

## Genetic Analysis and Mapping of Dominant Minute Grain Gene *Mi3(t)* in Rice

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**Abstract:** Grain size, determined chiefly by grain length, is one of the main factors affecting the grain yield in rice production. To study the trait of rice grain size, F<sub>1</sub> and F<sub>2</sub> populations were developed from crosses Shuhui 881/Y34 and Shuhui 527/Y34, and genetic analysis for minute grain was performed. The F<sub>1</sub> populations showed minute grains, and grain size segregations in the two F<sub>2</sub> populations were both in accordance with the ratio of 3:1, indicating that minute grain in Y34 was controlled by a completely dominant gene. By using the F<sub>2</sub> population from Shuhui 881/Y34, this dominant gene, tentatively designated as *Mi3(t)*, was mapped based on SSR markers in the interval between RM282 (genetic distance of 5.1 cM) and RM6283 (genetic distance of 0.9 cM) on the short arm of chromosome 3.

**Key words:** rice; minute grain; genetic analysis; gene location

Grain shape and size are of the main components in the formation of grain yield, and directly affect the appearance quality of milled rice. The grain length, width, thickness and ratio among them are the main targets for studies on the grain shape. Among the targets above mentioned, the grain length is the most important one, which mostly has effect on the grain shape of rice<sup>[1]</sup>. As the trait of the grain length is normally considered as the quantitative trait proved by much research, many QTLs have been detected on every chromosome of rice. To date, it has been found that chromosome 3 is the number one, on which the most QTLs were checked out<sup>[2,3]</sup>, and the number 2 is chromosome 2, followed by chromosomes 1, 6, 11, 5, 7, 9, 10 and 12<sup>[2-7]</sup> in order. With the help of the RIL (recombinant inbred line) developed from Zhenxian 97 and Minghui 63, a main QTL located between RG393 and C1087 on chromosome 3 was checked out in the years of 2000 and 2001<sup>[2,4]</sup>.

Furthermore, studies on rice grain-length gene were carried out with the mutants. Grain-length genes such as *lk1* (or *lk1.1*, or *lkna*), *lki1* (or *lk4*), *lki2* (or *lk5*), *Lkg*, *Lkf*, *Lk2* (or *Lknb*) and *Kr2* were studied in details with Gimbozu<sup>[8,9]</sup>, Roti<sup>[10]</sup>, Fusayoushi<sup>[11-15]</sup>, IRAT 13<sup>[11,12]</sup>, Nagayama 77402a (N179)<sup>[14,16]</sup> and

Nagayama 77402b<sup>[14,16]</sup>. Minute-grain genes such as *Mi* (or *Mi1*) and *mik* (or *mi2*) were studied with H346/L32 and Kitaake<sup>[1,11,17-21]</sup>. Short-round-and-minute-grain gene, *Kr1* (*Kr1.1*) was studied with club mutant from Sri Lanka<sup>[10]</sup>. In addition, other genes chaperoned by the minute-grain trait were also studied, including short-awn grain genes (*An6* and *An7*<sup>[10]</sup>), dwarf genes (*dwf37/d56*, *dwf33/d52/dK2*, *dwf2/d2*, *dwf3/d55/dK6*, *dwf3/d54/dK5*, *dwf1/d1* and *dwf3/d38*<sup>[16,22-25]</sup>), long twisted grain gene (*lgt*)<sup>[26]</sup>, and round-grain genes (*rk1* and *rk2*, whose alleles were *rk1.1* and *rk1.2*<sup>[23,27]</sup>, respectively).

Up to the present time, many grain genes have been located on rice chromosome: two incompletely dominant length genes (*Lkf* and *Lkg*), an incompletely dominant minute gene (*Mi*) and two recessive minute genes (*dwf33* and *dwf37*) were located on chromosome 3; a recessive length gene, *lk*, and a recessive round-minute gene (*rk1*) on chromosome 4; a recessive dwarf-minute gene (*dwf35*), a recessive dwarf-round-minute gene (*dwf2*) and a recessive round-big dwarf gene (*dwf36*) on chromosome 1; an incompletely dominant length gene (*Lk2*) and a recessive minute gene on chromosome 11; and two recessive dwarf-minute genes (*dwf1* and *dwf39*) and a recessive round-minute gene (*rk2*) were respectively located on chromosomes 5, 6 and 10. But other minute

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genes such as *lki1*, *lk5*, *Lk8*, *Kr2*, *Kr1* and *skr* have not been reported about their locations on chromosome. And the location of the completely dominant minute grain gene had not been reported yet.

To study the minute-grain trait, F<sub>1</sub> and F<sub>2</sub> populations were developed from Shuhui 881/Y34 and Shuhui 527/Y34. The rules of genetic segregation were analyzed and the completely dominant minute grain gene *Mi3(t)* was reported to be located on chromosome 3.

## MATERIALS AND METHODS

### Rice materials

A super-minute grain rice Y34 with the grain length of 0.641 cm and the 1000-grain weight of 15.22 g was used as male parent and two super-long grain rice Shuhui 881 and Shuhui 527 with the grain length of 1.113, 1.011 cm and the 1000-grain weight of 31.07, 32.66 g, respectively were used as female parents. All the materials mentioned above were provided by Rice Research Institute, Sichuan Agricultural University in China.

### Development and analysis of genetic groups

Y34, Shuhui 881 and Shuhui 527 were planted to develop two of F<sub>1</sub> crossbreeds (Shuhui 881/Y34 and Shuhui 527/Y34) in summer of 2003 at Wenjiang in Chengdu, China. In summer of 2004, F<sub>2</sub> populations were got at the same place due to the successful propagation of F<sub>1</sub> crossbreeds.

The grain-length trait of every parent, F<sub>1</sub> and F<sub>2</sub> populations were investigated to analyze the genetic segregation ratio, to ascertain the dominant-or-recessive relations between the long grain and the minute grain and to determine the number of genes controlling the minute-grain trait.

### Extraction of genome DNA

Referring to DNA extracting methods [2, 30-32], genome DNA of Shuhui 881, Shuhui 527 and recessive F<sub>2</sub> population from Shuhui 881/Y34 were extracted.

### SSR analysis and data statistic

There were 800 SSR primers employed in the

experiment, 700 of which were RM-series primers whose information could be got from the Gramene website (<http://www.gramene.org/microsat/>) and the other 100 were RP-series primers developed by Laboratory for bioinformatics at Rice Research Institute of Sichuan Agricultural University in May of 2003, whose information could be got from the SABEC website (<http://sabec.vicp.net:8080/sabec/modules/bioData/search/markerslist.php>). All the primers were synthesized by BioAsia Bio-Company in Shanghai of China.

By using 800 SSR primers, the polymorphism among parents were checked, the linkage relation among 26 recessive plants in F<sub>2</sub> populations from Shuhui 881/Y34 were also analyzed. The linkage markers checked out from the 26 recessive plants were then used to analyze the linkage relation among the whole plants of F<sub>2</sub> population from Shuhui 881/Y34.

Polymerase chain reaction (PCR) for SSR (simple sequence repeats) was performed in M.J. PTC-220 DNA Engine Dyrad Cycler. The volume of PCR system was 25 µL, which contained 2 µL of SSR primer (50 mol/L), 2 µL of dNTPs (2.5 mol/L), 2 µL of DNA template (50 ng/L), 2.5 µL of 10×buffer, 0.2 µL of *Taq*-DNA Polymerase (5 U/L) and 16.3 µL of super-purified water. The PCR profile was as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 40 s, 55°C for 30 s and 72°C for 1 min, and lastly 10 min at 72°C. After PCR, the products would undergo the electrophoresis in agarose gel under 4 V/cm for 2.5 h and then were stained with EB. Then band photos of the electrophoresis exposure were taken and the visualized bands were transformed to numerical values: marking '0' for the band types same to the recessive parent's, '2' for the ones same to the dominant parent's, '1' for the ones as same as the both parents' band and '3' for the blank band ones.

### Genetic mapping and gene localization

The transformed data from band photos was analyzed, the genetic distance (centimorgan, cM) was calculated and the genetic map was drawn with the help of MapMaker/ EXP software [31].

## RESULTS

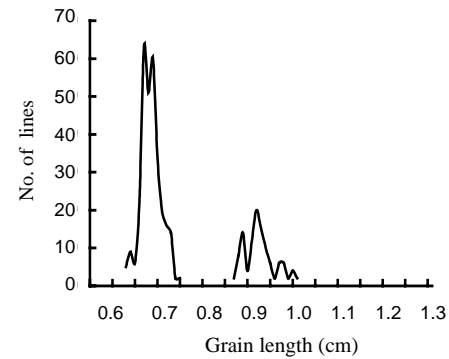
### Genetic analysis for minute-grain trait in Y34

It was concluded that the minute-grain trait was controlled by a dominant gene, which was based on the following facts (Table 1): the grain length in  $F_1$  crossbreeds from Shuhui 881/Y34 and Shuhui 527/Y34 was between the grain length of their parents and markedly partial to the minute-grain parent's. For example, the grain length in  $F_1$  from Shuhui 527/Y34 was about 0.642 cm, which was markedly partial to the minute-grain parent's (0.641 cm). The result could be also concluded from the presents of the grain length in  $F_1$  from Shuhui 881/Y34.

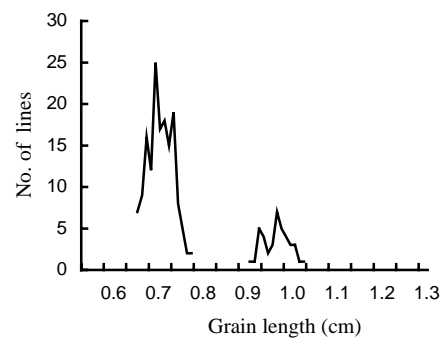
It was also concluded that the minute-grain trait was controlled by a completely dominant gene, which was based on the following facts (Fig. 1 and Fig. 2): the grain length in  $F_2$  populations developed by Shuhui 881/Y34 and Shuhui 527/Y34 was markedly partial to their minute-grain parents'. The frequency distributing maps of the grain-length traits were both with double peak and broken dots. Based on broken dots on the distributing map, two of groups distinguished with long grains and minute grains were classified and the segregation ratios were calculated according to  $\chi^2$  test, which were both accordance with 3:1. There were 306 minute-grain plants and 113 long-grain plants in  $F_2$  populations developed by Shuhui 881/Y34 with a less  $\chi^2$  value than the threshold ( $\chi^2 = 0.97 < \chi^2_{0.05, 1} = 3.84$ ). The same case could be observed in the  $F_2$  population by Shuhui 527/Y34 (158 minute-grain plants and 40 long-grain plants with a less  $\chi^2$  value,  $\chi^2 = 2.18 < \chi^2_{0.05, 1} = 3.84$ ).

**Table 1. Grain length in parents (Y34, Shuhui 527 and Shuhui 881) and  $F_1$ s.**

Combination	Grain length (cm)
Y34	0.641
Shuhui 527	1.113
Shuhui 881	1.011
Shuhui 527/Y34 $F_1$	0.642
Shuhui 881/Y34 $F_1$	0.650



**Fig. 1. Frequency distribution of the grain length for  $F_2$  derived from Shuhui 527/Y34.**



**Fig. 2. Frequency distribution of the grain length for  $F_2$  derived from Shuhui 881/Y34.**

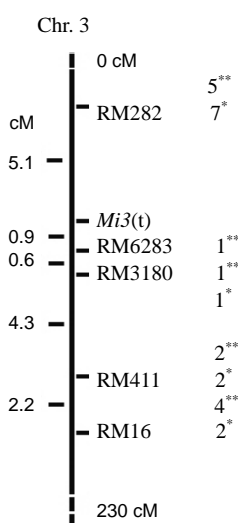
### SSR polymorphism examination, linkage analysis and gene localization

Among the 800 primers it was found that there were 65 primers, which could trace the polymorphism between Shuhui 881 and Y34. Furthermore, with the analysis for the whole recessive plants from the  $F_2$  population crossed from Shuhui 881/Y34, 5 primers among the 65 primers, namely RM282, RM6283, RM3180, RM411 and RM16, were found to be linked with the long-grain trait. The markers were located on chromosome 3 (Table 2).

As it's confirmed from the facts above, the data transformed from electrophoresis bands were analyzed with Mapmaker 3.0. The result was presented to draw out a conclusion: the dominant minute-grain gene was located between RM282 and RM6283 on the short arm of chromosome 3, with the distances of 5.1 cM and 0.9 cM to the marker, respectively. The dominant minute-grain gene is temporarily named *Mi3(t)* (Fig. 3.)

**Table 2. Information about RM282, RM6283, RM3180, RM411 and RM16 (www.gramene.org).**

Marker	Synonyms	Forward primer	Reverse primer	Genome positions(bps)	Germplasm	Chr.
RM282	CT787	CTGTGTCGAA AGGCTGCAC	CAGTCCTGTG TTGCAGCAAG	12395176-12395304	IR36	3
RM6283	MRG5283.IRRI5283	TGGAGACTGA GCTGATGCC	TCAGGTGGTC GGTTCCTTAC	16934043-16934135	Nipponbare	3
RM3180	MRG2180.HAU2180	GGGTCGGATA GCCACACAC	GAGGTAATCT CGCGGAGTTG	18230016-18230148	Nipponbare	3
RM411	-	ACACCAACTC TTGCCTGCAT	TGAAGCAAAA ACATGGCTAGG	21390246-21390356	Nipponbare	3
RM16	GA53	CGCTAGGGCA GCATCTAAA	AACACAGCA GGTACGCGC	23087067-23087234	IR36	3

**Fig. 3. Location of RM282, RM6283, RM3180, RM411, RM16 and *Mi3(t)* on chromosome 3.**

RM282, tracing seven single crossing-over and five double crossing-over plants; RM6283, tracing one single crossing-over plant; RM3180, tracing one single crossing-over and one double crossing-over plants; RM411, tracing two single crossing-over and two double crossing-over plants; RM16, tracing two single crossing-over and four double crossing-over plants.

\*\*No. of double crossing-over plants; \*No. of single crossing-over plants.

## DISCUSSION

Rice grain is the most important factor affecting rice production. And the grain-length gene is the main hereditary base of the grain size. Many reports showed that the grain-length trait was commonly the quantitative trait [32]. Some reports also showed that the grain-length trait observed from some exceptive materials was controlled by one [33], two [34] or more pairs of genes [35-37], which probably were completely

or partially dominant genes. To date, there have been four recessive long-grain genes, four incomplete long-grain genes, one recessive minute-grain gene, one incomplete minute-grain gene and twelve minute-concomitant genes were studied, some of them had been located on the chromosomes [8-12,17-41].

*Mi3(t)* was located on chromosome 3 in this study. There were three genes on chromosome 3, namely *dwf33*, *dwf37* and *Mi*, which had been located on Rice Morphologic Map 2000 drawn by Kinoshita since 1998, but have not been located on the molecular map yet. *Dwf33* and *dwf37* were both found from Kinmaze in Japan [35]. The former was named 'dwarf Kyushu 2' and located at 30 cM on Rice Morphologic Map 2000, which could result in dwarf plants with dark leaves and minute grain (but similar to normal grain) on the upper branches. The latter was named 'dwarf Kyushu 9' and located at 57 cM on Rice Morphologic Map 2000, which could result in dwarf plants with broad and erect leaves and round-minute grain. *Mi* (or *Mi1*) was an incomplete dominant gene located at 30 cM on Rice Morphologic Map 2000, which were tightly linked with *VI* and could shorten the grain length by 70–80% and lighten 1000-grain weight by 35.7–47.4% due to the extreme imbalance between the shortening in glume and ovary. However, *Mi3(t)* was from indica rice, which could shorten the grain length by 35.7–47.4% and lighten 1000-grain weight by 45.9–62.4%. Meanwhile, *Mi3(t)* mainly controlled the grain length to produce the minute grain without being associated with round grain, low seed-setting rate or late heading date.

As above-mentioned, *Mi3(t)* was a novel minute-grain gene reported as a completely dominant gene to be located on the molecular genetic map. The studying of fine location and cloning for *Mi3(t)* is tensely being carried on.

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