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Inheritance of grain shape and molecular tagging of the QTL for reduced grain width (*gw*) in rice (*Oryza sativa* L.)

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Abstract

Grain shape as determined by the ratios of grain length (L) to width (W) is an important trait affecting consumer preferences and the rice markets worldwide. Study of its inheritance and identification of linked molecular markers are therefore essential for rice breeding with desirable grain shapes. For this purpose, we used the Single Segment Substitution Lines (3-S Lines) developed by crossing HJX74 (medium grain) with Amol-3 (slender grain) and Basmati 370 (slender grain). Slender shape (L: W > 3) of grain was found to be monogenic recessive. The QTL for grain width (*gw8*) was mapped to the distal end of the chromosome 8 and was found to be linked with RM447 (0.0cM) and RM502 (2.5cM) which were located at a distance of 55kb on the same clone AP005529 of rice physical map. The study should facilitate map-based cloning of the gene (QTL) and marker-assisted selection (MAS) for slender shape of grain in rice.

Key words: Grain shape, grain width, QTL tagging, 3-S lines, rice

Introduction

Rice grain appearance is classified into two categories based on two physical parameters *viz*., size and shape. Size is a measure of the rice kernel in its greatest dimension while as shape is determined by the grain length: width ratio. Grain physical features *viz*., length (L), width (W) and the length: width ratio (L : W) are considered important traits, especially in quality rices. Secondly, rice kernel shapes also influences the milling or processing qualities. Therefore, grain shape is an important breeding objective. However, it is a complex trait from the genetic-control point of view. Most of the previous workers reported monogenic, digenic, trigenic and polygenic inheritance of grain length [1]. Redoza and McKill [2] reported detection of quantitative trait loci (QTL) on chromosomes 3, 4 and 7 that explained 45% of the variations observed in the ratio of grain length to width. Takamure and Kinoshita [3, 4] detected two recessive gene *lk-i* and *lk-i-2* for long grain character in variety IRAT 13. Tsunematusu *et al*. [5] used RFLP markers and a RIL population to map QTL for grain length onto the region delineated by C1677 and R19 on rice chromosome 3. Similarly, Kubo [6] mapped the QTL for long kernel *viz*., *lk3 (t)* onto the chromosome 3. Sobrizal and Yoshimura [7] mapped two QTL for slender kernel *viz*., sk1 (t) and sk2 (t) on to the chromosomes 2 and 5, respectively. Lin *et al*. [8] used molecular markers to map QTL for grain characteristics in rice. All such reports contrasting or inconclusive owing to complexity of the trait on one hand, and the weaknesses of the population and the processes employed for the study, on the other. In most of the above-mentioned studies, the mapping population used was F_2/F_3, BC_1F_1, DH or RIL. Genetic background of such populations is no less complex and hence affects the QTL mapping process. To overcome such problems, concept of Introgression Lines (ILs) was put forwarded by Eshed and Zamir [9]. However, the ILs is also not free from drawbacks as it usually contains more than one introgression-segment that affects the genetic background of the recipient parent. Recently, Talukdar and Zhang [10] and He *et al*. [11] reported the development of an improved version of Introgression Lines called Single Segment Substitution Lines (SSSL) or 3-S Lines, in short. The 3-S lines are developed by crossing the recipient parent with the donor following marker-assisted backcross breeding. So, unlike Introgression Line (IL) and Chromosome Segment Substitution Line (CSSL), the 3-S Lines contain only one small segment of chromosome from the donor. As such, the 3-S Line and

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the recipient parent are genetically identical except the single substituted segment of chromosome substituted (introgressed) from the donor parent. Hence, any variations between the 3-S Line and the recipient parent must be due to QTL located on the substituted segment of chromosome. This alternative population has effectively been used for mapping of gene(s) and analysis of QTL in rice [12]. In the present study, such 3-S Line and Simple Sequence Repeats (SSR) markers was used to map the QTL affecting grain shape in rice.

Material and methods

Plant materials

In the current study two Single Segment Substitution Lines (3-S Line) were used. To develop the 3-S Lines, two donors (Amol-3 and Basmati 370) with varying grain shape and sizes were crossed separately with the recipient parent Hua Jing Xian 74 (HJX74). The population was advanced through marker-assisted backcross breeding approach [16]. In every generation, the genome of the recipient parent was surveyed thoroughly with 258 polymorphic SSR markers. In the BC_2F_1, some plants were recovered that contained only one segment of chromosome introgressed (substituted) from one of the donor. In BC_2F_2, two such plants *viz.*, W2-8-7-5 and W11-10-6-5 from the cross combinations HJX74 x Amol 3 and HJX74 x Basmati 370, respectively segregated for grain shape. Both the plants contained a segment on chromosome 8 that got introgressed from their respective donor parent i.e. Amol 3 and Basmati 370. The introgressed segment was 9.6cM long and was represented by two SSR markers, RM 502 and RM 447. Heterozygous plants from these two plant populations were then selected separately and used to develop two mapping populations. The mapping population of Amol 3 (to be called as Amol 3 population) and Basmati 370 (to be called as Basmati population) consisted of 100 and 150 segregating plants, respectively. Both the mapping populations along with their parents were grown on the Experimental Field of South China Agricultural University, Guangzhou, China and the standard package of practices were followed for their management.

Phenotyping the population for grain shapes

For phenotypic study of the grains *viz.*, length (L), width (W) and L: W, protocol as described by Tan *et al*. [13] were used. The grading of grains as medium (L: W = 2.1 - 3.0) and slender (L: W > 3.0) was done as per scale followed at International Rice Research Institute (IRRI), Philippines. The number of plants under different classes (medium and slender) of grains was finally subjected to χ^2 analysis to see its goodness of fit.

Genotyping the populations

For genotyping the segregating plants, leaf samples were collected from every young plants separately. Extraction, purification and quantification of DNA from the leaf samples were done as per Zheng *et al*. [14]. The plants of both the populations were genotyped with the two SSR markers (RM502 and RM447) that represented the 9.6 cM long substituted segment. For PCR, gel-electrophoresis, silver staining and scoring of bands the protocols as described by Li *et al*. [15] were used.

Linkage map construction

For construction of the linkage map, the data for grain shape and corresponding molecular genotypic scores were subjected to the software MAPMAKER/EXP Version 3.0. The map distance between the QTL and the linked marker was adjusted following the Kosambi equation [16].

Results and discussion

Most of the traits that plant breeders are concerned with, are controlled by many genes having small effect, called Quantitative Trait Loci (QTL). Mapping of QTL with closely linked markers are essential to understand its effect on phenotype properly.

In recent past, considerable progress has been made in the field of QTL mapping in rice. Most of the agronomic and morpho-physiological characters have been mapped through different molecular markers. Subsequently, finer mapping and map-based cloning of the gene(s) have also been done at a rapid pace. Thus, it has been proved that QTL, even those with minor effect, can be dealt with as single Mendelian factor.

Breeding for desirable grain shape has gained importance worldwide. But the outcomes of these studies are not always applicable universally. Such variations in the findings are the result of using different experimental materials, sets of different markers, variable approaches and the complexity of the trait *per se.* Here we used the 3-S lines which are more powerful in detecting QTL [10, 11].

Inheritance of slender grain shape

Grain shape for the recipient (HJX74), two donors (Amol - 3 and Basmati 370), and individual plants of the two

mapping populations were recorded separately. Average grain length (L), width (W) and L : W for the HJX74 was 7.25 ±0.04, 2.65 ± 0.03 and 2.73 ± 0.03mm, respectively. Similarly, the data for Basmati 370 was 8.9 ± 0.04, 2.37 ± 0.05 and 3.75 ± 0.09 and for Amol 3 it was 8.5 ± 0.01, 2.33 ± 0.05 and 3.64 ± 0.07, respectively. The L : W ratios of the mapping populations were found to range between 2.79 and 3.64 in Amol-3 population (Fig. 1a) and between 2.65 and 3.75 in Basmati population (Fig. 1b). In both the populations, the distribution of plants with medium (L : W ≤ 3) and slender grains (L : W > 3) showed distinct classes (Figs. 1a & b).

In Amol-3 population, the number of plants with slender (23) and medium grains (73) was in good agreement with 1 : 3 monogenic ratio as supported by the χ^2 test (χ^2 = 0.12). Similarly, the genotypic segregation ratio of the allele for homozygous recurrent parent (A/A): heterozygous (A/B): homozygous donor parent (B/B) also showed a good fit to the 1:2:1 ratio for both the markers used (χ^2 = 0.38).

In case of the Basmati population also, the ratio of plants with slender (38) and medium grain (112) shape, and the genotypic ratio of allele for homozygous recurrent parent (A/A = 44): heterozygous (A/B = 68): homozygous donor parent (B/B = 38) showed a goodness of fit to the 1:3 (χ^2 = 0) and 1:2:1 (χ^2 = 1.79) ratios, respectively. The result from both the populations indicated that the slender shape of grain is controlled by a single recessive gene.

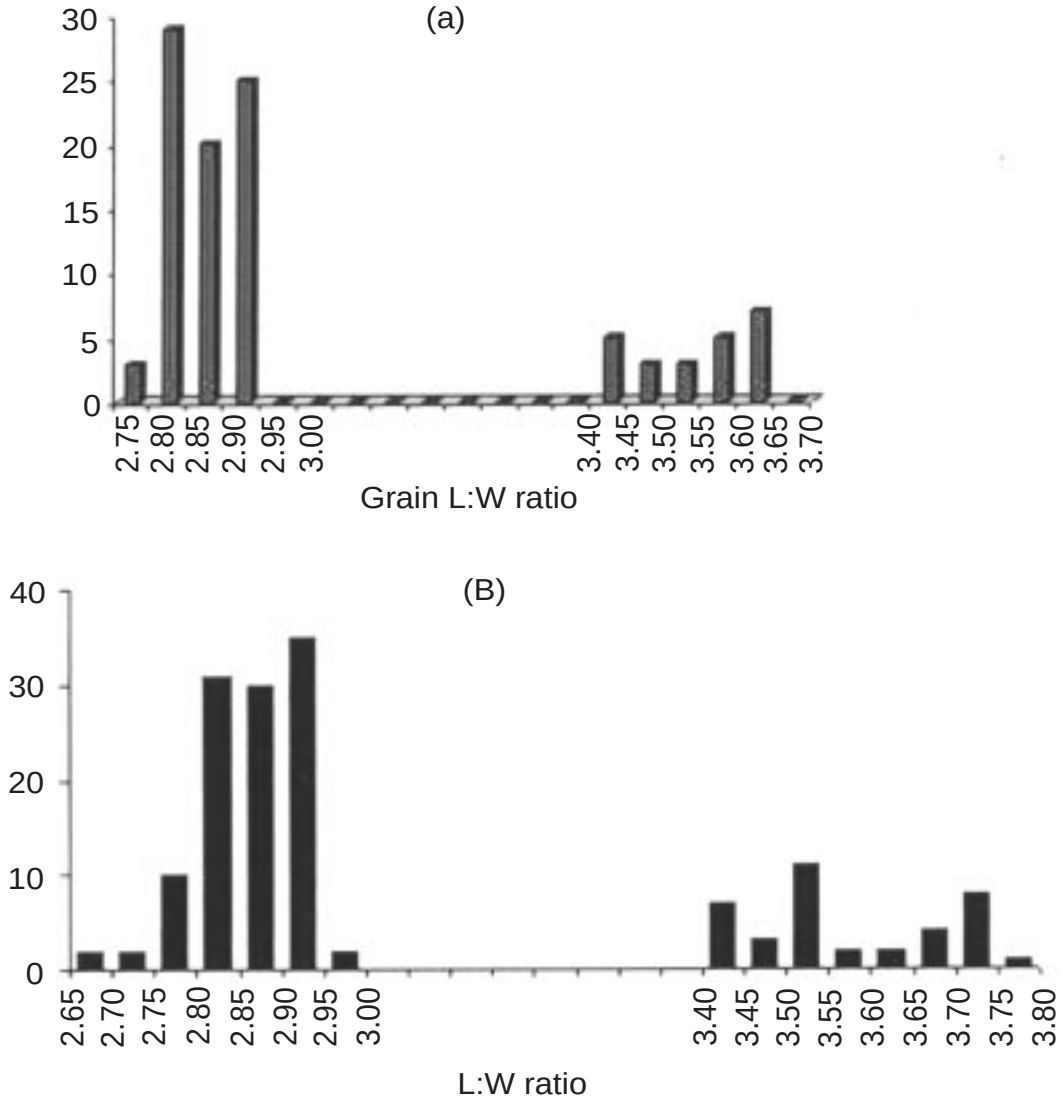

Fig. 1. Distribution of different classes of grains in (a) Amol-3 populations; (b) Basmati 370 population

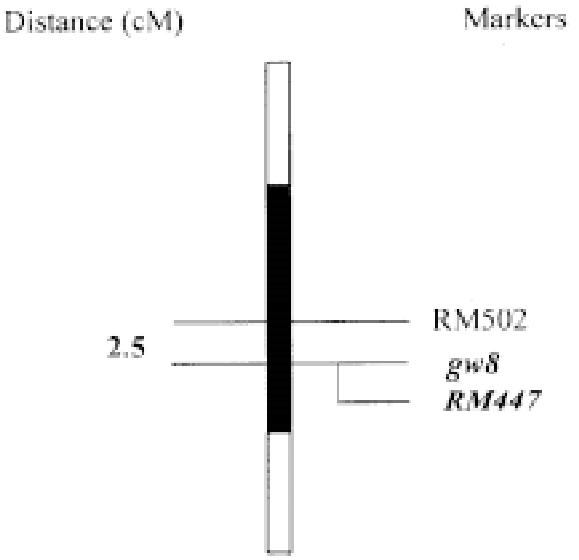

Fig. 2. Linkage map of QTL for grain shape *(gw8)* on chromosome 8 of rice. White block is recipient genome and the black block is the segment substituted from the donor (9.6cM long). The physical distance between RM447 and RM502 is 55kb

Grain shape as determined by QTL for (reduced) grain width

As grain shape is determined by the ratio of grain length and width, hence any variations in length and width or in both will lead to the change of grain shape. In the present study, it was observed in both the mapping populations that the width of the grains (2.31± 0.02mm in Amol 3 population and 2.42 ± 0.01 in Basmati population) was nearly similar to that of the donors but always less than the recipient parent (2.65 ± 0.03mm in HJX74). On the other hand, the length of the grains (7.37± 0.01mm in Amol 3 population and 7.39 ± 0.03mm in Basmati population) was either same as or little more than the recipient but in no cases was longer than the donors (8.5± .0.01 mm in Amol 3 and 8.9 ± 0.09mm in Basmati 370). It was thus obvious that the observed slenderness (L: W > 3) of the grains were primarily due to the decrease in grain width. The QTL for such decrease in grain width must be introgressed into HJX74 from Basmati 370 or Amol 3 through the substituted segment of chromosome 8. The QTL for reduced grain width identified here have been designated as *gw8* and was found to be linked to the markers RM447 and RM502. Zeng *et al*. [17] have found a QTL for grain width (*Gw-8*) in the same region of chromosome 8. They have also mapped one QTL for grain length (*Gl-3*) on chromosome 3. It is thus found that grain length and width are controlled by different QTLs. So, any breeding effort targeted to manipulate grain shape must be adjusted accordingly to manipulate the QTLs for grain length and width either singly or simultaneously.

Molecular map of the QTL for grain width

While analyzing the phenotypic and molecular genotypic data from both the populations, it was observed that no recombination was occurring between RM447 and the QTL for reduced grain width. However, a few recombinations (4 in Amol-3 population and 6 in Basmati population) were found to occur between the markers. Eventually, the linkage map constructed through MAPMAKER showed that RM 447 is cosegregating with the QTL for grain width (*gw8*) while RM 502 was located 2.5cM away from it (Fig. 2). The linkage analysis was done separately for both the population and the same linkage map was produced from the two. It was observed that no polymorphic markers were available on the other side of the QTL. It should not cause any hindrance to MAS, because both the markers were very closely linked with the QTL for grain width. Secondly, selection is equally effective with linked markers in one side of the QTL (gene), provided the marker is closely linked to the gene [18]. However, validation of any markers before its actual use in breeding program is always suggested.

Physical map of the QTL for grain width

As per the rice physical map (http://www.gramene.org), the markers RM447 and RM502 are located on the same clone AP005529 and the distance between them is only 55 kb. It will therefore be possible to construct the physical map of this QTL and proceed for map-based cloning to know its biochemical products, functions and regulatory pathways for possible manipulation. In present time, a number of gene(s) in rice have already been cloned and compared with gene(s) of similar effect in other plants.

With the availability of molecular markers linked with the grain length and width separately, breeding of rice for desirable grain shape and sizes should proceed at a speed faster than ever. Such efforts should help people get rice of their choices soon.

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