Supporting Information

Fujino et al. 10.1073/pnas.0805303105

SI Text: RT-PCR Analysis

DNAS

S A No

RT-PCR experiments were as described by Fujino *et al.* (1). Total RNA (0.5 μ g) was reverse transcribed by ReverTra Ace (TOYOBO) with an Oligo (dT)₂₀ primer. The PCR was performed using KOD-plus (TOYOBO). Each PCR (10 μ l) con-

1. Fujino K, et al. (2008) NARROW LEAF 7 controls leaf shape mediated by auxin in rice. Mol Genet Genomics 279:499–507. tained 0.5 μ l of cDNA template diluted fivefold. The specificity of the primer to the transgene of the functional *qLTG3–1* (F: CGGCTCATGCCCTAGTCC, R: GTGTCGATCGGGCA-TCTT) was confirmed by the sequencing of the PCR products. *Ubi2* was used as a loading control (2).

 Yang G, et al. (2005) A two-edged role for the transposable element Kiddo in the rice ubiquitin2 promoter. Plant Cell 17:1559–1568.



Fig. S1. Expression of *qLTG3–1* measured by semiquantitative RT-PCR analysis. RNA extracted from leaves (*A*), young panicles (*B*), and embryos after 1 day at 30°C (*C*) of Hayamasari (HY), Italica Livorno (IL), the NIL, the vector controls, and the transgenic lines were used. *Ubi2* was used as a loading control in the RT-PCR experiment.

DNAS



Fig. S2. Sequence alignment of qLTG3-1 and related proteins. Red bar indicates the 10 conserved amino acids.



Fig. S3. Phylogenetic tree of qLTG3–1 and related proteins. The phylogenetic tree was constructed using CLUSTAL W. Bootstrap analysis values are shown at the nodal branches. The indicated scale represents 0.05 aa substitutions per site. The accession numbers and plant species are shown.



Fig. S4. Characterization of the morphological changes in seed germination at 15°C. (*A*–*C*) Embryos incubated for 3, 5, and 3 days, respectively, at 15°C. (*D*–*F*) Close up view of an area of the epidermal-side of the epiblast. (*G*–*I*) Close up view of an area on the inner-side of the epiblast. Hayamasari; (*A*, *B*, *D*, *E*, *G*, and *H*), NIL; (*C*, *F*, and *I*).

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Fig. S5. Graphical genotype of the materials. (*A*) BIL116. (*B*) NIL. (*C*) Genotype of the NIL around *qLTG3–1*. The black, white, and hatched regions represent segments of the 12 chromosomes (listed as 1 to 12) derived from Italica Livorno, Hayamasari, and heterozygous, respectively. The mapped markers in panels *A* and *B* are positioned by the chromosome assignment from the high-density restriction fragment length polymorphism linkage map (1). The markers used for the genotype analysis were described in Fujino *et al.* (2). The physical map in panel *C* is based on RAP-DB (http://rapdb.lab.nig.ac.jp/index.html).

Table S1. List of the SSR and STS markers developed and used in this study

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Category	Туре	Marker	Marker position in mapping	Primer		
				Upper (5'-3')	Lower (5'-3')	Position
Fine mapping	SSR	SSR097627-33.1		CCAACAACAAGGCAATCGCG	CGAGGGGGAAAAGGGCTAGA	67,539
	SSR	SSR097627-22.1		TGAGTTTGGAGTGATTGGAT	CTCAAAGAATGACACCGATG	99,844
	SSR	GBR3001a	а	CCTCTTCCCTTCTTGTGTCA	GGGATTTTTTCATCGAAATT	117,791
	SSR	SSR125411-4.1	A	GATCGATCGACATTACACAC	GCATGCATGGACTAGTAATT	157,388
	SSR	SSR118673-3.1	D	CAATTAAGTTAACCCGATGA	GCTTGTTGCTGTTCTGTACT	196,244
	STS	STS73–28	I	GCTTATCCGATTCCGTCTGCGGTTA	TTGAGACATGCCTAATTAAGCGAAC	253,333
	SSR	SSR107224-21.1		TTAGGTAAAATTAAGGCACC	TCTGTTGTAGGTGTAGCAGC	359,550
	SSR	SSR107224-13.1	b	ATTTGTGTTGCTGCATGCAG	ACTCGATCTCGTGTGTGCCA	370,778
	SSR	SSR113930-23.1	с	GCAACTCTGCTAAACGAATT	TAGCCCCATGATAAGAGATT	514,861
	SSR	SSR105363-21.1		GCTCGCTCCCCACATTTTAA	GGCATCAGCAACAGCAGCTA	1,134,844
	SSR	GBR3002a	d	AGAGCATAACATCAAAGCCA	ATAGCTCCAATTCGATCTTC	1,322,466
High-resolution mapping	SSR	S70		AGGGCTAAGTCGGAAGAATCAT	GGAGTCGTGGGGGTCGGTGT	157,592
	SSR	S65		CATATTCAAAATAGCTAAGGGAGC	CGCACACATACAAGAGTTTTACT	160,269
	indel	S51a		TCAGCAAATATCATCTCCCA	GTGTCACCCTAGTGAAAAAATTT	165,027
	SNP	\$51b		TCAGCAAATATCATCTCCCA	GTGTCACCCTAGTGAAAAAATTT	165,053
	SSR	S57	В	CTCACATTCCCTTGCTATGCT	CCATCAATTAATTCTTCCGATC	17,349
	indel	S21a		TGAAAATACACGCATGGCTG	GAGAGCGAATGCGCTGCTTC	174,798
	SSR	S21b		TGAAAATACACGCATGGCTG	GAGAGCGAATGCGCTGCTTC	174,986
	SNP	S43	С	TTAATCCATGGAAGTTAAAGAATAT	GTCCATGATTAGCTATAAGTGCTAC	191,822
	indel	S103a	E	CAGCTAAGCTACCAAAAGCCCA	TTATCAGCCCATTCAGCACGTT	198,461
	SNP	\$103b		CAGCTAAGCTACCAAAAGCCCA	TTATCAGCCCATTCAGCACGTT	198,464
	SSR	S107	F	CGCACGCGTATATTTGAATG	CAGATTAAATGGTTAGTTAACCGGC	200,192
	SSR	S306	G	ACATGCATGCAGTGATTTCG	CTACTGCTCATCACTACAAAGAGTG	230,403
	SSR	S179	Н	CGGCGATGGTTAGTTAAATTATCC	CAAGCTAGGCAAAAGGTGGTATT	233,798
	SSR	S218		GTTAGTCATATCAGCCCCAAGAAC	ATTTGCCCATAAACTACCGCAC	253,122

Position indicates the location of the upper primer in IRGSP build 3 in RAP-DB. SSR, simple sequence repeat; STS, sequence-tagged site.