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Isolation of Individual Quantitative Trait Loci Causing Long Grain in Rice

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Abstract

To evaluate the gene action and interaction of quantitative trait loci (QTLs) as a single Mendelian factor, attempts were made in this study to isolate individual QTLs causing long grain in a large- grain rice cultivar 'BG 1', in other words, to establish a series of near-isogenic lines which had long-grain alleles derived from BG 1 at only one QTL and the short0grain alleles at the other QTLs. In the course of developing recombinant inbred lines from the cross between BG 1 and a short-grain cultivar Koshihikari, the lines were surveyed through backcrossing of the recombinant inbreds and by progeny test. Until the BC2F2 generation, a total of 8 lines, which obviously showed monogenic segregation within the respective progenies in which a single QTL was involved, were obtained. All of the long-grain alleles at the isolated QTLs were recessive to the short-grain alleles. The process of isolation of 2 different QTLs was described in detail as an example of the strategy adopted in this study. These isolines for isolated QTLs should contribute significantly not only to the characterization of individual QTLs and their combinations but also breeding programs as gene sources to be used directly.

Discipline: Plant breeding
Additional key words: gene action, near-isogenic line, progeny test, recombinant inbred line

Introduction

Most of the agronomic traits exhibit continuous variations in populations consisting of different genotypes, like genetically segregating populations after crossing. These traits are referred to as quantitative traits, and their genetic control is attributed to a number of loci which are designated as quantitative trait loci (QTLs), or polygenes. Generally, each of the QTLs is assumed to affect the trait cumulatively and less dominantly compared with non-genetic factors. As a result, it is very difficult to evaluate QTLs individually for their locations along chromosomes as well as their gene actions and interactions.

Some efforts have been made to localize the individual QTLs along chromosomes by detecting linkage relationships between QTLs and some suitable genetic markers[7,11]. Recent advances in molecular biology have provided many kinds of molecular markers which can cover all the chromosomes with a high density. Using these high-resolution molecular linkage maps with an adequate software, a large number of QTLs controlling various complex agronomic characters have been located in limited chromosomal regions in many crops[1,10]. Several kinds of software for the QTL mapping can also estimate some of the gene effects for each QTL, the interaction of QTL with the environment, etc., from the same mapping population[6,12].

From a plant breeder's standpoint, on the other hand, it is very important to evaluate the gene actions of individual QTLs as single Mendelian factors which can actually show monogenic segregation, and not as regions estimated on the linkage map by calculations under some assumptions. To achieve this kind of QTL evaluation, it is essential to isolate the individual QTLs through recombination and selection. This QTL isolation so far has not been sufficiently carried out, because it is a time-consuming procedure.

In this study special attention was paid to the grain length of rice as an example of quantitative trait, because this trait was generally considered to be controlled by a number of QTLs, as well as a single major gene, and had agronomic significance in determining the grain size and finally the grain yield in this crop. In the current study, then,

attempts were made to isolate the individual QTLs causing long grain length in a large-grain rice cultivar 'BG 1'. The final objective is to establish a series of near-isogenic lines with long-grain alleles derived from BG 1 at only one QTL and short-grain alleles at the other QTLs in relation to grain length. Although this objective has not been fully achieved yet, in this paper, the progress made and some of the results obtained are reported.

Materials and methods

A rice (*Oryza sativa* L.) cultivar BG 1 showing an extremely large (long) grain size[8] was crossed as a female parent with a cultivar 'Koshihikari' showing a small (short) grain size (or a standard grain size in Japanese cultivars). Fifty F_2-derived F_5 progeny lines, which corresponded nearly to a set of recombinant inbred lines, were developed by the single-seed descent method from randomly selected F_2 plants of this cross-combination. In the F_5 population, several recombinant inbred lines showing different grain lengths were selected and crossed as a male parent with a cultivar 'Gimbozu' which showed a nearly equal or shorter grain length compared with Koshihikari. The crosses between recombinant inbreds and Gimbozu aimed at estimating the number of QTLs related to grain length involved in these inbreds. In each F_2 population of these crosses, F_2-derived progeny lines were developed from several F_2 plants with different grain lengths. Among these F_2-derived lines, the candidates which seemed to have only one QTL carrying the allele for long grain from BG 1 were crossed with Gimbozu recurrently until monogenic segregation in grain length was clearly confirmed within the respective progeny lines. Allelism test for long grain length was conducted when different lines of interest were obtained in the course of the study.

Grain length of a plant was determined as the average length of 5 unhulled grains collected from the basal parts of the 3rd, 4th, and 5th primary branches from the top of a typical panicle of this plant. The length of an unhulled grain was measured as the distance between the top of the apiculus except for awn and the bottom of the glume up to the F_5 generation after the crossing of BG 1/Koshihikari. From the F_1 generation of the crosses between recombinant inbreds and Gimbozu, the distance between the base of the apiculus and the bottom of the palea was defined as grain length.

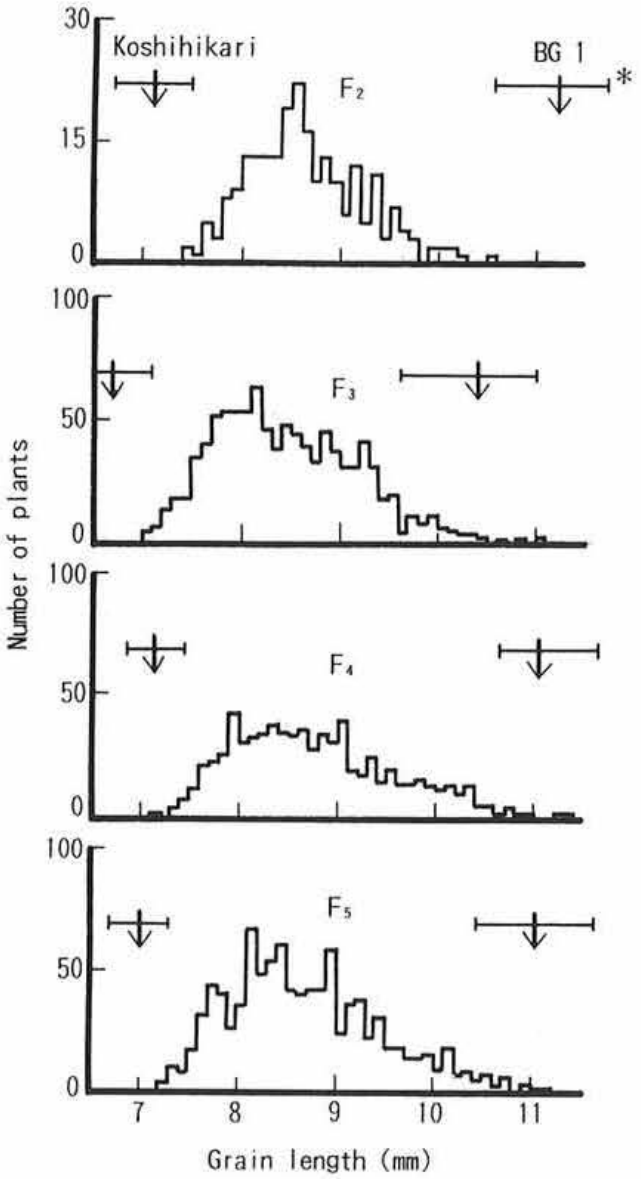

Fig. 1. Frequency distribution of grain length in F_2, F_3, F_4, and F_5 populations from the cross of BG 1/Koshihikari, developed by the single-seed descent method
*Means and ranges of Koshihikari and BG 1.

Results and discussion

Fig. 1 shows the changes in the frequency distribution for grain length with generation advancement after the crossing of BG 1/Koshihikari. In the F_2 and F_3 generations, typical continuous distributions were observed for this trait, indicating that the genetic difference in grain length between these 2 parents was due to a number of QTLs and not to a single locus. In the F_4 and F_5 generations, on the other hand, several peaks emerged in the distribution,

resulting in a multimodal distribution. In addition, several F_2-derived lines appeared to show within-family segregation in a monogenic manner in the F_3 and F_4 generations (data not shown). This monogenic-like segregation within a derived line had already been observed by Takita[9] in his materials. In all the generations, no plant exhibited extreme transgressive segregation beyond the range between the 2 parents.

These results suggest that every QTL for grain length converged into either of the parental genotypes, a large-grain homozygote (BG 1 type) or a short-grain homozygote (Koshihikari type). The population thus consisted of several and not numerous kinds of recombinant homozygotes showing different grain lengths by F_4 and F_5 generations. Among these homozygotes, there were genotypes where only one or a few QTLs carried the alleles for long grain derived from BG 1. Several recombinant inbred lines with different grain lengths were selected and crossed with a cultivar Gimbozu to estimate the number of QTLs carrying the long-grain allele in their genotypes and to initiate backcrossing. The use of Gimbozu instead of Koshihikari as a recurrent parent aimed at comparing other kinds of near-isogenic lines involving major genes for long grain under the same background as that of Gimbozu[4]. As an example of the following processes, the progenies of the cross between Gimbozu and a recombinant inbred T34 are described hereafter.

A part of Fig. 2 shows the distribution of grain length in the F_2 population of Gimbozu/T34. Apparently, a bimodal-like distribution was observed in this population, in contrast with the distribution in the F_2 population of BG 1/Koshihikari (Fig. 1). A total of 20 F_2 plants with different grain lengths

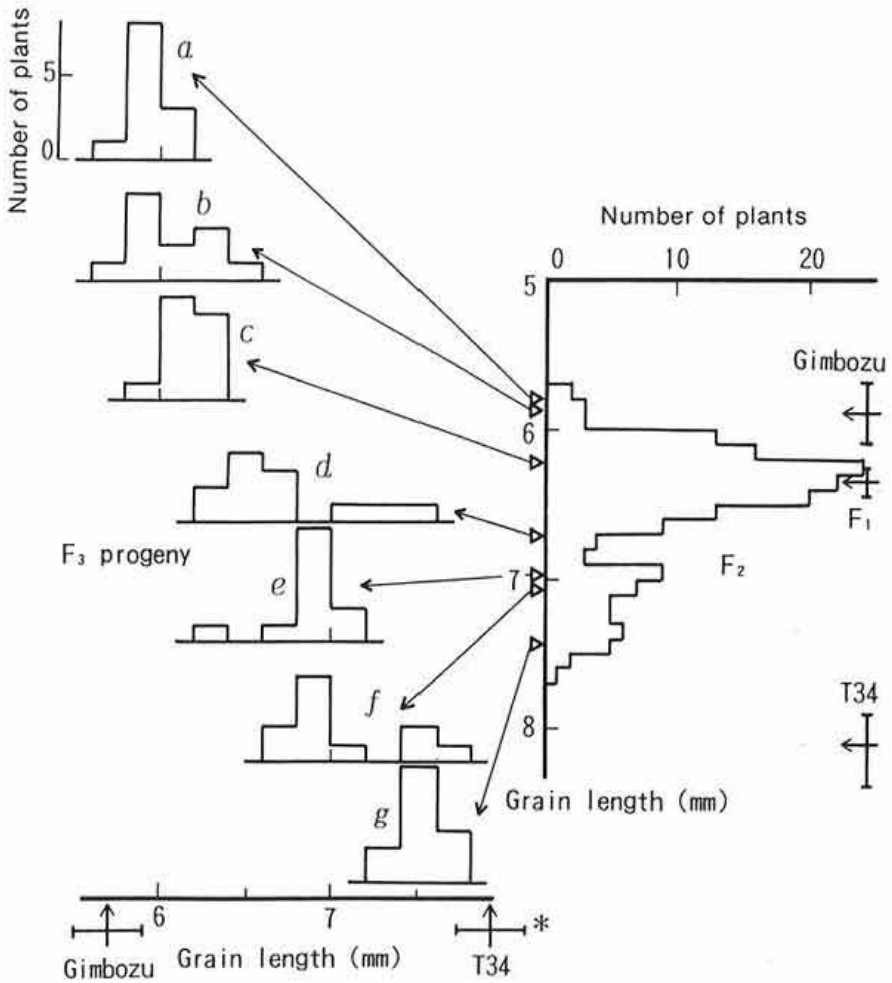

Fig. 2. Frequency distribution of grain length in the F_2 population and F_2-derived F_3 lines from the cross of Gimbozu/T34 (a recombinant inbred from BG 1/Koshihikari)
*Means and ranges of Gimbozu, T34, and their F_1.

was selected and F_2-derived F_3 progeny lines were developed for the progeny test of grain length. The other part of Fig. 2 shows the distributions in 7 F_3 lines out of 20, as examples. In this figure, several types of F_3 lines could be distinguished: non-segregating lines whose single modes of grain length were similar to that of either of the parents, Gimbozu (line *a* in Fig. 2) or T34 (*g*); non-segregating lines whose single modes showed either of the 2 intermediates between the 2 parents (*c* and *e*); and segregating lines which showed discontinuous distributions with 2 modes or more (*b*, *d*, and *f*). In the segregating F_3 lines, each of the modes in the distribution roughly corresponded to one of the modes of the parents and intermediates.

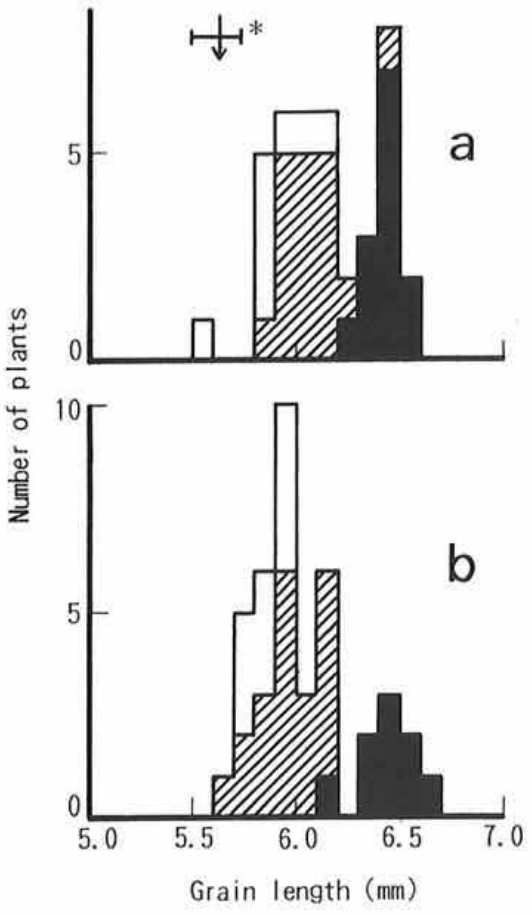

Fig. 3. Frequency distribution of grain length in the BC_1F_2 populations (Gimbozu/T34//Gimbozu)
The donor parents of the populations in Fig. 3-a and -b are the progenies of line *c* and line *e* in Fig. 2, respectively. Open, hatched, and closed segments refer to the BC_1F_2 plants generating the short-grain, segregating, and long-grain BC_1F_3 families, respectively.
*Mean and range of Gimbozu.

These results imply that a recombinant inbred line T34 received the alleles for long grain from BG 1 at 2 different QTLs, and the alleles for short grain from Koshihikari at the other QTLs. Each of the F_2-derived F_3 lines showing an intermediate grain length, i.e. line *c* and line *e* in Fig. 2, should have either of the 2 QTLs as above where the long-grain allele from BG 1 occurred under a homozygous condition, whereas the segregating lines, lines *b*, *d*, and *f*, had these QTLs under heterozygous conditions. These lines of intermediate grain lengths, therefore, might indeed be the final objectives in this study. After checking homozygosity for grain length in the F_4 generation, each F_4 progeny of these lines was crossed as a female donor parent with Gimbozu.

Fig. 3 shows the segregation of grain length in the BC_1F_2 populations (Gimbozu/T34//Gimbozu). The donor parents of the populations depicted in Fig. 3-a and -b were the progenies of line *c* and line *e* shown in Fig. 2, respectively. Obviously, a bimodal distribution in grain length was observed in each population. In Fig. 3 the results of the progeny test using BC_1F_3 families derived from individual BC_1F_2 plants were superimposed. The ratio of the short-grain, segregating, and long-grain families fitted well to the expected ratio of 1:2:1 ($x^2 = 1.51$ and 0.08 in Fig. 3-a and -b, respectively). The range of grain length of the short-grain group considerably overlapped with the range of Gimbozu in each population. These results strongly suggest that in both populations only one QTL should be involved in the segregation of grain length, and the short-grain allele at this QTL was dominant over the long-grain allele. Consequently, it is concluded that, in the long-grain segregants of each BC_1F_2 population, a single QTL should be isolated as a monogenically segregating factor from a number of QTLs causing long grain in BG 1. In each population, one of these segregants was subjected to successive recurrent backcrossings with Gimbozu, and also to allelism tests between these 2 QTLs.

Fig. 4 shows the segregation of grain length in the BC_2F_2 populations. Apparently, typical monogenic segregation with a ratio of 3 short- to 1 long-grain group was observed ($x^2 = 1.63$ and 0.01 in Fig. 4-a and -b, respectively), confirming that only one QTL carried the recessive allele for long grain in the long-grain segregants in each population. The average grain length of the long-grain group in Fig. 4-a was obviously shorter than that of the long-grain group in Fig. 4-b. Also, as observed

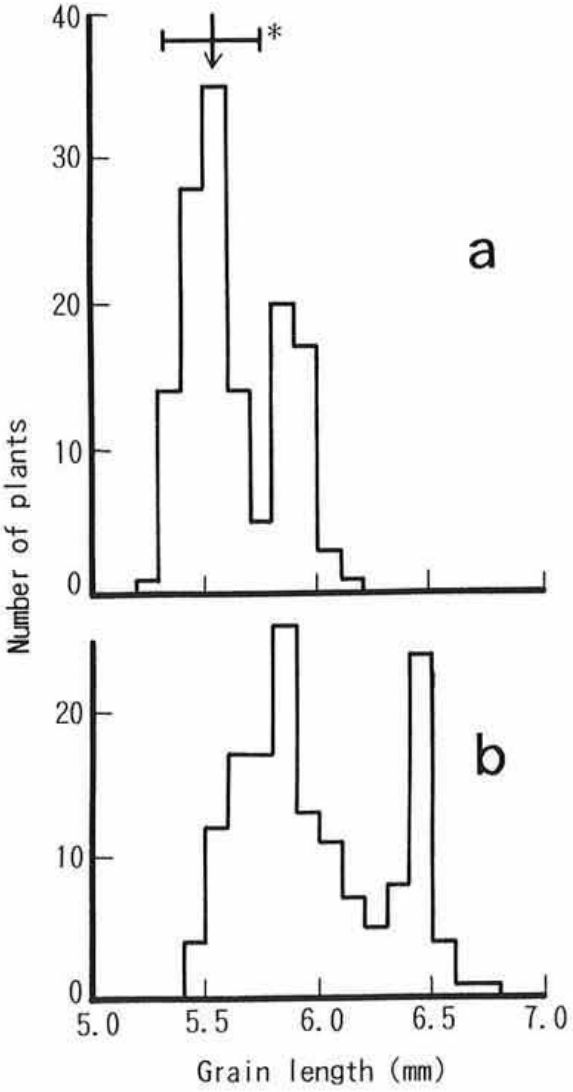

Fig. 4. Frequency distribution of grain length in the BC_2F_2 populations (Gimbozu/T34//2*Gimbozu)
The donor parents of the populations in Fig. 4-a and -b are the progenies of line *c* and line *e* in Fig. 2, respectively.
*Mean and range of Gimbozu.

in Fig. 2, the average grain length of line *c*, was shorter than that of line *e*. These results suggest that in the 2 isolated QTLs gene expression showed different magnitudes.

Fig. 5 shows the results of the allelism test between the 2 isolated QTLs as indicated above, where a continuous distribution with a wide range was observed in this F_2 population, although no transgressive segregant in the direction of shorter grain length was recorded. The results of the allelism test confirmed that the 2 isolated QTLs for long grain were different from each other. The isolated QTLs in the populations shown in Fig. 4-a and -b were,

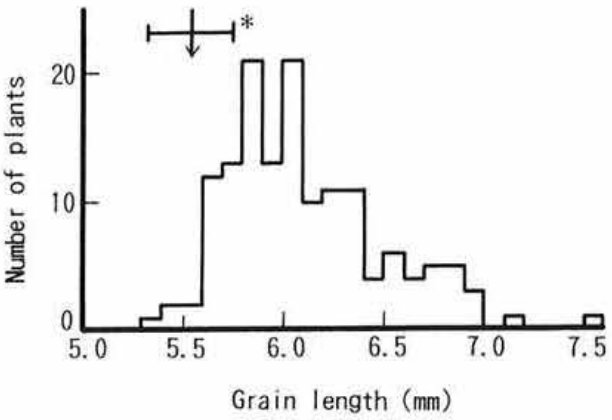

Fig. 5. Frequency distribution of grain length in the F_2 population from the cross between a long-grain segregant of BC_1F_2 shown in Fig. 3-a and that in Fig. 3-b
*Mean and range of Gimbozu.

thus, tentatively designated as 'QTL-A' and 'QTL-B', respectively. As shown in Fig. 5, there were many transgressive segregants beyond the grain length of the parent with longer grain (a long-grain segregant shown in Fig. 3-b) which should include the QTL-B. This fact indicates that QTL-A and QTL-B induced long grain in an additive manner. Hence, the genetic variation of grain length depicted in Fig. 5, and also in Fig. 2, might be attributed mainly to the segregation in QTL-B and partly to that in QTL-A.

Until the BC_2F_2 generation, 6 QTLs for long grain were isolated besides the QTL-A and QTL-B via other recombinant inbreds than T34, although allelism test among them has not been completed. In all of the isolated QTLs, the short-grain alleles were dominant over the corresponding long-grain alleles. Kato[3] conducted a diallel analysis of rice grain length using 6 cultivars including BG 1 and Koshihikari, the parents of the progenies used in this study. In this analysis, the genes conferring long grain were considered to be recessive, and BG 1 and Koshihikari were assigned to a completely recessive parent and a completely dominant parent, respectively. These results from the diallel analysis agreed well with the present results.

A series of isolines involving every single QTL, the final objective of the attempt as described above, corresponded to the "substitution lines" for chromosomal regions which are expected to be a very suitable tool for analyzing QTL action and location[5,13]. In rice, several QTLs related to heading date[13] and one QTL related to grain width[2]

had already been isolated as a form of near-isogenic lines through backcrossing by monitoring the graphical genotypes of the progenies. The actions and interactions were evaluated also for the QTLs of heading date using these isolines[14]. The present attempt will be continued until every QTL causing long grain, at least with sufficient gene action to be detected, is isolated. The tagging of individual QTLs with molecular markers should certainly facilitate the enumeration and handling of the QTLs in this study, whereas the detection of suitable markers has not fully progressed because of insufficient polymorphisms among the present materials. In any case, these isolines for individual QTLs may raise various issues about the nature of a quantitative trait. In addition, plant breeders could directly utilize these materials themselves as gene sources in their breeding programs. A larger number of QTLs should be isolated as a monogenic Mendelian factor in various agronomic traits for achieving these purposes.

References

1) Dudley, J. W. (1993): Molecular markers in plant improvement; Manipulation of genes affecting quantitative traits. *Crop Sci.*, **33**, 660-668.
2) Kajiya, H., Yano, M. & Sasaki, T. (1995): Molecular tagging of the gene *wgl*^{n}*(t)* conferring glume width in rice. *Breed. Sci.*, **45**(suppl. 1), 77 [In Japanese].
3) Kato, T. (1989): Diallel analysis of grain size of rice (*Oryza sativa* L.). *Jpn. J. Breed.*, **39**, 39-45.
4) Kato, T. (1997): Inheritance of grain size in rice. 6. Development of near-isogenic lines for major genes causing long grain. *Breed. Sci.*, **47**(suppl. 2), 232 [In Japanese].
5) Kearsey, M. J. & Pooni, H. S. (1996): Designer chromosomes. *In* The genetical analysis of quantitative traits. Chapman & Hall, London, 165-185.
6) Lander, E. S. et al. (1987): MAPMAKER; An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, **1**, 174-181.
7) Sax, K. (1923): The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics*, **8**, 552-560.
8) Takita, T. (1983): Breeding of a rice line with extraordinary large grains as a genetic source for high yielding varieties. *JARQ*, **17**, 93-97.
9) Takita, T. (1985): Inheritance of grain size and the relationship between grain size and other characters in rice. *Bull. Natl. Agric. Res. Cent.*, **3**, 55-71 [In Japanese with English summary].
10) Tanksley, S. D. (1993): Mapping polygenes. *Annu. Rev. Genet.*, **27**, 205-233.
11) Thoday, J. M. (1961): Location of polygenes. *Nature*, **191**, 368-370.
12) Tinker, N. A. & Mather, D. E. (1995): MQTL; Software for simplified composite interval mapping of QTL in multiple environments. *JQTL*, **1**, http://probe.nalusda.gov:8000/otherdocs/jqtl/.
13) Yano, M. & Sasaki, T. (1997): Genetic and molecular dissection of quantitative traits in rice. *Plant Mol. Biol.*, **35**, 145-153.
14) Yano, M., Yamamoto, T. & Sasaki, T. (1997): Epistatic interaction of expression among photoperiod-sensitive genes, *Hd-1*, *Hd-2* and *Hd-3* in rice. *Breed. Sci.*, **47**(suppl. 2), 224 [In Japanese].

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