

Deletion in a gene associated with grain size increased yields during rice domestication

Ayahiko Shomura^{1,5}, Takeshi Izawa^{2,5}, Kaworu Ebana³, Takeshi Ebitani⁴, Hiromi Kanegae³, Saeko Konishi² & Masahiro Yano³

The domestication of crops involves a complex process of selection in plant evolution and is associated with changes in the DNA regulating agronomically important traits. Here we report the cloning of a newly identified QTL, *qSW5* (QTL for seed width on chromosome 5), involved in the determination of grain width in rice. Through fine mapping, complementation testing and association analysis, we found that a deletion in *qSW5* resulted in a significant increase in sink size owing to an increase in cell number in the outer glume of the rice flower; this trait might have been selected by ancient humans to increase yield of rice grains. In addition, we mapped two other defective functional nucleotide polymorphisms of rice domestication-related genes with genome-wide RFLP polymorphisms of various rice landraces. These analyses show that the *qSW5* deletion had an important historical role in artificial selection, propagation of cultivation and natural crossings in rice domestication, and shed light on how the rice genome was domesticated.

As Charles Darwin said¹, domestication is a good example of evolution, as domesticated species and their corresponding wild species can be easily compared. This should be all the more true for domestication of crops with known genome information^{2–5}. However, crop domestication is a complex process of selection in which multiple agronomically important traits have been involved, and few details are known about how it has proceeded^{6,7}. Domestication-related genes for various traits have been cloned from several crops—especially cereals such as maize^{8,9}, wheat¹⁰, barley¹¹ and rice^{12,13}. The domestication traits are associated with grain size, grain number, panicle size, grain quality, flowering time, plant architecture and seed shattering. One important motive behind the domestication of rice was the increase of grain yield, as all accessions of the wild species of Asian rice, *Oryza rufipogon*, have thin panicles with thin grains and relatively low fertility.

To identify the genes involved in the increase in grain yield that occurred during rice domestication, we carried out a QTL

(quantitative trait locus) analysis for grain size in an F₂ cross population between Nipponbare (*japonica*) and Kasalath (*indica*) cultivars, which we thought might have distinct domestication histories. We detected several QTLs for grain width and focused on a major one, termed *qSW5* (QTL for rice seed width on chromosome 5), which explained 38.5% of natural variation in the F₂ population (Fig. 1a and Supplementary Fig. 1a online). Using SL22, a line with substitution of Kasalath chromosome 5 in a Nipponbare genetic background (see URLs section in Methods and Fig. 1b), and NIL(*qSW5*), a nearly isogenic line (NIL) that contained around 90 kbp of Kasalath fragments of the *qSW5* region in a Nipponbare background (Fig. 1c), for comparison, we first observed the appearance of rice grains (Fig. 1d,e) and found that the number of rows of specialized cells with rigidified walls in the upper epidermis—and especially of the outer glume (lemma)—of the rice flower were increased in Nipponbare but not in SL22 (Table 1), indicating that the primary cause of the increase in grain width was the increase in size of the outer glumes (Fig. 1f,g). The size of the rice glume is one of the determinants of rice endosperm size or grain size^{14,15}. We also counted the number of lower epidermis cells inside the rice glumes of SL22 (Fig. 1f and Table 1) and found that the number was higher in Nipponbare, indicating that the *qSW5* gene may control cell number of the outer glume of the rice flower. As the Nipponbare allele of *qSW5* behaves in a recessive manner in inheritance, it might have acquired a defect during domestication. Fine mapping of *qSW5* using F₃ and F₄ progeny of a F₂ plant, 94BC₃F₂-7 (Supplementary Fig. 1b), delimited the functional nucleotide polymorphisms (FNPs) for *qSW5* within a 2,263-bp fragment of Kasalath genomic region (Fig. 2 and Supplementary Fig. 1a). Compared with the corresponding region of Kasalath, the Nipponbare region harbored a 1,212-bp deletion and several SNPs (Fig. 2b–e). It is likely that this deletion is the FNP for *qSW5* (Fig. 2b–e). We next carried out a complementation test by transforming several Kasalath fragments covering the FNP region into Nipponbare (Fig. 2c). Only transformation of an 11.2-kbp fragment covering the deletion region resulted in thin rice grains; thus, we succeeded in cloning the *qSW5* gene (Fig. 2f and Supplementary

¹Institute of the Society for Techno-Innovation of Agriculture, Forestry, and Fisheries, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki 305-0854, Japan. ²Plant Genome Research Unit, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan. ³QTL Genomics Research Center, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan. ⁴Toyama Agricultural Research Center, 1124-1, Yoshioka, Toyama 939-8153, Japan. ⁵These authors contributed equally to this work. Correspondence should be addressed to T.I. (tizawa@nias.affrc.go.jp).

Received 7 April; accepted 12 May; published online 6 July 2008; doi:10.1038/ng.169

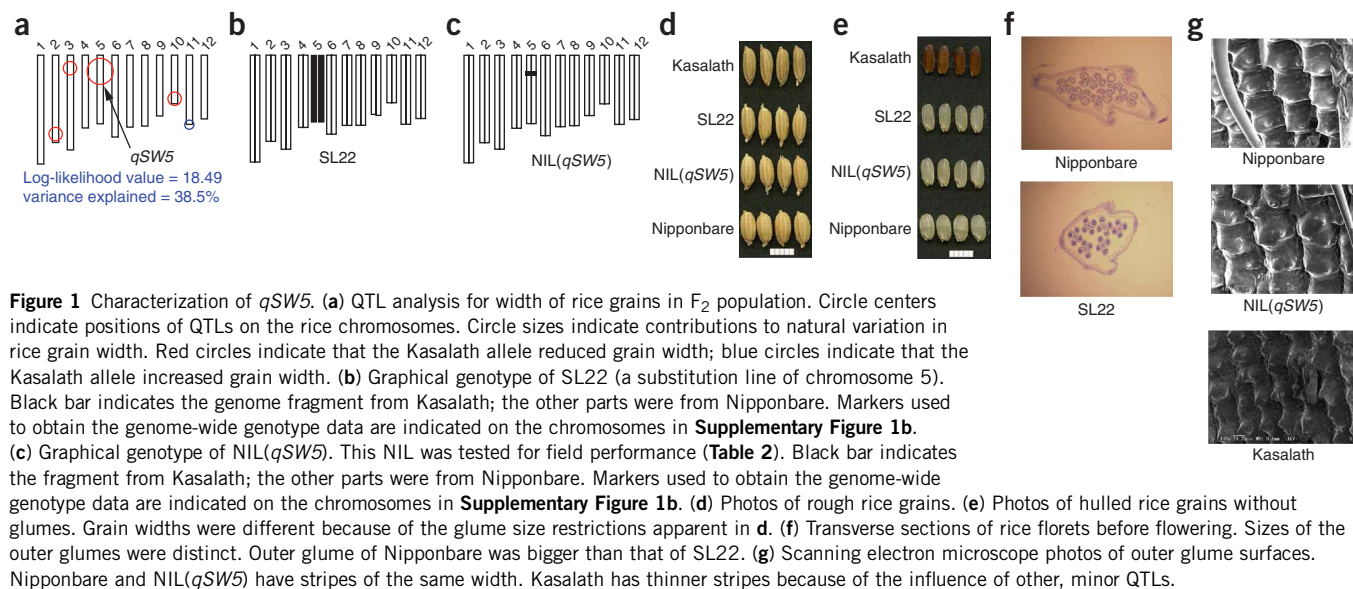


Fig. 2 online). We predicted three ORFs in the 11.2-kbp fragment. RT-PCR analysis of these ORFs identified a putative transcribed ORF, termed ORF1, for the *qSW5* gene product (**Fig. 2d**), although the sequence information of the ORF gave no clues as to the biochemical function of the *qSW5* gene (**Supplementary Fig. 3** online). To confirm that the transcript for the ORF was responsible for the phenotypic changes induced by *qSW5*, we transformed an RNAi construct for ORF1 (**Fig. 2d**) into Kasalath. The seed weight of T0 plants was increased in most of the RNAi transgenic lines (**Fig. 2h**), strongly suggesting that ORF1 is the *qSW5* gene product. We next sequenced the PCR-amplified *qSW5* regions of more than 100 rice landraces, including *japonica* and *indica*, and examined the grain width of each cultivar (**Supplementary Table 1** online). Several haplogroups were identified, and the deletion in the Nipponbare allele of *qSW5* was clearly associated with an increase in rice grain width (**Fig. 2g**), suggesting that the deletion was an FNP that might have been selected by ancient humans during rice domestication. From this finding, together with the evidence from the cloning of *qSW5*, we concluded that *qSW5* is a domestication-related gene in rice. Note that because of the complex population structure of rice, the association of DNA change with some trait changes alone was not enough to conclude that *qSW5* is a domestication gene.

Using the NIL(*qSW5*), we further carried out a field test in a paddy field in Japan (**Table 2**). We found that NIL(*qSW5*) showed more than 10% reduced grain yield (**Table 2**), possibly as a result of reduced grain width (**Fig. 1e**). On the other hand, most of the RNAi lines of ORF1 in Kasalath (**Fig. 2d**) produced seeds with increased grain weight (**Fig. 2h**), suggesting the possible use of the defective *qSW5* allele for a breeding program of new *indica* cultivars.

Two more FNPs of domestication-related genes of rice were identified previously. One FNP was found at the junction of the first exon and intron of the *Wx* (*Waxy*) gene, which encodes a granule-bound starch synthase and controls taste and texture of cooked rice grains¹⁶; natural variations in *Wx* genes have been extensively analyzed in landraces of rice^{17,18}. The other one was an SNP found at the RY-repeat *cis*-element in the promoter region of *qSH1* (*QTL for seed shattering in chromosome 1*), which played a critical role in the loss of seed shattering trait in temperate *japonica*

group¹³. As defective alleles were somehow selected during domestication in these cases, we could judge in current rice landraces whether the genotypes of these three FNPs were still original types or whether they were the defective types resulting from selection. Therefore, we mapped the genotypes of these defective FNPs in various rice landraces (in total 142 cultivars, including a few modern cultivars; **Supplementary Table 2** online) with the local origins (**Fig. 3a**). We further matched genome-wide RFLP data on the 142 rice landraces in order to elucidate the changes in genome structure during domestication (**Supplementary Figs. 4 and 5** online); each dataset contained one of three RFLP genotypes—Nipponbare, Kasalath or ‘other’—for 179 loci distributed genome-wide over the 12 chromosomes¹⁹ (**Supplementary Fig. 4**).

Examination of this genome distance map (**Supplementary Fig. 4**) led us to identify ‘heritage’ *japonica* landraces of rice—that is, landraces that have all the original alleles for the three genes (**Fig. 3b**)—as these FNPs were defective and had occurred as mutations in the past. Because the same mutation rarely occurred several times within the 10,000 years over which rice was domesticated^{6,7,13}, we can consider these mutations as single events in rice domestication. First, most tested *indica* landraces carried Kasalath-biased genome structures and all the original alleles, suggesting that the FNPs were not facilitated

Table 1 Phenotypes of SL22

Traits		Nipponbare (%)	SL22 (%)
Grain width (mm)		3.3 ± 0.1 (100)	2.8 ± 0.2 (85)
Grain length (mm)		7.2 ± 0.2 (100)	7.6 ± 0.2 (95)
Circumference (mm)	Lemma	5.7 ± 0.2 (100)	4.9 ± 0.1 (86)
	Palea	2.6 ± 0.2 (100)	2.5 ± 0.1 (96)
Cell row number of upper epidermis	Lemma	82.7 ± 2.5 (100)	61.5 ± 2.6 (74)
	Palea	32.9 ± 2.4 (100)	30.1 ± 2.1 (91)
Cell number in lemma	Upper	106.0 ± 3.6 (100)	79.3 ± 0.9 (75)
	Lower	157.7 ± 5.6 (100)	126.0 ± 2.4 (80)
Cell number in palea	Upper	44.0 ± 3.3 (100)	42.7 ± 3.1 (97)
	Lower	72.3 ± 2.6 (100)	61.3 ± 2.6 (85)

