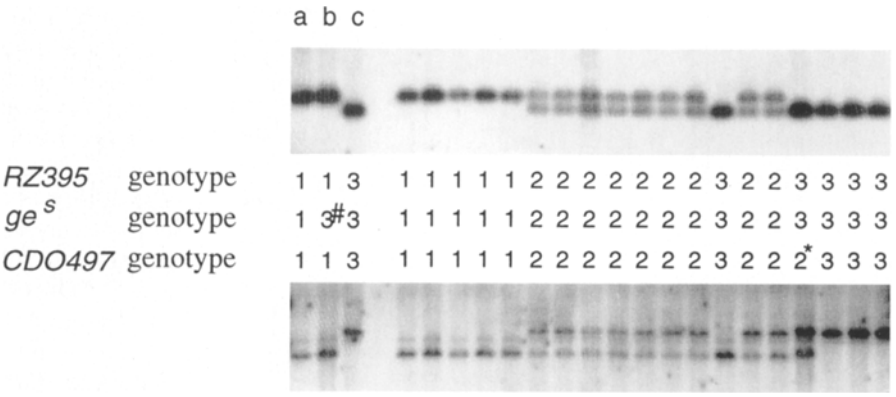

Fig. 4 Co-segregation of the ge^s gene and flanking RFLP clones, *RZ395* and *CDO497*, in an F_2 population. *Lane a* = Hwacheongbyeo-ge^s(ge^sge^s); *lane b* = Hwacheongbyeo (*GeGe*); *lane c* = Milyang 23(*GeGe*). *Genotype 1* = ge^sge^s; *genotype 2* = $Gege^s$; *genotype 3* = *GeGe*; * = recombinant ; # Hwacheongbyeo is the wild-type from which the ge^s mutant is derived

4.7%[1]. If the loci detected by *RZ395* and *CDO497* were both used for selection, the error rate would be negligible, and three quarters of the population could be eliminated before the plants matured and set seed. The two microsatellite markers flanking ge^s offer a more economical screening tool. Although they are farther from the ge^s locus, and therefore introduce a slightly greater chance of error, the use of *RM18* and *RM10* together (15–18% when used separately, or 2.7% when used together)[1] to screen for ge^sge^s genotypes offers an efficient alternative to RFLP, and permits earlier screening of lines than is possible using traditional phenotypic evaluation for this character.

Two loci clustered near the region of ge^s, *G287* and *G379*, showed a dominant/recessive banding pattern and segregated in a 3:1 (presence:absence) ratio. "Null alleles" were detected in both parents, depending on the marker, and lowered the mapping resolution for these loci because heterozygotes could not be distinguished from one of the homozygous classes. These markers provide evidence that disruptive mutational events, possibly involving insertions or deletions, must have occurred in this region of chromosome 7. A similar pattern was previously reported for *RG146* (McCouch and Tanksley 1991) which is located only 8 cM from *G287* and *G379* on the map presented in Fig. 3. Together, these observations raise the question of whether this region of the genome may be a hot spot for mutation in rice.

Among the three alleles that have been identified at the *ge* locus, induced by chemical mutagenesis of Hwacheongbyeo (Kim et al. 1992), a dominant-recessive relationship exists such that the smaller the embryo size the more dominant the allele controlling it (Koh, unpublished). The structure of the gene and the control of gene expression are of great interest to breeders and molecular geneticists alike, and the materials described in the present study provide an excellent starting point for a map-based cloning endeavor. The giant-embryo character contributes positive nutritional value and is currently being incorporated into the breeding program for quality improvement and diversification of rice in Korea.

Acknowledgements This work was supported in part by the Korean Science and Engineering Foundation and in part by the Rockefeller Foundation's International Rice Biotechnology Program. We are grateful to Hirofumi Uchimiya (Institute of Molecular and Cellular Biosciences, Tokyo, Japan) and to Takuji Sasaki (Rice Genome Project, Tsukuba, Japan) for clones used in this analysis, to Gary Kochert for pre-publication information about the map location of the UCH clones, to Bill Wilson and Clare Nelson for critical reviews, and to Carole Morehouse for formatting the manuscript.

[1] Lep p equal the crossover frequency between two loci A and B, such that the proportion of the phenotypic groups *aaB_* (a recombinant type) and *aabb* (a parental type having homozygous recessive alleles) in an F_2 population will be $p(2-p)/4$ and $(1-p)^2/4$, respectively. In this study, since the crossover frequency between *RZ395* and ge^s is 0.024 (2.4%), the proportion of each *aaGeGe* and *aage*^{s}-ge^s phenotypic group will be 0.00119 and 0.2381. Therefore, the possible selction error rate will be [0.0119/(0.0119+0.2381)]×100=4.7% when this marker is used alone. When used in combination with a marker showing a recombination frequency of 0.034 from the target gene, the selection error rate is 0.047×0.067=0.003(0.3%)

References

Ahn SN, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harritongton 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

Clark JK, Sheridan WF (1991) Isolation and characterization of 51 *embryo-specific* mutations of maize. Plant Cell 3:935–951

Coffman WR, Juliano BO (1987) Rice. In: Olson RA, Frey KJ (eds) Nutritional quality of cereal grains: genetic and agronomic improvement. American Society of Agronomy, Madison, Wisconsin, pp 101–131

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Heu MH, Park SZ (1990) Breeding strategies for quality diversification in rice. Proc Symp Rice Quality, Crop Experimental Station, RDA, Korea 99:41–58

Juliano BO (1985) Rice – chemistry and technology, AACC Monograph Series, pp 17–174

Kim KH, Heu MH, Park SZ, Koh HJ (1991) New mutants for rice grain quality. Korean J Crop Sci 36:197–203

Kim KH, Park SZ, Koh HJ, Heu HM (1992) New mutants for endosperm and embryo characters in rice: two dull endosperms and a giant embryo. Proc SABRAO Int Symp 'Impact of Biological Research on Agricultural Productivity', pp 125–131

Kinoshita T (1993) Current linkage maps. Rice Genet Newslett 10:17–32

Koh HJ, Park SZ, Won YJ, Heu MH (1994) Nutritional and agronomic characteristics of super-giant embryo mutant in rice. Korean J Crop Sci 38:537–544

Kosambi, DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kumamaru T, Satoh H, Iwata N, Omura T, Ogawa M, Tanaka K (1988) Mutants for rice storage proteins. 1. Screening of mutants for rice storage proteins of protein bodies in the starch endosperm. Theor Appl Genet 76:11–16

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, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma Y, 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

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

McCouch SR, Tanksley SD (1991) Development and use of restriction fragment length polymorphism in rice breeding and genetics. In: Khush GS, Toenniessen GH (eds) Rice biotechnology. CAB International and IRRI, Wallingford, Oxford, UK, pp 109–133

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

Michelmore RW, Paran I, Kesseli KV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Panaud O, Chen X, McCouch SR (1996) Development of rice SSLP markers and applications in breeding and evolutionary studies. Mol Gen Genet (in press)

Rutger JN (1981) Applications of induced and spontaneous mutation in rice breeding and genetics. Adv Agron 36:383–413

Satoh H, Iwata N (1990) Linkage analysis in rice. On three mutant loci for endosperm properties, *ge* (giant embryo), *du-4* (dull endosperm-4) and *flo-1* (floury endosperm-1). Japan J Breed 40 (Suppl 2):268–269

Satoh H, Omura T (1981) New endosperm mutations induced by chemical mutagen in rice, *Oryza sativa* L. Japan J Breed 31:316–326

Scanlon MJ, Stinard PS, James MG, Myers AM, Robertson DS (1993) Genetic analysis of 63 mutations affecting maize kernel development isolated from *Mutator* stocks. Genetics 136:281–294

Schaeffer GW, Sharpe FT (1987) Increased lysine and seed storage protein in rice plants recovered from calli selected with inhibitory levels of lysine plus threonine and S-(2-aminoethyl) cysteine. Plant Physiol 84:509–515

Singh K, Ishii T, Parco A, Huang N, Brar DS, Khush GS (1996) Centromere mapping in rice (*Oryza sativa* L.). Proc Natl Acad Sci USA (in press)

Suh HS, Heu MH (1978) The segregation mode of plant height in the cross of rice varieties. II. Linkage analysis of the semidwarfness of rice variety „*Tongil*". Korean J Breed 10:1–6