

# IDENTIFICATION AND MAPPING OF A GENE FOR RICE SLENDER KERNEL USING *Oryza glumaepatula* INTROGRESSION LINES

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## ABSTRACT

World demand for superior rice grain quality tends to increase. One of the criteria of appearance quality of rice grain is grain shape. Rice consumers exhibit wide preferences for grain shape, but most Indonesian rice consumers prefer long and slender grain. The objectives of this study were to identify and map a gene for rice slender kernel trait using *Oryza glumaepatula* introgression lines with *O. sativa* cv. Taichung 65 genetic background. A segregation analysis of BC<sub>4</sub>F<sub>2</sub> population derived from backcrosses of a donor parent *O. glumaepatula* into a recurrent parent Taichung 65 showed that the slender kernel was controlled by a single recessive gene. This new identified gene was designated as *sk1* (slender kernel 1). Moreover, based on the RFLP analyses using 14 RFLP markers located on chromosomes 2, 8, 9, and 10 in which the *O. glumaepatula* chromosomal segments were retained in BC<sub>4</sub>F<sub>2</sub> population, the *sk1* was located between RFLP markers *C679* and *C560* on the long arm of chromosome 2, with map distances of 2.8 and 1.5 cM, respectively. The wild rice *O. glumaepatula* carried a recessive allele for slender kernel. This allele may be useful in breeding of rice with slender kernel types. In addition, the development of plant materials and RFLP map associated with slender kernel in this study is the preliminary works in the effort to isolate this important grain shape gene.

[Keywords: *Oryza glumaepatula*, genetic maps, genetic markers, grain quality, slender kernel]

## INTRODUCTION

As the impact of human population and economic growth, the demand for superior rice grain quality as well as quantity tends to increase. The world demand for rice has been projected to increase by 25% from 2000 to 2025 (Smil 2005). In many countries the importance of improving rice quality is increasingly becoming priority to improve competitiveness. The efforts are also being made by developing new value-added and convenience rice foods. Therefore, combining the improvement for high yield and high grain quality is an important breeding objective for rice breeders. A high yielding rice cultivar will not be accepted by the consumers unless its quality is acceptable.

The primary components of rice grain quality include milling efficiency, appearance, cooking and edibility characteristics, and nutritional quality. The quality of appearance is determined by grain length, width, width-length ratio, grain shape and size, and translucency of the endosperm (Juliano and Villareal 1993). Most consumers in the tropics and subtropics prefer long to medium-long and slender grains. However, in temperate areas, short, bold and roundish grains are preferred (Khush 2001). Moreover, a survey conducted from April 2004 to March 2005 at three rice market centers in Indonesia, namely Subang, Karawang and Jakarta, showed that most of the available rice at the markets was long and slender kernels (Rachmat *et al.* 2006). To meet the preference of Indonesian rice consumers, the genetic resources of long and slender kernels are necessary. Grain size may be indicated by weight, volume or length, but grain length is the most adequate character for analyzing the inheritance of grain size because of the high heritability of the trait (Takeda 1991). Grain length affects grain shape since the grain shape is mostly expressed by width-length ratio.

The recent advances in high-density marker linkage maps of rice have provided powerful tools for elucidating the genetic basis and mapping of genes associated with important traits (Harushima *et al.* 1998; Causse *et al.* 1994). As a result, some qualitative and quantitative trait loci associated with grain size in rice have been identified and mapped by means of molecular markers. By using an interspecific advanced backcross population derived from a cross between *Oryza sativa* cv. V20A and *O. glaberrima* (Acc. IRGC 103544), Li *et al.* (2004) identified 11 QTLs associated with grain quality and grain morphology in six chromosomal regions, with the favorable alleles originated from *O. glaberrima* at eight loci. Favorable *O. glaberrima* alleles were associated with improvements in grain shape and appearance, resulting in an increase in kernel length, transgressive variation for thinner grains, and increase in length-width ratio. Tan

*et al.* (2000) identified the major locus for grain length located on the interval of RG393-C1087 on chromosome 3 using  $F_{2,3}$  and recombinant inbred line populations derived from a cross between Zhenshan 97 and Minghui 63, the parents of Shanyou 63, an elite hybrid rice in China. Tsunematsu *et al.* (1996) identified several QTLs for grain size using recombinant inbred lines derived from a cross of Asominori/IR24, and one of them was further analyzed by Kubo *et al.* (2001) resulting that the long kernel on chromosome 3 was controlled by one recessive gene designated as *lk3*.

To analyze the genetics of the traits specific to *O. glumaepatula* and to exploit the genetic potential of this species, a series of *O. glumaepatula* introgression lines with *O. sativa* cv. Taichung 65 genetic background was developed (Sobrizal *et al.* 1999). During the development of the introgression lines, they found that the plants segregated for slender kernel in a  $BC_4F_2$  population. The present study aimed to identify and map a gene for slender kernel of rice using  $BC_4F_2$  segregating population.

## MATERIALS AND METHODS

### Plant Materials

A Brazilian wild rice, *O. glumaepatula* (Acc. IRGC 105668) and a japonica cultivated rice, *O. sativa* cv. Taichung 65 were used as original crosses in the construction of *O. glumaepatula* introgression lines. The resultant  $F_1$  plants served as female parents were continuously backcrossed into the Taichung 65 recurrent parent to generate  $BC_4F_1$  populations. The japonica rice cv. Taichung 65 was selected as a recurrent parent because this variety grows well both in temperate and tropical rice growing areas due to its photoperiod insensitivity.

Molecular selection of  $BC_4F_1$  plants in the construction of introgression lines was previously described (Sobrizal *et al.* 1999). The RFLP genotypes of the  $BC_4F_1$  used in the previous study were used as reference data in this study. One of the  $BC_4F_2$  populations segregating for slender kernel was used as a mapping population and as materials for genetic analysis. This population consisted of 55 plants. Mapping and genetic analysis of the gene for slender kernel were conducted at the Plant Breeding Laboratory, Kyushu University, Fukuoka, Japan in 2002.

### Genomic DNA Extraction and RFLP Analysis

Genomic DNA from each plant was extracted from frozen leaf samples using a CTAB extraction method

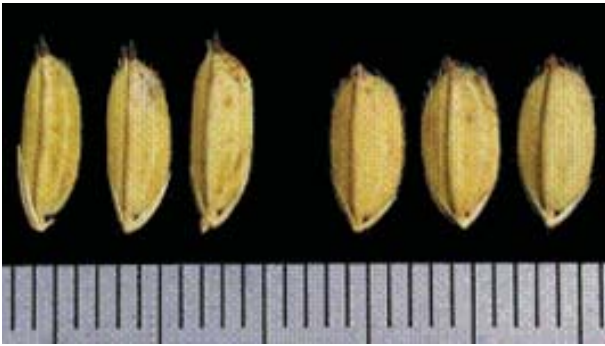
(Murray and Thompson 1980). The isolated DNA (2.5  $\mu$ g each) was digested with eight restriction enzymes (*ApaI*, *BamHI*, *BglII*, *DraI*, *EcoRI*, *EcoRV*, *HindIII*, and *KpnI*), separated by 0.8% agarose-gel electrophoresis and blotted onto Hybond N+ membranes (Amersham) by capillary transfer using 0.4 N NaOH solution. The blotted membranes were rinsed in 2x SSC, dried and baked at 120°C for 20 minutes. Fourteen DNA clones, previously mapped by Harushima *et al.* (1998), were used as DNA markers. DNA labeling, hybridization, and signal detection were conducted using the ECL detection system (Amersham).

### Data Analysis

The  $X^2$  test was performed to examine goodness of fit of the frequencies of the slender kernel plants against expectation from Mendelian segregation. The null hypothesis of the test was that progenies segregated in a 3:1 ratio of which the alleles were derived from the first and second parents, respectively. Recombination values were estimated with the maximum likelihood equation (Allard 1956). Obtained values were converted into map distances (cM) using the Kosambi function (Kosambi 1944). Calculations were conducted by using Microsoft Office Excel 2003 computer program.

## RESULTS AND DISCUSSION

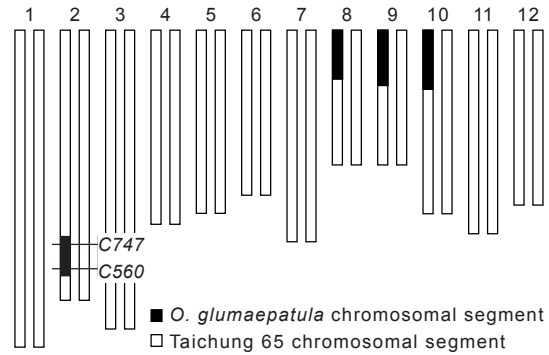
A wild rice *O. glumaepatula* (Acc. IRGC 105668) has long kernels with size of 10.0 mm x 2.5 mm, while *O. sativa* cv. Taichung 65 has medium-long kernels with size of 7.8 mm x 3.8 mm. In the process of development of *O. glumaepatula* introgression lines with Taichung 65 genetic background, we observed that the plants having normal and slender kernels segregated in  $BC_4F_2$  population. Since the genetic background of this population is Taichung 65, the plants with kernel size of 7.8 mm x 3.8 mm was grouped as normal kernel, and that with kernel size of 8.3 mm x 3.1 mm was grouped as slender kernel (Fig. 1).  $BC_4F_2$  population segregated into 34 normal and 21 slender kernel plants. The segregation ratio fitted the 3:1 ( $X^2 = 5.1$ ; nonsignificant at 1% level), indicating that the slender kernel was controlled by a single recessive gene. Several major genes for kernel length have also been reported such as *lk-f* (Takeda and Saito 1980), *lk-i* (Takamure 1994), *lk-na(t)* and *lk-nb(t)* (Takamure and Kinoshita 1996), and *lk3* (Kubo *et al.* 2001). The availability of various genetic resources for kernel length gives opportunity to create new varieties with various kernel sizes to meet the consumer preference.



**Fig. 1.** Phenotypes of slender (left) and normal (right) kernels of rice segregated in  $BC_4F_2$  population.

Result of the RFLP analysis to determine the chromosomal location of the gene controlling the slender kernel of the  $BC_4F_2$  population indicated that this gene was carried on the *O. glumaepatula* chromosomal segments 2, 8, 9 and 10 (Fig. 2). Result of the analysis using 14 RFLP markers located on these chromosomes revealed that the slender kernel gene was located on chromosome 2. Out of 21 plants with slender kernel, 20 plants were homozygous for *O. glumaepatula* allele at RFLP marker *C560* of chromosome 2, and one plant was heterozygous. Out of 34 plants with normal kernels, 10 plants were homozygous for Taichung 65 allele at *C560* and 24 plants were heterozygous (Table 1). These results confirmed that slender kernel was controlled by a single recessive gene, and this gene is tightly linked with RFLP marker *C560*. One slender kernel plant carrying heterozygous allele at *C560* was considered as a recombinant between the loci of slender kernel and RFLP marker *C560*. At this locus, *O. glumaepatula* has a recessive allele.

Several other major genes for kernel length have also been determined to be located on several rice chromosomes (Takeda and Saito 1980; Kubo *et al.* 2001). Moreover, some QTLs for long kernel have been detected on several chromosomes (Tsunematsu *et al.* 1996; Aluko *et al.* 2004; Li *et al.* 2004; Rabiei *et al.* 2004). Tsunematsu *et al.* (1996) identified some QTLs for grain length using recombinant inbred lines derived from a cross between Asominori and IR24. Among them, a major QTL located between *C1667* and *R19* on chromosome 3 was further analyzed by Kubo *et al.* (2001) using  $BC_3F_2$  of the IR24 chromosome segment substitution lines with genetic background of Asominori. The results demonstrated that long kernel on chromosome 3 was controlled by one recessive gene (*lk3*), and *lk3* was tightly linked with *An5* for awned spikelet. Since the *Lkf* gene for



**Fig. 2.** Graphical genotype of plant,  $BC_4F_1$  107-17, carrying heterozygous alleles on chromosomes 2, 8, 9, and 10. The self progenies of this plant ( $BC_4F_2$  population) were used in this study.

**Table 1. Relationship between slender kernel, normal kernel, and genotype at RFLP marker *C560* in  $BC_4F_2$  population.**

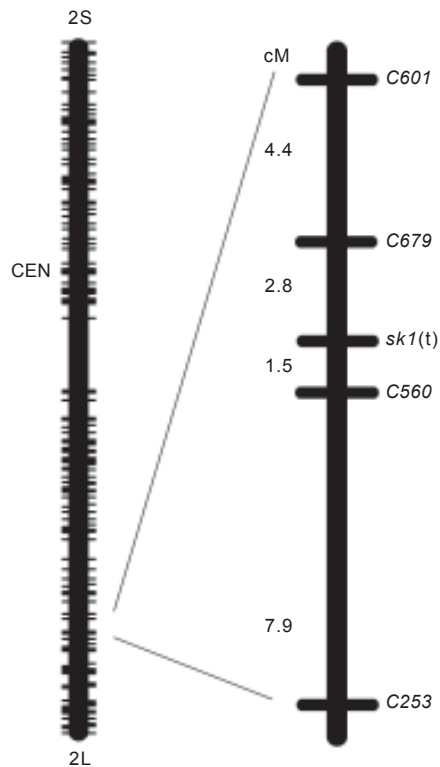
Phenotype	Genotype at <i>C560</i>		
	TT	TG	GG
Normal	10	24	0
Slender kernel	0	1	20

TT, TG, GG are Taichung 65 homozygous, heterozygous, and *O. glumaepatula* homozygous alleles, respectively.

long kernel was also located on chromosome 3 (Takeda and Saito 1980), and *Lkf3* was linked to *An3* (Takamure *et al.* 1991), the *lk3* and *An5* might be the same locus as *Lkf* and *An3*, respectively (Kubo *et al.* 2001).

So far, no single major gene for rice grain size on chromosome 2 has been reported. Moreover, only one QTL for grain length was detected on chromosome 2, by using  $F_2$  population derived from a cross between Zhenshan 97 and Minghui 63, the parents of the best hybrid rice in China, Shanyou 63. This QTL was located near RFLP marker *R1843*, around the centromere of chromosome 2 (Tan *et al.* 2000). On the contrary, the major gene for slender kernel identified in this study was tightly linked to RFLP marker *C560*, near the telomere of chromosome 2. Therefore, this slender kernel gene was considered as a newly identified gene and was designated as *sk1* (slender kernel 1).

To map the *sk1* gene on the RFLP linkage map, RFLP analyses were conducted between *sk1* and RFLP markers located around *C560*. The result revealed that *sk1* gene was located between RFLP markers *C679* and *C560* on the long arm of chromosome 2, with map distances of 2.8 and 1.5 cM, respectively (Fig. 3). This map information together with plant materials used in this study is the preliminary works



**Fig. 3.** Linkage map of chromosome 2 showing the location of *sk1*. Framework map is quoted from Harushima *et al.* (1998).

to perform the isolation of gene controlling grain size. Understanding the molecular mechanisms involved in grain size along with quantitative and qualitative genetic data will provide new strategies to develop rice varieties with desired level of grain size. In the  $BC_4F_2$  population used in this study, a recessive allele for slender kernel originated from a wild rice *O. glumaepatula*. Since most Indonesian rice consumers prefer long and slender grains, this allele might be a valuable new source for introgression and improvement of rice varieties having slender kernel.

### CONCLUSION

By using *Oryza glumaepatula* introgression lines, a novel gene for slender kernel was identified. The gene was designated as *sk1* and mapped between RFLP markers *C679* and *C560* on the long arm of chromosome 2, with map distances of 2.8 and 1.5 cM, respectively. This finding provides a new genetic resource for grain shape improvement of rice varieties, and the closely linkage markers that flank the identified gene should be very useful when transferring the gene in breeding program.

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